Kelt Reconditioning and Reproductive Success Evaluation Research

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Contents

List of Tables:

List of Figures:

Abstract

The Kelt Reconditioning and Reproductive Success Evaluation Project is a research, monitoring, and evaluation (RM&E) uncertainties category project, that was funded through the 2008 Columbia Basin Fish Accords. The objectives are to evaluate methodologies to produce viable artificially reconditioned repeat steelhead spawners and evaluate reproductive success, physiology, homing, and geographic differences. Our work occurs in both the Yakima and Snake river basins, additionally we make some contrasts and comparisons with the Kelt Reconditioning Project in the Upper Columbia River (Project 2008-458-00). We focused on collecting steelhead kelts at juvenile bypass facilities at Prosser, Lower Granite, and Little Goose dams. These kelts were reconditioned (given prophylactic treatments and fed a specially formulated diet) at Prosser and Dworshak National Fish (DNFH) hatcheries. Survival of long-term reconditioned kelts has been 44% (21 years) at Prosser Hatchery and 39% (10 years; 44% over the last 8 years) for mixed stock collections reconditioned at Nez Perce Tribal and Dworshak National Fish hatcheries. In total, we released 460 and 4,787 reconditioned kelt steelhead in the Snake and Yakima rivers since 2011 and 2000, respectively. In 2020, unmarked upstream "wild" migrant adult steelhead return counts were at the 9th lowest across the region, placing great importance on safety net programs like kelt reconditioning. Years with low runs typically translate into lower abundance of kelts but the value of each fish increases. In 2020, we collected 455 kelts in the Snake River. From this collection of 455, 318 were PIT tagged and released back to the river, 137 were taken to DNFH for reconditioning, and 66 reconditioned-mature (a combination of consecutive and skip spawners) fish were released in the Snake River below Lower Granite Dam on November 10, 2020. In 2020, we collected 471 kelts in the Yakima River. From this collection of 471, 8 were PIT tagged and released back to the river, 463 were taken to Prosser Hatchery for reconditioning, and 268 reconditioned-mature consecutive spawners were released in the Yakima River below Prosser Dam on October 20, 2020. Additionally, 52 reconditioned-immature kelts were trucked and released below Bonneville Dam on October 21, 2020. Reproductive success of reconditioned steelhead was confirmed in the Yakima River tributaries of Satus and Toppenish creeks once again, with genetic parentage assignments (584 samples from Satus and 646 samples from Toppenish in 2020) which are currently being tabulated and will be reported in our 2021 Annual Report to BPA. Based on cumulative sampling from 2013- 2019, lifetime reproductive success for female reconditioned kelt steelhead in the Yakima River is estimated as 2.49 relative to steelhead that successfully spawn once. Studies applying tools from fish physiology and endocrinology to issues in kelt reconditioning were continued in 2020. 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 2020, we sampled blood at DNFH, and provided maturation status of individual fish at DNFH and Winthrop to project managers so that consecutive and skip spawners could be managed appropriately. Kelts in the Prosser project were not sampled in 2020 due to the COVID-19 pandemic. The 2020 results were added to a comparison of the performance of the three Columbia River Basin kelt projects in terms of survival and maturation rates. We published the third of three linked studies using hatchery origin kelts at DNFH (Jenkins, et al. 2020)). The first two studies were published in 2018 and 2019 (Jenkins, et al. 2018; Jenkins, et al. 2019). Collectively, these studies advance scientific understanding of the physiology of repeat spawning and kelt reconditioning and address significant issues such as 1) a potential tradeoff between reproductive investment in maiden spawning versus repeat spawning, 2) the productivity benefit to be expected from releasing reconditioned consecutive and skip spawners to spawn naturally, and 3) the timing and physiological basis of decision underlying consecutive versus skip spawning. We published the results of laboratory work establishing assays for plasma insulin-like growth factor-1 (IGF-1) and growth hormone (GH), indicators of growth and metabolic status (Medeiros 2020). The ability to measure these hormones increases our understanding of physiologically important interactions between metabolic status and reproduction in steelhead kelts. This is illustrated in two studies in progress on the effect of nutritional restriction during the period after spawning in hatchery-origin steelhead kelts and a post-spawning rainbow trout kelt model. In the first, we show that metabolic status at spawning, as indicated by plasma GH level, and nutrition during the first 10 weeks after spawning interact to determine whether female hatchery-origin kelts adopt a consecutive or skip spawning life history. In the second, we show that plasma IGF1 level does not respond to nutritional status for the first 8 weeks after spawning, implying that there is a process of recovery from spawning that must occur before resources can be allocated to anabolic (muscle) growth. In addition, we are continuing data analysis in a study where we are combining the results of a genetic analysis enabling classification of Yakima River kelts by subpopulation, with survival, physiological, and migration data. From 2008 to 2020 we have detected conclusive evidence of 140 kelts showing repeat homing and upstream movement patterns of an additional 1,325 kelts that is consistent with repeat homing in the Yakima basin. Significant progress was made toward the realization of a dedicated kelt reconditioning facility for the Snake River 2020. Efforts included: forming a Facility Review team, selecting a facility design contractor, Facility Review team site visit, and development of 10% facility design documents. Due to COVID-19 restrictions, shutdowns of many conferences and travel has resulted in diminished opportunities for presentations, a list of past presentations is available in the appendices.

Acknowledgments

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Executive Introduction

Current iteroparity rates for interior Columbia River Basin steelhead are considerably less than lower-Columbia River populations, due largely to high mortality of downstream migrating kelts (post-spawn steelhead) at hydropower dams (Evans and Beaty 2001), and potentially inherent differences in iteroparity rate based on latitudinal and inland distance effects (Withler 1966; Bell 1980; Fleming 1998). The highest recent estimates of repeat spawners from the CRB were in the Kalama River (tributary of the un-impounded lower Columbia River), which exceeded 17% (NMFS 1996). A total of 8.3% of the adult steelhead from Snow Creek, WA were identified as repeat spawners based on scale samples (Seamons and Quinn 2010). In Hood River, repeat spawning summer run steelhead comprise on average 5.7% of the run based on scale pattern analysis (Olsen 2008). Iteroparity rates for Klickitat River steelhead were reported at 3.3% from 1979 to 1981 (Howell et al. 1985). Summer steelhead in the South Fork Walla Walla River expressed 2% to 9% iteroparity rates (J. Gourmand, ODFW, pers. comm.). Hockersmith et al. (1995) reported that repeat spawners composed 1.6% of the Yakima River wild run. Repeat spawners make up approximately 0.2% of the Snake River steelhead run based on the return of 26 out of 14,829 PIT tagged fish at Lower Granite Dam that were tagged by Nez Perce Tribe from 2009-2020.

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. In the 1st quarter of 2019, a new Biological Opinion (NMFS 2019) was produced by NOAA which recognized that our current kelt reconditioning program is the only steelhead hatchery action in the basin which is benefiting wild ESA-listed Columbia River steelhead population abundance and genetic diversity. In 2020 we continued to focus collections and reconditioning in the Snake basin on B-run fish. In future reports the A/B- run components will still be considered, but will likely focus on Major Population Groups (MPG) in the basin, since this is what is recognized under the Endangered Species Act listings that are targeted for mitigation/restoration actions (NMFS 2019).

The Independent Scientific Review Panel (ISRP), in 2014, issued a memorandum (ISRP 2014-9) reviewing the progress of project 2008-458-00, an independent, sister kelt reconditioning program managed/researched by the Yakama Nation 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 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 2 primary locations throughout the Columbia River Basin (CRB): The Chandler Juvenile Monitoring Facility (CJMF) in Prosser, WA (Yakima River) and the Lower Granite Dam (LGR), WA (Snake River). Collections of steelhead kelts also occurred from 2012-2016 at Dworshak National Fish Hatchery, 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. Another affiliated, but independent kelt operation is the one managed by the Yakama Nation in the upper Columbia River near Winthrop, WA that has been ongoing since 2010 (Abrahamse et al 2020). 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. 2013a, 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	-	-	-	1999-2020
2	Yakama Nation Prosser Fish Hatchery	Yakima River	RKM 75.6	-	Yes	Yes	-	1999-2020
3	Lower Granite Dam Juvenile Bypass (LGDJB)	Snake River	RKM 173	Yes	-	Yes	-	2009-2020
4	Dworshak National Fish Hatchery (DNFH)	Clearwater River	RKM 65	Yes (hatchery fish for experimental purposes) 2009-2018	Yes	-	-	2009-2020
5	Nez Perce Tribal Fish Hatchery (NPTH)	Clearwater River	RKM 38	No	Yes	-	-	2016-2019
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 National Fish 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	2008-2020
22	Toppenish Creek	Yakima River		-	-	-	Yes	2008-2020
23	Simcoe Creek	Yakima River		-	-	-	Yes	2008-2020
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
31	Lower Goose Dam Juvenile Bypass (LGSDJB)	Snake River	RKM	Yes	-	-	-	2020

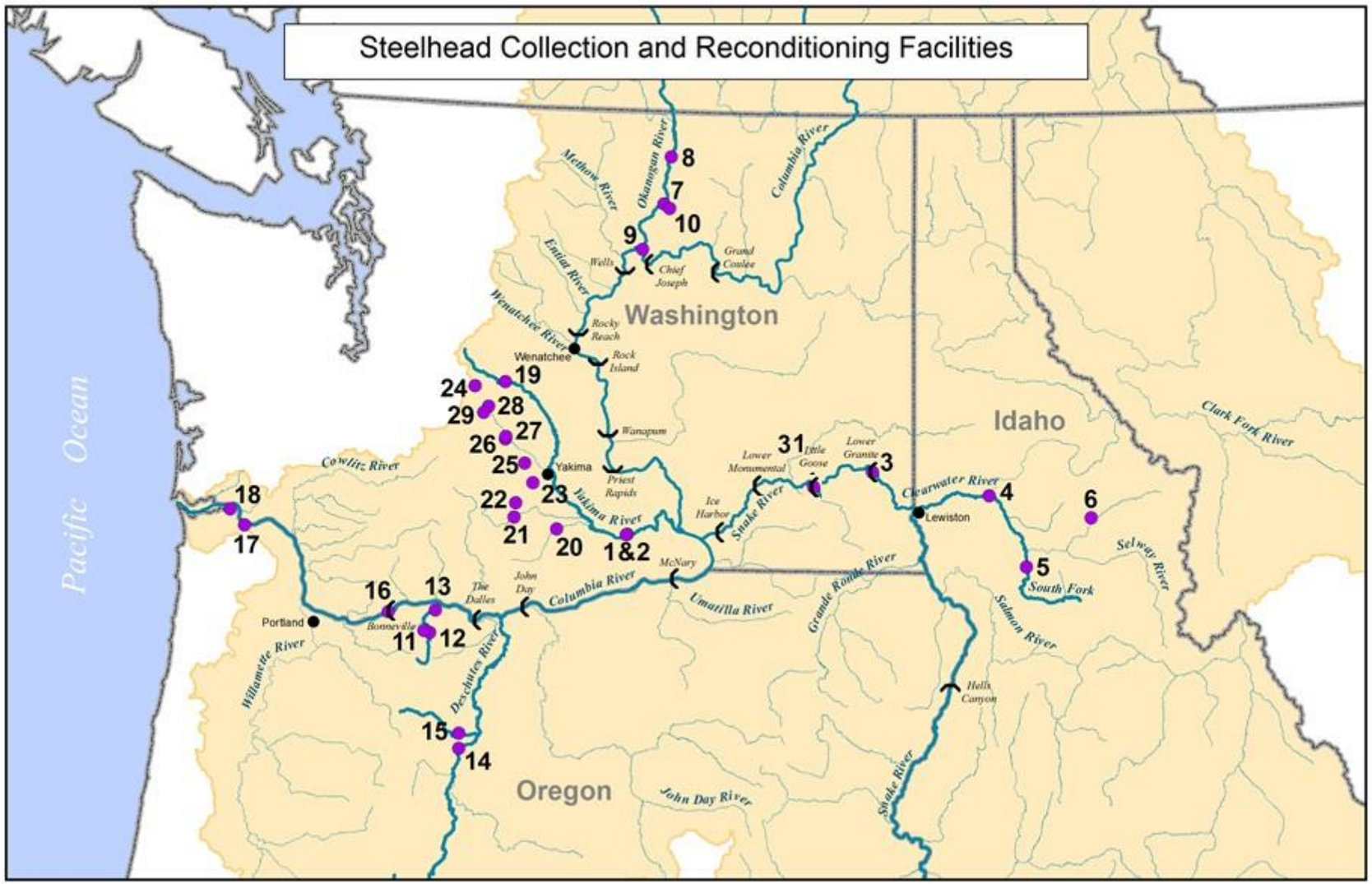


Figure 1: Map of Steelhead kelt Project area 2000-2020.

Yakima River Basin

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

Chandler Juvenile Collection Facility (Yakima River)

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 conducted at the CJMF.

Yakama Nation Prosser Hatchery

Prosser Hatchery is located on the Yakima River just downstream of Prosser Dam (RK 75.6) and adjacent to the CJMF. 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*).

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, the 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 35 km (22 miles) south of the town of Colfax, and 35 miles (56 km) north of Pomeroy (Wikipedia). 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) (RKM 173).

The Little Goose Juvenile Fish Facility

The Second Dam on the Snake River, the Little Goose 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 14km (9mi) northeast of the town of Starbuck, and 40km (25 mi) north of Dayton (Wikipedia). Steelhead kelts migrating from tributaries of the Snake River above Little Goose 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 Little Goose Dam (LGS) (RKM 113).

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 (RKM 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. Beginning in 2016 most of the kelts reconditioned at this location were hatchery fish that returned to the hatchery. They are then air spawned and reconditioned to learn more about kelt rematuration and how we can improve maturation and survival. Through 2019 besides the experimental groups surplus Lower Granite Dam captured “wild” kelts were held on site and trucked to NPTH in the fall. In 2020 all of the Lower Granite Dam and Little Goose kelts collected and then selected for reconditioning were trucked and reconditioned at this location due to technical issues at NPTH.

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. No fish were reconditioned here in 2020 due to technical issues that need to be resolved before reconditioning can resume (Hatch et al 2020). This is the future site of a permanent steelhead kelt reconditioning facility.

Chapter 1: Kelt Capture, enumeration, and reconditioning in the Yakima and Snake basins.

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 through 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 create 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 our 2020 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 for existing PIT-tags, and 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 is collected. The data is then uploaded to a central kelt database at CRITFC and any fish that is released back to the wild is entered into the PTAGIS 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 transferring to the 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 that were diverted into the receiving tank. Those held kelt steelhead, judged to be in good or better condition, with intact adipose fins, and >63cm are then trucked to DNFH for reconditioning. The transport truck had a 1.5-kiloliter tank fitted with supplemental, regulated, and 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.

Little Goose Dam

Collection and protocols followed are the same as Lower Granite Dam (Table 1, Site 31).

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). All kelt reconditioning conducted at Prosser Hatchery is primarily done in the 6.1m x 1.5m (20' x 5') circular tanks, with 4 smaller, 3m x 1.2m (10' x 4'), and one, 5.2m x 1.5m (17' x 5') circular tank also available. The water is spring fed, at 10-11^{0}C. Fish are held at DNFH in 4.6m diameter outdoor tanks, supplied with 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).

Life History Strategies: Consecutive vs. Skip Spawning

The steelhead reproductive physiology research conducted by the CRITFC has determined that both natural and artificially reconditioned kelts can pursue two alternative pathways toward rematuration and repeat spawning. One pathway is termed consecutive spawning where individuals remature and proceed to spawn in the next spawn cycle. The other pathway is termed skip spawning where individuals remature and proceed to spawn two years after their previous spawning. To illustrate, kelts collected in the spring of 2020 could spawn again in the winter/spring 2021 as consecutive spawners or wait until spring of 2022 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%) and will spawn the following winter/spring, while the majority of Snake River kelts follow the skip spawner life history (60-70%), which has them held for an additional 12 months after capture, with subsequent spawning the next winter/spring. Strategy choice is likely controlled by a combination of genetics and environment.

Additional study is needed to evaluate reconditioning strategies for skip spawners. Our past approach was 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 likely due to difficulties keeping intake water chilled adequately during the winter months. This is important as the temperature regime of the spring water utilized at Prosser can be approximately 5-8 degrees warmer than ocean/river water during the winter months. This warmer water seems to cause maturation synchronization issues due to the prolonged holding that is needed for skip spawners. Additionally, the cost of holding fish for an additional year should be compared to results from other scenarios. We plan to continue with experimental releases at least through 2021, until we can determine if it is a worthwhile strategy to convert immature spawners, using a combination of artificial and ocean reconditioning, into viable mature spawners. If results are promising this may continue as a cost-effective method to increase kelt spawner abundance in the basin.

Summary Research-Scale Efforts to Address RPA 33 and subsequent Biological Opinion (2019)

At DNFH in 2020 we continued to conduct research toward addressing Reasonable and Prudent Alternative 33 for the Hydro-system Biological Opinion. The RPA 33 required the Action Agencies to develop, in cooperation with regional salmon managers, implementation of 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 were tested and implemented including passage improvements and reconditioning kelt steelhead. The designation of B-run can be difficult to quantify in the Snake Basin. Historically size has been used to determine B-run fish populations, which has been determined by genetic analysis to not be the sole determinant of B-run populations, since genetic assignments have provided data that B-run populations do not meet specific size requirements or overlap with A-run populations. To accurately measure how kelts contributed towards the 6% RPA 33 rule NOAA, CRITFC, Nez Perce Tribe, and the federal Action Agencies (Bonneville Power Administration, U.S. Army Corps of Engineers, and the Bureau of Reclamation) devised a

system that would recognize kelt reconditioning actions and give credit for those fish we successfully reconditioned and released towards the 6% RPA value (Kelt Master Plan Document) .

In the spring of 2019, the NOAA published a new Hydrological Biological Opinion for the Columbia River Power System (NMFS 2019). The new rules effectively retired the 6% number and considered the current (2019) and all future Snake River reconditioning actions as the only hatchery actions that are appropriate for corrective mitigation for steelhead loss in the Snake River. We have continued to maintain this evaluation to gauge how much progress we are making reconditioning kelts in the Snake River Basin.

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 2002-2020 a total of 22,387 downstream migrating kelts at LGD, at LGS starting in 2020 a total of 71 kelts were collected, and 15,384 from at CJMF from 2000-2020 were collected and either released back to the river or retained for reconditioning. The Columbia River upriver steelhead run in 2019-20 was the 9th worst year since unclipped fish were counted (FPC Data). We collected 384, 49, and 71 kelts at LGD, LGS, and CJMF, respectively (Table 1.1 and Appendix A1a). In 2020, the kelt collection represented 3.5 %, and 32% of the upstream run in the Snake and Yakima rivers, respectively.

Reconditioning

Since 2011, 1,811 kelt steelhead have been retained for reconditioning from collections in the Snake River and 709 fish have survived to their first fall. Since 2000; 10,858 kelt steelhead were retained for reconditioning from collections at CJMF and 4,787 fish survived to the first fall of the annual collection period (Appendix A1a). Snake River collections were made at the LDG and LGS in 2020, however it should be noted that kelt collections have also come from the South Fork Clearwater River and Fish Creek (Lochsa River tributary) in previous years (Hatch et al. 2018).

Long-term reconditioning survival (from collection to the release in the fall) is variable from year to year but has averaged 44% at the Prosser Fish Hatchery (PFH) over the last 21 years. For the last 5 years, Yakima reconditioning has been just over 60.9% survival (Figure 1.1). The staff here has a number of years of reconditioning experience, so we generally observe small annual variations in survival (Figure 1.1). The reconditioning survival rate for wild Snake River kelts from 2011 through 2020 is 39.1%. 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. 2013a) and compounded by the inexperience of new hatchery staff. However, the past 5 years have seen marked improvement in survival rates, as water quality issues have been improved and staff gained additional experience in handling and caring for wild steelhead, with the survival rate for this last five-year period averaging 47.4% (Figure 1.2). In 2020 the kelt

collections were low (137 fish), but long-term survival was quite high (52%). Prevalence of the skip spawner life history is much higher in the Snake River than the Yakima River. On average skip spawners compose 60% of the kelt collection in the Snake River and approximately 40% in the Yakima River. This difference is likely the result of energy demands of longer migration distance in the Snake River (Keefer et al. 2008). Figures (1.1) and (1.2) show differences in the life history strategy by the proportion of retained fish (skip spawners) at the two facilities. Reconditioned steelhead kelt releases for the Yakima have been at historic lows due to poor river migration conditions (high temperatures and low flows), low ocean adult returns, and high water/flooding during collection periods. In spite of these challenging population and collection conditions we have continued to observe the reconditioned kelt population rebounding again in 2020. The improvement in Snake River releases have trended upwards in large part to successful retention and reconditioning of skip spawners (Figures 1.1 and 1.2) at the DNFH.

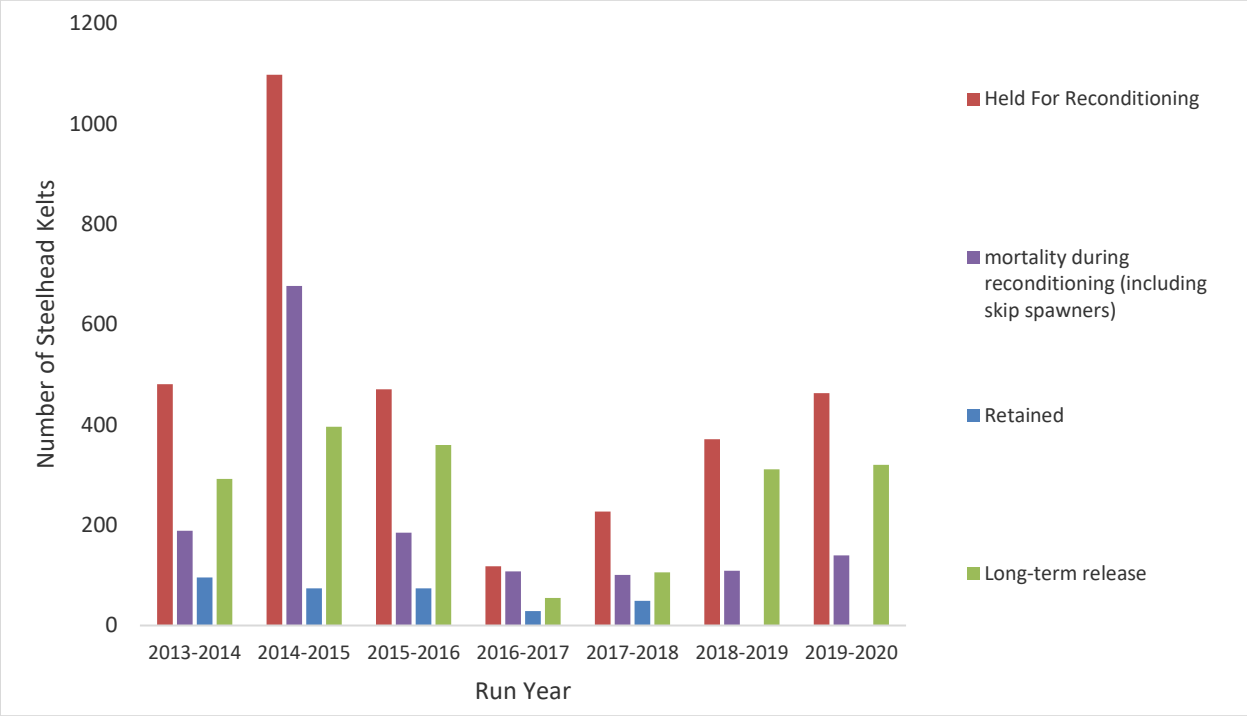


Figure 1. 1: Yakima River steelhead kelt collection for reconditioning and fate from 2013-2020. Beginning in 2019, kelts that would have been retained, were instead trucked and released below Bonneville Dam.

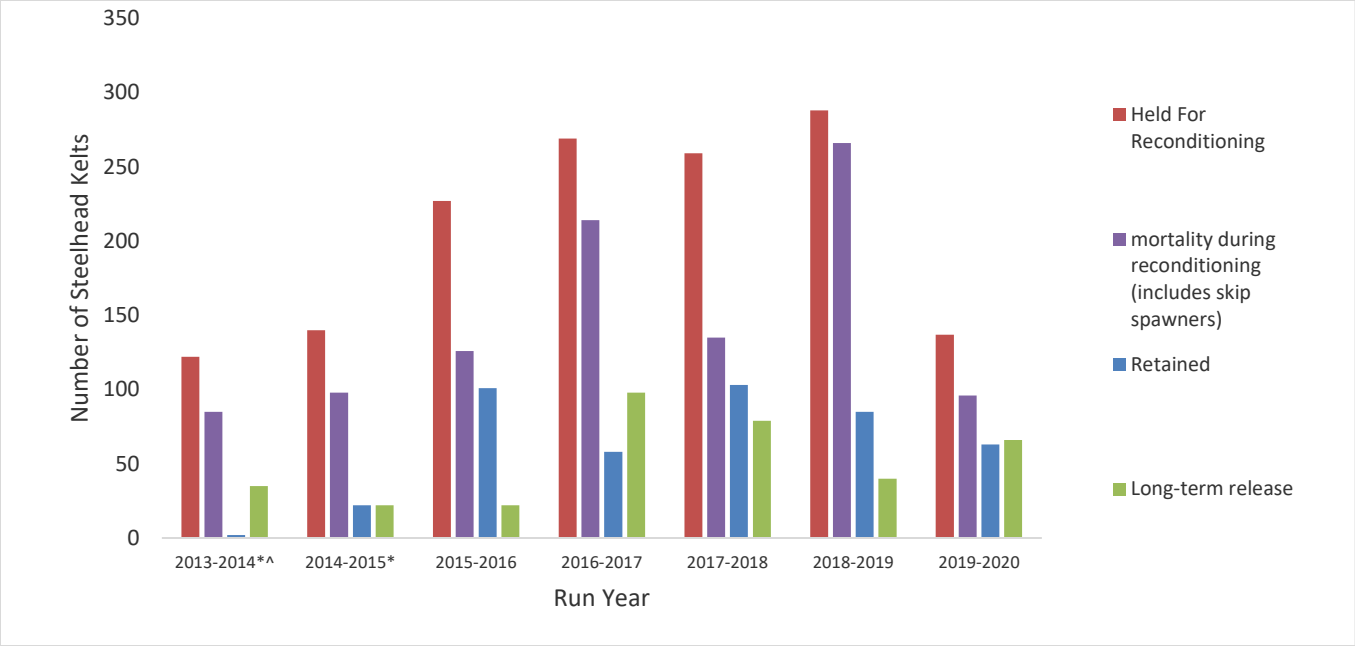


Figure 1. 2: Snake River steelhead kelt collection for reconditioning and fate from 2013-2020.

Table 1. 1 Summary of kelt collections and fish disposition in 2020 from the Snake and Yakima Rivers.

	Lower Granite	Little Goose	Snake River total	Yakima River total
Total Collection	384	71	455	471
# released back to river	296	22	318	8
# taken to reconditioning facility	88	49	137	463
# mature reconditioned fish released (consecutive spawners)	7	1	8	268
# immature reconditioned fish retained (skip spawners)	46	17	63	NA
# immature reconditioned fish released (skip spawners)	NA	NA	NA	52
# skip spawners released from previous collection			58	NA

Skip Spawner Management Strategy in the Yakima River

Reconditioned kelt steelhead demonstrate either consecutive- or skip-spawning life histories. Skip spawners make up approximately 40% of collection in the Yakima River and 60% of the collection in the Snake River. Our primary management strategy for skip spawners was to hold the fish in the reconditioning for an additional year, thus releasing the fish in the second fall of captivity. This strategy has been successful in the Snake River where second year survival and rematuration of skip spawners has been very high (60.5% survival and 26.1% rematuration). In the Yakima River, this management strategy of holding skip spawners for a second year has been much less successful in terms of skip spawner survival. This likely is a result of warmer water temperature that is used to rear skip spawners during the winter. The water source is from a well that is ideal for summertime rearing, but that same water is much warmer than the average ambient river/ocean water temperatures where kelts would naturally recondition. This water temperature fluctuation has likely had an impact on held skip spawner maturation.

Beginning in 2019, we experimented with transporting skip spawner kelts to the lower Columbia River and releasing them in the first fall concurrent with consecutive spawner releases in the Yakima River.

2019 Yakima River Lower Columbia River Release

We trucked and released 103 PIT-tagged, immature, reconditioned kelts below Bonneville Dam on 10/31/2019 to evaluate this management strategy. Thirty fish were detected moving upstream at the Bonneville Dam fish ladders within the first 30 days post release. Ten of these

30 fish had additional detections, four moving downstream and six moving upstream. The four downstream moving fish were detected either in the Bonneville Dam juvenile bypass or corner collector. Their migration patterns were consistent with post-spawn steelhead.

Final detection history for the six upstream moving fish include three fish last heard at mainstem dams (2 at The Dalles and 1 at John Day ladders) and three fish last detected in tributaries. Tributary detections included one fish in Satus Creek, a Yakima River tributary. This fish genotyped to the Yakima River GSI reporting group and was likely a male based on genetic analysis. One fish was last detected in Little Sheep Creek, a tributary of the Imnaha River. This fish genotyped to the MGILCS (mid-Columbia, Grande Ronde, Imnaha, lower Snake, lower Salmon, lower Clearwater) reporting group and was likely a male based on genetic analysis (Hess et al. 2020) Finally, one fish was detected in Fifteenmile Creek, located just upriver of the Dalles Dam, a tributary of the Columbia River. This fish genotyped to the MGILCS reporting group and also was likely male based on genetic analysis.

We will continue to observe for possible detections when we would anticipate skip spawning adult returns beginning as early as July of 2021.

2020 Yakima River Lower Columbia River Release

On October 21, 2020, we released 52 PIT-tagged, immature, reconditioned kelts below Bonneville Dam. Due to COVID-19 concerns, blood draws were not conducted at Prosser to determine maturation status. Instead visual maturation was determined by the hatchery manager. We initially observed 8 of these kelts moving upstream at Bonneville Dam. One fish has migrated back to Prosser already, which was a female, based on earlier visual calls when selected for reconditioning. Likely, this fish maturation status was called incorrectly and will be a consecutive spawner. We anticipate a that more thorough analysis will be ready in 2021, which will include genetic analysis to accurately determine sex and GSI origin.

We will continue to monitor PTAGIS for any possible return detections which we would anticipate beginning as early as July of 2021 possibly going into 2022.

Long term reconditioning mature kelt 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. The annual survival to release average ranged from 18% at the start of the program in the early 2000's to an annual high of 76% in 2016 and averaged 45% over the course of the study (2000-20) 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 1.3 and 1.4, 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 kelt, a fish caught in the Spring of 2014 would be released into the 2015/2016 run year. Figures 1.3 and 1.4 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. While the total number of returned fish may appear small compared to the overall run, especially in the Snake, many of these fish are contributing towards ESA-listed populations throughout the Yakima and Snake basins. In Figure 1.4, both Snake River A and B-run populations are counted. We primarily targeted B-run fish while selecting kelts for reconditioning although there were a small proportion of A-run fish that were also collected and reconditioned. The A-run population in the Snake is much larger than the B-run population, with most of these fish coming from the Grande Ronde basin (Hatch et al. 2019).

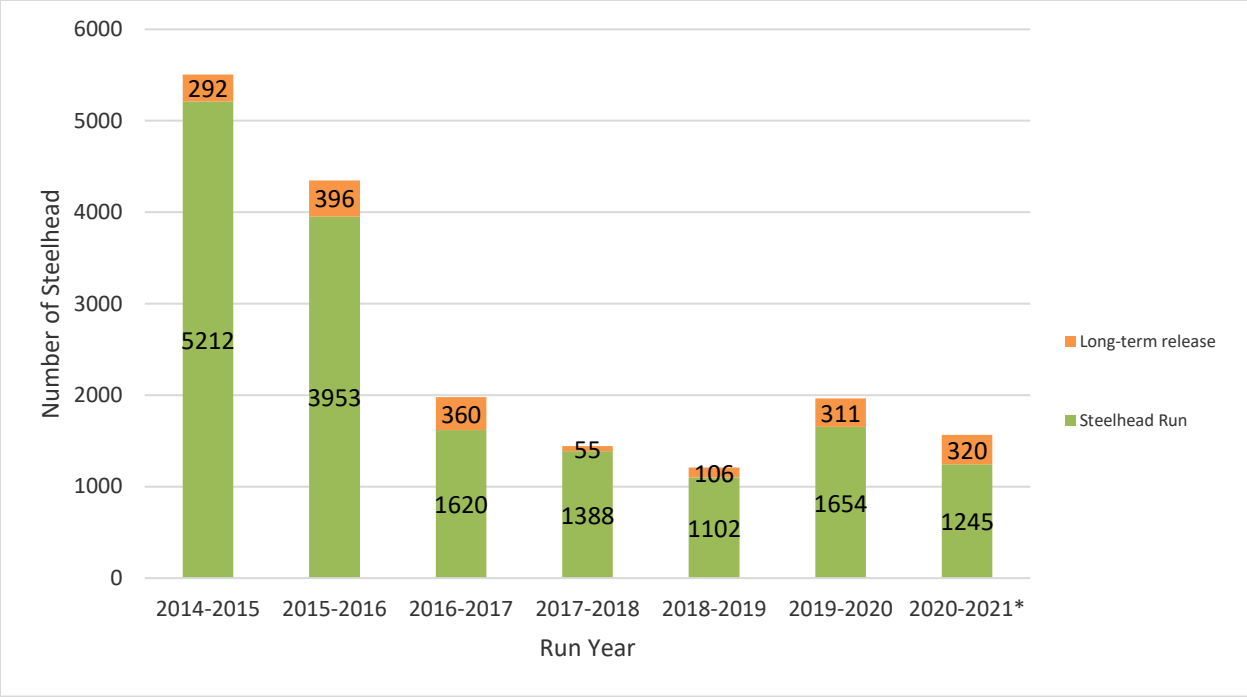


*Figure 1. 3: Contribution to steelhead run from reconditioned kelt release in Yakima Basin. * Run is still ongoing; value will be updated in 2021 steelhead kelt reconditioning annual report.*

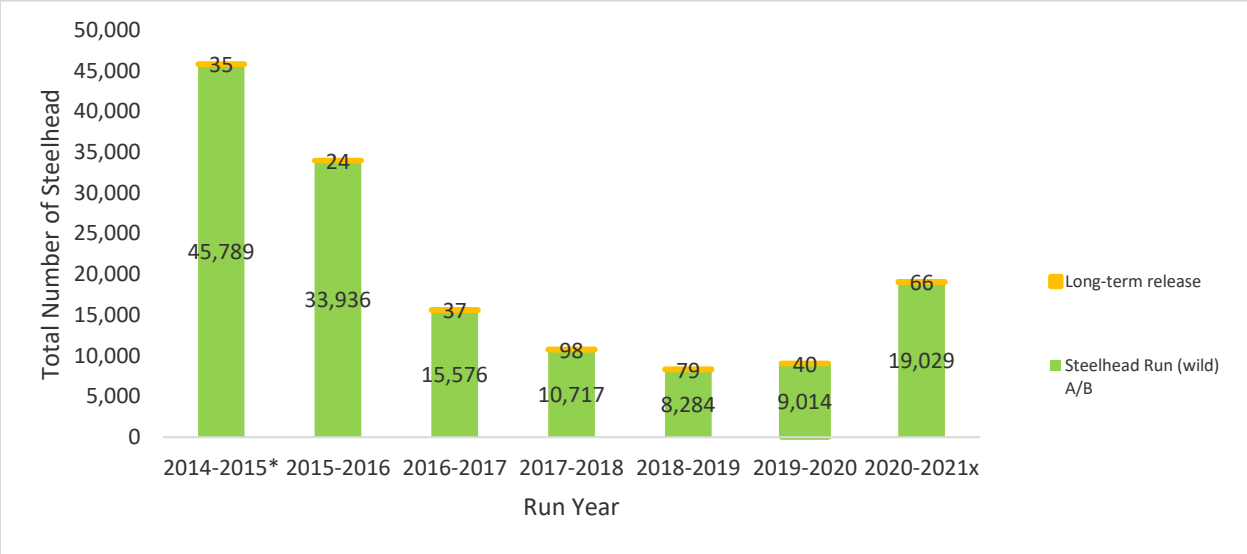


*Figure 1. 4: Contribution to steelhead run from reconditioned kelt release in Snake Basin at LGD. * includes Fish Creek and/or South Fork Clearwater Kelts*

The natural repeat spawner rate (or no intervention) measured as a return rate to Bonneville Dam, for the Snake River, is 0.27% and 2.90% for the Yakima River. The calculated benefit of reconditioning relative to leaving the fish in the river is 171.5 times for the Snake River and 15.2 for the Yakima River.

Summary Research-Scale Efforts to Address RPA 33 and subsequent Biological Opinion (2019)

Since operating at a research scale in the Snake Basin, as approved by the ISRP in the 2008 review, the capacity of our facility was 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 2020, we only reared fish at DNFH due to low collection numbers. Table (1.1) summarizes all collections for both A and B run, and releases associated with the RPA 33.

In 2013, we had a successful reconditioning and release at nearly 40% towards reaching the RPA 33 goal with 69 female fish released (Table 1.2). Our best year was in 2017, with releases just over 50% towards the RPA goal, at 98 mature female spawners released. In 2019, we had a lower number of mature kelts to release due to a mishap at the Nez Perce Tribal Hatchery, which resulted in a significant loss of retained skip spawner kelts from 2018. Nez Perce Tribe is taking actions to prevent such a catastrophe in the future, with protocols that should help to eliminate the chance that such an event will happen again. Since 2013, we have averaged 31% of the RPA goal, releasing an average of 56 mature female fish per year with a total of 393 mature female fish released from 2011-2020 (Table 1.2). Figure 1.5 is the schema that was devised by the Power Agencies and CRITFC to determine the credit that would be allocated towards collection and successful reconditioning of what was considered B-run kelt steelhead towards the larger B-run the year of release. The number represents the B-run steelhead released x estimated successful reproductive contribution. This is not representative of number of total kelts released and was generated under the direction of RPA 33. We will continue to estimate B-run steelhead kelt contributions in future reports for the benefit of management implications of kelt reconditioning in the Snake River Basin.

Table 1. 2 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 (subtotal)	Lower Granite Dam	**227**	**120**	**52.9%**	**19**	**101**	**77**	**37*^**
2017 Subtotal	Lower Granite Dam	**269**	**59**	**21.9%**	**21**	**58**	**29**	**98^**
2018 Subtotal	Lower Granite Dam	**259**	**177**	**68.3%**	**50**	**99**	**1**	**79^**
2019 Subtotal	Lower Granite Dam	**288**	**121**	**42.0%**	**39**	**85**	**58**	**40^**
2020	Lower Granite Dam	88	53	60.2%	7	46	TBD 2021	65^
2020	Little Goose Dam	49	18	36.8%	1	17	TBD 2021	1
2020 Subtotal		**137**	**71**	**51.8%**	**8**	**63**	**TBD 2021**	**66^**
Grand Total		**1811**	**709**	**39.1%**	**275**	**429**	**185**	**460**
				***includes Fish Cr. kelt skip spawners**				
				^Includes previous year kelt spawners from LGD				

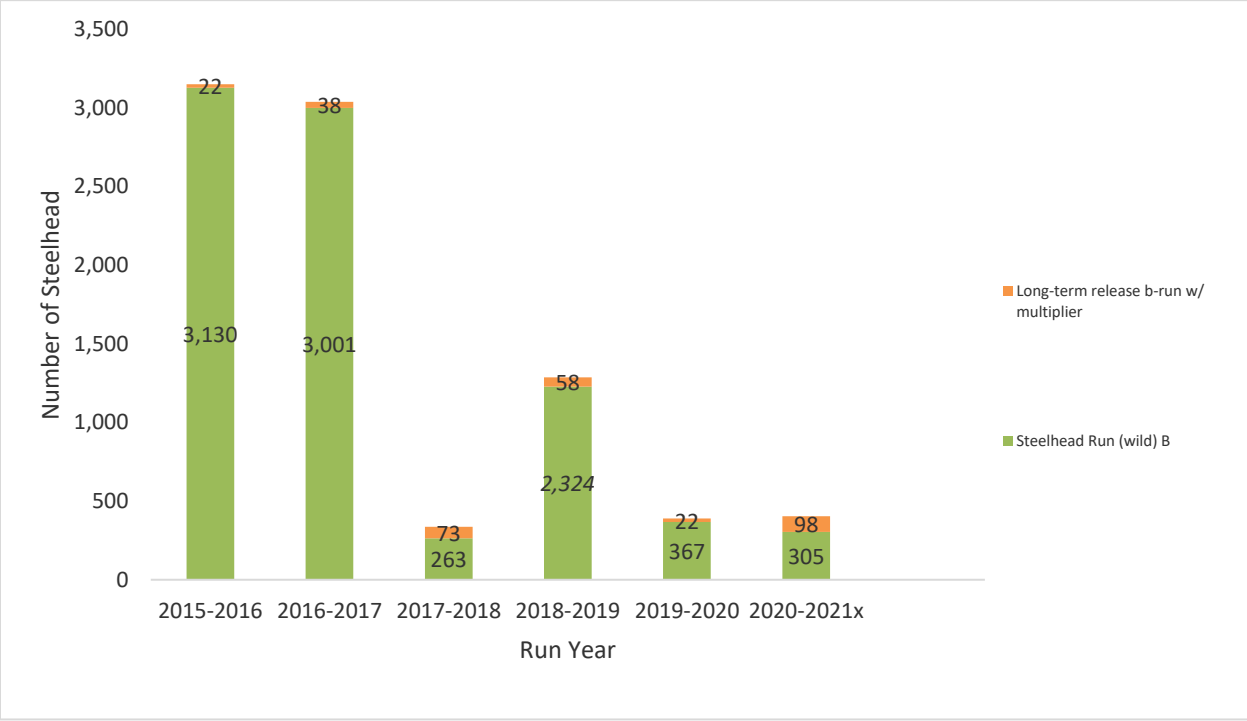


Figure 1. 5: Contribution to steelhead run from reconditioned kelt release in Snake River Basin. x2020-2021 value is an estimated run size and will be corrected in the 2021 Kelt Annual Report. B-run numbers utilize Kelt Master Plan definition for reconditioned female kelt spawners.

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 either 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.

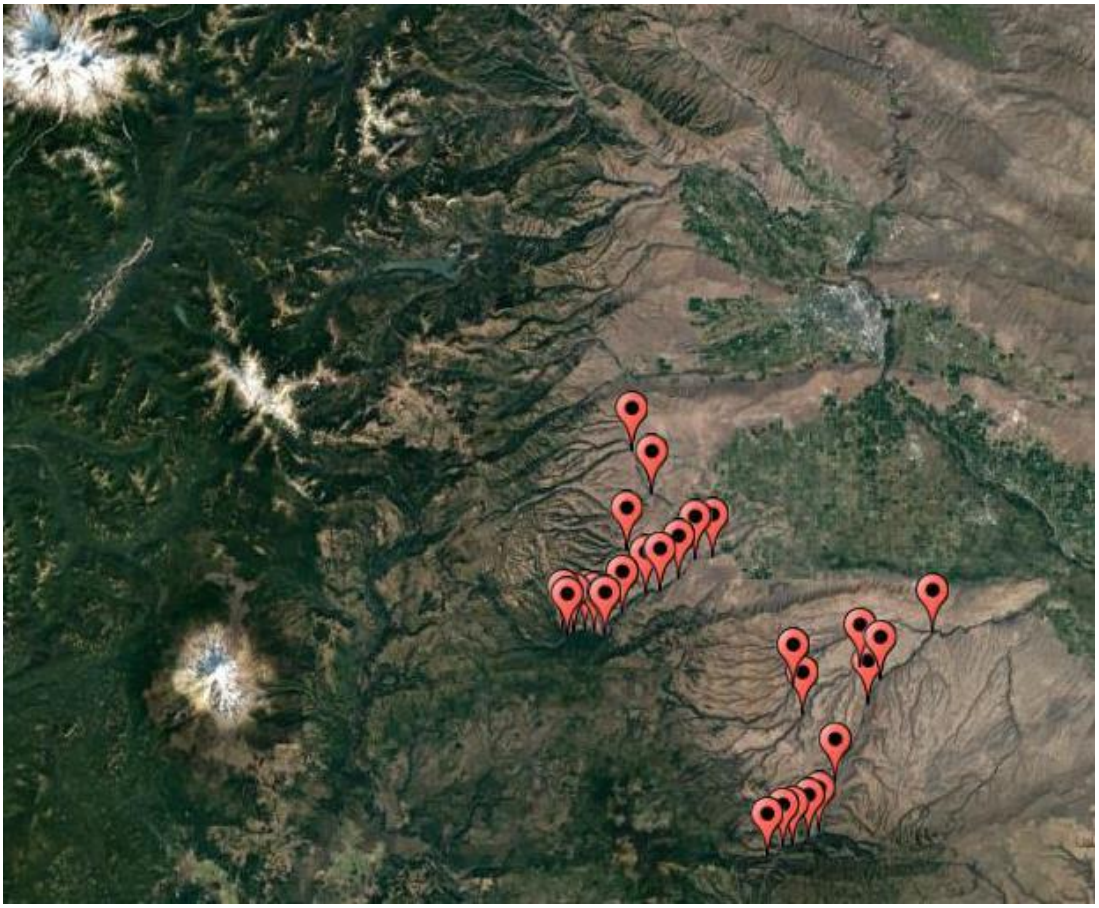


Figure 2. 1: juvenile sampling locations in Satus (lower right) and Toppenish creeks over 7- years 2013-2020.

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. Loci numbers utilized for parentage analysis varied between 172 and 239. 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 kelts, 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, relative reproductive success 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 annually 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 individual fish that spawned across multiple years, nor does it look at the same group of fish across 2 consecutive years (e.g. Maiden in 2013, reconditioned kelts in 2014). Rather, it adds the RRS estimates of fish spawning in the same calendar year. Male data is reported, but sample sizes severely limit statistical inference. Because of incomplete data, average LRS for males uses only years 2013 to 2016.

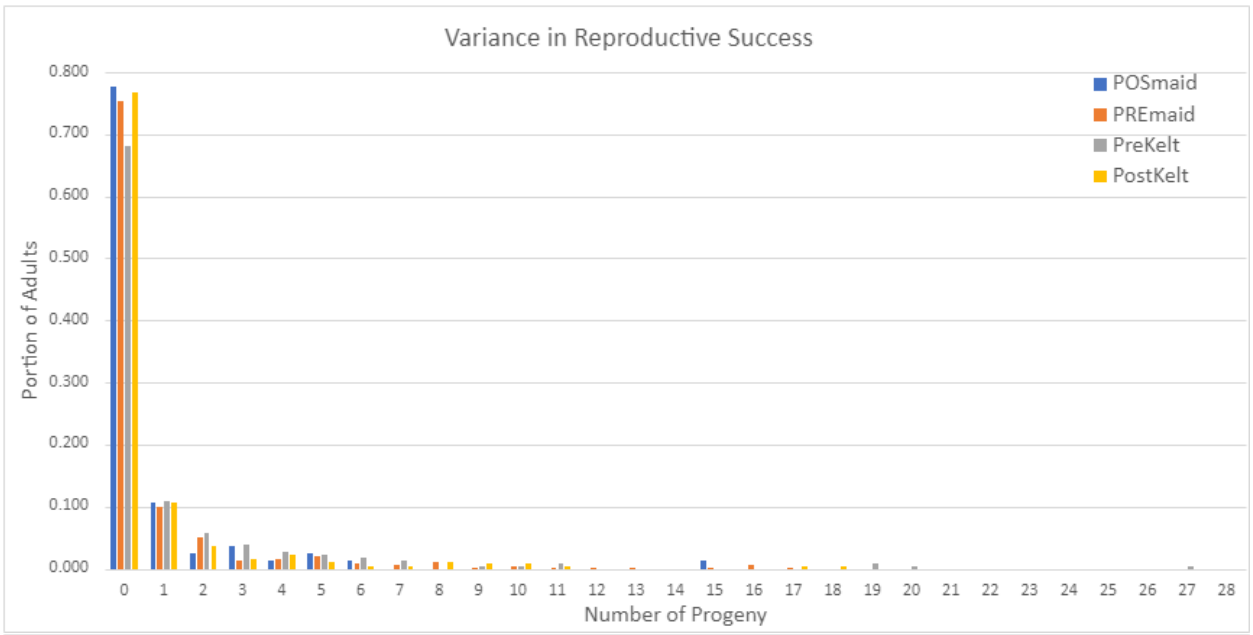
Results

The number of juveniles successfully genotyped at the tributary level, 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. In Figure (2.2), the largest number that fish assign to 0 fish, this does not necessarily indicate that fish are not reproducing but that finding progeny in nature is a difficult proposition and missing assignments appears to be equally distributed amongst all groups. This holds true for fish that had a single progeny assignment as well. The second part of the Figure (2.2) has a smaller y-axis to better show the variation between spawning event types. Kelts appear to have a larger number of progeny overall than maidens in general (Figure 2.2).

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%
2018	Genotyped	615	589
	Assigned	171	165
	% Assigned	28%	28%
2019	Genotyped	651	592
	Assigned	299	239
	% Assigned	46%	40%
Sum	Genotyped	2700	3087
	Assigned	836	1059
	% Assigned	31%	34%

Figure (2.2) shows the variance in reproductive success by spawning even type. The majority of adults have zero assignments, again due to the limited sampling protocols. It does not indicate that fish are not reproducing but rather that we did not sample all potential offspring across all possible locations. Missing assignments appears to be similarly distributed amongst all groups. This holds true for fish that had a single progeny assignment as well. The second part of the Figure (2.2) has a smaller y-axis to better show the variation between spawning events is still similar.



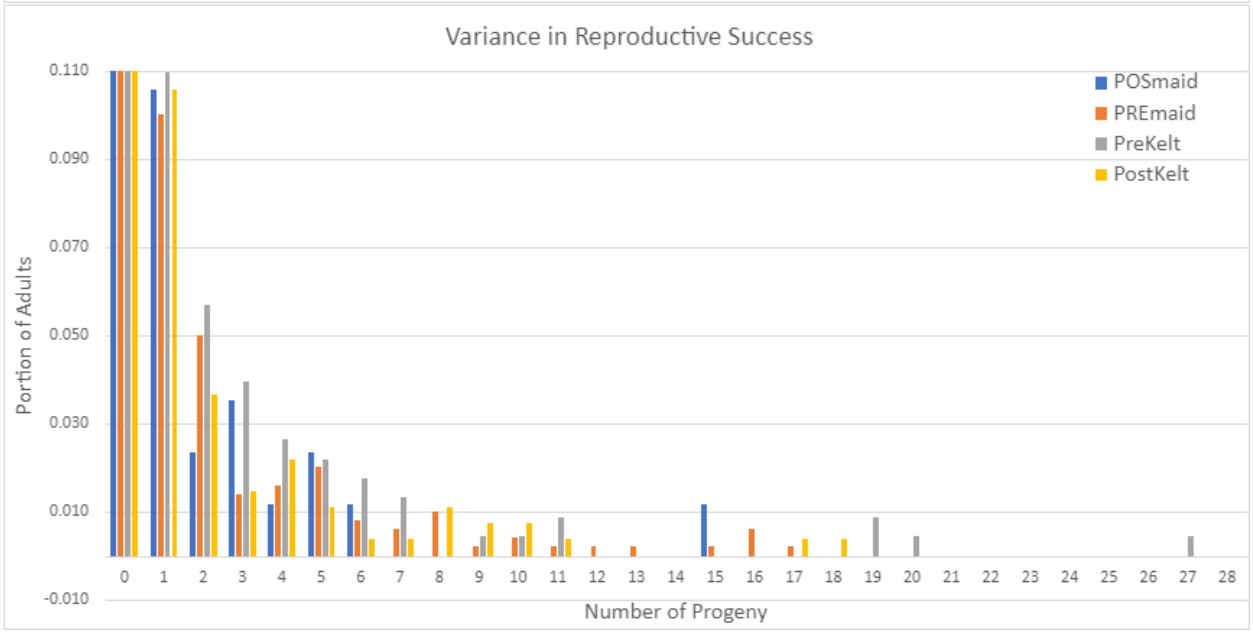


Figure 2. 2: Variation in reproductive success of maiden and kelt spawners. The first part of the figure demonstrates the number of progeny across the portion of adults genotyped. The second part of the figure shows the same details but with a truncated y-axis to better demonstrate the variability based on number of progeny. POSmaid= Post spawn maiden event, PREmaid= Maiden event collected as pre-spawner, PreKelt= first spawning event for reconditioned kelt, and PostKelt=second spawning event for reconditioned kelt see Methods, Genetic Analysis, Ch 2.

The number of genotyped parents confirmed to have entered either Satus or Toppenish creeks is shown in Table 2.2. Pre-spawn maidens have the greatest number of samples with a total of 253 males and 494 females. Post-spawn maidens have only 17 males and 84 females overall. Reconditioned kelts have 25 male and 228 female Event-1 detections and 30 male and 273 female Event-2 detections. The number of fish increase each year but are limited by the number of kelts that can be collected, and mortality seen during the reconditioning process.

The number of kelt males is likely due to low returns, and male spawners staying on spawning grounds until they likely perish due to low competition.

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

Class	Sex	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**All**
Pre-Spawn Maidens	Male	38	46	57	79	6	21	6	253
Post-Spawn Maidens	Male	4	1	7	2	1	1	1	17
Reconditioned Kelt Event-1	Male	3	13	7	1	0	0	1	25
Reconditioned Kelt Event-2	Male	5	3	13	8	1	0	0	30
Pre-Spawn Maidens	Female	88	70	92	141	46	37	20	494
Post-Spawn Maidens	Female	12	13	38	9	2	5	5	84
Reconditioned Kelt Event-1	Female	15	43	51	22	13	44	40	228
Reconditioned Kelt Event-2	Female	74	20	38	56	26	15	44	273

The number of progeny assigned to each class of fish is shown in Table 2.3. The majority of assignments are to pre-spawn maidens with 229 juveniles assigned to males and 428 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**	**2018**	**2019**	**All**
Pre-Spawn Maidens	Male	13	17	24	120	13	34	8	229
Post-Spawn Maidens	Male	1	0	0	4	7	1	0	13
Reconditioned Kelt Event-1	Male	5	6	4	1	0	0	3	19
Reconditioned Kelt Event-2	Male	3	4	1	0	7	NA	NA	15
Pre-Spawn Maidens	Female	43	41	26	165	76	42	35	428
Post-Spawn Maidens	Female	8	1	27	2	0	9	9	47
Reconditioned Kelt Event-1	Female	5	17	32	17	63	67	97	298
Reconditioned Kelt Event-2	Female	18	12	16	40	39	38	58	221

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	2018	2019	All
Pre-Spawn Maidens	Male	0.34	0.37	0.42	1.52	2.17	1.62	1.33	0.91
Post-Spawn Maidens	Male	0.25	0.00	0.00	2.00	7.00	1.00	0.00	0.76
Reconditioned Kelt Event-1	Male	1.67	0.46	0.57	1.00	NA	NA	3.00	0.76
Reconditioned Kelt Event-2	Male	0.60	1.33	0.08	0.00	7.00	NA	NA	0.50
Pre-Spawn Maidens	Female	0.49	0.59	0.28	1.17	1.65	1.14	1.75	0.87
Post-Spawn Maidens	Female	0.67	0.08	0.71	0.22	0.00	1.80	1.80	0.67
Reconditioned Kelt Event-1	Female	0.33	0.40	0.63	0.77	4.85	1.52	2.43	1.31
Reconditioned Kelt Event-2	Female	0.24	0.6	0.42	0.71	1.50	2.53	1.32	0.81

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	2018	2019	AVG
Pre-Spawn Maidens	Male	1	1	1	1	1	1	1	1
Pos-Spawn Maidens	Male	0.73	0	0	1.32	3.23	0.62	0	0.84
Reconditioned Kelt Event-1	Male	4.87	1.25	1.36	0.66	NA	NA	2.25	1.76
Reconditioned Kelt Event-2	Male	1.75	3.61	0.18	0.00	3.23	NA	NA	1.60
Pre-Spawn Maidens	Female	1	1	1	1	1	1	1	1
Pos-Spawn Maidens	Female	1.36	0.13	2.51	0.19	0	1.59	1.03	0.97
Reconditioned Kelt Event-1	Female	0.68	0.67	2.22	0.66	2.93	1.34	1.39	1.41
Reconditioned Kelt Event-2	Female	0.50	1.02	1.49	0.61	0.91	2.23	0.75	1.07

Lifetime reproductive success (LRS) of reconditioned kelts are shown in table 2.6. Male kelt LRS varied between 0.0 and 6.83 times that of fish sampled as pre-spawn maidens within the same year. Across years 2013-2016 male kelts have an LRS of 3.42. Female kelt LRS had annual variation between 1.18 and 3.84 with an average of 2.49.

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

Class	Sex	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**AVG**
Reconditioned Kelt Lifetime	Male	6.83	4.86	1.54	0.66	NA	NA	NA	3.42
Reconditioned Kelt Lifetime	Female	1.18	1.70	3.71	1.27	3.84	3.57	2.14	2.49

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 2.49 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 natural repeat spawners should scale with the number of breeding spawners. We specifically found that the relative reproductive success of the second spawning event for female reconditioned kelts (1.07) is similar or greater than that of putative first time spawners and demonstrates the potential to boost numbers additively over their first spawning event.

The 2019 spawning event was the seventh 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.

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. We anticipate that we will submit a manuscript on reproductive success in an accredited scientific journal in 2021.

Chapter 3. Kelt Reconditioning Physiology Studies

Introduction

Studies applying tools from fish physiology and endocrinology to issues in kelt reconditioning were continued in 2020. 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 2020, we sampled blood at DNFH, and provided maturation status of individual fish at DNFH and Winthrop to project managers so that consecutive and skip spawners could be managed appropriately (Chapter 3:Section A). Kelts in the Prosser project were not sampled in 2020 due to the COVID-19 pandemic. The 2020 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 published the third of three linked studies using hatchery origin kelts at Dworshak National fish hatchery (Appendix A.2; (Jenkins, et al. 2020). The first two these studies were published in 2018 and 2019 (Jenkins, et al. 2018; Jenkins, et al. 2019). Collectively, these studies advance scientific understanding of the physiology of repeat spawning and kelt reconditioning, and address significant issues such as 1) a potential tradeoff between reproductive investment in maiden spawning versus repeat spawning, 2) the productivity benefit to be expected from releasing reconditioned consecutive and skip spawners to spawn naturally, and 3) the timing and physiological basis of the decision underlying consecutive versus skip spawning. We published the results of laboratory work establishing assays for plasma insulin-like growth factor-1 (IGF-1) and growth hormone (GH), indicators of growth and metabolic status (Appendix A.2; Medeiros 2020). The ability to measure these hormones increases our understanding of physiologically important interactions between metabolic status and reproduction in steelhead kelts. This is illustrated in two studies in progress on the effect of nutritional restriction during the period after spawning in hatchery-origin steelhead kelts and a post-spawning rainbow trout kelt model. In the first (Chapter 3:Section B), we show that metabolic status at spawning, as indicated by plasma GH level, and nutrition during the first 10 weeks after spawning interact to determine whether female hatchery-origin kelts adopt a consecutive or skip spawning life history. In the second (Chapter 3:Section C), we show that plasma IGF1 level does not respond to nutritional status for the first 8 weeks after spawning, implying that there is a process of recovery from spawning that must occur before resources can be allocated to anabolic (muscle) growth. In addition, we are continuing data analysis on a completed study in which we are combining the results of a genetic analysis enabling classification of Yakima River kelts by subpopulation, with survival, physiological, and migration data (Chapter 3:Section D). These studies are ongoing, and laboratory analysis, statistical analysis, results, interpretations, and conclusions may change as additional work is completed.

Section 3.A: Reproductive status of wild kelt steelhead

Introduction

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

Iteroparous female salmonids have two major post-spawning life history trajectories (Chaput and Jones 2006; Keefer, et al. 2008; Rideout, et al. 2005; Rideout and Tomkiewicz 2011). After a spawning event, some fish are able to restore energy lost during migration and spawning, redevelop a mature ovary, and spawn the next year. These fish are termed consecutive spawners. Other fish do not initiate redevelopment of the ovary for the next spawning season, but instead skip a year. These fish are termed skip spawners. We hypothesize that these life history trajectories are the result of the effect of energy balance on maturation decisions made during seasonally defined critical periods. The influential critical period model of the first reproductive maturation (puberty) in salmonids posits that maturation is initiated during a decision window approximately one year prior to spawning (Campbell, et al. 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 (Satterthwaite et al. 2009; Thorpe 2007; Thorpe, et al. 1998). We hypothesize that a similar decision mechanism regulates rematuration in post-spawning steelhead. Consistent with this idea, we found that energy restriction affected reproductive development within 10 weeks after spawning in female rainbow trout (Caldwell, et al. 2013; Caldwell, et al. 2014). In post-spawning fish, energy driven decisions take place in the context of the extreme energy deficit incurred by migration and spawning (Penney and Moffitt 2014a, b, 2015). Threshold energy levels for maturation or rematuration are determined by the genetic makeup of the fish and subject to selection (Carlson and Seamons 2008; Hutchings 2011a).

Studies using female wild and hatchery steelhead kelts, as well as post-spawning female rainbow trout have established that blood levels of estradiol and vitellogenin diverge between rematuring and non-rematuring fish during reconditioning (Caldwell et al. 2013; Caldwell et al. 2014; Jenkins et al. 2019; Pierce, et al. 2017). 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 2020, we measured estradiol level in a large number of blood samples. We collected blood from fish in the reconditioning programs at Dworshak National Fish Hatchery, 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. Due to COVID-19 we were unable to collect plasma at Prosser, though fish were visually assessed for maturation prior to release. Additionally, 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. 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 (DNFH), Idaho, and Winthrop National Fish Hatchery (WNFH), Washington as described earlier in this report (Chapter 1) and elsewhere (Abrahamse and Murdoch 2013, 2014).

Sampling

Fish were blood sampled on the indicated dates (Table 3A.1, 3A.2, 3A.3). During blood sampling, blood (2 mL) was drawn from the caudal vein using heparinized syringes (ammonium heparin, 10 mg/ml) and centrifuged (5 min, 5000 g). Plasma was collected and frozen on dry ice in the field prior to storage at -80°C. In addition to blood sampling, the length, weight and sex of fish were recorded, and a reading of muscle lipid levels was taken with a Distell Fish Fatmeter (Distell Inc., West Lothian, Scotland), using the rainbow trout muscle lipid setting (Trout-1) at the two most anterior measurement sites recommended by the manufacturer (Colt and Shearer 2001; Crossin and Hinch 2005).

Table 3A. 1: Wild steelhead kelts being held for long-term reconditioning following the 2020 collection year. Prosser: Prosser Hatchery, DNFH: Dworshak National Fish Hatchery, WNFH: Winthrop National Fish Hatchery.

Location	Fish type	Total # Fish	Collected in 2020	Collected in 2019	Notes
Prosser	Wild kelts	463	463	N/A	
DNFH	Wild kelts	223	143	80	Overall condition of fish collected in 2020 was poorer than previous years
WNFH	Wild kelts	73	69	14	Condition was below average

Table 3A. 2: Wid steelhead kelts sampled during the fall of 2020. Prosser: Prosser Hatchery, DNFH: Dworshak National Fish Hatchery, WNFH: Winthrop National Fish Hatchery.

Location	Sample date	Fish type	# Fish	Notes
Prosser	10/20/20	Wild kelts	321	Fish were visually inspected for maturation status
DNFH	9/9/20	Wild kelts	139	Fish were collected in 2019 (n = 64) and 2020 (n = 75)
WNFH	9/23/20	Wild kelts	32	Fish were collected in 2019 (n = 8) and 2020 (n = 24)

Table 3A. 3: Wild steelhead kelts released during the fall of 2020. Prosser: Prosser Hatchery, DNFH: Dworshak National Fish Hatchery, WNFH: Winthrop National Fish Hatchery.

Location	Release date	Fish type	# Fish	Notes
Prosser	10/20/20	Wild kelts	269	Maturation rate = 83.8% (maturation status determined by visual inspection)
DNFH	11/10/20	Wild kelts	75	Includes 6 fish that were not sampled on 9-9-20 and 1 non-maturing fish deemed unlikely to survive
WNFH	11/10 and 11/13/20	Wild kelts	15	12 released on 11/10/20 and 3 released on 11/13/20

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 duplicate technical replicates assayed in the EIA according to the manufacturer's instruction manual provided with the kit.

Results

Plasma E2 levels were bimodally distributed in blood samples taken from female kelts in all projects at a pre-release sampling in the fall (Figs 3A.1, 3A.2). As found in previous years, the division between the lower and higher modes was approximately 1000 pg/ml E2 at DNFH, and WNFH. One Lower Granite fish with an E2 level close to 1000 pg/ml appeared to group with the lower mode but could be a fish maturing more slowly than the rest of the upper mode. In order to release all fish that might possibly be maturing, the division between modes was adjusted to include this fish as rematuring. The rematuration rate of female kelts as consecutive spawners in 2020 was high at Prosser; based on visual inspection, females rematured at a rate of 83.8%. However, since this was not based on plasma E2 levels as in previous years, caution should be used in comparing the Prosser 2020 maturation rate to previous years or other projects. Consecutive spawners from other programs on the Snake River and Upper Columbia River had lower rates of rematuration for 2020, with only 9.6% of the Snake River fish rematuring and 29.2% of the Upper Columbia River fish rematuring. As with previous years, the rematuration rate of female kelts held for a second year of reconditioning as skip spawners was very high for the Snake River fish (92.1%) as well as the Upper Columbia River fish (100%).

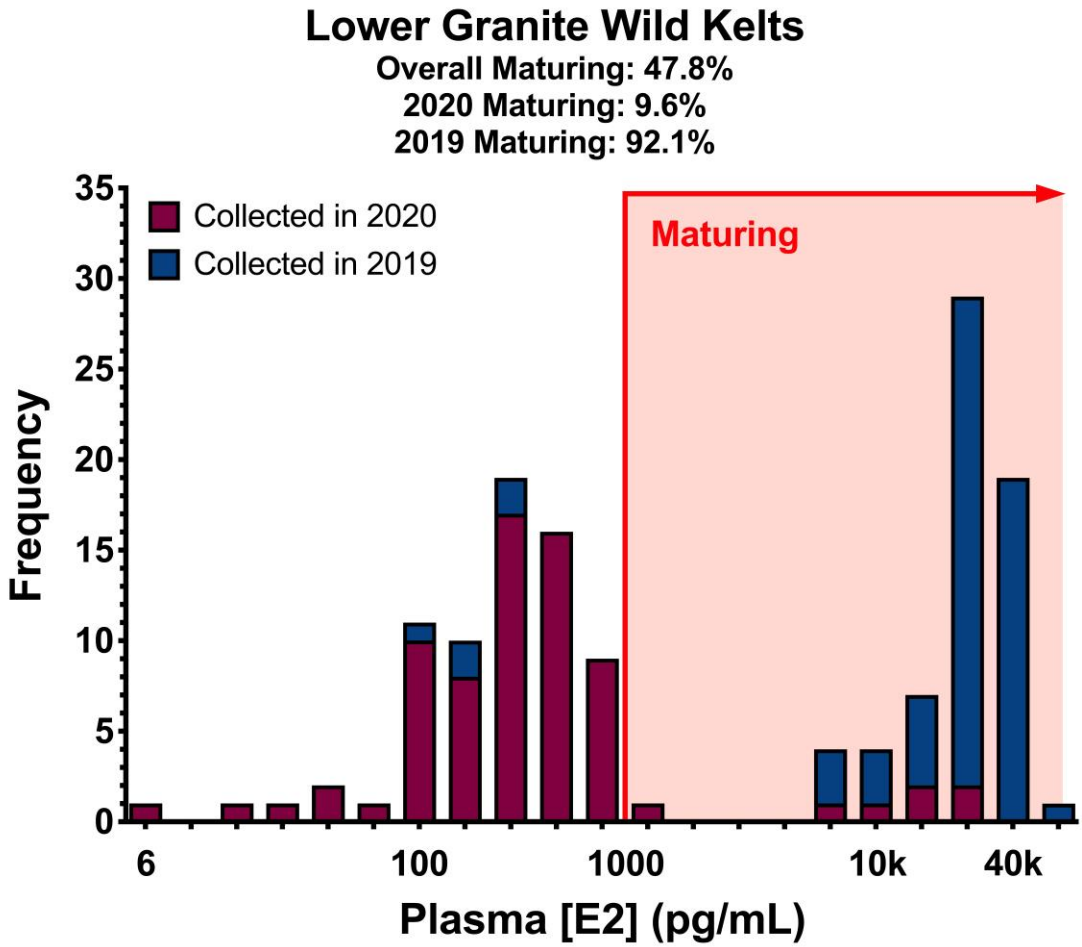


Figure 3A. 1: Plasma estradiol (E2) levels in wild female Snake River kelts sampled in fall of 2020.

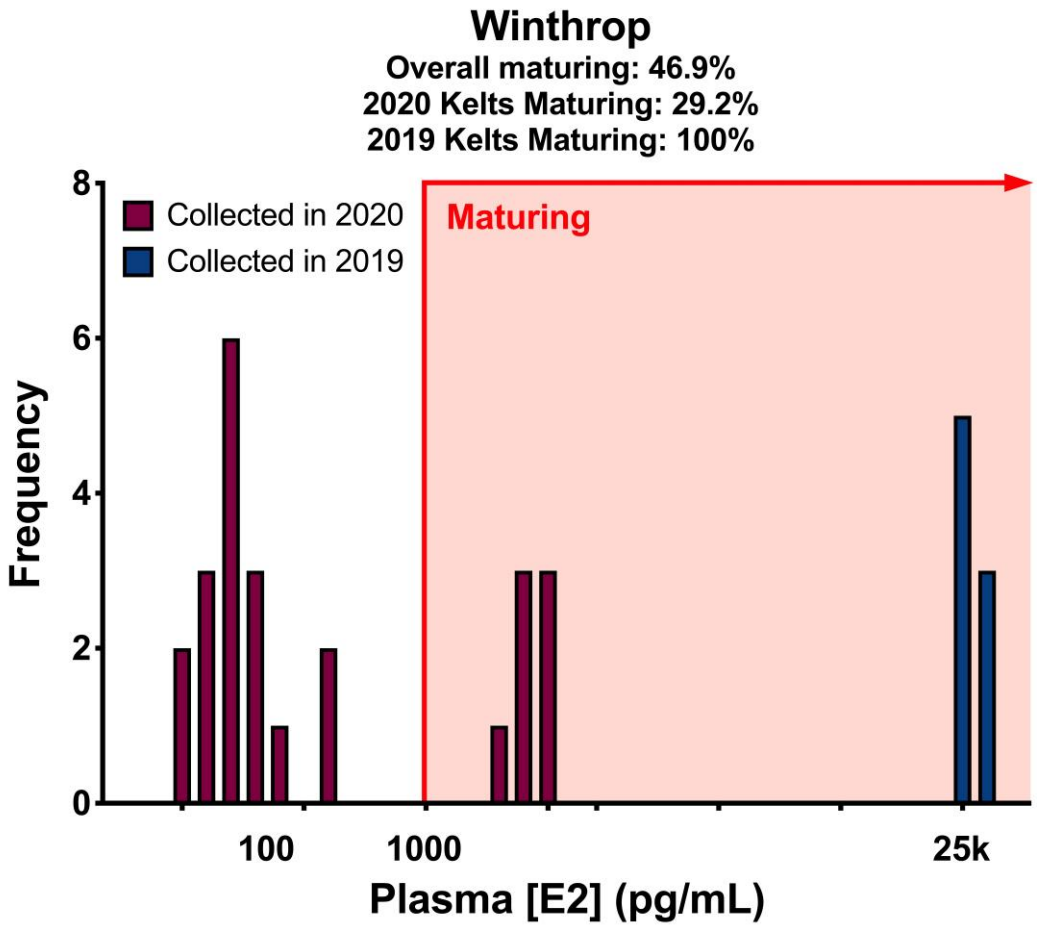


Figure 3A. 2: Plasma estradiol (E2) levels in female Upper Columbia River kelts sampled in fall of 2020.

Due to the relatively low survival rate for hold over fish at Prosser (averaging approximately 13.7%), all fish are now being released regardless of maturation status. Thus, there were no skip spawners held for a second year at Prosser. Fish believed to be maturing were released just downstream of Prosser Dam while immature fish were trucked downstream to below Bonneville Dam. Skip spawner survival for fish collected at Lower Granite in 2019 and held at DNFH was on parr with previous years (80%). Mortality of fish collected in 2020 was 30.2% at Prosser, 47.5% at DNFH, and 63.8% at WNFH.

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-June onward indicates maturation status (Jenkins et al. 2018; Jenkins et al. 2019; Pierce et al. 2017; Trammell, et al. 2016). Evidence in both steelhead kelts and post-spawning rainbow trout suggests that the initial decision to remature is made early, before mid-July for kelts and during the 10 weeks after spawning in rainbow trout (Bromage, et al. 1992; Caldwell et al. 2013; Caldwell et al. 2014; Hatch, et al. 2013; Jenkins et al. 2018; Jenkins et al. 2019; Pierce et al. 2017; Trammell et al. 2016). Plasma estradiol levels in rematuring and non-rematuring kelts for 2020 at all sites were similar to previous years and were similar between 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. With only 30% of fish rematuring after a year of reconditioning (on average), consecutive rematuration is observed in less than half of this 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. This year's higher mortality, coupled with the lower-than-average rate of consecutive rematuration, at WNFH and DNFH provides support for the idea that pre-capture environmental conditions are an important factor in determining rematuration. Should we observe a high rate of maturation in the skip spawners next year, it will provide further evidence that the reconditioning program is providing an (artificial) refuge for life histories that would otherwise be in decline, which is especially important during years with low adult returns.

Non-rematuring fish collected from the Snake River and Upper Columbia held for a second year rematured at very high rates (92.1% or higher) in 2020. 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, which will become increasingly important as run numbers show an overall decrease and the kelt survival rate increases. Together, these data point out the important role the program is playing in increasing the productivity and life history diversity of target steelhead populations.

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

Note: This section is currently being prepared for submission to a peer-reviewed journal. Please refer to the journal article for the definitive version. This study follows on to our previous project-funded studies using this hatchery kelt model (Jenkins et al. 2020; Jenkins et al. 2018; Jenkins et al. 2019).

Introduction

Consecutive (1-year spawning interval) and skip (2-year or greater spawning interval) spawning life histories are found in repeat spawning steelhead, both in natural repeat spawners and in artificially reconditioned fish (Jenkins et al. 2019; Keefer et al. 2008; Pierce et al. 2017). Consecutive spawning rates vary substantially between projects, and between years (Hatch, et al. 2019). The proportion of consecutive spawners in any given year has a major bearing on the both the impact and the operation of reconditioning projects. Only consecutive spawners (i.e. actively reproductive fish collected in the current year) 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 both repeat spawning life history types is greater than that of maidens, and productivity 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. 2005; Rideout and Tomkiewicz 2011). In salmonids, maturation is thought to be initiated based on energetic status (i.e. energy reserves or energy balance) 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 gonadal recrudescence or becoming reproductively active in subsequent years. Maturation is thought to be condition-dependent based on energetic status (McBride, et al. 2015). Maturation requires a fish to exceed genetic thresholds for energetic status (Thorpe 2007), where energetic status either exceeds or falls below a threshold, creating reaction norms (Hutchings 2011b), which predict whether a fish will mature. Summer steelhead incur a large energy deficit during their prolonged fasting return migration from the ocean and spawning, which likely interacts with the maturation decision.

The critical period for initiation of maturation (puberty) 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 gonadal recrudescence in steelhead kelts is not known in detail. In first-time spawning 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 to a maintenance ration after spawning resulted in reduced plasma estradiol (E2) levels within 10 weeks after spawning and resulted in no fish maturing as consecutive spawners (Caldwell et al. 2013). In repeat spawning hatchery-origin steelhead, growth was significantly elevated in consecutive versus skip spawners over the initial 10 weeks after spawning (Jenkins et al. 2019). However, in winter flounder, fish in better condition after spawning were more likely to spawn consecutively regardless of post-spawning feeding (Burton 1994). Consecutive spawning rates in steelhead kelt reconditioning programs vary from year to year despite constant conditions including feeding to satiation after capture, suggesting that pre-capture environmental conditions must play a role (Hatch et al. 2019; Jenkins et al. 2019; Pierce et al. 2017). Based on these findings, we hypothesize that gonadal recrudescence as a consecutive spawner may be determined during the 10 weeks after spawning by an interaction between energy reserves at spawning and post-spawning nutrition. In order to test this hypothesis, we conducted an experiment to assess the effects of fasting during this time period.

The growth hormone/insulin-like growth factor (GH/IGF) endocrine axis is the principal physiological system that regulates growth in salmonids, as in other vertebrates (Norbeck, et al. 2007; Perez-Sanchez, et al. 2018; Wood, et al. 2005). Under conditions that favor growth, GH secreted by the pituitary gland stimulates the liver to produce IGF1 and release it into the circulation. Circulating as well as locally produced IGF1 and the related IGF2 (in fish) stimulate anabolic (muscle) growth. However, during fasting and under other catabolic conditions, the role of GH switches from stimulation of growth to mobilization of stored energy (Bergan-Roller and Sheridan 2018; Norbeck et al. 2007). The endocrine mechanisms underlying this switch are liver GH resistance, in which liver production of IGF1 becomes resistant to stimulation by GH (Bergan-Roller and Sheridan 2018; Norbeck et al. 2007; Pierce, et al. 2011; Pierce, et al. 2005a), and negative feedback from circulating IGF1 on pituitary GH secretion (Fruchtman, et al. 2000; Rousseau, et al. 1998). During fasting and other catabolic states, GH resistance develops, resulting in reduced circulating IGF1 and strongly increased GH (Bjornsson, et al. 2018; Pierce, et al. 2005b). The reduction in circulating IGF1 results in reduced growth, and circulating IGF1 levels can be used under some circumstances as an indicator of growth status (Beckman 2011; Perez-Sanchez et al. 2018; Picha, et al. 2008a; Pierce, et al. 2001). The increase in circulating GH has a well-established role in the mobilization of stored lipids (Bergan-Roller and Sheridan 2018; Norbeck et al. 2007), and a more speculative role as permissive for protein catabolism (Bjornsson et al. 2018). Thus, plasma GH can be used as an indicator of catabolic status, although this application is less well developed than the use of IGF1 as a growth indicator (Perez-Sanchez et al. 2018; Picha et al. 2008a). The GH/IGF axis also interacts with the reproductive endocrine axis in fishes at the level of pituitary gonadotrophs, and likely other levels as well, which has been proposed to be a mechanism underlying the effect of energetic status on reproductive decisions (Baker, et al. 2000; Campbell et al. 2006; Huang, et al. 1998; Luckenbach, et al. 2010). To explore the role of the GH/IGF axis in reproductive decisions in

steelhead kelts, we used recently developed assays in our laboratory (Medeiros, et al. 2020) to measure plasma GH and IGF1 levels in this experiment.

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 2017 and 2018, and were held unfed in holding ponds supplied with river water. During February through April in 2017 and 2018, DNFH staff selected fully mature fish >74 cm for use as broodstock. Fish in good or fair condition (Hatch et al. 2013b), N=191 in 2017 and N=122 in 2018, were selected for this study and individually marked with passive integrated transponder (PIT) tags inserted into the pelvic girdle.

Spawning and Sampling

Fish were anesthetized using AQUI-S 20E (AquaTactics Inc., Kirkland, WA; 75mL $1000L^{-1}$ water) and were manually "air spawned" (Leitritz and Lewis 1980). Fish were non-lethally sampled for length (fork length, FL, cm), wet body mass (kg), muscle lipid level (ML, %) (Fish Fatmeter model 692, Distell Inc., West Lothian, UK), total egg mass, and blood. Samples of 25 eggs were retained and weighed for each fish to determine individual egg mass. These data were used to adjust mass at the time of spawning to account for for any residual eggs that were removed both at the 10-week sampling point and at terminal sampling. Non-lethal sampling occurred at spawning and at 10-week intervals thereafter (Jenkins et al. 2019), except for fish from 2017 spawning event 2, which were sampled 11-weeks after maiden spawning before resuming the schedule described. Sampling continued at 10-week intervals until fish were terminally sampled 30-weeks after spawning in September. During terminal sampling, all of the data and samples in non-lethal sampling were taken, and then cerebral percussion was employed to terminate anesthetized fish. Fish were dissected, ovary and liver weights recorded, and the number of residual eggs in the body cavity recorded.

Reconditioning husbandry

Fish were held at DNFH in 4.6m diameter outdoor tanks, supplied with 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 to control external *Saprolegnia* infestation (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*) via intraperitoneal injection: oxytetracycline (Durvet, Blue Springs, Missouri; 20 mg/kg body weight) and emamectin benzoate (Sigma-Aldrich, St. Louis, Missouri; 200ug/kg body weight), respectively, at maiden spawning and every 10 weeks thereafter (only as needed for copepods) as previously described (Jenkins et al. 2018).

Experimental Treatment

Fish from each spawning event (N=3 in 2017, N=2 in 2018) were randomly divided between two tanks. Due to limitations on the number of tanks available, fish from 2017 spawn event 2 and 3, which were one week apart, were combined into the same tanks. One tank from each spawning event was fed *ad libitum*, and the other tank was fasted. Fish from 2017 spawning events 1 and 3 were fasted for 10-weeks, and fish from 2017 spawning event 2 were fasted for 11 weeks. In 2018, fasting lasted 10-weeks for both spawning events. After fasting, fish were consolidated into one tank per spawning event (2017 spawning events 2 and 3 were combined), and all tanks were fed *ad libitum*. *Ad libitum* feeding consisted of offering a mixture of fish pellets (Biobrood, 6-mm pellet size, BioOregon, Longview, Washington) and boiled krill *Euphausia superba* (Atlantic Pacific Products, Kingston, Rhode Island) top-coated with menhaden oil (Argent Aquaculture, Redmond, Washington) and decapsulated Artemia cysts (American Brine Shrimp, Ogden, Utah) at least 5 times daily until feeding activity stopped (Jenkins et al. 2018).

Assays

Plasma estradiol-17 β (E2, ng/mL) concentration was measured by ELISA (Biosense, Cayman Chemical, Ann Arbor, MI), in duplicate technical replicates after solvent extraction as previously described (Jenkins et al. 2019). Plasma GH and plasma IGF-1 were measured by time-resolved fluoroimmunoassay in duplicate technical replicates as previously described (Medeiros et al. 2020). To remove interference from IGF binding proteins, plasma samples were treated by acid-ethanol cryoprecipitation, dried down, and reconstituted before assay for IGF-1 (Medeiros et al. 2020; Shimizu, et al. 2000).

Morphometric analysis

Mass specific growth rate (MSGR) and Fulton's condition factor (K) were calculated as follows:

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

$$(2)\quad K = 100 * \text{body mass (g)} * (\text{fork length (cm)})^{-3}$$

Before calculation of MSGR and K, body mass was adjusted to account for any eggs retained from maiden spawning in the body cavity (Jenkins et al. 2018). Length specific growth rate was calculated the same way as MSGR.

Statistical analysis

Reproductive status was assigned in early autumn, 30 weeks after maiden spawning, based on complete separation of fish into two E2 concentration groups (high levels = reproductive, low levels = non-reproductive), and necropsy by examining developing ovaries for large (vitellogenic) oocytes. Fed and fasted reproductive and non-reproductive groups were

compared at 10-week intervals in a time series starting at maiden spawning. Only fish positively identified by PIT tags through the entire experiment were included in the analysis.
Before further analyses, levels of E2 and GH were log_{10}-transformed, and ML was arcsine square root-transformed, in order to conform to assumptions of normality. E2, GH, and IGF-1 were then standardized to the average of the value for all fish at week 0 (standardized value = x * (average value at week zero)$^{-1}$) in each year to account for differences between years, which would be expected due to seasonal variations in conditions experienced by cohorts of fish before capture. Fasting treatment and reproductive status were combined into a single categorical variable (Fasting treatment – reproductive status) to simplify data presentation. For each response variable, potential differences between years were assessed by comparing the two years at each time point in two-way ANOVAs assessing the effects of fasting treatment-reproductive status, year, and the interaction of these two effects. Years were combined when significant year and interaction effects were absent. When significant year or interaction effects were found, they were examined to determine whether year or interaction effects changed the major results of interest, i.e. effects of group or time, and years were combined when the main result was not changed. Effects over time on each response variable were assessed by one-way ANOVA using the combined categorical variable followed by the Tukey test. Differences at each time point in the combined categorical variable were assessed by one-way ANOVA followed by the Tukey test. Effects of fasting treatment, reproductive status, and the interaction of these factors was assessed at each time point by two-way ANOVA.

All statistical analysis was conducted with PRISM software version 9.0 (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

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

Survival and Reproductive Status

Survival was 43.5% (83/191) in 2017 and 42.6% (52/122) in 2018 (Table 3.B.1), which was above average compared to that found in previous studies in steelhead trout kelts: 38%, 24% (Hatch et al. 2013b; Jenkins et al. 2019). Of the 2017 survivors, 29% (24/83), or 13% of the total fish collected, became reproductively active on a consecutive spawning schedule. In 2018, 60% (31/52), or 25% of fish collected, became reproductively active on a consecutive spawning schedule. Of the fully fed fish, 33% (14/42) became reproductively active in 2017 and 68% (17/25) in 2018. Of fasted fish, 24% (10/41) became reproductively active in 2017 and 52% (14/27) in 2018.

Table 3B. 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 2017	**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	65.4	13	4	76.5
	Fasted	29	15	48.3	10	4	71.4
Two, 2/20/2018	Fed	32	24	25.0	4	4	50.0
	Fasted	33	20	39.4	4	9	30.8
All 2018	**Fed**	**58**	**33**	43.1	**17**	**8**	68.0
	Fasted	**62**	**35**	43.6	**14**	**13**	51.9

E2

Significant year and interaction effects were found for E2 at week 30, but these were due to slight differences that did not affect the main results and years were combined. Fasting treatment and reproductive status significantly affected E2 (Table 3B.2). At spawning (0 weeks) there were no differences in E2 between groups: Fed-Reproductive, Fed-Non-reproductive, Fasted-Reproductive, and Fasted-Non-reproductive (Figure 3B.1). Apparent differences between Reproductive and Non-reproductive fish within fed and fasted groups are not significant (P = 0.1318, 0.3411 for Fed- and Fasted- respectively). In all groups, E2 decreased significantly from 0 weeks to 10 weeks. At 10 weeks, Fasted Non-reproductive fish had significantly lower E2 than Fed-Reproductive fish, while others showed no detectable differences.

Table 3B. 2: P-values from 2-way-ANOVAs testing the effects of experimental treatment (fasted or fed for the first 10 weeks after spawning) reproductive status at 30 weeks (reproductive or non-reproductive), and the interaction of these two factors on plasma estradiol and growth hormone levels through the course of the experiment. Significant effects are bolded.

	Estradiol			Growth Hormone		
Week	Experimental Treatment	Reproductive Status	Interaction	Experimental Treatment	Reproductive Status	Interaction
0	0.4816	**0.018**	0.4741	0.2017	**0.011**	**0.0032**
10	**0.0193**	0.0982	0.8092	**0.0001**	**0.0034**	0.344
20	0.1052	**0.0001**	0.0541	0.126	**0.0016**	0.0572
30	0.7235	**0.0001**	0.5866	0.9407	**0.0002**	0.2587

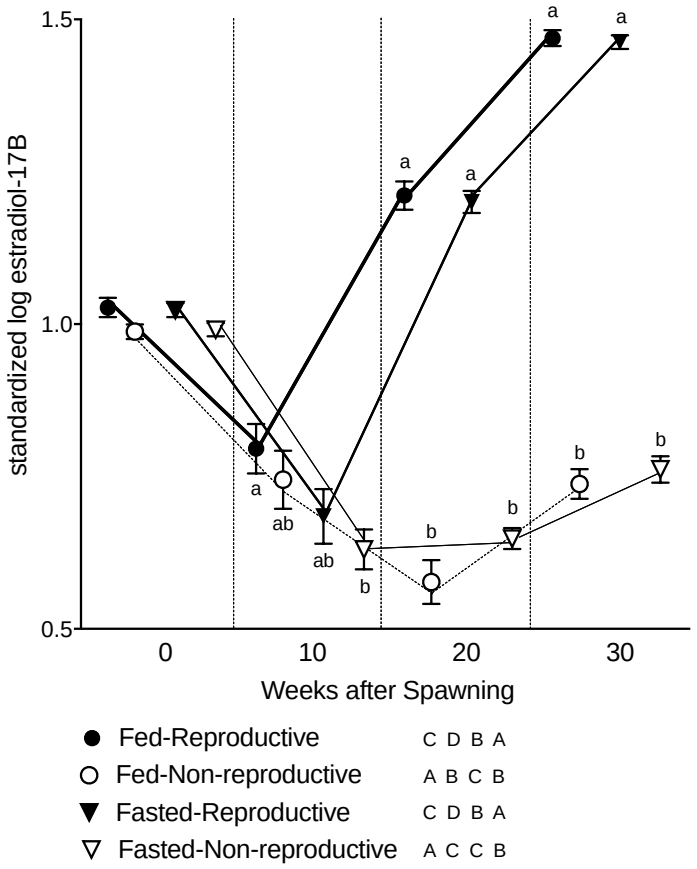


Figure 3B. 1: Estradiol-17B (E2) concentrations in female steelhead trout sampled at 10-week intervals starting at first spawning. Analysis included Fed-Reproductive and Fed-Non-reproductive (closed and open circles, n = 31, 35) and Fasted-Reproductive and Fasted-Non-reproductive (closed and open triangles, n = 24, 44). Symbols indicate mean and bars indicate SEM. At each sampling point, symbols sharing the same letter on the graph do not differ significantly. Rows of letters below the figure indicate significant differences over time within each treatment group.

At 20 weeks after spawning, Fed- and Fasted-Reproductive fish showed increased E2 from 10 weeks and reached significantly higher levels than Non-reproductive fish. From 10-20 weeks, E2

levels stayed the same for Fasted-Non-reproductive fish and continued to decrease from 10 weeks for Fed-Non-reproductive fish. At 30 weeks, all groups significantly increased from levels at 20 weeks. Reproductive fish remained significantly higher than Non-reproductive fish. Fed-Non-reproductive fish E2 levels returned to week 10 levels.

GH

No significant year or interaction effects were found for GH and years were combined. Fasting treatment, reproductive status, and the interaction of these factors affected GH (Table 3B.2). At the time of spawning, GH levels were lower in Fasted-Reproductive fish than in any other group (Figure 3B.2). At 10 weeks after spawning, GH increased significantly from spawning levels in both fasted groups, which no longer differed from each other, and reached higher levels than the two fed groups. GH was lower than both Fasted groups in Fed-Non-reproductive fish at 10 weeks and did not change from 0 weeks. GH significantly decreased in Fed-Reproductive fish from 0 to 10 weeks and became significantly lower than in any other group.

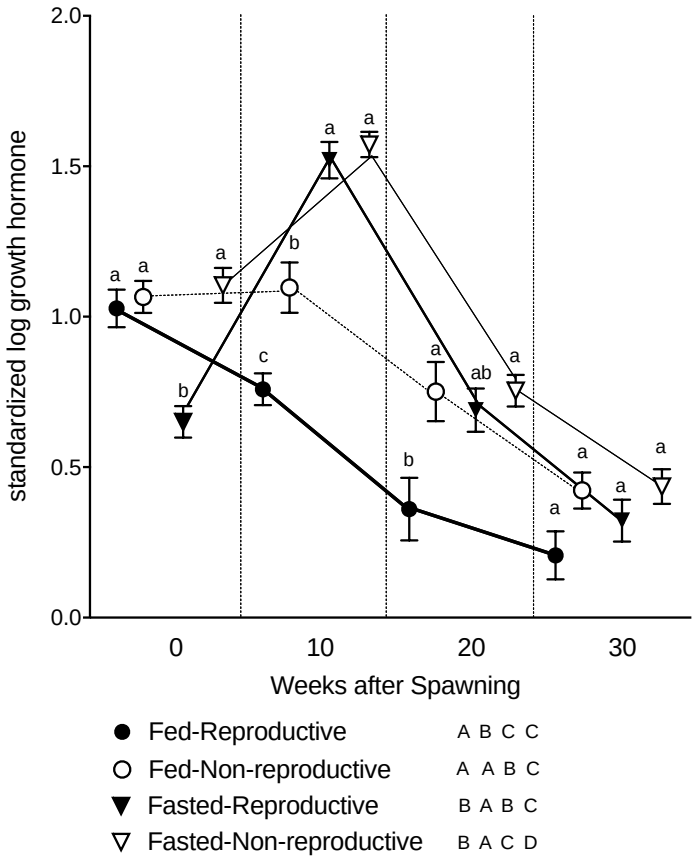


Figure 3B. 2: Growth Hormone in female steelhead trout sampled at 10-week intervals starting at first spawning. The meaning of the symbols is as described in Figure 3B.1.

At 20 weeks, all groups significantly decreased from GH levels at 10 weeks. GH levels did not differ between groups except for Fed-Reproductive, which continued to have the lowest average GH levels and was almost significantly lower than Fasted-Reproductive GH ($P = 0.0571$). Fasted-Reproductive returned to not detectably different from its GH level at spawning (0 weeks). At 30 weeks, all groups significantly decreased in GH level from 20 weeks except Fed-Reproductive. None were significantly different from each other, although GH was nearly significantly lower in Fed-Reproductive fish than in Non-reproductive fish: Fed ($P = 0.1143$) and Fasted ($P = 0.0553$).

IGF-1

No significant year or interaction effects were found for IGF1 and years were combined. There were no differences in IGF-1 between groups at the time of spawning (Figure 3B.3). At the end of the fasting treatment (10 weeks), Fed-Non-reproductive fish had higher IGF-1 than Fasted-Reproductive fish, which had the lowest average IGF-1. At 20 weeks, there were no differences observed between groups, though averages tended to be higher in Non-reproductive fish. IGF1 increased over spawning levels at 20 weeks in Non-reproductive fish regardless of fasting treatment, whereas IGF1 levels in Reproductive fish from both fasting treatments were not significantly different from spawning levels. In contrast, IGF1 levels increased between 20 and 30 weeks in Reproductive fish, at which point all groups reached levels above those at both 0 and 10 weeks. At 30 weeks, IGF-1 was nearly greater in Reproductive than in Non-reproductive Fed fish ($P = 0.1106$).

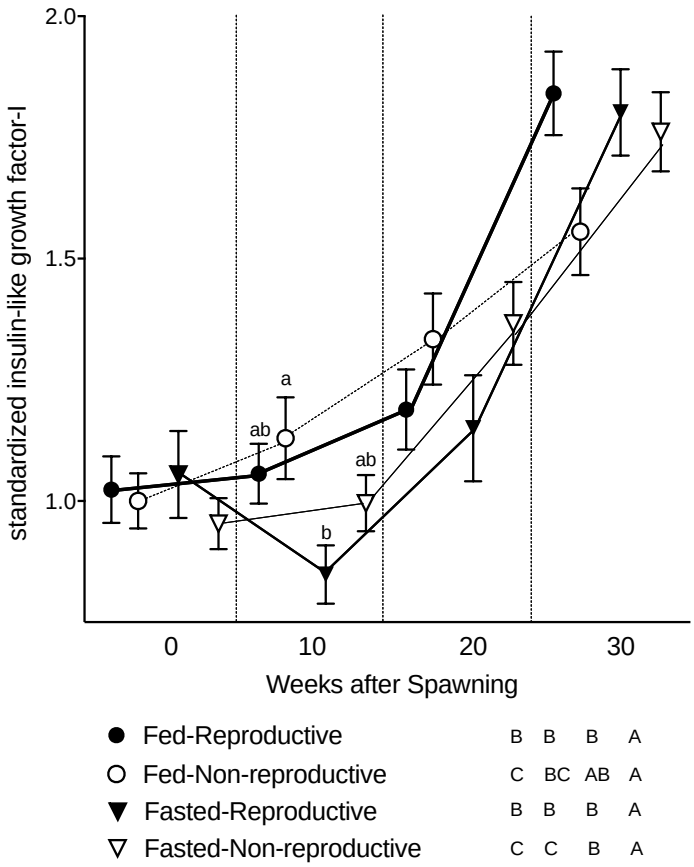


Figure 3B. 3: Insulin-like Growth Factor-I in female steelhead trout sampled at 10-week intervals starting at first spawning. The meaning of the symbols is as described in Figure 3B.1.

Growth

Fasting treatment, reproductive status, and the interaction of these factors significantly affected growth rates (Table 3B.3). During the first 10-week interval following spawning (0-10 weeks), the fasting period for fasted groups, Fed-Reproductive fish had greater growth rate in mass than all other groups (MSGR, Figure 3B.4), and Fasted-Non-Reproductive fish had lower growth than all other groups. All groups showed a significant increase in growth rate from the first (fasting) to the second (re-feeding) interval. During both the 10-20- and 20-30-week intervals, growth was lower in Fed-Non-reproductive fish than in all other groups. Fasted fish had greater growth than fed fish at 10-20 weeks (P = 0.0059, post-hoc Mann Whitney test; average MSGR = 0.2992 fasted, 0.2037 fed).

Table 3B. 3: P-values from 2-way-ANOVAs testing the effects of experimental treatment (fasted or fed for the first 10 weeks after spawning) reproductive status at 30 weeks (reproductive or non-reproductive), and the interaction of these two factors on growth rates through the course of the experiment. Significant effects are bolded.

	Specific Growth Rate in Weight			Specific Growth Rate in Length		
Week	Experimental Treatment	Reproductive Status	Interaction	Experimental Treatment	Reproductive Status	Interaction
0-10	**0.00001**	**0.00001**	**0.00076**	**0.00012**	**0.00006**	**0.02349**
10-20	**0.00029**	**0.00009**	0.1594	**0.00002**	**0.0011**	0.23653
20-30	0.06546	**0.00018**	0.08664	0.84482	**0.00005**	**0.01433**

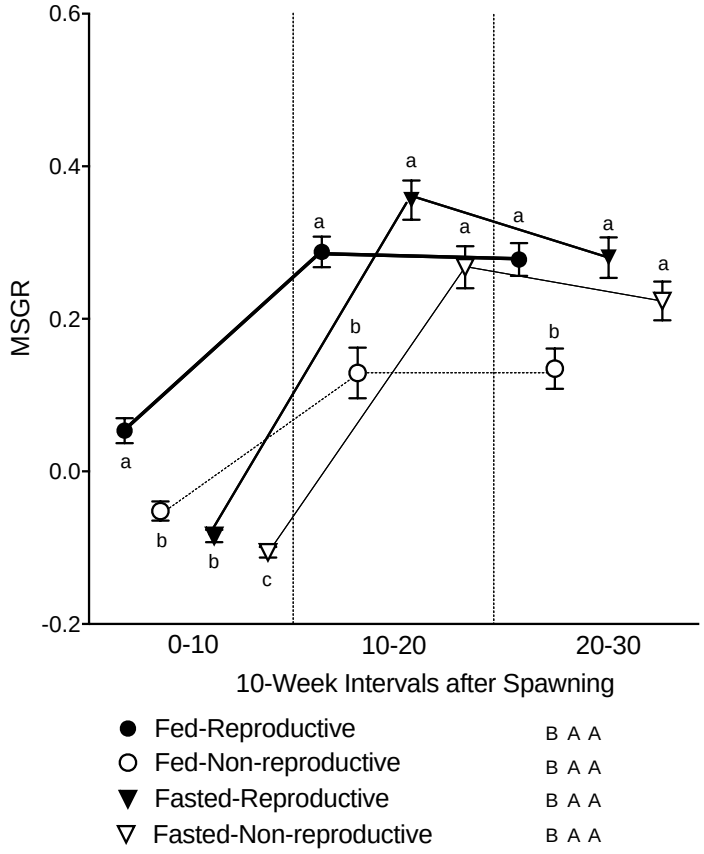


Figure 3B. 4: MSGR in female steelhead trout sampled at 10-week intervals starting at first spawning. The meaning of the symbols is as described in Figure 3B.1.

Growth in length (LSGR, Figure 3B.5) was significantly greater in Fed-Reproductive fish than other groups during the 0-10-week interval. From 10-20 weeks, the period following fasting, all groups significantly increased in growth rate from the 0-10-week interval. Similar to MSGR, Fasted groups were significantly higher than Fed-Non-reproductive fish, though not significantly different from Fed-Reproductive fish. From 20-30 weeks, there were no changes from 10-20

weeks, except the Fasted-Reproductive group decreased in LSGR. At 30 weeks, Fed-Reproductive fish had significantly higher LSGR than either of the Non-Reproductive groups.

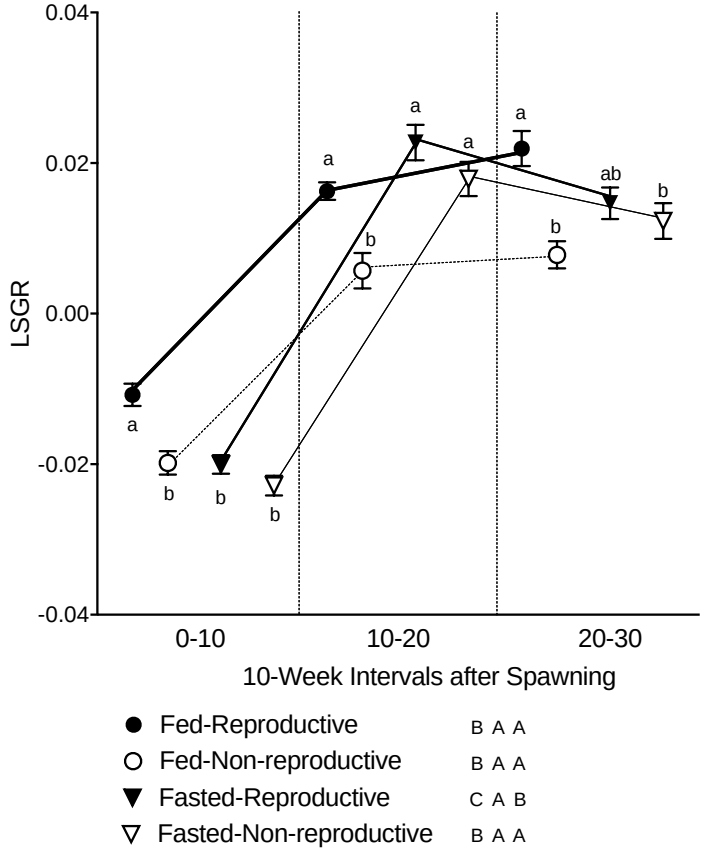


Figure 3B. 5: LSGR in female steelhead trout sampled at 10-week intervals starting at first spawning. The meaning of the symbols is as described in Figure 3B.1.

Condition and Lipid Levels

Fasting treatment, reproductive status, and the interaction of these factors significantly affected Fulton's condition factor K and muscle lipid level (Table 3B.4). There were no differences in Fulton's condition factor (K, Figure 3B.6) at spawning (0 weeks). At 10 weeks after spawning, the end of the fasting period, K was highest in Fed-Reproductive fish, and had increased from levels at 0 weeks. Fed-Non-reproductive fish had significantly lower K than Fed-Reproductive fish and did not change from 0 weeks. K decreased from 0-10 weeks in fasted fish (nearly significant for Fasted-Reproductive group, P = 0.0851), and was significantly lower than Fed groups in Fasted-Non-reproductive fish at 10 weeks.

Table 3B. 4: P-values from 2-way-ANOVAs testing the effects of experimental treatment (fasted or fed for the first 10 weeks after spawning) reproductive status at 30 weeks (reproductive or non-reproductive), and the interaction of these two factors on condition factor (K), and muscle lipid level (%) through the course of the experiment. Significant effects are bolded.

	Condition Factor, K			Muscle Lipid Level (%)		
Week	Experimental Treatment	Reproductive Status	Interaction	Experimental Treatment	Reproductive Status	Interaction
0	0.39103	0.21339	0.77762	0.37542	0.51104	0.8239
10	**0.00001**	**0.00015**	0.08823	**0.00001**	0.12489	0.28219
20	0.26456	**0.00001**	**0.01961**	0.18174	**0.00001**	**0.03201**
30	0.65193	**0.00001**	**0.00919**	0.27206	**0.00008**	0.09896

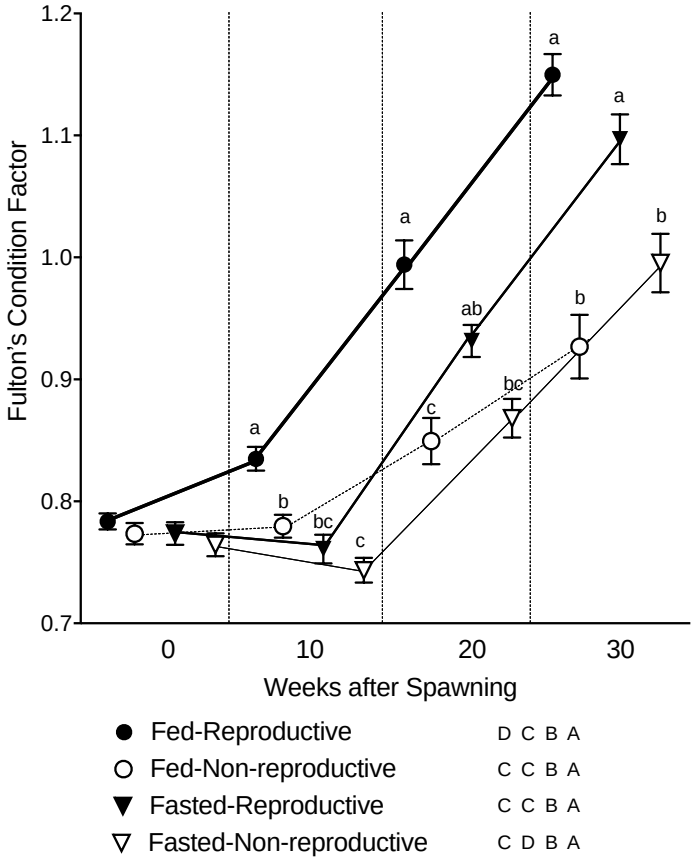


Figure 3B. 6: Condition (K) in female steelhead trout sampled at 10-week intervals starting at first spawning. The meaning of the symbols is as described in Figure 3B.1.

At 20 weeks, K increased in all groups from levels at 10 weeks. K was highest in Fed-Reproductive fish and lowest in Fasted-non-reproductive fish. K increased in all groups from 20 to 30 weeks. K was higher in Reproductive groups than Non-reproductive groups regardless of treatment.

As with K, there were no differences in muscle lipid level (Figure 3B.7) at 0 weeks. From 0-10 weeks, all groups except Fed-Reproductive decreased in fat. Fed-Reproductive had the highest fat, followed by Fed-Non-reproductive. Fasted-Non-reproductive fish had the least at 10 weeks. At both 20 and 30 weeks, all groups increased in fat from the previous time period and Reproductive groups had higher fat than Non-reproductive groups. At 20 weeks, fat was nearly significantly higher in fed-reproductive fish than in fasted-reproductive fish (P = 0.0764).

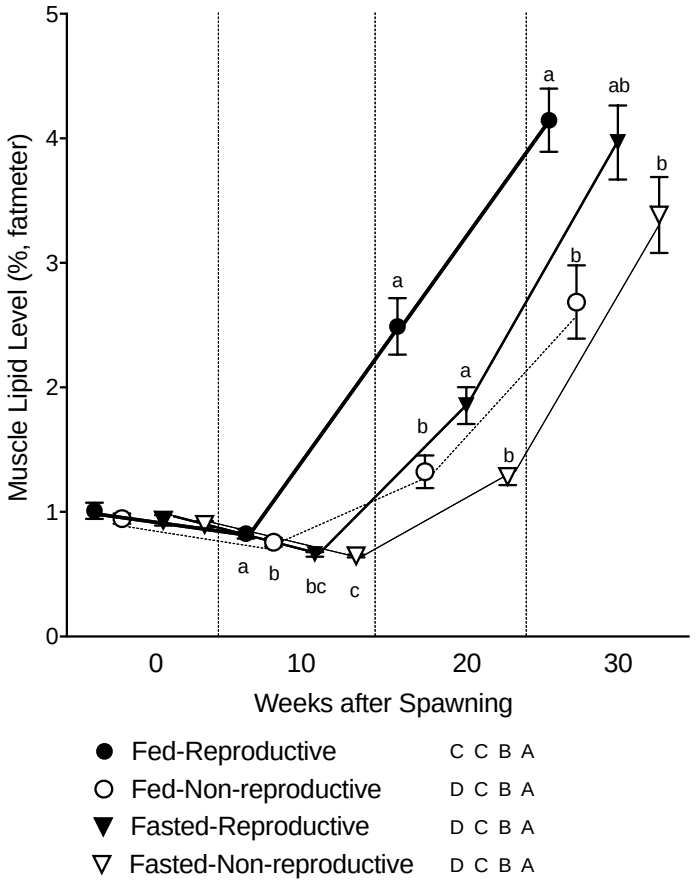


Figure 3B. 7: Muscle Lipid Level in female steelhead trout sampled at 10-week intervals starting at first spawning. The meaning of the symbols is as described in Figure 3B.1.

Discussion

Plasma GH levels were measured for the first time in post-spawning steelhead in this study. The resulting profiles show that plasma GH levels differed between reproductive and non-reproductive individuals at early time points, at spawning in the fasted fish and at 10 weeks after spawning in the fed fish. Plasma GH is an indicator of metabolic status that increases during fasting and other catabolic states, due to its role in the mobilization of stored energy, particularly lipid stores (Norbeck et al. 2007; Pierce et al. 2005b). Plasma GH levels were

significantly higher in non-reproductive fish versus reproductive fish at spawning in the fasted group, the earliest indicator of reproductive trajectory found to date. This suggests that fish in the fasted group that were more catabolic at spawning were less likely to become reproductive. However, in the fed group, no differences in plasma GH level were found between reproductive and non-reproductive fish at spawning. This shows that metabolic status at spawning and feeding conditions during the 10 weeks after spawning interact in determining consecutive versus skip spawning in steelhead kelts, and that feeding conditions during the 10 weeks after spawning had a stronger effect under our experimental conditions. In the fed group, non-reproductive fish first had significantly higher plasma GH levels than reproductive fish at 10 weeks post-spawning. This suggests that fish in the fed group that fed less and were more catabolic during the 10 weeks following spawning, despite being offered food, were less likely to become reproductive. This is consistent with the timing of the differences in plasma triglyceride level and weight growth rate found at 10 weeks between reproductive and non-reproductive fish in our previous study (Jenkins et al. 2019). Thus, multiple lines of evidence suggest that fish that consume more feed over the initial 10 weeks after spawning, resulting in increased growth rate, increased plasma triglyceride level, and decreased plasma GH level, are more likely to be consecutive spawners. Taken together, the plasma GH results in fasted and fed fish are consistent with a model in which both metabolic state at the time of spawning and feeding conditions over the 10 weeks after spawning influence the physiological decision underlying consecutive versus skip spawning. This implies that the "decision window" for the physiological decision underlying consecutive versus skip spawning extends to the period after spawning. In the management of kelt reconditioning projects, these results suggest that: 1) selection of fish in the best possible condition at capture, and 2) measures to maximize feeding of fish over the initial period after capture will maximize consecutive spawning rate. Assay of plasma GH levels at collection may provide a better indicator of condition than the categorization of fish based on visual appearance that is currently used (Hatch et al. 2013b).

The plasma GH results also show that the growth axis is responsive to nutritional status early during recovery from spawning. Plasma GH levels increased from week 0 to week 10 in fasted fish, both among reproductive and non-reproductive individuals, and this increase was not found in fed fish. Previous studies in immature salmonids have shown that plasma GH levels increase during fasting (Pierce et al. 2005b; Sumpter, et al. 1991). The present study shows that the growth axis responds similarly in the post-spawning steelhead kelt model, even after the approximately 6 month fasting spawning migration undertaken by these fish. This suggests that the growth axis continues to be involved in the mobilization of stored energy in post spawning steelhead kelts, even though energy stores and in particular lipid reserves are severely depleted in these fish (Jenkins et al. 2019; Penney and Moffitt 2014b). Whether the increase in GH level during post spawning fasting results in mobilization of remaining lipid stores or plays a role in accessing other energy stores such as protein remains to be determined. The state of GH-resistance, in which plasma GH levels are elevated while growth and plasma and tissue levels of insulin-like growth factor-1 (IGF1) are decreased, has recently been proposed to be permissive for protein catabolism in rainbow trout (Bjornsson et al. 2018). Measurement of circulating levels of metabolic fuels (e.g. triglycerides and amino acids) may provide additional insight in this area.

Plasma IGF1 levels did not respond strongly to fasting or show a clear relationship with reproductive status. The IGF system is highly complex (Bergan-Roller and Sheridan 2018; Norbeck et al. 2007; Shimizu and Dickhoff 2017; Wood et al. 2005), and other parts of the system, such as IGF2 and IGF binding proteins (IGFBPs) may have changed in response to the fast. There was a trend toward lower IGF1 levels in fasted fish versus fed fish after 10 weeks, which would be expected based on previous studies showing that IGF1 decreases during fasting (Bjornsson et al. 2018; Pierce et al. 2005b). IGF1 increased after spawning, attaining significantly higher levels than at spawning by 30 weeks in all groups. Similarly, in our study on post-spawning rainbow trout, IGF1 increased after spawning but did not respond to nutritional status until 8 weeks post-spawning (Section 3.C). Taken together, these results suggest that IGF1 is regulated by recovery from spawning rather than nutritional status in the post-spawning period. Plasma cortisol levels are highly elevated in spawning salmonids (Barry, et al. 2010; Dickhoff 1989; Mommsen, et al. 1999; Schreck, et al. 2001), and cortisol inhibits liver IGF1 production and decreases circulating IGF1 (Pierce et al. 2005a). In our previous study in post-spawning rainbow trout, liver igfbp1b mRNA expression, which responds directly to glucocorticoids, decreased over an approximately 12 week period after spawning (Caldwell et al. 2013; Pierce, et al. 2006). Plasma IGF1 levels may not be able to increase until liver IGF1 production is released from suppression by elevated cortisol levels. Thus, the timing of the increase in IGF1 after spawning may provide an indicator of recovery from spawning. Further studies including measurement of circulating cortisol and IGFBP levels during recovery from spawning are needed to clarify these issues. Unfortunately, measurement of basal circulating cortisol levels in steelhead kelts is complicated due to the rapid response of cortisol to stress and the logistical challenges involved in sampling these large and strong fish.

The profile of plasma E2 over time in reproductive and non-reproductive fish is similar to our previous studies in steelhead kelts (Jenkins et al. 2019; Pierce et al. 2017), and supports our previous conclusion that the physiological decision underlying consecutive versus skip spawning must be completed by 20 weeks post-spawning. Reproductive fish in both the fed and fasted groups had slightly elevated plasma E2 levels at spawning versus non-reproductive fish, and this difference was significant when the fasted and fed fish were pooled (Table 3B.2). However, the biological significance of this very small (4%) difference is not clear. A similar difference at the time of collection was found in one of three years in our previous study on wild fish (Pierce et al. 2017). Plasma E2 level did not differ in reproductive fish from the fed and fasted groups at weeks 20 or 30, providing no indication of an impact of the fasting period on reproductive development in fasted fish that initiated maturation as consecutive spawners. There was an effect of fasting on plasma E2 levels at week 10, with lower E2 levels in fasted fish (Table 3B.2), suggesting potentially more rapid clearance of E2 in the fasted group.

Fasting treatment reduced growth rates in the fasted group as expected over the period of food deprivation. Fasted fish displayed compensatory growth during refeeding in the second 10-week period, which continued over the third 10-week period in non-reproductive but not in reproductive individuals. Compensatory growth is often found after a period of feed deprivation in fishes, although to our knowledge this is the first instance of this phenomenon

that has been described in post-spawning fish (Ali, et al. 2003; Gabillard, et al. 2006; Picha, et al. 2008b). As found in our previous study using the DNFH hatchery kelt model, weight gain was near zero or negative, and fish decreased in length over the first 10 weeks after spawning (Jenkins et al. 2019). However, after fasting for the initial 10 weeks after spawning, both weight and length growth rates increased dramatically in fasted fish, and exceeded rates in fed fish over the second 10 weeks (Table 3B.3, Figs. 3B.4, 3B.5). This suggests that reduced growth rates over the initial 10 weeks are due to a process of recovery from spawning, and that this recovery process does not depend on feeding, since it occurred in the fasted group. One aspect of this recovery process may be recession of the kype, which explains the negative length growth over the period after spawning (Jenkins et al. 2019). Interestingly, no difference in survival was found between the fasted and fed groups, and there was not any trend toward a difference, suggesting that the lack of an effect of the fasting treatment on survival was not due to low statistical power (N=5). The bulk of the mortality that occurred in this experiment was during the initial 10-week period after spawning (data not shown). In the wild, steelhead would not begin feeding in the ocean until at least several weeks after spawning (Colotelo, et al. 2014). Thus, it appears that both 1) steelhead are adapted for a period of fasting after spawning, and 2) the small amount of feeding that does occur during this period significantly impacts their metabolic physiology and reproductive schedules. Further study of the physiology underlying the post-spawning recovery process may shed light on the proximate causes of post-spawning mortality in salmonids in general. In this regard, an examination of stress physiology during the period after spawning would be interesting (Barry et al. 2010; Dickhoff 1989; Schreck et al. 2001).

The energy storage metrics condition factor (K) and muscle lipid level responded to both fasting treatment and reproductive status. Consistent with the negative growth observed over the first 10 weeks after spawning, K and muscle lipid level remained constant or decreased from week 0 to week 10 fasted fish. Fed-non-reproductive fish were similar to the fasted group in that K and muscle lipid level remained constant or decreased. In contrast, K increased over the first 10 weeks in fed-reproductive fish, consistent with greater feeding in this group. Although compensatory growth occurred after the fasting period in both reproductive and non-reproductive fish, K and muscle lipid level showed different responses between reproductive and non-reproductive fish during refeeding. In reproductive fish, greater levels of both K and muscle lipids were maintained in fed versus fasted fish during refeeding. In non-reproductive fish, in contrast, K and muscle lipid levels appeared to show compensation in the fasted group, showing a trend toward greater levels than fed fish at 20 weeks and significantly higher levels at 30 weeks. This difference may relate to the need to accumulate lipids to fuel ovarian development in reproductive fish. Both K and muscle lipid levels increased to higher levels in reproductive than non-reproductive fish by these time points in our previous study (Jenkins et al. 2019).

Section 3.C: Effects of post-spawning ration restriction on reproductive development and the growth hormone/insulin-like growth factor-1 axis in female rainbow 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. This study follows on to our previous studies using a post-spawning rainbow trout model (Caldwell et al. 2013; Caldwell et al. 2014).

Introduction

The post-spawning period is critical in the survival and reproductive life-history trajectory of seasonally spawning fishes in general and steelhead kelts in particular. Energy reserves are at a low level due to the demands of migration, gonadal development, and spawning (Penney and Moffitt 2014b, 2015). Fish are in a profoundly catabolic state with elevated cortisol resulting in immunosuppression and breakdown of tissues to support metabolism, which results in programmed post-spawning mortality in the semelparous salmonids, and high mortality in the iteroparous anadromous species such as steelhead and Atlantic salmon (Barry et al. 2010; Barry, et al. 2001; Dickhoff 1989; Mommsen 2004; Mommsen et al. 1999). The gut is atrophied due to prolonged fasting, and gut function must be restored to access energy from feeding, which requires energy (Krogdahl and Bakke-McKellep 2005; Penney and Moffitt 2014a; Simpkins, et al. 2003; Zaldua and Naya 2014). Further, due to physiological constraints on gonadal development, fish must begin investment of energy into gonadal recrudescence at this time if they are to follow a consecutive repeat spawning life history schedule. However, little is known about the physiology of the post-spawning period. Fish must repair tissue breakdown and restore lost energy as they switch from a catabolic to an anabolic state, which likely involves the growth hormone/insulin-like growth factor (GH/IGF) endocrine axis, the principal physiological system regulating growth (Bergan-Roller and Sheridan 2018; Norbeck et al. 2007; Wood et al. 2005). We hypothesize that the GH/IGF axis interacts with the reproductive endocrine axis in the decision to remature as a consecutive spawner. However, experimental study of this time period in steelhead or Atlantic salmon kelts is logistically difficult and subject to high variability. Collaborations to access fish require extensive coordination, the fish are large and require substantial facilities for holding, mortality rates are high, and individual variability is large due to the differing histories and environmental conditions experienced by the fish before returning to hatcheries. Therefore, we have established post-spawning female rainbow trout as an experimental model to supplement studies using steelhead kelts (Section 3.B.; Caldwell et al. 2013; Caldwell et al. 2014).

In our previous studies using the rainbow trout model, we found that feeding a restricted ration resulted in 100% of females adopting a skip-spawning life history, and that differences in reproductive development were evident by approximately 10 weeks post-spawning (Caldwell et al. 2013; Caldwell et al. 2014). To examine this with greater resolution, we repeated the experimental design from this study with more frequent sampling. In addition, we used assays

recently established in our laboratory to examine interactions between the GH/IGF axis and reproductive development in the post spawning period (Medeiros et al. 2020).

Methods

Fish

Two-year-old maiden spawning female rainbow trout from the population maintained at the Aquaculture Research Institute (ARI) at the University of Idaho in Moscow, Idaho were used for this study. Fish were progeny from a previous experiment testing the effects of cortisol implantation on reproductive development (Medeiros, et al. 2016); however, only progeny from the control group were used.

Spawning and Sampling

Fish were checked weekly for ripeness and spawned on three dates (1/13/2017, 1/26/2017, and 2/23/2017). Ripe females were anesthetized (MS-222, buffered) and strip spawned. Fish were then implanted with PIT tags and sampled. Sampling consisted of recording the PIT tag code, length (mm) and weight (0.1g), and taking a non—lethal reading of muscle lipid level (%, Distell Fish Fatmeter, Fauldhouse, UK) for each individual at each sampling point. Fish from each spawning date were sampled at spawning (week 0) and after 2, 4, 6, 8, 10, 12, 14, 16, 20, and 30 weeks post-spawning. Blood was drawn from all fish at weeks 0, 20, and 30, and from half of the fish at the other sampling points. Blood sampling was staggered using individual PIT tag codes so that each individual received a blood draw every 4 weeks. During blood sampling, 1.5 ml of blood was drawn from the caudal vein using a heparinized (Sigma ammonium heparin, 10 mg ml^{-1}) 2 ml syringe and a 23 gauge needle. Plasma was separated by centrifugation (6000g, 5 min) and stored at -80 °C.

Experimental Treatment and Fish Husbandry

After spawning, fish were alternately distributed into 1130 L tanks to a maximum of 20 fish per tank, tanks alternately assigned to the fully fed or restricted treatment. Fish were held in two recirculating systems with a flow rate of 14 L per minute per tank, at a temperature ranging from 11.1 to 14.6 °C following a seasonal profile. Feeding followed the scheme established in our previous studies using this model (Caldwell et al. 2013; Caldwell et al. 2014). The fully fed group was fed a ration of 0.5% body weight, per day, and the restricted group was fed 0.1% body weight, per day. Fish were fed a commercial trout broodstock diet (6.4 mm pellets, Rangen Inc., Buhl ID). Fish were checked daily, mortalities removed, and feeding rates adjusted based on the most recent sampling.

Assays

Plasma estradiol-17 β (E2, ng/mL) concentration was measured by ELISA (Biosense, Cayman Chemical, Ann Arbor, MI), in triplicate technical replicates after solvent extraction as previously described (Jenkins et al. 2019). Plasma GH and plasma IGF1 were measured by time-resolved fluoroimmunoassay in triplicate technical replicates as previously described (Medeiros et al. 2020). To remove interference from IGF binding proteins, plasma samples were treated by

acid-ethanol cryoprecipitation, dried down, and reconstituted before assay for IGF1 (Medeiros et al. 2020; Shimizu et al. 2000).

Morphometric analysis

Mass specific growth rate (MSGR) and Fulton's condition factor (K) were calculated as follows:

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

$$(2) \qquad K = 100 * \text{body mass (g)} * (\text{fork length (cm)})^{-3}$$

Length specific growth rate was calculated the same way as MSGR.

Statistical analysis

Analysis was restricted to fish that were positively identified by PIT tag at each sampling point and that survived to the end of the experiment at week 30. Reproductive status was assigned based on plasma E2 level at 30 weeks after spawning. Fish with E2 levels less than 1000 pg/ml were classified as non-maturing based on our previous results using both rainbow trout and steelhead kelts (Caldwell et al. 2013; Caldwell et al. 2014; Jenkins et al. 2019; Pierce et al. 2017). The fully fed and restricted groups were compared at each time point for each response variable using T-tests. The effect of sampling time was assessed within the fully fed and restricted groups by one-way ANOVA followed by the Tukey test. Levels of E2 and GH were $\log_{10}$-transformed to conform to assumptions of normality. Statistical analysis was conducted with JMP Pro (SAS Institute Inc., Cary NC). 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

The results from this study are preliminary at this point, as laboratory assays and statistical analysis of results are ongoing. Updates will be provided in the 2021 Steelhead Kelt Annual Report.

Survival and Reproductive Status

85 fish survived to the end of the experiment at 30 weeks. Only 12 fish had E2 levels below 1000 pg/ml at week 30 and were classified as non-maturing, 10 from the restricted group and 2 from the fully fed group (Fisher's Exact Test, P=0.0135). Since this was not a sufficient number for statistical analyses based on ration x reproductive status, non-maturing fish were excluded

from further analyses. Thus, all data presented are for maturing females only (N=32 in the restricted group and N=41 in the fully fed group).

Growth

Specific growth rates in both weight and length were significantly elevated in the fully fed versus restricted group beginning at the interval week 2-4 and maintained through the rest of the experiment until week 30 (Fig 3C.1).

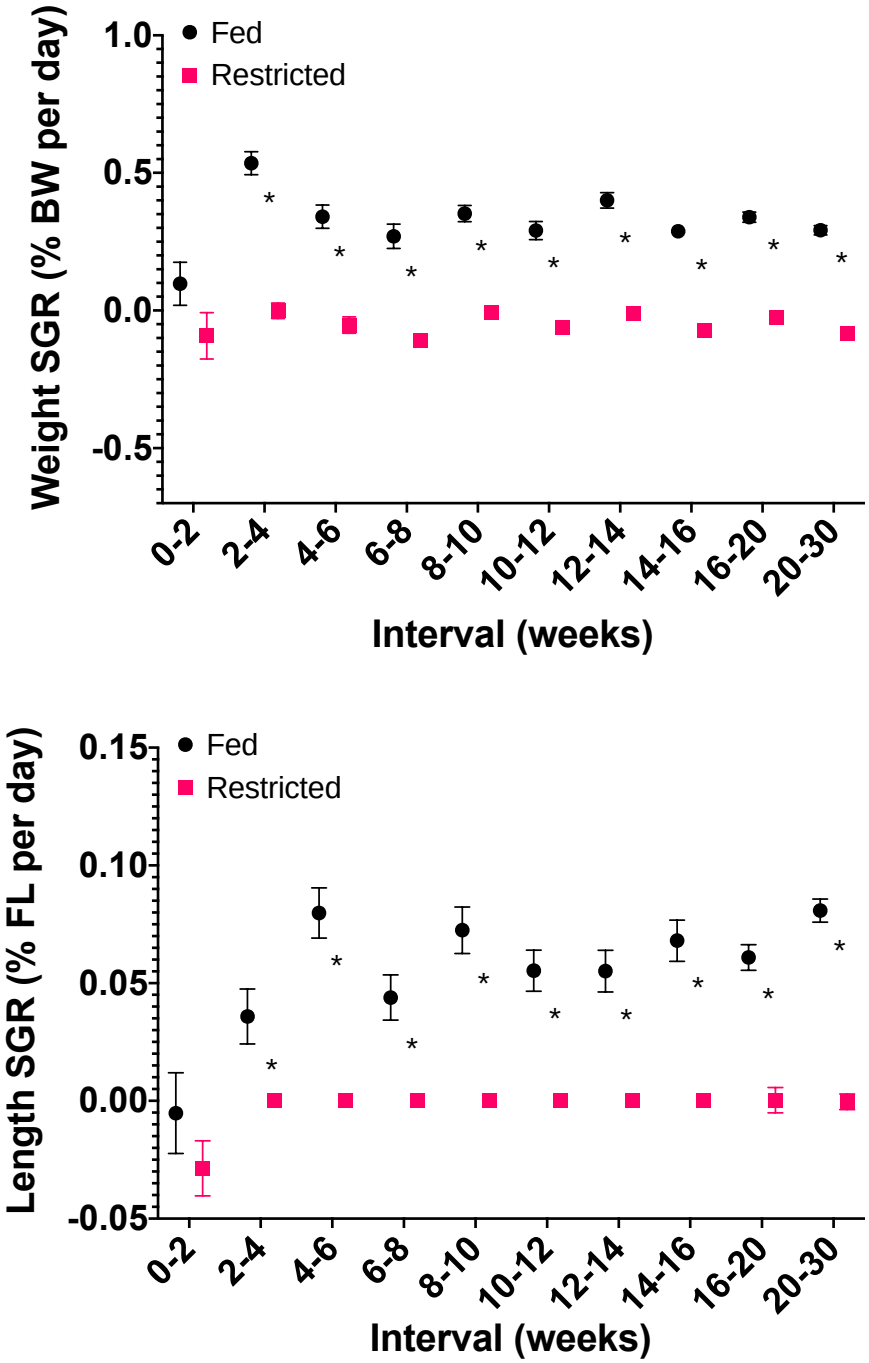


Figure 3C. 1: Specific growth rates in weight and length over the course of the experiment. Interval is growth interval in weeks beginning at spawning (week 0). All fish are maturing females. Symbols indicate mean and bars indicate SEM. Asterisks indicate significant differences between the fully fed (0.5% body weight per day) and restricted (0.1% body weight per day) treatment groups.

Condition and Lipid Levels

Condition factor was significantly elevated in the fully fed versus restricted group beginning at the week 4, and muscle lipid level at week 6. Levels of both remained significantly higher in the fully fed group through the rest of the experiment (Fig 3C.2).

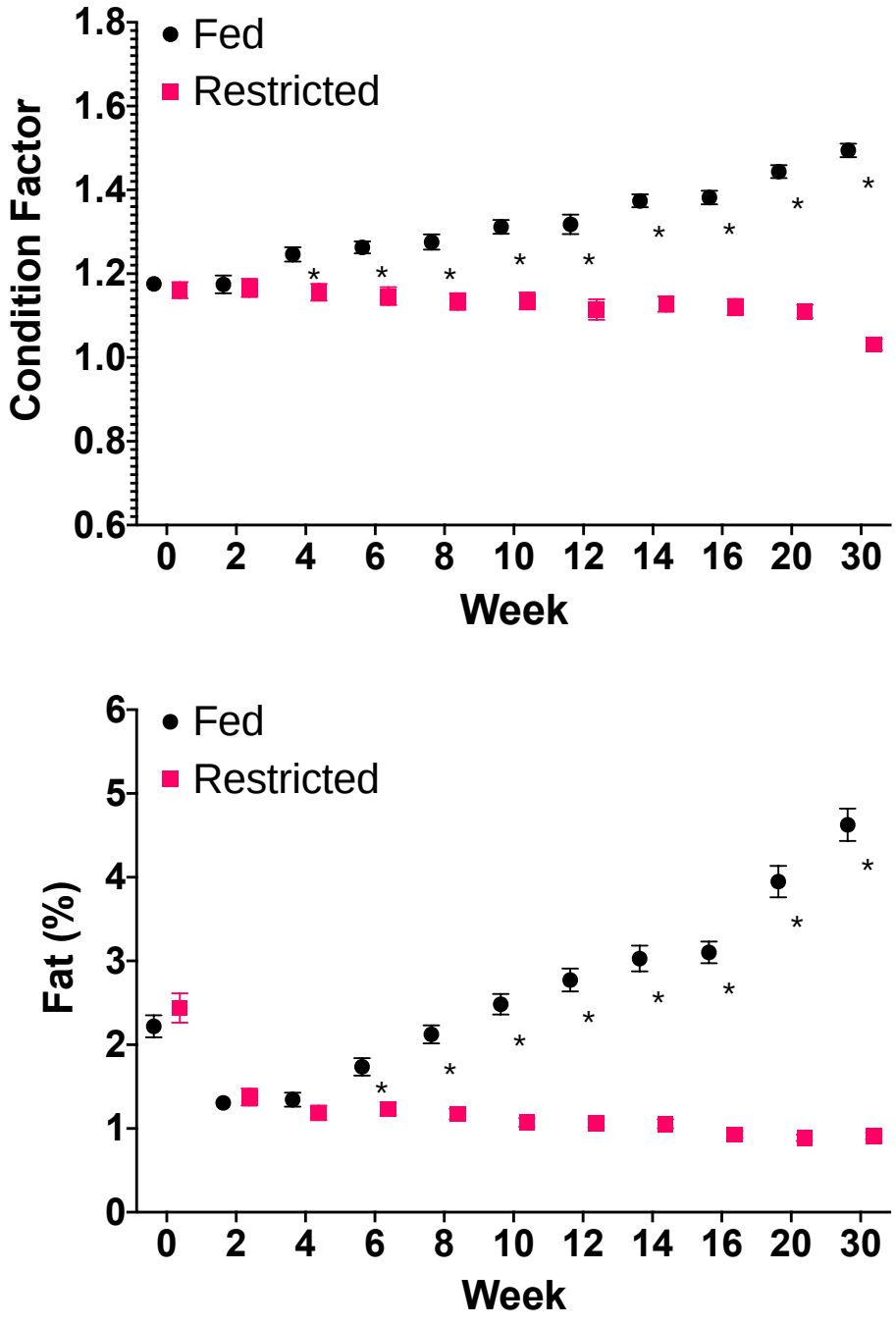


Figure 3C. 2: Fulton's condition factor (K) and muscle lipid level (%, measured non-lethally with the Fish Fatmeter) over the course of the experiment. The meaning of the symbols is as described in Figure 3C.1.

E2

Time and treatment group both significantly affected plasma E2 level (Fig. 3C.3). Plasma E2 decreased significantly from week 0 to week 10 in the fully fed group and week 12 in the restricted group, and then increased significantly to week 30 in both groups. E2 was significantly elevated in the fully fed versus restricted group beginning at week 12 and through the rest of the experiment, except at week 16 (p=0.1335).

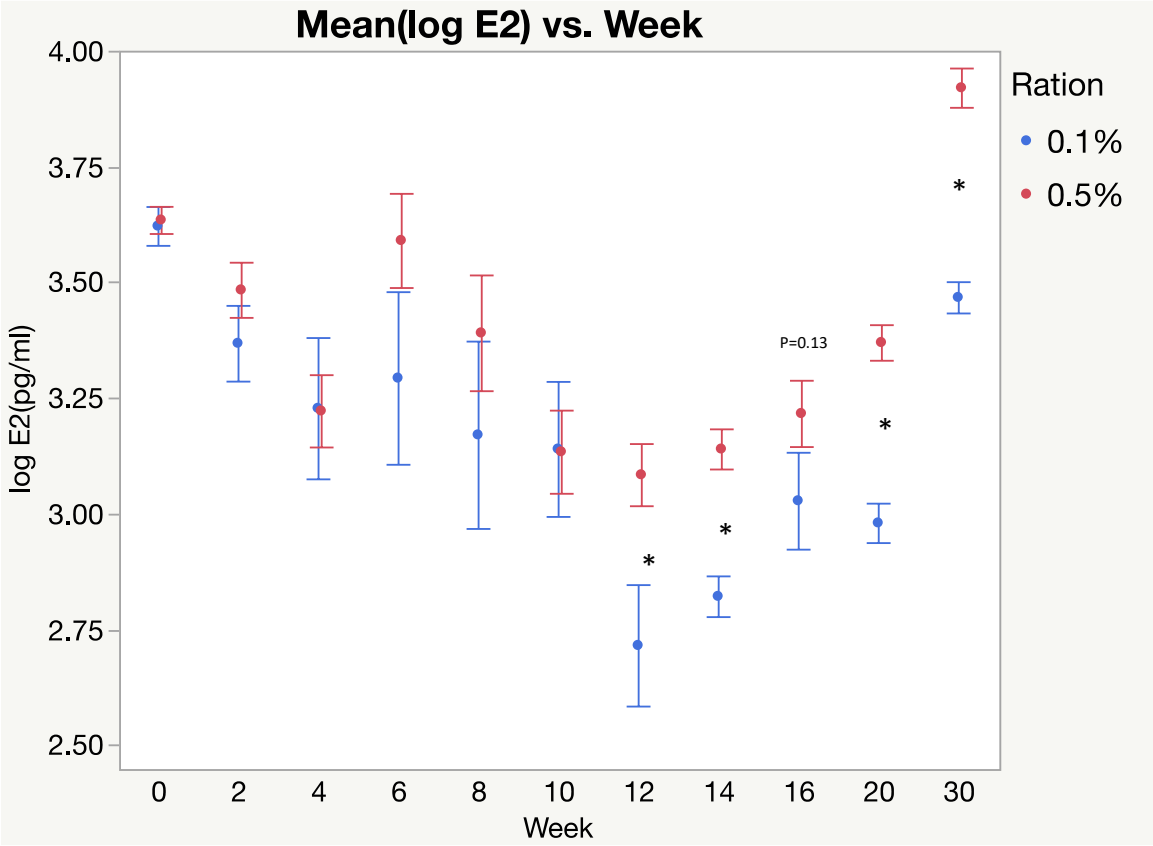


Figure 3C. 3: Plasma estradiol (E2) levels over the course of the experiment. The meaning of the symbols is as described in Figure 3C.1. Statistical analysis was performed on log-transformed values. The P values for one comparison that narrowly missed significance is indicated on the figure.

GH

Time and treatment group both significantly affected plasma GH level (Fig. 3C.4). Plasma GH decreased significantly from week 0 to week 4 in both the fully fed and restricted groups. There was a further significant decrease at week 30 in the fully fed group only. GH was elevated in the restricted versus fully fed group beginning at week 6 (p=0.08) and generally maintained through the rest of the experiment, except at week 14.

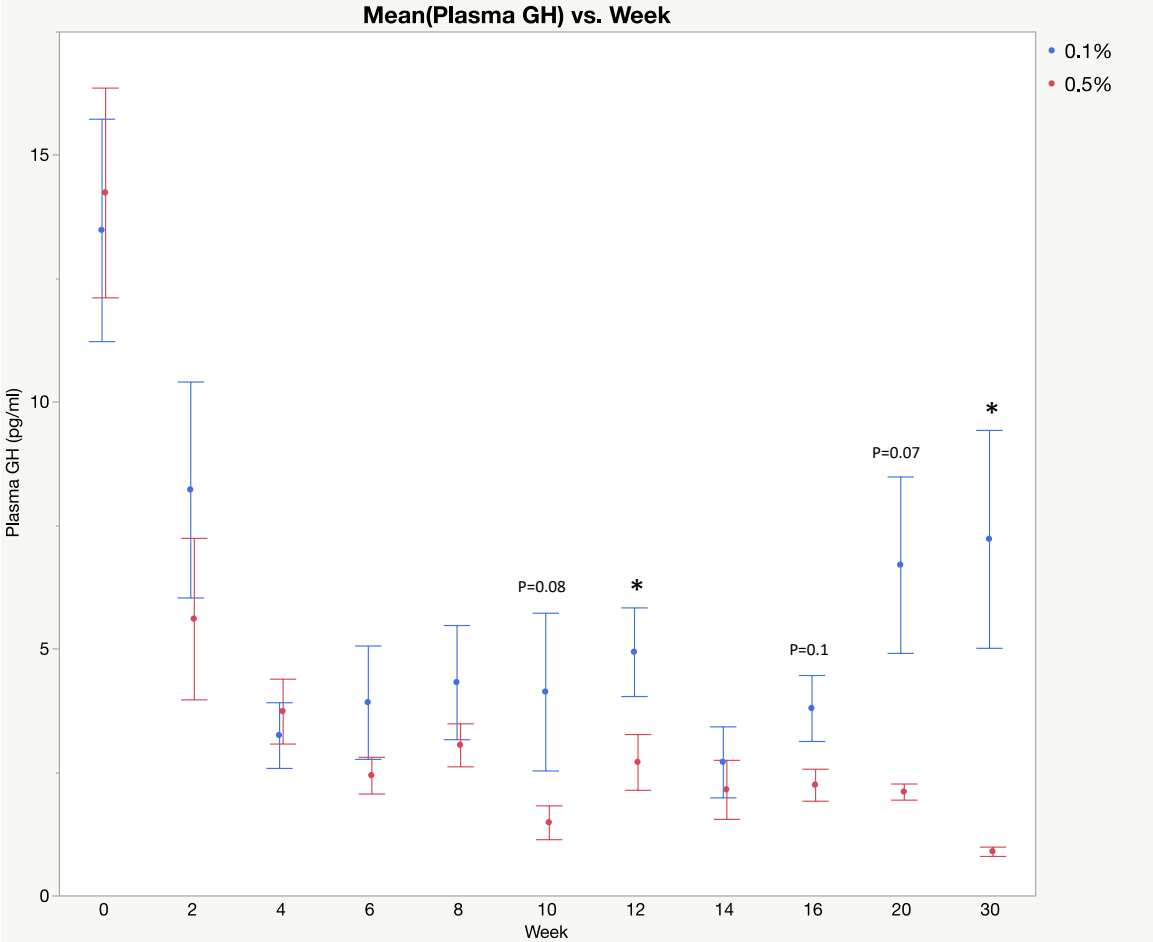


Figure 3C. 4: Plasma growth hormone (GH) levels over the course of the experiment. The meaning of the symbols is as described in Figure 3C.1. Statistical analysis was performed on log-transformed values. The P values for several comparisons that narrowly missed significance are indicated on the figure.

IGF-1

Time and treatment group both significantly affected plasma IGF1 level (Fig. 3C.5). Plasma IGF1 level increased significantly from week 0 to a peak at week 6 in the restricted group, and then declined significantly to week 20. In the fully fed group, IGF1 increased significantly from week 0 to a peak at week 8 and then declined significantly to week 30. Plasma IGF1 level was significantly higher in the fully fed vs restricted group beginning at week 8 and maintained through the rest of the experiment (P=0.07 at week 14).

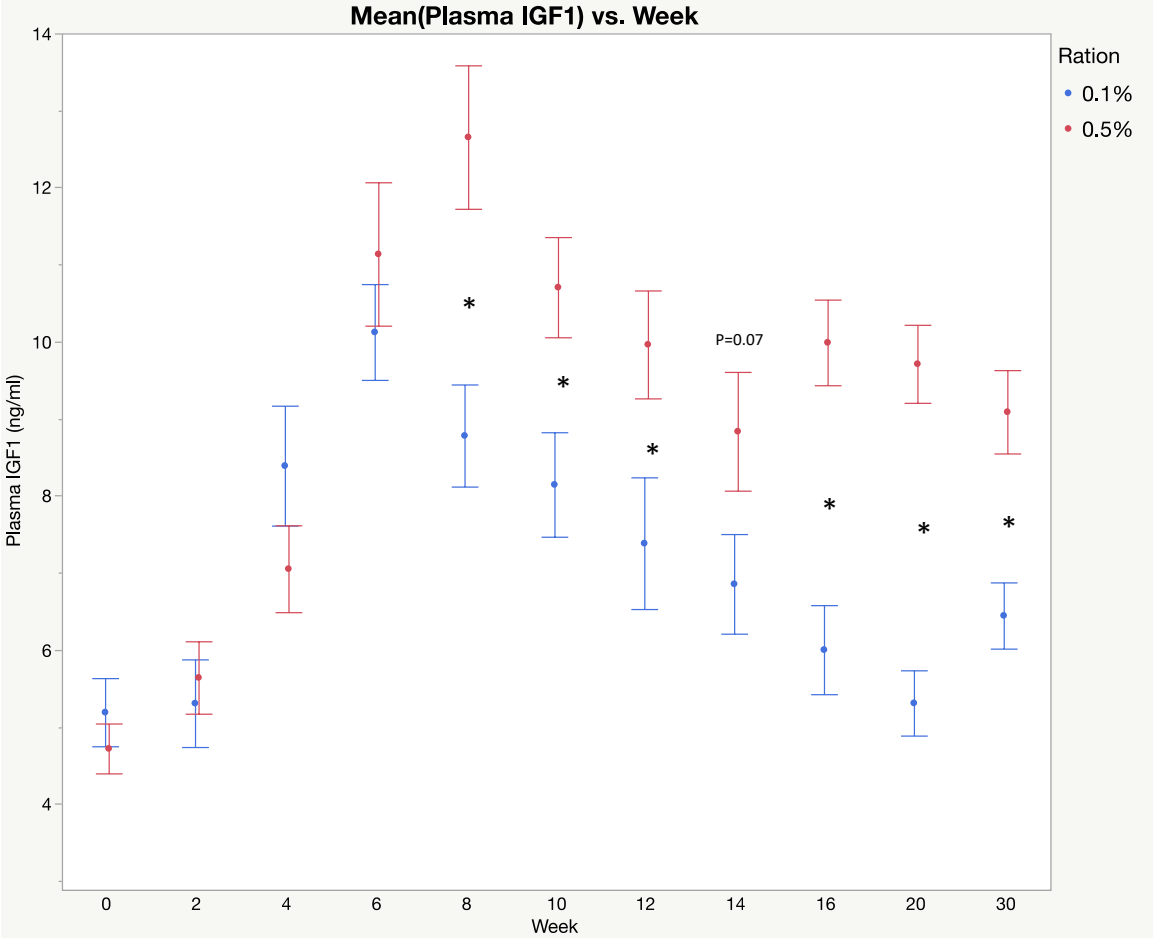


Figure 3C. 5: Plasma insulin-like growth factor-1 (IGF1) levels over the course of the experiment. The meaning of the symbols is as described in Figure 3C.1. The P values for one comparison that narrowly missed significance is indicated on the figure.

Discussion

The ration levels employed in this study permitted positive growth in both length and weight in the fully fed group, but kept growth rates near zero in the restricted group. Thus, the restricted ration was a maintenance ration, as found in our previous studies employing this experimental model (Caldwell et al. 2013; Caldwell et al. 2014). Growth was near zero and muscle lipid levels decreased from week 0 to week 2 in the fully fed group, suggesting that these fish continued to be in a negative energy balance in which lipid energy reserves were being mobilized to support metabolism. However, after 4 to 6 weeks, condition factor and muscle lipid levels began to increase in the fully fed group, indicating that these fish were in positive energy balance and replenishing lipid stores. Muscle lipid levels were quite low at spawning and immediately afterward in these rainbow trout, almost as low as levels measured in steelhead kelts (Section 3.B). Muscle lipid stores would be depleted to support ovarian development during vitellogenesis in both the rainbow trout and the steelhead, however, the steelhead fast for approximately 6 months and migrate before spawning whereas the rainbow trout do neither.

The similar muscle lipid levels at spawning in the two groups suggests that female rainbow trout are selected to retain approximately 1-2% muscle lipid levels at spawning.

Although the ration levels used in this study produced similar growth results to our previous studies using this experimental model, maturation was not prevented in the restricted group, in contrast to our previous results (Caldwell et al. 2013; Caldwell et al. 2014). The maturation rate in the restricted group was significantly lower than the fully fed group (76.2% vs 95.3%), indicating that the maintenance ration did impact maturation rate, but it was not zero, suggesting that energy reserves at spawning were sufficient to support rematuration in many of the fish in the restricted group. Since no fish in the restricted group rematured in our previous study, this suggests that either energy reserves or the threshold energy level for rematuration differed between the two studies, which is plausible because different strains of rainbow trout were used in the two studies. Similarly, in winter flounder, and our study in steelhead kelts, energy reserves at spawning were sufficient to support rematuration in spite of post-spawning nutritional restriction (Section 3.B.; Burton 1994; Rideout et al. 2005). This supports our hypothesis that rematuration is determined during the period immediately after spawning.

Plasma E2 levels decreased for 12 weeks after spawning, similar to results with 3-year-old of rainbow trout in our previous study but different from those with 2-year-old rainbow trout (Caldwell et al. 2013; Caldwell et al. 2014). The reasons for this difference are not clear, but they may relate to the much lower E2 levels immediately after spawning in the 2-year-old rainbow trout in our previous study. In all three profiles, E2 increased to over 1000 pg ml^{-1} at approximately 10-16 weeks post spawning, suggesting that the physiological decision to mature is completed in this time frame, similar to our findings in steelhead kelts (Jenkins et al. 2019; Pierce et al. 2017). Plasma E2 was also lower in the restricted group beginning at 12 weeks in the present study. This suggests that the restricted ration was inhibiting (but not preventing) ovarian development in the restricted group relative to the fully fed group. Consistent with this idea, measures of energy balance and energy reserves at 10 to 20 weeks after spawning were correlated with measures of reproductive investment at repeat spawning in steelhead kelts (Jenkins et al. 2020). Taken together, these results suggest a model in which, in individual females that have committed to consecutive rematuration, investment in ovarian development and aspects of reproductive performance such as fecundity and egg size are influenced by energy balance during the 10 to 20 weeks after spawning.

The GH/IGF axis responded to both recovery from spawning and nutritional status in the post-spawning period in this study. GH declined and IGF1 increased after spawning, but differences due to ration level did not develop until 8 (IGF1) to 12 (GH) weeks after spawning. This suggests that the GH/IGF axis is primarily regulated by recovery from spawning during this period. The stress hormone cortisol is elevated during spawning in salmonids, and cortisol suppresses IGF1 production and plasma IGF1 levels (Barton and Iwama 1991; Dickhoff 1989; Mommsen et al. 1999; Pierce et al. 2005a; Schreck et al. 2001). The increase in IGF1 after spawning may be due to release from suppression by cortisol. GH increases during fasting and other catabolic states in salmonids, and functions in these states to mobilize stored lipids and possibly promote protein catabolism, as does cortisol (Barton and Iwama 1991; Bergan-Roller

and Sheridan 2018; Bjornsson et al. 2018; Dickhoff 1989; Mommsen et al. 1999; Norbeck et al. 2007; Pierce et al. 2005b; Schreck et al. 2001). Thus, the decrease in GH and increase in IGF1 after spawning may reflect the switch from a catabolic state induced by spawning, with suppressed appetite and elevated cortisol, to an anabolic state in which muscle and bone growth is stimulated by the increased IGF1. This process of recovery from spawning took 4 to 8 weeks in the rainbow trout in the present study. The profile of IGF1 and GH during recovery from spawning may provide insight in delineating the time frame of recovery from spawning in steelhead kelts and other iteroparous salmonids.

Section 3.D 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. 2016), 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 assess 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 to which of the Yakima River subpopulations each individual fish assigns to, because PIT tag arrays are only in place for some of the subpopulations. In 2018 and 2019, 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. These results have been integrated with physiological data for corresponding fish. Analysis and classification of migration patterns based on PIT tag detection data is ongoing. An analysis of the relationships between release characteristics of fish and aspects of post-release migration such as detection entering a spawning tributary, spawning survival, and spawn timing will be presented in a future report.

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

PIT tag detections

To investigate homing in reconditioned kelt steelhead we compiled PIT tag 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. 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 tag detections at Prosser Dam and from recapturing post spawn fish that were previously released as reconditioned kelts. Prosser dam fish ladders were completely wired with PIT antennas by 2008, enabling us to use ladder detections to confirm upstream movement by reconditioned kelt steelhead. Additionally, we have PIT detections at the tributary level (Satus Creek, Toppenish Creek, Ahtanum Creek, Naches River, and Roza Dam) that also provide evidence of spawning behavior consistent with homing.

Genetic detections

Most fish collected as kelts are not PIT tagged until after collection at Chandler following their presumptive maiden spawning event. This limits the number of fish that are available to test for homing fidelity by comparison of spawning migration events across multiple years. To increase sample numbers at the maiden stage, we used parentage results as a proxy for PIT tag detections of the maiden spawning event.

Age-0 juveniles were collected in Satus and Toppenish Creek as part of the relative reproductive success project. Presence of progeny within either tributary was treated as the equivalent of a PIT tag detection of the parent within either tributary. Analysis of PIT detections to determine homing fidelity were then completed by reviewing complete tag history from PTAGIS. Eight fish previously identified in the Pit to Pit comparison were removed from the results leaving 70 fish with both a proxy maiden location and a post reconditioning PIT detection. All fish demonstrated fidelity to their maiden location with 34 returning to Satus Creek and 36 to Toppenish Creek. No fish were detected at other PIT tag arrays that would have indicated straying behavior.

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 59 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 this group of fish. 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. The third source of data that is consistent with homing comes from 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).

Table 4. 1: Observed and inferred homing from artificially reconditioned kelt steelhead in Omak Creek (2005-2013) and the Yakima River (2001 to 2019). 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. Column D are fish with PRO detections as repeat spawners. Column E are post-repeat spawn fish collected at CJFF a second time. Table updated on 1/21/21 with the 2019 kelt release and subsequent 2020 spawning season.

	Conclusive Evidence for Homing			Consistent with Homing					
Location	**A. Maiden/ Repeat Spawner Tag Detection**	**B. Repeat Spawner Tag Detection**	**C. Con 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**	**H. Repeat Spawner Trib Detection**	**Total Spawners: Consistent w/ Spawning Movement (Total of D +E+F+G+H)**
Yakima R	59	70	129	711	67	67	109	371	1325
Omak Cr	11	-	11						
Total	70	70	140	711	67	67	109	371	1325

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 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 facilities (both Lower Granite Dam and Chandler Juvenile Facility). A total of 553 steelhead kelts were released as controls between 2005 and 2011 for the purposes of this analysis. Control releases were discontinued in the Yakima River due to low numbers of available kelts effectively in 2017. All future long-term reconditioning and transport after 2016 will be compared against this baseline with a total of 894 fish released back to the Yakima River from 2005-2016. In-river releases are still ongoing at Lower Granite Dam with a total of 18,041 kelts released from 2002-2004, 2009-2020.

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.

Long-term reconditioning and transport (immature kelts).

At Prosser Hatchery, water quality can be an issue for holding kelts for long-periods of time (over winter). The water temperature is adequate through the summer months (11^0C) but is on the warmer side when compared against ocean temperatures during winter would be approximately 5.5 degrees cooler. We believe that this temperature shift can lead to rematuration or disease issues in the fish over the winter thus the lower survival rates of skip

spawners in the Yakama reconditioning effort at Prosser. This has led us to try releasing fish to the lower river to determine if this may have a better outcome than holding and reconditioning for an additional year at Prosser. In late October of 2019, 103 non-rematuring kelts were trucked from Prosser and released just below Bonneville Dam. Lower Columbia River releases continued in 2020 with 52 long-term reconditioned non-rematuring kelts trucked and released at the same location as 2019. These fish were visually determined to be immature unlike the previous year which utilized hormone assays to determine maturation. This was done out of concerns for COVID-19 disease transmission between staff that do not regularly work together and the logistics of processing samples from a qualitative standpoint. We anticipated that we would see results from the transport in July of 2020 which are included in this annual report and will be updated in the 2021 annual report.

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 (Figure 5.1). All upstream migrating fish at McNary Dam pass through PIT tag detection systems in a fish ladder.

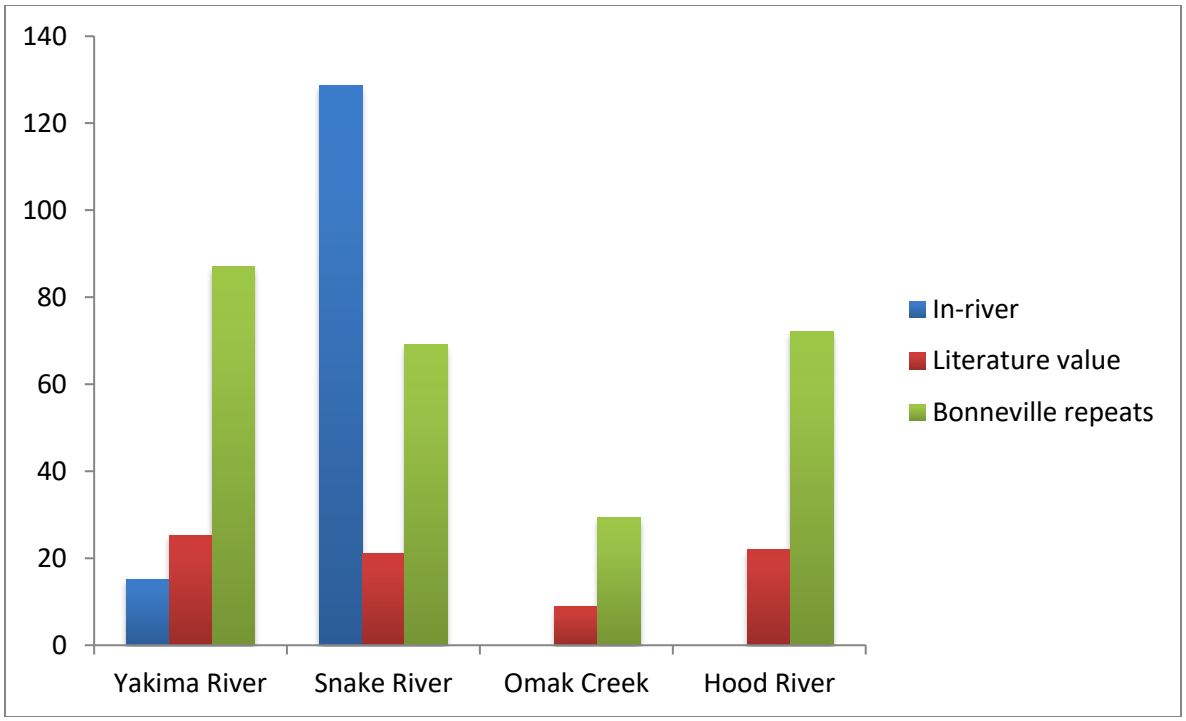


Figure 5. 1: Benefits of long-term reconditioning relative to 3 control metrics. In-river control groups were not available for Omak Creek or Hood River.

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 45% for the Yakima River, 28% for the Snake River, 15% for Omak Creek, and 36% for Hood River. The Yakima River is represented by 21 the Snake River 9, 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 128 times higher than fish left in-river.

Geographic Comparison of Reconditioning Programs

Survival and maturation data from Prosser, Winthrop, and Dworshak are shown in Figure 5.2. Survivals in the Prosser project increased beginning in 2012 and have been in the 70-80% range from 2016 to 2020. 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. The performance of the Prosser project over the past 5 years has been exceptional and is a tribute to the quality of the fish care in this project. The Prosser project also has the advantage of collecting fish at the reconditioning location, whereas fish must be transported from the collection location to the reconditioning location in other projects. Survival of kelts collected at Lower Granite Dam increased to levels similar to the Prosser project in 2018 and 2019 (not including the mortality event at NPTH in 2019), suggesting that survival rates similar to that found in the Prosser project are achievable with the Snake River fish. Survival of fish at both Winthrop and in the Snake River decreased in 2020, which can be attributed to the poorer overall fish condition in 2020 noted at both sites. 2020 was a low run year, and collection criteria were adjusted to maximize collection to include fish in poorer condition. Survivals of DNFH hatchery fish were somewhat lower than found for wild origin fish, which may be due to the effects of anesthesia and manual spawning at the hatchery. Further, hatchery returning steelhead have been lethally spawned at DNFH since the hatchery was established in the 1970s, which could have resulted in selection against iteroparity. Overall, results suggest that survival

rates above 50% are attainable in CRB kelt reconditioning, even in inland populations with a long migration.

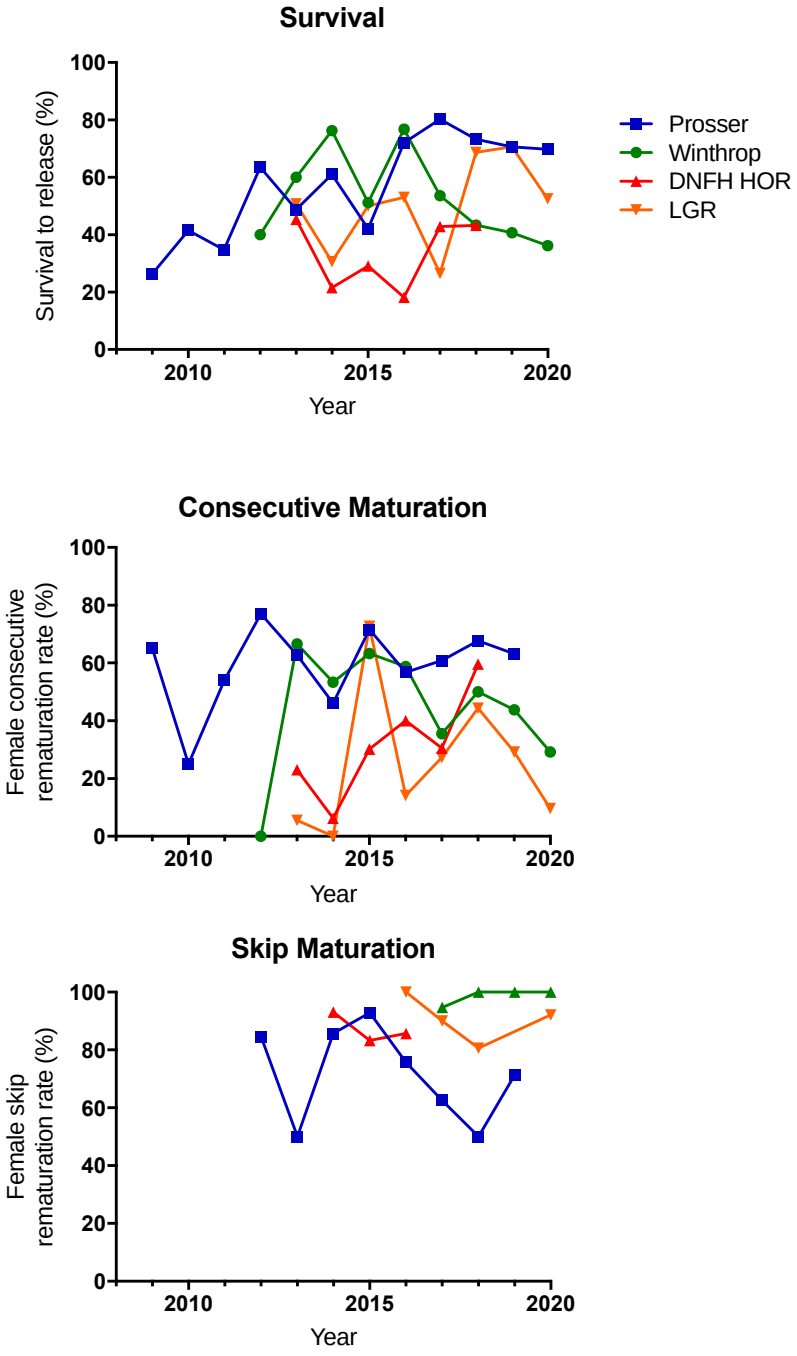


Figure 5. 2: Survival and female consecutive and skip maturation rates in CRB kelt reconditioning projects. Snake River fish include kelts collected at Lower Granite Dam (LGR) and air spawned hatchery origin kelts from the DNFH stock (DHFH HOR). Maturation data for skip spawners is from non-mature fish from the previous season held over for an additional year. Prosser 2020 consecutive maturation rates are not shown, because maturation was not assessed by plasma E2 level.

With a few exceptions, consecutive rematuration rates in the Prosser project have consistently been near 60%. Maturation rates for Snake River and Upper Columbia fish have generally been lower, and overall, the ranking over the past few years has been Prosser > Upper Columbia > Snake River, which is consistent with the hypothesis that fish with a longer and energetically more demanding migration tend to repeat spawn as skip spawners (Keefer, et al. 2008). The consecutive maturation rate for 2020 at Prosser is not shown because fish were not blood sampled due to the COVID-19 pandemic. Based on visual appearance, the 2020 Prosser maturation rate was 83.8%, but this should not be compared to other maturation rates as it is not based on plasma estradiol level. Some of the variation in maturation rates is attributable to conditions during reconditioning. For example, 2010 was a substantially higher collection year at Prosser than typical, resulting in high densities during reconditioning (Hatch, et al. 2013b), Winthrop fish were not given effective treatment for copepods in 2012, and there were issues with fish care on the Snake River project in 2014, 2016, and 2017. The pattern of low consecutive maturation rates in these years indicates that consecutive maturation rate is sensitive to husbandry conditions. However, there were no issues with fish care at Winthrop or in the Snake River in 2020, and yet consecutive maturation rates decreased at both sites. This can be attributed to the effects of pre-capture environmental conditions, and the collection of fish in poorer condition may have also played a role. The variation in consecutive maturation rate from year to year means that projects either need to be able to accommodate varying numbers of skip spawners, or alternative strategies such as transporting and releasing skip spawners need to be explored.

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 generally been lower than in the other projects. The reasons for this difference are not known, but it may relate to water quality during the winter at the different locations. 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 or 2017 in the Winthrop 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 (Jenkins, et al. 2018). Thus, skip spawners provide a source of steelhead spawners to seed habitat in years when the numbers of maiden spawners or survival and consecutive maturation rates of reconditioned kelts are low. Given the critically low number of steelhead spawners throughout the CRB in recent years, this is a resource worth supporting.

Chapter 6. Building a Snake River Kelt Reconditioning Facility

2020 Progress

Significant progress was made toward the realization of a dedicated kelt reconditioning facility for the Snake River 2020. Efforts included: forming a Facility Review team, selecting a facility design contractor, Facility Review team site visit, and development of 10% facility design documents.

Early in 2020, the Kelt Facility Review Team was formed. Team members include representatives from the Bonneville Power Administration, the Nez Perce Tribe, and the Columbia River Inter-Tribal Fish Commission. The team met and reviewed proposals from three design firms. The team agreed to accept the design proposal from R2 Resource Consultants, Inc located in Redmond, Washington. BPA quickly secured a contract with R2 Resource Consultants for design of the Snake River Kelt Facility.

A site visit with the Facility Review Team and R2 Resource Consultants occurred on September 1, 2020. BPA representatives participated via telephone and Nez Perce Tribe, R2 Resource Consultants, and CRITFC participated in person in the site visit. Discussions involved integration of the Kelt Facility with the existing Nez Perce Tribal Hatchery communications, alarm, and control systems; water system integration; kelt building location, footprint and services; and the seasonal water intake design and location.

R2 Resource Consultants distributed a 10% Design Report on October 29, 2020. The Review Team felt that this document was quite thorough and appropriate. Design modification discussion centered on formalin storage and delivery system and tank design. Both of these issues resulted in an agreed-on design. R2 Resource Consultants is currently working toward a 30% Design Report.

We anticipate that Final Design Documents will be completed in 2021 and we will begin steps toward construction of the Snake River Kelt Reconditioning Facility.

Background

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.

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.

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.
Clarification on why male kelts are not included in the proposed reconditioning program is needed.

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?

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.

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.

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.
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?
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 2019, discussions between CRITFC and BPA continued regarding soliciting for a design and construction firm and development of a M&E and O&M plan. Bonneville Power Administration solicited proposals for the design phase in November 2019. A review group was formed to select a design firm and received completed designs at the end of 2020. If the current schedule holds, construction will be completed in 2021 and facility use will begin in 2022.

Chapter 7: Kelt Reconditioning Genetic Studies

Introduction

To better understand Snake River steelhead kelt stock composition and how best to focus our efforts in such a large basin, we have been conducting methods to better manage populations utilizing genetic research and analysis.

Searching for Genetic Basis for Consecutive and Skip Spawner Life Histories: Genome re-sequencing of 3 years of kelts from Dworshak hatchery

Note: This work is in preliminary stages, and data is currently being tabulated to determine if there are any correlations within the data.

It is possible there are hereditary differences that influence sequential or non-sequential spawning in steelhead, such as differences in genes that underlie basal metabolism or reproductive cycling. To identify genetic differences, we have undertaken a whole genome re-sequencing approach that compares whole genome variation of known sequential and non-sequential spawning fish. To eliminate differences inherent to specific populations that may otherwise create false positives, the work relies on repeating this comparison in populations with significant genetic differentiation, thus making genetic commonalities within reproductive classes more prominent. To this end, we have sequenced whole genomic DNA from ~30 individuals across three year classes of each sequential and non-sequential spawning steelhead from the Dworshak National Fish Hatchery in the Clearwater River (Snake River sub-basin) and ~60 individuals across five year classes of sequential and non-sequential spawning steelhead collected at Prosser Dam in the Yakima River (upper Columbia River sub-basin). Illumina 'next-generation' sequencing was performed such that each population-class had a minimum coverage of 10 reads over a common 68% of the genome, which has permitted the identification of ~12 million genetic variants (SNPs) within and across populations. Analyses to identify variants strongly associated with reproductive patterns are ongoing.

We anticipate that we may have quality publishable data on these analyses and will provide an update in the 2021 Annual Steelhead Kelt Reconditioning and Reproductive Success Evaluation Research Final Draft in early 2022.

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 Reporting Group's (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 1,700 kelt steelhead were collected in the Snake River since 2012 and 697 of those fish were reconditioned and released back into the Snake River.

19. As a result of this project 7,868 kelt steelhead were collected in the Yakima River since 2008 and 3,416 of those fish were reconditioned and released back into the Yakima River.
20. 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.
21. 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. The National Marine Fisheries Service has issued a new Biological Opinion for operation of the Columbia River Hydrosystem (NMFS 2019) and plan to issue supplanting BiOp for the FCRPS in 2020.
22. Conducted and produced valuable kelt research on rematuration of steelhead kelts and how environmental factors play into rematuration and how we may be able to better identify sequential/skip spawners to address management of steelhead kelts in the Columbia River Basin See Appendices A2A and A2B.
23. New and improved redundancy systems and protocols are being put in place at Nez Perce Tribal Hatchery to prevent catastrophic loss at the facility until a dedicated facility is constructed, which will have better fail-safe systems in place.

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Appendices

A1.a Master Kelt Tracking Table

Strategy	Year	Location	# Collected	# Released	[redacted]	# @ Bonneville	Return Rate to Bonneville (%)	# @ Lower Granite Dam (or Prosser)	Return Rate to Lower Granite Dam (or Prosser) (%)	Transportation Benefit relative to in-river	Treatment benefit relative to Hockersmith 1.66	Transportation Benefit relative to Bonneville natural
In-river	2005	Prosser	67	67		3	4.5			1.54	2.70	25.61
In-river	2006	Prosser	51	51		1	2.0			0.67	1.18	3.16
In-river	2007	Prosser	53	53		3	5.7			1.95	3.41	9.28
In-river	2008	Prosser	88	88		4	4.5			1.56	2.74	6.64
In-river	2009	Prosser	58	58		3	5.2			1.78	3.12	11.54
In-river	2010	Prosser	155	154		2	1.3			0.44	0.78	3.74
In-river	2011	Prosser	85	85		3	3.5			1.21	2.13	7.01
In-river	2012	Prosser	59	59		2	3.4			1.17	2.04	3.15
In-river	2013	Prosser	52	52		0	0.0			0.00	0.00	0.00
In-river	2014	Prosser	45	45		3	6.7			2.29	4.02	11.52
In-river	2015	Prosser	121	121		0	0.0	0	0.0	0.00	0.00	0.00
In-river	2016	Prosser	56	56		2	3.6	2	3.6	1.23	2.15	57.50
In-river	2017	Prosser	5	5		0	0.0	0	0.0	0.00	0.00	0.00
In-river	2018	Prosser	0	0		NA	NA	NA	NA	NA	NA	NA
In-river	2019	Prosser	0	0		NA	NA	NA	NA	NA	NA	NA
In-river	2020	Prosser	0	0		NA	NA	NA	NA	NA	NA	NA
Total and weighted mean	13		***895***	***894***		***26***	***2.91***	***2***	***0.22***	***1.07***	***1.75***	***5.64***
In-river	2009	Lower Granite	178	176		2	1.1	2	1.1	4.67	0.68	1.96
In-river	2010	Lower Granite	1410	1399		5	0.4	4	0.3	1.47	0.21	0.42
In-river	2011	Lower Granite	1633	1613		3	0.2	3	0.2	0.76	0.11	0.10
In-river	2012	Lower Granite	2098	2098		4	0.2	3	0.1	0.79	0.11	0.10
In-river	2013	Lower Granite	840	827		3	0.4	2	0.2	1.48	0.22	0.37
In-river	2014	Lower Granite	2584	2571		11	0.4	9	0.4	1.77	0.26	0.50
In-river	2015	Lower Granite	1195	1193		0	0.0	0	0.0	0.00	0.00	0.00
In-river	2016	Lower Granite	1841	1837		4	0.2	2	0.1	0.90	0.13	2.11
In-river	2017	Lower Granite	824	821		0	0.0	0	0.0	0.00	0.00	0.00
In-river	2018	Lower Granite	868	863		3	0.3	1	0.1	1.44	0.21	0.31
In-river	2019	Lower Granite	1062	1034		0	0.0	0	0.0	0	0.00	0.00
In-river	2020	Lower Granite	296	284		TBD 2021	TBD 2021	TBD 2021	TBD 2021	TBD 2021	TBD 2021	TBD 2021

Total and weighted mean	**12**		***14829***	***14716***	[redacted]			***35***	***0.2***	***26***	***0.2***	***1.21***	***0.15***	***0.28***
In-river	2020	Little Goose	***22***	***22***				TBD 2021	TBD 2021	TBD 2021	TBD 2021	TBD 2021	TBD 2021	TBD 2021
Strategy	**Year**	**Location**	**# Collected**	**# Survived**	**Survival (%)**	**# Remature**	**Retained**	**Skip Remature**		[redacted]		**Transportation Benefit relative to in-river**	**Treatment benefit relative to Hockersmith 1.66**	**Transportation Benefit relative to Bonneville natural**
Long-term	2000	Prosser	512	91	17.77							NA	10.71	NA
Long-term	2001	Prosser	551	197	35.75							NA	21.54	NA
Long-term	2002	Prosser	420	140	33.33							NA	20.08	NA
Long-term	2003	Prosser	482	298	61.83							NA	37.24	NA
Long-term	2004	Prosser	662	253	38.22							NA	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							15.54	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				16.77	29.35	44.47
Long-term	2014	Prosser	481	292	60.71	149	96	22				9.11	36.57	104.90
Long-term	2015	Prosser	1098	396	36.07	382	74	37				12.41	21.73	69.96
Long-term	2016	Prosser	471	360	76.43	236	74	15				21.40	46.04	1230.57
Long-term	2017	Prosser	118	55	46.61	55	29	4				16.04	28.08	90.42
Long-term	2018	Prosser	227	103	45.37	103	49	5				15.62	27.33	67.76
Long-term	2019	Prosser	371	248	66.85	145	NA	NA				23.01	40.27	182.71
Long-term	2020	Prosser	463	320	69.11	268	NA	NA				23.79	41.64	134.07
Total and weighted mean	21		***10858***	***4787***	***44.1***	***2091***	***363***	***91***				***15.18***	***26.56***	***85.52***

Strategy	Year	Location	# Collected	# *Survived*	Survival (%)	Released	# Cons. remature	Retained	Skip remature	[redacted]	Transportation Benefit relative to in-river	Treatment benefit relative to Hockersmith 1.66	Transportation Benefit relative to Bonneville natural
Long-term	2011	Lower Granite	111	2	1.80	2	2	-	-		9.80	1.08	3.58
Long-term	2012	Lower Granite	124	10	8.06	10	10	0	-		42.30	4.86	7.49
Long-term	2013	Lower Granite/S.F. Clearwater	134	69	51.49	69	69	0	-		144.18	31.02	47.01
Long-term	2014	Lower Granite/Fish Cr.	122	37	30.33	35	35	2	2		71.24	18.27	52.41
Long-term	2015	Lower Granite/S.F. Clearwater/Fish Cr.	140	43	30.71	24	22	21	18		127.53	18.50	59.58
Long-term	2016	Lower Granite/S.F. Clearwater	227	120	52.86	37	19	101	77		243.30	31.85	851.10
Long-term	2017	Lower Granite	269	59	21.93	98	21	58	29		91.07	13.21	42.55
Long-term	2018	Lower Granite	259	177	68.34	79	50	99	1		197.73	41.17	102.05
Long-term	2019	Lower Granite	288	121	42.01	40	39	85	58		174.45	25.31	114.84
Long-term	2020	Lower Granite/Little Goose	137	71	51.82	66	8	63	***TBD 2021***		215.19	31.22	100.53
Total and weighted mean	10		***1811***	***709***	***39.1***	***460***	***275***	***429***	***185***		***162.56***	***22.96***	***75.94***

Strategy	Year	Location	# Collected	# released
Transported Immature (Fall Release @ Hamilton Is.)	2019	Prosser	***103***	***103***
Transported Immature (Fall Release @ Hamilton Is.)	2020	Prosser	***52***	***52***
Total	2		***155***	***155***

Strategy	Year	Location	# Collected	[redacted]	# @ Bonneville	Return Rate to Bonneville (%)
Natural repeat	2004	Bonneville Dam	1125		4	0.36
Natural repeat	2005	Bonneville Dam	572		1	0.17
Natural repeat	2006	Bonneville Dam	1452		9	0.62
Natural repeat	2007	Bonneville Dam	1967		12	0.61
Natural repeat	2008	Bonneville Dam	2630		18	0.68
Natural repeat	2009	Bonneville Dam	2454		11	0.45
Natural repeat	2010	Bonneville Dam	1740		6	0.34
Natural repeat	2011	Bonneville Dam	1391		7	0.50
Natural repeat	2012	Bonneville Dam	1486		16	1.08
Natural repeat	2013	Bonneville Dam	1278		14	1.10
Natural repeat	2014	Bonneville Dam	1728		10	0.58
Natural repeat	2015	Bonneville Dam	904		0	0.00
Natural repeat	2016	Bonneville Dam	1610		1	0.06
Natural repeat	2017	Bonneville Dam	837		0	0.00
Natural repeat	2018	Bonneville Dam	896		6	0.67
Natural repeat	2019	Bonneville Dam	820		3	0.37
Natural repeat	2020	Bonneville Dam	1222		4	0.33
Total			24112		122	***0.52***

Highlighted text is still considered draft data.

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

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Published in 2019

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Published in 2020

Jenkins, L.E., A.L. Pierce, Christopher C. Caudill, N.D. Graham, L.R. Medeiros, D.R. Hatch, J.J. Nagler. 2020. Effects of physiological condition on aspects of repeat spawning in female Steelhead Trout reconditioned in captivity. Transactions of the American Fisheries Society.

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A2.A: Effects of Physiological Condition on Aspects of Repeat Spawningin Female Steelhead Reconditioned in Captivity

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ARTICLE

Effects of Physiological Condition on Aspects of Repeat Spawning in Female Steelhead Reconditioned in Captivity

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Abstract

Physiological condition (hereafter shortened to "condition") influences survival, spawning schedules, and reproductive effort in salmonids. In iteroparous females, the effects of first spawning on condition could result in trade-offs with future reproduction, mediated by postspawning survival, repeat spawning schedule (i.e., consecutive or skip spawning), or reproductive effort. However, which aspects of condition affect these outcomes and when they are sensitive to condition are not well understood. These issues were examined in highly energy-depleted hatchery-origin female summer steelhead *Oncorhynchus mykiss* that were undergoing postspawning reconditioning in captivity. Measures of

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condition and reproductive characteristics (i.e., fecundity, egg size, and total egg mass [TEM]) at first spawning were examined for their effects on postspawning survival and future spawning schedules, and condition was tracked during reconditioning to examine effects on reproductive characteristics at repeat spawning. The levels of plasma osmolality and triglycerides measured at first spawning were positively correlated with survival probability, suggesting that survival depends on the ability to maintain homeostasis and access stored energy. Surprisingly, size-standardized TEM measured at first spawning was positively correlated with the probability of consecutive spawning, providing no support for the hypothesized trade-off between current and future reproduction. This finding instead suggests that both first-spawning reproductive effort and consecutive spawning may be influenced by condition at earlier points. Plasma triglycerides and growth rates at sampling points 10–20 weeks after first spawning were strongly correlated with size-standardized egg size and TEM at consecutive spawning, suggesting that reproductive effort and its allocation to egg size and fecundity depend on energetic status during early oogenesis. These results indicate that condition influences survival, repeat spawning schedules, and reproductive characteristics in female steelhead up to a year or more before repeat spawning. If more broadly applicable, these relationships could provide insight into the mechanisms that link environmental conditions with reproductive characteristics in salmonids and other species.

Physiological condition influences multiple aspects of salmonid life histories. Physiological condition, hereafter shortened to "condition," has been defined as "the relative capacity to maintain optimal functionality of all vital systems within the body" (Hill 2011), encompassing both the energy reserves and functional capacities of the individual. Salmonid spawning schedules and reproductive effort are condition-dependent. Puberty (i.e., initial maturation) has been hypothesized to be determined by energy reserves (i.e., absolute level or rate of change of body size and/or lipid reserves) during seasonally defined critical periods (Thorpe 1994, 2007; Campbell et al. 2006; Taranger et al. 2010). In Atlantic Salmon *Salmo salar*, it is postulated that maturation is initiated during a critical period approximately 1 year prior to spawning, and it can be arrested at a second critical period approximately 6 months later should energy reserves become insufficient (Thorpe 1994, 2007). However, the effect of condition on spawning schedules in repeat spawners has been much less studied in salmonids than has the effect of condition on initial maturation.

In iteroparous salmonids, repeat spawners can either spawn in consecutive years (consecutive spawners) or omit one or more cycles, resulting in skipping one or more years (skip spawners) (Bull and Shine 1979; Rideout et al. 2005; Rideout and Tomkiewicz 2011). The decision to spawn in consecutive years or to skip a reproductive cycle is thought to be related to condition in postspawning fish (Rideout et al. 2005; Rideout and Tomkiewicz 2011). For example, in Winter Flounder *Pleuronectes americanus*, individuals in better condition after spawning initiated ovarian recrudescence in consecutive years despite feed restriction (Burton 1994). The mechanisms that link postspawning condition with reproductive decisions in repeat spawners are likely to be similar to those that act during maturation, with the additional influence of the energetic cost of first spawning (McBride et al. 2015). In addition, condition affects reproductive effort and how it is allocated (i.e., TEM, egg size, and egg number; McBride et al. 2015), though the stage of ovarian development at which reproductive characteristics are sensitive to condition appears to vary between species.

The effects of condition on survival, spawning schedules, and reproductive effort could result in trade-offs between first-spawning reproductive effort and future survival and reproduction in iteroparous salmonids. Greater allocation of energy to reproduction at first spawning could result in reduced condition, leading to reduced odds of survival, reduced odds of initiating gonadal recrudescence as a consecutive spawner, or reduced allocation of energy to the next reproductive cycle (Stearns 1992). Determining when these outcomes are sensitive to condition and which components of condition are involved is necessary to evaluate the potential effects of any trade-offs. A study on winter-run steelhead found reduced reproductive success at initial spawning in successfully iteroparous individuals, consistent with a trade-off between energy allocation to reproduction and postspawning survival (Christie et al. 2018). However, to our knowledge, no studies have directly examined the relationships between first-spawning reproductive effort, condition, and outcomes in terms of survival, repeat spawning schedules, or reproductive effort and how it is allocated between fecundity and egg size at repeat spawning in iteroparous anadromous salmonids.

Although condition is defined in terms of functional capacities, most condition measures that are employed in fisheries studies are structural indices that are intended to measure energy reserves. Fork length, Fulton's condition factor, and muscle lipid levels are structural condition measures that are frequently used to estimate energy reserves, i.e., energetic condition (Koops et al. 2004; Quinn et al. 2011). However, these measures may not adequately capture the aspects of condition that are relevant to survival, spawning schedules, or reproduction. Additional rapid, nonlethal measures that reflect the functioning of physiological systems (i.e., functional condition measures) may provide better indices of condition that interact with energetic

condition and are also related to fitness. For example, the ability to maintain osmotic homeostasis can be assessed by measuring plasma osmolality (Jeffries et al. 2011) and the ability to mobilize stored energy can be assessed by measuring circulating levels of metabolic fuels (Simpkins et al. 2003; Congleton and Wagner 2006; Gauthey et al. 2015). On the other hand, these plasma variables may respond transiently to environmental conditions, whereas structural indices would be expected to be more stable. Empirical evaluation is required to determine which condition measures perform best in any given situation.

In this study, hatchery-origin female steelhead, returning to Dworshak National Fish Hatchery (DNFH) in Ahsahka, Idaho, were used to examine the relationships between first-time spawning reproductive effort, condition, postspawning survival, spawning schedules, and reproductive characteristics at repeat spawning. The fish were captured upon their return to the hatchery following an approximately 800-km freshwater, fasting spawning migration during which large amounts of stored energy are diverted into ovarian development, resulting in extreme energy depletion (Penney and Moffitt 2014b). Consequently, although steelhead are iteroparous, approximately 50% of the studied population survives beyond 10 weeks after spawning when individuals are reconditioned in captivity and fewer than half initiate ovarian recrudescence in consecutive years (Jenkins et al. 2018, 2019). This provides a unique opportunity to investigate condition-dependent outcomes and potential trade-offs. The objectives of this study were to determine (1) whether postspawning survival and spawning schedules were condition-dependent, (2) whether there are trade-offs with first-time spawning reproductive effort, (3) whether and when reproductive characteristics at second spawning were condition-dependent during ovarian recrudescence, and (4) which measures of condition were informative for each outcome.

METHODS

Fish.— First-time spawning female steelhead that had originated from DNFH were captured upon their return after ascending the DNFH adult ladder on the North Fork Clearwater River in Ahsahka, Idaho. The fish were captured during February through April in 2015 and 2016, and they were held unfed in holding ponds that were supplied with river water. During the capture periods, DNFH staff selected fully mature fish that were >70 cm fork length (FL) for use as brood stock. Of these, the fish that were in good or fair external condition, i.e., those lacking morphological damage, lesions, or fungal infection (Evans et al. 2004; Hatch et al. 2013), were selected for this study and individually marked with passive integrated transponder tags that were inserted into the pelvic girdle ($n = 150$ in 2015 and $n = 164$ in 2016).

Spawning.— The fish were anesthetized by using AQUI-S 20E (AquaTactics, Kirkland, Washington; 75 mL/1,000 L water) and were manually "air spawned" (Leitritz and Lewis 1976). The repeat spawners were checked weekly for ripeness during the spawning season and air spawned as above when they were ripe.

Reproductive performance measures.— At first and second spawning, individual egg mass (IEM), total egg mass (TEM), and fecundity were quantified gravimetrically (Fleming and Ng 1987; Jenkins et al. 2018). To enable comparisons of fish of different sizes, the measures for IEM, TEM, and fecundity were standardized based on fish mass (Jenkins et al. 2018).

Sampling.— At spawning, the fish were sampled for fork length (cm), wet body mass (kg), muscle lipid level (ML, %; Fish Fatmeter model 692, Distell, West Lothian, UK), blood, and the number of parasitic gill copepods *Salmincola californiensis* that were counted on all of the gills on the left side (gill parasite load, PL). After spawning, sampling occurred at 10-week intervals for all of the measures described.

Reconditioning husbandry.— The fish were held at DNFH in 4.6-m diameter outdoor tanks that were supplied with North Fork Clearwater River water at approximately 200 L/min and maintained at a water height of 1.5 m, with a seasonally varying temperature profile (4.9–11.0°C). The tanks were treated with formalin (Syndel USA, Portland, Oregon) as a flow-through treatment (1:6,000 dilution) for 1 h daily to control *Saprolegnia*. The fish were fed ad libitum and were prophylactically treated for bacterial infection with oxytetracycline (Durvet, Blue Springs, Missouri; 20 mg/kg body weight) and for parasitic gill copepods *Salmincola californiensis* with emamectin (Sigma-Aldrich, St. Louis, Missouri; 200 μg/kg body weight), both via intraperitoneal injection, at spawning and at 10-week intervals thereafter, with emamectin dispensed only when copepods were visible on the gills (Jenkins et al. 2018).

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

$$K = 100 \times \text{body mass(g)} \times [\text{fork length(cm)}]^{3} \quad (1)$$

$$\begin{aligned}\text{MSGR} &= \%\text{bodymassgain/d} \\ &= 100 \times [\ln(\text{bodymassfinal}) - \ln(\text{bodymassinitial})]/\text{d}.\end{aligned} \quad (2)$$

The values for LSGR were calculated in the same manner as was MSGR. Before calculation of K and MSGR, body mass was adjusted to account for any eggs that were retained from first spawning in the body cavity as follows: the mass of any eggs that were retained in the body cavity

following spawning but later removed at future sampling points and/or necropsy was subtracted from body mass before calculating K and MSGR (Jenkins et al. 2018).

Assays.—Plasma estradiol-17β (E2, ng/mL) concentration was measured by ELISA (Biosense, Cayman Chemical, Ann Arbor, Michigan; Jenkins et al. 2019). Plasma triglyceride (TG) concentration was measured by using a VetTest (Idexx, Westport, Maine) as validated for use in salmonids (Meador et al. 2006). Plasma osmolality (OS, mmol/kg) was measured by using a VAPRO Vapor Pressure Osmometer 5520 (WESCOR, Elitech, Puteaux, France).

Statistical analysis.—Survival in postspawning female steelhead over time in each year (2015 and 2016) was compared by using a log-rank test conducted in PRISM (version 8, GraphPad).

The probability of fish surviving after first spawning was assessed by a using multiple logistic regression analysis. Of the total number of fish that were spawned ($n = 314$), a total of 291 were included in the analysis due to missing values ($n = 2$) and the removal of outliers based on the ROUT outlier test ($n = 21$; $Q = 1\%$). Binomial response variables were defined as follows: survivors (i.e., fish that survived 70 d postspawning, assigned as "1") versus mortalities (i.e., fish that died prior to 70 d postspawning, assigned as "0"). The predictor variables ($n = 7$) that were measured at first spawning included K, ML (arcsine-square-root transformed), TG, OS, PL, TEM, and E2 ($\log_{10}$ transformed). The predictor variables were standardized in order to compare effect sizes ([x – mean]/SD) (e.g., Keefer et al. 2017) and were found to be independent, as no variable explained more than 22% of the variation in any other variable (Table 1). The data from 2015 and 2016 were examined for a year effect. Because the preliminary univariate tests revealed a year effect (in the survival analysis only, $P = 0.00393$), we tested for first-order interactions with year. The interaction effects were nonsignificant ($P > 0.29$), so they were dropped from the survival model. The multiple logistic regression equation was as follows:

$$\text{Survival} = \text{intercept} + \text{year} + \text{TG} + \text{ML} + \text{K} + \text{OS} + \text{PL} + \text{TEM} + \text{E2} + \text{error}. \quad (3)$$

The points representing the predicted probability of survival were plotted in 3-D space by using the R package plotly (Sievert et al. 2018).

To further explore predictors of survival, multiple linear regression was used to identify predictors of survival duration (survival_{70}) for individuals that died within the first 70 d following first spawning (mortalities, $n = 172$). A total of 156 mortalities were included in the analysis due to missing values ($n = 2$) and the removal of outliers ($n = 14$, $Q = 1\%$). The analysis used the same standardized predictor variables as were used for the multiple logistic regression on survival. Plasma triglyceride and OS were found to interact with year in the preliminary models and thus were included in the final model as follows:

$$\text{Survival}_{70} = \text{intercept} + \text{year} + \text{TG} + \text{ML} + \text{K} + \text{OS} + \text{PL} + \text{TEM} + \text{E2} + \text{year} \times \text{OS} + \text{year} \times \text{TG} + \text{error}. \quad (4)$$

The factors that were associated with reproductive status in the year following first spawning were assessed in a multiple logistic regression using the same standardized predictor variables as were used in the multiple logistic regression on survival. Reproductive status was determined in fish that survived to 30 weeks postspawning ($n = 73$) by using E2 concentrations. Previous studies have indicated that E2 levels in females at this point have diverged completely between reproductive (consecutive spawning schedule, E2 range 8,000–70,000 ng/ml) and nonreproductive (skip spawning schedule, E2 range 10–1,000 ng/ml) individuals, including the fish that were used in this study (Pierce et al. 2017; Jenkins et al. 2019). The fish with a high E2 concentration were classified as reproductive and assigned as "1," and fish with a low E2 concentration were classified as nonreproductive and assigned as "0." The probability of being reproductive in the year following first spawning was assessed. Of the fish that survived to 30 weeks, $n = 60$ fish were included in the analysis due to missing data points ($n = 2$) and removal of outliers based on the ROUT outlier test ($n = 11$, $Q = 1\%$). No evidence for first-order interactions with year was found in the preliminary models, so the interactions were dropped. The final model was

$$\text{Status} = \text{intercept} + \text{year} + \text{TG} + \text{ML} + \text{K} + \text{OS} + \text{PL} + \text{TEM} + \text{E2} + \text{error}. \quad (5)$$

The data were plotted in R by using the popbio package (Smart et al. 2004; Stubben and Milligan 2007).

To identify potential sensitive periods for the condition-dependent regulation of reproductive characteristics in consecutive spawners, simple linear regression was used to describe the relationships between the size-standardized reproductive metrics (IEM, TEM, and fecundity) at consecutive spawning and six independent variables, which were measured at six points (first spawning and at 10-week intervals thereafter out to 50 weeks). The independent variables were MSGR, LSGR, TG, E2, ML, and K. Consecutive spawners ($n = 12$) from the 2015 spawn year

TABLE 1. Correlation coefficients between parameters that were measured at spawning in female steelhead that were collected in 2015 and 2016 ($n = 314$). The P-values for the correlations are above the diagonal and the correlation coefficients are below the diagonal. The bolded r-values indicate significance ($P < 0.05$).

	Plasma triglycerides (mg/dL)	Muscle lipids (%)[a]	Condition K	Plasma osmolality (mmol/kg)	Parasite load (number of copepods)	Total egg mass (g)	Plasma E2 (ng/mL)[a]
Plasma triglycerides		**<0.0001**	**0.0141**	**<0.0001**	0.5933	**0.0004**	**<0.0001**
Muscle lipids	**0.384**		**0.0489**	**0.0154**	0.7491	**0.0202**	**<0.0001**
Condition K	**0.139**	**0.113**		0.2556	0.1960	**<0.0001**	0.1808
Plasma osmolality	**0.389**	**0.140**	0.065		0.2903	**<0.0001**	**0.0005**
Parasite load	−0.031	0.018	−0.074	−0.061		**0.0001**	0.1402
Total egg mass	**−0.201**	**−0.132**	**−0.244**	**−0.220**	**0.214**		0.1647
Plasma E2	**0.469**	**0.326**	0.076	**0.198**	−0.085	0.079	

[a]The correlations were based on transformed data (muscle lipids: arcsine square root; plasma E2: $\log_{10}$).

were included in the analysis because the most complete data set was available for these fish. Coefficients of determination (R^2) and an associated P-value were calculated for each predictor at each period (spawning, and at each 10-week sampling period or interval thereafter). Unless otherwise indicated, the statistical analysis was conducted in JMP (version 13, SAS Institute) and plotted in PRISM (version 8, GraphPad).

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

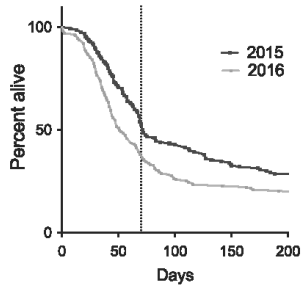

FIGURE 1. Survival of female steelhead ($N = 314$) following first spawning in 2015, indicated by the black line, and 2016, indicated by the gray line. The vertical dashed line at 70 d indicates the first sampling point after spawning.

RESULTS

Survival

Survival declined steeply during the first 70 d following first spawning and remained relatively constant during weeks 10 to 30 (Figure 1). Survival was significantly lower in 2016 than in 2015 (log-rank test, $\chi^2 = 9.335$, $P = 0.0022$). Survival at 70 d postspawning was 53% (80/150) in 2015 and 38% (62/164) in 2016, averaging 45% (142/314) in the 2 years combined (Table 2). Of all of the measures that were examined, OS and TG were the only ones that were strongly and significantly positively related to survival (Figure 2; from equation 3: $\chi^2 = 10.94$, 7.20; $P = 0.0009$ and 0.0073, respectively; Figure 3). The 95% confidence intervals of all of the other standardized coefficients overlapped zero and were thus without significant effect. Survival_{70} was significantly related to TG, OS, TG × year, and OS × year (from equation 4) (adjusted model $R^2 = 17.3\%$, Table 3).

Spawning Schedule

Approximately one third (34%) of the female steelhead that survived to 30 weeks (13/43, 30% in 2015; 12/30, 40% in 2016) and an average of 8% of all of the fish were reproductively active in the year following first spawning (2015: 9% [13/150], 2016: 7% [12/164]) (Table 2), so they were on schedule to spawn in consecutive years. The only significant predictor of consecutive reproductive status was TEM (Figure 4; from equation 5: $\chi^2 = 5.02$; $P = 0.0250$; Figure 5), and it positively predicted a consecutive spawning schedule. The 95% confidence intervals of all of the other standardized coefficients overlapped zero and were thus without significant effect.

Reproductive Characteristics

The consecutive spawners that had spawned for the first time in 2015 ($n = 12$) that were included in this analysis had an average TEM of 721.9 g (SD = 132.8), which can be broken down into an average IEM of 96.1 mg (SD = 13.5) and an average fecundity of 7,505 eggs (SD = 1,456) at second spawning. The reproductive characteristics at the second spawning were related to the condition of the fish early in

TABLE 2. Number (n [%]) by outcome category of female steelhead trout that were collected in 2015 and 2016 with major condition and reproductive metrics (mean [SD]). The terms are defined as follows: survived = alive at 70 d after spawning; mortality = died before 70 d after spawning; consecutive = consecutive spawner; and skip = skip spawner.

		Energetic status			Homeostatic ability		Reproductive effort	
	n (%)	Muscle lipids (%)	Condition K	Plasma triglycerides (mg/dL)	Plasma osmolality (mmol/kg)	Parasite load (number of copepods)	Total egg mass (g)	Plasma E2 (ng/mL)
2015								
Consecutive	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)
Skip	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)
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)
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								
Consecutive	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)
Skip	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)
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)
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)
All								
Consecutive	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)
Skip	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)
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)
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)

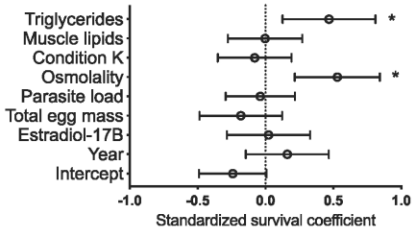

FIGURE 2. Standardized coefficients ([x – mean]/SD) with 95% confidence intervals of potential predictors in a multiple logistic regression model of survival of female steelhead to 70 d after first spawning in 2015 and 2016 ($n = 291$). Positive coefficients indicate a greater probability of postspawning survival. Coefficients with confidence intervals that do not overlap zero were statistically significant ($P < 0.05$) and are marked with an asterisk.

the interval between spawning events (10 to 20 weeks postspawning) (Table 4). Repeat spawning IEM was most strongly related to TG at 10 weeks ($R^2 = 0.5593$, Figure 6A). Repeat spawning TEM was most strongly related to MSGR during the 10–20-week interval ($R^2 = 0.5382$, Figure 6B). The strongest relationship between repeat spawning fecundity and condition, excluding the 50-week period

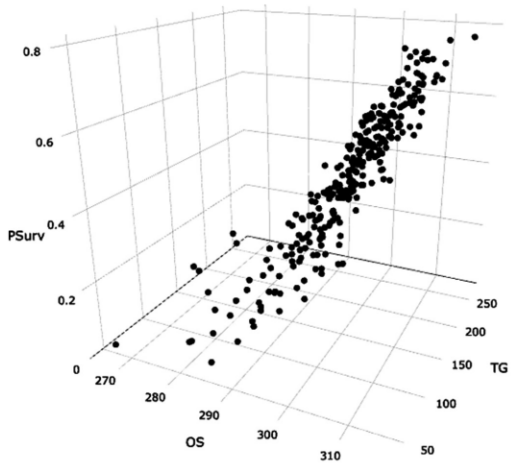

FIGURE 3. Postspawn survival probabilities (PSurv) to 70 d for female steelhead, as predicted by plasma triglcyerides (TG [mg/dL]) and plasma osmolality (OS [mmol/kg]) measured at first spawning in 2015 and 2016 ($n = 291$).

TABLE 3. Standardized parameter estimates resulting from the multiple linear regression analysis of postspawning survival duration within the first 70 d after first spawning in female steelhead ($n = 156$) that were collected in 2015 and 2016. The bolded Prob>|t|-values indicate significance ($P < 0.05$).

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
Plasma 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
Plasma 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
Plasma E2	−0.78957	1.455299	−0.54	0.5883
Year × plasma triglycerides	−3.2989	1.461476	−2.26	**0.0255**
Year × plasma osmolality	3.2818172	1.553802	2.11	**0.0364**

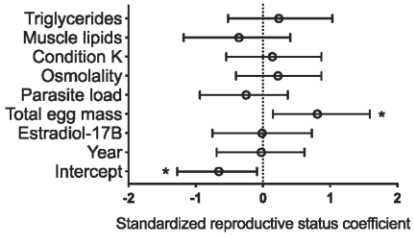

FIGURE 4. Standardized reproductive status coefficients ([*x* – mean]/SD) with 95% confidence intervals of potential predictors in a multiple logistic regression model of reproductive status in consecutive years of female steelhead ($n = 60$) following spawning in 2015 and 2016. Positive coefficients indicate greater probability of consecutive reproductive status. Coefficients with confidence intervals that do not overlap zero were statistically significant ($P < 0.05$) and are marked with an asterisk.

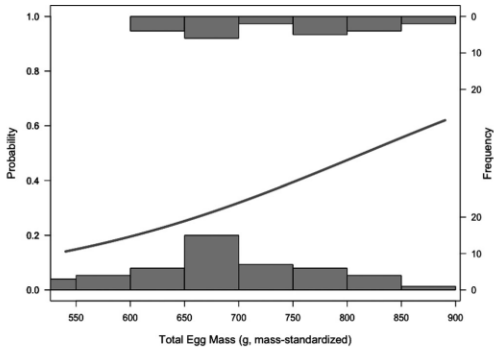

FIGURE 5. The probability of consecutive spawning reproductive status in female steelhead in relation to total egg mass at first spawning (g, mass-standardized, $n = 60$). The bars at the top and bottom of the graph represent the number of fish that became reproductively active (top) or remained nonreproductive (bottom) following first spawning, respectively, for each range of total egg mass.

that coincided with second spawning, was with TG at 20 weeks after first spawning ($R^2 = 0.3202$, Figure 6C), but this relationship was not significant ($P = 0.0551$).

DISCUSSION

Postspawning survival was condition-dependent based on functional measures of condition in female steelhead in this study. The fish with greater TG and OS at first spawning had a significantly increased probability of survival. In contrast, ML and *K*, structural measures of condition that reflect energy stores, were not significant predictors of survival. Reproductive effort at consecutive spawning and how it was allocated were condition-dependent during the period immediately after spawning. Individual egg mass was most strongly correlated with TG at 10 weeks after spawning, and TEM was most strongly correlated with growth rate during the 10–20-week interval following first spawning. Surprisingly, the consecutive spawning schedule was positively related to first-spawning reproductive effort (TEM), providing no support for a hypothesized trade-off between current and future reproduction. Consistent with this, first-spawning TEM and measures of condition at first spawning were weakly negatively correlated ($R^2 < 0.06$), providing little evidence for a biologically significant trade-off between first spawning reproductive effort and condition at first spawning. Instead, both first spawning reproductive effort and the decision to initiate ovarian recrudescence in consecutive years may depend on condition at points prior to first spawning.

Survival and Spawning Schedule

Survival in the present study was in the range that has been reported from other studies of steelhead and Atlantic

TABLE 4. Correlation coefficients (r) for the relationships between condition measures over time starting at first spawning and the reproductive characteristics that were measured at consecutive spawning in $n = 12$ female steelhead that first spawned in 2015. The bolded r-values indicate significance ($P < 0.05$).

Repeat reproductive performance	Sampling point	MSGR[a]	LSGR[a]	Plasma triglycerides	Plasma 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

[a]Data are shown for the mass- and length-specific growth rates (MSGR, LSGR) that occurred over the preceding interval.

Salmon kelts that were reconditioned in freshwater (Moffett et al. 1996; Hatch et al. 2013), and it was much higher than in-river survival to repeat spawning for steelhead in the Snake River (Keefer et al. 2008, 2017). The mortality profiles were similar in both years, with postspawning mortality leveling off by 10 weeks after spawning. Heavy mortality during the initial reconditioning period is typical in steelhead reconditioning (Hatch et al. 2013), which motivated the analysis of predictors of survival through this period. The consecutive spawning schedule was evident in 30–40% of the fish that survived to 30 weeks postspawning. Similarly, 38% of the wild steelhead in the Snake River that had been tagged as out-migrating adults at Lower Granite Dam during 2002–2004 returned as consecutive spawners (Keefer et al. 2008).

Predictors of Survival

Energy reserves.— Survival to 70 d after spawning and survival duration for fish that died during the first 70 d were positively related to TG levels at spawning, suggesting that survival depends on energy reserves at spawning. However, the structural energy reserve measures K and ML did not significantly predict survival, indicating that TG is a better measure of energy reserves under our experimental conditions. Growth over the initial 10 weeks after spawning was minimal, and TG levels tended to decrease from 0 to 10 weeks postspawning (Jenkins et al. 2019). Together with the present results, this suggests that survival over the first 10 weeks depends to a greater degree on the ability of fish to access stored energy than on their energy acquisition through feeding. Plasma TG level at spawning integrates both body levels of stored lipids and the functioning of the physiological systems that enable access to these energy stores. Plasma TG levels decrease during catabolic states in salmonids including fasting, energy expenditure by swimming, and spawning (Simpkins et al. 2003; Congleton and Wagner 2006; Gauthey et al. 2015). Plasma TGs at spawning would be used for energy rather than for synthesizing vitellogenin (Bon et al. 1997). Similar to our results, K at collection for reconditioning did not predict survival during captive reconditioning in Atlantic Salmon (Crim et al. 1992) or wild steelhead kelts in the Yakima River (Hatch et al. 2013). Although K is easy to measure, it is an imperfect index of energy reserves (Sutton et al. 2000; Trudel et al. 2005). Although ML levels were positively correlated with plasma TG levels (Table 1), as expected because plasma TG would be largely derived from muscle lipid stores (Sheridan 1988), the correlation was weak ($r = 0.384$) and ML levels were not significantly associated with survival. This suggests that functional measures of energetic status may be superior indicators of condition in circumstances where the functioning of physiological systems may be compromised.

Plasma osmolality.— Plasma osmolality was positively related to survival and survival duration. This association was particularly strong over the low range of the data such that fish with OS that was below approximately 290 mmol/kg were highly subject to mortality soon after first spawning.

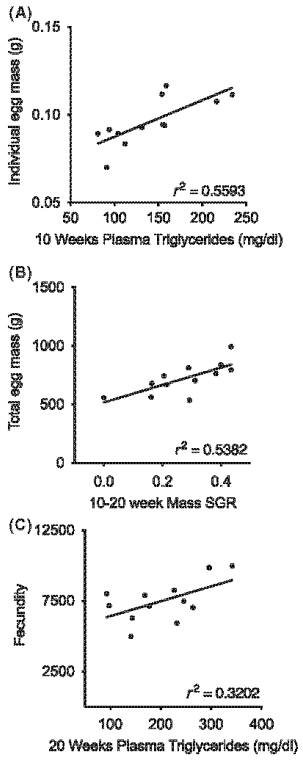

FIGURE 6. Relationships between reproductive characteristics at consecutive spawning, **(A)** individual egg mass, **(B)** total egg mass, and **(C)** fecundity, and plasma triglycerides or mass-specific growth rate (MSGR) during ovarian recrudescence in female steelhead.

Fish in freshwater must take up ions through the gill and excrete excess water through the kidney to maintain OS in a narrow range, approximately 308.5 ± 3.0 mmol/kg for Rainbow Trout in freshwater (Oguri and Ooshima 1977). A wider range of values was observed in the present study (survivors: 286–317 mmol/kg, mortalities: 265.5–312 mmol/kg). Low plasma osmolality in individual fish indicates impairment of the osmoregulatory system, which explains the association with mortality. Consistent with our study, decreased OS predicted mortality in prespawning Sockeye Salmon in freshwater, with fish becoming moribund below 280 mOsm/kg (Jeffries et al. 2011). In this study, the decrease in OS occurred before changes in metabolic factors, stress, or reproductive hormones, suggesting that failure of the osmoregulatory system may be a primary cause of mortality. The similarity in the role of OS as an early indicator of mortality in both iteroparous and semelparous *Oncorhynchus* species suggests that the physiological basis for mortality may be similar. In steelhead at spawning, OS and TG were positively correlated, consistent with the possibility that decreased osmoregulatory ability could both cause and/or result from energy depletion. Osmoregulation is estimated to consume as much as 28% of basal metabolism for salmonids in freshwater (Boeuf and Payan 2001).

First spawn reproductive investment.— This study provides an examination of whether a trade-off occurred between energy reserves that were allocated to reproduction at first spawning (measured as TEM) and survival postspawning, thought to be a classic life history trade-off. Postspawning survival was not significantly negatively correlated with TEM in the whole model, so the classic trade-off is not supported. Trade-offs between reproductive effort and survival are thought to be mediated by condition (Stearns 1992). However, correlations between TEM and condition measures at spawning were low, accounting for at most 6% of the variation in condition measures, suggesting that condition at spawning was not strongly influenced by TEM. Our results contrast with a proposed trade-off between first-spawning reproductive investment and survival to future spawning in wild winter-run steelhead from the Hood River, Oregon (Christie et al. 2018). However, the survival percentages were much lower in the wild fish study, consistent with stronger selection on postspawning energy reserves for fish that are attempting to return to the ocean. Thus, the manifestation of any underlying trade-off is likely to be context dependent. The present results suggest that survival during captive reconditioning does not select for fish with lower reproductive effort at first spawning for summer-run female steelhead.

Plasma estradiol and parasite load.— Plasma estradiol at spawning did not predict subsequent survival. Estradiol-17β was assessed as a predictor of survival because of its potential association with reproductive effort. However, E2 at spawning showed high variation and E2 over the year before second spawning was not significantly correlated with TEM or any reproductive characteristic at second spawning (Table 4), suggesting that E2 may not be a good proxy for reproductive effort. Gill copepod number (PL) at spawning also did not predict subsequent survival. Gill copepod number was assessed as a predictor of survival because of its potential association with overall parasite load and immune system function. However, it is understood that that parasite load results from a complex interaction of factors such as exposure and immune response and that other pathogens are likely present that could influence survival in adult salmonids (Jia et al. 2020).

First Spawning Reproductive Investment and Spawning Schedule

Consecutive spawning reproductive status was positively related to first-spawn TEM in female steelhead in this study. Because energy allocation for ovarian

development begins at least a year before spawning, this indicates the existence of a positive relationship between allocation of energy into current and future reproduction. This does not support the notion of the trade-off between current and future reproduction that is proposed in life history theory (Stearns 1992); instead, it suggests that both first-spawn reproductive effort and consecutive reproduction are positively regulated by condition at a point(s) before spawning. Similarly, evidence for trade-offs between secondary sexual characteristics, gonad size, and retained somatic energy were not found in Sockeye Salmon, which was attributed to variation in condition between individuals driving investment into these aspects of reproduction to a greater degree than do potential trade-offs (Hendry et al. 2000). The strong positive relationships that we found between condition measures early in the year before second spawning and reproductive effort at second spawning support the idea that first-spawning reproductive effort depends on condition before spawning. Evidence of the growth of a second cohort of developing oocytes prior to ovulation of the imminent cohort has been found in Rainbow Trout and steelhead (De Mones et al. 1989; Penney and Moffitt 2014a), which supports the idea that the decision to initiate ovarian recrudescence in consecutive years may occur before first spawning.

The present study did not find any significant relationship between the measures of condition at first spawning and consecutive reproductive status (the consecutive spawning schedule), suggesting that the reproductive decision is not condition-dependent at the time of spawning. In contrast, a recent study in anadromous Brown Trout *Salmo trutta* found that fish with higher K at the time of adult outmigration in the spring were more likely to return as consecutive spawners (Haraldstad et al. 2018). The difference may relate to when during their ovarian development the fish were sampled. Sea-run Brown Trout spawn in the fall, and most overwinter in freshwater before returning to the ocean. Thus, ovarian development would have been underway in the consecutive spawners when the Brown Trout were sampled in late April, which could account for the higher K.

Condition-Dependent Reproductive Characteristics at Consecutive Spawning

The reproductive characteristics at consecutive spawning were sensitive to the postspawning condition measures early in ovarian development up to approximately 9.5 months prior to second spawning. Individual egg mass was strongly positively correlated with growth rates (mass, FL) and TG at 10 weeks after first spawning. Total egg mass was strongly positively correlated with mass growth rate, TG, and K at 20 weeks after first spawning. Fecundity was strongly positively correlated (NS) with growth rates (mass, FL) and TG at 20 weeks after first spawning. These findings suggest that, among fish on the consecutive spawning trajectory, egg size is set first based on condition at approximately 10 weeks after first spawning and then fecundity (and consequently TEM) is set based on condition at approximately 20 weeks after first spawning. The relationships were quite strong, accounting for approximately 50% of the variation in IEM and TEM. A previous study found that feed restriction early during oogenesis reduces size-adjusted fecundity in salmonids (Henderson and Wong 1998). However, to our knowledge, no previous studies have directly demonstrated that energetic status early in oogenesis influences egg size in salmonids. Consistent with our results, size-adjusted egg size was reduced in consecutive-spawning Atlantic Salmon, which would be energy restricted early in oogenesis, compared with first-time spawners and skip spawners (Reid and Chaput 2012). Further research is required to determine whether these relationships are restricted to consecutive spawning summer-run steelhead or apply more broadly. The physiological mechanisms that are involved and whether and how the determination of egg size and fecundity by these mechanisms is adaptive are not clear at present.

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A2.B. Establishment of time-resolved fluoroimmunoassays for detection of growth hormone and insulin-like growth factor I in rainbow trout plasma

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Establishment of time-resolved fluoroimmunoassays for detection of growth hormone and insulin-like growth factor I in rainbow trout plasma

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ABSTRACT

The GH/IGF-I axis influences many aspects of salmonid life history and is involved in a variety of physiological processes that are related to somatic growth (e.g., reproduction, smoltification, and the response to fasting and stress). As such, fisheries studies utilize GH/IGF-I axis components as indicators of growth and metabolic status. This study established time-resolved fluoroimmunoassays (TR-FIAs) for rainbow trout plasma GH and IGF-I using commercially available reagents. For the GH TR-FIA, the ED_{80} and ED_{20} were 0.6 and 28.1 ng/mL, the minimum detection limit was 0.2 ng/mL, and the intra- and inter-assay coefficients of variation (%CV) were 4.1% and 13.4%, respectively. Ethanol remaining from acid-ethanol cryoprecipitation (AEC) of plasma samples to remove IGF binding proteins reduced binding and increased variability in the IGF-I TR-FIA. Drying down and reconstituting extracted samples restored binding and reduced variability. The extraction efficiency of IGF-I standards through AEC, drying down, and reconstitution did not vary over the working range of the assay. For the IGF-I TR-FIA, the ED_{80} and ED_{20} were 0.2 and 6.5 ng/mL, the minimum detection limit was 0.03 ng/mL, and the intra- and inter-assay %CV were 3.0% and 6.5%, respectively. Biological validation was provided by GH injection and fasting studies in rainbow trout. Intraperitoneal injection with bovine GH increased plasma IGF-I levels. Four weeks of fasting decreased body weight, increased plasma GH levels, and decreased plasma IGF-I levels. The GH and IGF-I TR-FIAs established herein provide a cost-comparable, non-radioisotopic method for quantifying salmonid plasma GH and IGF-I using commercially available reagents.

1. Introduction

In fishes, as in other vertebrates, the growth hormone (GH)/insulin-like growth factor-I (IGF−I) axis is the major endocrine system regulating growth, indicating strong evolutionary conservation across taxa (Bergan-Roller and Sheridan, 2018; Reindl and Sheridan, 2012). Indeed, mammalian and salmonid data are in agreement on all key aspects of the GH/IGF-I axis (Bjornsson, 1997; Bjornsson et al., 2018). The GH receptor is found in most tissues, with the highest density located in the liver (Bjornsson et al., 2002; Reindl and Sheridan, 2012). GH can thus stimulate tissue growth directly, but also does so indirectly through GH-induced production and release of IGF-I from the liver (Daughaday and Rotwein, 1989). In mammals and fishes alike, IGF-I acts via autocrine, paracrine, and endocrine mechanisms to mediate the growth promoting actions of GH as well as acting as a negative feedback signal on GH secretion from the pituitary (Duan, 1998; Perez-Sanchez and Le Bail, 1999; Perez-Sanchez et al., 1992; Tannenbaum, 1993; Wood et al., 2005). The effects of both local and circulating IGF-I are modulated by a complex suite of IGF binding proteins (IGFBPs) (Duan and Xu, 2005; Shimizu and Dickhoff, 2017).

It is not surprising then, that nutritional status is the primary environmental regulator of the GH/IGF-I axis – that is, growth must adjust according to nutritional conditions and, as the primary regulator of growth, this means that nutritional state will affect the GH/IGF-I axis (Duan, 1998; Moriyama et al., 2000; Pierce et al., 2001; Thissen et al., 1999). During fasting, growth ceases, and energy is mobilized from storage tissues to support metabolism and maintain homeostasis (Bar, 2014; Navarro and Gutierrez, 1995). These effects are mediated in part by changes in the GH/IGF-I axis. Fasting normally leads to increased plasma GH levels while IGF-I levels decrease, an endocrine condition known as acquired GH resistance (Jenkins and Ross, 1996; Thissen et al., 1994; Thissen et al., 1999). In fasted fish, changes in GH/IGF-I axis hormones are part of an adaptive response; low IGF-I suppresses growth and high GH stimulates lipolysis, thereby directing available nutrients to the physiological functions necessary for survival while also gaining access to energy reserves. This response is important in

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survival in the natural environment, as most fish undergo periods of fasting throughout their life history, while others, such as salmonids, undergo prolonged periods of fasting while migrating long distances (Bar, 2014; Mommsen, 2004; Navarro and Gutierrez, 1995). Thus, plasma GH and IGF-I levels can be used as indicators of nutritional status.

The GH/IGF-I axis interacts with other physiological systems, particularly those that regulate life history transitions. In salmonids, GH and IGF-I increase during smoltification, the suite of physiological, morphological, and behavioral changes that occur before migration to saltwater (McCormick, 2012). The GH/IGF-I axis interacts with the reproductive endocrine axis in the regulation of the initiation of puberty and other aspects of reproductive development in teleosts (Taranger et al., 2010). For example, the decision in salmonids to mature is believed to be linked to growth and lipid reserves several months prior to developmental changes in the gonad (Larsen et al., 2006; Medeiros et al., 2018; Silverstein et al., 1998; Thorpe, 2007). This is likely a consequence of the fact that maturation depends on parameters related to growth and metabolism, such as size, energy reserves, and growth rate, at a specific stage ("critical period") several months prior to histological indications of gonadal development (Taranger et al., 2010; Thorpe, 2007; Thorpe et al., 1998). Thus, the GH/IGF-I axis plays a role in the major life history transitions that define salmonid life histories. Due to these interactions, as well as the role of the GH/IGF-I axis in the regulation of growth and metabolism, GH/IGF-I axis components show promise for use as indicators in fisheries and aquaculture studies. Plasma IGF-I is a well-established indicator of growth rate (Beckman, 2011; Perez-Sanchez et al., 2018; Picha et al., 2008; Pierce et al., 2001), although, as reviewed by Beckman (2011), the correlation between IGF-I and growth ranges from reliable to non-discernable, reflecting the effects of a range of endogenous and exogenous factors on the GH/IGF-I axis. The complex and changeable suite of IGFBPs present in fish plasma interferes in IGF-I assays, resulting in a requirement for an extraction procedure to remove IGFBPs before assay (Shimizu 1999, Shimizu 2000, Shimizu 2006, Plisetskaya, 1998). Although less explored, GH also shows promise as an indicator of catabolic status during the extended periods of reduced feeding and growth that occur throughout the life histories of many fish (Picha et al., 2008; Pierce et al., 2005).

Understanding the life histories, growth, and metabolic status of wild and cultured salmonids is important in the management of salmonid populations. Measurement of GH/IGF-I axis components can provide insight in all of these areas. Time-resolved fluorimmunoassays (TR-FIAs) for GH and IGF-I enable measurement of protein hormone levels while avoiding the safety, regulatory, and radiolabel decay issues associated with radioimmunoassays. However, to our knowledge, no TR-FIA has yet been established for salmonid GH, and no existing TR-FIA for detecting plasma GH in fish is composed of commercially available reagents (Fukuda et al., 2015). Moreover, current protocols for IGF-I TR-FIAs require beginning the assay directly after completing the binding protein extraction procedure (Small and Peterson, 2005; Hevroy et al., 2013), which complicates the workflow and introduces high levels of ethanol remaining from extraction into the TR-FIA, resulting in suboptimal binding conditions. Therefore, the aims of this study were to establish and validate TR-FIA assays to detect GH and IGF-I in salmonid plasma, and to modify IGFBP extraction protocols to remove ethanol and allow storage of samples between the extraction and assay procedures. In addition, existing IGF-I assay protocols were modified to increase sensitivity. Biological validation was provided by GH injection and fasting studies in rainbow trout.

2. Materials and methods

2.1. Fish

Rainbow trout (*Oncorhynchus mykiss*) used in this study were acquired from a breeding population at the Aquaculture Research Institute (ARI) at the University of Idaho (Moscow, ID, USA) and cared for according to the ARI's standard operating procedure as described by Medeiros et al. (2016). All fish rearing and sampling procedures followed guidelines approved by the Institutional Animal Care and Use Committee at the University of Idaho.

2.2. Peptide, hormone, and antibody sources

Recombinant barramundi (*Lates calcarifer*) and tuna (*Thunnus maccoyii*) IGF-I as well as recombinant black bream (*Acanthopagrus butcheri*) GH were purchased from GroPep (Adelaide, Australia). Polyclonal rabbit anti-barramundi (*L. calcarifer*) IGF-I and anti-salmon/trout (genus *Oncorhynchus*) GH were also purchased from GroPep (Adelaide, Australia). Custom recombinant rainbow trout GH (*gh1*, GenBank Accession no. **P09538.2**) and gilthead seabream (*Sparus aurata*) IGF-I were purchased from Prospec (New Brunswick, NJ). Both the IGF-I and GH antibodies have been used previously, and the specificity tested and confirmed (Dyer et al., 2004; Le Bail et al., 1991; Shimizu et al., 2000; Small and Peterson, 2005). Native bovine GH (bGH) was purchased from US Biologicals (Salem, MA).

2.3. Peptide and hormone labeling

Europium (Eu)-labeled GH and IGF-I (Eu-GH and Eu-IGF-I, respectively) were prepared by PerkinElmer (Waltham, MA) via labeling of recombinant barramundi IGF-I and recombinant rainbow trout GH with DELFIA® Eu-N1 ITC lanthanide chelate (Ref 1244–302, PerkinElmer, Waltham, MA). Initial labeling of IGF-I under conditions favoring only N-terminal attachment of the europium chelate failed; therefore, this condition was relaxed.

2.4. Pituitary homogenate

For demonstration of competitive binding, a crude pituitary homogenate from rainbow trout was prepared by homogenizing 10 whole pituitaries in 1 mL of 100 mM ammonium bicarbonate (pH 7.8) containing 1 mM phenylmethylsulfonyl fluoride on ice for 5 min. The solution was stirred at 4 °C for 1 h and then centrifuged at 20,000 × g at 4 °C for 30 min. The supernatant was transferred to a clean microcentrifuge tube and considered to be at a concentration of 1 ×.

2.5. TR-FIA for GH

The assay was performed in goat anti-rabbit-coated yellow 96-well plates (PerkinElmer, Waltham, MA) and run over the course of 4 days, with 3 overnight incubations. The standard curve consisted of 8 serially diluted points, ranging from 500 to 0.03 ng/mL, with 4-fold dilutions. On the first day, the 96-well plate was washed 5× with 300 μL of Delfia® wash solution (PerkinElmer, Waltham, MA) per well before adding Delfia® assay buffer (DAB; PerkinElmer, Waltham, MA) and 20 μL of the rabbit anti-salmon/trout GH polyclonal antiserum (diluted 1:5000; GroPep, Adelaide, Australia). The non-specific binding (NSB) wells received 165 μL of DAB while the maximum binding (B_0), standard curve, and unknown sample wells received 145 μL of DAB plus 20 μL of the primary antibody. The plate was then sealed and incubated for 24 h at 4 °C with constant slow shaking on an orbital plate shaker (VWR, Radnor, PA). On the second day, the plate was washed 5 × with 300 μL of Delfia® wash solution per well and the standard curve and unknown samples were plated. Both the NSB and B_0 wells received 165 μL of DAB. The standard curve was generated by adding 125 μL of a known recombinant barramundi IGF-I concentration and 40 μL of DAB. The unknown plasma samples were determined by adding 125 μL of plasma diluted as necessary in DAB and 40 μL of DAB. The standard curve was run in duplicate and unknowns in triplicate. The plate was then sealed and incubated for 24 h at 4 °C with constant slow shaking

on an orbital plate shaker. On day 3, the plate was not washed and 20 μL of Eu-GH (diluted to 25 ng/mL with DAB) was added to all wells except the blank and total count (TC) wells. The plate was then sealed and incubated for 18 h at 4 °C with constant slow shaking on an orbital plate shaker. On the fourth and final day, the plate was washed 5× with 300 μL of Delfia® wash solution per well. 200 μL of Delfia® enhancement solution was added to all wells, and 5 μL of the Eu-GH label (made up the previous day) was added to the TC wells. The plate was then sealed and incubated for 10 min at room temperature with moderate shaking on an orbital plate shaker, and the time resolved fluorescence was measured with a Victor™ X4 Multilabel Plate Reader (PerkinElmer, Waltham, USA) using the Europium program. Briefly, the well is pulsed 1000 times per second with an excitation light of 340 nm, in the period between flashes the sample fluorescence is measured (europium fluoresces at 615 nm) for 400 μs after a delay time of 400 μs. Assay calculations were conducted as follows, using average fluorescence counts for replicate wells. The value for the blank wells was subtracted from all wells, and then the NSB value was subtracted from remaining wells. The B_0 (maximum binding) was calculated and standards and unknowns *(B)* expressed as $\%B_0$ ($(B/B_0) \times 100$). The standard curve was generated by plotting $\%B_0$ against known IGF-I concentrations on a linear y-axis and a log x-axis and fitting a curve using a four-parameter-logistic equation. Unknown sample values were interpolated from the standard curve; samples with percent binding values above 80% and below 20% were re-assayed. The total counts (TC) value was multiplied by 4 in order to account for the difference in label volume added to the total count well versus what was added to the sample wells. Percent total counts was calculated as (B_0/TC) × 100. The minimum detection limit of the assay was calculated using the mean count of the zero standard minus two standard deviations, expressed as B/B_0, and used to interpolate a value from the standard curve (Shimizu et al., 2006).

2.6. TR-FIA for IGF-I

2.6.1. IGF-I acid-ethanol-cryoprecipitation extraction procedure

The procedure was modified from Daughaday et al. (1980) and Shimizu et al. (2000) to remove ethanol and enable storage of extracted samples. Briefly, 125 μL of blood plasma was acidified with acid-ethanol (87.5% 200 proof ethanol and 12.5% 2 N HCl, at a ratio of 1,4 v:v). The solution was thoroughly mixed and incubated at room temperature for 30 min. Following incubation, samples were centrifuged at 1860 × *g* for 30 min. The resulting supernatant was decanted into a fresh tube and neutralized with 0.855 M Tris base at a ratio of 5:2 v:v, respectively. The samples were again thoroughly mixed and incubated at −20 °C for one hour, and then centrifuged at 1860 × *g* for 30 min. The resulting supernatant was decanted into a fresh 1.5 mL microcentrifuge tube, the amount decanted was noted, and then the sample was dried under vacuum for 4 h. Samples were reconstituted in nanopure water at the same volume that was transferred following the final centrifugation, vortexed thoroughly, and incubated in a water bath set at 37 °C for 30 min. Samples used in the extraction comparison assays were made up from recombinant barramundi IGF-I. The extracted and dried down samples followed the protocol outlined above, whereas the extracted samples followed the protocol up until the final centrifugation, and then the supernatant was transferred to a fresh tube and assayed immediately. The AEB standard curve samples were made up in neutralized extraction buffer but not subject to the extraction procedure. To account for the dilution factor inherent to this extraction process, the standard curve was prepared at 7-times the normal concentration. In both extraction procedures, the entire standard curve was prepared and then extracted. The extracted samples were run in triplicate.

2.6.2. TR-FIA for IGF-I

The IGF-I time-resolved fluoroimmunoassay (TR-FIA) protocol was based on Small and Peterson (2005) and Hevroy et al. (2013) with modifications. The incubation timing, volumes, and time resolved fluorescence detection employed in the IGF-I assay were identical to those in the GH assay. The standard curve consisted of 8 serially diluted points, ranging from 400 to 0.024 ng/mL, with 4-fold dilutions. The rabbit anti-barramundi IGF-I polyclonal antiserum was diluted 1:1500 (GroPep, Adelaide, Australia). The Eu-IGF-I label was diluted to 50 ng/mL. Unknowns were extracted and dried down plasma reconstituted in nanopure water as described above and diluted as necessary in DAB. Assay calculations and interpolation of unknowns were conducted as described for the GH assay.

2.7. In vivo experiments

2.7.1. IP injection experiment

To determine if exogenous GH increases plasma IGF-I in rainbow trout, twenty fish were intraperitoneally (IP) injected with either vehicle or vehicle containing bGH. While under anesthetic, fish were IP injected with either 1 μL vehicle × g $fish^{-1}$ (vehicle fish [145.3 ± 13.2 g, $n = 10$]) or with vehicle plus bGH at a dose of 2.5 μg bGH × 1 μL × g $fish^{-1}$ (bGH-injected fish [140.7 ± 9.8 g, $n = 7$]). The vehicle was sterile-filtered 0.9% NaCl containing 0.1% BSA. At $t = 12$ h, fish were lethally sampled, and a blood sample collected (see section 2.8 Sampling Procedures below). Fish were held in 595 L circular tanks under a simulated normal photoperiod. Tanks were supplied with 17–18 °C well water by a recirculating system that was composed of a bubble bead mechanical and biological filter and u-shaped settling basin. Flow rate was approximately 11 L min^{-1}. Fish were fasted for the duration of the experiment.

2.7.2. Fasting experiment

An experiment was conducted to assess the effects of fasting on the GH/IGF-I axis in rainbow trout. For the duration of the experiment, fish were held in 1130 L circular tanks under a simulated normal photoperiod. Tanks were supplied with 10 °C water by a recirculating system that was composed of a chiller, settling pool, sand filter, a UV treatment, and a biological filter. Flow rate was approximately 14 L min^{-1}. Fish were fed to satiation 5 days a week with a standard commercial trout diet (5.5 mm pellets; Skretting USA, Tooele, UT). At the onset of the experiment, 22 fish with an average mass of 686.3 ± 25.6 g were split into two groups ($n = 11$ per treatment). The control group (Fed) continued to be fed as described above, while the other group (Fasted) was fasted for the duration of the experiment. Fish were individually identified with passive integrated transponder (PIT) tags inserted into the body cavity (Biomark, Inc., Boise, ID). On March 20th, 2019 (4 weeks after the start of the experiment), all fish were lethally sampled (see Sampling Procedures below).

2.8. Sampling procedures

Lethal sampling consisted of recording (1) the PIT tag code, (2) total body weight and length and (3) collecting a blood sample. Briefly, fish were anesthetised in MS-222 (0.1 g l^{-1}; Western Chemical, Ferndale, WA) and blood sampled from caudal vessels using a 23G needle attached to a 1 mL syringe coated with heparin (10 mg/mL; Sigma-Aldrich, St. Louis, MO). Blood samples were centrifuged at 10,000 × g for 10 min, the plasma aspirated and frozen immediately on dry ice. Plasma samples were stored at −80 °C until laboratory analysis.

2.9. Statistical analyses

The experimental data were analyzed using Prism 8.2 (GraphPad Software, Inc., San Diego, CA). Plasma hormone concentrations were subjected to unpaired *t*-tests. Plasma parallelism was objectively evaluated by comparing hillslope values between individual curves and the standard curve for the respective assay using nonlinear regression

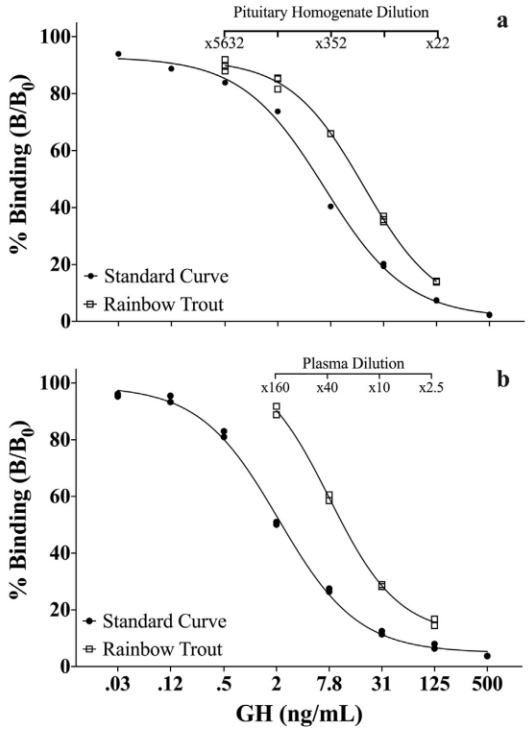

Fig. 1. Displacement curves for recombinant rainbow trout GH assay standards, serially diluted rainbow trout pituitary homogenate (a), and serially diluted rainbow trout blood plasma (b). Serial dilutions of pituitary homogenate and blood plasma from rainbow trout were parallel to the standard curve (p = .67 and 0.43, respectively).

analysis evaluated over the usable portion (20 to 80% of maximum binding) of the standard curve. For all analyses, the level of significance was evaluated at $P \leq .05$, and values are expressed as the average ± the standard error of the mean (S.E.M.).

3. Results

Eu-GH was labeled at a ratio of 7.6 europium chelate molecules per molecule of GH protein. At the label concentration employed, this gave approximately 120,000 counts per 20 µL. The primary antibody bound approximately 20% of the label in the B_0 wells at the concentration employed. Specific binding of the europium-labeled GH was displaced by increasing amounts of unlabeled GH (Fig. 1). Serial dilutions of pituitary homogenate (Fig. 1a) and blood plasma (Fig. 1b) from rainbow trout were parallel to the standard curve (p = .67 and 0.43, respectively). The ED_{80} and ED_{20} were 0.58 ± 0.06 ng/mL (n = 8) and 28.1 ± 1.1 ng/mL (n = 8), respectively. The minimum detection limit of the assay was 0.22 ± 0.08 ng/mL (n = 8). The intra- and inter-assay coefficients of variation were 4.1% and 13.4%, respectively (n = 23). Interspecies specificity of the primary antibody was tested by running a serial dilution of recombinant black bream GH, which was found to be not parallel to the standard curve (data not shown; $p < .05$). During development and validation, it was discovered that the assay required at least a 2-fold dilution of plasma prior to assay. This was deduced from observations that plasma run neat yielded %B below 20%, but when the same sample was diluted 2- to 10-fold the

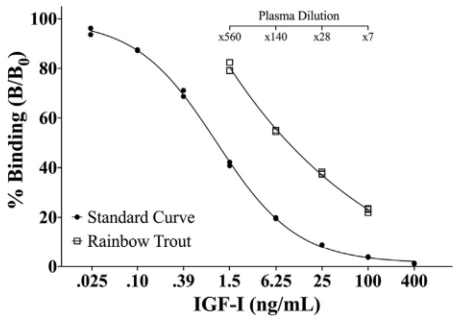

Fig. 2. Displacement curves for recombinant barramundi IGF-I standards and serially diluted rainbow trout blood plasma. Serial dilutions of blood plasma from rainbow trout were parallel to the standard curve (Fig. 2; p = .83).

results implied that the neat value should have been within the 20 to 80% binding range.

Eu-IGF-I was labeled at a ratio of 2.3 europium chelate molecules per molecule of IGF-I protein. At the label concentration employed in this study, this resulted in approximately 95,000 counts per 20 µL. The primary antibody bound approximately 25% of the label in the B_0 wells at the primary antibody concentration employed. Specific binding of the europium-labeled IGF-I was displaced by increasing amounts of unlabeled IGF-I and serial dilutions of blood plasma from rainbow trout were parallel to the standard curve (Fig. 2; p = .83). The ED_{80} and ED_{20} were 0.23 ± 0.02 ng/mL (n = 8) and 6.5 ± 0.4 ng/mL (n = 8), respectively. The minimum detection limit of the assay was 0.028 ± 0.008 ng/mL (n = 8). The intra- and inter-assay coefficients of variation were 3.0% and 6.5%, respectively (n = 7). Interspecies specificity of the primary antibody was further tested by running serial dilutions of recombinant tuna and gilthead seabream IGF-I, which were found to be parallel to the standard curve (data not shown; p = .22 and 0.97, respectively).

Maximum binding was reduced from near 100% to approximately 80% of B_0 in extracted standard curves or curves made up in neutralized acid/ethanol buffer versus the neat standard curve (Fig. 3; top parameter values from curve fit equation were significantly different,

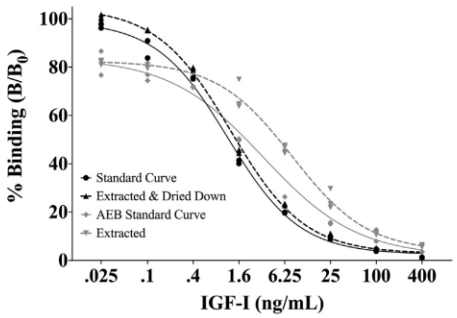

Fig. 3. Displacement curves for recombinant barramundi IGF-I standards that have been extracted and run using the extraction and TR-FIA procedures outlined in this study (black symbols and lines) compared to the displacement curves for recombinant barramundi IGF-I standards that have been extracted and run using the unmodified extraction protocol (gray symbols and lines), which does not remove the ethanol from the assay. Maximum binding was significantly reduced in curves containing neutralized acid/ethanol buffer when compared to curves produced using the modified extraction protocol (top parameter values from curve fit equation were significantly different, $p < .05$).

$p < .05$). Increased variability between replicates was also evident in curves containing extraction buffers. A similar reduction in percent B_0 and increase in variability between replicates was found when ethanol at the final concentration in neutralized extract (50%) was added to B_0 wells. Additionally, the reduction in percent B_0 and increase in variability decreased with decreasing ethanol concentration (data not shown). The reduction in percent B_0 and increased variability were eliminated when the standard curve was dried down after extraction and reconstituted. Curves reconstituted in ddH_20 were more similar to the neat standard curve than when reconstituted in DAB (data not shown). Based on a comparison of the extracted and dried down versus neat standard curves, the recovery of IGF-I after extraction, drying down, and reconstitution was 80% (based on values interpolated from 20, 50, and 80% binding), and recovery was similar across the working range of the assay. Based on these results, samples were dried down and resuspended in ddH20 after extraction before being run in the TR-FIA IGF-I assay.

Using the TR-FIA, the response of circulating IGF-I to IP injection with bGH was examined in rainbow trout. Twelve hours post-injection, fish injected with bGH experienced a nearly two-fold increase in plasma IGF-I levels when compared to fish injected with vehicle alone ($p < .01$, Fig. 4).

Following a four-week period of fasting, fasted fish weighed significantly less than the fed treatment group ($p < .001$), which corresponded to a 12.5% decrease in weight compared to their initial weight versus the 14.2% increase that the fed group experienced (Fig. 5). This expected decrease in weight was accompanied by a significant increase in plasma GH levels ($p < .01$, Fig. 6a) and a significant decrease in plasma IGF-I levels ($p < .01$, Fig. 6b).

4. Discussion

The present study developed and validated a TR-FIA for rainbow trout GH. This study also established modifications to the standard IGF binding protein extraction procedure, and validated modifications to an existing TR-FIA IGF-I for use in rainbow trout, resulting in an improved assay and more efficient workflow. The assays, including the modification to the extraction procedure, are intended to target the physiological range for plasma GH and IGF-I observed in salmonid species. As with other TR-FIAs, these assays provide a cost-effective, convenient alternative to radioimmunoassays (RIAs) that provide comparable sensitivity. Additionally, as the recombinant rainbow trout GH is available for purchase through Prospec, all components of the TR-FIAs are commercially available to any interested party with the laboratory resources to perform TR-FIAs. Availability of these assays will enable investigation of the role of the GH/IGF-I axis in studies on the life history, growth, reproduction, and response to environmental stressors

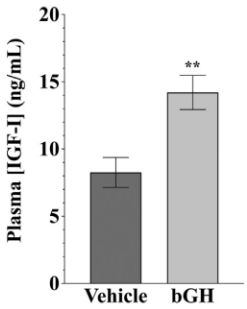

Fig. 4. Effect of vehicle alone or vehicle with bGH on plasma IGF-I levels 12 h post-injection. Values are expressed as means ± SEM ($n = 10$ and 7, respectively). Asterisks indicate significant differences ($p < .01$) between treatments.

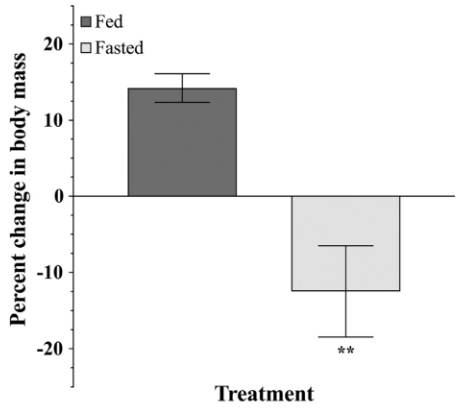

Fig. 5. Percent change in body mass of rainbow trout fasted for 4 weeks (mean ± SEM; $n = 11$). Fasting had a significant ($p < .01$) effect on body mass.

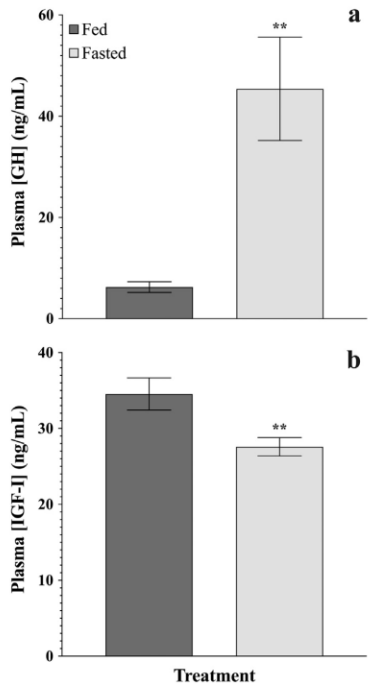

Fig. 6. Effect of fasting rainbow trout for 4 weeks on plasma GH (a) and IGF-I (b). Values are expressed as means ± SEM ($n = 11$). Asterisks indicate significant differences ($p < .01$) between treatments.

in rainbow trout and other salmonids, which are species of cultural, conservation, economic, and recreational importance.

The GH utilized in this study was a custom recombinant hormone constructed based on the rainbow trout gh1 sequence, that demonstrated similar displacement to native GHs employed by other studies (Le Bail et al., 1991; Wilkinson et al., 2006). This implies that the labeling process does not appear to have affected its ability to bind to the

antiserum. Plasma parallelism tests indicate that the GH TR-FIA can be used to detect GH in rainbow trout blood plasma, but it does require a small dilution. This is probably the result of plasma matrix interference due to the high ratio of sample to total well volume (125 μL in 165 μL total) added on the second day of the assay and remaining after addition of the label (20 μL) on the third day. This study found that a 2-fold dilution was enough to ameliorate the effects of the matrix interference in adult rainbow trout. A dilution series is therefore recommended for future studies to prevent matrix interference. The sensitivity of the GH TR-FIA was sufficient to measure plasma GH levels in rainbow trout after the 2-fold dilution, and was similar to the sensitivity of RIAs (Le Bail et al., 1991; Wilkinson et al., 2006) taking the dilution into account. Considering the quantification limits of GH RIAs validated for use in salmonids, this GH TR-FIA provides a safe, convenient, and cost-comparable alternative to traditional RIAs.

Specific binding proteins have been identified and characterized for both GH (GHBP) and IGF-I, which modulate their physiological effects (Duan and Xu, 2005; Wood et al., 2005). More information exists regarding the function and regulation of IGFBPs (reviewed by Duan and Xu, 2005) than that for GHBPs (Baumann, 2002; Fisker, 2006). The vast majority (approximately 99.7%) of IGF-I circulates bound to high affinity IGFBPs, which interfere in IGF-I assays, thus plasma IGF-I should not be quantified without first removing IGFBPs with a specific extraction procedure (Shimizu et al., 1999; Shimizu et al., 2000). In contrast, extraction procedures for GHBPs are not employed in salmonid GH assays due to lower amounts of GH bound to specific GHBPs (Bjornsson et al., 1994; Le Bail et al., 1991; Sohm et al., 1998; Wilkinson et al., 2006). In the present study, it was determined that the ethanol remaining from the standard acid-ethanol cryoprecipitation (AEC) IGFBP extraction method developed by Shimizu et al. (2000) reduced maximum binding to approximately 80% of B_0 and increased variability between replicates in the IGF-I TR-FIA. The effect of ethanol on the IGF-I TR-FIA is likely worse than in IGF-I RIAs because the proportion of extracted sample in the reaction volume is higher (125 of 165 μL in TR-FIA vs 10 of 160 μL or 36 of 350 μL in RIA). Therefore, ethanol was removed by drying down and reconstituting samples, which restored binding and reduced variability. This modification has the additional benefits of separating the days on which the samples are extracted and run in the TR-FIA (since samples in AEC extraction buffers cannot be stored) and enables concentration of samples.

In the current study, IGF-I extraction efficiency (including drying down and reconstitution) was calculated by interpolating the values at 20, 50, and 80% binding from an unextracted standard curve and also from a standard curve that had been extracted, dried down, and reconstituted, and then comparing the values. The extraction efficiency was found to be between 78 and 80% at all three points, implying that the loss is not concentration dependent. The loss of immunoreactive IGF-I is likely due to protein damage from exposure to low pH and ethanol during the extraction procedure, adsorption to surfaces, and precipitation in the pellet. The loss is probably less in fish plasma samples due to the protective nature of the plasma matrix, and thus the 80% value represents a minimal extraction efficiency. Fish IGF-I RIA and TR-FIA procedures do not include correction for extraction efficiency because this factor in fish plasma is not known (Ferriss et al., 2014; Hevroy et al., 2013; Shimizu et al., 2000; Small and Peterson, 2005). To provide results comparable to existing studies, we do not propose to correct IGF-I values measured with the present IGF-I extraction procedures and TR-RIA for extraction efficiency.

Extracted and reconstituted rainbow trout blood plasma and reconstituted recombinant tuna and sea bream IGF-I diluted in parallel with the IGF-I standard curve in the TR-FIA. This indicates that the assay is valid for use in rainbow trout and suggests that it will work in other fish species as well. These results were expected based on previous assays utilizing the same antibody (Dyer et al., 2004; Shimizu et al., 2000; Small and Peterson, 2005). Initial trials followed an established IGF-I TR-FIA protocol with addition of the primary antibody, standards/unknowns, and label on the first day, followed by a single overnight incubation (Small and Peterson, 2005). However, this was found to result in poor assay sensitivity (data not shown). Simultaneous incubation of the main assay reactants could result in loss of standards/unknowns and label in the following wash step. Therefore, the addition of reagents was separated, with overnight incubations between each step, similar to RIA protocols. This modification decreased the minimum detectable level by approximately 7-fold compared to other TR-FIAs for IGF-I. So, while the present TR-FIA requires a longer timeframe to complete it is provides improved sensitivity compared to other TR-FIAs and a reliably accurate, highly sensitive, convenient, and cost-effective alternative to RIA for IGF-I.

Biological validation of the IGF-I TR-FIA and the modifications to the IGFBP extraction procedure was provided by a GH injection study using rainbow trout. GH is a major positive regulator of liver IGF-I production, and increases circulating IGF-I under fed conditions (Bergan-Roller and Sheridan, 2018; Moriyama et al., 2000; Reindl and Sheridan, 2012; Wood et al., 2005). A sample collected 12 h post-injection confirmed that bGH elicited a significant increase in circulating levels of IGF-I, in agreement with previous studies in rainbow trout (Biga et al., 2005). This result confirms that the IGFBP extraction procedure and IGF-I assay can measure physiologically relevant changes in plasma IGF-I in rainbow trout. Biological validation of both assays and the extraction procedure was provided by a fasting study. Although GH stimulates liver IGF-I production and increases circulating IGF-I in fed animals, the situation is different during fasting. In general, for fish and mammals alike, fasting increases blood plasma GH (Bjornsson et al., 2018; Duan and Plisetskaya, 1993; Pierce et al., 2005; Small and Peterson, 2005) while at the same time blood plasma IGF-I is reduced (Bjornsson et al., 2018; Cohick and Clemmons, 1994; Moriyama et al., 1994; Pierce et al., 2001; Pierce et al., 2005). To determine whether these changes could be detected using the new assays, we conducted a 4-week fasting experiment using mature rainbow trout. As expected, over the course of the 4-week period, the body mass of the control fish increased whereas fasted fish experienced a decrease in body mass. After 4 weeks of fasting plasma GH was significantly increased and plasma IGF-I significantly reduced compared to controls. This provides evidence that physiologically relevant changes in plasma GH and IGF-I can be measured using these assays and the extraction procedure.

Although expected, the results of the present study add to the body of evidence on regulation of the GH/IGF-I axis in fishes. The increase in plasma IGF-I after GH injection adds to the many studies showing that GH is the major positive regulator of circulating IGF-I in the fed state. However, during fasting, a metabolic state termed acquired GH resistance develops, in which the liver becomes resistant to stimulation of IGF-I production by GH (Kelley et al., 2000). GH resistance appears to be due to both liver receptor downregulation and inhibition of post-receptor signaling pathways (Gray et al., 1990; Perez-Sanchez et al., 1995; Perez-Sanchez et al., 2018; Thissen et al., 1999). The resulting decrease in circulating IGF-I is thought to lead to increases in pituitary GH secretion via relaxation of negative feedback (Blaise et al., 1995; Fruchtman et al., 2000; Perez-Sanchez et al., 1992; Yamashita and Melmed, 1986). The pattern of increased GH and reduced IGF-I in response to fasting found in the present study is consistent with this model. This is believed to be an adaptive response of GH to nutritional conditions requiring catabolism of body tissues to support metabolism; GH's growth-promoting role is turned off and its lipolytic, energy mobilizing role is turned on (Bergan-Roller and Sheridan, 2018; Perez-Sanchez et al., 2018; Reindl and Sheridan, 2012).

Because of the central importance of the GH/IGF-I axis in both growth and energy mobilization, GH/IGF-I axis components can be used as indicators of growth and nutritional or metabolic status in fisheries and aquaculture studies. In addition, the GH/IGF-I axis interacts with other physiological systems, particularly those that regulate life history transitions linked to growth. Thus, quantification of plasma GH and IGF-I levels provides considerable insight into the physiological

state of individual fish, which can be used in studies on the conservation and management of fish populations.

Declaration of Competing Interest

None.

Acknowledgements

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Presentations 2019:

Hatch DR, Pierce AL, Branstetter R, Fast D, Bosch WJ, Blodgett J, Everett SR, and Graham ND*. Steelhead kelt reconditioning and reproductive success studies in the Columbia River Basin. Aquaculture 2019 Triennial Meeting, New Orleans, Louisiana 3/7/2019-3/11/2019.

Hatch DR., Fast D., Branstetter R*., Blodgett J., Bosch B., Pierce A., Hyun S-Y., Fiander B., Penney Z., and Everett S. Transport and Release of Yakima and Snake Origin Steelhead Kelts to the Lower Columbia River. Yakima Basin Science and Management Conference, Ellensburg, WA, 6/12/2019-6/13/2019.

Hatch DR*, Pierce AL, Branstetter R, Fast D, Bosch WJ, Blodgett J, Everett SR. Steelhead kelt reconditioning and reproductive success studies in the Columbia River Basin. 149TH Annual Meeting and Joint Conference with the Wildlife Society in Reno, Nevada, 09/29/2019-10/3/2019.

Jenkins LE*, Pierce AL, Graham ND, Medeiros LR, Branstetter R, Hatch DR, and Nagler JJ. Recovery from spawning, rematuration, and reproductive performance in repeat spawning reconditioned female steelhead. Aquaculture 2019 Triennial Meeting, New Orleans, Louisiana 3/7/2019-3/11/2019.

Medeiros LR* and Pierce AL. Establishment and biological validation of non-ratioactive assays for plasma growth hormone (GH) and insulin-like growth factor-1 (IGF1) in rainbow trout. Aquaculture 2019 Triennial Meeting, New Orleans, Louisiana 3/7/2019-3/11/2019.

Pierce AL*, Hatch DR, Jenkins LE, Graham ND, Medeiros LR, Caldwell LK, and Nagler JJ. Reproductive development in reconditioned female steelhead kelts: insights into consecutive and skip spawning. Aquaculture 2019 Triennial Meeting, New Orleans, Louisiana 3/7/2019-3/11/2019.

Pierce AL* et al. Factors influencing survival and consecutive rematuration in steelhead kelts. Yakima Basin Science and Management Conference, Ellensburg, WA 6/12/2019-6/13/2019.

Stephenson J., Fast D., Bosch B., Blodgett J., Branstetter R.*, Pierce A., Narum S., and Hatch D. Reproductive Success of Artificially Reconditioned Kelt Steelhead in the Yakima River. Yakima Basin Science and Management Conference, Ellensburg, WA, 6/12/2019-6/13/2019.

Presentations 2020

Pierce AL. Reproductive decisions in salmonids: skip spawners and minijacks. CRITFC Brown Bag Seminars, Portland, OR, July15, 2020.

A.3: List of Metrics and Indicators

Data Collection Methods

Air Spawning: https://www.monitoringresources.org/Document/Method/Details/5343
Coloration Rating: https://www.monitoringresources.org/Document/Method/Details/5302
Determining Adult Anadromous Salmonid Gender:
https://www.monitoringresources.org/Document/Method/Details/1429
Determining Sex of Adult Steelhead:
https://www.monitoringresources.org/Document/Method/Details/5334
DNA Extraction Kit Protocol:
https://www.monitoringresources.org/Document/Method/Details/1353
Downloading Data from PTAGIS:
https://www.monitoringresources.org/Document/Method/Details/4095
Electrofishing- Determine Electrofisher Settings:
https://www.monitoringresources.org/Document/Method/Details/115
Electrofishing - Fish Processing and Recovery:
https://www.monitoringresources.org/Document/Method/Details/117
Estimating Lipid Content in Muscle Tissue of Adult Salmonids:
https://www.monitoringresources.org/Document/Method/Details/4215
Extracting Fish Plasma to Measure Reproductive Development:
https://www.monitoringresources.org/Document/Method/Details/4239
Fish Wet Weight: https://www.monitoringresources.org/Document/Method/Details/1734
Genetic Sampling and Storage Using Chromatography Filter:
https://www.monitoringresources.org/Document/Method/Details/4087
Identifying Marks/Tags on Fish:
https://www.monitoringresources.org/Document/Method/Details/342
Identifying Steelhead Kelts:
https://www.monitoringresources.org/Document/Method/Details/5310
Measuring Fish Length- Fork length:
https://www.monitoringresources.org/Document/Method/Details/4041
Measuring Fish Length- Mid-orbital hypural length:
https://www.monitoringresources.org/Document/Method/Details/1549
Modified Assessment of Fish Condition:
https://www.monitoringresources.org/Document/CustomizedMethod/Details/22915
Modified Off Ladder Adult Trapping Procedures:
https://www.monitoringresources.org/Document/CustomizedMethod/Details/31061
Modified PIT Tag Marking Procedures:
https://www.monitoringresources.org/Document/CustomizedMethod/Details/22818
O. mykiss and O. tshawytscha SNP Marker Sets for PBT and GSI Use in the Columbia River Basin:
https://www.monitoringresources.org/Document/Method/Details/1356
SNP Genotyping on Fluidigm Platform:
https://www.monitoringresources.org/Document/Method/Details/1332

Tissue Sampling for Parentage Based Tagging:
https://www.monitoringresources.org/Document/Method/Details/1432
Tissue Sampling of Salmonids in Nature for Genetic Analysis:
https://www.monitoringresources.org/Document/Method/Details/933
Weights: Green Egg Mass
https://www.monitoringresources.org/Document/Method/Details/1457

Weights: Sampling for Green Egg Fecundity Estimate
https://www.monitoringresources.org/Document/Method/Details/1458

Data Analysis/Interpretation Methods

Assessing Genetic Population Structure Using Bayesian Clustering Methods
https://www.monitoringresources.org/Document/Method/Details/1351

Calculating a Mean, Variance and Standard Deviation
https://www.monitoringresources.org/Document/Method/Details/4245

Estimating Relative Reproductive Success (RRS)
https://www.monitoringresources.org/Document/Method/Details/696

Estradiol Assay of Fish Plasma Samples
https://www.monitoringresources.org/Document/Method/Details/5320

Fulton's Fish Condition Factor
https://www.monitoringresources.org/Document/Method/Details/952

Genetic Assignment Tests Using GeneClass2
https://www.monitoringresources.org/Document/Method/Details/487

Genetic Stock Mixture Analysis Using the Software Program BAYES
https://www.monitoringresources.org/Document/Method/Details/488

Modified Analysis of Variance Models
https://www.monitoringresources.org/Document/CustomizedMethod/Details/22904

Modified Binary Logistic Regression of Multi-Year Monitoring Data
https://www.monitoringresources.org/Document/CustomizedMethod/Details/31060

Modified Calculating the smolt to adult return rate (SAR)

https://www.monitoringresources.org/Document/CustomizedMethod/Details/22932

Modified Growth Rate for Individual Fish
https://www.monitoringresources.org/Document/CustomizedMethod/Details/31057

Modified Integrated Status and Effectiveness Monitoring Program Salmonid Life Cycle Modeling
https://www.monitoringresources.org/Document/CustomizedMethod/Details/31063

Monitor Survival and Mortality Rates of Fish
https://www.monitoringresources.org/Document/Method/Details/3992

Parentage Analysis Using Cervus
https://www.monitoringresources.org/Document/Method/Details/1430

Predicting the Accuracy of Genetic Stock Identification
https://www.monitoringresources.org/Document/Method/Details/1346

Solvent Extraction of Plasma Samples
https://www.monitoringresources.org/Document/Method/Details/5319

Tests of Significance: T-Test
https://www.monitoringresources.org/Document/Method/Details/5291

Vitellogenin Assay of Fish Plasma Samples
https://www.monitoringresources.org/Document/Method/Details/5335

Metrics

Title	Category	Subcategory	Subcategory Focus 1	Subcategory Focus 2
"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
"Maturation rate"	Fish	Condition of Life Stage: Fish (ID: 57)	Fish Life Stage: Adult - Returner	NA
"Reconditioned kelt condition"	Fish	Condition of Life Stage: Fish (ID: 57)	Fish Life Stage: Adult Fish	NA

"Kelt homing"	Fish	Distribution of Fish Species (ID: 62)	Fish Life Stage: RANGE: Adult to Adult	NA
"Fecundity"	Fish	Fecundity: Fish (ID: 68)	NA	NA
"Growth rate"	Fish	Growth Rate: Fish (ID: 73)	Fish Life Stage: RANGE: Juvenile to Adult	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
"Lipid content"	Fish	Lipid Content (ID: 200)	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
"Reconditioned kelt survival rate"	Fish	Survival Rate: Fish (ID: 99)	Fish Life Stage: Adult - Outmigrant	Fish Origin: Both
"Natural kelt survival rate"	Fish	Survival Rate: Fish (ID: 99)	Fish Life Stage: Adult - Outmigrant	Fish Origin: Both
"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

Indicators

Title	Category	Subcategory	Subcategory Focus 1
"Relative reproductive success of artificially reconditioned kelt steelhead"	Fish	Relative Reproductive Success (RRS) (ID: 88)	Fish Origin: Natural