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% (19 years) for Yakima River at Prosser Hatchery and 33% (8 years; 43% over the last 6 years) for mixed stock collections at Lower Granite Dam, and in previous years Fish Creek and the South Fork Clearwater River. In 2018 unmarked upstream migrant adult steelhead return counts were at the 5th lowest across the region since records were kept in this regard in the mid 1990's. These low return years typically translates into a lower abundance of kelts. Regardless, we collected a sizeable number of kelts at both the Snake and Yakima rivers due to lower water conditions which means that more kelts migrated via juvenile bypass systems. A total of 107 reconditioned B-run steelhead were released below Lower Granite Dam in 2018 to address Reasonable and Prudent Alternative 33 of the FCRSP Biological Opinion. A total of 106 reconditioned, remature steelhead were released in the Yakima River in 2018. Reproductive success of reconditioned steelhead was confirmed in the Yakima River once again with assignments of 46 juvenile fish to 27 unique kelt parents. Lifetime reproductive success for female reconditioned kelt steelhead was estimated as 2.36 relative to single time spawning steelhead. Using estradiol assays, we have established that steelhead rematuration rates vary annually and spatially and ranged from 41.7% for Snake River consecutive spawners while at 43.3% for upper Columbia and 65.2% for Prosser in 2018. Skip spawner rematuration rates in comparison are typically higher and where so at both the Snake and Upper Columbia sites 78.4% and 100% respectively, while they were lower than has been typically observed at Prosser (50%). Survival of skip spawners at Prosser has been very low and we are working on ways to improve survival of skip spawners at this location. Work continues with the Yakama Nation VSP study to determine plasma hormone levels of maiden fish so that we have a baseline with which to compare kelt to and tie these values to migration success, homing fidelity, spawn timing, and genetic stock index (GSI) structure in the basin. Differences in hormonal analysis for consecutive and skip spawners was compared. We discovered that consecutive spawners tended to recover a number of the hormones necessary for oogenesis much more rapidly than skip spawners. Skip spawners though did have much higher concentrations of muscle lipids, plasma triglycerides, and estradiol than consecutive spawners, which would allow for a greater reproductive investment. Post spawning survival was contingent on both high counts of triglycerids and plasma osmolality. Also, consecutive spawning was condition was dependent during the period immediately after spawning. Among fish on the consecutive spawning trajectory, egg size is set first based on condition at approximately 10 weeks after spawning, and then fecundity (and

consequently TEM) is set based on condition at approximately 20 weeks after spawning. Females with greater maiden reproductive investment were more likely to mature as consecutive spawners, suggesting that both reproductive investment and consecutive spawning may be condition-dependent traits, and raising the key question of when condition influences these outcomes. We also tested fasting of steelhead kelts during the first 10 weeks after spawning which did not result in significantly reduced maturation rate in this study. This supports that the critical period for the rematuration decision occurs before spawning in steelhead kelts. We continued to refine our plasma assays that detect IGF-I and GH concentrations that we utilize for evaluating kelt maturation. From 2008 to 2017 we have detected conclusive evidence of 342 kelts showing strong site fidelity from both aforementioned waterways. Most of 2018 implementation of the kelt master plan for the Snake River has been in process with Bonneville Power Administration. Development of a Snake River kelt reconditioning facility preliminary design has been ongoing with BPA in 2017 into 2019. Once the preliminary design is completed, we will move to the final stage of the ISRP 3-step process. Development of a kelt population model continues to make progress with simulations of kelt reconditioning in the Yakima 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 published 2 papers and gave 14 professional presentations in 2018/19.

Acknowledgments

We would like to thank our partners at the Upper Yakima Kelt Reconditioning Program, Tom Scribner, Keely Murdoch, and Matt Abrahamse for sharing data and sampling with our team. Many thanks to the Lower Granite Dam capture and sampling crews (Nez Perce Tribe, Idaho Department of Fish and Game (IDFG), University of Idaho, and Army Corps of Engineers Walla Walla District). Thank you to Stephanie Harmon and Megan Moore for diligent and reliable laboratory processing and collection of genotypic data. Data and information provided by Mike Ackerman and Craig Steele from IDFG made a substantial contribution to these analyses. Thank you to the Washington Department of Wildlife staff at the Genetics lab in Olympia for sharing samples with us. Also, we appreciate the help from Tim Resseguie and his team at Yakama Nation for helping us with electroshocking and genetic collection. We would like to thank our colleagues at CRITFC: Bobby Begay, Jeff Fryer, Denise Kelsey, Melissa Edwards, David Graves, Joe Nowinski, Scotty Riddle, Hazel Schaffer, Jayson FiveCrows, Agnes Strong, Aaron Ikemoto, Casey Justice, Seth White, Sara Thompson, Loretta Islas, Christine Golightly, and Chrissy Bynum. Yakama Hatchery and field staff (Bill Fiander, Zack Mays, T. Newsome, Michael Fiander, Charlie Strom, JJ, and OJ) WDFW personnel (Gabe Temple, Anthony Fritts, and Tim Webster). Many thanks to the LGD sampling crew at Nez Perce Tribe.

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 0.29% of the Snake River steelhead run based on the return of 28 out of 16,610 PIT tagged fish at Lower Granite Dam.

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 with five chapters addressing 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, this project's 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), the Dworshak National Fish Hatchery (DNFH) at Ahsahka, ID (Clearwater River). Collections of steelhead kelts also occurred from 2002-2013 at Omak Creek near Omak, WA, Powerdale Dam trap/East Fork Hood River near Hood River (upstream adult migrants), OR 2006-2012, Shitike Creek from 2005-2009 near Warm Springs, OR, and Fish Creek located in the Nez Perce-Clearwater National Forest, ID from 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.

Site Number	Site	Drainage	Location	Collection site	Reconditioning site	Release Site	Juvenile Sampling Location	Dates of use
1	Chandler Juvenile Monitoring Facility (CJMF)	Yakima River	RKM 75.6	Yes	-	-	-	1999-2018
2	Yakama Nation Prosser Fish Hatchery	Yakima River	RKM 75.6	-	Yes	Yes	-	1999-2018
3	Lower Granite Dam Juvenile Bypass	Snake River	RKM 173	Yes	-	Yes	-	2009-2018
4	Dworshak National Fish Hatchery	Clearwater River	RKM 65	Yes (hatchery fish for experimental purposes)	Yes	-	-	2009-2018
5	Nez Perce Tribal Fish Hatchery	Clearwater River	RKM 38	No	Yes	-	-	2016-2018
6	South Fork Clearwater	Clearwater River	RKM 0 - 100	Yes	-	-	-	2013, 2015
7	Fish Creek Weir	Lochsa River	RKM 0.8	Yes	-	-	-	2014, 2015
8	Omak Creek Weir	Okanogan River	RKM 0.8	Yes		-	Yes	2003-2013

9	Bonaparte Creek	Okanogan River	RKM 0.4	Yes		-	-	2003-2014
10	Cassimer Bar Hatchery	Okanogan R./ Columbia R.	RKM 0/ 859	-	Yes	Yes	-	2003-2010
11	St. Mary's Acclimation Ponds	Okanogan River	RKM 8.0	-	Yes	-	-	2011-2013
12	Powerdale Dam	Hood River	RKM 6.4	Yes	-	-	-	2006-2010
13	East Fork Weir	East Fork Hood River	RKM 20.1	Yes	-	-	-	2011-2013
14	Parkdale Hatchery	Middle Fork Hood River	RKM 5.6	-	Yes	-	-	2006-2013
15	Shitike Creek Weir	Deschutes River	RKM 0.7	Yes	-	-	-	2005-2008
16	Warm Springs Hatchery	Warm Springs River	RKM 16	-	Yes	-	-	2005-2008
17	Hamilton Island	Columbia River	RKM 231	-	-	Yes	-	2002-2008, 2010,2011, 2014
18	Westport	Columbia River	RKM 72	-	-	Yes	-	2010, 2011
19	Aldrich Point	Columbia River	RKM 75.6	-	-	Yes	-	2010, 2011
20	Cle Elum Spawning Channel	Yakima River		-	-	Yes (experimental group)	Yes	2015-2017
21	Satus Creek	Yakima River		-	-	-	Yes	2008-2018

22	Toppenish Creek	Yakima River		-	-	-	Yes	2008-2018
23	Simcoe Creek	Yakima River		-	-	-	Yes	2008-2018
24	Ahtanum Creek	Yakima River		-	-	-	Yes	2008-2016
25	Big Creek	Yakima River		-	-	-	Yes	2008-2016
26	Cowiche Creek	Yakima River		-	-	-	Yes	2008-2016
27	Little Rattlesnake Creek	Yakima River		-	-	-	Yes	2008-2016
28	Nile Creek	Yakima River		-	-	-	Yes	2008-2016
29	Quartz Creek	Yakima River		-	-	-	Yes	2008-2016
30	Bumping River	Yakima River		-	-	-	Yes	2008-2016

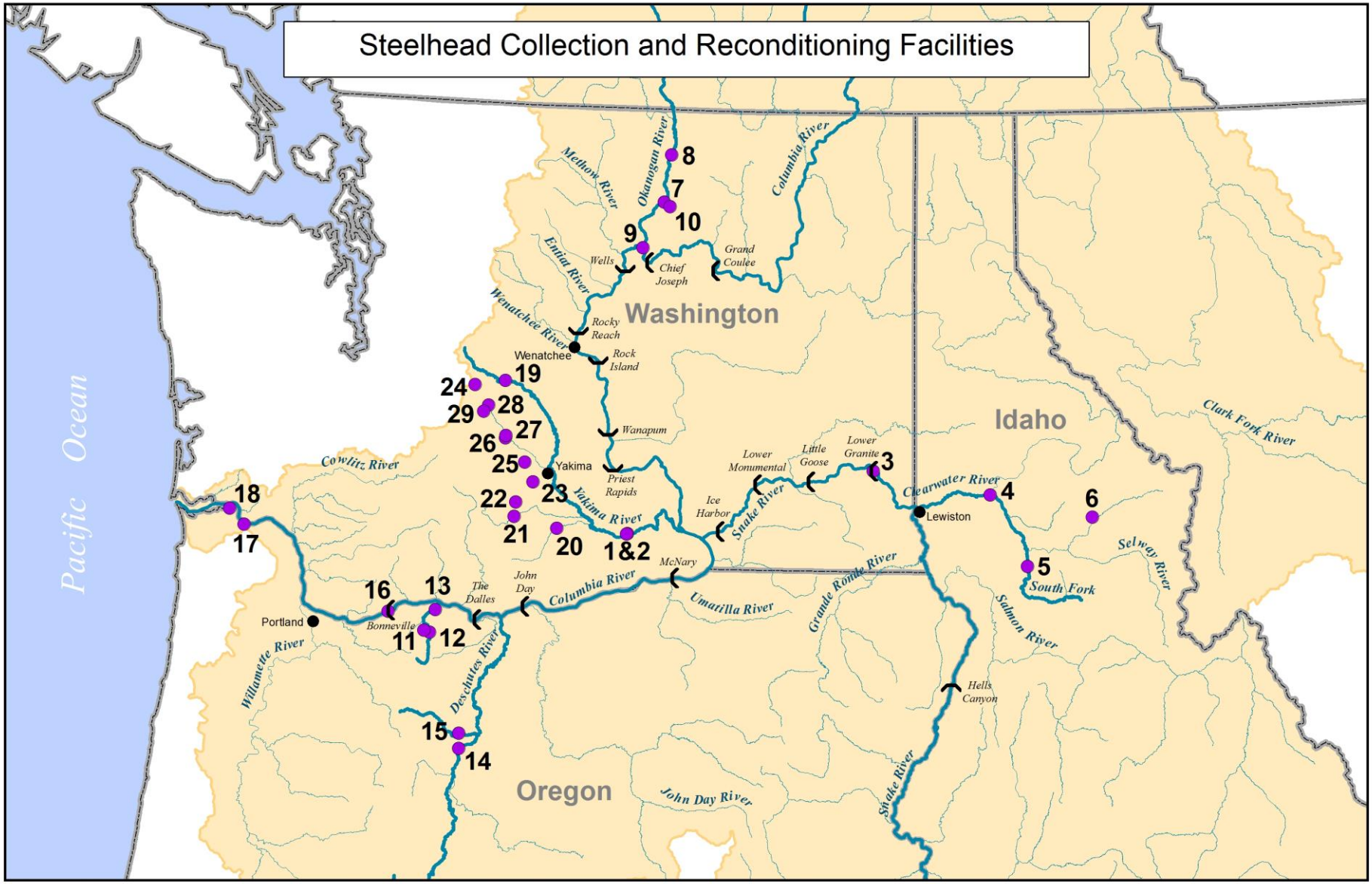

Figure 1: Map of Steelhead kelt Project area 2000-2018.

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

Yakima River Basin

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Chandler Juvenile Collection Facility (Yakima River)

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.

Snake River Basin

The Snake River watershed is the tenth largest among North American rivers and covers almost 280,000 km^2 in portions of six U.S. states: Wyoming, Idaho, Nevada, Utah, Oregon, and Washington, with the largest portion in Idaho. Most of the Snake River watershed lies between the Rocky Mountains on the east and the Columbia Plateau on the northwest. The largest tributary of the Columbia River, the Snake River watershed makes up about 41% of the entire Columbia River Basin. The Snake River enters the Columbia at RK 523. Its average discharge at the mouth constitutes 31% of the Columbia's flow at that point. The Snake River's average flow is 1,553 m^3/s. At Anatone, Washington, downstream of the confluences with the Salmon and Grand Ronde, but upstream of the Clearwater, the mean discharge is 979 m^3/s. Steelhead spawn naturally throughout the lower portion of the basin with the vast amount of "B-run" steelhead produced at the Dworshak National Fish Hatchery found on the Clearwater River.

The Lower Granite Juvenile Fish Facility

The third dam on the Snake River Lower Granite Lock and Dam is a concrete gravity run-of-the-river dam on the Snake River, in the U.S. state of Washington. The dam is located 22 miles (35 km) south of the town of Colfax, and 35 miles (56 km) north of Pomeroy. Steelhead kelts migrating from tributaries of the Snake River above Lower Granite Dam that do not emigrate via the Removable Spillway Weir (RSW) are directed by a large bypass system to the Juvenile Fish Facility (JFF) at Lower Granite Dam (LGR) (RK 173).

Dworshak National Fish Hatchery

Kelt reconditioning facilities are located at Dworshak National Fish Hatchery (DNFH) in Ahsahka, Idaho. DNFH is located at the confluence of the North Fork of the Clearwater River (RK 65). The Dworshak National Fish Hatchery is a "mitigation" hatchery constructed in 1969 by the Army Corps of Engineers, which presently is 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 return to the hatchery. They are then air spawned and reconditioned to learn more about kelt rematuration and how we can improve maturation and survival. 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 was 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 trucked here to be reconditioned.

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

Introduction

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

We define long-term reconditioning as holding and feeding post-spawn steelhead in a captive environment to increase kelt survival and additional spawning opportunities. The long-term steelhead reconditioning diet and care treatments were established from the studies conducted in 2001 and 2002 (Hatch et al. 2002 and Hatch et al. 2003b) and summarized in Hatch et al. 2013b. These fish are typically released in the fall to over-winter and return to the spawning sites volitionally. This chapter recaps 2018 kelt collection efforts for a broader review of specific fish culturing practices please 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, any fish released back to wild are entered into PITAGIS database.

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 dip netted for processing and input to reconditioning tanks at Prosser Hatchery (Table 1, site 2). Yakama Nation staff monitored the Chandler bypass separator during the kelt migration.

Lower Granite Dam
Steelhead kelts entering the juvenile bypass separator (Table 1, site 3) are collected by Army Corps of Engineer (COE) staff. Kelts are netted off the adult fish separator bars and moved to a fish hopper that 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 methane sulfonate (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 into the collection river as the run at large is returning from the ocean. These reconditioned fish generally mingle with the run at large and proceed to in-river, over-winter locations and spawning grounds in the spring. This strategy seeks to reduce mortality in the hydro system 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).

GSI analyses and tests of demographic difference

Genetic stock identification (GSI) analyses were performed using a reference baseline of 12 Snake River Steelhead Trout populations, distributed among four geographic regions associated with a predominant migratory phenotype (Matala et al. 2016; Table 1). Populations were genotyped at a panel of 391 single nucleotide polymorphism (SNP) loci (Hasselman et al. 2017; Hess et al. 2019). The actual number of SNPs used in these analyses is a subset of the total number genotyped, following removal of some loci based on quality control screening. Loci removed include those that failed to reach a genotyping success threshold ($\geq$85%) or exhibiting fixed allele frequencies (i.e. non-informative). Ultimately, analyses were based on 347 putative neutral loci and 13 adaptive loci that were developed for utility in differentiating run-timing (i.e. maturation phenotype) in *O. mykiss* (Hess et al. 2016; Micheletti *et al.* 2018). Over the course of these studies, we have employed an iterative process of updating the reference baseline, including the newly incorporated 13 run-time associated loci. The resolving power of the baseline to accurately identify population-of-origin was assessed using GENECLASS2 (Piry et al. 2004). Individual assignment likelihood scores (LS) were generated in a leave-one-out jackknife procedure, implementing the Bayesian method of Rannala and Mountain (1997). Accuracy was assessed as the proportion of individuals in each baseline population that correctly assigned to their respective known population of origin (self-assignment). Kelt origins were evaluated similarly, by comparing the genotype of each "unknown" mixture sample to the allele frequencies in each reference population using GENECLASS2. Ranked assignment likelihood scores were used to infer greatest genetic similarity between kelts of "unknown" origin and all possible references sources represented in the baseline. Fork length (mm) size data were recorded during kelt sampling and the data was used to calculate mean size and estimate size distributions of kelts assigned to each population and region. They include: Grande Ronde River – "GRROND", Imnaha River – "IMNAHA", lower Clearwater River – "LOCLWR", lower Salmon River – "LOSALM", lower Snake River – "LSNAKE, and upper Salmon River – "UPSALM". Four additional reporting groups are coincident with Snake River regions dominated by B-run populations (Narum et al. 2008; Ford et al. 2011; Matala et al. 2014), including: Middle Fork Salmon River – "MFSALM", South Fork Salmon River – "SFSALM", upper Clearwater River – "UPCLWR", and South Fork Clearwater River – "SFCLWR" (Figure 1B.1).

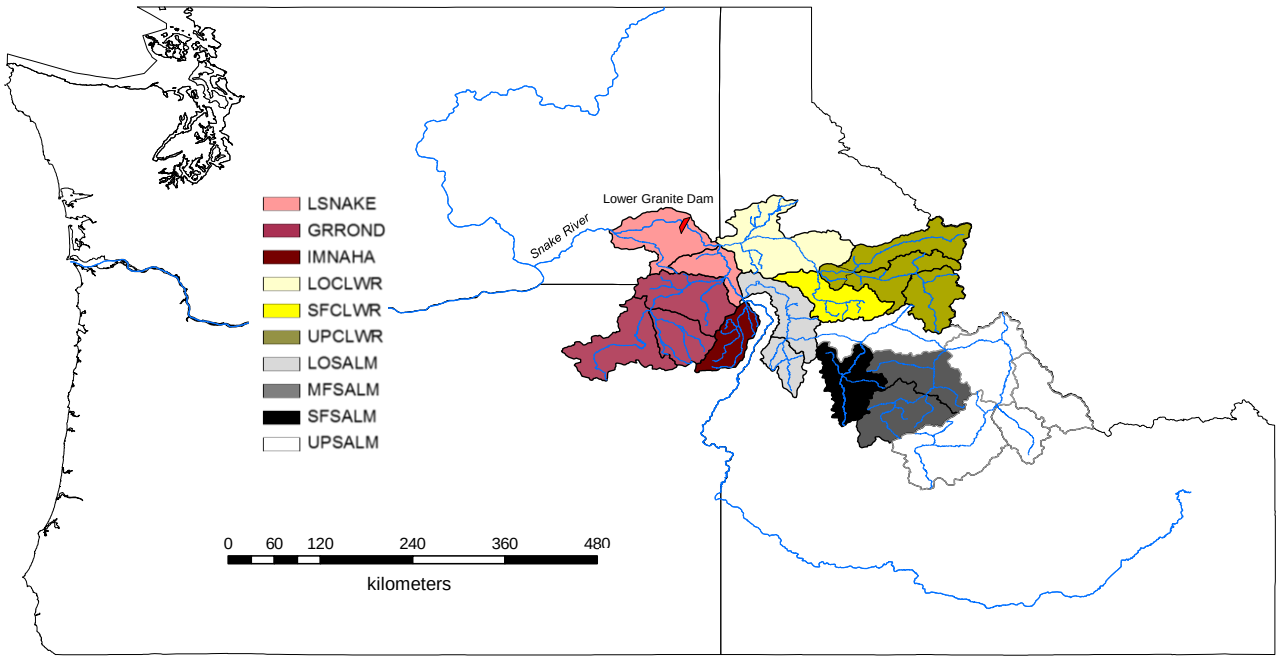

Figure1.1Map of GSI region and reporting groups established on the basis of 73 baseline O. mykiss populations (see Appendix 1). Lower Snake River (LSNAKE); Grande Ronde River (GRROND); Imnaha River (IMNAHA); Lower Clearwater River (LOCLWR); South Fork Clearwater

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

Results/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 2002-2018 we captured a total of 20,655 downstream migrating kelts at the LGD and 14,530 from the CJMF from 2000-2018. The Columbia River upriver steelhead run in 2017-18 was low, resulting in a smaller than average steelhead kelt collection at both CJMF (227 kelts) and LGD (1,095 kelts) (Appendix A1a). In 2018, the kelt collection represented 7% and 18% of the upstream run at LGD and CJMF, respectively. This compares to 2017 collections of 4.6% and 8% for LGD and CJMF, respectively.

Reconditioning

Since 2011, 1,232 kelt steelhead have been retained for reconditioning from collections at LGD and 418 fish have survived to their first fall. Since 2000, 10,024 kelt steelhead were retained for reconditioning from collections at CJMF and 4,219 fish survived to the first fall of that annual collection period (Appendix A1a). All Snake River collections were made at the LDG in 2018, however kelts were collected and reconditioned from the South Fork Clearwater and Fish Creek in previous years (Hatch et al. 2018).

Long-term reconditioning survival is variable from year to year but has averaged 42% at the Prosser Fish Hatchery (PFH) over the last 19 years. For the last 5 years Yakima reconditioning has been just over 50% survival (Figure 1.2). The staff here has a number of years of reconditioning experience, so we see generally small annual variations in survival (Figure 1.2). The reconditioning survival rate for wild Snake River kelts from 2011 through 2018 is 39%. Survival during the initial years at DNFH (2011-12) was compromised as a result of poor water quality detailed in previous reports (Hatch et al. 2012 and Hatch et al. 2013) and compounded by inexperience of new hatchery staff. Although, the past 5 years have seen marked improvement in survivals as water quality issues have been improved and staff gain experience in handling and caring for wild steelhead, rate for this five-year period has averaged 45% (Figure 1.3).

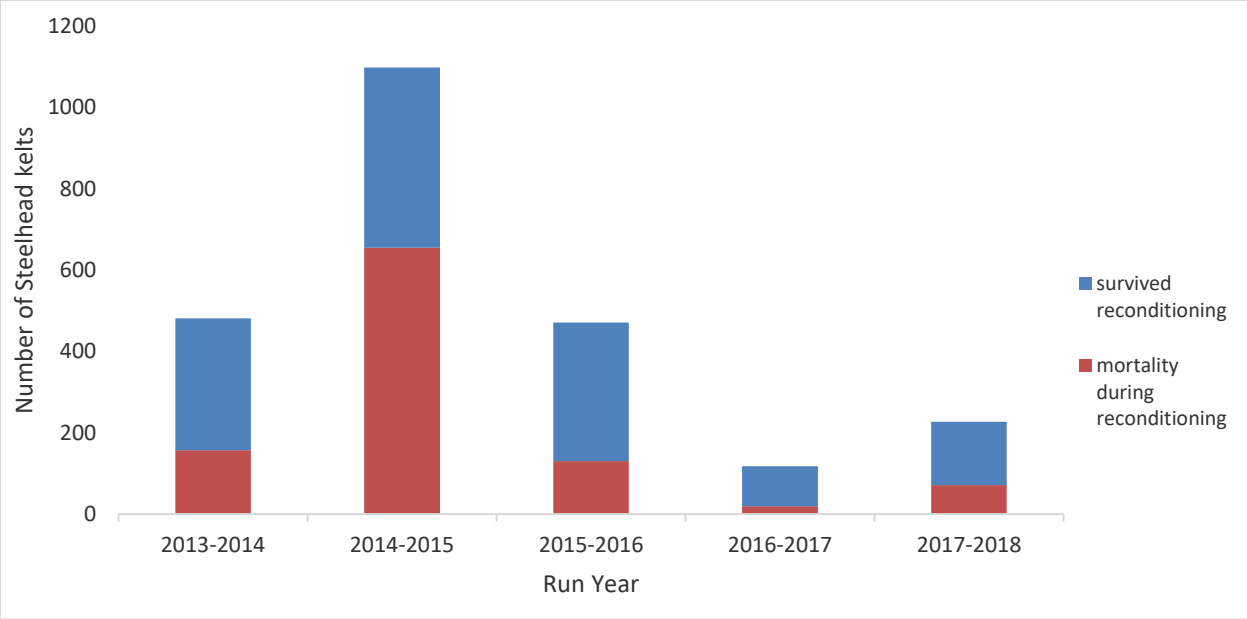

Figure1. 2: Yakima River steelhead kelt collection for reconditioning and fate from 2013-2018.

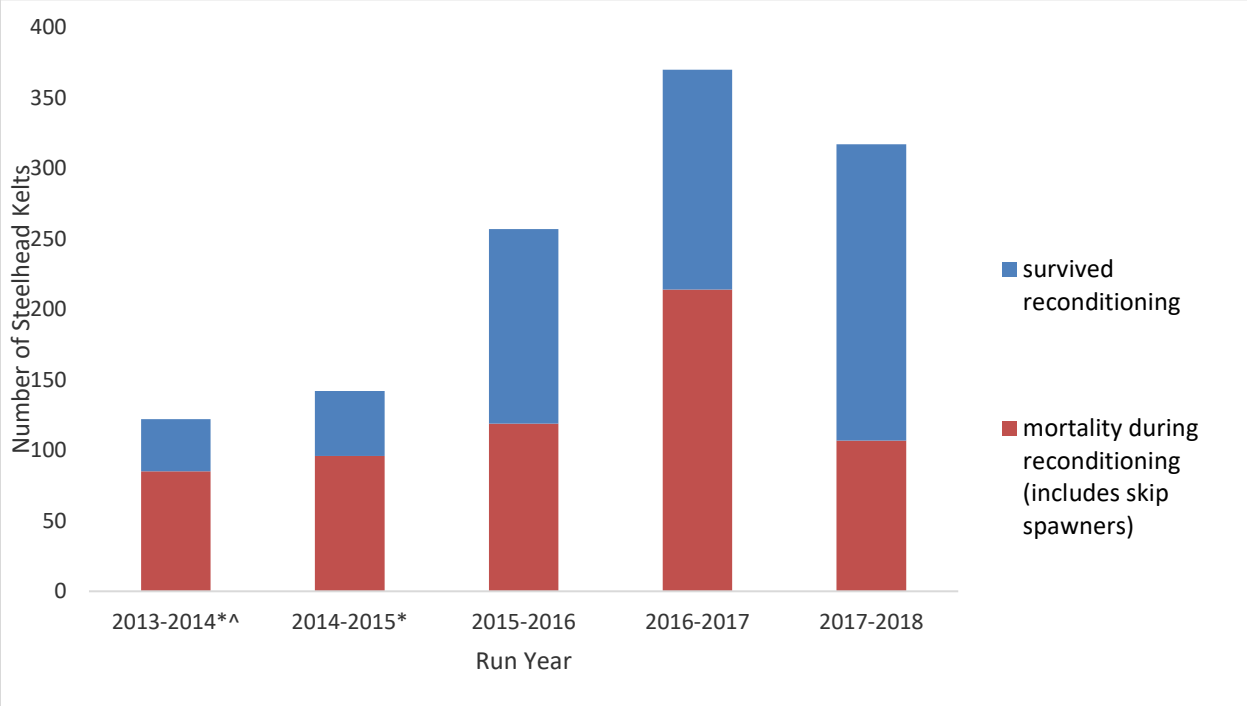

Figure1. 3: Snake River steelhead kelt collection for reconditioning and fate from 2013-2018.

Natural and artificially reconditioned kelts can pursue two alternative pathways toward rematuration and repeat spawning. One pathway is termed consecutive spawning where individual's remature and proceed to spawn in the next spawn cycle. The other pathway is termed skip spawning where individual's remature and proceed to spawn two years after their previous spawning. To illustrate, kelts collected in 2018 could spawn again in 2018/2019 as

consecutive spawners or wait until 2019/2020 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 (60-70%) while the majority of Snake River kelts follow the skip spawner life history (60-70%). Strategy choice is likely controlled by genetics and environment.

Release

We evaluated the traits and survival to release of reconditioned kelt steelhead *Oncorhynchus mykiss* in the Yakima River (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 (2000-18) 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.

In Figures 1.4 and 1.5, the numbers of female kelts reconditioned and released are added to the corresponding steelhead run. For example, a consecutive kelt that is collected in the Spring of 2014 (from 2013/2014 run) would be released into the following run year 2014/2015. For a skip spawner, which is typical of Snake River kelts, a fish caught in the Spring of 2014 would be released into the 2015/2016 run year. Figures 1.4 and 1.5 demonstrate that the contribution of reconditioned kelts to the overall runs in the Yakima and Snake rivers is measurable and quite substantial in comparison to no intervention. The natural repeat spawner rate (or no intervention), measured as a return rate to Bonneville Dam, for the Snake River is 0.29% and for the Yakima River is 2.90%. The calculated benefit of reconditioning relative to leaving the fish in the river is 14.5 times for the Yakima River and 131 times for the Snake River.

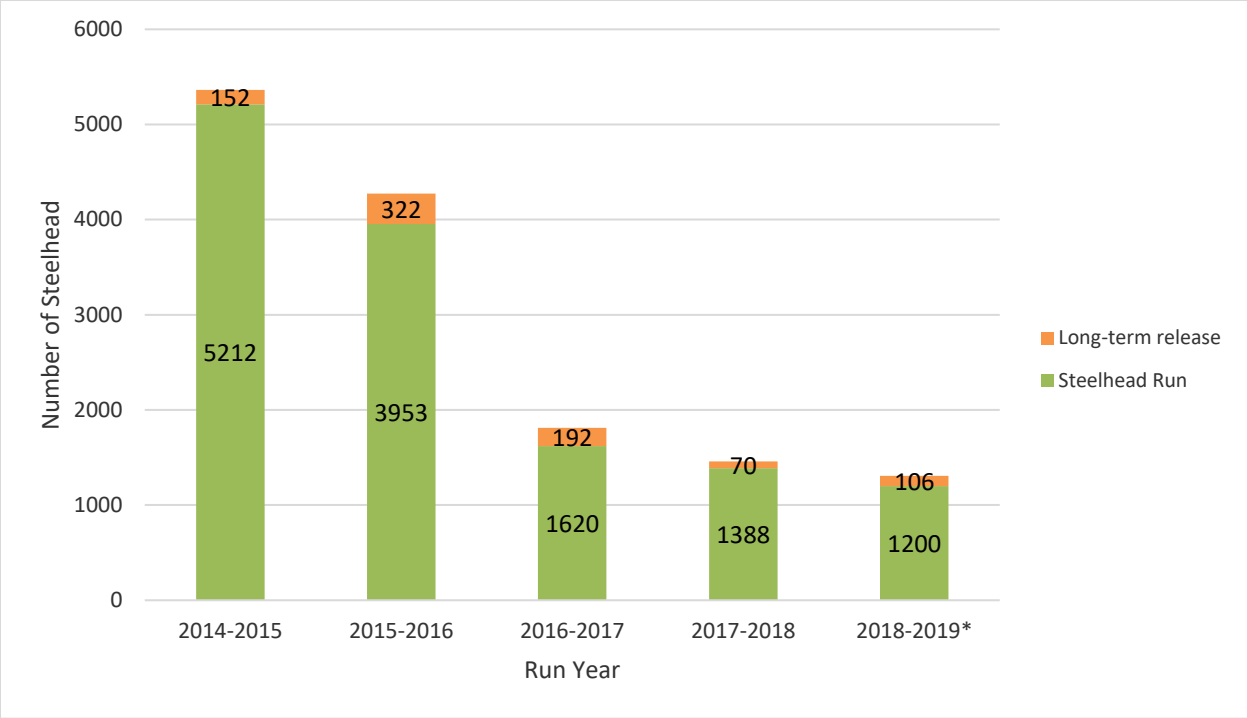

Figure1. 4:Contribution to steelhead run from reconditioned kelt release in Yakima Basin.

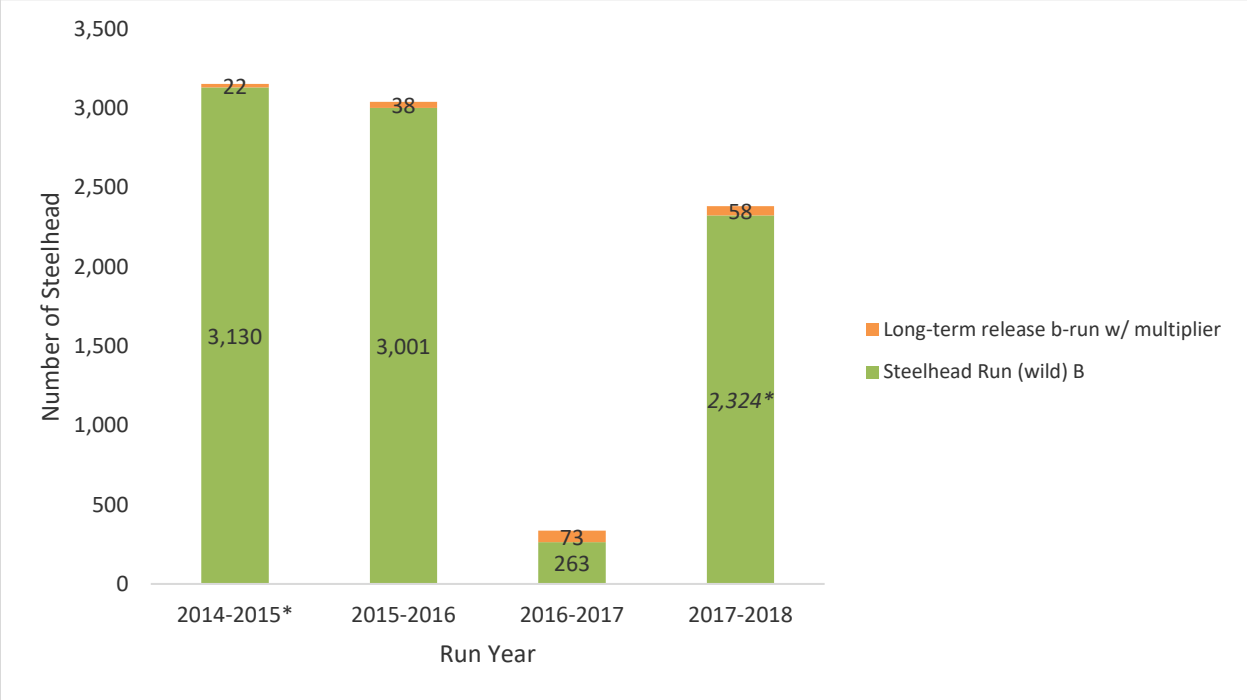

*Figure1. 5: Contribution to steelhead run from reconditioned kelt release in Snake River Basin. *2018 value is an estimated run size and will be corrected in the 2019 Kelt Annual Report. B-run numbers utilize Kelt Master Plan for reconditioned female kelt spawners*

Additional study is needed 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 well in the Snake River but is less successful in the Yakima River. Additionally, the cost of holding fish for an additional year should be compared to results from other scenarios. Other possible scenarios could include early release of skip spawners or transporting and releasing skip spawners past the hydroelectric dams.

Summary Research-Scale Efforts to Address RPA 33

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

Since we are operating at a research scale, as approved by the ISRP in the 2008 review, the capacity of our facility is much too small to meet the RPA 33 goal of increasing the LGR ladder count of B-run steelhead by 6%. However, we have demonstrated the feasibility of reaching the 6% goal. Releases of successfully reconditioned mature kelts began in 2011, but due to water quality issues in both 2011 and 2012 (Hatch et al. 2012 and 2013) these numbers were severely under representative of what we could accomplish at the initial site (Dworshak National Fish Hatchery) and with the inclusion of an additional temporary reconditioning site in 2016 (Nez Perce Tribal Hatchery). In 2013 we had a successful reconditioning and release at nearly 40% towards reaching the RPA 33 goal with 69 female fish released that year. Our best year was in 2017 with releases just over 50% towards the RPA goal at 98 mature female spawners released that year. Since 2013 we have averaged just over 32% of the RPA goal, releasing an average of 59 female fish per year with a total of 382 mature female fish released from 2011-2018. Table (1.1) summarizes all collections both A and B run, and releases associated with the RPA 33.

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

Year	Collection Location	Number of Fish Collected	Number of Fish that Survived Reconditioning	% Survival	Consecutive Spawner Release	Number of Fish Retained	Mature Skip Spawners Released (Capture Year)	Total Release by Year
2011	Lower Granite Dam	111	2	1.8%	2	-	-	-
2011	S.F. Clearwater	-	-	-	-	-	-	-
2011	Fish Creek	-	-	-	-	-	-	-
2011 (subtotal)		**111**	**2**	**1.8%**	**2**	**-**	**-**	**2**
2012	Lower Granite Dam	124	10	8.1%	10	-	-	-
2012	S.F. Clearwater	-	-	-	-	-	-	-
2012	Fish Creek	-	-	-	-	-	-	-
								-
2012 (subtotal)		**124**	**10**	**8.1%**	**10**	**-**	**-**	**10**
2013	Lower Granite Dam	110	57	51.8%	57	-	-	-
2013	S.F. Clearwater	24	12	50.0%	12	-	-	-
2013	Fish Creek	-	-	-	-	-	-	-
2013 (subtotal)		**134**	**69**	**51.5%**	**69**	**-**	**-**	**69**
2014	Lower Granite Dam	110	34	30.9%	34	-	-	-
2014	S.F. Clearwater	-	-	-	-	-	-	-
2014	Fish Creek	12	3	25.0%	1	2	2	-
2014 (subtotal)		**122**	**37**	**30.3%**	**35**	**2**	**2**	**35**

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*
2015 (subtotal)		**140**	**43**	**30.7%**	**22**	**21**	**18**	**24**
2016	Lower Granite Dam	227	120	52.86%	19	101	77	22^
2016 (subtotal)		**227**	**120**	**52.9%**	**19**	**101**	**77**	**37*^**
2017	Lower Granite Dam	269	59	21.9%	21	58	26	98^
2017 Subtotal		**269**	**59**	**21.9%**	**21**	**58**	**26**	**98^**
2018	Lower Granite Dam	259	177	68.3%	78	99	TBD 2019	107^
2018 Subtotal		**259**	**177**	**68.3%**	**78**	**99**	**TBD 2019**	**107^**
Grand Total		**1262**	**517**	**41.0%**	**256**	**281**	**123**	**382**
				***includes Fish Cr. kelt skip spawners**				
				^Includes previous year kelt spawners from LGD				

Snake River GSI 2015-2018

Utilizing genetic stock identification techniques, we ascertained the stock origins of steelhead kelts that utilized the LGR bypass, the collection of kelts held for reconditioning, and the group of released reconditioned kelts. This project is targeting B-run fish per RPA 33 and the B-run fish are components of the Clearwater and Salmon river GSI reporting groups.

Clearwater and Salmon river reporting groups represented 18% and 24% of the kelt run, respectively (Figure 1.6). Our collection for reconditioning contained 17% and 31% Clearwater and Salmon river groups, respectively (Figure 1.6). Our collections targeted larger fish that likely increased the Salmon River group. We did not observe a similar increase in the Clearwater group, likely because fish from the Clearwater are disproportionately observed in poorer condition at LGR (Table 1.2) than fish from any of the other GSI reporting groups and we do not collect fish in poor condition as reconditioning success is very low with that group (Hatch et al. 2013). This discrepancy in condition at LGR may be a result of geographic location. The Clearwater fish are much closer to LGR than other groups, so less overall migration mortality has occurred on Clearwater stocks relative to other groups. In other words, populations that migrate further likely lost members in poor condition to mortality prior to reaching LGR. This is further represented by the Salmon River group that faces a long migration from spawning grounds and demonstrates a much higher recondition rating at LGR than other groups (Table 1.2).

Fifty-one percent of the released reconditioned kelts were from B-run reporting groups (Clearwater and Salmon rivers). The proportion of fish from the Clearwater increased in proportion from collection to release and the fish from the other three groups (Salmon, Imnaha, and Grande Ronde stayed the same or decreased in proportion (Figure 1.6). Clearwater fish would likely be the largest fish in the tanks, which the increased survival may be a result of conspecific competition within the tanks with the larger Clearwater fish successfully obtaining feed over smaller fish from the other basins (Table 1.3). The fact remains that all of these groups of fish are listed under the ESA, concurrently, we have been successful at reconditioning and releasing a sizeable portion of B-run steelhead as well, with Salmon and Clearwater stocks comprising 51% of the kelt releases.

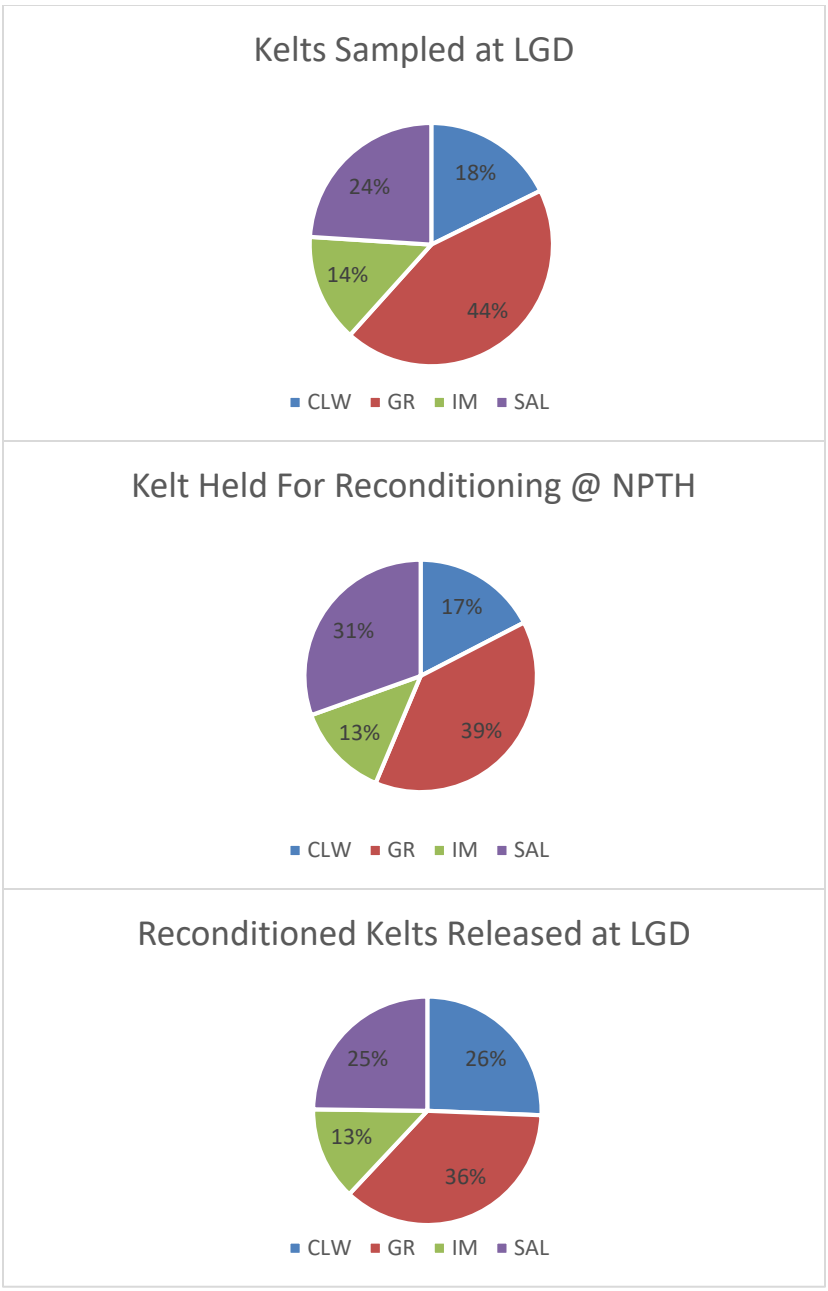

Figure1. 6 Kelt GSI at 3 different points in time and location. A. Kelt GSI at collection Lower Granite Dam 2015-2018. B. Selected Kelts for Reconditioning from Lower Granite Dam and GSI 2015-2018. C. Reconditioned Kelts from Lower Granite Dam Released GSI 2015-2018.

Table 1. 2: Condition of kelts sampled at Lower Granite Dam 2015-2018.

Condition	Sum of CLW	Sum of GR	Sum of IM	Sum of SAL
Poor	58 (13%)	94 (9%)	33 (9%)	96 (10%)
Fair	150 (34%)	316 (29%)	97 (27%)	247 (26%)
Good	174 (39%)	489 (45%)	157 (44%)	458 (48%)
Very Good	59 (13%)	194 (18%)	73 (20%)	161 (17%)

Table 1. 3: Condition of kelts sampled at Lower Granite Dam and average fork length based on condition factor 2015-2018.

Condition	CLW	GR	IM	SAL
Poor	683	646	635	651
Fair	736	663	633	645
Good	714	640	643	636
Very Good	720	639	634	643

2. Yakima River Kelt Reproductive Monitoring

Introduction

The reproductive success of long-term reconditioned kelts is being 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 sampled at the Chandler facility that did not go into the reconditioning program or that did not survive the reconditioning program are referred to as post-spawn maidens. Adults that were collected at the Chandler facility that were reconditioned and released in the fall are referred to as kelts. Kelt reproduction is subdivided between Event-1 (prior to reconditioning) or Event-2 (after reconditioning).

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 spawning redds detected. Technicians in the field were directed to target only age-0 juveniles. A 100 mm general maximum 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. Sampling locations for Juveniles are seen in figure 2.1. Some sampling sites were not sampled across all years due to access constraints.

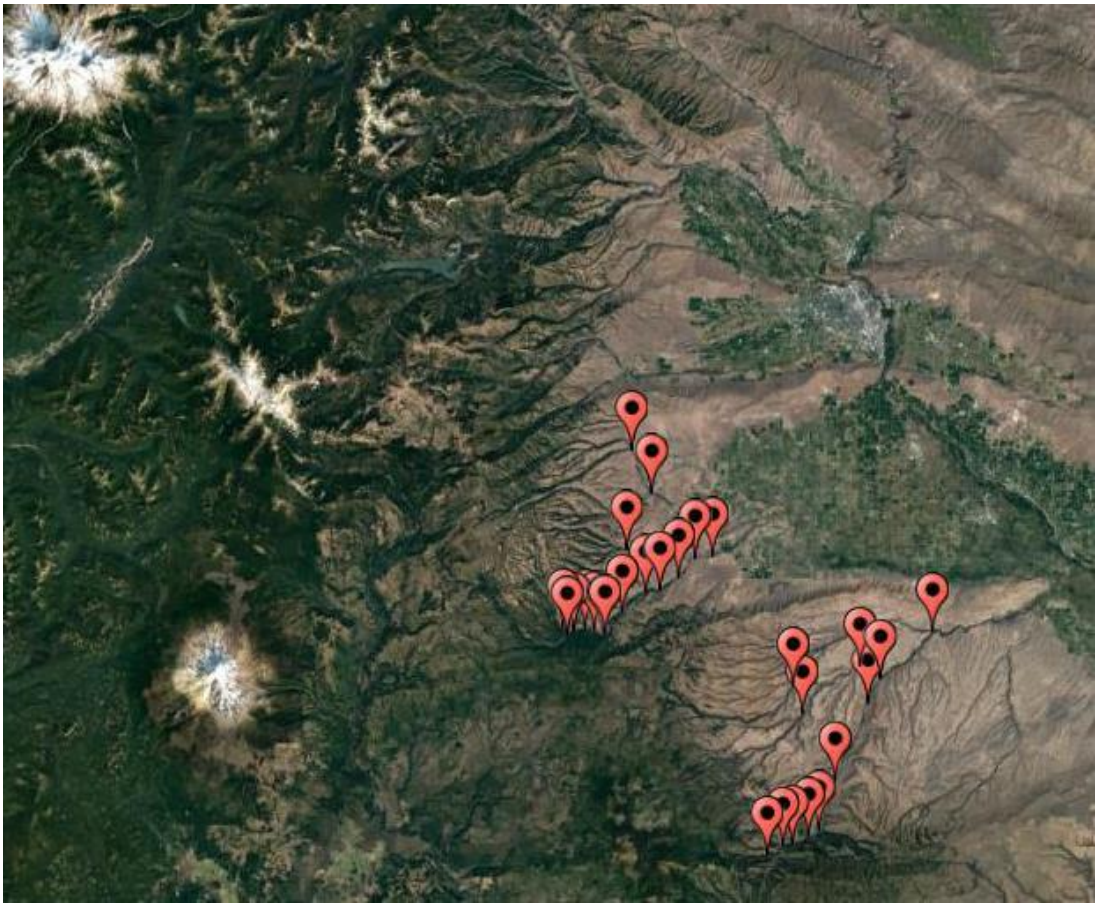

Figure 2.1: juvenile sampling locations in Satus (lower right) and Toppenish creeks over 5 years 2013-2017.

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 expanded marker panels 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, loci with genotyping rates less than 90%, and loci with minor allele frequency less than 0.05. 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 estimate 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 minimal single locus mismatches. This accounts for the presence of minor genotyping errors while minimizing the loss of parental assignment matches.

Parentage data and relative reproductive success was stratified by reporting reproductive success of four adult classes: 1) Maiden event of fish collected as pre-spawners, 2) Maiden event of fish collected as post-spawners, and 3) The first spawning event for reconditioned kelt, and 4) The second spawning event for reconditioned kelts following successful reconditioning and release. 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 in spawning tributaries. Juvenile assignments are reported here only for fish within Satus and Toppenish Creeks, although juvenile samples were previously genotyped in the Ahtanum, Big Creek, and Naches drainages, and adult samples included fish without PIT tag detections.

Relative reproductive success (RRS) was calculated between classes of fish by standardizing to the pre-spawn maiden class of adults. Lifetime reproductive success (LRS) estimates for kelts were calculated by adding the RRS of Event-1 and Event-2 estimates. 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. Because we had no information for male Event-1 in 2017, a placeholder of 1.00 was used to allow an LRS across all years. This number is conservative (lower) as compared to the average male Event-1 across the previous 4 years.

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 2.1. This table does not include locations dropped because of access issues, low sampling success, or lack of assignments to an anadromous offspring. The low apparent assignment rate is due to the low number of adults sampled compared to the relative number of unsampled anadromous steelhead and potential resident fish.

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

		Satus Cr.	Toppenish Cr.
2013	Genotyped	227	204
	Assigned	54	64
	% Assigned	24%	31%
2014	Genotyped	285	231
	Assigned	64	67
	% Assigned	22%	29%
2015	Genotyped	341	369
	Assigned	123	165
	% Assigned	36%	45%
2016	Genotyped	790	524
	Assigned	288	187
	% Assigned	36%	36%
2017	Genotyped	442	578
	Assigned	136	172
	% Assigned	31%	30%
Sum	Genotyped	2085	1906
	Assigned	665	655
	% Assigned	32%	34%

The number of genotyped parents confirmed to have entered either Satus or Toppenish Creek is shown in Table 2.2. Pre-spawn maidens have the greatest number of samples with a total of 225 males and 436 females. Post-spawn maidens have only 15 males and 75 females overall. Reconditioned kelts have 24 male and 145 female Event-1 detections and 30 male and 214 female Event-2 detections. Number of fishes increase each year but are limited by the number of kelts that can be collected, and mortality seen during the reconditioning process.

Table 2. 2: Number of genotyped adults with PIT detections.

Class	Sex	**2013**	**2014**	**2015**	**2016**	**2017**	**All**
Pre-Spawn Maidens	Male	38	46	57	78	6	225
Post-Spawn Maidens	Male	4	1	7	2	1	15
Reconditioned Kelt Event-1	Male	3	13	7	1	0	24
Reconditioned Kelt Event-2	Male	5	3	13	8	1	30
Pre-Spawn Maidens	Female	88	70	94	138	46	436
Post-Spawn Maidens	Female	12	13	39	9	2	75
Reconditioned Kelt Event-1	Female	15	44	51	22	13	145
Reconditioned Kelt Event-2	Female	74	18	40	56	26	214

The number of progeny assigned to each fish is shown in Table 2.3. The majority of assignments are to pre-spawn maidens with 185 juveniles assigned to males and 335 juveniles assigned to females. All other classes of fish have lower numbers assigned as a function of the lower representation in the number of adult fish both detected and genotyped.

Table 2. 3: Number of Progeny Assigned to each category of fish.

Class	Sex	**2013**	**2014**	**2015**	**2016**	**2017**	**All**
Pre-Spawn Maidens	Male	12	17	24	119	13	185
Post-Spawn Maidens	Male	0	0	0	4	7	11
Reconditioned Kelt Event-1	Male	3	6	4	1	NA	14
Reconditioned Kelt Event-2	Male	3	4	1	0	7	15
Pre-Spawn Maidens	Female	41	41	28	149	76	335
Post-Spawn Maidens	Female	8	1	27	2	0	38
Reconditioned Kelt Event-1	Female	3	21	32	17	65	138
Reconditioned Kelt Event-2	Female	17	12	18	40	39	126

The average number of progeny per parent is shown in Table 2.4. Numbers varied greatly between both years and between classes in within years. Males in particular had ranges between 0.00 and 7.00 progeny per parent.

Table 2. 4: Average number of progeny per parent for each category of fish.

Class	Sex	**2013**	**2014**	**2015**	**2016**	**2017**	**All**
Pre-Spawn Maidens	Male	0.32	0.37	0.42	1.53	2.17	0.82
Post-Spawn Maidens	Male	0.00	0.00	0.00	2.00	7.00	0.73
Reconditioned Kelt Event-1	Male	1.00	0.46	0.57	1.00	NA	0.58
Reconditioned Kelt Event-2	Male	0.60	1.33	0.08	0.00	7.00	0.50
Pre-Spawn Maidens	Female	0.47	0.59	0.30	1.08	1.65	0.77
Post-Spawn Maidens	Female	0.67	0.08	0.69	0.22	0.00	0.51
Reconditioned Kelt Event-1	Female	0.20	0.48	0.63	0.77	5.00	0.95
Reconditioned Kelt Event-2	Female	0.23	0.67	0.45	0.71	1.50	0.59

Relative reproductive success for each category of fish is seen in Table 2.5. Variance in the number's parallels that of the average number of progeny per parent with higher variance seen in males.

Table 2. 5: Relative reproductive success for each category of fish.

Class	Sex	**2013**	**2014**	**2015**	**2016**	**2017**	**AVG**
Pre-Spawn Maidens	Male	1	1	1	1	1	1
Pos-Spawn Maidens	Male	0.00	0.00	0.00	1.31	3.23	0.91
Reconditioned Kelt Event-1	Male	3.17	1.25	1.36	0.66	1.00*	1.61
Reconditioned Kelt Event-2	Male	1.90	3.61	0.18	0.00	3.23	1.78
Pre-Spawn Maidens	Female	1	1	1	1	1	
Pos-Spawn Maidens	Female	1.43	0.13	2.32	0.21	0.00	0.82
Reconditioned Kelt Event-1	Female	0.43	0.81	2.11	0.72	3.03	1.42
Reconditioned Kelt Event-2	Female	0.49	1.14	1.51	0.66	0.91	0.94

* No data was collected for 2017. 1.00 was inserted to allow for the subsequent estimate of LRS.

Lifetime reproductive success (LRS) of reconditioned kelts are shown in table 2B.6. Male kelt LRS varied between 0.66 and 5.07 times that of fish sampled as pre-spawn maidens within the same year. Across all years male kelts has an LRS of 3.27. Female kelt LRS had annual variation between 0.92 and 3.93 with an average of 2.36.

Table 2. 6: Lifetime reproductive success estimate for male and female Reconditioned kelts.

Class	Sex	**2013**	**2014**	**2015**	**2016**	**2017**	**AVG**
Reconditioned Kelt Lifetime	Male	5.07	4.86	1.54	0.66	4.23	3.27
Reconditioned Kelt Lifetime	Female	0.92	1.95	3.62	1.38	3.93	2.36

Discussion

The presence of kelt offspring demonstrates that reconditioned kelts successfully spawn in the wild. Lifetime reproductive success of female reconditioned kelts was calculated to be 2.36 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. While relative reproductive success of female reconditioned kelts following reconditioning (Event-2) is slightly lower (Average RRS=0.94) than that of pre-spawn, any spawning by a reconditioned kelt is additive to the population and demonstrates the potential to boost numbers.

The 2017 spawning event was the fifth consecutive year that we successfully assigned multiple progeny to reconditioned kelts. The methodology of focusing sampling efforts on age-0 fish in areas that anadromous spawning was expected to have occurred, and an increased sampling rate of juvenile has resulted in an increase in the number of successful assignments to both maiden and kelt fish. Future sampling will continue to focus on age-0 fish in areas that

spawning was expected to have occurred. This includes expanded sites in the upper Toppenish Creek Drainage that will allow us to cover a broader geographical region.

Reconditioned kelt steelhead have demonstrated that they are capable of spawning in the wild. With additional sampling in future years, including adult to adult estimates, 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 2018. 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 2018, we sampled blood and provided maturation status of individual fish to project managers so that consecutive and skip spawners could be managed appropriately (Section 3A). The 2018 results were added to a comparison of the performance of the three Columbia River Basin kelt projects in terms of survival and maturation rates (See Chapter 5). We completed a genetic analysis enabling classification of Yakima River kelts by subpopulation, which is being combined with survival, physiological, and migration data (Section 3B). We completed the analysis and writeup of three studies using hatchery origin kelts at Dworshak National fish hatchery. The first of these studies, on the reproductive performance of reconditioned kelts, was published in 2018 (Jenkins, et al. 2018b). The second study, on the development of the consecutive and skip spawning life histories, has been submitted for publication (Section 3C), and the third study, on factors predicting survival and rematuration in kelts, is being prepared for submission (Section 3D). We conducted the second and final year of a study on the effect of nutritional restriction during the period after spawning on life history trajectory in hatchery-origin kelts (Section 3E). Finally, 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 3F). Many of these studies are ongoing, and laboratory analysis, statistical analysis, results, interpretations, and conclusions may change as additional work is completed.

Section 3.A: Reproductive development in kelt steelhead 2018 Fish sampled and maturation rates

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. 2005a; Rideout and Tomkiewicz 2011). After a spawning event, some fish are able to restore energy lost during migration and spawning, redevelop a mature ovary, and

spawn the next year. These fish are termed consecutive spawners. Other fish do not initiate redevelopment of the ovary for the next spawning season, but instead skip a year. These fish are termed skip spawners. We hypothesize that these life history trajectories are the result of the effect of energy balance on maturation decisions made during seasonally defined critical periods. The influential critical period model of the first reproductive maturation (puberty) in salmonids posits that maturation is initiated during a decision window approximately one year prior to spawning (Campbell, et al. 2006; Satterthwaite, et al. 2009; Shearer and Swanson 2000; Thorpe 2007). This decision is made based on energy reserves. If maturation is initiated during this critical period, it may be arrested at a second critical period before the onset of exogenous vitellogenesis, if energy reserves are not sufficient (Yamamoto, et al. 2011). We hypothesize that a similar decision mechanism regulates rematuration in post-spawning steelhead. Consistent with this idea, we found that energy restriction affected reproductive development within 10 weeks after spawning in female rainbow trout (Caldwell, et al. 2013b; 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 1/4th of the cost of the vitellogenin assay.

During 2018, 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 National Fish Hatchery (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 (data not shown). 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 (Abrahamse and Murdoch 2013, 2014).

Sampling

Fish were blood sampled on the indicated dates (Table 3. A1). 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 3.A 1: Wild steelhead kelts sampled during the fall in 2018. Prosser: Prosser Hatchery, DNFH: Dworshak National Fish Hatchery, NPTH: Nez Perce Tribal Hatchery, WNFH: Winthrop National Fish Hatchery.

Location	Sample date	Fish type	# Fish	Notes
Prosser	9/13/18	Wild kelts	169	Fish were collected in 2017 (n = 165) and 2018 (n = 4)
DNFH and NPTH	10/3 and 10/4 of 2018	Wild kelts	217	Fish were collected in 2017 (n = 37) and 2018 (n = 180); all holdover fish held at DNFH
WNFH	10/11/18	Wild kelts	58	Fish were collected in 2017 (n = 8) and 2018 (n = 50)

Estradiol Assay

Fish plasma level of estradiol-17β (E2) is an indicator of reproductive development. Fish plasma samples must be solvent extracted prior to E2 assay to remove interfering substances. Plasma samples (250 µL) were extracted twice consecutively in 10 mL glass tubes with anhydrous diethyl ether (JT Baker, Avantor Performance Materials, Inc.; Center Valley, PA, USA). 2.0 mL diethyl ether was added to each tube and samples were vortexed for 1 m, and then frozen on dry ice. After 6-8 m, the aqueous phase was inspected to ensure that it was frozen solid, and the solvent fraction was then poured off into a 5 mL glass tube. Diethyl ether extracts were then placed in a 54°C water bath (OA-SYS™Heating System; Organomation Associates, Inc; Berlin, MA) and dried down under a gentle stream of N_2 directed *via* a nitrogen evaporator manifold (N-EVAP™ 112; Organomation Associates, Inc; Berlin, MA). A second extraction of the remaining aqueous fraction from each plasma sample was then performed, again using 2.0 mL diethyl ether, as described above; this second extract was pooled with the first extract. Dried extracts of fish plasma were resuspended in 250 µL assay buffer from the estradiol assay kit. Plasma E2 concentrations were assayed by an enzyme immunoassay 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 3. A1, 3. A2, 3. A3). 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. The rematuration rate of female kelts as consecutive spawners in 2018 was high at Prosser; females rematured at a 65.2% rate. Consecutive spawners from other programs on the Snake River and Upper Columbia River had lower rates of rematuration for 2018, with 41.7% of the Snake River fish rematuring and 43.3% 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 for the Snake River and Upper Columbia River fish (78.4% and 100%, respectively); however, it was lower than what is typically observed at Prosser (50%). The low rate of rematuration in hold over fish at Prosser is likely due to the low sample number (n = 4), which in turn reflects the relatively low survival rate for hold over fish at this location (11.1%).

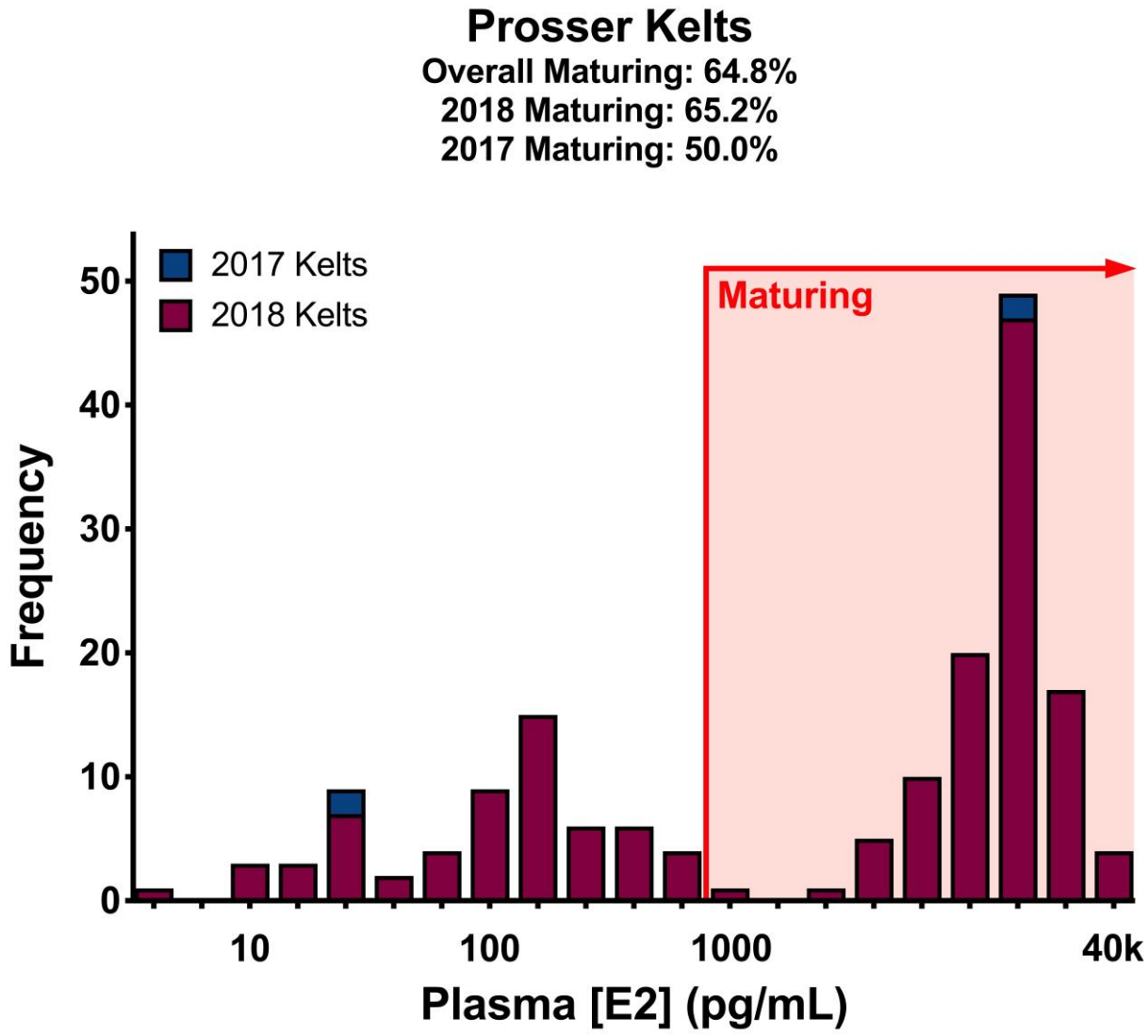

Figure 3.A 1: Plasma estradiol (E2) levels in wild Yakima River female kelts sampled in fall of 2018.

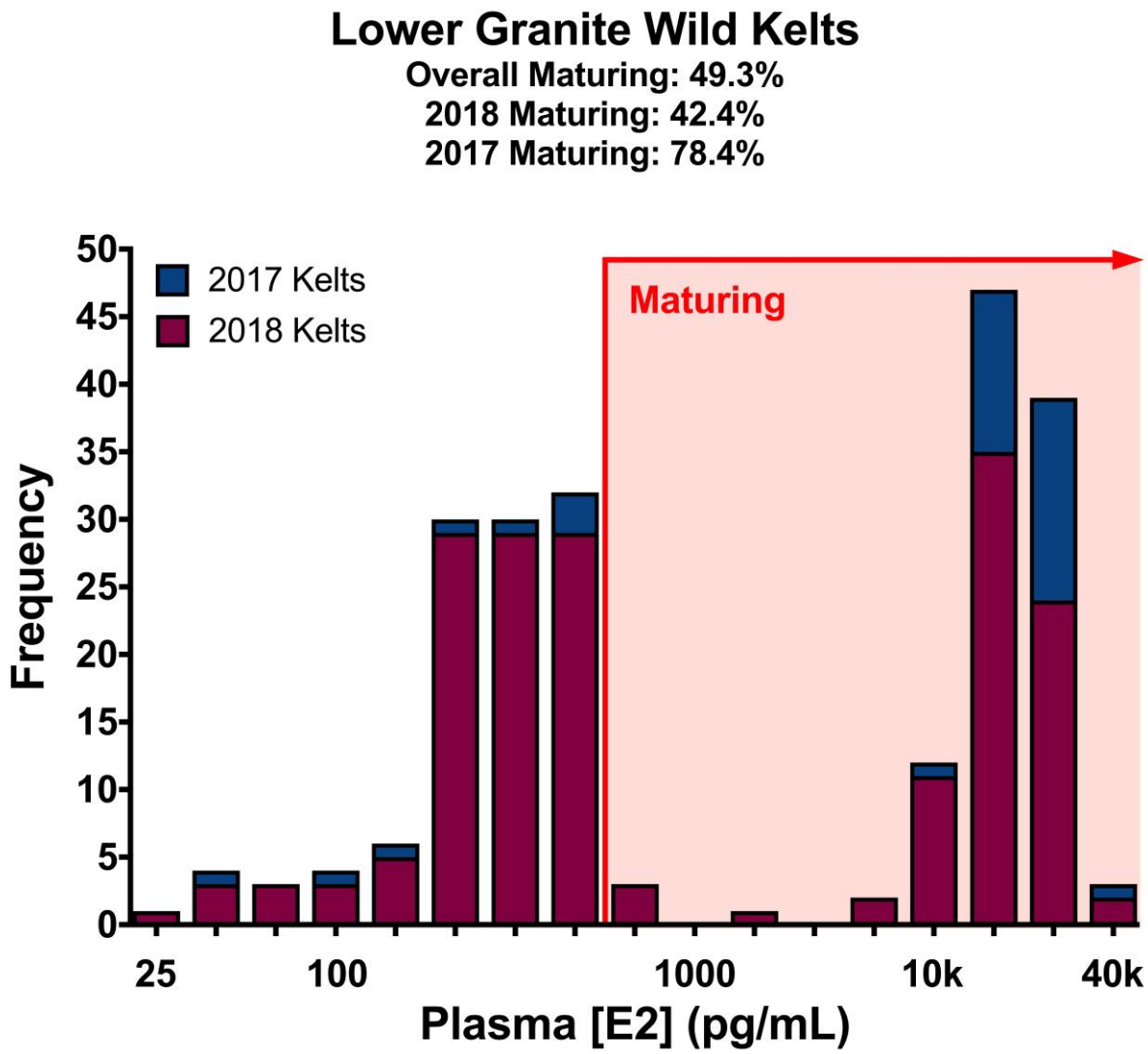

Figure 3.A 2: Plasma estradiol (E2) levels in wild female Snake River kelts sampled in fall of 2018.

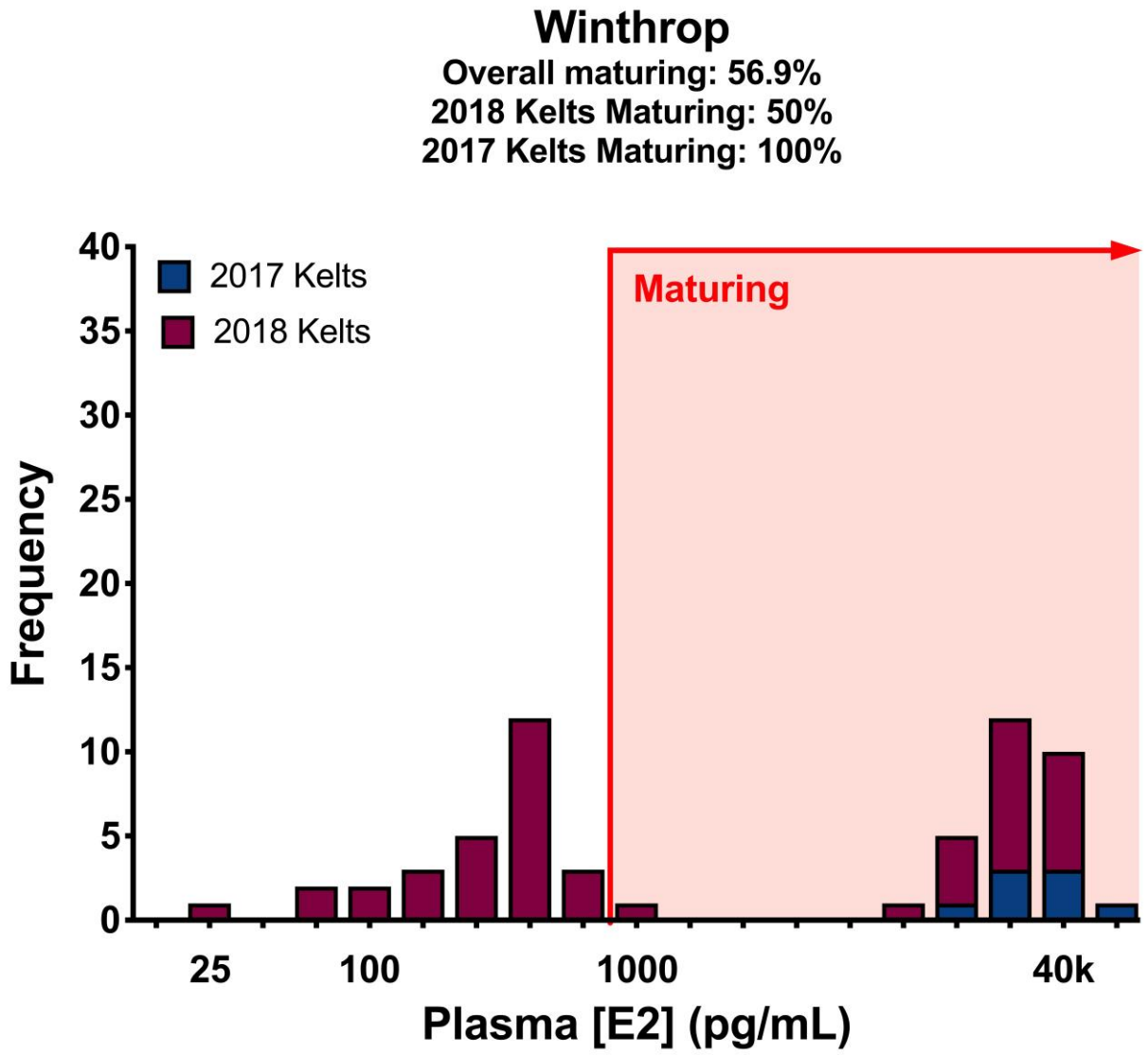

Figure 3.A 3: Plasma estradiol (E2) levels in female Upper Columbia River kelts sampled in fall of 2018.

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 before 10 weeks after spawning in rainbow trout (Bromage, et al. 1992; Caldwell et al. 2013b; Caldwell et al. 2014; Hatch, et al. 2013a; Jenkins et al. 2018b). Plasma estradiol levels in rematuring and non-rematuring kelts for 2018 at all sites were similar to previous years and were similar to those seen in other projects.

Female consecutive maturation rates were variable among the projects this season. It is possible that this relates to pre-capture environmental conditions. In previous years, the relatively low consecutive maturation rates found in Snake River kelts has been in line with what has been observed previously in Snake River steelhead, and steelhead from the Skeena and

Nass systems in British Columbia, which have a life history similar to Snake River B-run steelhead. These cohorts have been found to repeat spawn predominantly as skip spawners (Chudyk 1976; Keefer et al. 2008; Moore, et al. 1995). This has been hypothesized to be due to the longer migration and later spawn timing of these fish. And, while this year's consecutive maturation rate (41.7%) is relatively high (and similar to the rate observed in 2015), consecutive rematuration is still observed in less than half the population, implying that pre-capture environmental conditions may dictate the reproductive strategy employed. This could be the result of the warmer water temperatures the Columbia River Basin has been experiencing the past couple of summers, requiring a longer recovery period before the kelts are able to mature again (even with reconditioning). This is supported by the consistently high rates of maturation in the fish held for a second year of reconditioning.

Non-rematuring fish held for a second year rematured at very high rates (78.4% or higher) in 2018 at NPTH/DNFH and WNFH, and while 50% rematuration in surviving hold over fish at Prosser is lower it is at least on par with consecutive spawners. 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 result in greater relative reproductive success versus maidens or consecutive repeat spawners (Jenkins et al. 2018). The presence of skip spawners increases life history diversity, which would be expected to increase population stability in steelhead populations (Moore, et al. 2014; Schindler, et al. 2010). Moreover, whether and how much culture conditions can influence the proportion of consecutive and skip spawning kelts in captive reconditioning is not well understood. These considerations suggest that Columbia Basin kelt reconditioning programs should find ways to accommodate the skip spawner life history.

Hold over survival in Snake River fish is relatively high (typically over 60%), which is in contrast to the low survival experienced by holdover fish held at Prosser. The difference could be due to population-specific life history differences but could also be due to over winter water quality. The Prosser facility is located in an area that experiences heavy runoff. In addition to the low survival observed over the past three years (19.9% in 2016, 11.9% in 2017, and 11.1% in 2018), maturation in hold over fish has also declined (from 75.8% in 2016, to 62.5% in 2017, and then 50% for this year). In order to address this issue, steps should be taken to understand and correct the causes of mortality of hold over fish at Prosser, including an assessment of water quality during the winter and additional disease testing and treatment.

Section 3.B: Progress report: Reproductive development and migration success of maiden steelhead and reconditioned steelhead kelts in Yakima River steelhead subpopulations

We have been monitoring reproductive development in reconditioned steelhead kelts using plasma hormone levels. However, how these levels compare to those in maiden natural spawners is not known. To our knowledge, no information is available on plasma hormone levels during reproductive development and spawning migration in wild naturally spawning steelhead. During the fall of 2012, we began a collaboration with a VSP study on Yakima River steelhead (Frederiksen, et al. 2015; Frederiksen, et al. 2012; Hatch, et al. 2016a), which enabled us to obtain blood samples and biological data from upstream migrating maiden female steelhead at Prosser dam. In addition, we have relatively complete plasma hormone data on kelts released from Prosser Hatchery, immediately downstream from Prosser Dam, from 2012 through present. We would like to be able to analyze whether and how release hormone levels relate to the performance of the fish after release, such as migration success, homing fidelity, and spawn timing. In order to accomplish these objectives, however, it is necessary to know which of the Yakima River subpopulations each individual fish assigns to. In 2018, we completed a genetic STRUCTURE analysis of both the maiden and kelt samples which will allow us to assign the majority of samples to one of the Yakima River subpopulations. Integration of these results with physiological and PIT tag detection data is ongoing, and a full analysis will be presented in a future report.

Section 3.C: Elevated plasma triglycerides and growth are early indicators of rematuration in female steelhead trout (*Oncorhynchus mykiss*)

Note: This section is currently in review at a peer-reviewed journal. Please refer to the journal article for the definitive version.

Introduction

Skipped spawning is common in seasonally breeding iteroparous fishes (Rideout and Tomkiewicz 2011). After the first (*i.e.*, maiden) spawning event individuals may spawn again after a one-year interval (consecutive spawners) or skip one or more years (skip spawners). Many fish populations of conservation concern exhibit consecutive and skip spawning, which significantly impacts management, particularly of exploited stocks (Rideout et al. 2005a). Rematuration schedules are phenotypically plastic and respond to environmental conditions (Chaput and Benoit 2012; Rideout et al. 2005a; Thorpe, et al. 1998) suggesting that rematuration decisions and subsequent life history diversity will be sensitive to climate change. Energetic status is thought to be the main determinant of the decision to initiate rematuration of the gonad as a consecutive spawner or defer reproduction leading to skip spawning (Rideout

et al. 2005a; Thorpe 1994). Many of the fish species that exhibit skip spawning are capital breeders that fund reproduction from energy stores acquired prior to the majority of reproductive investment (McBride, et al. 2015). This has resulted in the idea of a threshold level of energy reserves required to successfully complete gonadal development, spawning, and associated activities such as migration. In this reaction norm framework an individual's condition or level of energy reserves interacts with a genetically determined threshold to generate a decision to either initiate maturation or rematuration, or to remain reproductively inactive for the given reproductive cycle (Hutchings 2011b). However, the proximate physiological mechanisms involved in the decision to initiate or defer rematuration are not fully understood.

The critical period hypothesis of maturation decisions was developed for Atlantic salmon (*Salmo salar*) and proposes that the initial maturation decision takes place during a seasonally defined critical period approximately one year before spawning, and is permissively gated by stores of metabolic fuels (Thorpe 2007; Thorpe et al. 1998). The aspect of metabolic fuel storage that gates entry into a reproductive cycle is not known, but is functionally defined as either the absolute level or the rate of change of body size (growth) and/or lipid reserves (Taranger, et al. 2010; Thorpe 2007). Triglycerides are the primary form in which lipids are stored for energy in fishes (Sheridan 1994) and are a reasonable representation of lipid energy reserves. Although considerable support for the critical period hypothesis exists, to our knowledge, the timing of the maturation decision window and the relative importance of lipid reserves and growth in the initiation of maturation have not been precisely delineated in any species. In addition, maturation decisions in fishes have been most studied in the context of puberty. Although it is reasonable to assume that similar mechanisms operate in rematuration and in puberty, the impact of energy depletion from the previous spawning event on rematuration decisions and reproductive development has not been examined.

The anadromous rainbow trout (*Oncorhynchus mykiss*, steelhead trout) provides a model species for studying the reproductive decisions underlying consecutive and skip spawning. Steelhead trout spawn in the spring in cold freshwater streams, the resulting juveniles migrate to the ocean to feed and grow, and mature adults then return to their natal stream to spawn (Burgner, et al. 1992; Quinn 2005). Steelhead trout display a diverse and phenotypically plastic suite of life histories, including freshwater resident and anadromous forms, variation in size and age at seaward migration, variation in size and age at initial maturation, and consecutive and skip repeat spawning (Courter, et al. 2013; Moore et al. 2014; Nielsen, et al. 2011). In coastal "winter-run" populations adults return to freshwater with fully developed gonads shortly before spawning. However, in "summer-run" populations, such as those in the interior Columbia River Basin (CRB), adults enter freshwater with immature gonads in late summer and complete gonadal development while fasting and migrating to spawning areas to spawn the following spring (Quinn, et al. 2016). Summer-run steelhead trout are considered capital breeders, with the energetic demands of migration, gonadal development, and spawning resulting in an extreme energy deficit and high post-spawning mortality (Penney and Moffitt 2014b). In interior CRB summer-run populations the incidence of skip spawning increased with migration distance (Keefer et al. 2008) consistent with regulation by energetic status.

Reconditioning of post-spawned steelhead trout (kelts) has been developed as a conservation measure for declining populations of interior summer-run CRB steelhead trout listed as threatened under the U.S. Endangered Species Act (ESA) (Hatch, et al. 2013b; Trammell, et al. 2016). Kelt reconditioning projects are being implemented at several locations in the interior CRB and aim to increase the productivity of steelhead populations by allowing wild-origin fish the opportunity to spawn again. Kelts are captured after spawning, held and fed in freshwater, and then released to migrate upstream and spawn again. Kelts collected for reconditioning are predominantly female, and some projects only recondition female fish (Hatch 2013, Hatch 2016). The consecutive and skip spawning life histories are observed in female reconditioned kelts and vary significantly in proportion by location and year (Hatch, et al. 2016b; Pierce, et al. 2017). Consecutive spawners are released after reconditioning over the summer, whereas skip spawners must be held for an additional year before release, adding complexity to the management of kelt reconditioning projects. Thus, understanding the timing and basis of rematuration decisions in steelhead trout will directly assist in the management of CRB steelhead kelt reconditioning projects. More generally, advances in understanding of the physiology of rematuration decisions in steelhead trout would be expected to lead to improvements in the management of other fish populations displaying consecutive and skip spawning.

To facilitate studies on steelhead trout kelt reconditioning, an experimental system was developed using non-ESA-listed hatchery-origin female summer-run steelhead trout returning to Dworshak National Fish Hatchery (DNFH) on the Clearwater River in Idaho, U.S.A. The spawning migration for this population (nearly 800 km) approaches the maximum for steelhead trout. DNFH steelhead trout fast from freshwater entry in August-September through spawning in February-April resulting in extreme energy depletion. Fish returning to DNFH are captured and held in tanks enabling repeated sampling to observe their recovery and reproductive development. Additional advantages of this system are that these fish are of uniform genetic stock, origin, and age; have uniform and known spawn timing, and demonstrate both consecutive and skip spawning life histories (Jenkins et al. 2018b). The objectives of this study were (i) to determine when the rematuration decision occurs, (ii) to determine how growth rates and lipid reserves relate to the rematuration decision, and (iii) to assess the impact of recovery from maiden spawning on rematuration.

Methods

Fish

Female steelhead trout *Oncorhynchus mykiss* were captured after returning on their maiden spawning migration and ascending the adult ladder trap at Dworshak National Fish Hatchery (DNFH), on the Clearwater River in Ahsahka, ID, USA. Fish were collected for up to several weeks prior to spawning and maintained in holding ponds supplied with North Fork Clearwater River water. Females were selected for spawning based on a minimum criterion of 70 cm fork length (FL). Fish in good and fair condition with no visible wounds were selected for this study (Hatch et al. 2016b; Hatch et al. 2013a). DNFH females are nearly universally spawned at age 4; age was confirmed for a subset of the study fish (L. Jenkins et al., unpublished data).

Spawning

In February-April 2015 and 2016, N=150 and 164 females, respectively, were selected for this study. Fish were anesthetized using AQUI-S 20E (AquaTactics Inc., Kirkland, WA; 75mL $1000L^{-1}$ water) and manually "air spawned". Air spawning consisted of inserting a 16-gauge pneumatic-hypodermic needle through the mid body-cavity wall just posterior to the pelvic fin, blowing 17.2-20.7 kpa oxygen into the body cavity, and collecting eggs from the urogenital opening (Leitritz and Lewis 1976). Fish were individually tagged using 12 mm passive integrated transponder (PIT) tags (Biomark Inc., Boise, ID) inserted near the pelvic girdle.

Sampling

At spawning and at 10-week intervals thereafter fish were sampled for fork length (FL, cm), wet mass (kg), muscle lipid level (ML, %), and blood. Wet mass at maiden spawning was taken after eggs were removed, and was corrected for any residual eggs remaining in the body cavity as previously described (Jenkins et al. 2018b), hereafter referred to as somatic mass. Subsequent measurements of body mass included any new ovarian growth. ML level was measured by microwave energy meter (Fish Fatmeter model 692, Distell Inc., West Lothian, UK) using the Trout-1 setting, as previously validated for rainbow trout (Caldwell et al. 2013). Blood (3mL) was taken from the caudal vein using a heparinized (ammonium heparin, 10 mg mL^{-1}, Sigma-Aldrich, St. Louis, MO) 20 gauge, 3.8cm needle and syringe. Blood was centrifuged at 8300G for 5 min. The plasma was removed, frozen on dry ice in the field, and then later stored at -80^{o}C. Sampling continued at 10-week intervals until spawning occurred again approximately 1 year later (50 weeks) for consecutive spawning 2015 fish, until approximately 1 year plus 30 weeks after spawning for 2015 fish that skipped spawning, or until 30 weeks after spawning for 2016 spawn year fish.

Fish 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 200 liters $minute^{-1}$ drawn from the North Fork Clearwater River, with a seasonally varying temperature profile (4.9 – 11.0 ^{o}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 *Artemia* cysts (American Brine Shrimp, Ogden, UT) for increased palatability. At spawning, fish were prophylactically treated for bacterial infection with oxytetracycline (Durvet, Blue Springs, Missouri; 20 mg kg^{-1} body mass) and for parasitic gill copepods (*Salmincola californiensis*) with emamectin (Sigma-Aldrich, St. Louis, Missouri; 200 μg kg^{-1} body mass), both via intraperitoneal injection. Oxytetracycline injections continued at 10-week intervals during sampling, with emamectin injections applied only when copepods were visible on the gills. Tanks were treated with formalin (Syndel USA, Portland, OR; flow through treatment, 1:6000 for 1 hour daily) to control *Saprolegnia*.

Survival
Mortality occurred during reconditioning as expected for steelhead trout kelts (Hatch et al. 2013b). On average, 55% of the mortality occurred within 10 weeks of spawning (47% and 62% for 2015 and 2016, respectively). Survival to 30 weeks after spawning was 29% in 2015 (43/150 fish), 18% in 2016 (30/163), 70% for 2015 skip spawners from 30 weeks after maiden spawning to 1 year plus 30 weeks (21/30), and 84% for 2015 skip spawners from 1 year after spawning to 1 year plus 30 weeks (21/25). A necropsy was performed on all mortalities. In November 2016, approximately 35 weeks after maiden spawning of the 2016 spawn fish and 15 weeks prior to repeat spawning of the 2015 skip spawners and 2016 consecutive spawners, all fish died due to an equipment malfunction.

Assays
Plasma estradiol-17β (E2) levels were measured by ELISA (Biosense, Cayman Chemical, Ann Arbor, MI). Steroids were extracted from plasma using ether extraction, re-suspended in assay buffer, and assayed in triplicate. The intra- and inter-assay coefficient of variation was 8.0% and 7.1%, respectively. Plasma triglyceride (TG) concentration was measured using a VetTest (Idexx, Westport, ME), as validated for use in *Oncorhynchus* spp. (Meador, et al. 2006).

Morphometric Analysis

Fulton's condition factor (K) was calculated as:
$$\mathrm{K} = 100 * \text{body mass (g)} * (\text{fork length (cm)})^{-3}$$
Mass specific growth rate (MSGR) was calculated as:
$$\%\ \text{body mass gain} * \text{day}^{-1} = 100 * (\ln(\text{body mass final}) - \ln(\text{body mass initial}))\text{days}^{-1}$$
Length specific growth rate (LSGR) was calculated in the same manner as that for mass.

Statistical Analysis
Rematuration status was assigned in early autumn, 30 weeks after spawning, based on complete separation of fish into two E2 concentration groups (high levels = maturing, low levels = not maturing), and confirmed by spawning of survivors (Jenkins et al. 2018b) or at necropsy for pre-spawn mortalities by examining developing ovaries for large oocytes. Based on these results fish were categorized as either rematuring and non-rematuring in the year following maiden spawning and were compared in a time series at 10-week intervals starting at spawning. Additionally, fish rematuring in either the first or second year after maiden spawning were categorized as either consecutive rematuring or skip rematuring fish, respectively, and compared at the same relative time points during the year prior to repeat spawning.

E2 levels were $\log_{10}$-transformed and ML levels were arcsine square root-transformed prior to analysis. Two-way ANOVA was employed to test for time, maturation group, and interaction effects on E2 levels, MSGR, LSGR, TG levels, and K. Where significant effects were found, one-way ANOVA was used to assess the effects of time and maturation group, followed by Tukey's HSD or a T-test. A Kruskal-Wallis test was used for ML level data due to truncation of the data, followed by Dunn's multiple comparison test or the Mann-Whitney test. The Rout Outlier Test

was used to detect and remove outliers. Unless otherwise indicated, all statistical analysis was conducted with PRISM software version 7.0 (GraphPad Inc., La Jolla, CA). Results are reported as significant when $P<0.05$.

Ethics

Fish care and sampling were conducted in accordance with a protocol reviewed and approved by the University of Idaho Animal Care and Use Committee.

Results

Rematuration

The proportion of fish that survived to 30 weeks after maiden spawning and matured in consecutive maturation years was 30% (13/43) in 2015 and 40% (12/30) in 2016. Of 2015 fish surviving to 1 year plus 30 weeks after spawning, 86% (18/21) rematured as skip spawners in 2016. No evidence of arrested maturation (*i.e.*, premature decreases in plasma E2 level) was detected in any individual. However, one of the 30 non-rematuring 2015 fish showed a distinctly non-representative negative E2 trajectory starting in early summer (20 weeks post-spawn), followed by death 1 year after spawning, and was excluded from the data.

Time Course of Recovery from Spawning

Two-Way ANOVA found significant effects of maturation group, time, and group*time interactions, except for group*time interaction effects for LSGR (both years) and MSGR in 2016 (Table 3.C1).

TG level was greater in rematuring than non-rematuring fish at 10 weeks in both years, remaining that way except for week 20 in 2016 and week 50 in 2015 (Figure 3.C1). TG increased in rematuring fish from 10 to 30 weeks in both years. In non-rematuring fish, TG decreased from 0-10 weeks (2015: 1.8-fold; 2016: 1.8-fold). After the 10-week time point, TG in non-rematuring fish returned to maiden levels at 20 (2016) or 30 (2015) weeks and for all following time points.

ML level was greater in rematuring than non-rematuring fish at week 20 and 30 (week 30, $P=0.0585$) in 2015 and at weeks 10-30 in 2016 (Figure 3.C2). At week 50 in 2015 ML level was greater in non-rematuring than in rematuring fish. ML level increased from week 10 to week 20 in both groups and years.

K was greater in rematuring than non-rematuring fish at weeks 20-40 in 2015 and at weeks 10-30 in 2016 (Figure 3.C3). K increased progressively from week 10 to week 30 in rematuring fish in both years. K increased progressively from 20 to 50 weeks (2015) and 10 to 30 weeks (2016) in non-rematuring fish.

Table 3.C 1: Two-way ANOVA test statistics for each dependent variable and time during the year following maiden spawning for consecutive and skip spawning fish from 2015 and 2016.

Measure	Year	Source of Variation	F (DF_n, DF_d)	P-value
Plasma TG	2015	Time	F (5, 225) = 33.60	P<0.0001
		Maturation	F (1, 225) = 25.35	P<0.0001
		Interaction	F (5, 225) = 7.950	P<0.0001
	2016	Time	F (3, 108) = 11.74	P<0.0001
		Maturation	F (1, 108) = 4.119	P=0.0449
		Interaction	F (3, 108) = 9.089	P<0.0001
ML level	2015	Time	F (5, 223) = 73.25	P<0.0001
		Maturation	F (1, 223) = 6.214	P=0.0134
		Interaction	F (5, 223) = 19.13	P<0.0001
	2016	Time	F (3, 106) = 72.39	P<0.0001
		Maturation	F (1, 106) = 12.36	P=0.0006
		Interaction	F (3, 106) = 4.239	P=0.0072
K	2015	Time	F (5, 227) = 57.09	P<0.0001
		Maturation	F (1, 227) = 18.77	P<0.0001
		Interaction	F (5, 227) = 2.835	P=0.0167
	2016	Time	F (3, 109) = 52.12	P<0.0001
		Maturation	F (1, 109) = 25.52	P<0.0001
		Interaction	F (3, 109) = 6.739	P=0.0003
MSGR	2015	Time	F (4, 183) = 47.53	P<0.0001
		Maturation	F (1, 183) = 5.751	P=0.0175
		Interaction	F (4, 183) = 9.478	P<0.0001
	2016	Time	F (2, 80) = 31.98	P<0.0001
		Maturation	F (1, 80) = 12.92	P=0.0006
		Interaction	**F (2, 80) = 0.0370**	**P=0.9637**
LSGR	2015	Time	F (4, 185) = 49.26	P<0.0001
		Maturation	F (1, 185) = 20.62	P<0.0001
		Interaction	**F (4, 185) = 1.811**	**P=0.1285**
	2016	Time	F (2, 79) = 63.17	P<0.0001
		Maturation	F (1, 79) = 4.35	P=0.0402
		Interaction	**F (2, 79) = 1.369**	**P=0.2603**
Plasma E2	2015	Time	F (5, 225) = 52.18	P<0.0001
		Maturation	F (1, 225) = 434.8	P<0.0001
		Interaction	F (5, 225) = 53.7	P<0.0001
	2016	Time	F (3, 109) = 38.61	P<0.0001
		Maturation	F (1, 109) = 156.5	P<0.0001
		Interaction	F (3, 109) = 54.76	P<0.0001

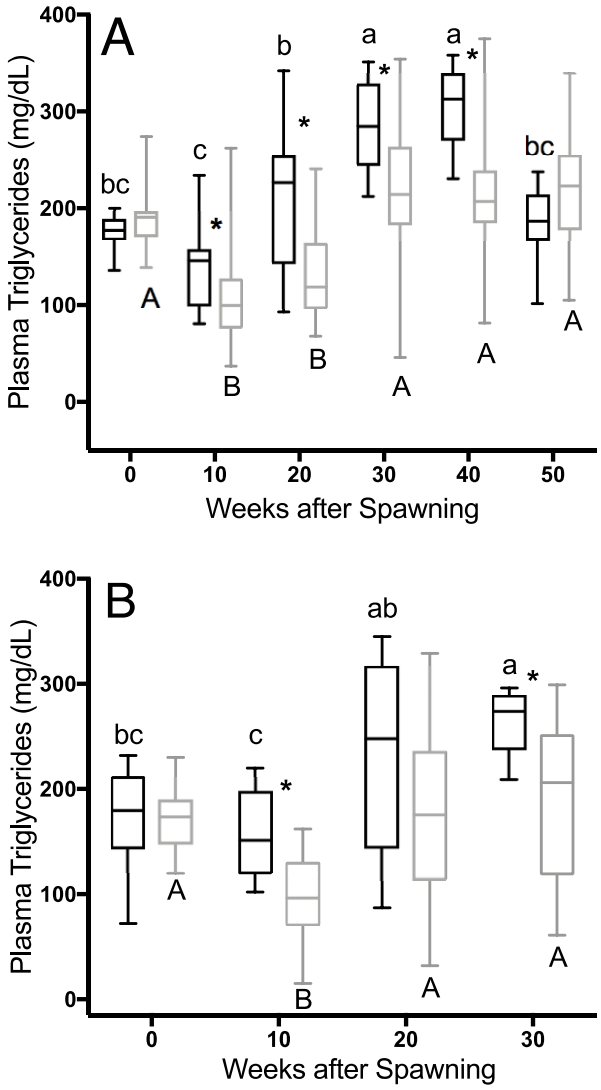

Figure 3.C 1: Plasma triglyceride concentrations in female steelhead trout from the Clearwater River, Idaho, sampled in 2015 (A) and 2016 (B) at 10-week intervals following spawning. Females were grouped as rematuring (black: 2015, N=13; 2016, N=12) or non-rematuring (grey: 2015, N=29; 2016, N=18). Box heights indicate interquartile range, horizontal lines within indicate the median, and whiskers show the data range. Time points within a rematuration group sharing the same letter do not differ significantly (One-Way ANOVA followed by Tukey's Multiple Comparison Test, P<0.05). Asterisks indicate significant differences between rematuration groups at each time point (T-test, P<0.05).

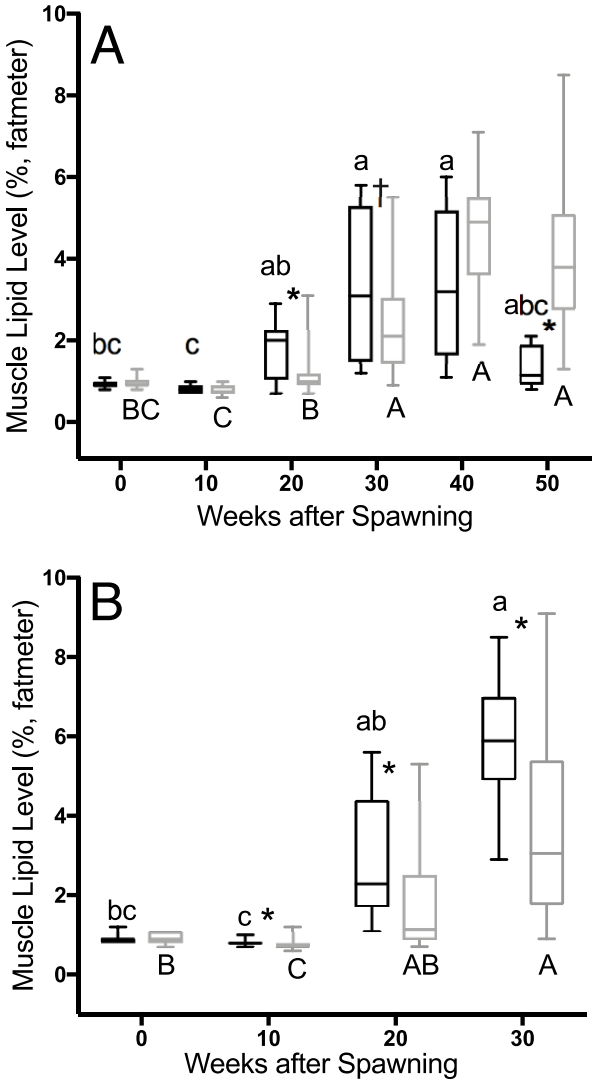

Figure 3.C 2: Muscle lipid levels in female steelhead trout from the Clearwater River, Idaho, sampled in 2015 (A) and 2016 (B) at 10-week intervals following spawning. Maturation groups, box and whisker plots, and significance indication are as in Figure 1. Due to truncation of the data, non-parametric statistical tests were used (time points: Kruskal-Wallis followed by Dunn's Multiple Comparison Test, maturation groups: Mann-Whitney Test, P<0.05). †Marginal non-significance at week 30 in 2015, Mann-Whitney Test: U = 199, P=0.0585.

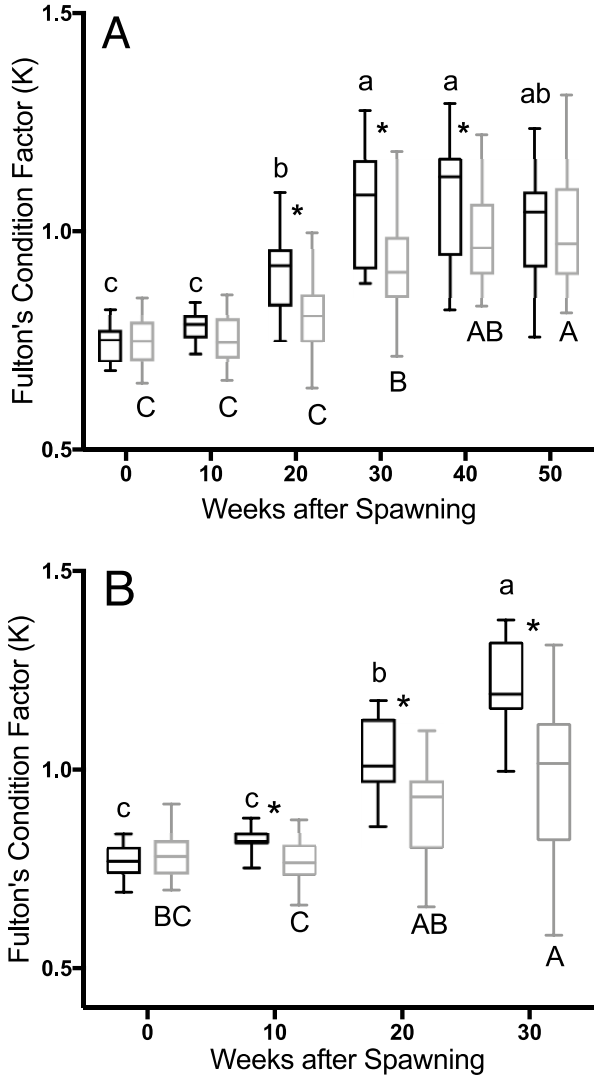

Figure 3.C 3: Fulton's condition factor (K) in female steelhead trout from the Clearwater River, Idaho, sampled in 2015 (A) and 2016 (B) at 10-week intervals following spawning. Maturation groups, box and whisker plots, significance indication, and statistical analyses are as in Figure 3.C1.

Mass specific growth rate (MSGR) was greater in rematuring than non-maturing fish during weeks 0-10 after maiden spawning in both years (Figure 3.C4), continuing for weeks 10-20 and weeks 20-30 in 2015. MSGR was positive for rematuring and negative for non-rematuring fish during weeks 0-10 in both years (Figure 3.C4). MSGR increased strongly from weeks 0-10 to weeks 10-20 in both rematuring and non-rematuring fish in both years and remained high through weeks 20-30. In 2015, MSGR declined progressively from weeks 20-30 through weeks 40-50 in rematuring fish, and from weeks 30-40 to weeks 40-50 in non-rematuring fish, returning to levels similar to weeks 0-10. MSGR was greater in non-rematuring fish than rematuring fish over weeks 40-50 in 2015.

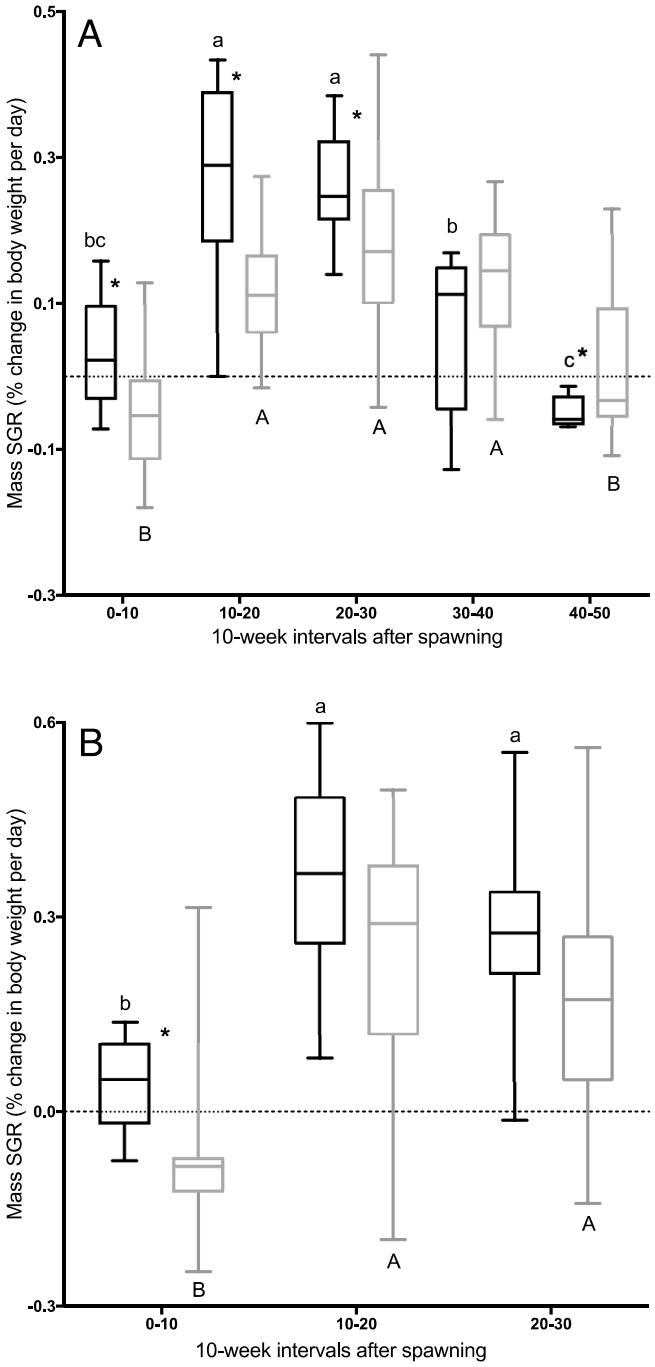

Figure 3.C 4: Mass specific growth rate (Mass SGR, % change in body weight per day) in female steelhead trout from the Clearwater River, Idaho, sampled in 2015 (A) and 2016 (B) calculated over 10-week intervals following spawning. Maturation groups, box and whisker plots, significance indication, and statistical analyses are as in Figure 1.

LSGR was negative during weeks 0-10 and increased during weeks 10-20 in both rematuring and non-rematuring fish in both years (Figure 3.C5). LSGR subsequently decreased from weeks

30-40 to 40-50 in non-rematuring fish in 2015. LSGR was greater in rematuring than non-rematuring fish at weeks 10-20, 20-30, and 40-50 in 2015 and weeks 20-30 in 2016.

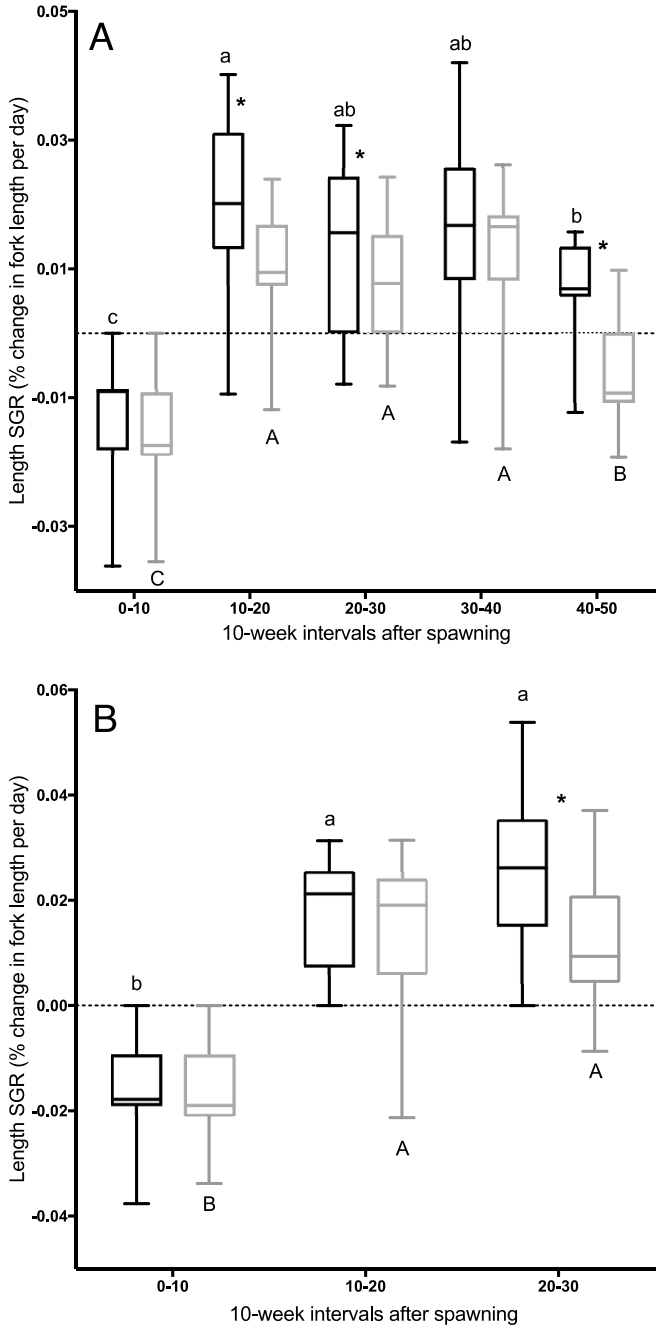

Figure 3.C 5: Length specific growth rate (Length SGR, % change in fork length per day) in female steelhead trout from the Clearwater River, Idaho, sampled in 2015 (A) and 2016 (B) at 10-week intervals following spawning. Maturation groups, box and whisker plots, significance indication, and statistical analyses are as in Figure 3.C1. In 2016, N=11 for rematuring fish.

E2 levels were greater in rematuring than non-rematuring fish starting at 20 weeks after maiden spawning, remaining that way for the study duration in both years (Figure 3.C6). E2 levels decreased (1.5-3-fold) from spawning to week 10 in both groups and years. In 2015, rematuring E2 levels increased progressively from week 10 to 40, and then decreased at week 50 to levels similar to week 0. The pattern was similar in 2016, except higher rematuring E2 levels were attained at week 20 and there was no subsequent increase at week 30. In non-rematuring fish E2 remained below spawning levels for the recovery year (both years), decreasing from weeks 10-20, but increasing from weeks 20-40 in 2015.

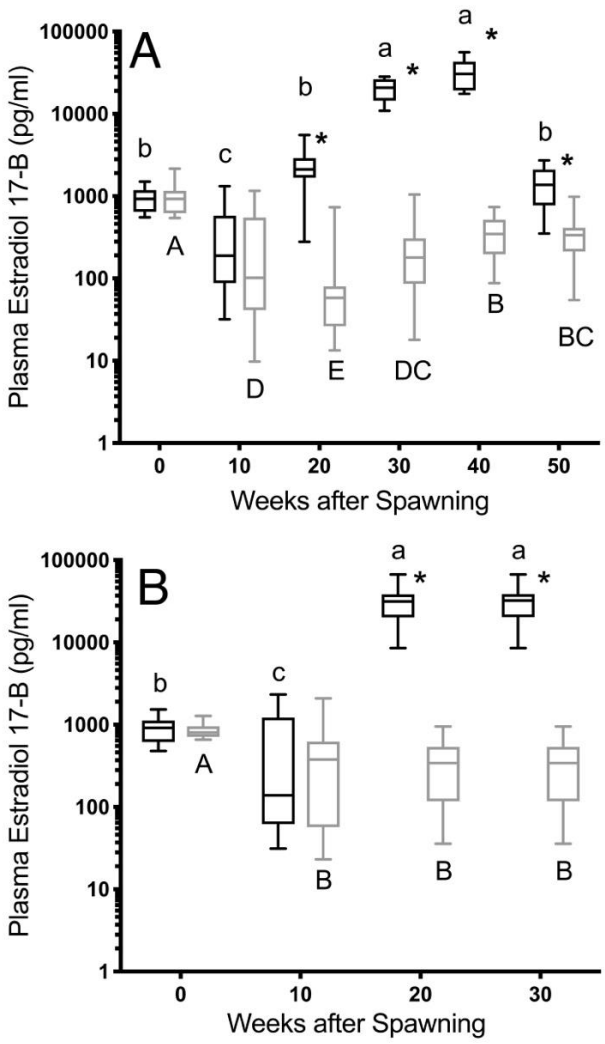

Figure 3.C 6: Plasma estradiol-17β concentrations in female steelhead trout from the Clearwater River, Idaho, sampled in 2015 (A) and 2016 (B) at 10-week intervals following maiden spawning. Females were grouped as rematuring (red: 2015, N=13; 2016, N=12) or non-rematuring (blue: 2015, N=29; 2016, N=18). Maturation groups, box and whisker plots, significance indication, and statistical analyses are as in Figure 3.C1.

Time Course of Reproductive Rematuration

Two-Way ANOVA found significant effects of maturation group, time, and group*time interactions (Table 3.C2).

Table 3.C 2: Two 2-way ANOVA test statistics for each dependent variable and time during the year of rematuration for consecutive and skip spawning fish from the 2015 maiden spawn year.

Measure	Source of Variation	F (DFn, DFd)	P-value
Plasma TG	Time	F (3, 107) = 26.02	P<0.0001
	Maturation	F (1, 107) = 38.48	P<0.0001
	Interaction	F (3, 107) = 8.622	P<0.0001
ML level†	Time	F (1, 223) = 6.214	P=0.0134
	Maturation	F (5, 223) = 73.25	P<0.0001
	Interaction	F (5, 223) = 19.13	P<0.0001
K	Time	F (3, 116) = 33.57	P<0.0001
	Maturation	F (1, 116) = 175.8	P<0.0001
	Interaction	F (3, 116) = 3.112	P=0.0290
MSGR	Time	F (2, 86) = 27.75	P<0.0001
	Maturation	F (1, 86) = 8.781	P=0.0039
	Interaction	F (2, 86) = 19.94	P<0.0001
LSGR	Time	F (2, 87) = 35.19	P<0.0001
	Maturation	F (1, 87) = 17.26	P<0.0001
	Interaction	F (2, 87) = 10.14	P=0.0001
Plasma E2	Time	F (3, 112) = 332.9	P<0.0001
	Maturation	F (1, 112) = 62.71	P<0.0001
	Interaction	F (3, 112) = 45.65	P<0.0001

TG levels were greater in skip than consecutive spawners at weeks 0 and 10 (Figure C3.7). TG levels increased progressively from week 10 to 30 in consecutive spawners (2.0-fold), whereas levels were constant in skip spawners from weeks 0 to 20, followed by a small (1.3-fold) but significant increase at week 30.

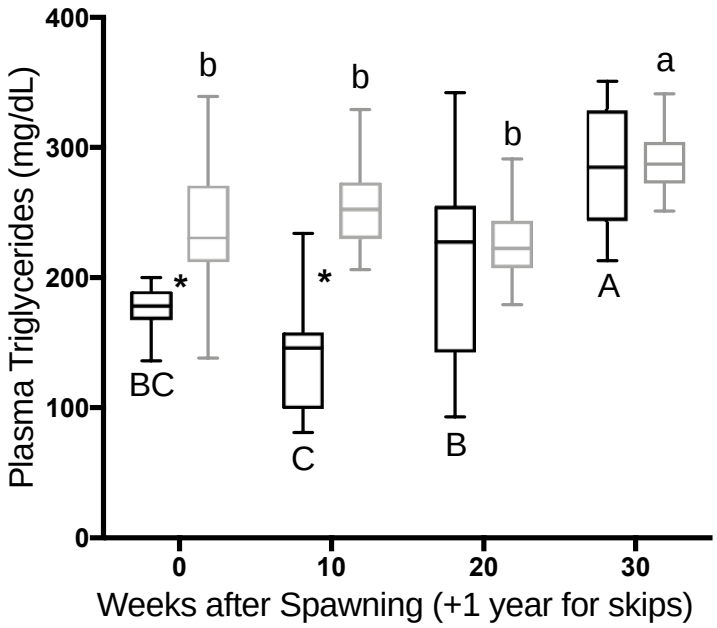

Figure 3.C 7: Plasma triglyceride concentrations during maturation for repeat spawning in female steelhead trout from the Clearwater River, Idaho. Fish from the 2015 cohort year were sampled in 2015 (black, N=13, rematured in year 1 as consecutive spawners) and in 2016 (grey, N=18, rematured in year 2 as skip spawners) at 10-week intervals. Box heights indicate interquartile range, horizontal lines within indicate the median, and whiskers show the data range. Time points within a group sharing the same letter do not differ significantly (one-Way ANOVA followed by Tukey's Multiple Comparison Test, P<0.05). Asterisks indicate significant differences between consecutive and skip spawners at each time point (T-test, P<0.05).

ML levels were greater in skip than consecutive spawners at all time points (Figure C3.8). ML levels increased at week 20 in skip and week 30 in consecutive spawners versus week 0 in the year prior to repeat spawning.

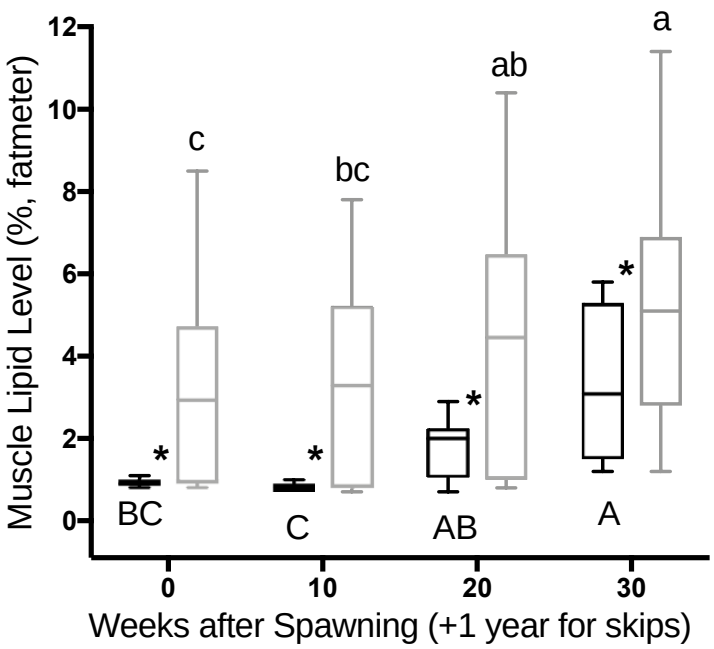

Figure 3.C 8: Muscle lipid levels during maturation for repeat spawning in female steelhead trout from the Clearwater River, Idaho. Spawning groups, box and whisker plots, and significance indication are as in Figure 7. Due to truncation of the data, non-parametric statistical tests were used (time points: Kruskal-Wallis followed by Dunn's Multiple Comparison Test, maturation groups: Mann-Whitney Test, P<0.05).

K was greater in skip than consecutive spawners at all time points in the year prior to repeat spawning (Figure 3.C9). K increased progressively in consecutive spawners from week 10 to 30 and in skip spawners from week 10 to 20.

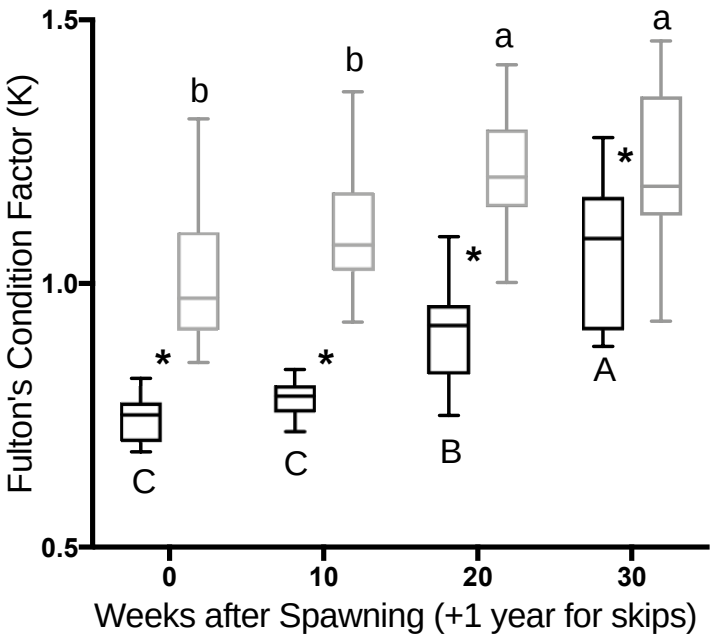

Figure 3.C 9: Fulton's condition factor (K) during maturation for repeat spawning in female steelhead trout from the Clearwater River, Idaho. Spawning groups, box and whisker plots, significance indication, and statistical analyses are as in Figure 3.C7.

MSGR was greater in skip spawners during weeks 0-10, but greater in consecutive spawners during weeks 20-30 (Figure C3.10). MSGR increased from weeks 0-10 to 10-20 in both consecutive and skip spawners. During weeks 20-30 MSGR in skip spawners decreased to levels similar to weeks 0-10.

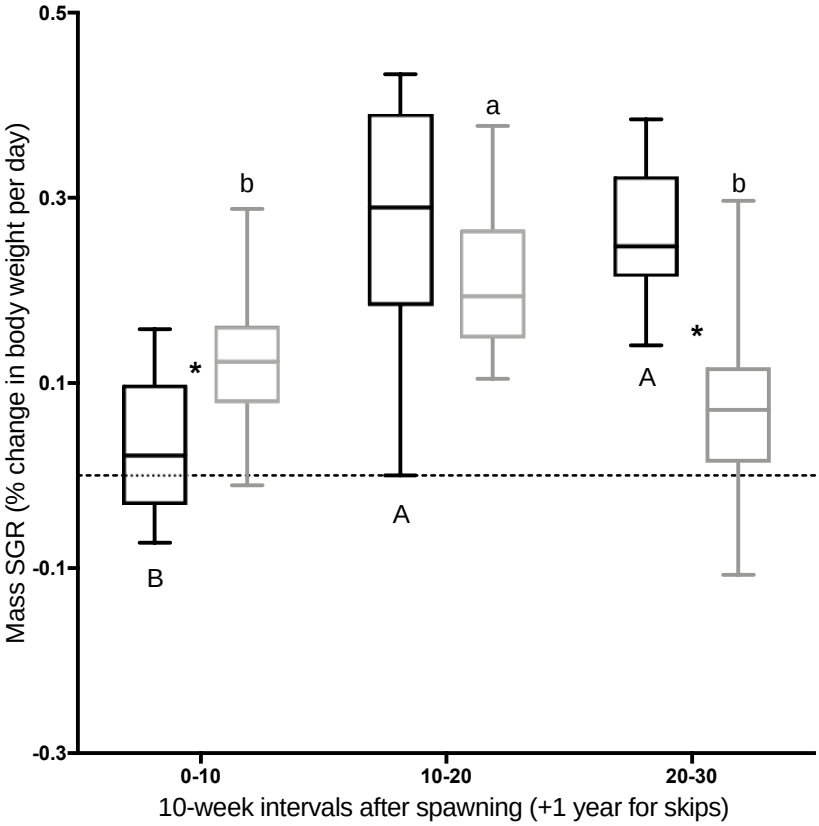

Figure 3.C 10: Mass specific growth rate (Mass SGR, % change in body weight per day) during maturation for repeat spawning in female steelhead trout from the Clearwater River, Idaho. Spawning groups, box and whisker plots, significance indication, and statistical analyses are as in Figure 3.C7.

LSGR was greater in skip than consecutive spawners (which had negative LSGR) during weeks 0-10 (Figure 3.C11). LSGR increased from weeks 0-10 to 10-20 in both consecutive and skip spawners.

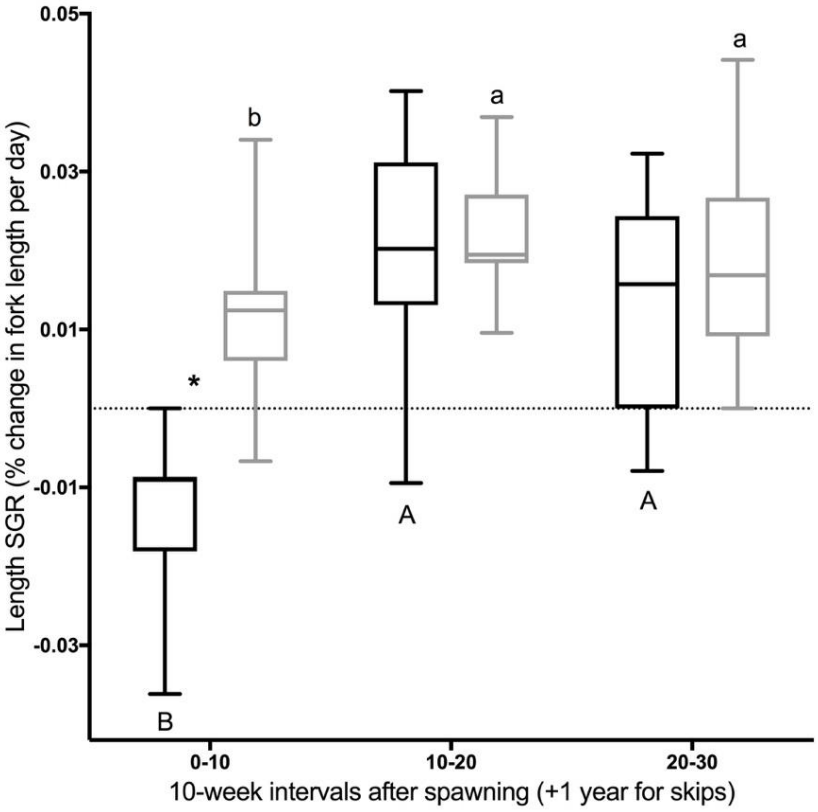

Figure 3.C 11: Length specific growth rate (Length SGR, % change in fork length per day) during maturation for repeat spawning in female steelhead trout from the Clearwater River, Idaho. Spawning groups, box and whisker plots, significance indication, and statistical analyses are as in Figure 7.

E2 levels were greater in consecutive than skip spawners at week 0, but greater in skip spawners from 10 to 30 weeks (Figure 3.C12). From 0-10 weeks E2 levels increased in skip and decreased in consecutive spawners. Thereafter, E2 levels increased for both groups.

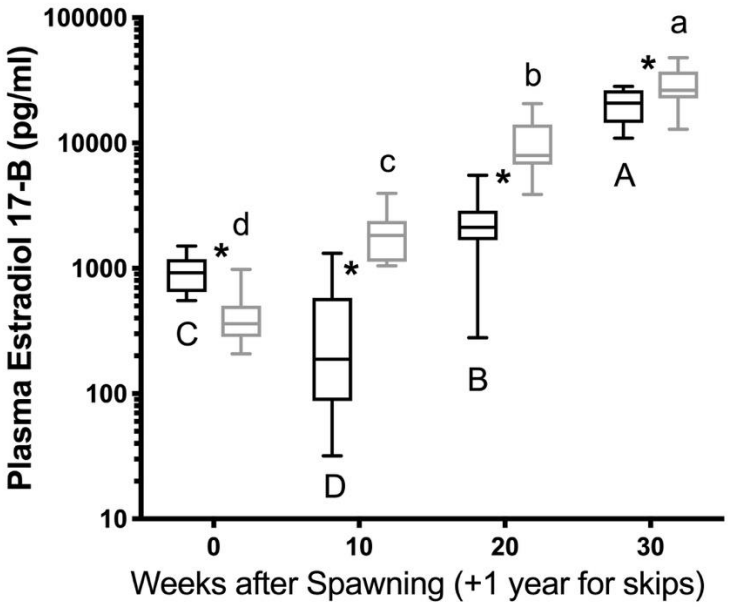

Figure 3.C 12: Plasma estradiol-17β concentrations during maturation for repeat spawning in female steelhead trout from the Clearwater River, Idaho. Spawning groups, box and whisker plots, significance indication, and statistical analyses are as in Figure 3.C7.

Discussion

The sequence of events over the year after maiden spawning tracked in this study (Figures 3.C1-C6) illustrates the timing of reproductive decisions and differences in energy acquisition and allocation between consecutive and skip spawning female steelhead trout. TG levels and growth in mass increased significantly over the first 10 weeks after spawning in consecutive versus skip spawners. This implies greater feeding in consecutive spawners over this time period and that the decision to enter the next reproductive cycle occurred before 10 weeks post-spawning. Consecutive spawners accumulated greater energy reserves and grew faster than skip spawners over the summer growing season in the year following maiden spawning, suggesting that maturation stimulated feeding. The sequence of events over the year prior to repeat spawning (Figures 3.C7-C12) illustrates the effect of recovery from maiden spawning on rematuration, energy reserves, and growth. The increase in E2 occurred earlier during the maturation year in skip spawners, suggesting that in consecutive spawners reproductive development was delayed by the energetic or physiological demands of maiden spawning. Skip spawners had substantially greater energy reserves (*i.e.* ML and TG) and E2 levels during oogenesis, which likely allowed for greater reproductive investment in skip versus consecutive spawners, as was found in our companion study (Jenkins et al. 2018b). This study provides the first mechanistic look at the timing and physiological factors involved in rematuration in repeat spawning female steelhead trout. These results will directly inform the management of kelt reconditioning conservation programs and advance knowledge about the underlying physiology of consecutive and skip spawning, an important issue in the management of many fish populations.

Energy Reserves

Energy reserves were assessed using three metrics, focusing on lipid reserves as these have been proposed as particularly important in salmonid maturation (Thorpe et al. 1998): TG representing short-term lipid energy availability, ML representing long term lipid energy stores, and Fulton's K, a measure of body shape used as a proxy for whole body lipid and other energy stores in fishes (Hanson, et al. 2010; Sutton, et al. 2000). Energy reserves generally increased more rapidly during recovery from spawning in consecutive spawners but attained higher levels during the extended reconditioning period in skip spawners.

Year after maiden spawning.

After spawning, TG levels were greater in rematuring fish from 10-40 weeks, with minor variation in significance between years, and then decreased immediately before spawning. The divergence in circulating TG levels at 10 weeks is most likely due to greater food intake, assimilation, and more rapid somatic recovery in consecutive spawners. However, we cannot exclude the possibility that lipid metabolism differed between maturing and non-maturing female steelhead trout during this period. Our interpretation that the increase in TG in rematuring fish was due to greater food intake is supported by the mass SGR results discussed below. If our interpretation is correct then physiological differences manifesting in the form of increased feeding motivation were found between rematuring and non-rematuring fish by 10 weeks after spawning, implying that the rematuration decision is linked to the difference in feeding motivation. This suggests two possible mechanisms: 1) the decision occurs late during the first ten weeks or afterward, with increased feeding early on after spawning causing improved energetic status that leads to the initiation of rematuration or 2) the decision occurs prior to or early during the first ten weeks, with the initiation of rematuration stimulating increased feeding and nutrient assimilation. The second possibility is supported by studies in Atlantic salmon and rainbow trout showing increased feed intake, growth, and plasma levels of insulin-like growth factor-1 in maturing fish during early stages of maturation approximately one year before spawning (Kadri, et al. 1996; Stead, et al. 1999; Wilkinson, et al. 2010). In addition, the presence of small ovarian follicles in rainbow trout and steelhead trout kelt ovaries immediately after ovulation (De Mones, et al. 1989; Penney and Moffitt 2014a) suggest that secondary oocyte growth begins prior to ovulation of the mature oocyte cohort. Elevations in TG level in consecutive spawners in this study at subsequent time points, as well as the decrease immediately before spawning, are consistent with both greater feeding and mobilization of lipid reserves during vitellogenesis. The major fates of circulating TGs would be expected to be storage in muscle, mesenteric, and liver lipid depots (Sheridan 1994) and incorporation into the developing ovary during exogenous vitellogenesis (Norberg and Haux 1985). This is supported by studies that have observed increases in plasma TG during exogenous vitellogenesis in reconditioned repeat spawning Atlantic salmon (Johnston, et al. 1987) and prior to peak vitellogenesis in rainbow trout (Bon, et al. 1997). In female brown trout (*Salmo trutta*), plasma TG levels decreased approximately 50% after spawning (Gauthey, et al. 2015), similar to the post-spawning decrease found in non-rematuring fish in the present study. In non-rematuring fish, TG levels increased to 200-300 mg dL^{-1} by week 30 and remained in this range through the following winter and summer, suggesting that plasma TGs are maintained in this range by homeostatic processes in actively feeding steelhead trout kelts.

ML levels and K increased more rapidly in consecutive than in skip spawning fish diverging at 20 weeks following the increase in TG levels. The greater ML levels and K in rematuring fish in the year following maiden spawning is consistent with reconditioned wild steelhead trout approximately 6 months after spawning (Pierce et al. 2017) and fully fed versus feed restricted rainbow trout over the first 20 weeks after spawning (Caldwell et al. 2013b). Similarly, K was greater in rematuring reconditioned repeat spawning Atlantic salmon from 18-34 weeks after spawning (Johnston et al. 1987). As spawning approached for consecutive spawning fish, ML levels tended to decrease (though not significantly) and ultimately became significantly lower than ML levels in skip spawning fish at week 50. This likely reflects mobilization of muscle lipids for incorporation into the ovary and reduced appetite.

Unlike plasma TG and ML levels, K did not decrease significantly in consecutive spawners over the period immediately before spawning. This was likely due to the presence of fully developed ovaries in the body cavity of the consecutive spawners. The much higher K levels at repeat versus maiden spawning in consecutive spawners is both because the mass of the eggs was not included in somatic mass at maiden spawning and because feeding and somatic growth continued through the fall prior to spawning in consecutive spawners, unlike in maidens. Similar results were observed in reconditioned Atlantic salmon kelts that were rematuring in consecutive years; these fish experienced a minor decline in K following spawning but remained well above maiden spawning levels (Johnston et al. 1987).

At maiden spawning, no differences in lipid reserve metrics were detected between subsequently rematuring and non-rematuring fish, providing no evidence for a determinative role of lipid reserves at spawning in the rematuration decision. This does not necessarily imply that the critical period hypothesis of salmonid maturation is incorrect, as the critical decision period may occur before spawning. It is also possible that the lipid reserve metrics employed in this study did not capture the relevant physiological signals. Signaling factors associated with energy reserves, rather than energy reserves directly, presumably directly interact with neuroendocrine mechanisms underlying the maturation decision (Wootton and Smith 2015). Future study measuring signaling factors associated with energy reserves and growth is required to elucidate mechanisms underlying maturation decisions in salmonids.

Year before repeat spawning.

Over the year prior to repeat spawning, TG levels were suppressed in consecutive versus skip spawners at weeks 0 and 10, which can be attributed to the costs of fasting, migration, spawning and recovery in consecutive spawners. A comparable effect was seen in juvenile rainbow trout, in which plasma TG levels decreased during fasting and this decrease was exacerbated by swimming (Simpkins, et al. 2003).

Over the summer before repeat spawning, ML levels continued to increase in skip spawners and remained higher than in consecutive spawners, indicating greater energy reserves in the skip spawners due to the much longer time for recovery from maiden spawning. K increased in both consecutive and skip spawners. Although the increase was steeper in consecutive

spawners, this was not sufficient to surpass the much higher K levels in skip spawners. As K is a measure of body shape, this likely reflects increased size and greater energy stores in a variety of tissues and organs in the skip spawners, including muscle tissue, visceral lipids, and the developing ovaries.

Growth

Year after maiden spawning.

MSGR diverged between rematuring and non-rematuring fish during the first 10 weeks after maiden spawning and remained elevated in rematuring versus non-rematuring fish through the summer growing season, although these differences were not always significant. Growth in length was generally similar to that in mass over the summer growing period, aside from the negative length growth over the first 10 weeks. Rematuring fish gained mass whereas non-rematuring fish lost mass over the first 10 weeks after spawning. As discussed above, this is almost certainly due to greater food intake and assimilation in rematuring versus non-rematuring fish. These results are consistent with the greater spring to fall growth found in rematuring versus non-rematuring reconditioned wild Yakima River female steelhead trout kelts (Pierce *et al.*, 2017), and with post-spawning mass gain in rematuring rainbow trout (Caldwell *et al.*, 2013).

Growth in both mass and length decreased compared to earlier time periods as spawn timing approached for both rematuring and non-rematuring fish. This is likely at least in part due to seasonal growth patterns dictated by water temperature and photoperiod (Burgner *et al.*, 1992). Decreases in growth as spawn timing approached were also reported in both maturing and non-maturing Atlantic salmon. And, similar to what was observed in this study, decreases were more dramatic in maturing fish (Kadri *et al.*, 1996). Additionally, Stead et al. (1999) found a correlation between decreased growth, increased levels of plasma sex steroids, and decreased food consumption during later stages of maturation in Atlantic salmon (Stead *et al.*, 1999). Negative mass growth (e.g., weight loss) in rematuring fish during the 10 weeks before spawning may reflect both reduced food consumption and the energetic cost of ovarian growth.

Length decrease over the immediate 10 weeks after maiden spawning was observed for both rematuring and non-rematuring fish. This length decrease (approximately 1cm) may be due to recession of the kype, a secondary sexual characteristic consisting of elongation of the lower jaw. Although kype development is more pronounced in male salmonids, it also occurs over the period before spawning in females (Vandenberghe *et al.*, 1989). Consistent with this possibility, length increase in rematuring fish exceeded that of non-rematuring fish over the 10 weeks preceding spawn timing.

Year before repeat spawning.

MSGR was greater in skip spawners than in consecutive spawners over weeks 0-10 during the year before repeat spawning. This difference can be attributed to the impact of prolonged fasting, migration, and maiden spawning on consecutive spawners. The gut is atrophied in post-spawning summer run steelhead trout and degenerative changes are found in the liver (Penney

et al., 2014a). The gut-somatic index decreases linearly over time in fasted juvenile rainbow trout with a loss of ~40% of the relative mass of the gut over 147 days of fasting (Simpkins *et al.*, 2003, Zaldua *et al.*, 2014). In Atlantic salmon fasted for 50 days, restoration of the gut upon refeeding required at least one week, during which feed intake was reduced (Krogdahl *et al.*, 2005). The duration of fasting and energetic demands of migration and ovarian development were substantially greater in the steelhead trout used in the present study than in the Atlantic salmon refeeding study, as indicated by a proportional lipid depletion of 93-98% observed from upstream to post-spawn migration (Penney *et al.*, 2014b) and less than 1% wet muscle lipid mass at spawning (this study). Thus, restoration of digestive function, feeding motivation, and feed intake would be expected to take at least several weeks in post-spawning steelhead trout kelts. LSGR was also greater in skip spawners than in consecutive spawners over weeks 0-10 during the year before repeat spawning, consistent with post-spawning kype reduction in consecutive spawners discussed above, as well as with the impact of fasting, migration and spawning on growth on consecutive spawners.

Estradiol-17β

Year after maiden spawning.

E2 decreased following spawning regardless of rematuration trajectory, increased to peak at 40 weeks post-spawning in rematuring fish before decreasing at repeat spawning, and diverged between rematuration trajectories at 20 weeks after maiden spawning. Post-ovulatory decreases in E2 over the month after spawning have been described in rainbow trout and Atlantic salmon (Andersson *et al.*, 2013, Caldwell *et al.*, 2014, De Mones *et al.*, 1989). This post-ovulatory decrease may be physiologically significant in that gonadal steroids and other gonadal factors suppress plasma follicle-stimulating hormone levels in post-ovulatory rainbow trout (Breton *et al.*, 1998, Chyb *et al.*, 1999). Thus, it is possible that clearance of these factors may be necessary before FSH stimulation of ovarian development can occur. The decrease in E2 late in oogenesis is consistent with previous studies in salmonids (Andersson *et al.*, 2013, Fostier *et al.*, 1978, Nagler *et al.*, 2012, Whitehead *et al.*, 1983) and likely reflects a steroidogenic shift from E2 to the maturation inducing steroid 17*α*, 20*β* dihydroxy progesterone induced by luteinizing hormone (Bobe *et al.*, 2006, Nagahama, 1994). The divergence of E2 levels 20 weeks after maiden spawning indicates that the rematuration decision was made prior to this time point. At approximately 5 months after spawning, this was somewhat slower than that observed in reconditioned wild-origin Yakima River steelhead trout (approximately 3 months) (Pierce *et al.*, 2017) and slower than in feed-restricted versus fully fed post-spawning rainbow trout (10 weeks) (Caldwell *et al.*, 2014). The differences in divergence timing may be due to fish origin and variation in metabolic rate. DNFH hatchery-origin steelhead trout are larger than Yakima River steelhead trout and much larger than rainbow trout and were held in colder water than in either of the previous studies. Both the size and temperature differences would be expected to result in a lower metabolic rate in the DNFH fish. The time between natural spawning and collection for reconditioning, as well as potential differences between natural and artificial spawning, could result in more rapid development in the Yakima River fish.

Year before repeat spawning.

E2 was low through the winter in skip spawners, increased from week 0 (+1 year)-week 10 to approximately 10-fold higher in skip spawners than consecutive spawners, comparable to levels in consecutive spawners at week 20. This indicates that the initiation of rematuration occurred before week 10 in skip spawners and suggests that reproductive development was accelerated in skip spawners versus consecutive spawners. Consistent with this idea, spawning was later in consecutive versus skip spawning steelhead trout (Jenkins *et al.*, 2018), as in consecutive versus maiden spawning Atlantic salmon (Pankhurst *et al.*, 2011). Plasma E2 levels remained greater in skip versus consecutive spawners as oogenesis proceeded through the summer growing season and levels increased. Similarly, plasma E2 levels were lower in repeat spawning Atlantic salmon versus maidens at sampling time points approximately 10 to 20 weeks after spawning (Pankhurst *et al.*, 2011). The higher E2 levels in skip versus consecutive spawners may have resulted in the 14% greater size-adjusted total egg mass found in these fish (Jenkins *et al.*, 2018). Both the delay in initiation of maturation in consecutive spawners and the greater reproductive investment observed in skip spawners can be attributed to the effects of recovery from maiden spawning on consecutive spawners. These effects were likely largely mediated by energetic status, as discussed above. However, in addition, reproductive development in consecutive spawners may have been directly affected by recovery from spawning, due to time required for clearance of steroids and other gonadal factors, continued steroid production by postovulatory follicles, and tissue resorption and remodeling of the postovulatory ovary (Caldwell *et al.*, 2014, Chyb *et al.*, 1999, De Mones *et al.*, 1989). The impact of maiden spawning on reproductive development in consecutive spawners illustrates the benefits of having time to recover from maiden spawning occur before the early stages of oogenesis.

Conclusions

Within the first 10 weeks after maiden spawning, growth and TG levels were greater in consecutive spawners than in skip spawners. This suggests that the rematuration decision takes place prior to 10 weeks after spawning in consecutive spawners. During the rematuration year, plasma E2 levels increased by 10 weeks in skip spawners, implying that the rematuration decision occurs prior to 10 weeks +1 year after spawning in skip spawners. The increase in plasma E2 was delayed by 10 weeks in consecutive spawners compared to skip spawners, suggesting that reproductive development was delayed due to the effects of maiden spawning. After maiden spawning, rematuring fish recovered more quickly than non-rematuring fish, consistent with stimulation of feeding by rematuration. Furthermore, while consecutive spawners sustained greater growth rates during oogenesis, skip spawners accumulated higher levels of energy reserves and E2, which may be causally related to the 14% greater reproductive investment found in skip spawners (Jenkins et al. 2018). Further studies using this experimental system should provide additional insights into consecutive and skip repeat spawning biology, as well as directly informing management of steelhead kelt reconditioning programs.

Section 3.D Condition-dependent survival, rematuration, and reproductive performance in reconditioned female steelhead trout (*Oncorhynchus mykiss*)

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

Physiological condition is thought to be a key predictor of many outcomes in life histories in fishes, including survival, migration, size and age at initial maturation, and reproductive mode and performance. Physiological condition has been defined as "…the relative capacity to maintain optimal functionality of all vital systems within the body…" (Hill 2011), which encompasses the energy reserves and health of an individual. However, how this should be quantified is not clear and the relevant aspects of physiological condition may vary in different situations. Survival is intuitively condition-dependent. An individual in positive energy balance and health is likely to survive, and an individual in negative energy balance and failing health is not. Migratory decisions have also been found to be condition-dependent, whereby a minimum condition threshold is required for initiating migration. Cyprinids with greater body condition were able to migrate away from areas of greater feed availability to escape areas of high predator density sooner than their feed restricted counterparts (Brodersen, et al. 2008). In the case of anadromous juvenile rainbow trout, larger individuals were more likely to migrate to locations with greater feed availability (Caisman 2015). Repeat spawning adult Atlantic salmon (*Salmo salar*) migrated upstream earlier in the season than their maiden spawning counterparts (Niemelä, et al. 2006), which was attributed to differing diets and the nearby locale of estuary feeding grounds. In many salmonid populations, the age at the onset of initial maturation (puberty) varies, and is regulated by aspects of individual condition such as growth rate, lipid stores, and feeding, as well as environmental factors such as temperature, photoperiod, and social cues (Taranger et al. 2010). The initiation of maturation in Atlantic salmon and other salmonids has been hypothesized to depend on energy stores during a critical period approximately one year before spawning associated with early stages of oogenesis (Thorpe 1994; Thorpe 2007). Consistent with this hypothesis, feed restriction early in oogenesis reduced the probability of completion of maturation in lake charr (*Salvelinus namaycush*) (Henderson and Wong 1998). In Atlantic salmon parr, males that reached a minimum lipid threshold at a younger age than their siblings matured precociously at smaller sizes than their siblings (Rowe and Thorpe 1990). Female salmonids reaching first maturity at younger ages than their siblings generally mature at smaller sizes and with reduced reproductive effort due to the positive correlations between fecundity and egg size with fork length (Crespi and Teo 2002; Quinn, et al. 2011).

Reproductive mode is also widely found to be condition-dependent. Alternative mating phenotypes of male dung beetles, bees, and fish, such as sneaking, female mimicry, and satellite males are subject to diversifying selection, with the alternative tactic resulting from a genotypic polymorphism associated with reduced diet, size, and growth (Gross 1996). The

selected tactic within the flexible strategy is expected to yield greater fitness (greater reproductive performance balanced with survival), but at some switch-point in condition either tactic is expected to yield equal fitness. Where reproductive mode is fixed, reproductive performance has been observed to be condition-dependent. For example, physiological condition impacted reproductive performance in both capital and income breeders (*i.e*., species that "fund" reproduction using energy stores (capital) gained beforehand versus using feed continually accessed during vitellogenesis (income) (McBride et al. 2015). Egg number and egg size were positively related to body condition in European plaice (*Pleuronectes platessa*; an income breeder) based on feed restriction during late vitellogenesis, indicating condition-dependent reproductive performance attributed to a relationship between feed availability and the rate of vitellogenesis (Kennedy, et al. 2008). In rainbow trout and lake charr (capital breeders), feed restriction reduced reproductive performance, but only for feed restriction during early oogenesis (Bromage et al. 1992; Henderson and Wong 1998). Condition impacted reproduction at different times during oogenesis for the two reproductive modes, likely due to the difference in funding strategies. These observations highlight how condition, reproductive mode, and reproductive performance interact.

In iteroparous species, individuals can reproduce either at the shortest interval of the reproductive cycle for that species, referred to as consecutive spawning for seasonally breeding iteroparous fishes that spawn at 1-year intervals, or at reduced frequencies, referred to as skip spawning for fishes that omit 1 or more reproductive cycles, spawning at intervals of 2 or more years (Bull and Shine 1979; Rideout et al. 2005a; Rideout and Tomkiewicz 2011). Skip spawning is particularly prevalent in capital breeders, due to the necessity to migrate to feeding locations following spawning for recovery of energy reserves (McBride et al. 2015), suggesting that rematuration depends on condition. However, the effect of condition on rematuration has been much less studied than the effect of condition on initial maturation (puberty). Mechanisms linking condition with rematuration are likely to be similar to those in initial maturation, but also influenced by energetic costs associated with maiden spawning. The decision to remature has been framed as condition-dependent, as in winter flounder (*Pleuronectes americanus*), where individuals in better condition rematured despite feed restriction (Burton 1994), and in Atlantic salmon, where both condition factor and rematuration rate were greater after a year of reproductive inactivity than in the year immediately following spawning (Crim, et al. 1992). Repeat maturation was also deferred as a result of poor condition in long distance migrating female albatross (*Thalassarche melanophris*) (Crossin, et al. 2012). Repeat maturation has alternately been framed in relation to energy availability in the environment and energy expenditure, presumably mediated by individual condition. Consecutive spawning Atlantic salmon were distributed in areas with high prey availability near river mouths (Chaput and Benoit 2012), whereas skip spawners travelled farther to feed. Finally, the effect of energy reserves on rematuration is not necessarily always positive. In Atlantic cod (*Gadus morhua*), skipping was associated with too little energy for rematuration, but also with abundant energy and growth opportunities, due to the potential increase in reproductive performance (Jorgensen, et al. 2006).

Despite extensive theory surrounding "condition-dependent" impacts on survival, behavioral, and reproductive outcomes, definitions of physiological condition are often vague. Measures used to capture condition range from body size (often one dimensional, such as body length), to condition indices incorporating mass and body size such as K, to measures of stored energy such as muscle and other tissue lipid levels. However, though these measures of condition indicate energetic status, they may not give a complete picture of an organism's physiological condition, and they do not directly interact with the physiological systems that regulate life history decisions. Integrated measures of the ability to maintain homeostasis, such as circulating levels of metabolic fuels, may be more relevant indicators of physiological condition by indicating the organism's ability to function, and may more directly interact with physiological systems that regulate life history decisions and outcomes. For example, K did not differ between survivors and mortalities of post-spawning Atlantic salmon, but upon follow-up investigation of mortalities, extensive evidence of parasites was discovered (Crim et al. 1992). As lipids are a major energy source and are primarily stored as triglycerides in poikilotherms (Sheridan 1994), circulating triglyceride level is a reasonable measure of the ability to maintain homeostasis by circulating of readily available energy. Growth rates in both length and mass are integrated measures of energy balance over time. However, measures of energy circulation and balance alone may fail to capture key aspects of the health of an individual, essential to the organism's ability to function. For fish, active maintenance of plasma solute concentration within a narrow range is required for the functioning of physiological systems, and osmoregulatory failure has been identified as an earlier indicator of senescence in sockeye salmon (*Oncorhynchus nerka*) (Jeffries, et al. 2011). Thus, plasma osmolality shows promise as an integrated indicator of the ability of an individual to maintain homeostasis. Individuals with weakened immune systems are subject to rapid colonization by ubiquitous pathogens. Quantification of parasite load can be used both as a proxy to measure immune system function and as an indicator of parasitic drain on energy reserves. Finally, quantification of reproductive investment at maiden spawning (total egg mass, E2) should provide insight into the relationship between reproductive investment and physiological condition. Reproductive investment has been theorized to tradeoff with survival (Abrahamse and Murdoch 2014; Christie, et al. 2018) and future reproductive decisions through effects on condition (Stearns 1992). Limited energy reserves resulting in poor physiological condition leaves fewer resources (energy, materials) available for allocation to physiological processes. Evaluation of relationships between maiden reproductive investment and condition, survival, rematuration, and reproductive investment at repeat spawning, are required to determine whether these tradeoffs occur, and the magnitude of any effects in our experimental system.

An experimental model system of post-spawning female steelhead was used to study intersections between maiden condition, maiden reproductive investment, and aspects of repeat spawning (survival, timing, and effort) in this study. Anadromous hatchery-origin female steelhead (*Oncorhynchus mykiss)* were obtained prior to spawning, manually spawned, maintained, sampled, and repeat spawned in captivity. This experimental model is more tractable than studies that use wild fish due to minimized variation between individuals: age, origin (Dworshak National Fish Hatchery, DNFH), size, and prior life history are not expected to vary as much as in wild populations. The hatchery kelt model provides a unique opportunity for

study of the energetic aspects of condition, due to the extreme energy deficit in these fish due to an approximately 800km freshwater fasting spawning migration, during which the fish divert large amounts of stored energy into ovarian development.

The objectives of this study were to determine whether and how post-spawning survival or rematuration were related to maiden reproductive investment or condition and whether reproductive investment at repeat spawning was condition-dependent during reconditioning, based on physiological condition measured early following maiden spawning. Within the first objective, there were four aims. Aim 1 was to determine whether post-spawning survival or the consecutive rematuration decision were condition-dependent. Aim 2 was to evaluate measures of physiological condition that indicated either the energetic status or the functional ability of an organism and to determine which were most relevant to outcomes in our study system. The third aim was to determine whether maiden reproductive investment was related to physiological condition at spawning, as reproductive life history trade-offs between maiden reproductive investment and post-spawning survival or the consecutive rematuration decision are hypothesized to be mediated by physiological condition. The fourth aim was to directly assess whether there was a reproductive life history trade-off between maiden reproductive investment and post-spawning survival or the consecutive rematuration decision, since the measures of condition used here may not capture the relevant aspects of physiological condition. It was hypothesized that (a) post-spawning survival, the consecutive rematuration decision, and reproductive investment at consecutive spawning would vary positively with condition, due to being condition-dependent traits, and (b) condition at maiden spawning, post-spawning survival, and the decision to remature in consecutive years would vary negatively with maiden reproductive investment, due to a trade-off resulting from limited energy reserves.

Methods

Fish

Maiden spawning female steelhead originating from Dworshak National Fish Hatchery (DNFH) were captured after ascending the adult ladder on the North Fork Clearwater River in Ahsahka, ID in February through April in 2015 and 2016, and were held in holding ponds supplied with river water. During February through April in 2015 and 2016, DNFH staff selected fully mature fish >70 cm for use as broodstock. Fish in good or fair condition (Hatch et al. 2013b), n = 150 in 2015 and 164 in 2016, were selected for this study and individually marked with passive integrated transponder (PIT) tags inserted into the pelvic girdle.

Spawning

Fish were anesthetized using AQUI-S 20E (AquaTactics Inc., Kirkland, WA; 75mL $1000L^{-1}$ water) and were manually “air spawned” (Leitritz and Lewis 1976). Repeat spawners were checked weekly for ripeness during the spawning season and air spawned as above when ripe.

Reconditioning husbandry

Fish were held at DNFH in 4.6m diameter outdoor tanks, supplied with a flow of North Fork Clearwater River water at 200 liter/minute, maintained at a water height of 1.5m, with a

seasonally varying temperature profile (4.9 – 11.0^{o}C). Tanks were treated with formalin (Syndel USA, Portland, OR; flow through treatment, 1:6000 for one hour daily). Fish were prophylactically treated for bacterial infection and parasitic gill copepods (*Salmincola californiensis*) both via intraperitoneal injection at maiden spawning and every 10 weeks thereafter (only as needed for copepods) and fed *ad libitum* (Jenkins et al. 2018b).

Sampling

Fish were sampled for length (fork length, FL, cm), wet body mass (kg), muscle lipid level (ML, %) (Fish Fatmeter model 692, Distell Inc., West Lothian, UK), and blood. Fish were sampled for parasitic gill copepods (*Salmincola californiensis*) counted on the left gill (PL). Sampling occurred at spawning and at 10-week intervals thereafter.

Morphometric analysis

Fulton's condition factor K, mass specific growth rate (MSGR), and length specific growth rate (LSGR) were calculated as follows:

$$K = 100 * \text{body mass (g)} * (\text{fork length (cm)})^{-3}$$

$$\text{MSGR} = \%\ \text{body mass gain} * \text{day}^{-1} = 100 * (\ln(\text{body mass final}) - \ln(\text{body mass initial}))\text{days}^{-1}$$

LSGR was calculated in the same manner as was MSGR. Body mass and K were adjusted to account for eggs retained from maiden spawning in the body cavity to represent somatic measures of body mass and K (Jenkins et al. 2018b).

Reproductive performance measures

Repeat spawning individual egg mass (IEM), total egg mass (TEM), and fecundity, were quantified gravimetrically (Fleming and Ng 1987; Jenkins et al. 2018b). To facilitate comparisons of fish of different sizes, TEM, IEM, and fecundity were standardized based on fish mass (Jenkins et al. 2018b).

Assays

Plasma estradiol-17 β (E2, ng/mL) concentration was measured by ELISA (Biosense, Cayman Chemical, Ann Arbor, MI) (Pierce et al. 2017). Plasma triglyceride (TG) concentration was measured using a VetTest (Idexx, Westport, ME) as validated for use in salmonids (Meador et al. 2006). Plasma osmolality was measured using a VAPRO Vapor Pressure Osmometer 5520 (WESCOR, Elitech, Puteaux, France).

Ethics

Fish care and sampling were conducted in accordance with a protocol reviewed and approved by the University of Idaho Animal Care and Use Committee.

Statistical analysis

The probability of survival post-spawning in female steelhead (hereafter, survival) was assessed for associations with potential predictor variables using univariate and multiple logistic regression. Binomial response variables were defined as survival to 70 days (1=survival) versus mortality prior to 70 days (0=mortality).

Seven continuous predictor variables measured at maiden spawning included K, ML (arc-sine square-root transformed), TG, OS, PL (number of gill copepods), TEM, and E2 (log_{10}-transformed). Predictor variables were standardized in order to compare effect sizes ([x-mean]/SD) (Keefer, et al. 2017).

Years (2015, 2016) were analyzed separately, as well as with the data combined. When data from both years were combined, year was included as an effect. Because preliminary univariate tests saw a year effect (in the survival analysis only, P=0.00393), a model was tested with all first order interactions with year. No evidence supported an interaction with year, so the interactions were dropped from the whole model.

To further explore potential predictors of survival, univariate and multiple linear regression were used to identify predictors of time survived ("survival duration") for mortalities within the first 70 days following maiden spawning, with the continuous response variable "days survived". The same 7 standardized predictor variables listed above were included (8 with year when data from both years were combined). When data from both years were included, interactions with year were explored as described above and included where identified as significant.

The probability of rematuration as a consecutive repeat spawner ("rematuration", vs. deferring rematuration for a future year) was assessed for associations with the 7 potential explanatory variables listed above, using univariate and multiple logistic regression models, as described above for the survival analysis. Maturation trajectory was determined using E2 measured 30 weeks post-spawning. Fish with high E2 were classified as rematuring and fish with low E2 concentration group were classified as non-rematuring (Section C3). Binomial response variables were defined as rematuring as a consecutive spawner (1=rematuring) or deferring rematuration (0=skipping). The same 7 standardized predictor variables were tested as described above. Year was included as an effect when data from both years were included. Though year was not a significant univariate predictor of maturation (P = 0.38819), a model was tested with all first order interactions with year. No evidence was found for interaction with year, so the interactions were dropped.

To identify potential sensitive periods for condition-dependent regulation of repeat reproductive investment ("reproductive investment"), simple linear regression was used to test for relationships between 6 potential predictor variables, measured at time points starting at maiden spawning, and 3 dependent variables of reproductive performance at repeat spawning. Potential predictor variables were: MSGR, LSGR, TG, E2, ML, and K. Dependent variables were: repeat spawning IEM, TEM, and fecundity. Consecutive spawners (N=12) from the 2015 spawn year were included in the analysis as the most complete dataset was available for these fish. Coefficients of determination (R) and an associated P value were calculated for each predictor at each time period (spawning, and at each 10-week sampling period or interval thereafter).

Results

Patterns of survival

Survival declined steeply during the first 70 days following maiden spawning and remained approximately constant between weeks 10-30 (Figure 3. D1) Survival was lower in 2016 than in 2015 (Log-rank test, $\chi^2 = 9.335$, P = 0.0022). Mortality decreased after 70 days, which coincided with the first sampling event (10 weeks). Survival rates to 70 days after spawning were 53% (80/150) in 2015 and 38% (62/164) in 2016, averaging 45% (142/314) in the two years combined (Table 3. D1).

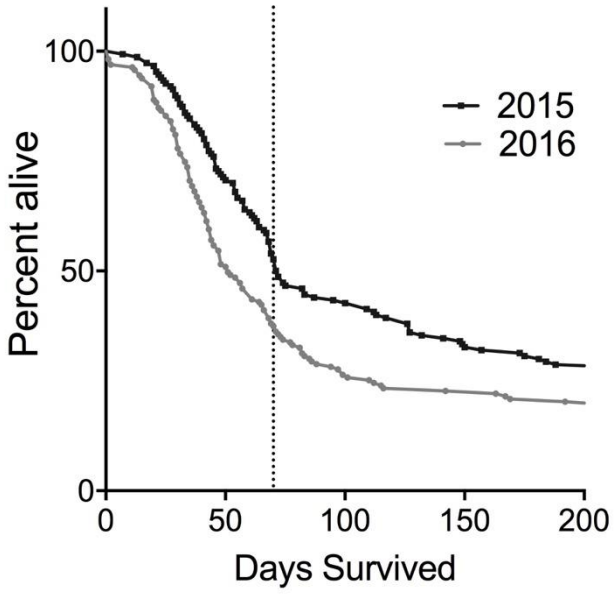

Figure 3.D 1Survival of female steelhead trout (N=314) following maiden spawning at Dworshak National Fish Hatchery in Ahsahka, Idaho in 2015 and 2016.

Table 3.D 1: Number (N(%)) of female steelhead trout collected for study in 2015 and 2016 by continuous covariate (mean(SD)) for outcomes: "survived" (to 70 days after spawning), "mortality" (before 70 days), "remature" (in consecutive years), and "skip".

			Energetic Status		Homeostatic Ability			Reproductive Investment	
		N (%)	Muscle Lipids (%)	Condition K	Plasma Triglycerides (mg/dL)	Plasma Osmolality (mmol/kg)	Parasite Load (# copepods)	Total Egg Mass (g)	Plasma Estradiol-17b (ng/mL)
2015	Survived	80 (53)	0.96 (0.12)	0.758 (0.05)	189.6 (28.4)	303.1 (5.7)	4.0 (2.7)	688.8 (97)	984.8 (327.6)
	Mortality	70 (47)	0.95 (0.13)	0.763 (0.05)	180.8 (33.4)	301.0 (6.1)	4.1 (2.7)	698.0 (116)	958.0 (292.6)
	Rematured	13 (9)	0.92 (0.10)	0.743 (0.04)	176.8 (17.4)	303.2 (5.6)	4.0 (2.6)	723.7 (89)	940.4 (312.9)
	Skipped	30 (20)	0.96 (0.13)	0.750 (0.05)	185.8 (19.4)	303.3 (4.8)	3.7 (2.4)	679.9 (80)	976.5 (380.9)
	All	150	0.96 (0.12)	0.760 (0.05)	185.5 (31.0)	302.1 (6.0)	4.0 (2.7)	693.0 (106)	972.4 (311.1)
2016	Survived	62 (38)	0.93 (0.14)	0.779 (0.05)	167.7 (36.1)	300.3 (5.6)	3.9 (2.8)	776 (129)	956.4 (382.5)
	Mortality	102 (62)	0.90 (0.11)	0.763 (0.05)	143.5 (40.2)	294.6 (9.2)	4.3 (2.5)	811.3 (127)	851.1 (307.5)
	Rematured	12 (7)	0.90 (0.11)	0.770 (0.04)	183.5 (33.3)	299.7 (5.5)	3.7 (1.8)	743.7 (68)	902.8 (318.8)
	Skipped	18 (11)	0.94 (0.14)	0.777 (0.06)	171.5 (32.0)	299.3 (6.4)	4.6 (3.7)	701.6 (92)	853.4 (181.2)
	All	164	0.91 (0.11)	0.769 (0.05)	152.5 (40.3)	296.7 (8.4)	4.2 (2.6)	798.0 (129)	891.7 (341.1)
Both Years	Survived	142 (45)	0.95 (0.12)	0.767 (0.05)	180.0 (33.6)	301.8 (5.8)	4.0 (2.7)	726.6 (119)	972.4 (351.6)
	Mortality	172 (55)	0.92 (0.12)	0.763 (0.05)	158.5 (41.7)	297.2 (8.6)	4.2 (2.6)	765.6 (135)	895.0 (305.2)
	Rematured	25 (8)	0.91 (0.11)	0.756 (0.05)	180.0 (25.8)	301.5 (5.7)	3.8 (2.2)	732.4 (80)	921.6 (309.5)
	Skipped	48 (15)	0.95 (0.13)	0.760 (0.05)	180.5 (25.5)	301.7 (5.8)	4.0 (3.0)	687.9 (84)	932.0 (326.3)
	All	314	0.93 (0.12)	0.765 (0.05)	168.2 (39.7)	299.3 (7.8)	4.1 (2.7)	747.8 (129)	930.5 (329.0)

Predictors of survival

In the survival dataset (N=314), several parameters were significantly but not strongly intercorrelated [uncorrected for 21 tests] ($R \leq 0.469$; Table 3.DS1). TG was weakly positively correlated with ML, K, OS, and E2 (r^2=0.148, 0.019, 0.151, and 0.220, respectively; P<0.0001, P=0.0141, P<0.0001, and P<0.0001, respectively), with relationships explaining less than 25% of the variability for each. ML was weakly positively correlated with K, OS, and E2 (r^2=0.013, 0.019, and 0.106; P = 0.0489, P = 0.0154, and P<0.0001). TEM was weakly negatively correlated with TG, ML, K and OS (r^2 = 0.041, 0.018, 0.060, and 0.049; P=0.0004, P=0.0202, P<0.0001, and P<0.0001, respectively), and weakly positively correlated with PL (r^2 = 0.046, P = 0.0001).

Table 3.DS 1: Correlation coefficients (R) (below diagonal) for all female steelhead trout collected for study in 2015 and 2016 (n = 314). Correlations were based on transformed data (MLL = arcsine-square root; Plasma E2 = log10). P-values for correlations are above diagonal. Significant correlations (P < 0.05) are bolded.

	Plasma Triglycerides (mg/dL)	Muscle Lipids (%)	Condition K	Plasma Osmolality (mmol/kg)	Parasite Load (number of copepods)	Total Egg Mass (g)	Plasma E2 (ng/mL)
Plasma Triglycerides	x	**<0.0001**	**0.0141**	**<0.0001**	0.5933	**0.0004**	**<0.0001**
Muscle Lipids	**0.384**	x	**0.0489**	**0.0154**	0.7491	**0.0202**	**<0.0001**
Condition K	**0.139**	**0.113**	x	0.2556	0.1960	**<0.0001**	0.1808
Plasma Osmolality	**0.389**	**0.140**	0.065	x	0.2903	**<0.0001**	**0.0005**
Parasite Load	-0.031	0.018	-0.074	-0.061	x	**0.0001**	0.1402
Total Egg Mass	**-0.201**	**-0.132**	**-0.244**	**-0.220**	**0.214**	x	0.1647
Plasma E2	**0.469**	**0.326**	0.076	**0.198**	-0.085	0.079	x

Significant univariate predictors of survival were found in 2015, 2016, and in years combined [uncorrected for 21 tests] (Table 3.DS2). OS was a significant univariate predictor of survival in 2015, 2016, and in years combined (Figure 3. D2(A)), (χ^2 = 4.40, 15.29, 23.90; P=0.0359, P<0.0001, P<0.0001, respectively). TG was a significant univariate predictor of survival in 2016 (χ^2 = 12.63, P=0.0004), in years combined (χ^2 = 20.88, P<0.0001) (Figure 3. D2(B)) and was nearly significant in 2015 (χ^2 = 2.89, P=0.0891).

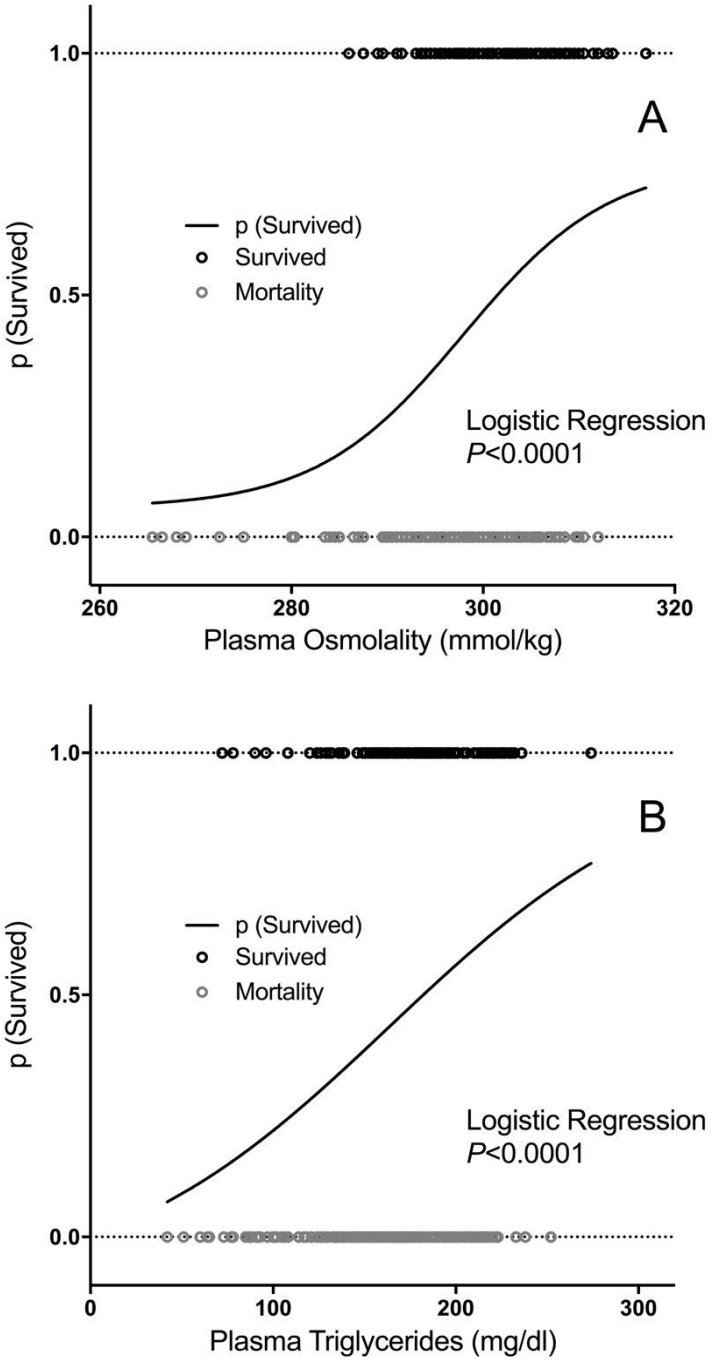

Figure 3.D 2: Plasma osmolality (A) and plasma triglycerides (B) as univariate predictors of survival to 70 days after maiden spawning in female steelhead trout at Dworshak National Fish Hatchery in Ahsahka, Idaho, 2015 and 2016 combined data.

K was significant univariate predictor of survival in 2016 only (χ^2 = 3.89, P=0.0486) (Table 3.DS2). E2 was nearly significant in 2016 (χ^2 = 3.24, P=0.0720). TEM was a significant negative predictor of survival in years combined (χ^2 = 6.97, P=0.0083, OR=0.396033), and was nearly significant in 2016 (χ^2 = 2.88, P=0.0897), but not in 2015 (χ^2 = 0.28, P=0.5954). ML was a nearly significant predictor of survival in 2016 and in years combined (χ^2 = 1.83, χ^2 = 3.39; P=0.1763,

P=0.0654 respectively), but was a slightly positive predictor in 2016 and a slightly negative predictor in years combined.

Table 3.DS 2: Results of univariate logistic regression models tested to predict female steelhead trout survival to 70 days after maiden spawning. Survival was coded as 1. Univariate models are shown when P < 0.25 for a univariate relationship in a given year and then for that same effect in each other year (or years combined) for comparison. Odds ratio values <1 represent "negative odds" or increased likelihood of non-rematuration as the effect decreases in value. Significant effects (P < 0.05) are bolded.

	Effect	df	χ^2	*P*	Odds ratio	95% CI
2015	Osmolality	1	4.40	**0.0359**	3.449132	0.5369-22.15779
	Triglycerides	1	2.89	0.0891	4.218233	0.385319-46.17863
	Condition K	1	0.43	0.5100	0.34912	0.041309-2.95056
	E2	1	0.15	0.6962	1.302279	0.132607-12.78914
	Total Egg Mass	1	0.28	0.5954	0.456986	0.055577-3.757627
	Muscle Lipids	1	0.29	0.5926	1.093447	0.186193-6.421422
2016	Osmolality	1	15.29	**<0.0001**	45.10924	3.138067-648.4385
	Triglycerides	1	12.63	**0.0004**	23.83648	1.856865-305.9875
	Condition K	1	3.89	**0.0486**	1.40327	0.133512-14.74894
	E2	1	3.24	0.0720	0.785226	0.036113-17.07343
	Total Egg Mass	1	2.88	0.0897	0.377656	0.05058-2.819756
	Muscle Lipids	1	1.83	0.1763	1.110909	0.200052-6.169004
Both Years	Osmolality	1	23.90	**<0.0001**	32.39213	4.125691-254.3211
	Triglycerides	1	20.88	**<0.0001**	15.4575	2.093281-114.1434
	Condition K	1	0.12	0.7279	0.621488	0.123711-3.12218
	E2	1	0.75	0.3878	1.180187	0.122063-11.41088
	Total Egg Mass	1	6.97	**0.0083**	0.396033	0.083927-1.868789
	Muscle Lipids	1	3.39	0.0654	0.984414	0.210247-4.609196
	Year	1	8.21	**0.0042**	1.3787	0.748598-2.539165

In the following model: "Survival = Intercept + Year + TG + ML + SK + OS + PL + TEM + E2" (Table 3. D2), OS and TG were both strongly and significantly positively related to survival (χ^2= 10.94, 7.20; P=0.0009, 0.0073, respectively). The 95% confidence intervals of all other standardized coefficients overlapped zero and are thus without significant effect (Figure 3. D3).

Table 3.D 2: Results for multiple logistic regression analysis of post-spawning survival in female steelhead trout collected for study in 2015 and 2016. Fish that died before the first sampling point at 70 days were classified as mortalities (coded as 0). Prior to analysis, covariates were standardized ([x-mean]/SD) in order to assess the relative effects of each parameter. N = 291 due to missing values (N = 7) and outlier removal (N = 16). Significant effects (P < 0.05) are bolded.

Effect	Parameter Estimate	Standard Error	χ^2	P-value
Intercept [Survival]	-0.24086217	0.1269621	3.60	0.0578
Year	0.1606668	0.1558857	1.06	0.3027
Triglycerides	0.4687915	0.1746524	7.20	**0.0073**
Muscle lipids	-0.00279237	0.1400080	0.00	0.9841
Condition K	-0.07991895	0.1383801	0.33	0.5636
Osmolality	0.5293059	0.1600113	10.94	**0.0009**
Parasite Load	-0.03816411	0.1305209	0.09	0.7700
Total Egg Mass	-0.18191859	0.1554757	1.37	0.2420
E2	0.0223342	0.1560571	0.02	0.8862

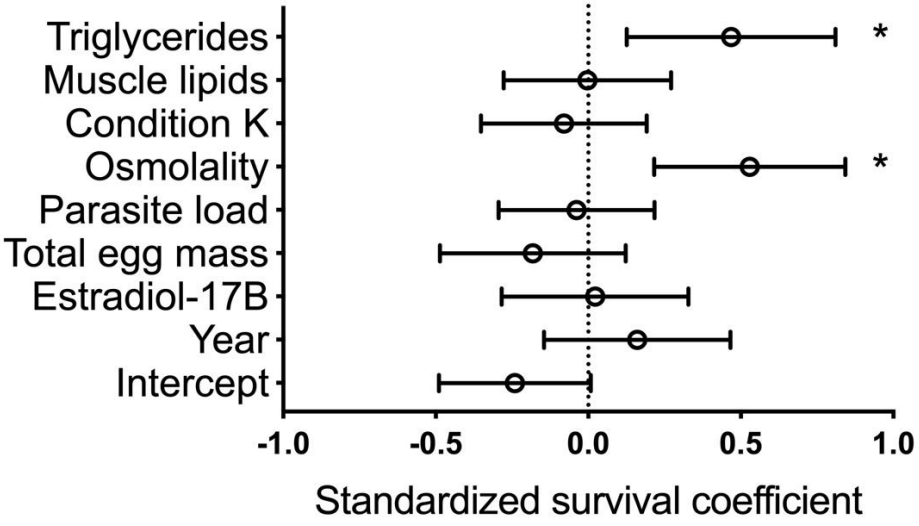

Figure 3.D 3: Standardized coefficients ([x-mean]/SD) with 95% confidence intervals of potential predictors in a multiple logistic regression model of survival of female steelhead trout to 70 days after maiden spawning in 2015 and 2016. Positive estimates indicate greater probability of post-spawning survival. Coefficients with confidence intervals that do not overlap zero were statistically significant (P < 0.05) and are marked with an asterisk. The intercept is nearly significant (P = 0.0578). N=291 of 314 due to missing values (N = 7) and removal of outliers (N = 16) based on the Rout Outlier Test (Q=1%).

Predictors of survival duration

For fish that were mortalities within the first 70 days after spawning (N=172), several parameters were significantly but not strongly intercorrelated [uncorrected for 21 tests] (Table 3.DS3). Consistent with the survival dataset, TG were weakly positively correlated with ML, OS, and E2 (r^2 = 0.093, 0.169, and 0.236, respectively; P<0.0001 for each). Relationships explained less than 25% of the variability for each. Also consistent with the survival dataset, TEM was weakly negatively correlated with TG, K and OS (r^2 = 0.078, 0.109, and 0.035; P=0.0002, P<0.0001, and P=0.0156, respectively), and weakly positively correlated with PL (r^2 = 0.035 and P=0.154).

Table 3.DS 3: Correlation coefficients (R) (below diagonal) for mortalities within 70 days after spawning in female steelhead trout (n = 172). Correlations were based on transformed data (MLL = asin-sqrt; Plasma E2 = log10). P-values for correlations are above diagonal. Significant correlations (P < 0.05) are bolded.

	Plasma Triglycerides (mg/dL)	Muscle Lipid Level (%)	Condition K	Plasma Osmolality (mmol/kg)	Parasite Load (number of copepods)	Total Egg Mass (g)	Plasma E2 (ng/mL)
Plasma Triglycerides	x	**<0.0001**	0.0590	**<0.0001**	0.7142	**0.0002**	**<0.0001**
Muscle Lipids	**0.304**	x	0.1943	0.2715	0.3574	0.0549	**0.0102**
Condition K	0.146	0.102	x	0.4566	0.2532	**<0.0001**	0.7932
Plasma Osmolality	**0.410**	0.086	0.058	x	0.6495	**0.0156**	**0.0007**
Parasite Load	-0.028	-0.072	-0.089	-0.035	x	**0.0154**	0.2357
Total Egg Mass	**-0.280**	-0.151	**-0.329**	**-0.187**	**0.188**	x	0.7530
Plasma E2	**0.485**	**0.201**	-0.021	**0.261**	-0.093	-0.025	x

Significant univariate predictors of survival duration (days) were found in 2015, 2016, and in both years combined [uncorrected for 21 tests] (Table 3.DS4). TG was significant in 2015, 2016, and in both years combined (t-ratio=3.35, 2.31, and 4.62; P=0.00133, P=0.0230, and P<0.0001, respectively) (Figure 3. D4(A)). OS was significant in 2016 and in both years combined (t-ratio=3.73, 4.06; P=0.0003, P<0.0001, respectively) (Figure 3. D4(B)).

Table 3.DS 4: Results of univariate linear regression models to predict survival duration (days) for female steelhead trout that died during the first 70 days after maiden spawning. Univariate models are shown when P < 0.25 for a univariate relationship in a given year and then for that same effect in each other year (or years combined) for comparison. Significant effects (P < 0.05) are bolded.

	Effect	df	t-ratio	*P*
2015	Triglycerides	68	3.35	**0.00133**
	Osmolality	68	0.21	0.83810
	Total Egg Mass	68	-1.16	0.25086
	E2	68	1.31	0.19395
	Parasite Load	67	0.71	0.48170
2016	Triglycerides	100	2.31	**0.0230**
	Osmolality	99	3.73	**0.0003**
	Total Egg Mass	97	-0.30	0.7680
	E2	97	0.82	0.4157
	Parasite Load	99	1.50	0.1360
Both Years	Triglycerides	169	4.62	**<0.0001**
	Osmolality	168	4.06	**<0.0001**
	Total Egg Mass	166	-1.96	0.0517
	E2	166	1.86	0.0645
	Parasite Load	167	1.43	0.1558
	Year	169	-2.71	**0.0075**

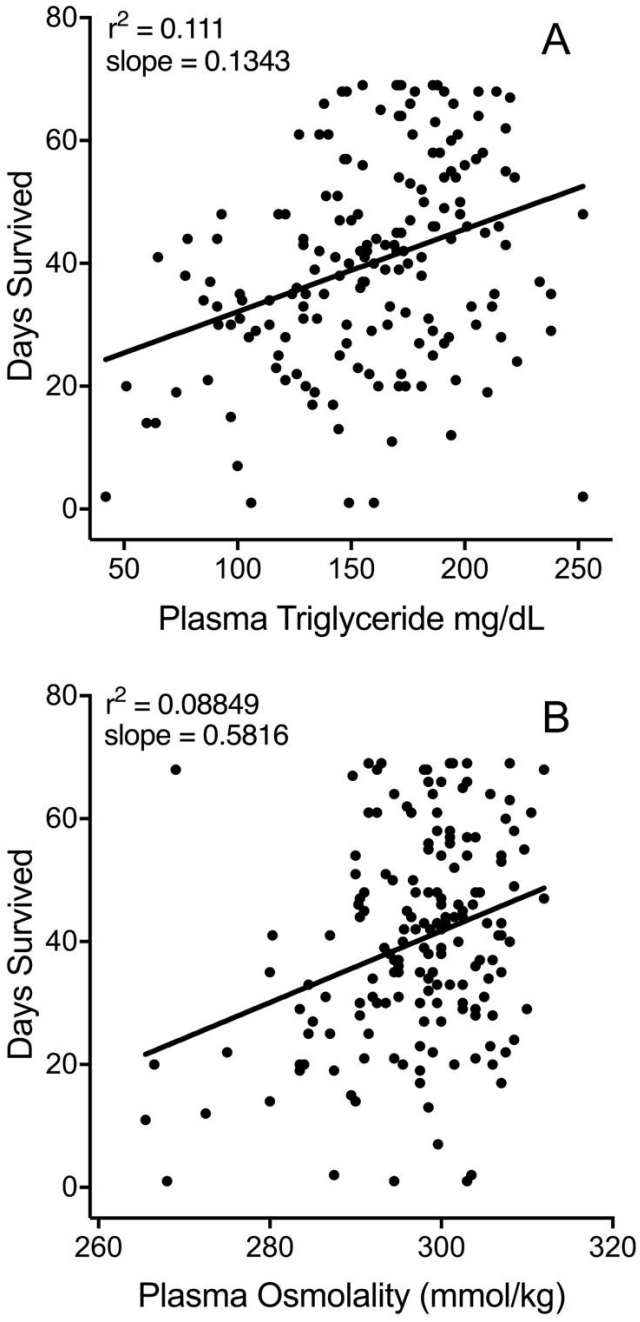

Figure 3.D 4: Plasma triglyceride level (A) and plasma osmolality (B) at maiden spawning as univariate predictors of survival duration in female steelhead trout during the first 70 days following spawning at Dworshak National Fish Hatchery in 2015 and 2016. Regressions are significant (P<0.0001).

E2 and TEM were nearly significant univariate predictors of survival duration in 2015 and in years combined (E2: t-ratio = 1.31 and 1.86, P = 0.19395 and 0.06450; total egg mass: t-ratio = -1.16 and -1.96, P = 0.25086 and 0.0517) (Table 3.DS4). PL was nearly significant in 2016 and in years combined (t-ratio=1.50 and 1.43, P=0.1360 and 0.1558).

Significant interactions between year and both TG and OS were included in the following model: "Survival days = Intercept + Year + TG + ML + SK + OS + PL + TEM + E2 + TG*Year + OS*Year" (Table 3. D3). TG (P=0.0059), OS (P=0.0312), TG*Year (P=0.0255), and OS*Year (P=0.0364) were significantly related to survival duration in the model. The model as a whole explained 22.7% of the variation in survival duration.

Table 3.D 3: Results for multiple linear regression analysis of post-spawning survival duration in female steelhead trout collected for study in 2015 and 2016. "Days lived" (within the first 70 days) was regressed against continuous predictor variables. Prior to analysis, covariates were standardized as in Table 3. D2. N=156 due to missing values (N = 13) and outlier removal (N = 3). Significant effects are bolded.

Effect	Parameter Estimate	Standard Error	t-ratio	Prob>|t|
Intercept [Days Survived]	40.224895	1.412952	28.47	**<0.0001**
Year	1.4297038	1.588125	0.90	0.3695
Triglycerides	4.4385188	1.586608	2.80	**0.0059**
Muscle lipids	2.4025687	1.289991	1.86	0.0646
Condition K	-0.590319	1.369398	-0.43	0.6671
Osmolality	3.1601919	1.452456	2.18	**0.0312**
Parasite Load	1.4043316	1.222431	1.15	0.2525
Total Egg Mass	-0.930838	1.472516	-0.63	0.5283
E2	-0.78957	1.455299	-0.54	0.5883
Year*Triglycerides	-3.2989	1.461476	-2.26	**0.0255**
Year*Osmolality	3.2818172	1.553802	2.11	**0.0364**

Patterns of rematuration

The proportion of fish that survived and rematured in the year following spawning was 9% (13/150) in 2015 and 7% (12/164) in 2016, averaging 8% average in years combined (Table 3. D1). Of the fish that survived to 30 weeks, 30% (13/43) rematured in 2015 and 40% (12/30) rematured in 2016, averaging 34% in years combined (Section 3C).

Predictors of rematuration

Of fish that survived to 30 weeks after maiden spawning (N=73), several parameters were significantly but not strongly intercorrelated [uncorrected for 21 tests] (Table 3.DS5). TG was weakly positively correlated with ML (r^2=0.302, P<0.0001), OS (r^2=0.063, P=0.0435), and E2 (r^2=0.155, P=0.0009). ML was also weakly positively correlated with E2 (r^2=0.266, P<0.0001). TEM and K were weakly negatively correlated (r^2=0.077, P=0.0206). TEM was also weakly negatively correlated with TG, ML, and OS, and positively correlated with PL, but unlike in the survival (complete) dataset (N=314), these relationships were not significant (P=0.3776, 0.3411, 0.2986, and 0.3958). Also, unlike the complete dataset, TG and PL were not negatively related, though this was not previously a significant correlation.

Table 3.DS 5: Correlation coefficients (R) (below diagonal) for female steelhead trout that survived to week 30 (n = 73). Correlations were based on transformed data (MLL = asin-sqrt; Plasma E2 = log10). P-values for correlations are above diagonal. Significant correlations (P < 0.05) are bolded.

	Triglycerides (mg/dL)	Muscle Lipid Level (%)	Condition K	Osmolality (mmol/kg)	Parasite Load (number of copepods)	Total Egg Mass (g)	E2 (ng/mL)
Plasma Triglycerides	x	**<0.0001**	0.5740	**0.0435**	0.5184	0.3776	**0.0009**
Muscle Lipids	**0.549**	x	0.4451	0.1166	0.5686	0.3411	**<0.0001**
Condition K	0.069	0.091	x	0.5153	0.2704	**0.0206**	0.4126
Plasma Osmolality	**0.251**	0.192	0.080	x	0.1999	0.2986	0.4662
Parasite Load	0.080	0.068	-0.132	-0.159	x	0.3958	0.8113
Total Egg Mass	-0.110	-0.116	**-0.278**	-0.132	0.105	x	0.1402
Plasma E2	**0.393**	**0.516**	0.099	0.090	-0.029	0.1820	x

Significant univariate predictors of maturation were found when data from both years were combined [uncorrected for 21 tests] (Table 3.DS6). TEM was a significant positive predictor of maturation in both years combined (χ^2 = 4.07, P=0.0437), and nearly significant in 2015 and 2016 (χ^2 = 2.36, χ^2 = 1.52; P=0.1241, P=0.2172, respectively) (Figure 3. D5). TG was a nearly significant negative predictor of maturation in 2015 (χ^2 = 1.83, P=0.1756), as was ML in years combined (χ^2 = 1.80, P=0.1795) (Table 3.DS6).

Table 3.DS 6: Results of univariate logistic regression models to predict consecutive rematuration of female steelhead trout after maiden spawning. Univariate models are shown when P < 0.25 for a univariate relationship in a given year and then for that same effect in each other year (or years combined) for comparison. Odds ratio values <1 represent "negative odds" or likelihood of non-rematuration as the effect increases in value. Significant effects (P < 0.05) are bolded.

	Effect	df	χ^2	*P*	Odds ratio	95% CI
2015	Total Egg Mass	1	2.36	0.1241	117.2602	1.077105-12765.65
	Muscle Lipids	1	0.96	0.3278	2.498202	0.019924-313.2479
	Triglycerides	1	1.83	0.1756	0.078502	0.000461-13.37439
2016	Total Egg Mass	1	1.52	0.2172	121.5049	0.272936-54091.28
	Muscle Lipids	1	0.63	0.4258	0.050838	0.000191-13.50347
	Triglycerides	1	0.92	0.3382	19.54283	0.059273-6443.432
Both Years	Total Egg Mass	1	4.07	**0.0437**	28.29456	1.521568-526.1559
	Muscle Lipids	1	1.80	0.1795	0.180527	0.00422-7.7122035
	Triglycerides	1	0.01	0.9340	2.851269	0.098832-82.2579

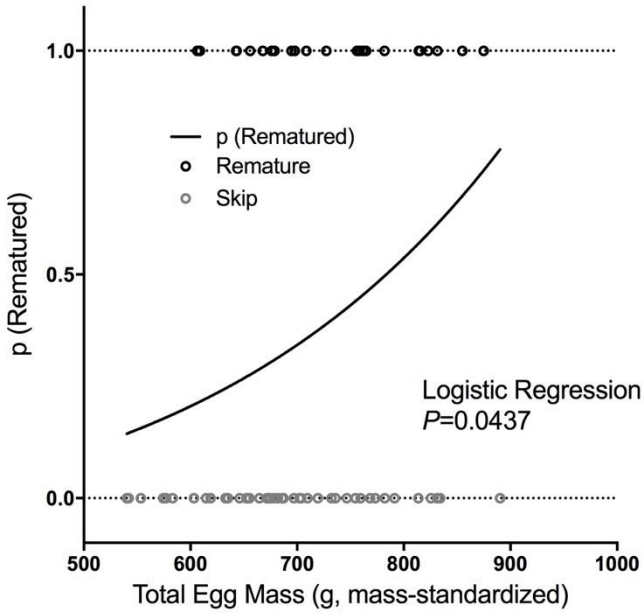

Figure 3.D 5: Total egg mass from maiden spawning as a univariate predictor of consecutive rematuration in female steelhead at Dworshak National Fish Hatchery in Ahsahka, Idaho, in 2015 and 2016.

In the following model: "Rematuration = Intercept + Year + TG + ML + SK + OS + PL + TEM + E2" (Table 3. D3), TEM was the only significant positive predictor of maturation (χ^2= 5.02; P=0.0250). The 95% confidence intervals of all other standardized coefficients overlapped zero and are thus without significant effect by comparison (Figure 3. D6).

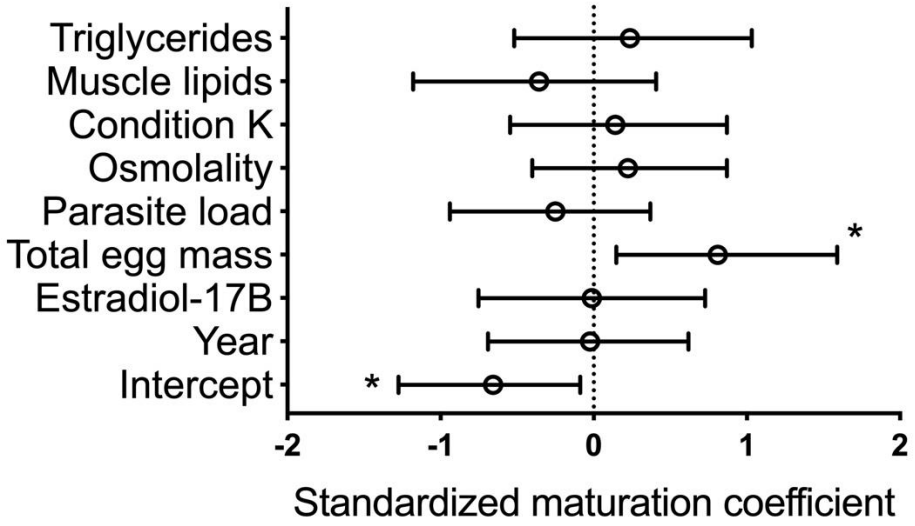

Figure 3.D 6: Standardized coefficients ([x-mean]/SD) with 95% confidence intervals of potential predictors in a multiple logistic regression model of consecutive rematuration of female steelhead trout after manual spawning in 2015 and 2016. Positive estimates indicate greater probability of consecutive rematuration. TG: plasma triglycerides, ML: muscle lipid level, K: Fulton's condition factor, OS: plasma osmolality, PL: parasite load (# copepods on left gill), TEM: mass standardized total egg mass, E2: plasma estradiol. Coefficients with confidence intervals that do not overlap zero were statistically significant (P < 0.05) and are marked with an asterisk. N = 60 of 72 due to missing values and removal of outliers based on the Rout Outlier Test (Q=1%).

Patterns of reproductive performance

Consecutive spawners of the maiden spawn year 2015 (N=12) included in this analysis had an average 96.1mg (SD=13.5) IEM, an average 721.9g (SD=132.8) TEM, and an average 7,505 eggs (SD=1,456) fecundity (Jenkins et al. 2018b).

Predictors of reproductive performance

Relationships were found between reproductive performance at repeat spawning (IEM, TEM, and fecundity) and growth, available energy, and condition at early time points (0-10 weeks and 10-20 weeks) during the year prior to repeat spawning (Table 3.DS7, Figure 3. D7). Relationships between IEM at consecutive repeat spawning with TG, MSGR, and LSGR peaked in strength at 10 weeks post-spawning (r^2=0.5593, 0.5247, and 0.4758, respectively (Figure 3. D7 A, D and G; Figure 3. D8 A, C, and E). Relationships between TEM and MSGR, K, and TG peaked in strength at 20 weeks post-spawning (r^2=0.5382, 0.4768, and 0.4595, respectively (Figure 3. D7 B, E, and H, Figure 3. D8 B, D, and F). Relationships between fecundity and MSGR, TG, and LSGR peaked in strength at 20 weeks post-spawning (r^2=0.41725, 0.56586, and 0.54093, respectively) (Figure 3. D7 C, F, and I), but these relationships were not quite significant (P=0.1772, 0.0551, 0.0693, respectively).

Table 3.DS 7: Correlation coefficient (R) of relationships between measures at time points starting at spawning with individual egg mass, total egg mass, and fecundity measured at repeat spawning in female steelhead trout. Bolded R values are significant (P < 0.05). Data shown for specific growth rate is over the preceding 10-week interval. N=12 consecutive repeat spawners from 2015.

Repeat Reproductive Performance	Sampling Point	MSGR	LSGR	Plasma TG	E2	Muscle Lipids %	Condition K
Individual Egg Mass	Spawning	-	-	-0.36919	0.04996	-0.49224	0.06760
	10 Weeks	**0.72436**	**0.68978**	**0.74786**	0.00072	0.35128	0.32573
	20 Weeks	0.35805	-0.11077	0.03912	0.26900	0.56018	0.47413
	30 Weeks	0.41316	0.23780	0.21166	0.15970	0.45935	0.57009
	40 Weeks	0.38704	0.52412	-0.23688	0.00200	0.49487	**0.58395**
	50 Weeks	-0.22154	-0.35100	**0.60166**	0.08780	0.52163	0.54065
Total Egg Mass	Spawning	-	-	-0.28775	0.00050	-0.51643	0.16214
	10 Weeks	0.32265	0.12227	0.18569	-0.48146	-0.10630	0.48000
	20 Weeks	**0.73362**	0.52602	**0.67786**	0.47021	0.56921	**0.69051**
	30 Weeks	0.06447	0.28773	0.46936	0.09101	0.38105	**0.59287**
	40 Weeks	0.45365	**0.73000**	0.39484	-0.07677	0.46271	**0.60605**
	50 Weeks	0.59565	0.22213	**0.61563**	0.43440	0.34322	**0.71099**
Fecundity	Spawning	-	-	0.02072	-0.13364	-0.11082	0.17516
	10 Weeks	-0.23117	-0.35285	-0.36111	-0.41122	-0.30188	0.15713
	20 Weeks	0.41725	0.54093	0.56586	0.06789	0.12526	0.26753
	30 Weeks	-0.19703	0.08621	0.28660	-0.18363	0.04873	0.12853
	40 Weeks	0.13119	0.28100	0.48765	-0.03984	0.09320	0.13004
	50 Weeks	0.66746	0.46033	0.12112	0.20698	-0.03676	0.24862

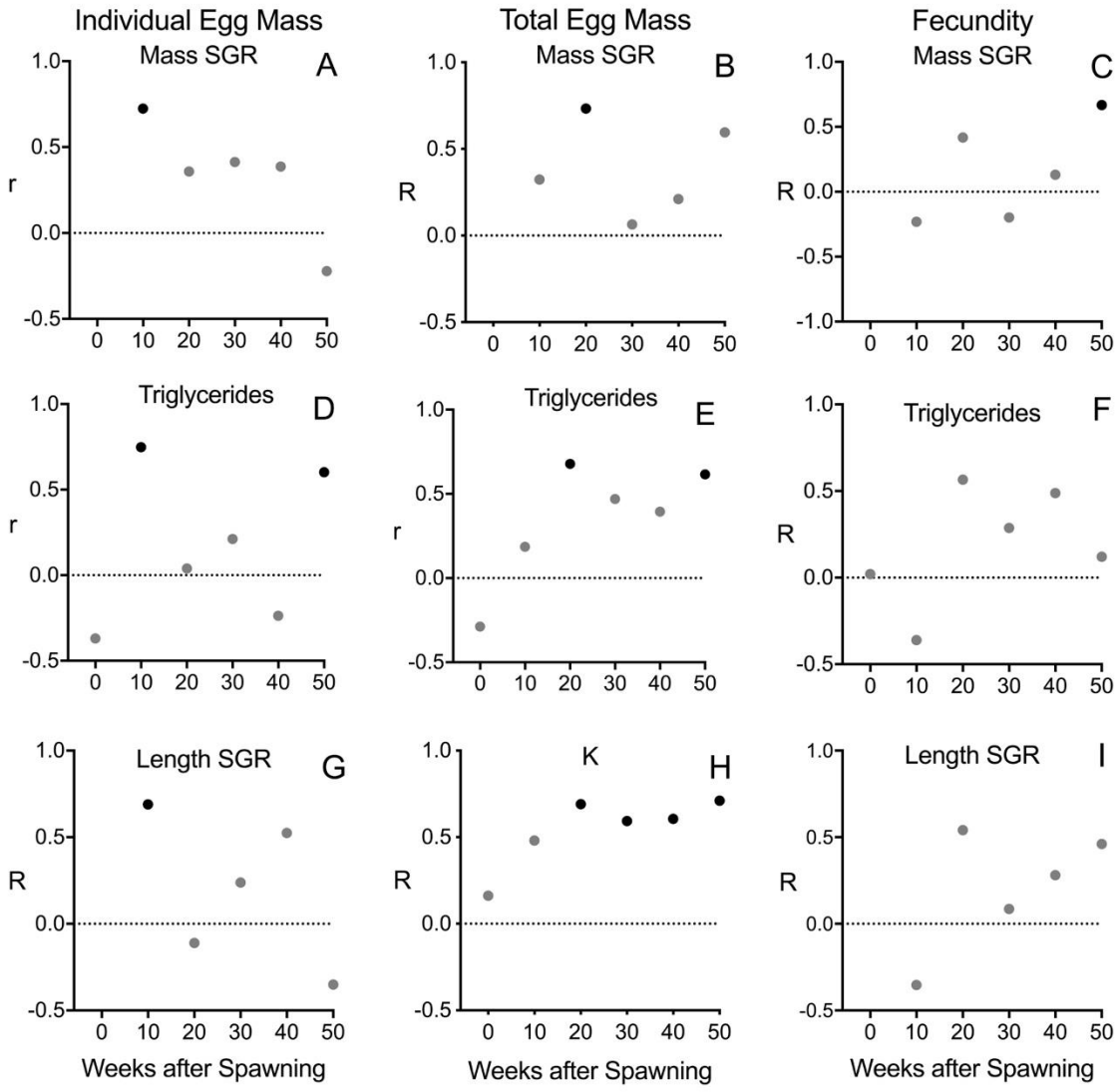

Figure 3.D 7: Correlation coefficients (R) over weeks after maiden spawning for relationships between individual egg mass (A, D, G), total egg mass (B, E, H), and fecundity (C, F, I), with mass specific growth rate (mass SGR), length specific growth rate (length SGR), plasma triglycerides, and Fulton's condition factor (K) in female steelhead trout. Specific growth rates were calculated over the preceding 10-week period. Black points indicate statistically significant linear regressions ($P < 0.05$), and grey points indicate non-statistically significant linear regressions.

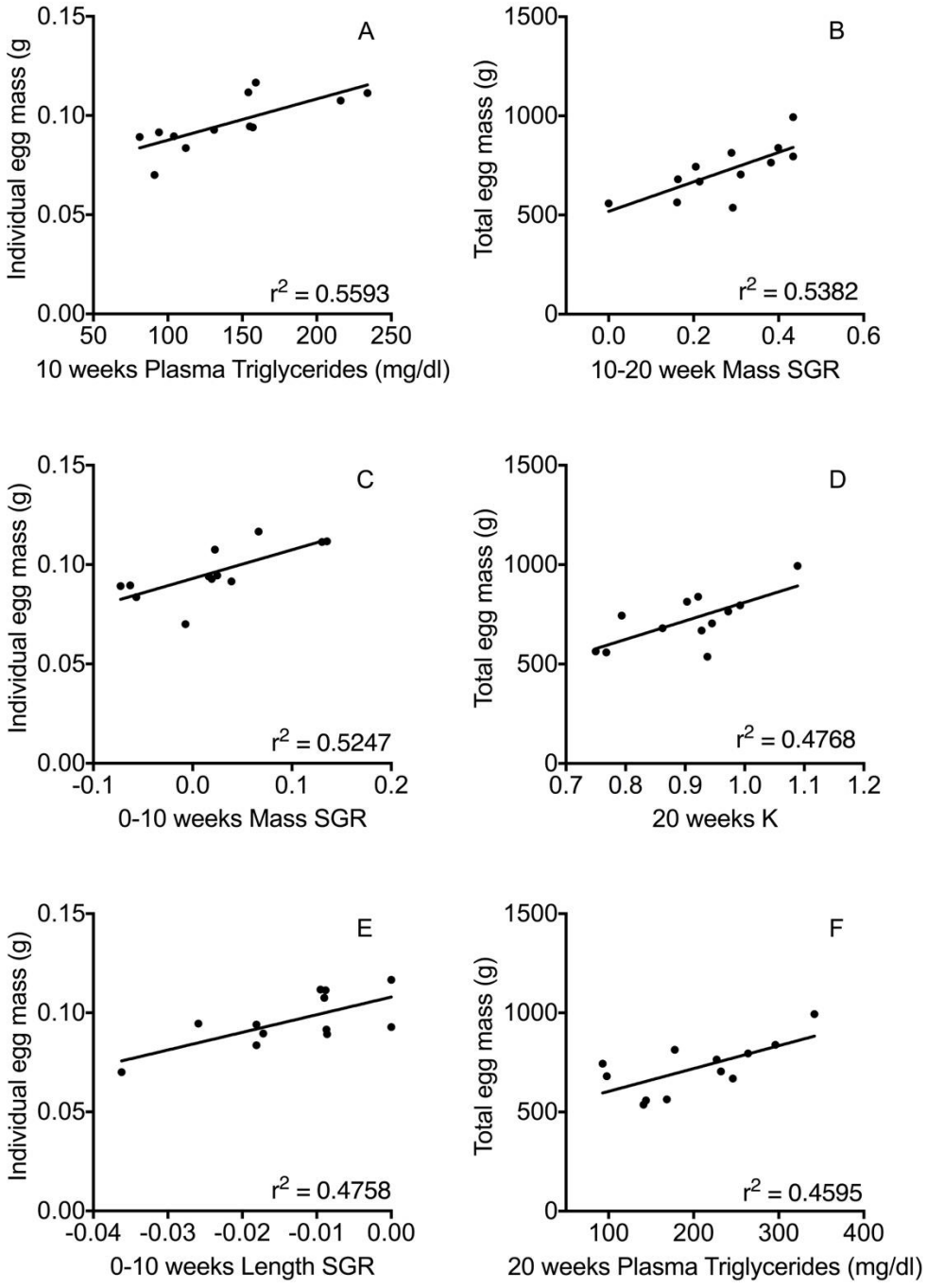

Figure 3.D 8: Relationships between repeat spawning individual egg mass (A, C, E) and total egg mass (B, D, F) at repeat spawning with plasma triglycerides (A, F), mass specific growth rate (B, C), length specific growth rate (E), or K (D) at times when the relationships were significant ($P < 0.05$) and first peaked in strength when compared over time.

Discussion

Evidence from this study indicates condition-dependent post-spawning survival and reproductive performance in repeat spawning female steelhead. Evidence herein is also consistent with a condition-dependent rematuration decision occurring prior to maiden spawning. Post-spawning survival was condition-dependent based on functional measures of physiological condition: fish with greater TG and OS at maiden spawning had an increased probability of survival. Reproductive performance at consecutive repeat spawning was condition-dependent during the period immediately after spawning: IEM was most strongly correlated with growth and TG during the first 10 weeks after spawning and TEM was most strongly correlated with growth, TG and K during the second 10-week interval after maiden spawning. The consecutive rematuration decision was positively related to maiden reproductive effort, consistent with the possibility that both reproductive investment at maiden spawning and consecutive rematuration depend on condition at time points before maiden spawning. Maiden reproductive performance (TEM) and measures of physiological condition at maiden spawning were statistically significant but weakly negatively correlated (r^2<0.06), providing little evidence for a biologically significant tradeoff between maiden reproductive investment and condition. Consistent with this, maiden reproductive performance was not negatively related to post-spawning survival nor to consecutive rematuration. Finally, measures of physiological condition indicating the organism's functional abilities, such as the ability to mobilize stored lipids as TG, maintain OS in an optimal range, and growth rates, were better predictors of survival and reproductive performance than physiological condition measures indicating energetic status, such as ML and Fulton's K.

Condition-Dependent Survival

Post-spawn mortality averaged >50% in both years, with the number of surviving females leveling off by 2.5 months. Post-spawning mortality is potentially due to a variety of factors: handling stress, injuries during migration or spawning, difficulty reactivating the digestive tract, inability to switch off the reproductive mode, diseases and parasites, and energetic costs associated with an approximately 6 months fasting freshwater spawning migration and ovarian development. Mortality in the present study was higher than that reported for Atlantic salmon kelts reconditioned in freshwater or brackish salt water (14-38%) (Crim et al. 1992; Dumas, et al. 1991; Johnston et al. 1987), but was in the range reported for other programs for steelhead and Atlantic salmon kelts reconditioned in freshwater (38-80%) (Hatch et al. 2013a; Moffett, et al. 1996). Surviving spawning and the concurrent trauma requires accessing stored energy and the ability to maintain homeostasis. This study employed condition measures indicating energy reserves (K, ML), as well as those more directly reflecting the ability to maintain homeostasis: plasma triglyceride (TG) level, osmoregulatory function measured as plasma osmolality (OS), and immune system function at the time of spawning as the ability to resist parasites (PL).

Significant relationships with survival were not found with the more traditional, energy reserve indices of condition (K, ML), nor was a negative relationship found with PL. In the present study, we measured condition factor at spawning as "somatic" mass excluding eggs, such that it was independent from total egg mass, one of our measures of reproductive investment. Similarly, in

a reconditioning study using Atlantic salmon, K was not found to be predictive of post-spawning survival (Crim et al. 1992). Upon later investigation, Crim et al. (1992) discovered parasites in many of the mortalities. It is possible that parasite load was not a significant predictor of survival in the present study because all study subjects were given an effective treatment for copepods at the time of spawning. The treatment, however, would only interfere with the impact of the copepod parasites following its application. Any impact of the copepods on individuals prior to spawning would remain as a potential impact on post-spawning mortality. The lack of a relationship with post-spawning mortality suggests that the copepods at the infestation levels observed did not substantially impact the health of these fish. Up to 120 adult *S. californiensis* per kg of fish were reported as a heavy but sublethal infestation in a captive population of rainbow trout (Roberts, et al. 2004), suggesting that levels observed in the present study (approximately 1-11 adult *S. californiensis* beneath one opercula) would not be expected to have lethal effects unless further exacerbated by interacting stressors. ML was very low in all fish spawned, averaging ~0.9-1%. However, levels of 0.5% or lower were measured in study fish, so this is not the minimum muscle lipid level detectable for the instrument used. In order to use muscle lipid stores to support metabolism, post spawning kelts must be able to mobilize these stores from the muscle into the circulation. Many mortalities during the first 70 days after spawning were observed with muscle lipid levels above the minimum detectable with the instrument used (data not shown). Thus, the ability to mobilize stored lipids from body stores (e.g., liver) may be a better indicator of physiological condition than a measure of muscle lipid reserves. This is supported by our finding of a significant relationship between TG and post-spawning survival.

Plasma Triglycerides.–

Survival and TG level were positively related. For mortalities during the first 70 days after maiden spawning, the relationship between survival duration and TG level appeared consistent over the range of the data, without any apparent threshold effects. TG on average was greater in survivors to 70 days than in mortalities within each year. The positive relationship between TG level and survival is most likely because fish with higher TG level showed a stronger ability to mobilize stored energy to meet the demands required to survive spawning (resume feeding, re-generate the digestive system, transition out of the reproductive mode, fight parasites, infection, stress, etc.) than fish with lower TG levels. TG levels on average for both survivors and mortalities were consistent with non-fasted, sedentary juvenile rainbow trout (Simpkins et al. 2003), and were greater than post-spawning TG levels in brown trout (Gauthey et al. 2015). Lipids measured in the plasma prior to vitellogenesis in rainbow trout were found to be used for energy (plasma triacylglycerols or triglycerides), as opposed to lipoproteins for vitellogenin synthesis, consistent with a relationship between circulating TG and survival (Bon et al. 1997).

Plasma Osmolality. –OS was positively associated with survival and with survival duration. This association was particularly strong over the low range of the data, such that fish with low OS were highly subject to mortality in the early days after maiden spawning. This suggests that there may be a threshold below which low OS indicates impending mortality. The body fluids of fish have greater solute concentration than found in freshwater, so they must take up ions through active transport mechanisms in the gill and excrete excess water through the kidney to maintain OS in a narrow range, approximately 308.5 +/- 3.9 mOsm/kg for rainbow trout in

freshwater (Oguri and Ooshima 1977). Low plasma osmolality in individual fish indicates failure of these systems, which explains the association with mortality. Slightly lower values than the normal biological range were observed in the present study: 295-303mmol/kg. The discrepancy in units is due to previous reporting of osmolality in different units: mOsm/kg. Standard International units of osmolality (mmol/kg) were adopted for increased accuracy as early as 1995, as mOsm/kg implies a linear relationship between solute concentration and dissolution, which is misleading due to residual attraction between dissolved ionic compounds (*i.e.*, NaCl) (WESCOR manual, Elitech, Puteaux, France). Consistent with our study, reduced osmoregulatory ability, measured as decreased OS, was associated with post-spawning senescence in sockeye salmon in freshwater (~20% reduction, ranging from 315- 235mOsm/kg, with fish becoming moribund below 280mOsm/kg) (Jeffries et al. 2011). Also consistent with our study, Jeffries et al. (2011) observed a time effect, whereby osmolality declined days in advance of changes in other variables associated with post-spawn mortality, suggesting that failure of the osmoregulatory system may be an early indicator of impending mortality. Similarly, plasma osmolality was used as a measure of physiological recovery in post-capture survival of gill-netted coho salmon (*Oncorhynchus kisutch*) in saltwater, and survival was positively associated with decreases in plasma osmolality (Farrell, et al. 2001).

Life history trade-off. –

This study also provides an examination of whether a reproductive life history trade-off occurred between energy reserves spent on maiden reproductive performance (TEM, E2) and post-spawning survival. Post-spawning survival was not significantly negatively correlated with TEM in the whole model, so a life history trade-off between reproductive performance and survival is not supported by the data. Although TEM was a significant predictor of post-spawning survival in the univariate model with years combined, this effect was largely driven by the ~15% greater TEM in 2016 versus 2015 in combination with higher survival in 2015. In the AICc comparison of the univariate model (TEM as a predictor of survival) with the whole model, there was no support for the univariate model (ΔAIC=48) [Analysis not shown]. Our data were not consistent with a reproductive life history trade-off between maiden reproductive investment and survival to future spawning that was proposed for a population of winter-run steelhead from the Hood River, Oregon, based on analysis of adult to adult returns (Christie et al. 2018). However, this study did not directly measure maiden reproductive investment, and there may be alternate explanations for the adult to adult return result. E2 level at spawning did not predict subsequent survival. During the year before repeat spawning, E2 levels increased to a peak 10 weeks before spawning, and then declined (Section 3C). It is possible that E2 levels at an earlier time point could indicate reproductive investment better than levels at spawning. However, E2 levels over the year before repeat spawning were not significantly correlated with any measure of reproductive performance at repeat spawning (Table 3.DS7), suggesting that E2 levels may not be a good proxy for reproductive investment.

Relationship between Maiden Reproductive Investment and Rematuration

Consecutive rematuration was 30% for fish surviving to 30 weeks post-spawning (40% in 2016). Similarly, rematuration was estimated at 38% in 2002-2004 for wild Snake River steelhead surviving to make a consecutive repeat spawning migration after being tagged as out-migrating

kelts at LGR (Keefer et al. 2008). Consecutive maturation averaging near 60% is typical of wild kelt reconditioning projects in the Columbia River Basin, somewhat higher than proportions found in DNFH-origin kelts (Hatch, et al. 2017).

The present study did not find any significant relationships between measures of physiological condition and consecutive rematuration, suggesting that the reproductive decision is not condition-dependent at time of spawning. There was also no support for a life history trade-off between consecutive rematuration and reproductive investment (TEM) at maiden spawning. Interestingly, consecutive rematuration was positively related to maiden TEM, consistent with the possibility that both TEM and consecutive maturation were positively regulated by condition at time points before spawning. Individual fish in better condition would both invest more in TEM and be more likely to have initiated rematuration as a consecutive spawner before maiden spawning. These fish would then have begun investment of energy into the second batch of developing oocytes before maiden spawning, which would differentially reduce energy stores versus skip trajectory fish, potentially resulting in no measurable difference in condition at spawning. Evidence of the growth of a second batch of developing oocytes prior to ovulation of the imminent batch has been found in rainbow trout and steelhead (De Mones et al. 1989; Penney and Moffitt 2014a). Support for part of this model is provided by the positive relationships found between energy reserves early in the year before repeat spawning and reproductive performance at repeat spawning (Section 3E). In contrast to our results, large scale analysis seeking to explain the evolution of senescence in Pacific salmonids found increased reproductive investment and egg size to be correlated with a decreased likelihood of repeat breeding (Crespi and Teo 2002).

Condition-Dependent Reproductive Performance at Repeat Spawning

Measures of homeostatic ability (TG and growth) were greater in skip than consecutive fish during the first 0-20 weeks of the year prior to repeat spawning, whereas measures of energetic reserves (ML, K) were greater in skip spawners over the entire maturation year (Section 3C). Skip spawners made substantially greater reproductive investment (TEM) than consecutive spawners (Jenkins et al. 2018b). Taken together, these results indicate that physiological condition during the year prior to repeat spawning is related to reproductive investment at repeat spawning. These findings are supported by the present analysis of condition-dependent reproductive performance at consecutive repeat spawning. IEM was most strongly positively correlated with growth rates (mass, FL) and TG at 10 weeks after maiden spawning, TEM was most strongly positively correlated with mass growth rate, TG, and K at 20 weeks after maiden spawning, and fecundity was most strongly positively correlated with growth rates (mass, FL) and TG at 20 weeks after maiden spawning, although the correlations with fecundity were not quite significant. These findings suggest that, among fish on the consecutive spawning trajectory, egg size is set first based on condition at approximately 10 weeks after spawning, and then fecundity (and consequently TEM) is set based on condition at approximately 20 weeks after spawning. A study in rainbow trout found that reproductive effort was impacted by feed restrictions in the period close to a year prior to spawning, but not at time periods approaching repeat spawning (Bromage et al. 1992). Similarly, feed restriction implemented prior to the theorized critical period for maturation negatively impacted

reproductive investment in lake charr in terms of both ovarian lipid density and fecundity (Henderson and Wong 1998), which was not the case for feed restriction implemented later during oogenesis. The present data suggest that not only is reproductive investment plastic based on physiological condition, but that physiological condition during early- to mid-oogenesis will impact the maternal and offspring generations differently through microevolutionary, intergenerational trade-offs (Stearns 1992). Restricted energy during early oogenesis will reduce egg size, potentially detrimental to both offspring survival and maternal fitness, yet restricted energy during mid-oogenesis will reduce offspring number, potentially detrimental to maternal fitness, but beneficial for the survival of individual offspring via reduced competition. In contrast to the present results, condition at breeding in female albatross was associated with rematuration decision but not reproductive performance (Crossin et al. 2012), likely due in part to different physiological mechanisms regulating energy allocation between reproductive and somatic processes than in the present model.

Management Implications

The ability to access energy and maintain osmotic balance, measured as TG and OS in steelhead in the weeks prior to spawning, are particularly important to post-spawning survival. Conditions in the river prior to spawning, as well as hatchery holding practices, can be expected to favor post-spawning survival and consecutive rematuration. The relationships between physiological condition, post-spawning survival, and rematuration may differ between summer-run steelhead, which hold in freshwater for many months prior to spawning, as compared to winter-run steelhead, which enter freshwater often just prior to spawning. Commonly utilized condition measures that serve to indicate energetic reserves, such as condition factor and muscle lipids, may not reliably predict post-spawning survival in various taxa as well as functional measures indicating the ability of the individual to access energy and/or maintain homeostasis, based on the present results. Increasing our understanding of condition-dependent traits, and relevant measures of condition, may provide clues as to how populations will react to a changing environment (Hutchings 2011b).

No evidence was observed for a trade-off between maiden reproductive investment and post-spawning survival (Stearns 1992), providing no support for the idea that iteroparity selects for fish with poorer maiden reproductive investment, as was hypothesized for winter-run steelhead in Hood River (Christie et al. 2018). Thus, kelt reconditioning would not be expected to select for fish with poorer reproductive investment, a concern which has been raised about reconditioning programs (Abrahamse and Murdoch 2014). Females with greater maiden reproductive investment were more likely to mature as consecutive spawners, suggesting that both reproductive investment and consecutive spawning may be condition-dependent traits, and raising the key question of when condition influences these outcomes. Effectively, kelt reconditioning as a recovery effort, and/or managing for increased rates of iteroparity, should not be expected to select for high nor low reproductive investment, nor either life history, but rather should increase life history diversity and increase population stability during bottleneck years (Moore et al. 2014).

This study provides evidence of modulation of reproductive investment, and modulation of energy allocation within reproduction, based on physiological condition early during oogenesis in steelhead. This provides a mechanism linking ocean and other environmental conditions with reproductive performance in salmonids and potentially other species of management interest. As with plasticity in maturation schedules (*i.e.*, consecutive and skip spawning), plasticity in reproductive effort (egg size, egg number) related to physiological condition during oogenesis should be incorporated into recruitment models, particularly in a changing environment.

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Section 3.E Effects of post-spawning fasting on growth, life history trajectory, and reproductive development in a hatchery model of steelhead kelt reconditioning (2018 update)

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. 2017). 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 (Jenkins et al. 2018). 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 2011). 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 2011), 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. 2006; 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. 1992). In repeat spawning rainbow trout, energy restriction after spawning resulted in reduced plasma estradiol (E2) levels within 10 weeks after spawning (Caldwell, et al. 2013a). 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 (Section 3.C) (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 and 2018, hatchery origin maiden female steelhead were air spawned at DNFH on five egg takes in February (Table 3. E1). 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.

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 in 2017, 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. In 2017, fish from takes one and three were fasted for 10 weeks, and fish from take 2 were fasted for 11 weeks. In 2018, both takes were fasted for 10 weeks. After 10 weeks, 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.

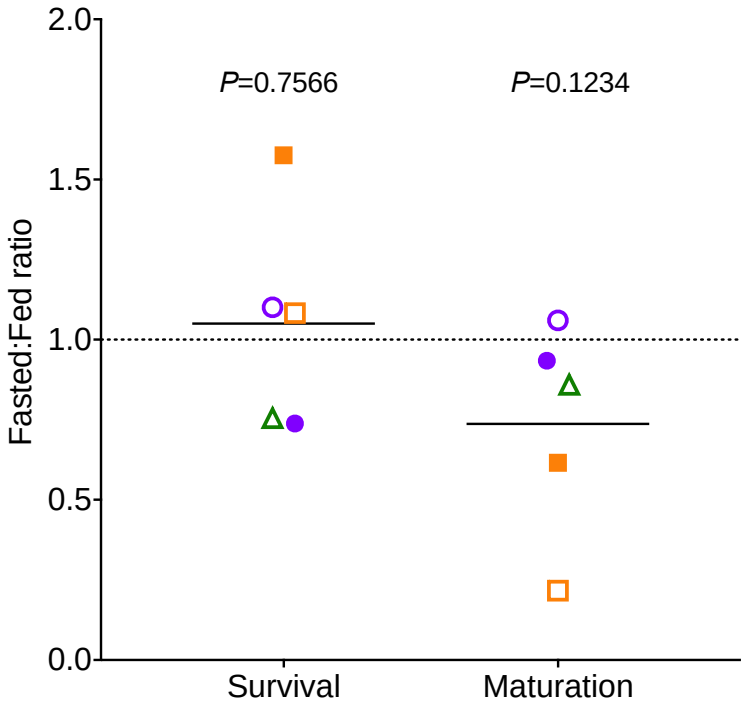
Survival decreased substantially from take 1 to take 3 in 2017 and take 1 to take 2 in 2018 (Table 3. E1). However, survival was not significantly affected by feeding treatment (Figure 3. E1, One-Sample T-test, $p=0.7566$). Maturation percentage did not differ substantially between fasted and fed fish for most replicates. Overall, the effect of fasting on maturation was not significant. However, there was a tendency toward decreased maturation in fasted treatments (Figure 3. E1, One-Sample T-test, $p=0.1234$).

Table 3.E 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%	6	14	30.0%
	Fasted	32	11	68.8%	7	15	31.8%
Two, 2/21/2017	Fed	32	20	37.5%	5	8	38.4%
	Fasted	32	19	40.6%	1	11	8.3%
Three, 2/28/2017	Fed	31	22	29.0%	3	6	33.3%
	Fasted	32	25	21.9%	2	5	28.6%
All	**Fed**	**95**	**54**	**44.2%**	**14**	**28**	**33.3%**
	Fasted	**96**	**55**	**42.7%**	**10**	**31**	**24.4%**

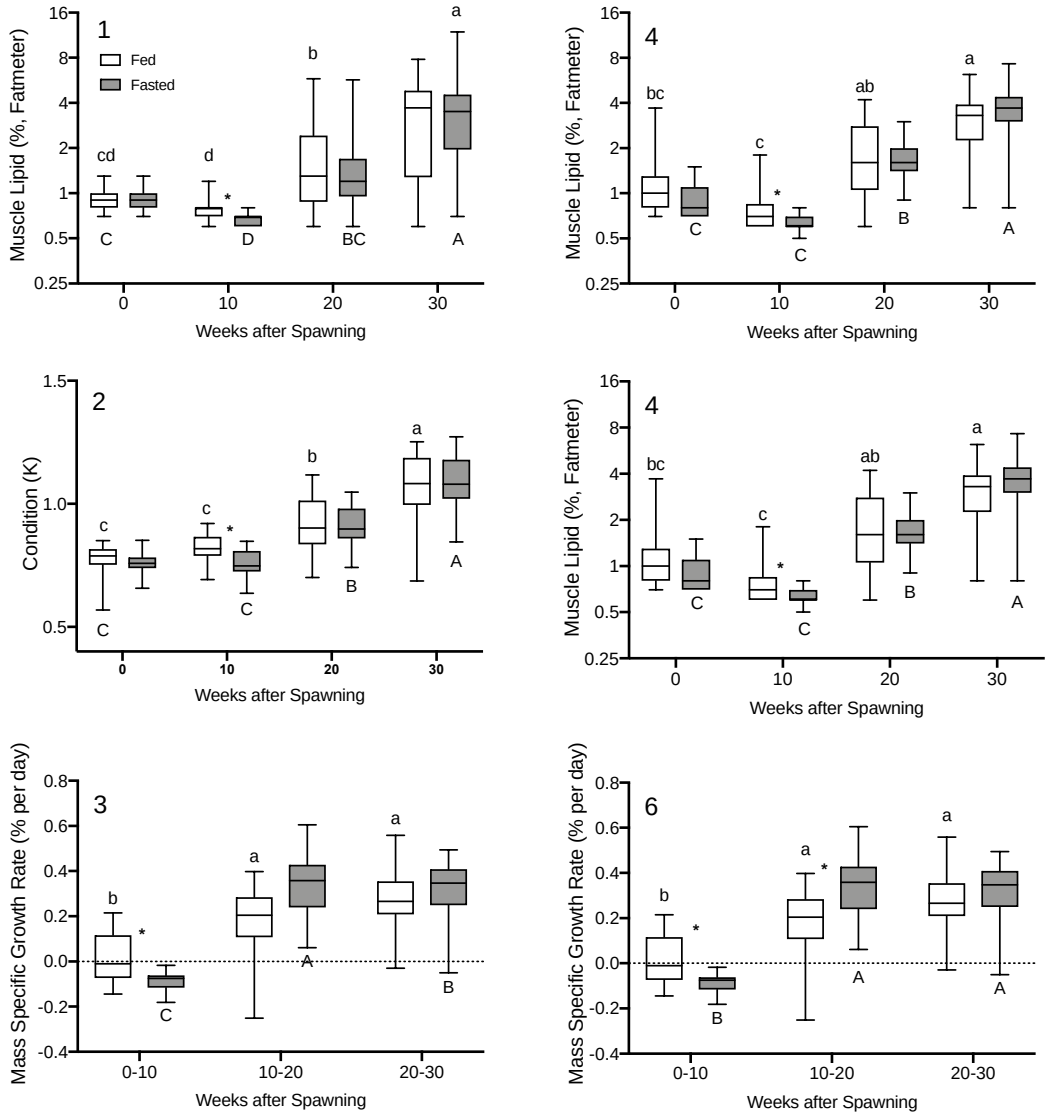
Take	Treatment	Fish #	Mortalities	Survival %	Maturing	Non-maturing	Maturation %
One, 2/6/2018	Fed	26	9	65.38%	13	4	76.47%
	Fasted	29	15	48.28%	10	4	71.43%
Two, 2/20/2018	Fed	32	24	25.00%	4	4	50.00%
	Fasted	33	20	39.39%	4	9	30.77%
All	**Fed**	**58**	**33**	43.10%	**17**	**8**	68.00%
	Fasted	**62**	**35**	43.55%	**14**	**13**	51.85%

Figure 3.E 1Ratios of survival and maturation percentages between fasted and fed treatments (one-sample t-test compared to 1). Spawning events are coded by shape (circle = take 1, square = take 2, triangle = take 3; open symbols = 2017, closed symbols = 2018).



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 3. E2). Growth rates in fasted fish were significantly elevated during weeks 10-20 after feeding was initiated in 2018, and a similar trend was observed in 2017 (t-test, p = 0.1297).

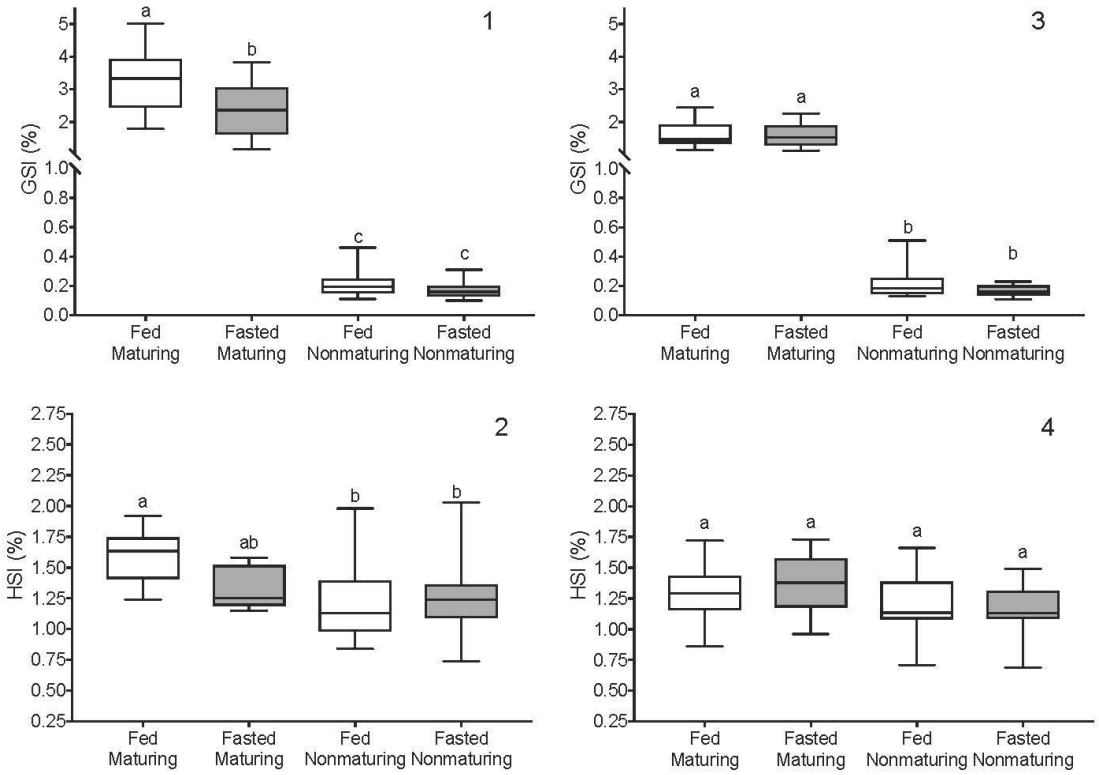
Figure 3.E 2: Adiposity metrics and growth in fully fed fish (white) and fish fasted for the first 10 weeks after spawning (grey) in 2017 (1-3) and 2018 (4-6). Box heights indicate interquartile range, horizontal lines indicate the median, and whiskers show the date range. Asterisks indicate significant differences at each time point (T-test, p < 0.05). Time points within treatment groups sharing the same letter do not differ significantly (One-Way ANOVA followed by Tukey's Multiple Comparison test, p < 0.05).



Fasted fish muscle lipid levels and Fulton's condition factor recovered to levels not significantly different from those of fed fish at weeks 20 and 30.

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 in 2017 but not in 2018 (Fig 3. E3). Maturity status and fasting treatment did not consistently affect hepatosomatic index (HSI).

Figure 3.E 3: Gonadosomatic index (GSI) and Hepatosomatic index (HSI) in fish that were either fully fed (white) or fasted (grey) from 2017 (1-2) and 2018 (3-4). All fish were 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 significantly reduced maturation rate 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. Although these results are surprising, they are consistent with the early increases in growth rates and plasma triglyceride levels reported in Section 3.C. We believe that a reasonable interpretation of these results is that the critical period for the rematuration decision occurs before spawning in steelhead kelts. 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). 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 caused arrest of rematuration in some fish, resulting in the trend toward decreased maturation rate that we observed. However, this effect was not nearly as strong as expected, consistent with the possibility that rematuration is chiefly regulated by energy reserves at some time point before spawning.

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, and in 2018, fasted fish exhibited compensatory growth after the period of feed restriction. Thus, there may be a physiological process of recovery from spawning that occurs over the first 10 weeks after spawning, during which feeding, and growth are reduced.

The effect of fasting during the 10-week period after spawning on GSI and HSI was not consistent between the two years of the study. In 2017, GSI was reduced to approximately 75% of the fed level in fasted fish, whereas in 2018, no differences were detected. The difference between years may relate to the stage of ovarian development at the final sampling where GSI was assessed. 2017 GSIs were in the 2-3+% range, versus less than 2% in 2018. Plasma estradiol levels may provide additional insight as to whether fasting impacted reproductive development and investment in rematuring fish. The elevated HSI in fed maturing fish in 2017 may be a result of greater vitellogenin production in these 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). Exogenous vitellogenesis would be expected to have begun near the end of this study. Measurement of plasma vitellogenin levels may provide additional insight as to whether the stage of vitellogenesis was different between the two years of the study or between fed and fasted fish.

Section 3F: Developing a time-resolved fluorescence assay for growth hormone (GH) in steelhead (*O. mykiss*) Progress report on assay development

Introduction

The growth hormone (GH)/insulin like growth factor (IGF) endocrine axis regulates growth in vertebrates. In addition, the GH/IGF axis interacts with the reproductive endocrine axis in the regulation of puberty. Components of the GH/IGF axis, particularly plasma levels of IGF1, are used as indicators of growth and metabolic status in salmonids and can be measured in a non-lethal blood sample. Therefore, we sought to establish assays for plasma GH and IGF1 levels in our laboratory. Establishment of the plasma IGF1 assay is now compete.

Steelhead fast during their spawning migration. Snake River steelhead kelts show significant atrophy of the digestive tract (Penney and Moffitt 2014a). Getting kelts to initiate feeding appears to be a critical step in reconditioning (Evans, et al. 2001). The stomach hormone ghrelin has been found to stimulate feeding in rainbow trout, and strongly stimulates pituitary GH secretion. In a previous experiment, we explored whether long term ghrelin or GH administration might stimulate appetite in kelt steelhead. To do this, we tested whether these treatments were effective in domestic rainbow trout. Our findings were limited by the lack of a properly validated assay that could determine plasma GH (Hatch, et al. 2018). Therefore, we began developing a competitive time-resolved fluorescence (TRF) immunoassay for rainbow trout GH. As such, work on the development of a TRF assay for GH in steelhead plasma began this year by working with PerkinElmer to obtain a europium-labeled GH protein for use in a TRF assay. The assay was developed to be run on the recently purchased Victor X4 (PerkinElmer). Assay optimization produced a highly repeatable and sensitive assay that detects levels down to 0.4 ng/mL and up to 20 ng/mL. Plasma and crude pituitary extract have been used to start validation, with biological validation using samples collected from ghrelin-injection and ghrelin mini-osmotic pump experiments to follow soon. What follows is a summary of the work conducted so far and the plan we have for completing validation of the assay.

Methods

Standard TRF assay methods were employed to begin development of the assay (adapted from protocols known to be working in several labs).

Europium Labeling

ProSpec produced a custom recombinant rainbow trout GH protein, which was sent to PerkinElmer for labeling with Europium. Europium labeling was performed at PerkinElmer's Boston location using the proprietary DELFIA Eu-N1 ITC lanthanide chelate (Ref 1244-302, PerkinElmer).

General Plan for Time Resolved Fluorescence Immunoassay:

After several attempts using a TRF protocol that was developed in the lab and is working failed to produce a repeatable standard curve, a step-wise protocol was developed that produced a

repeatable standard curve with higher sensitivity than expected. A brief summary of the optimized 4-day protocol is as follows.

Day 1: Wash plate prior to plating any reagents. Plate primary antibody (20 uL per well at 1:5000 dilution) and/or assay buffer into appropriate wells and incubate for 24 hours with slow shaking.

Day 2: Wash plate. Add 125 uL of standard curve and samples to appropriate wells. Incubate for 24 hours with slow shaking.

Day 3: Do not wash plate prior to label addition. Add 20 uL label (25 ng/mL) to appropriate wells. Incubate for 18 hours with slow shaking.

Day 4: Wash plate. Add 200 uL enhancement solution to all wells and 20 uL label to total count wells. Incubate for 10 minutes with moderate shaking. Read plate on Victor X4.

Results

Assay optimization resulted in finding that a 1:5,000 dilution of the primary antibody worked best for both the sensitivity of the assay and giving a total binding: total count (TB/TC) ratio of 33.1% (Fig 3. F1). The range of the assay at this dilution of primary antibody and concentration of label (25 ng/mL) is applicable to the physiological range experienced by salmonids and should detect even the lowest concentrations typically observed by healthy fish (Gomez, et al. 1996). Assay validation began with diluting kelt plasma and crude pituitary extract and detecting parallelism across the usable portion of the standard curve (between 20 and 80 percent binding; Figure 3. F2).

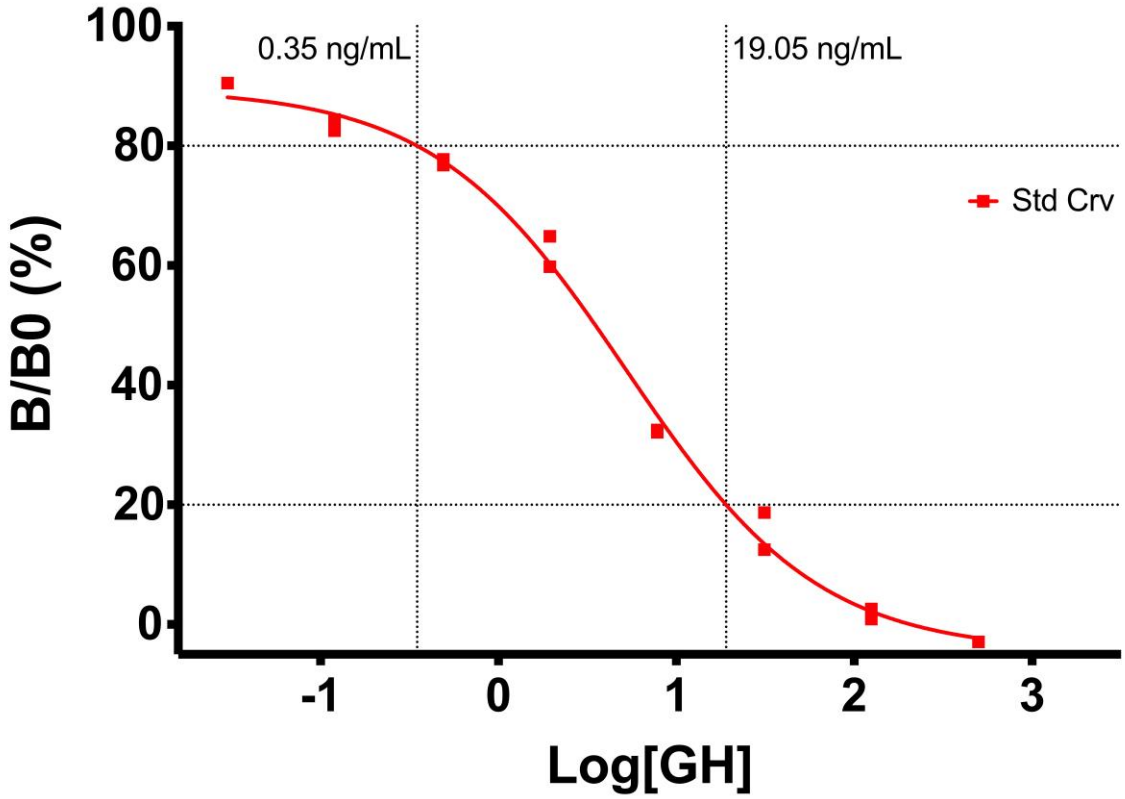


Figure 3.F 1: Optimized standard curve plot from GH TRF assay. The protocol uses the primary antibody (anti-salmon GH) at a 1:5,000 dilution and the labeled recombinant rainbow trout GH at a concentration of 25 ng/mL and employs 3 overnight incubations to increase the assay's sensitivity and repeatability.

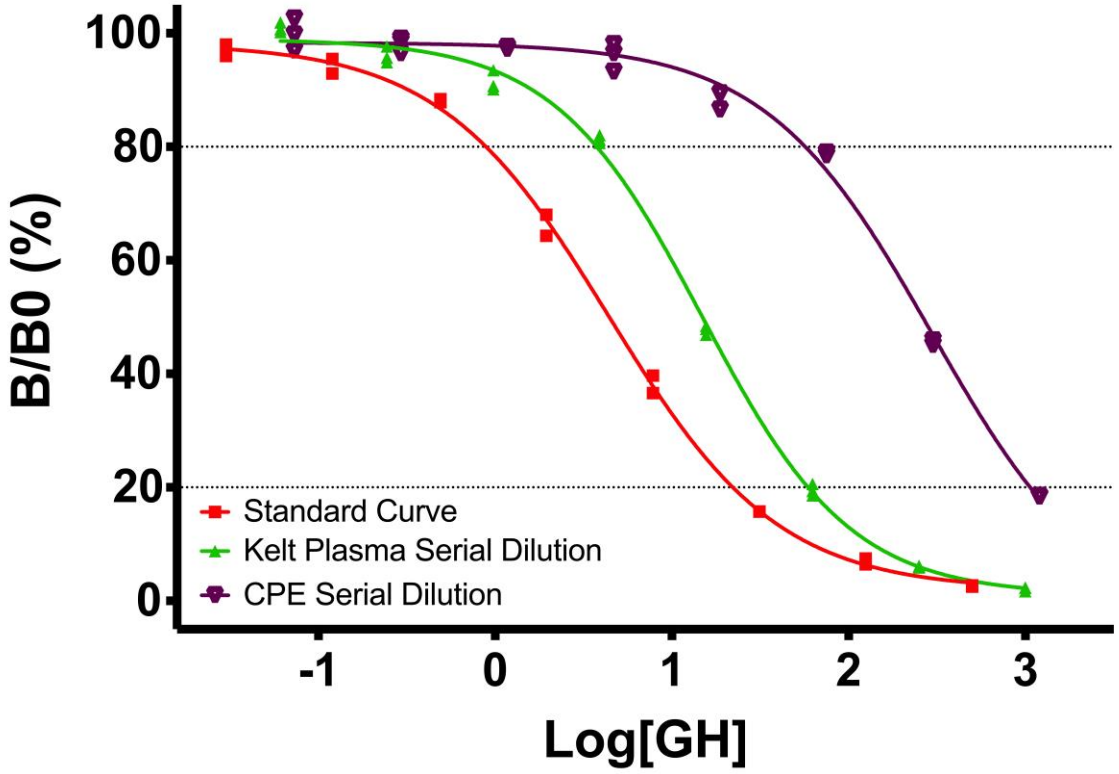


Figure 3.F 2: Initial efforts to validate the GH TRF assay for use with salmonid plasma included running the assay with serially diluted steelhead kelt plasma (purple symbols and line) and serially diluted crude pituitary extract (green symbols and line; collected from juvenile rainbow trout).

Discussion

Optimization of the GH TRF assay produced an assay with greater sensitivity than anticipated. The increased sensitivity, which is on par with mammalian GH TRF assays, will enable measurement of plasma GH levels in a greater variety of life stages and salmonid species. Assay optimization continued on to validating the assay for use with biological samples. We collected and produced a crude pituitary extract from juvenile rainbow trout and used samples collected from steelhead kelts to run in the assay. Both were serially diluted and run against the standard curve and were found to be parallel to the working portion (between 20% and 80% binding) of the curve.

Our future plans include continuing to complete biological validation of the assay with samples collected from the experiment outlined in section C3.D of the 2017 BPA report (Hatch et al. 2018) and an experiment from 2010 (Branstetter, et al. 2011). We will also be submitting a paper to a peer-reviewed journal.

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

Introduction

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

Methods

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

In-stream PIT arrays exist in both 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 49 fish that we detected as maiden and kelts returned to spawn in the same tributary or were detected again at Roza Dam

(Naches/Upper Yakima population). We have found no evidence of straying in these sampled fish. Second, PIT detections of reconditioned kelt steelhead at in-stream arrays in Satus, Toppenish, Ahtanum creeks, the lower Naches River, and Roza Dam (Upper mainstem Yakima River) accompanied by matching genetic stock identification 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.

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

Table 4. 1: Observed and inferred homing from artificially reconditioned kelt steelhead in Omak Creek and the Yakima River from 2001 to 2018. 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

	Conclusive Evidence for Homing			Consistent with Homing				
Location	**A. Maiden/ Repeat Spawner Tag Detection**	**B. Repeat Spawner Tag Detection + GSI confirmation**	**C. Conclusive Homing total A+B**	D. **Prosser Det. Only**	**E. Prosser Det./Sunnyside Instream Det (Operational: Nov. 2016)**	**F. Prosser Det./Out-migrating 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	49	313	362	655	56	65	107	883
Omak Cr	11	-	11					
Total	60	313	373	655	56	65	107	883

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

Introduction

In this section we evaluate kelt steelhead management options and we compare three geographically different long-term reconditioning programs. It is thought that downstream passage through the hydrosystem limits repeat spawner steelhead in the Columbia River (Wertheimer and Evans 2005; Wertheimer 2007). In recent years, there may be some evidence that emigrating kelt survival has improved as a result of smolt management actions (e.g. removable spillway weirs, mandated spill). Colotelo et al. (2014) reported that 27.3% of kelts tagged at or upstream of Lower Granite Dam (rkm 695) survived to Martin Bluff (rkm 126) passing 8 hydroelectric dams along the way. Collecting and transporting kelt steelhead around hydroelectric projects could improve emigration survival and result in increased repeat spawner abundance. Our goal is to compare the benefits of long-term reconditioning to alternate kelt management treatments like transporting kelts downstream of the hydropower system. Our team recently published a manuscript comparing kelt management options (Trammell et al. 2016).

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

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

And,

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

Methods

Kelt Treatments

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

In-river migration (control).

Fish were systematically chosen, taking every tenth fish that came into the facility. 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 (2004-2011) 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 kelt 2x (4/2=2) versus leaving the kelts in the river. Comparisons were made within each year and across years using weighted means to account for different sample sizes among years. We calculated benefits for long-term reconditioned kelts from the Yakima River, Omak Creek, Hood River, and Snake River in a similar manner. The reconditioning benefits calculation was the survival rate of long-term reconditioned kelts from each location divided by three different control groups. The control groups were: 1. Survival rates of in-river release groups to BON (the same as the treatment groups). 2. Literature values (Hockersmith et al. 1995). 3. The composition of repeat spawners in the run at large sampled at BON based on scale pattern analysis and prior PIT-tag history. None of these control groups are perfect comparisons, for example survival of the in-river release groups is to BON not the river of origin so these are biased high due to mortality that likely occurs between BON and the river of interest. However, the in-river groups are paired by year with the treatment groups reducing annual variation.

Results and Discussion

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

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

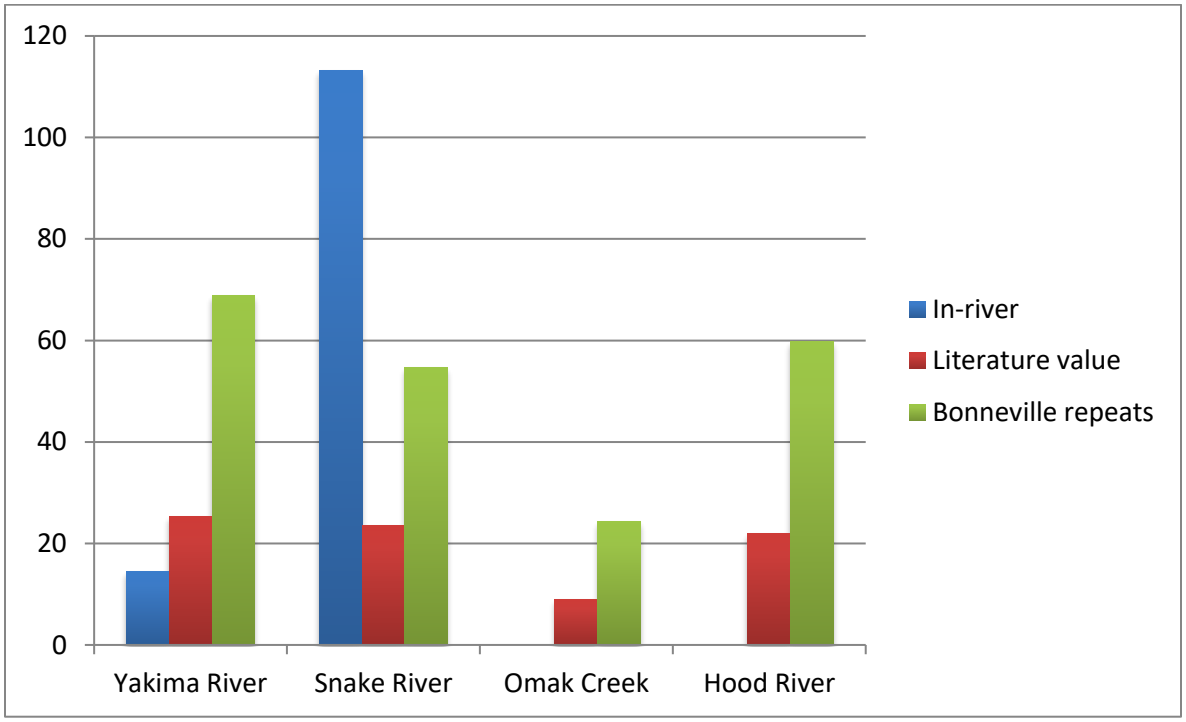


Figure 5. 1: Benefits of long-term reconditioning relative to 3 control metrics. In-river control groups were not available for Omak Creek or Hood River.

Geographic Comparison of Reconditioning Programs

Survival and maturation data from Prosser, Winthrop, and Dworshak and Nez Perce Tribal Hatchery are shown in Figure 5.2. Survivals in the Prosser and Winthrop projects from 2012 onward have consistently been in the 40 – 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. All CRB kelt projects now treat for copepods using emamectin benzoate by intraperitoneal injection. Survival of kelts collected at Lower Granite Dam increased to levels similar to the Prosser project in 2018, suggesting that survivals similar to those found in the Prosser project may be achievable with the Snake River fish.

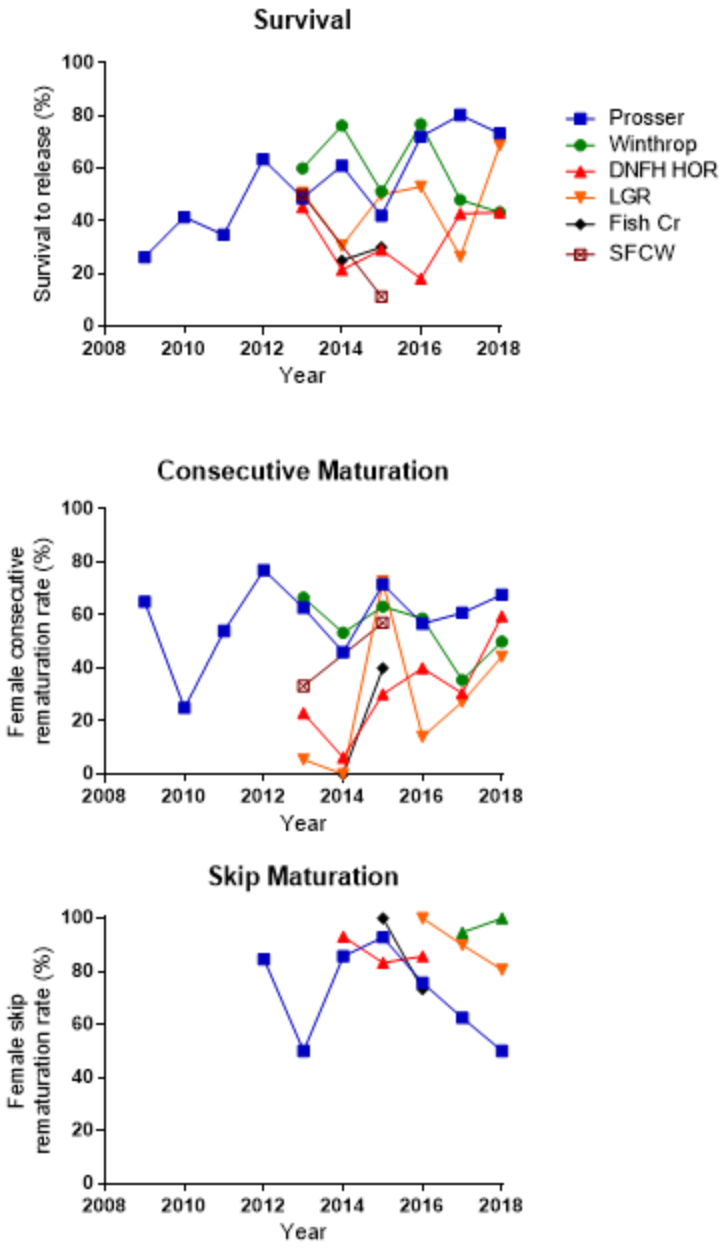


Figure 5. 2:Survival and female consecutive and skip maturation rates in CRB kelt reconditioning projects. Fish reconditioned in the Snake River project were housed at Dworshak and Nez Perce Tribal hatcheries and include air spawned hatchery origin kelts from the DNFH stock (DHFH HOR), kelts collected at Lower Granite Dam (LGR), and kelts collected at Fish Creek on the Lochsa River in 2014 and 2015 (Fish Cr), and air spawned South Fork Clearwater Fish (SFCW) in 2013 and 2015. Maturation data for skip spawners is from non-mature fish from the previous season held over for an additional year.

Survivals of DNFH hatchery fish have been somewhat lower than found for wild origin fish, may be due to stress due to fish anesthesia and processing at the hatchery. 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 a few exceptions, consecutive rematuration rates in the Prosser and Winthrop projects have consistently been in the 50 – 70% range. Maturation rates for Snake River fish have generally been lower. In 2018, consecutive maturation rates for both wild and hatchery origin Snake River kelts increased, to 44.4% in wild fish and 59.6% in hatchery fish. The increase in hatchery origin maturation rate occurred even though half of the fish were fasted for the first 10 weeks of reconditioning (Section C5 in the Physiology chapter). Thus, reasonably high rematuration rates appear to be possible for Snake River fish. The increased maturation rates in both wild and hatchery origin Snake River fish and wild Upper Columbia fish suggests that common environmental conditions experienced before capture may influence consecutive maturation rates.

Skip maturation rates in most CRB kelt reconditioning projects have been uniformly high, ranging from 80 to 100%. Surprisingly, however, skip maturation rates at Prosser, the most well established and longest running of the reconditioning projects, have been lower in the last two years. The reasons for this decrease should be investigated. In other projects, 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 most kelts that are not rematuring after one summer of reconditioning will mature as skip spawners the next year. In addition, skip spawners have larger eggs and are more fecund than maiden or consecutive spawners, and have greater energy reserves at release (Physiology chapter 3 section C, Jenkins 2018). Thus, skip spawners provide a valuable safety net in years when the numbers of maiden spawners or survival and maturation rates of reconditioned kelts are low.

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, Basin wide 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.

In 2018, discussions between CRITFC and BPA continued regarding soliciting for a design and construction firm and development of a M&E and O&M plan. We hope to complete a facility in time for the 2021 kelt migration.

Chapter 7: Accounting for measurement errors and life history stage variability in steelhead survival

Evaluation of steelhead kelt reconditioning effort needs to distinguish between the failure of kelts to repeat spawn, and the failure of smolts to survive to reproduction. Yakima River summer steelhead kelts have been reconditioned since 2000. Reconditioned kelts have returned to spawn in the Naches, Satus, and Toppenish drainages, as well as in the upper mainstem of the Yakima River. Smolts and adults have been enumerated at Prosser Dam, and spawners have been enumerated at each of the drainages. A proper separation of smolt production survivals has been hampered by the difficulty in measuring smolt out-migrating abundances. Flow variability, entrainment rate sensitivity to flow, and sampling rates have led to high uncertainty in smolt abundance estimates. By contrast, adult escapement abundances are less uncertain.

Incorporating estimates of these uncertainties into stock recruitment assessments would provide new perspectives on both the underlying productivity of the systems, as well as the relative benefits of kelt reconditioning. As part of the overall evaluation of steelhead productivity and kelt reconditioning in 2017, this project examined life cycle reconstructions of Snake River B-run steelhead populations. We used a state-space model that incorporated prediction and measurement uncertainties. In 2018 we pursue a similar course of analysis, reconstructing Yakima summer steelhead smolt production and life cycle survivals with a state space model that accounts for the uncertainties in spawner and adult abundance, and the uncertainty in the prediction of survival from stage to stage. We make use of environmental condition data to predict survival variability in ocean life stages. This development of a state-space life cycle model of Yakima summer steelhead in 2018 is a step toward reconciling uncertainties in abundance measurements, and variability driven by environmental factors. This chapter describes progress toward the end goal of capturing spatial diversity in tributary production patterns, but the progress described herein is a first step that uses Yakima River steelhead aggregate rather than the longer-term target of tributary specific production.

Data and Methods

We constructed a life cycle model that aggregates Naches, Satus, Toppenish, and upper Yakima steelhead into a single aggregate. We obtained age and abundance of adult returns and harvest from NOAA Salmon Population Summary tables from 1985 to 2010. Smolt abundances were obtained from Chandler counts reported in Frederickson *et al.* 2017 from 1985 to 2010. Smolt to adult return rates (SARs) were estimated using the brood year smolt outmigration and brood year adult returns. We obtained ocean environmental variables from NOAA records. We used the upwelling index (UPW) and the Pacific Decadal Oscillation (PDO) in the month of May.

Model

The life cycle model assumes that spawners are counted at Prosser Dam, and both pre-spawn mortality and smolt outmigration survival are implicit in the smolt production function, i.e., $M = M_{T\,sc,}$ where M is the number of smolts at Chandler, M_T is the number of smolts in tributaries, and s_c is the survival of out-migrating smolts from tributaries to the Chandler facility. Smolt production is a Ricker function (Ricker 1954) defined by $M_T = a\ B\ e^{-bB}$, where $B = N\ s_T$ are Prosser counts N surviving with survival rate to the tributaries s_T to become brood year spawners in the tributaries B. We modeled the entire life cycle from the point of reference of the Prosser Dam and Chandler facility such that smolts out-migrating in year t+2 $M_{t+2} = log\ (N_t) + log(a) - bN_t$. Subsequent to passing the Chandler facility, smolts are modelled such that outmigration to the ocean, survival and maturation in the ocean, and upstream migration back to Prosser as adults calculated such that the total recruits R_t to Prosser from brood year t would be $R_t = M_{t+2}\ SAR_t + \omega_t$, where $SAR_t = s_j s_a\ (s_{1,\,t+2}\ s_2\ \phi + s_{1,\,t+2}\ s_2\ s_3\ (1-\phi))$. s_j and s_a are juvenile and adult migration survivals, s_2 and s_3 are second- and third-year ocean survivals, and ϕ is the probability of maturation after the second year in the ocean. $\omega_t \sim N(\rho\omega_{t-1}, \sigma_R)$ is a normally distributed prediction error term where ρ is a lag-1 autocorrelation term and σ_R is the prediction error term. We simplified this formulation by fixing ocean survivals $s_2 = 0.7$ and $s_a = 0.8$, and by combining $s_j s_a$ into a compound migration survival capturing both juvenile and adult survivals in a single term s_m. To get adults, we subtracted the known catches in the run year such that $N_t = R_t - C_t$. We also predicted the individual age classes $A_{a,\,t,}$ where a = 4 or 5 years.

Environmental variability in early ocean survival was modeled such that two ocean variables were used to predict interannual fluctuations in $s_{1,\,t.}$ We used the upwelling index (UPW) and the Pacific Decadal Oscillation (PDO) in the month of May such that logit $(s_{1,\,t}) = \varepsilon_0 + \varepsilon_{UPW}\ UPW_t + \varepsilon_{PDO}\ PDO_{t,}$ where ε_0 is and estimated coefficient that predicts the average value of $s_{1,\,t}$ and ε_{UPW} and ε_{PDO} are estimated coefficients that predict the magnitude of the effect of UPW and PDO on $s_{1,\,t.}$

We implemented the life cycle model in JAGS (Plummer 2003), which is a Bayesian Gibbs sampling platform that allows models to be validated in such a way that uncertainties in predictions and measurements can be evaluated simultaneously. We implemented the model such that we evaluated the relative probability of predicting smolts and adult returns against the relative probabilities of the measured values of smolt and adult data, i.e., a state-space model. We estimated measurement errors in A, M, Estimating parameters in JAGS involves estimating demographic parameters a, b, s_o, ϕ, ε_0, ε_{UPW}, and $\varepsilon_{PDO,}$ as well as statistical parameters that describe the prediction errors and measurement errors. Estimated demographic parameters are shown in Table 7.2. We predicted one prediction uncertainty and five measurement uncertainty parameters shown in Table 7.3.

Table 7.2: Demographic parameters estimated. Descriptions show sampling distributions for the likelihoods. N (m, p) is normal distribution with mean m and precision p. U (l, u) is uniform with lower bound l and upper bound u). B (a, b) is beta distribution with shape parameters a and b.

Parameter	Description	Prior distribution and likelihood
a	Ricker a	$log(a) \sim N(0,.001)$, constrained in (0,6)
b	Ricker b	$b \sim U(0,0.1)$
s_m	Migration survival	$s_m \sim B(1,1)$, constrained in (0,1)
ϕ	Maturation rate	$\phi \sim B(1,1)$, constrained in (0,1)
$\varepsilon_{(i=0, UPW, PDO)}$	SAR	$\varepsilon \sim N(0,.001)$
ρ	Autocorrelation	$\rho \sim N(0,.001)$, constrained in [0,1]

Table 7.3: Statistical parameters estimated. Descriptions show the sampling distributions for the likelihoods. ~G (.001,.001) indicates sampling from a gamma distribution with precision 0.001. ~ LN(X,1/σX) indicates sampling from a log-normal distribution with precision 1/σX.

Parameter prior	Description	Statistical distribution and likelihood
$\sigma_R \sim G(.001,.001)$	Recruitment	Prediction error in $R_{obs} \sim LN(log(R_{pred}), 1/\sigma_R)$
$\sigma_M \sim G(.001,.001)$	Smolts	Measurement error in $M_{obs} \sim LN(log(M_{pred}), 1/\sigma_M)$
$\sigma_N \sim G(.001,.001)$	Adults	Measurement error in $N_{obs} \sim LN(log(N_{pred}), 1/\sigma_N)$
$\sigma_A \sim G(.001,.001)$	Age adults	Measurement error in $A_{a,\,obs} \sim LN(log(A_{a,\,pred}), 1/\sigma_A)$
$\sigma_{SAR} \sim G(.001,.001)$	SAR	Measurement error in $SAR_{obs} \sim LN(log(SAR_{pred}), 1/\sigma_{SAR})$

The JAGS life cycle model implementation was used to simulate samples from the posterior chain by simulating three chains of length 100,000 simulations after discarding 10,000 simulations from each chain. We retained 1000 samples from all three chains to illustrate the statistical validation of the model.

Results

Figure 7.3 shows the pattern of observed and predicted abundances of 4 and 5-year-old Yakima steelhead returning to Prosser Dam. The top panel shows a direct comparison of the returns, where predictions above the one to one line are overpredictions, and predictions below the line are underpredictions. There is no evidence of bias in the distribution of over and underpredictions. The lower panel shows the time series plot of predicted and observed abundances of 4 and 5-year-old Yakima steelhead. The uncertainty shown by the shaded region indicates more uncertainty in recent years.

Figure 7.4 shows temporal pattern in estimated survival. The SAR and $s_{1,\,t}$ follow closely the same pattern owing to the fact that the $s_{1,\,t}$ is used in the calculation of the SAR. During the period of time between 1985 and 2000, the model neither over estimated or underestimated the SAR. Following 2000, the model predicts lower SARs than the values reported in (Frederickson *et al.* 2015). The average SAR was slightly lower than 5%. Early ocean survival had no direct empirical comparison. The variability is driven by the UPW and PDO ocean variables,

with the magnitude of their effect predicted by estimated values of the ε_{UPW} and ε_{PDO} parameters.

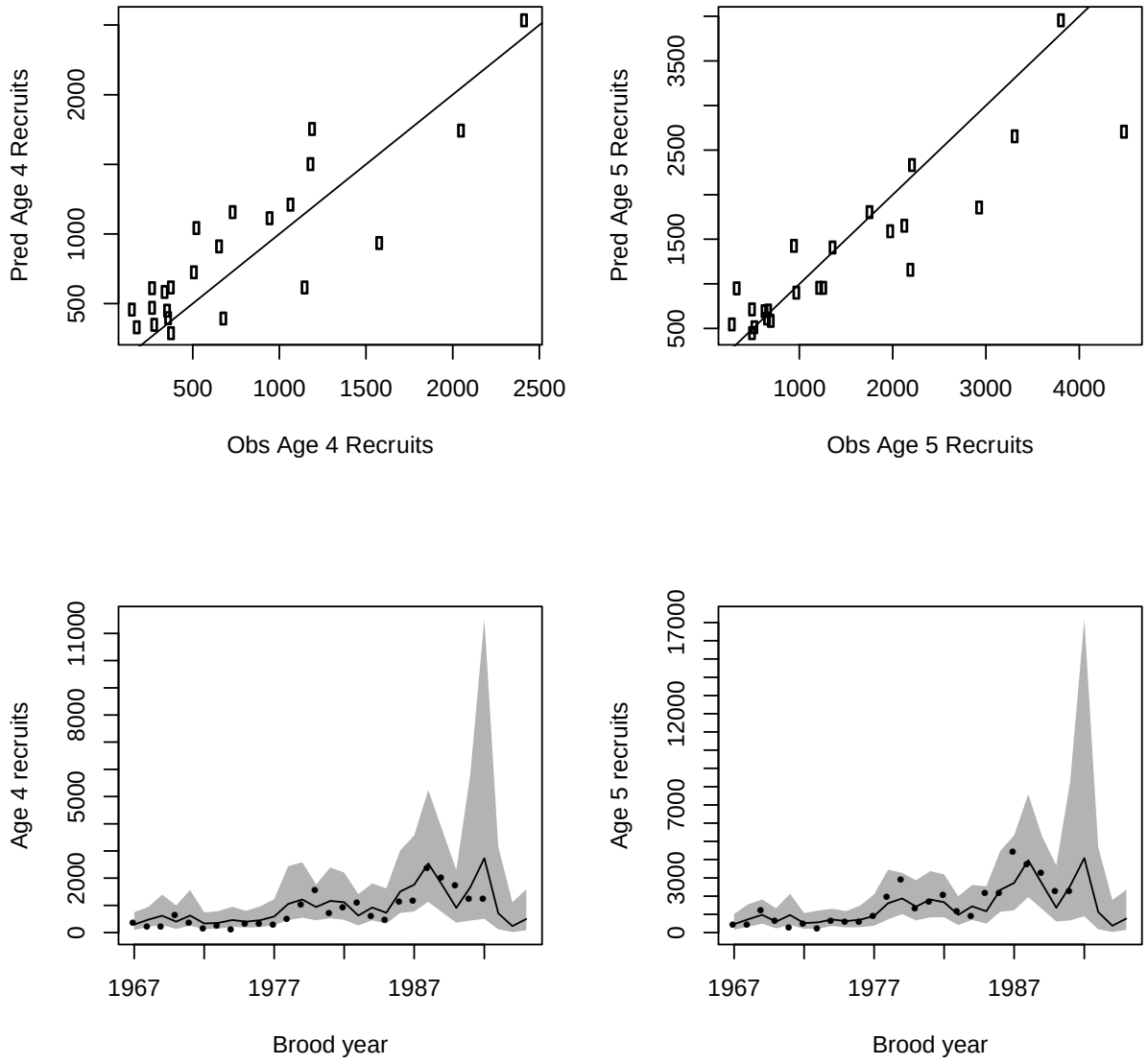


Figure 7.3: Observed and predicted age of return abundances. Top two panels show the 4 and 5-year-old predictions against the observed values. The diagonal line is the one to one line. The bottom two panels show the observed (points) and predicted median (lines) return abundances of 4 and 5-year-old Yakima steelhead. The shaded region represents the range between the 25th and 75th percentiles.

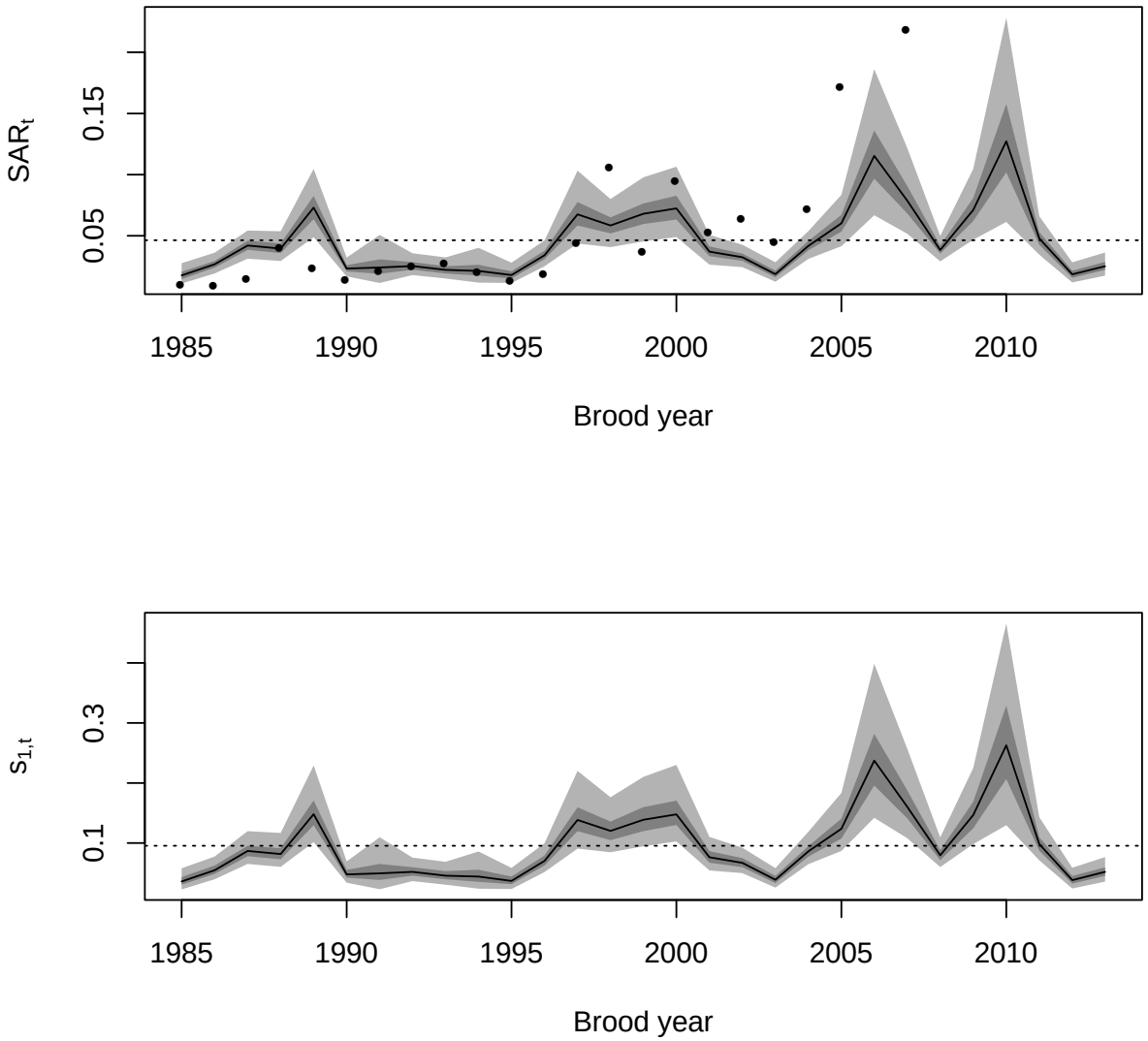


Figure 7.4: Time series plots of estimated SAR and early ocean survival. The upper panel shows the median SAR (line), the 25th to 50th percentile range (dark grey) and 95% confidence range (light grey). The lower panel shows the median early ocean survival (line), the 25th to 50th percentile range (dark grey) and 95% confidence range (light grey).

Figure 7.5 shows the variability in the estimated values of demographic and statistical parameters, as well as adult production, smolt production, and the spawner to smolt relationship. The top two rows show histograms of a sample of 1,000 values from 3 chains of length 100,000 of the sampled posterior distributions of each parameter. The Ricker a parameter shows a median value of approximately 60 smolts per spawner once converted from log space. The Ricker b median value is approximately 0.00045. The median Ricker a and b predict a maximum production of approximately 48,000 smolts.

The estimated value of ε_0 predicts approximately 8% early ocean survival in the absence of any effect from PDO and UPW. The estimated values of ε_{PDO} and ε_{UPW} both predict negative effects with positive deviations from the means of PDO and UPW. The migration survival parameter s_m has a mode at approximately 0.8 but shows considerable variability. With the exception of the prediction uncertainty σ_R, the measurement errors uncertainties for smolt abundance, brood year return adults, and SARs are approximately 0.6-0.7. The prediction uncertainty σ_R was much lower than the measurement errors at less than 0.1.

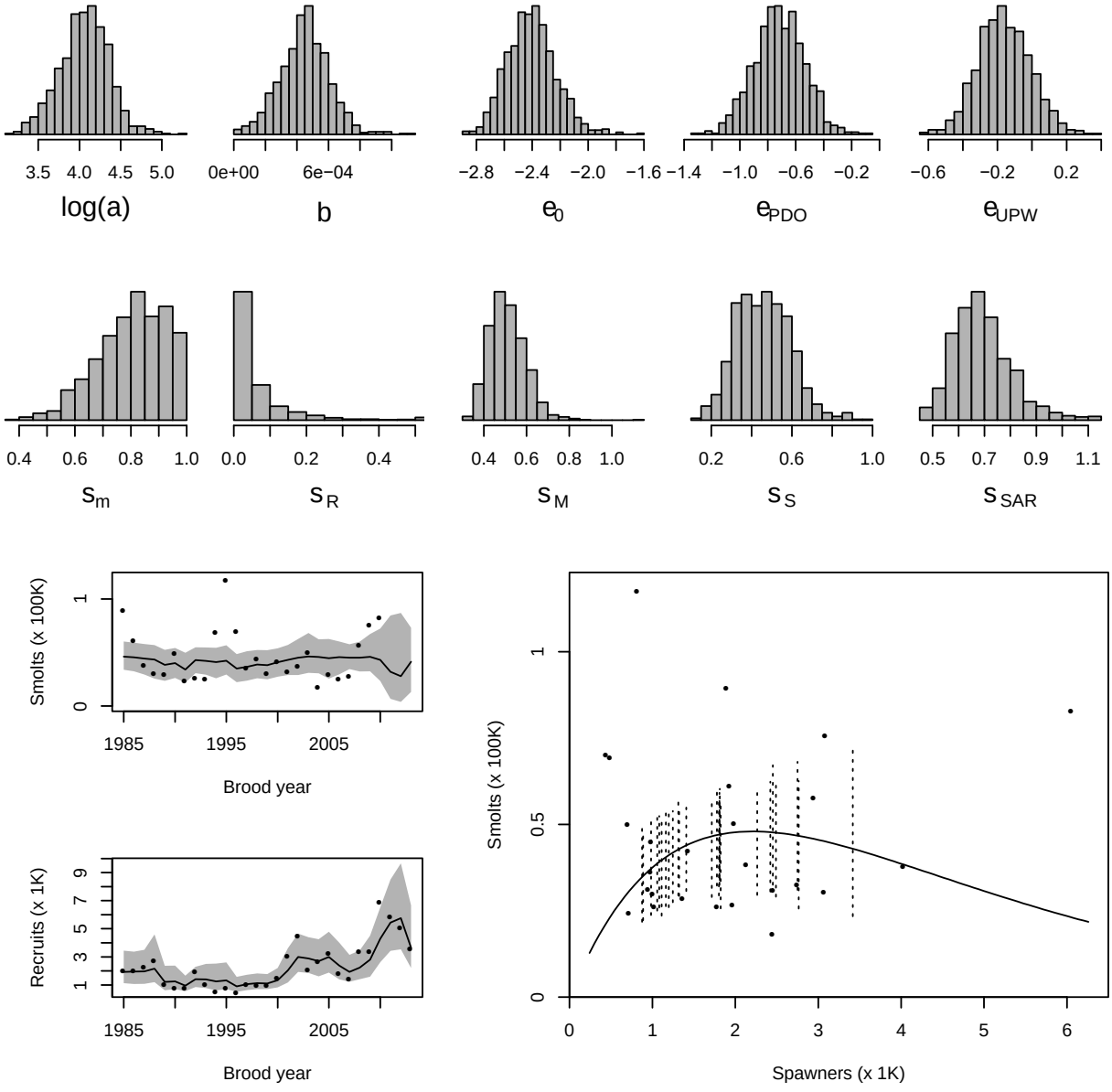


Figure 7.5: Statistical parameters and production. Top two rows show the posterior distributions of demographic and statistical parameters described in Tables 1 and 2. The large lower left figure shows the median predicted (line) and observed (points) wild catch and escapement combined. Predicted and observed smolts are also shown. The shaded regions are the 95% confidence regions surrounding the median prediction. The lower left-hand figure shows the observed and median predicted smolts using the median Ricker a and b parameters, with vertical dotted lines showing the 95% range of uncertainty around the predicted smolts occurring at the median predicted spawner abundance (observed spawners are shown with points).

The predicted total adult production including harvest (recruits) follows closely with the empirically observed total abundance. All empirical values fall within the 95% confidence region, with the median prediction tracking closely with many of the empirical observations. The predicted trend is consistent with the data. Several empirical observations of smolts fell outside the 95% prediction region, and the predictions did not track a pattern in observed values. The lower right figure shows the median predicted smolts per spawner (line) and the observes smolts per spawner (points), with the range of uncertain in the predicted smolts described at the predicted level of spawners (vertical dotted lines). The smolt per spawner relationship shows a median predicted maximum smolt production of approximately 50,000 smolts (asymptote of line). The vertical dotted lines show the variability around the median production line. There is considerable variability in empirically observed smolt production within defined ranges of spawner abundances. The maximum predicted smolt production of 50,000 smolts occurs at approximately 2,000 spawners, and yet the range of observed smolts at 2,000 spawner is between 25,000 and 90,000.

Discussion

We have constructed a state-space life cycle model of Yakima River summer steelhead by using Prosser as a reference point for spawner abundance and smolt production. Using a Bayesian approach, we have reconciled prediction and measurement uncertainty and estimated median and quantile ranges of demographic parameter values. There were known uncertainties in the data, which hampered our ability to predict trends, but the approach nonetheless arrived at estimates of demographic rates that are consistent with the understanding of the system. This analysis represents a step forward in understanding Yakima steelhead population dynamics. With some additional complexities added, and with additional data, better distinctions could be drawn between source of uncertainty, and the approach could then be extended to include a sensitivity analysis of the potential production benefit of kelt reconditioning.

A Ricker *a* productivity parameter value of 60 smolts per spawner needs to be put in perspective. This is referenced at Prosser and the Chandler facility, and the smolt production relationship implicitly captures pre-spawn mortality and smolt migration survival into the productivity parameter. Further, the 60 smolts per spawner is really 60 smolts that survived from the tributary to Chandler and were the progeny of adults following pre-spawn mortality. If pre-spawn mortality is 10% and smolt migration survival is 25%, then 60 smolts per spawner translates to 265 smolts per spawner at a tributary reference point.

A Ricker *a* value of 60 is also consistent with the data. At low spawning abundances, smolt production was observed to be in the range of 30 to 150 smolts per spawner. The density dependent parameter *b* is also consistent with the data. Only 4 brood years produced smolt abundances above 50,000. Passive Integrated Transponder (PIT) tag data have been used to estimate survival from tributaries to Prosser, and from Prosser to McNary detection arrays. Survival to Chandler detection has been estimated to be as low as 12% and as high as 30%

during the period 2010-2016 migration years, based on an aggregate of all smolts leaving Toppenish, Satus, and Naches drainages.

This analysis did not attribute any variation in migration survival to environmental or anthropogenic conditions. A logical extension of this analysis would be to formulate the Ricker *a* parameter in relation to environmental conditions (e.g.: river flow, temperature, and predator abundance in the Yakima mainstem). The same could be done with the s_o parameter using river conditions between Prosser and McNary dams. Both of these life stages could potentially explain some of the total life cycle variability, which would reduce the uncertainty in full life cycle survival prediction. Beyond adding complexity and environmental drivers, the model would benefit from additional data coming from the tributaries. Where available, screw trap juvenile abundance data and survival estimates from PIT tags should be incorporated.

This analysis demonstrated that the Yakima summer steelhead aggregate comprised of Naches, Toppenish, Satus and upper Yakima tributaries shows density dependent smolt recruitment from a Prosser/Chandler spawner and smolt reference point. The aggregate maximum smolt production median estimate is for approximately 50,000 smolts. This in no way suggests that that the total outmigration of all tributaries combined is limited to 50,000 smolts. The migration survival between tributaries and the Chandler detection facility has been reported at an average 20%. A substantial amount of the density dependence visible at the Prosser reference point may take place between the tributaries and the Chandler facility. Additionally, this analysis did not attribute density dependent survival in outmigration. Further analysis of Yakima steelhead would benefit from an investigation of density dependence in the tributary to Chandler and Prosser to McNary stages.

Adaptive Management & Lessons Learned

1. Columbia River steelhead populations upstream of Bonneville Dam are listed under ESA and need novel recovery strategies.
2. There is a relatively large abundance of kelt steelhead in the Columbia River Basin even in the upper most areas.
3. In general, repeat spawning steelhead make up a very small proportion of the spawning run.
4. Increasing repeat spawners in steelhead populations can have many positive effects on populations including increasing; genetic diversity, lifetime fecundity, and fitness since genes are distributed across generations.
5. Long-term reconditioning kelt steelhead provides 5 to over 100 times more repeat spawners than leaving the fish in the river.

6. Physiology studies have provided us with a much better understanding of energetic and physiological status of kelts, improved our understanding of alternative life histories in post-spawning fish, and improved survival and health of reconditioned fish.
7. Blood hormone assays are useful to classify consecutive and skip spawner steelhead. Future work needs to focus on optimizing strategies for skip spawner contributions.
8. There appears to be a reduction in the B-run steelhead composition between the maiden and kelt stage, but the B-run composition of repeat spawners is similar to the kelt composition. Underlying biological and behavioral factors contributing to such discrepancies are not well understood but likely warrant further investigation of potential causes. With more data including escapement comparisons, it may be possible to refine the confidence in estimated rates of iteroparity among RG's.
9. Age appears to be less of a factor in rates of iteroparity than size. While the A-run life history was observed to be present among all reporting groups, so too were the B-run life history.
10. Despite the understanding in recent years that the B-run life history is relatively uncommon outside the middle and south forks of both the Clearwater River and Salmon River, our results suggest otherwise. In fact, age 2-ocean fish were dominant among all 10 reporting groups. This finding has implications for management of steelhead populations in the basin and provides evidence that regionally based classifications of life history types or their distributions warrants reconsideration.
11. The upper Salmon River region produces a disproportionate number of Snake River kelt steelhead and is presumably an important factor in spawner abundance for that region. This result is mirrored among hatchery-origin fish.
12. Adding a production level kelt reconditioning facility at Nez Perce Tribal Hatchery will make achieving the goal RPA 33 possible, i.e. increase the abundance on adult b-run steelhead by 6%.
13. The Snake River Kelt Reconditioning Facility Master Plan was submitted and favorably review by the ISRP and recommended to proceed to final design by the NWPCC in December of 2016.
14. Reproductive success studies are underway at a variety of scales: hatchery analog, spawning channel, and natural river. Results are positive.
15. Artificially reconditioned kelt steelhead appear to repeat home with high fidelity. Data indicates that natural repeat spawners in the Snake River exhibited a 15% stray rate.
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.

19. Kelt Reconditioning, during years of low steelhead returns, effectively acts as a stop gap or safety net measure that should allow for a larger production of the juvenile population than normal under poor return years. This increase in juvenile production, should rearing and migration conditions improve, would translate into additional adult returns later, thus decreasing the time period for recovery after poor run years.
20. GSI analysis revealed that >50% of the reconditioned kelts released in the Snake are from B-run MPGs (Clearwater and Salmon rivers). These fish are important for meeting the goal of RPA 33.

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Appendices

A1.a Master Kelt Tracking Table

Last update
Branstetter
11/2018
Steelhead Kelt
Reconditioning
Treatments

lower Granite
Total (2002-
2018) 20655
Prosser total
(2000-2018) 14530
LGD total 2018 1095

Strategy	Year	Location	# Collected	# released	S @ release (%)	# remature	Retained	skip remature	# @ ocean	S @ ocean (%)	# @ Bonneville	Return Rate to Bonneville (%)	# @ Lower Granite Dam (or Prosser)	relative to in-river	Transportation (or treatment) Benefit relative to Hockersmith **1.66**	Treatment benefit relative to Bonneville natural	Transportation (or treatment) Benefit
In-river	2005	Prosser	67	67					3	4.48		1.54		2.70	25.61		
In-river	2006	Prosser	52	52					1	1.92		0.66		1.16	3.10		
In-river	2007	Prosser	53	53					3	5.66		1.95		3.41	9.28		
In-river	2008	Prosser	88	88					4	4.55		1.57		2.74	6.64		
In-river	2009	Prosser	58	58					3	5.17		1.78		3.12	11.54		
In-river	2010	Prosser	155	155					2	1.29		0.44		0.78	3.74		
In-river	2011	Prosser	85	85					3	3.53		1.22		2.13	7.01		
In-river	2012	Prosser	59	59					2	3.39		1.17		2.04	6.74		
In-river	2013	Prosser	52	52					0	0.00		0.00		0.00	0.00		
In-river	2014	Prosser	45	45					3	6.67		2.30		4.02	11.52		
In-river	2015	Prosser	121	121					0	0.00		0.00		0.00	0.00		
In-river	2016	Prosser	56	56					2	3.57		1.23	2.00	2.15	57.50		
In-river	2017	Prosser	5	5					0	0.00		0.00		0.00	0.00		
In-river	2018	Prosser	0	0					NA	NA		NA		NA	NA		
Total and weighted mean			***896***	***896***					***2.08***	***2.90***		***1.00***		***1.75***	***5.57***		
In-river	2002	Lower Granite*	1209	1209					8	0.66		2.28		0.40			
In-river	2003	Lower Granite*	865	865					3	0.35		1.19		0.21			
In-river	2004	Lower Granite*	1138	1138					10	0.88		3.02		0.53	1.49		
In-river	2009	Lower Granite	178	176					2	1.12		3.86		0.68	1.51		
In-river	2010	Lower Granite	1411	1399					5	0.35		1.22		0.21	0.62		
In-river	2011	Lower Granite	1633	1613					3	0.18		0.63		0.11	0.22		
In-river	2012	Lower Granite	2098	2098					1	0.05		0.16		0.03	0.03		
In-river	2013	Lower Granite	840	827					2	0.24		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	2017	Lower Granite	824	821			0	0.00		0.00	0.00	0.00		
In-river	2018	Lower Granite	868	863			3	0.35		1.19	0.21			
Total and weighted mean			***16684***	***16610***			***5.05***	***0.29***		***1.00***	***0.18***	***0.56***		
In-river	2002	John Day*	***287***	***287***			***28***	***9.76***		***1.00***	***5.88***	***18.72***		
Total and weighted mean														
Transported (Hamilton Island)	2002	Lower Granite*	750	750			19	2.53		3.83	1.53			
Transported (Hamilton Island)	2003	Lower Granite*	376	376			3	0.80		2.30	0.48			
Transported (Hamilton Island)	2004	Lower Granite*	982	982			7	0.71		0.81	0.43	2.00		
Transported (Hamilton Island)	2009	Lower Granite	71	68			0	0.00		0.00	0.00	0.00		
Transported (Hamilton Island)	2010	Lower Granite	301	301	13/108	12.04	0	0.00		0.00	0.00	0.00		
Transported (Hamilton Island)	2011	Lower Granite	109	109	3/47	6.38	0	0.00		0.00	0.00	0.00		
Total and weighted mean			***2589***	***2586***		***9.21***	***8.59***	***1.12***		***1.16***	***0.67***	***2.15***		
Transported (estuary release)	2010	Lower Granite	23	22	4/10	40.00	0	0.00		0.00	0.00	0.00		
Transported (estuary release)	2011	Lower Granite	91	90	14/46	30.43	0	0.00		0.00	0.00	0.00		
Total and weighted mean			***114***	***112***		***35.22***	***0.00***	***0.00***		***0.00***	***0.00***	***0.00***		
Transported	2002	John Day*	***271***	***271***			***34***	***12.55***		***1.29***	***7.56***	***24.08***		
Total and weighted mean														
Transported (unfed Hamilton Island)	2004	Prosser	75	63	15/28	53.57	5	6.67			4.02	18.75		
Transported (unfed Hamilton Island)	2005	Prosser	98	96	14/57	24.56	1	1.02		0.23	0.61	5.84		
Transported (unfed Hamilton Island)	2006	Prosser	55	49	31/49	63.27	2	3.64		1.89	2.19	5.87		
Transported (unfed Hamilton Island)	2007	Prosser	43	38	14/35	40.00	0	0.00		0.00	0.00	0.00		

Transported (unfed Hamilton Island)	2008	Prosser	100	100		26/49	53.06	3	3.00		0.66	1.81	4.38
Transported (unfed Hamilton Island)	2010	Prosser	124	123		27/59	45.76	1	0.81		0.16	0.49	2.34
Transported (unfed Hamilton Island)	2011	Prosser	100	100		16/47	34.04	1	1.00		0.78	0.60	1.99
Total and weighted mean			***595***	***569***			***44.89***	***1.86***	***2.18***		***0.75***	***1.32***	***4.19***
Transported (unfed estuary release)	2010	Prosser	113	113		13/60	21.67	1	0.88		0.69	0.53	2.57
Transported (unfed estuary release)	2011	Prosser	90	89		16/47	34.04	3	3.33		2.58	2.01	6.62
Total and weighted mean			***203***	***202***			***27.85***	***1.00***	***1.97***		***1.63***	***1.19***	***3.78***
Transported (fed Hamilton Island)	2002	Prosser	479	334				43	8.98			5.41	
Transported (fed Hamilton Island)	2003	Prosser	208	187				8	3.85			2.32	
Transported (fed Hamilton Island)	2004	Prosser	105	83		11/26	42.31	5	4.76			2.87	13.39
Transported (fed Hamilton Island)	2005	Prosser	106	96		6/56	10.71	0	0.00		0.00	0.00	0.00
Transported (fed Hamilton Island)	2006	Prosser	56	50		32/50	64.00	0	0.00		0.00	0.00	0.00
Transported (fed Hamilton Island)	2007	Prosser	40	38		19/27	70.37	1	2.50		0.44	1.51	4.10
Transported (fed Hamilton Island)	2008	Prosser	108	100		28/50	56.00	7	6.48		1.43	3.90	9.47
Total and weighted mean			***1102***	***888***			***48.68***	***21.40***	***5.81***		***2.00***	***3.50***	***11.15***
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***		***1.47***	***2.71***	***8.18***
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	101	28					9.11	36.57	104.90
Long-term	2015	Prosser	1098	396	36.07	322	74	37					12.43	21.73	69.21
Long-term	2016	Prosser	471	349	74.10	236	94	15					20.75	44.64	1192.97
Long-term	2017	Prosser	118	55	46.61	55	29	4					16.06	28.08	89.45
Long-term	2018	Prosser	227	103	45.37	103	50	TBD 19					15.64	27.33	87.08
Total and weighted mean			***10024***	***4208***	***41.98***	***1618***	***389***	***92***					***14.47***	***25.24***	***80.56***
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***	***10.10***
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***	***28.48***
Long-term	2006	Parkdale	1	1	100.00									60.24	161.33
Long-term	2007	Parkdale	13	1	7.69									4.63	12.61
Long-term	2008	Parkdale	14	7	50.00									30.12	73.06
Long-term	2009	Parkdale	9	4	44.44									26.77	99.15

Long-term	2010	Parkdale	15	4	26.67							16.06	77.33
Long-term	2011	Parkdale	23	5	21.74							13.10	43.20
Long-term	2012	Parkdale	21	13	61.90							37.29	57.49
Total and weighted mean			***96***	***35***	***36.46***							***21.96***	***69.97***

				# ***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	55.38
Long-term	2016	DNFH	164	30	18.29	12	18		18	62.90	11.02	294.51
Long-term	2017	DNFH	191	83	43.46	24	0		-	4.45	26.18	83.40
Long-term	2018	DNFH	122	52	42.62	31	0		-		25.68	
Total and weighted mean			***1081***	***293***	***25.13***	***98***	***112***		***63***	***86.40***	***15.14***	***48.23***

**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	59.32
Long-term	2015	Lower Granite	22	11	50.00	8	8	3	3	171.91	30.12	95.96
Long-term	2016	Lower Granite	227	120	52.86	19	19	101	77	243.30	31.85	851.10
Long-term	2017	Lower Granite	269	59	21.93	21	21	58	26	2.25	13.21	42.09
Long-term	2018	Lower Granite	259	184	71.04	81	81	103	***TBD 19***		29.07	
Total and weighted mean			***1232***	***477***	***38.72***	***232***	***135***	***265***	***106***	***116.65***	***20.34***	***65.11***
					46.43 (2013-2018)							
*subset of LGD fish**												
Long-term	2016	LGD @DNFH 9/25 to NPTH										
Long Term	2017	LGD NPTH to DNFH	***39***	***32***	82.05	***5***	***5***	***27***	***TBD 18***	115.11	49.43	157.47
Long Term	2018	LGD @DNFH to NPTH										
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	38.38
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***	-	***110.72***	***17.08***	***61.80***
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	57.81
Total and weighted mean			***95***	***28***	***29.47***	***11***	***10***	***17***	***17***	***101.34***	***17.76***	***56.56***

A.2: Publications

Publications:

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

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.

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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 2018.

Jenkins LE, Pierce AL, Graham N, Branstetter R, Hatch DR, and Nagler JJ. Reproductive performance and energy balance in consecutive and skip repeat spawning reconditioned female steelhead trout Oncorhynchus mykiss. Transactions of the American Fisheries Society, accepted 6/6/2018.

Published in 2019

Jenkins, L.E., A.L. Pierce, N.D. Graham, L.R. Medeiros, D.R. Hatch, J.J. Nagler. Elevated plasma triglycerides and growth are early indicators of rematuration in female steelhead trout (*Oncorhynchus mykiss*). Conservation Physiology. Submitted 7 Jan 2019.

In Preparation

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Presentations 2018:

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Hatch, DR. Steelhead kelt reconditioning and reproductive success studies in the Columbia River Basin. Western Division American Fisheries Society Annual Meeting, Anchorage, AK. Oral Presentation.

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Jenkins LE, Pierce AL, Graham N, Branstetter R, Hatch DR, and Nagler JJ. Reproductive performance and energy balance in consecutive and skip repeat spawning reconditioned female steelhead trout *Oncorhynchus mykiss*. Transactions of the American Fisheries Society, submitted 3/19/2018.

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Jenkins, LE et al. Condition-dependent survival and rematuration in consecutive and skip repeat spawning female steelhead trout *Oncorhynchus mykiss*. In preparation.

Jenkins LE. Life history diversity in repeat spawning reconditioned female steelhead in the Clearwater River, Idaho. Oral presentation. North Idaho Fly Fishing Expo, Lewiston ID 5/11/2018-5/12/2018.

Jenkins LE, Pierce AL, Graham ND, Medeiros LR, Branstetter R, Hatch DR, and Nagler JJ. Recovery, rematuration, and reproductive performance in repeat spawning reconditioned female steelhead. Oral presentation. Yakima Basin Science and Management Conference, Ellensburg WA 6/13/2018-6/14/2018.

Jenkins, LE et al. Condition-dependent survival and rematuration in consecutive and skip repeat spawning female steelhead trout Oncorhynchus mykiss. In preparation.

Jenkins, L.E. Reproductive Life History Decisions in a Long-Distance Migrating Iteroparous Fish Model. Public seminar at the University of Idaho. Moscow, ID. 2018.

Jenkins, L.E., A. Pierce, N. Graham, L. Medeiros, D. Hatch, and J. Nagler. Recovery from Spawning, Rematuration, and Reproductive Performance in Consecutive and Skip Repeat Spawning Reconditioned Female Steelhead *Oncorhynchus mykiss*. 13th International Congress on the Biology of Fish. Calgary, Alberta, Canada. 7/15/2018-7/19/2018.

Jenkins, L.E**.**, A. Pierce, N. Graham, L. Medeiros, R. Branstetter, D. Hatch, and J.J. Nagler. Recovery, rematuration, and reproductive performance in repeat spawning reconditioned female steelhead. Ellensburg, WA. 2018.

Jenkins, L.E. Life history diversity in repeat spawning reconditioned female steelhead in the Clearwater River, Idaho. North Idaho Fly Fishing Expo. Lewiston, ID. 2018.

Jenkins, LE. Reproductive life history decisions in a long-distance migrating iteroparous fish model. Oral presentation. Ph.D. Dissertation defense, Department of Biological Sciences, University of Idaho, Moscow, ID 11/1/2018.

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

Electro fisher Settings: https://www.monitoringresources.org/Document/Method/Details/115
Backpack Electrofishing: https://www.monitoringresources.org/Document/Method/Details/117
Parentage Analysis using Cervus: https://www.monitoringresources.org/Document/Method/Details/1430
Radio Tagging: https://www.monitoringresources.org/Document/CustomizedMethod/Details/23045
Lotek Receiver Download: https://www.monitoringresources.org/Document/Method/Details/4244

Habitat Monitoring

Piezometer Method: https://www.monitoringresources.org/Document/Method/Details/5478
McNeil Samples (Field Method): https://www.monitoringresources.org/Document/Method/Details/5397

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

Metrics

Title	Category	Subcategory	Subcategory Focus 1	Subcategory Focus 2
"Kelt abundance"	Fish	Abundance of Fish (ID: 46)	Fish Life Stage: Adult - Outmigrant	Fish Origin: Both
"Reconditioned Kelt abundance"	Fish	Abundance of Fish (ID: 46)	Fish Life Stage: Adult Fish	Fish Origin: Both
"Stock Composition"	Fish	Composition: Fish Species Assemblage (ID: 56)	Fish Life Stage: Adult - Outmigrant	Fish Origin: Natural
"Kelt Condition"	Fish	Condition of Life Stage: Fish (ID: 57)	Fish Life Stage: Adult - Outmigrant	NA
"Reconditioned Kelt condition"	Fish	Condition of Life Stage: Fish (ID: 57)	Fish Life Stage: Adult Fish	NA
"Maturation Status"	Fish	Condition of Life Stage: Fish (ID: 57)	Fish Life Stage: Adult - Returner	NA
"Fecundity"	Fish	Fecundity: Fish (ID: 68)	NA	NA
"Fry Growth"	Fish	Growth Rate: Fish (ID: 73)	Fish Life Stage: Juvenile - Fry/Parr	NA
"Fertilization Rate"	Fish	Hatchery Practices: Propagation (ID: 87)	Fish Origin: Both	NA
"Kelt length"	Fish	Length: Fish Species (ID: 75)	Fish Life Stage: Adult - Outmigrant	NA
"Reconditioned kelt length"	Fish	Length: Fish Species (ID: 75)	Fish Life Stage: Adult Fish	NA
"Mark Detection"	Fish	Mark/Tag Recovery or Detection (ID: 381)	NA	NA
"Parentage Analysis"	Fish	Relative Reproductive Success (RRS) (ID: 88)	Fish Origin: Both	NA
"Reproductive success"	Fish	Reproductive Success (Nb/N) (ID: 89)	Fish Origin: Natural	NA

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