Kelt Reconditioning and Reproductive Success Evaluation Research

Project Number 2007-401-00

Report covers work performed under BPA contract # 65416 and 69335

Report was completed under BPA contract #69335

Report covers work performed from: January, 2015 – December, 2015

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Report Created: March, 2016

“This report was funded by the Bonneville Power Administration (BPA), U.S. Department of Energy, as part of BPA's program to protect, mitigate, and enhance fish and wildlife affected by the development and operation of hydroelectric facilities on the Columbia River and its tributaries. The views in this report are the author's and do not necessarily represent the views of BPA.”

This report should be cited as follows:

Hatch, D., Branstetter R., Stephenson J., Pierce A., Matala A., Lessard R., Bosch W., Everett S., Newell J., Graham N., Medeiros, L., Jenkins L., Tall Bull, T., Elliott, M., Huggler, K., Cavileer T., Nagler, J., Fiander M., Frederickson C., Blodgett J., Fast D., Whiteaker J., Johnson R. Kelt Reconditioning and Reproductive Success Evaluation Research. 1/1/2015 - 12/31/2015 Bonneville Power Administration Annual Report, 2007-401-00.

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Abstract

The Kelt Reconditioning and Reproductive Success Evaluation Project is a research, monitoring, and evaluation (RM&E) uncertainties category project funded through the Columbia Basin Fish Accords. The objectives are to evaluate methodologies to produce viable artificially reconditioned repeat steelhead spawners and to determine the productivity of repeat spawners. Work occurs in the Yakima and Snake river basins. We focused on collecting steelhead kelts at juvenile bypass facilities in Prosser and Lower Granite dams, and additionally some fish were collected at Dworshak National Fish Hatchery, and the Fish Creek weir. These kelts were reconditioned (given prophylactic treatments and fed a specially formulated diet) at Prosser and Dworshak National fish hatcheries. Survival of long-term reconditioned kelts has been 40% (16 years) for Yakima River at Prosser Hatchery and at Dworskak Hatchery 28% (4 years) for hatchery origin fish, 32% (4 years) for mixed stock collections at Lower Granite Dam, 27% (2 years) for South Fork Clearwater collections, and 34% (2 years) for Fish Creek collections. Using estradiol assays, we have established that steelhead rematuration rates vary annually and spatially and range from 10.4% to 80.0%. We determined that kelts can remature as consecutive or skip spawners, typically returning to spawn in 5 or 6 months after kelting or 17 to 18 months later. We characterized the outmigrating Snake River kelt run as primarily composed of Salmon, Grand Ronde, and the Imnaha populations based on GSI analysis at Lower Granite Dam. A total of 24 reconditioned B-run steelhead were released below Lower Granite Dam in 2015 to address Reasonable and Prudent Alternative 33 of the FCRSP Biological Opinion. We air-spawned a group of maiden Dworshak Hatchery steelhead in 2015. These fish were then reconditioned and rematuring fish air-spawned as consecutive repeat spawners in 2015 to compare performance between maiden and repeat spawnings. Repeat spawners relative to maiden spawners had higher fecundity, larger eggs and similar fertilization rates. Reproductive success of reconditioned steelhead was confirmed in the Yakima River once again with assignments of 42 juvenile fish to 11 unique parents. Lifetime reproductive success for reconditioned kelt steelhead was estimated as 2.06 relative to single time spawning steelhead. Mature reconditioned steelhead kelts were stocked in the Cle Elum Hatchery Spawning Channel in 2015, to evaluate the feasibility of using the facility to evaluate reproductive success in a more controlled setting. A population model was further developed to provide a means to the management implications of a kelt reconditioning program. The model mimics iteroparity in ways explicit to body condition, reconditioning, and release method. We have shown that repeat spawners could contribute up to 10% of spawning if sufficient kelts are captured and reconditioned, consistent with existing data on survival and maturation rates and estimates of repeat spawner fecundity. This modeling tool provides the means to examine several questions regarding potential avenues for recovery, and management options for doing so. We conducted feed trials with cooperation of the USDA Aquaculture research group from Bozeman, MT and found that the feed produced shows promising results with kelts increasing in lipid levels. Our team has published 9 manuscripts, 1 manuscript is in press, and 3 papers are currently in review. Additionally, the team gave 11 professional presentations in 2015.

Acknowledgments

We would like to thank our partners at the Upper Yakima Kelt Reconditioning Program Tom Scribner, Keely Murdoch, and Matt Abrahamse for sharing data and sampling with our team. Many thanks to the Lower Granite Dam capture and sampling crews (Nez Perce, Idaho Department of Fish and Game (IDFG), University of Idaho, and Army Corps of Engineers). This study and report benefited from resources provided by Christine Moffitt, Jessica Beulow and Zach Penny. Thank you to Nick Hoffman, Stephanie Harmon and Megan Moore for diligent and reliable laboratory processing and collection of genotypic data. Data and information provided by Mike Ackerman and Craig Steele from IDFG, and Maureen Hess from CRITFC made a substantial contribution to these analyses. Thank you to the Washington Department of Wildlife staff at the Genetics lab in Olympia for sharing samples with us and to the field crews Anthony Fritts, Chad Stockton for their help in the Yakima Basin. Assistance was provided for radio tracking by Conan Northwind, Ryan Deknikker, Bill Flett, Rene Tule at Yakama Nation, Mark Nelson at USFWS, and Zack Mays at Washington Department of Fish and Wildlife. Also, we appreciate the help from Tim Resseguie and his team at Yakama Nation for helping us with electroshocking and genetic collection. We would like to thank our colleagues at CRITFC: Bobby Begay, Jeff Fryer, Denise Kelsey, Melissa Edwards, Nicole Tursich, Henry Franzoni, David Graves, Joe Nowinski, Scotty Riddle, Hazel Schaffer, Buck Jones, Jayson FiveCrows, Agnus Strong, and Percy Brigham. @ Yakama Hatchery and field staff (Zack Mays, T. Newsome,) WDFW personnel (Gabe Temple, Anthony Fritts, Chad Stockton, Tim Webster). Dr. Rick Barrows, Jason Frost, and Carley Brentenson at USDA Agricultural Research Services Bozeman, MT. Charlie Strom. Many thanks to the LGD sampling crew (Nez Perce, IDFG, University of Idaho).

Executive Introduction

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 CRB were in the Kalama River (tributary of the un-impounded lower Columbia River), which exceeded 17% (NMFS 1996). A total of 8.3% of the adult steelhead from Snow Creek, WA were identified as repeat spawners based on scale samples (Seamons and Quinn 2010). In Hood River, repeat spawning summer run steelhead comprise on average 5.7% of the run based on scale pattern analysis (Olsen 2008). Iteroparity rates for Klickitat River steelhead were reported at 3.3% from 1979 to 1981 (Howell et al. 1985). Summer steelhead in the South Fork Walla Walla River expressed 2% to 9% iteroparity rates (J. Gourmand, ODFW, pers. comm.). Hockersmith et al. (1995) reported that repeat spawners composed 1.6% of the Yakima River wild run.

The Kelt Steelhead Reconditioning and Reproductive Success Evaluation Project (BPA Project Number 2007-401-00) 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 (kelt) steelhead, first it assesses reconditioning processes and strategies, and second, it measures reproductive success of artificially reconditioned kelt steelhead. The project specifically addresses Reasonable and Prudent Alternatives (RPAs) 33 and 42 (NMFS 2008). RPA 33 requires the Action Agencies to develop and implement a Snake River steelhead kelt management plan designed to provide at least a 6% improvement in B-run population productivity. Toward that goal, a variety of approaches are being tested and implemented including passage improvements and reconditioning kelt steelhead. RPA 42 focuses on the reconditioning component and seeks to preserve and rebuild genetic resources through safety-net (kelt reconditioning) and mitigation actions to reduce short-term extinction risk and promote recovery.

The Independent Scientific Review Panel (ISRP) in 2014 issued a memorandum (ISRP 2014-9) reviewing the progress of project 2008-458-00, a sister kelt reconditioning program in the Upper Columbia region. The ISRP review listed five areas for research to address including:

1. Establish methods to assess how kelt reconditioning may benefit population growth, abundance, spatial structure and diversity;
2. Clarify how many juvenile and F1 adults should be sampled to detect meaningful differences in the breeding and reproductive success of HOR, NOR, and reconditioned NOR females;
3. Develop and implement methods to assess the fat levels, maturation timing, fecundity, egg size, and gamete viability of reconditioned kelts,
4. Monitor homing and straying rates of reconditioned kelts; and,

5. Experiments are needed to discover the best geographic locations and times of the year for release of the project's reconditioned fish.

We are organizing our report into five chapters using these topics deemed important by the ISRP to create a document that tracks progress in those areas and where appropriate we are integrating RM&E reportable work elements from our project 2007-401-00 statement of work. All of our RM&E work elements are uncertainties research.

Methods

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

Study Area

Steelhead Kelt Collection, Reconditioning, and Release Sites

Currently, steelhead kelt collections occur at 4 primary locations throughout the CRB: the Chandler Juvenile Monitoring Facility (CJMF) in Prosser, WA (Yakima River), Lower Granite Dam (LGR), WA (Snake River), Dworshak National Fish Hatchery (DNFH) at Ahsahka, ID (Clearwater River), and at Fish Creek weir (tributary of the Lochsa River). Collections of steelhead kelts also occurred from 2002-2013 at the Omak Creek weir near Omak, WA and from 2006-2012 steelhead were captured at the Powerdale Dam trap/East Fork Hood River weir near Hood River, OR, and at Shitike Creek 2005-2009 , those and other historic collection sites are reported in Table (1) and Figure (1). Generally, downstream moving kelts are captured in the juvenile bypass facilities such is the case at CJMF and LGR facilities or captured via weir-trap box in the case of Fish, Omak, and Shitike creeks, while maiden steelhead were captured in upstream traps at DNFH, Powerdale Dam, and the East Fork Hood River weir and air-spawned. The collections at DNFH, Powerdale Dam and the East Fork Hood River typically occur in January-March, while collection at the remaining sites (CJMF, LGR, Fish Creek, and Omak Creek) occur(ed) in the spring (late-March through early-June). With the exceptions of CJMF and DNFH all kelts are truck transported to reconditioning facilities. Releases have occur currently at Prosser just below Prosser Dam and in the Snake just below Lower Granite Dam. Historically, releases have been conducted in the Lower Columbia River, the Okanogan, and in Shitike Creek. For a more thorough description of both the current and historical collection, reconditioning, and release sites see Hatch et al. 2015, Hatch et al. 2013, Hatch et al. 2012, and Branstetter et al. 2008.

Table 1. Kelt steelhead collection, reconditioning, release, and juvenile collection sites used in this study.

Site Number	Site	Drainage	Location	Collection site	Reconditioning site	Release Site	Juvenile Sampling Location	Dates of use
1	Chandler Juvenile Monitoring Facility (CJMF)	Yakima River	RK 75.6	Yes	-			1999-2015
2	Yakama Nation Prosser Hatchery	Yakima River	RK 75.6	-	Yes	Yes		1999-2015
3	Lower Granite Dam Juvenile Bypass	Snake River	RK 173	Yes	-	Yes		2009-2015
4	Dworshak National Fish Hatchery	Clearwater River	RK 65	Yes (hatchery fish for experimental purposes)	Yes	-		2009-2015
5	South Fork Clearwater	Clearwater River	RK 0 - 100	Yes	-	-		2013, 2015
6	Fish Creek Weir	Lochsa River	RK 0.8	Yes	-	-		2014, 2015
7	Omak Creek Weir	Okanogan River	RK 0.8	Yes		-	Yes	2003-2013
8	Bonaparte Creek	Okanogan River	RK 0.4	Yes		-		2003-2014
9	Cassimer Bar Hatchery	Okanogan R./ Columbia R.	RK 0/ 859	-	Yes	Yes		2003-2010

10	St. Mary's Acclimation Ponds	Okanogan River	RK 8.0	-	Yes	-		2011-2013
11	Powerdale Dam	Hood River	RK 6.4	Yes	-	-		2006-2010
12	East Fork Weir	East Fork Hood River	RK 20.1	Yes	-	-		2011-2013
13	Parkdale Hatchery	Middle Fork Hood River	RK 5.6	-	Yes	-		2006-2013
14	Shitike Creek Weir	Deschutes River	RK 0.7	Yes	-	-		2005-2008
15	Warm Springs Hatchery	Warm Springs River	RK 16	-	Yes	-		2005-2008
16	Hamilton Island	Columbia River	RK 231	-	-	Yes		2002-2008, 2010,2011, 2014
17	Westport	Columbia River	RK 72	-	-	Yes		2010, 2011
18	Aldrich Point	Columbia River	RK 75.6	-	-	Yes		2010, 2011
19	Cle Elum Spawning Channel	Yakima River		-	-	Yes (experimental group)	Yes	2015
20	Satus Creek	Yakima River		-	-	-	Yes	2008-2015
21	Toppenish Creek	Yakima River		-	-	-	Yes	2008-2015
22	Simcoe Creek	Yakima River		-	-	-	Yes	2008-2015
23	Ahtanum Creek	Yakima River		-	-	-	Yes	2008-2015

24	Big Creek	Yakima River		-	-	-	Yes	2008-2015
25	Cowiche Creek	Yakima River		-	-	-	Yes	2008-2015
26	Little Rattlesnake Creek	Yakima River		-	-	-	Yes	2008-2015
27	Nile Creek	Yakima River		-	-	-	Yes	2008-2015
28	Quartz Creek	Yakima River		-	-	-	Yes	2008-2015
29	Bumping River	Yakima River		-	-	-	Yes	2008-2015

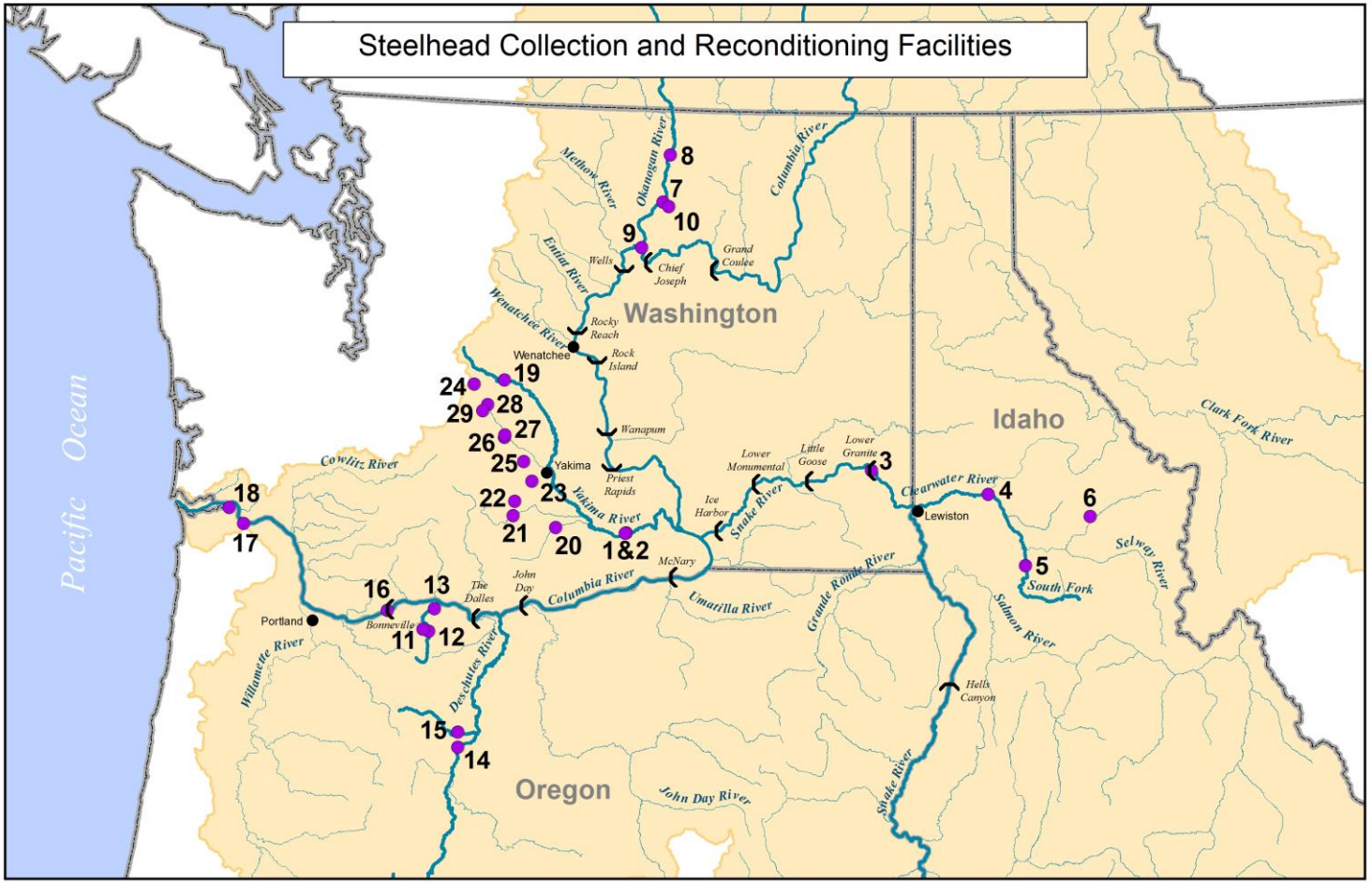


Figure 1. Map of Steelhead kelt Project area 2000-2015.

The Yakima River is approximately 344 km in length and enters the Columbia River at RK 539. The basin is 15,928 km² and average discharge is 99 m^3/s. Summer steelhead populations primarily spawn upstream from Prosser Dam in Satus Creek, Toppenish Creek, Naches River, and other tributaries of the Yakima River (TRP 1995).

Yakima River Basin

The Yakima River is approximately 344 km in length and enters the Columbia River at RK 539. The basin is 15,928 km² and average discharge is 99 m^3/s. Summer steelhead populations primarily spawn upstream from Prosser Dam in Satus Creek, Toppenish Creek, Naches River, and other tributaries of the Yakima River (TRP 1995).

Chandler Juvenile Collection Facility (Yakima River)

Post spawn steelhead migrating downriver are inadvertently collected by way of the Chandler Juvenile Monitoring Facility (CJMF a.k.a Chandler Juvenile Evaluation and Monitoring Facility CJEMF)) which diverts migratory fishes away from the irrigation canal.

Yakama Nation 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 as well as experimental rearing of white sturgeon *(Acipenser transmontanus)* and Pacific lamprey (*Entosphenus tridentate*).

Cle Elum Research Facility

The Cle Elum Supplementation and Research Facility (CESRF) was built in 1997 to research the effects of supplementation programs on the Upper Yakima near the town of Cle Elum, WA . In 2000, an artificial stream 127m x 7.9 m wide was built at the CESRF.

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. Steelhead spawn naturally throughout the lower portion of the basin with the vast amount of “B-run” steelhead produced at the Dworshak National Fish Hatchery found on the Clearwater River.

The Lower Granite Juvenile Fish Facility

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 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).

Dworshak National Fish Hatchery

Kelt reconditioning facilities are located at Dworshak National Fish Hatchery (DNFH) in Ahsahka, Idaho. 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 (USFWS 2009).

Fish Creek

Fish Creek is a tributary of the Lochsa River which is part of the greater Clearwater subbasin that feeds into the Snake River basin. This stream system is primarily dominated by both resident and anadromous O. mykiss (Copeland et al. 2013). The anadromous run are considered b-run type steelhead.

South Fork Clearwater River

Is a tributary of the Snake River and is part of the larger Clearwater River subbasin. Historically it was estimated that this was one of the largest salmon bearing streams in the Pacific Northwest. This subbasin also produces b-run type steelhead.

Chapter 1: Establish methods to assess how kelt reconditioning may benefit population growth, abundance, spatial structure and diversity.

1.A: Steelhead Kelt Collection and Reconditioning

Introduction

Kelt steelhead reconditioning process evaluations involve fish culturing practices, studying alternative management strategies, and implementing research scale reconditioning programs. Adding repeat spawner steelhead to the population through reconditioning can add stability through the portfolio effect (Moore et al. 2014) and increase population abundance by increasing lifetime reproductive success (Seamons and Quinn 2010). We established "control" groups in both the Snake and Yakima rivers. These control groups were downstream migrating kelts, systematically collected, PIT tagged and released back into the river each year. These fish are monitored via PITAGIS to determine how successfully they naturally recondition in the ocean.

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. 2002 and Hatch et al. 2003b) and summarized in Hatch et al. 2013b. These fish are typically released in the fall to over-winter and return to the spawning sites volitionally. This chapter recaps 2015 kelt collection efforts for a broader review of specific fish culturing practices see (Hatch et al. 2015).

Methods

Standard Data Collection

All captured steelhead are scanned and recorded for existing PIT-tags, biological data is collected which includes determination of kelt/maiden status, fork length, weight, condition factor (color and presence/absence of wounds/skin-body condition), coloration rating (bright, medium, dark), notation of clipped or non-clipped fins (typically adipose), and small (typically a 1 x 1 mm) tissue sample (caudal fin clip) is collected for genetic analysis. Steelhead without a PIT-tag will unless moribund, will receive a 12.5 mm PIT-tag injected into the pelvic girdle to track migration history and to determine reconditioning efficacy. All releases or mortalities are recorded, which

includes date of event, condition factor, and PIT-tag identifier. In the case of a lost PIT-tag, typically at time of release, fish are retagged and an additional genetic sample collected.

Steelhead Kelt Collection

Chandler Juvenile Monitoring Facility
Once diverted into the CJMF (Table 1, site 1), emigrating kelts are manually collected from a fish separation device (a device that allows smaller juvenile salmonids to "fall through" for processing in the juvenile facility while larger fish can be dipnetted for processing and input to reconditioning tanks at Prosser Hatchery (Table 1, site 2). Yakama Nation staff monitored the Chandler bypass separator during the kelt migration.

Lower Granite Dam
Steelhead kelts entering the juvenile bypass separator (Table 1, site 3) are collected by Army Corps of Engineer (COE) staff. Kelts are netted off the adult fish separator bars and moved to a fish hopper that led into the kelt receiving tank. Staff from the Nez Perce Tribe (NPT), University of Idaho (UI), and CRITFC processed fish diverted into the receiving tank by the USACE.

Both B-run ($\geq$ 70 cm) and A-run (<70 cm) steelhead are selected. Our determination differs from the TAC cutoff at 78cm (Busby et. al., 1996) based on evidence that this size distinction does not fit the size distribution of the population. This determination is reinforced, based on our own analysis of the kelt run length data, which suggests that a bimodal size distribution in kelts existed at 63cm (Hatch et al. 2015).

Fish Creek
A picket weir operated by IDFG (Table 1, site 6) is used to interrogate upstream and downstream migrants. The Nez Perce Tribe/CRITFC processed captured female kelts and then either transported kelts to Dworshak National Fish Hatchery for long-term reconditioning or released downstream of the trap.

South Fork Clearwater River
Fish are hook and line collected by volunteer fisherman (Table 1, site 5). Fishermen then store these fish in holding tubes that are then later collected by IDFG staff (Osborne 2015) and transported to Dworshak National Fish Hatchery.

Transport to Dworshak from Lower Granite Dam, Fish Creek, and South Fork Clearwater.
Fish destined for DNFH (Table 1, site 4) were dipped netted from the adult holding tanks at Lower Granite Dam (Table 1, site 3) and trap box at Fish Creek (Table 1, site 6) then placed in a transport truck. Nets were large enough to handle active adult steelhead and consisted of a soft cotton or natural fiber mesh. The transport truck had a 1.5-kiloliter tank fitted with supplemental regulated, compressed oxygen that was fed via air

stones; also a 12-volt powered tank aeration pump was used to circulate oxygenated water. Stress Coat® or PolyAqua® was used to replace the natural protective slime coating that may have been compromised by handling. In addition, salt was added to reduce osmo-regulatory 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. South Fork Clearwater (Table 1, site 5) fish were collected by IDFG and transported by truck to Dworshak National Fish Hatchery.

Dworshak National Fish Hatchery (Brood Air Spawning)
Fish either volitionally entered the adult ladder at the DNFH (Table 1, site 4) or were brought from the South Fork Salmon collection. They are then crowded mechanically into collection baskets and anesthetized in tricaine methanesulfonate (MS-222) or Aqui-S® (clove oil). However, several of the air-spawned fish had been anesthetized with carbon dioxide during the previous weeks for ladder counting and fish sorting. Carbon dioxide presents sub-lethal stresses that are likely to be adverse to survival of the kelts (Iwama et al 1989). 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.

Steelhead are air-spawned at the DNFH to augment the number of fish for reconditioning experiments (Section 3.B) (Monitoring Methods). Selected fish were transferred to an area set aside for the air-spawning procedure (Lietritz and Lewis 1976). Low-pressure compressed air was injected into the fish using a 20-gauge needle. Eggs were allowed to flow freely with some gently applied manual pressure to obtain the remainder. Each female's eggs were collected in a bucket with a distinct identification tag. Standard fish health sampling occurred on these fish to meet the DNFH spawning criteria routinely employed at the hatchery, this included ovarian fluid and genetic sampling. A majority of the eggs were fertilized and incorporated into DNFH production. Eggs not used by DNFH were treated with iodine, rinsed and frozen. Standard data collection procedures were followed with the addition of blood sampling and body lipid levels recorded.

Fish not selected for reconditioning were air-spawned, PIT tagged and released into the mainstem Clearwater River after a three day recovery period.

Long-term Reconditioning
Long-term reconditioning is a management strategy where emigrating kelt steelhead are collected and held in large tanks, given prophylactic treatments and fed a specially formulated diet for approximately 6 months (Hatch et al. 2013b). After 6 months, the "reconditioned" kelts are released back into the collection river as the run at large is returning from the ocean. These reconditioned fish generally mingle with the run at large and proceed to in-river, over-winter locations and spawning grounds in the spring. This strategy seeks to reduce mortality in the hydrosystem and ocean, providing another opportunity for fish to reproduce in the wild. Techniques used in kelt reconditioning

were initially developed for Atlantic salmon *Salmo salar* and Brown or Sea-trout *S. trutta*, and a review of these studies and others applicable to steelhead kelts are summarized in Evans et al. (2001).

Results/Discussion

Steelhead Kelt Collections

Large numbers of kelt steelhead are available for collection at many sites across the Columbia River Basin. These sites generally are associated with juvenile bypass systems or weirs. For example, from 2000-2015 we captured a total of 13,653 downstream migrating kelts at an CJMF, on average about 26% of each annual wild steelhead return. In 2015, steelhead kelts were quite abundant across the basin. We collected 1,098, 22, 83, and 35 at CJMF, Lower Granite Dam, Fish Creek Weir, South Fork Clearwater River, respectively (Appendix A1a). Additional fish were available at Lower Granite Dam but our reconditioning capacity was already met. Later in the year, we installed two 20' circular tanks at Nez Perce Tribal Hatchery to increase capacity.

Long-term reconditioning survival averaged 40% at the Prosser Fish Hatchery (PFH) over the last 16 years (Hatch et al. 2013b). The reconditioning survival rate has been more variable for the past 4 years at DNFH with an average of 28% for the hatchery fish, 32% for the fish captured at LGR, 27% for fish from the South Fork Clearwater, and 34% for the Fish Creek kelts. We conducted reconditioning experiments at other sites but subsequently discontinued efforts after completing objectives at the St. Maries site in Omak, WA, Shitike Creek at Warm Springs National Fish Hatchery, and the Parkdale Fish Facility (Hood River, WA), where long-term reconditioning survival averaged 15%, 5%, and 36%, respectively See Appendix A1.a for annual data.

Low survival at DNFH resulted from water quality issues in the early years and also obtaining/training staff that have experience with fish culturing skills and training them in reconditioning techniques. This site has had continuous improvement every year since its inception.

We evaluated the traits and survival to release of reconditioned kelt steelhead *Oncorhynchus mykiss* in the Yakima River (Washington State, USA) and published the analysis in the North American Journal of Fisheries Management in 2013 (Hatch et al. 2013b). Reconditioned steelhead kelts were predominantly (>92%) female. Annual survival to release ranged from 18% at the start of the program to an annual high of 62% and averaged 40% over the course of the study with surviving reconditioned kelts showing increases in fork length, weight, and Fulton's K condition factor. Kelts in good condition and those with bright coloration at the time of collection were more likely to survive. Post-release upstream migration timing of reconditioned kelts was spread out over several months and correlated well with run timing of upstream pre-spawn migrants. The empirical results we observed demonstrate the potential of kelt reconditioning to provide recovery benefits for ESA-listed, repeat spawning steelhead populations in highly developed river systems. See Appendix A1.a for annual data.

Summary Research-Scale Efforts to Address RPA 33

At DNFH we are conducting research detailed in other sections and working toward addressing RPA 33 for the Hydro system Biological Opinion. RPA 33 requires the Action Agencies to develop, in cooperation with regional salmon managers, and implement a Snake River steelhead kelt management plan designed to provide at least a 6% improvement in B-run population productivity (NMFS 2008, 2010, and 2014). Toward that goal, a variety of approaches are being tested and implemented including passage improvements and reconditioning kelt steelhead.

Since we are operating at a research scale, as approved by the ISRP in the 2008 review, the capacity of our facility is much too small to meet the RPA 33 goal of increasing the LGR ladder count of B-run steelhead by 6%. However, we have demonstrated the feasibility of reaching the 6% goal. In 2013, we released 69 reconditioned B-run steelhead (approximately 40% of RPA 33's goal). In 2015, we released 24 reconditioned B-run steelhead below Lower Granite Dam in association with RPA 33, an additional 21 fish were determined to be skip spawners and retained for release in 2016. Table (1A.1) summarizes all collections and releases associated RPA 33.

Table 1A.1. Summary of fish collections and releases in the Snake River associated with RPA 33.

Year	Collection Location	Number of Fish Collected	Number of Fish that Survived Reconditioning	% Survival	Consecutive Spawner Release	Number of Fish Retained	Mature Skip Spawners Released (Capture Year)	Total Release by Year
2011	Lower Granite Dam	111	2	1.80%	2*	-	-	-
2011	S.F. Clearwater	-	-	-	-	-	-	-
2011	Fish Creek	-	-	-	-	-	-	-
2011 (subtotal)		111	2	1.80%	2	-	-	2
2012	Lower Granite Dam	124	10	8.10%	10	-	-	-
2012	S.F. Clearwater	-	-	-	-	-	-	-
2012	Fish Creek	-	-	-	-	-	-	-
								-
2012 (subtotal)		124	10	8.06%	10	-	-	10

2013	Lower Granite Dam	110	57	51.80%	57	-	-	-
2013	S.F. Clearwater	24	12	50.00%	12	-	-	-
2013	Fish Creek	-	-	-	-	-	-	-
2013 (subtotal)		134	69	51.50%	69	-	-	69
2014	Lower Granite Dam	110	34	30.90%	34	-	-	-
2014	S.F. Clearwater	-	-	-	-	-	-	-
2014	Fish Creek	12	3	25.00%	1	2	2	-
2014 (subtotal)		122	37	30.30%	35	2	2	35
2015	Lower Granite Dam	22	11	50.00%	8	3	TBD	
2015	S.F. Clearwater	35	7	20.00%	4	3	TBD	
2015	Fish Creek	83	25	30.10%	10	15	TBD	12
2015 (subtotal)		140	43	30.70%	22	21	TBD	24
Grand Total		631	161	25.52%	138	23	TBD (2)	**140**

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

Introduction

Kelt is the term used to describe steelhead trout (*Oncorhynchus mykiss*) that survive after spawning. This ability is a necessary stage of an iteroparous life history and is unique to *O. mykiss* among all Pacific salmon. The demographic benefit of an iteroparous life history is realized when kelts migrate to the ocean and successfully complete one or more subsequent spawning migrations. Kelts are found throughout the Snake River Basin, but their spatial distribution or occurrence among watersheds is highly variable. Rates of iteroparity or repeat spawning in the Snake River are highest among populations characterized by smaller, 1-ocean age individuals (A-run). Conversely, repeat spawning is less frequent among B-run steelhead which are characterized as larger, 2-ocean age individuals (Narum et al. 2008). We used multilocus genotype data at single nucleotide polymorphism (SNP) loci to conduct an analysis of genetic stock composition among kelt steelhead sampled at Lower Granite Dam (LGD) between 2009 and 2013. The objective of this study was primarily to estimate stock proportions in a mixed stock sample, providing a better understanding of the origins of post-spawn steelhead among the major subbasins (e.g., Clearwater River, Salmon River, Grande Ronde) and major population groups (MPG's) within the Snake River Basin. Results will provide managers with valuable information about the relative behaviors and population demographics exhibited by genetically assigned kelt stocks.

Methods

Sampling and genotyping

The majority of kelt steelhead emigrating from tributaries of the Snake River pass downstream of Lower Granite Dam through a removable spillway weir (Colotelo et al. 2014). A large bypass system directs approximately 4-6% of the total annual kelt emigration into a juvenile fish facility (JFF). All kelts entering the JFF were sampled from 2009 to 2014, and sampling occurred over the entire emigration period from late March to late June (Hatch 2015). This included a total of 5,712 natural-origin kelts and 2,642 hatchery-origin kelts (Tables 1B.1 & 1B.2). Caudal fin or opercule punched tissue samples were stored dry on whatman paper (LaHood et al. 2008). Biological data recorded during kelt sampling included: tag detection and presence of physical marks (i.e. indicating hatchery origin), sample date, fork length (FL), gender and condition rating of "poor", "fair" or "good". The rating protocol (see Buelow 2011; Hatch et al. 2015) was predetermined with specific criteria, and uniformly scrutinized, based largely on the presence and severity of fungus and injury, along with other aspects of physical appearance.

Detailed methods for genomic DNA extraction, DNA amplification, and single nucleotide polymorphism (SNP) genotyping are described in Ackerman et al. (2012) and Hess et al. (2012). Individuals were genotyped at a sex-linked assay (identifying genetic sex), and three species diagnostic markers to identify Cutthroat Trout (*O. clarki*) or O. *mykiss* x O. *clarki* hybrids. Genotypes collected at an additional 188 autosomal SNPs (Hess et al. 2012) were used to conduct GSI analyses. Two comparable platforms were used to genotype SNP loci: 1) Fluidigm 96.96 Dynamic Array IFCs (chips), imaged on a Fluidigm EP1^{TM} system, and analyzed using Fluidigm SNP Genotyping Analysis Software, and 2) a next generation sequencing technique "GT-seq" developed by Campbell et al. (2015). Data quality and data compatibility between methods was verified through concordance testing using 90 randomly selected individuals, with side-by-side genotype score confirmation. Kelts were initially identified as natural-origin based on the absence of observed physical marks or tags (e.g., adipose fin clip, fin erosion, or coded wire tag detection). Where possible, origins were confirmed through parentage based tagging (PBT) analyses. A subset of 96 previously described SNPs were designated for use in PBT as described by Steele et al. (2012) and Steele et al. (2013). The PBT reference baseline includes all hatchery broodstocks in the Snake River, and involves tissue sampling and genotyping of every spawned parent pair among broodstocks. The method effectively "tags" all hatchery progeny, which can then be identified through their genotypes regardless of where or when (e.g. life stage). Because the PBT baseline was not established until broodyear 2008, its utility in the screening process was limited to broodyear 2011 and later (i.e. age3+ kelts). Parentage analyses were conducted using the program SNPPIT v1.0 (Anderson 2010a; https://github.com/eriqande/snppit/commits/master). Assignments between progeny and parent pairs were accepted at a threshold LOD>14 to ensure highest posterior probability, and were consistent with Mendelian compatibility (i.e., no mismatches) in assigned parent-offspring trios (Anderson 2010a). All fish identified as hatchery progeny via PBT analysis were deemed to be of "known" origins (i.e. broodstock program) regardless of initial LGR field identifications. Ocean ages of hatchery kelts were determined based on PBT parental brood year, and resulting size-at-age distributions were used to infer age of natural-origin kelts for which no age data were available.

Table 1B.1. Age and length statistics for hatchery-origin kelts (2011-2014). Known stock origins were determined via PBT, and ocean age was calculated based on parental brood year. The corresponding GSI reporting groups (RG) are shown for each hatchery stock. Age proportions are shown as % total, and fork length (FL) is the mean size at age. These values were used to infer age of natural-origin kelts.

		PBT assignment			1-ocean		2-ocean	
PBT stock	RG	(n)	%	mean FL	%total	FL	%total	FL
Female kelts								
CGRW	GRROND	203	0.11	59.6	69%	56.3	31%	67.3
WALL	GRROND	75	0.04	59.3	67%	56.5	33%	65.0
LSCR	IMNAHA	75	0.04	57.9	79%	55.6	21%	66.2
LYON	LSNAKE	5	0.00	56.4	**100%**	56.4	na	na
TUCW	LSNAKE	9	0.00	61.8	67%	56.8	33%	71.7
EFSW	UPSALM	78	0.04	61.1	65%	57.0	35%	68.5
OXBO	UPSALM	177	0.09	61.8	53%	57.1	47%	67.2
PAHH	UPSALM	567	0.30	57.6	84%	56.1	16%	65.5
SAWT	UPSALM	550	0.29	57.6	81%	55.7	19%	65.6
	subtotal/mean	*1739*	0.91	*58.5*	*76%*	*56.1*	*24%*	*66.3*
DWOR	SFCLWR	167	0.09	75.3	8%	61.5	91%	76.5
Male kelts								
CGRW	GRROND	55	0.08	57.9	98%	57.7	2%	65.0
WALL	GRROND	14	0.02	57.3	93%	57.1	7%	60.0
LSCR	IMNAHA	21	0.03	57.3	**100%**	57.3	na	na
LYON	LSNAKE	13	0.02	57.4	**100%**	57.4	na	na
TUCW	LSNAKE	2	0.00	62.5	50%	61.0	50%	64.0
EFSW	UPSALM	97	0.13	57.6	98%	57.6	2%	61.0
OXBO	UPSALM	53	0.07	57.8	94%	57.2	6%	68.7
PAHH	UPSALM	204	0.28	56.1	99%	55.9	1%	64.0
SAWT	UPSALM	208	0.28	56.5	99%	56.3	1%	68.0
	subtotal/mean	*667*	0.91	*56.8*	*98%*	*56.6*	*2%*	*65.2*
DWOR	SFCLWR	66	0.09	64.6	92%	63.3	8%	80.9

Table 1B.2. GSI results for natural-origin kelts (2009-2014) Origins were determined on the basis of highest ranked individual assignment likelihood scores (p).

RG	region	GSI assignment proportions						
		2009	2010	2011	2012	2013	2014	total
natural-origin kelts (n)		*262*	*1,362*	*1,111*	*1,121*	*437*	*1,419*	*5,712*
LOCLWR	A	6%	5%	8%	11%	8%	8%	8%
LOSALM	A	11%	7%	7%	7%	9%	7%	7%
GRROND	A	19%	15%	20%	24%	20%	22%	20%
IMNAHA	A	11%	13%	16%	13%	16%	15%	14%
LSNAKE	A	7%	9%	10%	12%	11%	14%	11%
UPSALM	A	19%	34%	18%	16%	21%	19%	22%
total/overall		**74%**	**83%**	**78%**	**82%**	**84%**	**85%**	**82%**
MFSALM	B	15%	10%	11%	8%	8%	6%	9%
SFSALM	B	5%	3%	4%	3%	3%	2%	3%
UPCLWR	B	3%	3%	3%	2%	3%	3%	3%
SFCLWR	B	3%	2%	4%	5%	2%	3%	3%
total/overall		**26%**	**17%**	**22%**	**18%**	**16%**	**15%**	**18%**

GSI analyses and tests of demographic difference

Assigning population-of-origin for individuals in a mixture sample has proven to be routinely less accurate than assignment to aggregates of genetically similar populations (e.g., McGlauflin et al. 2011; Campbell et al. 2012; Hess et al. 2014). For our GSI analyses we assembled population aggregates, or "reporting groups" (RG) from among 73 discrete steelhead reference populations (Hess et al. 2012, Ackerman et al. 2014). We defined ten RGs on the basis of observed genetic similarity and stream adjacency coincident with management unit boundaries (Matala et al. 2014). Although the A-run life history is observed throughout the Snake River Basin, six RGs named by subbasin or region have been deemed "A-run" for these analyses based on the traditional belief that they primary support populations of the A-run life history type (Busby et al. 1996; Narum et al. 2008; Ford et al. 2011; Ackerman et al. 2014). They include: Grande Ronde River – "GRROND", Imnaha River – "IMNAHA", lower Clearwater River – "LOCLWR", lower Salmon River – "LOSALM", lower Snake River – "LSNAKE, and upper Salmon River – "UPSALM". Four additional reporting groups are coincident with Snake River regions dominated by B-run populations (Narum et al. 2008; Ford et al. 2011; Matala et al. 2014), including: Middle Fork Salmon River – "MFSALM", South Fork Salmon River – "SFSALM", upper Clearwater River – "UPCLWR", and South Fork Clearwater River – "SFCLWR" (Figure 1B.1).

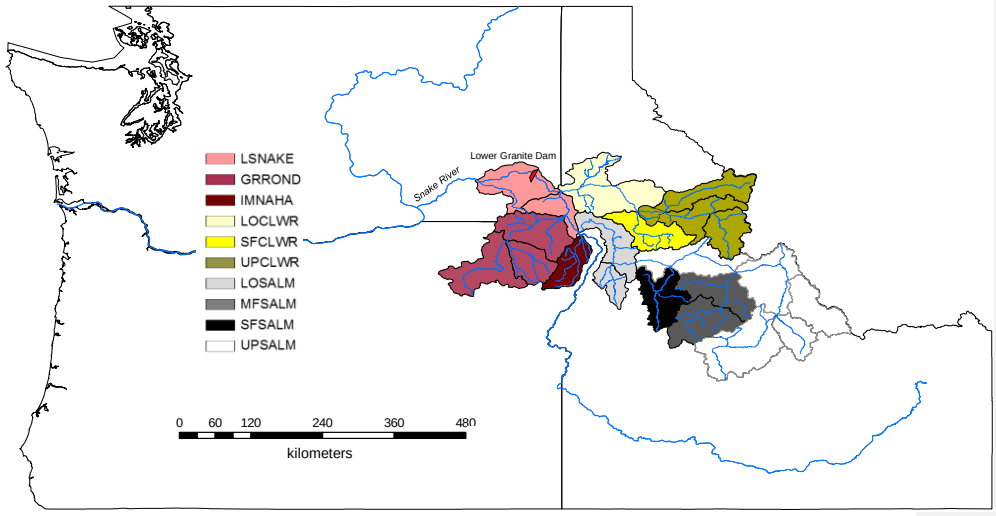


Figure 1B.1. Map of GSI region and reporting groups established on the basis of 73 baseline O. mykiss populations (see Appendix 1). Lower Snake River (LSNAKE); Grande Ronde River (GRROND); Imnaha River (IMNAHA); Lower Clearwater River (LOCLWR); South Fork Clearwater River (SFCLWR); Middle Fork Clearwater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

The degree of GSI assignment resolution or accuracy was assessed through a jackknife ('leave-one-out') procedure performed using the program GENECLASS2 (Piry et al. 2004), and implementing the Bayesian method of Rannala and Mountain (1997). Briefly, the proportion of correctly assigned individuals from each RG in the baseline, along with corresponding mean likelihood scores (*p*) provided a measure of expected confidence in GSI assignment accuracy for "unknown" mixture samples. The GSI analyses of emigrating kelt steelhead were performed implementing the same Bayesian approach in GENECLASS2. An RG-of-origin for each individual kelt in the LGR mixture was determined on the basis of its highest ranked assignment likelihood score (*p*), which indicates greatest genetic similarity among possible baseline references. Data and results from the GSI analyses of returning adult steelhead were the product of a separate but related study that was completed prior to, and independent of the kelt GSI analyses. The GSI assignment results were evaluated at the level of RG and also relative to putative run type region. This was done in order to evaluate the appropriateness of traditional regional distinctions, and to test for differences in proportions and associated demographic variation (size, age, condition, emigration/migration time) between groups. Two sample t-Tests of unequal variance were used to test for significant gender based differences in emigration timing (or migration timing) within

RGs and between run-type regions. Significant differences in condition rating of kelts (i.e. proportion "good") were tested using a two proportion z-test.

Results

GSI quality control and PBT analyses

The quality-control comparison of genotypes generated from Fluidigm and GT-seq methods yielded a concordance rate of 99.9% (17 discrepancies out of 16,928 comparisons), which meets accepted error limits reported for similar standardization exercises (Stephenson et al. 2009; Ackerman et al. 2014). Between 2011 and 2014 a total of 632 kelts (15% overall) that were initially identified as natural-origin at LGR were genetically identified as progeny of hatchery broodstocks through PBT screening. Hatchery-origin kelts from A-run RGs were dominated by age 1-ocean fish (male – mean 98% and female – mean 76%). The age 2-ocean proportion for A-run females ranged from 19% (SAWT) to 47% (OXBO), and only 14 total A-run males were older than age 1-ocean (Table 1). Male B-run kelts originating from DWOR hatchery were similarly dominated by age 1-ocean fish (92%), while female B-run kelts from DWOR were dominated by age 2-ocean (mean 91%). Over the course of these monitoring efforts, only five age 3-ocean hatchery kelts (all female) were sampled. Intuitively, fork length sizes of age 1-ocean fish were smaller than older age 2-ocean fish. The mean size of hatchery-origin kelts was small compared to natural-origin kelts among A-run stocks, and the disparity was greater among females (58.5cm vs. 63cm respectively). Conversely, hatchery B-run kelts (i.e. DWOR) were larger than natural-origin B-run kelts originating from the SFCLWR reporting group (75.6cm and 71.2cm for females respectively).

Kelt GSI proportions

The expected level of assignment resolution resulting from reference baseline testing varied depending on RG-of-origin and run type region (Table 1B.3). The accuracy of assignment to RG was weak to moderate within the putative A-run region, with proportions of correct assignments ranging from 43% in LSNAKE to 72% in IMNAHA (mean 59%). Corresponding likelihood scores ranged from *p*=66.9 to *p*=84.0 (mean *p*=76.4). For reporting groups in the putative B-run region the accuracy associated with RG assignments was relatively strong. Correct assignment proportions ranging from 86% in MFSALM to 93% in SFCLWR (mean 89%), and corresponding likelihood scores ranged from *p*=94.5 to *p*=96.7 (mean *p*=95.4). Assignment accuracy at the regional level (RGs combined by putative run-type region) was markedly improved among "A-run" RGs (mean correct assignment – 96%; mean score *p*=96.6), and moderately improved among "B-run" RGs. These results indicate a high level of confidence in assigning regional origins of "unknown" individuals (Winans et al. 2004; Ackerman et al. 2011; Ensing et al. 2013; Hess et al. 2014).

Table 1B.3. Reference baseline assignment accuracy/resolution, and kelt GSI assignment confidence. The proportion of correct self-assignments among baseline individuals (% self) and corresponding assignment likelihood scores (mean p) are shown for assignments at the level of RG-of-origin and traditional run type region.

RG	region	baseline (self)		baseline (mean *p*)		kelts (mean *p*)	
		RG	run	RG	run	RG	region
LOCLWR	A	58.0%	92.0%	80.4	95.6	64.0	94.0
LOSALM	A	55.0%	89.0%	81.9	94.0	63.0	92.0
GRROND	A	56.0%	98.0%	69.6	97.5	64.0	98.0
IMNAHA	A	72.0%	98.0%	84.0	96.6	75.0	97.0
LSNAKE	A	43.0%	97.0%	66.9	97.0	59.0	98.0
UPSALM	A	67.0%	97.0%	77.4	97.3	75.0	98.0
	total/mean	*59.0%*	*96.0%*	*76.4*	*96.6*	*67.0*	*96.0*
MFSALM	B	86.0%	87.0%	94.5	95.6	87.0	88.0
SFSALM	B	92.0%	94.0%	94.9	96.0	84.0	87.0
UPCLWR	B	91.0%	97.0%	96.7	98.3	92.0	96.0
SFCLWR	B	93.0%	98.0%	95.2	97.0	87.0	91.0
	total/mean	*89.0%*	*93.0%*	*95.4*	*96.7*	*87.0*	*90.0*

We observed moderate temporal variation in estimated GSI stock proportions for natural-origin kelts sampled between 2009 and 2014. Variation was highest in UPSALM among six RGs in the We observed moderate temporal variation in estimated GSI stock proportions for natural-origin kelts sampled between 2009 and 2014. Variation was highest in UPSALM among six RGs in the A-run region (18%) and in MFSALM (9%) among four RGs in the B-run region (Table 2). Overall GSI proportions were dominated by kelts from RGs in the putative A-run region (82%), particularly from UPSALM (22%) and GRROND (20%). The average proportion of kelts assigned to RGs in the B-run region was small, ranging from a total 15% to 26% of annual mixture samples. Modest statistical confidence was observed for RG assignments within the A-run region. Likelihood scores ranging from *p*=0.59 in LSNAKE to *p*=0.75 in both IMNAHA and UPSALM (mean *p*=0.67; Table 3). By comparison, RG assignments within the B-run region, were highly confident. Likelihood scores ranging from *p*=0.84 in SFSALM to *p*=0.92 in UPCLWR (mean *p*=0.87). On a more coarse scale, kelts assigned to the "correct" run-type region with a high degree of accuracy. Corresponding likelihood scores ranged from *p*=0.92 to *p*=0.98 (mean *p*=0.96) for the A-run region, and from *p*=0.87 to *p*=0.96 (mean *p*=0.90) for the B-run region. This represents a significant improvement in mean likelihood score (up 29%) between assignment to RG vs. assignment to putative run-type region or the A-run type.

Kelt demographics

The ratio of females to males among natural-origin kelts ranged from 66% to 80% among RGs in the A-run region, and from 70% to 85% among RGs in the B-run region (Table 1B.4). Female kelts were rated in significantly better overall condition (% "good") than males among RGs in the A-run region (P<0.001; Table 1B.4), with the exception of LOSALM. There was no difference in condition rating between males and females among RGs in the B-run region, or between A-run and B-run regions (e.g., 58% vs. 56% for females). Kelt emigration timing was variable, and females emigrated significantly earlier than males (range 5-11 days) among all 10 reporting groups. Kelts originating from SFCLWR were the first to be encountered at LGR during the emigration period (mean ordinal day 119) while the remaining three RGs in the B-run region exhibited the most delayed emigrations (Figure 1B.2). The emigration times for kelts from RGs in the B-run region (mean day 140.3) were significantly later than kelts from RGs in the A-run region (mean day 131.0; Table 1B.4), which includes some of the longest emigration distances to LGR.

Table 1B.4. Descriptive results for natural-origin kelts. Statistics are based on GSI assignment to RG: sample size (n), female proportion (%F), LGR sample date (ordinal day), and condition rating.

RG	region	total (n)	%F	LGR day			"good" condition		
				F	M	*P-value*	F	M	*P-value*
LOCLW	A	447	0.7	125	130	0.0027	0.5	0.3	0.0001
LOSAL	A	408	0.7	138	143	0.0059	0.5	0.4	0.0424
GRRON	A	1131	0.7	131	136	<0.000	0.5	0.4	<0.000
IMNAH	A	810	0.8	136	141	<0.000	0.5	0.3	<0.000
LSNAKE	A	620	0.6	126	132	0.0002	0.5	0.4	0.0001
UPSAL	A	1261	0.7	130	139	<0.000	0.6	0.4	<0.000
total/mean		*4,677*	*0.7*	*131.*	*136.*	*<0.000*	*0.5*	*0.4*	*<0.000*
MFSAL	B	618	0.7	152	159	<0.000	0.6	0.5	0.0189
SFSALM	B	197	0.8	149	160	0.0027	0.5	0.5	0.4669
UPCLW	B	200	0.7	141	149	0.0096	0.4	0.5	0.1464
SFCLWR	B	254	0.7	119	128	0.0006	0.4	0.3	0.1522
		1,269	*0.7*	*140.*	*149.*	*<0.000*	*0.5*	*0.5*	*0.0467*

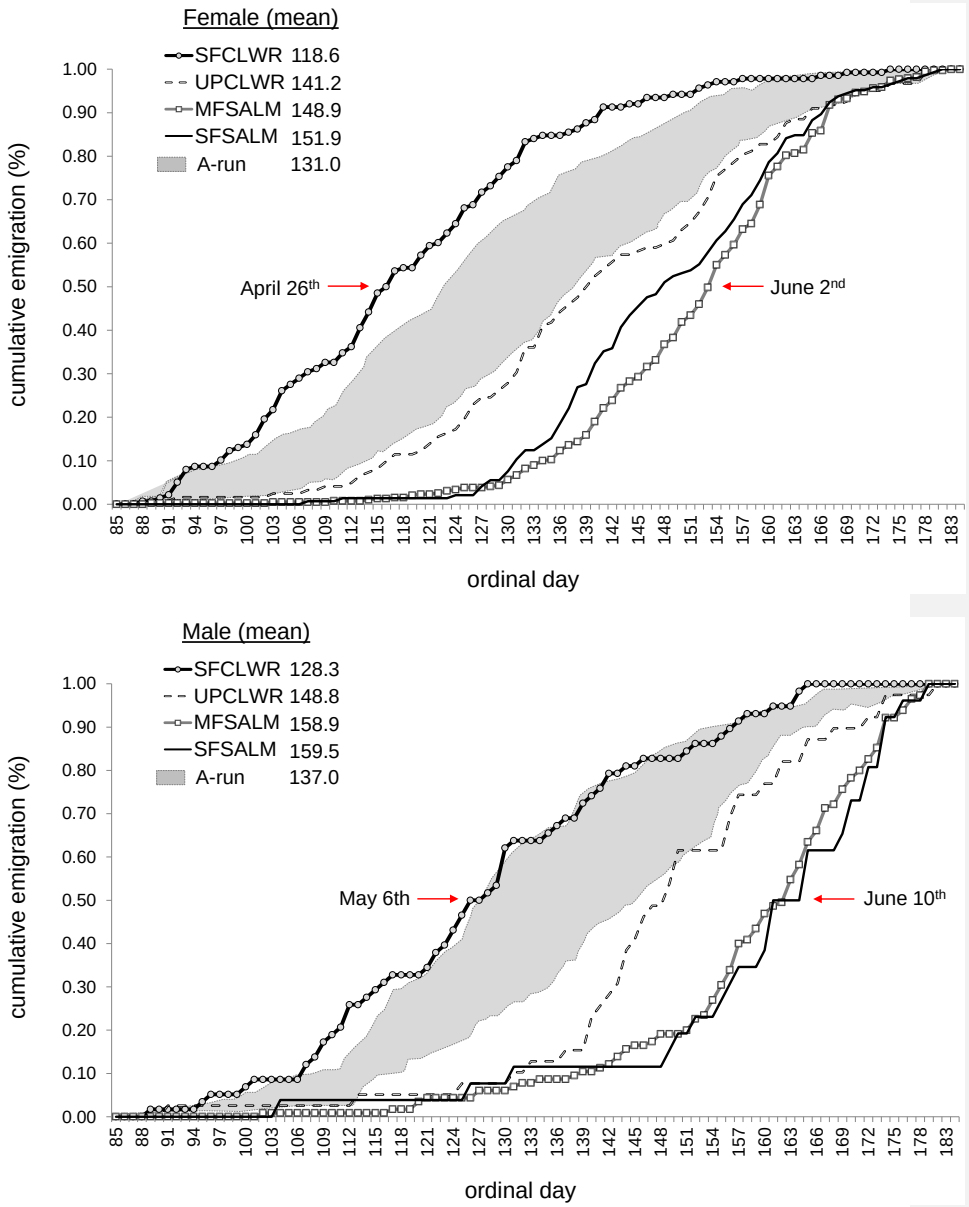
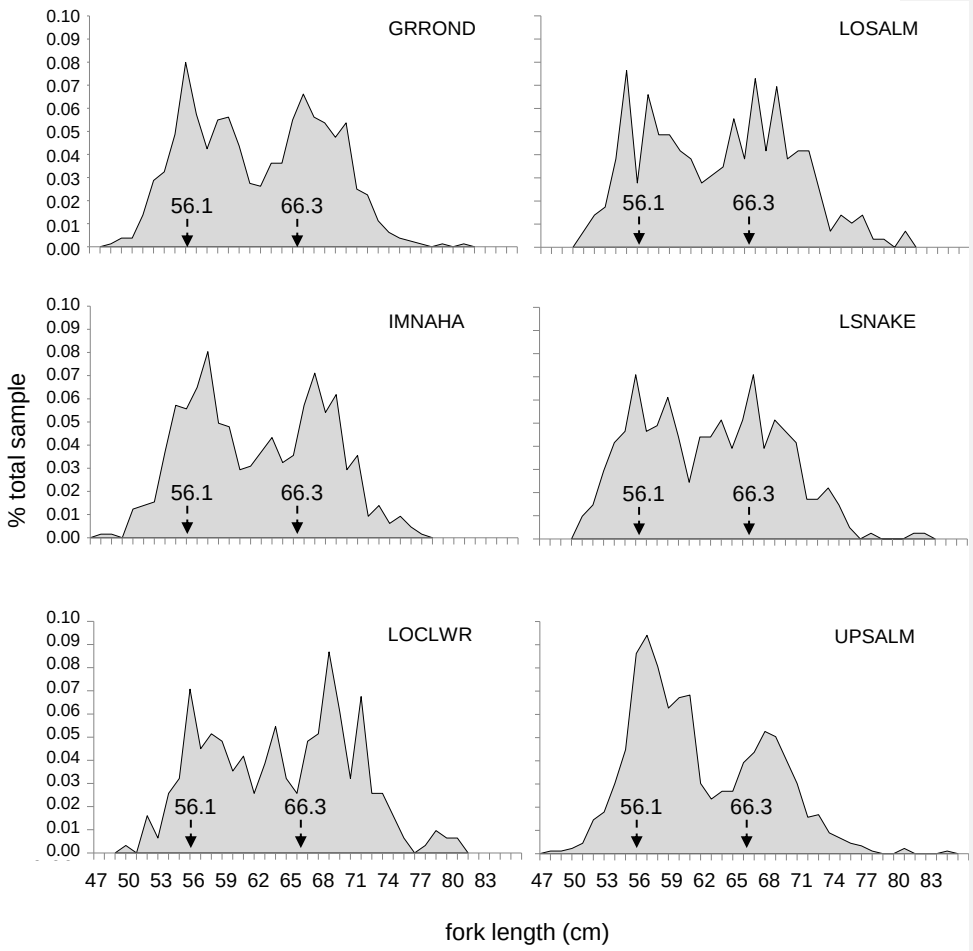


Figure 1B.2. Cumulative emigration time to LGR Time is shown as ordinal day (January 1st = day1). Arrows identify the earliest and most delayed emigration times (50% cumulative) among RGs. The A-run RGs, which exhibited minor differences, are shaded gray to highlight the B-run groups.

Female kelts were consistently larger than their male counterparts (Table 1B.5; Figure 1B.3), and the average size difference was greater among RGs in the B-run region (6.8cm) compared to the A-run region (4.3cm). The mean size of kelts from the B-run region was significantly larger than kelts from the A-run region for both males and females (P<0.001; Table 1B.5). On average, less than 1% of all kelts from RGs in the A-run region were larger than 78cm in length. The proportions of kelts from RGs in the B-run region that were larger than 78cm ranged from 8.7% (MFSALM) to 45.1% (UPCLWR) for females, and from 0% (SFSALM) to 15.4% (UPCLWR) for males. Natural-origin kelts from all 10 RGs exhibited bimodal size distributions (Figure 1B.3). Peaks were coincident with two age classes based on PBT results for hatchery kelts. The mean size-at-age of A-run hatchery kelts was 56.1cm (age 1-ocean), and 66.3 cm (age 2-ocean), and the mean size-at-age of larger B-run hatchery kelts was 61.5 cm (age 1-ocean) and 76.5 cm (age 2-ocean; Table 1B.1). The A-run natural-origin kelts occurred in near equal proportions of age 1-ocean and age 2-ocean fish with the exception of UPSALM, which was dominated by age 1-ocean fish. The size distributions of natural-origin kelts from B-run RGs indicated the presence of two age classes skewed toward age 2-ocean fish.

Table 1B.5. Fork length (FL) size statistics for kelts (2009-2014). Significant differences in size (individual size data) were tested using two sample t-Tests of unequal variance. All comparisons between run-types, RGs, genders (F - female, M - male) and between kelts and returns (*) were significant (P<0.0001).

RG	region	sample size (n)		mean FL (cm)		‡FL >78cm	
		F	M	F	M	F	M
LOCLWR	A	311	136	64.5	59.2	2.6%	0.7%
LOSALM	A	287	121	63.5	58.9	1.4%	0.0%
GRROND	A	802	329	62.8	58.8	0.4%	0.0%
IMNAHA	A	647	163	62.7	58.0	0.2%	0.6%
LSNAKE	A	409	211	62.9	59.1	0.7%	0.9%
UPSALM	A	892	369	61.8	58.3	0.4%	0.3%
total/mean		*3,348*	*1,329*	*63.0*	*58.7*	*0.7%*	*0.4%*
MFSALM	B	388	115	67.3	62.2	8.7%	2.6%
SFSALM	B	145	26	71.4	64.2	25.5%	0.0%
UPCLWR	B	122	39	73.9	66.6	45.1%	15.4%
SFCLWR	B	138	58	72.9	65.3	31.9%	6.9%
total/mean		*793*	*238*	*71.4*	*64.6*	*21.4%*	*5.5%*


GRROND
LOSALM
IMNAHA
LSNAKE
LOCLWR
UPSALM
56.1
66.3
% total sample
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
47 50 53 56 59 62 65 68 71 74 77 80 83
fork length (cm)

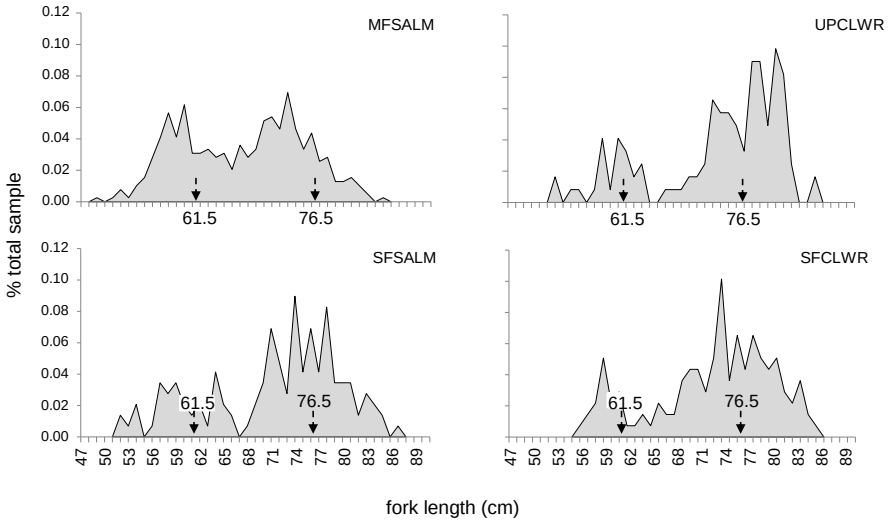


Figure 1B.3. Size distribution and inferred ocean age of female natural-origin kelts sampled at LGR. Arrows identify mean size of age 1-ocean and 2-ocean female hatchery-origin kelts (based on PBT broodyear).

Discussion

Our results substantiate differential rates of post-spawn survival and repeat spawning potential between putative run types characterized on the basis of size and age criteria, and to some extent on region of origin (Keefer et al. 2008; Narum et al. 2008). Compared to the genetic distinctiveness of putative B-run RGs, the moderate assignment resolution observed between putative A-run RGs suggests greater genetic similarity among the latter. The total proportion of A-run kelts in our six year sample (designated by reporting group) greatly exceeded the proportion of B-run kelts with regularity. Although our GSI results verify genetic differentiation between Snake River regions or reporting groups, a clear correlation on a demographic basis was not apparent. Therefore, the extent to which they align with alternative life history distinctions (i.e. run types) is called into question.

Our results support previously documented demographic or biological differences between A-run and B-run Snake River steelhead, in that we observed a predominant 2-ocean age class among B-run regions, significantly smaller A-run kelts compared to B-run kelts, and significantly smaller and less abundant male kelts for both run types (Bowersox et al. 2011; Copeland et al. 2013). Unlike published data, our results infer ages and size-at-age of kelts that do not conform to a size threshold criteria indicative of run-type (i.e. a 78cm cutoff), or a single predominant ocean age class. In fact, in most instances the sizes and inferred ages of A-run kelts aligned with two age classes (age 1-

ocean and 2-ocean) of relatively equal proportion. While size selectivity (exclusion) of larger kelts at the LGR juvenile fish facility cannot be unequivocally ruled out, it is not likely to have contributed to apparent size differences between upstream migrating steelhead and emigrating kelts. This conclusion is based on the fact that kelts sampled at the JFF included individuals as large as 74cm (averaging 67.3cm to 73.6cm among B-run RGs). Moreover, the size disparity observed between typically larger B-run kelts was mirrored by a comparable disparity among significantly smaller A-run kelts, which could not be explained by size selectivity at the JFF.

1.C: Steelhead Kelt Population Model (Yakima River Basin)

Introduction

In 2013 we constructed a prototype steelhead model to examine the management implications of kelt reconditioning. The model was designed to address the factors influencing kelt condition at capture, the effect of the capture rate of kelts on the overall recovery rate, the effects of the in-river release, transport unfed, transport fed, and long term reconditioning survival on recovery, and the proportion of kelts reconditioned, transported or released. The model was intended to be used to assess the effectiveness of alternate reconditioning strategies, and limitations of the reconditioning program by comparing the rate of population increase achievable when captured kelts are reconditioned under assumed potential capture rates and assumed survival rates. The model was intended to be a very general implementation, capable of reproducing multiple age classes of adult returns, and tracking all components of the kelt program. The model explicitly tracked groups of kelts of different conditions (fair, good, and poor condition) and of different release groups (in-river, fed-transported, and unfed-transported).

Methods

Model Description

The model assumes that iteroparity rates do not differ among years, but that the possible iteroparity rate for virgin spawners r_N is different from that of successful repeat spawners r_I due to variability in migration differences (distance and timing), bioenegertics, and size. 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 Beverton Holt function with productivity a_t and capacity b given by

$$M_t = \frac{a_t S_t}{1 + a_t S_t / b}$$

Where a_t is predicted by a functional relationship using flow as the independent variable. Average Yakima river daily flows are measured at Kiona[1]. The average flow Y_t between June and Sept is used as a surrogate for upstream tributary flow conditions that have the potential to influence juvenile rearing survival. Average flows are normalized by subtracting the average Y_t across years and dividing by the standard deviation of Y_t . The normalized flow index Z_t is used in a functional relationship to predict the yearly smolt productivity using the logit equation

$$a_t = 500/(1 + e^{-\alpha - \beta Z_t})$$

Where α is the logit productivity intercept parameter that predicts the productivity at the average flow (because $Z_t = 0$ at average Y_t), and β is the logit productivity slope parameter that predicts the change in productivity with changes in flow (where $Z_t \neq 0$). In this way, α and β are estimated parameters and a_t is a derived parameter.

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 .

[1] http://www.usbr.gov/pn/hydromet/yakima/yakwebarcread.html

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

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 two years $N_{5,t}$ 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 three years $N_{6,t}$ is the ocean adults multiplied by the maturation rate φ_3 after a third year in the ocean.

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

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

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

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

Data and Model Implementation

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

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

Initializing the model requires initial spawning abundances, which were available from 1985-2015. Smolt abundance data were also available for part of the same period (1985-2010). For the model to be able to predict future generations of fish, it must be able to predict smolts from spawners, adults in the ocean from smolts, and returning spawners from ocean adults. We obtained estimates of the Beverton Holt smolt production parameters by fitting the smolt abundances predicted by the Beverton Holt function to the observed smolt abundances. A Beverton Holt function was fit to the model using a process error model with a log-normal negative log-likelihood minimization. We used migration year total smolts two years after the spawning year as the estimated smolts from a spawning group. Since the majority of outmigrating juveniles are two years old, this value would be most robust to temporal variation.

The model fit estimates a Beverton Holt productivity intercept parameter of α=-1.04, a productivity slope parameter β=1.12, and a capacity parameter of 58,607. Figure 1C.1 shows the functional relationship that predicts the productivity as a function of flow. The logistic function predicts a mean productivity value of approximately 130 smolts per spawner at average flow conditions (the normalized flow equals zero at the average flow value). We can see that productivities vary from approximately 20 smolts per spawner to sometimes greater that 300 smolts per spawner. Note that a high productivity does not necessarily translate to high recruits per spawner. Density dependence can cause compensation at higher densities. Figure 1C.2 shows the same predicted productivities over time, and we see that productivity in recent years has averaged about 150 smolts per spawner at the origin. Figure 1C.3 shows the fit of the Beverton Holt model to the smolt data, and the predicted smolts per spawner using the parameterized model. Smolts per spawner are in the range of predominantly 20-50 smolts per spawner with a few higher return rates near 100, which translates to requiring approximately a 5-7% smolt to adult return rate for population stability. Using the smolt production parameters estimated by fitting the Beverton Holt function to the

spawner and smolt data, a second estimation was done to obtain the ocean survival in the first year. Second year ocean survival was set at 0.7.

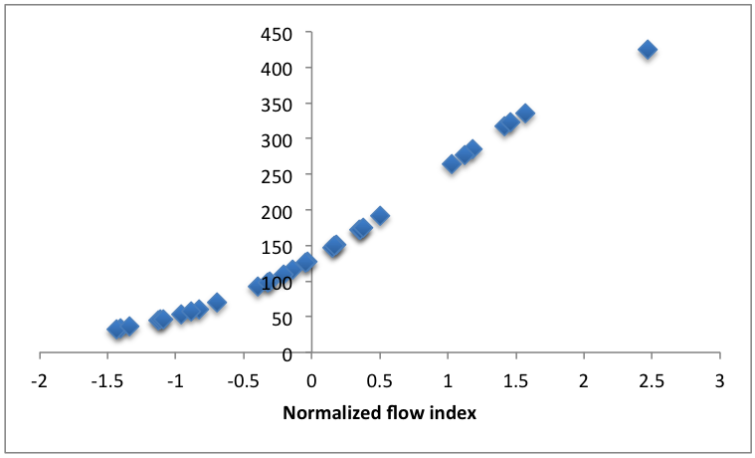


Figure 1C.1: Productivity relationship to flow conditions. Each point is a prediction of the productivity at a given flow level between 1985 and 2015.

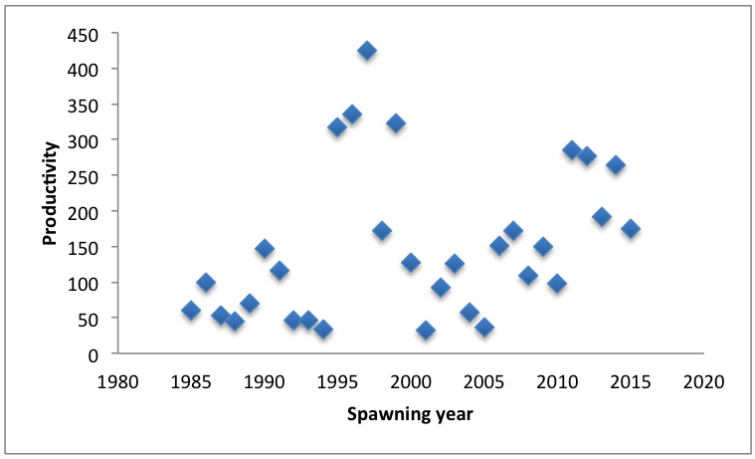


Figure 1C.2: Predicted yearly productivities. Each point represent the productivity predicted as a function of flow conditions and corresponds to a value in Figure 1.

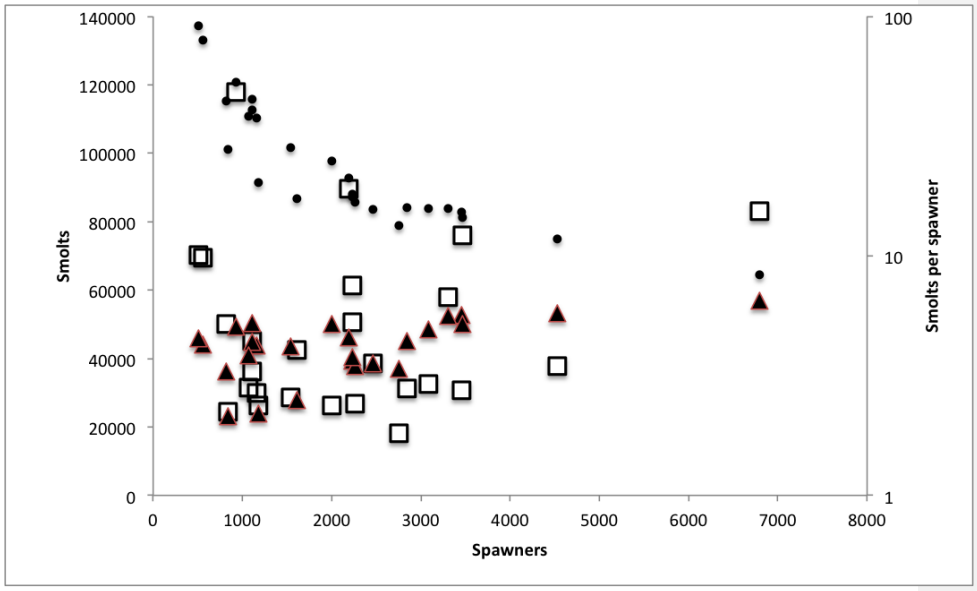


Figure 1C.3: Beverton Holt stock recruitment model fit of smolts to spawners. Open squares indicate observed smolt abundances. Closed triangles show the predicted smolts at a given observed number of spawners. Closed circles show the predicted smolts per spawner.

Because we did not have age structure data of returning adults, we assumed 10% of fish matured after one year in the ocean and that all remaining adults spent two years in the ocean. We fixed the maturation rate of first year ocean fish to 0.1 so that 10% would return after one year in the ocean, and we fixed the second year maturation rate to 1.0 so that all fish would return after the second year in the ocean. The model thus predicted no returns older than four years old. Additional assumptions in the life cycle parameterization included: 1. Ocean capacity terms of 1.e+12 (effectively eliminating density dependence in the ocean), 2. Kelt capture rates of 40%, repeat kelt rate of 25%, 6% in-river release portion of kelts, 5% transport unfed portion of captured kelts, 15% transport fed portion of kelts, and 73% long term recondition portion of kelts.

Results

The parameterization above produced a prediction of future spawning abundances, smolt abundances, and adult returns based on the parameters derived from acoustic tagged kelt capture and release analysis, the rates of tagging and release groups, and the Beverton Holt stock recruitment analysis. We further refined the model by fitting the predicted spawners to the observed spawners. We fit the model with a log-normal likelihood function, where the predicted spawners were compared to the estimated spawners. Note that since all fish returned as two-salt fish, only the product of the two is relevant, which is about 5%. Recalling that 5% is approximately the SAR required for

the population to be stable at about 20 smolts per spawner production levels, the estimate seems correct. The observed and predicted spawners from the fitting process are shown in Figure 1C.4.

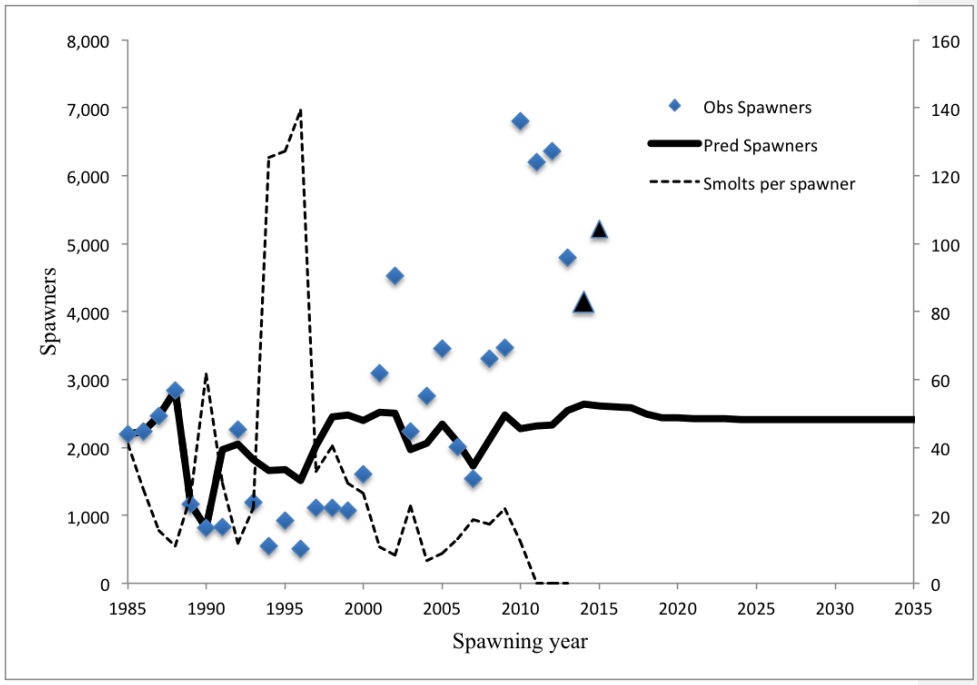


Figure 1C.4: Observed and predicted spawners from model fit. Black triangles show the most recent two years of empirical spawning abundance.

We can see in Figure 1C.4 that the predicted spawners overestimate spawning in the 1990's, then underestimate spawning in the 2000's. Figure 4 also shows the empirical pattern in smolts per spawner, with effective productivity declining in recent years. We further validated the Beverton Holt production parameter estimates by mapping out the likelihood that the parameter values could be responsible for seeing the spawning abundances empirically observed. Figure 1C.5 shows the negative log likelihood of the predicted versus observed spawners in relation to the log of the value of the Beverton-Holt b parameter.

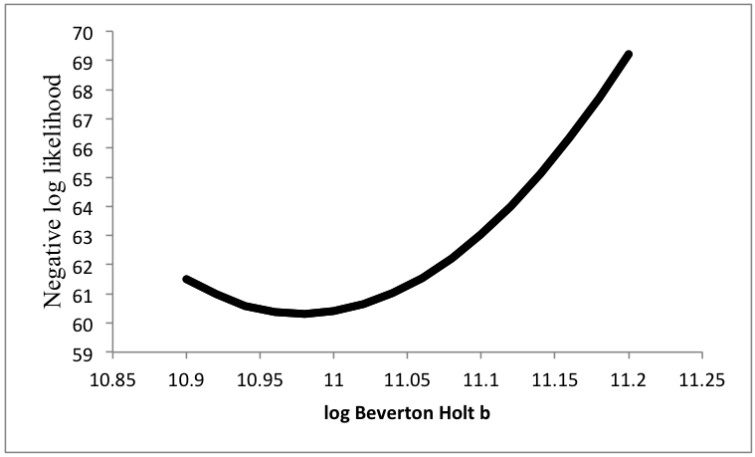


Figure 1C.5: Likelihood profile of the log of the Beverton Holt b parameter.

We repeated this same analysis with the Beverton Holt productivity intercept parameter. The likelihood profile of the intercept parameter from the spawning abundance likelihood is shown in Figure 1C.6.

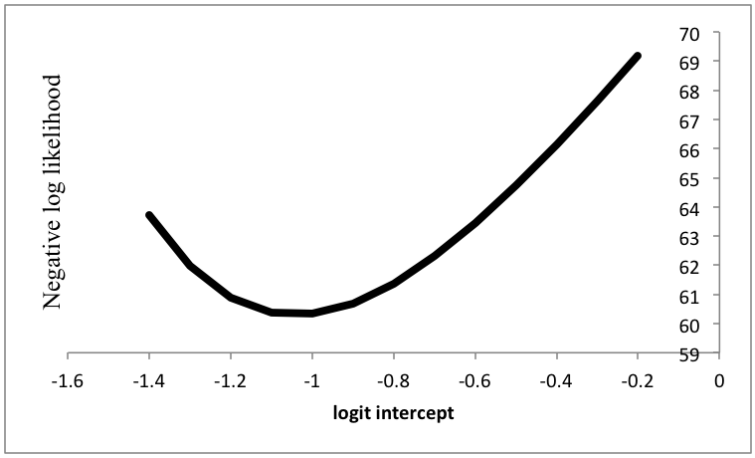


Figure 1C.6: Likelihood profile of the logit of the Beverton Holt productivity intercept parameter.

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

somewhere in the middle of the data (Figure 1C.7). It is worth noting that the population model predictions of smolts do not differ from empirical values with any noticeable bias. Figure 1C.8 shows the time series plot of observed and predicted smolts in the population model.

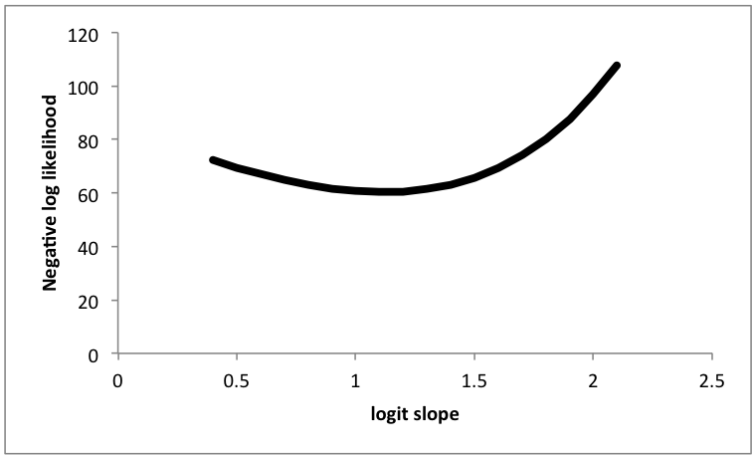


Figure 1C.7: Likelihood profile of the logit of the slope parameter that predicts smolt productivity.

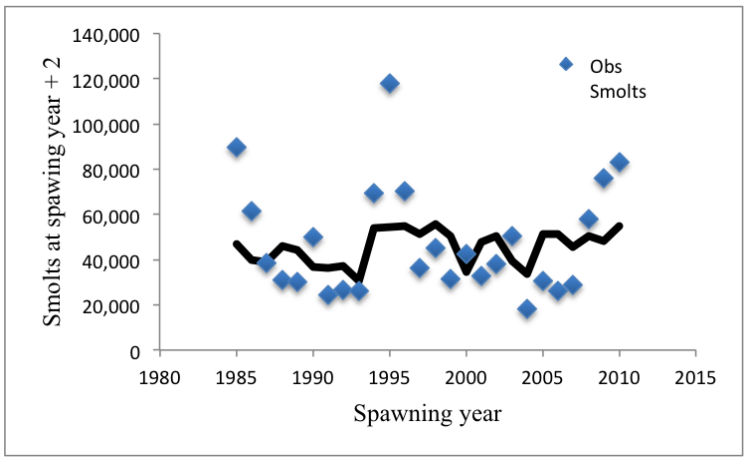


Figure 1C.8: Observed smolts and smolts predicted from the population model.

Having been statistically fit to empirical data, the model can be used as a population prediction tool. By predicting population trends with initial spawning abundances and known parameters (fixed and estimated), we can postulate the relative effect of altering a parameter of interest. An example of this is the kelt capture rate. The assumption in

the model as parameterized, was that 40% of kelt were captured, and the uncaptured kelts survived as in-river fish of good, fair and poor conditions. If the assumption is that capturing more kelts would ultimately result in more spawners, and if that would lead to an increase in the overall population size, then the obvious question is "How much will the additional collection contribute to spawning?". To answer this question, we used the predicted returning kelts and returning spawners over a fifteen year period, and calculated the average portion of spawners that were returning kelts. Figure 1C.9 shows the relative change in the portion of kelts at different collection rates.

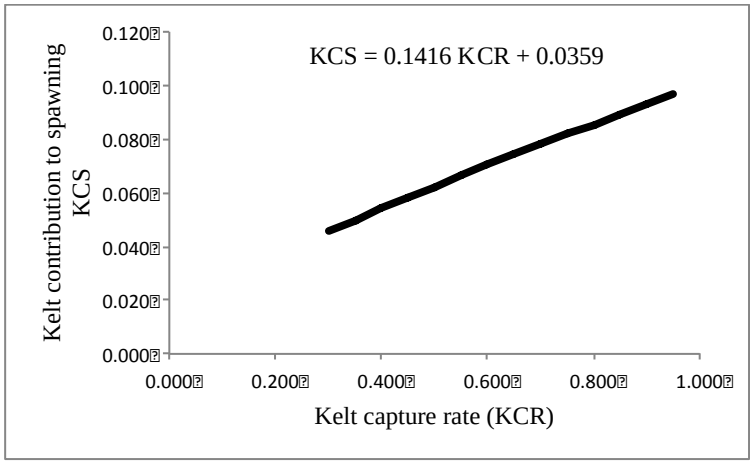


Figure 1C.9: Relative contribution of kelts to spawning abundance.

We also wanted to look at the possible impact of kelt collection on the overall returning abundance of spawners. Figure 1C.10 shows the returning spawning abundance in 2020 predicted by a given kelt capture rate. We see that an increase in the kelt capture rate from 40% (assumed to be current capture rate) to 80% would yield a predicted 120 more spawners. While this may not seem like much, two things must be noted: 1. The population fit to empirical trends appears to underestimate recent production, so it would be reasonable to assume the number could be higher than 120, and most importantly 2. There is no decline in the rate of production from increasing kelt capture rates, i.e., there is no apparent diminishing returns in doing so.

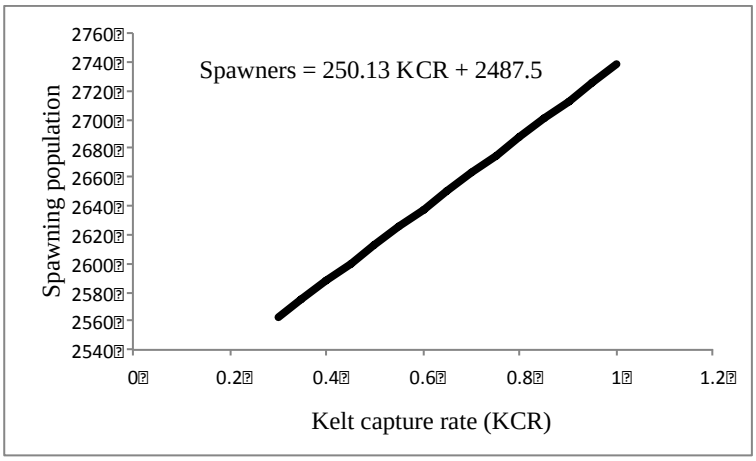


Figure 1C.10: Predicted increase in spawning population with increase in kelt capture rate.

Discussion

A model was developed for the purpose of examining population recovery from the perspective of the kelt reconditioning program. We have shown that kelts contribute up to 10% of spawning if sufficient kelts are captured and reconditioned. Further, high rates of kelt reconditioning can lead to higher overall spawning abundances, approximately an increase of 200 spawners.

The model was parameterized with constant kelt survival rates that were derived from tagging data, variable Beverton Holt spawner to smolt productivity predicted from flow conditions, and constant capacity. Given that there was a systematic increase in spawning abundance during the period of time that the population model was used to fit the spawning abundance data, the best fit resulted in overestimation of spawners in the 1990's, and an underestimation of spawners between 2000 and 2013. As a result of the recent underestimation, the projected spawning abundance is potentially biased low. This could be for a number of reasons. First, it's possible that some life stage survival rates have been better than average in recent years. This seems like a likely scenario since empirical spawning numbers have increased despite smolt numbers being constant or declining since 2000. This is consistent with recent population increases in Columbia River salmonid populations, and points to improved mainstem or ocean survival conditions. It is likely that the model is simultaneously underestimating smolt capacity and adult rearing survival.

If an argument can be made that model projections relying on the constant rates are underestimating spawning abundance in recent years, and if the cause of this is that smolt to adult survival is higher than average currently, then it stands to reason that

performance measures are conservative if based on this model parameterization. The obvious solution is to characterize the cause of the systematic change in survival, which was attempted using variability in smolt productivity to be described by changes in flow conditions, but additional variation in survival rates can be achieved by implementing similar mechanisms with smolt capacity or ocean survival.

We have been able to demonstrate a useful comparison of production levels with simple alteration of a key parameter: the kelt capture rate. This same type of comparison can be implemented on other key variables, such as pre-spawn mortality, repeat kelt rate, sensitivity of production parameters to environmental variation, sensitivity of kelt survival to body condition, and sensitivity of body condition to environmental conditions and density.

Chapter 2. Steelhead Kelt Reproductive Success

2. A: Cle Elum Spawning Channel

Introduction

We tested the Cle Elum spawning channel as a method of demonstrating reproductive success of reconditioned kelt steelhead. Long term hypotheses include 1. Reconditioned kelt steelhead can build redds, find mates and successfully spawn in a spawning channel; 2. Reconditioned kelt steelhead have reproductive metrics similar to maiden steelhead; 3. Spawning behaviors of reconditioned kelt steelhead are similar to maiden steelhead. Because this system had not previously been used for steelhead, 2015 was treated as a feasibility study.

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

Methods

Channel Description and Modification

The Cle Elum spawning channel was originally designed with optimal spring Chinook spawning conditions in mind (Schroeder et al. 2008). Since spring Chinook on average are larger than steelhead, gravel sizes were slightly too large for some of our smaller female kelts based on Fredle index (Lotspeich and Everest 1981). All 3 agencies involved decided that making enhancements to the spawning gravels to better optimize them for steelhead spawning would be prudent before the study started. Gravel sizes 19, 38, and 50 millimeters were introduced into the channel.

Since two populations that are closely related would be used in the same channel we divided the channel into three major sections to prevent populations from mingling since progeny would be released back to their streams of parental origin.

Adult Collections and Stocking

Fish were collected at the Chandler Juvenile Monitoring Facility (CJMF) and reconditioned as kelts in the reconditioning program (long-term reconditioning). Fish were separated by prior PIT-tag detections at the time of release and retained until it was determined that river conditions for spawning were occurring. This included water temperatures and monitoring migration of fish over Roza Dam. Fish were collected from reconditioning tanks by net and placed into a large tanker truck and transported to the Cle Elum fish facility. Fish were tempered by chilling water in reconditioning tanks and mixing water on the way to Cle Elum. Fish were netted from tanks and placed into either the Naches or Upper Yakima sections based on PIT-tag histories.

Juvenile Collections

Juvenile samples were collected at multiple stages. The primary collection method was using box traps with netted tubes. Traps were checked twice daily and every tenth fry was lethally sampled for fin tissue. Samples were recorded separately for each section. Our goal was 500 samples. Barring not enough collection from trap boxes, the remainder of juveniles will be collected by electrofishing the remaining juveniles from the channel. Juvenile fish will then be released to an area near Nelson Springs for Naches fish and upstream of the hatchery for Upper Yakima fish.

Testing Channel Hydrology and Substrate Composition

Hydrology

Methods for installing and assessing vertical hydraulic gradient (VHG) were modified from a previous study (C. Baxter, Hauer, and Woessner 2003). VHG is a dimensionless metric indicating relative strength of upwelling (positive values) or downwelling (negative values) of subsurface flow. After installation and initial measurement, each minipeizometer was visited multiple times in approximate 10 min intervals to ensure water levels had come to equilibrium.

Substrate

We collected samples using a McNeil core sampler to collect sediment samples at each sample locations. The sampler was driven into the streambed to a depth of 20 cm, or until the base of the collection barrel is flush with the streambed surface. Extraction of the gravel is done by hand and transferred to a 2 gallon bucket. Samples were then warmed to slowly remove all moisture weight from the samples. After the removal of moisture, samples were then placed into a mechanical sifter to separate particles by the following sizes: 63, 31.5, 16, 11.2, 8, 6.3, 4, 3.35, 2, 0.85, 0.355, and .125 millimeters. Each collection size was then weighed in grams and the percentages of the total weight was determined (Justice 2012).

Genetic Analysis

To identify individual fish (parents and progeny) 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 chelex beads. Genotyping efforts utilized 192 Single Nucleotide Polymorphism (SNP) markers and GTseq methods using an Illumina Hiseq1500 instrument. Three cutthroat diagnostic and one sex-determining marker were not used for parentage analysis. 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.

Results and Discussion

Adult Collections and Stocking

On February 19, 2015 we released 25 steelhead from the Prosser reconditioning program into the Cle Elum Hatchery Spawning Channel. The spawning channel was separated into two sections and in the upper section 15 Upper Yakima River origin reconditioned kelts were released. These 15 fish were composed of 9 maturing females, 1 immature female, and 5 males. In the lower section of the spawning channel, 10 Naches origin reconditioned kelts were released. These 10 fish were all females of which 5 were mature, based on plasma hormone assays. To reduce handling and stress on the fish, we did not separate the mature and non-mature fish. The WDFW and Yakama Nation obtained 12 resident males through hook and line, electroshocking, and hydro-bypass collection from the Naches and 15 from the Upper Yakima that were placed into the spawning channel to supplement the low number of reconditioned male kelts.

Redd Construction

There were a number of test or small redds constructed in each portion of the channel and much larger ones as well. We observed 2 large redds in the Naches and 7 in the Upper Yakima sections. There were numerous smaller redds that were constructed but in the experience of our observer these were likely test digs. Figure 2A.1 shows were the larger redds were constructed. The first large redd was constructed in the Upper Yakima section on February 28, 2015 in section 1-1(l). The first redd constructed in the Naches section occurred on March 24, 2015 at section 2-3(a). The final redd in the Naches section was constructed 6 days later in section 2-2(c). In the Upper Yakima section 5 redds alone were constructed in section 1-2 (j) at differing dates over a 2 month period. The final redd in the Upper Yakima section was constructed on May 4, 2015.

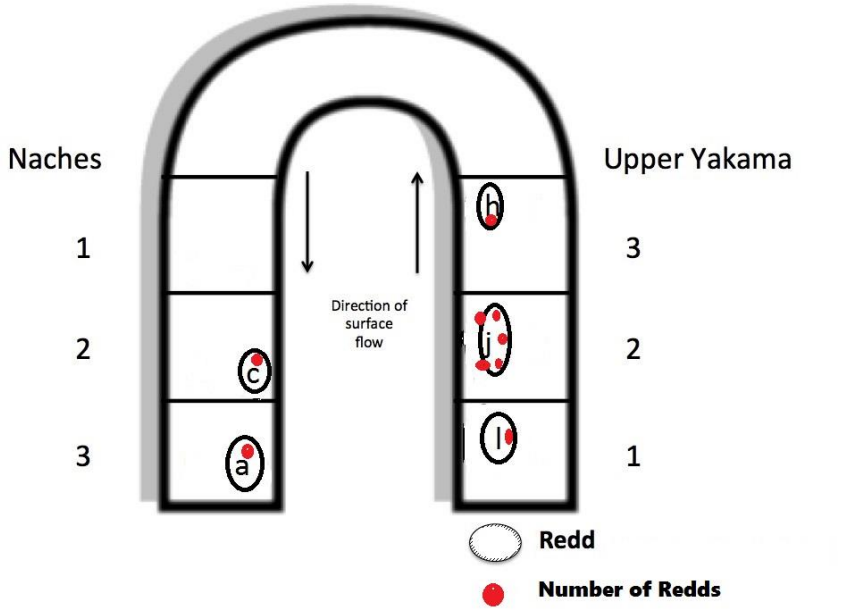


Figure 2A.1. Site of redd locations at Cle Elum spawning channel 2015. Circled areas represent redd locations while red dots represent individual redds found in that location.

Juvenile Collection

All traps were removed on August 11th with the remaining fish collected using electrofishing methods. As with the traps, every tenth fish sampled for genetic tissue. Because we had not collected our minimum target of 500 offspring, additional samples were collected from the juvenile holding tanks. After the tank subsampling effort, remaining fish were collected with and electrofishing backpack. All surviving juvenile fish were released back to either the Naches or Upper Yakima depending on section collected.

Genetic Analysis

Genotypes were generated for all 52 fish stocked in the channel including 43 potential spawners comprising 11 mature female reconditioned kelts, 5 male reconditioned kelts and 27 resident males. Fish that died prior to redd construction and immature fish (screened by estradiol and confirmed with lack of progeny assignments) were omitted from parentage analysis. While 521 juveniles were extracted, 27 had incomplete genotypes and were not used for parentage analysis. Two of these fish had genotypes consisted with pure cutthroat trout at the three diagnostic markers. Of the 494 fish with quality genotypes, only 2 fish failed to assign back to stocked adults. These 2 fish along

with the 2 cutthroat juveniles are thought to have entered the spawning channel from the river water intake to the hatchery. This is consistent with reports of juvenile trout being seen when the channel was used for chinook spawning.

All 492 fish that assigned to a parent assigned to two parents, one male and one female (Table 2A.1). In all cases, the assignment was to a pair that were both stocked into the same section of the channel. While 1 male was found as a carcass in the elbow section, there is no other evidence that adult fish were able to move between their stocking locations. The collection source for each of the 492 offspring is shown in table 2A.2. Assignments to parent classes are shown in table 4. Of note, 88% (230/260) of the offspring collected in the Naches section were assigned to parents from the Upper Yakima section of the channel. As both parents were from the Upper Yakima section, it's likely that small fish are able to escape the trap, bypass the screens, or travel through the gravel.

Table 2A.1: Juvenile sampling and collection method.

Channel section	Method	Number	Sum
Upper Yakima	Trap	124	
Upper Yakima	Electrofish	48	
Upper Yakima	Tank Resample	49	221
Naches	Trap	127	
Naches	Electrofish	92	
Naches	Tank Resample	41	260
Elbow	Electrofish	9	
End	Electrofish	2	11

Table 2A.2: Juvenile sampling, collection method, and parentage assignment.

Channel section	Method	Number	Sum	Upper Yakima Parents	Sum	Naches Parents	Sum
Upper Yakima	Trap	124		124		0	
Upper Yakima	Electrofish	48		48		0	
Upper Yakima	Tank Resample	49	221	49	221	0	0
Naches	Trap	127		115		12	
Naches	Electrofish	92		79		13	
Naches	Tank Resample	41	260	36	230	5	30
Elbow	Electrofish	9		9		0	
End	Electrofish	2	11	1	10	1	1

All potential spawners in the Upper Yakima section are listed in Table (2.A3). Progeny were assigned to 4 of the 9 females. Of the 5 females without progeny assignments, 2 were collected as mortalities with less than 500 eggs retained, 1 had no eggs sampled with a tight skein located nearby and 2 had no additional records. Progeny were assigned to 4 of the 5 male reconditioned kelts and 7 of the 15 resident males (Table

2A.3). The single male reconditioned kelt that did not reproduce was detected as a mortality in the elbow section on March 29th, but it is unknown when it escaped from the Upper Yakima section.

Table 2A.3: Fish stocked into Upper Yakima section of channel and spawning status.

PIT tag	Sex	Male Source	FL	Stock Date	Mort Date	Status
3D9.1C2E0D7996	F		560	2/19/2015	4/14/2015	Successful
3DD.0077494335	F		560	2/19/2015	4/5/2015	Successful
3D9.1C2E0DA182	F		700	2/19/2015	3/12/2015	Successful
3D9.1C2DF0D728	F		510	2/19/2015		Successful
3DD.0077495EEB	F		580	2/19/2015	3/23/2015	
3D9.1C2DF0B048	F		710	2/19/2015	3/29/2015	
3DD.007748BD25	F		630	2/19/2015		
3D9.1C2DBE62B2	F		760	2/19/2015	4/5/2015	
3DD.0077490E37	F		530	2/19/2015		
3DD.0077498507	M	Chandler kelt	550	2/19/2015		Successful
3DD.0077490F88	M	Chandler kelt	610	2/19/2015		Successful
3D9.1C2DB9D9AE	M	Chandler kelt	570	2/19/2015		Successful
3D9.1C2DF446BA	M	Chandler kelt	570	2/19/2015	4/23/2015	Successful
3DD.0077488804	M	Chandler kelt	590	2/19/2015	3/29/2015	
3DD.00774937A4	M	EBURG MSYR34	342	2/17/2015		Successful
3DD.0077484639	M	REC5	329	2/17/2015		Successful
3DD.007747C7E9	M	REC5	274	2/17/2015		Successful
3DD.007748FD2D	M	REC5	269	2/17/2015		
3DD.0077491CE9	M	REC5	257	2/17/2015		
384.3B23A47006	M	REC5	257	2/17/2015		
3D9.1C2E0EA32B	M	REC5	318	2/17/2015		
3DD.00774889B9	M	WIL39	223	2/17/2015		Successful
3DD.0077496204	M	WIL39	238	2/17/2015		
3DD.0077491DB0	M	Bullfrog MSYR96	469	2/19/2015		
3DD.007748B9D5	M	Bullfrog MSYR96	354	2/19/2015		
3DD.007748C7D5	M	Roza Adult Trap	445	2/23/2015	5/7/2015	Successful
3DD.00774DD17A	M	Roza Adult Trap	420	2/25/2015		
3DD.00774DCE6E	M	Roza Adult Trap	480	2/26/2015		Successful
3DD.00774DC7B5	M	Roza Adult Trap	460	2/27/2015	5/7/2015	Successful

All potential spawners in the Naches section of the channel are shown in Table 2A.4. Progeny were assigned to 1 of the 2 potential female spawners. The female lacking progeny assignments was collected as a mort and found to have unripe eggs in a tight skein.

Table 2A.4: Fish stocked into Naches section of channel and spawning status.

PIT tag	Sex	Male Source	FL	Stock Date	Mort Date	Status
3D9.1C2D7F5169	F		700	2/19/2015	4/5/2015	Successful
3D9.1C2E0EBF13	F		640	2/19/2015	4/6/2015	
3DD.0077483A92	M	TIET17	211	2/18/2015		Successful
3DD.0077497CEF	M	TIET17	222	2/18/2015		
3DD.007748C69C	M	Buckskin Slough	218	2/24/2015		Successful
3DD.0077486481	M	Buckskin Slough	222	2/24/2015		
3DD.007748E506	M	Buckskin Slough	246	2/24/2015		
3DD.007749ABCC	M	Buckskin Slough	221	2/24/2015		
3DD.00774AA56F	M	NACH13	336	2/24/2015		
3DD.0077491028	M	OAK10	215	2/24/2015		
3DD.007748760B	M	NACH13 HSR OS	323	3/25/2015		
3DD.007748B5F2	M	NACH16 HSL ECU	328	3/25/2015		
3DD.00774AD1E1	M	NACH16 RM SCB	327	3/25/2015		
3DD.007749E97C	M	NACH8 RM	257	3/25/2015		

The number of crosses and progeny for each successful adult are seen in Table 2A.5. All females with progeny detected were found with at least 3 males in the Upper Yakima section, and 2 males in the Naches section. One female crossed with 6 different males with a total of 149 progeny detections. This female crossed with 2 male reconditioned kelts and 4 residents. Males crossed with a maximum of 2 females and had between 1 and 76 progeny detections.

Table 2A.5: Number of crosses and progeny for individual adult fish.

PIT tag	Sex	Male Source	FL	Stock Date	Mort Date	# of Crosses	# of Progeny
3DD.0077494335	F		560	2/19/2015	4/5/2015	3	47
3D9.1C2E0DA182	F		700	2/19/2015	3/12/2015	4	149
3D9.1C2E0D7996	F		560	2/19/2015	4/14/2015	3	114
3D9.1C2DF0D728	F		510	2/19/2015		6	149
3D9.1C2DF446BA	M	Chandler kelt	570	2/19/2015	4/23/2015	2	55
3DD.0077498507	M	Chandler kelt	550	2/19/2015		1	23
3D9.1C2DB9D9AE	M	Chandler kelt	570	2/19/2015		1	76
3DD.00774DC7B5	M	Roza Adult Trap	460	2/27/2015	5/7/2015	2	54
3DD.007747C7E9	M	REC5	274	2/17/2015		1	63
3DD.00774DCE6E	M	Roza Adult Trap	480	2/26/2015		2	57
3DD.00774889B9	M	WIL39	223	2/17/2015		2	64
3DD.0077484639	M	REC5	329	2/17/2015		1	26
3DD.007748C7D5	M	Roza Adult Trap	445	2/23/2015	5/7/2015	2	10
3DD.0077490F88	M	Chandler kelt	610	2/19/2015		1	30
3DD.00774937A4	M	EBURG MSYR34	342	2/17/2015		1	1
3D9.1C2D7F5169	F		700	2/19/2015	4/5/2015	2	31
3DD.0077483A92	M	TIET17	211	2/18/2015		1	4
3DD.007748C69C	M	Buckskin Slough	218	2/24/2015		1	27

Table 2A.6 shows the percentage of each collection type assigned to individual fish. Note that each column sums to 200% as it includes both the male and female contribution. For each fish you can see differential proportions of each group. Large variances were seen. For example, in the upper Yakima section progeny assignments from female 3D9.1C2E0DA182 comprised 9% of the trap samples but 56% of the electrofishing samples.

Table 2A.6: Percentage of each collection type and assignment to individual parent.

PIT tag	# of Progeny	Naches Section Trap	Naches Section EF	Naches Section Tank	Upper Yakima Trap	Upper Yakima EF	Upper Yakima Tank	Elbow EF	End EF
# of progeny detections		254	184	82	246	96	96	18	4
3DD.0077494335	47	9%	18%	5%	9%	4%	4%	11%	0%
3D9.1C2E0DA182	149	30%	35%	37%	9%	56%	46%	33%	50%
3D9.1C2E0D7996	114	30%	23%	27%	22%	6%	19%	56%	0%
3D9.1C2DF0D728	149	21%	10%	20%	60%	33%	31%	0%	0%
3D9.1C2DF446BA	55	15%	11%	12%	10%	6%	10%	11%	0%
3DD.0077498507	23	6%	2%	2%	4%	6%	8%	0%	0%
3D9.1C2DB9D9AE	76	13%	4%	12%	33%	15%	8%	0%	0%
3DD.00774DC7B5	54	9%	16%	7%	13%	6%	10%	11%	0%
3DD.007747C7E9	63	6%	21%	24%	2%	25%	23%	11%	0%
3DD.00774DCE6E	57	13%	7%	17%	15%	4%	15%	0%	0%
3DD.00774889B9	64	15%	17%	7%	7%	13%	10%	67%	50%
3DD.0077484639	26	8%	4%	5%	2%	13%	4%	0%	0%
3DD.007748C7D5	10	2%	1%	0%	3%	0%	4%	0%	0%
3DD.0077490F88	30	3%	2%	0%	12%	13%	6%	0%	0%
3DD.00774937A4	1	1%	0%	0%	0%	0%	0%	0%	0%
3D9.1C2D7F5169	31	9%	14%	12%	0%	0%	0%	0%	50%
3DD.0077483A92	4	2%	2%	0%	0%	0%	0%	0%	0%
3DD.007748C69C	27	8%	12%	12%	0%	0%	0%	0%	50%
		2	2	2	2	2	2	2	2

Testing Channel Hydrology and Substrate Composition

Hydrology

On 6 August 2015, fifteen (15) minipeizometers were installed near the downstream end of each section at previous redd locations, and at locations across the channel from redds (Figure 2A.2). In Naches section 1, no redds were observed so minipeizometers were installed on the inside and outside bend of the channel at the downstream end of that section. Fourteen of the fifteen minipeizometers came to equilibrium, most stabilizing in 40 min or less with the exception of one location (*g*) in Naches and two locations in Upper Yakama (*n* and *o*) that were measured by Cle Elum hatchery staff the following day. One minipeizometer (*l* in Upper Yakama section 2) became irrecoverably clogged and data should be considered anomalous; however, the clogging is potentially indicative of low subsurface flow connectivity and poor substrate conditions (see Summary). Although sample size is small and caution should be taken in interpreting results, plotting values of VHG revealed a downwelling trend at redd locations, in the Upper Yakama channel, and on the inner bends of the channel (Figure 2A.3).

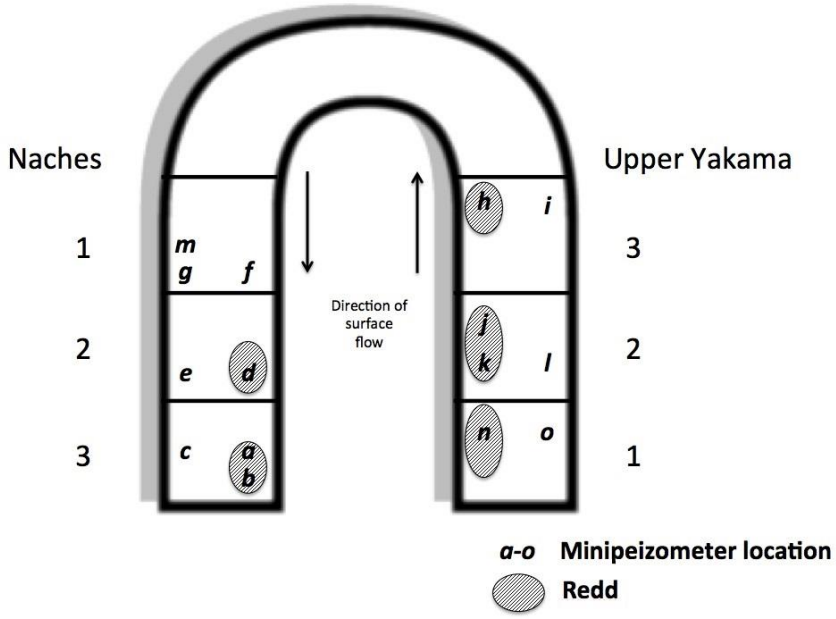


Figure 2A.2. Channel sectional layout with redd locations and minipeizometer locations.

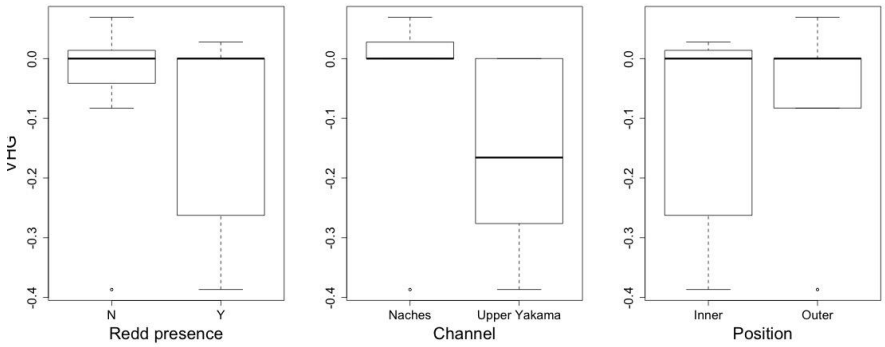


Figure 2A.3. Vertical hydraulic gradient (dh/dl) according to redd presence (n = 7 present, 7 absent) channel (n = 8 Naches, 6 Upper Yakama), and lateral position in channel (n = 8 inner, 6 outer).

In terms of selection of salmon spawning sites, the sample size of five redd clusters distributed among six stream sections should be considered extremely small, so all subsequent conclusions are tentative. Trends in negative VHG at sites where redds were

observed confirms results from an existing study demonstrating spawning bull trout preference for localized downwelling, in theory to increase oxygenation to incubating eggs (C. V. Baxter and Hauer 2000). Another study indicated that fall Chinook Salmon selected upwelling locations (Geist 2000) although the contradictory results could be explained by differences in the spatial scale of observation. Salmonids are thought to select upwelling locations at the reach scale to benefit from colder water temperatures, while simultaneously selecting downwelling sites at channel unit scales to maximize oxygenation through redds (C. V. Baxter and Hauer 2000). The fine spatial scale of this study approximates that of the channel unit scale, since comparisons were made within meters of one another. Downwelling tended to be more prevalent in the Upper Yakama where—again based on a small sample size—spawning was described as more abundant. Downwelling was more prevalent on the inner bend of the spawning channel where all spawning was observed.

Long equilibration times indicated low subsurface flow conductivity throughout most sections of the spawning channel, a value that was not measured precisely but gleaned from the long waiting times, with some piezometers requiring revisiting the following day. Two of the three piezometers requiring overnight equilibration were in the Upper Yakama section, where another piezometer was irrecoverably clogged, indicating that section may have substantial concentrations of fine sediment or decaying organic matter which can retard subsurface flow. A preliminary conclusion is that the spawning channel has very high levels of fine sediment which are suboptimal for spawning, but this interpretation should be considered alongside that of the substrate size composition evaluation that occurred simultaneously.

Substrate

Two sites were chosen in each section, both a redd, and non-redd samples were collected for a total of 12 samples for the entire channel (Figure 2A.4). The only exception to the redd/non-redd sampling was in Naches section 2-1 which had no redds, but still collected two samples so that we had an index for that section. All 9 redds were constructed at tailouts at the end of each of the sections on the inside portion of the channel (Figure 2A.4 and Table 2A.7). The Upper Yakima had the most redds with 7 and 2 in the Naches which were constructed over a period of approximately 2 months (early-March through early-May) (Table 2A.8). McNeil samples suggested that the Upper Yakima section had more fines deposition (particle sizes less than .85mm) than the Naches section.

Figure 2A.4. Channel sectional layout with McNeil samples and redd locations circled.

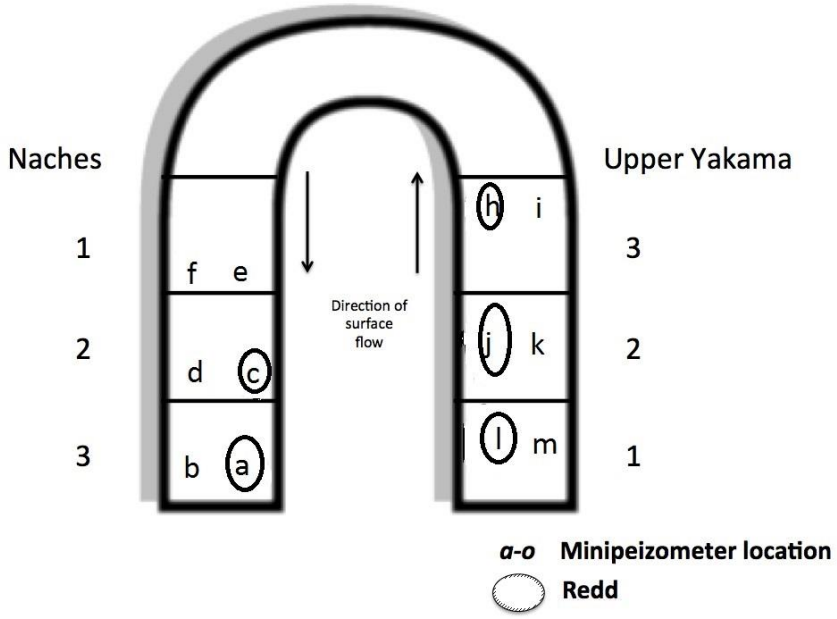


Table 2A.7. Redd locations and approximate build date.

Upper Yakima	Section redd construction	Date of redd construction	Naches	Section redd construction	Date of redd construction
Redd #1Y	Sec. 1-1(l)	3/2/2015	Redd #3N	Sec 2-3(a)	3/24/2015
Redd #2Y	Sec. 1-2(j)	3/8/2015	Redd #4N	Sec 2-2(c)	3/30/2015
Redd #5Y	Sec. 1-3(h)	4/1/2015			
Redd #6Y	Sec. 1-2(j)	4/1/2015			
Redd #7Y	Sec. 1-2(j)	4/28/2015			
Redd #8Y	Sec. 1-2(j)	5/4/2015			
Redd #9Y	Sec. 1-2(j)	5/4/2015			

Table 2A.8. McNeil Sample locations and percentages of substrate sizes (green=redd location and red =fines which can negatively effect egg to fry survival.

Upper Yakima Section

M: Upper Yakima Sec 1-1 Non Redd/Right Bank

size (mm)	weight (g)	% Comp	
63	1152.6	8.6%	
31.5	4951.2	37.1%	
16	4527.1	33.9%	
11.2	436.2	3.3%	
8	412.4	3.1%	
6.3	178.4	1.3%	
4	168.1	1.3%	
3.35	26.4	0.2%	
2	30	0.2%	
0.85	29.6	0.2%	
0.355	95.1	0.7%	**Total Fines**
0.125	1346.8	10.1%	10.8%
Total	13353.9	100.0%	

L:Upper Yakima Sec 1-1 Redd/Left Bank

size (mm)	weight (g)	% Comp	
63	0	0.0%	
31.5	4821.1	37.2%	
16	6176.6	47.6%	
11.2	751.4	5.8%	
8	198.3	1.5%	
6.3	73.1	0.6%	
4	51.8	0.4%	
3.35	6.5	0.1%	
2	9.2	0.1%	
0.85	23.4	0.2%	
0.355	52.1	0.4%	**Total Fines**
0.125	807.4	6.2%	6.6%
Total	12970.9	100.0%	

Naches Section

F: Naches Sec 2-1 Non Redd/Right Bank

size (mm)	weight (g)	% Comp	
63	0	0.0%	
31.5	2489.8	18.8%	
16	7508.7	56.7%	
11.2	859.1	6.5%	
8	553.8	4.2%	
6.3	360.4	2.7%	
4	761.9	5.8%	
3.35	196.7	1.5%	
2	104.8	0.8%	
0.85	25.4	0.2%	
0.355	9.3	0.1%	**Total Fines**
0.125	363.3	2.7%	2.8%
Total	13233.2	100.0%	

E: Naches Sec 2-1 Non-Redd/Left Bank

size (mm)	weight (g)	% Comp	
63	577	4.9%	
31.5	3709.4	31.6%	
16	5621.6	47.9%	
11.2	980	8.4%	
8	458.2	3.9%	
6.3	111.2	0.9%	
4	49.5	0.4%	
3.35	4.7	0.0%	
2	4.1	0.0%	
0.85	1.4	0.0%	
0.355	2	0.0%	**Total Fines**
0.125	217.1	1.8%	1.9%
Total	11736.2	100.0%	

J: Upper Yakima Sec 1-2 Redd/Left Bank

size (mm)	weight (g)	% Comp	
63	922.8	6.5%	
31.5	4060.7	28.8%	
16	4925.4	34.9%	
11.2	802.3	5.7%	
8	773.3	5.5%	
6.3	440.9	3.1%	
4	690.4	4.9%	
3.35	178.9	1.3%	
2	140.6	1.0%	
0.85	68.9	0.5%	
			Total
0.355	78	0.6%	**Fines**
0.125	1033.2	7.3%	7.9%
Total	14115.4	100.0%	

C: Naches Sec 2-2 Redd/Left Bank

size (mm)	weight (g)	% Comp	
63	0	0.0%	
31.5	4527.8	41.4%	
16	4813.5	44.0%	
11.2	977.8	8.9%	
8	493.8	4.5%	
6.3	102.4	0.9%	
4	24.2	0.2%	
3.35	0.2	0.0%	
2	T	#VALUE!	
0.85	T	#VALUE!	
			Total
0.355	0.2	0.0%	**Fines**
0.125	4.8	0.0%	0.0%
Total	10944.7	100.0%	

I:Upper Yakima Sec 1-3 Non-Redd/Right Bank

size (mm)	weight (g)	% Comp	
63	0	0.0%	
31.5	4794.9	31.6%	
16	6375	42.0%	
11.2	1240	8.2%	
8	894.8	5.9%	
6.3	504.3	3.3%	
4	793	5.2%	
3.35	208.7	1.4%	
2	113.4	0.7%	
0.85	38.1	0.3%	
			Total
0.355	11.1	0.1%	**Fines**
0.125	200.4	1.3%	1.4%
Total	15173.7	100.0%	

b: Naches Sec 2-3 Non Redd/Right Bank

size (mm)	weight (g)	% Comp	
63	0	0.0%	
31.5	3643.6	30.7%	
16	6175.1	52.0%	
11.2	1270.5	10.7%	
8	549.6	4.6%	
6.3	102.4	0.9%	
4	27.9	0.2%	
3.35	0.7	0.0%	
2	1	0.0%	
0.85	0.7	0.0%	
			Total
0.355	1.9	0.0%	**Fines**
0.125	99.4	0.8%	0.9%
Total	11872.8	100.0%	

H:Upper Yakima Sec 1-3 Redd/Left Bank			
size (mm)	weight (g)	% Comp	
63	0	0.0%	
31.5	4073.3	37.1%	
16	5306.9	48.3%	
11.2	688.3	6.3%	
8	394.1	3.6%	
6.3	150.7	1.4%	
4	114.5	1.0%	
3.35	18.6	0.2%	
2	10.3	0.1%	
0.85	2.7	0.0%	
			Total
0.355	58.5	0.5%	**Fines**
0.125	167.9	1.5%	2.1%
Total	10985.8	100.0%	

A:Naches Sec 2-3 Redd/Left Bank			
size (mm)	weight (g)	% Comp	
63	348.8	2.6%	
31.5	3961	29.8%	
16	5711.9	42.9%	
11.2	1498.9	11.3%	
8	1033.2	7.8%	
6.3	343	2.6%	
4	207.5	1.6%	
3.35	22.7	0.2%	
2	20.6	0.2%	
0.85	3.9	0.0%	
			Total
0.355	3	0.0%	**Fines**
0.125	152.9	1.1%	1.2%
Total	13307.4	100.0%	

We do not have data for channel composition and how much fines (<.855mm) were present before steelhead kelts were constructing redds. Based on photos and notes we can assume that there were some major inputs of organic fine materials that were transported into the channel beginning on March 16, 2015 and stopping sometime in late May. It is likely that redds in the upper Yakima sections 1-1 and 1-2 may have had lower egg to fry rates than other redds in the channel since they are approaching the 10% fines that based on the literature (Jensen et al. 2009) can halve egg-to-fry rates. This fine organic sedimentation possibly could have translated into a 33-39.5% reduction in egg to fry production at these two sites. This would mean that the Naches fish had the best spawning habitat while the Upper Yakima fish would have had the less desirable gravels. Another thing to also consider is that behavior could have been driving the locations of the redd placement. The middle berm appears to provide more dramatic shading on those sides depending on the time of day (morning/afternoon). Downwelling along the inside portion of the channel may be a driving component of redd site selection.

Channel Improvements

Based on this evidence we will attempt to reduce the sediment load by trying to use less effluent from the raceways and/or, employing more splash boards or logs at the first section to capture sediment. Also, we will consider rethinking of splitting the channel in half and instead consider splitting the channel based on number of mature kelts from each population. That way if we cannot implement any changes to reduce sediment we could close section 1-1 and open part of the elbow so the uppermost section would function to trap sediment.

Summary
Reconditioned kelt steelhead were successful in spawning in the Cle Elum artificial channel. As a feasibility study the 2015 effort was successful, however, there are several ways we can improve the channel prior to implementing a full study: 1. Fish stress during transport and holding will need to be improved. High mortalities were seen during and shortly after stocking the fish. To address this we will sedate fish utilizing a low level of anesthetic. Also, additional cover was added to the channel to provide holding and resting areas for fish which should reduce stress and encourage spawning. 2. Deposition of fine sediments within the channel will be addressed by increasing flows when fish are spawning so that sediment should not settle out as readily into the channel. Sampling of the hydrology and substrate should be continued to determine how effective kelt channel improvements will be and also the differences in observed sedimentation (pers. comm. with Chad Stockton) that occur at the Cle Elum hatchery from year-to-year. Samples will be collected just prior to kelt release/watering the channel sometime in early/mid-February (weather permitting), a mid-season sampling in the elbow section (June), and another following final expected redd emergence in August/September to monitor the amount of total fine sediment buildup. This also could help explain any differences in juvenile representation seen in the genetic sampling. 3. Trap efficiency and slippage between the sections will be addressed by improving design of the trap boxes and to use an additional trap box at the outflow to insure that we are collecting all juveniles. 4. Additionally, collection of every tenth juvenile sample should continue at both the trap and electrofishing stages. Re-sampling at the tank stage will only be used as a backup as it may contain mortality based biases. 5. Finally, all lengths of offspring will continue to be collected. It may be impossible to differentiate between variable sizes of target progeny and non-target juveniles that enter through the hatchery intake.

2.B: Yakima River Kelt Reproductive Monitoring

Introduction

The reproductive success of long-term reconditioned kelts needs to be explored to assess the net benefit of the kelt reconditioning program. Specific questions regarding the success of artificially reconditioning kelt steelhead include: 1) Do reconditioned kelts produce viable offspring that contribute to recruitment, 2) How does artificially reconditioned kelt reproductive success compare with natural repeat spawner success, and 3) How does artificially reconditioned kelt reproductive success compare with first time spawner success? In this study we utilize DNA markers and pedigree analysis to address these questions for kelt steelhead in tributaries of the Yakima River Basin.

Methods

Sample Collection

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

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

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 chelex beads Qiagen® DNeasy™ extraction kits. Current genotyping efforts utilize the 192 Single Nucleotide Polymorphism (SNP) markers and methods described in Hess et al. (2012). Samples were genotyped using either a fluidigm Ep1 platform, or GTseq techniques on an Illumina Hiseq 1500. Prior to parentage analysis, 40 loci were removed from the dataset. Dropped Loci included the sex-determining marker (OmyY1_2SEX), three loci diagnostic for cutthroat, one locus with poor genotypes, and 35 loci with low minor allele frequency.

Confirmed duplicate samples, samples with incomplete genotypes, and non-target species samples were omitted and are not included in the results.

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 annually to determine a 99.0% confidence LOD value. Individual parentage assignments were included if they had a minimum of 90% Loci comparisons, met the critical LOD value and had no more than a single locus mismatch. This accounts for the presence of minor genotyping errors, while still incorporating most genotyped samples.

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

Relative reproductive success (RRS) was calculated between classes of fish by standardizing to the maidens collected at the pre-spawn stage. Lifetime reproductive success (LRS) of reconditioned kelts was calculated by adding the RRS of post spawn maidens to the RRS of reconditioned kelts. This estimate of LRS does not look at individual fish that spawned across multiple years, nor does it look at the same group of fish across 2 consecutive years (e.g. Maiden in 2013, reconditioned kelts in 2014). Rather, it adds the RRS estimates of fish spawning in the same calendar year.

Results

The number of progeny collected at individual sites, and the corresponding number and percentage of samples assigned to at least one adult parent is shown in table 2B.1. Numbers for 2013 varied between zero assignments for offspring collected in Willy Dick Creek (Tributary to Toppenish Creek), and 36 assignments in Toppenish Creek above the three way. Numbers for 2014 varied between zero assignments at multiple locations to 21 assignments at Toppenish Creek upstream of Wesley Road. Average assignment rate across both years varied between 0% and 100% of juveniles assigning to at least one adult parent. Across all sites, 25.0% of the juveniles in 2013 and 23.3% of the juvenile in 2014 assigned to at least one of the anadromous parents for which a genetic tissue was taken.

Table 2B.1. Number of juveniles genotyped and assigned in 2013 and 2014 and average assignment rate across both years for each collection site

	Genotyped in 2013	assigned to parent	Genotyped in 2014	assigned to parent	Average Assignment Rate
Satus Creek Screw Trap			1	0	0.0%
Satus Creek-Dry mouth	11	8			72.7%
Satus-Logy Creek at Swamps			17	4	23.5%
Satus Creek-Above Logy Creek	3	2			66.7%
Satus Creek at Holwegners Ranch	21	5			23.8%
Satus - Dry Creek at Elbow Crossing	88	8	46	6	10.4%
Satus Creek below High bridge	75	21	68	20	28.7%
Satus Creek above High bridge	36	14			38.9%
Satus Creek below Wilson Charlie Cr			52	7	13.5%
Satus Creek above Wilson Charlie Cr			38	9	23.7%
Satus Creek below county line bridge	14	1	64	18	24.4%
Toppenish Creek Upper Screwtrap	50	14	37	12	29.9%
Toppenish Creek at Signal Peak Rd			23	9	39.1%
Toppenish Creek above Wesley Rd	100	36	64	21	34.8%
Toppenish Creek Near Olney Diversion			43	7	16.3%
Toppenish Creek at Swim hole	10	4			40.0%
Toppenish - Willy Dick Creek	46	0	8	0	0.0%
Toppenish Creek above Willy Dick			24	9	37.5%
Toppenish Creek near camp creek			19	5	26.3%
Toppenish - Simcoe Creek at Simcoe Rd.	*45*	*16*	45	16	35.6%
Toppenish - Simcoe NF SF confluence	49	8			16.3%
Toppenish - Agency Creek			13	0	0.0%
Ahtanum Creek by Fullbrite			10	3	30.0%
Ahtanum Creek at upper WIP			19	3	15.8%
Big Creek			14	0	0.0%
Cowiche and/or Crow Creek			30	7	23.3%
Naches - Little Rattlesnake Creek			23	0	0.0%
Naches - Nile Creek			53	0	0.0%
Naches - Quartz Creek			13	13	100.0%
Naches - Bumping River			2	0	0.0%
Naches - Lower Nile Creek			16	4	25.0%

The number of genotyped parents is shown in Table 2B.2. Pre-spawn maidens have the greatest number of samples with a total of 312 males and 905 females. The number of Post-spawn maidens was lower with only 78 males and 625 females. The lowest number of samples is seen in the reconditioned kelts detected moving upstream of Prosser dam. Across both years, only

24 males and 321 females have been sampled and genotyped. This number will increase incrementally with additional years of data, but will remain the smallest class due to the limited number of kelts that can be collected, and the expected mortality seen during the reconditioning process.

Table 2B.2. Number of genotyped parents by class.

Class	Sex	2013	2014	Both
Pre-spawn maidens	Male	145	167	312
Post-spawn maidens	Male	23	55	78
Reconditioned kelts	Male	16	8	24
Pre-spawn maidens	Female	451	454	905
Post-spawn maidens	Female	330	295	625
Reconditioned kelts	Female	225	96	321

Table 2B.3 shows the number of parents with progeny assigned to them, the percentage of each class with progeny assignments, the number of progeny assigned to each class, the number of progeny per parent, RRS of each class, and LRS of the reconditioned class. The number of parents with progeny assigned to them is expected to be much lower than the true number of successful parents as we sampled across a relatively small portion of the spawning habitat and the total juvenile numbers within any brood year. Detection as a percentage of all individuals within a class was lowest in the Pre-spawn maidens at 5.1% of the male fish and 5.6% for female fish. Detection rates in the post-spawn maidens were higher at 12.8% for males and 10.1% in females. The higher rate in the post spawn maidens is probably because these fish are known to have not suffered from prespawn mortality. Because ripe females are not taken into the program, female fish likely spawned prior to interrogation as post spawn kelts in at the Chandler facility. While the 6.0 % of female reconditioned kelts with progeny detected was similar to that of pre-spawn maidens (5.1%), 16.7% of the male reconditioned kelts had progeny detected while only 5.1% of the male pre-spawners had progeny detected.

The RRS of male post-spawners and reconditioned kelts were both over triple that of pre-spawners Table 2B.3. This led to a LRS of 6.82 that of fish collected as pre-spawners. While female post-spawn collection RRS was 1.37 times that of pre-spawn collection, reconditioned kelt RRS was slightly lower at 0.69. This gave a LRS of 2.06.

Table 2B.3. Number and percentage of parents detected in each class, Average number of progeny assigned to an individual, relative reproductive success (RRS), and lifetime reproductive success (LRS) for each group of individuals.

		Parents detected		Progeny Assigned			
Class		N	%	N	Per	RRS	LRS
Pre-spawn maidens	Male	16	5.1%	33	0.11	1.00	
Post-spawn maidens	Male	10	12.8%	27	0.35	3.27	
Reconditioned kelts	Male	4	16.7%	9	0.38	3.55	6.82
Pre-spawn maidens	Female	33	5.6%	101	0.17	1.00	
Post-spawn maidens	Female	55	10.1%	128	0.23	1.37	
Reconditioned kelts	Female	17	6.0%	33	0.12	0.69	2.06

Discussion

The 2014 spawning event was the second consecutive year that we successfully assigned multiple progeny to reconditioned kelts. A total of 42 juveniles are attributed to a spawning event following successful reconditioning of a kelt. We have currently assigned 310 progeny to at least one anadromous parent. This reflects the methodology of focusing sampling efforts on age-0 fish in areas that spawning was expected to have occurred. Additional years will add to this number, and we plan to increase the number of potential offspring sampled and genotyped.

Future sampling will continue to focus on age-0 fish in areas that spawning was expected to have occurred. Locations that fail to provide adequate sample numbers or have few assignments to anadromous adults across multiple years will be dropped. The only site that has currently been dropped for this reason is Willy Dick Creek in the Toppenish drainage which was excluded from sampling in 2015.

The presence of progeny shows that reconditioned kelts are able to successfully spawn in the wild. While relative reproductive success of female reconditioned kelts was lower than that of pre-spawn, any spawning by a reconditioned kelt is additive to the population and should be considered a success. Due to the higher RRS of fish from the post-spawn collections, Lifetime reproductive success of female reconditioned kelts was calculated to be 2.06 times that of the pre-spawn maidens. This is similar to findings by Seamons and Quinn (2010) who theorized and found that lifetime reproductive success of repeat spawners should scale with the number of breeding spawners.

Reconditioned kelt steelhead have demonstrated that they are capable of spawning in the wild. With additional sampling in future years we hope to have more accurate numbers and modeling potential. Current data shows that reconditioned kelt steelhead contribute to the productivity of the natural population on a scale similar to that of natural kelts, helping to preserve this important life history.

Chapter 3. Develop and implement methods to assess the fat levels, maturation timing, fecundity, egg size, and gamete viability of reconditioned kelts.

Introduction

Studies applying tools from fish physiology and endocrinology to issues in kelt reconditioning were continued in 2015. 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. In 2015, we assessed maturation status in blood samples from kelts taken in 2015 and earlier years (Section 3A). We conducted a study using hatchery origin kelts at Dworshak National fish hatchery to assess the effect of reconditioning on egg quality and other aspects of reproductive performance (Section 3B). We conducted a diet study using Prosser kelts (Section 3C). We continued studies comparing reproductive development and energy reserves in reconditioned kelts and maiden spawning steelhead in the Yakima and Snake Rivers (Sections 3D and 3E). We began laboratory work to establish an assay for plasma insulin-like growth factor-1, an indicator of growth and metabolic status (Section 3F). Many of these studies are ongoing, and laboratory analysis, results, interpretations, and conclusions may change as additional work is completed.

Section 3.A: Reproductive development in kelt steelhead

Introduction

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

Iteroparous female salmonids have two major post-spawning life history trajectories (Chaput and Jones 2006; Keefer, et al. 2008; Rideout, et al. 2005; Rideout and Tomkiewicz 2011). After a spawning event, some fish are able to restore energy lost during migration and spawning, redevelop a mature ovary, and spawn the next year. These fish are termed consecutive spawners. Other fish do not initiate redevelopment of the ovary for the next spawning season, but instead skip a year. These fish are termed skip spawners. We hypothesize that these life

history trajectories are the result of the effect of energy balance on maturation decisions made during seasonally defined critical periods. The influential critical period model of the first reproductive maturation (puberty) in salmonids posits that maturation is initiated during a decision window approximately one year prior to spawning (Campbell, et al. 2006b; Satterthwaite, et al. 2009; Shearer and Swanson 2000; Thorpe 2007). This decision is made based on energy reserves. If maturation is initiated during this critical period, it may be arrested at a second critical period before the onset of exogenous vitellogenesis, if energy reserves are not sufficient (Yamamoto, et al. 2011). We hypothesize that a similar decision mechanism regulates rematuration in post-spawning steelhead. Consistent with this idea, we found that energy restriction affected reproductive development within 10 weeks after spawning in female rainbow trout (Caldwell, et al. 2013; Caldwell, et al. 2014). In post-spawning fish, energy driven decisions take place in the context of the extreme energy deficit incurred by migration and spawning (Penney and Moffitt 2014a, b, 2015). Threshold energy levels for maturation or rematuration are determined by the genetic makeup of the fish and subject to selection (Carlson and Seamons 2008; Hutchings 2011).

Studies conducted in 2009-2011 established that blood levels of estradiol and vitellogenin diverge between rematuring and non-rematuring fish during reconditioning. Estradiol is the principal female gonadal steroid in fishes, which regulates many aspects of reproductive development, and vitellogenin is a phospholipoprotein produced by the liver under regulation by estradiol which provides most of the material for ovarian development. Estradiol indicates maturation earlier than vitellogenin, and the cost of the estradiol assay is about 1/4th of the cost of the vitellogenin assay.

During 2015, we measured estradiol level in a large number of blood samples. We collected blood from fish in the reconditioning programs at Prosser and Dworshak, ran plasma estradiol assays, and provided maturation status to project managers so that rematuring fish could be released and non-rematuring fish retained for further reconditioning. We collaborated with colleagues in the Upper Columbia reconditioning project at Winthrop National Fish Hatchery to measure estradiol levels in samples they collected from their reconditioned kelts, and in maiden spawners they sampled at Wells dam. For the first time, we measured plasma 11-ketotestosterone levels in male kelts reconditioned at Prosser, and found that rematuring and non-rematuring males are produced by the project. Laboratory assays and data analysis are ongoing. Preliminary results are presented here, with the caveat that they may change as more assays and analysis are completed.

Methods

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 (Ch. 1, Section 1) (Abrahamse and Murdoch 2013, 2014).

Sampling

Fish were blood sampled on the indicated dates (Table 3A.1). During blood sampling, blood (2 mL) was drawn from the caudal vein using heparinized syringes (ammonium heparin, 10 mg/ml) and centrifuged (5 min, 5000 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 were 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).

Table 3A.1: *Steelhead kelts sampled during the fall in 2015. DNFH: Dworshak National Fish Hatchery, WNFH: Winthrop National Fish Hatchery, Prosser: Prosser Hatchery. Additional hatchery origin kelts sampled for our reproductive performance study are described in section C2, and maiden steelhead sampled at Lower Granite Dam are described in section C5.*

Location	Sample date	Fish type	# Fish	Notes
Prosser	9/16/2015	Wild kelts	573	Includes males.
DNFH	9/22/15	Wild kelts	45	Kelts collected at Fish Cr, Lower Granite Dam, and South Fork of the Clearwater River.
WNFH	10/8/2015	Wild kelts	31	
Wells Dam	9/29/2015 to 10/5/2015	Hatchery and wild maidens	13	

Estradiol Assay

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

plasma samples were appropriately diluted and duplicate technical replicates assayed in the EIA according to the manufacturer's instruction manual provided with the kit.

11-ketotestosterone assay

11-ketotestosterone (11-KT) is the principal androgen in male teleosts, and increases during reproductive maturation in male salmonids. Plasma samples from all Prosser fish categorized as male at intake or at the 9/16/2015 sampling were assayed for 11-KT, and an additional random selection of female plasma samples were assayed. Plasma samples were ether extracted following the same protocol as for the estradiol assay. Plasma 11-KT concentrations were then assayed in reconstituted samples using an EIA kit specific for 11-KT (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.

Results

Plasma E2 levels were bimodally distributed in blood samples taken from female kelts in all projects at a pre-release sampling in the fall (Figs 3A.1, A.2, A.3). The division between the lower and higher modes was approximately 1000 pg/ml E2 at Prosser and DNFH, as found in previous years. However, at WNFH, several fish with E2 levels of 1000-3000 pg/ml appeared to group with the lower mode, and consequently the division between modes was adjusted upward. Plasma E2 levels in maiden Upper Columbia River steelhead sampled at Wells dam were similar to those of rematuring Upper Columbia kelts. The rematuration rate of female kelts as consecutive spawners was high in all projects in 2015. Prosser females rematured at a 71.6% rate, whereas females at DNFH and Winthrop rematured at rates closer to 50% overall. DNFH kelts from Fish Creek on the Lochsa River had a somewhat lower maturation rate (40%) than fish collected at Lower Granite Dam (72.7%) or the South Fork of the Clearwater River (57.1%). The rematuration rate of female kelts held for a second year of reconditioning was very high, 92.9% at Prosser and 100% at DNFH. Most male kelts at Prosser had plasma E2 levels similar to those of non-rematuring females, however, a few had elevated E2 levels in the rematuring female range.

Figure 3A.1: *Plasma estradiol (E2) levels in Prosser kelts sampled in fall of 2015.*

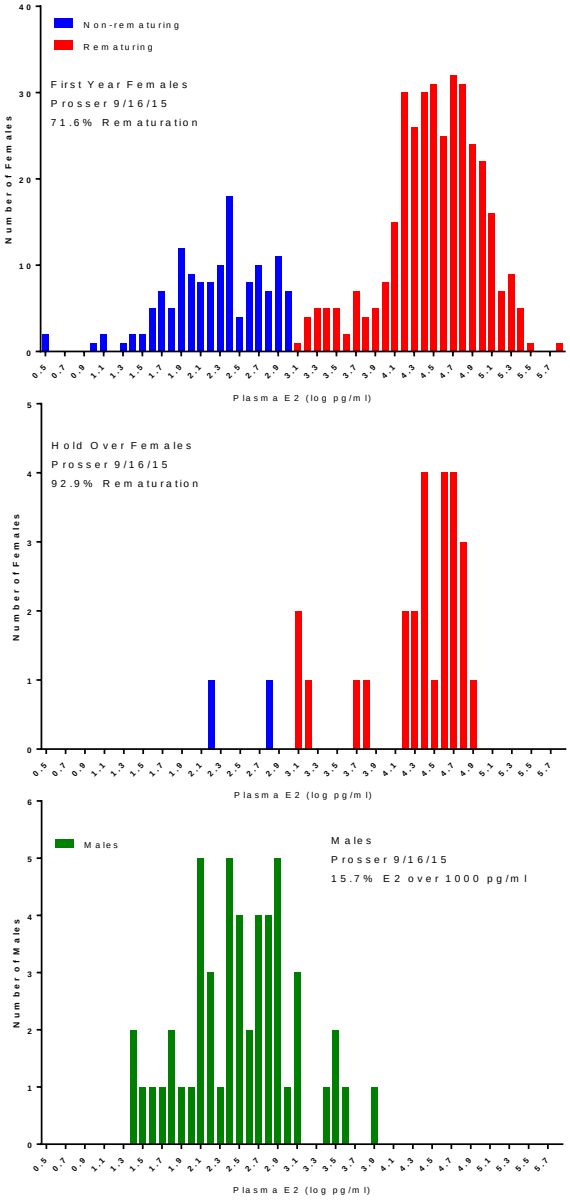


Figure 3A.2: *Plasma estradiol (E2) levels in female DNFH kelts sampled in fall of 2015.*

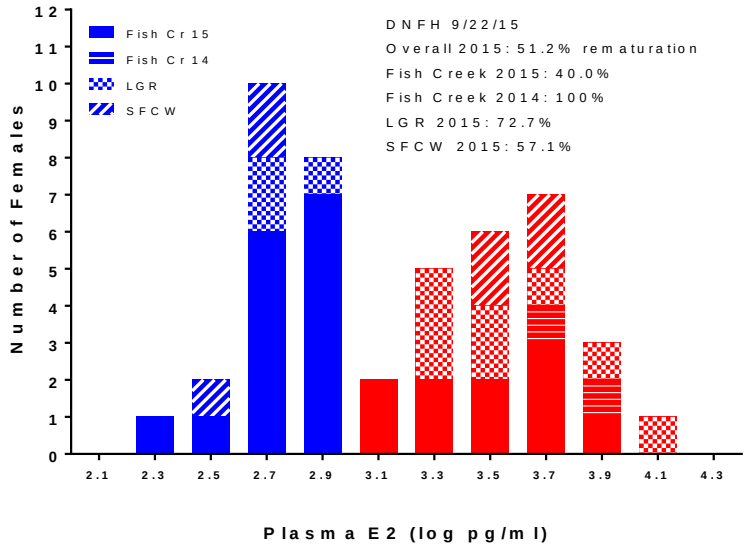
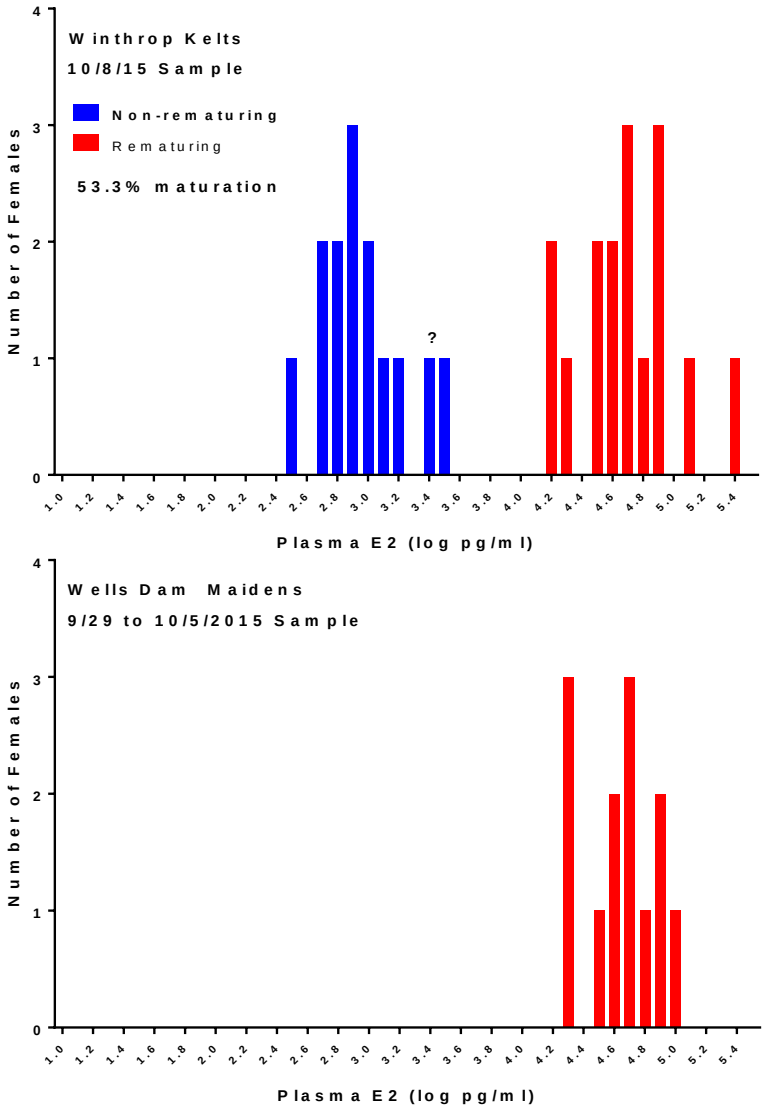
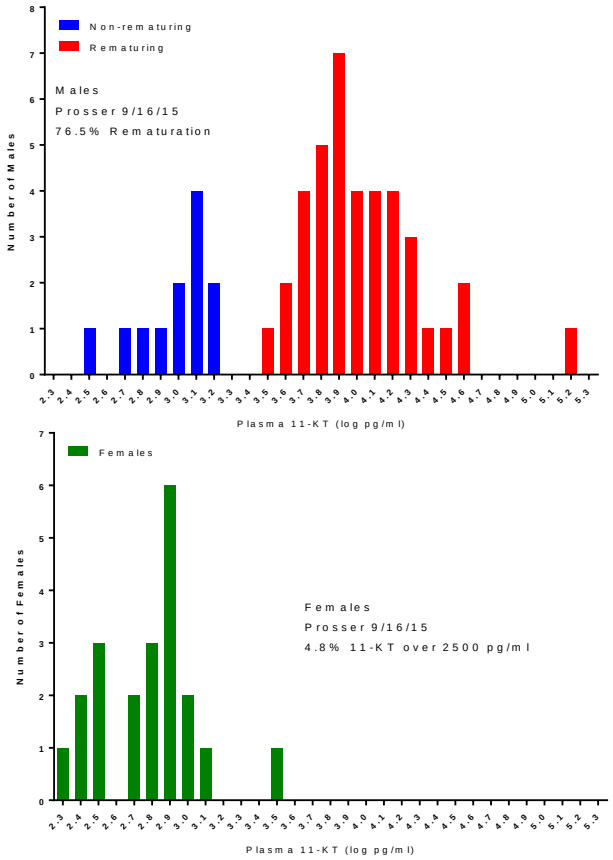


Figure 3A.3: *Plasma estradiol (E2) levels in female Upper Columbia kelts and maiden spawners sampled in fall of 2015.*



Plasma 11-KT levels were bimodally distributed in blood samples taken from male kelts at Prosser in the fall (Fig. 3A.4). The division between higher and lower modes was approximately 2500 pg/ml 11-KT. The maturation percentage of Prosser males (76.5%) was similar to that of Prosser females. Almost all fish categorized as female based on physical appearance had plasma 11-KT levels in the range of non-rematuring males.

Figure 3A.4: *Plasma 11-ketotestosterone (11-KT) levels in Prosser kelts sampled in fall of 2015.*



Discussion

It is now well established that some female steelhead kelts remature after a summer of reconditioning, whereas other fish do not, and that plasma estradiol level from mid-August onward indicates maturation status. Evidence in both steelhead kelts and post-spawning

rainbow trout suggests that the initial decision to remature is made early, before mid-July for kelts and during the 10 weeks after spawning in rainbow trout (Bromage, et al. 1992; Caldwell et al. 2013; Caldwell et al. 2014; Hatch, et al. 2013). Plasma estradiol levels in rematuring and non-rematuring kelts for 2015 at Prosser and Dworshak were similar to previous years. A shift upward in the high mode is probably due to the later sampling date. At DNFH, many Fish Creek females had E2 levels in the 700-900 pg/ml range. Since the spawn timing of Fish Creek fish is very late, it is possible that these fish, which were classified as non-rematuring, were actually early rematuring fish. These fish are being held for further reconditioning, so maturation status will become clear as the season advances. Plasma E2 levels were shifted upward in both the low and high modes in Winthrop kelts. The reasons for this are not known, but may relate to the genetic stock or physiological condition of the fish. Winthrop kelts had some of the highest muscle lipid levels and condition factors ever observed in any Columbia Basin kelt project (M. Abrahamse, personal communication). The similarity in E2 levels between reconditioned Winthrop kelts and maiden steelhead at Wells dam suggests that reproductive development is on track in the rematuring kelts. All Winthrop kelts were released this fall as the facility is being prepared for year round fish holding.

Female consecutive maturation rates were high at all projects this season. It is possible that this relates to pre-capture environmental conditions common to the three projects. The high consecutive maturation rates found in Snake River kelts was particularly notable. Previously, Snake River steelhead, and steelhead from the Skeena and Nass systems in British Columbia, which have a life history similar to Snake River B-run steelhead, have been found to repeat spawn predominantly as skip spawners (Chudyk 1976; Keefer et al. 2008; Moore, et al. 2014). This has been hypothesized to be due to the longer migration and later spawn timing of these fish. The present results shows that high consecutive rematuration rates are possible for Snake River steelhead in captive reconditioning. Captive kelts do not undergo the long return migration during warming river temperatures that natural repeat spawning Snake River steelhead must undergo. Obtaining kelts soon after spawning, and proper husbandry and feeding protocols to reverse the energy deficit incurred by migration and spawning are likely reasons for the improved results with Snake River kelts this season.

Non-rematuring fish held for a second year rematured at very high rates (90% or higher) in 2015 at both Prosser and DNFH. This adds to a growing body of data showing that non-rematuring females will remature as skip spawners if held for a second year. Skip spawning is a natural life history in Columbia Basin steelhead. Increased size, fecundity, and energy reserves in skip spawners would be expected to result in greater relative reproductive success versus maidens or consecutive repeat spawners. The presence of skip spawners increases life history diversity, which would be expected to increase population stability in steelhead populations (Moore et al. 2014; Schindler, et al. 2010). Moreover, whether and how much culture conditions can influence the proportion of consecutive and skip spawning kelts in captive reconditioning is not well understood. These considerations suggest that Columbia Basin kelt reconditioning programs should find ways to accommodate the skip spawner life history.

Plasma 11-KT levels were bimodally distributed in male steelhead kelts sampled in the fall at

Prosser, indicating that rematuring and non-rematuring individuals are present. This result is not surprising: during necropsy of mortalities at Prosser, male fish with no evidence of maturation in the testis have been found. Because the energy required for reproductive maturation is lower in males than in females, one might hypothesize that consecutive maturation rates for males would be higher than those for females. On the other hand, males remain in spawning tributaries longer than females and expend more energy (Quinn and Myers 2004), which could lead to a lower consecutive rematuration rate. The present finding of similar male and female consecutive rematuration rates does not strongly favor either hypothesis.

A few male kelts had plasma E2 levels in the range of rematuring females. However, all of these males were rematuring based on 11-KT level. During maturation in male salmonids, the testes produces large amounts of testosterone. Testosterone is converted to 11-KT by enzyme systems in the Leydig cells of the testes, however, testosterone can also be converted to E2 by aromatase activity, which is found in a number of tissues including brain and fat. Elevated E2 levels in some rematuring males is likely due to conversion of circulating testosterone to estradiol by tissue aromatase activity. Only one female kelt had an elevated 11-KT level. This fish also had a rematuring 11-KT level. Prosser kelts were classified as male or female based on appearance for the analysis reported here. It is possible that some fish may have been incorrectly classified. The female with elevated 11-KT may actually be a male. Additional ongoing work will enable us to identify the sex of each fish using genetic markers. Combined with plasma E2 and 11-KT levels, this will enable us to classify all fish as rematuring or non-rematuring males or females.

Section 3.B: Reproductive performance in hatchery origin maiden female steelhead and reconditioned kelts at Dworshak National Fish Hatchery (DNFH)

Introduction

In their recent review of the Upper Columbia Kelt Reconditioning Program, ISRP recommended that: "Methods to assess the fat levels, maturation timing, fecundity, egg size, and gamete viability of the project's reconditioned kelts need to be developed and implemented…" (ISRP Memorandum 2014-9, Qualification 3). To address ISRPs recommendation, we are conducting an experiment to assess reproductive performance in hatchery origin kelts at DNFH.

It is difficult to directly to assess egg quality and fecundity in wild fish, because wild fish spawn naturally before collection, and reconditioned wild fish are released to spawn naturally. The DNFH hatchery origin kelt model provides a unique opportunity to directly assess egg quality and fecundity in a large number of maiden spawners. If these fish can be successfully reconditioned, egg quality and fecundity in the first spawning can be directly compared to the second spawning. Production of high quality eggs is necessary for reconditioned kelts to contribute to listed Snake River steelhead populations. If issues with egg quality are identified, they will need to be addressed in order for the project to succeed. On the other hand, fecundity increases with body size in salmonids (Quinn 2005), suggesting that reconditioned kelts should have higher fecundity than maiden fish. The production of eggs that can be fertilized and develop successfully is a necessary but not sufficient condition for reproductive success of reconditioned kelts in the wild. However, if egg quality and spawning success are equal, then the relative fecundity of reconditioned kelts can provide an estimate of the productivity of reconditioned kelts versus maiden steelhead. Thus, assessment of egg quality and fecundity in reconditioned kelts is a step toward our goal of measuring the relative reproductive success of reconditioned kelts.

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

patterns and return proportions of consecutive and skip spawners (Chaput and Benoit 2012), suggesting that post-spawning life history is plastic and depends on feeding conditions in the ocean. This is supported by studies in steelhead showing that maturation is associated with growth in the marine environment (Quinn, et al. 2011).

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

Methods

In 2013- 2015, hatchery origin maiden female steelhead were air spawned at DNFH (Table 3B.1). Air spawning was conducted as previously described (Hatch, et al. 2014). In both years, after air spawning, lengths and weights of fish were recorded, and a non-lethal measure of muscle lipid content was taken using a Fish Fatmeter (Distell Inc., Midlothian, UK). In 2014-2015, the total weight of eggs collected from each female was recorded, and a subsample of approximately 100 eggs from each female was taken for transport to our laboratory at the University of Idaho. The total weight of eggs was used as ovary weight for calculation of gonadosomatic index. Milt from several males remaining from DNFH production spawning was also collected and transported to the University of Idaho. Milt samples were not pooled. At the University of Idaho, the motility of milt from each male was assessed, and a male was selected with confirmed motility and sufficient volume to fertilize all of the eggs collected. The weight of a random subsample of 25 eggs from each female was recorded for calculation of egg weight. Eggs were fertilized and incubated for 12 h. After 12 h, approximately 25 eggs from each female were fixed in Stockard's solution and stored (Stoddard, et al. 2005). The percentage of eggs successfully fertilized was measured as the percentage of fixed eggs showing cleavage (cell division) in the embryo by examination under a dissecting microscope. This method is less variable than assessments of egg quality further along in development, and eggs lots with reduced viability are clearly evident at the 12 hour time point (Stoddard et al. 2005).

Table 3B.1: *Hatchery origin female steelhead artificially spawned and reconditioned at Dworshak National Fish Hatchery in 2013-2015.*

Spawn Year	Fish Air Spawned	8/9/2013		8/28/2014		9/22/2015	
		Alive (%)	Rematuring (%)	Alive (%)	Rematuring (%)	Alive (%)	Rematuring (%)
2013	163	74 (45.4)	16 (21.6)	29 (50)	27 (93.1)	-	-
2014	149	-	-	32 (21.5)	2 (6.3)	6 (20)	5 (83.3)
2015	148	-	-	-	-	43 (29.1)	13 (30.2)

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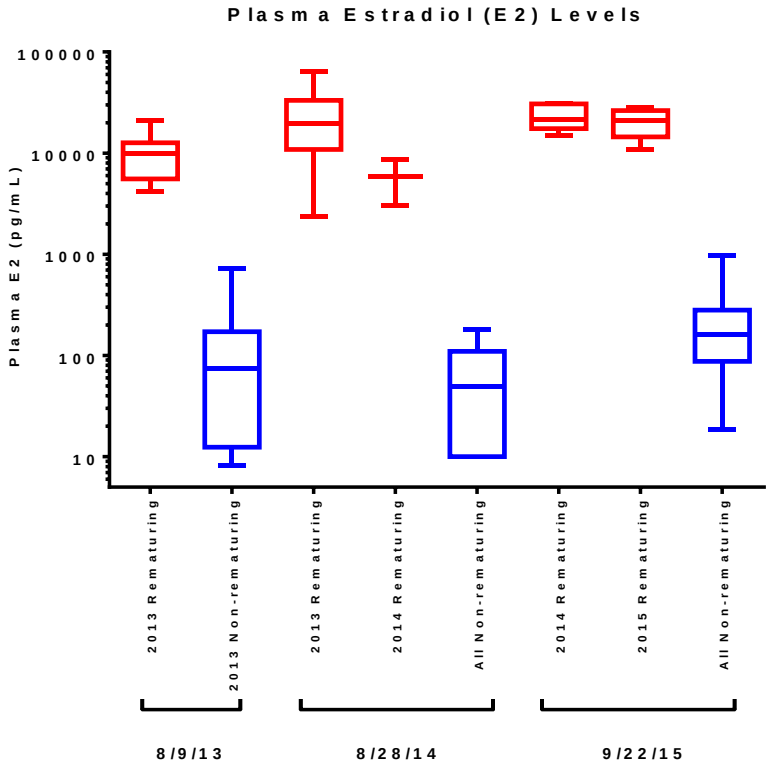
Fish were reconditioned as described (Hatch et al. 2014)(Methods: Long-term Reconditioning: Dworshak National Fish Hatchery: Dworshak Reconditioning Facility and Treatment pg. 35). Fish were sampled at approximately 6 week intervals. During sampling, length, weight, and muscle lipid levels were measured and blood was drawn for hormone assays. Laboratory analysis of these samples is ongoing. Results are reported for assays that have been completed. Plasma estradiol was assayed as described (Methods: Kelt Reconditioning Physiology Studies)(Hatch et al. 2014). Rematuring kelts were checked for ripeness weekly beginning in early February. Fish were air spawned when ripe. Eggs quality and fecundity were assessed as described above.

Results

Plasma E2 levels were elevated in rematuring kelts by late summer to fall (Fig. 3B.1). Median E2 levels were approximately 50-200 pg ml^{-1} in non-rematuring fish, versus 6000-20000 pg ml^{-1} in rematuring fish, with higher levels at later sampling points. Consecutive maturation rates ranged from 6.3% to 30.2%, whereas surviving fish matured as skip spawners at much higher rates of 83.3 to 93.1%.

Figure 3B.1: Plasma estradiol levels in rematuring and non-rematuring 2013-15 spawn year hatchery origin kelts at late summer to fall sampling.



Individual egg weight, total egg weight, and fecundity increased with increasing length in maiden spawners and reconditioned steelhead kelts (Fig 3B.2). Increases in all three factors with length were steeper in reconditioned kelts than in maiden steelhead. Maiden and kelt

reproductive performance are compared in Fig. 3B.3. Kelt eggs were significantly larger than those of maidens (consecutive and skip spawners 1.13 and 1.20 fold that of maidens, respectively). Kelt fecundity was significantly greater than maiden fecundity (consecutive 1.39; skip; 1.38 fold maiden fecundity). No differences were detected in GSI between spawning groups. Fertilization success did not differ significantly between maiden and consecutive spawners. However, median fertilization success was significantly reduced (.815 fold) in skip spawners versus maiden fertilization success. Consecutive and skip spawning steelhead were spawned mean 1.6 and 2.8 weeks earlier than the date of their maiden spawning, respectively (Fig. 3B.4).

Figure 3B.2: Length versus individual egg weight, total egg weight, and fecundity in maiden, consecutive repeat spawning, and skip repeat spawning reconditioned hatchery origin steelhead kelts. Kelt and maiden regression lines differ significantly.

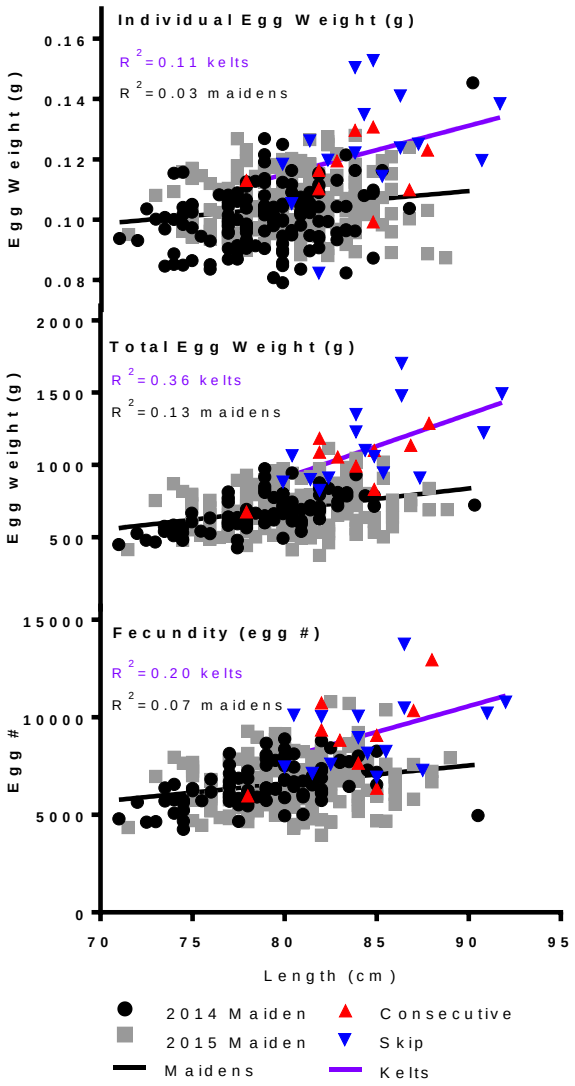


Figure 3B.3: Reproductive performance in maiden, consecutive repeat spawning, and skip repeat spawning reconditioned steelhead kelts. Bars not sharing a letter differ significantly (ANOVA followed by Tukey or Kruskal-Wallis test).

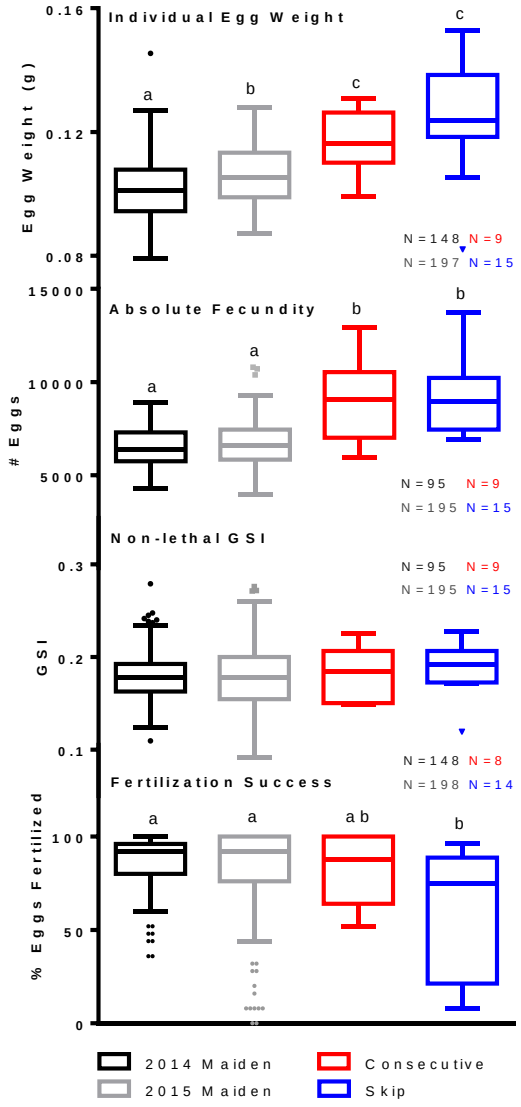
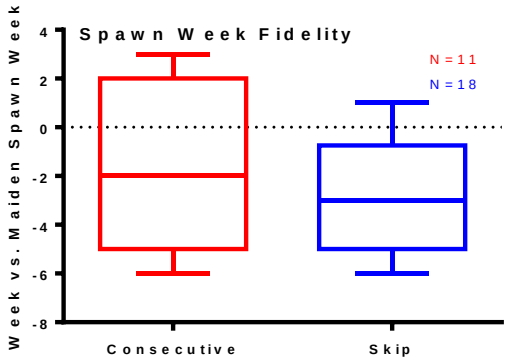


Figure 3B.4: Spawn week fidelity versus maiden spawning in consecutive and repeat spawning reconditioned hatchery steelhead kelts.



Kelts did not increase in length during the first 6 months of reconditioning, but did grow during the second 6 months (Fig. 3B.5). Growth in weight was greater in consecutive than skip spawning kelts over the first 6 months of reconditioning. Over the second 6 months, the pattern was reversed, with greater growth in skip spawners. Consecutive and skip spawners did not differ detectably in muscle lipid content at intake into reconditioning (Fig 3B.6). However, by 6 months post-spawning, muscle lipid content had increased, and was elevated in consecutive versus skip spawners. Muscle lipid decreased during the 6 months prior to spawning in consecutive spawners. In skip spawners, muscle lipid levels continued to increase to maximum levels at 18 months after spawning, and then they too declined during the final 6 months before the fishes second spawning.

Figure 3B.5: *Specific Growth Rates (SGR) in length and weight over the first and second six months of reconditioning in consecutive and skip repeat spawning reconditioned hatchery steelhead kelts. Bars not sharing a letter differ significantly (ANOVA followed by Tukey's test, p < 0.05).*

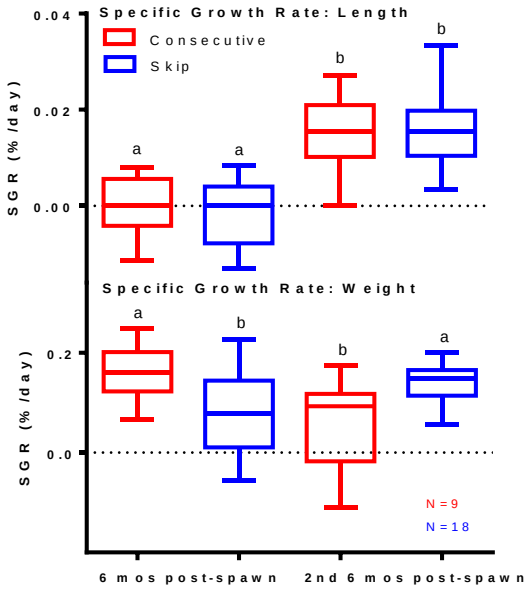
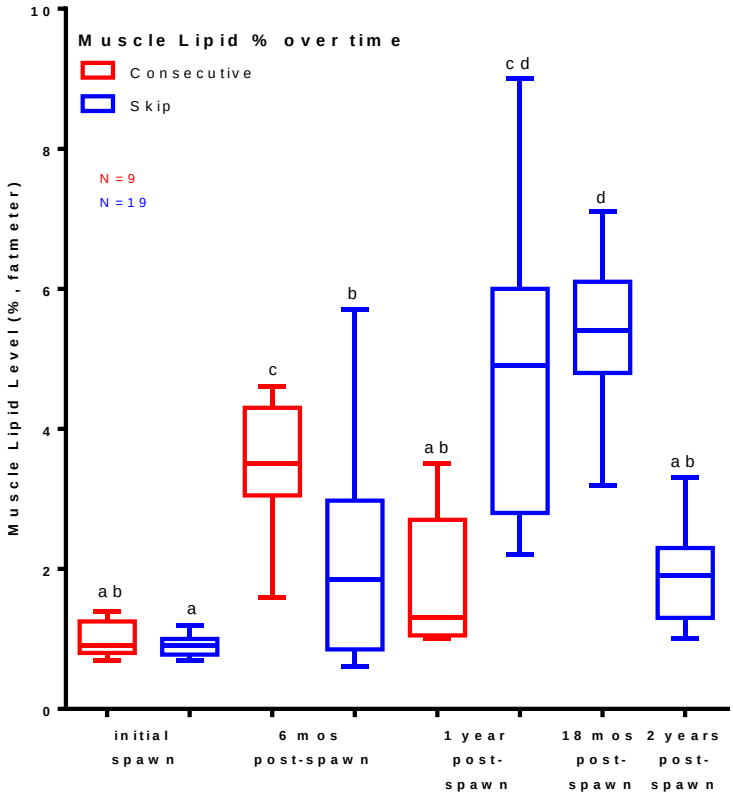


Figure 3B.6: *Muscle lipid levels over time in consecutive and skip repeat spawning reconditioned hatchery origin steelhead kelts. Bars not sharing a letter differ significantly (ANOVA followed by Tukey's test, p < 0.05).*



Discussion

Survival and maturation rates of hatchery origin kelts improved in 2015 versus 2014. The reasons for this likely include better fish condition at intake and more consistent fish care. Maturation percentage in years without issues with fish condition at intake or fish care have been in the 20-30% range, which may reflect what is possible for the genetic background and migration conditions of the DNFH hatchery stock under our current culture conditions. Survival was lower than our maximum survival rate of 45% obtained in 2013. This may be due to head injuries in a proportion of fish. These injuries were determined to be due to fish getting their heads caught between the sides of the tanks and PVC sheaths along the tank walls installed to

hold oxygen probes. No new injuries were observed after the oxygen probe sheaths were removed partway through the season.

Maturation rates of kelts held for a second year of reconditioning have been consistently high, indicating that fish most that do not remature as consecutive spawners after one summer of reconditioning are actually skip spawners which will remature the following season. However, survival of kelts over the second year has not been as good as anticipated, ranging from 50% to 20%. Survival over the second year is measured over a time period twice as long as survival to the late summer to fall sampling, which accounts for part of the difference. We believe that survival rates for skip spawners can be improved with improved holding facilities.

Plasma estradiol level have consistently shown complete separation between rematuring and non-rematuring DNFH hatchery kelts by August to September, similar to our results in wild kelts at Prosser (Branstetter et al. 2011; Hatch et al. 2013; Hatch et al. 2012). Estradiol levels in rematuring kelts were in the 6,000-20,000 pg/ml range, similar to Prosser kelts and considerably higher than maximal levels in maturing rainbow trout. Estradiol levels increase to a peak approximately four months before spawning in rainbow trout (Prat, et al. 1996; Tyler and Sumpter 1996; Tyler, et al. 1990), so levels in kelts sampled in August to September are likely less than maximal. The high E2 levels in rematuring kelts suggest a high level of reproductive investment in these fish.

Egg weight, GSI, and fecundity increased with length in both maidens and kelts, as expected (Quinn 2005; Quinn et al. 2011). The steeper slope of the regression lines in kelts versus maidens indicates a greater increase in reproductive investment per unit increase in length in kelts than in maidens. We hypothesize that this is due to better nutritional condition in kelts resulting in less adjustment to reproductive effort by atresia. Consistent with this hypothesis, GSI was not reduced in kelts versus maidens, even though kelts had much higher condition factors and lipid stores at spawning than maidens (data not shown), resulting in a greater somatic weight. This suggests that reproductive investment in steelhead may be determined by mass at spawning, rather than length.

Kelts were generally superior to maidens in measures of reproductive performance, suggesting that kelts released to spawn naturally should be more productive than maidens. The increase in fecundity of kelts suggests that they should produce approximately 1.4 fold the number of offspring of maidens, and the increase in egg size suggests that kelt offspring will begin life with a size advantage that should result in increased survival versus maidens. The greater fecundity of Atlantic salmon repeat spawners results in a disproportionate contribution to population productivity (Halttunen 2011; Moore, et al. 1995; Niemela, et al. 2006a). Skip spawning reconditioned kelts were expected to have even higher fecundity than consecutive spawning fish, however, this was not found in the comparison of spawning categories conducted here. However, as this study progresses, the analysis will be further refined by directly quantifying changes in fecundity and egg size in the maiden and kelt spawnings of the same fish, which should help clarify things. Fertilization success in consecutive repeat spawning reconditioned kelts was not significantly different from maiden spawners. However, fertilization success was

significantly reduced in skip spawners versus maidens. A number of skip spawners were observed to have infections in the body cavity at their second spawning, which may account for the reduced fertilization success. Further study is required to determine the reasons for reduced fertilization success in skip spawners and correct the problem. However, since skip spawning is a normal life history in steelhead, it is not anticipated that this problem is inherent in the biology of the fish.

The ovulation timing of consecutive and skip spawning reconditioned kelts was 1-3 weeks earlier than their maiden ovulation. How closely this corresponds with the shift in spawn timing in kelts released to spawn naturally is not known. One would expect that females would be able to adjust ovulation timing to some degree based on environmental conditions and the presence or absence of ripe males. Consistent with the present results, Atlantic salmon repeat spawners have been found to ascend rivers earlier than maiden spawners (Niemela, et al. 2006b). Results suggest that spawn timing was not substantially altered by artificial reconditioning. Given the broad spawn timing of steelhead, we would not expect an advance of 1-3 weeks would cause synchronization issues with finding mates or fry emergence timing.

Kelts did not increase in length over the first six months of reconditioning, but did increase during the second six months. This was the case for both consecutive and skip spawners, suggesting that the fish first replenish muscle and lipid tissue lost due to the demands of migration and spawning (Penney and Moffitt 2014a, b, 2015), and then initiate skeletal growth. Weight growth rate was elevated in consecutive versus skip spawners from intake to August. This is before ovarian growth would be expected to substantially contribute to increases in weight. Similar to these results, elevated growth rates and increased late summer to fall muscle lipid levels have been consistently found in rematuring Prosser kelts. The consistent and strong association of growth rate and maturation suggests that 1) increased growth rate stimulates maturation, and/or 2) maturation stimulates growth. Evidence exists for both of these possibilities. Growth rate has been found to greatly impact divergent maturation within populations of other salmonids, such as Chinook (Shearer, et al. 2006), and body growth has been found to influence oocyte development rate during the critical period for initiation of maturation in Coho (Campbell, et al. 2006a; Campbell et al. 2006b). On the other hand, the earlier stages of the maturation process stimulate feed intake and growth in Atlantic salmon (Kadri, et al. 1996; Stead, et al. 1999), which may be due to growth stimulatory effects of reproductive steroids and other gonadal factors (Bhatta, et al. 2012).

Muscle lipid levels showed a clear pattern of increase up to six months prior to spawning followed by decrease during the final six months in both consecutive and skip spawners. The decrease in muscle lipid stores in rematuring fish during the final six months is likely due to mobilization to support ovarian development. During exogenous vitellogenesis, which occurs during the final six months of ovarian development, stored lipids are mobilized and transported to the ovary, where they are incorporated into the eggs (Lubzens, et al. 2010; Tyler and Sumpter 1996). Significantly greater muscle lipid levels in consecutive spawners versus skip spawners after the first six months of reconditioning may be due to appetite stimulation during early maturation. One year after their maiden spawning, skip spawning kelts had increased

lipid levels to much higher than kelts at intake in the spring, which may account for the much higher rematuration percentage of fish held for a second year.

Section 3.C: Trial of a custom formulated semi-moist diet for kelt reconditioning

Introduction

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 sources of readily available lipids may be effective at increasing muscle lipid levels in reconditioned kelts. The feeding motivation of kelts is low at intake into reconditioning. Previously Cyclopeeze (Argent) was utilized (Hatch et al 2013a and Hatch et al. 2014) by topcoating feed along with fish oil. This technique showed great promise but supplies of this resource became scarce and unreliable to obtain (Hatch et al. 2013). We contacted Dr. Rick Barrows of the U.S. Department of Agriculture Aquaculture Research group to assist us in producing a better feed that we could tailor to the needs of steelhead kelts. He suggested that we utilize a similar product, artemia cysts or brine shrimp, and that improved diet conditions could be incorporated more effectively into the pellet by producing a semi-moist pellet that incorporated the feed into the pellet. This would have the effect of fish more readily consuming the additive and not producing as much waste from the topcoating being removed when placed into the water column.

Methods

Kelt steelhead arriving at Prosser during the spring of 2015 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 two small tanks (tanks S1-S4, 12' diameter, 19-21 first time reconditioned female fish per tank), and four large tanks (tanks C1-C4, 20' diameter, 102-105 first time reconditioned female fish per tank). Tanks S2, S4, and C2 were randomly assigned to the USDA semi-moist diet (Appendix A1.c), and the rest of the tanks with the exception of C1 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 semi-moist 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.

Results and Discussion

Analysis was restricted to female kelts positively identified by PIT tags from intake into reconditioning to the 9/16/2015 sampling. Tank C2 was excluded from analysis because this tank went off of and back onto the Barrows diet several times during the season when supplies of the experimental diet ran low. However, the four S tanks (S1-S4) were able to be maintained on the Barrows diet for the entire season. Fish were stocked into the S tanks on 4/13/2015 and 4/14/2015, so that all fish were fed the Barrows and Standard diets over the same period of time, and potential bias due to spawn timing and spawning subpopulation were minimized. With only two replicate tanks for each diet, it was not possible to conduct a 2-way ANOVA to assess the effects of diet and tank on response variables due to insufficient degrees of freedom. Therefore, we analyzed the results using 1-way ANOVA (Table 3C.1). Where results of the 1-way ANOVA were significant, the pattern was always consistent between the two replicate tanks within each treatment, suggesting that results can probably be attributed to the diets and not to tank effects. However, more replicate tanks are needed before this possibility can be excluded.

Table 3C.1. Results of 1-way ANOVA analysis of the effect of diet on fish performance metrics.

Response	DF	F	P	R^2	Mean Barrows	Mean Standard
K	1	9.833	**0.0026**	0.131	1.005	0.901
Fatmeter	1	1.652	0.2033	0.025	3.866	3.3
SGRL	1	2.227	0.1406	0.034	0.0201	0.0138
SGRW	1	8.021	**0.0062**	0.11	0.301	0.196
Rematuring E2 (log)	1	2.4	0.1269	0.041	4.788	4.619
Rematuring E2 (log) outliers excluded	1	7.442	**0.0086**	0.123	4.873	4.656

Fish fed the Barrows diet had significantly greater Fulton's condition factor (K) and weight growth rate over the reconditioning period than fish fed the Standard diet (Fig. 3C.1). Trends

were similar but non-significant for Fatmeter readings at the 9/16/2015 sampling and length growth rate. Plasma E2 level was greater in rematuring females fed the Barrows diet than the Standard diet, and this difference was significant when three outliers (2 Barrows diet, 1 Standard diet) with E2 levels in the 2000-6000 pg/ml range were excluded (Fig. 3C.2). After reverse transformation, mean plasma E2 levels in fish fed the Barrows and Standard diet were approximately 75,000 and 45,000 pg/ml, respectively. Female consecutive maturation percentage in the experimental tanks was: S1 Standard 80%, S2 Barrows 84.2%, S3 Standard 73.3%, and S4 Barrows 100%.

Figure 3C.1. Effects of diet on condition and growth metrics.

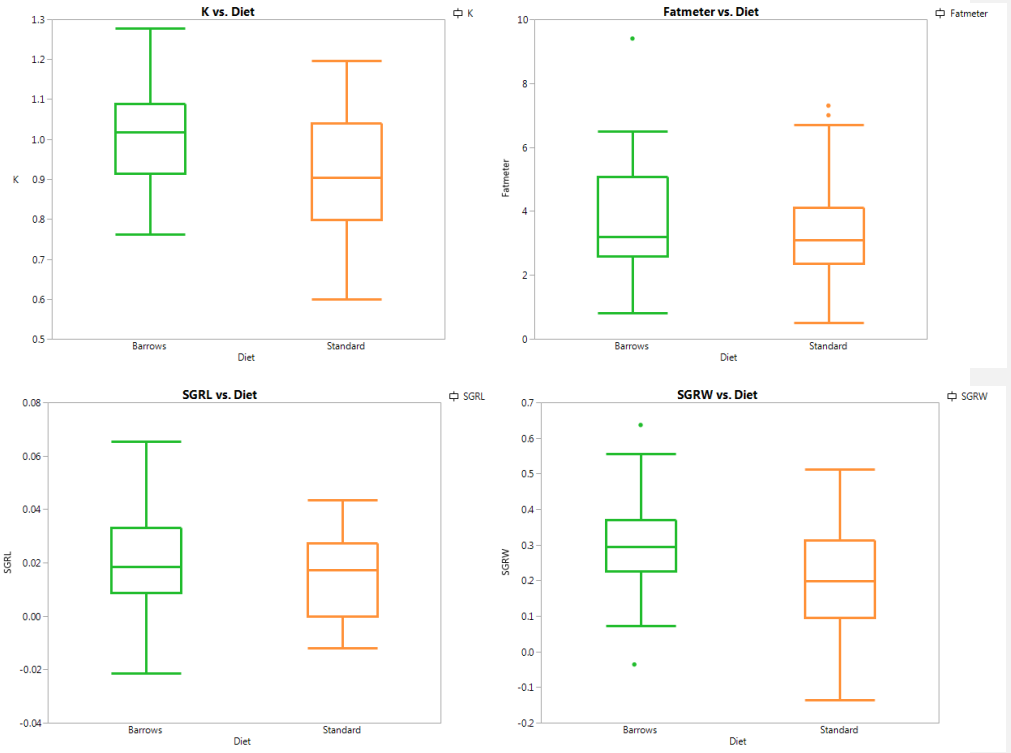
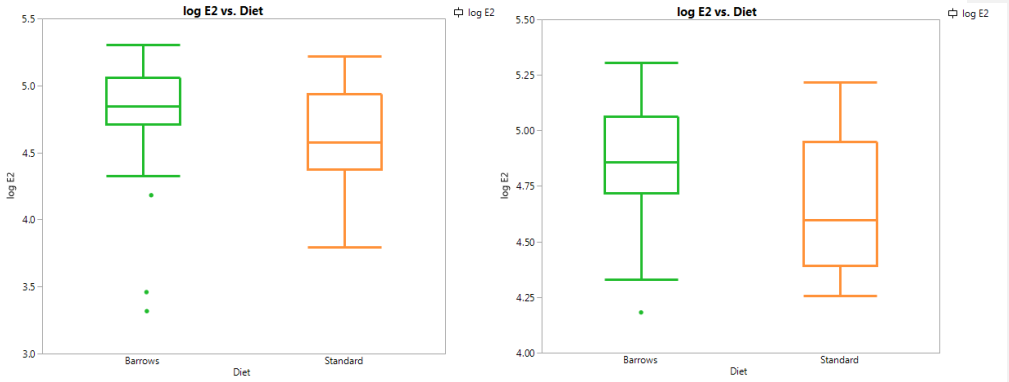


Figure 3C.2. Effects of diet on 9/16/2015 plasma estradiol level in rematuring females.



With outliers

Outliers excluded

The results of the preliminary diet trial were encouraging. Fish fed the semi-moist Barrows diet increased in weight at about 1.5 fold the rate of fish on the Standard diet, and had substantially higher condition factors and plasma E2 levels in the fall. This suggests that energy reserves and investment in ovarian growth were greater in the Barrows diet fish. Data are insufficient at this point to statistically evaluate the effect of diet on maturation rate, however, a trend toward a higher maturation rate in the Barrows diet was found. All of these results point to a potential benefit of the semi-moist diet. This result should be confirmed with further trials.

Section 3.D: Comparison of reconditioned kelt steelhead and spawning steelhead sampled during upstream migration at Prosser dam

Introduction

Since 2009, 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.

During the fall of 2012, we began a collaboration with a VSP study on Yakima River steelhead (Frederiksen, et al. 2015; Frederiksen, et al. 2012), which enabled us to obtain blood samples and biological data from upstream migrating maiden female steelhead 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. Our goals were to compare maiden spawners to fish reconditioned at Prosser in terms of: 1) reproductive status using plasma levels of estradiol and vitellogenin; 2) energy reserves using condition factor and muscle lipid levels measured with the Fatmeter; and 3) migration and spawning success using PIT tag detection and radio tracking. Additional laboratory and data analysis was completed in 2015 and is reported here as a progress report. However, further analysis remains to be completed, and the results and interpretations may change.

It is important to note that reconditioned kelts are not necessarily expected to be identical to maiden spawners. In terms of the effect of captive reconditioning on fish performance, the relevant comparison would be between reconditioned kelts and natural repeat spawning steelhead. However, the number of natural repeat spawners is so low that is difficult to obtain samples from natural repeat spawners for this purpose. Our goal was to obtain information which will help us to establish and quantify the benefit of the kelt reconditioning program, by quantifying differences between natural maiden spawners and reconditioned kelts at a similar point in their spawning migration.

Methods

Fish were trapped, sedated, sampled, PIT tagged, and radio tagged at the Prosser Denil as described for the radio tracking study (Frederiksen et al. 2015; Frederiksen et al. 2012). In addition, blood was drawn and muscle lipid levels were measured. During blood sampling, blood (2 mL) was drawn from the caudal vein using heparinized syringes (ammonium heparin, 10 mg/ml) and centrifuged (5 min, 5000 g). Plasma was collected and frozen in the field prior to storage at -80°C. 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). 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. Reconditioned kelts were sampled on 10/10/2012 and released on 10/29/2012. 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, including radio tagging, except that a second blood draw was not taken. In 2013, reconditioned fish were blood sampled and radio tagged during the release sampling on 10/23/13, immediately prior to release.

Plasma E2 concentrations were assayed by an enzyme immunoassay (EIA) 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. 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.

Fulton's condition factor (K) was calculated as 100*weight (g)/length (cm)^3. Response variables in maiden steelhead were checked for seasonal trends by linear regression versus Julian day. Where significant regressions were found, response variables were adjusted to the kelt release date for that year using the regression equation. Differences between kelts and maidens were assessed by 2-way ANOVA followed by Tukey's post-hoc test as appropriate. Migration success was categorized by PIT tag detection at arrays at the mouths of Satus and Toppenish Creeks, or by detection at Roza Dam. Fish detected at one of these sites were categorized as confirmed migration success, whereas fish that were not detected were categorized as unconfirmed. Factors predicting migration success were analyzed by univariate logistic regression.

Results

During the 2013 and 2014 spawn year migration season, blood samples were collected from female maiden and kelt steelhead during the period immediately before and during kelt migration through Prosser dam (Table 3D.1). Results from telemetry of radio tagged maiden and kelt steelhead are reported elsewhere (Frederiksen et al. 2015; Hatch, et al. 2015). All maiden female fish were maturing based on plasma estradiol and vitellogenin levels.

Table 3D.1. Maiden and kelt steelhead blood samples collected during the fall of 2012 and 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.

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	4	142	4	
10/14/2012	29		20	
10/21/2012	24		20	
10/28/2012	41		7	10
11/4/2012	23		7	
11/11/2012	2		3	
11/18/2012	2		2	
2013 Spawn Year total	**132**	**142**	**112**	**10**
10/6/2013	29		5	
10/13/2013	6		0	
10/20/2013	6	77	4	70
10/27/2013	8		5	
11/3/2013	1		1	
11/10/2013	4		4	
2014 Spawn Year total	**54**	**77**	**19**	**70**

Year, kelt versus maiden status, and the interaction significantly influenced many of the response variables measured (Table 3D.2). Effect size showed that fish size and condition factor were most strongly influenced by kelt versus maiden status. The interaction term strongly influenced plasma estradiol level, indicating differences between kelts and maidens varied between years, whereas year had the strongest effect on plasma vitellogenin level.

Table 3D.2. Results of 2-way ANOVA analyses of the effects of year, kelt versus maiden status, and interactions on response variables. Effect sizes greater than 10% are bolded.

Response	Effect	P	R^2
Length	Year	0.0546	0.0093
	Kelt vs Maiden	<.0001	**0.1418**
	Year x Kelt vs Maiden	0.0001	0.0371
Weight	Year	0.0025	0.0230
	Kelt vs Maiden	<.0001	**0.3131**
	Year x Kelt vs Maiden	0.0327	0.0116
Condition Factor	Year	0.0057	0.0193
	Kelt vs Maiden	<.0001	**0.3284**
	Year x Kelt vs Maiden	0.0322	0.0116
Muscle Lipid	Year	<.0001	0.0443
	Kelt vs Maiden	<.0001	0.0545
	Year x Kelt vs Maiden	**<.0001**	0.0746
Plasma E2	Year	**<.0001**	**0.1559**
	Kelt vs Maiden	<.0001	0.0445
	Year x Kelt vs Maiden	<.0001	**0.1867**
Plasma VG	Year	<.0001	**0.1175**
	Kelt vs Maiden	0.9261	0.0000
	Year x Kelt vs Maiden	0.1184	0.0064

Rematuring reconditioned kelt steelhead were significantly longer and heavier than maidens in both years (Fig. 3D.1). No significant differences in body size were detected between years in rematuring reconditioned kelts, however, maidens were significantly shorter and lighter in the 2014 than the 2013 spawn years. Condition factor was significantly higher in rematuring kelts in both years, and kelts had significantly higher muscle lipid levels versus maidens in the 2013 but not the 2014 spawn years (Figure 3D.2).

Figure 3D.1: *Fork length and weight in rematuring reconditioned kelts and maiden female steelhead sampled at the Prosser Denil ladder in 2012 and 2013. Bars with different letters are significantly different (ANOVA followed by Tukey's multiple comparison test, $p < 0.05$). Boxes represent the interquartile range, the median is indicated by a line, and whiskers indicate data range.*

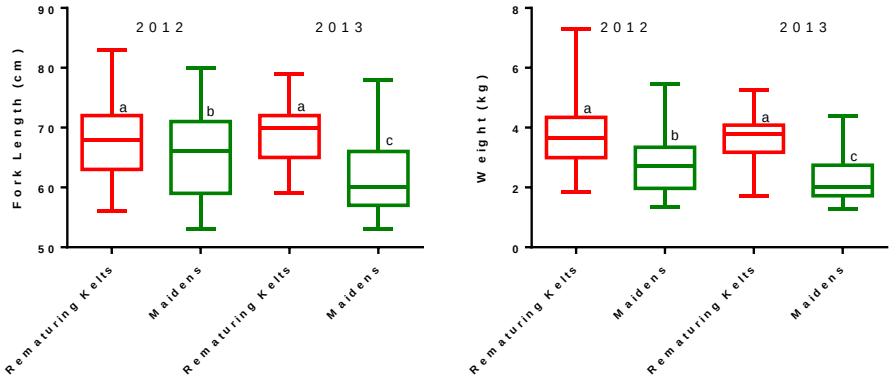
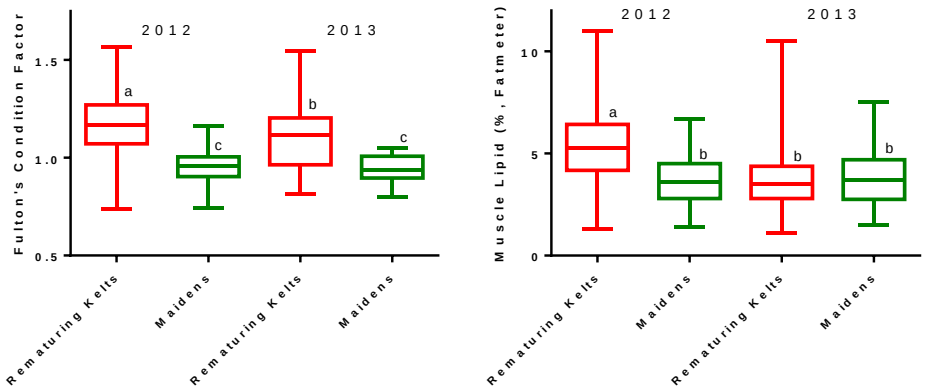
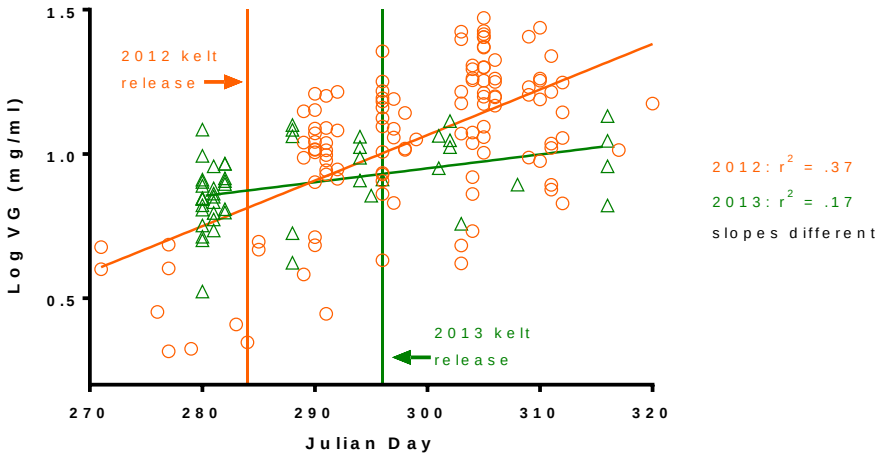


Figure 3D.2: *Fulton's condition factor and muscle lipid levels in rematuring reconditioned kelts and maiden female steelhead sampled at the Prosser Denil ladder in 2012 and 2013. Bars with different letters are significantly different (ANOVA followed by Tukey's multiple comparison test, $p < 0.05$). Boxes represent the interquartile range, the median is indicated by a line, and whiskers indicate data range.*



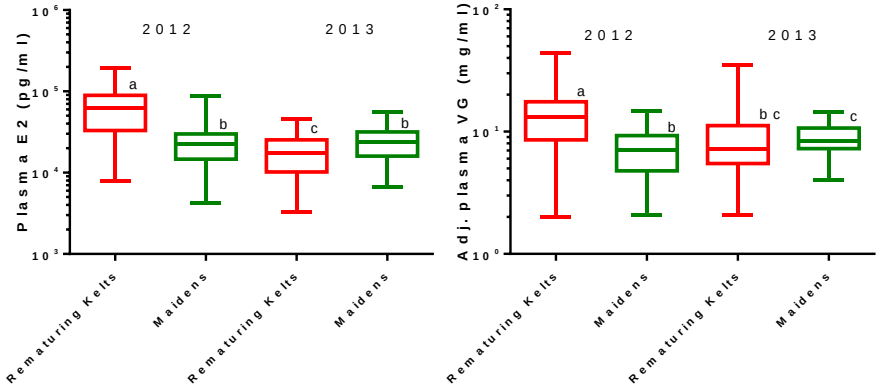
Plasma vitellogenin level increased with sampling date in maiden fish in both years (Fig. 3D.3). Regressions were significant in both years ($p < 0.05$). Maiden vitellogenin levels were adjusted to the kelt release dates using the linear regression equation. Significant seasonal trends were not found in other response variables.

Figure 3D.3: *Seasonal changes in plasma vitellogenin (VG) levels in maiden female steelhead sampled at the Prosser Denil ladder in 2012 and 2013.*



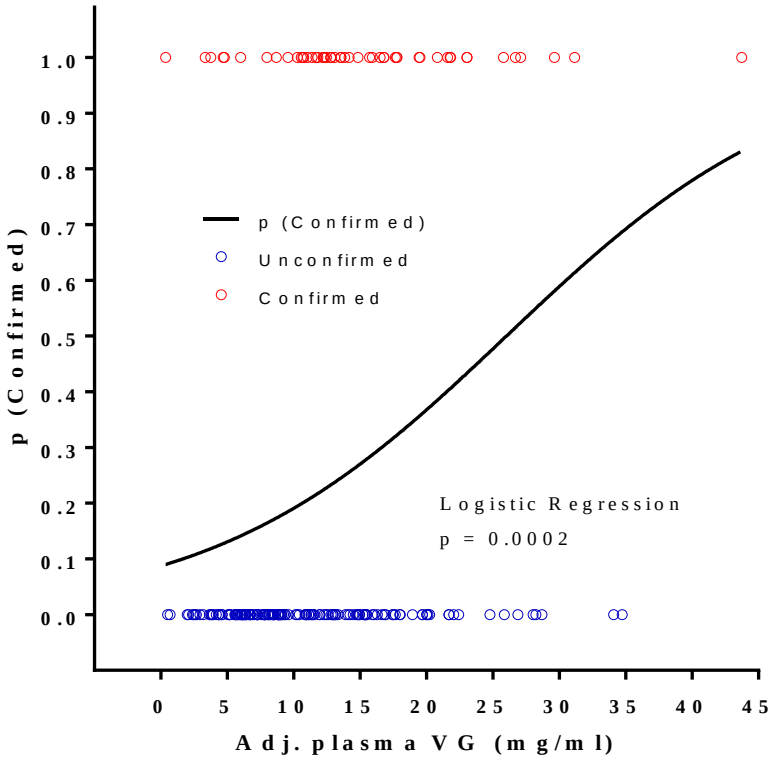
Plasma estradiol levels were similar between years in maiden steelhead at 15,000-30,000 pg ml^{-1} (Fig. 3D.4). Rematuring reconditioned kelts had significantly and substantially higher plasma estradiol levels of 30,000-90,000 pg ml^{-1} at release in the 2013 spawn year, and significantly but slightly lower plasma estradiol levels of 10,000-25,000 pg ml^{-1} at release in the 2014 spawn year. Combining years, kelts had significantly higher plasma estradiol levels versus maidens. Plasma vitellogenin levels were significantly lower in maidens in the 2014 versus 2013 spawn year. Rematuring reconditioned kelt vitellogenin levels were elevated versus maidens in the 2013 spawn year and similar to maidens in the 2014 spawn year. Combining years, kelts had significantly higher vitellogenin levels versus maidens.

Figure 3D.4: *Plasma estradiol (E2) and vitellogenin (VG) levels in rematuring reconditioned kelts and maiden female steelhead sampled at the Prosser Denil ladder in 2012 and 2013. Bars with different letters are significantly different (ANOVA followed by Tukey's multiple comparison test, $p < 0.05$). Boxes represent the interquartile range, the median is indicated by a line, and whiskers indicate data range.*



Univariate logistic regressions were conducted as a first step toward building a multivariate logistic regression model of factors potentially predicting migration success in reconditioned kelts. The only factor measured at release that was significantly related to confirmed migration success was plasma vitellogenin level (Fig. 3D.5). Kelts with higher plasma vitellogenin level at release were more likely to be detected entering spawning tributaries.

Figure 3D.5: Relationship between kelt plasma vitellogenin (VG) level at release and the probability of migration success as inferred by PIT tag detection in Satus Creek, Toppenish Creek, or at Roza Dam (confirmed migration success), versus fish that were not detected (unconfirmed migration success). Combined data from 2012 and 2013 was analyzed by univariate logistic regression.



Discussion

It makes sense that rematuring reconditioned kelts were larger than maiden spawning steelhead, because kelts have an additional year of growth. The size of maiden spawners varies between years due to differences in age structure and ocean feeding conditions, which likely explains the size difference in maidens between the 2013 and 2014 spawn years. The larger size of reconditioned kelts as compared to maidens would be expected to result in an increase in fecundity. Therefore, reconditioned kelts would be expected to contribute more to steelhead productivity in the Yakima on a per-fish basis than maidens. A length-fecundity

relationship for Yakima River steelhead is required to quantify the expected productivity increase.

The significantly greater condition factor and muscle lipid levels found in reconditioned kelts versus maiden spawners indicates that kelts have greater energy reserves than maidens. This is likely due to energy expenditure by maidens but not kelts on migration to Prosser dam. The middle 50% of maiden fish had condition factors and muscle lipid levels in a quite narrow range in both years, with a condition factor of 0.9-1.0, and muscle lipid levels of 2.8-4.7%. This suggests that the energy reserves associated with these ranges are what is required to spawn successfully in the Yakima River, under the conditions to which the fish are adapted. The greater energy reserves found in reconditioned kelts suggests that the kelts have more than sufficient energy reserves for migration and spawning. However, other factors in addition to energy reserves may influence migration and spawning success. Further work is required to determine the relative migration, spawning, and reproductive success of reconditioned kelts and maidens in the river.

The significant seasonal increase found in plasma vitellogenin but not estradiol during our fall sampling in maiden steelhead is probably due to differences in the seasonal profile of the two factors during ovarian development over the year before spawning. Plasma vitellogenin increases up to a peak one or two months before spawning in rainbow trout, whereas plasma estradiol levels peak approximately four to six months before spawning (Bromage et al. 1992; Prat et al. 1996; Tyler and Sumpter 1996; Tyler et al. 1990; Whitehead, et al. 1983; Wilkinson, et al. 2010). Therefore, plasma vitellogenin would be expected to be increasing during the period covered by our sampling. In contrast, plasma estradiol may be near maximal levels, and consequently not change detectably over time. Fish sampled for our study also presumably had spawn timing distributed across the range for Yakima River steelhead, which would put fish at different points on the average seasonal profile.

Overall, rematuring reconditioned kelts had higher plasma estradiol and vitellogenin levels than maiden spawners, although differences between years were found. This suggests that kelts may be investing more energy into development of the ovary than maidens at this point in their migration. This may be due to the greater energy reserves found in the kelts. Maidens but not kelts had to migrate over 500 RKM up the Columbia River to Prosser dam, and swimming has been found to suppress ovarian development in rainbow trout (Palstra, et al. 2010).
Our preliminary analysis found a relationship between plasma vitellogenin level at release in the fall and confirmed migration success in reconditioned kelts. This is potentially an exciting finding, but more work needs to be done to complete the analysis. During the years of the study, no PIT tag array was in place on the Naches River, a major Yakima River spawning tributary. Many of the fish classified as unconfirmed migration success were likely Naches spawners, and some were likely fish that spawned in the Yakima River mainstem or smaller tributaries without PIT tag arrays. We plan to use genetic stock identification to restrict the analysis to fish that genotype to spawning tributaries with a PIT array in place during the years of the study. It may be surprising that plasma vitellogenin predicts migration success, but condition factor and muscle lipid reserves do not. However, it makes some sense for plasma

vitellogenin level to be a stronger predictor. During the exogenous vitellogenesis stage of ovarian maturation, which would be occurring during the fall in our fish, vitellogenin is produced in very high amounts by the liver, and is present at very high levels in the plasma, basically taking the place of plasma albumin. Therefore, plasma vitellogenin level during exogenous vitellogenesis may be a measure of the ability of the fish to synthesize protein. Ability to synthesize protein would be a good measure of the overall health and energetic status of the fish.

Section 3.E: Comparison of reconditioned kelt steelhead and spawning steelhead sampled during upstream migration at Lower Granite Dam (LGR)

Introduction

For several years, we have been monitoring the reproductive status and factors indicating energy stores of female kelts in the reconditioning project at Dworshak National Fish Hatchery using measurement of blood hormone levels and morphometric measurements. The results of these studies have been interpreted based on existing data on reproductive development in captive rainbow trout. To our knowledge, no comparison exists of plasma hormone levels and energy reserves during spawning migration in first time (maiden) spawning steelhead and reconditioned kelts for this population.

Upstream migrating steelhead are sampled and PIT-tagged at Lower Granite Dam's Adult Fish Ladder. Reconditioned kelts are released below Lower Granite Dam each fall. PIT tag detections at Lower Granite Dam allow monitoring of whether reconditioned kelts actively migrate toward spawning grounds. In the fall of 2014 and 2015, we obtained blood samples, length, weight, Fatmeter readings, and DNA from upstream migrating female steelhead. Our goals are to compare maiden spawners from the ocean to fish reconditioned at Dworshak National Fish Hatchery in the fall in terms of: 1) plasma reproductive hormone levels; 2) physiological condition and energy reserves; and 3) using PIT arrays, determine how reproductive hormone levels, physiological condition, and energy reserves are related to upstream migration success for maiden spawners. The study is ongoing, and interpretation of the results may change as further analysis is completed.

Reconditioned kelts are not expected to be identical to maiden spawners. For example, rematuring reconditioned hatchery origin kelts are continually fed throughout the fall, whereas maiden spawners have ceased feeding. Additionally, reconditioned kelts are made up of both reproductively active (re-mature) fish, and skip spawners, represented separately in the analysis. Finally, a small representation of reproductively active (re-mature) fish skipped spawning the previous year, so spent longer in captivity resting and gathering energy. Our goal for this portion of the study is to determine whether reproductively active reconditioned kelts are physiologically on par with maiden spawning steelhead in terms of reproductive hormone levels, energy stores, and condition. Understanding how these factors impact steelhead migration, spawning, and reproductive success will be important for demonstrating and quantifying the benefit of the kelt reconditioning program. Analysis to date focuses on a comparison of condition and energy stores of maiden steelhead and kelts.

Methods

Adult steelhead were trapped at the Lower Granite Dam Adult Fish Trap through the run, sedated, sampled (length, weight, fatmeter %, DNA, blood), and PIT-tagged. In 2014, lengths <64 cm (unclipped) and 68 cm (clipped) (2014) and 72 (clipped) (2015) were excluded. A few smaller fish were sampled and included in the analysis. Phenotypic males were excluded to the

best of the sampling crew's ability. Restrictions served to focus sampling on fish comparable to reconditioned female "B-run" kelts.

Reconditioned kelts were sampled on 8/28/14 and 11/06/14 (length, weight, fatmeter %, blood). Maturation was determined by plasma analysis of Estradiol-17b (E2) levels. All wild kelts were non-mature in 2014, and were released below Bonneville Dam in early November. In 2015, blood samples collected on 9/22/15 were analyzed for E2. Mature wild kelts were released on 11/20/15. On 12/2/15, remaining kelts were sampled (hatchery-origin and wild non-mature) (Table C5.1). PITs of some 2015 fish have been detected migrating upstream. Fish Creek kelts were radio tagged at release in 2015.

Fulton's condition factor "K" was calculated for each fish (K=W/L^3). Analysis of plasma estradiol levels and energy stores in maiden spawners and re-maturing kelts, as well as the analysis of factors in maiden fish over time and by distance to tributary origins, is ongoing. Blood samples may be analyzed for vitellogenin, IGF-1, salmon growth hormone, whole plasma lipid content, and osmolality. Collaborating with IDFG, DNA samples will be analyzed for genetic stock identification and parentage-based analysis, which will allow increased precision for the comparisons between maiden fish and reconditioned kelts.

Results and Discussion

Condition (K) in maiden spawners was similar across years (2014 and 2015) and origins (wild and hatchery) (p=0.3, Dunn's multiple comparison test for non-parametric data). Wild medians were 0.98 and 0.97 (0.8-1.46 range), and hatchery medians were 0.98 each year (range 0.78-1.15) (Figure C5.1a).

Muscle lipid levels in maiden spawners from the ocean were similar across years within origin, except that 2014 hatchery and wild maidens were different (Dunn's multiple comparison test for non-parametric data). The reason for the difference between wild and hatchery maidens in 2014 is not known, although a different minimum size for hatchery fish was used in 2014 than in 2015. Wild median had 5.1% muscle lipid level each year (1.5% to 13.1% range), and hatchery medians had 4.4% and 5.1% in 2014 and 2015 respectively (1.5% to 11.3% range) (Figure C5.1b).

Upper Snake River steelhead have presumably been selected to store sufficient energy reserves for upstream migration, completion of ovarian development, spawning, and kelt migration. Therefore, the energy reserves of maiden spawners at Lower Granite Dam are presumably sufficient for the fish to complete these tasks successfully. Condition factor (K) and muscle lipid level (%) of reconditioned fish were compared with upstream migrating fish in 2014. K was either significantly greater (hatchery-origin) or not significantly different (wild-origin) in reproductively active reconditioned kelts than in maiden spawners (Figure C5.2). This is not surprising as maiden spawners have presumably ceased feeding upon river entry, and kelts continue to feed and not use energy for migration. K was either significantly less (wild-origin) or not significantly different (by second sampling) in non-reproductively active hatchery-origin kelts than in maiden spawners. It is unclear whether depressed condition is the cause of

reproductive inactivity (skipped spawning) or the result. Across years and origins, muscle lipid level was either significantly lower or not significantly different in kelts than maidens (Figure C5.3). Overall, rematuring reconditioned kelts had equal or greater energy reserves than maiden spawners as measured by condition factor, but equal or lower muscle lipid levels as measured with the Fatmeter. The lower muscle lipid levels in Snake River kelts contrasts with the higher muscle lipid levels in reconditioned kelts versus maidens found in Yakima River kelts (Section C4), and the very high muscle lipid levels found in Upper Columbia kelts (M. Abrahamse, personal communication). Kelt rearing temperatures are significantly higher at both the Prosser and Winthrop projects than the Dworshak project due to the use of well water at these sites. The effect of water temperature on feeding and growth could be a reason for the difference in muscle lipids storage in Snake River kelts. Measures to increase muscle lipid levels during reconditioning, such as warmer water and a lipid supplemented diet, are likely to decrease the disparity in muscle lipids levels between Snake River kelts and maidens.

Table C5.1. Blood samples collected from fall of 2014 and fall of 2015. Maiden blood samples were collected at Lower Granite Dam Adult Trap. Kelt blood samples were collected prior to release (wild fish) or prior to second captive spawning (hatchery fish).

Week	Maiden Blood Samples		Kelt Blood Samples	
	Hatchery	Wild	Hatchery	Wild
8/28/14			63	42
9/1/14	13	15		
9/4/14	5	7		
9/9/14	8	12		
9/13/14	5	15		
9/20/14	21	29		
9/27/14	26	26		
10/4/14	36	24		
10/11/14	42	18		
10/18/14	44	22		
10/25/14	0	3		
11/6/14			42	37 (release)
2014 Spawn Year total	**200**	**171**	**63**	**42**
9/7/15	16	24		
9/14/15	6	21		
9/21/15	28	32		
9/22/15			49	45 (23 released)
9/28/05	38	31		
10/5/15	37	31		
10/12/15	37	30		
10/19/15	13	15		
10/26/15	11	16		
12/2/15			44	20 (1 release)
2015 Spawn Year total	**186**	**200**	**49**	**45**

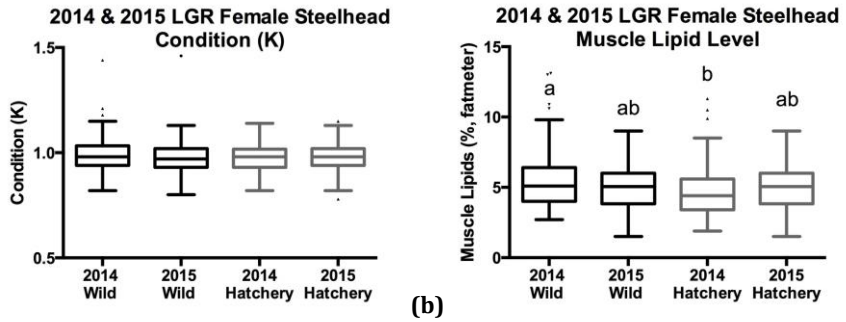
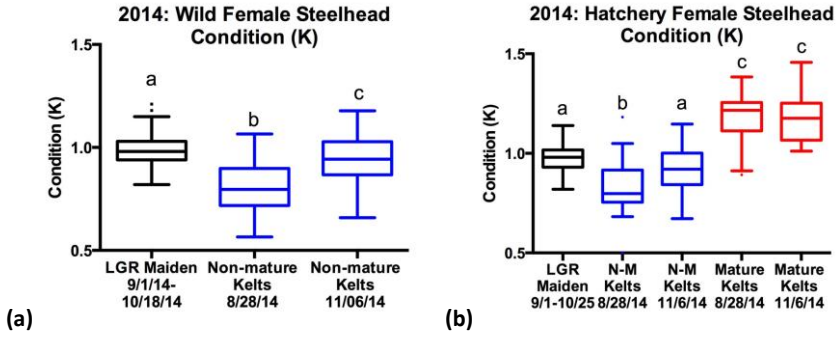
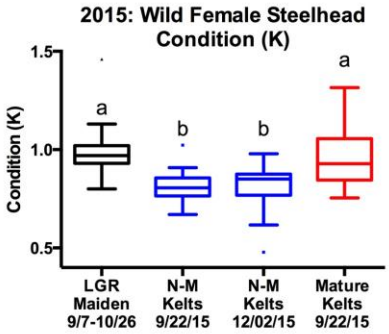
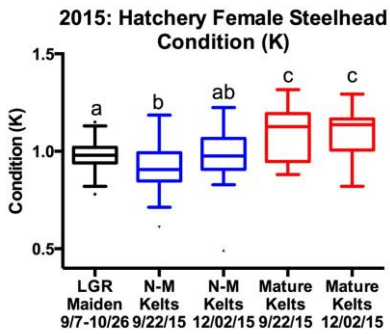


Figure C5.1: *Fulton's condition factor "K" (a) and muscle lipid levels (b) in maiden steelhead sampled at Lower Granite Dam adult fish ladder from the during fall of 2014 and 2015. Bars with different letters are significantly different (ANOVA followed by Mann Whitney multiple comparison test, $p < 0.05$). Boxes represent the interquartile range, the median is indicated by a line, and whiskers indicate a Tukey range.*



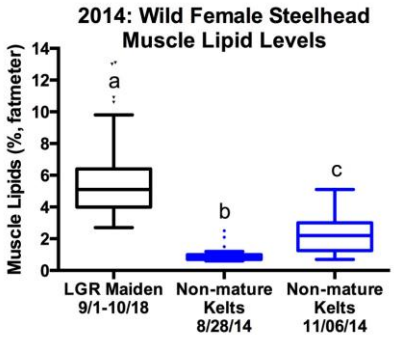


(c)

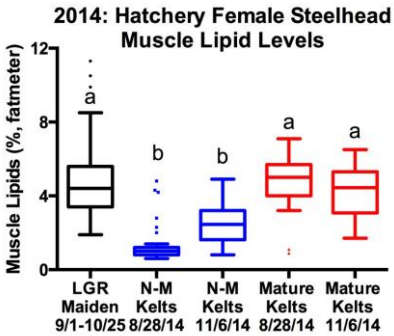


(d)

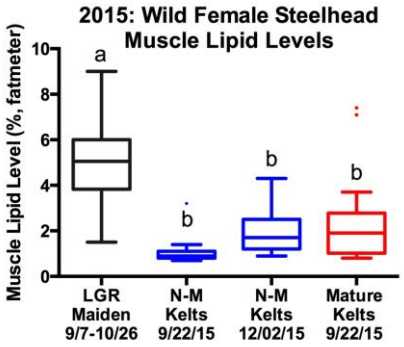
Figure C5.2: *Condition (K) in wild and hatchery maidens and kelts in 2014 and 2015. Condition was higher in wild maiden steelhead sampled from 9/1/14-10/18/14 than in non-mature wild kelts sampled on 8/28/14 and 11/06/14 (Tukey's multiple comparisons test) (a). Condition was higher in maiden hatchery steelhead than in non-mature hatchery kelts sampled on 8/28/14, and lower than mature hatchery kelts sampled on 8/28/14 and 11/06/14 (Dunn's multiple comparisons test) (b). Condition was higher in wild maiden steelhead sampled from 9/7/15-10/26/15 than non-mature wild kelts sampled on 9/22/15 and 12/02/15 (Dunn's multiple comparisons test) (c). Condition was higher in hatchery maiden steelhead sampled from 9/7/15-10/26/15 than non-mature hatchery kelts sampled on 9/22/15, and lower than mature hatchery kelts sampled on 9/22/15 and 12/02/15 (Dunn's multiple comparisons test) (d).*



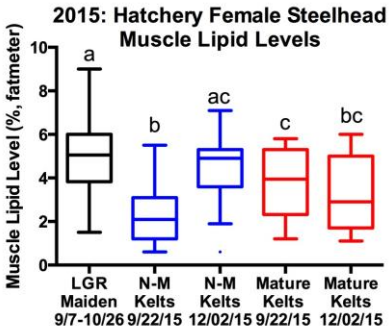
(a)



(b)



(c)



(d)

Figure C5.3: *Muscle lipids levels (fatmeter, %) in wild and hatchery maidens and kelts in 2014 and 2015. Fat was higher in wild maiden steelhead sampled from 9/1/14-10/18/14 than in non-mature wild kelts sampled on 8/28/14 and 11/06/14 (Dunn's multiple comparisons test) (a). Fat was higher in maiden hatchery steelhead than in non-mature hatchery kelts sampled on 8/28/14 and 11/06/14 (Dunn's multiple comparisons test) (b). Fat was higher in wild maiden steelhead sampled from 9/7/15-10/26/15 than non-mature wild kelts sampled on 9/22/15 and 12/02/15, and mature wild kelts sampled on 9/22/15 (Dunn's multiple comparisons test) (c). Fat was higher in hatchery maiden steelhead sampled from 9/7/15-10/26/15 than non-mature hatchery kelts sampled on 9/22/15 and mature hatchery kelts sampled on 9/22/15 and 12/02/15 (Tukey's multiple comparisons test) (d).*

Section 3.F: Developing a radioimmunoassay for plasma insulin-like growth factor 1 (IGF-1) in steelhead (O. mykiss)

Introduction

Female steelhead kelts have the capability to be iteroparous. Presently, there is no means available to know whether post-spawned steelhead kelt will immediately enter another reproductive cycle. A biomarker that indicates this physiological capability (intent) would be a valuable tool for managing captive fish for re-conditioning programs. Our premise is that plasma IGF-1 could fill the role as a biomarker, and could potentially provide information on whether a kelt will enter a reproductive cycle before plasma estradiol levels begin to increase (the currently employed biomarker). IGF-1 increases several months before increases in plasma steroids are detected in maturing rainbow trout (Taylor, et al. 2008). Developing a radioimmunoassay (RIA) for IGF-1 in steelhead plasma began this year by testing iodination protocols and antibody binding, with the goal being to develop a fully functional RIA by the end of next year.

Methods

Standard methods were employed to begin testing the protocol (adapted from a protocol known to be working in another lab).

Iodination

Preparation:

Desalting columns were prepared to remove the excess unincorporated I^{125} from the sample. This was done by pouring a 1x20 cm column of Sephadex G25, allowing the gel to flow by gravity. The column was washed with 2 column volumes of 1% BSA-PBS and then capped until used following the iodination reaction.

GroPep recombinant salmon IGF-1 (rsIGF-1; GroPep.com, Australia) was dissolved in 0.01 N Acetic acid (1ug/uL). It was then aliquoted into 0.5 mL polypropylene microfuge tubes in either 1 or 10 ug aliquots, dried in speed vacuum, and store desiccated at -20C. On the day of an iodination reaction, a 10 ug aliquot of rsIGF-1 was dissolved in 50 uL 10 mM HCl. This was allowed to incubate at room temperature for 30 minutes and then 50 uL 0.5 M phosphate buffer was added to the solution.

Cloramine T (0.4 mg/mL) and Sodium Metabisulfite (0.6 mg/mL) were prepared in 0.5 M sodium phosphate. These solutions were prepared fresh every time an iodination reaction was performed.

The following was then placed in the fume hood (Fig. C6.1):

Figure 3F.1: *Hood setup for efficient and safe iodination reaction. Not pictured is the fraction collector, which sits off to the side near the desalting column.*

The column to be used for desalting, 0.2% BSA-PBS (about 25 mL), NaI^{125} (Amersham, 1.0 mCi in 10 uL of NaOH, 100 mCi/mL), plastic test tubes in a rack for collecting fractions (80 tubes will collect 80 fractions, which is enough to collect the entire reaction and is advisable when testing the protocol), 0.5 mL microfuge tube for reaction, protein for iodination, chloramine T and sodium metabisulfite, any necessary pipettes and tips.

Iodination reaction:

10 uL of NaI^{125} (containing 1.0 mCi) was added to the tube containing rsIGF-1 (10 ug/100 uL). 20 uL of chloramine T (0.4 mg/mL) was then added to the tube and then gently mixed. The reaction was intermittently vortexed and allowed to proceed for 3 minutes.

The reaction was stopped by adding 20 uL sodium metabisulfite (0.6 mg/mL), mixed, and incubated at room temperature for 5 minutes. Approximately 200 uL of elution buffer (0.2% BAS-PBS) was added to the reaction before being transferred to column for desalting. The entire reaction was allowed to enter the column before adding more elution buffer.

The reaction was eluted with 0.2% BSA-PBS using gravity flow. The elution was performed by adding 10 mL to the top of the column (care should be taken not to disturb the column gel) and collecting effluent into 12x75 mm tubes via the fraction collector.

Counting fractions:

10 uL of each fraction was transferred to a borosilicate tube and counted in a gamma ray counter. The fractions expected to contain iodinated protein were kept and mixed 1:1 with glycerol and stored at -20 C in a lead pig.

Prior to freezing, a TCA precipitation was performed to ascertain if the fraction contained intact protein. Ideally, IGF1 label with peak binding and acid precipitable counts >95% should be used.

Antibody dilution testing

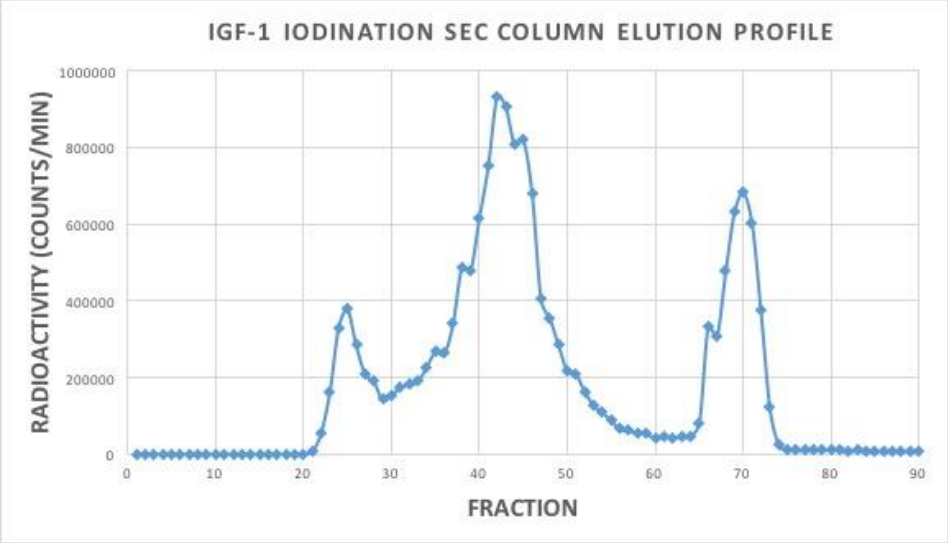
For every fraction kept, the following procedure was executed:

1. Standards (made up from rsIGF-1 previously prepared) were aliquoted into labelled 12 x 75 mm plastic tubes. 50 ul PBS-BSA-TTX were added to the NSB and B0 tubes, but nothing was added to the TOTAL tubes. Fractions being tested were pipetted into appropriately labeled assay tubes.
2. 50 ul of primary antibody (rabbit anti-Barramundi IGF-1; GroPep.com, Australia) in RIA buffer (1% weight/vol PBS-BSA with 0.5% vol/vol triton X-100) was added to all tubes except NSBs and TOTALS. NSBs get 50 ul of PBS-NRS-BSA-TTX without primary. A multitude of antibody concentrations was tested in order to obtain approximately 30% binding based on the radiation counts versus the B0s (which represent maximum binding). The tubes were then vortexed and incubated for 24 hours at 4°C.
3. After 24 hours, 100 ul 0.5 % pansorbin was added to all tubes except TOTALs. The reaction was then gently vortexed and incubated overnight.
4. TOTALs were capped and set aside for counting. All other tubes received 250 ul PBS, were vortexed, and then centrifuged at 3000 rpm for 30 minutes. The supernatant was then carefully poured off and counted in gamma counter.

Results

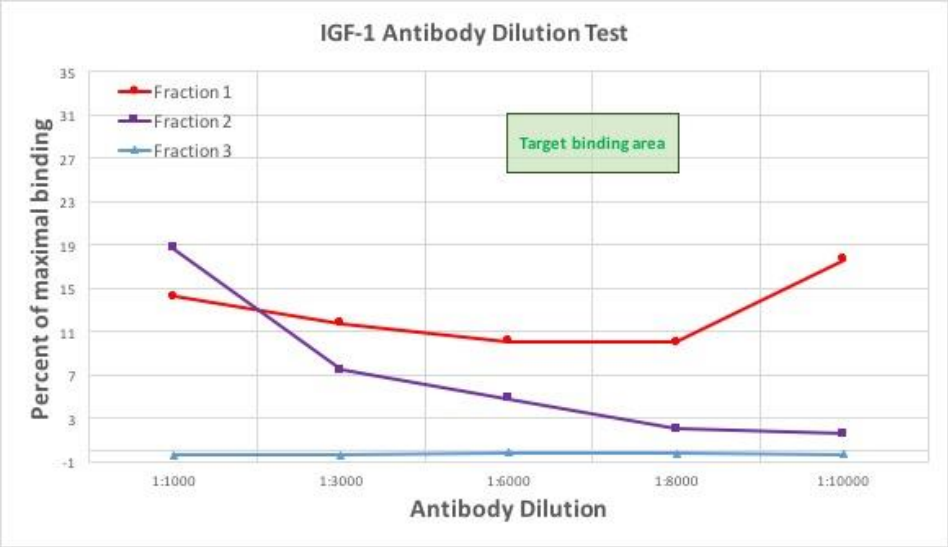
The iodination reaction typically resulted in the expected profile (Fig. 3F.2). Three distinct peaks can be seen, with the middle peak expected to contain the iodinated rsIGF-1 monomer. The peak on the left is expected to contain iodinated aggregates of rsIGF-1, which are not expected to bind to the antibody well (or at least unpredictably). The salt peak on the right is free I^{125} and is not expected to exhibit any binding. TCA precipitation tests indicated that some of the peak 2 fractions (in our multiple iodination reactions) had lower iodinated protein concentrations than is desirable (a range from 80-100% was observed), but all of the fractions were still considered good enough to proceed with for testing purposes.

Figure 3F.2: *iodination reaction profile*



Concentration-dependent antibody binding was achieved with Fraction 2 (Fig. C6.3), indicating that this fraction contains iodinated rsIGF-1 monomer. However, even at the highest antibody concentration tested, binding was less than ideal for use in a RIA. Previously, antibody dilutions of 1:3000 to 1:6000 have been used to achieve the target 30% binding. This suggests that Fraction 2 still contains considerable I^{125} which cannot bind the antibody, or that our antibody has lost activity. Trials with different antibody lots gave similar results, suggesting that the label is the issue. We believe that we are encountering issues with iodinated protein quality, which is lowering our measurable binding. From our troubleshooting efforts, we know that it is not the reagents being used, and most likely the protein itself (*i.e.*, the rsIGF-1) has formed aggregates over time, or the iodination reaction is creating aggregates. This would explain why were are seeing high precipitable iodinated protein counts in the TCA precipitation, but low antibody binding.

Figure C6.3: Concentration-dependent antibody binding



Discussion

We have made progress in developing an IGF-1 RIA, by establishing iodination protocols and demonstrating antibody binding. While further work needs to be done, we have also hit a few roadblocks due to issues with suppliers and facilities. The company that produces rsIGF-1 is no longer producing the protein. However, they are producing recombinant IGF-1 from other species of teleost, and we have been in contact with them to test a few of these species. We now have IGF-1 from two other species and will test it in our next run through, before we run out of rsIGF-1 so that the different species can be compared. Additionally, the facilities we use to run this radioactive procedure have essentially shut down due to budget cuts at WSU and the departure of key laboratory personnel. We are in contact with another lab that has agreed to let us run our tests, and will also receive additional help from the lab. This lab has a lot of experience iodinating proteins and, moreover, possess a HPLC machine set aside for use with radiolabeled proteins. This will enable us to separate out iodinated IGF-1 monomer with much greater resolution than the column method, and indeed separate individual iodinated IGF-1 monomer species. This will ensure that we know exactly where the I^{125} is bound on the IGF-1 and, as there are multiple binding sites, which I^{125} binding site on the IGF-1 protein produces the best antibody binding in the RIA. We believe the help we will be getting in 2016 will produce a quality IGF-1 label in a short period of time. And, once the protocol is developed, it will be highly reproducible due to the specificity the HPLC allows. In addition, we would like to begin work on development of an IGF-1 time resolved immuno-flourometric assay, so that use of radioactive label will not be required.

Chapter 4: Monitor homing and straying rates of reconditioned kelts.

Introduction

In spawning migrations of fishes, three types of homing are recognized (McCleave 1967): 1) natal homing: the return of adults to spawn in the same location in which they were hatched, termed "reproductive, parent stream, or natal homing" by Lindsey et al. (1959); 2) repeat homing: the return of adults to spawn in subsequent breeding seasons at the location of initial spawning; and 3) in-season homing: the return of adults within the same breeding season to the location of initial choice after displacement. With respect to reconditioned kelt steelhead, some data exists regarding natal homing, and much more data demonstrates repeat homing.

Methods

To investigate homing in reconditioned kelt steelhead we compiled data providing conclusive evidence for homing, data consistent with homing, and compared with homing / straying data on natural repeat spawners. Installation of in-stream PIT arrays provides us with data on individual fish's spawning runs at the stock level.

In-stream PIT arrays exist in Satus and Toppenish creeks in the Yakima River basin. Conclusive evidence for homing was obtained when maiden fish outfitted with PIT tags were detected by an in-stream PIT array and following reconditioning these same fish were detected on their repeat spawning run by the same in-stream PIT array. Additional conclusive evidence for homing was derived by recording the repeat spawner stream location for reconditioned kelts by in-stream PIT array detection and comparing that stream with results from genetic stock identification information that is sensitive to differences between Status and Toppenish creek stocks. Further conclusive evidence for homing was obtained from kelts collected in Omak Creek were detected at the Omak Creek weir following reconditioning and release in the Okanogan River during the previous fall.

Steelhead behavior consistent with homing was obtained from PIT detections at Prosser Dam and from recapturing post spawn fish that previously were released as reconditioned kelts. All ladders of Prosser Dam were wired with PIT antennas by 2008, so detection of reconditioned kelt steelhead at the ladders on their repeat spawning run is consistent with homing.

Results and Discussion

The following sources provide conclusive data confirming repeat homing of reconditioned kelt steelhead (Table 4.1). First, in the Yakima River, steelhead tagged (radio or PIT) prior to their first spawning event and detected in tributary streams exhibiting behavior consistent with spawning were later collected as kelts at the CJMF and reconditioned. Detection (radio and/or PIT) of these fish in the same tributaries during repeat spawning events provides conclusive data confirming repeat homing. In the Yakima River all 27 fish that we detected as maiden and kelts returned to spawn in the same tributary, thus exhibiting no straying. Second, PIT detections of reconditioned kelt steelhead at in-stream arrays in Satus and Toppenish creeks in the Yakima River basin accompanied by genetic stock identification of the same kelts to Satus or Toppenish creeks provides additional conclusive data on repeat and natal homing. The third

conclusive data source is from Omak Creek (Okanogan River tributary), where kelt steelhead were collected at a weir migrating out of the stream and following reconditioning were released near the mouth of the Okanogan River, and later detected at the Omak Creek weir on their repeat spawning run. Our last conclusive data source for repeat homing is from the upper Yakima River, where all adult fish crossing Roza Dam are sampled and PIT tagged. Fish initially tagged at Roza Dam that participate in the reconditioning program and are detected at Roza Dam on a repeat spawning run provide conclusive data on repeat homing.

In addition to the conclusive data on repeat homing, we also have collected data that is consistent with homing but is at a broader scale and thus is not as conclusive (Table 4.1). First, reconditioned kelt steelhead released downstream of Prosser Dam (PRO) are detected crossing PRO. The fish were all collected in Yakima River as kelts and their initial upstream movement after reconditioning is consistent with repeat homing. Second, a number of steelhead collected as pre-spawners in the South Fork Clearwater River were air-spawned, reconditioned, radio-tagged, and released. Subsequent detections of these fish in the South Fork Clearwater River is consistent with repeat homing. Third, some steelhead reconditioned and released in the Yakima program have been collected as post-spawners a second time at the CJMF. These fish spawned upstream of PRO on their initial and subsequent spawning run thus providing data consistent with repeat homing.

Lastly we compared the spawning location of natural origin steelhead tagged at LGR against the reporting group identified by genetic stock identification for each tagged individual. Out of a candidate list of over 20,000 PIT-tagged steelhead, we were able to assign 8,711 individuals to a spawning location by assessing the dates they entered and emigrated from a given tributary, the timing of that period relative to the period of spawning, and subsequent detections. Of those individuals 2,096 had accompanying GSI assignments exceeding 80% probability. Scale analysis identified 20 of these individuals as repeat spawners based on the presence of a spawning check with the remaining 1,945 individuals classified as first-time spawners. Three of the 20 repeat spawners (15%) spawned in a location that was not in agreement with their accompanying GSI assignment, whereas 330 of the 1,945 of the first-time spawners (17%) spawned in a location that was not in agreement with their GSI assignment. Based on this analysis, natural origin repeat spawners do not stray at a higher rate than natural-origin first-time spawners.

Table 4.1*. Observed and inferred homing from artificially reconditioned kelt steelhead in Omak Creek and the Yakima River from 2001 to 2015. Column A consists of fish with tag detections (PIT or Radio) in spawning tributaries as maiden and repeat spawners. Column B are fish with tag detections in tributaries as repeat spawners and consistent GSI conformation of reporting group (pending). Column D are fish with PRO detections as repeat spawners. Column E are post-repeat spawn fish collected at CJMF a second time.*

Location	Conclusive Evidence for Homing			Consistent with Homing		
	A. Maiden/ Repeat Spawner	B. Repeat Spawner Tag Detection +	C. Conclusive Homing total	D. Repeat spawner PIT	E. Post Spawn Repeat Spawner	F. Consistent with homing,

	Tag Detection	GSI conformation	A+B	Detection at Prosser	Recaptured at CJMF	some fish are in both D and E
Yakima R	27	200	227	561	103	629
Omak Cr	11	-	11	-	-	-
Total	38	200	238	561	103	629

Chapter 5: Evaluating Steelhead Kelt Treatments to Increase Iteroparous Spawners in the Columbia River Basin

Introduction

In this section we evaluate kelt steelhead management options and we compare three geographically different long term reconditioning programs. It is thought that downstream passage through the hydrosystem limits repeat spawner steelhead in the Columbia River (Wertheimer and Evans 2005; Wertheimer 2007). In recent years, there may be some evidence that emigrating kelt survival has improved as a result of smolt management actions (e.g. removable spillway weirs, mandated spill). Colotelo et al. (2014) reported that 27.3% of kelts tagged at or upstream of Lower Granite Dam (rkm 695) survived to Martin Bluff (rkm 126) passing 8 hydroelectric dams along the way. Collecting and transporting kelt steelhead around hydroelectric projects could improve emigration survival and result in increased repeat spawner abundance. Our goal is to compare the benefits of long term reconditioning to alternate kelt management treatments like transporting kelts downstream of the hydropower system. Our team recently published a manuscript comparing kelt management options (Trammell et al. in press).

There are three kelt reconditioning projects in the Columbia River Basin, in the Yakima, Snake, and Upper Columbia rivers. Fish in the three projects experience similar conditions in the ocean and lower Columbia River, but different conditions during the final portions of upstream migration, spawning, and kelt migration. In addition, fish in the three projects are from different genetic stocks, which have differing migration timing and express different life histories. In order to assess the degree to which common and unique factors influence the fish, we have begun compiling information from the three projects. Our goal is to use this time series to assess the effects of environmental and biological factors on kelt performance in reconditioning projects.

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

and,

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

Methods

Kelt Treatments

To compare kelt management options, we evaluated 4 treatments: 1. In-river control, where fish were PIT tagged and released back to the river; 2. Collect and transport fish around the

hydrosystem and release them downstream of Bonneville Dam; 3. Collect, short-term reconditioning and transport; and, 4. Long term reconditioning.

In-river migration (control).

Fish were systematically chosen, taking every tenth fish that came into the facility. A total of 553 steelhead kelts were released as controls between 2005 and 2011 for the purposes of this analysis. Control releases continue with a total of 835 fish released back to the Yakima River from 2005-2015.

Collect and Transport.
Fish were collected sequentially on a predetermined schedule. Fish were usually held for 3-5 days until a predetermined quota was met (generally 50-100 fish) and then trucked to below Bonneville Dam and released. A total of 798 fish were included in this treatment.

Short-term reconditioning and transport.
This treatment was implemented from 2002 until 2008, with a pooled total of 1,142 kelts. These fish were collected sequentially based on a predetermined time schedule typically earlier in the collection period (March-April) so that kelts could recondition sufficiently. Fish used for this option were held in reconditioning tanks (see long-term reconditioning) for three to eleven weeks before being trucked below Bonneville Dam for release. While being held, kelts were offered the same feed diet (krill) as the long-term fish in order to reinitiate the feeding response.

Long-term reconditioning.
The long-term reconditioning program was fully described in Hatch et al. (2013a) and consisted of a total of 4,917 kelts evaluated for the period 2002-2011. Fish were collected for long-term reconditioning throughout the kelt run. See section Long term Reconditioning.

Evaluation
Using the kelt collection opportunity at the CJMF, we assessed the return rate of Yakima steelhead by PIT tag detections at Prosser Dam. This analysis was a collect-to-return rate and therefore included all mortality incurred through all treatments. Poor condition fish (N=22) were excluded prior to analysis to remove potential biases due to selection of good and fair condition fish for some treatments. Exclusion of poor condition fish did not alter our estimate of the natural repeat spawning rate by PIT tag detections. Male kelts were also excluded because they were only placed in the long-term reconditioning treatment. In addition, we evaluated the natural repeat spawning rate using scales collected at Prosser Dam.

Fish from all four release groups were assumed to be actively migrating to the spawning grounds and representative of repeat spawners if their PIT tags were detected at Prosser Dam. Prior to 2005, PIT detections at Prosser Dam were only available for fish that migrated upstream through the adult trap on the right bank ladder that were sampled manually using the

FS2001 system (Biomark, Inc., Boise, ID). Therefore, the actual numbers of upstream migrant detections at Prosser Dam were not available for any release group prior to 2005, and also were not available for the long-term release group prior to 2008 (because fish were released upstream of the dam as noted above). Because of these limitations, we chose to use extrapolations as described below to expand the data set available for evaluation. Active upstream migration of repeat spawners from the three release groups that reconditioned in the ocean (transport, short-term recondition with transport, and control release) was determined by querying the PTAGIS database for post-release detections of PIT-tags at McNary Dam on the mainstem Columbia River (Table 1 and Figure 1). All upstream migrating fish at McNary Dam pass through PIT tag detection systems in a fish ladder.

An alternate analysis compares the net survival benefit for the two transport treatment groups by dividing the return rates to BON for the treatment by control groups. This yields a number that represents the relative positive or negative benefit of the treatment. For example if your treatment return rate to BON 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 benefits for long-term reconditioned kelts from the Yakima River, Omak Creek, Hood River, and Snake River 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 BON (the same as the treatment groups). 2. Literature values (Hockersmith et al. 1995). 3. The composition of repeat spawners in the run at large sampled at BON 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 BON not the river of origin so these are biased high due to mortality that likely occurs between BON and the river of interest. However, the in-river groups are paired by year with the treatment groups reducing annual variation.

Results and Discussion

Long-term reconditioning demonstrated significantly higher return rates of repeat spawners (11-18%) than other treatments (1-3%) (Table 5.1). This result was supported in spite of variation in river, ocean, and fish condition between years that was incorporated into the error term in our analysis. The data extrapolation required in our analysis does not account for variation in environmental or fish conditions between years. However, this method does provide a best and worst case interpolation of data for earlier years in the long-term reconditioned group, thereby strengthening our ability to draw conclusions among the four treatments. For more in-depth analysis see Trammell et al. (in press).

Table 5.1. Sample size (N), mean, and grouping output for Tukey post-hoc test from ANOVA of PIT tag detections at Prosser Dam.

Treatment	N	Mean	Grouping
Long-term min	10	11.5	A

Long-term max	10	17.6	A
Short-term	7	3.2	B
Transport	7	0.9	B
Control	7	2.7	B

Survival to release of long-term reconditioned kelt steelhead averaged 40% for the Yakima River, 32% for the Snake River, 15% for Omak Creek, and 36% for Hood River. The Yakima River is represented by 16 the Snake River 4, Omak Creek 9, and Hood River 7 years of data. Figure 5.1 shows relative to control groups, long-term reconditioning groups benefited more than any control group chosen. The highest benefit was to Snake River steelhead kelts in long-term reconditioning were over 80 times higher than fish left in-river.

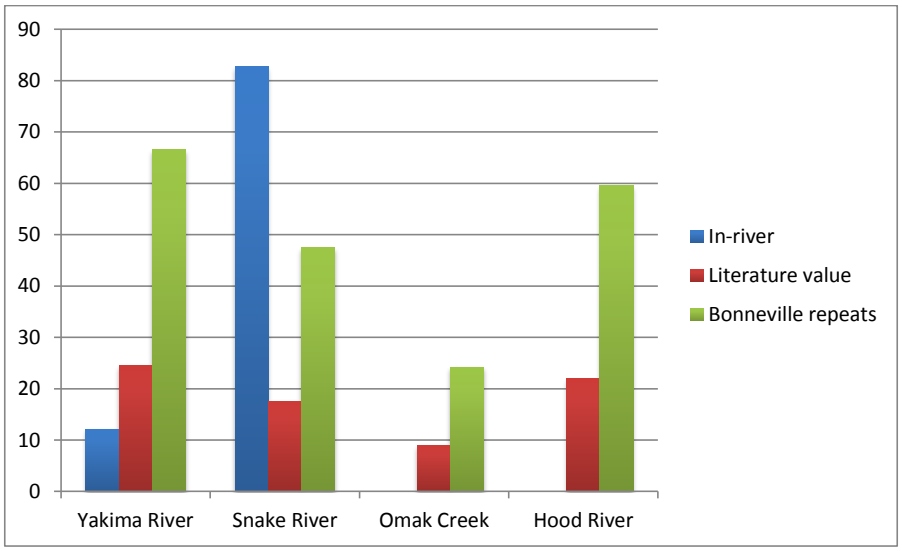


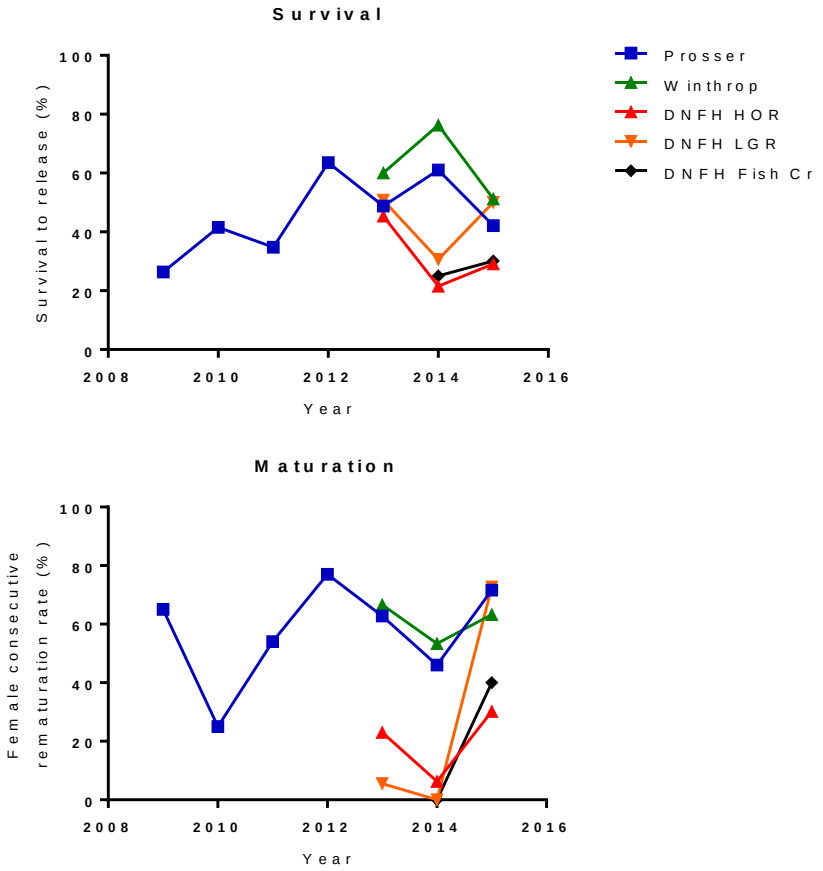
***Figure 5.1**: Benefits of long-term reconditioning relative to 3 control metrics. In-river control groups were not available for Omak Creek or Hood River.*

Geographic Comparison of Reconditioning Programs

Survival and maturation data from Prosser, Winthrop, and Dworshak are shown in Figure 5.2. Since our main interest is in identifying trends due to common environmental conditions, we have not included data from years where results were compromised by problems with fish holding facilities or disease. The Dworshak project was compromised by water quality issues in 2011 and 2012 (chlorine in the water supply and kelts placed on effluent water, respectively), and the Winthrop project was compromised by fish not receiving effective copepod treatment

during their first year of operation in 2012. Results at DNFH in 2014 may have been compromised by issues with formalin treatment and fish care.

Fig. 5.2: Survival and female consecutive maturation rates in CRB kelt reconditioning projects. Fish reconditioned at the Dworshak project include air spawned hatchery origin kelts from the DNFH stock (DHFH HOR), kelts collected at Lower Granite Dam (DNFH LGR), and kelts collected at Fish Creek on the Lochsa River in 2014 and 2015 (DNFH Fish Cr).



Survivals in the Prosser and Winthrop projects from 2012 onward have consistently been in the 50 – 80% range, and survivals of kelts collected at Lower Granite Dam have been similar at Dworshak in 2 of 3 years. In 2012, the Prosser project began treating all kelts with emamectin benzoate by intraperitoneal injection for copepod infestation. Previous treatment had been with ivermectin by gavage. We attribute the increased survival to the change to a less toxic treatment. Results suggest that survivals above 50% are attainable in CRB kelt reconditioning.

With the exception of 2010, consecutive rematuration rates in the Prosser and Winthrop projects have consistently been near 60%. Maturation rates at Dworshak have been lower. However, in 2012, 4 of 5 (80%) of surviving hatchery origin kelts at Dworshak were rematuring when lethally sampled in the fall, and in 2015, a 73% maturation rate was attained with kelts collected at Lower Granite Dam and reconditioned at Dworshak. Thus, high rematuration rates appear to be possible for Snake River fish. Overall, results suggest that rematuration rates averaging near 60% can be expected in CRB kelt reconditioning projects.

Interestingly, both survival and rematuration rates in the Prosser and Winthrop projects appear to be varying together over the three comparable years, suggesting that common environmental conditions prior to capture may influence fish performance in captive reconditioning. Additional years of data are required before conclusions can be drawn on this topic.

Adaptive Management & Lessons Learned

1. Columbia River steelhead populations upstream of Bonneville Dam are listed under ESA and need novel recovery strategies.
2. There is a relatively large abundance of kelt steelhead in the Columbia River Basin even in the upper most areas.
3. In general, repeat spawning steelhead make up a very small proportion of the spawning run.
4. Increasing repeat spawners in steelhead populations can have many positive effects on populations including increasing; genetic diversity, lifetime fecundity, and fitness since genes are distributed across generations.
5. Long-term reconditioning kelt steelhead provides 5 to over 100 times more repeat spawners than leaving the fish in the river.
6. Physiology studies have provided us with a much better understanding of energetic and physiological status of kelts, improved our understanding of alternative life histories in post-spawning fish, and improved survival and health of reconditioned fish.
7. Blood hormone assays are useful to classify consecutive and skip spawner steelhead. Future work needs to focus on optimizing strategies for skip spawner contributions.
8. There appears to be a reduction in the B-run steelhead composition between the maiden and kelt stage, but the B-run composition of repeat spawners is similar to the kelt composition. Underlying biological and behavioral factors contributing to such discrepancies are not well understood but likely warrant further investigation of potential causes. With more data including escapement comparisons, it may be possible to refine the confidence in estimated rates of iteroparity among RG's.
9. Age appears to be less of a factor in rates of iteroparity than size. While the A-run life history was observed to be present among all reporting groups, so too were the B-run life history.
10. Despite the understanding in recent years that the B-run life history is relatively uncommon outside the middle and south forks of both the Clearwater River and Salmon River, our results suggest otherwise. In fact age 2-ocean fish were dominant among all 10 reporting groups. This finding has implications for management of steelhead populations in the basin, and provides evidence that regionally based classifications of life history types or their distributions warrants reconsideration.
11. The upper Salmon River region produces a disproportionate number of Snake River kelt steelhead, and is presumably an important factor in spawner abundance for that region. This result is mirrored among hatchery-origin fish.
12. Adding a production level kelt reconditioning facility at Nez Perce Tribal Hatchery will make achieving the goal RPA 33 possible, i.e. increase the abundance on adult b-run steelhead by 6%. We are submitting a Facility Master Plan to the ISRP to proceed with building a kelt reconditioning facility.
13. Reproductive success studies are underway at a variety of scales: hatchery analog, spawning channel, and natural river. Results are positive.

14. Artificially reconditioned kelt steelhead appear to repeat home with high fidelity. Data indicates that natural repeat spawners in the Snake River exhibited a 15% stray rate.
15. Fish on the "Barrows" diet appeared to perform better than fish on the standard kelt diet. We will continue to work with USDA to improve the diet.

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Appendices

A1.a Master Kelt Tracking Table

Strategy	Year	Location	# Collected	# released	S @ release (%)	# @ ocean	S @ ocean (%)	# @ Bonneville	Return Rate to Bonneville (%)	Transportation (or Treatment) 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	1.56	2.70	25.61
In-river	2006	Prosser	52	52				1	1.92	0.67	1.16	3.10
In-river	2007	Prosser	53	53				3	5.66	1.97	3.41	9.28
In-river	2008	Prosser	88	88				4	4.55	1.58	2.74	6.64
In-river	2009	Prosser	58	58				3	5.17	1.80	3.12	11.54
In-river	2010	Prosser	155	155				2	1.29	0.45	0.78	3.74
In-river	2011	Prosser	85	85				3	3.53	1.23	2.13	7.01
In-river	2012	Prosser	59	59				2	3.39	1.18	2.04	6.74
In-river	2013	Prosser	52	52				0	0.00	0.00	0.00	0.00
In-river	2014	Prosser	45	45				3	6.67	2.32	4.02	6.09
In-river	2015	Prosser	121	121				0	0.00	0.00	0.00	0.00
Total and weighted mean			***835***	***835***				***2.46***	***2.87***	***1.00***	***2.02***	***4.72***
In-river	2002	Lower Granite*	1209	1209				8	0.66	1.88	0.40	
In-river	2003	Lower Granite*	865	865				3	0.35	0.99	0.21	
In-river	2004	Lower Granite*	1138	1138				10	0.88	2.50	0.53	1.49
In-river	2009	Lower Granite	178	176				2	1.12	3.20	0.68	1.51
In-river	2010	Lower Granite	1411	1399				5	0.35	1.01	0.21	0.62
In-river	2011	Lower Granite	1633	1613				3	0.18	0.52	0.11	0.22
In-river	2012	Lower Granite	2098	2098				1	0.05	0.14	0.03	0.06
In-river	2013	Lower Granite	840	827				2	0.24	0.68	0.14	0.13
In-river	2014	Lower Granite	2584	2571				8	0.31	0.88	0.19	0.17
In-river	2015	Lower Granite	1195	1193				0	0.00	0.00	0.00	0.00
Total and weighted mean			***13151***	***13089***				***5.05***	***0.35***	***1.00***	***0.21***	***0.58***
In-river	2002	John Day*	***287***	***287***				***28***	***9.76***	***1.00***	***5.88***	***16.02***
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	0.00	0.00
Transported (Hamilton Island)	2011	Lower Granite	109	109	3/47	6.38		0	0.00		0.00	
Total and weighted mean			***2589***	***2586***		***9.21***		***8.59***	***1.12***	***1.74***	***0.67***	***1.84***
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***	***20.61***

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	1.99
Total and weighted mean			***595***	***569***		***44.89***	***1.86***	***2.18***	***0.76***	***1.32***	***3.59***
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	6.62
Total and weighted mean			***203***	***202***		***27.85***	***1.00***	***1.97***	***1.63***	***1.19***	***3.24***
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***	***2.02***	***3.50***	***9.54***
Transported (Fed Hamilton Island)	2014	Lower Granite	***34*****	***34*****			***0.00***	0.00			
			34	***34***			***0.00***	***0.00***			
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	180	146		26/54	48.15	10	5.56		3.35	15.63
Transported (pooled groups)	2005	Prosser	204	192		20/113	17.70	1	0.49	0.11	0.30	2.80
Transported (pooled groups)	2006	Prosser	111	99		63/99	63.64	2	1.80	0.94	1.09	2.91
Transported (pooled groups)	2007	Prosser	83	76		33/62	53.23	1	1.20	0.21	0.73	1.97
Transported (pooled groups)	2008	Prosser	208	200		54/99	54.55	10	4.81	1.06	2.90	7.02
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	4.18
Total and weighted mean			***1710***	***1470***			***45.14***	***15.86***	***4.26***	***1.48***	***2.71***	***7.00***
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	65.17
	Long-term	2012	Prosser	550	340	61.82				18.24	37.24	57.41
	Long-term	2013	Prosser	546	266	48.72				16.95	29.35	44.47
	Long-term	2014	Prosser	481	292	60.71				9.11	36.57	104.90
	Long-term	2015	Prosser	1098	396	36.07				12.55	21.73	59.23
	Total and weighted mean			***9208***	***3701***	***40.19***				***13.98***	***27.49***	***66.01***
	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***	***8.64***
	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	39.74
	Long-term	2012	Omak Cr	65	4	6.15					3.71	5.72
	Long-term	2013	Omak Cr	49	4	8.16					4.92	
	Total and weighted mean			***283***	***42***	***14.84***					***8.94***	***24.37***
s	Long-term	2006	Parkdale	1	1.0	100.00					60.24	161.33
s	Long-term	2007	Parkdale	13	1.0	7.69					4.63	12.61
s	Long-term	2008	Parkdale	14	7	50.00					30.12	73.06
s	Long-term	2009	Parkdale	9	4	44.44					26.77	99.15
w	Long-term	2010	Parkdale	15	4	26.67					16.06	77.33
w	Long-term	2011	Parkdale	23	5	21.74					13.10	43.20
w	Long-term	2012	Parkdale	21	13	61.90					37.29	57.49
	Total and weighted mean			***96***	***35.0***	***36.46***					***21.96***	***59.88***

Strategy	**Year**	**Location**	**#Collected**	**#Survived Recon.**	**S @ Release**	**# Remature**	**Retained**	**Skip Rematures**	**# @Bonn.**	**Trans. (or treatment) Benefit relative to in-river**	**Treatement benefit relative to Hockersmith**	**Transportation (or treatment) Benefit relative to Bonn. Nat.**
Long-term	2012	DNFH	143	5	3.50	4	-	-		73.36	2.11	3.25
Long-term	2013	DNFH	163	61	37.42	14	47	22		157.18	22.54	34.16
Long-term	2014	DNFH	149	19	12.75	2	17	5		41.19	7.68	22.03
Long-term	2015	DNFH	149	43	28.86	13	30	TBD		82.15	17.38	47.40
Total and weighted mean			***604***	***66***	***28.13***	***33***	***94***	***27***		***80.08***	***12.77***	***46.20***
Long-term	2011	Lower Granite	111	2	1.80	2	-	-				
Long-term	2012	Lower Granite	124	10	8.06	3	-	-		169.19	4.86	7.49
Long-term	2013	Lower Granite	110	57	51.82	3	-	-		217.64	31.22	47.30
Long-term	2014	Lower Granite	110	34**	30.91	0	-	-		99.84	18.62	50.76
Long-term	2015	Lower Granite	22	11	50.00	8	3	TBD		116.45	24.64	67.19
Total and weighted mean			***234***	***112***	***25.05***	***16***	***3***	**TBD**		***91.03***	***18.11***	***52.52***
Long-term	2013	S.F. Clearwater	24	12	50.00	4	-	-		210.00	30.12	45.64
Long-term	2015	S.F. Clearwater	35	7	20.00	4	3	TBD		32.53	6.88	18.77
Total and weighted mean			***59***	***19***	***32.20***	***8***	***3***	***TBD***		***77.20***	***16.34***	***44.54***

Long-term	2014	Fish Creek	12	3	25.00	1	2	2			80.75	15.06	43.20
Long-term	2015	Fish Creek	83	25	30.12	10	15	TBD			85.74	18.14	49.47
Total and weighted mean			***95***	***28***	***29.47***	***11***	***17***	***2(TBD)***			***83.90***	***17.76***	***48.41***
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	0.50%			
Natural repeat	2012	Bonneville Dam	1486						16	1.08%			
Natural repeat	2013	Bonneville Dam	1278						14	1.10%			
Natural repeat	2014	Bonneville Dam	1728						10	0.58%			
Natural repeat	2015	Bonneville Dam	904						2	0.22%			
			18727						***110***	***0.59%***			

* Lower Granite and John Day data from Evans, A.F., R.H. Wertheimer, M.L. Keefer, C.T. Boggs, C.A. Peery, and K. Collis. 2008. Transportation of steelhead kelts to increase iteroparity in the Columbia and Snake Rivers.
North American Journal of Fish Management 28:1818-1827.

**Same group. After long-term reconditioning they were transported and released below Bonneville Dam and released at Hamiltion Is.

A1.b. GSI Snake River reference baseline.

reporting group (RG)		reference population					sample size	
code	Subbasin	code	tributary	year	lat.	long.	adult	juv.
LSNAKE	Lower Snake R.	ALPW	Alpowa Cr.	2010	46.4076	-117.2198	98	
	Lower Snake R.	ASOW	Asotin Cr.	2008	46.3228	-117.1368	99	
	Lower Snake R.	GEORGE	George Cr.	2008	46.3228	-117.1368	95	
	Lower Snake R.	TUCAN	Lower Granite Dam	2010	46.6583	-117.4336	106	
LOCLWR	Lower Clearwater R.	BBER	Big Bear Cr.	2007	46.6336	-116.6552	99	
	Lower Clearwater R.	EFPOT	EF Potlatch R.	2008	46.7984	-116.4235	158	
	Lower Clearwater R.	LAPWAI	Lapwai Cr.	2013	46.3355	-116.6045		32
	Lower Clearwater R.	LBER	little Bear Cr.	2007	46.6336	-116.6552	151	
	Lower Clearwater R.	MISSION	Mission Cr.	2013	46.3355	-116.6045		60
	Lower Clearwater R.	SWEET	Sweetwater Cr.	2013	46.3355	-116.6045		50
	Lower Clearwater R.	WEBB	Webb Cr.	2013	46.3355	-116.6045		16
	Lower Clearwater R.	WFPOT	WF Potlatch R.	2009	46.8055	-116.4190	84	
SFCLWR	S. F. Clearwater R.	CLEARCR	Clear Cr.	2000	46.0486	-115.7817		45
	S. F. Clearwater R.	CROOKSF	Crooked R.	2007	45.8212	-115.5279	106	30
	S. F. Clearwater R.	LOLO	Lolo Cr.	2012	46.2905	-115.9342	9	85
	S. F. Clearwater R.	NEWSOME	Newsome Cr.	2012	45.8366	-115.6156		99
	S. F. Clearwater R.	TENMILE	Tenmile Cr.	2000	45.8053	-115.6818		47
UPCLWR	M. F. Clearwater R.	BEAR	Bear Cr.	2000	46.0191	-114.8381		70
	M. F. Clearwater R.	COLT	Colt Cr.	2000	46.4311	-114.5395		47
	M. F. Clearwater R.	CFLR	Crooked Fork Lochsa R.	2000	46.5250	-114.6777		44
	M. F. Clearwater R.	EFMOOSE	EF Moose Cr.	2012	46.1742	-114.8861		44
	M. F. Clearwater R.	FISHCLR	Fish Cr.	2010	46.3336	-115.3481	83	17
	M. F. Clearwater R.	GEDNEY	Gedney Cr.	2000	46.0583	-115.3141		45
	M. F. Clearwater R.	LAKECLR	Lake Cr.	2010	46.3336	-115.3481	17	30
	M. F. Clearwater R.	LtlCLR	Little Clearwater R.	2008	45.7534	-114.7749		59
	M. F. Clearwater R.	UPSEL	upper Selway R.	2008	45.7534	-114.7749		78
	M. F. Clearwater R.	WtCAP	White Cap Cr.	2008	45.7534	-114.7749		110
	M. F. Clearwater R.	NFMOOSE	NF Moose Cr.	2012	46.1740	-114.9001		94
	M. F. Clearwater R.	3LINK	Threelinks Cr.	2000	46.0481	-115.5169		81
	M. F. Clearwater R.	OHARA	O'Hara Cr.	2000	46.0770	-115.5168		85
	M. F. Clearwater R.	STORM	Storm Cr.	2000	46.4694	-114.5415		38
GRROND	Grande Ronde R.	CATH	Catherine Cr.	2011	45.2406	-117.9220	91	
	Grande Ronde R.	GRCROOK	Crooked Cr. Wenaha R.	2001	45.9775	-117.5548		97
	Grande Ronde R.	Wenaha	Wenaha R.	2001	45.9775	-117.5548		94
	Grande Ronde R.	Joseph	Elk Cr. Joseph Cr.	2000	45.7036	-117.1571	79	18

	Grande Ronde R.	UPGR	Grand Ronde R.	2009	45.4965	-117.9244	65	
	Grande Ronde R.	LtlMIN	Little Minam R.	2000	45.3997	-117.6731		48
	Grande Ronde R.	Wallowa	Lostine R.	2000	45.5500	-117.4886	72	45
	Grande Ronde R.	MENAT	Menatchee Cr.	1999	46.0111	-117.3664		73
IMNAHA	Imnaha R.	BIGSH	Big Sheep Cr.	2001	45.5494	-116.8442	14	77
	Imnaha R.	GUMBOOT	Gumboot Cr.	2011	45.1780	-116.8775	39	
	Imnaha R.	LIGHT	Lightning Cr.	2000	45.6556	-116.7263		39
	Imnaha R.	LtlSHEEP	Little Sheep Cr.	2011	45.4724	-116.9624	16	77
	Imnaha R.	MAHOG	Mahogany Cr.	2012	45.1780	-116.8775	14	
LOSALM	Lower Salmon R.	BOUL	Boulder Cr.	2000	45.2019	-116.3113		47
	Lower Salmon R.	RAPIDsal	Rapid R.	2000	45.3586	-116.3877	100	
	Lower Salmon R.	SLATE	Slate Cr.	2000	45.6396	-116.2732		75
SFSALM	S. F. Salmon R.	EFSF	EFSF Salmon R.	2000	45.0128	-115.7131	9	37
	S. F. Salmon R.	JOHNSON	Johnson Cr.	2010	44.9349	-115.4857		89
	S. F. Salmon R.	LAKE	Lake Cr.	2010	45.3465	-115.9457	7	43
	S. F. Salmon R.	LICK	Lick Cr.	2010	45.3465	-115.9457		63
	S. F. Salmon R.	SECESH	Secesh R.	2010	45.3465	-115.9457	4	91
	S. F. Salmon R.	SFSR	Lower Granite Dam	2010	46.6583	-117.4336	11	34
MFSALM	M. F. Salmon R.	BRVC	Bear Valley Cr.	2010	44.4146	-115.4672		81
	M. F. Salmon R.	BIGC	Big Cr.	2000	45.1509	-115.3015	40	184
	M. F. Salmon R.	CAMAS	Camas Cr.	2000	44.8918	-114.7222		97
	M. F. Salmon R.	CAPEH	Cape Horn Cr.	2009	44.3929	-115.1710		77
	M. F. Salmon R.	CHAMB	Chamberlain Cr.	2000	45.4523	-114.9310		95
	M. F. Salmon R.	WFCHAMB	W. F. Chamberlain Cr.	2011	45.4543	-114.9359		94
	M. F. Salmon R.	ELKMF	Elk Cr. MF Salmon R.	2010	44.4146	-115.4672		92
	M. F. Salmon R.	LOON	Loon Cr.	2000	44.5974	-114.8121		131
	M. F. Salmon R.	MARSH	Marsh Cr.	2009	44.4471	-115.2283		118
	M. F. Salmon R.	PISTOL	Pistol Cr.	2000	44.7214	-115.1545		58
	M. F. Salmon R.	RAPIDMF	Rapid R.	2000	44.7214	-115.1545		75
	M. F. Salmon R.	SULPHUR	Sulphur Cr.	2000	44.5451	-115.3070		94
UPSALM	Upper Salmon R.	HAYDEN	Hayden Cr.	2010	44.8616	-113.6319	7	79
	Upper Salmon R.	HERD	Herd Cr.	2010	44.1115	-114.2574		85
	Upper Salmon R.	MORG	Morgan Cr.	2000	44.6206	-114.1822		61
	Upper Salmon R.	NFSALM	NF Salmon R.	2010	45.4200	-113.9945	100	
	Upper Salmon R.	PAH	Pahsimeroi R.	2006	44.6801	-114.0353	97	
	Upper Salmon R.	SAW	Sawtooth Hatchery	2011	46.6583	-117.4336	63	45
	Upper Salmon R.	VALL	Valley Cr.	2010	46.6583	-117.4336	14	80
	Upper Salmon R.	WFYANK	W. F. Yankee Fork	2010	46.6583	-117.4336	41	76

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A1.c. USDA Kelt Feed Formulation

Product Code: 923 worm- kelt

TRIAL FORMULA (Rounded):

Code	Ingredient Name	Lbs.	Pct.
784	Fish meal SeaPro 75	27.65	27.650
92	Poultry meal, IDF	26.39	26.390
42	Wheat gluten meal	20.00	20.000
69	Soy Protein Concentrate, Profine	20.00	20.000
55	Fish Oil, whittinQ triminQs oil	4.00	4.000
76	Vitamin Premix ARS 702	1.50	1.500
78	Stay-C	0.20	0.200
87	Astazanthin, pink	0.16	0.160
77	Trace min premix ARS 1520	0.10	0.100
	Formula Totals:	100.00	

NUTRIENT COMPOSITION: (Ali Nutrients)

Number	Nutrient Name	Amount	Units
2	DRY MATTER	93.20	PCT
3	MOISTURE	6.80	PCT
4	PROTEIN, CRUDE	71.40	PCT
5	FAT, CRUDE	11.61	PCT
6	FIBER, CRUDE	0.87	PCT
7	CALCIUM	0.69	PCT
8	PHOS. TOTAL	0.89	PCT
9	ASH	3.54	PCT
10	Phosphorus, diQestib	0.68	PCT
11		31.77	PCT
12		18.85	PCT
13	Protein, DiQestible	16.20	PCT
14	Fat, DiQestible	4.08	PCT
16		0.00	
17		0.00	
18		1,330.40	kcal/kQ
19		0.00	
20		0.00	kcal/kQ
21		0.00	
22		0.00	
24		0.00	
31	METHIONINE	1.64	PCT
32	CYSTINE	0.79	PCT
33	LYSINE	4.70	PCT
134	TRYPTOPHAN	0.62	PCT
35	THREONINE	3.08	PCT
36	ISOLEUCINE	4.29	PCT
37	HISTIDINE	1.72	PCT

38	VALINE	3.67	PCT
39	LEUCINE	5.80	PCT
40	ARGININE	4.59	PCT
41	PHENYLALAN INE	3.38	PCT
42	Taurine	0.00	PCT
43	-	0.00	
46	CAROTENE	0.00	MG/KG
47	VITAMIN A	0.00	IU/GM

A.2: Publications

Publications:

Buelow, J., C.M. Moffitt. 2014. Physiological Indices of Seawater Readiness in Postspawning Steelhead Kelts. 2014. Ecology of Freshwater Fish.

Caldwell, L.K., A.L. Pierce, and J.J. Nagler. 2013. Metabolic endocrine factors involved in spawning recovery and rematuration of iteroparous female rainbow trout (Oncorhynchus mykiss). General and Comparative Endocrinology 194: 124-132.

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

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

Hernandez, K., Copeland, T., Wright, K. Quantitative Assessment of Scale Resorption in Migrating and Spawning Steelhead of the Snake River Basin. Transactions of the American Fisheries Society 143:1562-1568, 2014.

Penney, Z.L. and C.M. Moffitt. 2013. Histological assessment of organs in sexually mature and post-spawning steelhead trout and insights into iteroparity. Reviews in Fish Biology and Fisheries 23(4).

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

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

In Submission 2015/2016.

Caldwell et al. submitted
Penney et al. submitted
Pierce et al. submitted
Matala et al. submitted

Trammell, J.L.J., D.E. Fast, D.R. Hatch, W.J. Bosch, R. Branstetter, J.W. Blodgett, A.L. Pierce, and C.R. Frederiksen. In press. Evaluating steelhead management scenarios to increase iteroparous spawners in the Yakima River Basin. North American Journal of Fisheries Management.

Presentations:

2015 Annual Meeting of the Idaho Chapter of the American Fisheries Society, Boise, ID, March 4-6, 2015.

- Presentation by Matala, A.P., S.R. Narum, D. Hatch, M. Ackerman, S. Everett, and M. Ackerman. "Genetic stock identification (GSI) to evaluate stock-of-origin from a mixed sample of kelt steelhead at Lower Granite Dam"
- Presentation by Stephenson, J., D. Hatch, D. Fast, R. Branstetter, and A. Pierce. Columbia Basin Steelhead Reconditioning Studies.

2015 Yakima Basin Science and Management Conference, Ellensburg, WA, June 15-16, 2015

- Jeff Stephenson: Reproductive Success of Reconditioned Kelt Steelhead (Oral).
- Andrew Pierce: Reproductive Development in Reconditioned Female Steelhead Kelts (Oral).
- Ryan Branstetter: A Pilot Study of Reconditioned kelt Steelhead Spawning in an Artificial Channel (Oral).

National AFS Meeting Portland, OR, August 16-20, 2015.

- Doug Hatch (CRITFC), *Steelhead Kelt Reconditioning and Reproductive Success Studies in the Columbia River Basin* (Oral)

- Andy Pierce (CRITFC/UI), *Comparison of Reproductive Hormone Levels and Energy Reserves Between Reconditioned Female Yakima River Steelhead kelts and Migrating Natural Spawners* (Oral)
- Jeff Stephenson (CRITFC), *Reproductive Success of Reconditioned Kelt Steelhead in the Yakima River Basin* (Oral)
- Ryan Branstetter (CRITFC), *Movement Patterns of Artificially Reconditioned Kelt Steelhead Following Release in the Yakima River Basin* (Poster)
- Laura Jenkins (University of Idaho), *Effects of Captive Reconditioning and Post-Spawning Life History on Reproductive Performance in Female Steelhead Oncorhynchus mykiss Kelts* (Poster)
- Neil Graham (CRITFC), *Hatchery Steelhead Kelt Reconditioning at Dworshak National Fish Hatchery: A Model for B Run Steelhead* (Poster)
- Jeff Trammell (Yakama Nation), Evaluating Steelhead Management Scenarios to Increase Iteroparous Spawners in the Yakima River Basin (Poster).
- Scott Everett (Nez Perce Tribe) An Updated Summary of Steelhead Kelts Collected at Lower Granite Dam and Reconditioned at Dworshak National Fish Hatchery (2011-14) (Poster).

66th Fish Culture Conference, Wilsonville, OR, December 1-3, 2015.

- Mike Murphy (Nez Perce Tribe) An Updated Summary of Steelhead Kelts Collected at Lower Granite Dam and Reconditioned at Dworshak National Fish Hatchery (2011-14) (Poster).

A.3: List of Metrics and Indicators (Optional)

Protocol:

Kelt Reconditioning and Reproductive Success Evaluation:

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

Methods

Kelt Collection

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

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

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

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

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

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

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

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

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

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

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

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

Genetic Stock Identification (GSI)

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

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

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

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

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

In-River Release

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

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

Kelt Reconditioning Physiology Studies

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

Reproductive Success of Artificially Reconditioned Kelt Steelhead

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

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

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

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

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

Habitat Monitoring

Piezometer Method
https://www.monitoringresources.org/Document/Method/Details/5478

McNeil Samples (Field Method)
https://www.monitoringresources.org/Document/Method/Details/5397

McNeil Samples (Lab Processing Method)
https://www.monitoringresources.org/Document/CustomizedMethod/Details/25591

Metrics

Title	Category	Subcategory	Subcategory Focus 1	Subcategory Focus 2
Kelt abundance"	Fish	Abundance of Fish (ID: 46)	Fish Life Stage: Adult - Outmigrant	Fish Origin: Both

Reconditioned Kelt abundance"			Fish Life Stage: Adult Fish	Fish Origin: Both
"Stock Composition"		Composition: Fish Species Assemblage (ID: 56)	Fish Life Stage: Adult - Outmigrant	Fish Origin:Natural
Maturation Status"		Condition of Life Stage: Fish (ID: 57)	Fish Life Stage: Adult - Returner	NA
"Kelt Condition"			Fish Life Stage: Adult - Outmigrant	NA
Reconditioned Kelt condition"			Fish Life Stage: Adult Fish	NA
Fecundity"		Fecundity: Fish (ID: 68)	NA	NA
Fry Growth"		Growth Rate: Fish (ID: 73)	Fish Life Stage: Juvenile - Fry/Parr	NA
"Fertilization Rate"		Hatchery Practices: Propagation(ID: 87)	Fish Origin: Both	NA
"Kelt length"		Length: Fish Species (ID: 75)	Fish Life Stage: Adult - Outmigrant	NA
"Reconditioned kelt length"			Fish Life Stage: Adult Fish	NA
"Mark Detection"		Mark/Tag Recovery or Detection (ID: 381)	NA	NA
"Parentage Analysis"		Relative Reproductive Success (RRS) (ID: 88)	Fish Origin: Both	NA
"Reproductive success"		Reproductive Success (Nb/N) (ID: 89)	Fish Origin: Natural	NA
"Mark application"		Stock Identity (ID: 95)	Fish Life Stage: Adult - Outmigrant	NA
"Kelt Survival"		Survival Rate: Fish (ID: 99)	Fish Life Stage: Adult - Outmigrant	Fish Origin: Both

"Collection Date"		Timing of Life Stage: Fish (ID: 101)	Fish Life Stage: Adult - Outmigrant	NA
"Release Date"			Fish Life Stage: Adult Fish	NA
"Kelt Weight"		Weight: Fish (ID: 206)	Fish Life Stage: Adult - Outmigrant	Fish Origin: Both
"Reconditioned Kelt weight"			Fish Life Stage: Adult Fish	Fish Origin: Bot