Kelt Reconditioning and Reproductive Success Evaluation Research

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Douglas R. Hatch, Columbia River Inter-Tribal Fish Commission, Portland, OR

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# Contents

## 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 both 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. 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 42% (17 years) for Yakima River at Prosser Hatchery and at Dworshak Hatchery 21% (5 years) for hatchery origin fish, 33% (6 years; 46% over the last 4 years) for mixed stock collections at Lower Granite Dam, 32% (2 years) for South Fork Clearwater collections, and 29% (2 years) for Fish Creek collections. Using estradiol assays, we have established that steelhead rematuration rates vary annually and spatially and ranged from 14.1% to 77.8%. 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. A total of 37 reconditioned B-run steelhead were released below Lower Granite Dam in 2016 to address Reasonable and Prudent Alternative 33 of the FCRSP Biological Opinion. We air-spawned a group of maiden Dworshak Hatchery steelhead in 2016. These fish were then reconditioned and the rematuring fish air-spawned as consecutive repeat spawners in 2016 to compare performance between maiden and repeat spawnings. Repeat spawners relative to maiden spawners had higher fecundity, larger eggs and similar fertilization rates. A total of 247 reconditioned, remature steelhead were released in the Yakima River in 2016. Reproductive success of reconditioned steelhead was confirmed in the Yakima River once again with assignments of 55 juvenile fish to 29 unique parents. Lifetime reproductive success for reconditioned kelt steelhead was estimated as 2.33 relative to single time spawning steelhead. Mature reconditioned steelhead kelts were stocked in the Cle Elum Hatchery Spawning Channel in 2016, to evaluate the feasibility of using the facility to evaluate reproductive success in a more controlled setting. 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. We drafted a Snake River Basin steelhead kelt reconditioning facility master plan, which was approved by the Northwest Power and Conservation Council (NPCC) in December 2016. This master plan summarizes our research effort, identifies fish collection locations, reconditioning locations, and provides a conceptual construction plan for a reconditioning facility. Next we plan to develop a final design and construction documents, complete environmental compliance requirements and return to the NPCC for a final recommendation. Our team has published 12 manuscripts to date, with four in 2016. Additionally, the team gave 6 professional presentations in 2016.

## Acknowledgments

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## 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 3 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). 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 occur currently at near Prosser just below Prosser Dam into the Yakima River and into the Snake River just below Lower Granite Dam. Prior releases have been conducted in the Lower Columbia (rkm 135) and Okanogan rivers (confluence of Columbia and Okanogan), and also into Shitike Creek near Warm Springs, OR. For a more thorough description of both the current and prior 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 | - | | | 1999-2016 |
| 2 | Yakama Nation Prosser Hatchery | Yakima River | RK 75.6 | - | Yes | Yes | | 1999-2016 |
| 3 | Lower Granite Dam Juvenile Bypass | Snake River | RK 173 | Yes | - | Yes | | 2009-2016 |
| 4 | Dworshak National Fish Hatchery | Clearwater River | RK 65 | Yes (hatchery fish for experimental purposes) | Yes | - | | 2009-2016 |
| 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, 2016 |
| 20 | Satus Creek | Yakima River | | - | - | - | Yes | 2008-2016 |
| 21 | Toppenish Creek | Yakima River | | - | - | - | Yes | 2008-2016 |
| 22 | Simcoe Creek | Yakima River | | - | - | - | Yes | 2008-2016 |
| 23 | Ahtanum Creek | Yakima River | | - | - | - | Yes | 2008-2016 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 24 | Big Creek | Yakima River | | - | - | - | Yes | 2008-2016 |
| 25 | Cowiche Creek | Yakima River | | - | - | - | Yes | 2008-2016 |
| 26 | Little Rattlesnake Creek | Yakima River | | - | - | - | Yes | 2008-2016 |
| 27 | Nile Creek | Yakima River | | - | - | - | Yes | 2008-2016 |
| 28 | Quartz Creek | Yakima River | | - | - | - | Yes | 2008-2016 |
| 29 | Bumping River | Yakima River | | - | - | - | Yes | 2008-2016 |

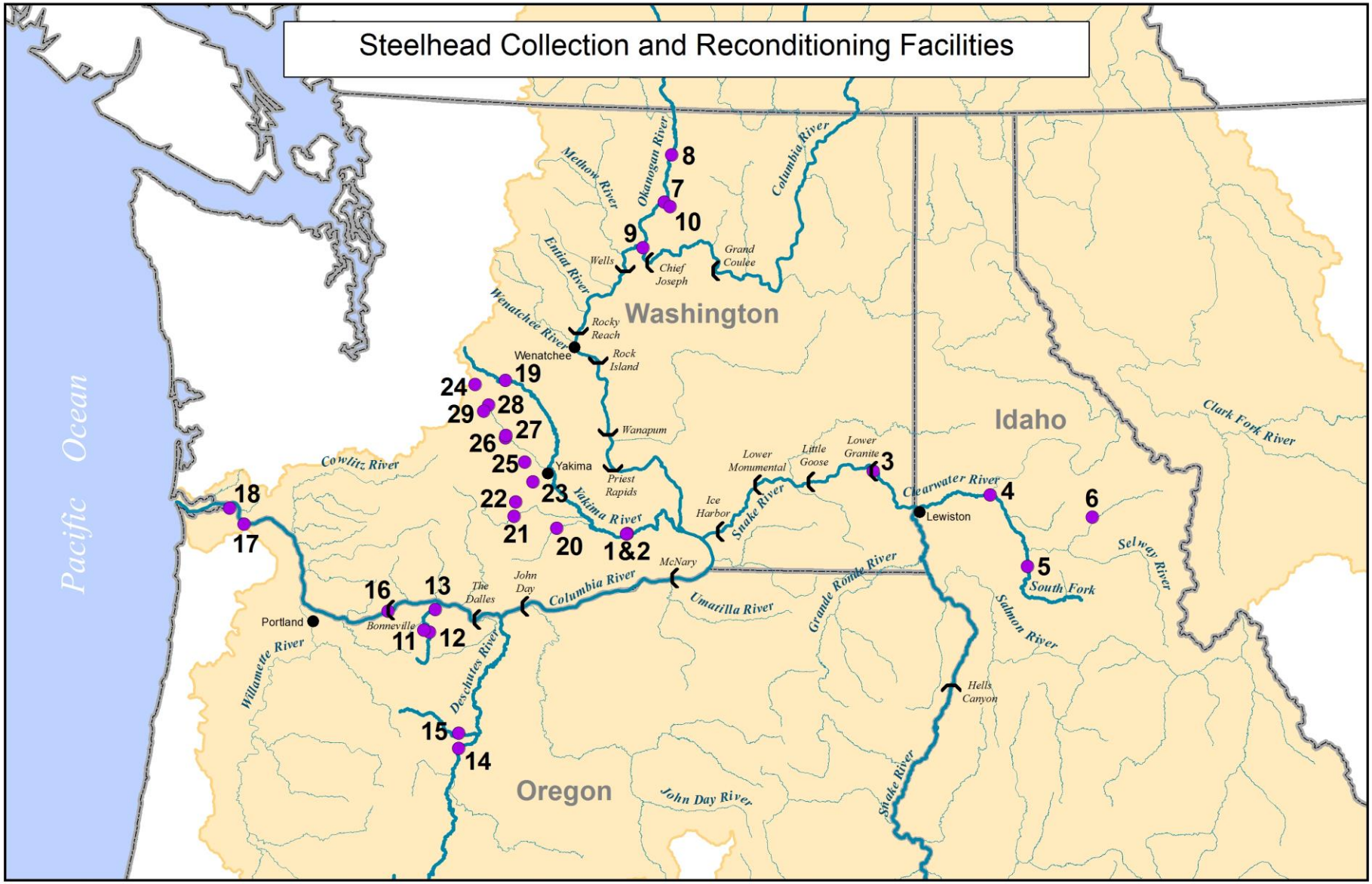


*Figure 1. Map of Steelhead kelt Project area 2000-2016.*

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

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### 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.

## 1A: 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 2016 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 2000-2016 we captured a total of 13,421 downstream migrating kelts at the CJMF, on average representing 22% of each annual wild steelhead return. In 2016, steelhead kelt collections were depressed at Prosser but were up at LGD. We collected 686 and 227, at the CJMF and Lower Granite Dam, respectively (Appendix A1a). There were no collections at South Fork Clearwater River and Fish Creek in 2016. Additional kelts were available at Lower Granite Dam but our reconditioning capacity was met.

Long-term reconditioning survival averaged 42% 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 5 years at DNFH with an average of 21% for the hatchery fish, 33% for the fish captured at LGD. 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 did have a setback with a power outage at DNFH that caused a malfunction in our formalin system that caused mortality in our DNFH hatchery fish and small portion of the LGD group.

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 continue to be predominantly (>92%) female. Annual survival to release ranged from 18% at the start of the program to an annual high of 76% in 2016 and averaged 42% over the course of the study (2000-16) 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 | 3 | 8 |
| 2015 | S.F. Clearwater | 35 | 7 | 20.00% | 4 | 3 | 0 | 4 |
| 2015 | Fish Creek | 83 | 25 | 30.10% | 10 | 15 | 15 | 12* |
| | | | | | | | | |
| **2015 (subtotal)** | | **140** | **43** | **30.70%** | **22** | **21** | **18** | **24** |
| | | | | | | | | |
| 2016 | Lower Granite Dam | 227 | 120 | 52.86% | 19 | 101 | TBD | 22^ |
| | | | | | | | | |
| **2016 (subtotal)** | | **227** | **120** | **52.86%** | **19** | **101** | **TBD** | **37*** |
| **Grand Total** | | **858** | **281** | **32.75%** | **157** | **124** | **20** | **177** |
| | | | | ***includes Fish Cr. kelt skip spawners** | | | | |
| | | | | **^Includes previous year kelt spawners from LGD** | | | | |

# Chapter 2. Steelhead Kelt Reproductive Success

# 2. A: Cle Elum Spawning Channel

## Introduction

We tested the feasibility of using the Cle Elum spawning channel to demonstrate reproductive success of reconditioned kelt steelhead. The spawning channel provides a semi-natural system where there is more control of variables relative to natural streams. The Cle Elum Spawning Channel was previously 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. Our current effort focuses on adapting the channel for steelhead, which so far has included modifying gravel size and adding cover. Because, steelhead adults and juveniles will need to spend significantly more time in the channel than chinook they likely are exposed to more predation events, fluctuating natural conditions (flood events and low water years), sedimentation, etc. 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. Long-term study 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. In 2015 kelts successfully spawned in the channel and collection of progeny that assigned back to all mature adult fish placed in the channel. In 2016 we continued to explore the Cle Elum spawning channel as a means to better understand kelt spawning in a controlled environment. A small group of maiden fish were included to test the feasibility of collecting, transporting, and stocking in the channel to determine their utility as comparisons to reconditioned kelts.

## Methods

### Channel Description and Modification

The Cle Elum spawning channel was originally designed for optimal spring Chinook spawning conditions (Schroeder et al. 2008). Some additional enhancements were made after conducting substrate samples that suggested that fine sediments could be having a negative impact on egg survival. A large log was placed into the channel to trap sediments in the uppermost section (1-1). Additional cover was provided for fish with the construction of bank overhanging covers for each group and added an extra floating cover to each section which complemented the already existing 2 that we had in place from last year. These should help to reduce possible incidences of predation and lower fish stress.

Since the two populations (Naches and upper Yakima) are closely related they were used for this experiment. Still we divided the channel into two similar sections to prevent or reduce the populations from mingling since the intent is that progeny would be released back to their streams of parental origin.

**Adult Collections and Stocking**

Kelt steelhead were collected at the Chandler Juvenile Monitoring Facility (CJMF) and placed in the long-term reconditioning program. Upon entry into the program, all kelts were scanned for PIT tags and those that were identified as Upper Yakima or Naches origin fish based off their juvenile detection history or when captured at the Prosser denil trap migrating upstream as "maidens" became candidates for the channel. These channel candidate fish were isolated into one circular tank at Prosser Hatchery, on September 16, 2015 and held until February 19, then trucked to the Cle Elum Channel. Naches origin fish were released into the upper channel section and the upper Yakima origin fish released into the lower channel section. Angling for anadromous "maiden" steelhead (includes males and females) and resident fish (only males) started on February 1st and ended March 31st. Maiden and resident fish were stocked on 2/4, 2/23, 2/25, 3/22, 3/30, and 3/31 of 2016.

**Juvenile Collections**

Juvenile samples were passively collected using box traps with netted tubes, located at the downstream end of the two channel sections. Traps were checked twice daily and collected fish were retained in 6-foot diameter circular tanks (one tank for each fish stock). Juveniles collections were systematically lethally sampled (every tenth fish) and tissue used for genetic parentage analysis. At the end of the study period, the fish remaining in the channel were actively collected using electrofishing. All Naches origin juvenile fish were released near river kilometer 6.4 of the Naches River and fish collected from upper Yakima section were released just upriver of the hatchery.

**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. 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.

**Substrate**

Two sites were chosen in each section, both redd, and non-redd (in the case of the elbow section both were non-redd index sites) samples were collected for a total of 14 samples from the channel (Figure 2A.1). We do not have data for channel composition and how much fines (<.855mm) were present before steelhead kelts began constructing redds in 2015. In 2016 we measured the amount of fine sediment in the channel at the beginning (February), mid-point (June), and end of the study (August). We used a McNeil core sampler to collect sediment samples at 14 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 placed into a Preisser Air Drying Oven to remove all moisture weight from the samples. After the removal of moisture, samples were 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 weighed in grams and the percentages of the total weight was determined (Justice 2012).

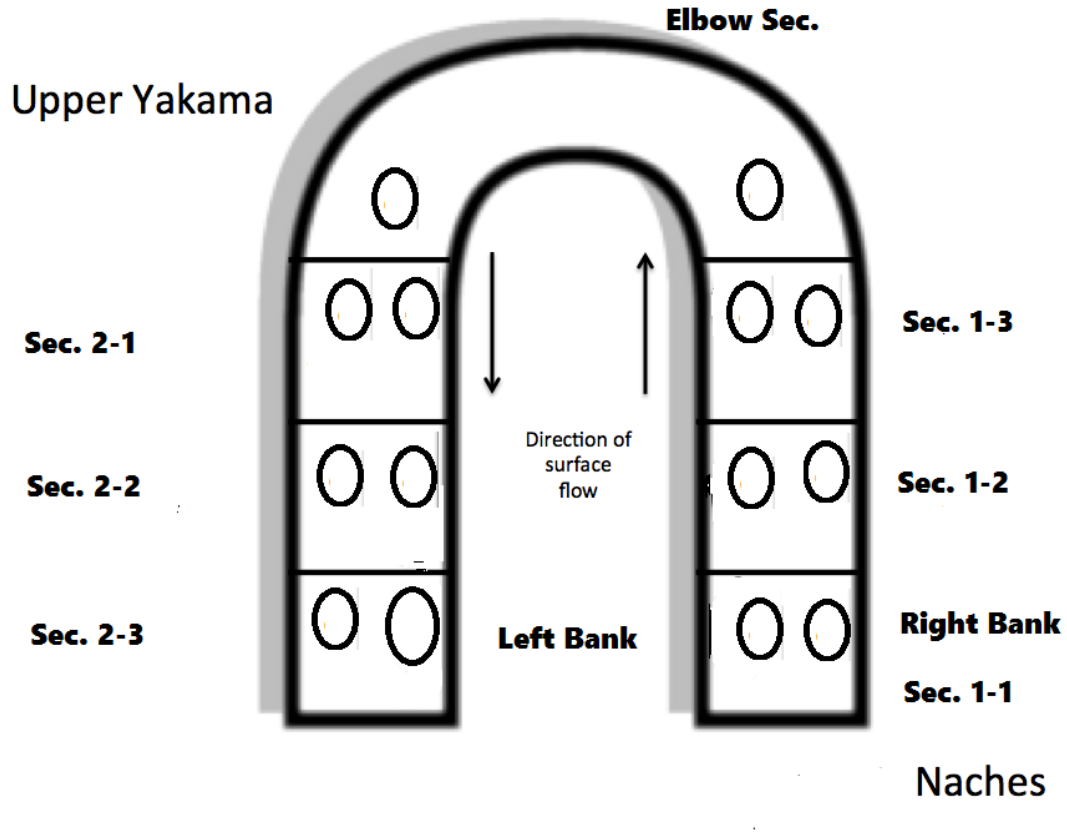



*Figure 2A.1 Cle Elum spawning channel McNeil sampling locations.*

## Results

### Adult Collections, Stocking, and Recoveries

On February 19, 2016 we released 18 steelhead from the Prosser reconditioning program into the Cle Elum Hatchery Spawning Channel. All females were identified as mature by blood hormone analysis. The spawning channel was separated into two sections and in the upper section 16 Naches origin reconditioned kelts were released. These 16 fish were composed of 14 maturing females and 2 males. In the lower section of the spawning channel, 2 female upper Yakima River origin reconditioned kelts were released. In February, 8 resident males were angled or electroshocked from the Naches and 4 from the Upper Yakima River. In late March, the WDFW and Yakama Nation angled 5 male and 4 female anadromous maiden steelhead from that Naches River and placed in the upper channel section.

Throughout the study, 7 Naches kelts (5 females and 2 males) and both upper Yakima kelts were recovered as mortalities. All of the anadromous Naches maiden females with 2 of the maiden males were recovered as mortalities. Only 2 large resident males were recovered in upper Yakima section. No evidence could be obtained as to the fate of the 12 unrecovered fish and no progeny were found associated with these fish.

### Redd Construction

We observed 5 large redds in the Naches section and 2 in the Upper Yakima section. There were numerous smaller redds that were constructed but in the experience of our observer these were likely test digs. Figure 2A.2 shows the location of the larger redds. The Naches fish spawned over a 2 month period, with the first large redd constructed on February 29, 2016 and the final redd in the Naches section was constructed April 27, 2016 by a maiden fish. The first redd constructed in the Upper Yakima section occurred on March 21, 2016 and the final redd on April 1, 2016.

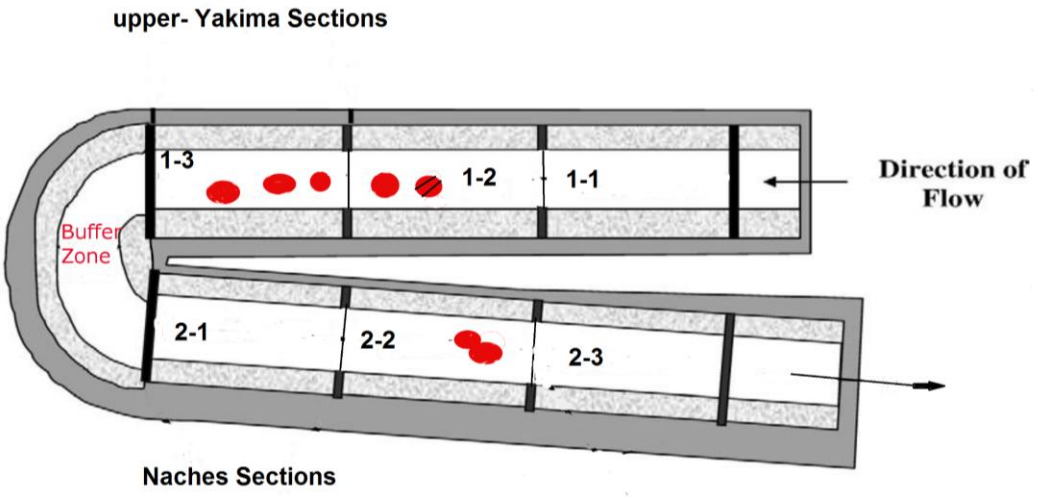



*Figure 2A.2. Site of redd locations at Cle Elum spawning channel 2016. Circled red dots areas represent redd locations. The red dot with black lines represents maiden spawn area. Sections start with flow direction 1-1 through 1-3 in upper 3 sections and 2-1 through 2-3 in lower 3 sections. The thick lines at the top of 1-1 and bottom of 1-3 and sections 2-1 and 2-3 represent areas that adult fish should not be able to pass through.*

**Juvenile Collection**

Traps were set on June 2, 2016 and removed on August 1st with the remaining juveniles were collected using electrofishing methods over the following week. All juvenile fish were released back to either the Naches or Upper Yakima depending on section collected.

**Genetic Analysis**

Genotypes were generated for all fish stocked in the channel including 39 potential spawners comprising 16 mature female reconditioned kelts, 4 maiden steelhead, 2 male reconditioned kelts, 5 anadromous males and 12 resident males. Fish that died prior to redd construction and were omitted from parentage analysis. Of 797 juveniles with quality genotypes, a single fish failed to assign back to the stocked adults. This single fish is thought to have entered the spawning channel from the river water intake to the hatchery. This is consistent with reports of juvenile trout also seen when the channel was used for chinook spawning (Schroder et al. 2008).

All 797 juveniles that assigned to a parent, were successfully assigned to two adults (Table 2A.1). In all cases, the juvenile fish were assigned to parents that were both stocked in the same section of the channel. There is no other evidence that adult fish were able to move between their stocking locations. Assignments to parent classes are shown in table 2A.1. Of note, a majority of the offspring collected in the Upper Yakima section were assigned to parents from the Naches section of the channel. Since all of these juvenile fish had parents from the

Naches section, it is presumed that they traveled downstream by escaping the trap, bypassing the screens, or traveling through the gravel.

*Table 2A.1 Juvenile assignments to parent origin by channel section in 2016 Entries in red indicate a sample that was collected.*

| Section | assigned to Naches Parent | assigned to Up Yak Parent |
|---|---|---|
| Elbow | 24 | 0 |
| End | 1 | 1 |
| Unknown | 9 | 0 |
| Naches | 400 | 0 |
| Yakima | 183 | 179 |

Progeny were assigned to 5 of the kelts (1 Upper Yakima, 4 Naches (2 males and 2 females)) and 5 of the maiden fish (4 females and 1 male). No progeny were assigned to fish not accounted for at the end of the study.

**Substrate**

Based on photos and notes we believe that major inputs of organic fine materials were transported into the channel beginning sometime around March 16, 2016 and ended sometime in final weeks of May 2016. This fine deposition is occurring during the time that kelts are constructing redds. Most redds were constructed at tail outs of each of the sections on usually favoring the inside portion of the channel. These areas typically had lower fine sediment deposition than the upper portion of the sections. McNeil samples reveal that the upper most Naches section 1-1 RB had the most fines deposition (particle sizes less than .85mm) (Table 2A.2). For the most part, the Upper Yakima section had the lowest amounts of fines deposition. This was likely a result of being further downstream from the outflow and the log placement upstream that helped trap fine sediment. It is interesting that in 2016, which was an above average flow year, that sediment deposition was not distinctly higher than 2015 which was an extremely low water year, which typically translates into lower sediment transport (Table 2A.3).

*Table 2A.2 Fine sediment deposition change from February 2016 to August 2016.*

| Section | % change |
|---|---|
| **1-1 RB** | 6.9% |
| **1-1 LB** | 4.8% |
| **1-2 RB** | -2.1% |
| **1-2 LB** | 4.4% |
| **1-3 RB** | 3.3% |
| **1-3 LB** | 0.5% |
| **Lower Elbow** | 0.6% |
| **Upper Elbow** | 4.3% |
| **2-1 RB** | 3.5% |
| **2-1 LB** | 2.4% |
| **2-2 RB** | 0.9% |
| **2-2 LB** | 0.4% |
| **2-3 RB** | -0.1% |
| **2-3 LB** | 1.3% |
| **Avg. change.** | 2.22% |

*2A.3 Fines change from August of 2015 to August of 2016*

| | |
|---|---|
| **1-1 RB** | 0.18% |
| **1-1 LB** | 1.49% |
| **1-2 RB** | 0.23% |
| **1-2 LB** | -1.96% |
| **1-3 RB** | 1.96% |
| **1-3 LB** | 0.41% |
| **Lower Elbow** | NA |
| **Upper Elbow** | NA |
| **2-1 RB** | 1.19% |
| **2-1 LB** | 1.72% |
| **2-2 RB** | 1.35% |
| **2-2 LB** | 0.85% |
| **2-3 RB** | -0.06% |
| **2-3 LB** | 0.40% |
| | |
| **Avg. change** | 0.65% |

The only section which was above the detrimental 10% fines (Jensen et al. 2009) deposition in 2016 was section 1-1 RB. The other side 1-1 LB was close at 8.3%. These high levels of fine sediments in this section likely explains why no redds were constructed there.

## Discussion

This pilot study was initiated to determine if it was feasible to use the Cle Elum spawning channel to quantitatively evaluate reproductive performance in reconditioned kelt steelhead. We have had some success in both 2015 and 2016 with both kelts and maidens producing progeny from the channel and we collected our target number (500) of juvenile fish. Our habitat improvements ((appropriate gravel sizes in 2015, fine sediment collection log placement in 2016, and covers (2015 and 2016)) seem to have encouraged spawning in both the upper Yakima and Naches spawners. We successfully managed to assign almost all juveniles back to parents that were placed in the channel with the exception of a single fish.

We have not yet accounted for the disposition of reconditioned kelts that were stocked but not detected again as mortalities or survivors. None of these fish were successful in producing juvenile progeny and we theorize that they suffered from predation. Predators (otters, herons etc.) were seen in the area and are a likely causing both a direct effect by removing fish from the system and an indirect effect by increasing stress levels in the adults.

Another issue which complicates our ability to accurately compare kelts and maiden fish is that maiden spawn timing was extremely truncated and delayed as compared to the kelts. This was likely due to the maidens being collected much later in the year (late March) and possibly from a specific related sub-population whereas our kelts may originate from multiple sub-populations from throughout the Naches watershed. Additionally, this truncated spawn timing probably helped maidens due to the lack of exposure they would have faced from predation unlike the kelts which had been in the channel for approximately a month and a half before the maidens. Because the reconditioned kelts are in the system for a longer time period, they are likely to suffer from both increased predation and increased stress relative to the maiden fish.

Many Naches origin juveniles were collected in the lower channel section, indicating that either the fish traps were not 100% effective or they were installed after fish had emerged and moved downstream. This also suggests that we likely had juveniles produced in the lower section move downstream and out of the system undetected. This issue is particularly problematic for a future quantitative study. In 2017, to further assess the problem we will place additional traps in the lowermost section to maximize catch. Additionally, we will install the traps sooner (3 weeks after 1$^{st}$ redd construction) to eliminate the chance of early emerging fry from moving downstream prior to trap installation.

We will attempt to remedy predation and trapping issues in 2017 so that we will be able to increase the accuracy of the data with which to compare relative reproductive success of kelts.

# 2. B: Yakima River Kelt Reproductive Monitoring

## Introduction

The reproductive success of long-term reconditioned kelts is 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 targeted using electrofishing techniques (NMFS 2000 Electrofishing Guidelines) during the late summer and 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 minimum length was used in addition to the judgment of those collecting the samples based on the time of year. Fork length was recorded for additional analysis of length outliers.

### 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. Past genotyping efforts have utilized a Fluidigm ep1 platform and the 192 Single Nucleotide Polymorphism (SNP) markers and methods described in Hess et al. (2012). All genotyping efforts in 2015 used an expanded marker panel and GTseq protocols 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 minimizing the loss of parental assignment matches.

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 for adult fish known to have been upstream of Prosser Dam. Juvenile assignments are reported here only for fish within Satus and Toppenish Creeks, although samples were previously genotyped in the Ahtanum, Big Creek, and Naches drainages.

Relative reproductive success (RRS) was calculated between classes of fish by standardizing to the pre-spawn maiden class of adults. Lifetime reproductive success (LRS) was calculated by adding the RRS of post spawn maidens to the RRS of reconditioned kelts. This estimate of LRS does not look at individuals of 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 successfully genotyped 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. All locations sampled in 2015 provided parental assignments varying between 4 at Satus Creek Above high bridge to 57 seen in Satus Creek above Wilson Charlie Creek. Across all sites, 24.9% of the juveniles in 2013, 25.5% of the juveniles in 2014 and 44.7% of the juveniles in 2015 assigned to at least one anadromous parent.

*Table 2B.1. Number of individuals genotyped and assigned at each site annually, and average assignment rate over three years.*

| | 2013 | | 2014 | | 2015 | | |
|---|---|---|---|---|---|---|---|
| **Location** | Genotyped | Assigned | Genotyped | Assigned | Genotyped | Assigned | Average Assignment Rate |
| Satus Cr. Screw Trap | | | 1 | 0 | | | 0.0% |
| Satus Cr.-Dry mouth | 11 | 8 | | | | | 72.7% |
| Satus-Logy Cr. at Swamps | | | 17 | 4 | | | 23.5% |
| Satus Cr.-Above Logy Cr. | 3 | 2 | | | | | 66.7% |
| Satus Cr. at Holwegners Ranch | 21 | 5 | | | | | 23.8% |
| Satus - Dry Cr. at Elbow Crossing | 88 | 8 | 46 | 6 | | | 10.4% |
| Satus Cr. Below High Bridge | 75 | 21 | 68 | 20 | 39 | 16 | 31.3% |
| Satus Above high bridge | 36 | 14 | | | 37 | 4 | 24.7% |
| Satus Cr. Above Kusshi | | | | | 26 | 14 | 53.8% |
| Satus Below Wilson Charlie aka green rope | | | 52 | 7 | 29 | 13 | 24.7% |
| Satus Cr. above Wilson Charlie Cr. | | | 38 | 9 | 110 | 57 | 44.6% |
| Satus Cr. Above Wilson Charlie Cr. Site 2 | | | | | 75 | 24 | 32.0% |
| Satus Cr. Below County Line | 14 | 1 | 64 | 18 | 51 | 9 | 21.7% |
| Topp Cr. Upper Screwtrap | 50 | 14 | 37 | 12 | | | 29.9% |
| Topp Cr. at Signal Peak Rd | | | 23 | 9 | | | 39.1% |
| Topp Cr. above Wesley Rd. | 100 | 36 | 64 | 21 | 55 | 13 | 32.0% |
| Topp Cr. Near Olney Diversion | | | 43 | 7 | 52 | 31 | 40.0% |
| Topp Cr. Near Wildlife Gate | | | | | | | |
| Topp Cr Above Swim Hole | 10 | 4 | | | 16 | 10 | 53.8% |
| Topp - Willy Dick Cr. | 46 | 0 | 8 | 0 | | | 0.0% |
| Topp Cr. Just above Willy Dick Canyon | | | | | 29 | 17 | 58.6% |
| Topp Above Willy Dick starting at Washout | | | 24 | 9 | 58 | 19 | 34.1% |
| Topp Cr. at Camp Cr. | | | 19 | 5 | 57 | 21 | 34.2% |
| Topp Cr. Below NF confluence | | | | | | | |
| Topp NF Toppenish | | | | | | | |
| Topp SF Toppenish | | | | | | | |
| Topp Simcoe Cr. At Simcoe Rd | *45* | *16* | 45 | 16 | 53 | 36 | 47.6% |
| Topp Cr. Simcoe Cr. NF SF confluence | 49 | 8 | | | 49 | 18 | 26.5% |
| Topp - Agency Cr. | | | 13 | 0 | | | 0.0% |

The number of genotyped parents is shown in Table 2B.2. Pre-spawn maidens have the greatest number of samples with a total of 581 males and 1753 females. The number of Post-spawn maidens was lower with only 226 males and 1874 females. The lowest number of samples is seen in the reconditioned kelts detected moving upstream of Prosser dam. Across both years, only 44 males and 413 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 adults genotyped.*

| Class | Sex | 2013 | 2014 | 2015 | All |
|---|---|---|---|---|---|
| Pre-spawn maidens | Male | 145 | 167 | 269 | 581 |
| Post-spawn maidens | Male | 23 | 55 | 148 | 226 |
| Reconditioned kelts | Male | 16 | 8 | 20 | 44 |
| Pre-spawn maidens | Female | 306 | 287 | 579 | 1753 |
| Post-spawn maidens | Female | 307 | 240 | 1101 | 1874 |
| Reconditioned kelts | Female | 194 | 88 | 72 | 413 |

Table 2B.3 shows the number of parents with progeny assigned to them. 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 4.8% of the male fish and 5.0% for female fish. Detection rates in the post-spawn maidens were higher at 9.3% for males and 8.3% in females. The higher rate in the post spawn maidens is partially attributable to the fact that by collecting after the spawning period, they by default cannot have 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 6.8 % of female reconditioned kelts with progeny detected was similar to that of pre-spawn maidens (4.8%), 11.4% of the male reconditioned kelts had progeny detected while only 4.8% of the male pre-spawners had progeny detected.

*Table 2B.3. Number and percentage of adults with at least one progeny assignment.*

| Class | | All | n | Successful Adults % |
|---|---|---|---|---|
| Pre-spawn maidens | Male | 581 | 28 | 4.8% |
| Post-spawn maidens | Male | 226 | 21 | 9.3% |
| Reconditioned kelts | Male | 44 | 5 | 11.4% |
| Pre-spawn maidens | Female | 1753 | 59 | 5.0% |
| Post-spawn maidens | Female | 1874 | 137 | 8.3% |
| Reconditioned kelts | Female | 413 | 24 | 6.8% |

Relative reproductive success (RRS) for each group of individuals, and calculated lifetime reproductive success (LRS) of reconditioned kelts are shown in table 2B.4. The RRS of male post-spawners and reconditioned kelts were both higher than pre-spawners leading to an LRS of 3.71 that of males collected as pre-spawners. While female post-spawn collection RRS was

1.48 times that of pre-spawn collection, reconditioned kelt RRS was slightly lower at 0.85 for a LRS of 2.33 in reconditioned kelts.

*Table 2B.4. Average number of offspring assigned per individual in each class, relative reproductive success (RRS) for each group of individuals, and calculated lifetime reproductive success (LRS) of reconditioned kelts.*

| Class | | Genotyped Adults | Progeny Assigned N | Per | RRS | LRS |
|---|---|---|---|---|---|---|
| Pre-spawn maidens | Male | 581 | 71 | 0.12 | 1.00 | |
| Post-spawn maidens | Male | 226 | 51 | 0.23 | 1.85 | |
| Reconditioned kelts | Male | 44 | 10 | 0.23 | 1.86 | 3.71 |
| Pre-spawn maidens | Female | 1753 | 176 | 0.15 | 1.00 | |
| Post-spawn maidens | Female | 1874 | 367 | 0.22 | 1.48 | |
| Reconditioned kelts | Female | 413 | 45 | 0.13 | 0.85 | 2.33 |

## Discussion

The 2015 spawning event was the third consecutive year that we successfully assigned multiple progeny to reconditioned kelts. A total of 55 juveniles from either Satus or Toppenish Creek are attributed to a spawning event following successful reconditioning of a kelt. We have currently assigned 582 progeny to at least one anadromous parent. This reflects the methodology of focusing sampling efforts on age-0 fish in areas that anadromous spawning was expected to have occurred. Additional years will add to this number.

Higher sample numbers were taken in 2016 along with additional sites in the upper Toppenish drainage. 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 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 2.33 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. Kelt Reconditioning Physiology Studies

# Introduction

Studies applying tools from fish physiology and endocrinology to issues in kelt reconditioning were continued in 2016. 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 2016, we assessed maturation status in blood samples from kelts and provided maturation status of individual fish to project managers so that consecutive and skip spawners could be managed appropriately (Section C1). We continued 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 C2). We conducted a diet study using Prosser kelts (Section C3). We continued laboratory work to establish assays for plasma insulin-like growth factor-1 (IGF-1) and growth hormone (GH), indicators of growth and metabolic status (Section C4). Finally, we present a brief summary and comparison of the performance of the three Columbia River Basin kelt projects (Section C5). 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; Pierce, et al. 2016; Rideout, et al. 2005; Rideout and Tomkiewicz 2011). After a spawning event, some fish are able to restore energy lost during migration and spawning, redevelop a mature ovary, and spawn the next year. These fish are termed consecutive spawners. Other fish do not initiate redevelopment of the ovary for the next spawning season, but instead skip a year. These fish are termed skip spawners. We hypothesize that these life history trajectories are the result of the effect of energy balance on maturation decisions made during seasonally defined critical periods. The influential critical period model of the first reproductive maturation (puberty) in salmonids posits that maturation is initiated during a decision window approximately one year prior to spawning (Campbell, et al.

2006; Satterthwaite, et al. 2009; Shearer and Swanson 2000; Thorpe 2007). This decision is made based on energy reserves. If maturation is initiated during this critical period, it may be arrested at a second critical period before the onset of exogenous vitellogenesis, if energy reserves are not sufficient (Yamamoto, et al. 2011). We hypothesize that a similar decision mechanism regulates rematuration in post-spawning steelhead. Consistent with this idea, we found that energy restriction affected reproductive development within 10 weeks after spawning in female rainbow trout (Caldwell, et al. 2013; Caldwell, et al. 2014). In post-spawning steelhead, 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 (Pierce et al. 2016). 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. Significant differences are found after several months of reconditioning, and diagnostic separation of rematuring and non-rematuring fish is possible from mid-August onward. Estradiol indicates maturation earlier than vitellogenin, and the cost of the estradiol assay is about 1/4$^{th}$ of the cost of the vitellogenin assay.

During 2016, we measured estradiol level in a large number of blood samples. We collected blood from fish in the reconditioning programs at Prosser, Nez Perce Tribal Hatchery (NPTH), and Dworshak (DNFH), 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 (WNFH) to measure estradiol levels in samples they collected from their reconditioned kelts, and in maiden spawners they sampled at Wells dam. We also measured plasma 11-ketotestosterone levels in male kelts reconditioned at Prosser, and confirmed our findings from last year 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 | 9/15/2016 | Wild kelts | 379 | Includes males. |
| DNFH | 9/21/2016 | Wild kelts | 28 | Kelts collected at Fish Cr, Lower Granite Dam, and South Fork of the Clearwater River. |
| NPTH | 9/20/2016 | Wild kelts | 132 | Kelts collected at Fish Cr, Lower Granite Dam, and South Fork of the Clearwater River. |
| WNFH | 9/28/2016 | Wild kelts | 51 | |
| Wells Dam | 9/13/2016 to 9/21/2016 | Hatchery and wild maidens | 31 | |

**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 49°C 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 (EIA) using an acetylcholinesterase linked estradiol tracer (Cayman Chemical, Ann Arbor, MI). Extracted plasma samples were appropriately diluted and triplicate 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 9/15/2016 sampling were assayed for 11-KT. Plasma samples were ether extracted following the same protocol as for the estradiol assay. Following reconstitution and dilution in assay buffer, plasma 11-KT concentrations were assayed using an EIA kit specific for 11-KT (Cayman Chemical, Ann Arbor, MI). Extracted plasma samples were assayed as tripicate technical replicates 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, 3A.2, 3A.3). The division between the lower and higher modes was approximately 1000 pg/ml E2 at Prosser, NPTH, and DNFH (as found in previous years). However, several fish with E2 levels of 1000-3000 pg/ml appeared to group with the lower mode, but could represent a group of fish maturing more slowly than the rest of the upper mode. Project managers wish to err on the side of releasing any fish that might possibly be rematuring. Consequently, the division between modes was adjusted to include these fish as rematuring so that the fish could be released. All plasma E2 levels in maiden Upper Columbia River steelhead sampled at Wells dam were in the maturing range. However, rematuring Upper Columbia kelts had significantly higher plasma E2 levels than maiden Upper Columbia River steelhead (data not shown). The rematuration rate of female

kelts as consecutive spawners in 2016 was high in programs in the Upper Columbia River and Prosser. Prosser females rematured at a 56.8% rate, whereas females at Winthrop rematured at a 58.8% rate. Consecutive spawners at DNFH and NPTH had relatively low rates of rematuration for 2016, with only 14.1% rematuring. Overall, the rematuration rate of female kelts held for a second year of reconditioning was higher than consecutive spawners, 75.7% at Prosser and 77.8% at DNFH and NPTH (which included 11 rematuring fish from Fish Creek on the Lochsa River and 3 collected at Lower Granite Dam). 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 female Prosser kelts sampled in fall of 2016.*

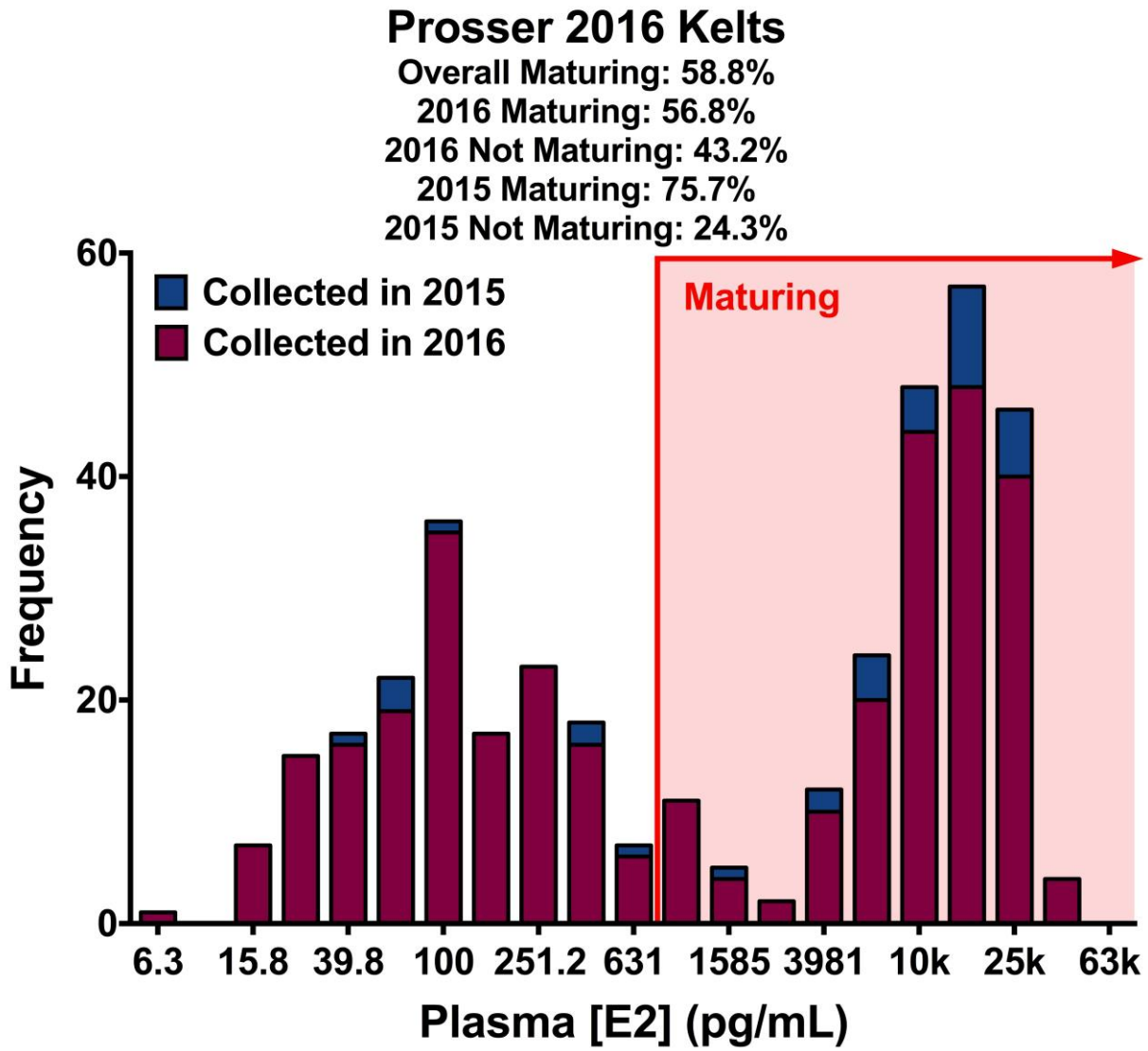


***Figure 3A.2:*** *Plasma estradiol (E2) levels in female Upper Columbia kelts and maiden spawners sampled in fall of 2015.*

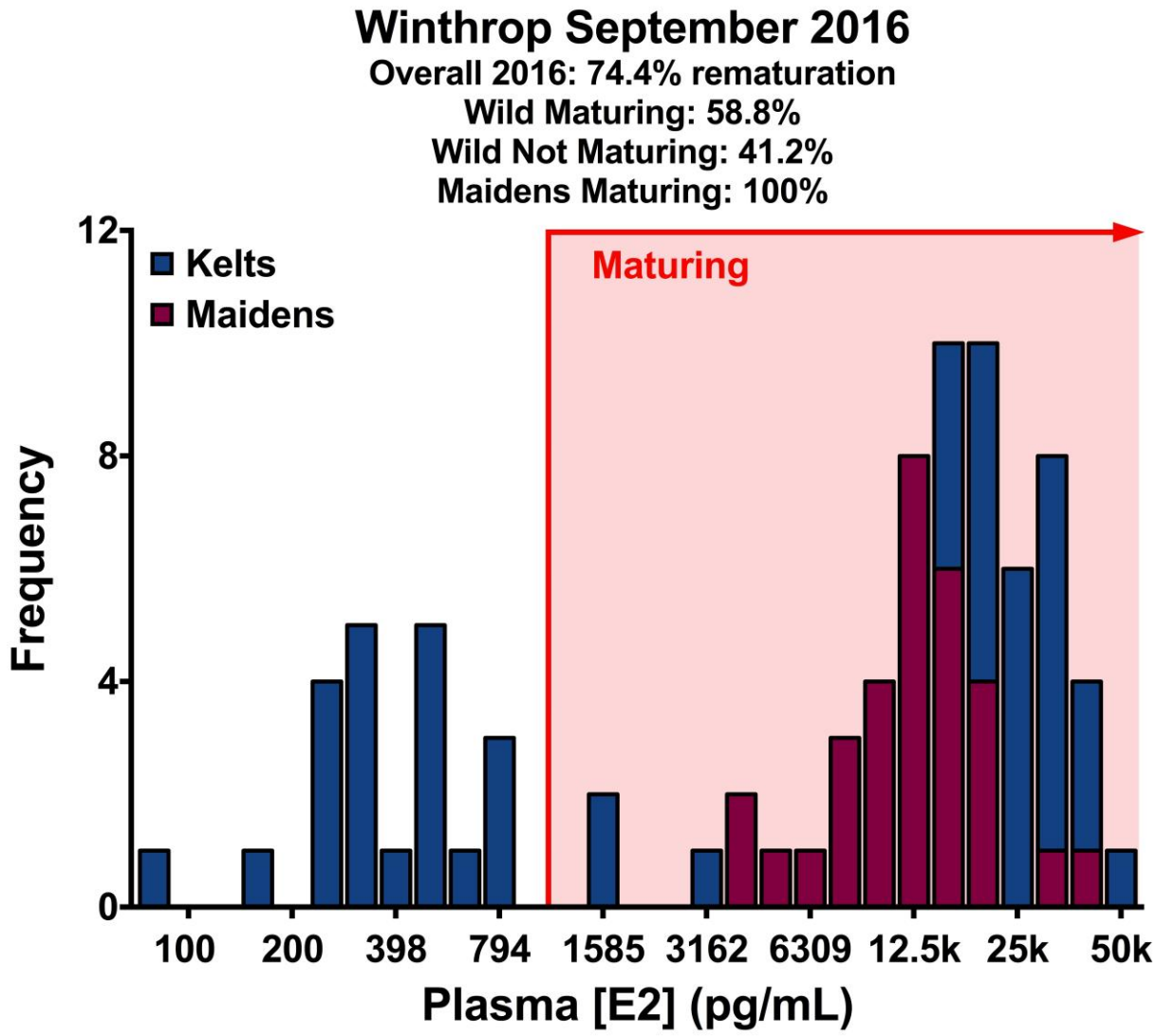
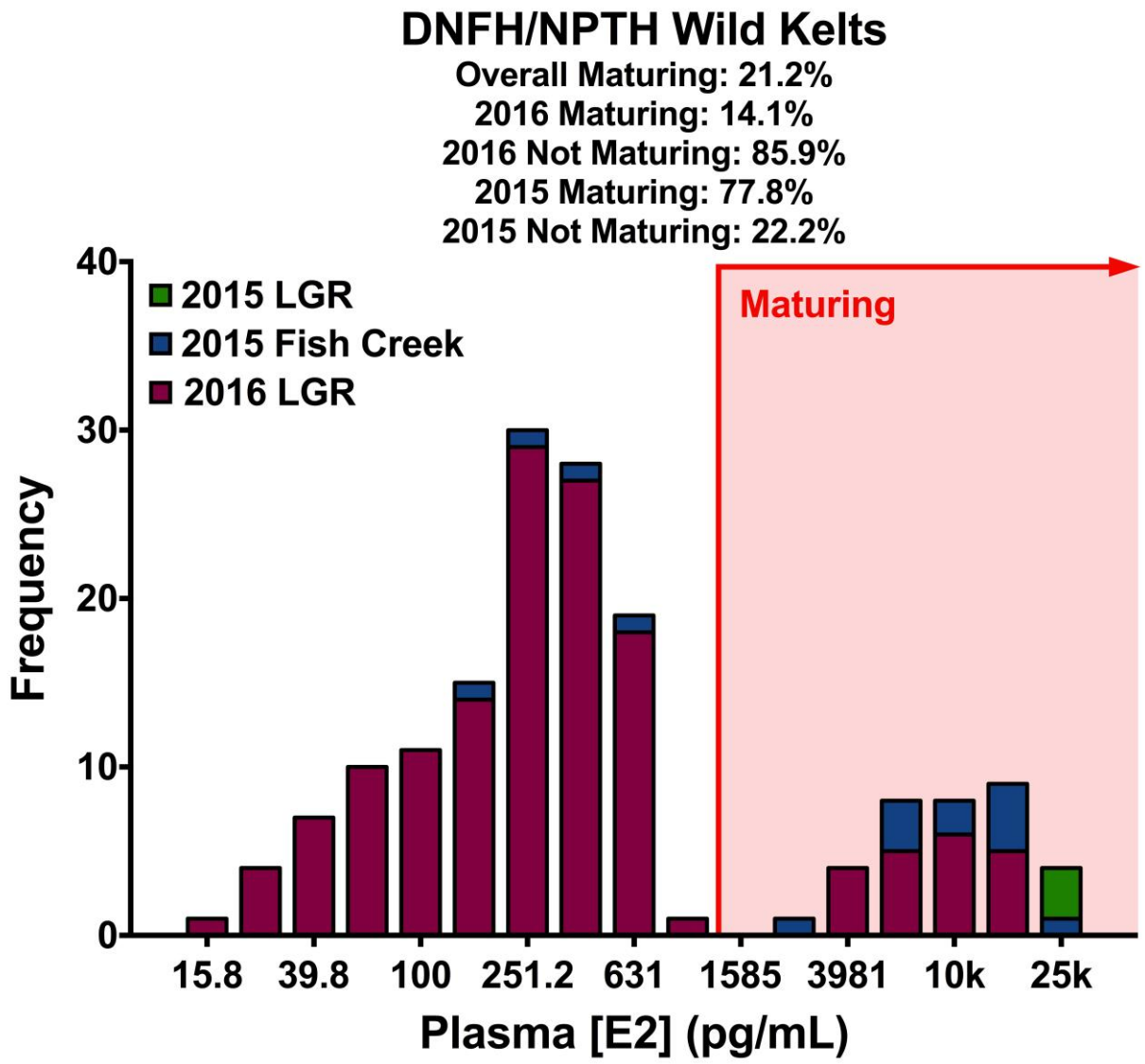
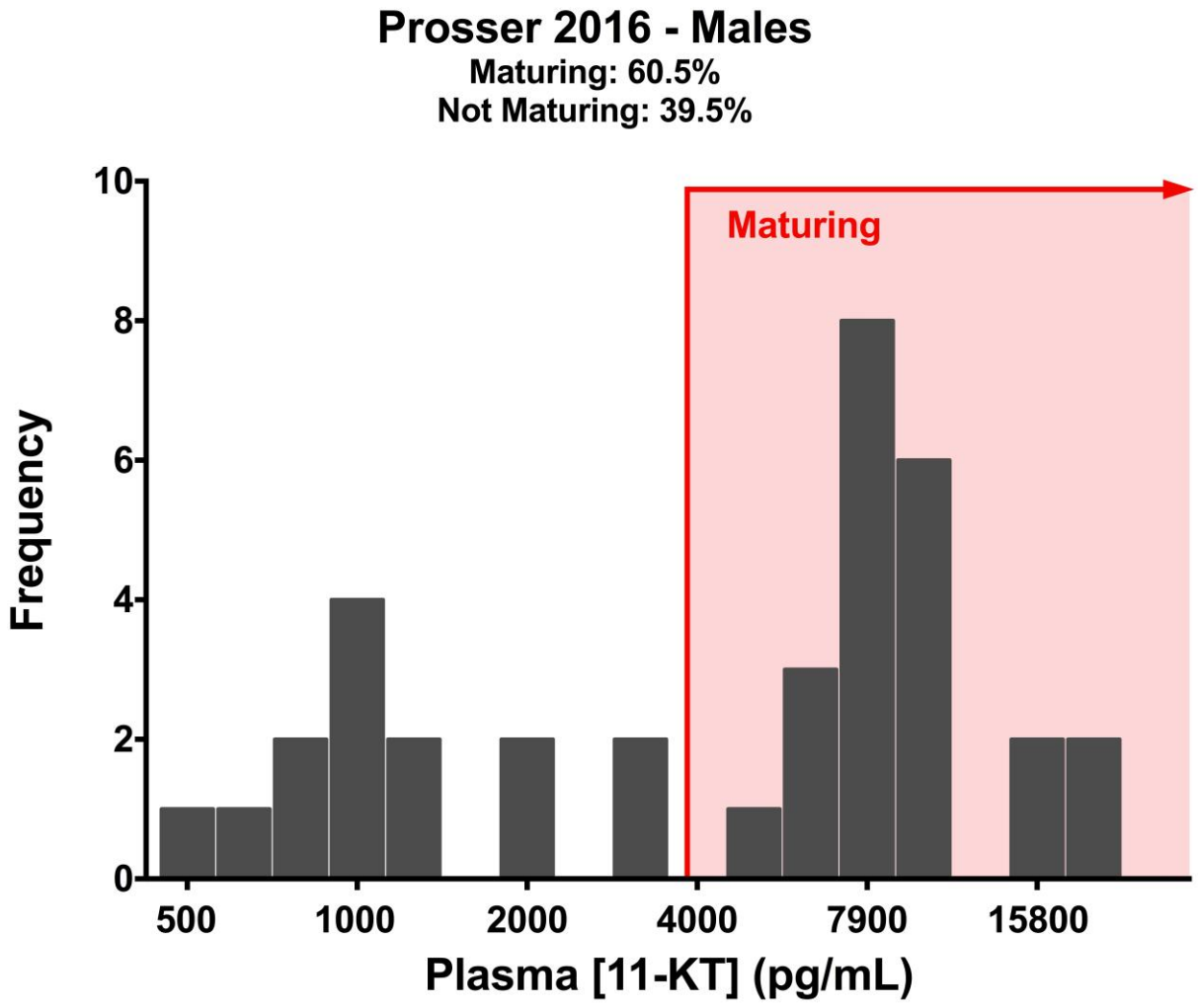


***Figure 3A.3:*** *Plasma estradiol (E2) levels in wild female kelts held at DNFH and NPTH sampled in fall of 2016.*




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 3980 pg/ml 11-KT. The maturation percentage of Prosser males (60.5%) was similar to that of Prosser females.

***Figure 3A.4:*** *Plasma 11-ketotestosterone (11-KT) levels in Prosser male kelts sampled in fall of 2016.*




**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. 2013a; Pierce et al. 2016). Plasma estradiol levels in rematuring and non-rematuring kelts for 2016 at Prosser and Dworshak were similar to previous years. A shift upward in the high mode is probably due to the later sampling date. In 2015, 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 was though that it was possible that these fish, which were classified as non-rematuring, were actually early rematuring fish. These fish were held for

further reconditioning, so maturation status would clarify as the season advances. As of 2016, it is clear that these fish were not rematuring and the call to keep them was correct. Eleven of the fifteen Fish Creek fish held for further recondition rematured in 2016, while the remaining fish were classified as not maturing. Average plasma E2 levels were (again) shifted upward in both the low and high modes in Winthrop kelts, though the cutoff remained similar to those seen at the other projects (implying a more narrow range of plasma E2 values for both the low and high modes). 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 significantly greater E2 levels in reconditioned Winthrop kelts as compared with maiden steelhead at Wells dam is similar to findings in Prosser reconditioned kelts in 2012, and suggests that investment of energy into ovarian development may be greater in kelts than in maidens.

Female consecutive maturation rates were variable among the projects this season. It is possible that this relates to pre-capture environmental conditions. The relatively low consecutive maturation rates found in Snake River kelts is in line with what has been observed previously in 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. These cohorts 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 2015 results, which had high rates of consecutive spawning, show that high consecutive rematuration rates are possible for Snake River steelhead in captive reconditioning; however, this year's low consecutive rematuration rates imply that pre-capture environmental conditions may dictate the reproductive strategy employed. This could be the result of the warmer water temperatures the Columbia River Basin has been experiencing the past couple of summers, requiring a longer recovery period before the kelts are able to mature again (even with reconditioning). This is supported by the consistently high rates of maturation in the fish held for a second year of reconditioning.

Non-rematuring fish held for a second year rematured at very high rates (73.3% or higher) in 2016 at both Prosser, NPTH, 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. Only one male kelt had an elevated E2 level, but also 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 male with elevated E2 may actually be a female. 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. 2013a; 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- 2016, 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 2014-2016, 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 in place of 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 from male O. mykiss raised at University of Idaho's Aquaculture Research Institute (ARI) were often used in place of DNFH milt in 2016.) 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 number. 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 2013-2016.*

| Spawn Year | Fish Air Spawned | 8/9/13 | | 8/28/14 | | 9/22/15 | | 9/23/16 | |
|---|---|---|---|---|---|---|---|---|---|
| | | Alive | Re-maturing | Alive | Re-maturing | Alive | Re-maturing | Alive | Re-maturing |
| | | (%) | (%) | (%) | (%) | (%) | (%) | (%) | (%) |
| 2013 | 163 | 74 | 16 | 29 | 27 | - | - | - | - |
| | | 45.4 | 21.6 | 50 | 93.1 | | | | |
| 2014 | 149 | - | - | 32 | 2 | 6 | 5 | 1 | 1 |
| | | | | 21.5 | 6.3 | 20 | 83.3 | 100 | 100 |
| 2015 | 148 | - | - | - | - | 43 | 13 | 21 | 18 |
| | | | | | | 29.1 | 30.2 | 70 | 85.7 |
| 2016 | 165 | - | - | - | - | - | - | 30 | 12 |
| | | | | | | | | 18.2 | 40 |

Fish were reconditioned as described (Hatch et al. 2014) Methods: Long-term Reconditioning: Dworshak National Fish Hatchery: Dworshak Reconditioning Facility and Treatment. Fish were sampled at approximately 10 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.

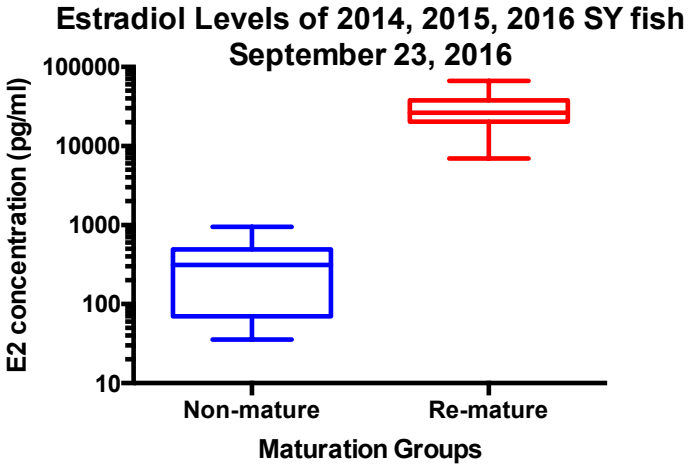
**Results**

Mortality of all fish in the hatchery study occurred on Nov 9, 2016 (Table 3B.2). The cause of the mortality appears to have been a malfunction of the formalin pump due to a power outage at the hatchery. Maturation status of the fish was known at the time of mortality, based on the previous sampling (Fig 3B.1). Full data was collected on mortalities, which will enable analysis of factors predicting maturation as a consecutive or skip spawner and factors predicting survival, and construction of a profile of the physiology of consecutive and skip spawners over time. However, data on reproductive performance of these fish at their second spawning was lost.

*Table 3B.2 Number of fish lost in November 2016 mortality. All hatchery origin kelts except 4 were found dead on the morning of November 9th, 2016. Remaining fish were then lethally sampled on Monday, November 14th. Complete data was taken off of fish mortalities including length, weight, fatmeter (%), gonad weight (ovaries with consistently un-ovulated eggs), and liver weight. Otoliths and blood were also collected.*

| | | | Data Lost at November 9th, 2016 Mortality | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Objective 1 | Objective 2 | | | Objective 3 | | | |
| | | | Maturation | Tracking Physiology | | | Reproductive Output | | | |
| | | | Spawn-Sept | 10 Week Periodic Sampling Points | | | Egg size, egg #, spawn date, E2, GSI – Fish | | | |
| | | | | Year | | | | | | |
| SY | Alive | Mature | | 1 | 2 | 3 | intake | consecutive | skip | skip x 2 |
| 2014 | 1 | 1 | 0 | 0 | 0 | 2 | 0 | 0/2 | 0/2 | 1 |
| 2015 | 21 | 18 | 0 | 0 | 2 | 5 | 0 | 0/12 | 18 | 3 |
| 2016 | 30 | 12 | 0 | 2 | 5 | - | 0 | 12 | 18 | - |

***Figure 3B.1:*** *Plasma estradiol levels in rematuring and non-rematuring 2014 -2016 spawn year hatchery origin kelts at late summer to fall sampling. N= 49 mature, 15 non-mature.*




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 10000-20000 pg*ml-$^{1}$ in rematuring fish. Significant elevations in plasma E2 levels occurred by July in consecutive spawners, and by April in skip spawners during the year before spawning (Fig 3B.2). Muscle lipid levels diverged by April to July. Specific growth rate in weight was greater in consecutive spawners than skip spawners over the first 10 week period after spawning, whereas both consecutive and skip spawners decreased in length over this time period.

***Figure 3B.2:*** *Profile Over Time – E2; Specific Growth Rate – Length, Weight; Muscle Lipid Level*

***3B.2a:*** *E2 levels of 2015 spawn year kelts tracked over time from initial spawning (Feb15) to repeat spawning (Feb16) for fish maturing in year one (consecutive spawners, red) or through maturation determination for fish that skipped spawning in 2016. Data were log transformed. Asterisks indicate differences between maturing and non-maturing fish at each time point, and letters track differences over time within each group (maturing - red, non-maturing - blue). A significant difference was first observed in July 2015 based on a Mann Whitney test for non-parametric data. All time-points were compared this way except for February, April, and September of 2015 where data was normally distributed and unpaired t-tests were used. Significant differences were found in April 2015 and September 2015. In 2016, significant differences were found at all time points using Mann Whitney test. N=3 for non-mature 2015 SY fish in 2016 (**** = p < 0.0001, ** = p <0.0). E2 levels were significantly elevated over levels at spawning in fish maturing in Sept 2015 and July 2016.*

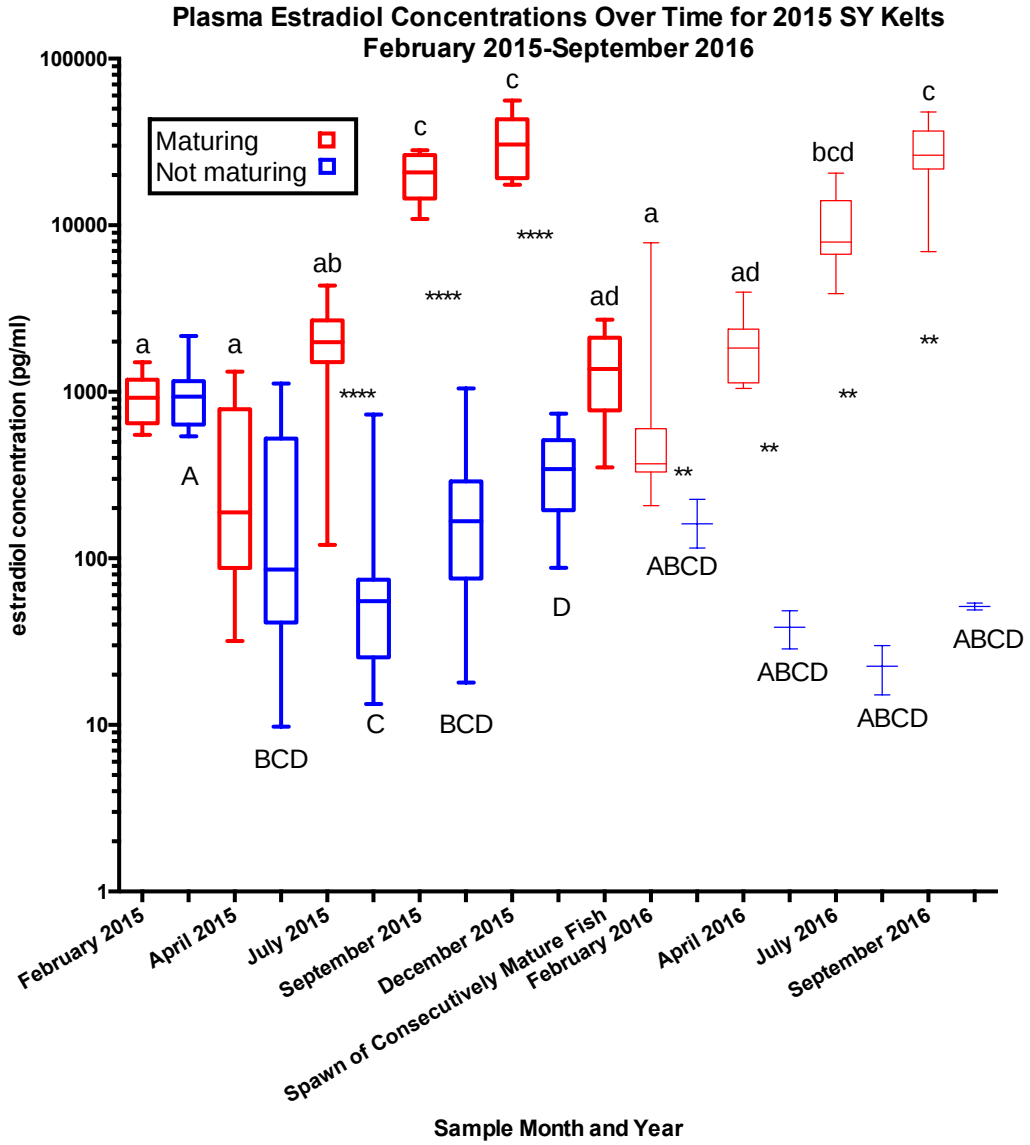


***Figure 3B.2b*** *E2 levels for 2016 spawn year (SY) kelts from initial spawning (Feb16) through maturation determination in September 2016. Data were log-transformed. Asterisks indicate significant difference between maturing and non-maturing fish at each time point, with significant differences occurring first in July based on unpaired t-tests. (**** p < 0.0001) Letters indicate difference over time within maturing and non-maturing fish. All levels were different within maturing fish based on 1-way ANOVA and Tukey's Multiple Comparison test. For non-maturing fish, all levels were significantly different from estradiol levels at spawning based on Kruskal-Wallis test and Dunn's Multiple Comparison.*

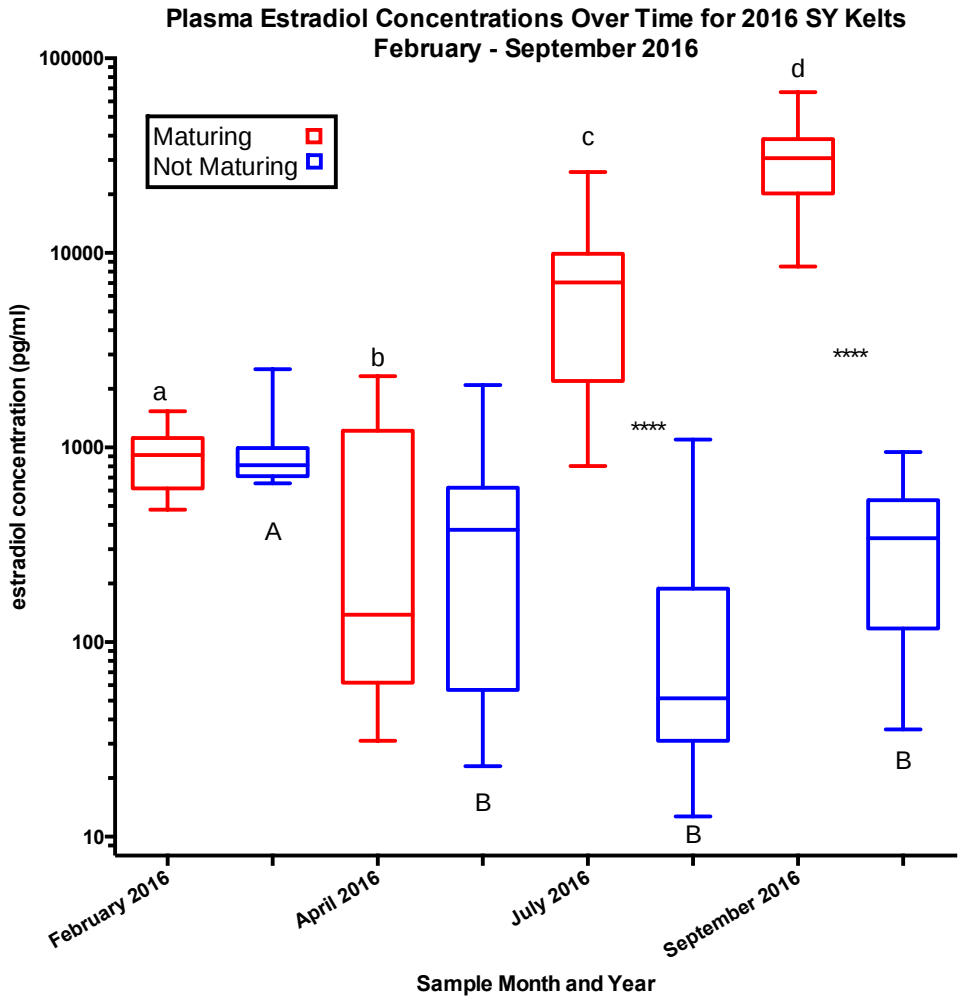


***Figure 3B.2c:*** *Muscle lipid levels over time in consecutive and skip repeat spawning reconditioned hatchery origin steelhead kelts of the spawn year 2015 (a) and 2016 (b). Data were arc sin square root transformed.*

a. SY 2015

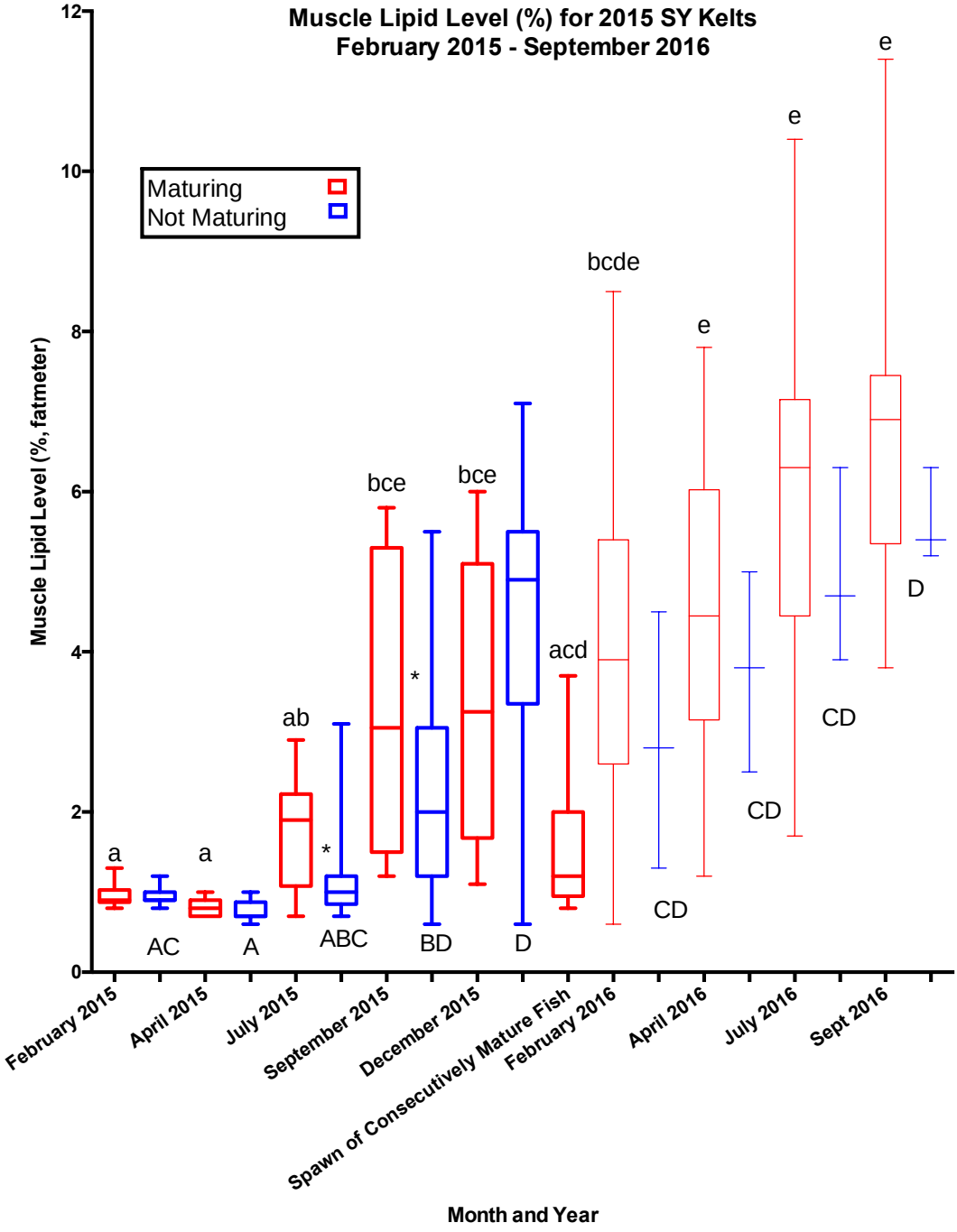
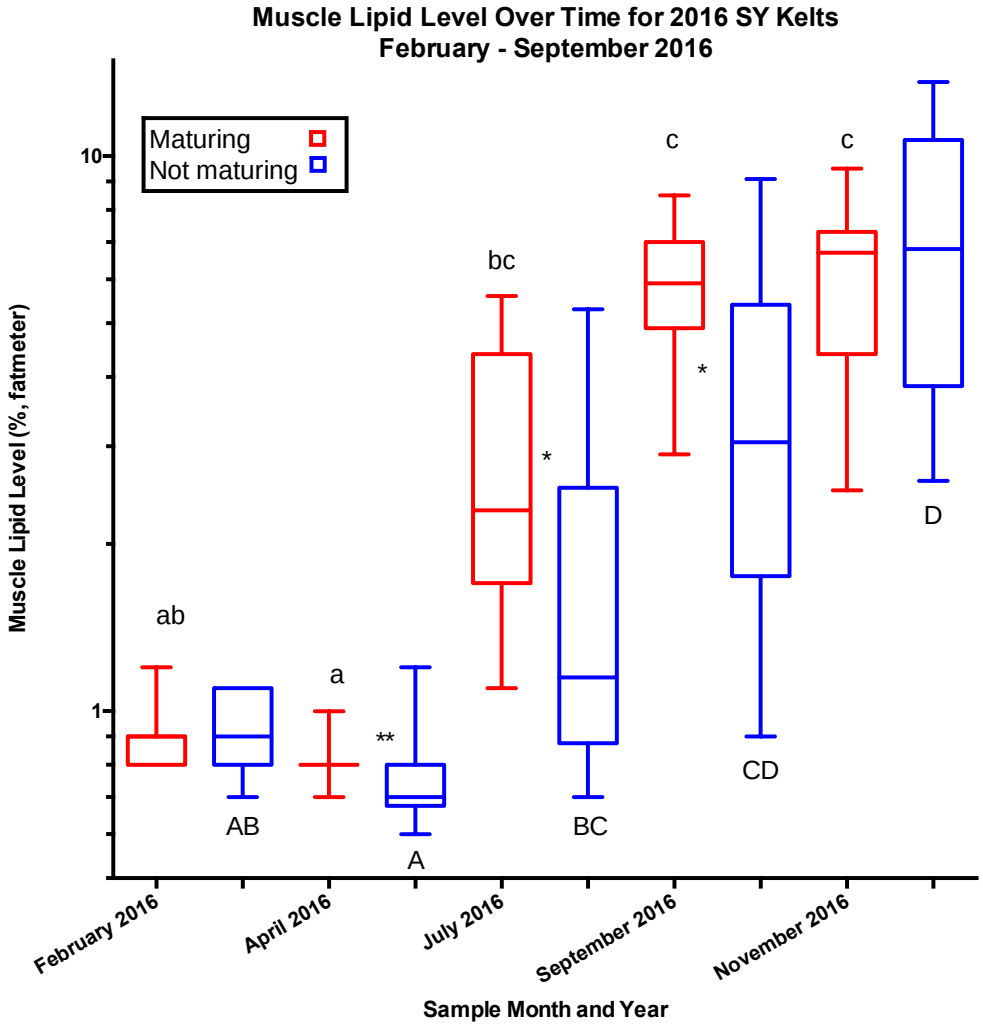
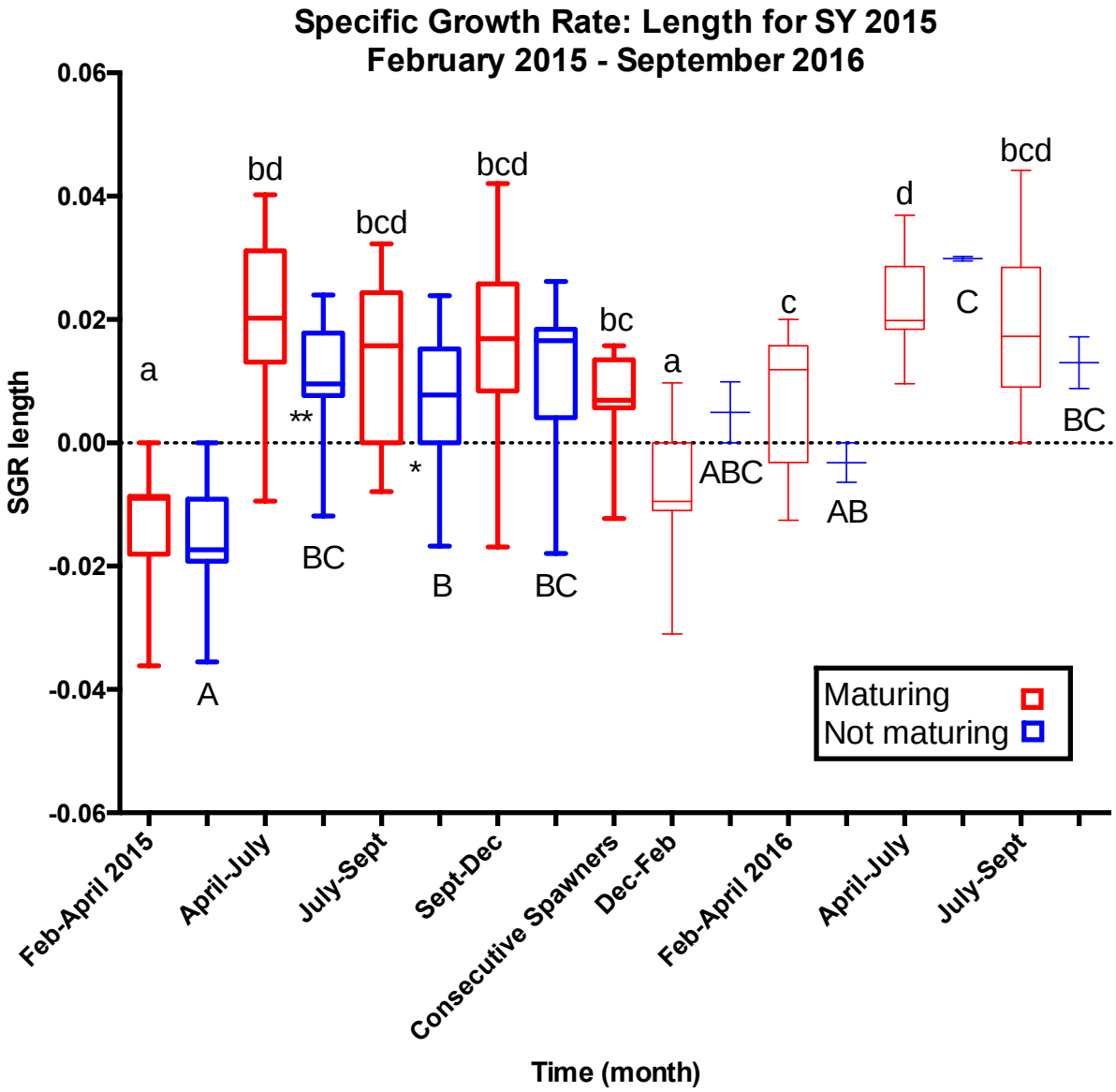


***Figure 3B.2d*** *SY 2016. Data were arc sin square root transformed. Asterisks indicated significant difference between maturing and non-maturing fish at each time point. Letters indicate significance over time within maturing or non-maturing fish. Levels are significantly different between maturing and non-maturing fish in April based on Mann Whitney test for non-parametric data (** p < 0.01). Levels are different in July and September based on Unpaired T-test (** P < 0.01). Muscle lipid levels are significantly elevated over intake level in September for maturing fish, significantly decreased in April for non-maturing fish, and significantly elevated in September for non-maturing fish based on Kruskal-Wallis test followed by Dunn's multiple comparison test for non-parametric data.*

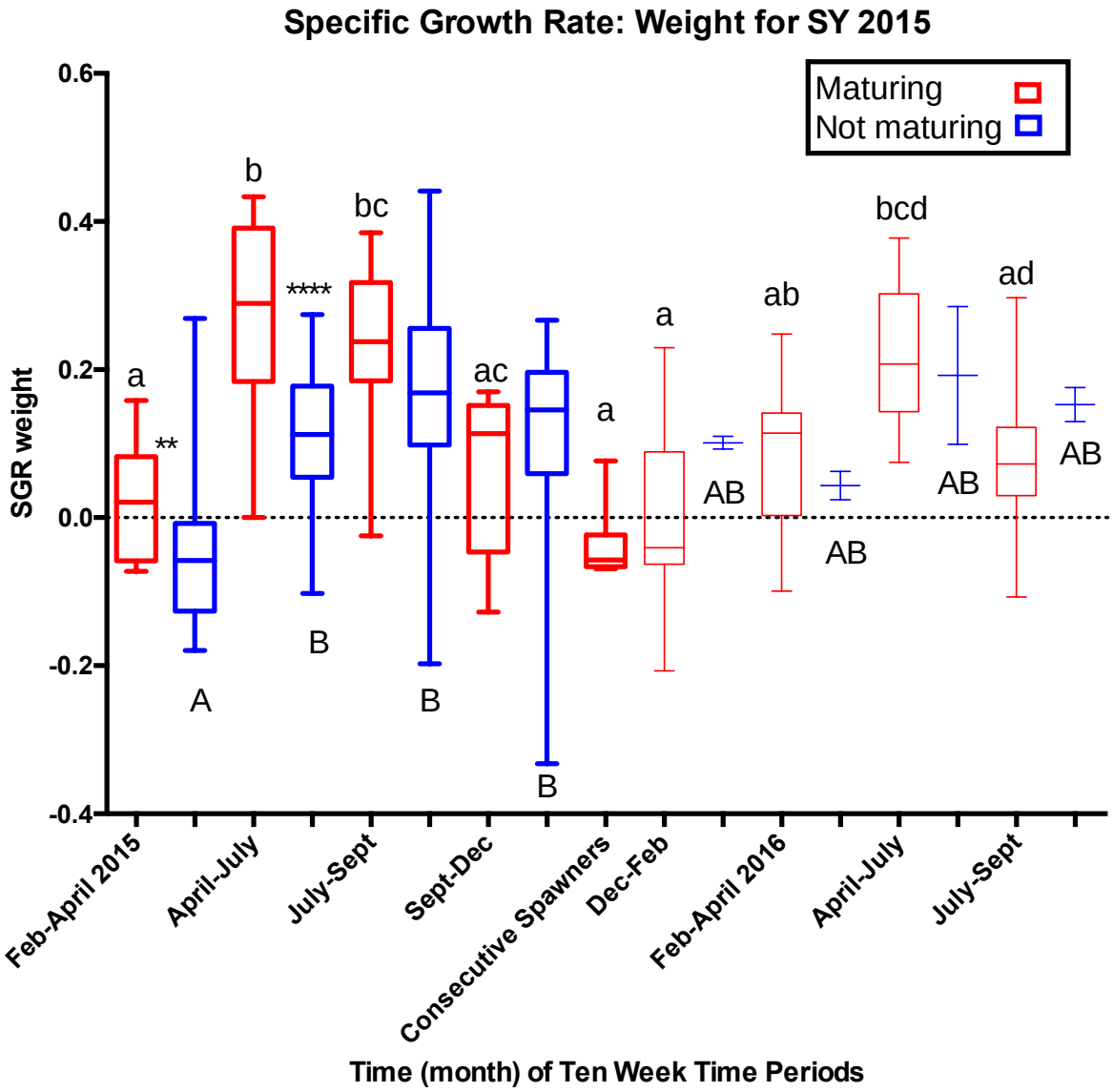


***3B.2e:*** *Specific Growth Rates (SGR) in length (a) and weight (b) over 20 months of reconditioning for 2015 SY fish (a, b) and 8 months of reconditioning for 2016 SY fish (c, d) in consecutive and skip repeat spawning reconditioned hatchery steelhead kelts from spawn year kelts. Samples were taken approximately every 10 weeks.*

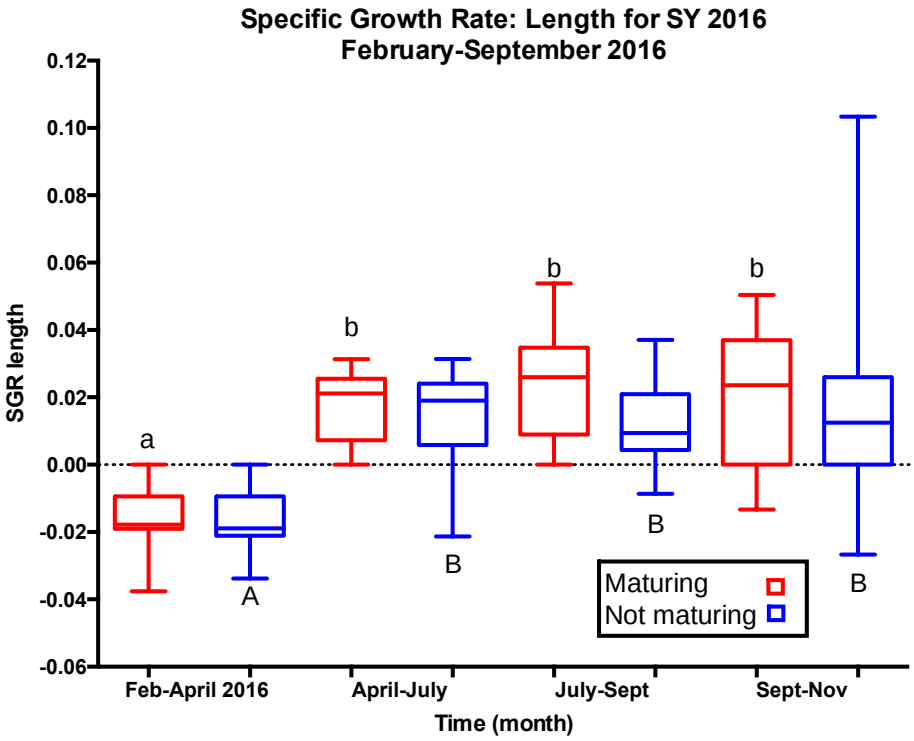
a. Letters indicate significant differences within maturing and non-maturing fish over time. Bars not sharing a letter differ significantly (One-way ANOVA followed by Tukey's test). Asterisks indicate significant differences between maturing and non-maturing fish within time points (** $p < 0.01$, * $p < 0.05$ based on un-paired t-test). Differences in growth rates in length between maturing and non-maturing fish were found for the period between April and July of 2015 and between July and September of 2015.



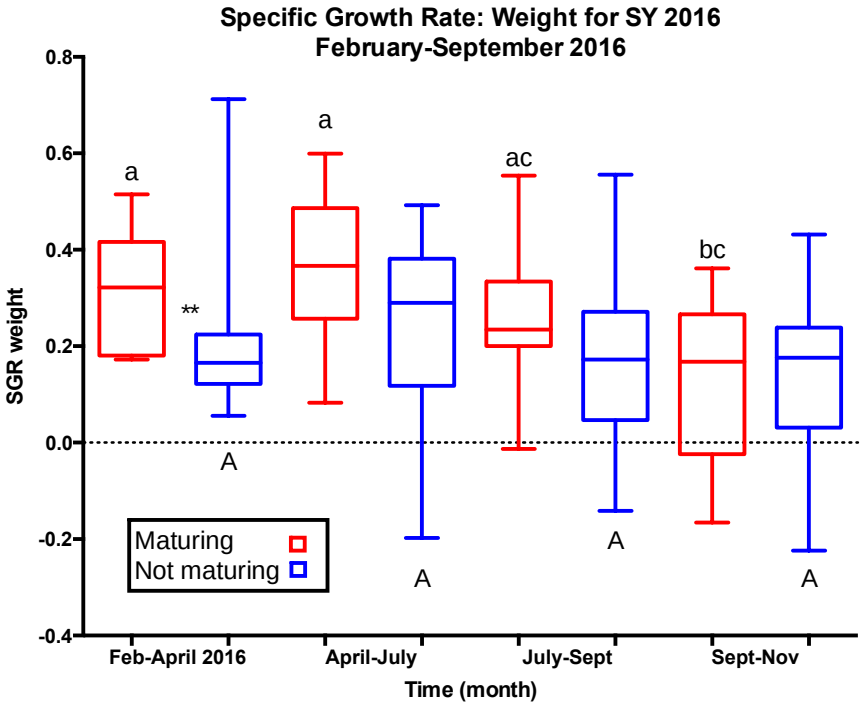
b. Letters indicate significant differences within maturing and non-maturing fish over time. Bars not sharing a letter differ significantly (Kruskal Wallis followed by Dunn's multiple comparison test, $p < 0.05$). Asterisks indicate significant differences between maturing and non-maturing fish within time points (** $p < 0.01$, **** $p < 0.0001$) based on un-paired t-test). Differences in growth rates in weight between maturing and non-maturing fish were found for the period between February and April of 2015 and between April and July of 2015.



c. Specific growth rate for length changes over time in SY 2016 fish.




d. Specific growth rate for weight changes over time in SY 2016 fish.



Statistical analysis of intake factors potentially predicting maturation trajectory is ongoing (Figs 3B.3, 3B.4, and 3B.5). Consecutive spawners tended to be longer than skip spawners, had more copepod parasites, and had greater plasma osmolality. Reproductive factors showed a pattern of greater reproductive effort at the initial spawning in consecutive versus skip spawners.

***Figure 3B.3:*** *Factors indicating size, condition, and homeostatic ability as predictors of maturation from SY 2015 and 2016. For parasite load, >10 is represented as 11.*

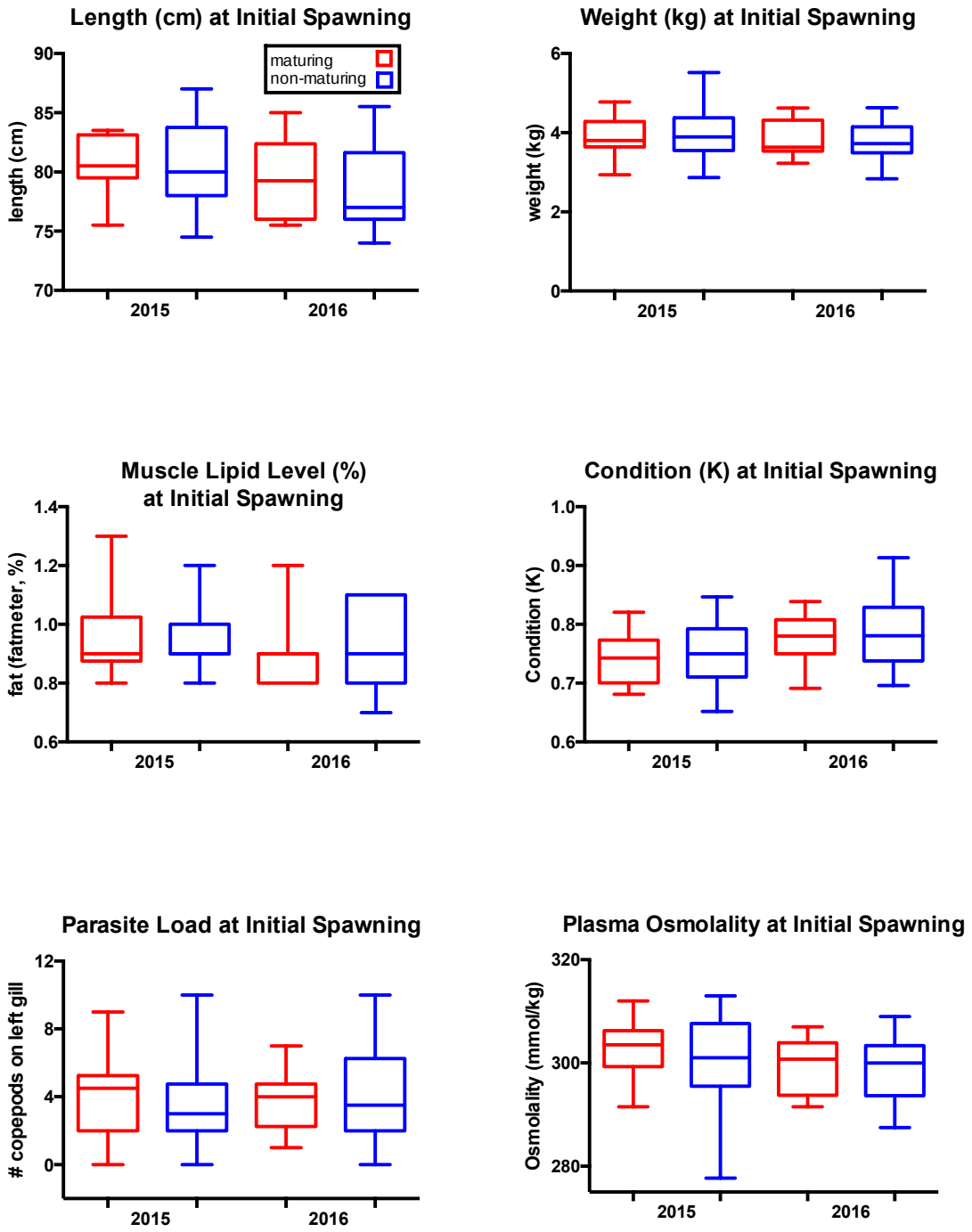
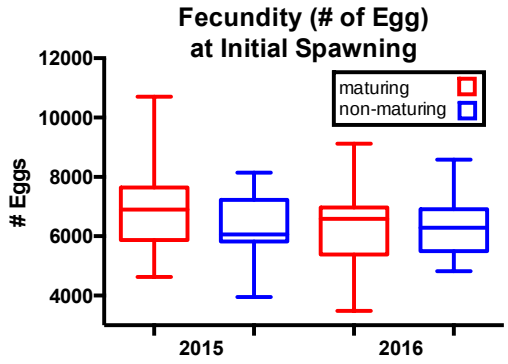
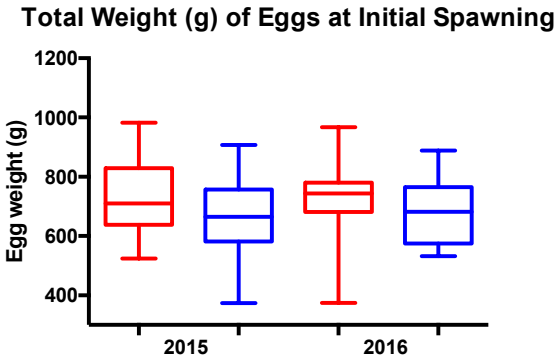
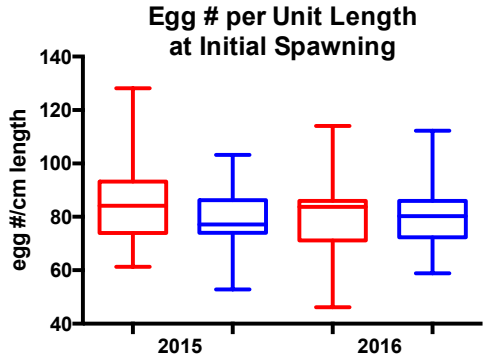
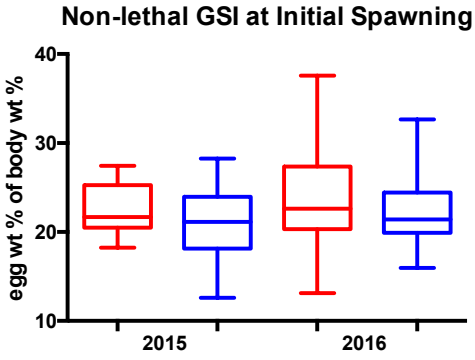


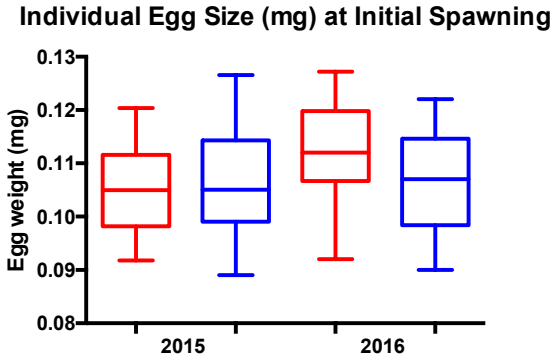
***Figure 3B.4:*** *Reproductive factors measured at initial spawning as maturation predictors, SY 2015-2016.*



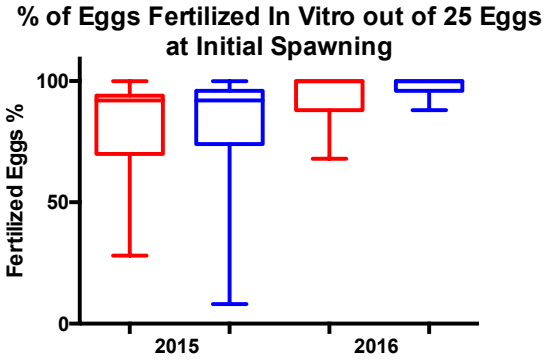
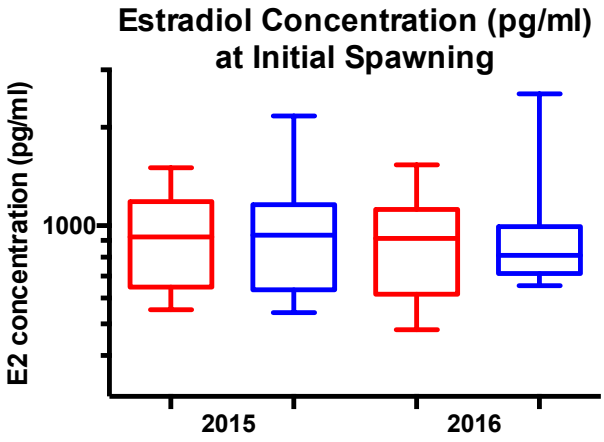
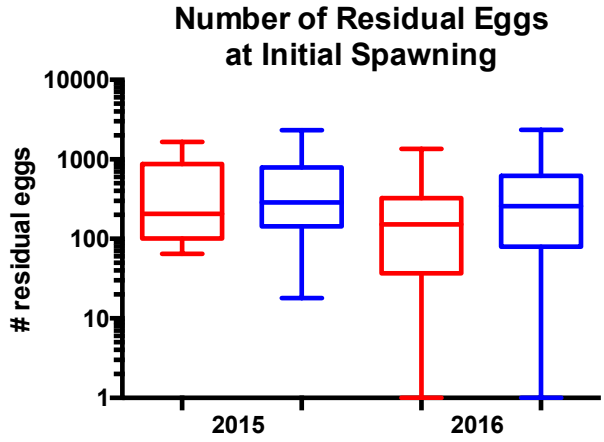
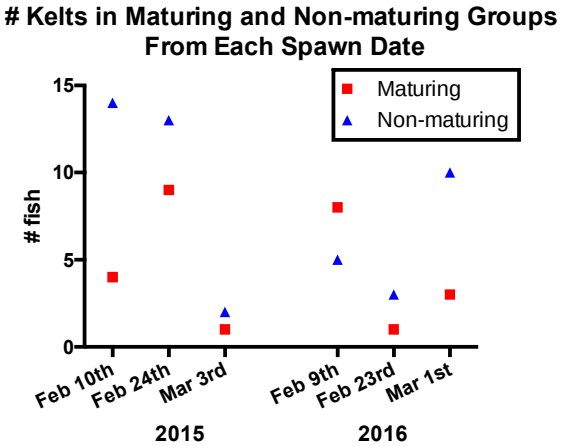



***Figure 3B.5:*** *Additional reproductive factors measured at initial spawning as predictors of maturation from SY 2015 and 2016.*



Statistical analysis of intake factors potentially predicting survival is ongoing (Figs 3B.6, 3B.7, and 3B.8). Fish that survived for 10 weeks tended to be shorter than mortalities, had fewer copepods, and had higher plasma osmolality. However, reproductive factors showed a pattern of reduced reproductive effort at the maiden spawning in survivors versus mortalities.

***Figure 3B.6:*** *Factors indicating size, condition, and homeostatic ability as predictors of survival from SY 2014, 2015, and 2016. For parasite load, a count of >10 is represented as 11.*

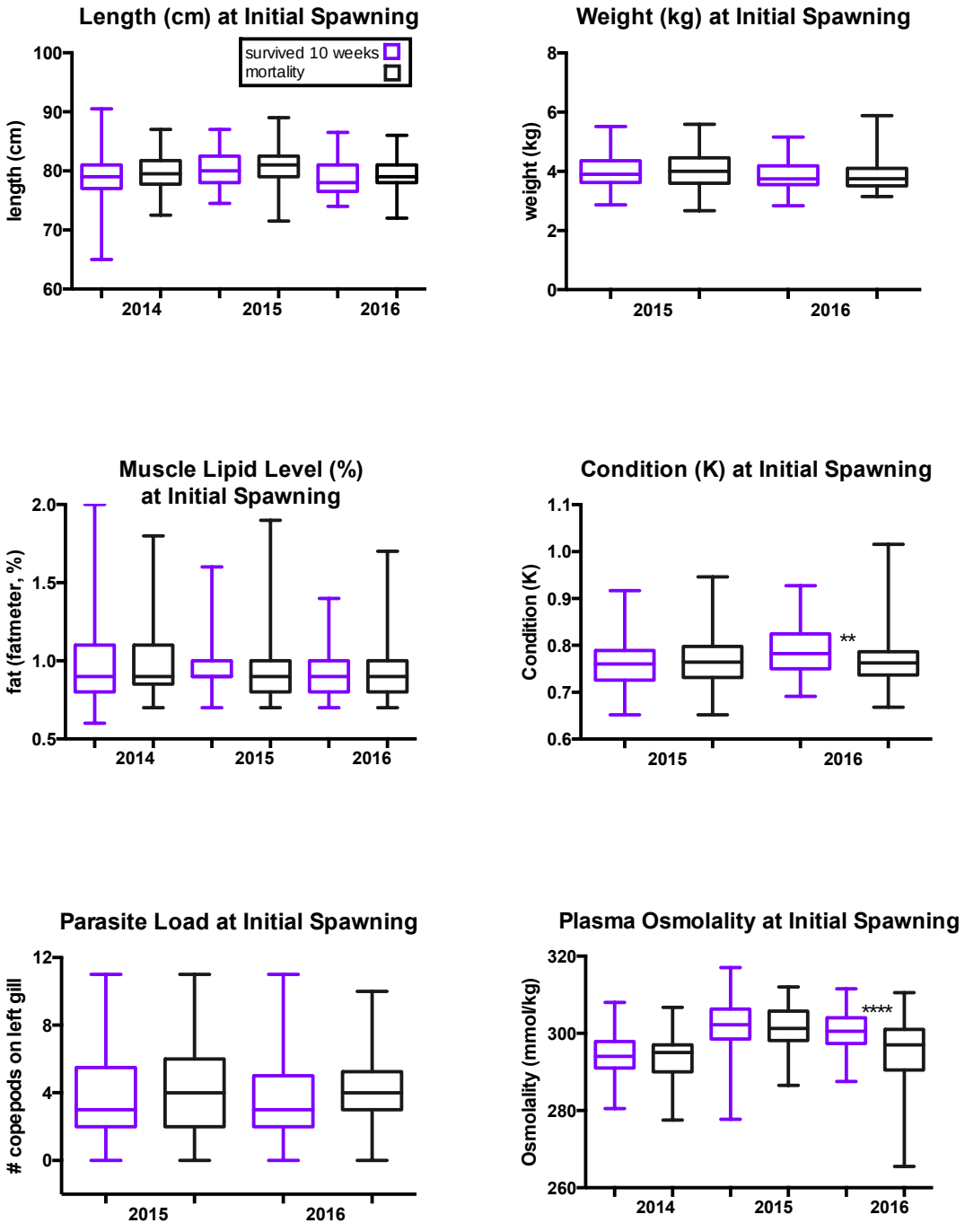


***Figure 3B.7:*** *Reproductive factors measured at initial spawning as survival predictors, SY 2014-2016.*

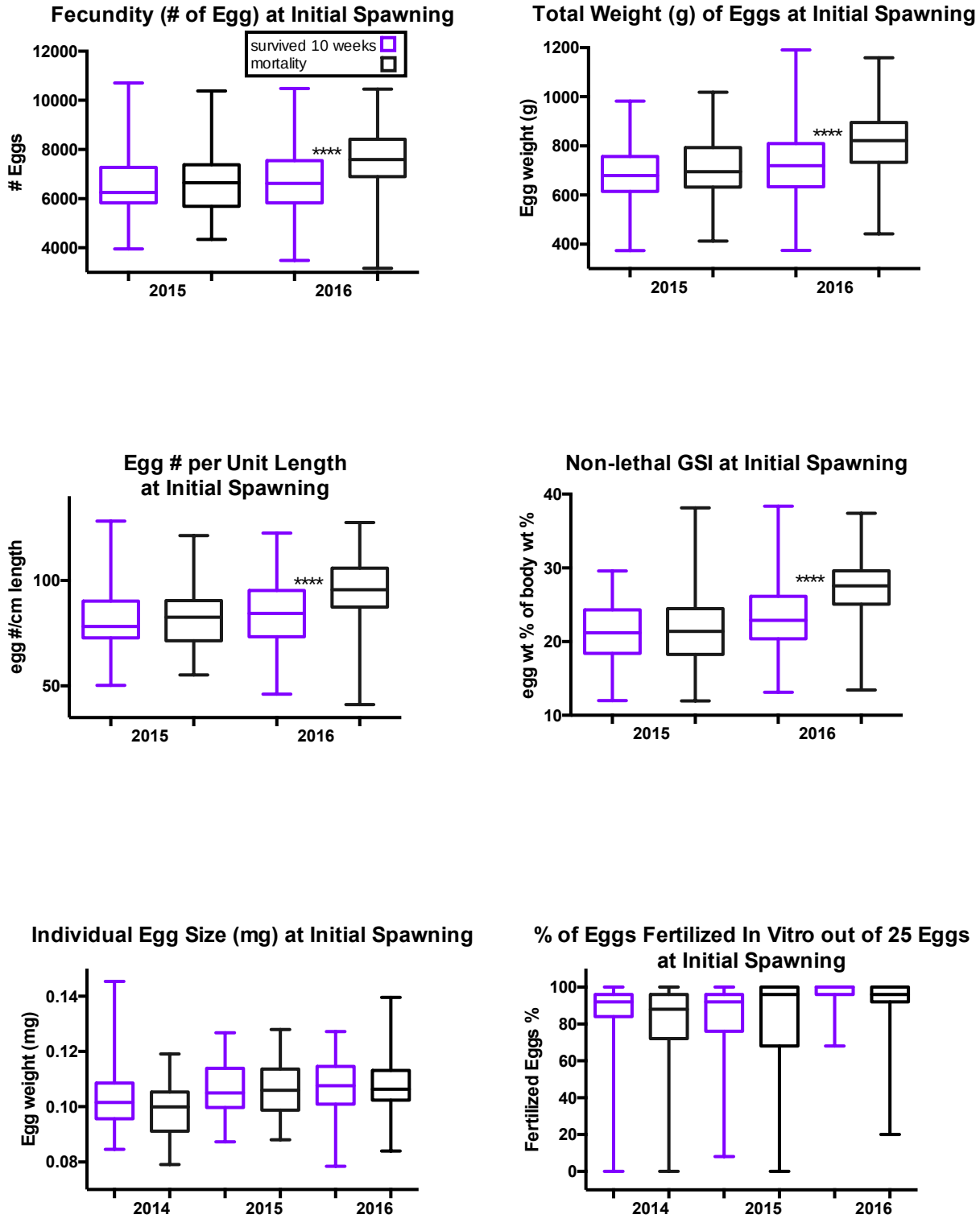
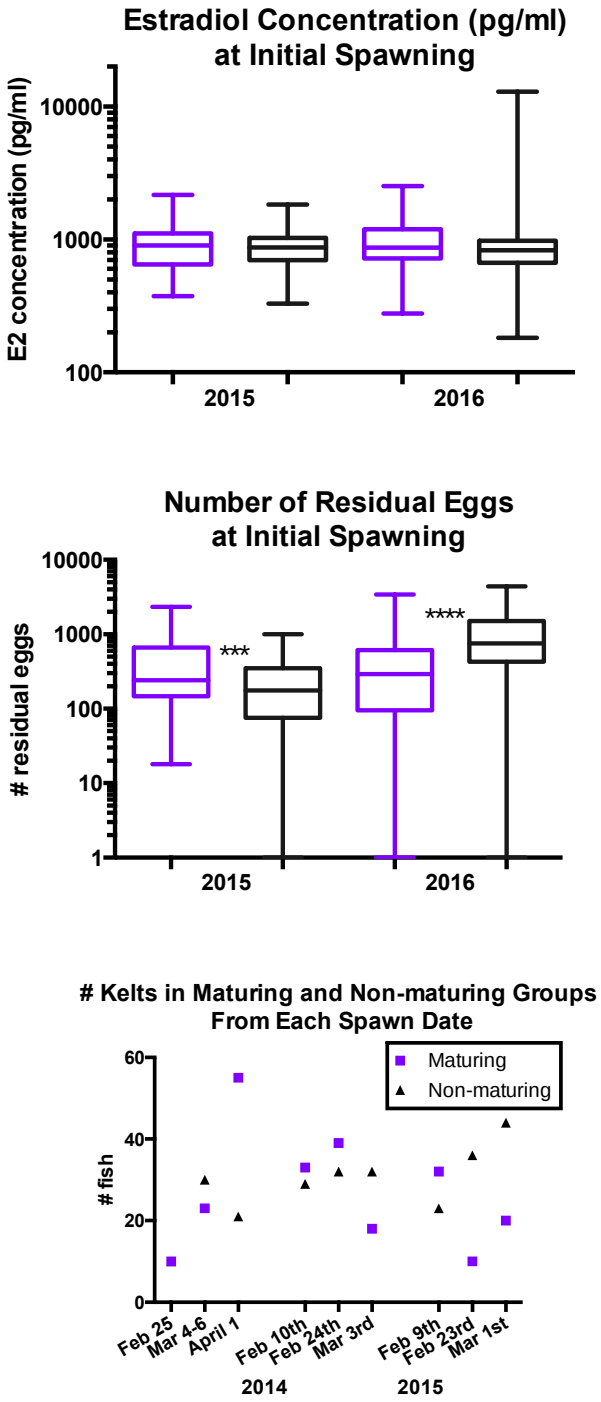
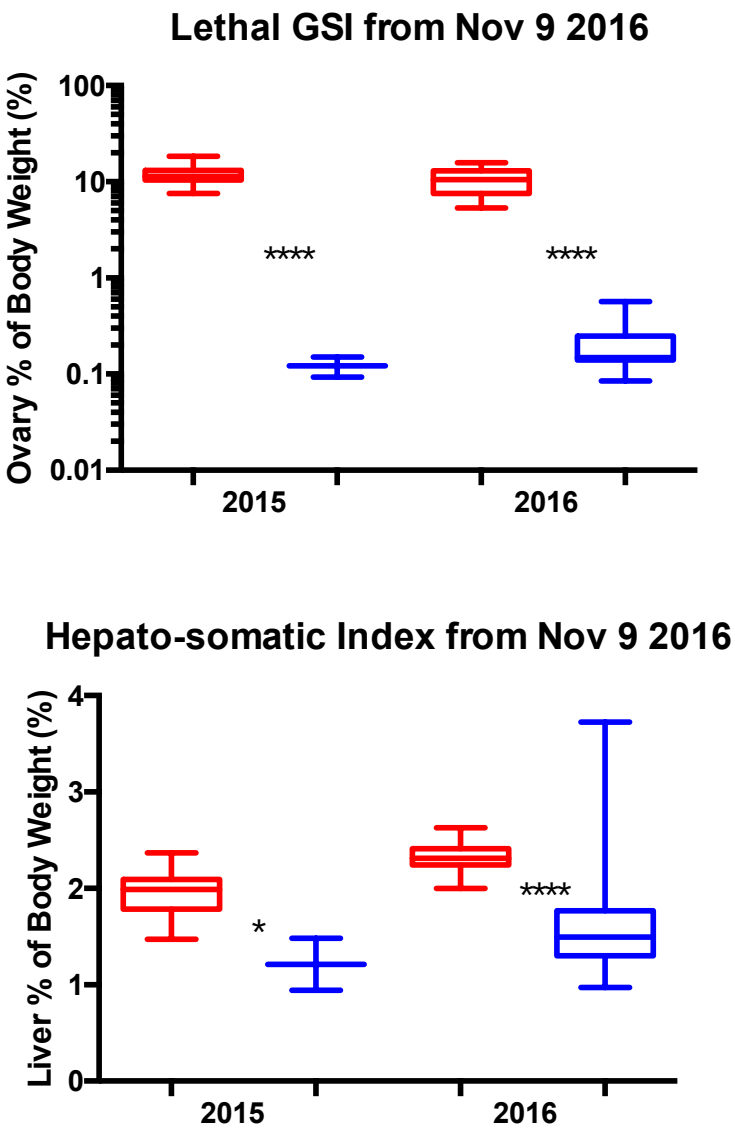


***Figure 3B.8:*** *Additional reproductive factors measured at initial spawning as predictors of survival from SY 2015 and 2016.*




Both GSI and hepato-somatic index (HSI) were significantly greater in maturing kelts at the Nov 9 2016 mortality event (Fig 3B.9).

***Figure 3B.9:*** *Lethal GSI and HSI in maturing and non-maturing kelts from SY 2015 and 2016.*




In a comparison of all fish spawning as maiden, consecutive, and skip spawners, egg weight and fecundity were greater in reconditioned consecutive and skip spawners versus maidens (Figs 3B.10, 3B.11). Fertilization success did not differ between maidens and consecutive spawners, but was decreased in skip spawners (Fig 3B.12). GSI was similar in all spawning groups (Fig 3B.13).

***Figure 3B.10:*** *Egg size in maiden, consecutive repeat spawning, and skip repeat spawning reconditioned steelhead kelts. Bars not sharing a letter differ significantly (ANOVA followed by Kruskal-Wallis test).*

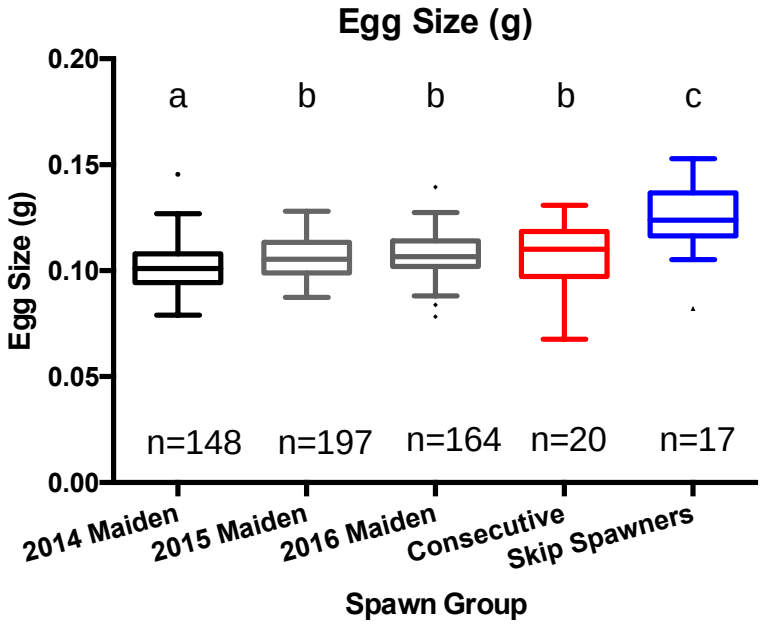



***Figure 3B.11:*** *Fecundity in maiden, consecutive repeat spawning, and skip repeat spawning reconditioned steelhead kelts. Bars not sharing a letter differ significantly (ANOVA followed by Kruskal-Wallis test).*

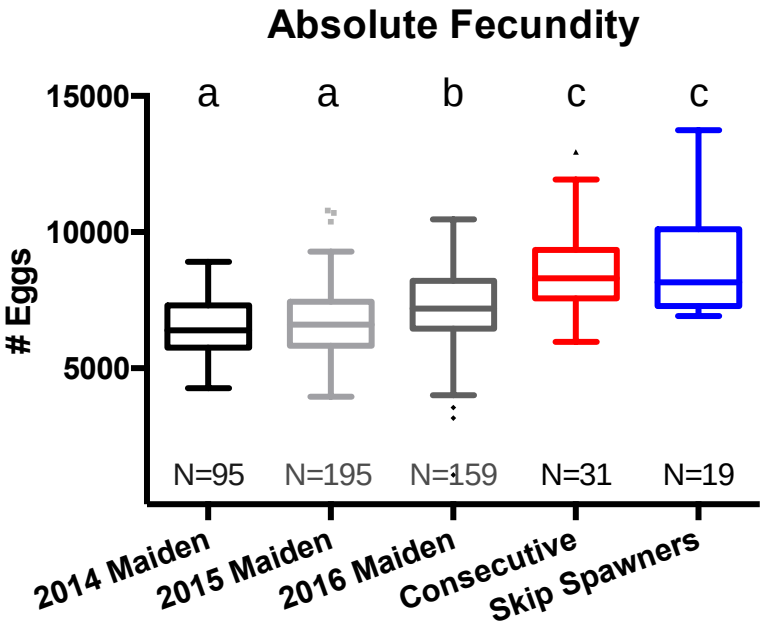



***Figure 3B.12:*** *Fertilization success in maiden, consecutive repeat spawning, and skip repeat spawning reconditioned steelhead kelts. Bars not sharing a letter differ significantly (ANOVA followed by Kruskal-Wallis test).*

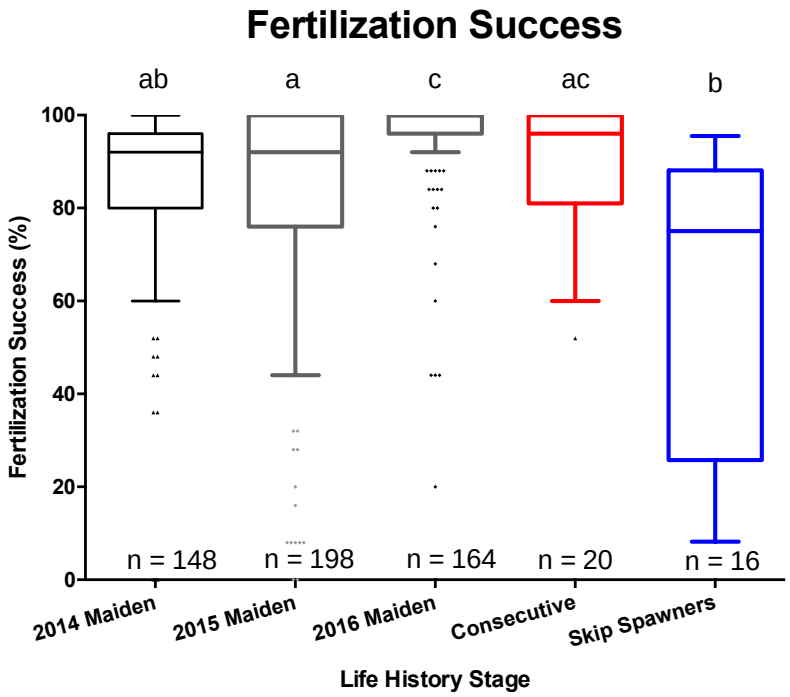
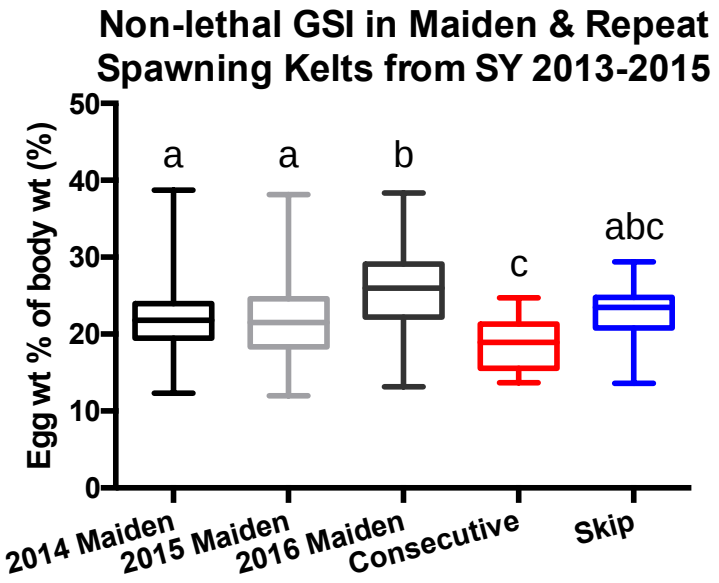



***Figure 3B.13:*** *Non-lethal GSI in maiden, consecutive repeat spawning, and skip repeat spawning reconditioned steelhead kelts. Bars not sharing a letter differ significantly (One-way ANOVA followed by Tukey's multiple comparison test).*




In a direct comparison of reproductive performance at the second spawning versus the maiden spawning in individual kelts using all available data, performance in terms of fecundity, total egg weight, fertilization success, and eggs per unit length was greater at the reconditioned kelt spawning for both consecutive and skip spawners (Fig 3B.14), whereas egg size was only greater in skip spawners, and GSI was slightly decreased in 2015 consecutive spawners. Skip spawning

kelts spawned a few weeks earlier than their maiden spawning, whereas consecutive spawners spawned at the same week (Fig 3B.15).

**Figure 3B.14:** Changes in reproductive performance from initial to repeat spawning for SY 2014-2015.

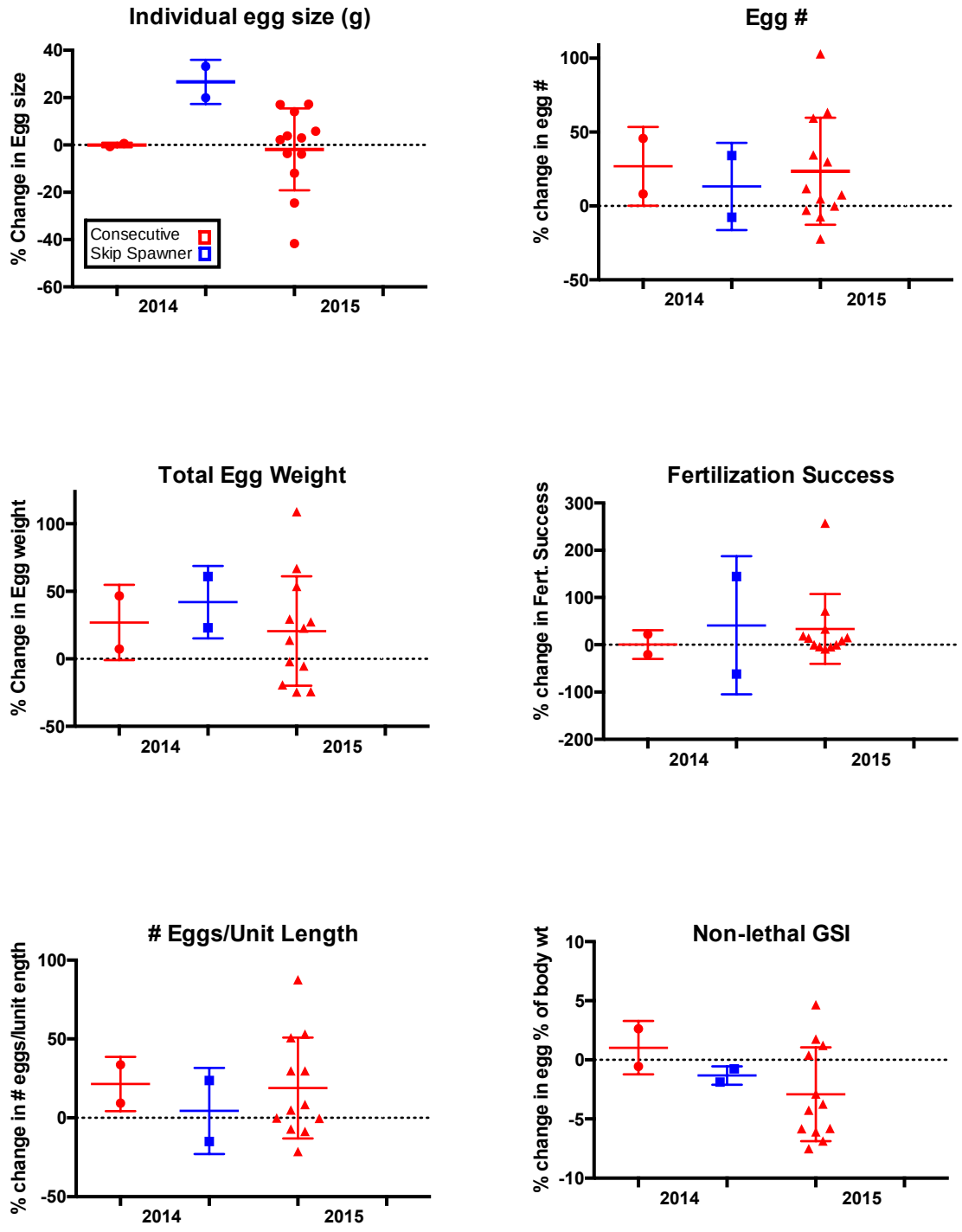
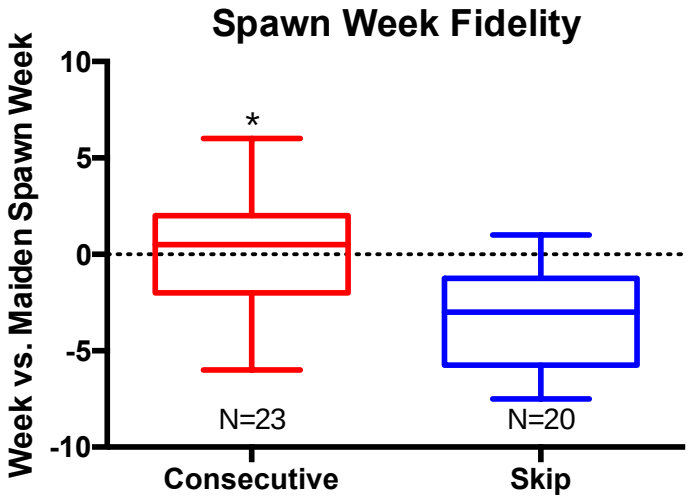


**Figure 3B.15:** Spawn week fidelity versus maiden spawning in consecutive and repeat spawning reconditioned hatchery steelhead kelts from 2013-2015.




**Lower Granite Dam Maiden Sampling**

Measurement of plasma factors in Lower Granite Dam maiden samples is ongoing, and genetic stock identification for fish collected in fall 2015 is in progress.

**Discussion**

Survival of DNFH hatchery kelts has ranged from approximately 20% to 50%, which is generally less than survival rates for wild kelts. This may be due to stresses associated with artificial spawning of the hatchery kelts, or selection against iteroparity by lethal spawning practices at the hatchery. In addition, wild kelts are collected post spawn, and only good condition fish are collected for reconditioning, whereas all hatchery fish spawned on a given day are reconditioned. Maturation rates for DNFH hatchery kelts have ranged from approximately 5% to 40%, with the highest rate yet found (40%) in 2016. The range in maturation rates is probably due to a combination of fish condition at spawning and fish husbandry conditions. 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. The few fish that do not remature after being held over are often found to be individuals with a disease or injury.

Plasma E2 levels were elevated in rematuring consecutive spawners by July in both 2015 and 2016, indicating that maturation decisions are made prior to this time. Interestingly, growth rate in weight was consistently elevated in consecutive spawners over the first 10 weeks of reconditioning, suggesting that consecutive spawners feed more actively during this time

period. This supports our previous finding in rainbow trout that feed restriction results in a decrease in plasma E2 levels within 10 weeks after spawning (Caldwell refs), and suggests that maturation decisions are made within a 10 week window after spawning in both the rainbow trout and hatchery steelhead model. We are planning a study to test the effects of feed restriction during the 10 weeks after spawning on maturation in DNFH kelts during the 2017 season.

In 2015 SY skip spawners, plasma E2 increased in April to levels similar to those found in July in consecutive spawners, suggesting that seasonal increases are delayed due to energy limitation in the consecutive spawners.

A statistical analysis of factors predicting maturation in DNFH kelts will need to be completed before conclusions can be drawn. However, an initial examination of the results shows some interesting patterns. Consecutive spawners were longer at the maiden spawning than skip spawners, which might be because longer fish have greater energy reserves (Penney and Moffitt 2014b). The finding that consecutive spawners had more copepods might just be because they are larger fish. More interestingly, consecutive spawners appeared to have a consistent pattern of greater reproductive investment at the maiden spawning in terms of fecundity, total egg weight, eggs per unit length, and GSI. This suggests that both reproductive effort at the maiden spawning and subsequent consecutive maturation may be positively related to physiological condition at spawning.

Similar to maturation, we are at an early stage in analysis of factors predicting survival for the first 10 weeks in DNFH kelts. Greater survival in smaller fish is similar to findings in Prosser kelts (Hatch, et al. 2013b). Interestingly, plasma osmolarity was greater in survivors in 2015 and 2016, suggesting that loss of osmoregulatory ability may be an early sign of impending mortality in steelhead kelts, as found in sockeye (Jeffries, et al. 2011). There appeared to be a negative relationship between reproductive investment at the maiden spawning and survival, which would support the hypothesis that kelts that allocate less energy to ovarian development and retain more somatic energy reserves after spawning survive at higher rates.

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 decrease in fertilization success in skip spawners was only seen in 2014 SY fish. A number of these fish were observed to have infections in the body cavity at their second spawning, which may account for the reduced fertilization success. Unfortunately, quantification of the reproductive performance of reconditioned kelts at their second spawning was impacted by the loss of approximately 50% of our anticipated data points due to the November 2016 mortality.

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

There is a strong relationship between dietary lipid levels and carcass lipid levels in salmonids (Halver 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 as a palatability enhancer (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 frozen pellet that incorporated the artemia 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 and 2016 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). In 2015, tanks S1, S3, and C2 were randomly assigned to the USDA semi-moist diet (Appendix A1.c Hatch et al. 2016), and the rest of the tanks were fed the standard diet. Tanks were identified incorrectly in

our previous report due to an error in the database; this has been corrected in the current report. In 2016, tanks S2 and S4 were fed the USDA semi-moist diet, and the rest of the tanks were fed the standard diet. Fish were stocked into the S tanks on 4/13/2015 and 4/14/2015, and 5/2/2016 and 5/9/2016, so that all fish were fed the USDA and Standard diets over the same period of time, and potential bias due to spawn timing and spawning subpopulation were minimized in these tanks. All fish were treated with oxytetracycline and emamectin at intake. Fish were fed ad libitum with at least 3 feedings per day. All fish were fed krill for an initial period of approximately one month before pellets were introduced. Fish were transitioned from krill to pellets by feeding a mixture of the two following standard procedures established at Prosser. Mortality was recorded daily. Only female fish being reconditioned for the first time were included in the analysis (i.e. no males, fish held over the winter, or recaptured fish). Only fish positively identified by PIT tag from intake to exit (mortality or release) were included in the analysis. Muscle lipid levels were measured with the Fatmeter, and specific growth rate in weight (SGRW) was calculated as $\frac{\ell n\left(mass_2/mass_1\right)}{daysbetweenmeasuremets} \text{ x } 100$. Detections of fish after release were obtained by queries of the PTAGIS database.

**Results**

In 2015, tank C2 went off of and back onto the USDA diet several times during the season when supplies of the experimental diet ran low. This tank, and the pooled other C tanks, are included in the data to illustrate the consistent pattern; however, statistical analysis was restricted to the S tanks.

In both 2015 and 2016, there was a consistent trend toward lower condition factor, muscle lipid level, and specific growth rate in weight over the reconditioning period in fish fed the USDA diet (Figs C3.1 and C3.2). Results were consistent for each diet between the larger C tanks and the smaller S tanks. In 2015, no pattern in maturation rate was evident between the diets; however, almost all tanks exhibited very high maturation rates in 2015. In 2016, a trend toward a decreased maturation rate was evident in the tanks fed the USDA diet. In 2015, fish fed the USDA diet had slightly lower rematuring estradiol levels at the fall sampling versus fish fed BioDiet pellets, whereas in 2016 fish fed the USDA diet had slightly higher rematuring estradiol levels.

In an analysis of tank average levels for each metric (S tanks only), pooling the 2015 and 2016 data, fish fed the USDA diet had significantly decreased condition factor, muscle lipid level, and specific growth rate in weight (Table C3.1).

*Table C3.1. Analysis of the effects of diet on fish growth and energy reserves metrics, using tank averages for S-tanks used in the experiment in both years.*

| Response | DF | N | F | p | Mean Standard | Mean USDA |
|---|---|---|---|---|---|---|
| K | 1 | 4 | 15.6443 | 0.0075 | 0.980 | 0.886 |
| Fatmeter | 1 | 4 | 10.1414 | 0.0190 | 4.240 | 2.585 |
| SGRW | 1 | 4 | 6.885 | 0.0394 | 0.259 | 0.156 |

**Discussion**

The trial conducted in 2015 was repeated in 2016 due to an error in notation in the database indicating which tanks were fed the USDA diet. After correcting this error, the results of the two years of diet trials are consistent, and indicate that the USDA semi-moist diet has a negative effect on fish performance compared to the standard BioDiet Brood pellets that have been used in the project. The decreases in growth rate and muscle lipid level are especially concerning, since both of these metrics are positively related to rematuration (Pierce, et al. 2016). A decrease in maturation rate was evident in the tanks fed the USDA diet in 2016.

The reasons for decreased performance with the semi-moist are likely due to a limitation on the amount of lipid that can be incorporated into this type of diet. Semi-moist diets have been reported to enhance growth performance versus dry diets in salmonids, which may be due to greater palatability (Ham, et al. 2015). Project personnel reported that fish responded well to the semi-moist diet, suggesting that palatability was not an issue. However, the semi-moist diet contained a rather low amount of lipid (12%, Appendix A1.C), compared to the standard BioDiet Brood pellets (20%). Lipid inclusion in the semi-moist diet was the maximum that can be incorporated into this type of diet (R. Barrows, personal communication). In comparison, typical semi-moist diet formulations such as the Oregon Moist Pellet contain 6-7% lipid (Hardy and Barrows 2002). During migration and spawning, kelt steelhead deplete nearly all of their stored lipids (Penney and Moffitt 2014, 2015). Lipid reserves play an important role in maturation decisions in salmonids (Campbell, et al. 2006; Kendall, et al. 2015), and muscle lipid levels increase dynamically during the early stages of reconditioning and then decrease during ovarian development in steelhead kelts (Section C2). Therefore, restoration of lipid reserves is of paramount importance in kelt reconditioning. Unfortunately, the limitation on lipid content in semi-moist diets may mean that this type of diet is not suitable for kelt reconditioning, in spite of superior palatability. In addition, semi-moist diets have lower energy density than dry pellets, due in part to the incorporation of approximately 30% water in the pellet. It is possible that this resulted in decreased energy consumption due to stomach fullness.

Consistent with the present results, a previous trial using a different semi-moist diet for steelhead kelt reconditioning also did not show improved performance compared to a different commercial broodstock diet used at the time (Hatch, et al. 2003). These studies suggest that semi-moist diets are not optimal for kelt reconditioning. Further trials of diets for kelt reconditioning should use diet formulations that can incorporate higher amounts (20% or

greater) of high quality marine lipids, along with palatability enhancers, a sink rate adjusted to match that of krill, and a nutrient profile to support recovery from spawning and ovarian development.

Figure C3.1. Effects of diet on condition and growth metrics in 2015. Tanks fed the standard BioDiet Brood pellets are indicated in blue, and tanks fed the semi-moist USDA diet are indicated in green.

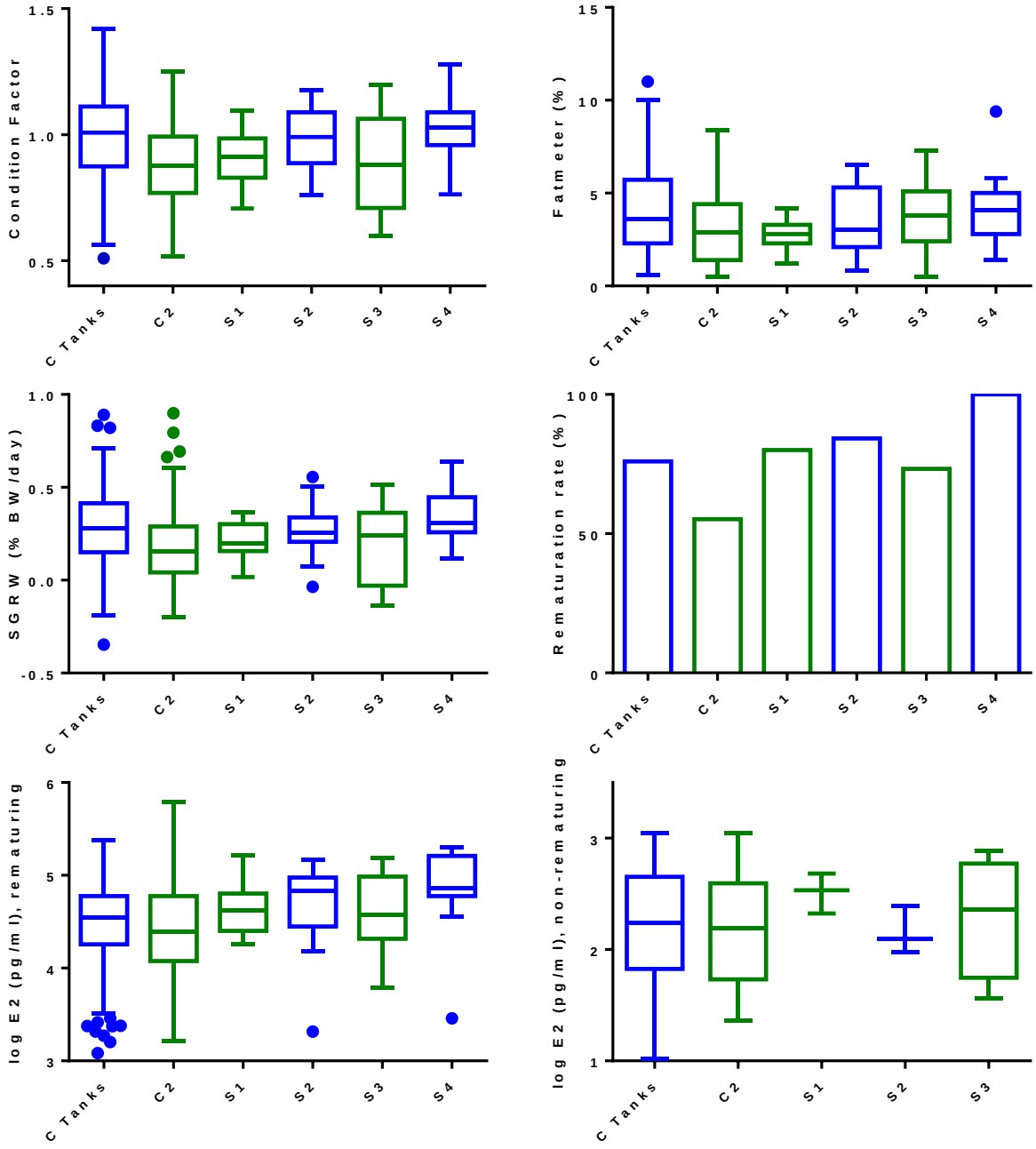
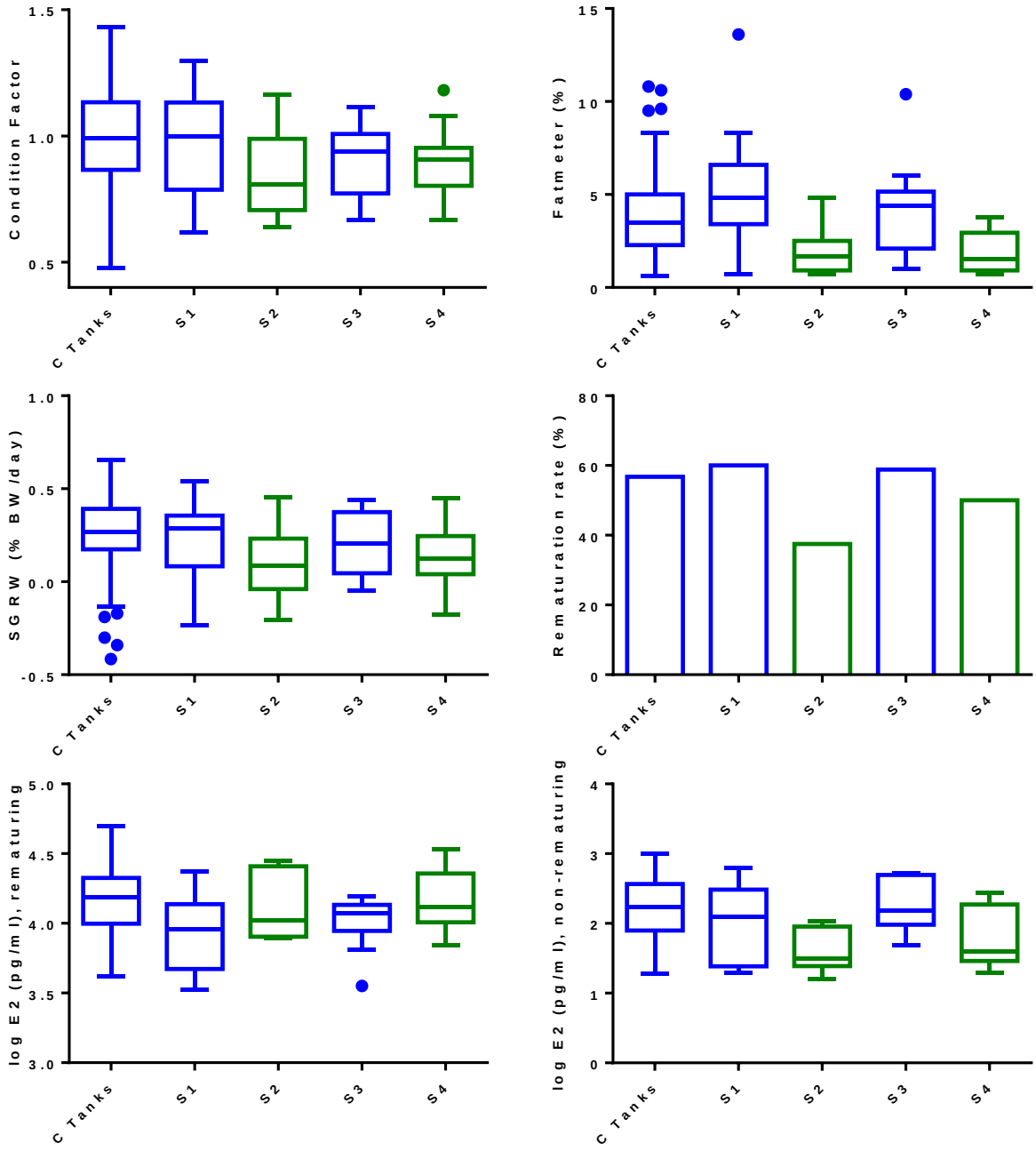


Figure C3.2. Effects of diet on condition and growth metrics in 2016. Tanks fed the standard BioDiet Brood pellets are indicated in blue, and tanks fed the semi-moist USDA diet are indicated in green.



# Section 3.D: Developing tools to assess growth/reproduction interactions in steelhead kelts: establishment and validation of assays for plasma insulin-like growth factor-1 and growth hormone and evaluation of the effect of ghrelin and GH administration in rainbow trout

## Introduction

Growth and reproduction interact in steelhead kelts and other fishes (Reinecke 2010b; Taranger, et al. 2010). The principal physiological system that regulates growth in fishes, as in other vertebrates, is the growth hormone (GH)/insulin-like growth factor-I (IGF-I) endocrine axis. Pituitary GH stimulates the liver to produce IGF-I, which mediates the growth stimulatory effects of GH (Moriyama, et al. 2000; Reinecke 2010a; Wood, et al. 2005). In addition, GH stimulates appetite and immune system function (Bjornsson 1997; Devlin, et al. 1994; Yada 2007), and enhances the mobilization and metabolism of stored lipids (Bjornsson, et al. 2002; Sheridan 1988). The prolonged fast and energetically demanding migration that steelhead undertake before spawning would be expected to result in profound changes in the GH/IGF axis. Strong increases in GH occur during fasting, whereas fasting decreases plasma IGF-1 level and suppresses anabolic growth (Pierce, et al. 2005). These seemingly paradoxical changes occur because the liver becomes resistant to the effects of GH during fasting (Gray, et al. 1992), and may be adaptive insofar as increased GH stimulates mobilization of stored energy, while decreased IGF-I reduces investment in anabolic growth. When fish begin feeding again after spawning, these changes are reversed, and growth resumes (Gabillard, et al. 2006). Changes in the GH/IGF-1 system are hypothesized to play a role in the gating of the reproductive endocrine axis. Plasma IGF-I increases several months before increases in plasma steroids are detected in maturing rainbow trout (Taylor, et al. 2008), suggesting that elevations in IGF-1 may provide a signal to the reproductive endocrine axis that energy reserves are sufficient to initiate maturation. Consistent with this idea, IGF-1 has been found to enhance the secretion of pituitary FSH (Baker, et al. 2000; Luckenbach, et al. 2010). Increases in FSH approximately one year before spawning are thought to be the initial signal to the ovary to begin development (Campbell et al. 2006; Pankhurst 2008; Wootton and Smith 2015). In order to track recovery from spawning and the effect of refeeding on reproductive decisions, we would like to be able to measure plasma levels of GH and IGF-1 in steelhead kelts.

Establishment of assays for plasma GH and IGF-1 require biological validation, which involves showing that levels change as expected based on established regulatory interactions. GH stimulates liver IGF-1 gene expression and increases plasma IGF-1 levels, so the IGF-1 response to GH treatment is appropriate as biological validation for an IGF-1 assay. The stomach hormone ghrelin strongly stimulates secretion of GH by the pituitary in fishes as in mammals. Ghrelin is highly conserved within vertebrates, and commercially available mammalian ghrelins have been shown to be effective at stimulating GH secretion in several fish species. Therefore, we will use ghrelin administration as biological validation for our GH assay. We can use existing

samples for part of this validation. In mammals, ghrelin strongly stimulates appetite (Kojima and Kangawa 2005); however, in fishes, data on the effect of ghrelin on appetite are mixed, showing both increased and decreased feed consumption (Jonsson, et al. 2007; Jonsson, et al. 2010; Riley, et al. 2005; Shepherd, et al. 2007; Unniappan and Peter 2004, 2005). In a previous experiment, we explored whether long term ghrelin or GH administration would stimulate appetite in rainbow trout, and potentially in steelhead kelts (Branstetter, et al. 2010). To supplement this long-term administration study, we are conducting an experiment using acute administration of ghrelin and GH.

## Methods

### Assay Development and Validation

#### *IGF-I Assay Development and Validation*

Previously, work was done to develop a radioimmunnoassay for IGF-I; however, this required the use of radioactivity and the subsequent reliance on an external laboratory for use of their equipment and radiation license. In the spring of 2016, we acquired a plate reader capable of reading time-resolved fluorescence (TRF, Perkin Elmer Victor X4), which enabled us to develop assays for protein hormones such as IGF-1 that do not require the use of radioactive tracers. We began work on the development of a TRF assay for IGF-I in steelhead plasma his year. Recombinant barramundi IGF-I (rbIGF-I) for tracer and standard and anti-rbIGF-I primary antibody were obtained from GroPep (GroPep.com, Brisbane, Australia). These reagents have been validated for use in rainbow trout in a TRF assay (Small 2005). The rbIGF-I was sent for custom labeling with europium $N^1$-(p-isothiocyanatobenzyl)-diethylenetriamine-$N^1$,$N^2$,$N^3$,$N^3$-tetraacetic acid (DTTA) chelate to Perkin Elmer's custom labeling laboratory (Perkin Elmer, Boston, MA). Europium DTTA chelate-labeled proteins and peptides are used with the DELFIA system reagents and a TRF capable plate reader in a TRF immunoassay.

#### *Plasma Growth Hormone Assay*

A commercially available salmonid growth hormone enzyme-linked immunosorbent assay (ELISA) was tested for determination of GH concentrations in plasma samples (Salmon somatotropin ELISA kit, catalog number MBS288370, MyBioSource, San Diego, CA).

### Ghrelin and GH administration experiment

Juvenile rainbow trout (80, approximately 150 g body weight) will be obtained from and housed at the Aquaculture Research Institute at the University of Idaho. The fish will be injected intraperitoneally (27G needle) with acylated rat ghrelin (Tocris; either 0.033 or 0.25 ug/g body weight in 0.9% saline + 0.1% bovine serum albumin, depending on the time point), bovine growth hormone (USA Biologicals; 2.5 ug/g body weight in 0.9% saline + 0.1% bovine serum albumin), or vehicle (0.9% saline + 0.1% bovine serum albumin) alone. A final group will not be injected and act as a double control. A t = 0, an initial blood sample will be collected from all fish immediately prior to intraperitoneal injection of their respective treatment. Fish from the 0.25 ug/g body weight ghrelin-injected group will be sampled at 1 and 3 hours, whereas the 0.033 ug/g body weight will be sampled at 12 hours post-injection. Fish from the growth hormone-injected group will only be sampled at 12 hours post-injection. All fish will be lethally

sampled to obtain blood samples for hormone (growth hormone and IGF-1) analysis and liver tissue for qPCR (IGF-1 mRNA) analysis. The injection concentrations and time courses are based on previously published literature in the same or similar species (Kaiya, et al. 2003; Riley, et al. 2002; Shepherd et al. 2007). All other aspects of the husbandry will follow the Standard Operating Practices for rainbow trout (*e.g.*, those used by the ARI).
Details of fish handling:

- Blood Sampling: Fish will be anesthetized in buffered (pH 7.0) 100 mg/l MS-222. Blood (1.5 – 2 ml) will be drawn from the caudal vessels of anesthetized fish using 21 gauge needles fitted to heparinized 3 ml syringes.
- Terminal Sampling: Fish will be euthanized in buffered (pH 7.0) 250 mg/l MS-222. A liver tissue sample (~200 g) will be removed for analysis.

## Results

### Assay Development and Validation

#### *IGF-I Assay*

In europium DTTA chelate protein and peptide labeling, the chelate moiety attaches to exposed amine groups, which include the N-terminus of the protein chain and lysine residues. Because assay performance is optimal with one molecule europium DTTA chelate per peptide molecule, in our first rbIGF-I labeling, we restricted labeling -I to the N-terminus (as per Small and Peterson, 2005). However, this attempt produced a large percentage (55%) of unlabeled peptide, and PerkinElmer could not purify the final product to separate labeled and unlabeled product. We determined that this label was not suitable for use in a TRF immunoassay, because the mixture of labeled and unlabeled rbIGF-I in the label would complete for binding to the primary antibody, resulting in a substantial reduction in the detection range of the assay. After extensive back and forth with Perkin Elmer, we determined that a more recent successful labeling of fish IGF-I did not restrict labeling to the N-terminus (Ferriss, et al. 2014), and Perkin Elmer agreed to waive the charge for relabeling. We are currently awaiting the results of the second labeling attempt, without the N-terminal restriction.

#### *GH Assay*

In the MyBioSource salmonid GH assay, serial dilution of rainbow trout plasma was parallel to the standard curve (Fig 3D.1). In fish injected with bovine GH, an elevation in plasma GH level, as measured with the MyBioSource assay, was detected after 12 hours, indicating that the primary antibody cross reacts with bovine GH (Fig 3D.2). An increase in plasma GH was not found in the ghrelin injected fish. The assay was sensitive enough to measure levels in rainbow trout plasma. However, rainbow trout plasma GH levels measured using this assay were approximately 10 fold higher than levels measured using other established plasma GH assays. In addition, MyBioSource was not able to provide any information on whether cross-reactivity with other pituitary hormones had been tested. Eventually, we found that the kit is actually manufactured by EIAab (Wuhan, China), and determined that cross-reactivity with other pituitary hormones had not been tested.

*Figure 3D.1. Plasma Parallelism of serially diluted rainbow trout plasma compared to the standard curve provided in the MyBioSource salmonid growth hormone ELISA.*

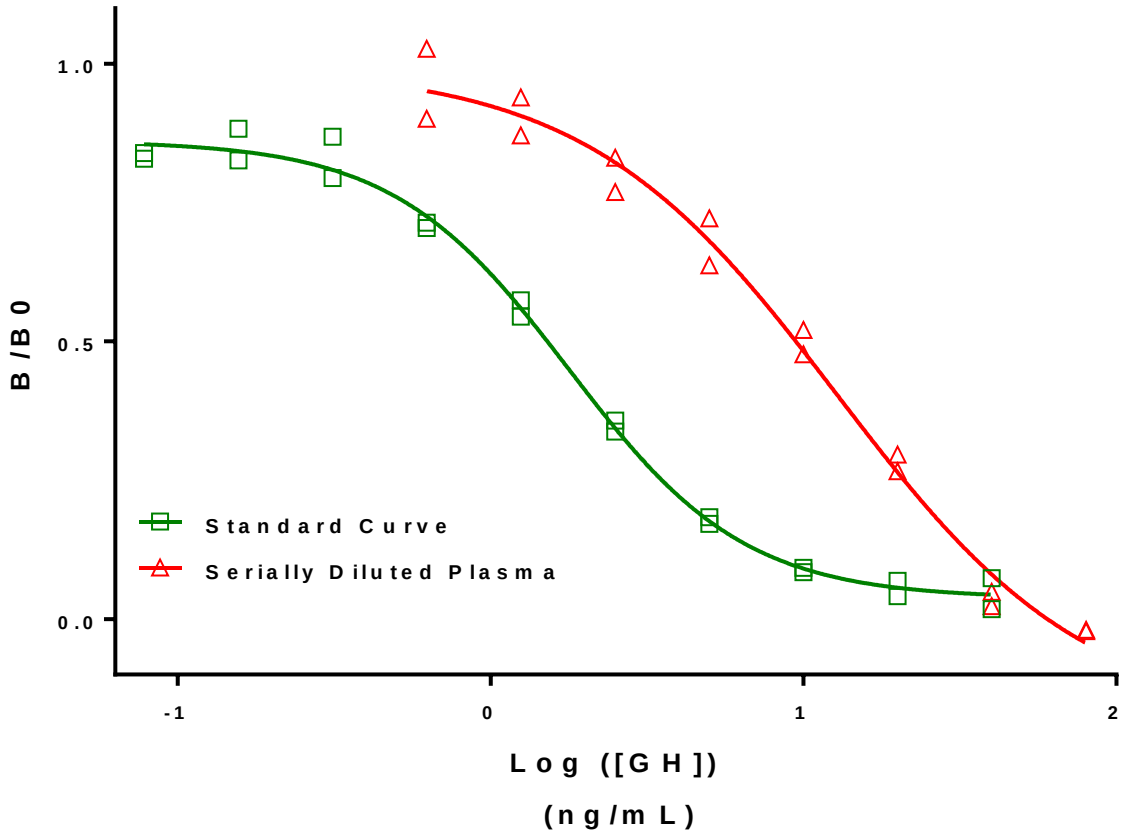
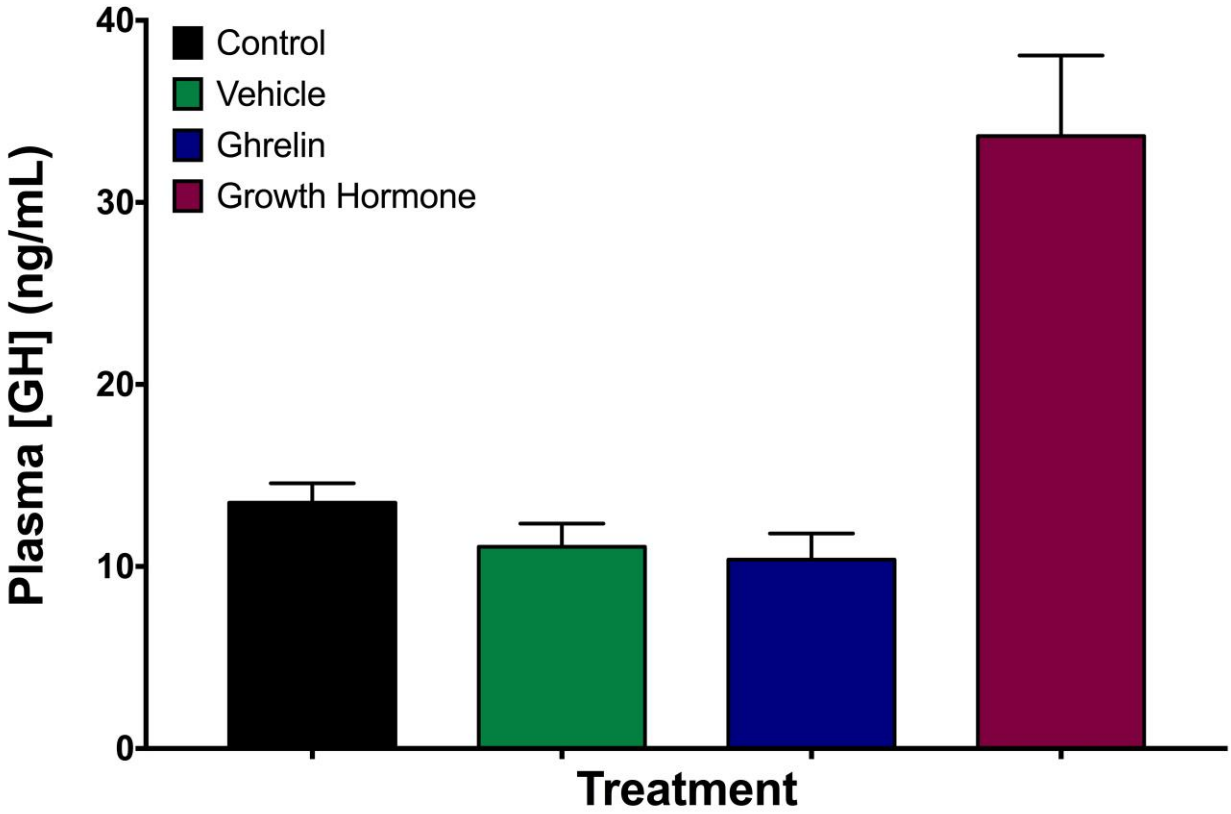


Figure 3D.2. Rainbow trout plasma GH levels measured with the MyBioSource salmonid GH assay in samples taken 12 hours after injection.



## Experiment Progress

We have completed the ghrelin-injection and GH-injection experiments for the 12 hours post-injection time point and will complete the shorter time course injections for ghrelin in January of 2017.

## Discussion

Substantial progress has been made toward development of an assay for steelhead and rainbow trout plasma IGF-I. All components required for an IGF-I assay have now been obtained, and the assay has been validated for rainbow trout (Small and Peterson 2005). Although setbacks in assay development are the norm, our laboratory has extensive experience in assay development and troubleshooting, and we expect to begin measuring plasma IGF-I levels soon.

Our initial trials with the MyBioSource GH assay were mixed. The increase in plasma GH seen in bovine GH injected trout indicates that the assay does measure GH-family proteins. It is not surprising that ghrelin injection did not stimulate an increase in plasma GH in this experiment, because any increase is likely to be transient due to the short half-life of ghrelin in the plasma. While the assay does appear to measure plasma levels of a GH-related protein, the specificity of the assay cannot be verified. In our view, the assay is suitable for use in measurement of GH-like immunoreactivity for preliminary trials. However, we are concerned that there would be issues with assay validation if we attempted to publish results from this assay in a peer-reviewed journal. In addition, the MyBioSource assay is expensive ($570 per 96 well plate). A GH primary antibody that has been validated and published for use in a radioimmunoassay for salmonid GH is commercially available (GroPep, Brisbane, Australia) (Wilkinson, et al. 2006). Thus, the only additional component needed for a GH TRF immunoassay is purified salmonid GH. Unfortunately, recombinant salmon GH is no longer available from GroPep. We are currently exploring options for obtaining recombinant or native salmonid GH for use in a TRF assay. Ultimately, this should save time and money, and result in a more reliable assay.

Fish injection experiments have been or are being completed, and will provide confirmation that assays are working properly. We hope to combine these results with our previous long-term ghrelin and GH administration experiment to produce a manuscript for submission to a peer-reviewed journal.

# 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 them 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 comparing reconditioned kelts in-stream PIT array detections with results from genetic stock identification information that is sensitive to differences between the genetically distinct populations of Status and Toppenish creek stocks. Further conclusive evidence for homing was obtained from kelts collected in Omak Creek. These fish 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 were previously released as reconditioned kelts. All fish ladders of Prosser Dam were wired with PIT antennas by 2008 Reconditioned kelt steelhead are released below the dam, enabling us to use ladder detections as further evidence that 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 evidence of repeat homing. In the Yakima River, all 27 fish that we detected as maiden and kelts returned to spawn in the same tributary. We have found no evidence of straying in these

sampled fish. 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 from 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 entered into the reconditioning program and are later 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. 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.

***Table 4.1**. Observed and inferred homing from artificially reconditioned kelt steelhead in Omak Creek and the Yakima River from 2001 to 2016. 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.*

| | Conclusive Evidence for Homing | | | Consistent with Homing | | |
|---|---|---|---|---|---|---|
| Location | A. Maiden/ Repeat Spawner Tag Detection | B. Repeat Spawner Tag Detection + GSI confirmation | C. Conclusive Homing total A+B | D. Repeat spawner PIT Detection at Prosser | E. Post Spawn Repeat Spawner Recaptured at CJMF | F. Consistent with homing, some fish are in both D and E |
| Yakima R | 38 | 294 | 332 | 562 | 105 | 779 |
| Omak Cr | 11 | - | 11 | - | - | - |
| Total | 49 | 294 | 343 | 562 | 105 | 779 |

# 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. 2016).

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 to act as a control measure for reconditioning. Control releases continue with a total of 835 fish released back to the Yakima River from 2005-2016.

**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**

Survival to release of long-term reconditioned kelt steelhead averaged 42% for the Yakima River, 33% for the Snake River, 15% for Omak Creek, and 36% for Hood River. The Yakima River is represented by 17 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.

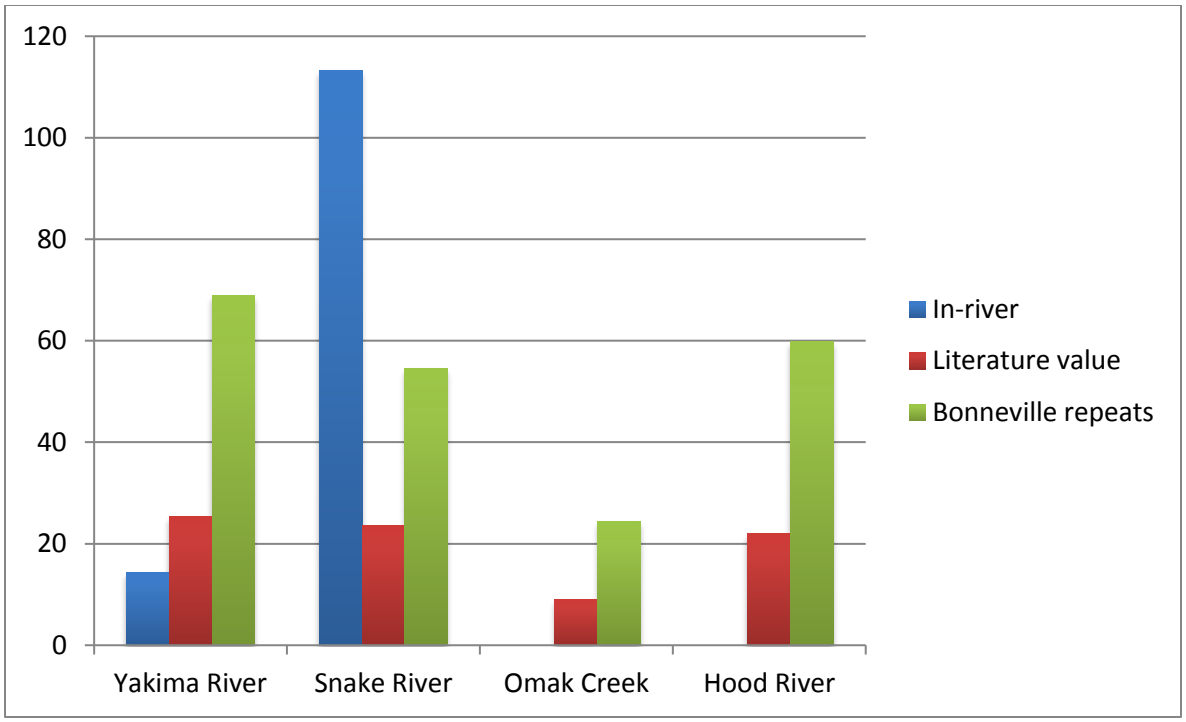
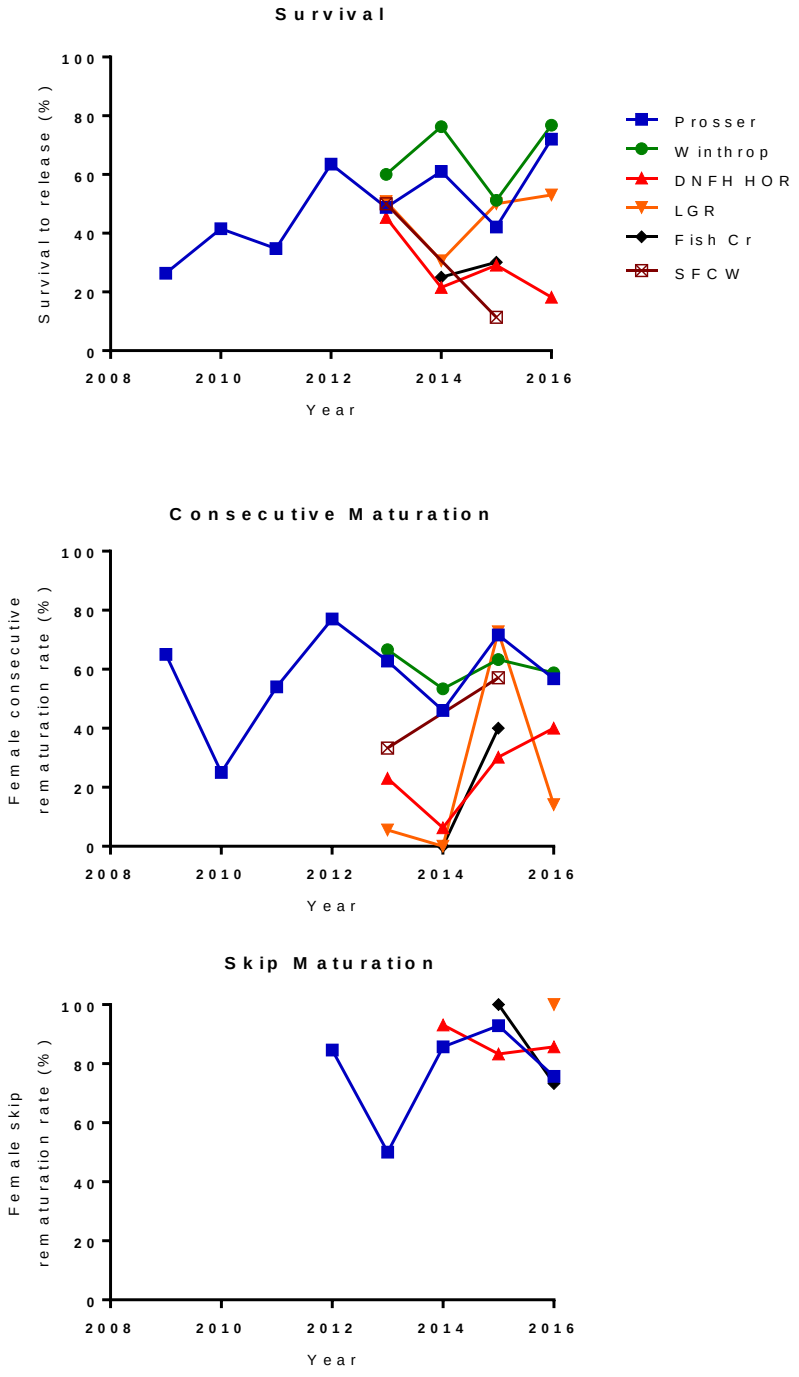



***Figure 5.1**: Benefits of long-term reconditioning relative to 3 control metrics. In-river control groups were not available for Omak Creek or Hood River.*

**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 known 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 and skip maturation rates in CRB kelt reconditioning projects. Fish reconditioned in the Snake River project were housed at Dworshak and Nez Perce Tribal hatcheries, and include air spawned hatchery origin kelts from the DNFH stock (DHFH HOR), kelts collected at Lower Granite Dam (LGR), and kelts collected at Fish Creek on the Lochsa River in 2014 and 2015 (Fish Cr), and air spawned South Fork Clearwater fish (SFCW) in 2013 and 2015. Maturation data for skip spawners is from non-mature fish from the previous season held over for an additional year.*



Survivals in the Prosser and Winthrop projects from 2012 onward have consistently been in the 50 – 80% range. 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. Survivals of kelts collected at Lower Granite Dam have been comparable to the Prosser and Winthrop projects in 3 of 4 years. However, survivals of fish collected at the Fish Creek weir, and of hatchery-origin fish air spawned at DNFH have been lower than survivals at other projects. The lower survival of DNFH hatchery fish may be due to the effects of fish anesthesia and processing at the hatchery, in particular carbon dioxide anesthesia. Further, hatchery returning steelhead have been lethally spawned at DNFH since the hatchery was established in the 1970s, which may have resulted in selection against iteroparity. The lower survival of kelts collected at the Fish Creek weir is puzzling. It is possible that collection further downstream results in enrichment of the proportion of kelts that are capable of survival. However, as these results are based on only two years of data, further study on reconditioning of fish collected at weirs immediately below spawning locations is required before conclusions can be drawn. Overall, results suggest that survivals above 50% are attainable in CRB kelt reconditioning, even in inland populations with a long migration.

With the exception of 2010, consecutive rematuration rates in the Prosser and Winthrop projects have consistently been near 60%. Maturation rates for Snake River fish 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, 73% of fish collected at Lower Granite Dam and 57% of fish collected from the South Fork of the Clearwater River were rematuring at the time of release. Thus, high rematuration rates appear to be possible for Snake River fish. Some of the variation in maturation rate in the Snake River project may be due to operation at less than ideal temporary facilities at Dworshak and Nez Perce Tribal hatcheries. Additional years of data on Snake River fish held under optimal culture conditions is needed to determine the range of maturation rates that can be expected for these fish. Overall, results suggest that consecutive 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 four comparable years, This relationship is nearly significant with the current dataset (linear regression survival $p = 0.0560$, $r^2 = 0.8911$; consecutive maturation $p = 0.1634$. $r^2 = 0.6998$). Both of these projects use well water, and fish have been given the same disease treatments and fed the same diets over the past four years. Thus, culture conditions are constant, which suggests that common environmental conditions prior to capture may influence fish performance in captive reconditioning. This relationship implies that both survival and consecutive maturation depend on fish condition at intake into reconditioning.

Skip maturation rates in all of the CRB projects have been uniformly high, ranging from 73 to 100%, with the exception of 2013 at Prosser, which is based on only four fish. Skip maturation rates have been high even in years with a low consecutive maturation rate, such as 2014 and 2016 in the Snake River project. These results indicate that nearly all kelts that are not rematuring after one summer of reconditioning will remature as skip spawners the next year. Skip spawning is a normal life history in steelhead (Keefer, et al. 2008; Pierce, et al. 2016). Natural skip spawners increase life history diversity, which enhances population stability in salmonids (Moore, et al. 2014; Schindler, et al. 2010). These considerations suggest that proper management of skip spawners can increase the benefits of reconditioning programs to target populations. However, additional research on the costs and benefits of various management options for handling skip spawners is needed.

# Chapter 6. Building a Snake River Kelt Reconditioning Facility

In the Columbia Basin Fish Accord Agreement that CRITFC is party to, $2M was included for capital construction of a Snake River Kelt Reconditioning Facility. The Northwest Power and Conservation Council (NWPCC) three-step review process is triggered for any artificial production initiative that involves the construction of new production facilities. In 2016, we drafted a Master Plan, reviewed the plan with co-managers and action agencies and submitted it the NWPCC for review by the Independent Science Review Panel (ISRP). In December 2016, the NWPCC accepted our Master Plan and recommended that we proceed to final design of the facility. This Master Plan would result in the fabrication of new facilities at an existing propagation facility. Given its eligibility for the three-step review process, this Master Plan must address a number of questions, which are bulleted below along with a reference (italicized) to the location in this Master Plan that addresses the information need.

- Address the relationship and consistencies of the proposed project to the six scientific principles (see 2014 Columbia River Basin Fish and Wildlife Program, Part Three, Section II (Step 1). *See Section 1.1.*

- Describe the link of the proposal to other projects and activities in the adopted subbasin and the desired end-state condition for the target subbasin (Step 1). *See Introduction.*

- Define the principles, goals and biological objectives associated with this proposed project (see 2014 Columbia River Basin Fish and Wildlife Program, Part Three, Section III (Step 1). *See Section 6.*

- Define the expected project benefits, for example, preservation of biological diversity, fishery enhancement, water optimization, and habitat protection (Step 1). *See Sections 1.6, 2, and 6.3.*

- Ensure that cost-effective alternate measures are not overlooked and include descriptions of alternatives for resolving the resource problem that the project or action being proposed is addressing, including a description of other management activities in the subbasin, province and basin (Step 1). *See Sections 5 and 7.*

- Provide the historical and current status of anadromous and resident fish and wildlife in the subbasin most relevant to the proposed project (Step 1). *See Section 4.*

- Describe current and planned management of anadromous and resident fish and wildlife in the subbasin (Step 1). *See Section 6.*

- Demonstrate consistency of the proposed project with National Marine Fisheries Service recovery plans and other fishery management and watershed plans (Step 1). *See Introduction Section.*

- Describe the status of the comprehensive environmental assessment (Step 1 and 2). *See Section 1.2.*

- Describe the monitoring and evaluation plan (see 2000 Columbia River Basin Fish and Wildlife Program, Basinwide Provisions, Section D.9) (Step 1, 2 and 3). *See Section 1.2.*

- Describe and provide specific items and cost estimates for the project's cost-to-date and a minimum of 10 Fiscal Years for operation and maintenance (see 2014 Columbia River Basin Fish and Wildlife Program, Part Six, Section III, and Appendix P) and monitoring and evaluation (Step 1, 2 and 3). In addition, include replacement costs for assets that have distinct value and the anticipated decommissioning costs at the end of the project's life cycle to be included (Step 3). See Section 8.

- Address the relationship to the fish propagation principles and measures (Columbia River Basin Fish and Wildlife Program, Part Three; Section IV; B, and C1, 2, 4, 5 and 6) (Step 1). *See Section 1.3.*

- Provide a completed Hatchery and Genetic Management Plan (HGMP) for the target population (s) (Step 1). *See Section 1.2.*

- Describe the harvest plan (see 2014 Columbia River Basin Fish and Wildlife Program, Part Two, Section II) (Step 1). *See Section 1.4*

- Provide a conceptual design of the proposed facilities, including an assessment of the availability and utility of any existing facilities (Step 1). *See Sections 6-8.*

- Provide a preliminary design, including an appropriate value engineering review, of the proposed facilities (Step 2). *See Section 8 and 1.2.*

- Provide a final design of the proposed facilities consistent with previous submittal documents and preliminary design (Step 3). See Section 1.2.

The initial review of the Master Plan by the ISRP was completed in May, 2016. The ISRP response is summary was:

> "The Master Plan is well written and contains an excellent summary of the extensive steelhead reconditioning work that has occurred in the Basin. Moreover, we compliment the proponents for investigating and addressing the many difficulties associated with steelhead reconditioning. Numerous challenges associated with fish culture had to be addressed, including establishing appropriate holding and rearing environments, formulating diets, and developing disease control protocols. The effects of long-term reconditioning on gamete viability, fidelity to natal streams, and ability to reproduce in nature were investigated. Comparisons that evaluated the potential

benefits of various kelt treatments that ranged from simple direct transportation past downstream dams to long-term reconditioning lasting from 6 to 20 months were also conducted. In general, the results of these assessments indicated that long-term reconditioning of kelts appears to be a promising approach that might lead to a viable conservation strategy for steelhead.

The proponents acknowledge that the submitted Master Plan does not yet have all the necessary components for a Step 1 review. It currently lacks a Hatchery Genetic Management Plan (HGMP), and work is needed on the program's Research, Monitoring and Evaluation Plan and Comprehensive Environmental Assessment. Before producing these elements of the Master Plan, the proponents requested that the ISRP determine if the program's preferred location for a long-term reconditioning facility, for Snake River B-run steelhead, is appropriate.

More information is needed before a decision about the location of the proposed long-term reconditioning facility can be reached. Specifically, information on the following issues is requested in the updated Step 1 Master Plan. Additional comments provided in the ISRP's full report should also be considered in the revision.

1 The biological and ecological rationale for annually increasing B-run steelhead escapement by 180 reconditioned female kelts needs to be explained in the Master Plan.
2 Clarification on why male kelts are not included in the proposed reconditioning program is needed.
3 The biological escapement goals for B-run steelhead populations in the Snake River subbasin should be in the Master Plan along with a description of what project "success" entails. To what extent, for example, are reconditioned kelts expected to contribute to the rebuilding of natural steelhead populations and eventually to fisheries?
4 If available, information on the abundance and status and trends of B-run steelhead populations in the Clearwater and Salmon River subbasins should be provided in the Master Plan. Current spawning levels of B-run steelhead in the Snake River Basin should also be described with reference to numerical objectives for natural spawning steelhead. Additionally, a brief overview of the factors limiting each of these populations should be added to the Plan.
5 Substantial hatchery and habitat restoration actions affecting B-run steelhead are occurring in the Snake River subbasin. The Master Plan should briefly describe these programs and indicate how the proponent's goal of annually releasing 180 reconditioned kelts will be coordinated with ongoing habitat restoration and existing hatchery programs.
6 As it is currently designed, the kelt reconditioning program will recondition female B-run steelhead kelts without targeting specific populations. It would seem that capturing, reconditioning, and releasing kelts from populations that have the potential to accommodate additional spawners would be a more efficient and productive way of directing this strategy. The Master Plan should

explain why a more focused program was not considered.

7 The Master Plan should discuss the infrastructural needs of a more focused and integrated reconditioning program. If the project, for instance, were to narrow its focus on B-run populations that could benefit from the addition of reconditioned kelts, would facilities at Dworshak National Fish Hatchery be adequate to meet these new escapement objectives?

8 The Master Plan should compare the benefits and drawbacks of increasing B-run steelhead escapements by modifying harvest regulations, by long-term reconditioning for adult release, and long-term reconditioning for captive breeding and smolt release.

Some discussion of the genetic risks that may accompany reconditioning (e.g., heritable epigenetic effects and domestication selection) needs to be added to the Master Plan or incorporated into the Plan's HGMP."

We revised the Master Plan and submitted the document to the ISRP in July, 2016 and received "meets scientific review criteria (qualified)" recommendation on September 27, 2016.

At the November 2016 NWPCC meeting in Coeur d' Alene, we presented our Master Plan to the Council's Fish Committee. The Fish Committee received the plan favorably and recommended that it be presented to the full Council in December. At the December Council meeting we again presented the Master Plan and received a recommendation from the Council to proceed to the Final Design stage of the 3-step process.

In 2017, we plan to complete the 3-Step process, gain a positive Council recommendation, and begin the construction phase of the project.

## 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%.
13. The Snake River Kelt Reconditioning Facility Master Plan was submitted and favorably review by the ISRP and recommended to proceed to final design by the NWPCC in December of 2016.

14. Reproductive success studies are underway at a variety of scales: hatchery analog, spawning channel, and natural river. Results are positive.
15. 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.

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# Appendices

## A1.a Master Kelt Tracking Table

Steelhead Kelt Reconditioning Treatments

| Strategy | Year | Location | # Collected | # released (survived) | S @ release (%) | # remature | Retained | skip remature | # @ ocean | S @ ocean (%) | # @ Bonneville | Return Rate to Bonneville (%) | Return to #### | Return Rate to # |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | # @ Prosser Dam | Return Rate to P |
| In-river | 2005 | Prosser | 67 | 67 | | | | | | | 3 | 4.48 | | |
| In-river | 2006 | Prosser | 52 | 52 | | | | | | | 1 | 1.92 | | |
| In-river | 2007 | Prosser | 53 | 53 | | | | | | | 3 | 5.66 | | |
| In-river | 2008 | Prosser | 88 | 88 | | | | | | | 4 | 4.55 | | |
| In-river | 2009 | Prosser | 58 | 58 | | | | | | | 3 | 5.17 | | |
| In-river | 2010 | Prosser | 155 | 155 | | | | | | | 2 | 1.29 | | |
| In-river | 2011 | Prosser | 85 | 85 | | | | | | | 3 | 3.53 | | |
| In-river | 2012 | Prosser | 59 | 59 | | | | | | | 2 | 3.39 | | |
| In-river | 2013 | Prosser | 52 | 52 | | | | | | | 0 | 0.00 | | |
| In-river | 2014 | Prosser | 45 | 45 | | | | | | | 3 | 6.67 | | |
| In-river | 2015 | Prosser | 121 | 121 | | | | | | | 0 | 0.00 | | |
| In-river | 2016 | Prosser | 56 | 56 | | | | | | | 2 | 3.57 | 2 | |
| *Total and weighted mean* | | | ***891*** | ***891*** | | | | | | | ***2.10*** | ***2.92*** | | |

| Strategy | Year | Location | # Collected | # released (survived) | S @ release (%) | # remature | Retained | skip remature | # @ ocean | S @ ocean (%) | # @ Bonneville | Return Rate to Bonneville (%) | # @ Lower Granite Dam | Return R Lower Granite |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| In-river | 2002 | Lower Granite* | 1209 | 1209 | | | | | | | 8 | 0.66 | | |
| In-river | 2003 | Lower Granite* | 865 | 865 | | | | | | | 3 | 0.35 | | |
| In-river | 2004 | Lower Granite* | 1138 | 1138 | | | | | | | 10 | 0.88 | | |
| In-river | 2009 | Lower Granite | 178 | 176 | | | | | | | 2 | 1.12 | | |
| In-river | 2010 | Lower Granite | 1411 | 1399 | | | | | | | 5 | 0.35 | | |
| In-river | 2011 | Lower Granite | 1633 | 1613 | | | | | | | 3 | 0.18 | | |
| In-river | 2012 | Lower Granite | 2098 | 2098 | | | | | | | 1 | 0.05 | | |
| In-river | 2013 | Lower Granite | 840 | 827 | | | | | | | 2 | 0.24 | | |
| In-river | 2014 | Lower Granite | 2584 | 2571 | | | | | | | 8 | 0.31 | | |
| In-river | 2015 | Lower Granite | 1195 | 1193 | | | | | | | 0 | 0.00 | 0 | |
| In-river | 2016 | Lower Granite | 1841 | 1837 | | | | | | | 2 | 0.11 | 1 | |
| *Total and weighted mean* | | | ***14992*** | ***14926*** | | | | | | | ***4.27*** | ***0.29*** | | |
| In-river | 2002 | John Day* | ***287*** | ***287*** | | | | | | | ***28*** | ***9.76*** | | |
| *Total and weighted mean* | | | | | | | | | | | | | | |
| Transported (Hamilton Island) | 2002 | Lower Granite* | 750 | 750 | | | | | | | 19 | 2.53 | | |
| Transported (Hamilton Island) | 2003 | Lower Granite* | 376 | 376 | | | | | | | 3 | 0.80 | | |
| Transported (Hamilton Island) | 2004 | Lower Granite* | 982 | 982 | | | | | | | 7 | 0.71 | | |
| Transported (Hamilton Island) | 2009 | Lower Granite | 71 | 68 | | | | | | | 0 | 0.00 | | |
| Transported (Hamilton Island) | 2010 | Lower Granite | 301 | 301 | | | | | 13/108 | 12.04 | 0 | 0.00 | | |
| Transported (Hamilton Island) | 2011 | Lower Granite | 109 | 109 | | | | | 3/47 | 6.38 | 0 | 0.00 | | |
| *Total and weighted mean* | | | ***2589*** | ***2586*** | | | | | | ***9.21*** | ***8.59*** | ***1.12*** | | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Transported (estuary release) | 2010 | Lower Granite | 23 | 22 | | | | 4/10 | 40.00 | 0 | 0.00 |
| Transported (estuary release) | 2011 | Lower Granite | 91 | 90 | | | | 14/46 | 30.43 | 0 | 0.00 |
| *Total and weighted mean* | | | ***114*** | ***112*** | | | | | ***35.22*** | ***0.00*** | ***0.00*** |
| | | | | | | | | | | | |
| Transported | 2002 | John Day* | ***271*** | ***271*** | | | | | | ***34*** | ***12.55*** |
| *Total and weighted mean* | | | | | | | | | | | |
| | | | | | | | | | | | |
| Transported (unfed Hamilton Island) | 2004 | Prosser | 75 | 63 | | | | 15/28 | 53.57 | 5 | 6.67 |
| Transported (unfed Hamilton Island) | 2005 | Prosser | 98 | 96 | | | | 14/57 | 24.56 | 1 | 1.02 |
| Transported (unfed Hamilton Island) | 2006 | Prosser | 55 | 49 | | | | 31/49 | 63.27 | 2 | 3.64 |
| Transported (unfed Hamilton Island) | 2007 | Prosser | 43 | 38 | | | | 14/35 | 40.00 | 0 | 0.00 |
| Transported (unfed Hamilton Island) | 2008 | Prosser | 100 | 100 | | | | 26/49 | 53.06 | 3 | 3.00 |
| Transported (unfed Hamilton Island) | 2010 | Prosser | 124 | 123 | | | | 27/59 | 45.76 | 1 | 0.81 |
| Transported (unfed Hamilton Island) | 2011 | Prosser | 100 | 100 | | | | 16/47 | 34.04 | 1 | 1.00 |
| *Total and weighted mean* | | | ***595*** | ***569*** | | | | | ***44.89*** | ***1.86*** | ***2.18*** |
| | | | | | | | | | | | |
| Transported (unfed estuary release) | 2010 | Prosser | 113 | 113 | | | | 13/60 | 21.67 | 1 | 0.88 |
| Transported (unfed estuary release) | 2011 | Prosser | 90 | 89 | | | | 16/47 | 34.04 | 3 | 3.33 |
| *Total and weighted mean* | | | ***203*** | ***202*** | | | | | ***27.85*** | ***1.00*** | ***1.97*** |
| | | | | | | | | | | | |
| Transported (fed Hamilton Island) | 2002 | Prosser | 479 | 334 | | | | | | 43 | 8.98 |
| Transported (fed Hamilton Island) | 2003 | Prosser | 208 | 187 | | | | | | 8 | 3.85 |
| Transported (fed Hamilton Island) | 2004 | Prosser | 105 | 83 | | | | 11/26 | 42.31 | 5 | 4.76 |
| Transported (fed Hamilton Island) | 2005 | Prosser | 106 | 96 | | | | 6/56 | 10.71 | 0 | 0.00 |
| Transported (fed Hamilton Island) | 2006 | Prosser | 56 | 50 | | | | 32/50 | 64.00 | 0 | 0.00 |
| Transported (fed Hamilton Island) | 2007 | Prosser | 40 | 38 | | | | 19/27 | 70.37 | 1 | 2.50 |
| Transported (fed Hamilton Island) | 2008 | Prosser | 108 | 100 | | | | 28/50 | 56.00 | 7 | 6.48 |
| *Total and weighted mean* | | | ***1102*** | ***888*** | | | | | ***48.68*** | ***21.40*** | ***5.81*** |
| | | | | | | | | | | | |
| Transported (Fed Hamilton Island) | 2014 | Lower Granite | ***36*** | ***36*** | | | | | | ***0.00*** | 0.00 |
| | | | ***36*** | ***36*** | | | | | | ***0.00*** | ***0.00*** |
| | | | | | | | | | | | |
| Transported (pooled groups) | 2002 | Prosser | 479 | 334 | | | | | | 43 | 8.98 | |
| Transported (pooled groups) | 2003 | Prosser | *208* | 187 | | | | | | 8 | 3.85 | |
| Transported (pooled groups) | 2004 | Prosser | 180 | 146 | | | | 26/54 | 48.15 | 10 | 5.56 | |
| Transported (pooled groups) | 2005 | Prosser | 204 | 192 | | | | 20/113 | 17.70 | 1 | 0.49 | |
| Transported (pooled groups) | 2006 | Prosser | 111 | 99 | | | | 63/99 | 63.64 | 2 | 1.80 | |
| Transported (pooled groups) | 2007 | Prosser | 83 | 76 | | | | 33/62 | 53.23 | 1 | 1.20 | |
| Transported (pooled groups) | 2008 | Prosser | 208 | 200 | | | | 54/99 | 54.55 | 10 | 4.81 | |
| Transported (pooled groups) | 2010 | Prosser | 237 | 236 | | | | 40/119 | 33.61 | 2 | 0.84 | 2 |
| Transported (pooled groups) | 2011 | Prosser | 190 | 189 | | | | 32/94 | 34.04 | 4 | 2.11 | 1 |
| *Total and weighted mean* | | | ***1900*** | ***1659*** | | | | | ***43.56*** | ***14.68*** | ***4.26*** | |
| | | | | | | | | | | | | |
| Long-term | 2000 | Prosser | 512 | 91 | 17.77 | | | | | | | |
| Long-term | 2001 | Prosser | 551 | 197 | 35.75 | | | | | | | |
| Long-term | 2002 | Prosser | 420 | 140 | 33.33 | | | | | | | |
| Long-term | 2003 | Prosser | 482 | 298 | 61.83 | | | | | | | |
| Long-term | 2004 | Prosser | 662 | 253 | 38.22 | | | | | | | |
| Long-term | 2005 | Prosser | 386 | 86 | 22.28 | | | | | | | |
| Long-term | 2006 | Prosser | 279 | 85 | 30.47 | | | | | | | |
| Long-term | 2007 | Prosser | 422 | 221 | 52.37 | | | | | | | |
| Long-term | 2008 | Prosser | 472 | 269 | 56.99 | | | | | | | |
| Long-term | 2009 | Prosser | 510 | 140 | 27.45 | 91 | | | | | | |
| Long-term | 2010 | Prosser | 1157 | 404 | 34.92 | 101 | | | | | | |
| Long-term | 2011 | Prosser | 680 | 223 | 32.79 | 120 | | | | | | 54 |
| Long-term | 2012 | Prosser | 550 | 340 | 61.82 | 275 | | | | | | 222 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Long-term | 2013 | Prosser | 546 | 266 | 48.72 | 166 | 41? | 8 | | | 93 |
| | Long-term | 2014 | Prosser | 481 | 292 | 60.71 | 149 | 96 | 22 | | | 109 |
| | Long-term | 2015 | Prosser | 1098 | 396 | 36.07 | 382 | 74 | 37 | | | 216 |
| | Long-term | 2016 | Prosser | 471 | 341 | 72.40 | 210 | 94 | TBD 17 | | | 139 |
| | *Total and weighted mean* | | | ***9679*** | ***4042*** | ***41.96*** | ***1494*** | ***264*** | ***67*** | | | **833** |
| | | | | | | | ***64.99%*** | | | | | |
| | Long-term | 2005 | Shitike Cr | 9 | 1 | 11.11 | | | | | | |
| | Long-term | 2006 | Shitike Cr | 4 | 0 | 0.00 | | | | | | |
| | Long-term | 2007 | Shitike Cr | 14 | 1 | 7.14 | | | | | | |
| | Long-term | 2008 | Shitike Cr | 11 | 0 | 0.00 | | | | | | |
| | *Total and weighted mean* | | | ***38*** | ***2*** | ***5.26*** | | | | | | |
| | Long-term | 2005 | Omak Cr | 17 | 3 | 17.65 | | | | | | |
| | Long-term | 2006 | Omak Cr | 27 | 2 | 7.41 | | | | | | |
| | Long-term | 2007 | Omak Cr | 43 | 8 | 18.60 | | | | | | |
| | Long-term | 2008 | Omak Cr | 32 | 9 | 28.13 | | | | | | |
| | Long-term | 2009 | Omak Cr | 17 | 2 | 11.76 | | | | | | |
| | Long-term | 2010 | Omak Cr | 13 | 6 | 46.15 | | | | | | |
| | Long-term | 2011 | Omak Cr | 20 | 4 | 20.00 | | | | | | |
| | Long-term | 2012 | Omak Cr | 65 | 4 | 6.15 | | | | | | |
| | Long-term | 2013 | Omak Cr | 49 | 4 | 8.16 | | | | | | |
| | *Total and weighted mean* | | | ***283*** | ***42*** | ***14.84*** | | | | | | |
| s | Long-term | 2006 | Parkdale | 1 | 1 | 100.00 | | | | | | |
| s | Long-term | 2007 | Parkdale | 13 | 1 | 7.69 | | | | | | |
| s | Long-term | 2008 | Parkdale | 14 | 7 | 50.00 | | | | | | |
| s | Long-term | 2009 | Parkdale | 9 | 4 | 44.44 | | | | | | |
| w | Long-term | 2010 | Parkdale | 15 | 4 | 26.67 | | | | | | |
| w | Long-term | 2011 | Parkdale | 23 | 5 | 21.74 | | | | | | |
| w | Long-term | 2012 | Parkdale | 21 | 13 | 61.90 | | | | | | |
| | *Total and weighted mean* | | | ***96*** | ***35*** | ***36.46*** | | | | | | |

| | | | | | ***# Survived*** | | | | | **Total Rematuring** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Long-term | 2012 | DNFH | 143 | 5 | 3.50 | 4 | 0 | - | 4 | | | | | |
| | Long-term | 2013 | DNFH | 163 | 61 | 37.42 | 12 | 47 | 22 | 34 | | | | | |
| | Long-term | 2014 | DNFH | 149 | 19 | 12.75 | 2 | 17 | 5 | 7 | | | | | |
| | Long-term | 2015 | DNFH | 149 | 43 | 28.86 | 13 | 30 | 18 | 31 | | | | | |
| | Long-term | 2016 | DNFH | 165 | 30 | 18.18 | 12 | 18 | TBD 17 | 12 | | | | | |
| | *Total and weighted mean* | | | ***769*** | ***158*** | ***20.55*** | ***43*** | ***112*** | ***45*** | ***88*** | | | | | |
| | | | | | | | | | | ***55.70%*** | | | | | |

| | | | | | ***# Survived*** | | **Released** | **# remature** | **Retained** | **skip remature** | **total release** | # @ Lower Granite Dam | Return F Lower Granite |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Long-term | 2011 | Lower Granite | 111 | 2 | 1.80 | 2 | **-** | **-** | **-** | 2 | | |
| | Long-term | 2012 | Lower Granite | 124 | 10 | 8.06 | 10 | 3 | 0 | **-** | 10 | | |
| | Long-term | 2013 | Lower Granite | 110 | 57 | 51.82 | 57 | 3 | 0 | **-** | 57 | | |
| | Long-term | 2014 | Lower Granite | 110 | 34 | 30.91 | 34 | 0 | 0 | **-** | 34 | | |
| | Long-term | 2015 | Lower Granite | 22 | 11 | 50.00 | 8 | 8 | 3 | 3 | 11 | 8 | |
| | Long-term | 2016 | Lower Granite | 227 | 120 | 52.86 | 19 | 19 | 101 | ***TBD 17*** | ***19*** | 8 | |
| | *Total and weighted mean* | | | ***704*** | ***234*** | ***33.24*** | ***130*** | ***33.0*** | ***104*** | ***3*** | ***133*** | **16** | |
| | | | | | | | | ***18.59%*** | | | | | |

| | | | | | | | **Released** | **# remature** | **Retained** | **skip remature** | **total release** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Long-term | 2013 | S.F. Clearwater | 24 | 12 | 50.00 | 12 | 4 | 0 | - | 12 | |
| | Long-term | 2015 | S.F. Clearwater | 35 | 7 | 20.00 | 4 | 4 | 3 | - | 4 | 2 |
| | *Total and weighted mean* | | | ***59*** | ***19*** | ***32.20*** | ***16*** | ***8*** | ***3*** | 0 | ***16*** | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | ***11.43%*** | | | | | | |
| | | | | | | **Cons. Released** | **# remature** | **Retained** | **skip remature** | **total release** | | | |
| Long-term | 2014 | Fish Creek | 12 | 3 | 25.00 | 1 | 0 | 2 | 2 | 3 | | | 0 |
| Long-term | 2015 | Fish Creek | 83 | 25 | 30.12 | 10 | 10 | 15 | 11 | 25 | | | 4 |
| *Total and weighted mean* | | | ***95*** | ***28*** | ***29.47*** | ***11*** | ***10*** | ***17*** | ***13*** | ***28*** | | | **4** |
| | | | | | | | | | | | | | |
| *All Natural SNR Fish* | | | ***858*** | ***281*** | ***29.00*** | ***157*** | ***41.73%*** | ***124*** | ***16*** | ***177*** | | | |
| | | | | | | | | | | | | | |
| 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 | | | | | | | | | | | |
| Natural repeat | 2016 | Bonneville Dam | | | | | | | | | | | |
| | | | 17823 | | | | | | | | | ***0.61*** | |

## 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.

**Published in 2016.**

Pierce A. P., J.W. Blodgett, T.D. Cavileer, L.R. Madeiros, J. Boyce, L.K. Caldwell, W.J. Bosch, R. Branstetter, D.E. Fast, D.R. Hatch, and J.J. Nagler. 2016. Reproductive development in captive reconditioned female steelhead kelts: evidence for consecutive and skip spawning life histories. Canadian Journal of Fisheries and Aquatic Sciences.

Penney, Z.L., C.M. Moffitt, B. Jones, B. Marston. 2016. Physiological comparisons of steelhead kelts emigrating from the Situk River, AK and Clearwater River, ID. Environmental Fish Biology Vol. 99 No. 4.

Matala A.P., D.R. Hatch, S. Everett, M.W. Ackerman, B. Bowersox, M. Campbell, and S. Narum. 2016. What goes up does not come down: the stock composition and demographic characteristics of upstream migrating steelhead differ from post-spawn emigrating kelts. ICES Journal of Marine Science

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. 2016. Evaluating steelhead management scenarios to increase iteroparous spawners in the Yakima River Basin. North American Journal of Fisheries Management.

## Presentations:

2016 Pacific Coast Steelhead Management Meeting March 8-10, 2016.

- Zach Penney: Do we need to manage for iteroparity in steelhead trout (Oral).
- Doug Hatch: Steelhead Kelt Reconditioning and Reproductive Success Studies in the Columbia River Basin (Oral).

2016 Yakima Basin Science and Management Conference, Ellensburg, WA, June 15-16, 2016.

- Laura Jenkins: Reproductive performance in reconditioned female steelhead kelts (Oral).
- Ryan Branstetter: Yakima River Reconditioned Steelhead Reproductive Success: Spawning Channel and Natural Setting (Oral).

Northwest Power and Conservation Council, Fish Committee November 2016.

- Doug Hatch: The Snake River Basin Steelhead Kelt Reconditioning Facility Master Plan (Oral).

Science After Hours, Moscow, ID, November 3, 2016.

- *Laura Jenkins:* What is kelt reconditioning and why is it done on the Snake River?

67th Fish Culture Conference, Centralia, WA, December 6 and 7th, 2016.

- Neil Graham: Reconditioning Snake River B-run Steelhead Kelts A Research Report (Poster).

Northwest Power and Conservation Council, December 2016:

- Doug Hatch: The Snake River Basin Steelhead Kelt Reconditioning Facility Master Plan (Oral).

## A.3: List of Metrics and Indicators

# Protocol:

Kelt Reconditioning and Reproductive Success Evaluation:
https://www.monitoringresources.org/Document/Protocol/Details/2051

# Methods

### Kelt Collection

Kelt ID: https://www.monitoringresources.org/Document/Method/Details/5310
Sex ID: https://www.monitoringresources.org/Document/Method/Details/5334
Coloration Rating: https://www.monitoringresources.org/Document/Method/Details/5302
Measuring Fork Length: https://www.monitoringresources.org/Document/Method/Details/4041
Fish Weight: https://www.monitoringresources.org/Document/Method/Details/1734
Measuring Mid-Orbital Hypural Length: https://www.monitoringresources.org/Document/Method/Details/1549
Fish Condition Rating: https://www.monitoringresources.org/Document/CustomizedMethod/Details/22915
PIT Tagging: https://www.monitoringresources.org/Document/Method/Details/1736
Genetic Sampling: https://www.monitoringresources.org/Document/Method/Details/4087
Blood Sampling: https://www.monitoringresources.org/Document/Method/Details/4239
Estimating Lipid Content: https://www.monitoringresources.org/Document/Method/Details/4215
Air-Spawning: https://www.monitoringresources.org/Document/Method/Details/5343

### GSI

Tissue Sampling for PBT: https://www.monitoringresources.org/Document/Method/Details/1432
SNP Marker Sets: https://www.monitoringresources.org/Document/Method/Details/1356
SNP Genotyping: https://www.monitoringresources.org/Document/Method/Details/1332
Genetic Assignment using GeneClass2: https://www.monitoringresources.org/Document/Method/Details/487
Predicting Accuracy of GSI: https://www.monitoringresources.org/Document/Method/Details/1346

### In-River Release

PIT Tagging: https://www.monitoringresources.org/Document/CustomizedMethod/Details/22818
Downloading Data from PTAGIS: https://www.monitoringresources.org/Document/Method/Details/4095

### Kelt Reconditioning Physiology Studies

Estradiol Assay: https://www.monitoringresources.org/Document/Method/Details/5320

### Reproductive Success of Artificially Reconditioned Kelt Steelhead

Electrofisher Settings: https://www.monitoringresources.org/Document/Method/Details/115
Backpack Electrofishing: https://www.monitoringresources.org/Document/Method/Details/117
Parentage Analysis using Cervus: https://www.monitoringresources.org/Document/Method/Details/1430
Radio Tagging: https://www.monitoringresources.org/Document/CustomizedMethod/Details/23045
Lotek Receiver Download: https://www.monitoringresources.org/Document/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 | Abundance of Fish (ID: 46) | Fish Life Stage: Adult Fish | Fish Origin: Both |
| "Stock Composition" | Fish | Composition: Fish Species Assemblage (ID: 56) | Fish Life Stage: Adult - Outmigrant | Fish Origin: Natural |
| "Kelt Condition" | Fish | Condition of Life Stage: Fish (ID: 57) | Fish Life Stage: Adult - Outmigrant | NA |
| "Reconditioned Kelt condition" | Fish | Condition of Life Stage: Fish (ID: 57) | Fish Life Stage: Adult Fish | NA |
| "Maturation Status" | Fish | Condition of Life Stage: Fish (ID: 57) | Fish Life Stage: Adult - Returner | NA |
| "Fecundity" | Fish | Fecundity: Fish (ID: 68) | NA | NA |
| "Fry Growth" | Fish | Growth Rate: Fish (ID: 73) | Fish Life Stage: Juvenile - Fry/Parr | NA |
| "Fertilization Rate" | Fish | Hatchery Practices: Propagation (ID: 87) | Fish Origin: Both | NA |
| "Kelt length" | Fish | Length: Fish Species (ID: 75) | Fish Life Stage: Adult - Outmigrant | NA |
| "Reconditioned kelt length" | Fish | Length: Fish Species (ID: 75) | Fish Life Stage: Adult Fish | NA |
| "Mark Detection" | Fish | Mark/Tag Recovery or Detection (ID: 381) | NA | NA |
| "Parentage Analysis" | Fish | Relative Reproductive Success (RRS) (ID: 88) | Fish Origin: Both | NA |
| "Reproductive success" | Fish | Reproductive Success (Nb/N) (ID: 89) | Fish Origin: Natural | NA |

| | | | | |
|---|---|---|---|---|
| "Mark application" | Fish | Stock Identity (ID: 95) | Fish Life Stage: Adult - Outmigrant | NA |
| "Kelt Survival" | Fish | Survival Rate: Fish (ID: 99) | Fish Life Stage: Adult - Outmigrant | Fish Origin: Both |
| "Collection Date" | Fish | Timing of Life Stage: Fish (ID: 101) | Fish Life Stage: Adult - Outmigrant | NA |
| "Release Date" | Fish | Timing of Life Stage: Fish (ID: 101) | Fish Life Stage: Adult Fish | NA |
| "Kelt Weight" | Fish | Weight: Fish (ID: 206) | Fish Life Stage: Adult - Outmigrant | Fish Origin: Both |
| "Reconditioned Kelt weight" | Fish | Weight: Fish (ID: 206) | Fish Life Stage: Adult Fish | Fish Origin: Both |