

Kelt Reconditioning and Reproductive Success Evaluation Research

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## Abstract

The Kelt Reconditioning and Reproductive Success Evaluation Project is a research, monitoring, and evaluation (RM&E) uncertainties category project funded through the Columbia Basin Fish Accords. The objectives are to evaluate methodologies to produce viable artificially reconditioned repeat steelhead spawners and to determine the productivity of repeat spawners. Work occurs in 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% (18 years) for Yakima River at Prosser Hatchery and 38% (6 years; 46% over the last 4 years) for mixed stock collections at Lower Granite Dam, and in previous years Fish Creek and the South Fork Clearwater River. 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 98 reconditioned B-run steelhead were released below Lower Granite Dam in 2017 to address Reasonable and Prudent Alternative 33 of the FCRSP Biological Opinion. A total of 70 reconditioned, remature steelhead were released in the Yakima River in 2017. Mature reconditioned steelhead kelts were stocked in the Cle Elum Hatchery Spawning Channel in 2017, to evaluate the feasibility of using the facility to evaluate reproductive success in a more controlled setting. Evidence of reproduction was again confirmed but we continued to have difficulty capturing all juveniles in spawning sections. This was the last year that we will conduct the study in the channel and instead focus on reconditioned kelt wild reproduction. 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. Estradiol sampling of kelts has shown that they metabolically “decided” to consecutively remature or skip spawn after approximately 2 to 5 months after spawning. Consecutive and skip spawners due to the rigors of spawning and oogenesis, egg size is decreased but that energy is directed towards investment into individual offspring. Additionally, we discovered when fasting a portion of the fish that the “decision” to remature is hardwired into them and may occur even before spawning or shortly after. Fasting did reduce overall growth of fish and reduced the size and number of eggs that were produced. We continue to refine our plasma assays that detect IGF-I concentrations that we utilize for evaluating kelt maturation. We investigated if kelts could have cortisol signaling blocked, low sample sizes prevented obtaining statistically valid results but we plan to continue to investigate in 2018. Evidence of homing fidelity to natal streams after reconditioning continues to be compiled for Yakima River and Omak Creek kelts based on both PIT-tag history and genotyping. From 2008 to 2017 we have detected conclusive evidence of 324 kelts showing strong site fidelity from both aforementioned waterways. Kelt reconditioning survival is good at multiple locations where it is being conducted, we continue to investigate how best to utilize the skip spawning portion of the life history.. 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. Development of a Snake River kelt reconditioning facility final design has been ongoing with BPA in 2017 into 2018, once this has been completed the next steps in the ISRP 3 step process will be progressed. Development of a kelt population model continues to make progress with simulations of kelt reconditioning in the Snake River. The results of these simulations are preliminary and are built on extremely limited data sets so results should not be considered definitive. The CRITFC and its member Tribes steelhead kelt reconditioning program continues to forward the science and inform the management of iteroparous *O. mykiss* in the Columbia River Basin. An extensive list of our work is compiled in the Adaptive Management and Lessons Learned section of this report. Also, our team produced 2 papers and given 11 professional presentations in 2017.

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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. Repeat spawners make up approximately XX% of the Snake River steelhead run (xxx 20xx).

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 Columbia River Basin (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, Shitike Creek 2005-2009, and Fish Creek 2014-2015. The previously mentioned 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.*

<b>Site Number</b>	<b>Site</b>	<b>Drainage</b>	<b>Location</b>	<b>Collection site</b>	<b>Reconditioning site</b>	<b>Release Site</b>	<b>Juvenile Sampling Location</b>	<b>Dates of use</b>
<b>1</b>	Chandler Juvenile Monitoring Facility (CJMF)	Yakima River	RK 75.6	Yes	-	-	-	1999-2017
<b>2</b>	Yakama Nation Prosser Hatchery	Yakima River	RK 75.6	-	Yes	Yes	-	1999-2017
<b>3</b>	Lower Granite Dam Juvenile Bypass	Snake River	RK 173	Yes	-	Yes	-	2009-2017
<b>4</b>	Dworshak National Fish Hatchery	Clearwater River	RK 65	Yes (hatchery fish for experimental purposes)	Yes	-	-	2009-2017
<b>5</b>	Nez Perce Tribal Fish Hatchery	Clearwater River	RK 38	No	Yes	-	-	2016-2017
<b>6</b>	South Fork Clearwater	Clearwater River	RK 0 - 100	Yes	-	-	-	2013, 2015
<b>7</b>	Fish Creek Weir	Lochsa River	RK 0.8	Yes	-	-	-	2014, 2015
<b>8</b>	Omak Creek Weir	Okanogan River	RK 0.8	Yes		-	Yes	2003-2013

<b>9</b>	Bonaparte Creek	Okanogan River	RK 0.4	Yes		-	-	2003-2014
<b>10</b>	Cassimer Bar Hatchery	Okanogan R./ Columbia R.	RK 0/ 859	-	Yes	Yes	-	2003-2010
<b>11</b>	St. Mary's Acclimation Ponds	Okanogan River	RK 8.0	-	Yes	-	-	2011-2013
<b>12</b>	Powerdale Dam	Hood River	RK 6.4	Yes	-	-	-	2006-2010
<b>13</b>	East Fork Weir	East Fork Hood River	RK 20.1	Yes	-	-	-	2011-2013
<b>14</b>	Parkdale Hatchery	Middle Fork Hood River	RK 5.6	-	Yes	-	-	2006-2013
<b>15</b>	Shitike Creek Weir	Deschutes River	RK 0.7	Yes	-	-	-	2005-2008
<b>16</b>	Warm Springs Hatchery	Warm Springs River	RK 16	-	Yes	-	-	2005-2008
<b>17</b>	Hamilton Island	Columbia River	RK 231	-	-	Yes	-	2002-2008, 2010,2011, 2014
<b>18</b>	Westport	Columbia River	RK 72	-	-	Yes	-	2010, 2011
<b>19</b>	Aldrich Point	Columbia River	RK 75.6	-	-	Yes	-	2010, 2011
<b>20</b>	Cle Elum Spawning Channel	Yakima River		-	-	Yes (experimental group)	Yes	2015-2017
<b>21</b>	Satus Creek	Yakima River		-	-	-	Yes	2008-2017

<b>22</b>	Toppenish Creek	Yakima River		-	-	-	Yes	2008-2017
<b>23</b>	Simcoe Creek	Yakima River		-	-	-	Yes	2008-2017
<b>24</b>	Ahtanum Creek	Yakima River		-	-	-	Yes	2008-2016
<b>25</b>	Big Creek	Yakima River		-	-	-	Yes	2008-2016
<b>26</b>	Cowiche Creek	Yakima River		-	-	-	Yes	2008-2016
<b>27</b>	Little Rattlesnake Creek	Yakima River		-	-	-	Yes	2008-2016
<b>28</b>	Nile Creek	Yakima River		-	-	-	Yes	2008-2016
<b>29</b>	Quartz Creek	Yakima River		-	-	-	Yes	2008-2016
<b>30</b>	Bumping River	Yakima River		-	-	-	Yes	2008-2016



## **Yakima River Basin**

The Yakima River is approximately 344 km in length and enters the Columbia River at RK 539. The basin is 15,928 km<sup>2</sup> and average discharge is 99 m<sup>3</sup>/s. Summer steelhead populations primarily spawn upstream from Prosser Dam in Satus Creek, Toppenish Creek, Naches River, and other tributaries of the Yakima River (TRP 1995).

### **Chandler Juvenile Collection Facility (Yakima River)**

Some post spawn steelhead (approximately 20%) migrating downriver are entrained in an irrigation canal and collected at the Chandler Juvenile Monitoring Facility (CJMF a.k.a. Chandler Juvenile Evaluation and Monitoring Facility CJEMF)) that screens migratory fishes away from the canal. The entire kelt collection for the Yakima River is made at CJMF.

### **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*). All kelt rearing is conducted at Prosser Hatchery in 20' x 5' circular tanks.

### **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. The facility is located on the Yakima River near the town of Cle Elum, WA (Figure 2). In 2000, an artificial stream 127m x 7.9 m wide was built at the CESRF to research salmon in the Yakima Basin. We evaluated the suitability of this channel for testing reproductive success of kelt steelhead.



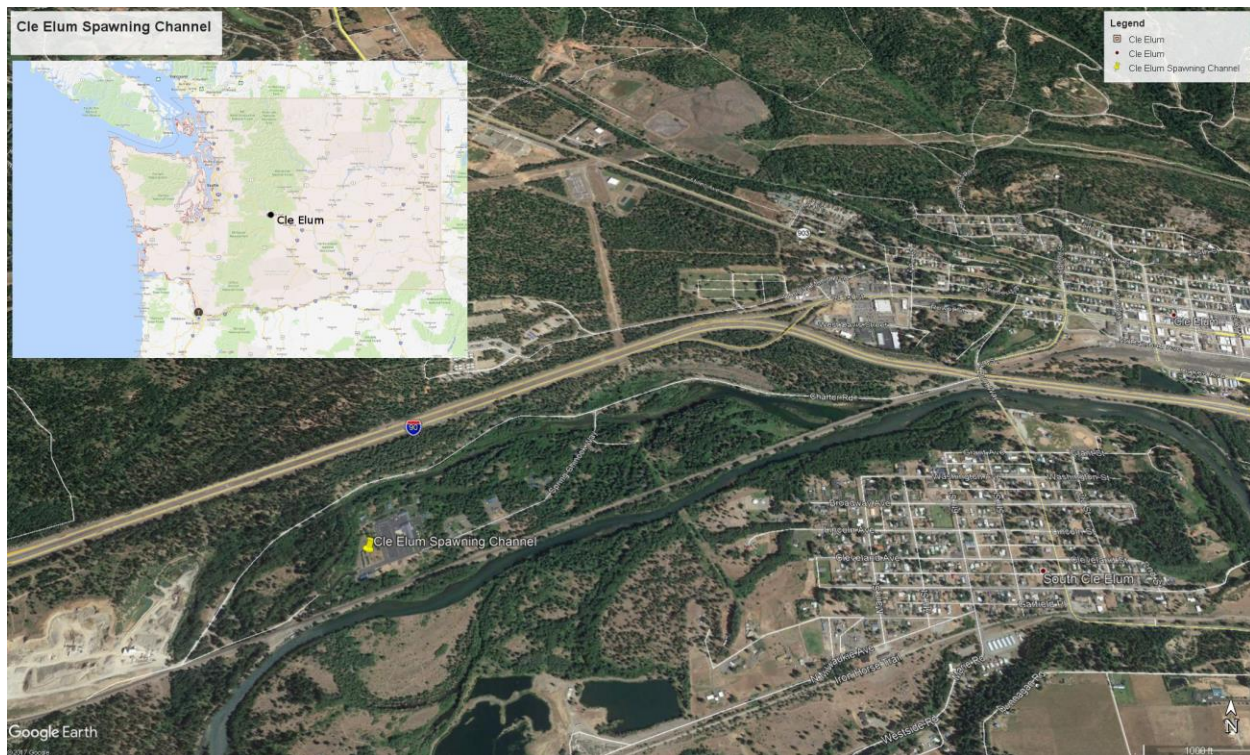


Figure 2: Cle Elum Spawning Channel and Cle Elum, WA.

## Snake River Basin

The Snake River watershed is the tenth largest among North American rivers, and covers almost 280,000 km<sup>2</sup> 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<sup>3</sup>/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<sup>3</sup>/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

Lower Granite Lock and Dam is a run-of-the-river dam on the Snake River (RK 173), 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 the 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) where we collect them.

#### 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). Kelts from Lower Granite and hatchery origin fish have been reconditioned at this facility since 2009. As of 2016 most of the kelts reconditioned at this location are hatchery fish that returned to the hatchery. These fish are surrogates for wild fish and are used for physiology experiments associated with rematuration and kelt life history. A small portion of the Lower Granite Dam kelts depending on capacity at Nez Perce Tribal Hatchery, are trucked and reconditioned at this location.

#### Nez Perce Tribal Hatchery

Starting in mid-2016 kelt reconditioning tanks were established at the Nez Perce Tribal Fish Hatchery site situated at Nez Perce Tribal allotment site 1705, located 38 km above the mouth of the Clearwater River. This Nez Perce Tribe managed facility was constructed in 2002 and primarily used to supplement spring and fall chinook (*O. tshawytscha*) in the Clearwater River. The majority of steelhead kelts captured at Lower Granite Dam are reconditioned here.

# **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, passive integrated transponder (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 2017 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 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 including 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. All data are uploaded to a central kelt database at CRITFC.

#### **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 monitor 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 leads into the kelt receiving tank. Staff from the Nez Perce Tribe (NPT), processed fish diverted into the receiving tank. Kelt steelhead judged to be in good or better condition, with intact adipose fins, and >63cm are collected and trucked to NPTH for reconditioning. 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.

#### Dworshak National Fish Hatchery (Brood Air Spawning)

Fish volitionally entered the adult ladder at the DNFH (Table 1, site 4), crowded mechanically into collection baskets, and anesthetized in tricaine methanesulfonate (MS-222) or Aqui-S®. However, several of the air-spawned fish had been anesthetized with carbon dioxide during the previous weeks for ladder counting and fish sorting. Unfortunately, the use of carbon dioxide presents sub-lethal stresses that are likely to be adverse to survival of the kelts (Iwama et al 1989). Sorted steelhead were placed on to a large stainless-steel table 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.

## **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 to 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 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. From 2000-2017 we captured a total of 19,526 downstream migrating kelts at the LGD and 12,403 at CJMF. The Columbia River steelhead run in 2016-17 was low, consequently in 2017, steelhead kelt collections were lower at CJMF (133 kelts) and LGD (1,091 kelts) ([Appendix A1a](#)).

Since 2011, 973 kelt steelhead were retained for reconditioning from collections at LGD and 293 fish survived to the first fall. Since 2000, 9,797 kelt steelhead were retained for reconditioning from collections at CJMF and 4,116 fish survived to the first fall. All Snake River collections were made LDG in 2017, however an additional 154 kelts were collected and reconditioned from the South Fork Clearwater and Fish Creek were used in previous years.

Long-term reconditioning survival averaged 42% at the Prosser Fish Hatchery (PFH) over the last 18 years. The reconditioning survival rate for wild Snake River kelts from 2013 through 2017 is 38%. These data indicate that the Snake River kelts are capable of surviving reconditioning and repeat spawning despite a longer migration and larger body size that was previously suggested might hamper reconditioning efforts (Keefer et al. 2008). Survival during the initial years (2011-12) was compromised as a result of poor water quality detailed in previous reports (Hatch et al. 2012 and Hatch et al. 2013).

Natural and artificially reconditioned kelts can pursue two alternative pathways toward rematuration and repeat spawning. One pathway is termed consecutive spawning where individuals remature and proceed to spawn in the next spawn cycle. The other pathway is termed skip spawning where individuals remature and proceed to spawn two years after their previous spawning. To illustrate, kelts collected in 2017 could spawn again in 2018 as consecutive spawners or wait until 2019 and spawn as skip spawners. The proportion of consecutive and skip spawners in a cohort varies annually and is detailed in Chapter 3, but in general Yakima River fish predominately follow the consecutive spawner pathway and the majority of Snake River kelts follow the skip spawner life history.

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 (2000-17) 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.

Additional study is prudent to evaluate reconditioning strategies for skip spawners. Our current approach is to hold the fish for an additional year in the hatchery and then release them. This scenario works, but there is additional mortality (5 to 40%) during the second year in the hatchery as well as continued facility operational costs. Investigations should focus on 1.) further development on techniques to screen fish for life history preference so skip spawners could be identified as earlier as possible in the reconditioning phase; and, 2.) investigate varying release strategies such as transporting fish to the estuary for release in the fall and in the spring of year following the first summer of reconditioning.

### **Summary Research-Scale Efforts to Address RPA 33**

We are 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. Twenty-two fish were released in 2016 and 98 fish were released in 2017. The 2017 release of 98 remature fish was composed of 77 skip spawners, which have fecundities approximately 1.51 times maiden fish, and 21 consecutive spawners that have fecundities approximately 1.27 times maiden fish ([Chapter 2.B](#)). Thus the 98-fish released, in

productivity terms equaled approximately 143 maiden fish. Table (1A.1) summarizes collections and releases associated with RPA 33.

Table (1A.1) summarizes all collections and releases associated 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	1.8%	2	-	-	-
2011	S.F. Clearwater	-	-	-	-	-	-	-
2011	Fish Creek	-	-	-	-	-	-	-
<b>2011 (subtotal)</b>		<b>111</b>	<b>2</b>	<b>1.8%</b>	<b>2</b>	<b>-</b>	<b>-</b>	<b>2</b>
2012	Lower Granite Dam	124	10	8.1%	10	-	-	-
2012	S.F. Clearwater	-	-	-	-	-	-	-
2012	Fish Creek	-	-	-	-	-	-	-
								-
<b>2012 (subtotal)</b>		<b>124</b>	<b>10</b>	<b>8.1%</b>	<b>10</b>	<b>-</b>	<b>-</b>	<b>10</b>
2013	Lower Granite Dam	110	57	51.8%	57	-	-	-
2013	S.F. Clearwater	24	12	50.0%	12	-	-	-
2013	Fish Creek	-	-	-	-	-	-	-

<b>2013 (subtotal)</b>		<b>134</b>	<b>69</b>	<b>51.5%</b>	<b>69</b>	<b>-</b>	<b>-</b>	<b>69</b>
2014	Lower Granite Dam	110	34	30.9%	34	-	-	-
2014	S.F. Clearwater	-	-	-	-	-	-	-
2014	Fish Creek	12	3	25.0%	1	2	2	-
<b>2014 (subtotal)</b>		<b>122</b>	<b>37</b>	<b>30.3%</b>	<b>35</b>	<b>2</b>	<b>2</b>	<b>35</b>
2015	Lower Granite Dam	22	11	50.0%	8	3	3	8
2015	S.F. Clearwater	35	7	20.0%	4	3	0	4
2015	Fish Creek	83	25	30.1%	10	15	15	12*
<b>2015 (subtotal)</b>		<b>140</b>	<b>43</b>	<b>30.7%</b>	<b>22</b>	<b>21</b>	<b>18</b>	<b>24</b>
2016	Lower Granite Dam	227	120	52.9%	19	101	77	22^
<b>2016 (subtotal)</b>		<b>227</b>	<b>120</b>	<b>52.9%</b>	<b>19</b>	<b>101</b>	<b>77</b>	<b>37*^</b>

2017	Lower Granite Dam	269	59	21.9%	21	58	TBD 2018	98^
<b>2017 Subtotal</b>		269	59	21.9%	21	58	TBD 2018	98^
<b>Grand Total</b>		1003	340	28.2%	178	182	97	140
				*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**

An important point in ISRP memorandum (ISRP 2014-9) was to clarify how many juvenile and F1 adults should be sampled to detect meaningful differences in the breeding and reproductive success of HOR, NOR, and reconditioned NOR females. It is very difficult to conduct these highly quantitative evaluations in natural systems, therefore 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). This effort tested the utility the spawning channel to conduct a similar experiment to observe spawning behavior of artificially reconditioned kelt steelhead.

Several elements are different between evaluating chinook salmon and steelhead reproductive success, for steelhead substrate size is smaller and fish must be contained in the channel almost 4 times longer than for chinook salmon. This extra holding time required creating more adult fish holding areas and allowed for much more sedimentation, predation events, and fluctuating natural conditions (flood events and low water years) than was experienced in the chinook salmon experiments.

Our effort focused on two phases. First, adapting the channel for steelhead, including reducing gravel size and adding cover for adult holding along with other logistical elements to ensure that the system could be used for a quantitative experiment. The second phase was conducting the quantitative reproductive success experiment. We developed a study design and obtained 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 included: U.S. Fish and Wildlife Service, BPA, WDFW, and NOAA through the Cle Elum technical team approval process. Long-term study hypotheses included 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; and, 3. Spawning behaviors of reconditioned kelt steelhead are similar to maiden steelhead.

#### **Study Site**

[Please see Study Area section for site description.](#)



## Methods

### Channel Description and Modifications

The Cle Elum spawning channel was originally designed for optimal spring Chinook spawning conditions (Schroeder et al. 2008) (Figure 2A.1). Since spring Chinook on average are larger (length and weight) than steelhead, the gravel sizes were slightly too large for some of our smaller female kelts based on the Fredle index (Lotspeich and Everest 1981). Gravel sizes 19, 38, and 50 millimeters in diameter were introduced into the channel (Hatch et al. 2016). Some additional enhancements were made after conducting substrate samples that suggested that fine sediments could be having a negative impact on egg survival (Hatch et al. 2016). A large log was placed into the channel to trap sediments in the uppermost section (1-1) (Figure 2A.2). Additional cover was provided for fish with the construction of bank overhanging covers for each group and the addition of extra floating covers to the sections which complemented the already existing 2 that we had in place since 2015. Resistance boards were placed in higher position to help catch any additional sediment. We also expected that the habitat improvements besides helping produce juveniles would have the added benefit of helping adult fish recover and reduce stress after handling and being held in an artificial channel.



Figure #: Cle Elum Spawning Channel.

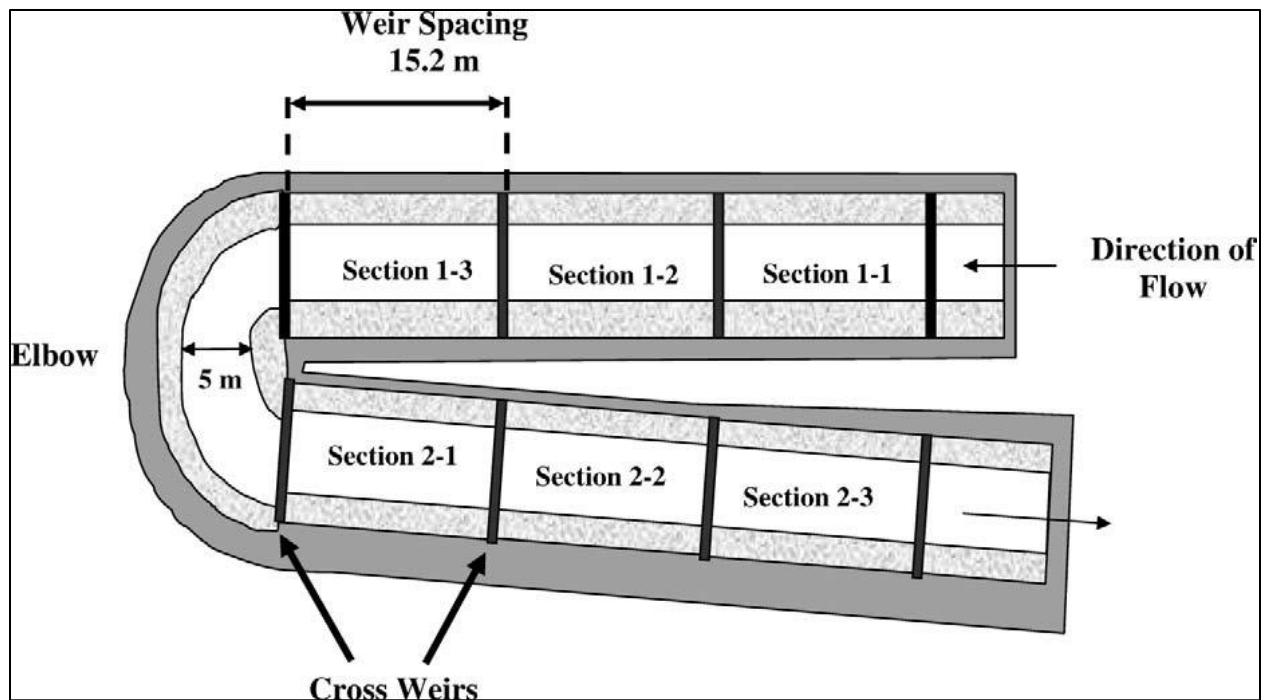


Figure 2A.2: Cle Elum spawning channel setup. Figure used with permission from North American Journal of Fisheries Management.

### Adult Collections and Stocking

One of our study goals was to eventually compare reproductive success of presumably-maiden steelhead with artificially-reconditioned kelt steelhead. Because Cle Elum Hatchery is located in the upper Yakima River basin, to prevent any potential transgression from fish held in the channel and the river population, we restricted our source fish to upper Yakima River or Naches River stocks. Further we segregated upper Yakima River fish from Naches River fish in the spawning channel by weir and fish trap placement.

We angled to collect maiden steelhead from the Naches River (males for 2015-2017, and females only in 2016) and to collect resident male fish from both the Naches and Upper Yakima rivers. Angling began on or near the transfer date to the channel, which typically happens in early to mid-February and lasted through most of March in most years.

Kelt steelhead were collected at the Chandler Juvenile Monitoring Facility ([CJMF](#)) and placed in the Prosser [long-term reconditioning](#) program. Upon entry into the reconditioning program, all kelts were scanned for PIT tags. Fish identified as either Upper Yakima or Naches origin fish, based off their juvenile or adult detection histories, became candidates for the channel. Since the two populations (Naches and upper Yakima) are closely related genetically (Frederiksen Yakima VSP project presentation) 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 was that progeny would be released back to their streams of parental origin. Only

females that were identified as mature by blood hormone assay analysis were placed into the channel. In both 2015 and 2016 fish were separated only by the elbow section with Naches fish in the lowermost sections 2-1 through 2-3 and Yakima fish in the uppermost sections 1-1 through 1-3 (Figure 2A.3). This gave each group 3 sections available for spawning. In 2017, the upper Yakima origin fish were limited to the upper channel section 1-1 and 1-2, while Naches origin fish were restricted to the channel sections 2-1 and 2-2 (Figure 2A.4). Separation of the channel into two sections was done so that we could accommodate multiple juvenile traps meant to increase collection efficiency and determine if juveniles could be kept separately.

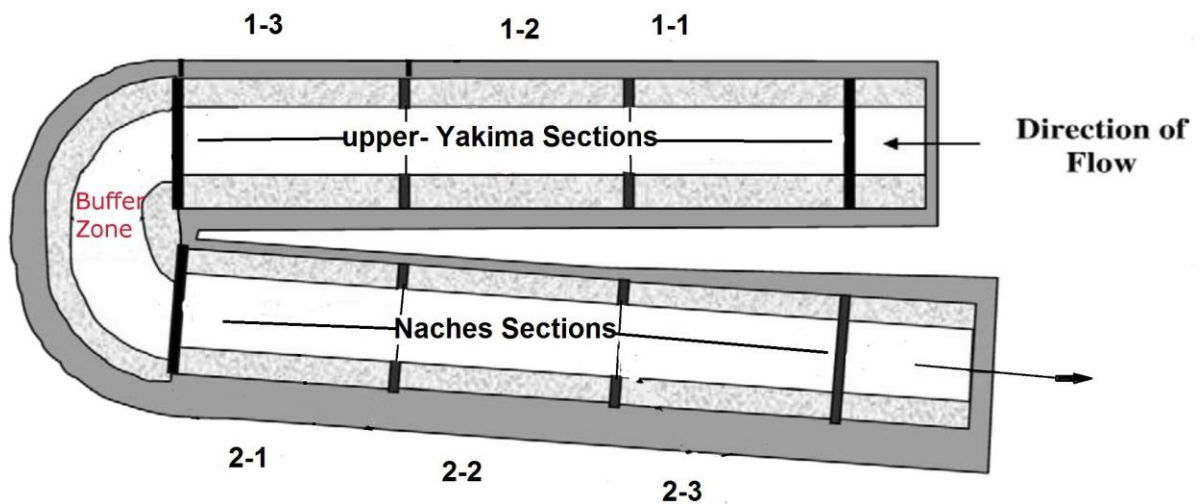


Figure 2A.3: Steelhead kelt placement into channel 2015-16.

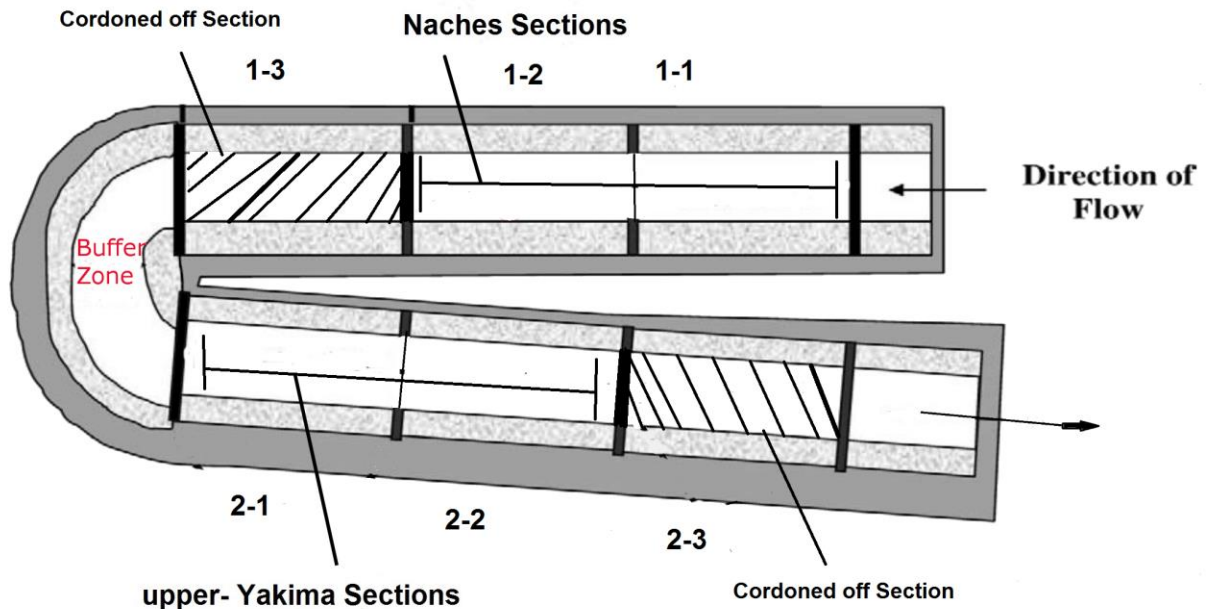
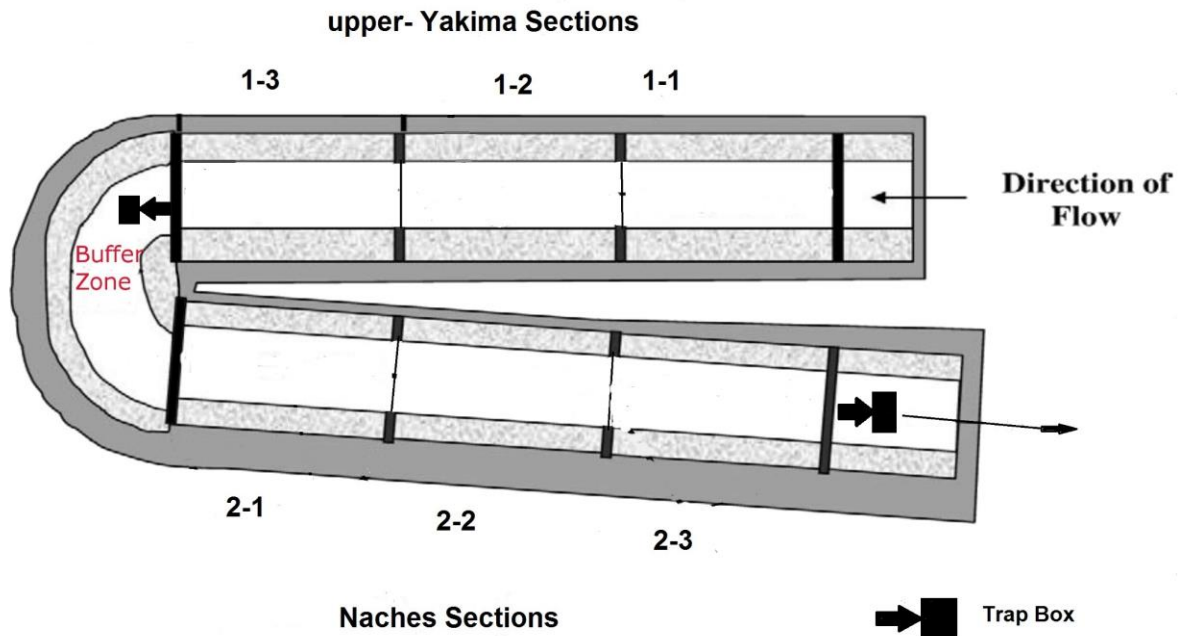


Figure 2A.4: Steelhead kelt placement into channel 2017.

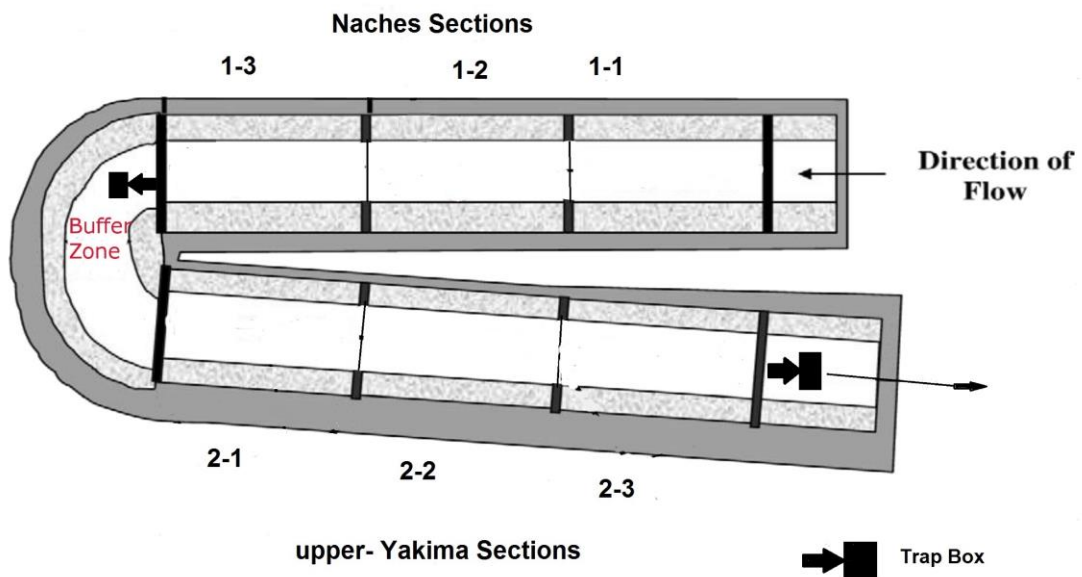
All anadromous mortalities were necropsied and biopsy of internal organ tissues sampled to determine cause of mortality. Remaining eggs were quantified to help in evaluating spawning success. Surviving maiden or resident males were returned near stream of origin and any recovered kelts in good shape were returned to Prosser for additional reconditioning.

### Juvenile Collections

Juvenile samples were passively collected using box traps with netted tubes, located at the downstream end of the two channel sections (Figure 2A.5). These boxes were initially designed for the capture of juvenile chinook. In 2016 we constructed and installed a new set of trap boxes that were better designed for capturing smaller steelhead progeny. In 2017, an additional trap in section 2-3 was installed to capture any fish which may have managed to bypass the first two traps (Figure 2A.5). The traps were installed to coincide with fry emergence of the first redd observed in the channel, based on available thermal units which came from intake temperatures. 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.

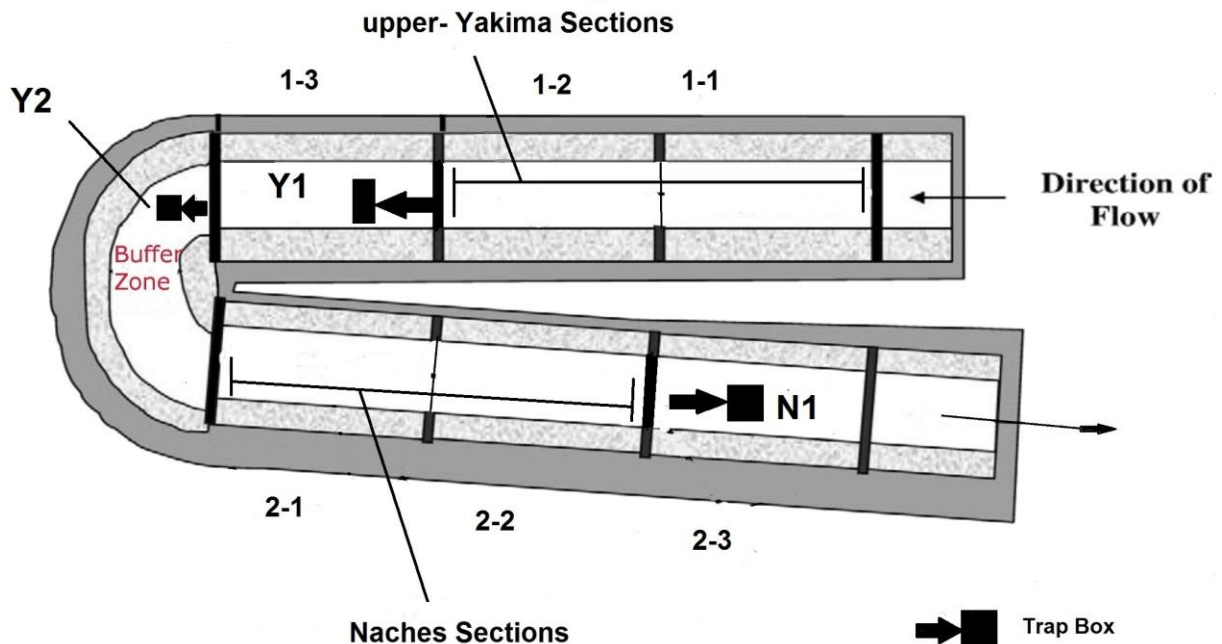


2015



2016





2017

Figure 2A.5: Trap box setup 2015-2017.

### Genetic Analysis

Fin tissue samples were collected and stored dry on whatman paper, paper slips in coin envelopes, or in ethanol vials for the preservation of DNA. Genetic analysis was conducted at the Hagerman Fish Culture Experiment Station in Hagerman, ID. The DNA was then extracted from tissue samples using chelex beads. Genotyping efforts utilized 192 Single Nucleotide Polymorphism (SNP) markers and GTseq methods (Campbell et al 2015) using an Illumina Nextseq 500 instrument. 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 Logarithm of odds (LOD) value.

### Substrate and Hydrology

#### Substrate Composition

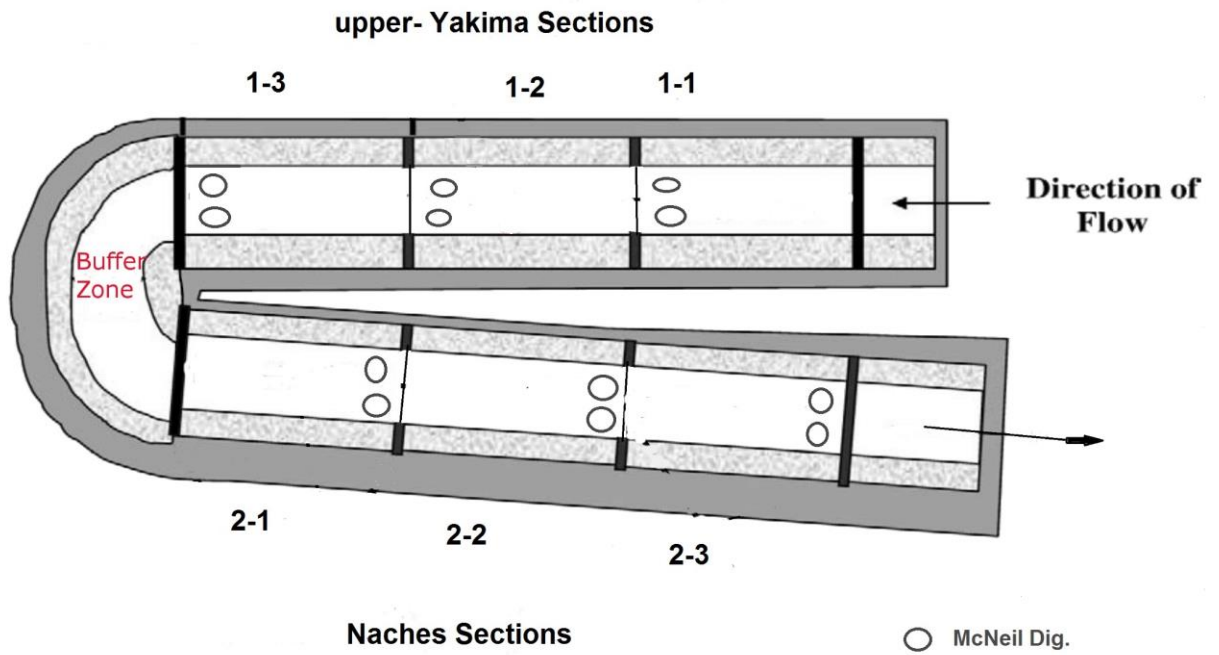
After the initial trial in 2015 began, there was concern amongst the group (Yakama Nation /CRITFC/Washington Department of Fish and Wildlife) that perhaps the uppermost portion of the channel may have high levels of fine sediments that may be deposited on eggs during and after spawning had occurred. This part of the Yakima River has high sediment levels during the freshet and no method exists to filter sediment at the facility. Much of literature regarding acceptable levels of fine sediments, defined as those greater than .855mm, suggests that negative impacts to eggs begins when the fine sediments exceed more than 10% of the total

substrate (Tappel and Bjornn 1983; Jensen et al. 2009). We began collecting substrate samples utilizing a McNeil sampler utilizing methods developed by McNeil and Ahnell 1964.

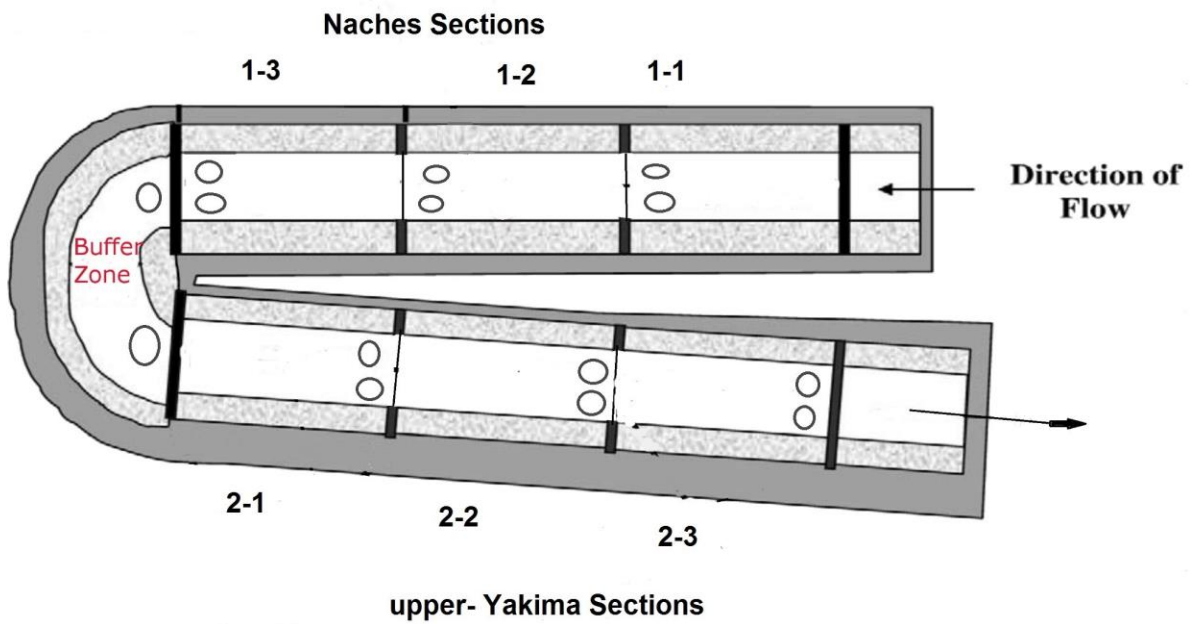
Two sites were chosen in each section 1-3 and 2-1, both redd, and non-redd (in the case of the elbow section both were non-redd index sites) samples were collected for a total of 6 samples from the channel (Figure 2A.6). We do not have data for channel composition and how much fines (<.855mm) were present before steelhead kelts began constructing redds in 2015. In 2017, we continued to measure the amount of fine sediment in the channel at the beginning (February), mid-point (June), and end of the study (August). The McNeil core sampler was used 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).

### Hydrology

We also looked at trying to determine the amount of upwelling and downwelling utilizing mini-peizometers in the first year of the experiment (2015). Unfortunately, due to an impermeable barrier that lies underneath the gravel we learned that upwelling and downwelling does not have an appreciable measure on eggs while in the channel. Methods for installing and assessing vertical hydraulic gradient (VHG) were modified from a previous study (C. Baxter, Hauer, and Woessner 2003). VHG is a dimensionless metric indicating relative strength of upwelling (positive values) or downwelling (negative values) of subsurface flow. After installation and initial measurement, each minipeizometer was visited multiple times in approximate 10 min intervals to ensure water levels had come to equilibrium.

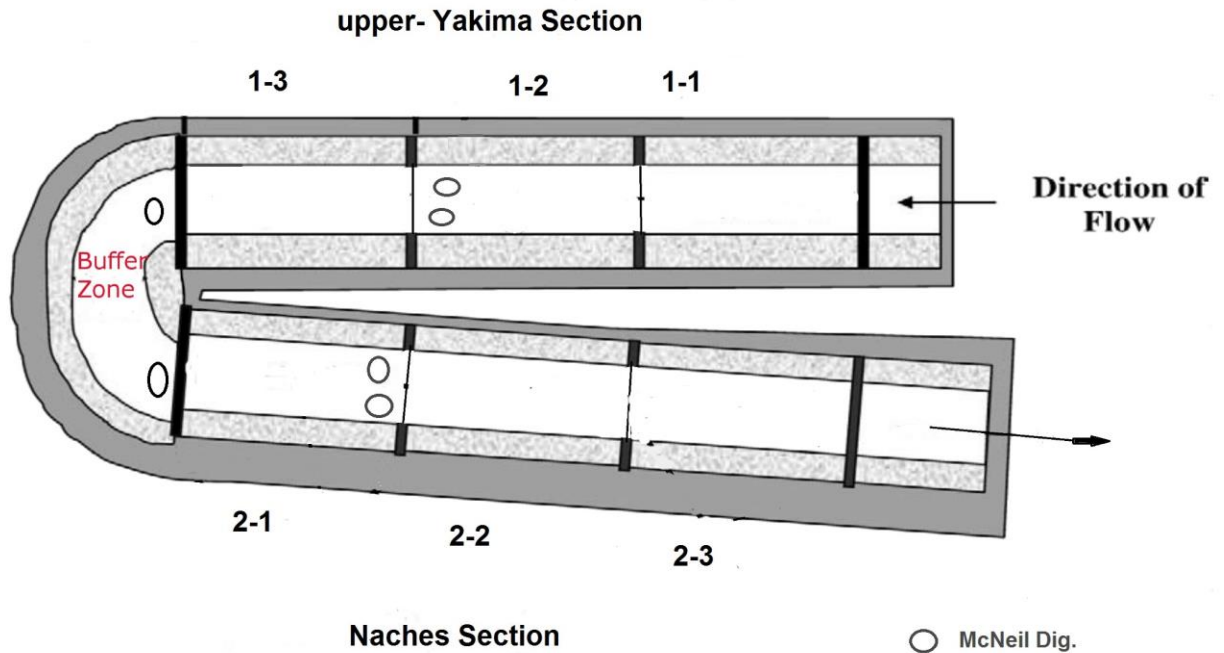


2015



2016





**2017**

Figure 2A.6: Cle Elum spawning channel McNeil sampling locations 2015-2017.

### Water Temperature

Water temperatures were tracked utilizing intake thermometer records and a HOBO temperature station located at the head of the channel (section 1-1). We used water temperature to estimate when juvenile fish were likely to emerge from eggs and effectively time the installation of capture gear to sample emerging juvenile fish. We installed juvenile fish traps after the first observed redd had received 1000-1200 thermal units. (Quinn 2005; Burton and Little 1997).

## Results

### Adult Collections, Stocking, and Recoveries

2015-2017

We stocked 16 to 20 remature female kelts in the channel each of 3 years (Table 2A.1). Additional maiden and resident fish were stocked at various levels during the study (Table 2A.1). Stocking locations remained consistent during 2015 and 2016, with fish confined to smaller areas to assist with determination of trap efficiencies

Table 2A.1: Release of fish by year, treatment type, origin, sex, and total number released.

Study Year	2015											
	Reconditioned Kelts				Maiden				Resident			
Release Group	Naches		Yakima		Naches		Yakima		Naches		Yakima	
Origin												
Sex	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Number Released	10 (5) *	0	10 (1) *	5	-	-	-	-	-	12	-	15

Study Year	2016											
	Reconditioned Kelts				Maiden				Resident			
Release Group	Naches		Yakima		Naches		Yakima		Naches		Yakima	
Origin												
Sex	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Number Released	14	2	2	-	4	5	-	-	-	8	-	4

Study Year	2017											
	Reconditioned Kelts				Maiden				Resident			
Release Group	Naches		Yakima		Naches		Yakima		Naches		Yakima	
Origin												
Sex	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Number Released	11(1) *	2	9(1) *	1	-	8	-	-	-	9	-	10
*(includes known immature fish)												

Most fish expired by the end of the spring with only 12% of fish surviving on average annually by June. In 2015 and 2016 prespawn mortality was nearly identical among years with it averaging around 29% of the total mature female spawners but in 2017 it was much higher at around 61%. We are not certain which conditions may have led to an increase in the amount of prespawn mortality but it significantly cut our ability to contribute towards a quantitative analysis. Necropsies resulted in no direct line of mortality (USFWS Fish Health Reports 2015-2017). We observed that approximately 24% of the mortalities in 2015-16 had fungal infections present, while we had an increase in infections in 2017 with about 41% of the observable mortalities having fungal infections present at time of mortality. In the prespawn mortalities, with the exception of immature fish, typically there were eggs present in varying levels as low as a couple hundred grams of eggs still in the skein to over 700 grams of eggs that had dropped from the skein and were ready to be deposited. In what we considered post spawn mortalities, female fish were observed to have at least one skein deposited or an extremely small number of eggs present (couple of hundred to as low as 30 remaining)

## Spawning

Successful redd construction by reconditioned kelts was observed in all years (2015-2017) and also including maidens in 2016 (Table 2A.2). Spawning started as early as late February for both Yakima and Naches kelts and ended as early as late March but typically ended by late April/early May for both groups.

Table 2A.2: Redd counts by year and first- last build date.

Year	2015		2016		2017	
Section	Naches	Yakima	Naches	Yakima	Naches	Yakima
<b>Redds Counted</b>	2	7	5*	2	6	2
<b>1st Redd Const.</b>	24-Mar	28-Feb	29-Feb	21-Mar	12-Mar	16-May
<b>Last Redd Const</b>	30-Mar	4-May	27-Apr	1-Apr	10-May	19-May

- Includes maiden fish redds.

Redds tended to be constructed primarily in the middle area of each section (1-2 and 2-2) in 2015 and 2016 and all spawning was confined to those areas in 2017 (Figure 2A.7,8, &9).

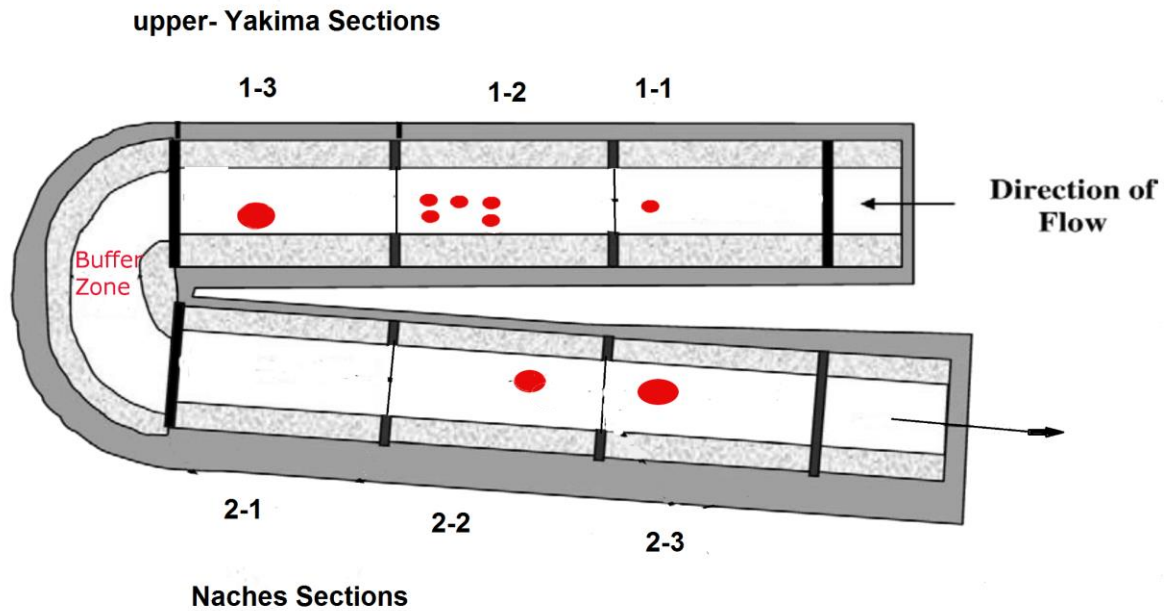


Figure 2A.7. Site of redd locations at Cle Elum spawning channel 2015. Circled areas represent redd locations while red dots represent individual redds found in that location. Sections start with flow direction 1-1 through 1-3 in upper 3 sections and 2-1 through 2-3 in lower 3 sections. The thick lines 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.

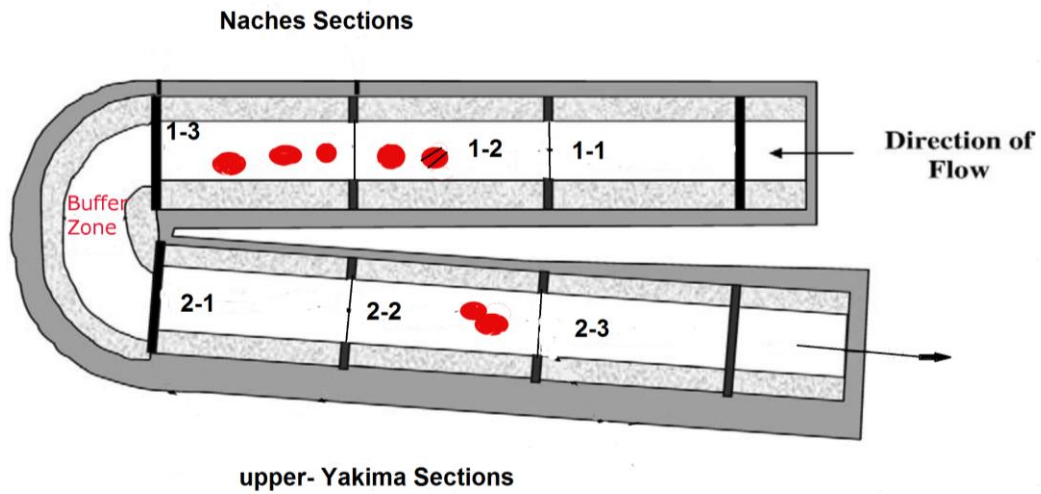


Figure 2A.8. Site of redd locations at Cle Elum spawning channel 2016. Circled red dots areas represent redd locations. The red dot with black lines represents maiden spawn areas.

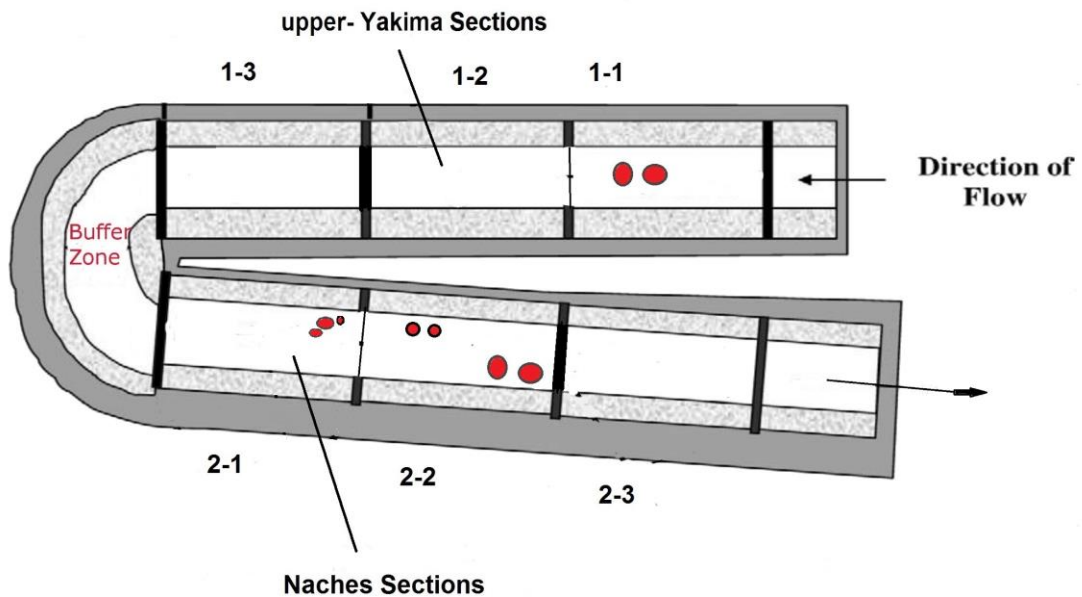


Figure 2A.9. Site of redd locations at Cle Elum spawning channel 2017. Circled red dots areas represent redd locations.

**Juvenile Collection and Release**

Juvenile traps were installed on June 1, June 2, and April 25 in 2015, 2016, and 2017, respectively (Table 2A.3). We more than doubled the number of collections of fish in from 2015 to 2016 and 2017 (Table 2A.3). This was likely due the construction of new trap boxes that were put into place in 2016. We set up a 3<sup>rd</sup> trap in 2017 at the lowermost end of the channel and found that we captured 14% of the total number of fish captured in the channel. This is probably still only a portion of what was in the channel and we likely still had fish that were able to leave the channel through the outflow. We attempted to install an outflow trap in 2016 and 2017 with limited success due to the high flow and deep channel. We managed to trap a small number of juvenile fish but our inability to stop fish from moving interstitially through the gravel kept us from being able to sample a complete representative sample of all juvenile fish.

Table 2A.3. Trap collections of juvenile by year and section.

<b>Year</b> <b>Trap Set Date</b> <b>Channel Section</b> <b>Numbers of Juveniles</b> <b>Collected in traps</b>	2015 1-Jun		2016 2-Jun		2017 25-Apr		
	Yakima	Naches	Yakima	Naches	Yakima 1	Yakima 2	Naches
	555	874	3284	3792	1203	1724	3091

Juvenile collection and holding mortalities were much reduced from previous years (28% in 2015 and 12% in 2016) and only 3.6% of the total collected fish perishing in 2017. Traps were removed on August 2<sup>nd</sup>, with the remaining juveniles collected using electrofishing methods over the following day.

All surviving Cle Elum channel juveniles were released in August just after final collections. Fish that we believed to have originated from the Upper Yakima were released just downstream of the hatchery and all Naches origin fish were released just above Parker Dam, near the confluence of the Yakima and Naches rivers.

## Genetic Analysis

The first two years we successfully were able to genotype all fish placed in the channel, although in 2017 some samples from the males were missing, mostly resident fish. These missing fish did not contribute toward parentage (no samples had unidentified parents) so these DNA collection failures did not negatively impact the study. Genotypes were generated for fish stocked in the channel including all potential spawners comprising 48 mature female reconditioned kelts, 4 maiden steelhead, 8 male reconditioned kelts, 12 anadromous males and 48 resident males (Table 2A.4). Fish that died prior to redd construction were omitted from final parentage analysis.

Table 2A.4. Genotyped potential spawners by study year.

Genotypes	Study year		
	2015	2016	2017
Kelts ♀	11	16	21
Kelts ♂	5	2	1
maiden ♀	-	4	-
maiden ♂	-	5	7
Resident ♂	27	12	9
total	43	39	38

Of 1,936 *O.mykiss* 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 offspring were successfully assigned to two adults (Table 2A.5). 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.5. Of note, offspring collected in the Yakima section were frequently 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.5 Juvenile assignments to parent origin by channel section in 2015-2017.

Year	2015		2016		2017	
Capture location	Upper Yakima Parents	Naches Parents	Upper Yakima Parents	Naches Parents	Upper Yakima Parents	Naches Parents
Yakima	221	0	179	183	178	0
Yakima 2	-	-	-	-	82	12
Elbow	9	0	0	24	2	0
Naches	230	30	0	400	4	378
End	1	1	1	1	0	0

Progeny were assigned to a total of 10 the female kelts (6 Upper Yakima, 6 Naches) and 4 anadromous maidens. For successful males there were a total of 7 kelt males (5 upper Yakima kelts, 2 Naches kelts), 12 male anadromous maiden fish (Naches) and 19 resident males (12 upper Yakima and 7 Naches) (Table 2A.6.). In the years when anadromous males (including kelts) and resident males were mixed, (Yakima 2015, Naches 2016, and both sections 2017), the anadromous males generally had the largest contribution to the genotyped juvenile production but only marginally (10% difference between resident anadromous) so with the upper Yakima population whereas anadromous males in the Naches juvenile contribution was much more disparate (anadromous contributions well over 80%). No progeny were assigned to fish not accounted for at the end of the study.

Table 2A.6. Successful adult spawners 2015-2017.

Year	2015				2016				2017			
Parent Origin	Upper Yakima Parents		Naches Parents		Upper Yakima Parents		Naches Parents		Upper Yakima Parents		Naches Parents	
Sex	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Number spawners	4K	4AK,7R	1K	2R	1K	1R	2K,4M	4R	1K	1AK,4R	3K	3AM, 1R
Female K=Kelt, M=Maiden												
Male (AK=Anadromous Kelt, AM=Anadromous Male (maiden), R=Resident												

**Substrate**

Based on previous years we believe that most of the major inputs of organic fine materials were transported into the channel beginning sometime during the freshet which typically occurs in early/mid-June (Figure 2A.10). This fine deposition is occurring during the time that kelts are constructing redds, March-May, though based on 2017 fine sediment data this was the lowest year of deposition with no sections with fish spawning above the detrimental 10% fines (Jensen et al. 2009). Kelts continued to construct the majority of redds at the inside portion of the channel tail outs. These areas typically collect lower fine sediment deposition than the outer bank portion of the sections. McNeil samples reveal that the Naches section 1-2 LB had the most fines deposition (particle sizes less than .85mm) (Table 2A.7). 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. Section 1-1 in both 2015 and 2016 were over the 10% total fines deposition on average by 1% (11% total). This section of the channel had a lone redd in 2015 (Table #). In 2017, which was an above average flow year even more so than 2016, sediment deposition was not distinctly higher than in 2015, which was an extremely low water year, which typically translates into lower sediment transport (Table 2A.3 and 2A.4). Likely the setup of the channel by utilizing the water level boards at the upper sections (1-1) helped to trap sediments and prevent it from being as big an issue as was initially feared when the project began in 2015.

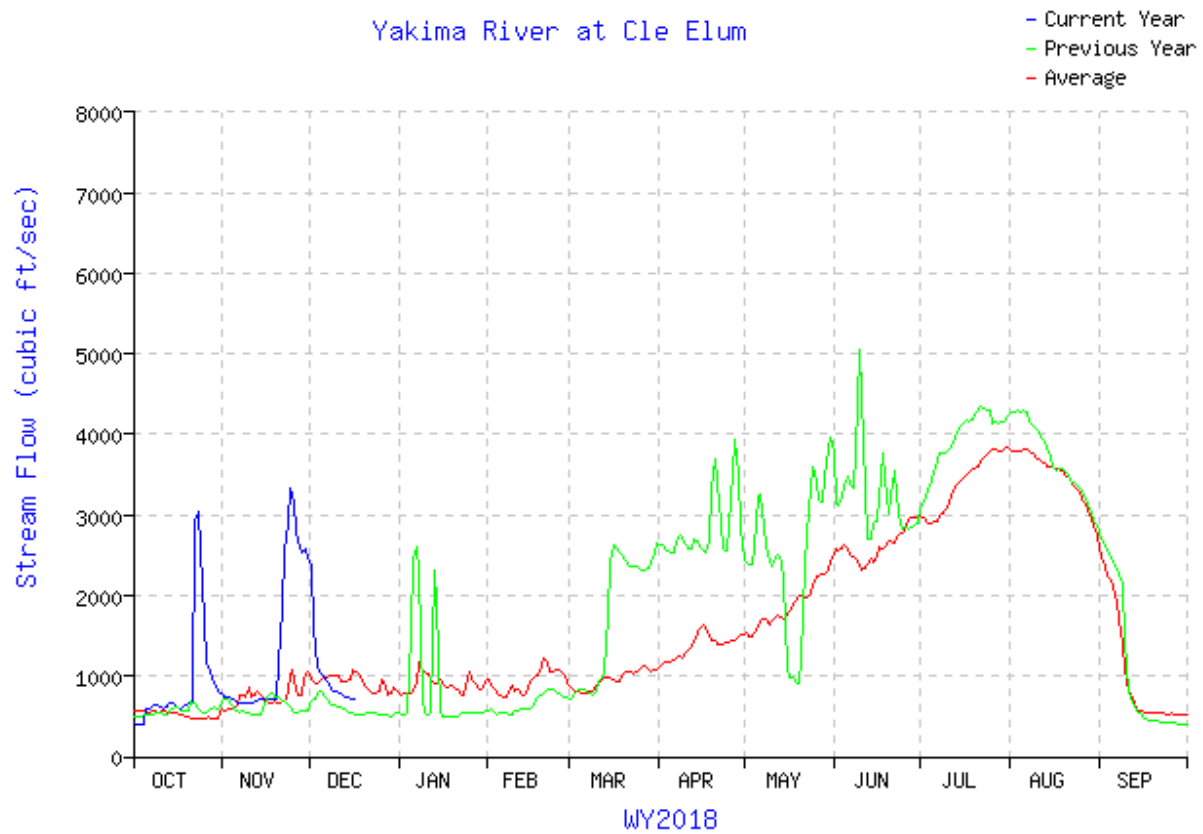


Figure 2A.10: Stream Flow of the Yakima River 2016-2017. Source US. Geological Survey.

Table 2A.7 Fine sediment deposition change from February 2017 to August 2017.

Section	
1-2 RB	-0.9%
1-2 LB	3.0%
Lower Elbow	0.8%
Upper Elbow	2.5%
2-1 RB	1.9%
2-1 LB	1.4%
avg change	1.90%

Table :2A.8 Fine sediment deposition change from August of 2015 to August of 2017

Section	
1-2 RB	-0.5%
1-2 LB	-4.4%
Lower Elbow	NA
Upper Elbow	NA
2-1 RB	2.0%
2-1 LB	1.8%
avg change	-0.3%

Table:2A.9 Fine sediment deposition change from August of 2016 to August of 2017

Section	
1-2 RB	0.3%
1-2 LB	-2.5%
Lower Elbow	-0.1%
Upper Elbow	-1.5%
2-1 RB	-2.1%
2-1 LB	-1.8%
avg change	-1.3%

## Water Temperature

Water temperatures were colder than they were in 2015 and 2016 by approximately 2 degrees in 2017, which likely lead to the later spawning than in previous years. Typically, kelts emerge from the egg and begin to move around sometime around 1000 Temperature units. Utilizing the hobo temperatures, we believe that we were successful getting traps placed before juvenile

fish emerged from the gravels. In 2015 and 2016 based on the latent HOBOT data it appears that we were likely a couple of weeks from sampling the earliest emerging fish. We also have anecdotal evidence that juveniles were already present when traps went in during those years. Whether we missed sampling these fish or not with the late trap install is difficult to parse out but our largest concern is how accurate our representative sample is. If it is not wholly representative it is difficult to quantitatively discern how much each spawner contributes.

## **Discussion**

ISRP memorandum (ISRP 2014-9) stated a need to clarify how many juvenile and F1 adults should be sampled to detect meaningful differences in the breeding and reproductive success of HOR, NOR, and reconditioned NOR females. It is very difficult to conduct these highly quantitative evaluations in natural systems, therefore 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). This effort tested the utility of the spawning channel to conduct a similar experiment to observe spawning behavior of artificially reconditioned kelt steelhead. Our investigation focused on three main areas including: 1. habitat needs, modification and monitoring; 2. fish collection and stocking; and, 3. juvenile collection and assignment.

To improve the spawning habitat we placed gravel sizes which favored steelhead instead of chinook. The following year, logs were placed in the upper most part of the channel to reduce flow and promote sediment deposition to reduce the amount of sediment in the spawning areas. Plywood sheets and floats were also placed in the channel to provide cover for holding adults. Additionally, we monitored fine sediment composition by sampling at the beginning and end of each season. Water temperature and flow was also continuously monitored. The channel was pressure washed and cleaned at the end of each field season so that spawning fish had a clean channel and that sediment buildup would not be an issue for eggs.

The Cle Elum Spawning Channel out flow connects to the upper Yakima River. Upper Yakima River steelhead are closely associated with Naches River steelhead and best management practice is to maintain this distinctness. Therefore, we limited stocked fish to ones from the Upper Yakima or from the Naches River, based on capture location or Genetic Stock Index (GSI). The upper Yakima River steelhead population is the lowest abundance of the major spawning aggregates (Fredrickson et al. 2015) in the Yakima River. All adult steelhead in the upper Yakima River pass over Roza Dam where they are trapped and PIT tagged, therefore, kelts from the upper Yakima River can be identified in mixed collections. Due to low steelhead escapement in the upper Yakima, kelt collections at CJFMF range from 10 to 30 fish annually. We used this pool of kelts for our spawning channel experiments. Since the upper Yakima supply of kelts is low and limited, we augmented the study with Naches River kelts identified by either PIT tag or GSI. Using fish from different streams necessitated us to maintain separation of the two stocks in the spawning channel. We were able to maintain this separation of adult fish in the channel, but some unexplained losses occurred, these were likely due to predation and

prespawn mortality which also occurred. Water temperature differences between Prosser Hatchery where the kelts were reconditioned, and the upper Yakima River, presented some difference in maturation schedules complicating the use of anadromous maiden fish in the channel. The additional timing that was needed to hold kelts at Prosser Hatchery and periodic shutdowns of the canal which feeds river water to the hatchery complicates matters. Ordinarily kelts are released in the fall which coincides with the arrival of maiden fish so these fish can synchronize maturation. Unfortunately, we needed to hold kelts over the winter at Prosser to have enough fish which we initially thought would be adequate from the Yakima and Naches for the experiment. Usually we start seeing flash flooding in mid-January which typically causes canal shutdowns, this source of water is which feeds our kelt tanks and has the appropriate tempered water. When this source of water is shutdown we need to augment with well water which is anywhere to 10-15 degrees warmer than Yakima water temperatures. This may cause asynchronization between maiden and kelt spawners which may impair pairing between females and males. It should be mentioned that these spawning times are not out of the norm for these populations (Temple et al 2015). Additionally, handling and holding stressors may have contributed to depressed immune system response and resulted in prespawn mortality via secondary fungal infections. Though even in natural settings, prespawn mortality is significant (Bowerman et al. 2016). Pre-spawn mortality was significant in this study, as evidenced by the number of fish with developed eggs still in skeins.

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. The biggest issue which complicates our ability to accurately compare kelts and maiden fish is that maiden spawn timing was extremely truncated and compared to the kelts which were much longer in spawning duration. 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/harassment 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. In 2017, we scaled back with placing maiden fish in the channel and focused on trying to improve the juvenile collections.

In 2015, reconditioned kelts successfully spawned in the channel and produced progeny that assigned back to spawners. That year we used juvenile traps that previously were used in a spring chinook salmon study. The efficiency of those traps was low as evidenced by a large proportion of progeny from the upper channel section being caught in the lower channel section trap as evidenced by genetic parentage assignments. Also, no evidence could be found that the parents had bypassed barriers and spawned in the lowermost sections This was done due to odd results of genotyped juveniles and parentages that suggest that juvenile traps may have been placed too late or that fish were making their way around barriers and traps.

Minimizing the “leaking” progeny is important because we had a very limited number of adults, either reconditioned kelts or maiden fish, that could be used for this experiment. To address this leaking issue, we fabricated new traps used in 2016 and 2017. The new traps caught much higher numbers of juveniles (Figure 2A.5) (Table 2A.3 and 2A.5), however we continued observing leaking. Therefore, in 2017 we placed a series of two traps below the upper section and a single trap in the lower section. We again found progeny from the upper section captured in each of the three traps. This leaking makes quantifying reproductive success from specific channel sections very difficult because of increased error and makes it very difficult to compare groups from the upper and lower channel sections, particularly when spawner sample sizes are low. This inability to maintain separation between stocks in the upper and lower sections of the spawning channel and the low abundance of upper Yakima River kelts makes the current system infeasible for conducting a quantitative reproductive success study. The next chapter details our findings from investigating reproductive success *in situ* in Satus and Toppenish Creeks. We believe this work will adequately address the ISRP questions and benefit from being in the natural environment.

A difficult issue that we had was trying to ensure that the majority of juveniles were captured so that we would have a representative sampling of progeny produced by spawning kelts. Many Naches origin juveniles were collected in the lower channel section traps, which indicates that the fish barriers and traps were not close to being 100% effective likely due to steelhead progeny moving interstitially through the substrate. Additionally, in 2015 and 2016 due to location of water temperatures the traps were likely installed after fish had emerged and had moved downstream, based on hobo vs. water intake temperatures. This also suggests that the lower section juveniles likely moved downstream and out of the channel undetected. This issue makes it problematic for conducting any future quantitative study. Ideally, we could go with extremely small mesh size to prevent fish from moving interstitially through the gravel, unfortunately due to the fine sediments and detritus this is not feasible as nets would easily become blocked within hours and water would flow around them allowing for juveniles to move around nets anyhow. Keeping nets clean would require an extremely labor intensive hourly cleaning regime. This issue is further compounded due to kelts not spawning necessarily along the same time lines, likely due to differentiations on an individual and possible subpopulation level. This is probably due to fish coming from differing subpopulations even within subbasins and thus partially responsible in the differing spawn timing. Another side effect of the differing spawn timing is that it may increase the exposure of fish to predation events and eggs deposited into redds into higher sedimentation rates. We feel we had adequately addressed the sedimentation issues by the result of measures taken to capture and reduce sediment load in the channel. Predation was a more difficult issue to deal with due to difficulties installing a cover near the channel to prevent mostly avian predators from harassing fish causing them undue stress.

Lastly, we did not adequately anticipate the amount of prespawn mortality that we would have. With already small numbers of fish losing fish from this has a detrimental effect on our statistical power. We thought that adding Naches fish would alleviate this but even this was

too low a number. Ideally, we would have double or triple the number of kelt spawners in the channel but due to inability to easily determine fish origin getting enough candidate fish was difficult. Also, due to poor river conditions for migration in conjunction with poor conditions for collection at CJMF we had lower than usual collection for the past 3 years. Additionally, since Cle Elum Hatchery is located in the upper Yakima River, it is necessary to use upper Yakima River origin kelt steelhead for the study and those fish are quite rare and under poor migration and collection conditions the small number of fish became even smaller. Yakama Nation and CRITFC staff also felt that not utilizing these kelts to help stabilize this already heavily impacted population for this study was not the best use of the resource.

We determined that maiden and reconditioned kelt steelhead could reproduce in the channel. In all 3 years of our feasibility study, kelts that did not perish from prespawn mortality were represented at varying levels of reproduction. As noted in the results kelt male progeny were well represented in the juvenile genotypes, while residents did not fare as well except in the case of upper Yakima grouping that did relatively well in 2015 and 2017. However, we determined that the channel was not adequate for a quantitative reproductive success study with steelhead because of our inability to collect all fry produced and the length of time steelhead needed to reside the channel that subjected them to predation, sedimentation, and other negative forces. In the end, sample sizes were too small, which leads to lack of statistically significant data with which to draw sound conclusions. In order to overcome the lack of numbers we would need to use a source with a larger spawning population. The largest source of kelts comes from both Satus and Toppenish creeks, but usage of genetically distinct fish from populations outside of the upper Yakima is not a feasible option.

Since both Satus and Toppenish creeks are our greatest source of kelt spawners in the Yakima River basin, we feel that project efforts will best be focused towards production monitoring occurring in these natural populations. Ideally, statistically valid data we obtain from natural spawning will better answer the kelt reproduction questions posited by the ISRP, then how well they perform in an artificial system and attempting to translate that into a natural setting.



## **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 100 mm 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.

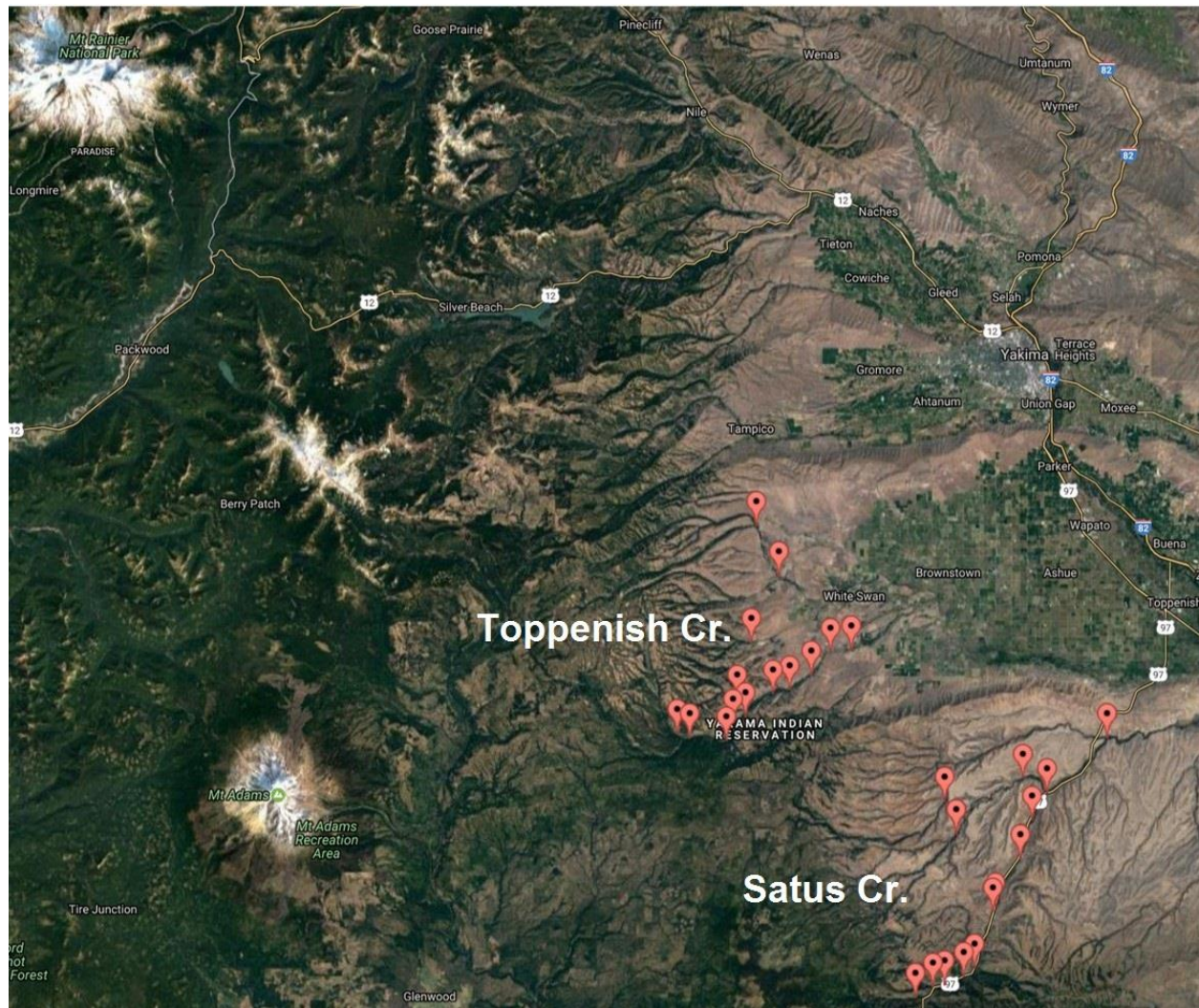


Figure: Juvenile sampling locations in Satus (lower right) and Toppenish creeks over 4 years 2013-2016.

### 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 Qiagen® DNeasy™ extraction kits or chelex extractions modified from Casquet et al (2012). 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). Genotyping efforts from 2015 on used an expanded marker panel and GTseq protocols (Campbell et al 2015) on an Illumina HiSeq 1500 or NextSeq 500 Sequencer. Prior to parentage analysis, Poor loci were removed from the dataset. Dropped loci included the sex-determining marker (OmyY1\_2SEX), three loci diagnostic for cutthroat, one loci with poor genotypes, and 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 detected at PIT-tag arrays upstream Prosser Dam. In the past years reports detections at Prosser Dam were used which provided a larger number of successfully genotyped adults. This provides more power for effective genotyping but since only Satus and Toppenish are effectively sampled for juveniles these fish have been dropped in favor of using PIT-tag detections in the aforementioned systems to accurately report relative reproductive success thus the differences in this year's report versus previous values provided in previous reports (Hatch et al. 2014, 2015, and 2016) 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 juveniles successfully genotyped at individual sites, and the corresponding number and percentage of samples assigned to at least one anadromous adult parent is shown in table 2B.1. Assignments have steadily improved over time, which at least may be attributable in Toppenish, to dropping the Willy Dick site that consistently had 0 assignments.

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

Year	2013			2014			2015			2016			Sum		
Location	Genotyped	Assigned	% Assigned	Genotyped	Assigned	% Assigned	Genotyped	Assigned	% Assigned	Genotyped	Assigned	% Assigned	Genotyped	Assigned	% Average Assignment Rate
Satus Cr.	248	59	0.24	286	64	0.22	367	137	0.37	790	288	0.37	1691	548	0.32
Toppenish Cr.	300	78	0.26	276	79	0.29	369	165	0.45	524	187	0.36	1469	509	0.35

The number of genotyped parents confirmed to have entered either Satus or Toppenish Creek is shown in Table 2B.2. Pre-spawn maidens have the greatest number of samples with a total of 228 males and 440 females. The number of Post-spawn maidens was lower with only 25 males and 136 females. Across all years, reconditioned 29 males and 196 females have been sampled and genotyped. This number will increase each year, but is limited by the number of kelts that can be collected, and mortality seen during the reconditioning process.

Table 2B.2. Number of adults genotyped.

Class	Sex	2013	2014	2015	2016	All
Pre-spawn maidens	Male	41	46	62	79	228
Post-spawn maidens	Male	3	13	7	2	25
Reconditioned kelts	Male	5	4	13	7	29
Pre-spawn maidens	Female	94	72	119	155	440
Post-spawn maidens	Female	21	44	47	24	136
Reconditioned kelts	Female	75	24	39	58	196

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. Kelt males have the lowest progeny assignments, this effect is due to the naturally low number of these individuals which do not comprise a large number of the kelts, which can sway the percentage strongly one way or the other.

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

Class	Sex	All	n	Successful Adults
				%
Pre-spawn maidens	Male	228	176	77.2%
Post-spawn maidens	Male	25	14	56.0%
Reconditioned kelts	Male	29	8	27.6%
Pre-spawn maidens	Female	440	274	62.3%
Post-spawn maidens	Female	136	73	53.7%
Reconditioned kelts	Female	196	86	43.9%

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 2.854 that of males collected as pre-spawners. While female post-spawn collection RRS was 1.135 times that of pre-spawn collection, reconditioned kelt RRS was slightly lower at 0.937 for a LRS of 2.072 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	Sex	Progeny Assigned				
		Genotyped Adults	N	Per	RRS	LRS
Pre-spawn maidens	Male	228	176	0.772	1.000	
Post-spawn maidens	Male	25	14	0.560	1.616	
Reconditioned kelts	Male	29	8	0.276	1.239	2.854
Pre-spawn maidens	Female	440	274	0.623	1.000	
Post-spawn maidens	Female	136	73	0.537	1.135	
Reconditioned kelts	Female	196	86	0.439	0.937	2.072

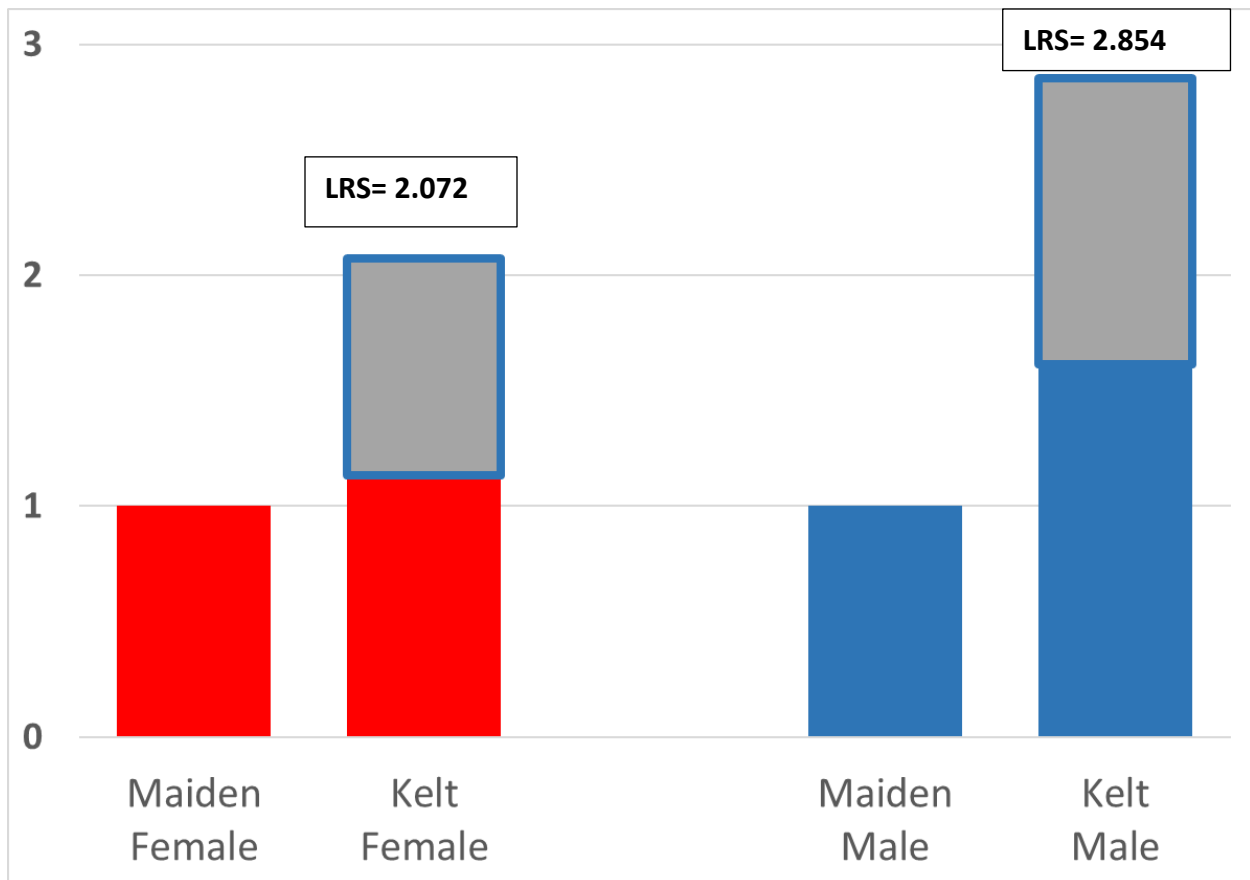


Figure 2B.1: Cumulative Lifetime Reproductive Success (CLRS) of maiden and kelt steelhead by sex.

## Discussion

The 2016 spawning event was the fourth consecutive year that we successfully assigned multiple progeny to reconditioned kelts. A total of 94 juveniles from either Satus or Toppenish Creek are attributed to a spawning event following successful reconditioning of a kelt. We have currently assigned 1,058 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. After the 2017 analysis is completed near the end of spring of 2018 we anticipate publishing the results of this research in a peer reviewed journal.

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 slightly 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 2.072 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 2017. 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. Screening of kelts for maturation status using plasma estradiol levels has become an essential part of the project. In 2017, we sampled blood and provided maturation status of individual fish to project managers so that consecutive and skip spawners could be managed appropriately (Section C1). We completed a study using hatchery origin kelts at Dworshak National fish hatchery to assess the effect of reconditioning on reproductive performance (Section C2). We conducted the initial year of a study on the effect of nutritional restriction during the period after spawning on life history trajectory (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 (Sections C4). We completed an initial pilot study on the effect of blocking cortisol signaling on recovery from spawning in rainbow trout (Section C5). A comparison of the performance of the three Columbia River Basin kelt projects in terms of survival and maturation rates is presented in Chapter 5. Many of these studies are ongoing, and laboratory analysis, statistical analysis, results, interpretations, and conclusions may change as additional work is completed.



## 3.A: Reproductive Development in Kelt Steelhead

### Introduction

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

Iteroparous female salmonids have two major post-spawning life history trajectories (Chaput and Jones 2006; Keefer, et al. 2008; Rideout, et al. 2005b; Rideout and Tomkiewicz 2011b). After a spawning event, some fish are able to restore energy lost during migration and spawning, redevelop a mature ovary, and spawn the next year. These fish are termed consecutive spawners. Other fish do not initiate redevelopment of the ovary for the next spawning season, but instead skip a year. These fish are termed skip spawners. We hypothesize that these life history trajectories are the result of the effect of energy balance on maturation decisions made during seasonally defined critical periods. The influential critical period model of the first reproductive maturation (puberty) in salmonids posits that maturation is initiated during a decision window approximately one year prior to spawning (Campbell, et al. 2006b; Satterthwaite, et al. 2009; Shearer and Swanson 2000; Thorpe 2007). This decision is made based on energy reserves. If maturation is initiated during this critical period, it may be arrested at a second critical period before the onset of exogenous vitellogenesis, if energy reserves are not sufficient (Yamamoto, et al. 2011). We hypothesize that a similar decision mechanism regulates rematuration in post-spawning steelhead. Consistent with this idea, we found that energy restriction affected reproductive development within 10 weeks after spawning in female rainbow trout (Caldwell, et al. 2013; Caldwell, et al. 2014). In post-spawning fish, energy driven decisions take place in the context of the extreme energy deficit incurred by migration and spawning (Penney and Moffitt 2014a, b, 2015). Threshold energy levels for maturation or rematuration are determined by the genetic makeup of the fish and subject to selection (Carlson and Seamons 2008; Hutchings 2011b).

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

During 2017, 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. 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, Nez Perce Tribal Hatchery, Idaho, and Winthrop National Fish Hatchery, Washington as described elsewhere ([Section 1A](#)) (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 C1.1. Steelhead kelts sampled during the fall in 2017. 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.

<b>Location</b>	<b>Sample date</b>	<b>Fish type</b>	<b># Fish</b>	<b>Notes</b>
Prosser	9/21/17	Wild kelts	110	Includes fish collected in 2016 and 2017
DNFH	9/25/17	Wild kelts	32	Kelts collected at Lower Granite Dam; fish held at DNFH were collected in 2017
NPTH	9/14/17	Wild kelts	125	Lower Granite Dam; fish held at NPTH were collected in 2016 and 2017
WNFH	10/12/17	Wild kelts	50	Fish were collected in 2016 and 2017
Wells Dam	9/27/17 to 10/19/17	Hatchery and wild maidens	36	Fish were collected in 2017; N = 1 fish was removed from the analysis due to not having a blood sample

### **Estradiol Assay**

Fish plasma level of estradiol-17 $\beta$  (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  $\mu$ L) 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 54°C water bath (OA-SYS™ Heating System; Organomation Associates, Inc; Berlin, MA) and dried down under a gentle stream of N<sub>2</sub> 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  $\mu$ L assay buffer from the estradiol assay kit. Plasma E2 concentrations were assayed by an enzyme immunoassay using an acetylcholinesterase linked estradiol tracer (Cayman Chemical, Ann Arbor, MI). Extracted plasma samples were appropriately diluted and triplicate technical replicates assayed in the EIA according to the manufacturer's instruction manual provided with the kit.

### **Results**

Plasma E2 levels were bimodally distributed in blood samples taken from female kelts in all projects at a pre-release sampling in the fall (Figs 3A.1, 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. Consequently, the division between modes was adjusted to include these fish as rematuring so that the fish could be released. Plasma E2 levels in maiden Upper Columbia River steelhead sampled at Wells dam were similar to those of rematuring Upper Columbia kelts. The rematuration rate of female kelts as consecutive spawners in 2017 was high at Prosser; females rematured at a 60.9% rate. Consecutive spawners from other programs on the Snake River and Upper Columbia River had relatively low rates of rematuration for 2017, with only 27.3% of the Snake River fish rematuring and 35.5% of the Upper Columbia River fish rematuring. As with previous years, the rematuration rate of female kelts held for a second year of reconditioning was higher than consecutive spawners, 62.5% at Prosser and 90% at DNFH and NPTH, and 94.7% at WNFH.

## Prosser 2016 Kelts

Overall Maturing: 61.1%

2017 Maturing: 60.9%

2016 Maturing: 62.5%

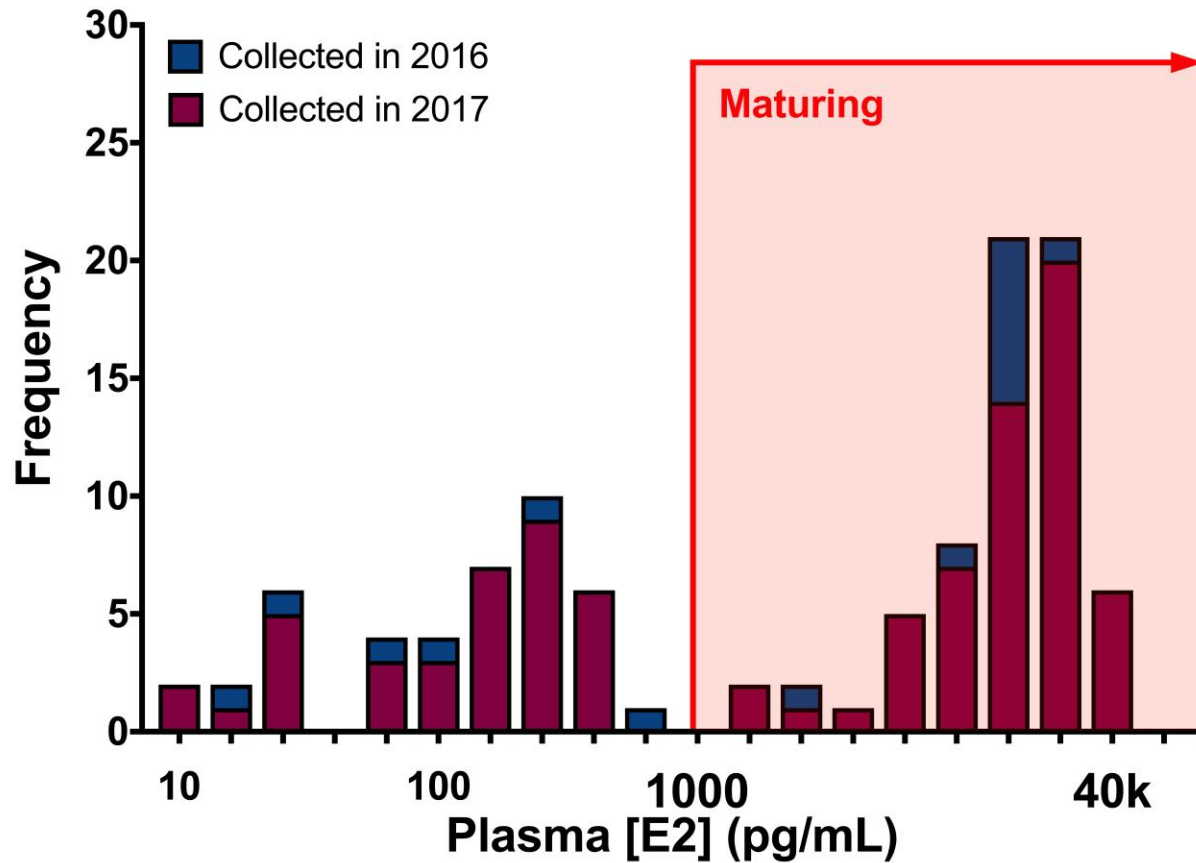


Figure 3A.1: Plasma estradiol (E2) levels in wild female Prosser kelts sampled in fall of 2017.

## Lower Granite Wild Kelts

Overall Maturing: 59.2%

2017 Maturing: 27.3%

2016 Maturing: 90%

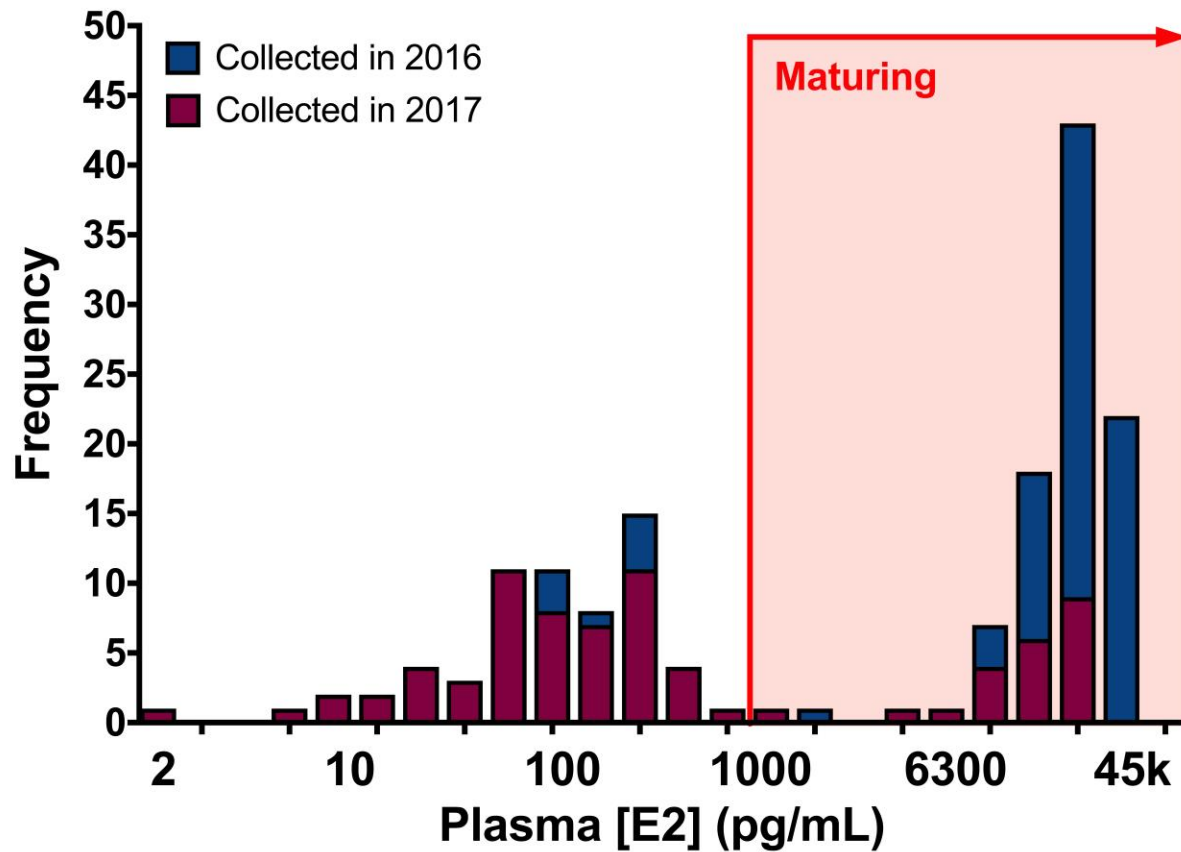


Figure 3A.2: Plasma estradiol (E2) levels in wild female kelts held at DNFH and NPTH sampled in fall of 2017.

## Winthrop September 2016

Overall maturing: 73.3%  
 2016 Kelts Maturing: 35.5%  
 2017 Kelts Maturing: 94.7%  
 Maidens Maturing: 94.4%

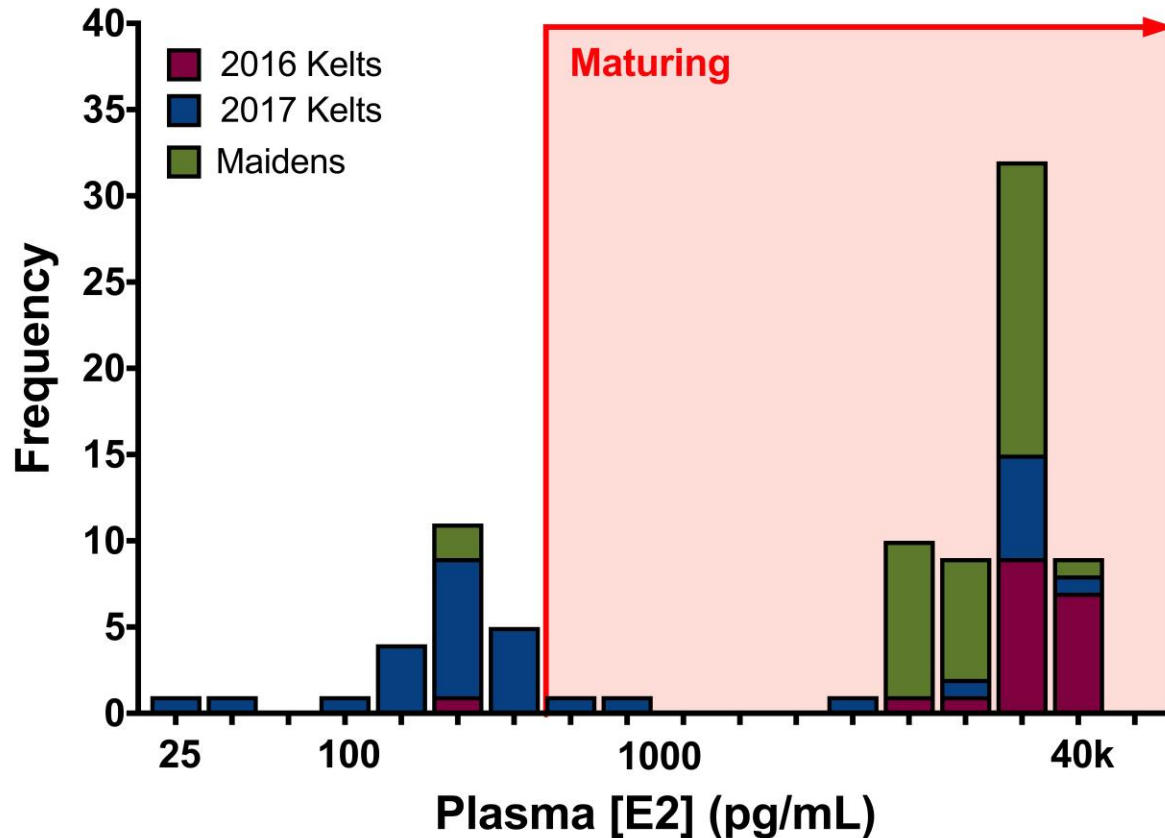


Figure 3A.3: Plasma estradiol (E2) levels in female Upper Columbia kelts and maiden spawners sampled in fall of 2015.

Wild fish collected in 2017 at Lower Granite Dam were held at either NPTH or DNFH due to tank size limitations at NPTH. Over the course of the reconditioning period (spring 2017 thru sampling in September 2017), NPTH experienced high mortality that reduced their numbers from the 268 collected to 45 at the time of sampling (16.8% survival). Fish held at DNFH did not experience this high rate of mortality; of the 39 transported to DNFH, 32 survived to sampling (82% survival). Wild 2016 fish held at NPTH did not experience the same rate of mortality experienced by the 2017 fish, with 80 of the 103-fish held over surviving until sampling (77.7% survival).

### 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. 1992b; Caldwell et al. 2013; Caldwell et al. 2014; Hatch, et al. 2013a). Plasma estradiol levels in rematuring and non-rematuring kelts for 2017 at Prosser, DNFH, and NPTH were similar to previous years. Average plasma E2 levels were similar to those seen in previous year and in other projects. The similarity in E2 levels between reconditioned Winthrop kelts and maiden steelhead at Wells dam suggests that reproductive development is on track in the rematuring kelts. The two-maiden fish sampled at Wells dam that had non-maturing E2 levels may have been males. 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 Upper Columbia and Snake River kelts is consistent with an environmental effect associated with migration conditions. Upper Columbia and Snake River steelhead have a longer migration and tend to spawn later than Yakima River steelhead, and river flows during the 2017 spawning season were higher than normal in both the Upper Columbia and Snake River drainages. Additional years of data and analysis are required to uncover relationships between environmental conditions and maturation rate at the three Columbia River Basin projects.

Non-rematuring fish held for a second year rematured at very high rates (up to 95% at WNFH) in 2017 at Prosser, NPTH and DNFH, and WNFH. 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. 2014a; 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.

Of the 98-fish released into the Snake River on October 24, 2017, 69 were maturing skip spawners. Undoubtedly the relative of amount of consecutive to skip spawners was affected by the low survival of wild 2017 fish. However, considering the high rate of maturation in skip spawning fish on the Snake River, the low survivorship will also have a large impact on the number of maturing fish released next year. It is likely that the holdover fish experienced lower mortality due to greater energy reserves and resistance to stressors.



### **3.B: Reproductive performance and energy balance in reconditioned female steelhead trout (*Oncorhynchus mykiss*) from the Clearwater River, Idaho exhibiting diverse life histories**

Note: This section is currently being prepared for submission to a peer-reviewed journal. Please refer to the journal article for the definitive version.

#### **Introduction**

Life history strategies with diverse tactics should be selected to adjust reproductive performance at individual breeding episodes, in order to maximize lifetime reproductive success and fitness in individuals. Individual developmental pathways are made up of sequential reproductive life history tactics, influenced by energy availability from the environment, physiological efficiency in energy acquisition and conversion, physiological condition, and a genetic threshold for energy stores needed to mature (McBride, et al. 2015; Thorpe 2007; Thorpe 1998). Energy stores must then be spent, or allocated, between somatic processes: survival, maintenance (retained somatic energy), and condition, growth, locomotion; and reproduction-related processes: migration, gonadal investment (current and future reproduction, size versus number of offspring); and when limited, energy allocation may tradeoff between these processes (Stearns 1992). Allocation life history theories can be tested by examining energy allocation (i.e. reproductive performance, retained somatic energy) in organisms differing in environmentally available energy and somatic condition throughout reproductive development.

In salmonids, reproductive investment is genetically regulated and phenotypically plastic. Oogenesis takes place over a year, and is comprised of primary (pre-vitellogenic) and secondary oocyte development. Secondary oocyte development is characterized by bulk transfer of energy from soma to gonad during vitellogenesis (Lubzens, et al. 2010), during which oocyte number is not expected to significantly change, whereas oocyte volume will increase up to 98%, or >50-fold, with increasing gonadosomatic index (GSI) to approximately 20% of the soma (Tyler, et al. 1990). However, the relationship between the timing of energy reserve acquisition and the allocation of reserves to somatic and reproductive processes is not fully understood and is a key question in salmonid biology (Bromage, et al. 1992a; Campbell, et al. 2006a).

Anadromous rainbow trout (steelhead, *Oncorhynchus mykiss*) have broad diversity and plasticity of life history patterns (Moore, et al. 2014b). Steelhead are capital breeders, using energy reserves gained in the ocean to make return migrations into freshwater streams to spawn (McBride et al. 2015) after one or multiple years at sea, returning either many months prior to spawning, i.e. “summer” steelhead, as in this study, or closer to the time of spring spawning, i.e. “winter” steelhead. In either case, additional years at sea prior to spawning allow for increased energy acquisition, growth, body size, and reproductive performance in steelhead, as fecundity and egg size are generally positively correlated with body length (Crespi and Teo 2002; Quinn, et al. 2011). Repeat spawning (iteroparity) also occurs over different intervals, where post-spawn fish spend one or more years reconditioning, typically at sea,

before returning to repeat spawn as either consecutive or skip spawners (Keefer et al. 2008; Moore et al. 2014b; Nielsen, et al. 2011; Rideout, et al. 2005a; Rideout and Tomkiewicz 2011a). Maturation over different intervals in repeats likely has implications for somatic growth and reproductive performance as it does in maidens. Somatic reserves spent on migration and maiden reproduction will need to be replenished in order to spawn a second time. Consecutive spawners spend only a few months recovering somatic reserves used during maiden spawning, while simultaneously initiating ovarian development and gathering energy reserves for reproduction and upstream migration. Skip spawners recondition for a year longer than consecutive spawners, first recovering somatic reserves used during maiden spawning, and then resuming allocation of energy to reproductive development and migration. How and when investment into individual offspring (egg size), overall reproductive performance, and maternal body size are determined in the context of energy balance and allocation during oogenesis in maiden and repeat spawning steelhead of diverse life histories is not known. Kelt reconditioning programs in the Columbia River Basin aim to enhance and maintain current populations of ESA-listed steelhead trout. Post-spawning female steelhead (kelts) are fed and treated prophylactically in captivity until release for repeat spawning in the wild, currently occurring on the Yakima River, WA, the Methow River, WA, in the Snake River Basin above Lower Granite Dam with increasing success (Hatch, et al. 2017), at the Coleman Fish Hatchery in California (Null, et al. 2012), and for Atlantic salmon in their native range (Moffett, et al. 1996). Alternate post-spawning life histories have been observed in kelt reconditioning (Crim, et al. 1992; Hatch, et al. 2013b; Moffett et al. 1996; Pierce, et al. 2016b). In order to validate kelt reconditioning in a given population, studies must (i) determine whether reconditioned kelts can generate viable reproductive tissue and (ii) quantify the benefit of kelt reconditioning by estimating potential productivity (reproductive performance) of released consecutive and skip spawners as compared to maiden spawners.

Hatchery origin multi-sea winter female summer steelhead trout (>70cm) returning to Dworshak National Fish Hatchery (DNFH) on the Clearwater River, Idaho migrate more than 800 km from the ocean, an extreme migration distance near the edge of the range for this species. We used DNFH steelhead to compare reproductive performance and energy balance in maiden, consecutive and skip spawners, and to investigate the effects of energy restriction and availability at different stages of ovarian development on the allocation of energy to somatic and reproductive investment.

## **Methods**

### **Fish**

Returning maiden hatchery-origin female steelhead trout (*Oncorhynchus mykiss*) were captured after ascending the adult ladder at Dworshak National Fish Hatchery (DNFH), Ahsahka, ID. Fish were maintained for up to several weeks in holding ponds supplied with water from the North Fork Clearwater River before spawning.

### **Spawning and Sampling**

During February-April 2013-2016 ripe females (163, 148, 150, and 164, respectively) were selected for spawning. Fish were anesthetized using AQUI-S (AquaTactics Inc., Kirkland, WA; 75mL 1000L<sup>-1</sup> water) and air spawned. Air spawning consisted of gently blowing oxygen into the body cavity via a 16-gauge pneumatic-hypodermic needle inserted through the mid body-cavity wall and collecting ovulated eggs from the genital opening. After spawning, fish were prophylactically treated for bacterial infection with oxytetracycline (Durvet, Blue Springs, Missouri; 20 mg kg<sup>-1</sup> body weight) and for parasitic gill copepods (*Salmincola californiensis*) with emamectin (Sigma-Aldrich, St. Louis, Missouri; 200 µg kg<sup>-1</sup> body weight) via intraperitoneal injection. Fish were individually tagged using passive integrated transponder (PIT) tags inserted into the pelvic girdle, and sampled for length, body weight, and physiological metrics to be reported later. Physiological sampling continued at 10-week intervals, and included oxytetracycline injections and emamectin injections when copepods were visible on the gills. Surviving reconditioned kelts were evaluated weekly during the spawning season (Jan 12-Apr 1), and air spawned as above when ripe.

### **Reconditioning Husbandry**

Fish were held in 4.6m diameter outdoor tanks, with a water height of 1.5m located at DNFH. Tanks were supplied with a flow of approximately 190-liter minute<sup>-1</sup> drawn from the North Fork Clearwater R., with a seasonally varying temperature profile (4.9 – 11.0°C). Fish were fed *ad libitum* a mixture of boiled krill (*Euphausia superba*, Atlantic Pacific Products Inc., Kingston, RI) and pellets (Biobrood 6mm pellet size, BioOregon Inc., Longview, WA) top coated with menhaden oil (Argent Aquaculture LLC, Redmond, WA), and freeze-dried decapsulated brine shrimp eggs (*Artemia* cysts, American Brine Shrimp, Ogden, UT) for increased palatability. Tanks were treated with formalin (Syndel USA, Portland, OR; flow through treatment, 1:6000 for 1 hour daily).

### **Reproductive Performance Measures**

Reproductive performance was quantified at maiden spawning for 2014-2016 females. This data was not collected for the 2013 maiden fish. Reproductive performance was quantified in females surviving to their second spawning (i.e., 1 year later = consecutive spawner or 2 years later = skip spawner) during the 2014-2016 spawning seasons. Due to an equipment malfunction, all remaining 2015 and 2016 fish died in November 2016. Necropsies were performed to determine maturation status in these mortalities.

Individual egg mass, total egg mass and fecundity were calculated gravimetrically (Fleming and Ng 1987). Dry egg mass was calculated by weighing 25 unfertilized eggs after desiccation at 110°C in an oven to a constant mass (24 hours) (Brosset, et al. 2016).

Not all eggs were removed from maiden fish by the air spawning technique employed. At ten weeks after initial spawning, maiden fish were anesthetized and residual eggs were removed by holding the fish vertically with the head up and gently massaging the ventral surface from anterior to posterior. Eggs were collected and enumerated. Additionally, at the time of death,

whether prior to or at repeat spawning, fish were necropsied and any remaining eggs were enumerated. Total egg mass, fecundity, and somatic mass measured at spawning were corrected for residual eggs. Somatic mass refers to the mass of the fish without eggs. Fertilization success was determined using an *in vitro* fertilization assay developed for rainbow trout (J. W. Stoddard, et al. 2005). After visual confirmation of motility, sperm was added to a subsample of 25-50 unfertilized eggs from each female, sperm activation solution was added, the fertilized eggs were incubated for 12-14-hours at 9-14.5 °C in vertical stack incubators, and then eggs were fixed in Stockard's solution. Fixed eggs were observed under a stereomicroscope and fertilization scored as the percentage with embryo cleavage. Egg lots from three 2013 consecutive spawners froze prior to the fertilization assay and were not included.

### Statistical Analysis

Differences in the body shape, and differences in the relationship between somatic mass or fork length and reproductive performance measures in maiden and repeat spawners were evaluated by ANCOVA, with a significant interaction between the categorical and continuous independent variables indicating a difference in slope between categories. In the analysis of fork length versus somatic mass, maiden spawners of different years did not differ significantly in slope ( $p=0.0967$ ), and consecutive and skip repeat spawners did not differ significantly in slope ( $p=0.8651$ ), and were thus pooled. In the analyses of reproductive performance measures, three consecutive and six skip spawners (spawn year 2013) were discovered to be infected at necropsy and were excluded from further analysis, resulting in  $n=38$  for repeat spawners. For the dry egg mass analysis, one 2013 consecutive was excluded as an outlier, resulting in  $n=37$ . The data were not available for one 2015 maiden and two 2016 maidens, resulting in  $n=456$  for maidens (for the dry egg mass analysis only).

Somatic mass, fork length, reproductive performance measures, and fertilization success were examined using univariate generalized linear models (GLM). Following significant whole model GLM results, differences between spawning categories (maiden, consecutive, and skip) were tested using contrasts (JMP 13, SAS Institute Inc., Cary, NC). In addition, due to the value of absolute reproductive performance measures in evaluating the benefit of kelt reconditioning programs, reproductive performance data was analyzed both absolute and standardized to a standard somatic mass. Somatic mass was selected for standardization because relationships between somatic mass and reproductive performance measures differed less between maiden and repeat spawners than those between fork length and reproductive performance measures. Standardization employed the slope of the pooled maiden regression relationship between somatic mass and each reproductive performance measure, and the pooled average somatic mass of a maiden spawner (3.847kg), where the standardized value = original value of individual – [(somatic mass of individual – 3.847kg) \* slope]. Two 2014 maidens (lengths 65cm and 90.5cm) and one 2016 maiden (mass 5.88kg) were excluded based on the Rout Outlier test ( $Q=1.0\%$ ) from this and all further analysis, resulting in  $n=459$  for maidens,  $n=25$  consecutives,  $n=22$  skips. Fertilization success data were arcsine-square root transformed prior to analysis. Two consecutive spawner egg lots from 2013 froze and could not be fertilized. One maiden egg lot was not collected in 2016. Fertilization success was not size-standardized.

Spawn week fidelity was evaluated by subtracting the repeat spawning week from the maiden spawning week for individual fish, where week was enumerated beginning January 1 of each year. Spawn week was compared to zero using a one-sample t-test (GraphPad Inc., La Jolla CA: <https://www.graphpad.com/quickcalcs/OneSampleT1.cfm>), and consecutive and skip repeat spawners were compared using a two-sample t-test. Maiden spawn dates were not available for four 2013 repeat spawners, including two consecutive and two skip spawners.

Unless otherwise indicated, all statistical analysis was conducted with PRISM 7.0 (GraphPad Inc., La Jolla, CA). Results are reported as significant when  $p < 0.05$ .

## **Results**

### **Survival**

The survival (2013-2015) for female steelhead trout reconditioned to the point of repeat spawning at DNFH was 10% (19%-2013; 3%-2014; 8%-2015). On average, 49% of mortality occurred within 10 weeks of spawning (39%, 47%, 62% for 2014-2016 respectively). Skip spawner survival in year two of captivity ranged from 56% (2013) to 17% (2014). At the November 2016 necropsy, approximately 3 months prior to repeat spawning, 84% (21/25) of skip spawners had survived from one year after spawning, of which 86% (18/21) were rematuring based on a developing ovary containing large oocytes.

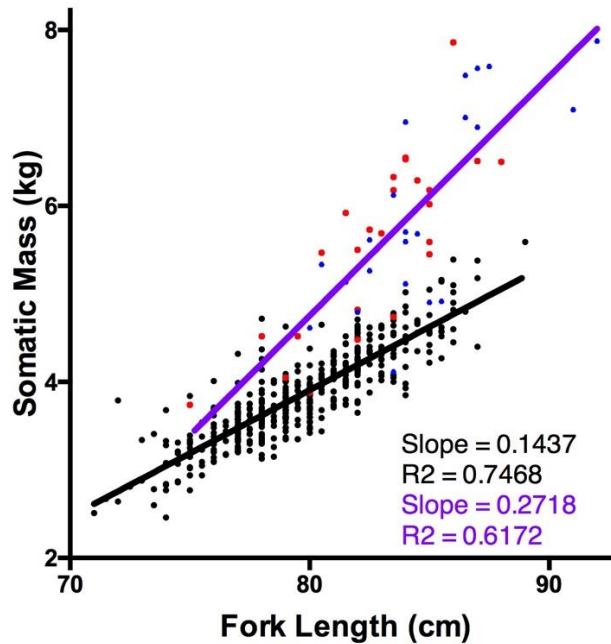
### **Body Size and Shape**

Repeat spawners were significantly larger than maidens in both somatic mass and fork length (Table 3B.1). Additionally, skip spawners were significantly larger than consecutive spawners in both somatic mass and fork length.

**Table 3B.1.** Somatic mass and fork length of female steelhead trout from the Clearwater R., Idaho. Mass and length were examined using univariate GLM, and, where significant, followed by independent contrasts of spawning categories (maiden, consecutive, and skip). Groups sharing a letter do not differ significantly ( $p>0.05$ ). Combined data for all repeat spawners, as well as data by maiden spawn year, is listed for reference but was not included in the statistical analysis.

	N	Somatic mass (kg)		Fork Length (cm)	
		Mean	Standard Dev	Mean	Standard Dev
<b>Maidens (all)</b>	<b>459</b>	<b>3.847<sup>C</sup></b>	<b>0.512</b>	<b>79.60<sup>C</sup></b>	<b>3.089</b>
2014	146	3.721	0.512	79.07	3.105
2015	150	3.985	0.555	80.50	3.178
2016	163	3.835	0.436	79.22	2.777
<b>Consecutives (all)</b>	<b>25</b>	<b>5.562<sup>B</sup></b>	<b>1.006</b>	<b>82.76<sup>B</sup></b>	<b>2.941</b>
2013	11	5.266	1.009	82.27	3.849
2014	2	5.270	1.061	82.25	3.889
2015	12	5.882	0.982	83.29	1.852
<b>Skips (all)</b>	<b>22</b>	<b>5.977<sup>A</sup></b>	<b>1.132</b>	<b>84.75<sup>A</sup></b>	<b>3.011</b>
2013	20	6.014	1.184	84.90	3.114
2014	2	5.610	0.014	83.25	1.061
Repeats (all)	47	5.756	1.075	83.69	3.108

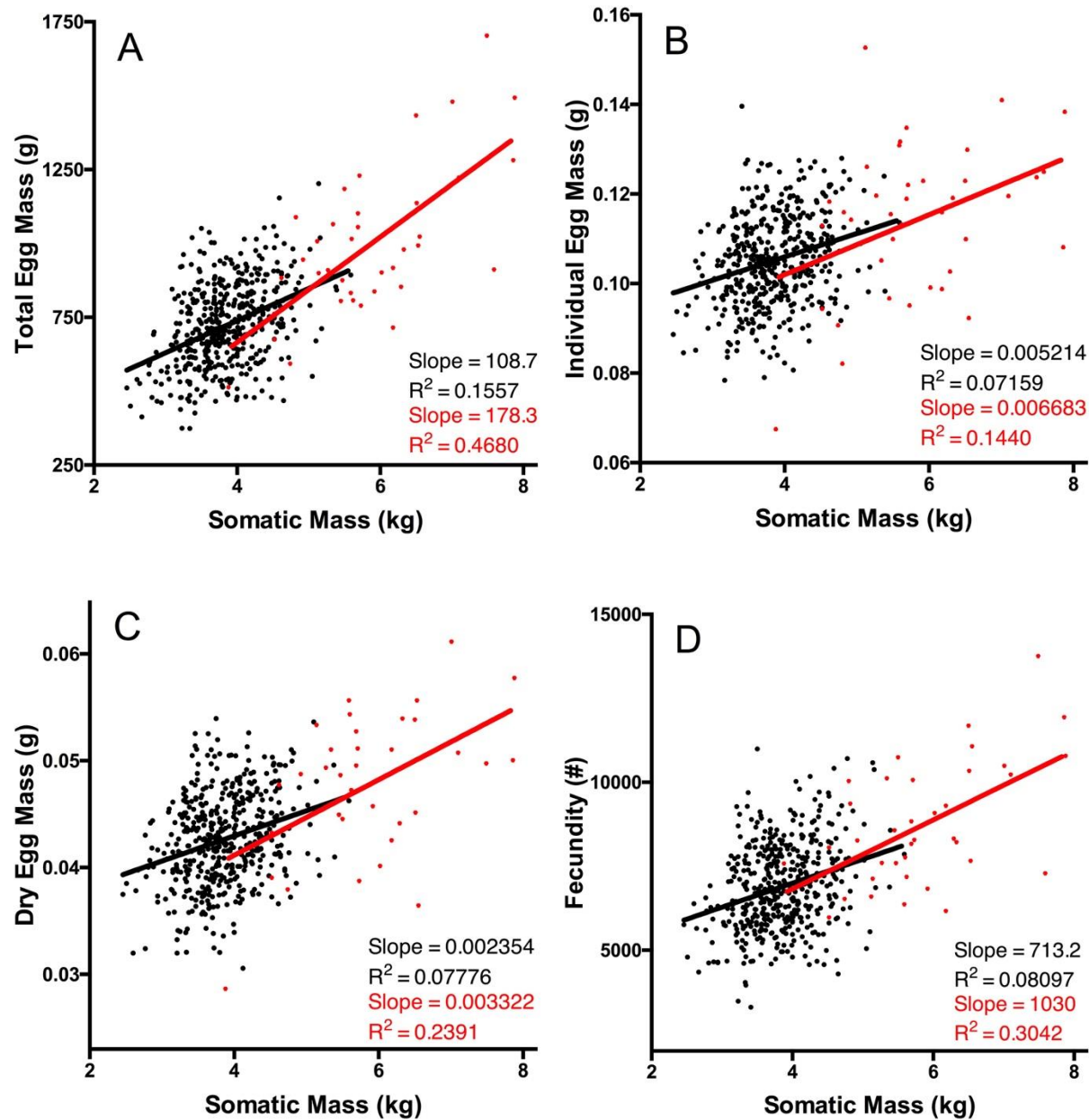
Linear regressions of somatic mass with fork length showed significantly steeper slopes (ANCOVA:  $F_{1,502} = 65.4976$ ,  $p<0.0001$ ) in repeat spawners than in maiden spawners (Figure 3B.1). Consecutive and skip repeat spawners, however, did not differ significantly in slopes (ANCOVA:  $F_{1,43} = 0.0333022$ ,  $p=0.8561$ ).



**Figure 3B.1.** Relationship between fork length and somatic mass in female steelhead trout from the Clearwater R., Idaho, sampled between 2014-2016. Females were grouped as maiden (black circles, N=459), consecutive (red, N=25), and skip spawners (blue, N=22). Maiden and repeat slopes differed significantly (ANCOVA:  $F_{1,502} = 65.4976$ ,  $p < 0.0001$ ), whereas consecutive and skip spawner slopes were similar (ANCOVA:  $F_{1,43} = 0.0333022$ ,  $p = 0.8561$ ). The combined regression line for consecutive and skip spawners is indicated by a purple line.

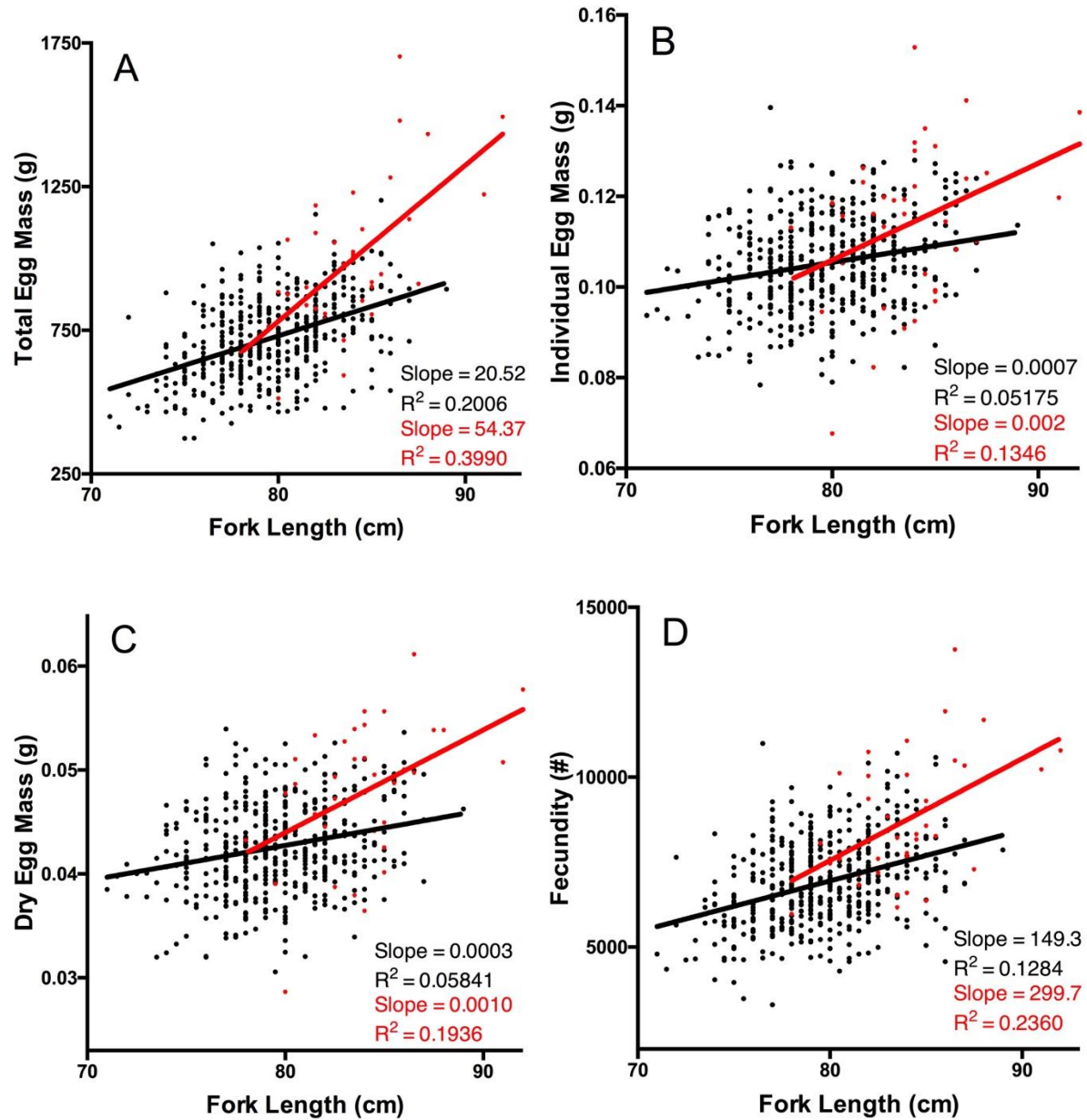
### Gonadosomatic Relationships

Linear regression of total egg mass with somatic mass showed a significantly steeper slope in repeat spawners than in maiden spawners (ANCOVA:  $F_{1,493} = 7.17975$ ,  $p = 0.0076$ ) (Figure 3B.2). Non-significant tendencies towards steeper slopes were present in individual egg mass, dry egg mass, and fecundity when regressed with somatic mass. Linear regressions of total egg mass, individual egg mass, dry egg mass, and fecundity with fork length showed significantly steeper slopes (ANCOVA:  $F_{1,493} = 19.2713$ ,  $p < 0.0001$ ;  $F_{1,493} = 5.4403$ ,  $p = 0.0201$ ;  $F_{1,487} = 6.42565$ ,  $p = 0.0116$ ;  $F_{1,493} = 4.41612$ ,  $p = 0.0361$ ) in repeat than in maiden spawners (Figure 3B.3).



**Figure 3B.2.** Relationship between somatic mass and total egg mass (A), individual egg mass (B), dry egg mass (C), and fecundity (D) in maiden and repeat spawning female steelhead trout. Females were grouped as maiden (black circles) and repeat spawners (red circles) (A, B, D: maiden N=459, repeat N=38; C: maiden N=456, repeat N=35). Slopes differed significantly in A (ANCOVA:  $F_{1,493} = 7.17975$ ,  $p=0.0076$ ), but not in B, C, or D (ANCOVA:  $F_{1,493} = 0.556268$ ,  $p=0.4561$ ,  $F_{1,487} = 1.91341$ ,  $p=0.1672$ , and  $F_{1,493} = 1.71762$ ,  $p=0.1906$ , respectively).





**Figure 3B.3.** Relationship between fork length and total egg mass (A), individual egg mass (B), dry egg mass (C), and fecundity (D) in maiden and repeat spawning female steelhead trout. Females were grouped as maiden (black circles) and repeat spawners (red circles) (A, B, D: maiden N=459, repeat N=38; C: maiden N=456, repeat N=35). The slopes differed significantly between maidens and repeat spawners for all measures (A-D) (ANCOVA:  $F_{1,493} = 19.2713$ ,  $p < 0.0001$ ;  $F_{1,493} = 5.4403$ ,  $p = 0.0201$ ;  $F_{1,487} = 6.42565$ ,  $p = 0.0116$ ;  $F_{1,493} = 4.41612$ ,  $p = 0.0361$ , respectively).

### **Reproductive Performance**

Total egg mass was significantly greater in repeat spawners than in maiden spawners, and greater in skip spawners than in consecutive spawners (Table 3B.2). Size-standardized total egg mass was greater in skip spawners than maiden and consecutive spawners (Table 3B.3).

Individual egg mass was significantly greater in skip spawners than in consecutive and maiden spawners (Table 2). Size-standardized individual egg mass was significantly greater in skip spawners than in consecutive and maiden spawners. However, maidens had significantly greater size-standardized individual egg size than consecutive spawners (Table 3B.3).

Dry egg mass was significantly greater in repeat spawners than in maiden spawners, and greater in skip spawners than in consecutive spawners (Table 3B.2). As with size-standardized individual egg mass, skip spawners had significantly greater dry egg mass than consecutive and maiden spawners, and maidens had greater dry egg mass than consecutive spawners, however, this difference was marginally non-significant ( $p=0.0518$ ; Table 3B.3).

Fecundity was significantly greater in repeat spawners than in maiden spawners (Table 3B.2), and similar between groups when standardized for size (Table 3B.3).

**Table 3B.2.** Reproductive performance across life history groups in female steelhead trout from the Clearwater R., Idaho. Total egg mass, individual egg mass, dry egg mass, and fecundity were examined using univariate GLM, and, where significant, followed by independent contrasts of spawning categories (maidens, consecutives, skips). Groups sharing a letter do not differ significantly ( $p>0.05$ ). Combined data for all repeat spawners, as well as data by maiden spawn year, is listed for reference but was not included in the statistical analysis.

	N	Total Egg		Individual Egg		Dry Egg		Fecundity	
		Mass (g)		Mass (g)		Mass (g)		(#)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
<b>Maidens (all)</b>	<b>459</b>	<b>722.2<sup>C</sup></b>	<b>141.0</b>	<b>0.105<sup>B</sup></b>	<b>0.010</b>	<b>0.0426<sup>C</sup></b>	<b>0.0043</b>	<b>6887<sup>B</sup></b>	<b>1283</b>
Maiden 2014	146	665.3	128.8	0.101	0.001	0.0400	0.0041	6612	1149
Maiden 2015	150	702.7	123.4	0.107	0.009	0.0430	0.0039	6620	1218
Maiden 2016	163	791.3	138.9	0.108	0.001	0.0440	0.0039	7378	1315
<b>Consecutives (all)</b>	<b>22</b>	<b>918.9<sup>B</sup></b>	<b>221.2</b>	<b>0.108<sup>B</sup></b>	<b>0.0151</b>	<b>0.0455<sup>B</sup></b>	<b>0.0070</b>	<b>8557<sup>A</sup></b>	<b>1753</b>
Consecutive 2013	8	1049.0	228.3	0.119	0.0084	0.0501	0.0056	8858	2069
Consecutive 2014	2	830.0	99.0	0.097	0.0034	0.0400	0.0008	8559	726
Consecutive 2015	12	847.3	200.3	0.102	0.0155	0.0440	0.0072	8355	1727
<b>Skips (all)</b>	<b>16</b>	<b>1092.0<sup>A</sup></b>	<b>265.2</b>	<b>0.123<sup>A</sup></b>	<b>0.0161</b>	<b>0.0518<sup>A</sup></b>	<b>0.0039</b>	<b>8922<sup>A</sup></b>	<b>1924</b>
Skip 2013	14	1118.0	271.4	0.123	0.0168	0.0520	0.0039	9130	1971
Skip 2014	2	910.0	147.1	0.123	0.0132	0.0510	0.0050	7463	414
Repeats (all)	38	992.0	252.6	0.114	0.0171	0.0479	0.0067	8710	1811

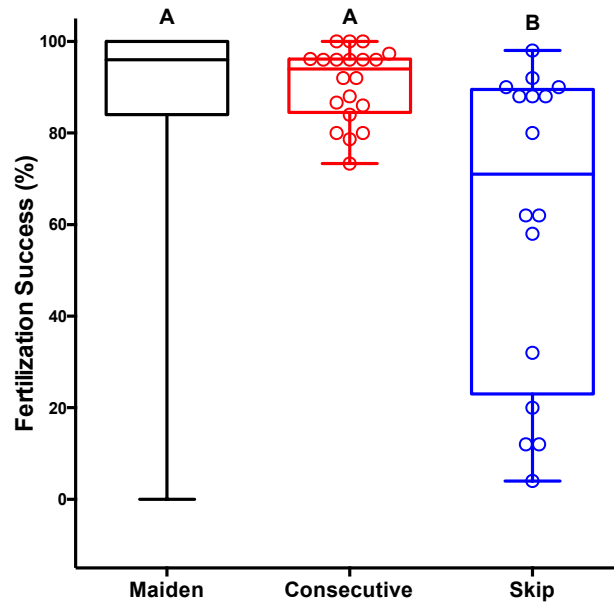
Note: For dry egg mass, N=149 (Maiden 2015) and N=161 (Maiden 2016), therefore N=456 (Maidens (all)). N=7 (Consecutives 2013), therefore N=37 (Repeats).

**Table 3B.3.** Size-standardized reproductive performance across life history groups in female steelhead trout from the Clearwater R., Idaho. Data were standardized to the average somatic mass of a maiden spawner (3.847 kg), using the linear regression for each metric from combined maiden spawner data. Total egg mass, individual egg mass, dry egg mass, and fecundity were examined using univariate GLM, and, where significant, followed by independent contrasts of spawning categories (maidens, consecutives, skips). Groups sharing a letter do not differ significantly ( $p>0.05$ ). Combined data for all repeat spawners, as well as data by maiden spawn year, is listed for reference but was not included in the statistical analysis.

	N	Total Egg		Individual Egg		Dry Egg		Fecundity	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
		Mass (g)	Mass (g)	Mass (g)	Mass (g)	Mass (g)	Mass (g)	(#)	(#)
<b>Maidens (all)</b>	<b>459</b>	<b>722.1<sup>B</sup></b>	<b>129.6</b>	<b>0.1051<sup>B</sup></b>	<b>0.0096</b>	<b>0.04260<sup>B</sup></b>	<b>0.00414</b>	<b>6886</b>	<b>1230</b>
Maiden 2014	146	679.1	122.7	0.1014	0.0094	0.04044	0.00398	6703	1119
Maiden 2015	150	687.6	105.6	0.1059	0.0089	0.04301	0.00371	6521	1128
Maiden 2016	163	792.5	126.5	0.1078	0.0094	0.04419	0.00384	7386	1256
<b>Consecutives (all)</b>	<b>22</b>	<b>709.9<sup>B</sup></b>	<b>172.2</b>	<b>0.0979<sup>C</sup></b>	<b>0.0138</b>	<b>0.04072<sup>B</sup></b>	<b>0.00626</b>	<b>7182</b>	<b>1501</b>
Consecutive 2013	8	845.6	190.8	0.1093	0.0080	0.04541	0.00494	7523	1870
Consecutive 2014	2	674.8	16.7	0.0894	0.0022	0.03621	0.00171	7537	35.04
Consecutive 2015	12	625.4	109.7	0.0916	0.0132	0.03875	0.00599	6895	1371
<b>Skips (all)</b>	<b>16</b>	<b>865.0<sup>A</sup></b>	<b>196.1</b>	<b>0.1122<sup>A</sup></b>	<b>0.0150</b>	<b>0.04657<sup>A</sup></b>	<b>0.00328</b>	<b>7426</b>	<b>1631</b>
Skip 2013	14	886.0	197.1	0.1121	0.0157	0.04657	0.00323	7601	1670
Skip 2014	2	717.7	148.6	0.1133	0.0132	0.04661	0.00505	6197	424
Repeats (all)	38	775.2	196.1	0.1039	0.0159	0.04306	0.00597	7284	1540

Note: For dry egg mass, N adjusted as in Table 2.

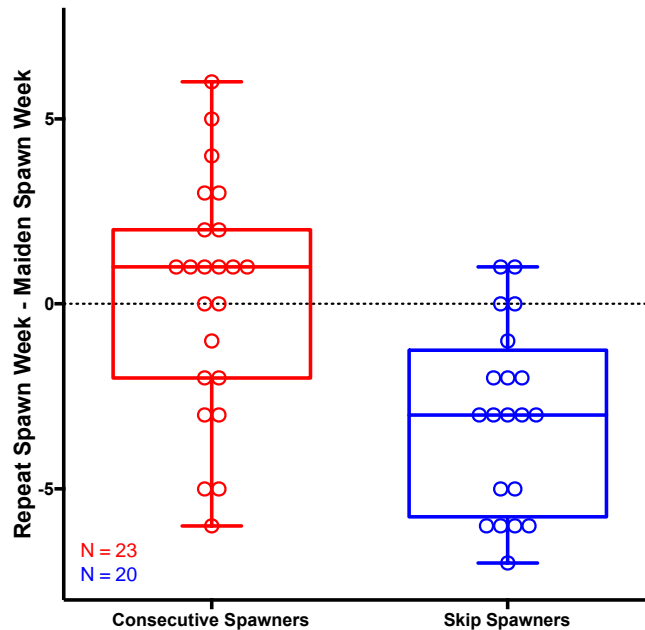
Fertilization success was significantly greater on average in maiden and consecutive spawners than in skip spawners (Figure 3B.4).



**Figure 3B.4.** Fertilization success in female steelhead trout from the Clearwater River, ID spawned between 2013-2016. Females were grouped as maiden (black: N=458), consecutive (red: N=20), or skip (blue: N=16). Boxes indicate the interquartile range, the line indicates the median, and whiskers show the data range. All points are shown for repeat spawners. Groups not sharing the same letter are significantly different (univariate GLM followed by independent contrasts).

### Spawn Date Fidelity

Consecutive spawning females on average spawned the same week they spawned as maiden spawners (one-sample t-test,  $t_{22} = 0.2630$ ,  $p=0.7950$ ). Skip spawning females spawned significantly earlier than they did as maiden spawners (one-sample t-test,  $t_{19} = 5.4978$ ,  $p<0.0001$ ), on average spawning 3 weeks earlier. Spawn date fidelity significantly differed between consecutive and skip spawners (Figure 3B.5, two-sample t test,  $t_{41}=3.671$ ,  $p=0.0007$ ) when individuals were compared back to their original spawn dates.



**Figure 3B.5.** Spawn week fidelity in repeat spawning female steelhead trout from the Clearwater River, ID sampled between 2013-2015. Boxes indicate the interquartile range, the line indicates the median, whiskers show the data range, and all points are shown. Repeat spawn week did not differ significantly from maiden spawn week in consecutive spawners (one sample t-test,  $t_{22} = 0.2630$ ,  $p = 0.7950$ ), but was significantly earlier in skip spawners (one-sample t-test,  $t_{19} = 5.4978$ ,  $p < 0.0001$ ), and repeat spawn week was significantly earlier in skip spawners than consecutive spawners (two-sample t test,  $t_{41} = 3.671$ ,  $p = 0.0007$ ).

## Discussion

### Body Size and Shape

Maiden and repeat spawners differed in body size (somatic mass, fork length) and shape (mass-length relationship), likely attributable to differences in acquisition and allocation of energy stores during the year prior to spawning. Somatic mass was greater in repeat spawners than maiden spawners. Maiden spawners fast during the 6-9 month freshwater period prior to spawning in this population of summer steelhead, allocating somatic energy to maintenance, survival, and converting stored energy to reproductive mass through vitellogenesis, which occurs during this time period for captive and anadromous *O. mykiss* (Pierce et al. 2016b; Tyler et al. 1990). Repeat spawners operated off of a steady energy income (satiation feeding) during the entire year prior to spawning, and grew during reconditioning, resulting in the larger sizes measured on average. Growth patterns will be detailed elsewhere (Jenkins et al. 2018). Repeat spawners also did not spend energy on migration or ecological interactions due to the artificial reconditioning environment.

Somatic mass was greater in skip spawners than in consecutive spawners, likely because skip spawners entered the year prior to spawning (oogenesis) with fully recovered energy stores, and then continued acquiring energy through the oogenic year on steady energy income. Following maiden spawning, both consecutive and skip spawners would have been in considerable energy deficit from spawning, fasting, and migration, and would be directing energy stores to survival, maintenance and condition prior to initiation of any growth. Energy acquisition would likely have been delayed by time required for re-activation of the gut following extended fasting (Penney and Moffitt 2014a). As a result, consecutive specifically would have had a shorter overall time for somatic mass acquisition following spawning, overlapping recovery and oocyte development (detailed below), resulting in a reduced overall size compared to skip spawners.

Length was 5% greater on average in repeat spawners than maiden spawners, which was significant but 10-fold lesser than the increase in somatic mass, perhaps due to relaxed selection for length and costliness of structural growth. Nearly 4.5 decades of lethal spawning have perhaps relaxed selective pressure for length increases previous applied by the need to defend territory for redds, compete for mates, move substrate to build redds, etc. Hatchery fish in this population need only reach a minimum size (78 cm) to guarantee spawning assuming they successfully return to the hatchery. Selective pressure for length in repeat spawners may also be relaxed by having already spawned once, as structural body growth requires energy and nutrients that cannot perhaps be easily remobilized into reproductive tissue. Further increases in length may actually cost more energy than the returns provided by increased fecundity often correlated with length. Skip spawners had significantly greater length than consecutive, likely due to increased time to acquire resources following maiden spawning, likely due to increased time to acquire resources and a lack in overlap in the timing of somatic recovery and oocyte development, as described for somatic mass. Wild Atlantic salmon returning to the Miramichi R., Canada, also differed significantly in length increase by post-spawn life history, with skips (21%) and consecutive (8%) greater in length than 2 sea winter maidens (Chaput and Benoit 2012), attributed to different foraging areas (nutrient acquisition).

Repeat spawners had 50% greater somatic mass than maiden spawners on average, but only 5% longer, resulting in different body shapes. The same was found in Atlantic salmon kelt reconditioning, where repeats were 53% larger (mass), but only 3.3% longer (Moffett et al. 1996). Comparing physiological measures tracked over time in consecutive and skip spawners in both the year following maiden spawning and in the year prior to repeat spawning will be essential to better understanding how somatic conditions develop over time in diverse post-spawn life histories.

### **Somatic Size and Reproductive Performance Relationships**

Relationships between reproductive and somatic measures were similar between maiden and repeat spawners when related with somatic mass, but differed when related with fork length. As a difference in body shape emerged between maiden and repeat spawners due to a large difference in somatic mass and a smaller difference in fork length in this study, reproductive performance measures maintained a similar relationship with somatic mass, and not with fork

length. Although reproductive performance measures (i.e. fecundity, egg size) are traditionally correlated with length rather than mass in salmonids (Crespi and Teo 2002) including *O. mykiss* (Quinn et al. 2011), this is likely due to length being recorded more often than mass in field fisheries datasets. Our data, however, indicate that somatic mass is more closely related to reproductive performance measures, and is likely a better indicator of energy stores available for reproductive investment than length.

### **Reproductive Performance – Size-Standardized**

When standardized for size, reproductive performance (total egg mass) was greater in skip spawners than maiden and consecutive spawners. Total egg mass integrates individual egg mass (egg size) and fecundity (egg number), as it is a product of the two measures. Individual egg mass was reduced in consecutive spawners as compared to maiden and skip spawners. This resulted in a trend towards slightly reduced total egg mass, while maintaining fecundity. Reduced egg size is likely due to a disproportionate allocation of energy to somatic recovery in consecutive spawners during early oogenesis in the months immediately following spawning, as compared to maiden and skip spawners. In maiden steelhead, oocyte development, occurring during the year prior to spawning, is fueled by energy intake occurring during the early part of the year (at sea), and energy transfer occurring later in the year during freshwater fasting. Maiden spawners spend the early part of oogenesis at sea, actively acquiring energy stores essential for allocation to upstream migration, maintenance of the soma during freshwater fasting, ecological interactions, and reproductive development. Vitellogenesis, or bulk energy transfer to the gonads, occurs roughly during the latter two thirds of the year prior to spawning in anadromous *O. mykiss* (Pierce et al. 2016b) (annual reports). Reduced proportional egg size, despite greater somatic mass in consecutives, suggests that egg size is determined early in oogenesis, despite the continuous, steady energy supply that consecutives receive right up until spawning. The energy they continue to receive results in an increased body size (discussed above), but does not increase egg size. Thus, egg size seems to be determined based on energy stores physiologically evaluated during early oogenesis. This early oogenenic, pre-vitellogenic period in consecutives, consumed mostly by recovery energy for the soma coincides with a period when maiden spawners would have been feeding at sea, and skip spawners would have been feeding to satiation in captivity. Consistent with this idea, skip spawners had greater size-standardized individual egg mass than maiden spawners.

How/why might such a mechanism evolve and/or be maintained? This population has historically overwintered in energy-poor freshwater streams, where shutting down the gut was more cost-effective than continuing to seek food, especially as compared to the energy resources available in the ocean. It's plausible that egg size would be set in the early stages of oogenesis when no additional energy would be expected to be gained later. Additionally, because *O. mykiss* is an iteroparous (repeat spawning) organism, it's reasonable to believe that if energy were to be gained post-vitellogenic onset, that energy might be useful for survival and downstream migration post-spawning.

Size-standardized fecundity did not differ significantly among life history groups. This indicates that changes in reproductive effort were largely modulated by quantities of energy stores



invested into individual offspring through the processes of vitellogenesis, rather than by modulating offspring number. This is consistent with previous accounts that fecundity is set early in vitellogenesis in rainbow trout (Tyler et al. 1990). In contrast to these results, reconditioned Atlantic salmon kelts showed reduced mean fecundity in repeat spawners, but similar egg diameter (Moffett et al. 1996). However, consistent with our study, size-standardized fecundity was defended at the expense of egg size in maiden spawning 2- and 3-ocean hatchery-origin steelhead (Quinn et al. 2011), attributed to nutrient acquisition time (ocean age). Also, consecutive spawning wild Atlantic salmon in the Miramichi River, Canada had significantly smaller diameter eggs, but not significantly different fecundity than maiden and skip spawners when standardized by length; although when standardized by mass, fecundity was greatest and most varied in consecutive spawners (Reid and Chaput 2012).

### **Absolute Reproductive Performance**

Reproductive performance (total egg mass) increased with somatic size and age when compared between groups in the absolute form. Individual egg mass was significantly (~15%) greater in skip spawners than in maiden and consecutive spawners. Also, despite a reduction in proportional egg size in consecutive spawners as compared to maiden spawners, absolute egg size was not smaller. Larger egg size is expected to lead to greater survival in salmonids due to larger size at first feeding, larger gape for feeding, competitive advantage, and reduced surface-area to volume ratio potentially reducing chances of contracting infection or disease (Bromage et al. 1992a). The benefit of egg size is difficult to quantify without a complex understanding of the contextual ecology and habitat (substrate size, conspecifics, nutrient availability for juveniles, etc.), though larger egg size has been empirically linked to environments of decreasing quality (Rollinson and Hutchings 2013). Fecundity was greater in repeat spawners than maiden spawners, suggesting that reconditioned kelts have the potential to produce more offspring than maiden spawners.

### **Fertilization Success**

Maiden and consecutive spawners had significantly greater fertilization success on average than skip spawners. Skip spawning is a normal life history for *O. mykiss*, and there is no reason to associate subfertility with skip spawning as a life history. Rather, likely subfertility in skip spawners resulted from misidentification of ripeness and or undetected infection. Maiden and consecutive spawner averages exceeded 80% fertilization success, consistent with the mean survival to eyeing in *O. mykiss*, reported as early as 1953 (Bromage et al. 1992a). Below 80% success, spawners are referred to as “subfertile”. Subfertility was found in 50% of skip spawners, which lowered averages significantly below other groups despite a wide variation. Subfertility was also found in maiden spawners: 2014 (20%), 2015 (36%), and 2016 (4%) and consecutive spawners (10%). Manual stripping of ovulated eggs can cause modest to significant reduction in fertilization success in the days following ovulation, particularly as number of days post-ovulation and water temperature increase (Bromage et al. 1992a). Stripping a fish that was only partially ovulated caused additional logistical problems (quantifying additional reproductive performance measures), so spawning individuals too early was a concern as well. Misidentification of ripeness (spawning fish just barely too early or too late) may have resulted in the median group of fish with approximately 60% fertilization success. Undetected infection

(in addition to N=8 removed due to observable infection) may explain group of fish with approximately 0-30% fertilization. Further fertilization success study in repeat spawners in this population with greater N are required to gain confidence in results regarding skip spawners. Subfertility in *O. mykiss* has previously been associated with stress occurring early in oogenesis (Medeiros, et al. 2016), which would be consistent with our findings had greater subfertility been found in consecutive spawners than in skip spawners. Atlantic salmon reconditioning efforts in Canada reported high fertilization success (93.7-95.6%) (Crim et al. 1992). Fry survival (64.5%) to 780 days after fertilization was similar to maidens (72.3%) (Moffett et al. 1996). More recently, wild Atlantic salmon offspring survival to “just before hatching” was significantly reduced (88.3%) in consecutive spawners compared to maiden and skip spawners (94%), which were similar (Reid and Chaput 2012).

### **Spawn Date Fidelity**

Skip spawners spawned earlier on average than their initial spawning dates and earlier than consecutive spawners. The earlier spawning, however, was not outside the average range of spawning for maiden spawners. Skip spawners migrated earlier than maiden spawners in wild Atlantic salmon (Niemelä, et al. 2006). Similar to Clearwater steelhead, diversity of run timing found some large multisea winter fish migrating earlier, likely due to longer distances historically (or currently) traveled.

### **Conclusions**

It has previously been established that fecundity is set prior to the onset of vitellogenesis in rainbow trout (Tyler et al. 1990), particularly in a controlled (laboratory) setting. Results from this study suggest that egg size is also set early in oogenesis, similar to fecundity, as (consecutive) spawners forced to allocate greater quantities of energy to somatic recovery showed smaller egg size, despite access to energy throughout the entire oogenic period. Fish in all groups defended fecundity at the expense of egg size, which suggests that fecundity is decoupled from physiologically perceived energy stores during early oogenesis, and rather that spawners in this population modulate reproductive performance by modulating investment into individual offspring, rather than into the number of offspring they develop.

### **Management Implications**

Repeat spawners are important to steelhead populations (Moore et al. 2014b), increasing stability during bottleneck years by having multiple cohorts of available to spawn with each other that cannot all be wiped out by one poor ocean year. Releasing wild reconditioned repeat spawners, particularly with larger eggs, more eggs, and greater total egg mass per fish should increase productivity. Larger eggs should be beneficial in the event of decreased quality of environment (discussed above), and more eggs should increase the productivity of reconditioned spawners in terms of smolts and adult returns. Having fish with a variety of spawn timings is also beneficial for the fishery. Efforts to maintain diversity of migration timing already exist on the Clearwater. The average diversity of spawn timings observed was not actually outside of the normal (maiden) spawning season, but rather should reinforce efforts to spread out opportunities for fishing. Our finding that continued feeding during the fall did not result in increased reproductive performance suggests that the current strategy of releasing

wild reconditioned kelts in the fall does not compromise reproductive performance, versus holding fish for a later release. Our finding that reproductive effort and egg size are determined early in oogenesis suggests that poor ocean years (winters) may reduce proportional egg size and total reproductive effort in maiden spawners. This environmental effect on productivity could be incorporated into population models.

### **Future work**

In general, more data is needed on skip spawners for this populations. This is particularly true of fertilization success, but for all factors, our understanding would be enriched by more years of data on repeat spawners. This is challenging data to collect and requires keeping large animals alive for 1-2 years in captivity.

Tracking physiological factors such as growth and condition over both the year following maiden spawning and the year prior to repeat spawning, compared between consecutive and skip spawners, will allow a deeper look at the trends we are observing in this study. Analysis of physiological factors indicating energy stores, condition, and growth prior to spawning would give more information about when the decision to spawn consecutively or skip years is made. Additionally, intentionally modulating feed in kelts at different time periods following spawning and then checking for correlates between these treatments, spawning life history, reproductive performance, body size would be highly informative towards deepening our understanding of life history questions regarding energy balance between the soma and gonads during oogenesis

### **3.C: Effects of post-spawning fasting on growth, life history trajectory, and reproductive development in a hatchery model of steelhead kelt reconditioning**

#### **Introduction**

The consecutive (1 year spawning interval) and skip (2 year spawning interval) spawning life histories are found in repeat spawning steelhead, both in natural repeat spawners and in artificially reconditioned fish (Keefer et al. 2008; Pierce, et al. 2016a). Consecutive spawning rates vary substantially between projects, and between years (Hatch et al. 2017). The proportion of consecutive spawners in any given year has a major impact on the both the impact and the operation of reconditioning projects. Only maturing consecutive spawners and skip spawners held from the previous year are released to spawn and contribute to steelhead production for a given year. Moreover, the productivity of the two life history types is greater than that of maidens, and increases further from consecutive to skip spawners (Section C2). This results in variation from year to year in the productivity benefit to be expected from reconditioning projects. Non-maturing potential skip spawners must be reconditioned for an additional year, requiring additional project resources. For these reasons, we seek an improved understanding of the physiological decision mechanisms underlying the consecutive and skip spawning life histories.

Skip spawning is common in seasonally breeding iteroparous fish (Rideout et al. 2005b; Rideout and Tomkiewicz 2011b). In salmonids, maturation is thought to be initiated based on energy reserves during seasonally defined critical periods (Satterthwaite et al. 2009; Thorpe 2007). Fish that do not initiate maturation during a certain period of time will skip reproduction for that cycle. This is likely the same process for repeat maturations. Maturation is thought to be condition-dependent based on energetic levels (McBride et al. 2015). Maturation requires a fish to exceed genetic thresholds for energy (Thorpe 2007), where energy either exceeds or falls below a threshold, creating reaction norms (Hutchings 2011a), which predict whether a fish will mature. Fasting steelhead use energy reserves gained in the ocean to make return migrations from the ocean and spawn, which they will need to replenish in order to spawn again.

The critical period for initiation of maturation in salmonids is thought to occur approximately one year before spawning (Campbell et al. 2006b; Satterthwaite et al. 2009; Thorpe 2007). However, the timing of the critical period for initiation of maturation in steelhead kelts is not known in detail. In maiden rainbow trout, energy restriction during the first third of the year prior to spawning resulted in a reduced proportion of maturing fish (Bromage et al. 1992b). In repeat spawning rainbow trout, energy restriction after spawning resulted in reduced plasma estradiol (E2) levels within 10 weeks after spawning (Caldwell et al. 2013). In our previous study in hatchery origin steelhead kelts, growth was significantly elevated in consecutive versus skip spawners over the initial 10 weeks after spawning (Hatch et al. 2017). Based on these findings, we hypothesize that rematuration as a consecutive spawner may be determined by

energetic status during the first 10 weeks after spawning. In order to test this hypothesis, we conducted an experiment to test the effects of energy restriction during this time period.

## Methods

In 2017, hatchery origin maiden female steelhead were air spawned at DNFH on three egg takes in February (Table 3C.1). Air spawning was conducted as previously described (Hatch, et al. 2014). After air spawning, fish were individually PIT tagged, 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). Fish were prophylactically injected with oxytetracycline to control bacterial infections and emamectin to control copepods and blood sampled. The total weight of eggs collected from each female was recorded, and a subsample of approximately 25 eggs from each female was taken in order to determine individual egg weight.

**Table 3C.1:** Survival and maturation in air spawned DNFH female steelhead fasted or fed during the initial 10 weeks after spawning.

Take	Treatment	Fish #	Mortalities	Survival %	Maturing	Non-maturing	Maturation %
One, 2/7/2017	Fed	32	12	62.5%	7	13	35.0%
	Fasted	32	11	65.6%	7	14	33.3%
Two, 2/21/2017	Fed	32	20	37.5%	5	7	41.7%
	Fasted	32	19	40.6%	1	12	7.7%
Three, 2/28/2017	Fed	31	22	29.0%	3	6	33.3%
	Fasted	32	25	21.9%	2	5	28.6%
All	<b>Fed</b>	<b>95</b>	<b>54</b>	<b>43.2%</b>	<b>15</b>	<b>26</b>	<b>36.6%</b>
	<b>Fasted</b>	<b>96</b>	<b>55</b>	<b>42.7%</b>	<b>10</b>	<b>31</b>	<b>24.4%</b>

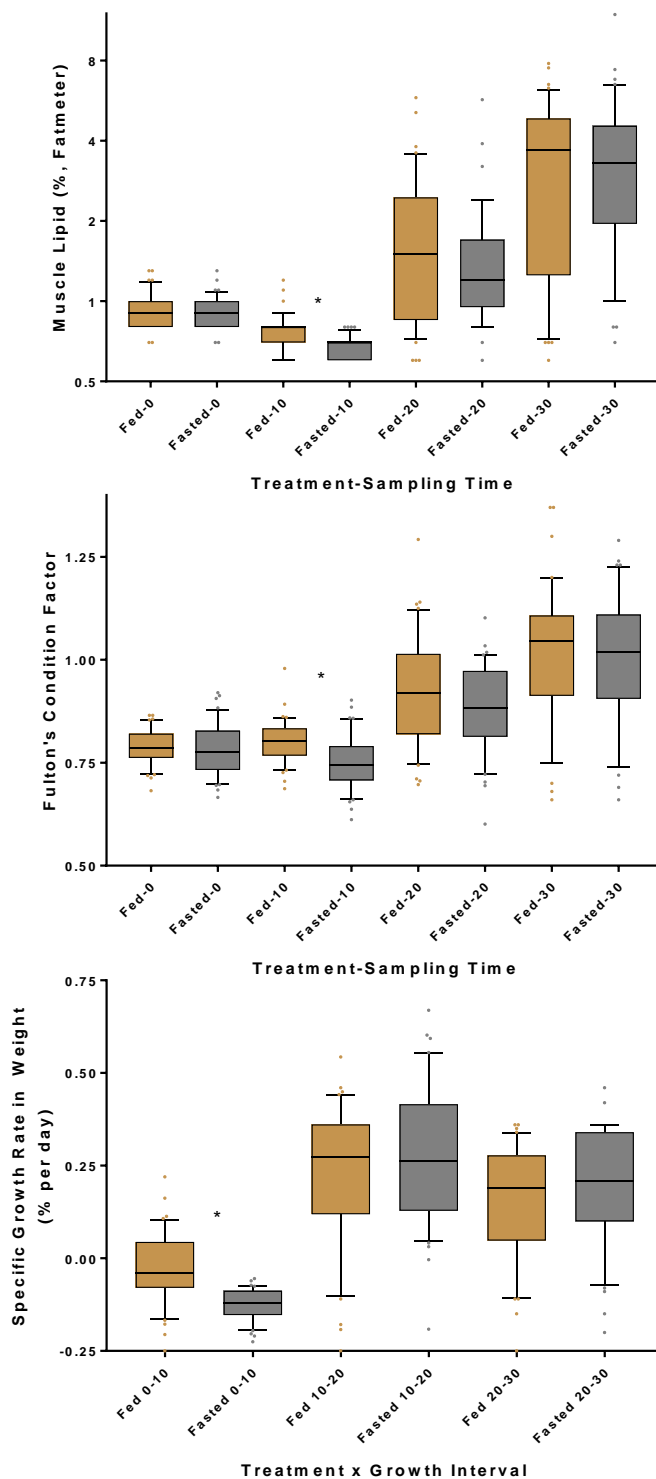
Fish from each take were randomly divided between two tanks. Due to limitations on the number of tanks available, fish from the second two takes, which were one week apart, were combined into the same tanks. One tank from each take was fed a mixture of krill and pellets to satiation, and the other tank was fasted (Hatch et al. 2014). Fish from takes one and three were fasted for 10 weeks, and fish from take 2 were fasted for 11 weeks. After 10 weeks (11 weeks for take 2), all fish were sampled, fish were consolidated into one tank per take, and all tanks were fed to satiation. Sampling continued at 10-week intervals until fish were terminally sampled in September. During non-lethal sampling, fish were anesthetized, length and weight were recorded, a Fatmeter reading was taken, a blood sample was taken, and fish were injected with oxytetracycline and emamectin. During lethal sampling, in addition, fish were killed, dissected, and ovary and liver weights recorded. Mortalities were recorded daily. Only fish positively identified by PIT tags through the entire experiment were included in the analysis. Growth rates and organo-somatic indices were calculated as previously described (Hatch et al. 2017). Because plasma estradiol levels have not yet been assayed, females with a September gonadosomatic index greater than 1% were classified as maturing.

## Results

The results from this study are preliminary at this point, as laboratory assays and statistical analysis of results are ongoing.

Survival decreased substantially from take 1 to take 3 (Table 3C.1). However, survival was not significantly affected by feeding treatment (Chi-Square test,  $p > 0.999$ ). Maturation percentage did not differ substantially between fasted and fed fish from take 1 and take 3. However, there was a large difference in take 2, with fed fish maturing at a 41.7% rate, versus 7.7% in fasted fish. Overall, the effect of fasting on maturation was not significant (Chi-Square test,  $p = 0.2292$ ). Fasting reduced growth rate over the initial 10 week fasting period, and muscle lipid levels and condition factor at the 10-week sampling point (Fig 3C.1). Fed fish maintained higher muscle lipid levels and condition factors versus fasted fish at subsequent time points, however, these differences were not significant.

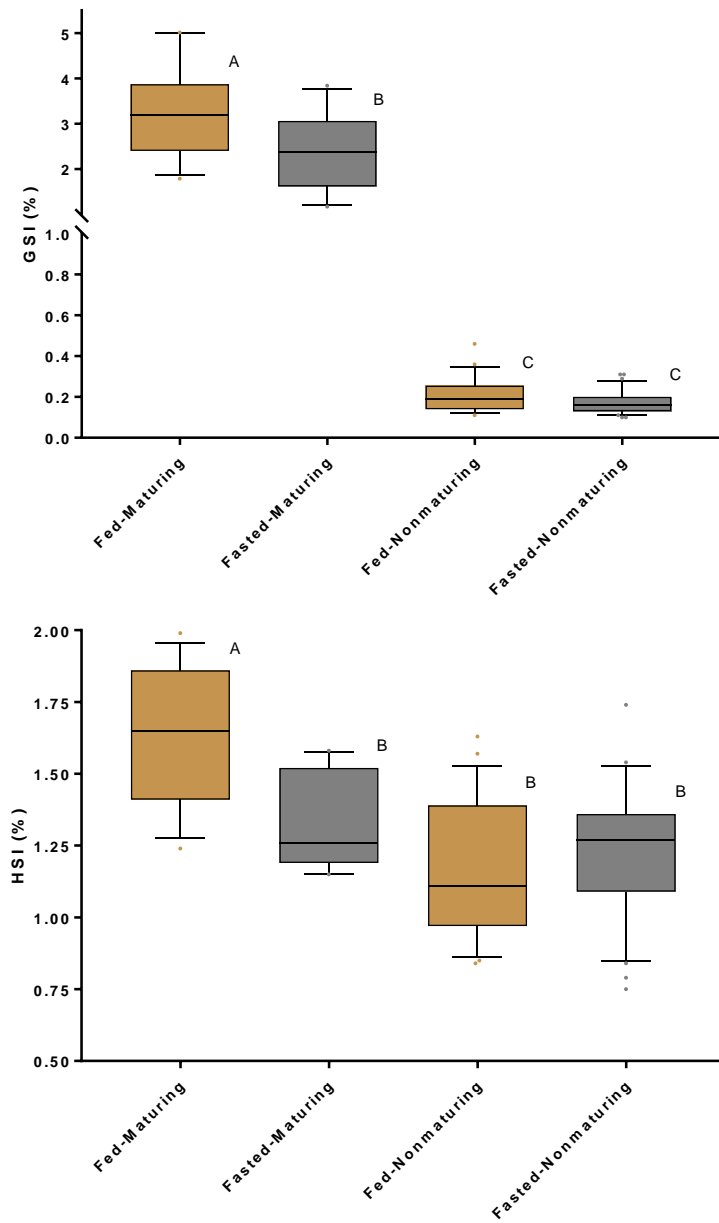
**Figure 3C.1:** Growth and adiposity metrics in full fed fish and fish fasted for the first 10 weeks after spawning. Asterisks indicate significant differences between groups (T-test,  $p < 0.05$ ). Differences over time have not yet been analyzed.



Fasting treatment, maturity status, and the interaction of these factors all significantly affected gonadosomatic index (GSI). As expected, GSI was significantly greater in maturing females. GSI was significantly reduced in maturing females that were fasted for the initial 10 weeks after spawning versus fully fed females (Fig 3C.2). GSI in non-maturing females was also lower in the fasted group, but this difference was marginally non-significant (T-test,  $p = 0.0507$ ). Maturity status and the interaction of maturity status and fasting treatment significantly affected hepatosomatic index (HSI), however, the effect of fasting treatment was not significant apart from the interaction ( $p = .0764$ ). HSI was significantly reduced in fasted maturing females versus fully fed maturing females, but no difference was detected between the fasting treatments in non-maturing females.



**Figure 3C.2:** Gonadosomatic index (GSI) and Hepatosomatic index (HSI) in maturing and non-maturing fish lethally sampled 30 weeks after spawning. Bars not sharing a letter differ significantly (ANOVA followed by Tukey's test,  $p < 0.05$ ).



## Discussion

Fasting during the first 10 weeks after spawning did not result in reduced maturation rate in two of the three takes used as experimental replicates in this study. These results do not support our hypothesis that the critical period for rematuration as a consecutive spawner occurs during the period immediately after spawning in female steelhead kelts. However, in take 2, maturation rate did appear to be suppressed by fasting. Take 2 was fasted for one week longer than the other takes, due to limitations on the number of tanks available for the study. However, given that these fish would have not fed for approximately 5 months before they returned to the hatchery, it seems unlikely that this extra week of fasting would have made a difference. Due to the inconsistency in results between takes, this experiment should be repeated.

The critical period model of salmonid maturation has led to a large number of modeling studies, but relatively few experimental studies have directly tested the key elements of the model. In particular, the exact timing of the critical period for initiation of maturation is vague. It has been proposed to be in April in models of maturation in steelhead, but this is not based on any physiological evidence (Satterthwaite et al. 2009). It is possible that the critical period for initiation of maturation occurs before spawning in steelhead kelts. While prolonged nutritional restriction will result in the arrest of maturation (e.g. Yamamoto et al. 2011), it is possible that the 10 week fast employed in this study was not sufficient to cause arrest of maturation. Survival did not differ between fed and fasted fish in this study, suggesting that feeding during the 10 weeks after spawning is not determinative of survival. Fasting did result in growth suppression and a reduction in energy stores after 10 weeks, indicating that the treatment had an effect. However, growth rates were near zero in fed fish over the first 10 weeks, and the differences in muscle lipid level and condition factor was due to greater decreases from spawning to week 10 in fasted fish than in fed fish. In contrast, when food was made available to the fasted group beginning 10 weeks after spawning, growth rates and energy reserves increased substantially over the following 10-week period. Thus, there may be a physiological process of recovery from spawning that must occur before feeding and growth can take off. Fasting during the 10-week period after spawning significantly reduced the GSI in maturing fasted fish to approximately 75% of that in maturing fed fish. Energy transfer into the developing ovary takes place during exogenous vitellogenesis, during which the liver produces large amounts of the egg protein vitellogenin (Lubzens et al. 2010; Tyler et al. 1990). The increased HSI in maturing fed fish is likely due to greater vitellogenin production. The reductions in GSI and HSI in fasted fish are consistent with the possibilities that total reproductive investment was reduced in fasted fish, or that reproductive development in fasted fish was delayed relative to fed fish. Our study on reproductive performance in DNFH hatchery fish (Section 3B) suggests that energy reserves during early ovarian development determine reproductive investment measured at spawning. Continued feeding during the second half of ovarian development, from approximately September to February in this stock, did not result in increased reproductive investment. Based on these results, we believe that it is unlikely that ovarian development in fasted fish in the present study would be able to catch up with the fed fish after September. Therefore, we believe the most reasonable interpretation of the reduced

GSI in fasted fish is reduced total reproductive investment, which would be expected to result in fewer and/or smaller eggs at spawning.

### **3.D: Development of time-resolved fluorescence immunoassays for salmonid plasma insulin-like growth factor 1 (IGF-I) and growth hormone (GH)**

#### **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. 2005b). 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. 2006b; 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.

Previously, work was done to develop a radioimmunoassay for IGF-I; however, due to our acquisition of a plate reader capable of reading time-resolved fluorescence (TRF), more recent work has focused on developing a competitive TRF immunoassay, which will alleviate the need for radioactivity and the subsequent reliance on an external lab for use of their equipment. As such, work on the development of a TRF assay for IGF-I in steelhead plasma began this year by working with PerkinElmer to produce a custom, europium-labeled IGF-I peptide for use in a TRF assay, to be run on the recently purchased Victor X4 (PerkinElmer). During 2017, we were able to get this assay fully validated, and started work on developing a TRF assay for salmonid GH.

## **Methods**

### **IGF-I Assay**

#### ***Europium Labeling***

##### **Preparation:**

As requested by PerkinElmer, 400 ug of lyophilized GroPep recombinant barramundi IGF-I (rbIGF-I; GroPep.com, Australia) was sent to PerkinElmer's Boston lab for custom europium labeling.

Standards were prepared by dissolving rbIGF-I in 0.01 N Acetic acid (1ug/uL). The resulting solution was then aliquoted into 0.5 mL polypropylene microfuge tubes in 1 ug aliquots, dried in speed vacuum, and store dessicated at -20C. Prior to use, standards will be reconstituted in DELFIA assay buffer (1244-111, Perkin Elmer) at a concentration of 0.4-30 ng/mL.

##### **Labeling reaction:**

Europium labeling was performed at PerkinElmer's Boston location using the proprietary DELFIA Eu-N1 ITC lanthanide chelate (Ref 1244-302, PerkinElmer).

#### **Extraction and Assay Protocols**

**The following protocols have been optimized and validated to be working. The protocol is divided into two steps: (1) acid/ethanol Extraction of the plasma samples and (2) running the extracted samples in the IGF-1 TRF assay.**

##### ***(1) Acid/Ethanol Extraction Protocol***

Overview: This protocol provides a description of how to perform acid/ethanol extractions on plasma samples to remove IGF-I binding proteins prior to evaluating the level of IGF-I in the sample and is based on the protocol developed by Shimizu, et al. (2000).

##### **Solutions and reagents needed:**

- 12 N (concentrated) HCl
- 200 proof EtOH
- 0.855 M Tris Base Solution
- ddH<sub>2</sub>O
- 12x75 Borosilicate glass tubes
- Centrifuge at 4C, can run @ 1860-10000 g

#### Notes:

- Samples should be acid/ethanol extracted prior to being run in the IGF-I TR-FIA. Please refer to the IGF-I Time-resolved Fluorescent Immunoassay protocol for necessary reagents, instructions, and timeline
- Make up the acid/ethanol Mixture fresh every day that it is needed -and- make up enough to perform the IGF-I TR-FIA
- For all steps, including preparing the acid/ethanol mixture, work in hood

#### Preparing Acid/ethanol (A/E) mixture:

1. Prepare 2N HCl by slowly adding 166 mL of 12N HCl to 834 mL of ddH<sub>2</sub>O
  - a. Can be stored in cabinet under hood
2. Measure 437.5 mL 200 proof EtOH into a 500 mL glass bottle, slowly add 62.5 mL 2N HCl to EtOH
  - a. Should be 12.5% 2N HCl to 87.5% 200 proof EtOH
  - b. For 12 mL, use 1.5 mL 2N HCl and 10.5 mL 200 proof EtOH

#### Preparing 0.855 M Tris Base:

1. Weigh out 10.35 g Tris base
2. Add to 100 mL ddH<sub>2</sub>O
3. Keep refrigerated

#### Extracting IGF-I binding proteins:

1. Turn on centrifuge and set to 4C. Load all four buckets on the SX4400 rotor, regardless of sample size. When loading, balance appropriately between at least two of the buckets (*i.e.*, it is not necessary to balance between all four buckets, only two need to be used). Set to maximum *g* (4255 or something like that)
2. Label three sets of 12x75 borosilicate tubes with appropriate sample numbers, plus extraction efficiency
3. Pipet out 125  $\mu$ L of each sample into the first set of tubes
  - a. Amount can be increased if trying to concentrate sample – the nature of this extraction process results in a 7-fold dilution
4. Add 500  $\mu$ L A/E mixture to each tube
  - a. Amount of sample used can change – just adjust the amount of A/E mixture used so that it's always 4x the amount of the sample being extracted
5. Mix thoroughly by vortexing
6. Cover tubes with parafilm to prevent evaporation
7. Let stand at room temperature for 30 min
8. Centrifuge at 4C for 30 min
9. Transfer or decant supernatant (probably around 420  $\mu$ L) into fresh set of tubes, making note of how much supernatant is in new tubes
10. Add 168  $\mu$ L of 0.855 M Tris base and mix
  - a. Can increase or decrease depending on volume of plasma extracted – should always be a 5:2 ratio of sample+A/E mix:0.855 M Tris base
11. Cover tubes with parafilm to prevent evaporation
12. Store tubes at -20C for 1 h
13. Turn on N-Evap water bath and set to 35C
14. Centrifuge at 4C for 30 min
15. Transfer or decant supernatant (probably around 500  $\mu$ L) to fresh tubes

16. Place tubes in N-Evap and dry down until liquid is evaporated
  - a. Will leave a gel-like film on bottom of tube
17. Store in freezer (-20 or -80) if need be
18. Reconstitute in 500 uL (or amount transferred at final decant) of DELFIA assay buffer
  - a. Can be reconstituted in less than was transferred if sample needs to be concentrated, though amount of plasma extracted should be increased so that the amount reconstituted is enough to run sample in triplicate

## ***(2) IGF-1 Time-Resolved Fluorescence Assay***

Overview: This protocol will ensure that you prepare reagents correctly and run the DELFIA IGF-I TR-FIA effectively. Protocol modified from Small and Peterson (2005) and Hevroy, et al. (2013), "Handling of GroPep Bioreagents IGF-I, IGF-II and IGF Analogs" (GroPep), and the infosheet for Barramundi IGF-I antiserum (GroPep).

### Solutions needed:

- Barramundi Eu-IGF-I, 16 ng/mL
- Anti-Barramundi IGF-I, 1:2072
- Barramundi IGF-I, 100 ug vial
- 10 mM HCl
- 0.9% saline + 0.5% RIA-grade BSA buffered with 50 mmol/L Tris-HCl
- A/E Blank Solution (1:2:4 assay buffer, Tris, A/E extraction mixture)
  - A/E extraction mixture is 12.5% 2N HCl and 87.5% 200 proof EtOH
- PerkinElmer DELFIA Assay Buffer (1244-111)
- Perkin Elmer DELFIA Wash Concentrate (1244-114)
- Perkin Elmer DELFIA Enhancement Solution (1244-105)
- Adhesive film for microplates (VWR 89087-690)
- ddH<sub>2</sub>O
- Plate washer x 2 (Room Temp & 4C)
- PerkinElmer goat anti-rabbit plates (AAAND-0004)

### Notes:

- Samples should be acid/ethanol extracted prior to being run. Please see the Acid/Ethanol Extraction protocol for necessary reagents and instructions.
- Prepare all reagents, standard curve, and samples the day of the assay (unless otherwise noted)
  - Reconstitute and dilute samples as needed before beginning assay
- Do not use phosphated buffers at any point
- Do not use the DELFIA assay buffer to reconstitute the IGF-I for the standard curve (prior to diluting the aliquots)
- **Directions are for one plate. If you need to run more plates on the same day, prepare the appropriate amount of reagents**

### Reconstituting the standards and making aliquots:

1. Add 100 uL 10 mM HCl + 400 uL 0.9% saline with 0.5% BSA buffered with 50 mmol/L Tris-HCl to the 100 ug vial of recombinant barramundi IGF-I
  - a. Produces a concentrated stock of 27.36 uM
2. Vortex gently

3. Transfer solution to a small falcon tube, making sure to note how much you transfer
4. Dilute the concentrated stock 1:10 (e.g., 450 uL concentrated stock in 4050) with the 0.9% saline solution
  - a. Produces a diluted stock of 2.736 uM
5. Vortex gently
6. Pipet 50 uL aliquots into 0.5 mL microcentrifuge tubes
7. Store in labeled box in -20C

Making up the standard curve from diluted aliquots:

1. Label eight 1.5 mL microcentrifuge tubes S1-S8
2. Add 1980 uL of DELFIA assay buffer to S1
3. Add 1000 uL of DELFIA assay buffer to S2-S8
4. Add 20 uL of diluted stock (2.736 uM) to S1
5. Gently vortex S1
6. Add 500 uL of S1 to S2
7. Gently vortex
8. Repeat steps 6-7 for S3-S8
  - a. Lowest detectable limit is 0.2 ng/mL

Reconstituting the antibody and making aliquots (1 to 51.8 dilution):

1. Add 518 uL of 0.9% saline solution to vial
2. Pipet 60 uL aliquots into 0.5 mL microcentrifuge tubes
  - a. Will make ~8 aliquots
3. Store in labeled box at -20C

Making a working solution of the antibody (1 to 2072 dilution):

1. Thaw 1 diluted stock aliquot (1:51.8 dilution)
2. Once antibody is thawed, vortex gently and then spin briefly in a mini centrifuge
3. Add 2120 uL DELFIA assay buffer (DAB) into a 12x75 glass tube
4. Remove 53 uL of DAB from what you just pipeted
5. Pipet 53 uL of antibody into 12x75 glass tube (for a total of 2120 uL)
6. Vortex GENTLY

Initial dilution and aliquoting to make stock (1 ug/uL):

1. Pipet 10 uL of label (concentrated stock – 225 ug/mL) into 12x75 glass tube and add 2240 uL of 0.9% saline solution
2. Vortex gently
3. Aliquot 50 uL into 0.5 mL microcentrifuge tubes
4. Store in labeled box at -20C

Making a working solution of label (16 ng/mL):

1. Thaw 1 diluted stock aliquot (1 ug/mL) and vortex gently
2. Add 2250 uL DELFIA assay buffer (DAB) into a 12x75 glass tube
3. Remove 36 uL of DAB from what you just pipeted
4. Pipet 36 uL into 12x75 glass tube (for a total of 2250)
5. Vortex gently

Preparing the plate wash buffer:



DELFIA Wash Solution is provided as a 25x concentrate; a full plate requires 150 mL, so make up (or make sure there is) enough (150 mL x # of plates). The following instructions prepare 2 L of wash. Stable for 2 weeks at 2-25C

1. Pour 80 mL of DELFIA Wash Concentrate into a clean container
2. Add 1920 mL of ddH<sub>2</sub>O

Immediately prior to running the assay:

- Allow all reagents to reach room temperature
- Ensure all reagents are mixed by gently mixing
- Avoid foaming
- Reconstitute samples, saving left over if sample needs to be diluted

Assay Protocol:

*Addition of the reagents*

*Standard Curve*

Add 125 uL from S8 to both of the lowest standard wells. Add 125 uL from S7 to each of the next to standard wells. Continue with this procedure until all the standards are pipeted. The same pipette tip should be used to pipet all the standards. Before pipetting each standard, be sure to equilibrate the pipette tip in that standard and then gently vortex.

*DELFIA Assay buffer (DAB)*

Add 145 uL of DAB to NSB wells and 125 uL DAB to B<sub>0</sub> wells.

*Samples*

Add 125 uL of sample per well in triplicate (preferred) or duplicate.

*Label*

Add 20 uL of label to each well *except* the Blk wells.

*Antibody*

Add 20 uL of antibody to each well *except* the NSB and Blk wells.

*Incubation of the Plate*

Cover the plate with an adhesive film and incubate overnight (~16 hours) at 4C with slow shaking

*Washing the plate*

Aspirate each well and wash, repeating the process three times for a total of 5 washes. Wash by filling each well with diluted DELFIA Wash Solution (~300 uL). Good aspiration of all liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot clean.

*Development of the plate*

1. Add 200 ul of delfia enhancement solution to each well
2. Cover the plate with an adhesive film and shake slowly at room temperature for 5 min
3. Read the plate immediately

*Reading the plate*

1. Wipe the bottom of the plate with a clean kimwipe to remove fingerprints, dirt, etc
2. Carefully remove the adhesive film

3. Note: any loss of enhancement solution could affect the absorbance readings. If any solution is present on the cover, use a pipette to transfer the solution back into the well. If too much solution has splashed on the cover to easily redistribute back into the wells, wash the plate five times with diluted delphia wash buffer and repeat the development with fresh delphia enhancement solution.
4. Read the plate immediately using the victor x4 microplate reader and the europium program

### *Calculation of Results*

1. Average the Blank wells and subtract value from all wells ← "Corrected Absorbance"
2. Average the readings from the NSB wells
3. Average the readings from the B0 wells
4. Subtract NSB average from the B0 average ← "Corrected B0" or maximum binding
5. Calculate the B/B<sub>0</sub> for the remaining wells  
To do this, subtract the average NSB from the corrected absorbance for each well and divide by the corrected B0. Multiply by 100 to calculate the %B/B<sub>0</sub>
6. Plot %B/B<sub>0</sub> for standards S1-S8 versus the somatotropin concentration using linear (y) and log (x) axes and perform a 4-parameter logistic (4-PL) curve fit using GraphPad Prism
7. Interpolate sample concentration based on the %B/B<sub>0</sub>
8. Samples with %B/B<sub>0</sub> values greater than 80% and less than 20% should be re-assayed

### **GH Assay**

We provided ProSpec with the amino acid sequence of rainbow trout (*O. mykiss*) GH. They then produced a custom recombinant rainbow trout GH protein (rtGH) for us. We sent 400 ug of rtGH to Perkin Elmer for labeling with Europium chelate. Europium labeling was performed at PerkinElmer's Boston location using the proprietary DELFIA Eu-N1 ITC lanthanide chelate (Ref 1244-302, PerkinElmer). 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). ProSpec's recombinant rtGH will be used as standard in the assay.

### **Biological Validation Experiment**

Juvenile rainbow trout (80, approximately 150 g body weight) were obtained from and housed at the Aquaculture Research Institute at the University of Idaho. The fish were 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 was not injected and acted as a double control. A t = initial blood sample was 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 were sampled at 1, 3, whereas the 0.033 ug/g body weight were sampled at 12 hours post-injection. Fish from the growth hormone-injected group were only sampled at 12 hours post-injection. All fish were lethally sampled to obtain blood samples for hormone (GH and IGF-1) analysis and liver tissue for qPCR (IGF-1 mRNA) analysis. The injection concentrations and time courses were 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 followed the Standard Operating Practices for rainbow trout (*e.g.*, those used by the ARI).

Details of fish handling:

- Blood Sampling: Fish were anesthetized in buffered (pH 7.0) 100 mg/l MS-222. Blood (1.5 – 2 ml) was drawn from the caudal vessels of anesthetized fish using 21-gauge needles fitted to heparinized 3 ml syringes.
- Terminal Sampling: Fish were euthanized in buffered (pH 7.0) 250 mg/l MS-222. The entire liver was collected for analysis.

## **Results**

### **IGF-I Assay Development**

Basing our initial optimization and troubleshooting efforts on Small and Peterson (2005) *and* Hevroy et al. (2013) gave us a very good starting point. Even using several dilutions for the antibody still resulted in us confirming that the 1:2072 dilution published by Small and Peterson (2005) is the correct one as it results in about 30% of maximum binding (Fig. C4.1), though we decided to test 1:4144 as well when moving on to determine the correct standard curve to use. We confirmed that the standard curve range in Small and Peterson (2005) was appropriate for us (200 – 0.09 ng/mL) and worked well with our reagents, and also that the 1:2072 antibody dilution was the best for that standard curve (Fig. C4.2). We proceeded with running samples (extracted according to the protocols cited by the aforementioned manuscripts), but started encountering some variation in the assay that led us to believe that the high level of ethanol in the assay was eliciting variation in the standard curve and samples which may lead to us not being able to make interassay comparisons (Fig. C4.3). Because the only samples that were parallel to a standard curve were those that did not contain any ethanol, we decided to develop an extraction method that would not leave any ethanol in the samples so we would not have to have any in the assay. We modified the existing protocol to involve a step that dried the samples down under nitrogen and then tested the reconstituted samples in the assay. This produced curves that were parallel to the standard curve and the results were repeatable (Fig. C4.4). We then performed an interspecies comparison and confirmed that the assay can be applied, after validation, to a range of species (Fig. C4.5). We then proceeded to extract and analyze the final samples from vehicle- and GH-injected fish. The GH-injected fish experienced a significant increase in plasma IGF-1, relative to the vehicle-injected fish, 12 hours post-injection (Fig. C4.6), providing biological validation of the assay.

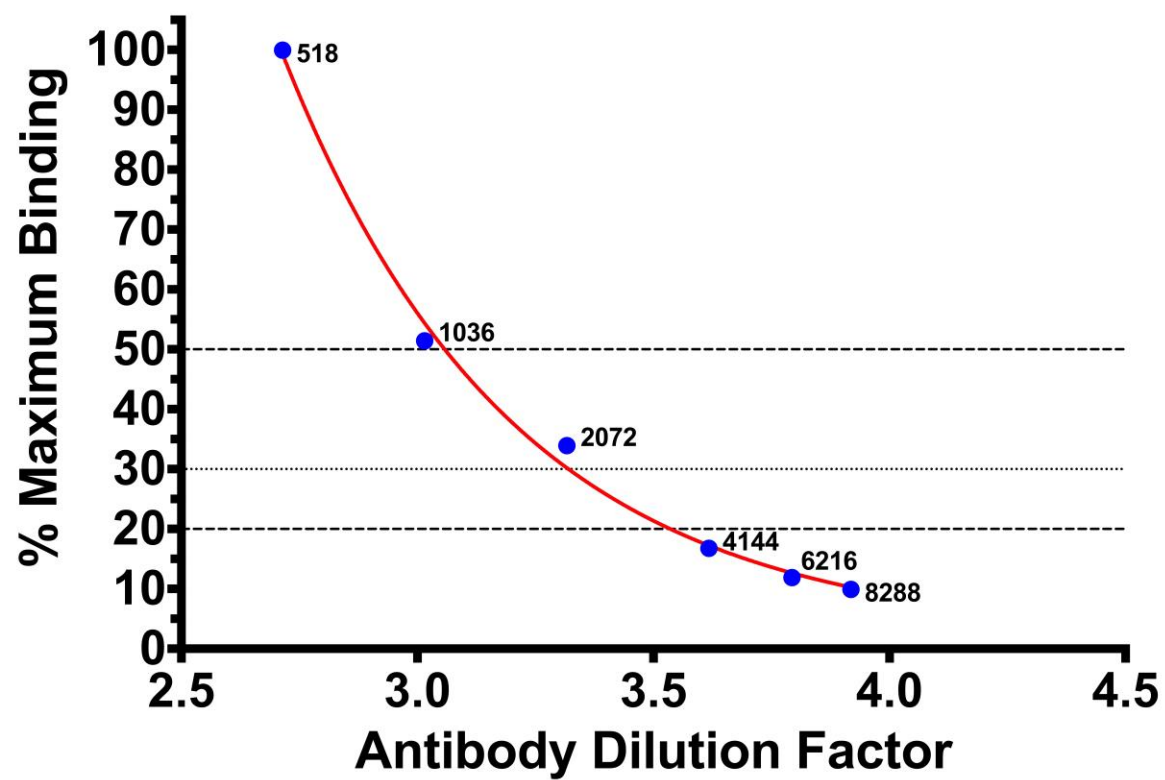


Figure C4.1. Antibody dilution test to determine which antibody dilution produced 30% of maximum binding (relative to binding observed at the lowest dilution).

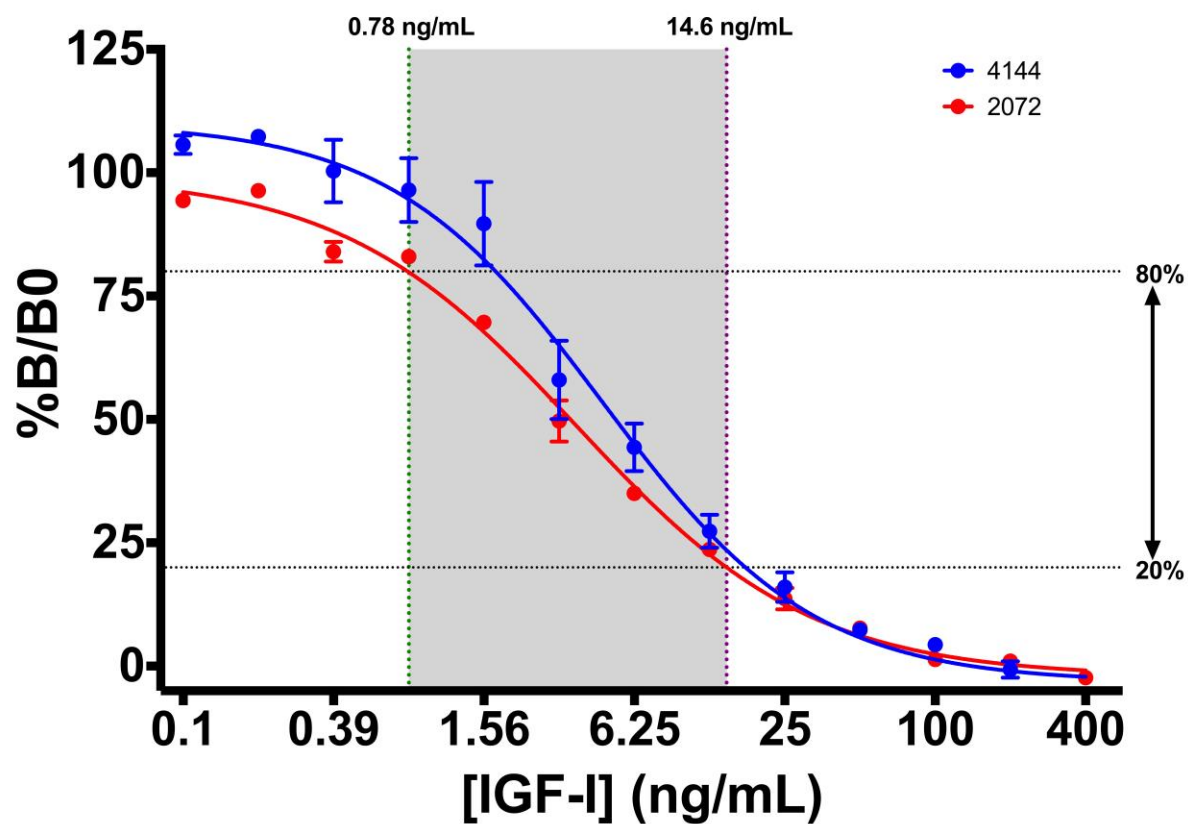
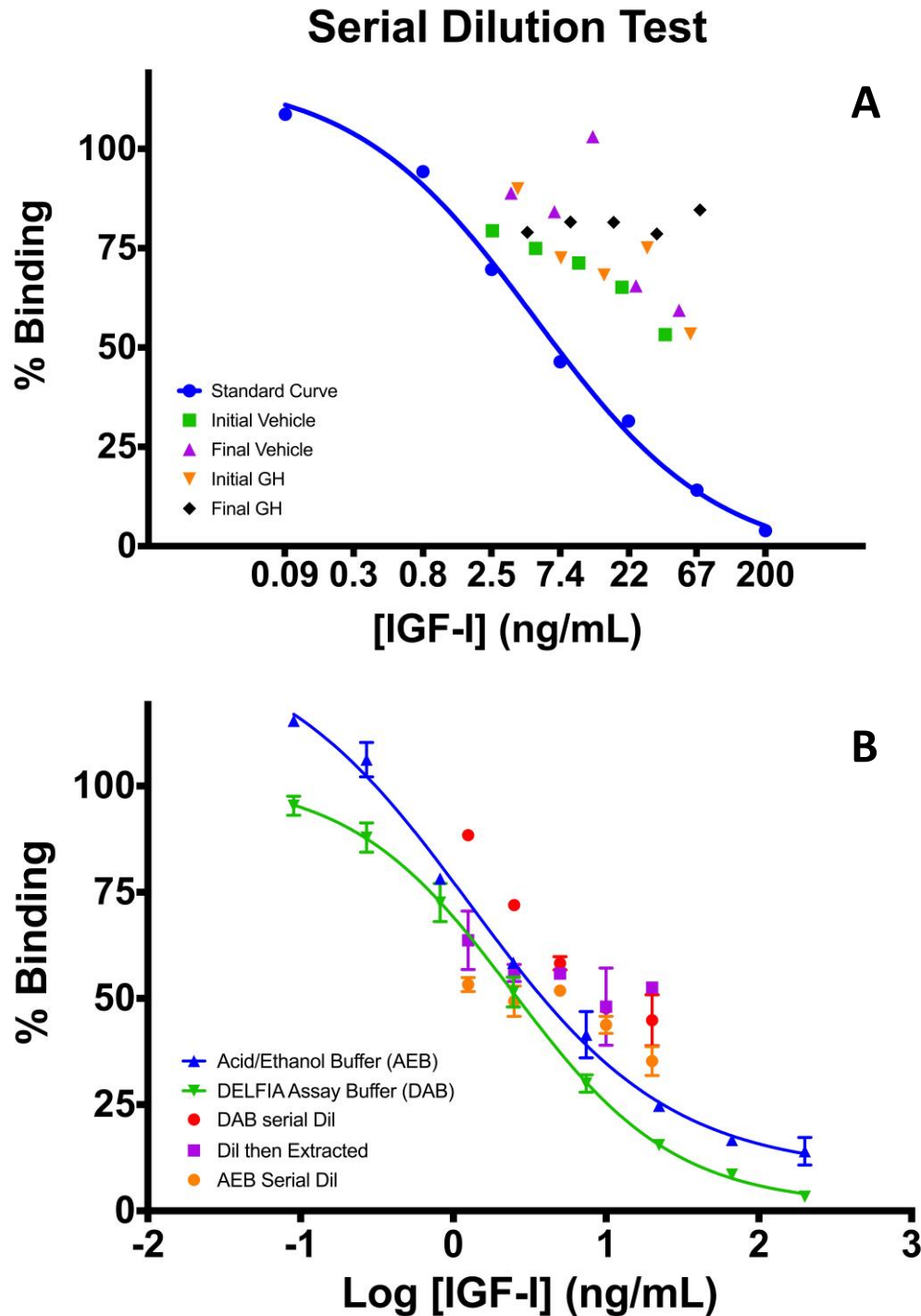


Figure C4.2. Standard curve comparison between two antibody dilution options. The 1:2072 dilution (red points and line) produced the lowest variation and highest  $R^2$  value for a 4-parameter multiple logistic regression. The linear portion of the curves (outlined in the grey square) corresponds to the useable portion of the curve.



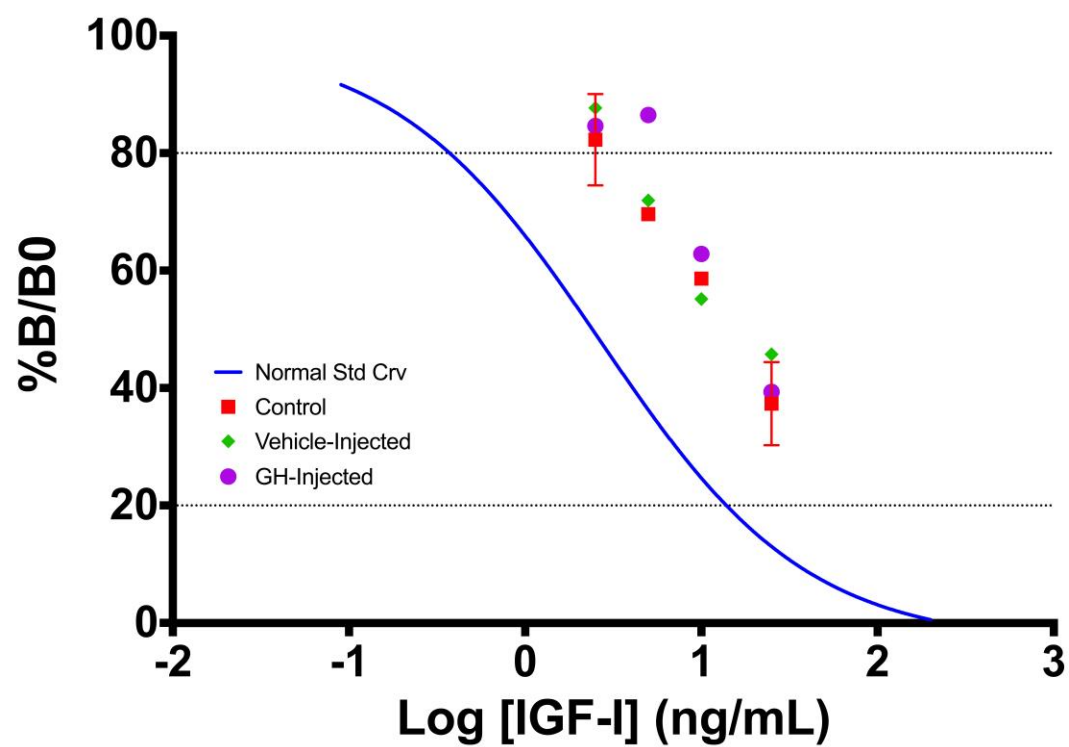


Figure C4.4. Samples that were extracted and dried down compared to a standard curve that did not contain any ethanol. Note that all diluted samples (red, green and purple points) are parallel to the linear portion of the standard curve (blue line).

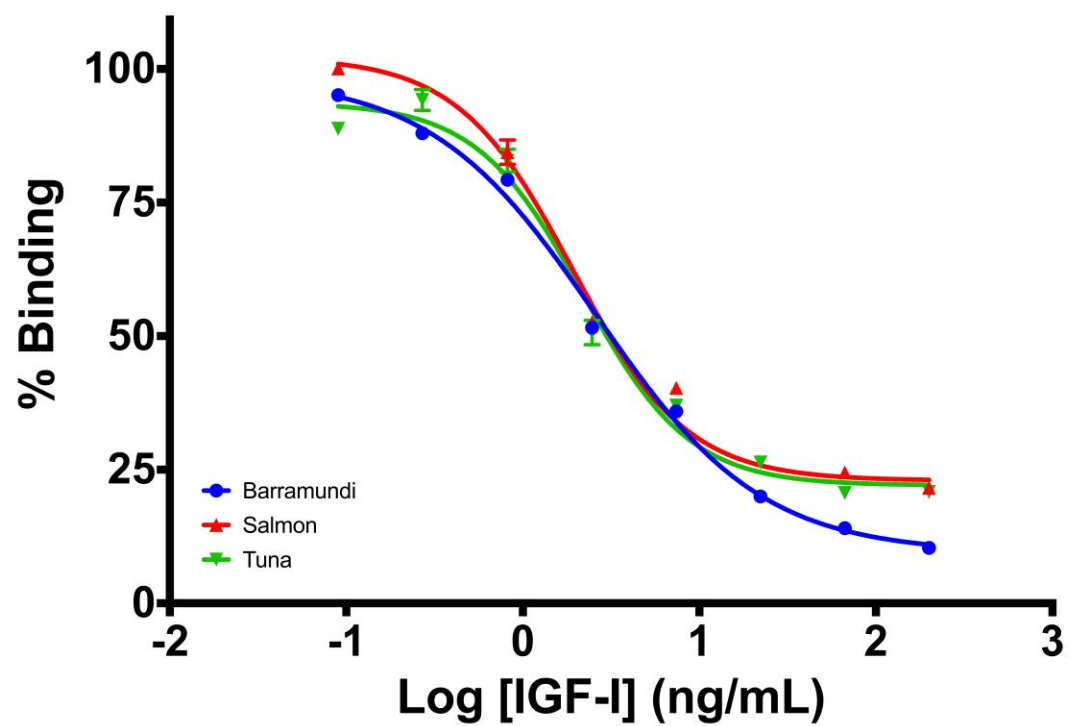
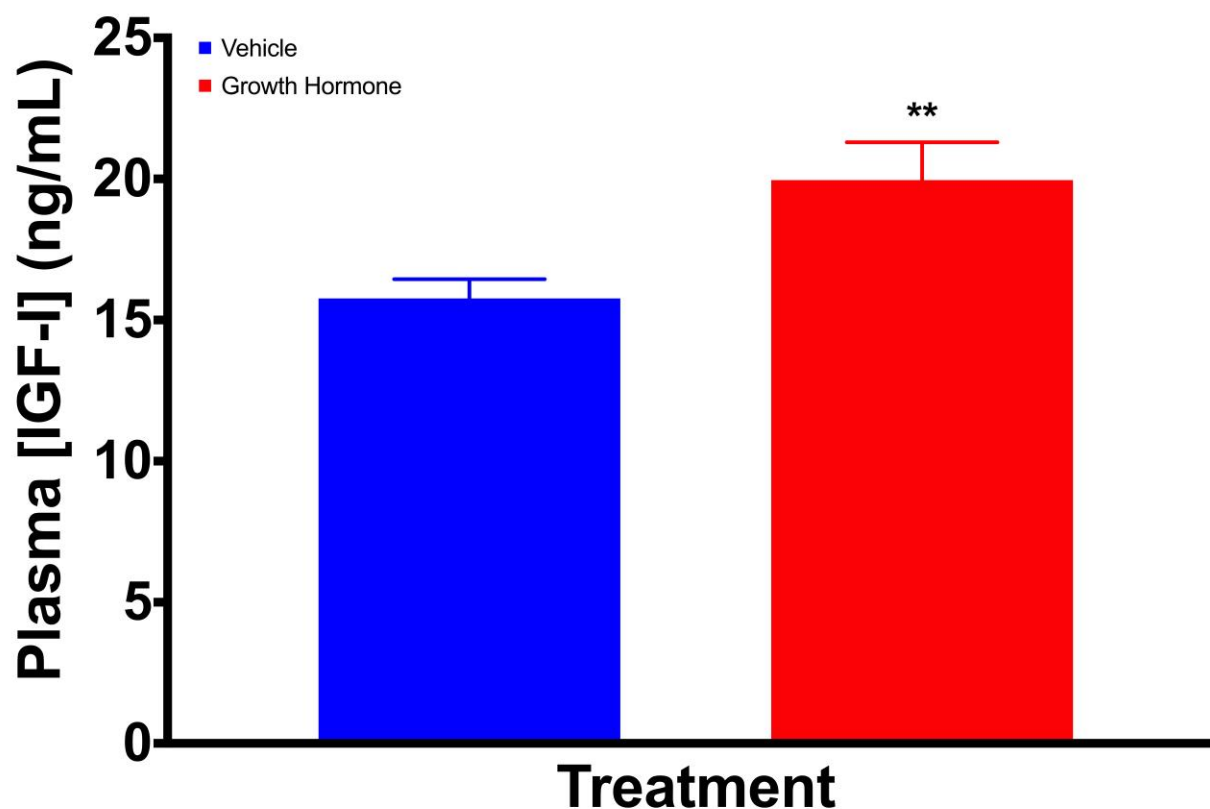


Figure C4.5. Interspecies comparison. While maximum and minimum binding may vary slightly between the species, the linear portion of the curve is nearly the same between all three-species tested. This speaks to the highly conserved nature of the IGF-1 peptide and the repeatability of the assay itself.





**Figure C4.6.** Plasma IGF-1 concentrations of vehicle- and GH-injected fish 12 hours following the injection. As expected, the GH-injected fish had significantly higher plasma IGF-1 concentrations compared to the vehicle-injected fish. This provides biological validation the IGF-1 TRF Assay is working.

#### GH Assay Development

Our initial rtGH labelling attempt failed. We determined that the rtGH we obtained from ProSpec was aggregated, and worked with ProSpec to resolve the issue. They provided us with a new lot of rtGH (free of charge), which was successfully labelled by Perkin Elmer (also free of charge). We now have all the key reagents needed to proceed with the assay.

#### Discussion

We have completed development and validation of an assay that detects IGF-I concentrations in salmonid plasma. The samples we have run provide biological validation of the assay as we saw a significant increase 12 hours post GH-injection, and it is generally accepted that GH elicits an increase in plasma IGF-1 in salmonids (Pierce, et al. 2005a; Small and Peterson 2005). This assay will be further vetted by continuing to analyze samples collected in earlier studies. Furthermore, removing the ethanol from the assay has allowed us to store extracted samples at -80 C and continue extracting more samples before proceeding to the assay. This substantially

streamlines the process and speed up the analysis versus previous protocols, which required samples be assayed for IGF-I immediately after extraction.

After experiencing some difficulty with vendors, we have now obtained all of the reagents necessary for the GH assay. The biological validation experiment we conducted will also be used for the GH assay, since ghrelin injection increases plasma GH in salmonids (Shepherd et al. 2007).

### **3.E: Effects of long-term administration of a glucocorticoid receptor antagonist on feed intake and growth in post-spawning rainbow trout**

#### **Introduction**

Reproduction, including gonadal development and gametogenesis, is under control of the hypothalamic-pituitary-gonadal (HPG) axis in fishes (Nagahama, et al. 1993). The HPG axis controls these processes using gonadotropin releasing hormones, gonadotropic hormones and sex steroids released from the hypothalamus, pituitary and gonads, respectively. Successful reproduction is dependent upon many different factors, both environmental and physiological, controlled by a complex neuroendocrine system (Billard, et al. 1981; Zohar, et al. 2010). One of the deciding factors is stress; activation of the stress axis is believed to inhibit the HPG axis (Barton 2002; Donaldson 1990).

The stress axis is more formally referred to as the hypothalamic-pituitary-interrenal (HPI) axis, part of which comprises the central endocrine pathway of the stress response in fish (Barton 2002; Wendelaar Bonga 1997). Activation of the HPI axis results in an increase in levels of corticotropin-releasing factor (Medeiros, et al. 2014; Pepels, et al. 2002; Pepels, et al. 2004), adrenocorticotrophic hormone (Medeiros et al. 2014; Rotllant, et al. 2001; Sumpter, et al. 1986), and corticosteroids, such as cortisol (Medeiros, et al. 2010; Wendelaar Bonga 1997). Resting levels of cortisol vary naturally with endogenous diurnal and seasonal cycles, with high levels being observed during the final stages of gonadal maturation and during spawning (Laidley and Leatherland 1988).

Cortisol, the primary corticosteroid secreted from the interrenal cells, has a broad activity spectrum. Although there is no comprehensive model integrating these functions, it is now accepted that cortisol initially plays an adaptive function during stress, but that chronic elevations of cortisol contribute to the deleterious effect of chronic stress (Barton and Iwama 1991). When released, cortisol alters intermediary metabolism (e.g., inhibiting protein synthesis) (van der Boon, et al. 1991), reduces gut motility and increases liver glycogen concentration (Mommsen, et al. 1999; Wendelaar Bonga 1997). Increased levels of cortisol in the circulation also affect carbohydrate, protein and lipid metabolism in an effort to reallocate energy to compensate for the energy demands of the stress. This reduces the performance capacity of the fish during chronic stress as well as the recovery phase following stress (Schreck 1981, 1990).

Elevated cortisol levels and the resulting catabolism, anorexia, and suppression of immune function are hypothesized to cause post-spawning death in semelparous *Oncorhynchus* species (Dickhoff 1989). Getting kelts to initiate feeding appears to be a critical step in reconditioning (Evans, et al. 2001). Therefore, we propose to explore treatments that may block cortisol signaling, ultimately stimulating appetite and feeding in kelt steelhead. In teleosts, many long-term adaptive changes in response to cortisol are mediated by cytosolic glucocorticoid receptors (GRs) located in target tissues. The GR antagonist mifepristone (RU486) has been

shown to effectively block the physiological reaction to cortisol in salmonids and other teleosts (McDonald and Wood 2004; McDonald, et al. 2004; Medeiros et al. 2014; Medeiros and McDonald 2013; Rodela, et al. 2009), interrupting the cortisol signaling cascade that leads to depressed appetite and the other undesirable side effects of chronic stress. To determine if long term RU486 administration effectively blocks cortisol signaling, and thus may stimulate appetite in kelt steelhead, we tested if this treatment was effective in stimulating the feeding response and recovery from spawning in post-spawning female rainbow trout.

## Methods

Post-spawning (less than 1 week after being stripped) female rainbow trout (N = 24, avg weight = 779.6 g) were obtained and housed in the ARI lab at UI. On day zero of the experiment (2/1/2017), fish were anesthetized, PIT tagged, length and weight were recorded, a fat meter reading taken, blood sampled, and fish were intraperitoneally injected with vehicle alone or vehicle mixed with RU486. The vehicle consisted of equal parts vegetable oil to vegetable shortening, and solidifies after injection to act as a slow release vehicle in rainbow trout. RU486 was prepared in the vehicle mix at a concentration of 100 mg/mL and administered at a rate of 2  $\mu$ l per g body weight (*i.e.*, 200 mg/kg). Blood was collected, spun down in a centrifuge for 5 min at 8,000 x *g*, the plasma drawn off, and stored at -80C until further analysis could be performed. Fish from each treatment were randomly distributed between 2 tanks, with each tank having approximately equal numbers of each treatment and initially 12 fish per tank. Fish were fed Rangen 4 mm pellets at 9:30 AM and 3:00 PM for 30 minutes daily, uneaten food was collected at the end of the feeding period. Fish were nonlethally sampled 21 days after the initial injection, and terminally sampled 42 days after initial injection. During the nonlethal sampling at day 21, fish were anesthetized, PIT tag number, length and weight recorded, a fat meter reading recorded, injected with another dose of their initial treatment, blood sampled, and returned to the tank. During terminal sampling, fish were anesthetized, PIT tag number, length and weight recorded, a fat meter reading recorded, and blood drawn for plasma hormone assays. Fish were then killed, the weight of the liver and ovary were recorded, a liver tissue sample was collected for mRNA analysis, and a 1 x 1 x 1 cm<sup>3</sup> ovary tissue sample collected for histological analysis. Organo-somatic indices were calculated as  $100 \times [(\text{organ weight}) / (\text{body weight of intact fish})]$ . Individual fish growth rates in length and weight were calculated by the method of Ricker (Ricker 1979).

## Results

After the initial implantation on 2/1/2017, fish began to succumb to post-spawning stress at a higher rate than was expected (most likely due to the added stress of the handling necessary for sampling). Seven fish died within 24 hours of the initial injection, with approximately equal numbers from each treatment. By the nonlethal sampling at day 21 (2/22/2017), 17 fish remained, still with approximately equal numbers in each treatment. Sampling proceeded as described and fish were combined into one tank. Over the course of the rest of the experiment, an additional 5 fish died leaving a total of 12 remaining for the final sampling on 3/15/2017. Preliminary results of the morphometric and organosomatic indices suggest that the treatment did not affect any of those growth-related indicators; however, plasma hormone analysis and histological evaluation of the ovary is on-going and may provide more insight. Additionally,

considering the state of the fish, we may discover that the level of RU486 injected was not sufficient to block all cortisol signaling. This is supported by the fact that mortality was observed at equal rates in both treatment groups, implying that the RU486 treatment did not cause any harm nor lend any benefits to the fish. Further analysis is required to confirm this hypothesis, which may suggest that more experimentation is needed.

### **Discussion**

This experiment was compromised by low fish numbers that were not sufficient to allow for post-spawning mortality. Excessive mortality occurred during the 42 days of the experiment. Further analysis of the plasma and ovary tissue is needed before any conclusions regarding the efficacy of the treatment can be made.

## **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. A portion of these fish are detected at Prosser Dam and then further upstream at both Sunnyside Dam, while another group are detected at Prosser with mainstem detections in the spring that are indicative of post-spawning behavior, the last group are kelts that may have had a detection at Prosser as upstream migrants and were later recaptured at the CJMF consistent with timing and body condition as post spawn fish. 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 G are post-repeat spawn fish collected at CJMF a second time.

Location	Conclusive Evidence for Homing			Consistent with Homing				
	A. Maiden/ Repeat Spawner Tag Detection	B. Repeat Spawner Tag Detection + GSI confirmation	C. Conclusive Homing total A+B	D. Prosser Det. Only	E. Prosser Det./Sunnyside Instream Det (Operational: Nov. 2015)	F. Prosser Det./Outmigrating Columbia Mainstem Dam Det. (Spring)	G. Post Spawn Repeat Spawner Recaptured at CJMF	Total Spawners: Consistent w/ Spawning Movement (Total of D +E+F+G)
Yakima R	41	272	313	641	48	62	105	856
Omak Cr	11	-	11					
Total	52	272	324	641	48	62	105	856



# **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 survival rates are similar spatially and temporally;**

and,

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

## **Methods**

### **Kelt Treatments**

To compare kelt management options, we evaluated 4 treatments: 1. In-river control, where fish were PIT tagged and released back to the river; 2. Collect and transport fish around the hydrosystem and release them downstream of Bonneville Dam; 3. Collect, short-term reconditioning and transport; and, 4. Long term reconditioning.

#### **In-river migration (control).**

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

#### **Collect and Transport.**

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

#### **Short-term reconditioning and transport.**

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

#### **Long-term reconditioning.**

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

### **Evaluation**

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

because they were only placed in the long-term reconditioning treatment. In addition, we evaluated the natural repeat spawning rate using scales collected at Prosser Dam.

Fish from all four release groups were assumed to be actively migrating to the spawning grounds and representative of repeat spawners if their PIT tags were detected at Prosser Dam. Prior to 2005, PIT detections at Prosser Dam were only available for fish that migrated upstream through the adult trap on the right bank ladder that were sampled manually using the FS2001 system (Biomark, Inc., Boise, ID). Therefore, the actual numbers of upstream migrant detections at Prosser Dam were not available for any release group prior to 2005, and also were not available for the long-term release group prior to 2008 (because fish were released upstream of the dam as noted above). Because of these limitations, we chose to use extrapolations as described below to expand the data set available for evaluation. Active upstream migration of repeat spawners from the three release groups that reconditioned in the ocean (transport, short-term recondition with transport, and control release) was determined by querying the PTAGIS database for post-release detections of PIT-tags at McNary Dam on the mainstem Columbia River ([Table 5.1](#) and [Figure 5.1](#)). 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  $2\times$  ( $4/2=2$ ) versus leaving the kelts in the river. Comparisons were made within each year and across years using weighted means to account for different sample sizes among years. We calculated benefits for long-term reconditioned kelts from the Yakima River, Omak Creek, Hood River, and Snake River in a similar manner. The reconditioning benefits calculation was the survival rate of long-term reconditioned kelts from each location divided by three different control groups. The control groups were: 1. Survival rates of in-river release groups to BON (the same as the treatment groups). 2. Literature values (Hockersmith et al. 1995). 3. The composition of repeat spawners in the run at large sampled at BON based on scale pattern analysis and prior PIT-tag history. None of these control groups are perfect comparisons, for example survival of the in-river release groups is to BON not the river of origin so these are biased high due to mortality that likely occurs between BON and the river of interest. However, the in-river groups are paired by year with the treatment groups reducing annual variation.

## **Results and Discussion**

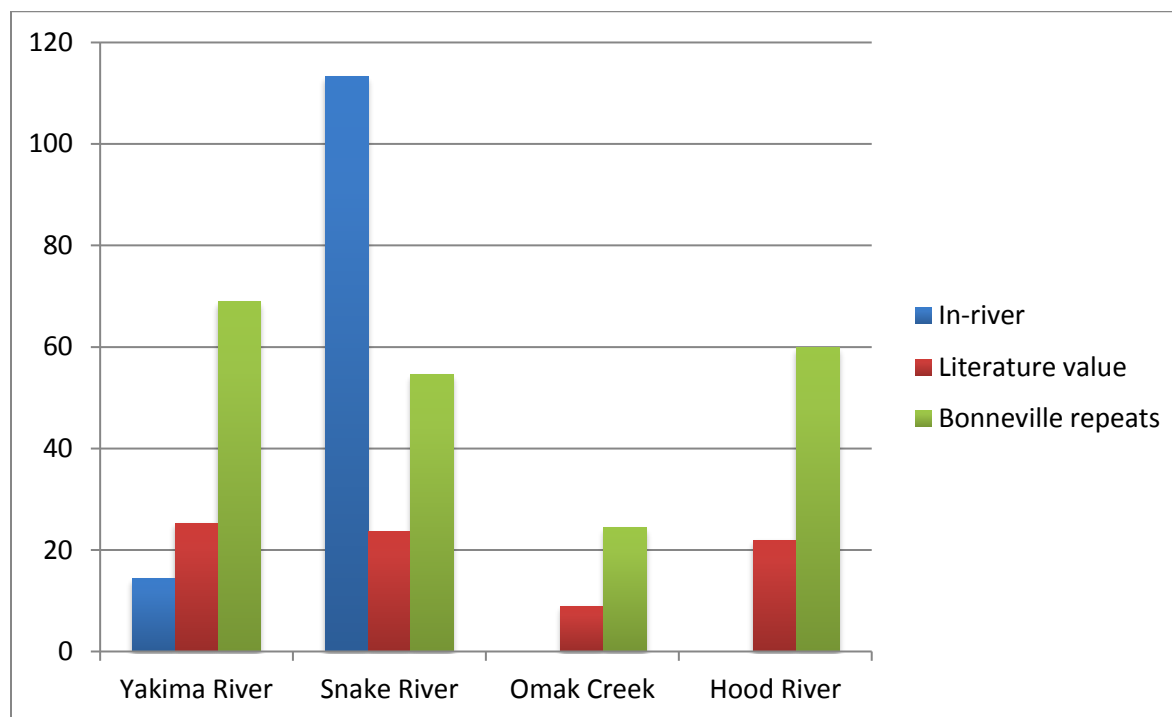
Long-term reconditioning demonstrated significantly higher return rates of repeat spawners (11-18%) than other treatments (1-3%) (Table 5.1). This result was supported in spite of variation in river, ocean, and fish condition between years that was incorporated into the error term in our analysis. The data extrapolation required in our analysis does not account for variation in environmental or fish conditions between years. However, this method does provide a best and worst-case interpolation of data for earlier years in the long-term

reconditioned group, thereby strengthening our ability to draw conclusions among the four treatments. For more in-depth analysis see Trammell et al. 2016.

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

Treatment	N	Mean	Grouping
Long-term min	10	11.5	A
Long-term max	10	17.6	A
Short-term	7	3.2	B
Transport	7	0.9	B
Control	7	2.7	B

Survival to release of long-term reconditioned kelt steelhead averaged 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.

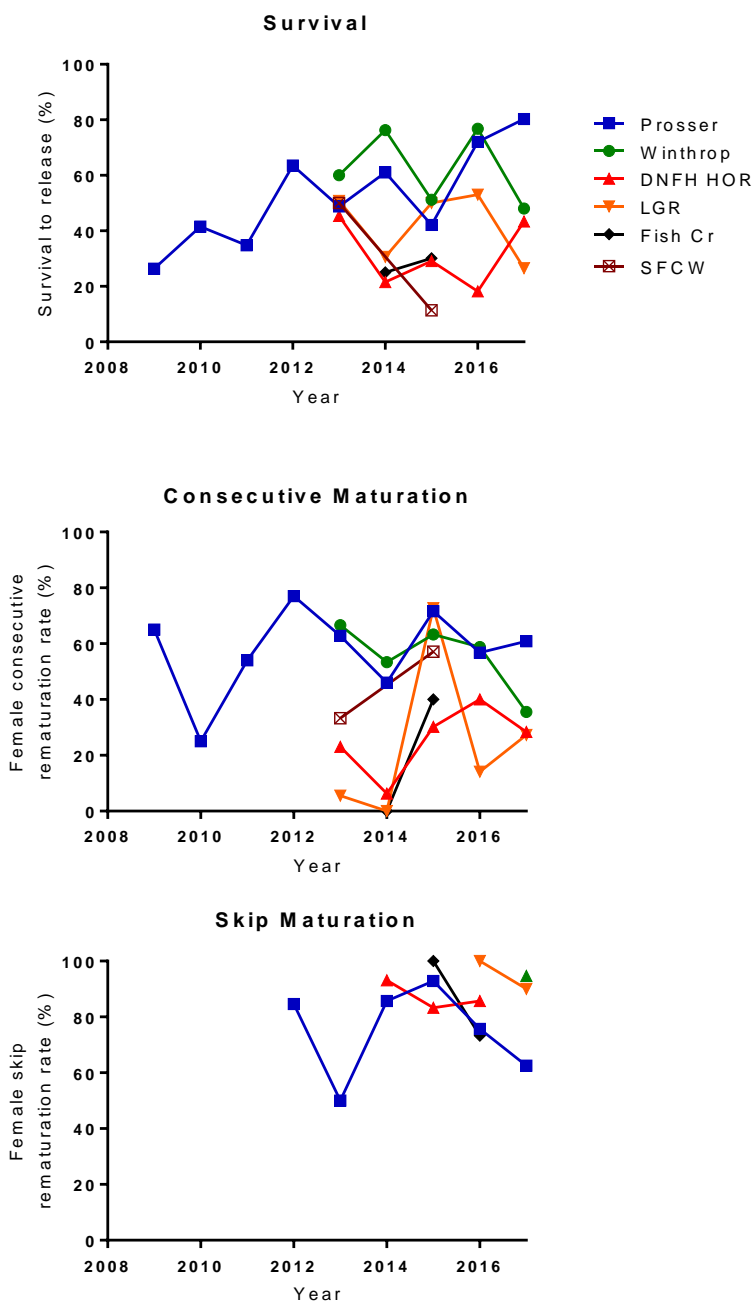


**Figure 5.1:** Benefits of long-term reconditioning relative to 3 control metrics. In-river control groups were not available for Omak Creek or Hood River.

## **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 and NPTH in 2017 may have been compromised by issues with formalin treatment and fish care.

Figure. 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 (DNFH 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 5 years. Survival of LGR kelts in 2014 and 2017 was compromised by issues with fish care and the water source. Survival of DNFH hatchery origin fish has been variable, with levels approaching those of wild kelts in the more established projects in some years, and lower survival in other years. The sometimes-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. 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 usually been near 60%, and vary together in most years. 2017 was an exception to these trends due to lower maturation in Winthrop fish. Maturation rates for Snake River fish have usually 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.

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 were high at Winthrop and for Snake River fish in 2017, but lower at Prosser. It is possible that the lower rate at Prosser could be due to unrecognized males in the dataset. Prosser is the only project that collects males for reconditioning. Males will usually have low plasma E2 levels, and be classified as non-maturing females if they are not noted as males when sampled. 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, advancements were made in drafting a Monitoring and Evaluation (M&E) Plan and environmental compliance documents. Also in 2017, we met with BPA and determined that BPA would solicit through a Request for Proposals (RFP) for a firm to design and build the kelt facility. The pace of this action has been slow and there are several components that must be completed. These include a Memorandum of Understanding (MOU) for construction, Operation and Maintenance (O&M) funding plans, Facility Designs, completion of the Northwest Power and Planning Council's Step 3, and construction of the facility.

# **Chapter 7: A state-space life cycle model effectiveness monitoring framework for the evaluation of Snake River B-run steelhead kelt reconditioning**

## **Introduction**

Populations of steelhead (*Oncorhynchus mykiss*) in the Snake River basin declined substantially from the 1960's through the 1970's during the construction of hydroelectric dams in the Snake and Columbia rivers. Declines have been attributed to varying degrees to hydrosystem development, habitat loss, and habitat degradation. Measures to improve steelhead returns have included habitat improvement, hatchery production and supplementation, and alterations to hydrosystem operations. Abundance monitoring efforts differ in scale for various individual tributary populations, reporting groups, and Major Population Groups (MPGs) throughout the Snake river. The entire Snake River Distinct Population Segment (DPS) aggregate returns, however, are quantified at Lower Granite (LGR) dam.

Two size categories of Snake river wild steelhead are considered separately for some management purposes: small steelhead less than 78 cm and larger steelhead greater than 78 cm. Smaller fish are considered A-run and larger fish B-run. Daily aggregate escapement to LGR of all Snake river steelhead was sampled by Idaho Department of Fish and Game (IDFG) and National Oceanic Atmospheric Administration Fisheries (or aka NMFS) from 2009 until 2016, and assigned to reporting group and age class by means of PIT tags, scale analysis, and genetic stock identification (GSI) (Camacho et al. 2017). B-run steelhead predominantly return to the Upper Clearwater, South Fork Clearwater, South Fork Salmon, and Middle Fork Salmon reporting groups. A-run fish also return to most of the same reporting groups, so assignments do not reflect exclusively B-run fish.

In this analysis, we perform a statistical reconstruction of 2009-2016 age class returns of reporting groups containing B-run populations using a state-space life cycle model based on Fleischmann et al. (2013). Our reconstructions of reporting group (RG) age class returns provide estimates of spawning abundance from 2002 until 2008, and estimates process and observation uncertainties, as well as temporal autocorrelation. We use parameter estimates from statistically validated models to perform a Management Strategy Evaluation (MSE) of kelt reconditioning. We use the validated model to simulate population trends 50 years into the future, and evaluate the potential for kelt reconditioning to recover or enhance production of wild B-run steelhead. We examine this potential from two distinct perspectives. The first perspective is that of *targeted* collection at the watershed, with kelts collected at a weir and released directly into the system. The second perspective is that of *aggregate* kelt collection at

LGR, with kelts from individual RGs being collected in proportion to the previous year's relative abundances, and returning to individual watersheds from a common release at LGR upon successful reconditioning.

The motivation for this analysis comes from the Federal Columbia River Power System FCRPS Biological Opinion's, Reasonable and Prudent Alternative (RPA) Action 33, which details in its key considerations the need to evaluate the potential for long-term kelt collection and reconditioning to increase the number of viable females on the spawning ground, and the need to perform research to accomplish this goal. This analysis, being a stock assessment based MSE, is an effectiveness monitoring tool. It is a work in progress, in that it begins to explore the relative expected benefits of two contrasting perspectives of kelt collection (*targeted* and *aggregated*). It is also subject to the limitations of the short time series of data provided by the genetic sampling, as well as any inherent biases resulting from the mixture of A-run and B-run fish in each of the reporting groups, and the assignment errors of fish to reporting groups. The analysis is nonetheless a significant step toward understanding the potential effects of kelt reconditioning, and can be built upon for further investigation into RG specific environmental drivers affecting production, as well as further tactical alternatives for kelt collection and release. The analysis does however, fall short of providing definitive answers about RG responses to collection, but we expect that continued monitoring and evaluation using this tool as it is further developed would be constructive to long term Research Monitoring and Evaluation (RM&E) project.

## Data

We examined 5 RGs. The Upper Clearwater, the South Fork Clearwater, the Middle Fork Salmon, and the South Fork Salmon, are five wild steelhead reporting groups that have wild B-run steelhead (Camacho et al. 2017). Fish Creek is a tributary of the Lochsa River in the Upper Clearwater subbasin that has B-run steelhead (Copeland et al. 2017). The Upper Grande Ronde is an A-run RG, and was examine for contrast only, but the remaining RGs are all contain B-run steelhead. We used GSI assignments of LGR samples from spawning years 2009 until 2016 to as the basis for age class recruits of each RG (Table 4 in Camacho et al. 2017). For environmental effects, we associated each spawning year with the Pacific Decadal Oscillation two years following spawning.

## Methods

We constructed a life cycle model using a technique developed by Fleischmann et al. (2017), which implements a Bayesian population model that accounts for uncertainty in predictions and observations, and estimates unobserved spawning abundance in years prior to observed returns to LGR.

The models are implemented in JAGS (Plummer 2003), using a lognormal AR (1) recruitment likelihood. The recruitment model is  $\ln(R_y) = \ln(S_y) + \ln(\alpha_y) - \beta S_y + \omega_y$ , with  $\omega_y \sim$

$N(\phi\omega_{y-1}, \sigma_v)$  AR(1) autocorrelation, where  $R_y$  is the recruitment from brood year  $y$ ,  $S_y$  is spawners in brood year  $y$ ,  $\alpha_y$  is the productivity parameter in year  $y$ ,  $\beta$  is the capacity parameter, and  $\sigma_v$  is the process error term. We assume that  $\alpha_y = \bar{\alpha} + \gamma PDO_{y+2}$  where PDO is the Pacific Decadal Oscillation in the month of May two years following spawning. The number of fish returning in year  $y$  of age  $a$  in the range 3 to 7 years old is predicted by  $N_{y,a} = R_{y-a} p_{y-a,a}$ , where  $p_{y-a,a}$  is the proportion of recruits from brood year  $y-a$  that return as age  $a$  fish in year  $y$ . The vector of proportions at age for brood year  $y$  is modeled as a latent variable distributed with a Dirichlet distribution with mean values  $\pi$  equal to the probabilities of returning at ages 3 to 7. The predicted  $S_y = \sum_{a=3}^7 N_{y,a}$  are treated as observed with error such that  $\ln(S_y/S_{observed_y}) = w_y \sim N(0, \sigma_w)$ , where  $\sigma_w$  is the observation error term. Uninformative priors were used for  $\sigma_v, \sigma_w, \gamma$ , and  $\pi$ .  $\phi$  and  $\beta$  were limited to be positive. Parameters for each individual RG were estimated by sampling 3 chains of 50,000 simulations after an initial burn-in of 10,000 samples. The sampling routine is a Markov Chain Monte Carlo simulation routine that uses an adaptive Gibbs Sampling method to produce samples from the joint posterior density. The posterior chains were then combined into a single chain of 150,000 samples, from which summary statistics were derived describing the variability in parameter estimates.

We constructed 6 variants of the estimation model that differ in complexity. The models include or exclude the following features: 1. Estimation of observation error parameter  $\sigma_w$ , 2. Estimation of process error parameter  $\sigma_v$ , 3. Estimation of autoregressive parameter  $\phi$ , and 4. Inclusion of PDO effect by estimating  $\gamma$ . The primary model (OEPEXAR) implements all features. The remaining model's complexities are outlined in Table 7.1.

Table 7.2 List of model forms used.

Model	Process error $\sigma_v$	Observation error $\sigma_w$	AR (1) effect $\phi$	PDO effect $\gamma$
OEPEXAR	x	x	x	x
OEPEX	x	x		x
PEX	x			x
OEPE	x	x		
OEPEAR	x	x	x	
PEAR	x		x	

We used the estimates of parameters for each RG to simulate future abundance by initializing the model for each RG with the most recent 7 years of spawning abundances at LGR and simulating forward with random samples from the posterior chain. We further added variability to the future simulations by adding variability to the predictions with  $\omega_y$ , as calculated from the mean  $\sigma_v$  and  $\phi$  from the posterior chain samples, i.e., we simulated  $\ln(R_y) = \ln(S_y) + \ln(\alpha_y) - \beta S_y + \omega_y$  using random draws for  $\bar{\alpha}, \beta, \pi$ .

We used these simulated future time series to characterize the spawning abundance of B-run fish that would be predicted on average over a 50-year period both with and without spawning enhancement via kelt reconditioning. We simulated each RG 10,000 times for 50 years into the future, and evaluated the response to spawning enhancement from reconditioned kelt abundances ranging from 0 to 500 kelts, i.e.,  $\ln(R_y) = \ln(S_y + K_y) + \ln(\alpha_y) - \beta(S_y + K_y) + \omega_y$ , where  $K_y$  is the kelts in spawning year  $y$ . The mean 50-year spawning abundance was calculated for each of the 10,000 simulations, and then averaged at each kelt addition level in increments of 50 kelts. This was done for each RG such that kelt additions were independent among RGs, thus demonstrating the potential benefit of *targeted* kelt reconditioning at the RG level if kelts were collected and released from each watershed, not at LGR as a *combined* collection.

We exploited the potential effect of *combined* kelt collection and release by assuming that kelts from all RGs could be collected at LGR and released above LGR to return to individual drainages. We assumed that up to 1,000 kelts could be collected at LGR and would be collected in proportion to the abundances of the 5 RGs containing B-run fish. We assumed that the proportion of all kelts composed of fish from reporting group  $p$  would be  $K_{y,p} = K_y S_{y,p} / \sum_p S_{y,p}$ , where  $K_y$  is the total number of kelts collected in year  $y$ . We further assumed that 30% of kelts would return the following year, and that 70% would return to spawner two years later. The mean 50-year spawning abundance was calculated for each of the 10,000 simulations, and then averaged at each kelt addition levels between 0 and 1,000 kelts at LGR, resulting in  $K_{y,p}$  for each RG.

For both *targeted* and *aggregate* assessments, the response to repeat spawners needs to be interpreted in relation to the effective sex ratio of kelts, i.e., kelts as modeled are spawners, not specifically females. We make no adjustments about sex, fecundity, or pre-spawn mortality, so the “kelt treatment level” needs to be considered in light of those assumptions, e.g., one female kelt would effectively be the same as two spawners at an even sex ratio.



## Results

Figure 7.1 shows the predicted spawner trends of each of the B-run RGs, showing both observed and predicted spawners using the OEPEXAR model. Spawners are also predicted for the 7 years prior to observations beginning. Shown along the same time frame is the predicted recruits per spawner at each brood year, also using the OEPEXAR model. Also shown in Figure 7.1 are the predicted recruitment vs spawners, with error bars indicating the predicted process error at the predicted number of spawners, i.e., the 95% confidence interval in predicted recruitment. The fitted line represents the spawner-recruit relationship at the median  $\bar{\alpha}, \bar{\beta}$  values across a range of spawners. Owing the relatively short range of spawner data, and the even shorter range of complete brood year returns, there is considerable uncertainty in predictions. Nonetheless, the relationships were estimated, and show a degree of density dependence that reflects the observed range of spawners.

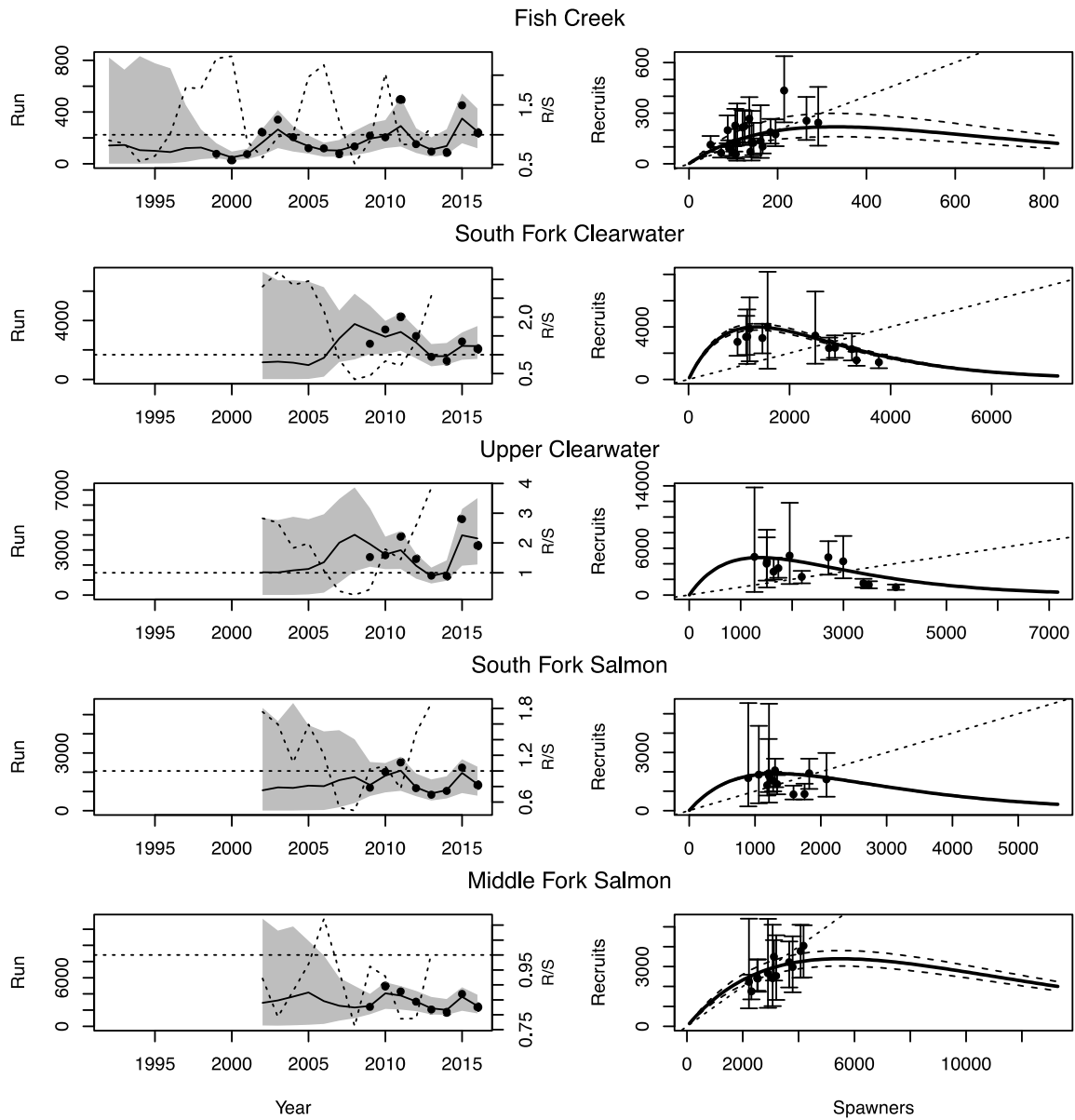


Figure 7.1: Results of fitting each population model to the age-class return spawner data collected at LGR using the OEPEXAR model. Solid lines in the left column represent predicted spawners. Filled circles represent observed spawners. The dotted line shows the predicted recruits per spawner. The right column shows the predicted recruits per spawner displayed with 95% confidence intervals at the median predicted spawner level. The solid curve shows the predicted recruits using median productivity and capacity estimates. The dashed lines surrounding the curve represent the relationship using a productivity value 1 standard deviation above and below the mean.

Figure 7.2-7.6 show the predicted spawner trends of each of the B-run RGs, the predicted recruits per spawner at each brood year, and the predicted recruitment vs spawners, using all of the models listed in Table 7.2. Most reporting groups do not demonstrate much sensitivity to the model form used. It is worth noting that models not incorporating observation error (no OE) display the recruit/spawner pairs at the empirically observed abundance, whereas OE models adjust spawners to account for error in measurement, i.e., GSI assignment error, pre-spawn loss, sampling bias, etc.... Nonetheless, all models estimated roughly the same carrying capacity parameter  $\beta$ . This raises a concern about the limited time series of age-class return data. There is very little contrast in recruitment across spawner abundances, because there are only a few years of complete brood year returns. Without observing recruitment at higher spawning abundance, the model fitting procedure can't identify if recruitment will increase past the highest spawning abundances observed, i.e., you can't estimate a higher capacity without observing recruitment at higher spawning abundances.

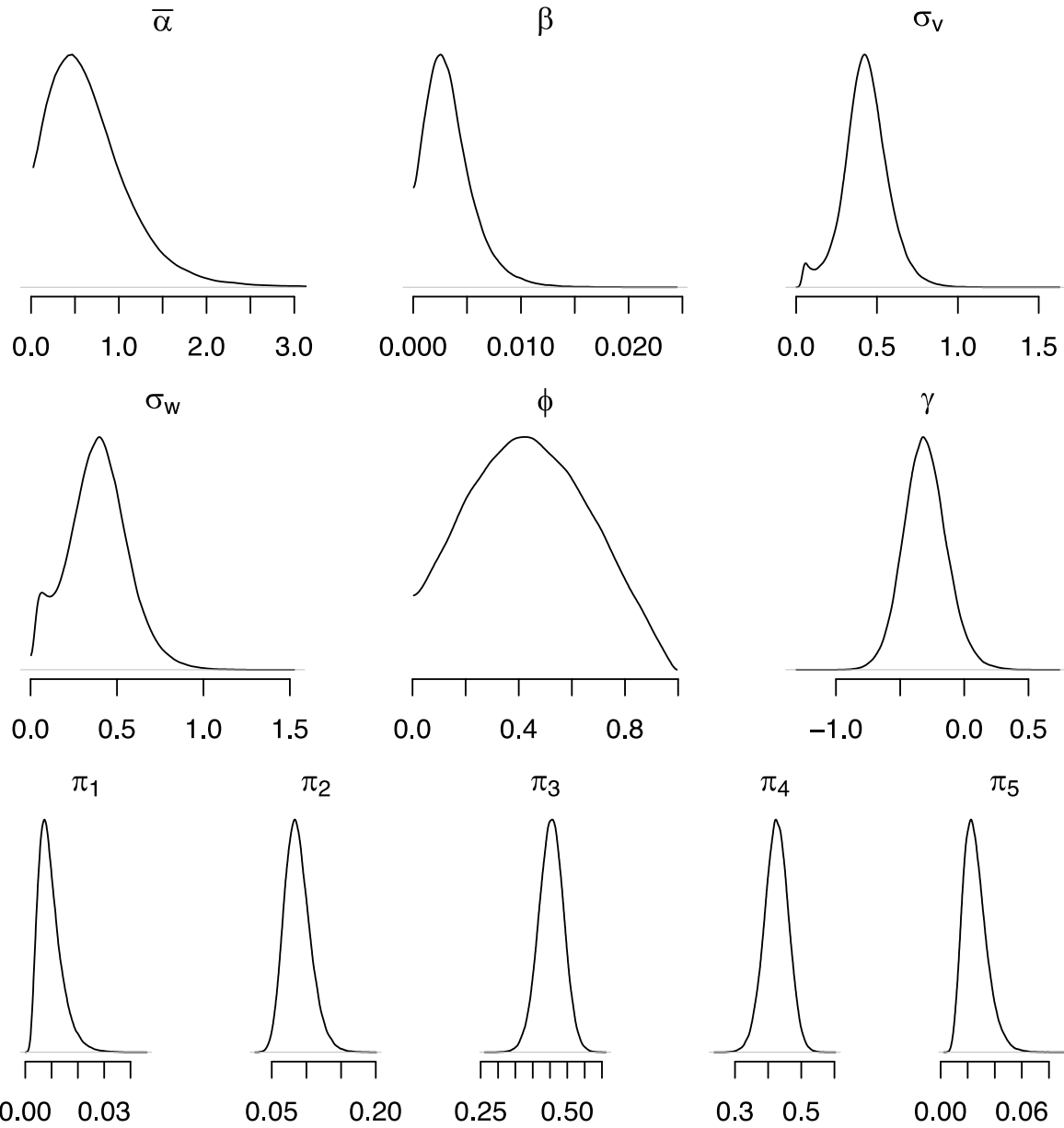


Figure 7.2 Densities of posterior parameter samples from Fish Creek simulations. Each histogram represents the density of the combined 3 chains of 50,000 samples.

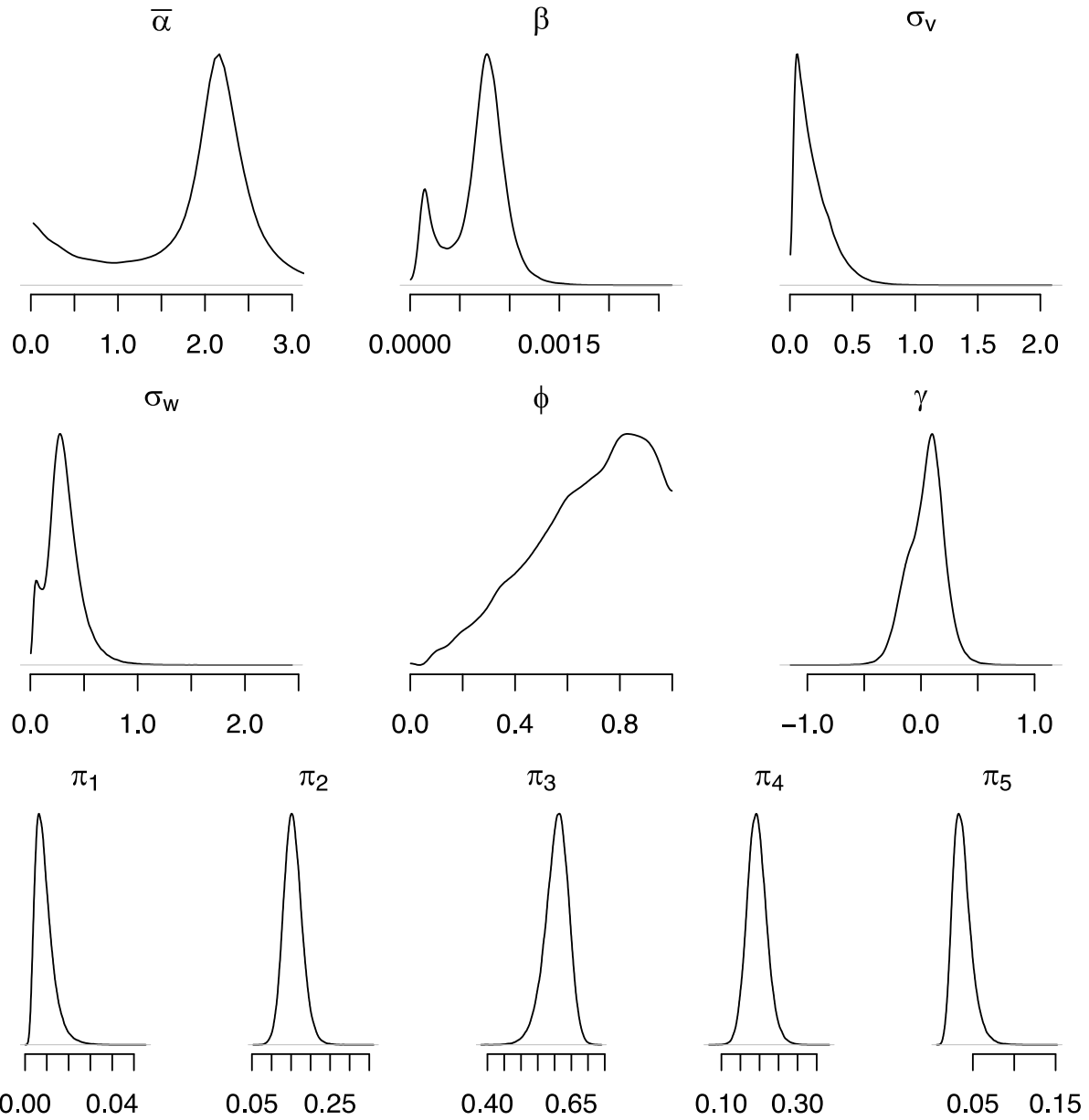


Figure 7.3 Densities of posterior parameter samples from South Fork Clearwater River simulations. Each histogram represents the density of the combined 3 chains of 50,000 samples.

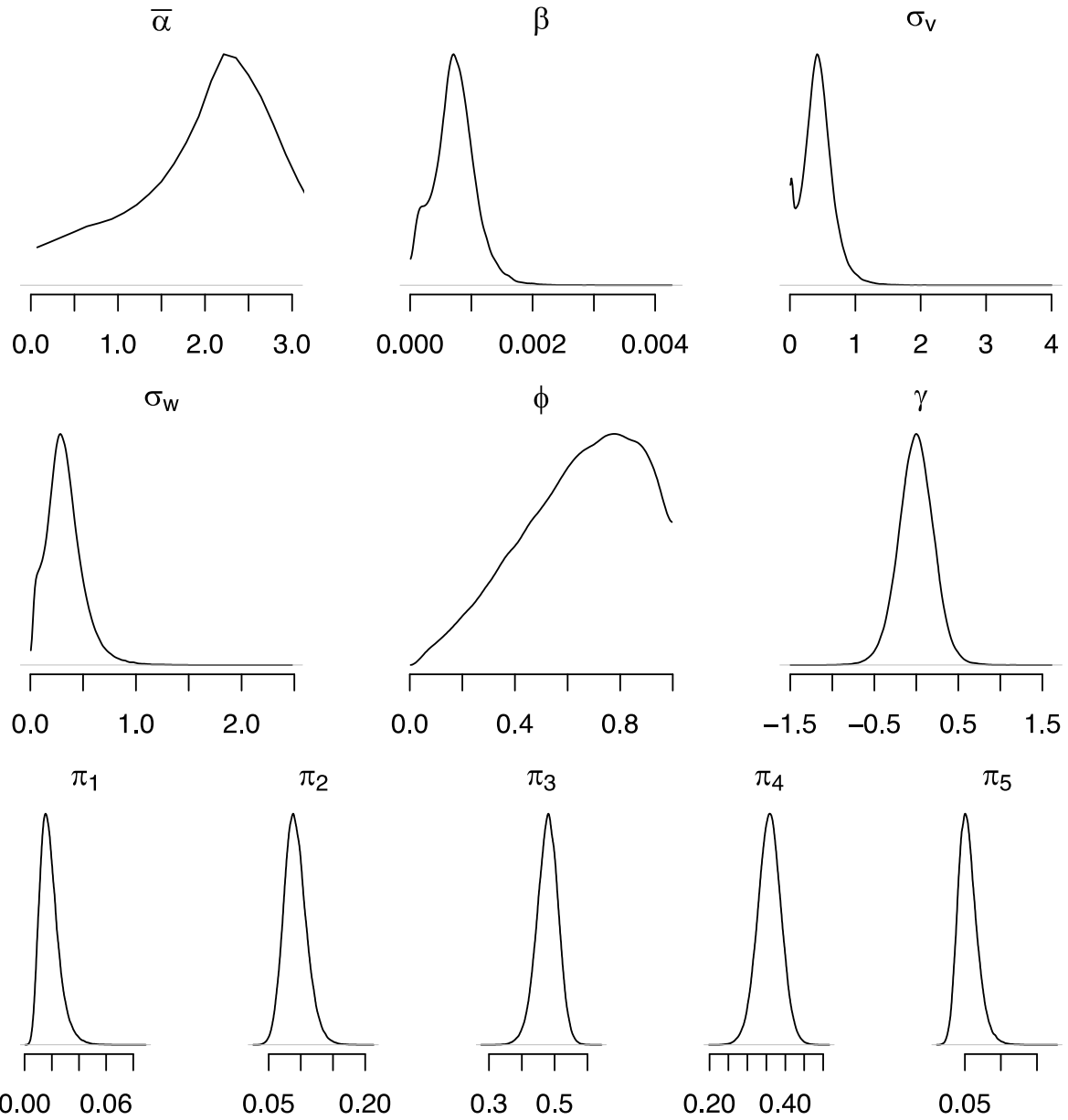


Figure 7.4 Densities of posterior parameter samples from Upper Clearwater River simulations. Each histogram represents the density of the combined 3 chains of 50,000 samples.

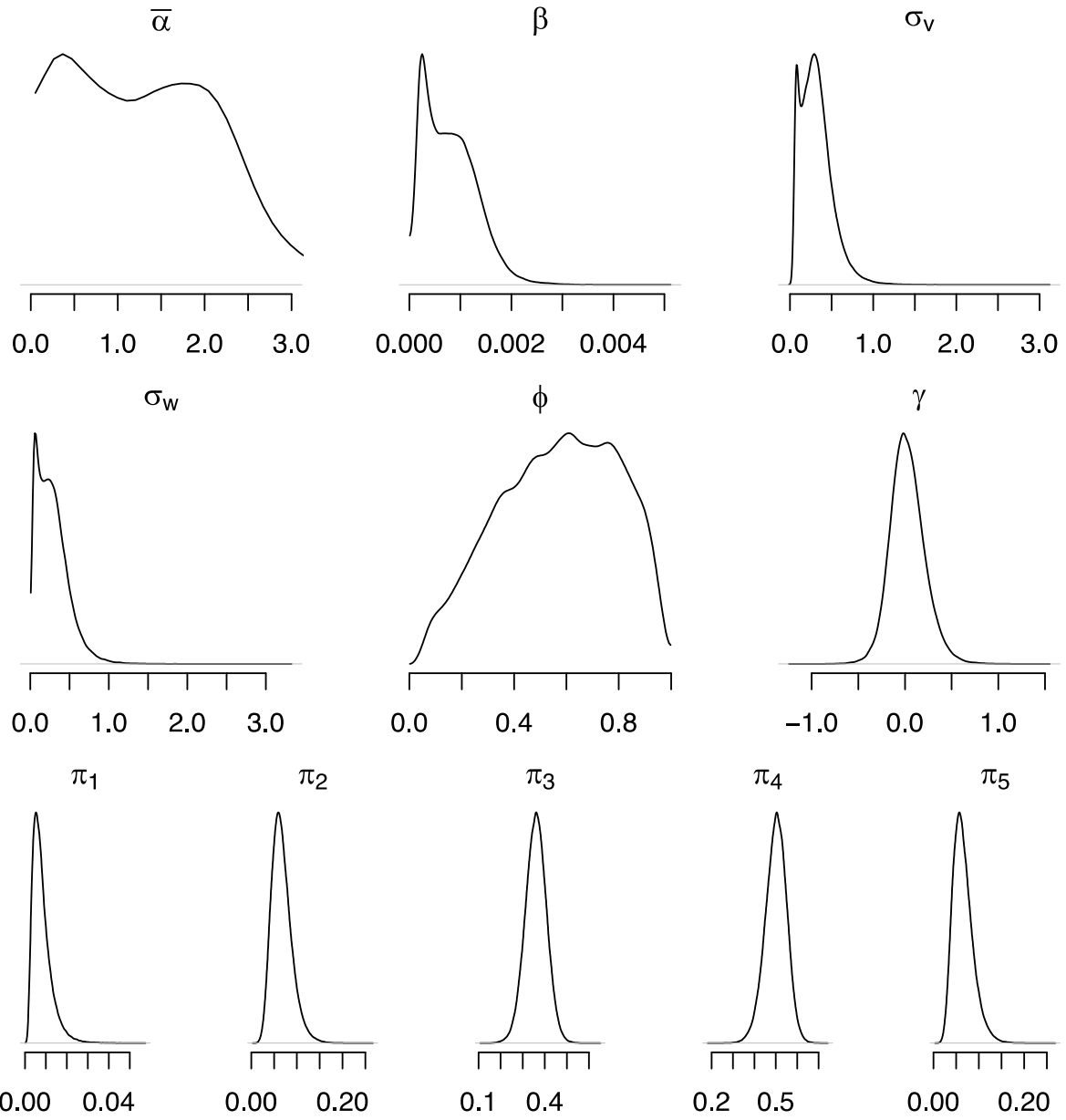


Figure 7.5 Densities of posterior parameter samples from South Fork Salmon River simulations. Each histogram represents the density of the combined 3 chains of 50,000 samples.

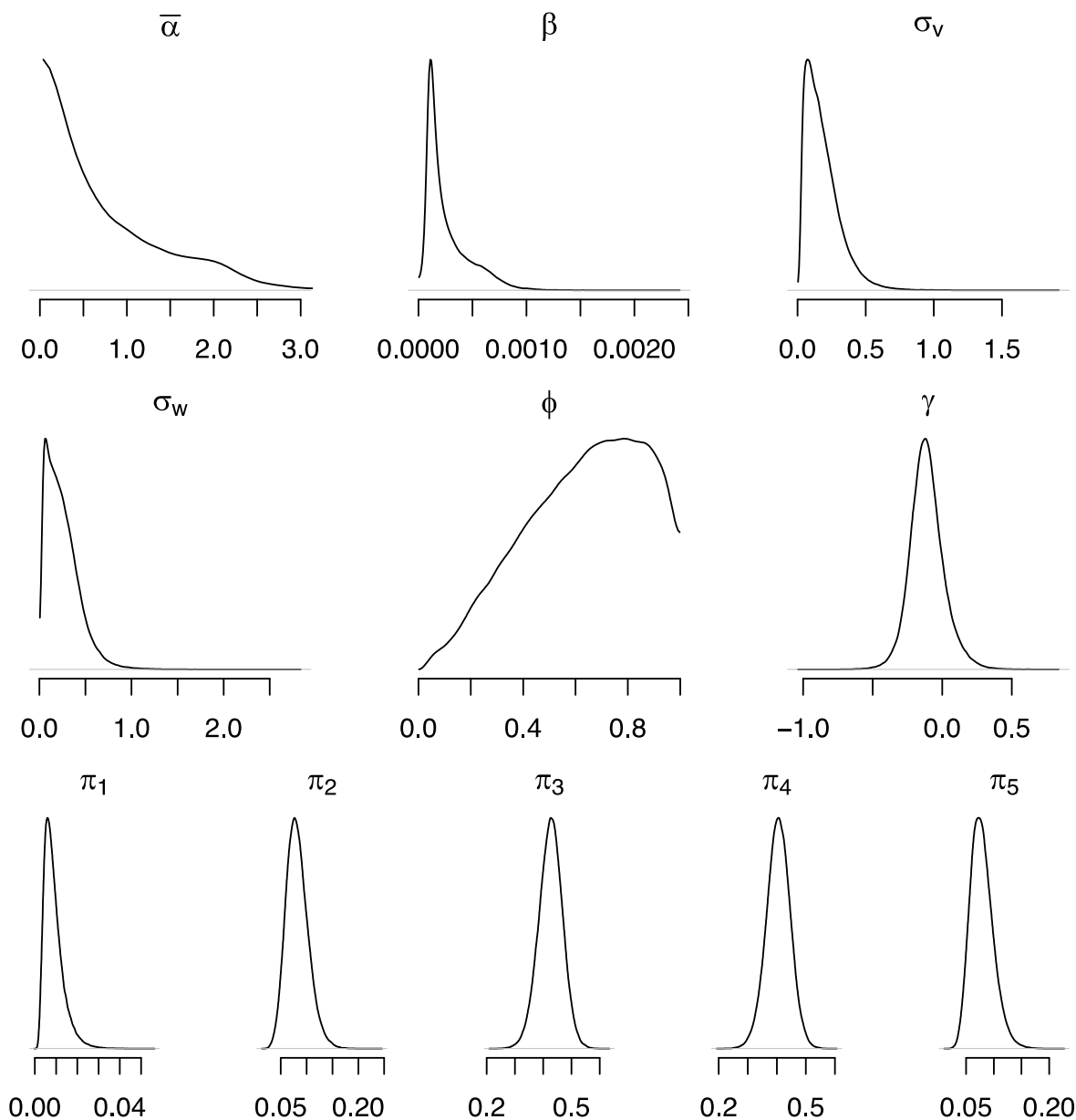


Figure 7.6 Densities of posterior parameter samples from Middle Fork Salmon River simulations. Each histogram represents the density of the combined 3 chains of 50,000 samples.

Figures 7.7-7.11 show the posterior densities of parameters for each RG, evaluated from the combined 3 chains of 50,000 samples after a burn-in of 10,000 simulations using the OEPEXAR model. The distributions that data were uninformative in some cases, most notably the lack a central tendency in the autocorrelation coefficient for all but the Fish Creek case. This may be due to the fact that the Fish Creek data set spanned twice as many years, but in some cases, the PDO coefficient is near zero, implying that if autocorrelation were left out of the analysis, the PDO coefficient may have been better determined. It is also work noting that the productivity



parameter  $\bar{\alpha}$  is not well determined for both the Middle Fork and South Fork Salmon cases. The age distribution parameters  $\pi$  were relatively well determined. The density dependence parameter  $\beta$  shows a large amount of uncertainty, owing to the fact that recruitment was not observed across a wide range of spawning abundances.

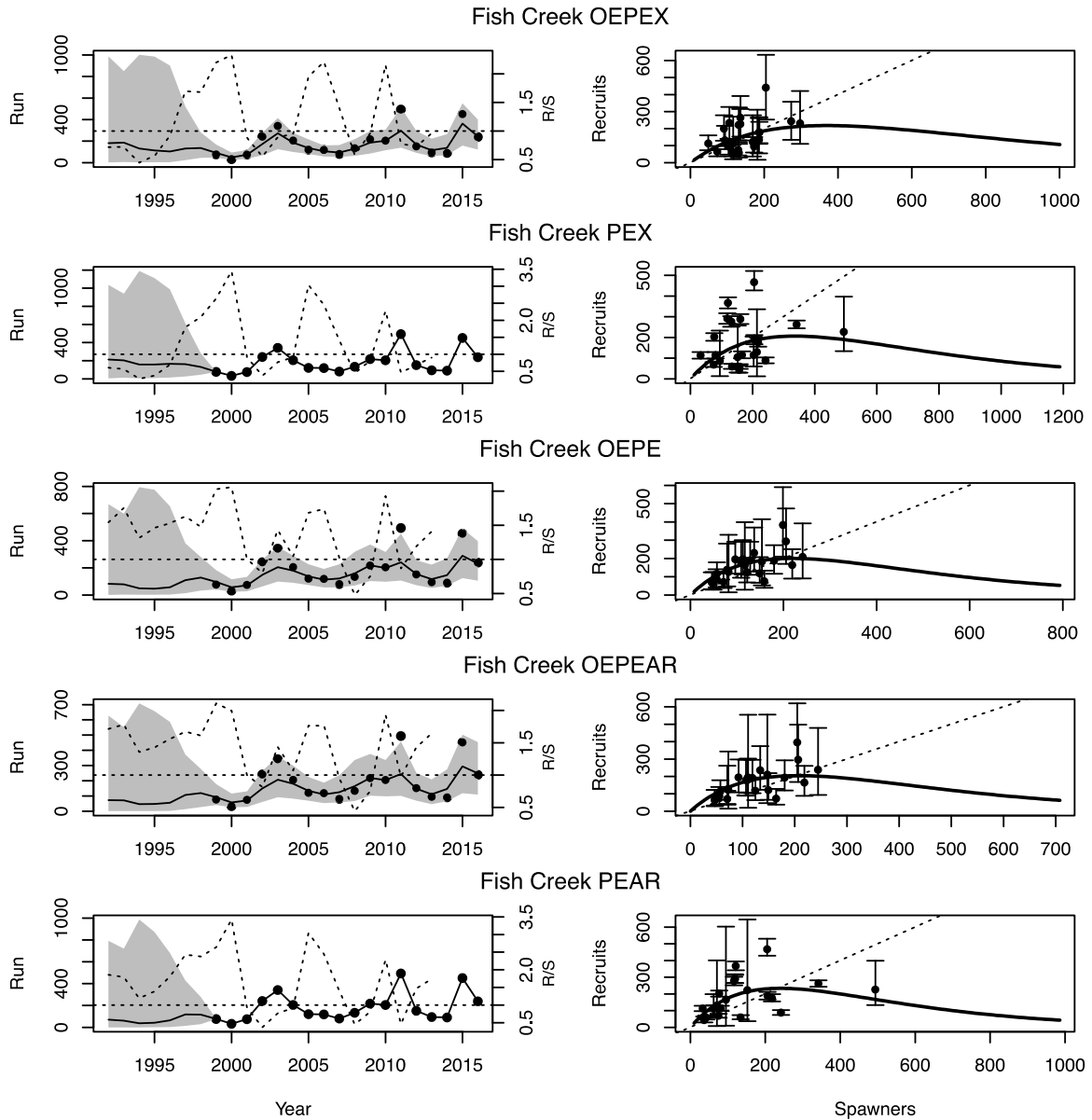


Figure 7.7 Results of fitting models list in Table 7.2 to Fish Creek age-class return spawner data. Solid lines in the left column represent predicted spawners. Filled circles represent observed spawners. The dotted line shows the predicted recruits per spawner. The right column shows the predicted recruits per spawner displayed with 95% confidence intervals at the median predicted spawner level. The solid curve shows the predicted recruits using median productivity and capacity estimates.

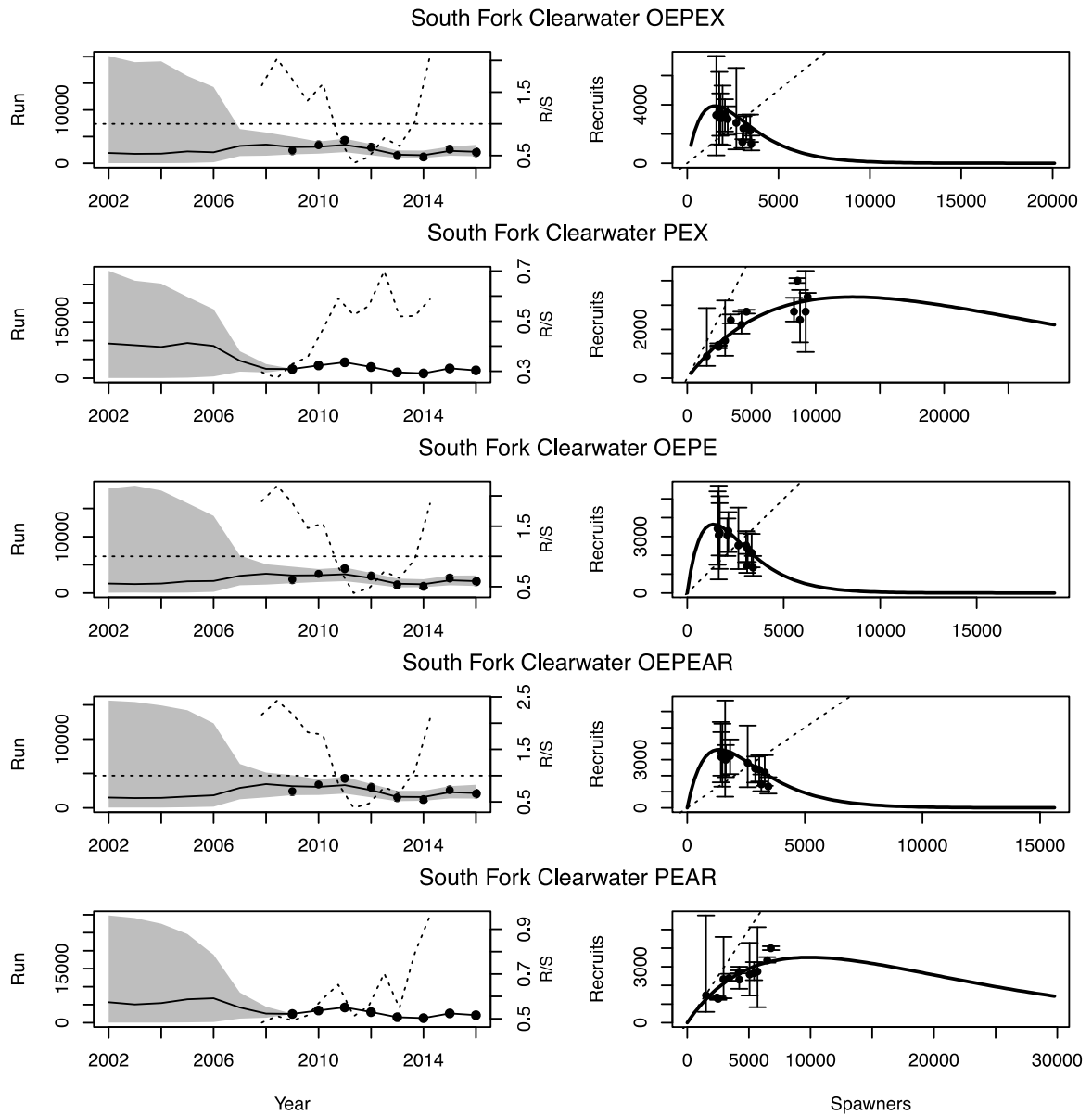


Figure 7.8 Results of fitting models list in Table 7.2 to South Fork Clearwater age-class return spawner data collected at LGR. Solid lines in the left column represent predicted spawners. Filled circles represent observed spawners. The dotted line shows the predicted recruits per spawner. The right column shows the predicted recruits per spawner displayed with 95% confidence intervals at the median predicted spawner level. The solid curve shows the predicted recruits using median productivity and capacity estimates.

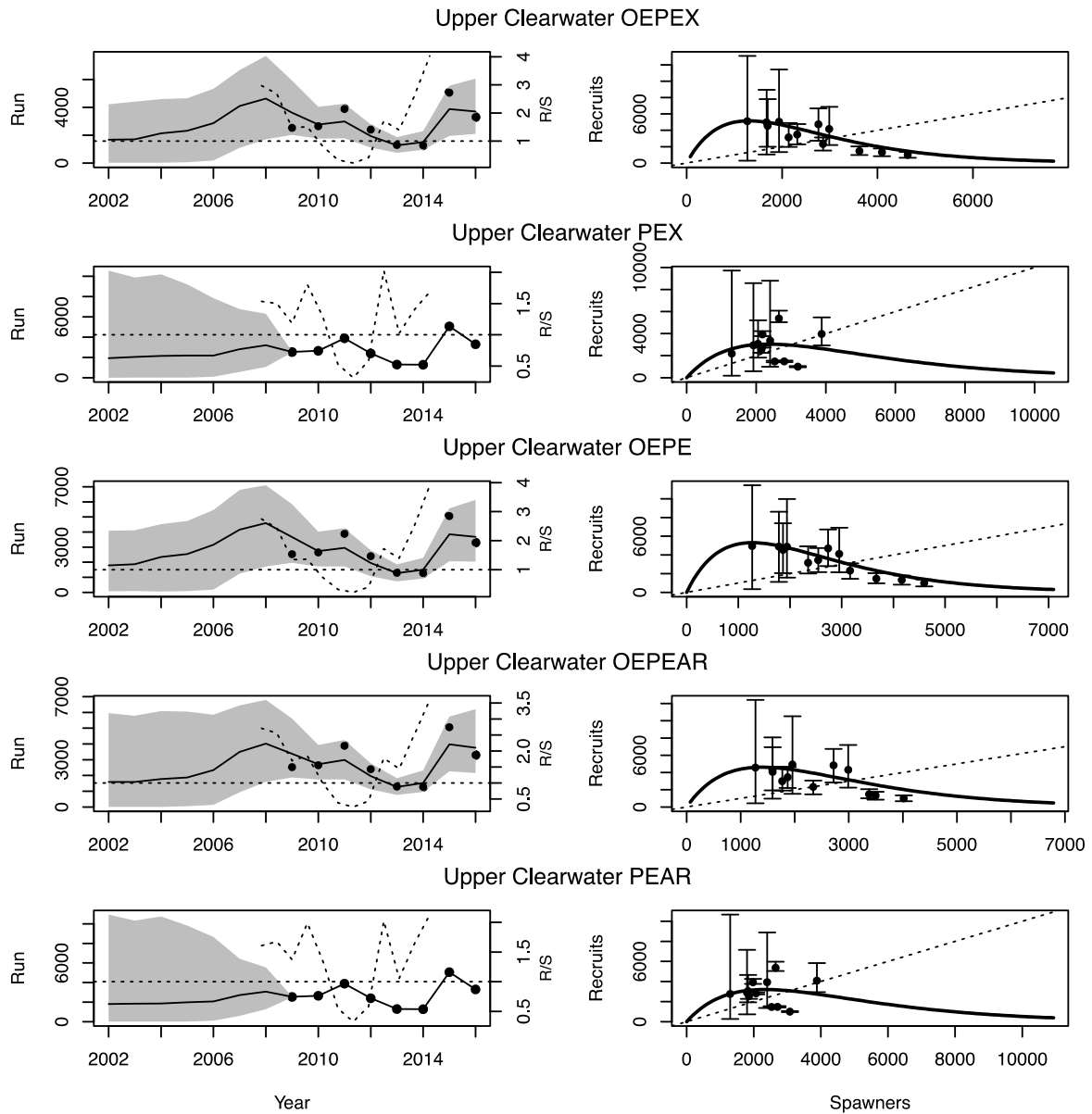


Figure 7.9 Results of fitting models list in Table 7.2 to Upper Clearwater age-class return spawner data collected at LGR. Solid lines in the left column represent predicted spawners. Filled circles represent observed spawners. The dotted line shows the predicted recruits per spawner. The right column shows the predicted recruits per spawner displayed with 95% confidence intervals at the median predicted spawner level. The solid curve shows the predicted recruits using median productivity and capacity estimates.

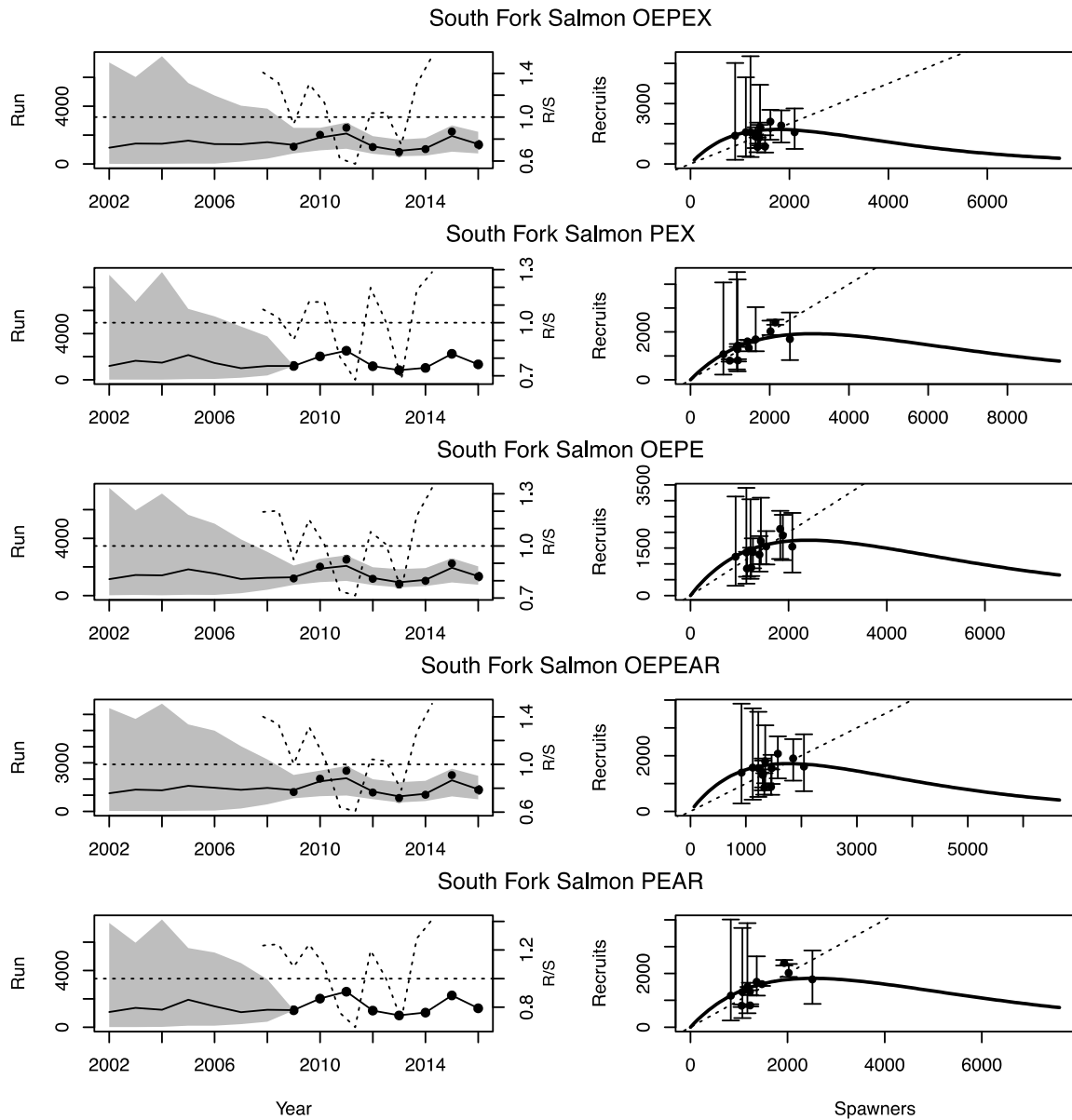


Figure 7.10 Results of fitting models list in Table 7.2 to South Fork Salmon age-class return spawner data collected at LGR. Solid lines in the left column represent predicted spawners. Filled circles represent observed spawners. The dotted line shows the predicted recruits per spawner. The right column shows the predicted recruits per spawner displayed with 95% confidence intervals at the median predicted spawner level. The solid curve shows the predicted recruits using median productivity and capacity estimates.

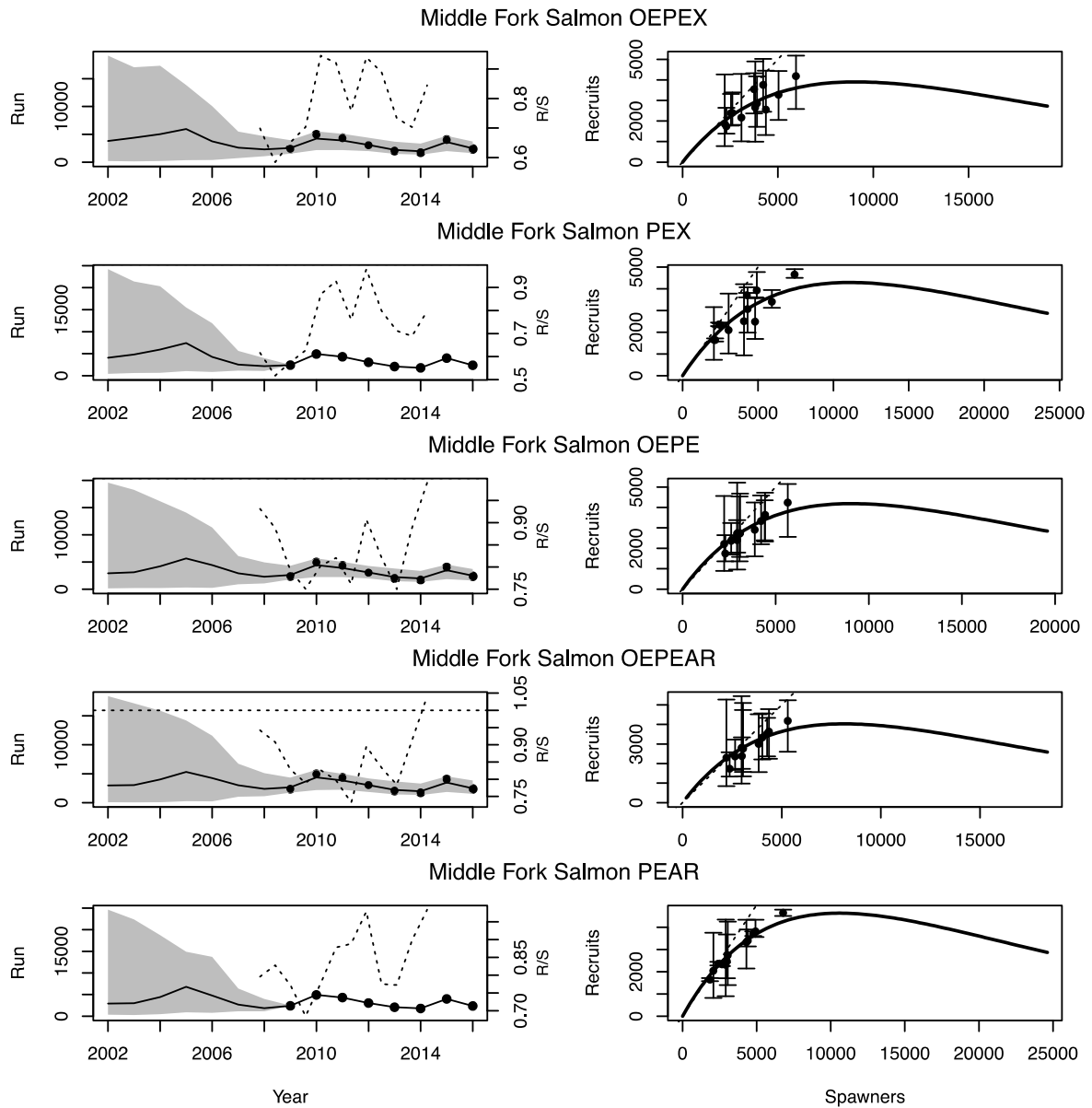


Figure 7.11 Results of fitting models list in Table 7.2 to Middle Fork Salmon age-class return spawner data collected at LGR. Solid lines in the left column represent predicted spawners. Filled circles represent observed spawners. The dotted line shows the predicted recruits per spawner. The right column shows the predicted recruits per spawner displayed with 95% confidence intervals at the median predicted spawner level. The solid curve shows the predicted recruits using median productivity and capacity estimates.

Figures 7.12-7.17 show the *targeted* effects of kelt reconditioning of B-run steelhead using model OEPEXAR. We show this result only for the OEPEXAR model, not the remaining models in Table 7.2. We show 5 simulation time series to give a sense of how much variability is predicted by drawing random parameter values and adding AR (1) process error. The average response from 10,000 simulations over a range of kelt reconditioning levels is shown, and the change

relative to no conditioning is also shown. Fish Creek achieves 6% increase in spawning abundance after the simulated reconditioning of approximately 20 kelts annually, and reaches a 15% increase near 80 kelts. The mean abundance without kelts is approximately 200 spawners, which is expected from the mean estimated density dependence parameter. The South Fork Clearwater indicates a negative response to the simulated reconditioning of kelts, converging on a 10% decrease in spawner abundance with the addition of 500 kelts. The Upper Clearwater shows no net response to the simulated reconditioning of kelts, but interestingly does not show a decline despite being at or near estimated capacity. This is owing to the fact that the estimated intrinsic productivity is very high, allowing the RG to produce a lot of recruits per spawner when simulated abundance are low, therefore evening out the losses at high abundance. The South Fork Salmon similarly shows no response to the simulated reconditioning of kelts. The Middle Fork Salmon shows nearly a 10% increase in average simulated spawner abundance with the simulated reconditioning of 500 kelts. For contrast, we also looked at the Grande Ronde A-run RG, where we predict approximately a 15% increase in spawner abundance on average with the addition of 2000 kelts, and 10% with 1000 kelts (see Figure 7.7).

We attempted to perform an evaluation of individual RG responses to simulated aggregate kelt collection and reconditioning, but found that the level of uncertainty in parameter estimates of each RG made any determination of relative benefits difficult. The overall pattern, similar to the *targeted* case, was that some RGs showed a positive response to simulated reconditioning, depending on how close spawning abundances were to estimated capacity. Since the returning reconditioned kelts are comprised of 30% of kelts reconditioned the year prior to repeat spawning and 70% of kelts reconditioned two years prior, proportions to each RG are subject to the variabilities across more than a single year, and not solely from variability in the target RG. The structure of the evaluation of RG within an aggregate would otherwise be an effective way

of evaluating overall benefits of reconditioning at LGR, but without more precise estimates of RG parameters, the results is just that populations increase to their capacities as expected.

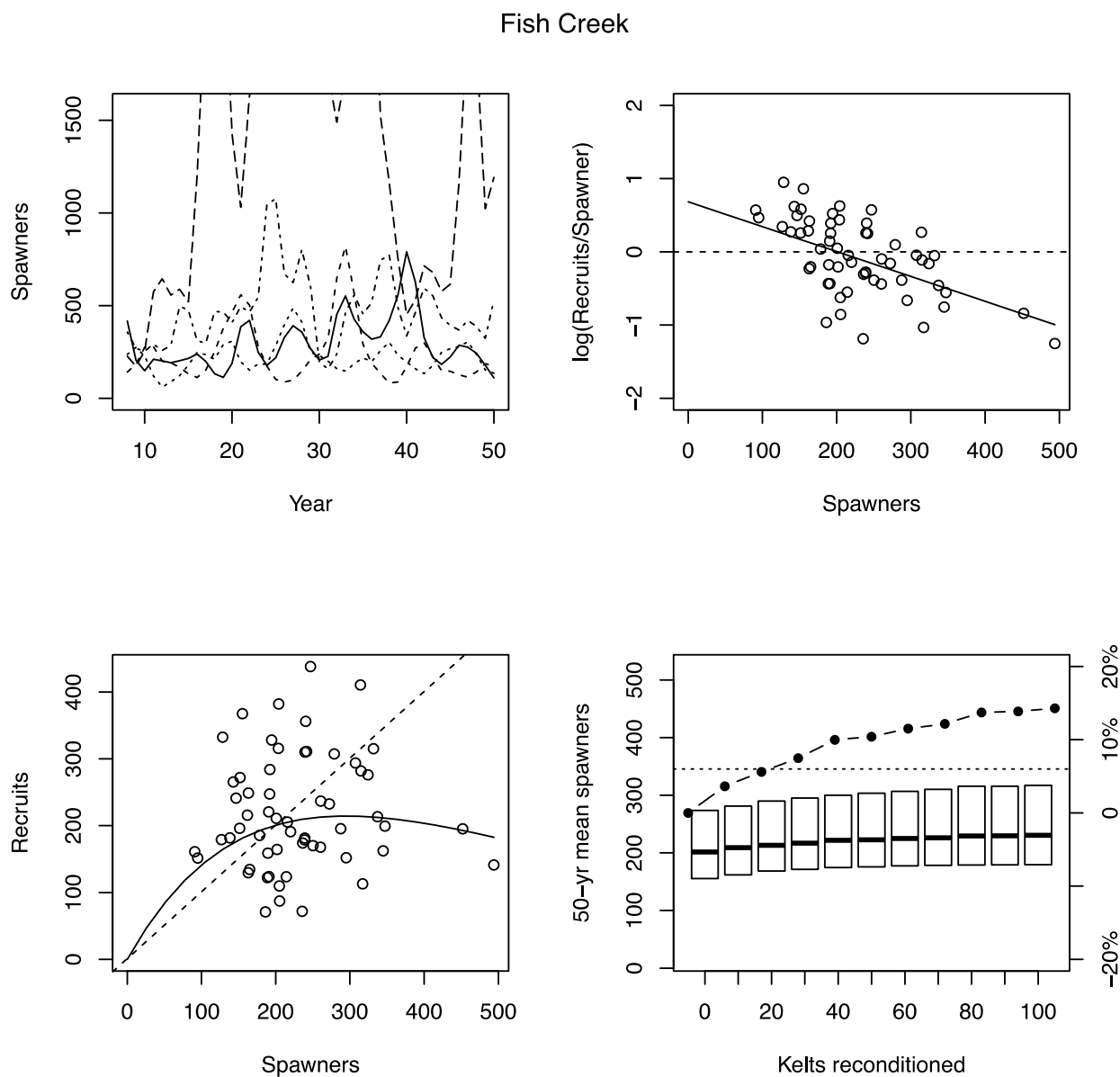


Figure 7.12 Simulated response of kelt reconditioning in Fish Creek. Upper left panel shows 5 of 10,000 50 year simulated returning spawner abundances drawing random productivity, capacity, and age class parameters, and adding temporally autocorrelated process error using mean process uncertainty and autocorrelation parameters. Upper right panel shows a single simulated log of recruit per spawner vs spawners with fitted line. Lower left panel shows the predicted recruits at mean productivity and capacity for given spawners. The replacement line is shown (dotted). Lower right panel shows the average 50 year mean return abundance at a given level of reconditioned kelts for 10,000 simulations.

### South Fork Clearwater

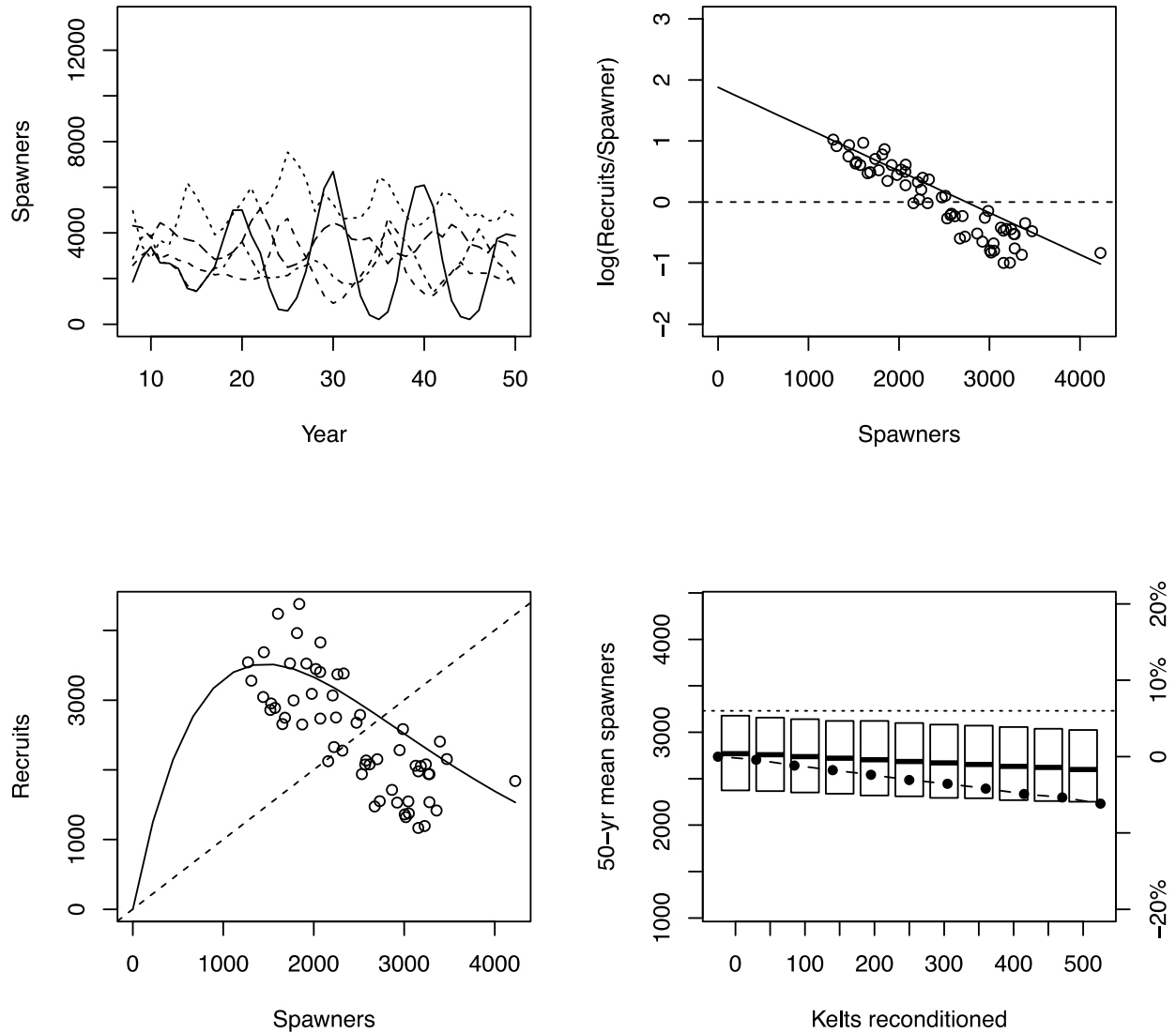


Figure 7.13 Simulated response of kelt reconditioning in the South Fork Clearwater. Upper left panel shows 5 of 10,000 50 year simulated returning spawner abundances drawing random productivity, capacity, and age class parameters, and adding temporally autocorrelated process error using mean process uncertainty and autocorrelation parameters. Upper right panel shows a single simulated log of recruit per spawner vs spawners with fitted line. Lower left panel shows the predicted recruits at mean productivity and capacity for given spawners. The replacement line is shown (dotted). Lower right panel shows the average 50 year mean return abundance at a given level of reconditioned kelts for 10,000 simulations.



### Upper Clearwater

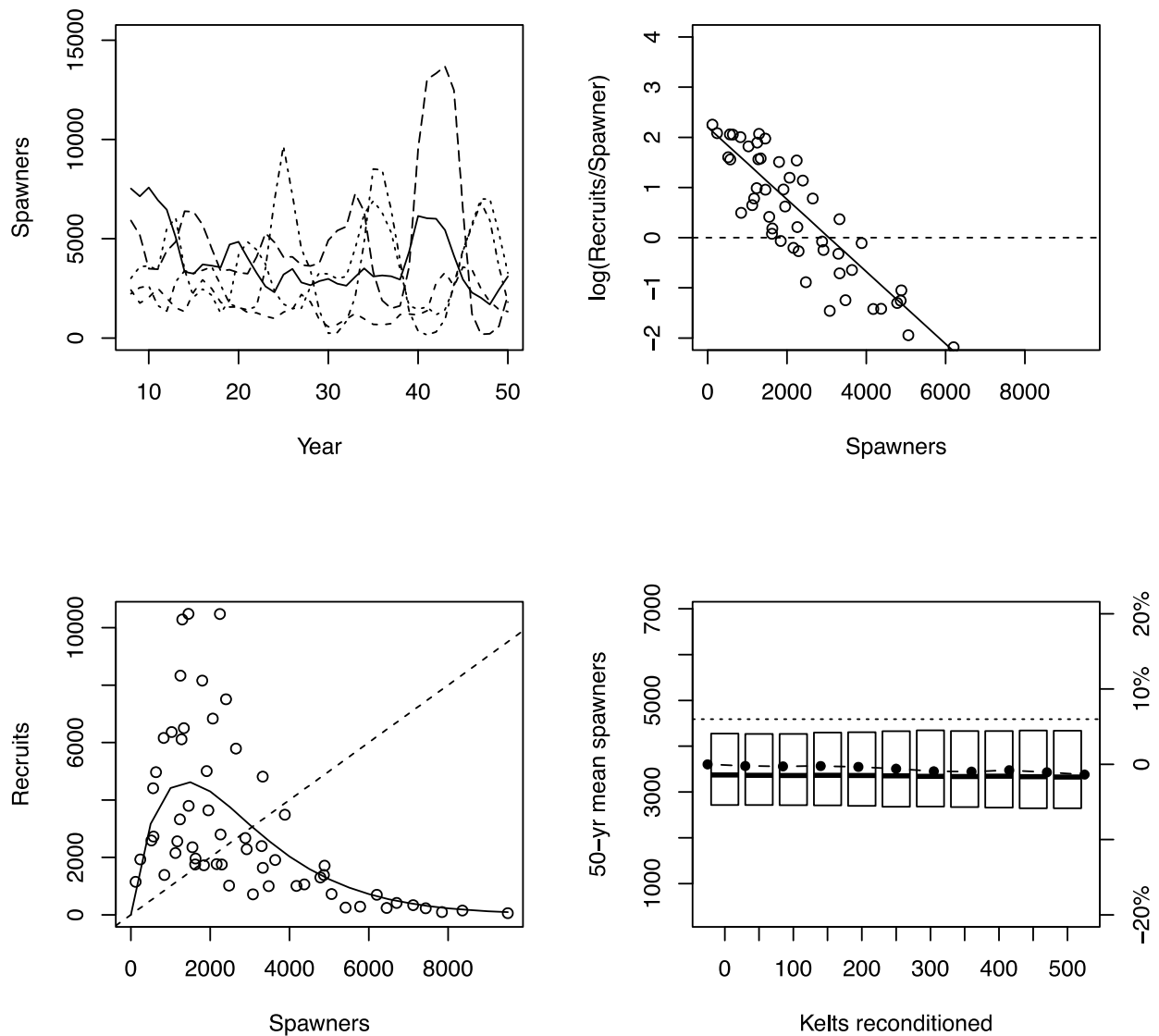


Figure 7.14 Simulated response of kelt reconditioning in the Upper Clearwater. Upper left panel shows 5 of 10,000 50 year simulated returning spawner abundances drawing random productivity, capacity, and age class parameters, and adding temporally autocorrelated process error using mean process uncertainty and autocorrelation parameters. Upper right panel shows a single simulated log of recruit per spawner vs spawners with fitted line. Lower left panel shows the predicted recruits at mean productivity and capacity for given spawners. The replacement line is shown (dotted). Lower right panel shows the average 50 year mean return abundance at a given level of reconditioned kelts for 10,000 simulations.

### South Fork Salmon

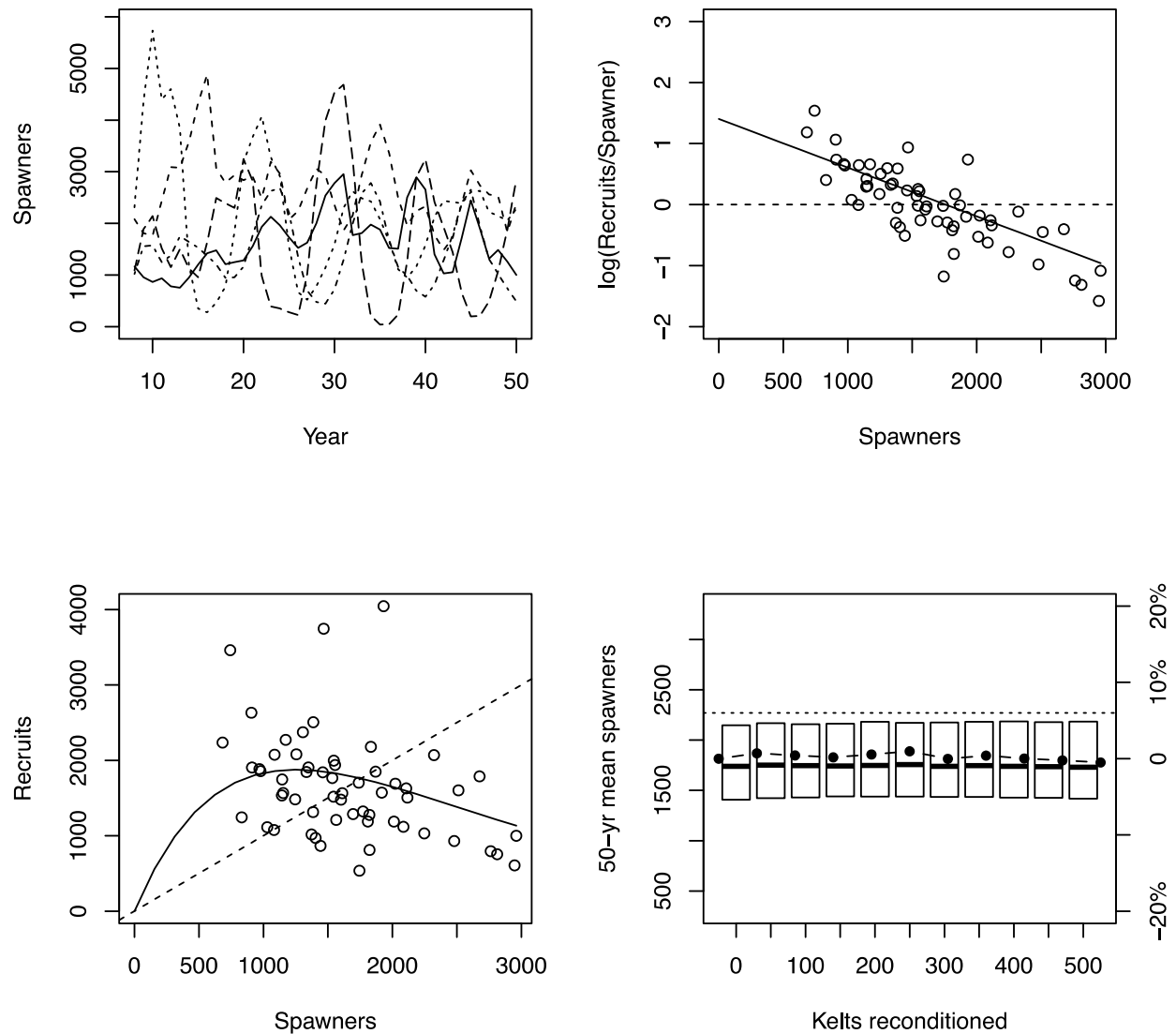


Figure 7.15 Simulated response of kelt reconditioning in the South Fork Salmon. Upper left panel shows 5 of 10,000 50 year simulated returning spawner abundances drawing random productivity, capacity, and age class parameters, and adding temporally autocorrelated process error using mean process uncertainty and autocorrelation parameters. Upper right panel shows a single simulated log of recruit per spawner vs spawners with fitted line. Lower left panel shows the predicted recruits at mean productivity and capacity for given spawners. The replacement line is shown (dotted). Lower right panel shows the average 50 year mean return abundance at a given level of reconditioned kelts for 10,000 simulations.

# Middle Fork Salmon

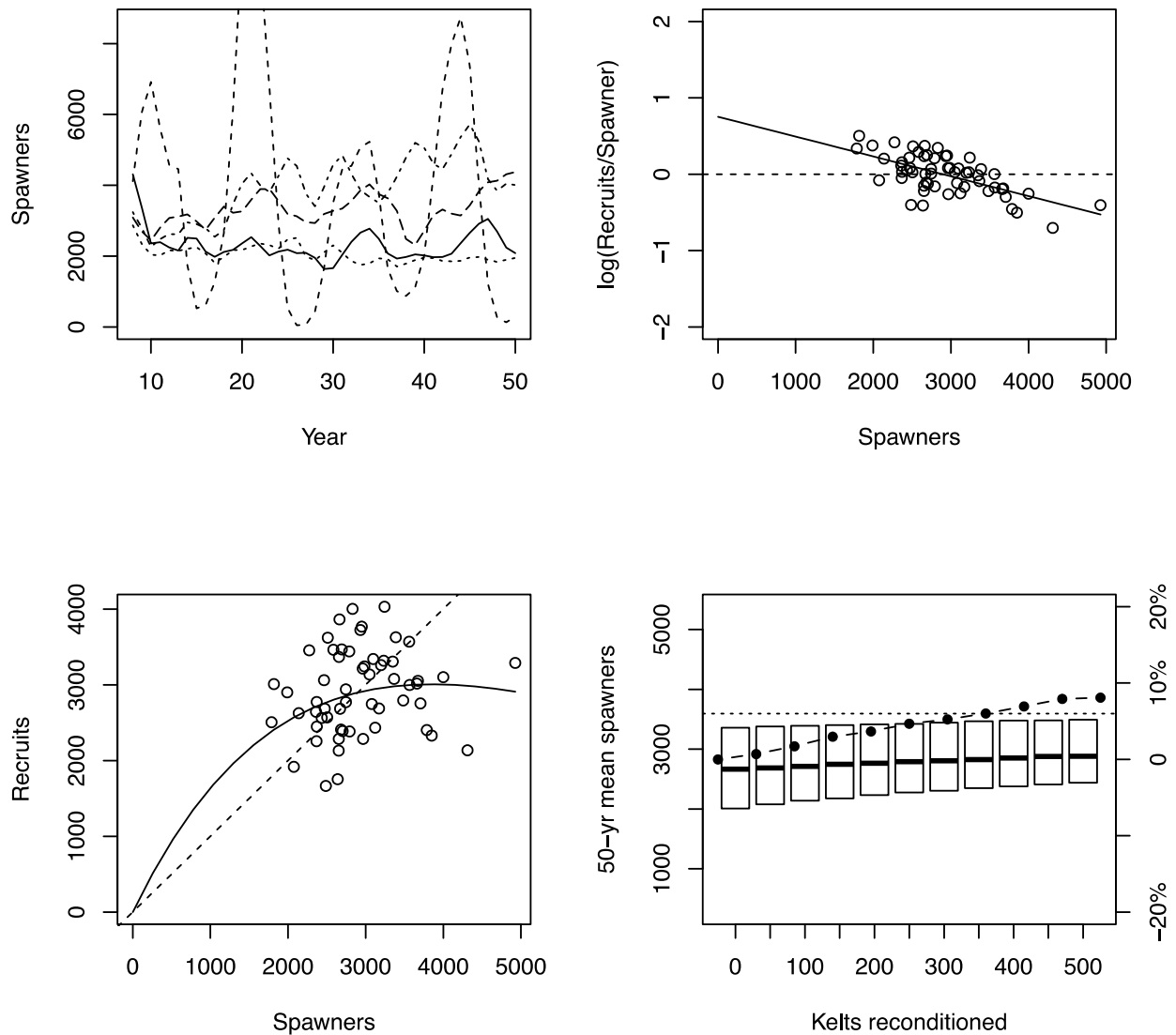


Figure 7.16 Simulated response of kelt reconditioning in the Middle Fork Salmon. Upper left panel shows 5 of 10,000 50 year simulated returning spawner abundances drawing random productivity, capacity, and age class parameters, and adding temporally autocorrelated process error using mean process uncertainty and autocorrelation parameters. Upper right panel shows a single simulated log of recruit per spawner vs spawners with fitted line. Lower left panel shows the predicted recruits at mean productivity and capacity for given spawners. The replacement line is shown (dotted). Lower right panel shows the average 50 year mean return abundance at a given level of reconditioned kelts for 10,000 simulations

# Grande Ronde

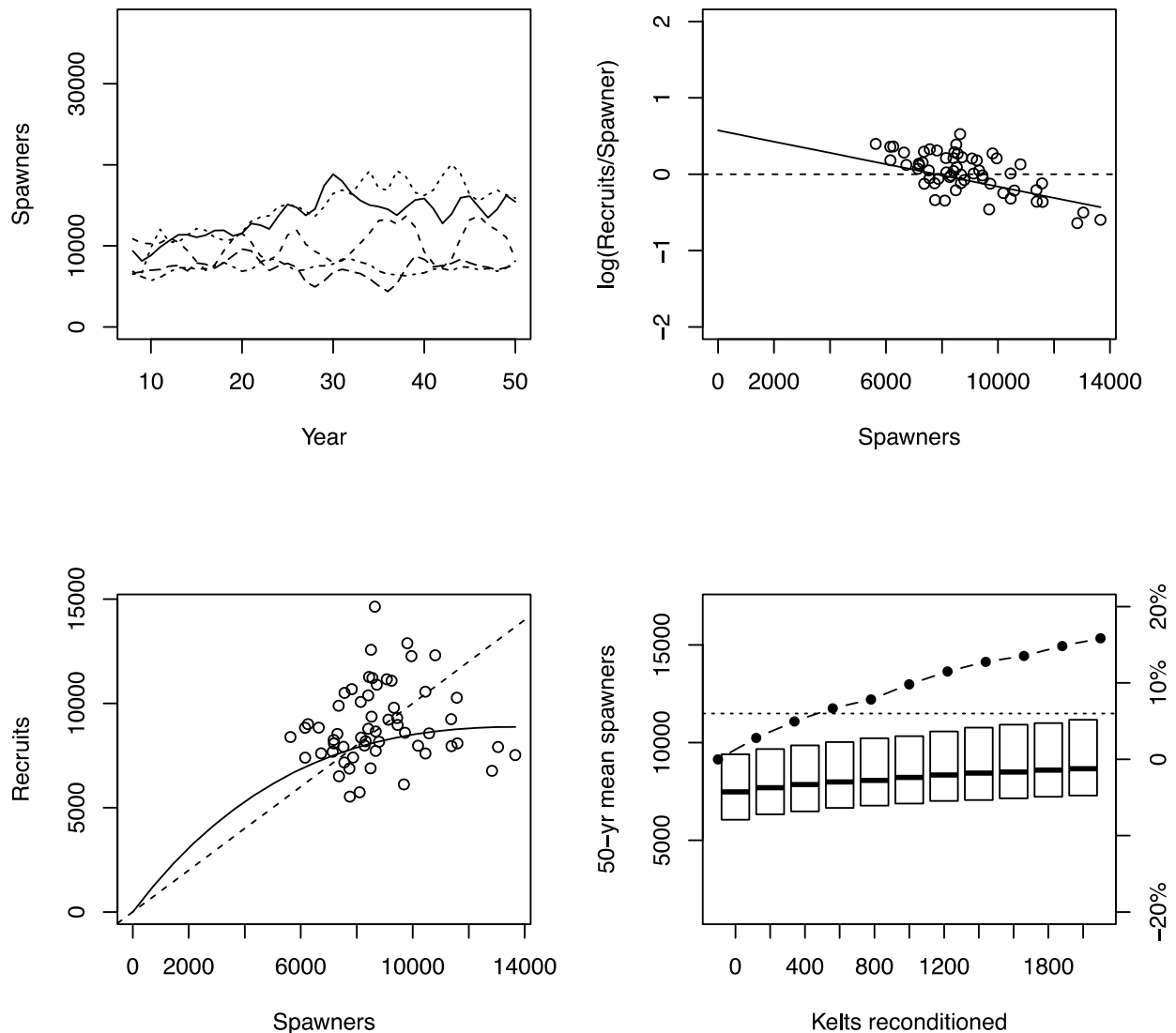


Figure 7.17 Simulated response of kelt reconditioning in the Grande Ronde. Upper left panel shows 5 of 10,000 50 year simulated returning spawner abundances drawing random productivity, capacity, and age class parameters, and adding temporally autocorrelated process error using mean process uncertainty and autocorrelation parameters. Upper right panel shows a single simulated log of recruit per spawner vs spawners with fitted line. Lower left panel shows the predicted recruits at mean productivity and capacity for given spawners. The replacement line is shown (dotted). Lower right panel shows the average 50 year mean return abundance at a given level of reconditioned kelts for 10,000 simulations

## Discussion

We have statistically reconstructed the trends of 5 B-run wild Steelhead RGs and one A-run RG. The reconstruction estimates the number of spawners in years preceding observed returns to LGR, the Ricker productivity and capacity terms, and the fractions of returns of each age class. To varying degrees of certainty, we were able to determine productivity and capacity parameters, and the underlying structure of uncertainty in prediction and measurement. With only 8 years of data for all but the Fish Creek population, it is not surprising that estimates of parameters spanned fairly wide ranges. The broad range of parameter estimates added to the uncertainty in simulated results, but nonetheless provided a perspective on the relative benefits that might be expected to the individual RGs. We emphasize that because observed spawner abundances did not span a wide range, there is no way of determining if capacity is higher than the estimated value from the limited range of data. We expect that continued monitoring and evaluation across a wider range of observations will provide further insight into the underlying spawner/recruit relationships.

Although reconditioning benefits can be predicted from estimated production parameters, the degree to which interactions at an aggregate scale at LGR might affect individual RGs is difficult to surmise without accounting for population interaction and uncertainties. We attempted to include an environmental effect with the PDO, but we also attempted to account for autocorrelation in process errors. Only Fish Creek showed a significant PDO effect. All other RGs showed a near zero effect, but also a strong autocorrelation effect at approximately  $\phi = 0.7$ . It is worth noting that an autocorrelation coefficient of approximately 0.7 produces approximately decadal cycles at process variations in the range of 0.3-0.6, so it may be confounded with a PDO effect if one is present.

We have shown that kelt reconditioning can have benefits if recruitment is not regulated by density, but the limited number of years of data was not sufficient to narrow the ranges of uncertainty in parameter estimates. Beyond the limited number of years of data, the RG level assessments were further biased by the assumption that data assigned to reporting groups were representative of the B-run component of interest. Furthermore, the assignments to reporting groups have uncertainty, so the number of spawners presumed may not have been the actual number that returned if some were incorrectly assigned. The observation error component of the assessment is meant to account for this. Further development of this methodology should involve a pooled approach to estimating the observation error component such that the assignment error is a single shared estimate instead of multiple individual assessments.

The objective of this analysis was to develop a framework to explore the relative potential benefits of kelt reconditioning of Snake river wild B-run reporting groups. The adult counts and genetic identification of LGR samples provided a means to explore contrasts in B-run

production parameters under the assumptions that genetic assignments were accurate, and that the samples were a reflection of the B-run component. Further analysis into estimation of production parameters should include a pooled analysis, where the common ocean temporal trend is separated from the individual freshwater spawning and rearing temporal trends in variability.

The mechanics of collection and reconditioning, and the dynamics of the interactions between populations, as well as the nature of independent variability and shared variability among populations, should be explored further. The evaluation tool developed can benefit from various sources of additional information to separate sources of variability to specific life stages, e.g., spawner to smolt production as a function of RG environmental covariates and smolt to adult return rates as a function of hydrosystem and ocean conditions. This kelt reconditioning assessment is based on a methodology that remains a work in progress, but has not yet included additional data sources that could reduce the level of uncertainty. It nonetheless represents a significant first step toward a statistically validated and objective effectiveness monitoring tool that can be used to evaluate effectiveness of RPA actions identified in the 2008 BiOp.

## **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. Currently working with BPA to complete design of reconditioning
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.
16. Concluded with the Cle Elum spawning channel.
17. Kelt biophysiological decision to remature is made soon after spawning.
18. As a result of this project an additional 164 (an additional 4 immature were released in the Yakima River) remature wild, adult steelhead were released back into river systems in 2017. A total of 98 were released into the Snake River, below Lower Granite Dam, and 66 were released into the Yakima River below Prosser Dam. Our studies indicate that these will repeat home to streams where they previously spawned with high fidelity and successfully spawn and produce offspring. Reconditioning kelt steelhead in the Yakima River provides approximately 14 times as many repeat spawners than return naturally and in the Snake River the reconditioning benefit is more than 96 times the natural repeat spawner rate.



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**Appendices**

A1.a Master Kelt Tracking Table

Steelhead Kelt  
Treatments  
Table 2017

Strategy	Year	Location	# Collected	# released	S @ release (%)	# remature	Retained	skip remature	# @ ocean	S @ ocean (%)	# @ Bonneville	Bonneville (%)	# @ Lower Granite Dam (or Prosser)	Return Rate to Lower Granite Dam (or Prosser) (%)	Transportation (or treatment)		Transportation (or treatment)	
															Benefit relative to in-river	Treatment benefit relative to Hockersmith 1.66		Benefit relative to Bonneville natural
In-river	2005	Prosser	67	67												1.54	2.70	25.61
In-river	2006	Prosser	52	52												0.66	1.16	3.10
In-river	2007	Prosser	53	53												1.95	3.41	9.28
In-river	2008	Prosser	88	88												1.57	2.74	6.64
In-river	2009	Prosser	58	58												1.78	3.12	11.54
In-river	2010	Prosser	155	155												0.44	0.78	3.74
In-river	2011	Prosser	85	85												1.22	2.13	7.01
In-river	2012	Prosser	59	59												1.17	2.04	6.74
In-river	2013	Prosser	52	52												0.00	0.00	0.00
In-river	2014	Prosser	45	45												2.30	4.02	11.52
In-river	2015	Prosser	121	121												0.00	0.00	0.00
In-river	2016	Prosser	56	56									2.00			1.23	2.15	57.54
In-river	2017	Prosser	5	5												0.00	0.00	0.00
Total and weighted mean			896	896							2.08	2.90				1.00	1.75	4.77
In-river	2002	Lower Granite*	1209	1209												2.28	0.40	
In-river	2003	Lower Granite*	865	865												1.19	0.21	
In-river	2004	Lower Granite*	1138	1138												3.02	0.53	1.49
In-river	2009	Lower Granite	178	176												3.86	0.68	1.51
In-river	2010	Lower Granite	1411	1399												1.22	0.21	0.62
In-river	2011	Lower Granite	1633	1613												0.63	0.11	0.22
In-river	2012	Lower Granite	2098	2098												0.16	0.03	0.03
In-river	2013	Lower Granite	840	827												0.82	0.14	0.13



In-river	2014	Lower Granite	2584	2571			8	0.31		1.06	0.19	0.32
In-river	2015	Lower Granite	1195	1193			0	0.00		0.00	0.00	0.00
In-river	2016	Lower Granite	1841	1837			4	0.22		0.75	0.13	2.11
In-river Total and weighted mean	2017	Lower Granite	824	821			0	0.00		0.00	0.00	0.00
			15816	15747			5.05	0.29		1.00	0.18	0.48
In-river Total and weighted mean	2002	John Day*	287	287			28	9.76		1.00	5.88	16.02
Transported (Hamilton Island)	2002	Lower Granite*	750	750			19	2.53		3.83	1.53	
Transported (Hamilton Island)	2003	Lower Granite*	376	376			3	0.80		2.30	0.48	
Transported (Hamilton Island)	2004	Lower Granite*	982	982			7	0.71		0.81	0.43	2.00
Transported (Hamilton Island)	2009	Lower Granite	71	68			0	0.00		0.00	0.00	0.00
Transported (Hamilton Island)	2010	Lower Granite	301	301	13/108	12.04	0	0.00		0.00	0.00	0.00
Transported (Hamilton Island)	2011	Lower Granite	109	109	3/47	6.38	0	0.00		0.00	0.00	0.00
Total and weighted mean			2589	2586		9.21	8.59	1.12		1.16	0.67	1.84
Transported (estuary release)	2010	Lower Granite	23	22	4/10	40.00	0	0.00		0.00	0.00	0.00
Transported (estuary release)	2011	Lower Granite	91	90	14/46	30.43	0	0.00		0.00	0.00	0.00
Total and weighted mean			114	112		35.22	0.00	0.00		0.00	0.00	0.00
Transported Total and weighted mean	2002	John Day*	271	271			34	12.55		1.29	7.56	20.61
Transported (unfed Hamilton Island)	2004	Prosser	75	63	15/28	53.57	5	6.67			4.02	18.75
Transported (unfed Hamilton Island)	2005	Prosser	98	96	14/57	24.56	1	1.02		0.23	0.61	5.84

Transported (unfed Hamilton Island)	2006	Prosser	55	49	31/49	63.27	2	3.64	1.89	2.19	5.87
Transported (unfed Hamilton Island)	2007	Prosser	43	38	14/35	40.00	0	0.00	0.00	0.00	0.00
Transported (unfed Hamilton Island)	2008	Prosser	100	100	26/49	53.06	3	3.00	0.66	1.81	4.38
Transported (unfed Hamilton Island)	2010	Prosser	124	123	27/59	45.76	1	0.81	0.16	0.49	2.34
Transported (unfed Hamilton Island)	2011	Prosser	100	100	16/47	34.04	1	1.00	0.78	0.60	1.99
Total and weighted mean			595	569		44.89	1.86	2.18	<b>0.75</b>	<b>1.32</b>	<b>3.59</b>
Transported (unfed estuary release)	2010	Prosser	113	113	13/60	21.67	1	0.88	0.69	0.53	2.57
Transported (unfed estuary release)	2011	Prosser	90	89	16/47	34.04	3	3.33	2.58	2.01	6.62
Total and weighted mean			203	202		27.85	1.00	1.97	<b>1.63</b>	<b>1.19</b>	<b>3.24</b>
Transported (fed Hamilton Island)	2002	Prosser	479	334			43	8.98		5.41	
Transported (fed Hamilton Island)	2003	Prosser	208	187			8	3.85		2.32	
Transported (fed Hamilton Island)	2004	Prosser	105	83	11/26	42.31	5	4.76		2.87	13.39
Transported (fed Hamilton Island)	2005	Prosser	106	96	6/56	10.71	0	0.00	0.00	0.00	0.00
Transported (fed Hamilton Island)	2006	Prosser	56	50	32/50	64.00	0	0.00	0.00	0.00	0.00
Transported (fed Hamilton Island)	2007	Prosser	40	38	19/27	70.37	1	2.50	0.44	1.51	4.10
Transported (fed Hamilton Island)	2008	Prosser	108	100	28/50	56.00	7	6.48	1.43	3.90	9.47
Total and weighted mean			1102	888		48.68	21.40	5.81	<b>2.00</b>	<b>3.50</b>	<b>9.54</b>
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		5.41	
Transported (pooled groups)	2003	Prosser	208	187					8	3.85		2.32	
Transported (pooled groups)	2004	Prosser	180	146			26/54	48.15	10	5.56		3.35	15.63
Transported (pooled groups)	2005	Prosser	204	192			20/113	17.70	1	0.49	0.11	0.30	2.80
Transported (pooled groups)	2006	Prosser	111	99			63/99	63.64	2	1.80	0.94	1.09	2.91
Transported (pooled groups)	2007	Prosser	83	76			33/62	53.23	1	1.20	0.21	0.73	1.97
Transported (pooled groups)	2008	Prosser	208	200			54/99	54.55	10	4.81	1.06	2.90	7.02
Transported (pooled groups)	2010	Prosser	237	236			40/119	33.61	2	0.84	0.16	0.51	2.45
Transported (pooled groups)	2011	Prosser	190	189			32/94	34.04	4	2.11	1.63	1.27	4.18
Total and weighted mean			1710	1470				45.14	15.86	4.26	<b>1.47</b>	<b>2.71</b>	<b>7.00</b>
Long-term	2000	Prosser	512	91	17.77							10.71	
Long-term	2001	Prosser	551	197	35.75							21.54	
Long-term	2002	Prosser	420	140	33.33							20.08	
Long-term	2003	Prosser	482	298	61.83							37.24	
Long-term	2004	Prosser	662	253	38.22							23.02	107.49
Long-term	2005	Prosser	386	86	22.28					4.98		13.42	127.44
Long-term	2006	Prosser	279	85	30.47					15.84		18.35	49.15
Long-term	2007	Prosser	422	221	52.37					9.25		31.55	85.84
Long-term	2008	Prosser	472	269	56.99					12.54		34.33	83.27
Long-term	2009	Prosser	510	140	27.45	91				5.31		16.54	61.24
Long-term	2010	Prosser	1157	404	34.92	101				27.06		21.03	101.26
Long-term	2011	Prosser	680	223	32.79	120				9.29		19.76	65.17
Long-term	2012	Prosser	550	340	61.82	275				18.24		37.24	57.41
Long-term	2013	Prosser	546	266	48.72	166	41?	8		16.79		29.35	44.47
Long-term	2014	Prosser	481	292	60.71	149	96	22		9.11		36.57	104.90
Long-term	2015	Prosser	1098	396	36.07	382	74	37		12.43		21.73	59.23
Long-term	2016	Prosser	471	360	76.43	236	74	15		21.40		46.04	1231.34
Long-term	2017	Prosser	118	55	46.61	55	29	TBD 18		16.06		28.08	76.55
Total and weighted mean			9797	4116	42.01	1575	273	82		<b>14.48</b>		<b>25.31</b>	<b>69.00</b>
						63.61%		66.92%					
Long-term	2005	Shitike Cr	9	1	11.11							6.69	63.56
Long-term	2006	Shitike Cr	4	0	0.00							0.00	0.00
Long-term	2007	Shitike Cr	14	1	7.14							4.30	11.71
Long-term	2008	Shitike Cr	11	0	0.00							0.00	0.00

Total and weighted mean			38	2	5.26					3.17	8.64	
Long-term	2005	Omak Cr	17	3	17.65					10.63	100.94	
Long-term	2006	Omak Cr	27	2	7.41					4.46	11.95	
Long-term	2007	Omak Cr	43	8	18.60					11.21	30.50	
Long-term	2008	Omak Cr	32	9	28.13					16.94	41.09	
Long-term	2009	Omak Cr	17	2	11.76					7.09	26.25	
Long-term	2010	Omak Cr	13	6	46.15					27.80	133.85	
Long-term	2011	Omak Cr	20	4	20.00					12.05	39.74	
Long-term	2012	Omak Cr	65	4	6.15					3.71	5.72	
Long-term	2013	Omak Cr	49	4	8.16					4.92		
Total and weighted mean			283	42	14.84					8.94	24.37	
s	Long-term	2006	Parkdale	1	1	100.00					60.24	161.33
s	Long-term	2007	Parkdale	13	1	7.69					4.63	12.61
s	Long-term	2008	Parkdale	14	7	50.00					30.12	73.06
s	Long-term	2009	Parkdale	9	4	44.44					26.77	99.15
w	Long-term	2010	Parkdale	15	4	26.67					16.06	77.33
w	Long-term	2011	Parkdale	23	5	21.74					13.10	43.20
w	Long-term	2012	Parkdale	21	13	61.90					37.29	57.49
Total and weighted mean			96	35	36.46					21.96	59.88	
			# Survived									
Long-term	2012	DNFH	143	5	3.50	4	0	-		73.36	2.11	3.25
Long-term	2013	DNFH	163	61	37.42	12	47	22		157.18	22.54	34.16
Long-term	2014	DNFH	149	19	12.75	2	17	5		41.19	7.68	22.03
Long-term	2015	DNFH	149	43	28.86	13	30	18		99.22	17.38	47.40
Long-term	2016	DNFH	164	30	18.29	12	18	18		62.90	11.02	294.70
Long-term	2017	DNFH	191	83	43.46	24	0	-		4.45	26.18	71.37
Total and weighted mean			959	241	25.13	67	112	63		86.40	15.14	41.27
*reconditioned at DNFH												
			# Survived		Released	# remature	Retained	skip remature				
Long-term	2011	Lower Granite	111	2	1.80	2	-	-	-			
Long-term	2012	Lower Granite	124	10	8.06	10	3	0	-	169.19	4.86	7.49
Long-term	2013	Lower Granite	110	57	51.82	57	3	0	-	217.64	31.22	47.30
Long-term	2014	Lower Granite	110	34	30.91	34	0	0	-	99.84	18.62	50.76
Long-term	2015	Lower Granite	22	11	50.00	8	8	3	3	171.91	30.12	82.12
Long-term	2016	Lower Granite	227	120	52.86	19	19	101	77	243.30	31.85	851.63
Long-term	2017	Lower Granite	269	59	21.93	21	21	58	TBD 18	2.25	13.21	36.02

Total and weighted mean			973	293	30.11	151	54	162	80			<b>103.54</b>	<b>20.34</b>	<b>49.46</b>
subset of LGD fish*														
Long-term	2016	LGD @DNFH 9/25 to NPTH LGD @DNFH 9/25 to												
Long Term	2017	NPTH	39	32	82.05	5	5	27	TBD 18			115.11	49.43	134.76
Long-term	2013	S.F. Clearwater	24	12	50.00	12	4	0	-			210.00	30.12	45.64
Long-term	2015	S.F. Clearwater	35	7	20.00	4	4	3	-			68.77	12.05	32.85
Long-term	2016	S.F. Clearwater	8	0	0.00	-	-	-	-			0.00	0.00	0.00
Total and weighted mean			59	19	32.20	16	8	3	-			<b>110.72</b>	<b>17.08</b>	<b>52.89</b>
Long-term*	2014	Fish Creek	12	3	25.00	1	0	2	2			80.75	15.06	43.20
Long-term*	2015	Fish Creek	83	25	30.12	10	10	15	15			103.56	18.14	49.47
Total and weighted mean			95	28	29.47	11	10	17	17			<b>101.34</b>	<b>17.76</b>	<b>48.41</b>
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	905							0	0.00			
Natural repeat	2016	Bonneville Dam	1611							1	0.06			
Natural repeat	2017	Bonneville Dam	838							0	0.00			
highlighted values in yellow are			21177								0.61			

considered  
draft.

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

## 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.
- 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.

**Published in 2017:**

Bosch, B., J. Trammell, and D. Hatch. 2017. Kelt reconditioning giving wild steelhead a boost in the Yakima Basin. The Osprey No. 86:16-17.

Pierce, A.L., J.W. Blodgett, T.D. Cavileer, L.R. Medeiros, J. Boyce, L.K. Caldwell, W.J. Bosch, R. Branstetter, D.E. Fast, D.R. Hatch, and J.J. Nagler. 2017. Reproductive development in captive reconditioned female steelhead kelts: evidence for consecutive and skip spawning life histories. Canadian Journal of Fisheries and Aquatic Sciences 74(7): 1049-1060.

**Presentations 2017:**

Bosch, W.J., Are recent increases in Columbia Basin natural-origin steelhead adult return abundance a response to restoration actions? Oregon Chapter of the American Fisheries Society. March 2017. Oral Presentation.

Graham, N., Jenkins LE, Everett SR, Branstetter, R. Peirce A. Hatch D.R. Reconditioning Snake River B-Run Steelhead Kelts A Research Report. 68th Annual NW Fish Culture Conference. December 5-7, 2017, in Redding, California. Poster Presentation.

Hatch, D.R. Steelhead Kelt Reconditioning. Columbia River Inter-Tribal Fish Commission Meeting. March 2017. Oral Presentation.

Hatch, D.R. Steelhead Kelt Reconditioning. Columbia Chapter of the Coastal Conservation Association. May 2017. St. Helens, OR. Oral Presentation.

Jenkins L.E. Reconditioned female steelhead trout (*Oncorhynchus mykiss*) in the Clearwater River, Idaho exhibiting different life history trajectories. Kelly Creek Flycasters. December 2017. Lewiston, ID. Oral Presentation.

Jenkins, I., A. Pierce, N. Graham, L. Medeiros, R. Branstetter, S. Everett, D. Hatch, and J. Nagler. 2017. Life History Diversity in Post-Spawn Female Steelhead Trout Assessed Using Plasma Estradiol-17 $\beta$ : Relationship with Growth and Energy Reserves. American Fisheries Society Western Division and Montana Chapter Annual Meeting, Missoula, MT, May 2017.

Jenkins LE, Pierce AL, Graham ND, Medeiros LR, Everett SR, R,Hatch DR, and Nagler JJ. Post Spawning Diversity in Reconditioned Female Clearwater River Steelhead Trout (*Oncorhynchys mykiss*) Kelts assessed Using Plasma Estradiol-17 $\beta$ . International Congress of Comparative Endocrinology. June 4-9, 2017. Chateau Lake Louise, Banff National Park, Alberta, Canada. Poster Presentation.



- Jenkins LE, Pierce AL, Graham ND, Everett SR, Medeiros LR, Branstetter R, Hatch DR, and Nagler JJ. Life history diversity in anadromous post-spawn female steelhead trout assessed using plasma estradiol-17 $\beta$ : relationship with growth and energy reserves. American Fisheries Society Annual National Meeting, Aug 20-24 2017, Tampa FL. Oral Presentation.
- Pierce, A.L. Hatch DR, Fast D. Everett S. Abrahamse, M. Nagler J. Jenkins L. Madieros L. Caldwell L. Cavalier T. Graham N. Branstetter R. Stephenson J. Begay B. Newell J. Blodgett J. Bosch B. Frederiksen C. Jackson N. Tall Bull T. USFWS and NPT Dworshak NFH staff and many others. Columbia Basin Steelhead Kelt Reconditioning Research. Western Division American Fisheries Society. May 22-25, 2017. Missoula, MT.
- Pierce, A.L. Hatch DR, Fast D. Everett S. Abrahamse, M. Nagler J. Jenkins L. Madieros L. Caldwell L. Cavalier T. Graham N. Branstetter R. Stephenson J. Begay B. Newell J. Blodgett J. Bosch B. Frederiksen C. Jackson N. Tall Bull T. USFWS and NPT Dworshak NFH staff and many others. Columbia Basin Steelhead Kelt Reconditioning Research. Conference June 14-15, 2017. Ellensburg, WA. Oral Presentation.
- Stephenson, J. Dave Fast, Bill Bosch, Joe Blodgett, Ryan Branstetter, Andy Pierce, Shawn Narum, Doug Hatch D.R. Reproductive Success of Artificially Reconditioned Kelt Steelhead Spawning in the Yakima River Basin. Yakima Basin Science and Management Conference June 14-15, 2017. Ellensburg, WA. Oral Presentation.

## 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>

Juvenile Tissue Sampling: <https://www.monitoringresources.org/Document/Method/Details/933>

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"	<a href="#">Fish</a>	<a href="#">Abundance of Fish</a> (ID: 46)	<a href="#">Fish Life Stage: Adult - Outmigrant</a>	<a href="#">Fish Origin: Both</a>
"Reconditioned Kelt abundance"	<a href="#">Fish</a>	<a href="#">Abundance of Fish</a> (ID: 46)	<a href="#">Fish Life Stage: Adult Fish</a>	<a href="#">Fish Origin: Both</a>
"Stock Composition"	<a href="#">Fish</a>	<a href="#">Composition: Fish Species Assemblage</a> (ID: 56)	<a href="#">Fish Life Stage: Adult - Outmigrant</a>	<a href="#">Fish Origin: Natural</a>
"Kelt Condition"	<a href="#">Fish</a>	<a href="#">Condition of Life Stage: Fish</a> (ID: 57)	<a href="#">Fish Life Stage: Adult - Outmigrant</a>	NA
"Reconditioned Kelt condition"	<a href="#">Fish</a>	<a href="#">Condition of Life Stage: Fish</a> (ID: 57)	<a href="#">Fish Life Stage: Adult Fish</a>	NA
"Maturation Status"	<a href="#">Fish</a>	<a href="#">Condition of Life Stage: Fish</a> (ID: 57)	<a href="#">Fish Life Stage: Adult - Returner</a>	NA
"Fecundity"	<a href="#">Fish</a>	<a href="#">Fecundity: Fish</a> (ID: 68)	NA	NA
"Fry Growth"	<a href="#">Fish</a>	<a href="#">Growth Rate: Fish</a> (ID: 73)	<a href="#">Fish Life Stage: Juvenile - Fry/Parr</a>	NA
"Fertilization Rate"	<a href="#">Fish</a>	<a href="#">Hatchery Practices: Propagation</a> (ID: 87)	<a href="#">Fish Origin: Both</a>	NA
"Kelt length"	<a href="#">Fish</a>	<a href="#">Length: Fish Species</a> (ID: 75)	<a href="#">Fish Life Stage: Adult - Outmigrant</a>	NA
"Reconditioned kelt length"	<a href="#">Fish</a>	<a href="#">Length: Fish Species</a> (ID: 75)	<a href="#">Fish Life Stage: Adult Fish</a>	NA
"Mark Detection"	<a href="#">Fish</a>	<a href="#">Mark/Tag Recovery or Detection</a> (ID: 381)	NA	NA
"Parentage Analysis"	<a href="#">Fish</a>	<a href="#">Relative Reproductive Success (RRS)</a> (ID: 88)	<a href="#">Fish Origin: Both</a>	NA
"Reproductive success"	<a href="#">Fish</a>	<a href="#">Reproductive Success (Nb/N)</a> (ID: 89)	<a href="#">Fish Origin: Natural</a>	NA
"Mark application"	<a href="#">Fish</a>	<a href="#">Stock Identity</a> (ID: 95)	<a href="#">Fish Life Stage: Adult - Outmigrant</a>	NA

"Kelt Survival"	<a href="#">Fish</a>	<a href="#">Survival Rate: Fish</a> (ID: 99)	<a href="#">Fish Life Stage:</a> <a href="#">Adult -</a> <a href="#">Outmigrant</a>	<a href="#">Fish Origin:</a> <a href="#">Both</a>
"Collection Date"	<a href="#">Fish</a>	<a href="#">Timing of Life Stage:</a> <a href="#">Fish</a> (ID: 101)	<a href="#">Fish Life Stage:</a> <a href="#">Adult -</a> <a href="#">Outmigrant</a>	NA
"Release Date"	<a href="#">Fish</a>	<a href="#">Timing of Life Stage:</a> <a href="#">Fish</a> (ID: 101)	<a href="#">Fish Life Stage:</a> <a href="#">Adult Fish</a>	NA
"Kelt Weight"	<a href="#">Fish</a>	<a href="#">Weight: Fish</a> (ID: 206)	<a href="#">Fish Life Stage:</a> <a href="#">Adult -</a> <a href="#">Outmigrant</a>	<a href="#">Fish Origin:</a> <a href="#">Both</a>
"Reconditioned Kelt weight"	<a href="#">Fish</a>	<a href="#">Weight: Fish</a> (ID: 206)	<a href="#">Fish Life Stage:</a> <a href="#">Adult Fish</a>	<a href="#">Fish Origin:</a> <a href="#">Both</a>