Kelt Reconditioning and Reproductive Success Evaluation Research

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Abstract

The Kelt Reconditioning and Reproductive Success Evaluation Project is a research, monitoring, and evaluation (RM&E) uncertainties category project funded through the 2008 Columbia Basin Fish Accords. The objectives are to evaluate methodologies to produce viable artificially reconditioned repeat steelhead spawners and to determine the productivity of repeat spawners. Work occurs in both the Yakima and Snake river basins. We focused on collecting steelhead kelts at juvenile bypass facilities at both Prosser and Lower Granite dams. These kelts were reconditioned (given prophylactic treatments and fed a specially formulated diet) at Prosser, Nez Perce Tribal, and Dworshak National Fish hatcheries. Survival of long-term reconditioned kelts has been 43% (20 years) at Prosser Hatchery and 40% (9 years; 47% over the last 7 years) for mixed stock collections from Lower Granite Dam and reconditioned at Nez Perce Tribal and Dworshak National Fish hatcheries. In 2019, unmarked upstream "wild" migrant adult steelhead return counts were at the 8th lowest across the region since records were kept in this regard since the mid 1990's. These low return years typically translate into lower abundance of kelts but also means that successful capture/reconditioning of kelts has a bigger contribution towards improving low population returns. We were still able to collect more kelts at both the Snake and Yakima rivers in 2019 likely due to larger numbers of steelhead migrants which means more available kelts in the both subbasins. A total of 40 reconditioned B-run steelhead were released below Lower Granite Dam in 2019 to address Reasonable and Prudent Alternative 33 of the 2008 FCRSP Biological Opinion. A total of 145 reconditioned, remature steelhead were released in the Yakima River in 2019. We have observed that consecutive spawners comprise 59% of the kelt population in the Yakima River, 60% in the Methow River and 25%in the Snake River. Likely this is a result of the bio-energetic expenditures of longer migration. Skip spawners in the Snake River are held an extra 12 months relative to consecutive spawners with excellent results. Survival during the final 12 months of reconditioning averages 70%. At Prosser Hatchery well water used in the kelt tanks is much warmer than river water in the winter and results in poor survival (23%) of skip spawners. At Prosser Hatchery well water used in the kelt tanks is much warmer than river water in the winter and results in poor survival of skip spawners. We are continuing to work on ways to improve survival of skip spawners at this location. In 2019, we began an experiment to transport skip spawners in the fall and release them below Bonneville Dam. On November 1, 2019, we trucked and released 103 skip spawners from Prosser Hatchery. We will monitor movement of these fish with PIT detections at fishways. Collaborative work continues with the Yakama Nation VSP study to establish baseline plasma hormone levels of maiden fish and compare with reconditioned kelts in terms of maturation status, migration success, homing fidelity, spawn timing, and genetic stock index (GSI) structure in the basin. Reproductive success of reconditioned steelhead was confirmed in the Yakima River tributaries of Satus and Toppenish creeks once again, with assignments (1029 samples from Satus and 598 samples from Toppenish in 2018) which are currently being tabulated and will be reported in our 2019 Annual Report to BPA. Based on cumulative sampling from 2013- 2018, lifetime reproductive success for female reconditioned kelt steelhead is estimated as 2.57 relative to single time spawning steelhead. Using estradiol assays, we have established that steelhead rematuration rates vary annually and spatially. To understand the maturation trigger system, we tested fasting of steelhead kelts during the first 10 weeks after spawning, which did not result in significantly reduced maturation rate in this study. This supports that the critical period for the rematuration decision (physiological pathway) occurs before spawning in steelhead kelts. We continued to refine our plasma assays that detect IGF-I and GH concentrations that we utilize for evaluating kelt maturation. The results are currently in draft form from this experiment and we anticipate publishing sometime in 2020. From 2008 to 2018 we have detected conclusive evidence of 373 kelts showing repeat homing in the Yakima basin. We finalized an MOU with BPA, the Nez Perce Tribe and CRITFC to guide the process of designing and constructing a dedicated kelt reconditioning facility in the Snake River. The CRITFC and its member tribes steelhead kelt reconditioning program continues to forward the science and inform the management of iteroparous *O.mykiss* in the Columbia River Basin. An extensive list of our work will be compiled in the Adaptive Management and Lessons Learned section of this report. The CRITFC team successfully published 2 papers in professional journals and gave 7 presentations at fisheries science and management conferences in 2019, a list is available in the appendices.

Acknowledgments

We would like to thank our partners at the Upper Yakima Kelt Reconditioning Program, Tom Scribner, Keely Murdoch, and Matt Abrahamse for sharing data and sampling with our team. Many thanks to the Lower Granite Dam capture and sampling crews (Nez Perce Tribe, Idaho Department of Fish and Game (IDFG), University of Idaho, and Army Corps of Engineers Walla Walla District). Thank you to Stephanie Harmon and Megan Moore for diligent and reliable laboratory processing and collection of genotypic data. Data and information provided by Mike Ackerman and Craig Steele

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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 14,4332 PIT tagged fish at Lower Granite Dam that were tagged by Nez Perce Tribe from 2009-2019.

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. Since we began 2019 with the intent of focusing collections and reconditioning in the Snake basin on b-run fish, we are considering 2019 a transition year and will continue to report on the impact the kelt reconditioning had on these specific populations in that basin. Future reports will still consider the a/b- run components but will likely focus on Major Population Groups or MPG in the basin as this is what is recognized as the population groupings under the Endangered Species Act listings that are targeted for mitigation/restoration actions.

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

1. Establish methods to assess how kelt reconditioning may benefit population growth, abundance, spatial structure and diversity;
2. Clarify how many juvenile and F1 adults should be sampled to detect meaningful differences in the breeding and reproductive success of HOR, NOR, and reconditioned NOR females;
3. Develop and implement methods to assess the fat levels, maturation timing, fecundity, egg size, and gamete viability of reconditioned kelts;
4. Monitor homing and straying rates of reconditioned kelts; and,
5. Experiments are needed to discover the best geographic locations and times of the year for release of the project's reconditioned fish.

We are organizing our report with 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. The previously mentioned and other historic collection sites are reported in Table (1) and Figure (1). Generally, downstream moving kelts are captured in the juvenile bypass facilities such is the case at CJMF and LGR facilities or captured via weir-trap box in the case of Fish, Omak, and Shitike creeks, while maiden steelhead were captured in upstream traps at DNFH, Powerdale Dam, and the East Fork Hood River weir and air-spawned. The collections at DNFH, Powerdale Dam and the East Fork Hood River typically occur in January-March, while collection at the remaining sites (CJMF, LGR, Fish Creek, and Omak Creek) occur(ed) in the spring (late-March through early-June). With the exceptions of CJMF and DNFH all kelts are truck transported to reconditioning facilities. Releases occur currently at near Prosser just below Prosser Dam into the Yakima River and into the Snake River just below Lower Granite Dam. Prior releases have been conducted in the Lower Columbia (rkm 135) and Okanogan rivers (confluence of Columbia

and Okanogan), and also into Shitike Creek near Warm Springs, OR. For a more thorough description of both the current and prior collection, reconditioning, and release sites see Hatch et al. 2015, Hatch et al. 2013, Hatch et al. 2012, and Branstetter et al. 2008.

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

Site Number	Site	Drainage	Location	Collection site	Reconditioning site	Release Site	Juvenile Sampling Location	Dates of use
1	Chandler Juvenile Monitoring Facility (CJMF)	Yakima River	RKM 75.6	Yes	-	-	-	1999-2019
2	Yakama Nation Prosser Fish Hatchery	Yakima River	RKM 75.6	-	Yes	Yes	-	1999-2019
3	Lower Granite Dam Juvenile Bypass	Snake River	RKM 173	Yes	-	Yes	-	2009-2019
4	Dworshak National Fish Hatchery	Clearwater River	RKM 65	Yes (hatchery fish for experimental purposes)	Yes	-	-	2009-2019
5	Nez Perce Tribal Fish Hatchery	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 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	2008-2019

22	Toppenish Creek	Yakima River		-	-	-	Yes	2008-2019
23	Simcoe Creek	Yakima River		-	-	-	Yes	2008-2019
24	Ahtanum Creek	Yakima River		-	-	-	Yes	2008-2016
25	Big Creek	Yakima River		-	-	-	Yes	2008-2016
26	Cowiche Creek	Yakima River		-	-	-	Yes	2008-2016
27	Little Rattlesnake Creek	Yakima River		-	-	-	Yes	2008-2016
28	Nile Creek	Yakima River		-	-	-	Yes	2008-2016
29	Quartz Creek	Yakima River		-	-	-	Yes	2008-2016
30	Bumping River	Yakima River		-	-	-	Yes	2008-2016

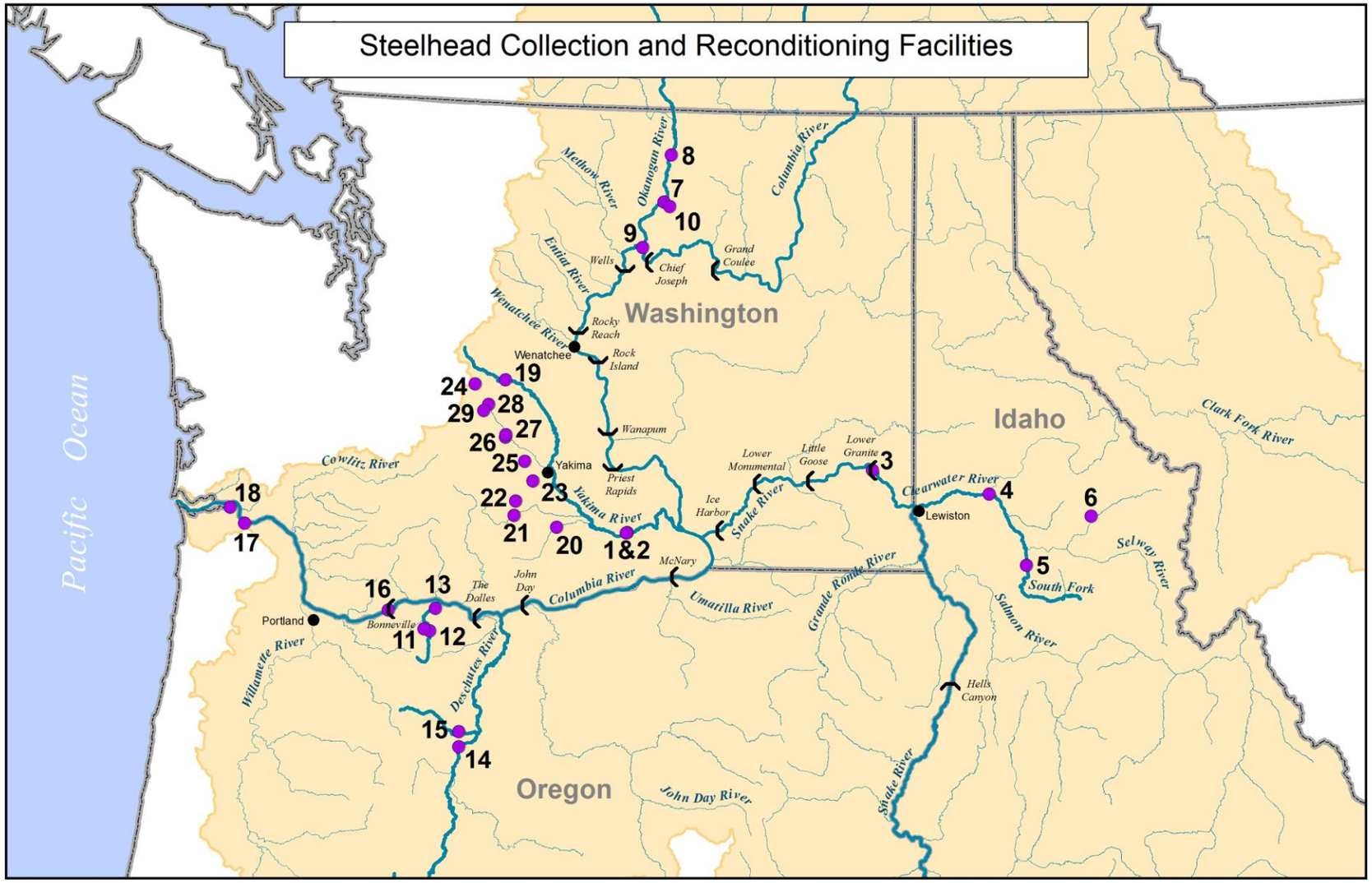


Figure 1: Map of Steelhead kelt Project area 2000-2018.

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

Yakima River Basin

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 m3/s. Summer steelhead populations primarily spawn upstream from Prosser Dam in Satus Creek, Toppenish Creek, Naches River, and other tributaries of the Yakima River (TRP 1995).

Chandler Juvenile Collection Facility (Yakima River)

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

Yakama Nation Prosser Hatchery

Prosser Hatchery is located on the Yakima River just downstream of Prosser Dam (RK 75.6). This facility is part of the The Yakima/Klickitat Fisheries Project, a supplementation project designated by the NPPC as the principle means of protecting, mitigating, and enhancing the anadromous fish populations in the Yakima and Klickitat Subbasins. Prosser Hatchery was constructed in 1994 with the primary function of rearing, acclimating, and releasing fall chinook salmon (*O. tshawytscha)*. It is also used for rearing coho salmon (*O. kisutch)* prior to acclimation and release in the upper Yakima River Basin as well as experimental rearing of white sturgeon *(Acipenser transmontanus)* and Pacific lamprey (*Entosphenus tridentate*). All kelt rearing is conducted at Prosser Hatchery primarily in the 20' x 5' circular tanks, with 4 smaller 10' x 4' and one 17' x 5' circular tanks also available.

Snake River Basin

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

The Lower Granite Juvenile Fish Facility

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

Dworshak National Fish Hatchery

Kelt reconditioning facilities are located at Dworshak National Fish Hatchery (DNFH) in Ahsahka, Idaho. DNFH is located at the confluence of the North Fork of the Clearwater River (RK 65). The Dworshak National Fish Hatchery is a "mitigation" hatchery constructed in 1969 by the Army Corps of Engineers, which presently is co-managed by the U.S. Fish and Wildlife Service and the Nez Perce Tribe (USFWS 2009). Kelts from Lower Granite and hatchery origin fish have been reconditioned at this facility since 2009. As of 2016 most of the kelts reconditioned at this location are hatchery fish that return to the hatchery. They are then air spawned and reconditioned to learn more about kelt rematuration and how we can improve maturation and survival. A small portion of the Lower Granite Dam kelts depending on capacity at Nez Perce Tribal Hatchery, are trucked and reconditioned at this location.

Nez Perce Tribal Hatchery

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

Chapter 1: 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 via PITAGIS to determine how successfully they naturally recondition in the ocean.

We define long-term reconditioning as holding and feeding post-spawn steelhead in a captive environment to increase kelt survival and additional spawning opportunities. The long-term steelhead reconditioning diet and care treatments were established from the studies conducted in 2001 and 2002 (Hatch et al. 2002 and Hatch et al. 2003b) and summarized in Hatch et al. 2013b. These fish are typically released in the fall to over-winter and return to the spawning sites volitionally. This chapter recaps 2019 kelt collection efforts for a broader review of specific fish culturing practices please see (Hatch et al. 2015).

Methods

Standard Data Collection

All captured steelhead are scanned and recorded for existing PIT-tags, biological data is collected which includes determination of kelt/maiden status, fork length, weight, condition factor (color and presence/absence of wounds/skin-body condition), coloration rating (bright, medium, dark), notation of clipped or non-clipped fins (typically adipose), and small (typically a 1 x 1 mm) tissue sample (caudal fin clip) is collected for genetic analysis. Steelhead without a PIT-tag receive a 12.5 mm PIT-tag injected into the pelvic girdle to track migration history and to determine reconditioning efficacy. All releases or mortalities are recorded including date of event, condition factor, and PIT-tag identifier. In the case of a lost PIT-tag, typically at time of release, fish are retagged, and an additional genetic sample collected. All data are uploaded to a central kelt database at CRITFC, any fish released back to wild are entered into PITAGIS database.

Steelhead Kelt Collection

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

Lower Granite Dam
Steelhead kelts entering the juvenile bypass separator (Table 1, site 3) are collected by Army Corps of Engineer (COE) staff. Kelts are netted off the adult fish separator bars and moved to a fish hopper that leads into the kelt receiving tank. Staff from the Nez Perce Tribe (NPT), processed fish diverted into the receiving tank. Kelt steelhead judged to be in good or better condition, with intact adipose fins, and >63cm are collected and trucked to NPTH for reconditioning. The transport truck had a 1.5-kiloliter tank fitted with supplemental regulated, compressed oxygen that was fed via air stones; also, a 12-volt powered tank aeration pump was used to circulate oxygenated water. Stress Coat® or PolyAqua® was used to replace the natural protective slime coating that may have been compromised by handling. In addition, salt was added to reduce osmo-regulatory stress. Temperature and dissolved oxygen levels were monitored during transport. Loading densities were kept to a minimum; no more than 20 kelts were transported at one time.

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).

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 2002-2019 a total of 21,876 downstream migrating kelts at LGD and 14,901 from at CJMF from 2000-2019. The Columbia River upriver steelhead run in 2018-19 was the 8th worst year since unclipped fish were counted. We collected 351 and 1,223 kelts at CJMF and LGD, respectively (Appendix A1a). In 2019, the kelt collection represented 10% and 32% of the upstream run at LGD and CJMF, respectively. This compares to 2018 collections of 9% and 18% for LGD and CJMF, respectively. This demonstrates the value of the kelt reconditioning program as a safety net action to boost wild fish abundance and maintain genetic and life history diversity especially in low steelhead abundance years.

Reconditioning

Since 2011, 1,520 kelt steelhead have been retained for reconditioning from collections at LGD and 611 fish have survived to their first fall. Since 2000; 10,375 kelt steelhead were retained for reconditioning from collections at CJMF and 4,559 fish survived to the first fall of the annual collection period (Appendix A1a). All Snake River collections were made at the LDG in 2019, however it should be noted that kelt collections have come from both the South Fork Clearwater River and Fish Creek in some previous years (Hatch et al. 2018). Collections from other sites such as Little Goose Dam and perhaps targeted smaller rivers or streams like South Fork Clearwater River/Fish Creek may be considered again in the near future to fill the future Snake River Reconditioning facility to capacity.

Long-term reconditioning survival is variable from year to year but has averaged 43% at the Prosser Fish Hatchery (PFH) over the last 20 years. For the last 5 years, Yakima reconditioning has been just over 55% survival (Figure 1.1). The staff here has a number of years of reconditioning experience, so we see generally small annual variations in survival (Figure 1.1). The reconditioning survival rate for wild Snake River kelts from 2011 through 2019 is 40%. Survival during the initial years at DNFH (2011-12) was compromised as a result of poor water quality detailed in previous reports (Hatch et al. 2012 and Hatch et al. 2013) and compounded by inexperience of new hatchery staff. Although, the past 5 years have seen marked improvement in survivals as water quality issues have been improved and staff gain experience in handling and caring for wild steelhead, rate for this five-year period has averaged just over 48% (Figure 1.2). Unfortunately, in 2019 there was a pump failure at Nez Perce Tribal Hatchery and redundancy system alerts failed so we lost 141 in the reconditioning program (88 skip spawners and 53 kelts collected in 2019) consequently there was a larger than average mortality of Lower Granite Fish in 2019 (Figure 1.2). In the Snake River skip spawning seems to be more prevalent in the population than consecutive spawning which is a larger component of the Yakima River Basin. This difference is likely the result of the length of migration which Snake River fish must endure from either the amount energy exerted for migration which must be replaced or that the trigger for rematuration may be inherently higher from these fish due to

the long distances they must travel to return to spawning grounds. In both figures (Figures 1.1 and 1.2) the differences in this rematuration is exhibited in the proportion of retained fish at the two facilities. Releases for the Yakima have been at historic lows but have begun to rebound in 2019 while Snake River returns have trended upwards in part to successful retention and reconditioning of skip spawners (Figures 1.1 and 1.2) and would have continued to do so if not for the aforementioned loss of fish in 2019.

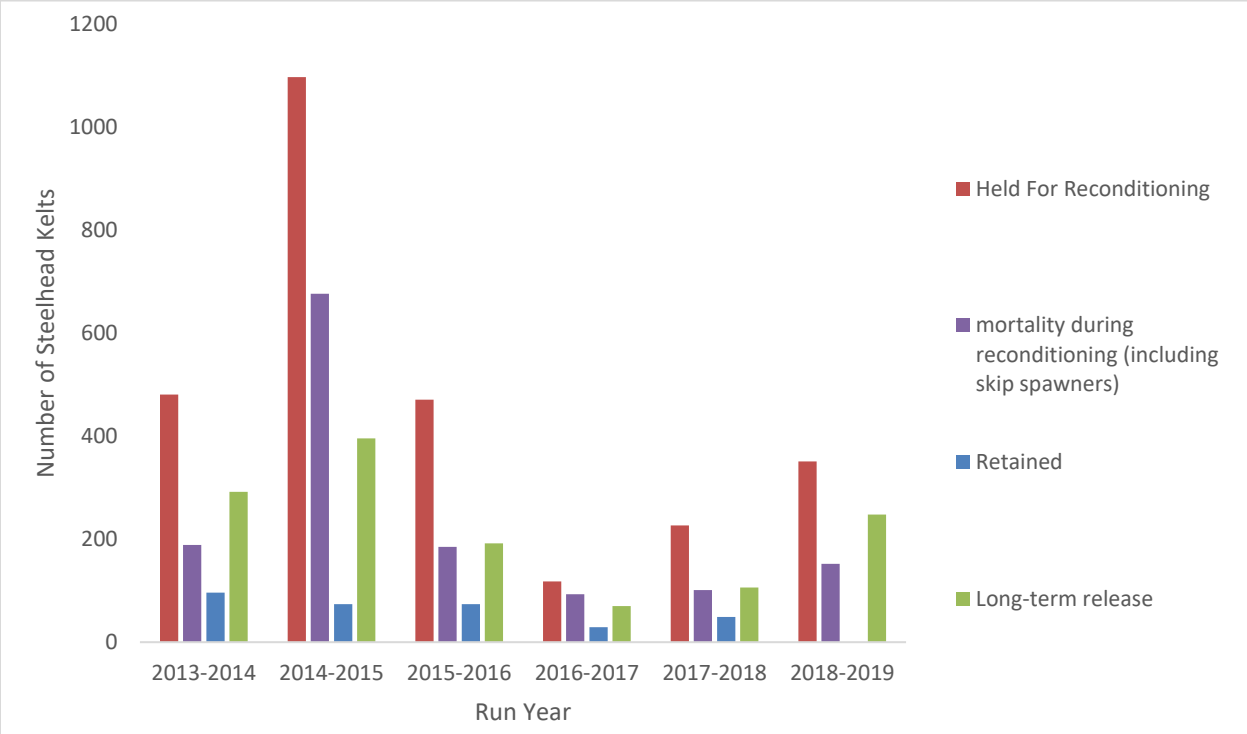


Figure 1. 1: Yakima River steelhead kelt collection for reconditioning and fate from 2013-2019.

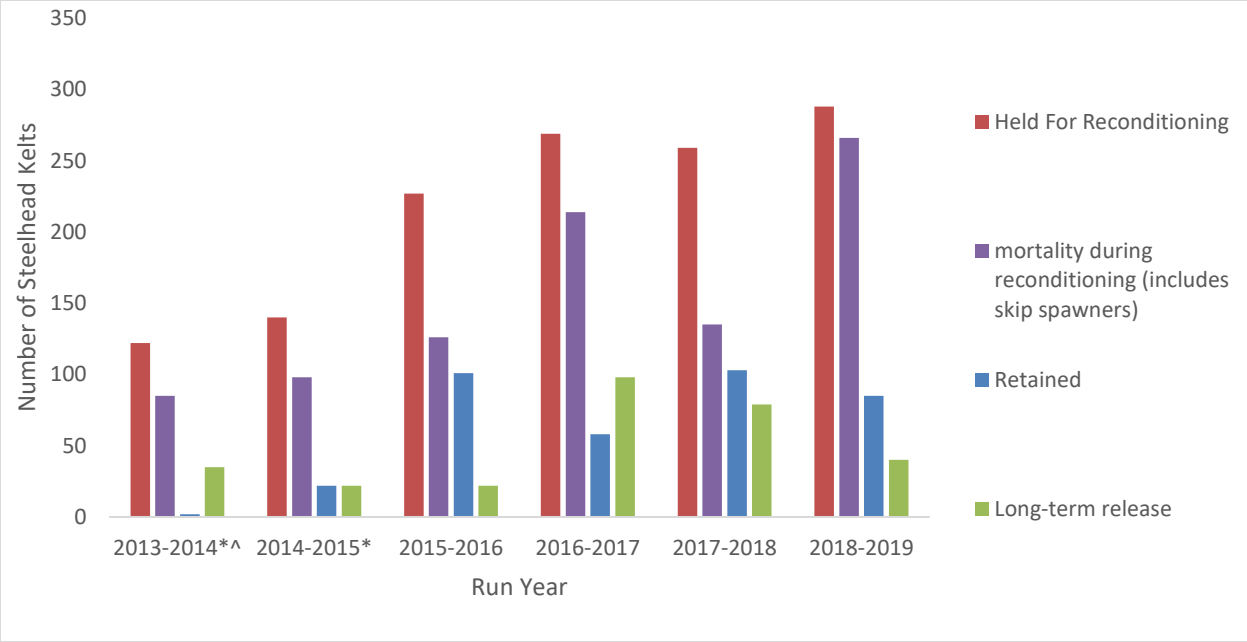


Figure 1. 2: Snake River steelhead kelt collection for reconditioning and fate from 2013-2019.

Life History Strategies: Consecutive vs. Skip spawning

The CRITFC through its steelhead reproductive physiology research, 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 2019 could spawn again in the winter/spring 2020 as consecutive spawners or wait until spring of 2021 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 being held for an additional 12 months after capture spawning the next winter/spring. Strategy choice is likely controlled by 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 water chilled adequately during winter months, which is needed due to the temperature regime of the spring water which is utilized at Prosser, which seems to cause maturation issues with the prolonged holding needed for skip spawners. Additionally, the cost of holding fish for an additional year should be compared to results from other scenarios. At the end of 2019 we released 103 immature reconditioned kelts below Bonneville Dam to determine if this management strategy would be better than the average for in-river returns. We will be

observing for possible results when we would anticipate adult returns beginning as early as July of 2020 or into 2021. If results are promising, we plan to continue with releases experimentally until we can determine if it is a worthwhile strategy to convert immature spawners into viable mature spawners. Add that these fish were all pit tagged.

Release

We evaluated the traits and survival to release of reconditioned kelt steelhead *Oncorhynchus mykiss* in the Yakima River (Hatch et al. 2013b). Reconditioned steelhead kelts continue to be predominantly (>92%) female. Annual survival to release ranged from 18% at the start of the program to an annual high of 76% in 2016 and averaged 43% 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 kelts, 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 overall number of returned fish may appear small compared the overall run especially in the Snake, many of these fish are contributing towards ESA-listed populations throughout both the Yakima and Snake basins. In Figure 1.4 both a and b-run populations are counted, while we primarily targeted b-run fish while selecting kelts for reconditioning there were a-run fish that were also collected and reconditioned but their numbers were a smaller proportion of the reconditioned population. The a-run population in the Snake is a much larger than the b-run population with most of these fish coming from the Grande Ronde basin.

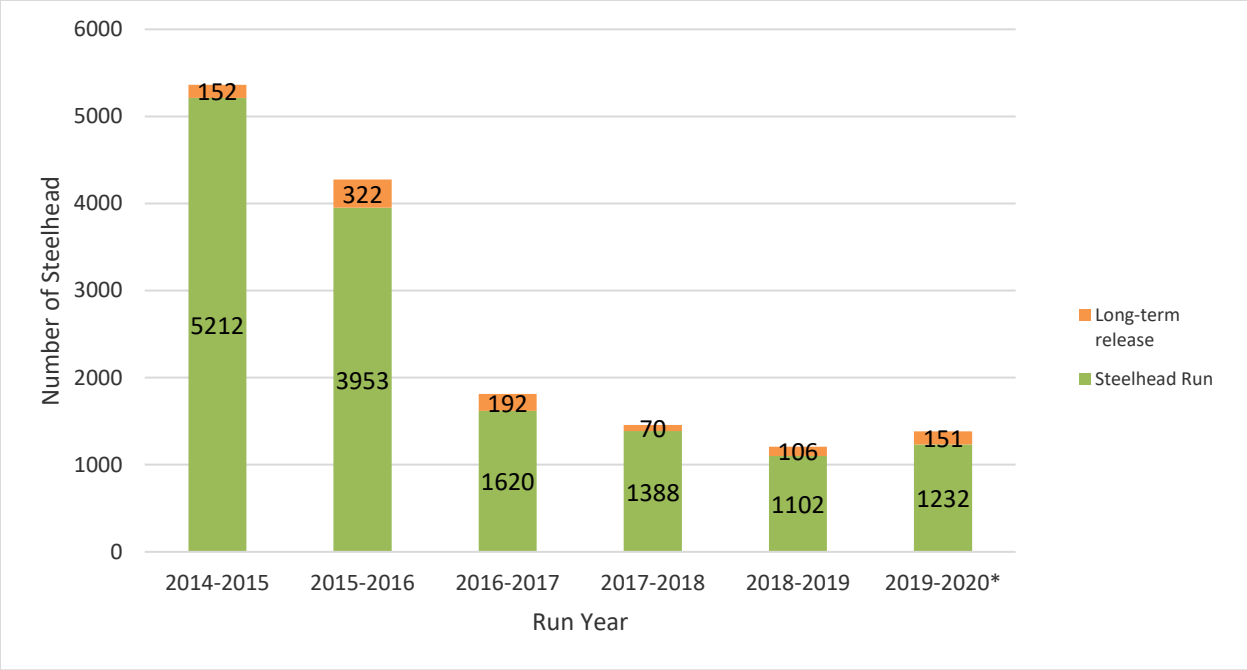


*Figure 1. 3: Contribution to steelhead run from reconditioned kelt release in Yakima Basin. * Run is still ongoing, value will be updated in 2020 kelt annual report.*

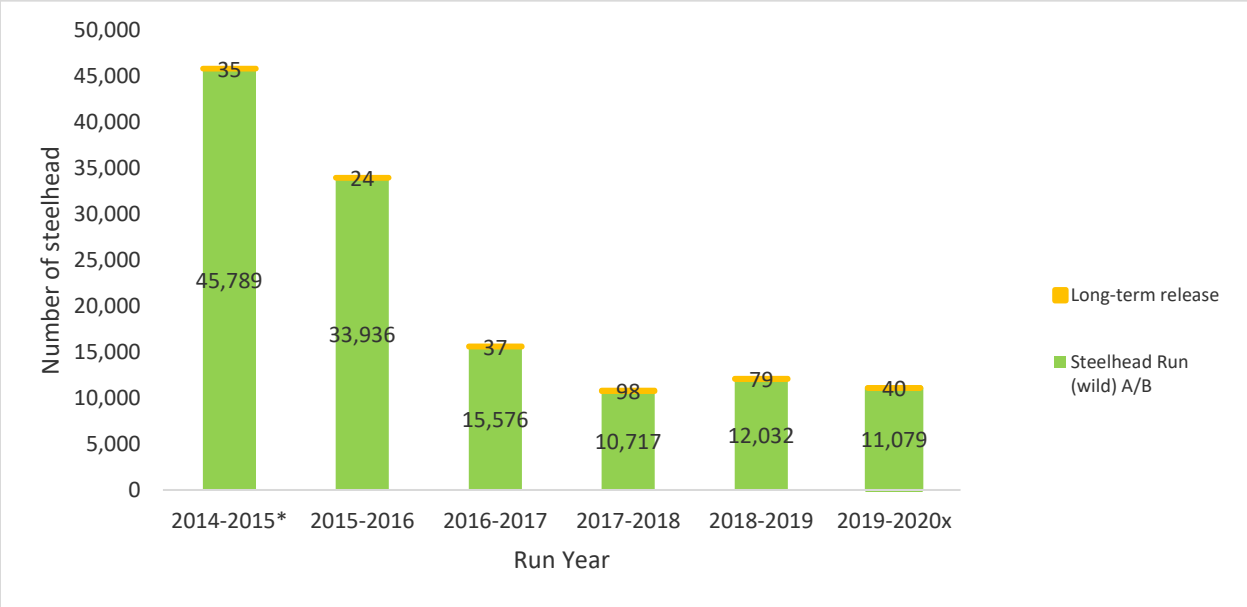


Figure 1.4: Contribution to steelhead run from reconditioned kelt release in Snake Basin. x Run is still ongoing, value will be updated in 2020 kelt annual report.

The natural repeat spawner rate (or no intervention), measured as a return rate to Bonneville Dam, for the Snake River is 0.29% and for the Yakima River is 2.90%. The calculated benefit of reconditioning relative to leaving the fish in the river is 14.8 times for the Yakima River and 136.8 times for the Snake River.

Summary Research-Scale Efforts to Address RPA 33 and subsequent Biological Opinion (2019)
At DNFH in 2019 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, to implement a Snake River steelhead kelt management plan designed to provide at least a 6% improvement in B-run population productivity (NMFS 2008, 2010, and 2014). Toward that goal, a variety of approaches 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. Size, while historically used to determine b-run fish, has been determined to not be solely the designator of b-run populations as some fish that have been genetically assigned to b-run populations do not meet 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 .

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 2013 we had a successful reconditioning and release at nearly 40% towards reaching the RPA 33 goal with 69 female fish released that year. Our best year was in 2017 with releases just over 50% towards the RPA goal at 98 mature female spawners released that year. 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 2011-2019 (Table 1.1). Table (1.1) summarizes all collections for both A and B run, and releases associated with the RPA 33. 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 management implications of kelt reconditioning in the Snake River Basin.

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

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

2015	Lower Granite Dam	22	11	50.0%	8	3	3	8
2015	S.F. Clearwater	35	7	20.0%	4	3	0	4
2015	Fish Creek	83	25	30.1%	10	15	15	12*
2015 (subtotal)		**140**	**43**	**30.7%**	**22**	**21**	**18**	**24**
2016	Lower Granite Dam	227	120	52.86%	19	101	77	22^
2016 (subtotal)		**227**	**120**	**52.9%**	**19**	**101**	**77**	**37*^**
2017	Lower Granite Dam	269	59	21.9%	21	58	29	98^
2017 Subtotal		**269**	**59**	**21.9%**	**21**	**58**	**29**	**98^**
2018	Lower Granite Dam	**259**	**177**	**68.3%**	**50**	**99**	**1**	**78^**
2018 Subtotal		**259**	**177**	**68.3%**	**50**	**99**	**1**	**78^**
2019	Lower Granite Dam	**288**	**121**	**42.0%**	**39**	**85**	**TBD 2020**	**40^**
2019 Subtotal	Lower Granite Dam	**288**	**121**	**42.0%**	**39**	**85**	**TBD 2020**	**40^**
Grand Total		**1550**	**638**	***41.2%***	**267**	**366**	**127**	**393**
				***includes Fish Cr. kelt skip spawners**				
				^Includes previous year kelt spawners from LGD				

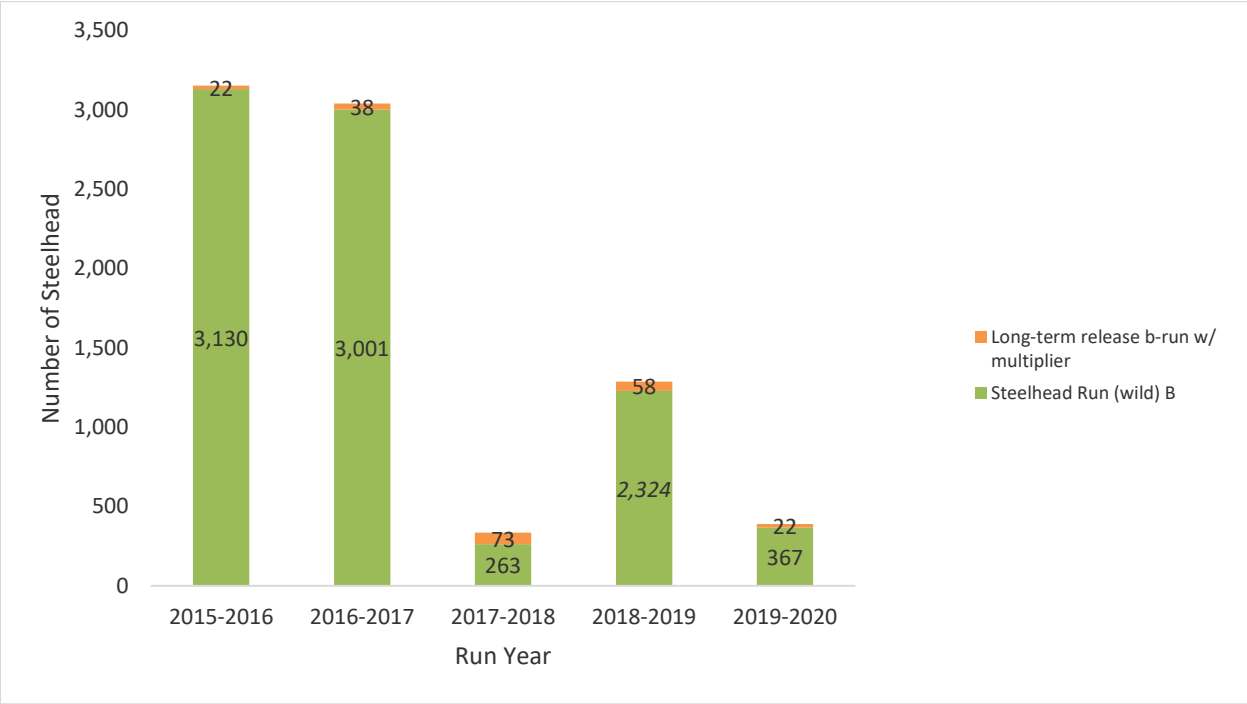


*Figure 1. 5: Contribution to steelhead run from reconditioned kelt release in Snake River Basin. *2019-2020 value is an estimated run size and will be corrected in the 2020 Kelt Annual Report. B-run numbers utilize Kelt Master Plan definition for reconditioned female kelt spawners.*

In the spring of 2019, the NOAA published a new Hydrological Biological Opinion for the Columbia River Power System (NMFS 2019). The new publication effectively retired the 6% number and considered current (2019) and future Snake River reconditioning actions as the only hatchery actions that appropriate corrective mitigation for steelhead loss in the Snake River.

2. Yakima River Kelt Reproductive Monitoring

Introduction

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

Methods

Sample Collection

Anadromous adult steelhead were collected as upstream migrants at Prosser Dam or downstream migrants at the Chandler Juvenile Monitoring Facility. Samples collected as upstream migrants at Prosser Dam were treated as maidens and referred to as pre-spawn maiden collections. Post-spawn adults sampled at the Chandler facility that did not go into the reconditioning program or that did not survive the reconditioning program are referred to as post-spawn maidens. Adults that were collected at the Chandler facility that were reconditioned and released in the fall are referred to as kelts. Kelt reproduction is subdivided between Event-1 (prior to reconditioning) or Event-2 (after reconditioning).

Age-0 juveniles (juveniles collected in the same calendar year as the spawning event) were targeted using electrofishing techniques (NMFS 2000 Electrofishing Guidelines) during the late summer and fall in natal tributaries. Sampling was targeted near areas where steelhead spawning has been observed or spawning redds detected. Technicians in the field were directed to target only age-0 juveniles. A 100 mm general maximum length was used in addition to the judgment of those collecting the samples based on the time of year. Fork length was recorded for additional analysis of length outliers. Sampling locations for Juveniles are seen in figure 2.1. Some sampling sites were not sampled across all years due to access constraints.

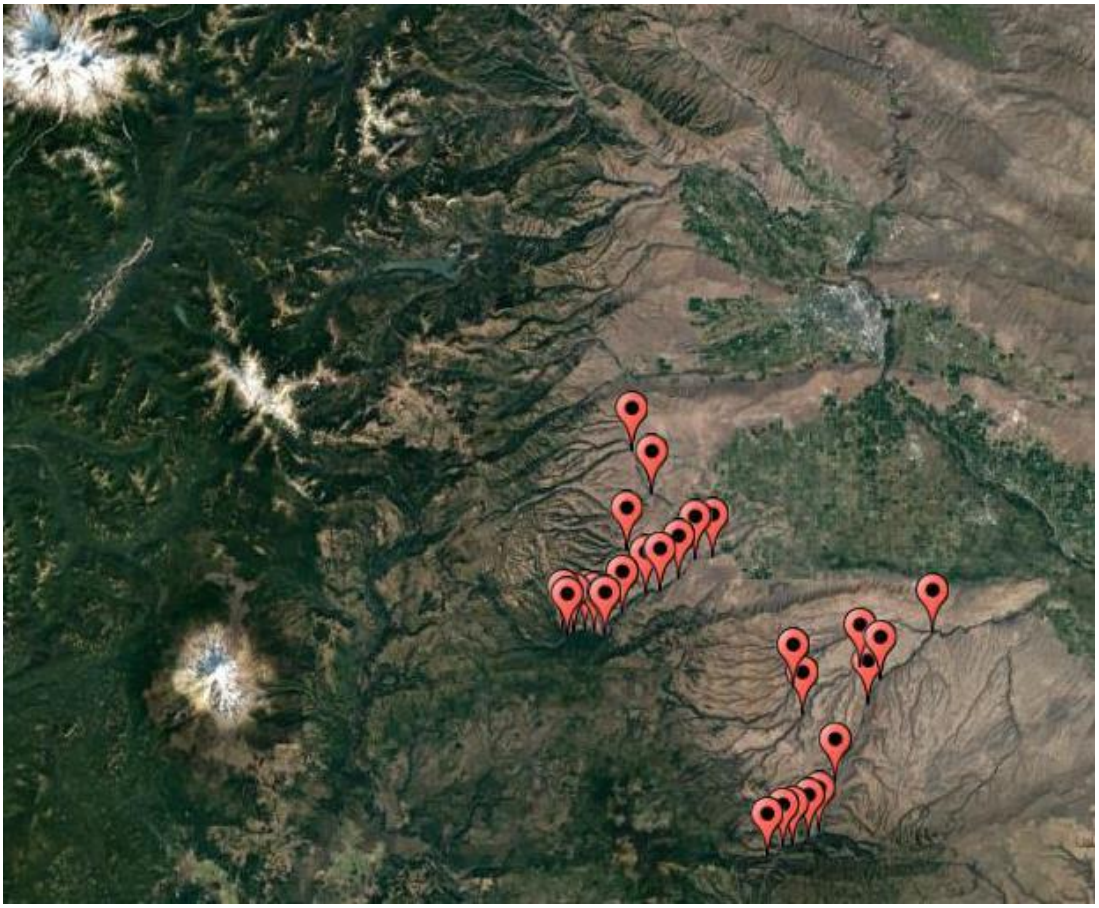


Figure 2.1: juvenile sampling locations in Satus (lower right) and Toppenish creeks over 5 years 2013-2017.

Genetic Analysis

Fin tissue samples were collected and stored dry on Whatman paper, or paper slips in coin envelopes for preservation of DNA. Genetic analysis was conducted at the Hagerman Fish Culture Experiment Station in Hagerman, ID. DNA was extracted from tissue samples using Qiagen® DNeasy™ extraction kits or chelex extractions modified from Casquet et al (2012). Past genotyping efforts have utilized a Fluidigm ep1 platform and the 192 Single Nucleotide Polymorphism (SNP) markers and methods described in Hess et al. (2012). Genotyping efforts from 2015 on used expanded marker panels and GTseq protocols (Campbell et al 2015) on an Illumina HiSeq 1500 or NextSeq 500 Sequencer. Prior to parentage analysis, Poor loci were removed from the dataset. Dropped loci included the sex-determining marker (OmyY1_2SEX), three loci diagnostic for cutthroat, loci with genotyping rates less than 90%, and loci with minor allele frequency less than 0.05. Confirmed duplicate samples, samples with incomplete genotypes, and non-target species samples were omitted and are not included in the results.

Parentage analysis was performed using CERVUS v 3.0 (Marshall et al. 1998, Kalinowski et al. 2007). Information on fish gender was not included in the analysis. To minimize incorrect assignments, simulations were performed annually to estimate a 99.0% confidence LOD value. Individual parentage assignments were included if they had a minimum of 90% loci comparisons, met the critical LOD value and had minimal single locus mismatches. This

accounts for the presence of minor genotyping errors while minimizing the loss of parental assignment matches.

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

Relative reproductive success (RRS) was calculated between classes of fish by standardizing to the pre-spawn maiden class of adults. Lifetime reproductive success (LRS) estimates for kelts were calculated by adding the RRS of Event-1 and Event-2 estimates. This estimate of LRS does not look at individuals of fish that spawned across multiple years, nor does it look at the same group of fish across 2 consecutive years (e.g. Maiden in 2013, reconditioned kelts in 2014). Rather, it adds the RRS estimates of fish spawning in the same calendar year. Because we had no information for male Event-1 in 2017, a placeholder of 1.00 was used to allow an LRS across all years. This number is conservative (lower) as compared to the average male Event-1 across the previous 4 years.

Results

The number of juveniles successfully genotyped at individual sites, and the corresponding number and percentage of samples assigned to at least one anadromous adult parent is shown in table 2.1. This table does not include locations dropped because of access issues, low sampling success, or lack of assignments to an anadromous offspring. The low apparent assignment rate is due to the low number of adults sampled compared to the relative number of unsampled anadromous steelhead and potential resident fish.

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

		Satus Cr.	Toppenish Cr.
2013	Genotyped	227	204
	Assigned	54	64
	% Assigned	24%	31%
2014	Genotyped	285	231
	Assigned	64	67
	% Assigned	22%	29%

2015	Genotyped	341	369
	Assigned	123	165
	% Assigned	36%	45%
2016	Genotyped	790	524
	Assigned	288	187
	% Assigned	36%	36%
2017	Genotyped	442	578
	Assigned	136	172
	% Assigned	31%	30%
2018	Genotyped	615	589
	Assigned	171	165
	% Assigned	28%	28%
Sum	Genotyped	2700	2495
	Assigned	836	820
	% Assigned	31%	33%

The number of genotyped parents confirmed to have entered either Satus or Toppenish Creek is shown in Table 2.2. Pre-spawn maidens have the greatest number of samples with a total of 247 males and 474 females. Post-spawn maidens have only 16 males and 80 females overall. Reconditioned kelts have 24 male and 188 female Event-1 detections and 30 male and 229 female Event-2 detections. Number of fishes increase each year but are limited by the number of kelts that can be collected, and mortality seen during the reconditioning process. The number of kelt males has been low over the last two years, 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**	**All**
Pre-Spawn Maidens	Male	38	46	57	79	6	21	247
Post-Spawn Maidens	Male	4	1	7	2	1	1	16
Reconditioned Kelt Event-1	Male	3	13	7	1	0	0	24
Reconditioned Kelt Event-2	Male	5	3	13	8	1	0	30
Pre-Spawn Maidens	Female	88	70	94	138	46	38	474
Post-Spawn Maidens	Female	12	13	39	9	2	5	80
Reconditioned Kelt Event-1	Female	15	44	51	22	13	43	188
Reconditioned Kelt Event-2	Female	74	18	40	56	26	15	229

The number of progeny assigned to each fish is shown in Table 2.3. The majority of assignments are to pre-spawn maidens with 219 juveniles assigned to males and 394 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.

Class	Sex	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**All**
Pre-Spawn Maidens	Male	12	17	24	119	13	34	219
Post-Spawn Maidens	Male	0	0	0	4	7	1	12
Reconditioned Kelt Event-1	Male	3	6	4	1	0	0	14
Reconditioned Kelt Event-2	Male	3	4	1	0	7	0	15
Pre-Spawn Maidens	Female	43	41	26	165	76	43	394
Post-Spawn Maidens	Female	8	1	27	2	0	9	47
Reconditioned Kelt Event-1	Female	5	21	32	17	63	66	204
Reconditioned Kelt Event-2	Female	18	12	16	40	39	38	163

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

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**	**All**
Pre-Spawn Maidens	Male	0.32	0.37	0.42	1.53	2.17	0.62	0.89
Post-Spawn Maidens	Male	0	0	0	2	7	1	0.75
Reconditioned Kelt Event-1	Male	1	0.46	0.57	1	NA	NA	0.58
Reconditioned Kelt Event-2	Male	0.6	1.33	0.08	0	7	NA	0.50
Pre-Spawn Maidens	Female	0.49	0.59	0.28	1.17	1.65	1.13	0.83
Post-Spawn Maidens	Female	0.67	0.08	0.69	0.22	0	1.8	0.59
Reconditioned Kelt Event-1	Female	0.33	0.48	0.63	0.77	4.85	1.53	1.09
Reconditioned Kelt Event-2	Female	0.24	0.6	0.41	0.71	1.5	2.53	0.71

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	AVG
Pre-Spawn Maidens	Male	1	1	1	1	1	1	1
Pos-Spawn Maidens	Male	0	0	0	1.31	3.23	1.62	1.03
Reconditioned Kelt Event-1	Male	3.17	1.25	1.36	0.66	1.00*	0	1.29
Reconditioned Kelt Event-2	Male	1.9	3.61	0.18	0	3.23	0	1.49
Pre-Spawn Maidens	Female	1	1	1	1	1	1	1
Pos-Spawn Maidens	Female	1.43	0.13	2.32	0.21	0	1.59	0.95
Reconditioned Kelt Event-1	Female	0.43	0.81	2.11	0.72	3.03	1.36	1.41
Reconditioned Kelt Event-2	Female	0.49	1.14	1.51	0.66	0.91	2.24	1.16

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

Lifetime reproductive success (LRS) of reconditioned kelts are shown in table 2B.6. Male kelt LRS varied between 0.0 and 5.07 times that of fish sampled as pre-spawn maidens within the same year. Across all years male kelts has an LRS of 2.73. Female kelt LRS had annual variation between 1.18 and 3.84 with an average of 2.57.

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

Class	Sex	2013	2014	2015	2016	2017	2018	AVG
Reconditioned Kelt Lifetime	Male	5.07	4.86	1.54	0.66	4.23	0	2.73
Reconditioned Kelt Lifetime	Female	1.18	1.84	3.67	1.27	3.84	3.6	2.57

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 2.57 times that of the pre-spawn maidens. This is similar to findings by Seamons and Quinn (2010) who theorized and found that lifetime reproductive success of repeat spawners should scale with the number of breeding spawners. While relative reproductive success of female reconditioned kelts following reconditioning (Event-2) is slightly lower (Average RRS=0.95) than that of pre-spawn, any spawning by a reconditioned kelt is additive to the population and demonstrates the potential to boost numbers.

The 2018 spawning event was the sixth consecutive year that we successfully assigned multiple progeny to reconditioned kelts. The methodology of focusing sampling efforts on age-0 fish in areas that anadromous spawning was expected to have occurred, and an increased sampling rate of juvenile has resulted in an increase in the number of successful assignments to both maiden and kelt fish. Future sampling will continue to focus on age-0 fish in areas that

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

Reconditioned kelt steelhead have demonstrated that they are capable of spawning in the wild. With additional sampling in future years, including adult to adult estimates, we hope to have more accurate numbers and modeling potential. Current data shows that reconditioned kelt steelhead contribute to the productivity of the natural population on a scale similar to that of natural kelts, helping to preserve this important life history. We anticipate that we will successfully publish this data in an accredited scientific journal in 2020 or early 2021.

Chapter 3. Kelt Reconditioning Physiology Studies

Introduction

Studies applying tools from fish physiology and endocrinology to issues in kelt reconditioning were continued in 2019. 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 2019, we sampled blood and provided maturation status of individual fish to project managers so that consecutive and skip spawners could be managed appropriately (Chapter 3.A). The 2019 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 second and third of three linked studies using hatchery origin kelts at Dworshak National fish hatchery (Jenkins, et al. 2020; Jenkins, et al. 2019). The first of these studies was published in 2018 (Jenkins, et al. 2018). 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 completed laboratory analysis of plasma hormone levels, and an initial analysis of the results for a study on the effect of nutritional restriction during the period after spawning on life history trajectory in hatchery-origin kelts (Chapter 3:Section3B). We completed laboratory work to establish assays for plasma insulin-like growth factor-1 (IGF-1) and growth hormone (GH), indicators of growth and metabolic status, and a draft manuscript describing this work for submission for publication (Chapter 3:Section C). Finally, we made progress on 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). Many of these studies are ongoing, and laboratory analysis, statistical analysis, results, interpretations, and conclusions may change as additional work is completed.

Section 3.A: Reproductive 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; Jenkins et al. 2019; Keefer, et al. 2008; Pierce, et al. 2017; 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. 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 or the rate of energy gain/loss, i.e. energy balance. If maturation is initiated during this critical period, it may be arrested at a second critical period before the onset of exogenous vitellogenesis, if energy reserves are not sufficient (Yamamoto, et al. 2011). We hypothesize that a similar decision mechanism regulates rematuration in post-spawning steelhead. Consistent with this idea, we found that energy restriction affected reproductive development within 10 weeks after spawning in female rainbow trout (Caldwell, et al. 2013; Caldwell, et al. 2014). In post-spawning fish, energy driven decisions take place in the context of the extreme energy deficit incurred by migration and spawning (Penney and Moffitt 2014a, b, 2015). Threshold energy levels for maturation or rematuration are determined by the genetic makeup of the fish and subject to selection (Carlson and Seamons 2008; Hutchings 2011a).

Studies conducted in 2009-2011 established that blood levels of estradiol and vitellogenin diverge between rematuring and non-rematuring fish during reconditioning (Pierce et al. 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 2019, we measured estradiol level in blood samples collected from steelhead kelts. We collected blood from fish in the reconditioning programs at Prosser, Nez Perce Tribal Hatchery (NPTH), and Dworshak National Fish Hatchery (DNFH). We collaborated with colleagues in the Upper Columbia reconditioning project at Winthrop National Fish Hatchery (WNFH) to obtain samples they collected from their reconditioned kelts. We ran plasma estradiol assays, and provided maturation status to project managers so that rematuring fish could be released. Non-rematuring fish are usually retained for further reconditioning. This year, non-rematuring fish were held at DNFH and WNFH; however, the non-rematuring fish from Prosser were transported and released to begin to evaluate this as a potential management strategy.

Methods

Fish Collection and Husbandry

Steelhead kelts were collected and reconditioned at Prosser Hatchery, Washington, Dworshak National Fish Hatchery (DNFH), Idaho, Nez Perce Tribal Hatchery (NPTH), Idaho, and Winthrop National Fish Hatchery (WNFH), Washington as previously described (Abrahamse and Murdoch 2013, 2014; Hatch, et al. 2019; Hatch, et al. 2013).

Sampling

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

Table 3A.1. Wild steelhead kelts sampled during the fall in 2019. Prosser: Prosser Hatchery, DNFH: Dworshak National Fish Hatchery, WNFH: Winthrop National Fish Hatchery.

ocation	Sample date	Fish type	# Fish	Notes
Prosser	9/18/19	Wild kelts	313	Includes 2019 males (n = 27) and 2018 hold-over fish (n = 7)
DNFH	9/26/19	Wild kelts	120	All females collected at LGR in 2019
NPTH	-	Wild kelts	0	Not sampled due to mortality on 8/25/2019. All 2018 Snake River hold-over fish were held at NPTH.
WNFH	10/1/19	Wild kelts	36	Fish were collected in 2018 (n = 4) and 2019 (n = 32)

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 (EIA) using an acetylcholinesterase linked estradiol tracer (Cayman Chemical, Ann Arbor, MI). Extracted

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

Results

Plasma E2 levels were bimodally distributed in blood samples taken from female kelts in all projects at a pre-release sampling in the fall (Figs 3A.1, 3A.2, 3A.3). As found in previous years, the division between the lower and higher modes was approximately 1000 pg/ml E2 at Prosser, DNFH, and WNFH. However, several fish with E2 levels of 700-1000 pg/ml appeared to group with the lower mode, but could represent a group of fish maturing more slowly than the rest of the upper mode. Consequently, the division between modes was adjusted to include these fish as rematuring so that the fish could be released. The rematuration rate of female kelts as consecutive spawners in 2019 was high at Prosser; females rematured at a 63.2% rate. Consecutive spawners from other programs on the Snake River and Upper Columbia River had lower rates of rematuration for 2018, with only 20.8% of the Snake River fish held at DNFH rematuring and 43.8% of the Upper Columbia River fish rematuring. As in previous years, the rematuration rate of female kelts held for a second year of reconditioning was higher than consecutive spawners: 100% of Upper Columbia River fish and 71.4% of Prosser fish held for a second year rematured. The rematuration rate of Snake River fish held for a second year is not known due to loss of these fish at NPTH before blood sampling (Chapter 1). Instead of being held for a second year, immature fish from Prosser were trucked downstream to below Bonneville Dam and released.

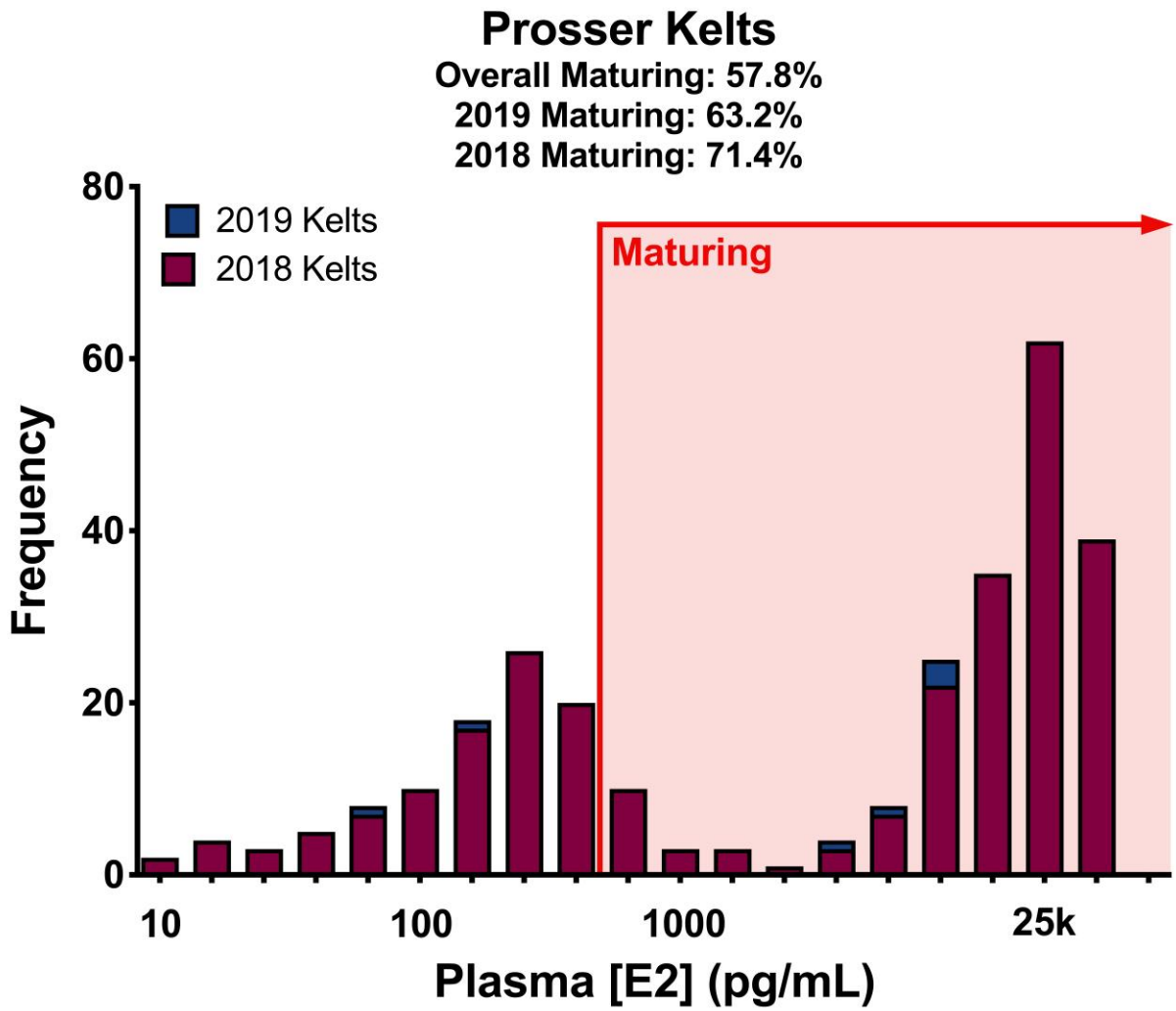


Figure 3A.1 Plasma estradiol (E2) levels in wild Yakima River female kelts sampled in fall of 2019.

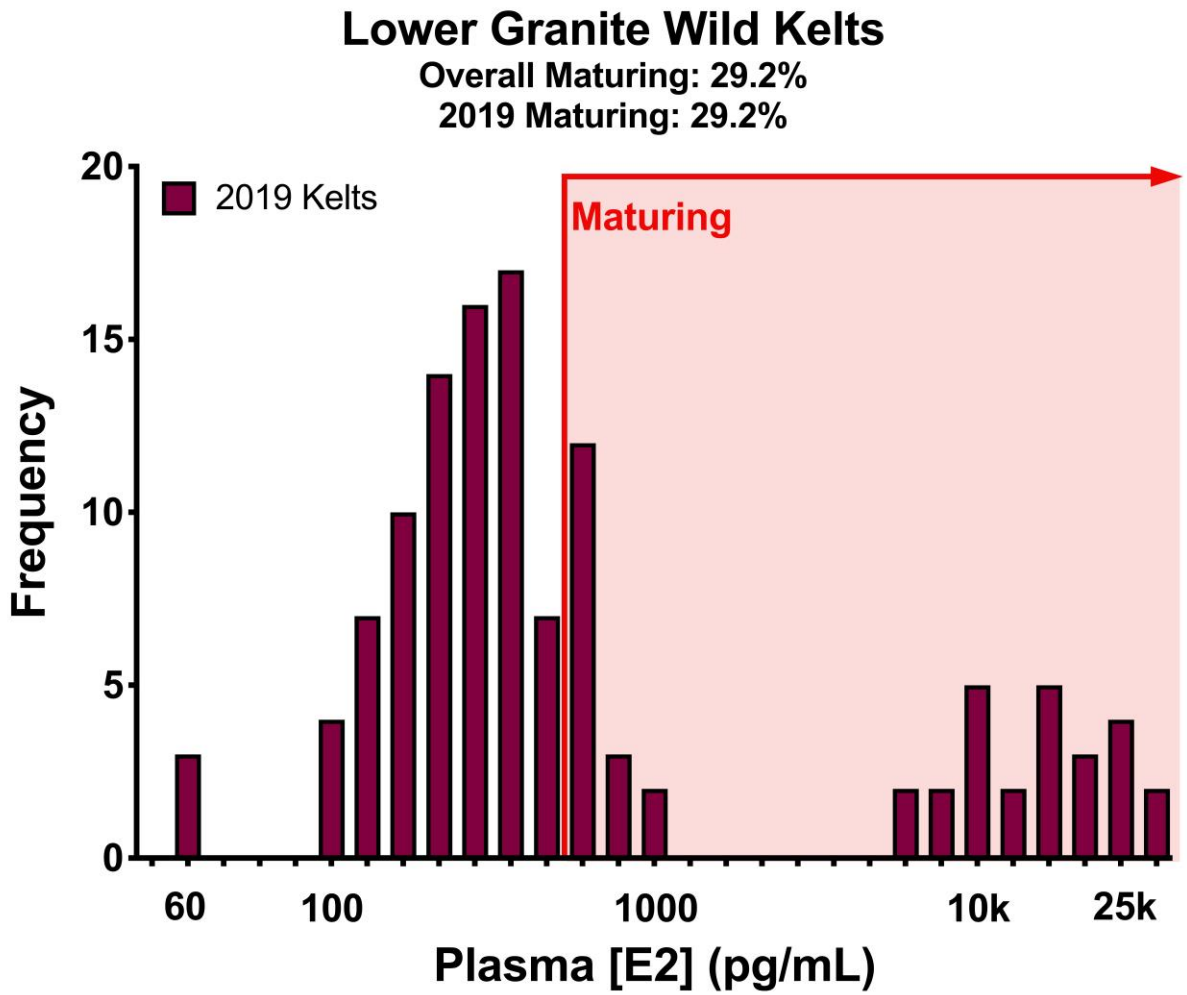


Figure 3A.2: Plasma estradiol (E2) levels in wild female Snake River kelts sampled in fall of 2019.

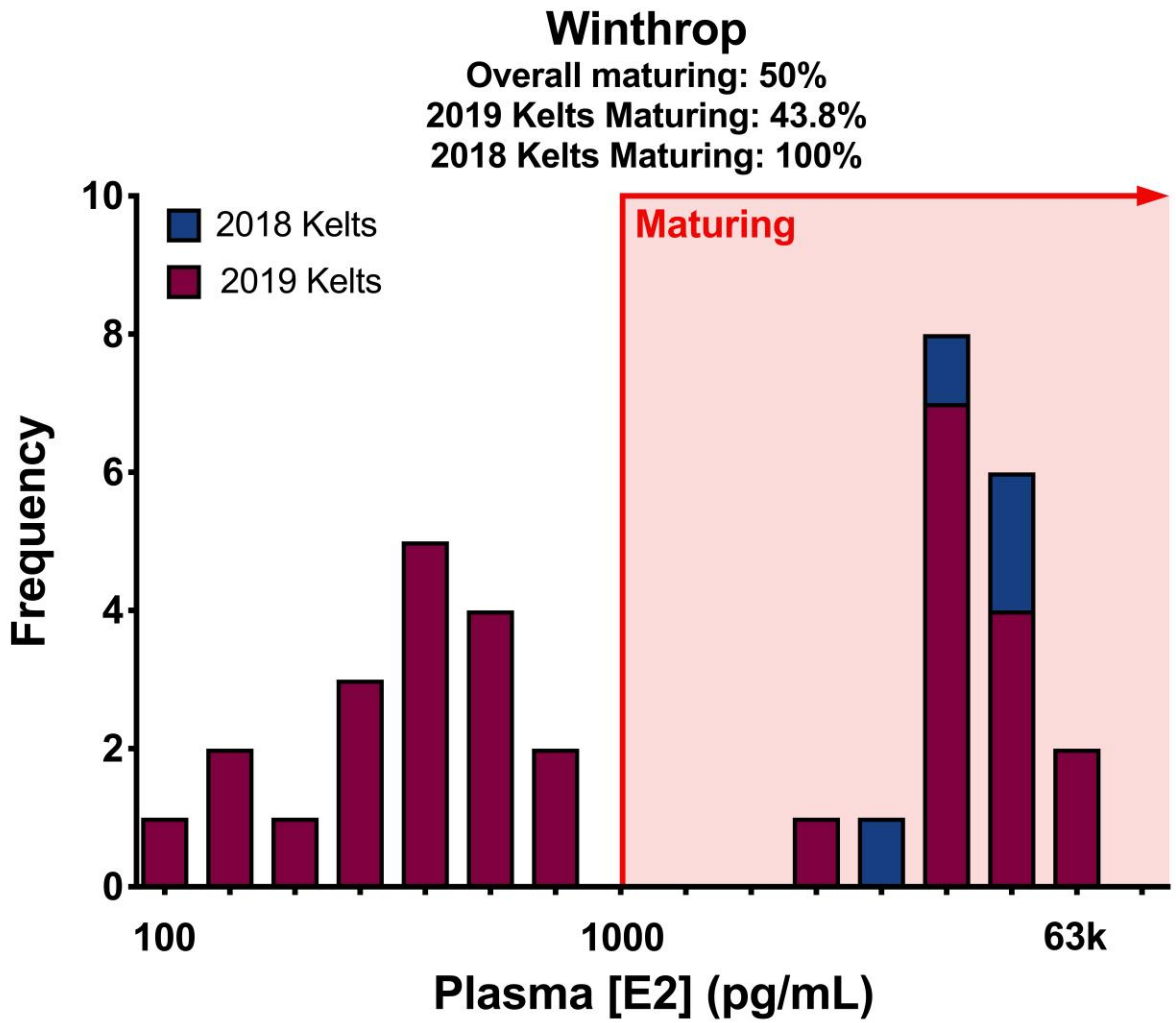


Figure 3A.3: Plasma estradiol (E2) levels in female Upper Columbia River kelts sampled in fall of 2019.

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). 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). Plasma estradiol levels in rematuring and non-rematuring kelts for 2019 at all sites were similar to previous years and were similar between projects.

Female consecutive maturation rates were variable among the projects this season, with a higher rate found at Prosser than at the Snake River and Upper Columbia River projects. It is possible that this relates to migration distance. The relatively low consecutive maturation rates

found in Snake River kelts is in line with what has been observed previously in naturally repeat-spawning 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 populations 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. Variation in consecutive maturation rate between projects and between years may also be due to variation in pre-capture environmental conditions. The warmer water temperatures the Columbia River Basin has been experiencing the past several seasons would be expected to result in greater energy depletion, requiring a longer recovery period before kelts are able to remature.

Non-rematuring fish held for a second year rematured at very high rates (71.4% or higher) in 2019. This adds to a growing body of data showing that non-rematuring females will remature as skip spawners if held for a second year. Skip spawning is a natural life history in Columbia Basin steelhead. Increased size, fecundity, and energy reserves in skip spawners result in greater relative reproductive success versus maidens or consecutive repeat spawners (Jenkins et al. 2018). The presence of skip spawners increases life history diversity, which would be expected to increase population stability in steelhead populations (Moore, et al. 2014; Schindler, et al. 2010). Moreover, whether and how much culture conditions can influence the proportion of consecutive and skip spawning kelts in captive reconditioning is not well understood. These considerations suggest that Columbia Basin kelt reconditioning programs should find ways to accommodate the skip spawner life history.

Hold-over survival in Upper Columbia and Snake River fish is relatively high (typically over 60%), which is in contrast to the low survival sometimes experienced by hold-over fish at Prosser. The difference could be due to population-specific genetic differences but could also be due to winter water quality or disease. The Prosser facility is located in an area that experiences heavy runoff. To evaluate the potential benefit of releasing Prosser hold-over fish in the lower river to facilitate access to the ocean, non-rematuring fish were transported to below Bonneville Dam and released in the fall of 2019. The migration patterns and repeat spawning rate of these fish will be evaluated by PIT tag detections in subsequent years.

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, two of which were published in 2019 (Jenkins et al. 2020; Jenkins et al. 2019), and one in 2018 (Jenkins et al. 2018).

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 (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 the two 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. Fasting steelhead use energy reserves gained in the ocean to make return migrations from the ocean and spawn, which they will need to replenish in order to spawn again.

The critical period for initiation of maturation (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 after spawning resulted in reduced plasma estradiol (E2) levels within 10 weeks after spawning (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). Based on these findings, we hypothesize that gonadal recrudescence as a consecutive spawner may be determined by energetic status during the first 10 weeks after spawning. In order to test this hypothesis, we conducted an experiment to assess the effects of energy restriction 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. 2005a). 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 increased GH (Bjornsson, et al. 2018; Pierce, et al. 2005b), associated with mobilization of energy reserves. Due to these relationships GH/IGF axis components are used as indicators of energetic status in fisheries studies (Beckman 2011; Perez-Sanchez et al. 2018; Picha, et al. 2008a). The GH/IGF axis 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 the 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 (Section 3.C) to measure plasma GH and IGF1 levels in this experiment.

Methods

In 2017 and 2018, hatchery-origin first-time spawning female steelhead were air spawned at DNFH on five egg takes in February (Table 3B.1). Air spawning was conducted as previously described (Hatch, et al. 2014). After air spawning, fish were individually PIT tagged, lengths and weights of fish were recorded, and a non-lethal measure of muscle lipid content was taken using a Fish Fatmeter (Distell Inc., Midlothian, UK). Fish were blood sampled and prophylactically injected with oxytetracycline to control bacterial infections and emamectin to control copepods. The total weight of eggs collected from each female was recorded, and a subsample of approximately 25 eggs from each female was taken in order to determine individual egg weight.

Table 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

Fish from each spawning event (hereafter, take) were randomly divided between two tanks. Due to limitations on the number of tanks available, fish from the second two takes in 2017, which were one week apart, were combined into the same tanks. One tank from each take was fed a mixture of krill and pellets to satiation, and the other tank was fasted. In 2017, fasted fish from takes one and three were fasted for 10 weeks, and fish from take two were fasted for 11 weeks. In 2018, fasting lasted for 10 weeks for designated fish from both takes. After 10 weeks, all fish were sampled, fish were consolidated into one tank per take (takes 2 and 3 remained consolidated in 2017), and all tanks were fed to satiation. Sampling continued at 10-week intervals until fish were terminally sampled in September. During non-lethal sampling, fish were anesthetized, length and weight were recorded, a Fatmeter reading was taken, a blood sample was taken, and fish were injected with oxytetracycline and emamectin. During lethal sampling, in addition, fish were terminated, dissected, and ovary and liver weights recorded. Mortalities were recorded daily. Only fish positively identified by PIT tags through the entire experiment were included in the analysis. Fulton's condition factor (K), specific growth rates, and organo-somatic indices were calculated as previously described (Jenkins et al. 2020; Jenkins et al. 2019). Plasma estradiol (E2) levels were assayed and reproductive status of individual fish was classified at 30 weeks as previously described (Jenkins et al. 2019). Plasma growth hormone (GH) levels were assayed as described in Section 3.C. The effects of experimental treatment

and reproductive status on endocrine, growth, and energy metrics were analyzed by 2-way-ANOVAs at each time point. Differences within a time point were analyzed by T-tests, and differences among time points were analyzed by Tukey tests. Years and takes were pooled for analysis of plasma hormone levels and growth and energy metrics. Plasma E2 and GH levels were log transformed and body lipid levels were arcsine-square-root transformed prior to analysis.

Results

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

Survival decreased substantially from take 1 to take 3 in 2017 and take 1 to take 2 in 2018, and maturation rates also varied between takes (Table 3B.1). To account for this variation, the ratio of survival rates and maturation rates between fasted and fed treatments was calculated for each take and compared to 1 using one sample T-tests (n=5). Survival was not significantly affected by feeding treatment (average fasted:fed ratio 1.051; one-sample T-test, p=0.7566). There was a tendency toward decreased maturation in fasted treatments, however this effect did not reach statistical significance (average fasted:fed ratio 0.737; one-sample T-test, p=0.1234).

Both feeding treatment and reproductive status affected plasma E2 levels (Table 3B.2). The data are presented to illustrate the effect of reproductive status (Figure 3B.1). As expected, E2 was elevated in reproductive versus non-reproductive fish at 20- and 30-weeks post-spawning. In contrast to previous results, there was a slight but significant difference in plasma E2 levels at spawning, but this was only found in the fed treatment. Plasma E2 levels decreased from spawning to 10 weeks, and then diverged between reproductive and non-reproductive groups, increasing substantially in reproductive fish while remaining low in non-reproductive fish.

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

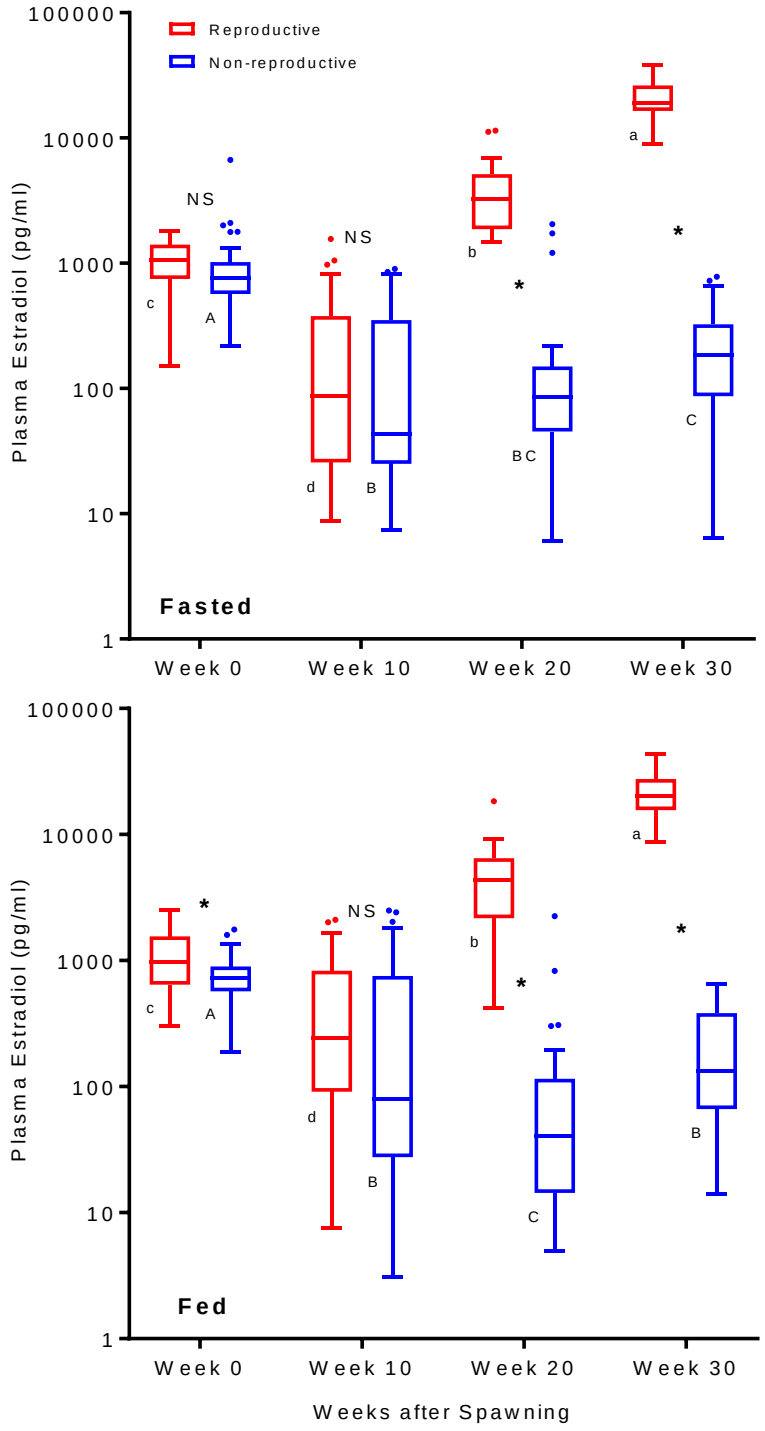


Figure 3B.1: *Plasma estradiol levels over the course of the experiment in reproductive and non-reproductive fish. Fish that were fasted over the first 10 weeks and those that were fed are shown separately. Asterisks or NS indicate significant or non-significant differences, respectively, at each time point. Letters indicate differences over time within each group.*

Feeding treatment, reproductive status, and the interaction of these two factors affected plasma GH levels (Table 3B.2). The data are presented to illustrate the effect of reproductive status (Figure 3B.2). In the fasted fish, plasma GH levels were significantly elevated in non-reproductive versus reproductive individuals at spawning. In contrast, in the fed fish, there was no difference or any trend toward a difference in GH between reproductive and non-reproductive individuals at spawning. This difference accounts for the significant interaction between feeding treatment and reproductive status at spawning. Fasting increased GH at 10 weeks after spawning in both reproductive and non-reproductive individuals. GH was significantly elevated in non-reproductive versus reproductive individuals from 10 weeks onward in the fed fish. A trend in this direction was found in fasted fish, but the differences were not significant until 30 weeks. After 10 weeks, GH decreased over time in all groups. Feeding treatment, reproductive status, and the interaction of these two factors affected growth rates (Table 3B.3). The data are presented to illustrate the effect of feeding treatment (Figure 3B.3). Fasting reduced weight growth rate over the fasting period. Fasted fish then showed elevated weight growth versus fed fish over the 10-20-week period after food was made available. The elevated growth in the fasted group continued over the 20-30-week period in non-reproductive fish, but was not significant in reproductive fish. Length growth was similar. Growth was low or negative (negative for length) over the first 10 weeks after spawning in all groups and increased strongly from the first 10 weeks to the second 10 weeks for both weight and length.

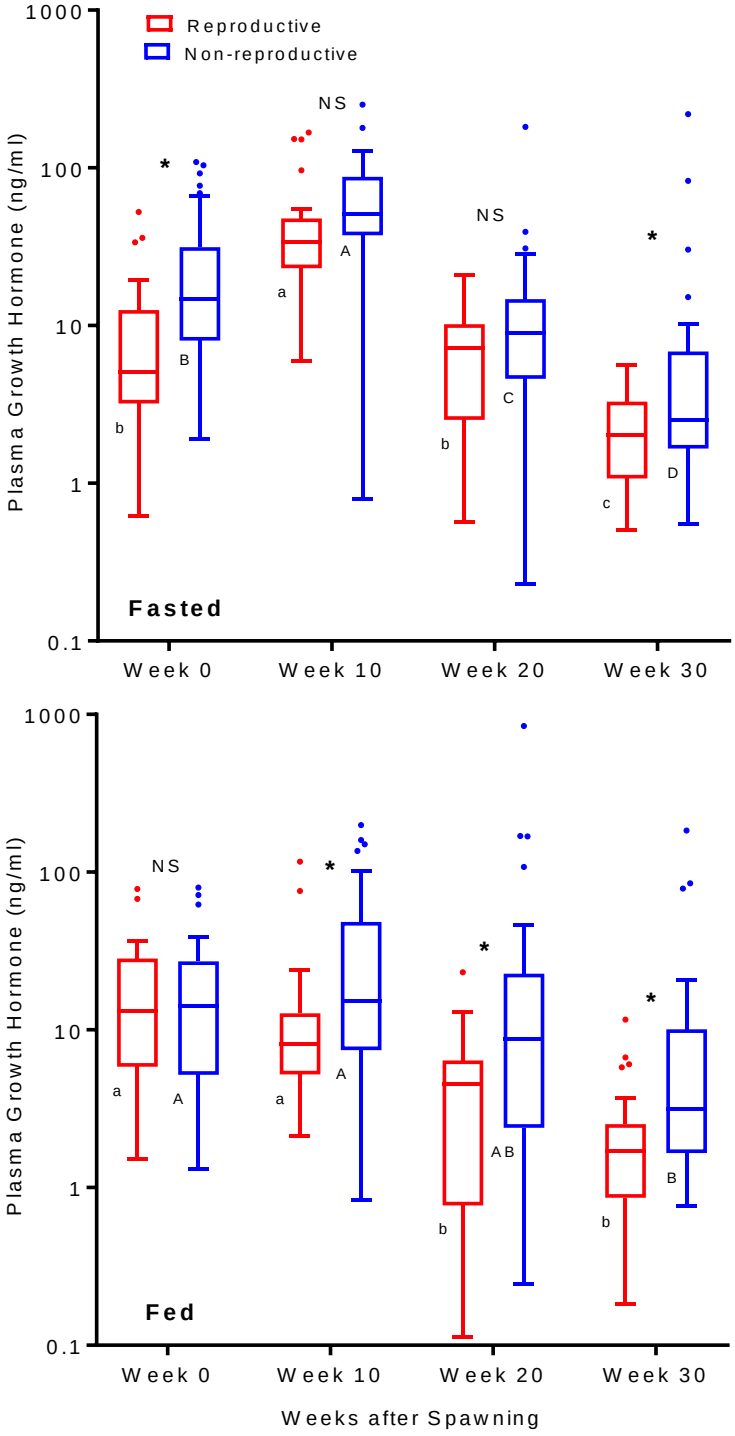


Figure 3B.2: *Plasma growth hormone (GH) levels over the course of the experiment in reproductive and non-reproductive fish. Fish that were fasted over the first 10 weeks and those that were fed are shown separately. Asterisks or NS indicate significant or non-significant differences, respectively, at each time point. Letters indicate differences over time within each group.*

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**

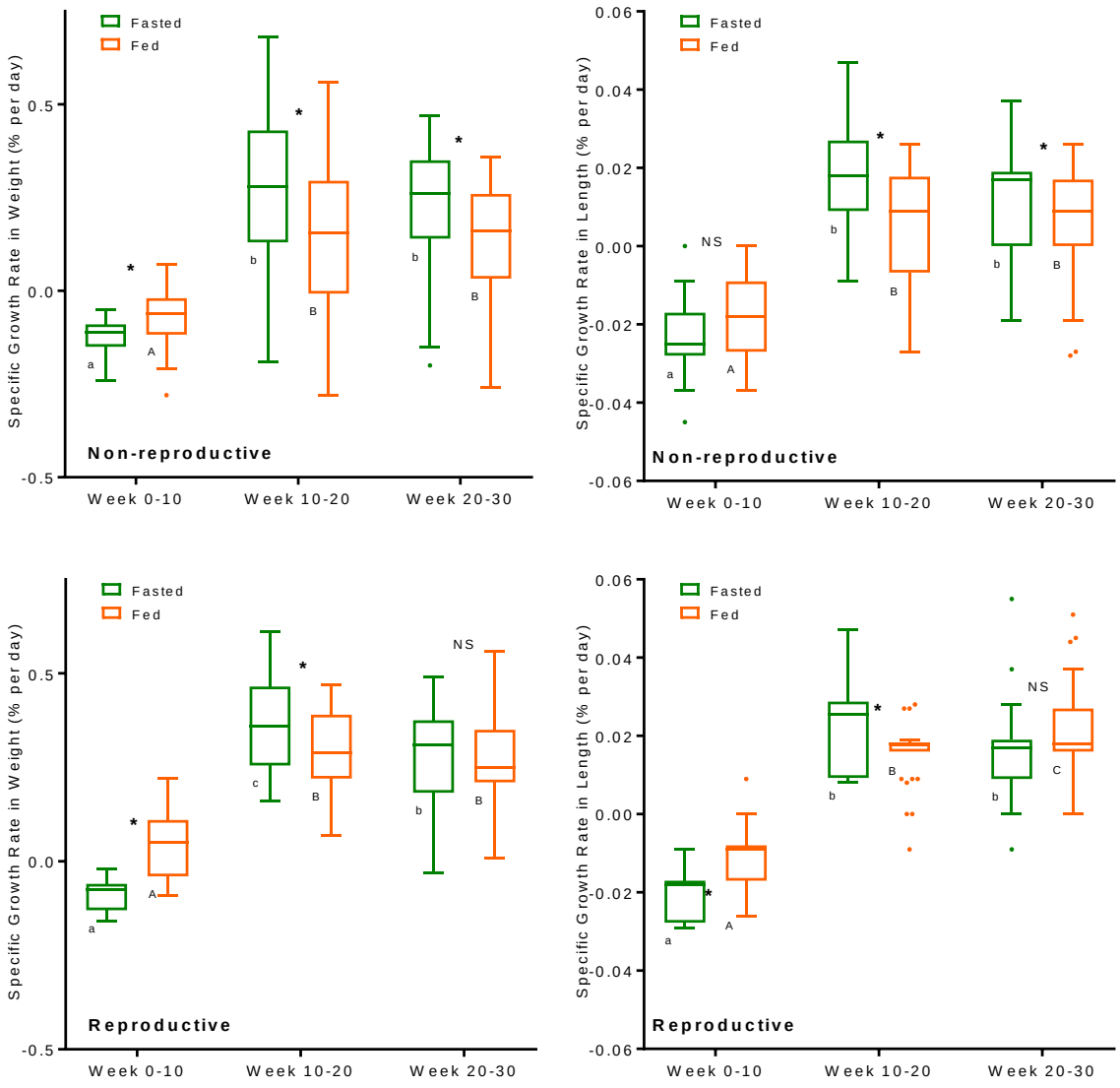


Figure 3B.3: *Growth rates over the course of the experiment in fasted and fed fish. Fasted fish were fasted over the first 10 weeks of the study. Reproductive and non-reproductive fish based on plasma E2 at 30 weeks are shown separately. Asterisks or NS indicate significant or non-significant differences, respectively, at each time point. Letters indicate differences over time within each group.*

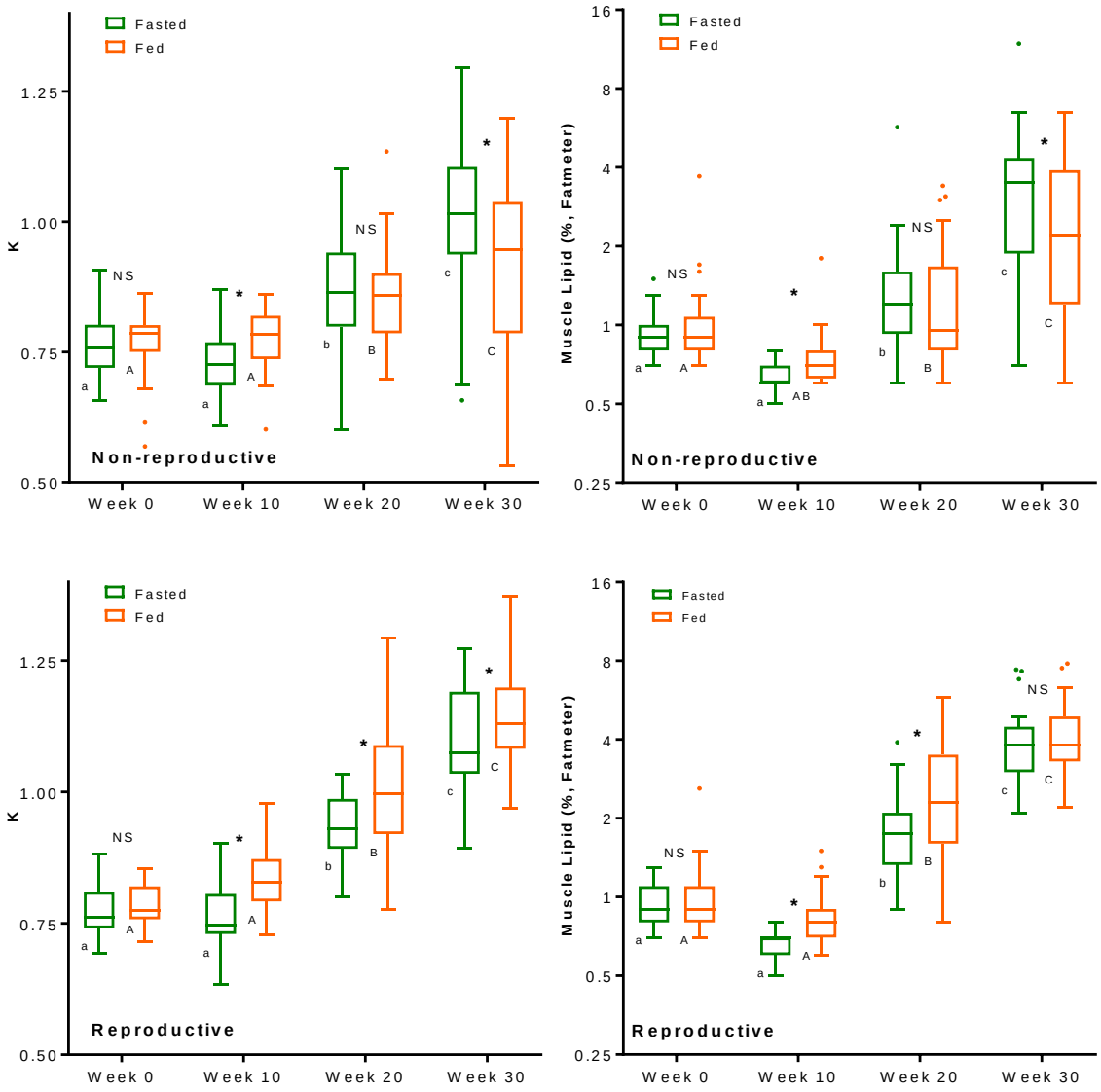
Feeding treatment, reproductive status, and the interaction of these two factors affected the energy reserve metrics condition factor (K) and muscle lipid level (Table 3B.4). The data are presented to illustrate the effect of feeding treatment (Figure 3B.4). Both energy reserve metrics were reduced in fasted versus fed fish at 10 weeks, among both reproductive and non-reproductive fish. In reproductive fish, fed fish maintained significantly higher levels of K and muscle lipid levels than fasted fish after the fasting period had ended, except that the difference in muscle lipid level at 30 weeks was not significant. Both energy metrics showed a trend toward compensation among non-reproductive fish, increasing to greater levels in fasted

than fed fish after the fasting period had ended, although these differences were not always significant.

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.4: *Condition factor (K) and muscle lipid level (%, measured using the Fatmeter) through the course of the experiment in fed and fasted fish. Fasted fish were fasted over the first 10 weeks of the study. Reproductive and non-reproductive fish based on plasma E2 at 30 weeks are shown separately. Asterisks or NS indicate significant or non-significant differences, respectively, at each time point. Letters indicate differences over time within each group.*



Discussion

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 the fed group had slightly elevated plasma E2 levels at spawning versus non-reproductive fish, with a similar trend in fasted fish. 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), and non-significant trends toward increased E2

at the 10-week time point have sometimes been apparent (Jenkins et al. 2019) (this study). These early differences have typically been quite small, and consequently not useful from a practical standpoint for predicting maturation rate or classifying fish. Their biological significance, if any, is not clear.

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. In contrast, in the fed group, non-reproductive fish first had significantly higher plasma GH levels at 10 weeks post-spawning, and this difference was maintained through the rest of the experiment. This suggests that fish that fed less and were more catabolic during the 10 weeks following spawning, despite being offered food, were less likely to become reproductive. Elevated GH at 10 weeks in non-reproductive fish is the earliest hormonal indicator of consecutive versus skip spawning that has been identified to date, and matches 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.

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). Assay of plasma IGF1 levels from this experiment is currently underway.

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. 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. Although the reduction in consecutive spawning rate in fasted fish was not statistically significant, in this case this was likely due to low statistical power. Further analysis using a logistic regression statistical model is needed here. 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 clearly responded to fasting treatment, showing a reduction versus fed fish at 10 weeks. Consistent with the minimal growth observed over the first 10 weeks after spawning, K and muscle lipid level did not increase from week 0 to week 10 in fed fish. 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: Development and validation of time-resolved fluorimmunoassays for salmonid plasma growth hormone and insulin-like growth factor-I

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

Introduction

In teleosts, as in other vertebrates (including mammals), the growth hormone (GH)/insulin-like growth factor (IGF-I) axis is the major endocrine system regulating growth, indicating a 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 (GHR) 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 teleost 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; Kato, et al. 2002; Lupu, et al. 2001; Perez-Sanchez and Le Bail 1999; Perez-Sanchez, et al. 1992; Tannenbaum 1993; Wood, et al. 2005b).

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 axis. Fasting normally leads to increased GH 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 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 an important component in 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 1998; Mommsen, et al. 1980; Navarro and Gutierrez 1995).

As the primary mediator of the growth-promoting effects of GH, IGF-I affects many biological processes, making it a good candidate as a growth index (reviewed by Perez-Sanchez et al. 2018). However, as reviewed by Beckman (2011), the correlation between IGF-I and growth ranges from reliable to non-discernable, reflecting a changing and sometimes confounding range of endogenous and exogenous factors. For instance, the true effects of different photoperiods on growth and plasma IGF-I level, and how the two are related, are difficult to discern even in well studied species of trout and salmon (Beckman, et al. 1998; Pierce et al. 2001; Taylor, et al. 2005). Consequently, IGF-I is believed to be a reliable growth index only over precisely defined groups of fishes. The importance of growth in fish life histories, and the role of GH and IGF-I in growth, suggests that GH and IGF-I may be important regulators of other physiological systems such as the corticotropic and reproductive axes (Benedet, et al. 2010; Bjornsson, et al. 1994; Campbell et al. 2006; Taylor, et al. 2008; Xu, et al. 2017). For example, the decision in salmonids to become smolts or mature precociously is believed to be linked to growth and fat depositions several months prior to the necessary biological transformations (Larsen, et al. 2006; Medeiros, et al. 2018; Silverstein, et al. 1998; Thorpe 2007). This is likely a consequence of the fact that life history decisions are not fixed and often depend on critical size and sufficient energy at a specific stage ("critical period") several months prior to biological transformations (Taranger, et al. 2010; Thorpe 2007; Thorpe, et al. 1998).

Having an understanding of the GH/IGF-I axis at specific points in a fish's life history will help aid in determining how best to manage that species. While major steps have been achieved in understanding this complex endocrine system, knowledge of some key aspects is still lacking. Exploring the relationships between energy balance and reproduction requires a thorough understanding of the GH/IGF-I axis and how it functions in different situations; however, investigations are currently hindered by the lack of commercially available assays to determine plasma GH and IGF-I in rainbow trout. The development of such assays will accelerate studies aimed at elucidating the role of the GH/IGF-I axis in various physiological functions of rainbow trout and other salmonids. Thus, the aims of this study were to develop, optimize, and validate non-radioisotopic time-resolved fluorimmunoassays (TR-FIAs) for determination of blood plasma GH and IGF-I in rainbow trout. Further validation was provided by examining the effect of fasting on plasma GH and plasma IGF-I in rainbow trout.

Materials and methods

Fish

Fish from several sources were utilized throughout the work presented in this manuscript. Rainbow trout 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). Spring Chinook salmon used in this study were progeny of fish spawned in 2016 at the Cle Elum Supplementation Research Facility (CESRF) in Cle Elum, Washington, and were cared for as described by Medeiros et al. (2018). All fish rearing and sampling procedures followed

guidelines approved by the Institutional Animal Care and Use Committee at the University of Idaho.

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 (Australia). Polyclonal rabbit anti-barramundi (*L. calcarifer*) IGF-I and anti-salmon/trout (genus *Oncorhynchus*) GH were also purchased from GroPep (Australia). Custom recombinant rainbow trout GH (*gh1*, GenBank Accession number P09538.2) and gilthead seabream (*Sparus aurata*) IGF-I was purchased from Prospec (Israel). 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).

Peptide and hormone labeling

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

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×.

TR-FIA for GH

The assay was performed in goat anti-rabbit-coated yellow 96-well plates (PerkinElmer) and run over the course of 4 days, with 3 overnight incubations. The standard curve consisted of 8 serially diluted points, ranging from 500-0.03 ng/mL, with 4-fold dilutions. On the first day, the 96-well plate was washed 5x with 300 μL of Delfia® wash solution (PerkinElmer) before adding Delfia® assay buffer (DAB; PerkinElmer) and 20 uL of the rabbit anti-salmon/trout GH polyclonal antiserum (diluted 1:5000; GroPep, 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 (GeneMate). On the second day, the plate was washed 5x with 300 μL of Delfia® wash solution 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 5x with 300 μL of Delfia® wash solution. 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 Inc., 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 $\%B_0$ ($(B/B_0)\times100$). 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 over the 80% to 20% binding range. 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 $(B_0/TC)\times100$.

TR-FIA for IGF-I

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 -20ºC 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 hours. 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 37ºC for 30 min. Samples used in the extraction comparison assays were made up from recombinant barramundi IGF-I. The Extracted & Dried Down samples followed

the protocol outlined above, whereas the Extracted samples followed the protocol up until the final centrifugation, and then the supernatent 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. The standard curve was prepared at 7-times the normal concentration to account for the dilution factor inherent to this extraction process. In both extraction procedures, the entire standard curve was prepared and then extracted. The extracted samples were run in triplicate.

TR-FIA for IGF-I

The protocol for this IGF-I time-resolved fluoroimmunoassay (TR-FIA) is based on Small and Peterson (2005), Ferriss, et al. (2014), 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 400-0.024 ng/mL, with 4-fold dilutions. The rabbit anti-barramundi IGF-I polyclonal antiserum was diluted 1:1500 (GroPep, 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.

In vivo experiments

Injection Experiment

To determine if exogenous GH increases plasma IGF-I in rainbow trout, fish were intraperitoneally injected with either vehicle or vehicle containing bGH. While under anesthetic, fish were intraperitoneally injected with either 1 μL vehicle×g fish^{-1} alone (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* = 10]). 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 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.

Fasting Experiment

To assess the effects of fasting on the GH/IGF axis in rainbow trout, an experiment was conducted. For the duration of the experiment, fish were held in 1,130 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} L. Fish were fed to satiation 5 days a week with a standard commercial trout diet (5.5 mm pellets; Skretting USA, Tooele, UT). On February 20th, 2019, 22 fish with an average mass of 686.3 ± 25.6 g were split into two groups (*N* = 11 each

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. On March 20th, 2019 (4 weeks after the start of the experiment), all fish were lethally sampled (see Sampling Procedures below). Throughout the experiment, fasted fish were observed closely for signs of opportunistic infections and pathogens. Any signs of unusual behavior, discoloration of the integument, and/or lesions were evaluated. It was not necessary to employ early euthanasia for any study fish.

Sampling Procedures

Lethal sampling consisted of recording (1) total body weight and length and (2) 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 (Sigma, 10 mg/mL). Fish were individually PIT tagged at the initial sampling, and PIT tag codes recorded at all samplings. 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 -80ºC until laboratory analysis.

Statistical Analyses

The experimental data were analyzed using Prism 8.2 (GraphPad Software, Inc; www.graphpad.com). 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 analysis evaluated over the usable portion (20-80% of maximum binding) of the standard curve in Prism 8.2. For all analyses, the level of significance was evaluated at $P \leq 0.05$, and values are expressed as the average ± the standard error of the mean (S.E.M.).

Results

Eu-GH was labeled to a ratio of 7.6 europium chelate molecules per molecule of GH protein. This gave approximately 120,000 counts at the label concentration employed. The primary antibody bound approximately 20% of the label in the B0 wells at the primary antibody concentration employed, and after some primary antibody loss that presumably occurred due to the initial step of incubation of the plate with the primary antibody followed by washing. Specific binding of the europium-labeled GH was displaced by increasing amounts of unlabeled GH (Fig. 1). Serial dilutions of pituitary homogenate (Fig. 3C.1) and blood plasma (Fig. 3C.2) from rainbow trout were parallel to the standard curve (p = 0.10 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, defined based on the mean count of the zero standard minus two standard deviations and then expressed as B/B_0 and used to interpolate a value from the standard curve, was 0.22 ± 0.08 ng/mL (n = 8). The intra- and inter-assay coefficients of variation were 4.1% and 13.4%, respectively. Interspecies specificity of the primary antibody was further 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; $p < 0.05$). During development and validation, it was discovered that the assay requires 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 plasma sample was run repeatedly at small dilutions (2-10-fold), interpolated values were consistent after adjustment for dilution, and this GH value should yield a %B over 20% when run neat.

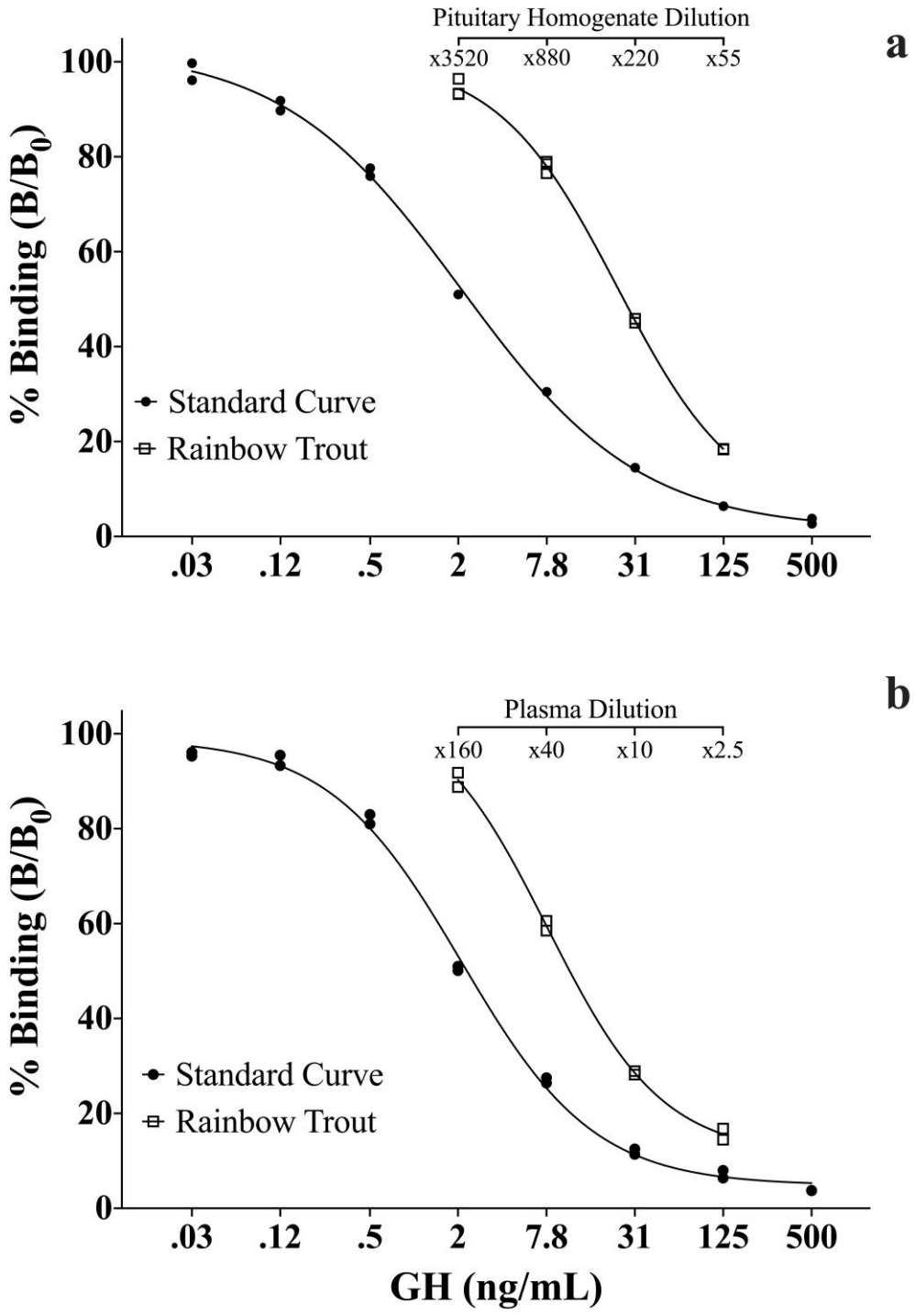


Figure 3C.1. Displacement curves for recombinant rainbow trout GH assay standards, serially diluted rainbow trout pituitary homogenate (a), and serially diluted rainbow trout blood plasma (b).

Eu-IGF-I was labeled to a ratio of 2.3 europium chelate molecules per molecule of IGF-I protein. This gave approximately 95,000 counts at the label concentration employed. The primary

antibody bound approximately 25% of the label in the B0 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. 3C.2; $p = 0.83$). 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. 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; $p = 0.22$ and 0.97, respectively).

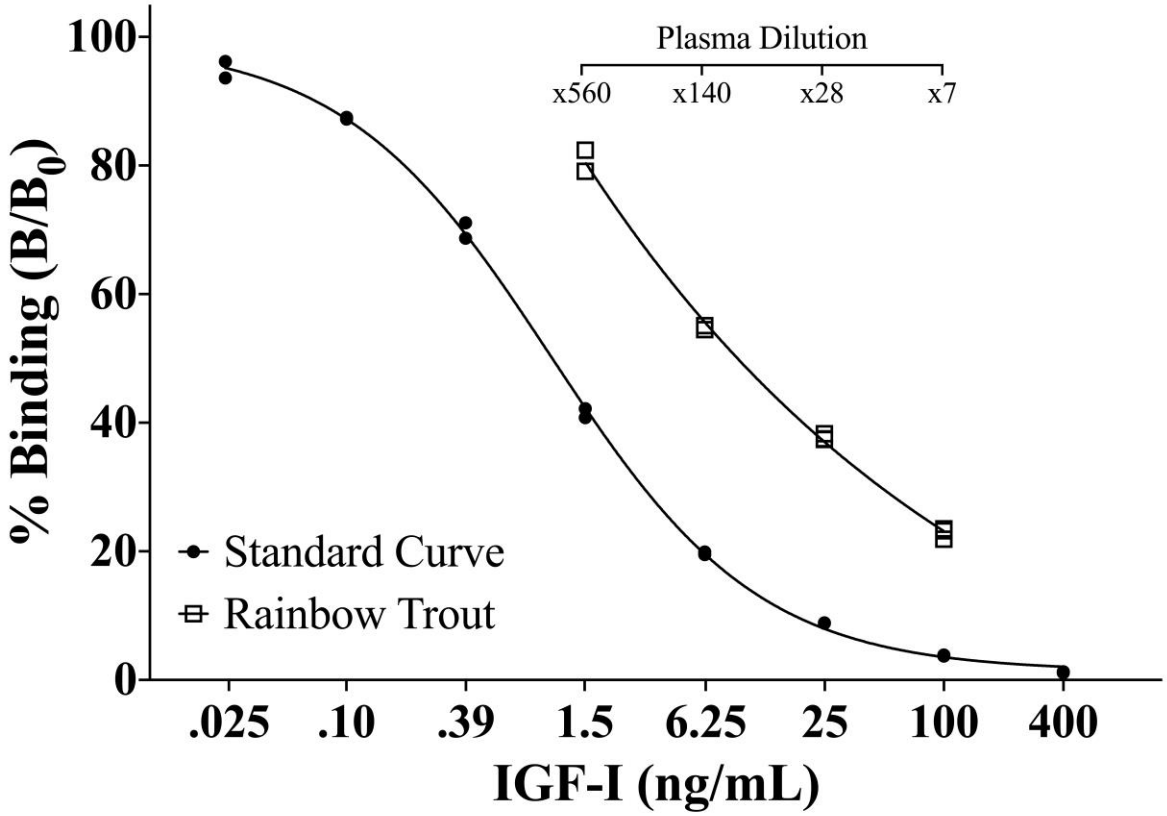


Figure 3C.2. Displacement curves for recombinant barramundi IGF-I standards and serially diluted rainbow trout blood plasma.

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. 3C.3; top parameter values from curve fit equation were significantly different, $p < 0.05$). 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), likely due to the doubling of the concentration of buffer chelating agents and ions in when reconstituting in DAB. 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.

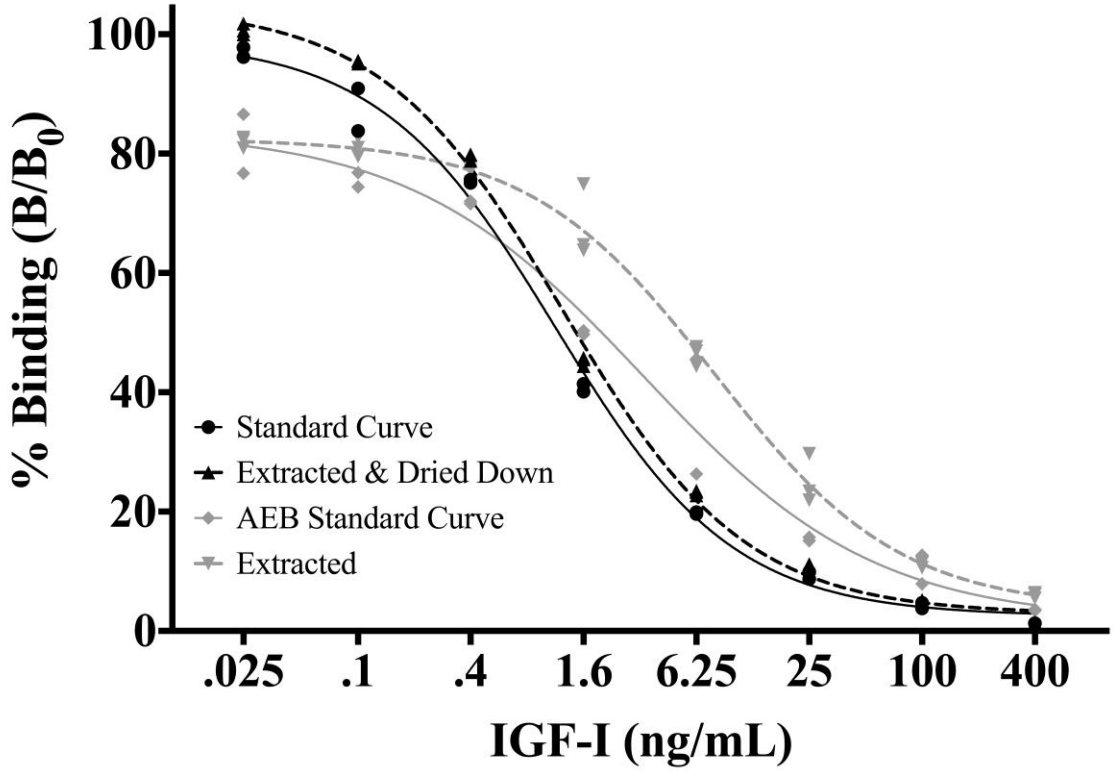


Figure 3C.3. Displacement curves for recombinant barramundi IGF-I standards that have been extracted and run using the extraction and TR-FIA procedures outlined in this study (black symbols and lines) compared to the displacement curves for recombinant barramundi IGF-I standards that have been extracted and run using the unmodified extraction protocol (grey symbols and lines), which does not remove the ethanol from the assay.

Using the TR-FIA, the response of circulating IGF-I to intraperitoneal (IP) injection with bovine GH (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 ($p < 0.01$, Fig. 3C.4).

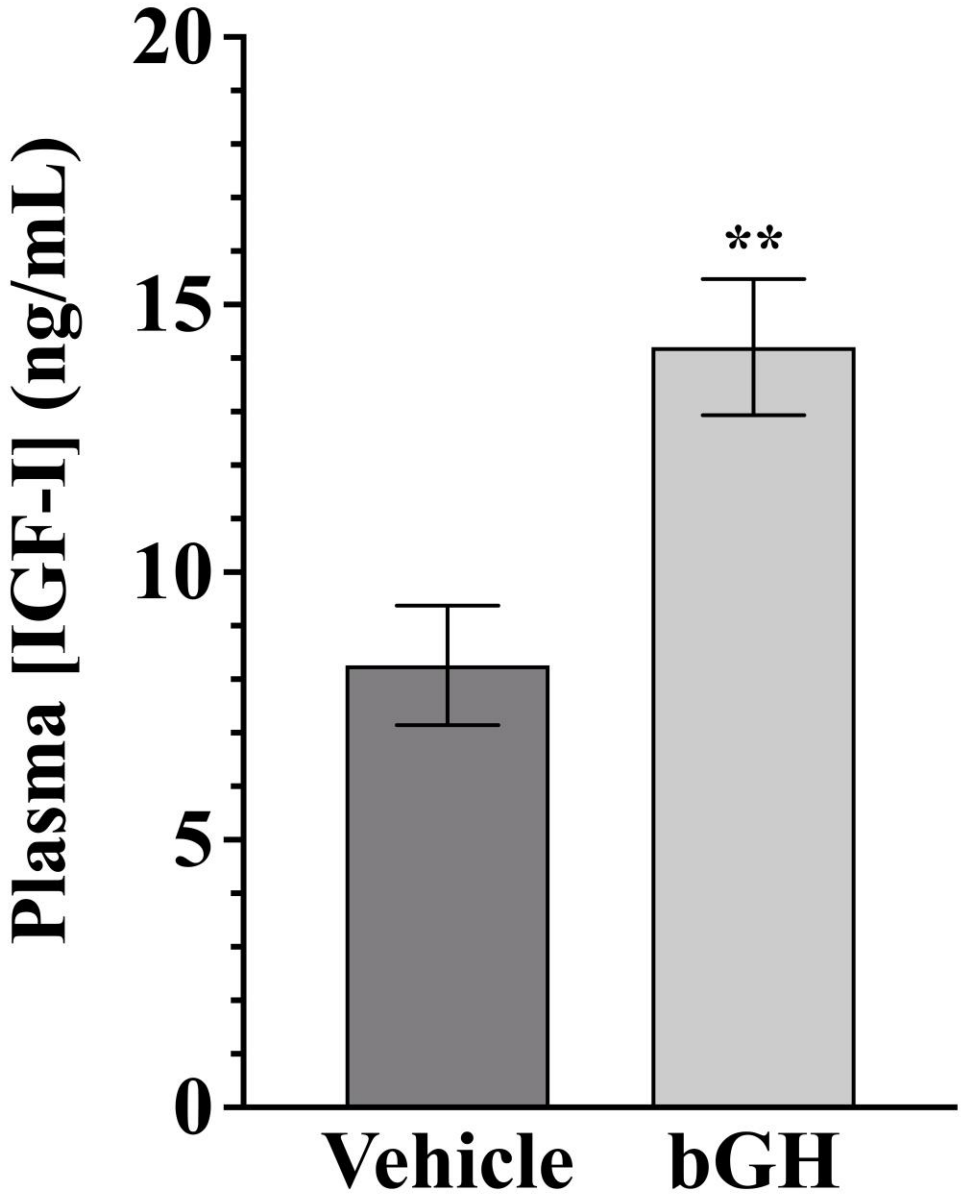


Figure 3C.4. Effect of vehicle alone or vehicle with bGH on plasma IGF-I levels 3 hours post-injection. Values are expressed as means ± SEM (n = 10). Asterisks indicate significant differences (p < 0.01) between treatments.

Following a four-week period of fasting, fasted fish weighed significantly less than the fed treatment group ($p < 0.001$) which corresponded to a 12.5% decrease in weight compared to their initial weight versus the 32.9% increase that the fed group experienced (Fig. 3C.5). This expected decrease in weight was accompanied by a significant increase in plasma GH levels ($p < 0.01$, Fig. 3C.6a) and a significant decrease in plasma IGF-I levels ($p < 0.01$, Fig. 3C.6b).

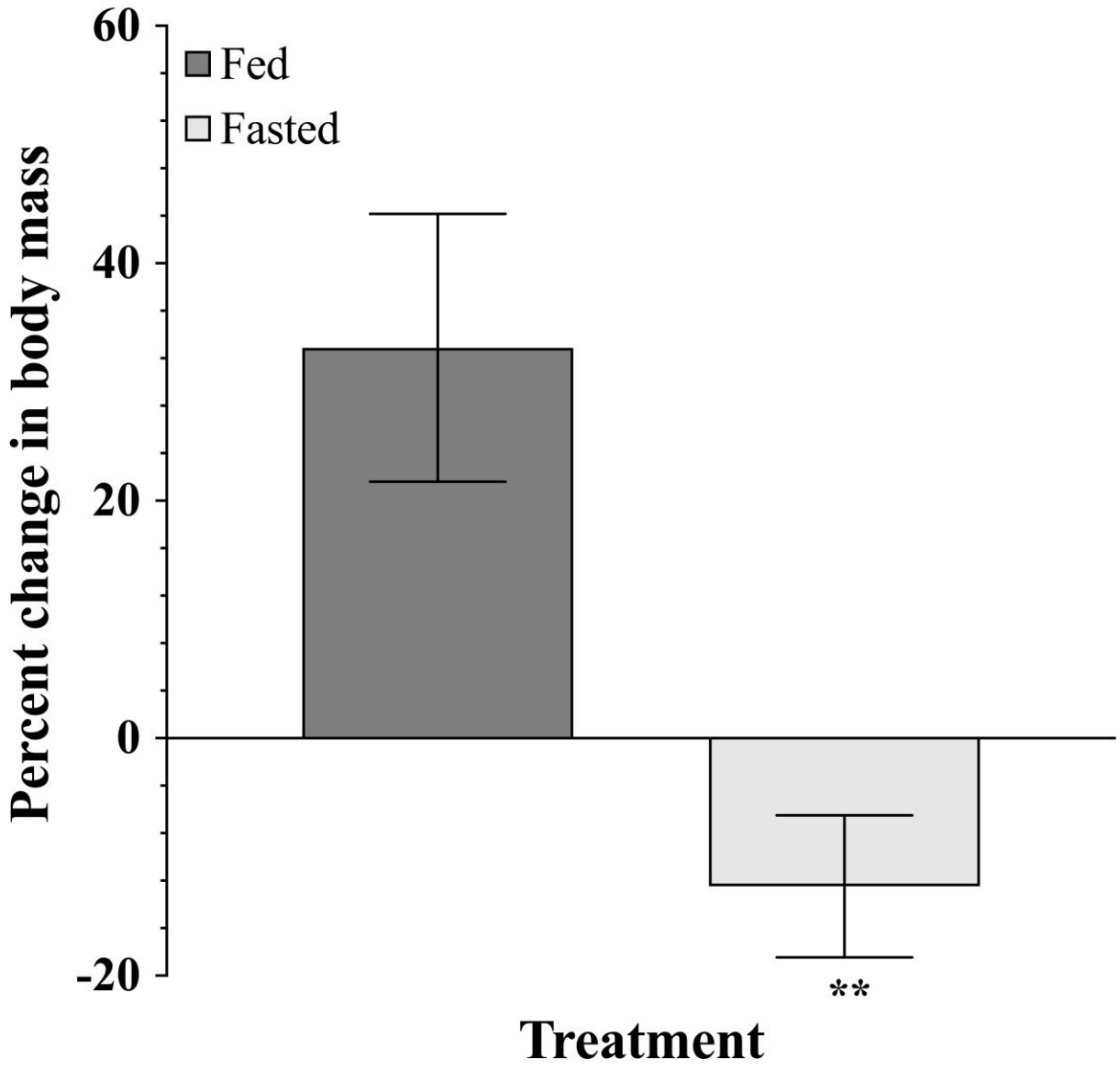


Figure 3C.5. Percent change in body mass of rainbow trout fasted for 4 weeks (mean ± SEM; n = 9). Fasting had a significant ($p < 0.01$) effect on body mass.

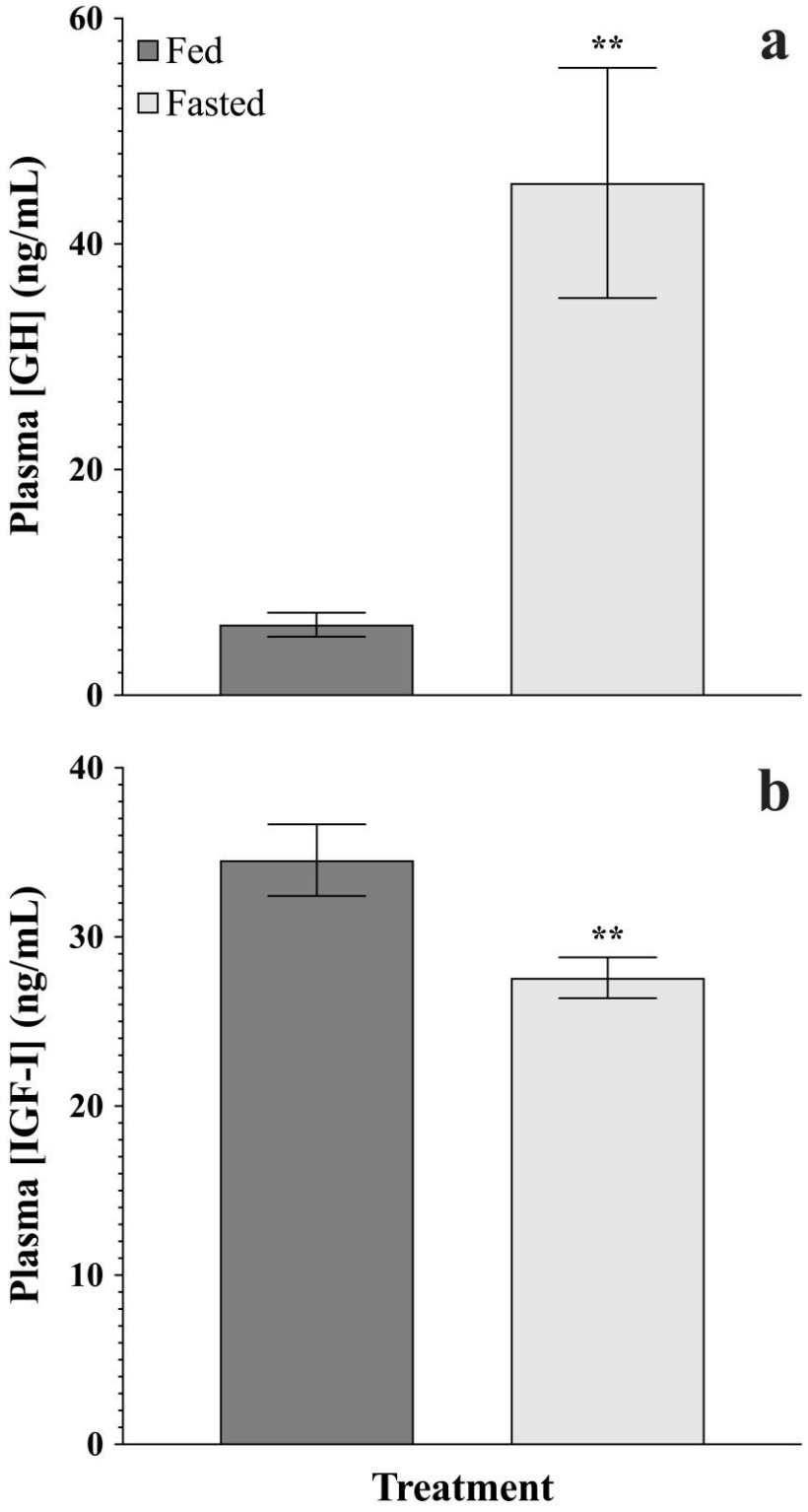


Figure 3C.6(a&b). Effect of fasting rainbow trout for 4 weeks on plasma GH (a) and IGF-I (b). Values are expressed as means ± SEM (n = 9). Asterisks indicate significant differences (p < 0.01) between treatments.

Discussion

The present study developed and validated a TR-FIA for rainbow trout GH which, to the best of our knowledge, is the first GH TR-FIA validated for use in teleosts. This study also established modifications to the standard IGF binding protein extraction procedure, and modifications to an existing TR-FIA IGF-I for use in rainbow trout, resulting in an improved assay and more efficient work flow. 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 low-cost, safe alternative to radioimmunoassays (RIAs) that provide comparable sensitivity. 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 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 indicated 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. Thus, the interference could be due to interactions of plasma components with the primary antibody or label. Before assaying new types of samples, a dilution series should be performed to ascertain the dilution necessary to ameliorate the matrix interference. This study found that a 2-fold dilution was enough to ameliorate the effects of the matrix interference in adult rainbow trout. While no interference has been previously described, RIA protocols employing the same antiserum (Le Bail et al. 1991; Wilkinson et al. 2006) incorporate at least a 4-fold plasma dilution and would thus not have observed the interference if present. The sensitivity of the assay was sufficient to handle the 2-fold dilution in the plasma samples employed, and was similar to the sensitivity of the RIAs taking the dilution into account. Considering the similar assay quantification limits of RIAs validated for use in teleosts, this GH TR-FIA provides a safe, convenient, and cost-comparable alternative.

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. 2005b). Quite a bit of information exists regarding the function and regulation of IGFBPs (reviewed byDuan and Xu 2005), while far less exists for GHBPs (Baumann 2002; Fisker 2006). The vast majority (99%+) of IGF-I circulates bound to IGFBPs, which interfere in IGF-I assays, thus plasma IGF-I cannot be quantified without first removing IGFBPs (Shimizu et al. 2000). In contrast, extraction procedures for GHBP are not employed in salmonid GH assays. In the present study, it was determined that 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 B0 and increased variability between replicates in the IGF-I TR-FIA.

Therefore, ethanol was removed by drying down and reconstituting samples, which restored binding and reduced variability. This modification has the additional benefits of enabling separation of the days on which the samples are extracted and run, since samples in extraction buffers cannot be stored, and enabling concentration of samples. 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.

In the current study, extraction efficiency was calculated by interpolating the values at 20, 50, and 80% binding from an unextracted versus an extracted and reconstituted standard curve and 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 acid 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. Because the extraction efficiency in fish plasma is not known, fish IGF-I RIA and TR-FIA procedures do not include correction for extraction efficiency. 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, showing that the assay is valid for use in rainbow trout and suggesting that it will work in the other 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 using addition of the primary antibody, standards/unknowns, and label on the first day, followed by a single overnight incubation (Hevroy et al. 2013; 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. However, the increased sensitivity requires a longer time to run the assay. Even so, the present IGF-I TR-FIA provides 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. GH is a major positive regulator of liver IGF-I production, and increases circulating IGF-I under fed conditions (Moriyama et al. 2000; Norbeck et al. 2007; Wood et al. 2005a). A sample collected 12 hours post-injection confirmed that bGH elicited a significant increase in circulating levels of IGF-I, in agreement with a previous study 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 teleosts and mammals alike, fasting increases blood plasma GH (Bjornsson et al. 2018; Duan and Plisetskaya 1993; Pierce et al. 2001; Pierce et al. 2005b; 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. 2005b). 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 by an average of 32% whereas fasted fish experienced an average 12% 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 (Bergan-Roller and Sheridan 2018; Kelley, et al. 2000; Norbeck et al. 2007). 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; Rousseau et al. 1998; Tannenbaum, et al. 1983; Uchida, et al. 2003; Yamashita and Melmed 1986). The pattern of increased GH and reduced IGF1 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 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. The use of plasma IGF-I level as an indicator of growth status is well established (Beckman 2011; Perez-Sanchez et al. 2018; Picha et al. 2008a; Pierce et al. 2001). Plasma GH level is less used for this purpose, but shows promise as an indicator of metabolic status under catabolic conditions. GH increases continuously during fasting, and may indicate stages of nutrient mobilization in the fasting response, whereas the decrease in IGF-I plateaus (Bar 2014; Bjornsson et al. 2018; Larsen, et al. 2001; Pierce et al. 2005b). However, plasma GH levels are more variable than IGF-I levels due to episodic pituitary GH secretion (Gomez, et al. 1996). A positive role for IGF-I in the gating of puberty in fishes is supported by both in vivo associative studies and in vitro mechanistic studies showing a linkage at the level of pituitary gonadotropin secretion status (Baker et al. 2000; Benedet et al. 2010; Campbell et al. 2006; Huang et al. 1998;

Luckenbach et al. 2010; Taylor et al. 2008; Wilkinson, et al. 2010). GH is a major regulator of smoltification in salmonids (McCormick 2012), as well as the principal regulator of hypoosmoregulation in fishes (Takei and McCormick 2012). Finally, the corticotropic axis interacts with the GH/IGF-I axis at multiple levels (Sadoul and Vijayan 2016). 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.

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, 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

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

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

Steelhead behavior consistent with homing was obtained from PIT detections at Prosser Dam and from recapturing post spawn fish that were previously released as reconditioned kelts. All fish ladders of Prosser Dam were wired with PIT antennas by 2008 Reconditioned kelt steelhead are released below the dam, enabling us to use ladder detections as further evidence that is consistent with homing.

Results and Discussion

The following sources provide conclusive data confirming repeat homing of reconditioned kelt steelhead (Table 4.1). First, in the Yakima River, steelhead tagged (radio or PIT) prior to their first spawning event and detected in tributary streams exhibiting behavior consistent with spawning, were later collected as kelts at the CJMF and reconditioned. Detection (radio and/or PIT) of these fish in the same tributaries during repeat spawning events provides conclusive evidence of repeat homing. In the Yakima River, all 49 fish that we detected as maiden and kelts returned to spawn in the same tributary or were detected again at Roza Dam

(Naches/Upper Yakima population). We have found no evidence of straying in these sampled fish. Second, PIT detections of reconditioned kelt steelhead at in-stream arrays in Satus, Toppenish, Ahtanum creeks, the lower Naches River, and Roza Dam (Upper mainstem Yakima River) accompanied by matching genetic stock identification provides additional conclusive data on repeat and natal homing. The third conclusive data source is from Omak Creek (Okanogan River tributary), where kelt steelhead were collected at a weir migrating out of the stream and following reconditioning were released near the mouth of the Okanogan River, and later detected at the Omak Creek weir on their repeat spawning run.

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

Table 4. 1: Observed and inferred homing from artificially reconditioned kelt steelhead in Omak Creek and the Yakima River from 2001 to 2018. Column A consists of fish with tag detections (PIT or Radio) in spawning tributaries as maiden and repeat spawners. Column B are fish with tag detections in tributaries as repeat spawners and consistent GSI conformation of reporting group (pending). Column D are fish with PRO detections as repeat spawners. Column

	Conclusive Evidence for Homing			Consistent with Homing				
Location	**A. Maiden/ Repeat Spawner Tag Detection**	**B. Repeat Spawner Tag Detection + GSI confirmation**	**C. Conclusive Homing total A+B**	D. **Prosser Det. Only**	**E. Prosser Det./Sunnyside Instream Det (Operational: Nov. 2016)**	**F. Prosser Det./Out-migrating Columbia Mainstem Dam Det. (Spring)**	**G. Post Spawn Repeat Spawner Recaptured at CJMF**	**Total Spawners: Consistent w/ Spawning Movement (Total of D +E+F+G)**
Yakima R	49	313	362	655	56	65	107	883
Omak Cr	11	-	11					
Total	60	313	373	655	56	65	107	883

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

Introduction

In this section we evaluate kelt steelhead management options and we compare three geographically different long-term reconditioning programs. It is thought that downstream passage through the hydrosystem limits repeat spawner steelhead in the Columbia River (Wertheimer and Evans 2005; Wertheimer 2007). In recent years, there may be some evidence that emigrating kelt survival has improved as a result of smolt management actions (e.g. removable spillway weirs, mandated spill). Colotelo et al. (2014) reported that 27.3% of kelts tagged at or upstream of Lower Granite Dam (rkm 695) survived to Martin Bluff (rkm 126) passing 8 hydroelectric dams along the way. Collecting and transporting kelt steelhead around hydroelectric projects could improve emigration survival and result in increased repeat spawner abundance. Our goal is to compare the benefits of long-term reconditioning to alternate kelt management treatments like transporting kelts downstream of the hydropower system. Our team 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 to provide statistically valid data after 2016 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 17,544 kelts released from 2002-2004, 2009-2019.

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 water quality can be an issue for holding kelts for long-periods of time (over winter). The issue is that cold water availability is the largest issue. The water temperature is adequate through the summer months (11^0C) but is on the warmer side when compared against ocean temperatures during winter which should be about 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. At the end of October of 2019 103 non-rematuring kelts were trucked from Prosser and released just below Bonneville Dam. We anticipate that we should see results from the transport in July of 2020 which we will report in the 2020 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 (Table 5.1 and Figure 5.1). All upstream migrating fish at McNary Dam pass through PIT tag detection systems in a fish ladder.

An alternate analysis compares the net survival benefit for the two transport treatment groups by dividing the return rates to BON for the treatment by control groups. This yields a number that represents the relative positive or negative benefit of the treatment. For example, if your treatment return rate to BON was 4% and the control rate was 2%, the treatment would benefit kelt 2x (4/2=2) versus leaving the kelts in the river. Comparisons were made within each year and across years using weighted means to account for different sample sizes among years. We calculated benefits for long-term reconditioned kelts from the Yakima River, Omak Creek, Hood River, and Snake River in a similar manner. The reconditioning benefits calculation was the survival rate of long-term reconditioned kelts from each location divided by three different control groups. The control groups were: 1. Survival rates of in-river release groups to BON (the

same as the treatment groups). 2. Literature values (Hockersmith et al. 1995). 3. The composition of repeat spawners in the run at large sampled at BON based on scale pattern analysis and prior PIT-tag history. None of these control groups are perfect comparisons, for example survival of the in-river release groups is to BON not the river of origin so these are biased high due to mortality that likely occurs between BON and the river of interest. However, the in-river groups are paired by year with the treatment groups reducing annual variation.

Results and Discussion

Long-term reconditioning demonstrated significantly higher return rates of repeat spawners (11-18%) than other treatments (1-3%) (Table 5.1). This result was supported in spite of variation in river, ocean, and fish condition between years that was incorporated into the error term in our analysis. The data extrapolation required in our analysis does not account for variation in environmental or fish conditions between years. However, this method does provide a best and worst-case interpolation of data for earlier years in the long-term reconditioned group, thereby strengthening our ability to draw conclusions among the four treatments. For more in-depth analysis see Trammell et al. 2016.

Table 5. 1: Sample size (N), mean, and grouping output for Tukey post-hoc test from ANOVA of PIT tag detections at Prosser Dam.

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

Survival to release of long-term reconditioned kelt steelhead averaged 42% for the Yakima River, 33% for the Snake River, 15% for Omak Creek, and 36% for Hood River. The Yakima River is represented by 17 the Snake River 4, Omak Creek 9, and Hood River 7 years of data. Figure 5.1 shows relative to control groups, long-term reconditioning groups benefited more than any control group chosen. The highest benefit was to Snake River steelhead kelts in long-term reconditioning were over 80 times higher than fish left in-river.

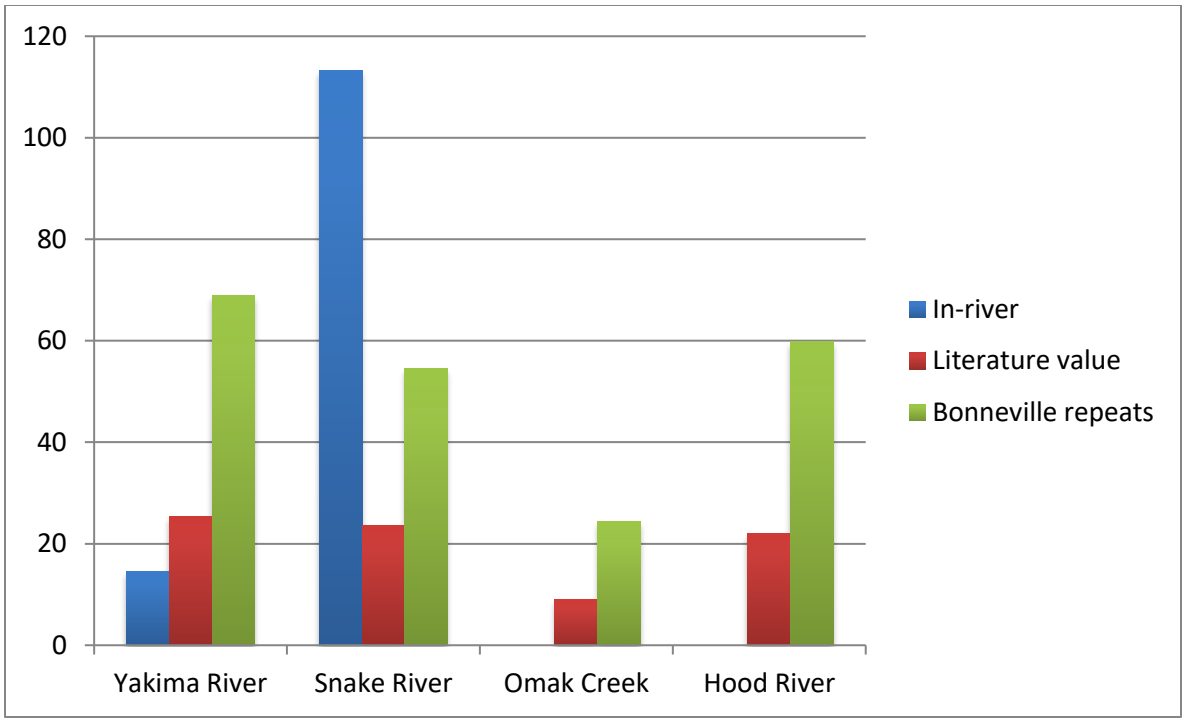


Figure 5. 1: Benefits of long-term reconditioning relative to 3 control metrics. In-river control groups were not available for Omak Creek or Hood River.

Geographic Comparison of Reconditioning Programs

Survival and maturation data from Prosser, Winthrop, and Dworshak 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 2019. 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 4 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, suggesting that survival rates similar to that found in the Prosser project may be achievable with the Snake River fish. This does not include the mortality event that occurred at NPTH in 2019, but only fish held at DNFH. Survival of fish held at Winthrop was similar to Prosser from 2013 to 2016, but has decreased relative to Prosser for the past 3 seasons. The reasons for this change are not known. 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 survivals above 50% are attainable in CRB kelt reconditioning, even in inland populations with a long migration.

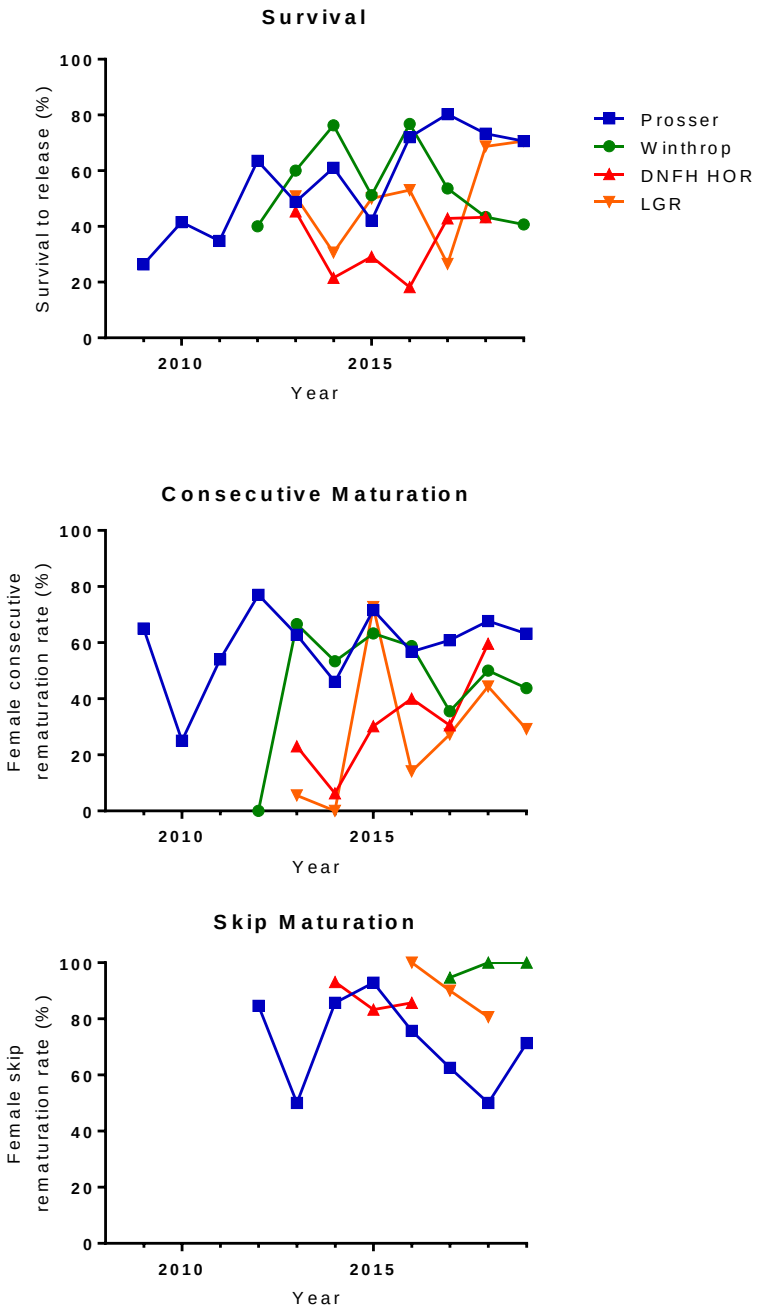


Fig. 5.2: Survival and female consecutive and skip maturation rates in CRB kelt reconditioning projects. Fish reconditioned in the Snake River project were housed at Dworshak and Nez Perce Tribal hatcheries, and include kelts collected at Lower Granite Dam (LGR) and air spawned hatchery origin kelts 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. LGR survival and consecutive maturation rates for 2019 are only for fish held at Dworshak hatchery, and skip maturation data for LGR fish are not available for 2019, due to mortality of skip spawners and 2019 collected fish at Nez Perce Tribal hatchery in 2019.

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). 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. 2013), 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.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

"The Master Plan is well written and contains an excellent summary of the extensive steelhead reconditioning work that has occurred in the Basin. Moreover, we compliment the proponents for investigating and addressing the many difficulties associated with steelhead reconditioning. Numerous challenges associated with fish culture had to be addressed, including establishing appropriate holding and rearing environments, formulating diets, and developing disease

control protocols. The effects of long-term reconditioning on gamete viability, fidelity to natal streams, and ability to reproduce in nature were investigated. Comparisons that evaluated the potential benefits of various kelt treatments that ranged from simple direct transportation past downstream dams to long-term reconditioning lasting from 6 to 20 months were also conducted. In general, the results of these assessments indicated that long-term reconditioning of kelts appears to be a promising approach that might lead to a viable conservation strategy for steelhead.

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

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

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 we anticipate completed designs in 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

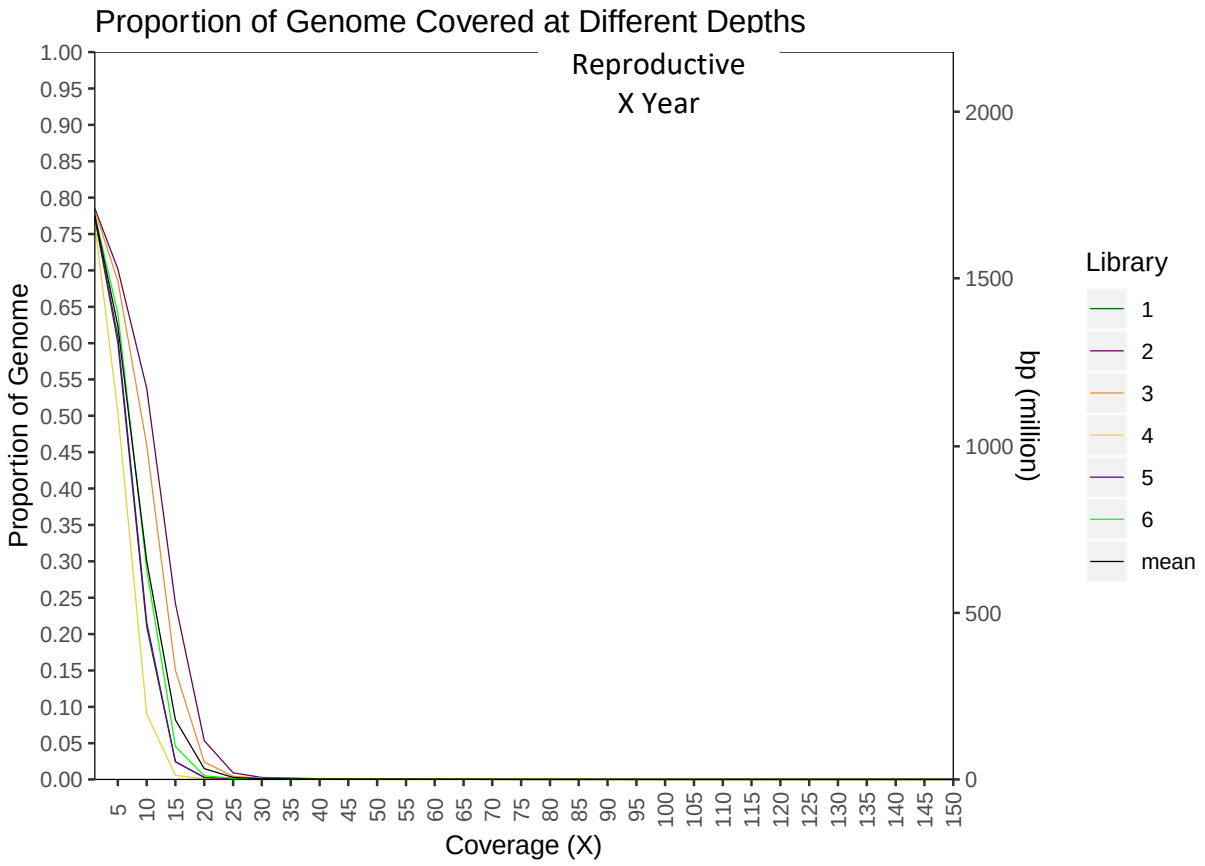
To better understand Snake River steelhead kelt stock composition and how best to focus our efforts in such a large basin, we have been conducting methods to better manage populations utilizing genetic research and analysis.

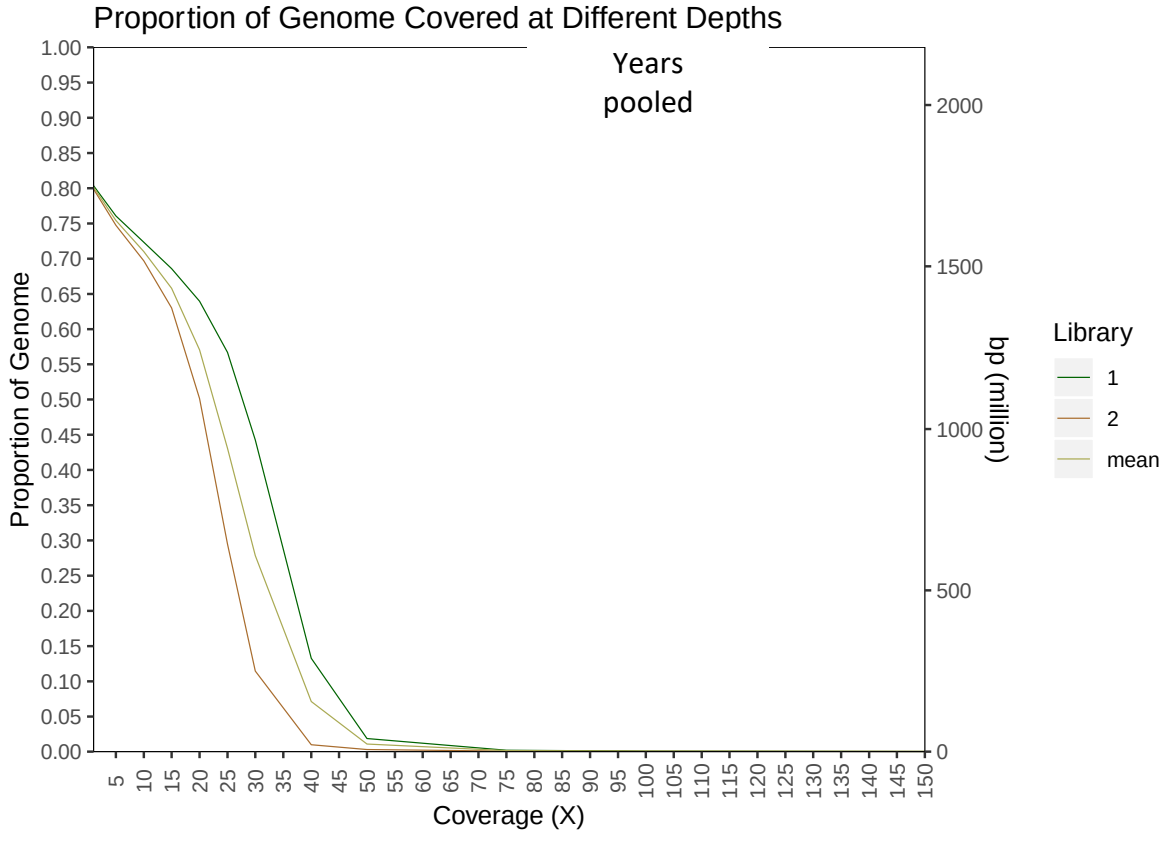
Searching for Genetic Basis for Consecutive and Skip Spawner Life Histories: Genome re-sequencing of 3 years of kelts from Dworshak hatchery

Sampling and coverage statistics

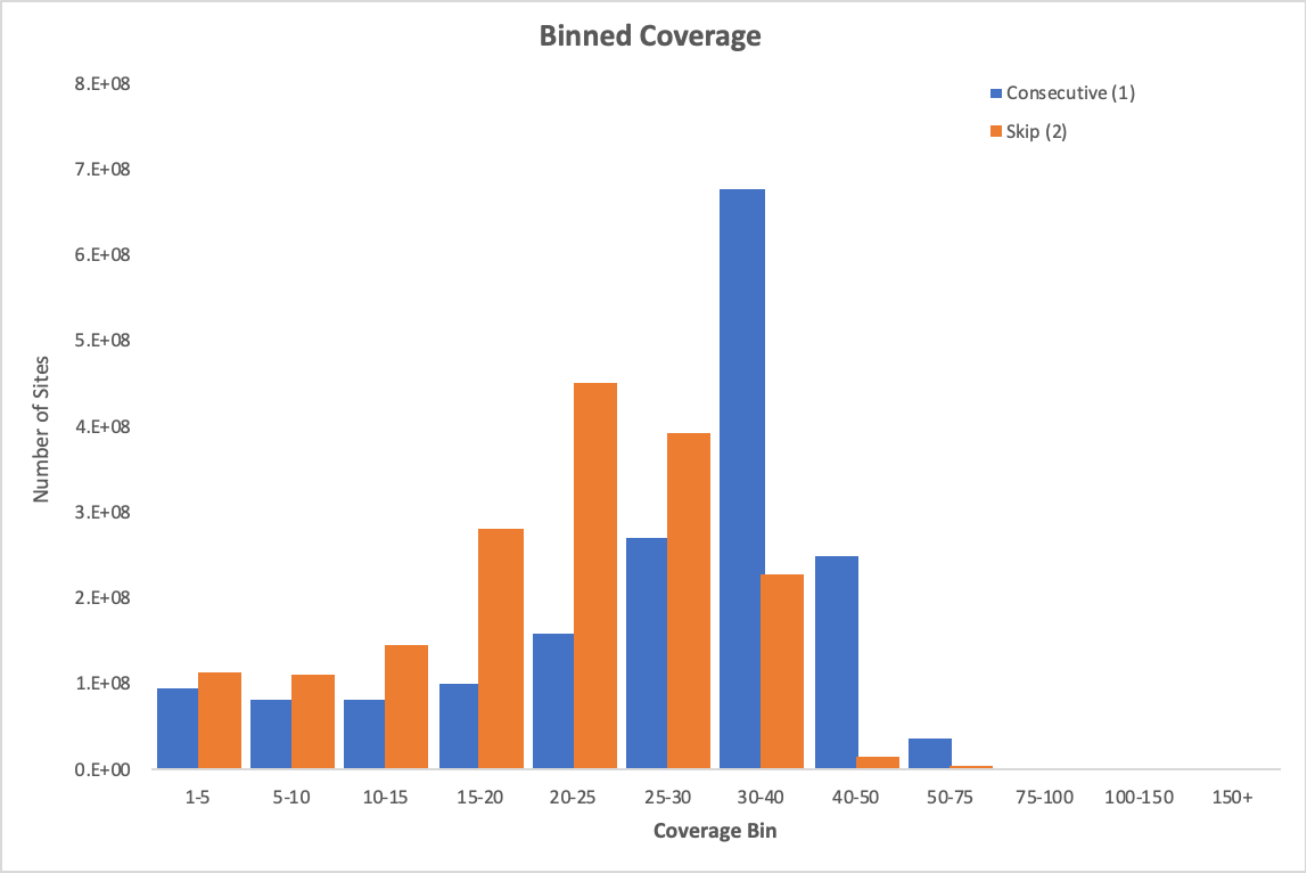
68 individuals, selected to only include **4-year old females**, were individually-barcoded and sequenced on the Illumina Nextseq, producing a total of **~1.13x10^9 paired reads** approximately evenly distributed between the six reproductive status (**skip-spawn vs. consecutive**) by year (**2013, 2015, 2016**) classes (mean 1.88x10^8, stdev 2.68x10^7). Reads attributed to barcoded individuals varied more widely, with a mean of 1.66x10^7 and standard deviation of 7.6x10^6 reads.

Records of reads mapped to the *O. mykiss* genome indicated that, when divided into the six RepxYear classes, each class was surveyed within coverage limits ($2 \leq X \leq 250$) at **~74% of the genome** with a **mean depth of 8.9 reads**, although across all populations only **~67% of the genome was surveyed in common**. After confirming relatively little year-class structure in the data (see below), years were pooled into reproductive classes, which showed that **~78% of the genome was covered in common** at an average filtered depth of **25 reads** (+/- 4). Proportion of the genome covered at various depths was similarly improved by pooling across years.

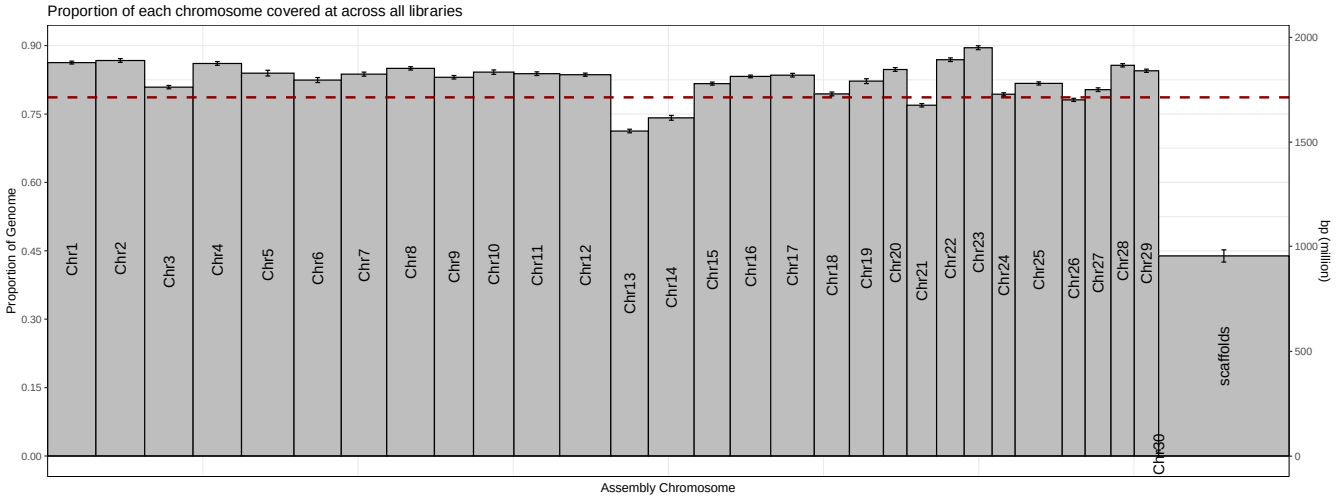

Proportion of Genome Covered at Different Depths
Reproductive
X Year
Proportion of Genome
Coverage (X)
bp (million)
Library
1
2
3
4
5
6
mean


Proportion of Genome Covered at Different Depths
Years
pooled
Proportion of Genome
Coverage (X)
bp (million)
Library
1
2
mean

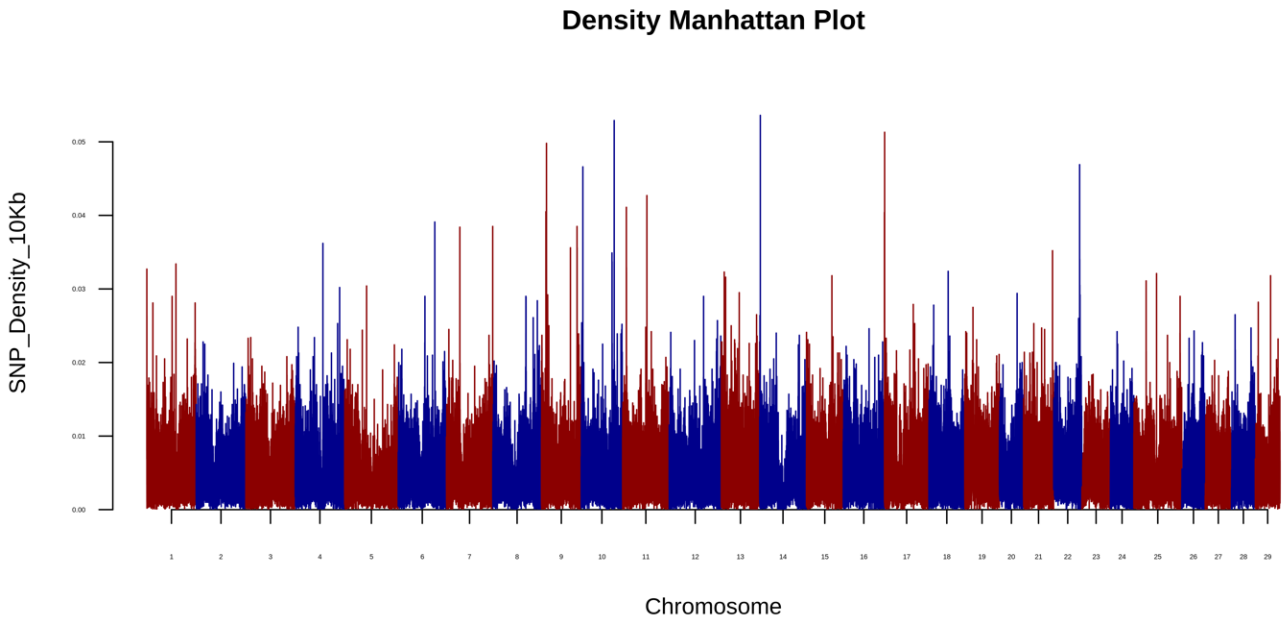
Tallying the number of sites with a given coverage indicated that **20-40** was an approximate modal value for each class pool, which suggests that **200** is an appropriate cutoff to filter most sites with paralogous reads in these pooled samples.



Coverage was relatively even across the 29 *O. mykiss* chromosomes, with some deviations that are typical for this genome assembly (Chr13+14; unanchored scaffolds).

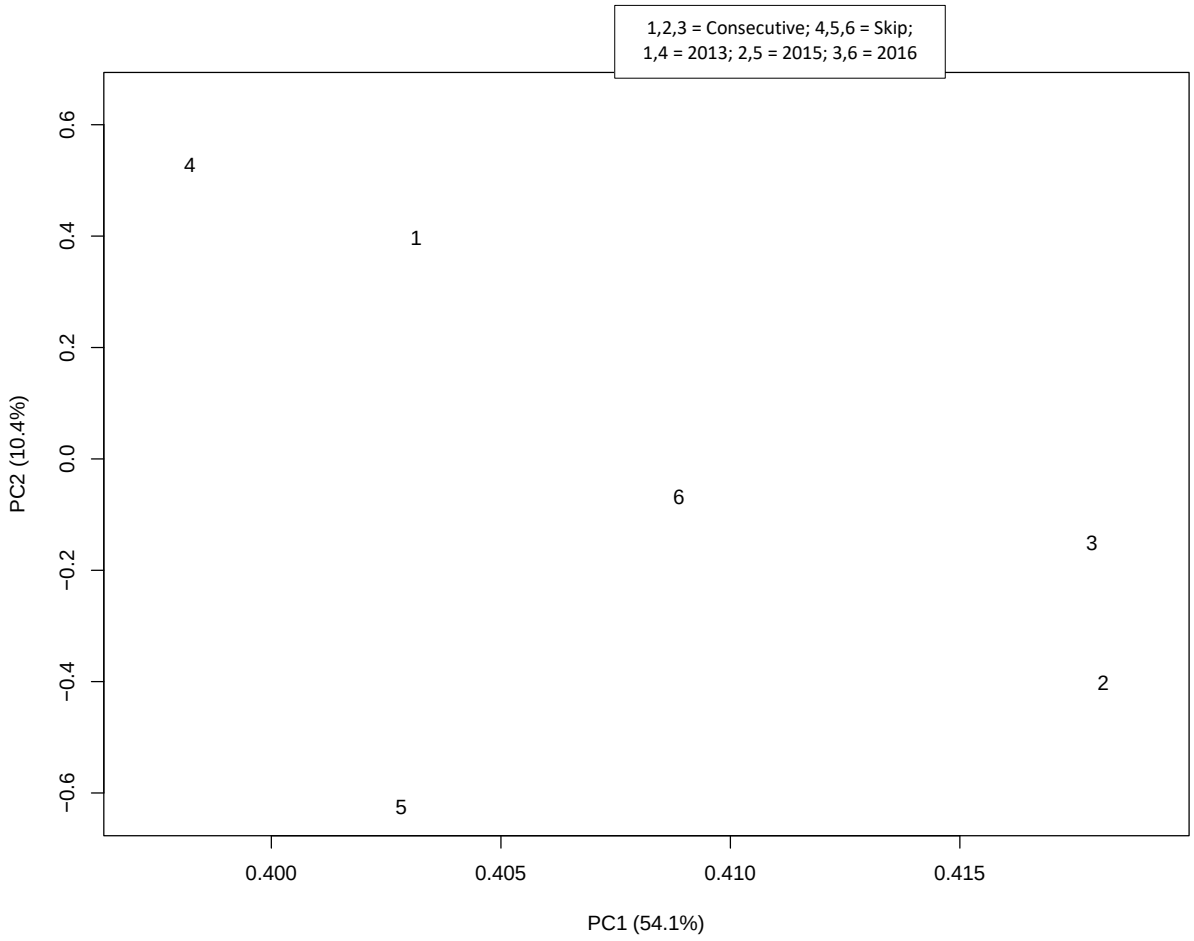


Density of the **9,441,019 SNPs** scored from these sequences was similarly even across the 29 chromosomes (after filtering by a minimum depth of 12 and representation by a minimum of 3 individuals from each library). The number of SNPs analyzed reflects the observation that these are polymorphisms segregating in samples from a single population.

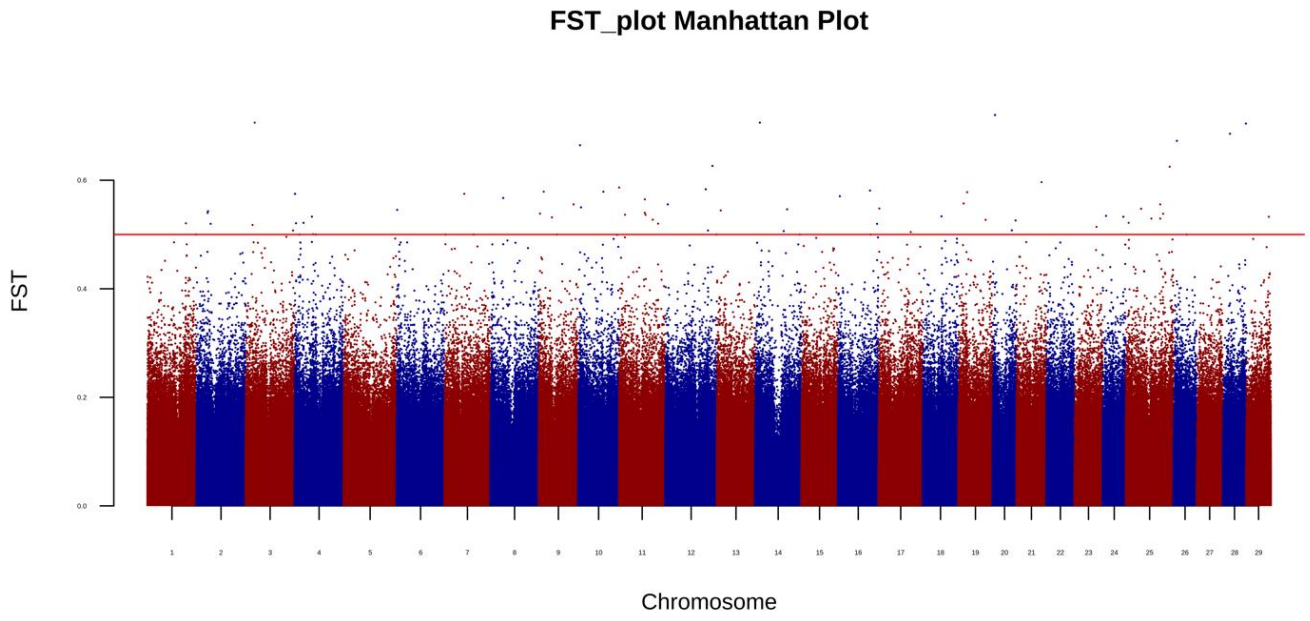


Analytical Results

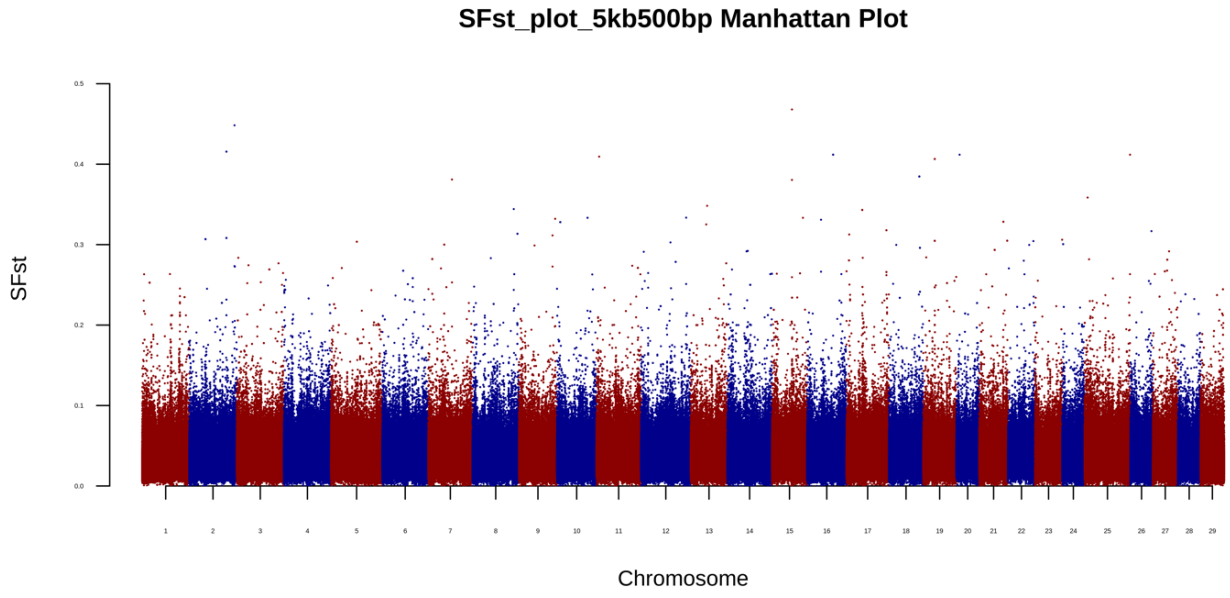
A principal components analysis of the six reproductive status x year pools indicated the strongest component of variation was not explained by any obvious class membership, while the second PC showed some modest structure by year. Because this modest structure is not expected to interfere with genetic association tests when each class is approximately equally represented, **analytical results represent normalized data pooled across year** (min. depth 12, min. representation 3 individuals) except analyses which explicitly incorporate population replicates (CMH+FLK) or as otherwise noted.


1,2,3 = Consecutive; 4,5,6 = Skip;
1,4 = 2013; 2,5 = 2015; 3,6 = 2016
PC2 (10.4%)
0.6
0.4
0.2
0.0
−0.2
−0.4
−0.6
4
1
6
3
2
5
0.400
0.405
0.410
0.415
PC1 (54.1%)

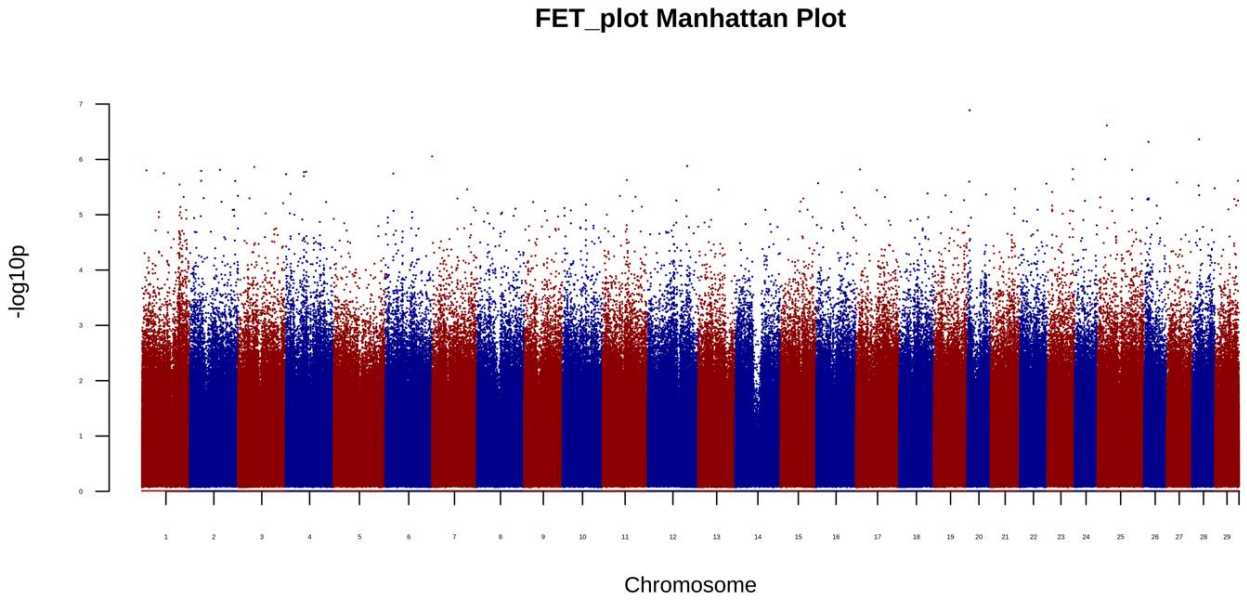
Global SNP-wise F_{ST}



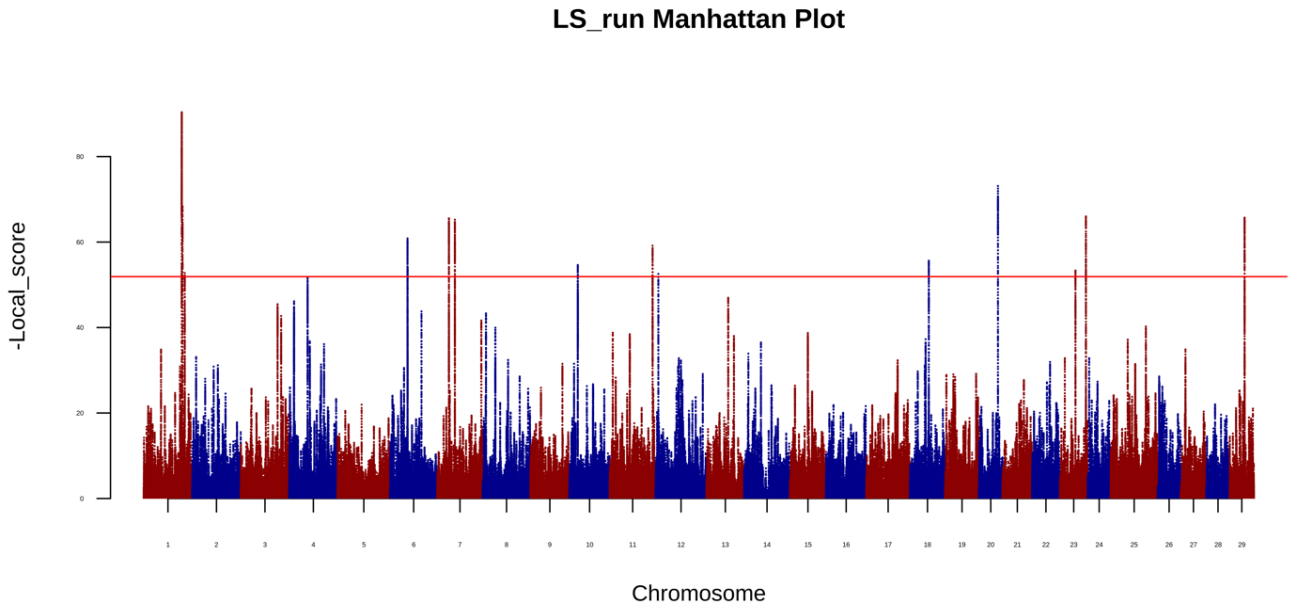
Sliding window Global F_{ST} (5Kb windows in 500bp steps)



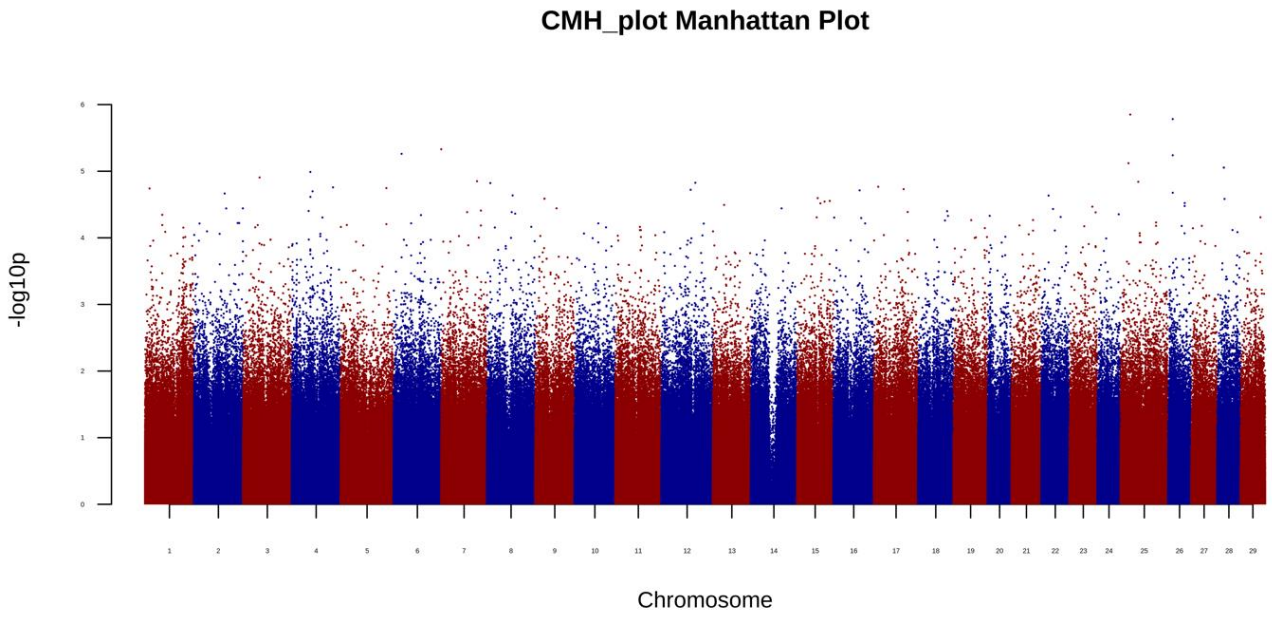
Fisher's exact test (log10 p-value)



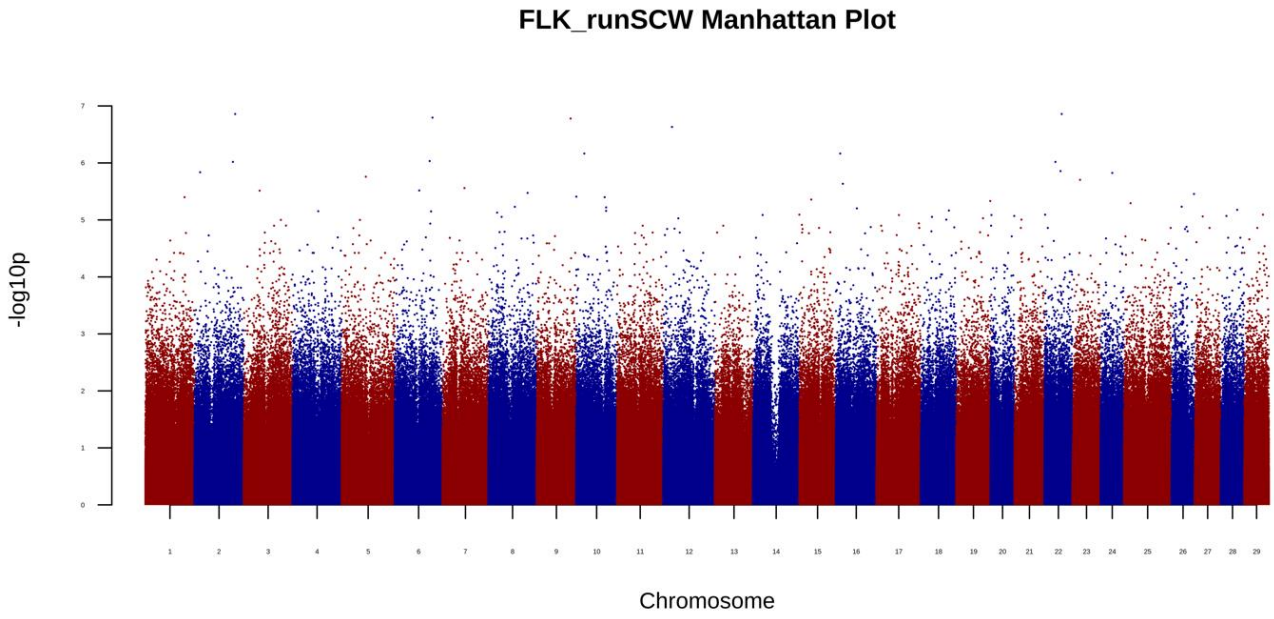
Local score (optimized ξ of 0.777 [85th percentile] for non-uniform data)



CMH test of paired population data (reproductive status by year; min. depth 6 and min. representation 2 individuals per class).



FLK test of population data (reproductive status by year; min. depth 6 and min. representation 2 individuals per class; mid-point rooted).



Interpretation

Many individual SNP sites had relatively high divergence between groups (F_{ST}>0.5) or significant individual tests, but few of these stood out in the more powerful tests, e.g sliding window F_{ST} or CMH. There were several regions that were strongly distinct in the local score analyses, which 'smooth' the background to identify linked SNPs with strong divergence. With increasing values of ξ (where the local score $X_M = -\log_{10}(p\text{-value}) - \xi$), which more strongly 'smooths' background divergence by considering only SNPs with significance beyond that threshold, two regions on Chromosome 1 were consistently significant at $\alpha < 0.05$ (see Appendix). One of these two regions was similarly significant in local score analysis of separate 6-class (reproductive status by year) data, although with a higher ξ value this region was no longer significant at $\alpha < 0.05$ (note that the threshold specified by a given ξ value, and its significance, are not comparable across data configurations because of concomitant changes in background divergence; i.e. ξ of 1 is more stringent in the 6-class data than pooled data). While the association of this region with the phenotype of interest should be interpreted with caution considering that it was not similarly distinct across the other tests, it was noted that the ~100Kb region indicated by the pooled dataset contained part or all of two genes (calcium/calmodulin dependent protein kinase II gamma-2C, camk2g, and potassium voltage-gated channel subfamily KQT member 5-like-2C, LOC110528511) and a purported pseudo gene (LOC110532615), while the larger ~400Kb region indicated for the 6-class dataset contained these loci and two other genes (zinc finger SWIM domain-containing protein 8-like-2C, LOC110528425, and bifunctional heparan sulfate N-deacetylase/N-sulfotransferase 2-like, LOC110528470).

Adaptive Management & Lessons Learned

1. Columbia River steelhead populations upstream of Bonneville Dam are listed under ESA and need novel recovery strategies.
2. There is a relatively large abundance of kelt steelhead in the Columbia River Basin even in the upper most areas.
3. In general, repeat spawning steelhead make up a very small proportion of the spawning run.
4. Increasing repeat spawners in steelhead populations can have many positive effects on populations including increasing; genetic diversity, lifetime fecundity, and fitness since genes are distributed across generations.
5. Long-term reconditioning kelt steelhead provides 5 to over 100 times more repeat spawners than leaving the fish in the river.
6. Physiology studies have provided us with a much better understanding of energetic and physiological status of kelts, improved our understanding of alternative life histories in post-spawning fish, and improved survival and health of reconditioned fish.
7. Blood hormone assays are useful to classify consecutive and skip spawner steelhead. Future work needs to focus on optimizing strategies for skip spawner contributions.
8. There appears to be a reduction in the B-run steelhead composition between the maiden and kelt stage, but the B-run composition of repeat spawners is similar to the kelt composition. Underlying biological and behavioral factors contributing to such discrepancies are not well understood but likely warrant further investigation of potential causes. With more data including escapement comparisons, it may be possible to refine the confidence in estimated rates of iteroparity among RG's.
9. Age appears to be less of a factor in rates of iteroparity than size. While the A-run life history was observed to be present among all reporting groups, so too were the B-run life history.
10. Despite the understanding in recent years that the B-run life history is relatively uncommon outside the middle and south forks of both the Clearwater River and Salmon River, our results suggest otherwise. In fact, age 2-ocean fish were dominant among all 10 reporting groups. This finding has implications for management of steelhead populations in the basin and provides evidence that regionally based classifications of life history types or their distributions warrants reconsideration.
11. The upper Salmon River region produces a disproportionate number of Snake River kelt steelhead and is presumably an important factor in spawner abundance for that region. This result is mirrored among hatchery-origin fish.
12. Adding a production level kelt reconditioning facility at Nez Perce Tribal Hatchery will make achieving the goal RPA 33 possible, i.e. increase the abundance on adult b-run steelhead by 6%.
13. The Snake River Kelt Reconditioning Facility Master Plan was submitted and favorably review by the ISRP and recommended to proceed to final design by the NWPCC in December of 2016.
14. Reproductive success studies are underway at a variety of scales: hatchery analog, spawning channel, and natural river. Results are positive.
15. Artificially reconditioned kelt steelhead appear to repeat home with high fidelity. Data indicates that natural repeat spawners in the Snake River exhibited a 15% stray rate.
16. Concluded with the Cle Elum spawning channel.
17. Kelt biophysiological decision to remature is made soon after spawning.
18. As a result of this project 1,554 kelt steelhead were collected in the Snake River since 2012 and 392 of those fish were reconditioned and released back into the Snake River.

19. As a result of this project 6,661 kelt steelhead were collected in the Yakima River since 2008 and 3,145 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 facility until dedicated facility is constructed which will should have better fail-safe systems in place.

References

Abrahamse MS & Murdoch KG 2013 Upper-Columbia River Steelhead Kelt Reconditioning Project: 2012 Annual Report to the U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2008-485-000. Portland, OR: Prepared by Yakama Nation Fisheries Resource Managment.

Abrahamse MS & Murdoch KG 2014 Upper-Columbia River Kelt Reconditioning Program Update. Portland, OR: Prepared by Yakama Nation Fisheries Resource Managment.

Ackerman, M. W., C. Habicht, and L. W. Seeb. 2011. Single-nucleotide polymorphisms (SNPs) under diversifying selection provide increased accuracy and precision in mixed-stock analyses of sockeye salmon from Copper River, Alaska. Transactions of the American Fisheries Society 140:865–881.

Ackerman, M. W., J. McCane, C. A. Steele, M. R. Campbell, A. P. Matala, J. E. Hess, and S. R. Narum. 2012. Chinook and steelhead genotyping for genetic stock identification at Lower Granite Dam. 2011 Annual Report. Submitted to: U.S. Department of Energy, Bonneville Power Administration, Division of Fish and Wildlife, P.O. Box 3621 Portland, OR Contract #53239; Project #2010-026-00. Available at: https://pisces.bpa.gov/release/documents/documentviewer.aspx?doc=P128035

Ackerman, M. W., J. McCane, C. A. Steele, N. V. Vu, M. R. Campbell, A. P. Matala, J. E. Hess, and S. R. Narum. 2014. Chinook and steelhead genotyping for genetic stock identification at Lower Granite Dam. 2013 Annual Report. Submitted to: U.S. Department of Energy, Bonneville Power Administration, Division of Fish and Wildlife, P.O. Box 3621 Portland, OR Contract #57343; Project #2010-026-00. Available at: https://pisces.bpa.gov/release/documents/documentviewer.aspx?doc=P128035

Ali M, Nicieza A & Wootton RJ 2003 Compensatory growth in fishes: a response to growth depression. Fish and Fisheries 4 147-190.

Anderson, E. C. 2010. Computational algorithms and user-friendly software for parentage-based tagging of Pacific salmonids. Final report submitted to the Pacific Salmon Commission's Chinook Technical Committee (US Section). 46 p.

Baker DM, Davies B, Dickhoff WW & Swanson P 2000 Insulin-like growth factor I increases follicle-stimulating hormone (FSH) content and gonadotropin-releasing hormone-stimulated FSH release from coho salmon pituitary cells in vitro. Biology of Reproduction 63 865-871.

Bar N 2014 Physiological and hormonal changes during prolonged starvation in fish. Canadian Journal of Fisheries and Aquatic Sciences 71 1447-1458.

Barry TP, Marwah A & Nunez S 2010 Inhibition of cortisol metabolism by 17 alpha,20 beta-P: Mechanism mediating semelparity in salmon? General and Comparative Endocrinology 165 53-59.

Baumann G 2002 Growth hormone binding protein. The soluble growth hormone receptor. Minerva Endocrinol 27 265-276.

Baxter, Colden, F. Richard Hauer, and William W. Woessner. 2003. “Measuring Groundwater–Stream Water Exchange: New Techniques for Installing Minipiezometers and Estimating Hydraulic Conductivity.” Transactions of the American Fisheries Society 132 (3): 493–502. doi: 10.1577/1548-8659(2003)132<0493: MGWENT>2.0.CO; 2.

Baxter, C. V., and R. F. Hauer. 2000. “Geomorphology, Hyporheic Exchange, and Selection of Spawning Habitat by Bull Trout (Salvelinus confluentus).” Canadian Journal of Fisheries and Aquatic Sciences 57: 1470–81.

Beckman BR 2011 Perspectives on concordant and discordant relations between insulin-like growth factor 1 (IGF1) and growth in fishes. General and Comparative Endocrinology 170 233-252.

Beckman BR, Larsen DA, Moriyama S, Lee-Pawlak B & Dickhoff WW 1998 Insulin-like growth factor-I and environmental modulation of growth during smoltification of spring chinook salmon (Oncorhynchus tshawytscha). General and Comparative Endocrinology 109 325-335.

Bell, G. 1980. The costs of reproduction and their consequences. The American Naturalist 116(1):45-76.

Benedet S, Andersson E, Mittelholzer C, Taranger GL & Bjornsson BT 2010 Pituitary and plasma growth hormone dynamics during sexual maturation of female Atlantic salmon. General and Comparative Endocrinology 167 77-85.

Bergan-Roller HE & Sheridan MA 2018 The growth hormone signaling system: Insights into coordinating the anabolic and catabolic actions of growth hormone. General and Comparative Endocrinology 258 119-133.

Bhatta S, Iwai T, Miura C, Higuchi M, Shimizu-Yamaguchi S, Fukada H & Miura T 2012 Gonads directly regulate growth in teleosts. Proceedings of the National Academy of Sciences of the United States of America 109 11408-11412. Bobe J, Montfort J, Nguyen T & Fostier A 2006 Identification of new participants in the rainbow trout (Oncorhynchus mykiss) oocyte maturation and ovulation processes using cDNA microarrays. In Reprod Biol Endocrin.

Biga PR, Peterson BC, Schelling GT, Hardy RW, Cain KD, Overturf K & Ott TL 2005 Bovine growth hormone treatment increased IGF-I in circulation and induced the production of a specific immune response in rainbow trout (Oncorhynchus mykiss). Aquaculture 246 437-445.

Bjornsson BT 1997 The biology of salmon growth hormone: from daylight to dominance. Fish Physiology and Biochemistry 17 9-24.

Bjornsson BT, Einarsdottir IE, Johansson M & Gong NP 2018 The Impact of Initial Energy Reserves on Growth Hormone Resistance and Plasma Growth Hormone-Binding Protein Levelsin Rainbow Trout Under Feeding and Fasting Conditions. Frontiers in Endocrinology 9.

Bjornsson BT, Johansson V, Benedet S, Einarsdottir IE, Hildahl J, Agustsson T & Jonsson E 2002 Growth hormone endocrinology of salmonids: regulatory mechanisms and mode of action. Fish Physiology and Biochemistry 27 227-242.

Bjornsson BT, Taranger GL, Hansen T, Stefansson SO & Haux C 1994 The Interrelation between Photoperiod, Growth-Hormone, and Sexual-Maturation of Adult Atlantic Salmon (Salmo-Salar). General and Comparative Endocrinology 93 70-81.

Blaise O, Weil C & Le Bail PY 1995 Role of IGF-I in the control of GH secretion in rainbow trout (Oncorhynchus mykiss). Growth Regulation 5 142-150.

Bobe J, Montfort J, Nguyen T & Fostier A 2006 Identification of new participants in the rainbow trout (Oncorhynchus mykiss) oocyte maturation and ovulation processes using cDNA microarrays. In Reprod Biol Endocrin.

Bon E, Corraze G, Kaushik S & LeMenn F 1997 Effects of accelerated photoperiod regimes on the reproductive cycle of the female rainbow trout: I—Seasonal variations of plasma lipids correlated with vitellogenesis. Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology 118 183-190.

Bonneville Power Administration and U.S. Army Corps of Engineers 2014. 2013 Snake River Kelt Management Plan. www.salmonrecovery.org. (April 2015).

Bowersox, B. J., R. Banks and N. Davids. 2011. Potlatch River steelhead monitoring and evaluation project, Annual Report 2010. IDFG #12-103, April 2012.

Branstetter R, Stephenson J, Pierce AL, Hatch DR, Bosch B, Fast D, Blodgett J, Everett SR, Paddlety J, Dasher R, et al. 2011 Steelhead Kelt Reconditioning and Reproductive Success. 2011 Annual Report to the U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2007-401-000. Portland, OR: Prepared by the Columbia River Inter-Tribal Fish Commission.

Branstetter R, Stephenson J, Pierce AL, Hatch DR, Bosch B, Fast D, Blodgett J, Everett SR, Paddlety J, Dasher R, et al. 2010 Steelhead Kelt Reconditioning and Reproductive Success. 2010 Annual Report to the U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2010-401. Portland, OR: Prepared by the Columbia River Inter-Tribal Fish Commission.

Breton B, Govoroun M & Mikolajczyk T 1998 GTH I and GTH II secretion profiles during the reproductive cycle in female rainbow trout: relationship with pituitary responsiveness to GnRH-A stimulation. General and Comparative Endocrinology 111 38-50.

Brodersen J, Nilsson PA, Hansson LA, Skov C & Bronmark C 2008 Condition-dependent individual decision-making determines cyprinid partial migration. Ecology 89 1195-1200.

Bromage N, Jones J, Randall C, Thrush M, Davies B, Springate J, Duston J & Barker G 1992 Broodstock management, fecundity, egg quality and the timing of egg-production in the rainbow trout (Oncorhynchus mykiss). Aquaculture 100 141-166.

Buelow, J. L. 2011. Physiological Characteristics of Steelhead Kelt steelhead (Oncorhynchus mykiss) in the Snake River, Idaho. Master's thesis, University of Idaho, Moscow.

Bull JJ & Shine R 1979 Iteroparous animals that skip opportunities for reproduction. American Naturalist 114 296-303.

Burgner RL, Light JT, Margolis L, Okazaki T, Tautz A & Ito S 1992 Distribution and origins of steelhead trout (Oncorhynchus mykiss) in offshore waters of the North Pacific ocean. Vancouver, B.C.: International North Pacific Fisheries Commission.

Busby, P. J., T. C. Wainwright, G. J. Bryant, L. J. Lierheimer, R. S. Waples, F. W. Waknitz and F. W. Lagomarsino. 1996. Status review of west coast steelhead from Washington, Idaho, Oregon, and California. U.S. Department of Commerce, NOAA Technical Memorandum NMFS-NWFSC-27. Available at: http://www.nwfsc.noaa.gov/publications/techmemos/tm27/tm27.htm

Burton MPM 1994 A critical period for nutritional control of early gametogenesis in female winter flounder, Pleuronectes americanus (Pisces, Teleostei). Journal of Zoology 233 405-415.

Caisman JM 2015 Partial migration in steelhead (Oncorhynchus mykiss): Identifying factors that influence migratory behavior and population connectivity across a watershed. In College of Graduate Studies, p 80. Moscow, Idaho: University of Idaho.

Caldwell LK, Pierce AL & Nagler JJ 2013a Metabolic endocrine factors involved in spawning recovery and rematuration of iteroparous rainbow trout (Oncorhynchus mykiss). General and Comparative Endocrinology 194 124-132.

Caldwell LK, Pierce AL & Nagler JJ 2013b Metabolic endocrine factors involved in spawning recovery and rematuration of iteroparous female rainbow trout (Oncorhynchus mykiss). Gen Comp Endocrinol 194 124-132.

Caldwell LK, Pierce AL, Riley LG, Duncan CA & Nagler JJ 2014 Plasma nesfatin-1 is not affected by long-term food restriction and does not predict rematuration among iteroparous female rainbow trout (Oncorhynchus mykiss). PLoS One 9 e85700.

Campbell B, Beckman BR, Fairgrieve WT, Dickey JT & Swanson P 2006a Reproductive investment and growth history in female Coho salmon. Transactions of the American Fisheries Society 135 164-173.

Campbell B, Dickey J, Beckman B, Young G, Pierce A, Fukada H & Swanson P 2006b Previtellogenic oocyte growth in salmon: relationships among body growth, plasma insulin-like growth factor-1, estradiol-17beta, follicle-stimulating hormone and expression of ovarian genes for insulin-like growth factors, steroidogenic-acute regulatory protein and receptors for gonadotropins, growth hormone, and somatolactin. Biology of Reproduction 75 34-44.

Campbell, M. R., C. C. Kozfkay, T. Copeland, W. C. Schrader, M. W. Ackerman, and S. R. Narum. 2012. Estimating abundance and life history characteristics of threatened wild Snake River steelhead stocks by using genetic stock identification. Transactions of the American Fisheries Society 141:1310–1327.

Campbell, NR, Harmon SA, Narum SR. 2015. Genotyping-in-Thousands by sequencing (GT-seq): a cost effective SNP genotyping method based on custom amplicon sequencing. Molecular Ecology Resources 15(4): 855–867.

Carlson SM & Seamons TR 2008 A review of quantitative genetic components of fitness in salmonids: implications for adaptation to future change. Evolutionary Applications 1 222-238.

Chapman, D. W. Critical review of variables used to define effects of fines in redds of large salmonids. Trans. Am. Fish. Soc., 117: 1–21 (1988).

Chaput G & Benoit HP 2012 Evidence for bottom-up trophic effects on return rates to a second spawning for Atlantic salmon (Salmo salar) from the Miramichi River, Canada. ICES Journal of Marine Science 69 1656-1667.

Chaput G & Jones R 2006 Reproductive rates and rebuilding potential for two multi-sea-winter Atlantic salmon (Salmo salar L.) stocks of the Maritime provinces. Ed FaO Canada: Canadian Science Advisory Secretariat.

Christie MR, McNickle GG, French RA & Blouin MS 2018 Life history variation is maintained by fitness trade-offs and negative frequency-dependent selection. Proceedings of the National Academy of Sciences of the United States of America 115 4441-4446.

Chudyk WE 1976 The life history of adult steelhead sampled in the Tyee test fishery in the Skeena river esturary and comparisons with other steelhead stocks in British Columbia. Ed BCFaW Branch.

Chyb J, Mikolajczyk T & Breton B 1999 Post-ovulatory secretion of pituitary gonadotropins GtH I and GtH II in the rainbow trout (Oncorhynchus mykiss): regulation by steroids and possible role of non-steroidal gonadal factors. Journal of Endocrinology 163 87-97.

Cohick WS & Clemmons DR 1994 Enhanced Expression of Dihydrofolate-Reductase by Bovine Kidney Epithelial-Cells Results in Altered Cell Morphology, Igf-I Responsiveness, and Igf Binding Protein-3 Expression. Journal of Cellular Physiology 161 178-186.

Colotelo, A. H., R. A. Harnish, and B. W. Jones, and 10 other authors. 2014. Passage Distribution and Federal Columbia River Power System Survival for Steelhead Kelt steelhead Tagged Above and at Lower Granite Dam, Year 2. PNNL-23051, prepared for the U.S. Army Corp of Engineers, Walla Walla District, Walla Walla Washington, by Pacific Northwest National Laboratory, Richland, Washington. http://www.salmonrecovery.gov/Files/Comprehensive%20Evaluation/Colotelo-tal_2013_-%20Kelt steelhead-Passage-Distribution-Survival_PNWD-22101.pdf.

Colt J & Shearer KD 2001 Evaluation of migrational delays on the reproductive success of adult hatchery spring Chinook salmon in the Columbia and Snake Rivers (objective 2 only). 2001 Report to the U.S. Army Corps of Engineers, Contract W66QKZ00805700. Seattle: Northwest Fisheries Science Center, National Marine Fisheries Service.

Copeland, T., R.V. Roberts, B.N. Oldemeyer, and K.A. Apperson. 2013. Idaho steelhead monitoring and evaluation studies, Annual Report 2012. IDFG 13-07.

Courter II, Child DB, Hobbs JA, Garrison TM, Glessner JJG & Duery S 2013 Resident rainbow trout produce anadromous offspring in a large interior watershed. Canadian Journal of Fisheries and Aquatic Sciences 70 701-710.

Crespi BJ & Teo R 2002 Comparative phylogenetic analysis of the evolution of semelparity and life history in salmonid fishes. Evolution 56 1008-1020.

Crim LW, Wilson CE, So YP, Idler DR & Johnston CE 1992 Feeding, reconditioning, and rematuration responses of captive Atlantic salmon (Salmo salar) kelt. Canadian Journal of Fisheries and Aquatic Sciences 49 1835-1842.

Crossin GT & Hinch SG 2005 A nonlethal, rapid method for assessing the somatic energy content of migrating adult pacific salmon. Transactions of the American Fisheries Society 134 184-191.

Crossin GT, Phillips RA, Trathan PN, Fox DS, Dawson A, Wynne-Edwards KE & Williams TD 2012 Migratory carryover effects and endocrinological correlates of reproductive decisions and reproductive success in female albatrosses. General and Comparative Endocrinology 176 151-157.

Daughaday WH, Mariz IK & Blethen SL 1980 Inhibition of access of bound somatomedin to membrane receptor and immunobinding sites: a comparison of radioreceptor and radioimmunoassay of somatomedin in native and acid-ethanol-extracted serum. J Clin Endocrinol Metab 51 781-788.

Daughaday WH & Rotwein P 1989 Insulin-Like Growth Factor-I and Factor-Ii - Peptide, Messenger Ribonucleic-Acid and Gene Structures, Serum, and Tissue Concentrations. Endocrine Reviews 10 68-91.

De Mones A, Fostier A, Cauty C & Jalabert B 1989 Ovarian early postovulatory development and estrogen production in rainbow trout (Salmo gairdneri R.) from a spring-spawning strain. General and Comparative Endocrinology 74 431-441.

Dickhoff WW 1989 Salmonids and annual fishes: Death after sex. In Development, maturation, and senescence of neuroendocrine systems: A comparative approach, pp 253-266. Eds MP Schreibman & CG Scanes. New York: Academic Press.

Duan C & Plisetskaya EM 1993 Nutritional Regulation of Insulin-Like Growth Factor-I Messenger-Rna Expression in Salmon Tissues. Journal of Endocrinology 139 243-252.

Duan CM 1998 Nutritional and developmental regulation of insulin-like growth factors in fish. Journal of Nutrition 128 306s-314s.

Duan CM & Xu QJ 2005 Roles of insulin-like growth factor (IGF) binding proteins in regulating IGF actions. General and Comparative Endocrinology 142 44-52.

Dumas J, Barriere L, Blanc D, Godard J & Kaushik SJ 1991 Reconditioning of Atlantic salmon (Salmo salar) kelts with silage-based diets - growth and reproductive performance. Aquaculture 96 43-56.

Dyer AR, Upton Z, Stone D, Thomas PM, Soole KL, Higgs N, Quinn K & Carragher JF 2004 Development and validation of a radioimmunoassay for fish insulin-like growth factor I (IGF-I) and the effect of aquaculture related stressors on circulating IGF-I levels. Gen Comp Endocrinol 135 268-275.

Ensing, D., Crozier, W.W., Boylan, P., O'Maoil_eidigh, N. & McGinnity, P. (2013) An analysis of genetic stock identification on a small geographical scale using microsatellite markers, and its application in the management of a mixed-stock fishery for Atlantic salmon Salmo salar in Ireland. Journal of Fish Biology 82:2080–2094.

Evans, A.F., R.E. Beaty, D.R. Hatch, J. Blodgett, & D. Fast. 2001. Kelt reconditioning: A research project to enhance iteroparity in Columbia Basin steelhead (Oncorhynchus mykiss). 2000 Annual Report to U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2000-017. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland, OR.

Everest, F. L., R. L. Beschta, J. C. Scrivener, K. V. Koski, J. R. Sedell, and C. J. Cederholm. Fine sediment and salmonid production: A paradox. In: Streamside Management: Forestry and Fishery Interactions (E. O. Salo and T. W. Cundy, Eds., pp. 98–142). Seattle: College of Forest Resources, University of Washington (1987).

Farrell AP, Gallaugher PE, Fraser J, Pike D, Bowering P, Hadwin AKM, Parkhouse W & Routledge R 2001 Successful recovery of the physiological status of coho salmon on board a commercial gillnet vessel by means of a newly designed revival box. Canadian Journal of Fisheries and Aquatic Sciences 58 1932-1946.

Ferriss BE, Trudel M & Beckman BR 2014 Regional and inter-annual trends in marine growth of juvenile salmon in coastal pelagic ecosystems of British Columbia, Canada. Marine Ecology Progress Series 503 247-261.

Fisker S 2006 Physiology and pathophysiology of growth hormone-binding protein: methodological and clinical aspects. Growth Horm IGF Res 16 1-28.

Fleming, I. A. 1998. Pattern and variability in the breeding system of Atlantic salmon (Salmo salar), with comparisons to other salmonids. Canadian Journal of Fisheries and Aquatic Sciences 55 (Suppl. 1), 59–76.

Ford, M. J. (ed.). 2011. Status review update for Pacific salmon and steelhead listed under the Endangered Species Act: Pacific Northwest. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-113, 281 p.

Fostier A, Weil C, Terqui M, Breton B & Jalabert B 1978 Plasma estradiol-17-beta and gonadotropin during ovulation in rainbow trout (Salmo gairdneri R). Annales De Biologie Animale Biochimie Biophysique 18 929-936.

Frederiksen CR, Fast DE, Bosch WJ & Temple GM 2015 Yakima Steelhead VSP Project: Yakima River Steelhead Population Status and Trends Monitoring, 10/15/2013-10/14/2014 Annual Report, 2010-030-00. Bonneville Power Administration.

Frederiksen, C.R., D.E. Fast, W.J. Bosch, and G.M. Temple. Yakima Steelhead VSP Project: Yakima River Steelhead Population Status & Trends Monitoring, 10/15/2011 - 10/14/2013 Annual Report, 2010-030-00

Frederiksen DR, Fast D & Temple G 2012 Yakima Steelhead Viable Salmonid Population (VSP) Status &Trends Monitoring. Yakima Steelhead VSP Project Annual Report 2011, Project No. 201003000. Toppenish, WA: Prepared by Yakama Nation Fisheries and Washington Department of Fish and Wildlife.

Fruchtman S, Jackson L & Borski R 2000 Insulin-like growth factor I disparately regulates prolactin and growth hormone synthesis and secretion: studies using the teleost pituitary model. Endocrinology 141 2886-2894.

Gabillard JC, Kamangar BB & Montserrat N 2006 Coordinated regulation of the GH/IGF system genes during refeeding in rainbow trout (Oncorhynchus mykiss). Journal of Endocrinology 191 15-24.

Gauthey Z, Freychet M, Manicki A, Herman A, Lepais O, Panserat S, Elosegi A, Tentelier C & Labonne J 2015 The concentration of plasma metabolites varies throughout reproduction and affects offspring number in wild brown trout (Salmo trutta). Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology 184 90-96.

Geist, David R. 2000. "Hyporheic Discharge of River Water into Fall Chinook Salmon (Onchorhynchus tshawytscha) Spawning Areas in the Hanford Reach, Columbia River." Canadian Journal of Fisheries and Aquatic Sciences 57: 1647–56.

Gomez JM, Boujard T, Fostier A & LeBail PY 1996 Characterization of growth hormone nycthemeral plasma profiles in catheterized rainbow trout (Oncorhynchus mykiss). Journal of Experimental Zoology 274 171-180.

Gray ES, Young G & Bern HA 1990 Radioreceptor assay for growth hormone in coho salmon (Oncorhynchus kisutch) and its application to the study of stunting. J Exp Zool 256 290-296.

Gross MR 1996 Alternative reproductive strategies and tactics: Diversity within sexes. Trends in Ecology & Evolution 11 92-98.

Guidelines for Electrofishing Waters Containing Salmonids Listed under the Endangered Species Act (Backpack Electrofishing Guidelines, NMFS, June 2000)

Halttunen E 2011 Staying alive - the survival and importance of Atlantic salmon post-spawners. In Department of Arctic and Marine Biology: University of Tromso, Norway.

Halver JE & Hardy RW 2002 Fish Nutrition. San Diego: Academic Press.

Ham BR, Myrick CA, Barrows FT, Yeoman CJ, Duff GC, Maskill MG & Sealey WM 2015 Feed Characteristics Alter Growth Efficiency of Cutthroat Trout. Journal of Fish and Wildlife Management 6 83-91.

Hanson KC, Ostrand KG, Gannam AL & Ostrand SL 2010 Comparison and validation of nonlethal techniques for estimating condition in juvenile salmonids. Transactions of the American Fisheries Society 139 1733-1741.

Hardy RW & Barrows FT 2002 Diet Formulation and Manufacture. In Fish Nutrition, pp 506-600. Eds JE Halver & RW Hardy. San Diego: Academic Press.

Hatch DR, Branstetter R, Stephenson J, Pierce A, Lessard R, Newell J, Matala A, Bosch W, Everett S, Graham N, et al. 2019 Kelt Reconditioning and Reproductive Success Evaluation Research. 1/1/2018 - 12/31/2018 Bonneville Power Administration Annual Report, 2007-401-00. Portland, OR: Prepared by the Columbia River Inter-Tribal Fish Commission.

Hatch, D. R., P. J. Anders, A. F. Evans, J. Blodgett, B. Bosch, D. Fast, & T. Newsome. 2002. Kelt reconditioning: A research project to enhance iteroparity in Columbia Basin steelhead (Oncorhynchus mykiss). Project 2000-017-00, Annual Report the Bonneville Power Administration, Portland, OR.

Hatch, D.R., R. Branstetter, J. Blodgett, B. Bosch, D. Fast, & T. Newsome. 2003b. Kelt reconditioning: A research project to enhance iteroparity in Columbia Basin steelhead (Oncorhynchus mykiss). 2002 Annual Report to U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2000-017. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland, OR.

Hatch, D.R., R. Branstetter, J. Stephenson, A.L. Pierce, A. Matala, R. Lessard, W. Bosch, L.K. Caldwell, S.R. Everett, J. Newell, N. Graham, L. Jenkins, M. Elliot, T. Cavileer, J. Nagler, M. Fiander, J. Blodgett, C. Frederiksen, D. Fast, K. J.M. Whiteaker, R. Johnson. 2015. Kelt Reconditioning and Reproductive Success Evaluation Research. 1/1/2014-12/31/2014 Annual Report to the U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2007-401-000. Portland, OR: Prepared by the Columbia River Inter-Tribal Fish Commission.

Hatch DR, Branstetter R, Stephenson J, Pierce AL, Matala A & Newell J. 2013b. Steelhead Kelt Reconditioning and Reproductive Success, 2012 Annual Report to the U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2007-401-000. Portland, OR: Prepared by the Columbia River Inter-Tribal Fish Commission.

Hatch DR, Branstetter R, Stephenson J, Pierce AL, Whiteaker JM & Bosch B 2012 Steelhead Kelt Reconditioning and Reproductive Success, 2011 Annual Report to the U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2007-401-000. Portland, OR: Prepared by the Columbia River Inter-Tribal Fish Commission.

Hatch DR, Branstetter R, Stephenson J, Pierce AL, Matala A, Whiteaker JM, Lessard R, Caldwell LK, Everett SR, Bosch B, et al. 2014. Kelt Reconditioning and Reproductive Success Evaluation Research, 2013 Annual Report to the U.S. Dept. of Energy, Bonneville Power

Administration, Project No. 2007-401-000. Portland, OR: Prepared by the Columbia River Inter-Tribal Fish Commission.

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

Hatch, D., R. Branstetter, J. Stephenson, A. Pierce, A. Matala, R. Lessard, W. Bosch, S. Everett, J. Newell, N. Graham, L. Medeiros, L. Jenkins, T. Tall Bull, M. Elliott, K. Huggler, T. Cavileer, J. Nagler, M. Fiander, C. Frederickson, J. Blodgett, D. Fast, J. Whiteaker, and R. Johnson. 2016a. Kelt Reconditioning and Reproductive Success Evaluation Research: 2015 Annual Technical Report. Bonneville Power Administration Annual Report, 2007-401-00, 156 p.

Hatch DR, Branstetter R, Stephenson J, Pierce AL, Newell J, Bosch B, Everett S, Graham N, Medeiros L, Jenkins L, et al. 2017 Kelt reconditioning and reproductive success evaluation research, 1/1/2016 - 12/31/2016 Bonneville power administration annual technical report. Portland, OR: Columbia River Inter-Tribal Fish Commission, 2007-401-00.

Hatch DR, Branstetter R, Stephenson J, Pierce AL, Newell J, Bosch WJ, Graham N, Medeiros LR, Jenkins LE, Hoffman B, et al. 2018 Kelt Reconditioning and Reproductive Success Evaluation Research. Portland, OR: Bonneville Power Administration Annual Report, 2007-401-00 (Prepared by the Columbia River Inter-Tribal Fish Commission).

Henderson BA & Wong JL 1998 Control of lake trout reproduction: role of lipids. Journal of Fish Biology 52 1078-1082.

Hess, J. E., N. Campbell, A. P. Matala and S. R. Narum. 2012. Genetic assessment of Columbia River Stocks: 2011 annual report. Submitted to Bonneville Power Administration. Contract #41224; Project # 2008-907-00 https://pisces.bpa.gov/release/documents/documentviewer.aspx?doc=P120015

Hess, J. E., J. M. Whiteaker, J. K. Fryer and S. R. Narum. 2014. Monitoring Stock-Specific Abundance, Run Timing, and Straying of Chinook salmon in the Columbia River Using Genetic Stock Identification (GSI). Transactions of the American Fisheries Society 34:184–201.

Hevroy EM, Hunskar C, de Gelder S, Shimizu M, Waagbo R, Breck O, Takle H, Sussort S & Hansen T 2013 GH-IGF system regulation of attenuated muscle growth and lipolysis in Atlantic salmon reared at elevated sea temperatures. Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology 183 243-259.

Hill GE 2011 Condition-dependent traits as signals of the functionality of vital cellular processes. Ecology Letters 14 625-634.

Hockersmith, E., J. Vella, L. Stuehrenberg, R. Iwamoto, & G. Swan. 1995. Yakima River radio telemetry study: steelhead, 1989-93. Prepared for Bonneville Power Administration, P.O. Box 3621, Portland, OR. Project Number 89-089. Seattle, WA: Coastal Zone and Estuarine Studies Division, Northwest Fisheries Science Center, National Marine Fisheries Service, NOAA.

Howell, P., K. Jones, D. Scarnecchia, L. LaVoy, W. Kendra. & D. Ortmann. 1985. Stock assessment of Columbia River anadromous salmonids Volume II: steelhead stock

summaries stock transfer guidelines – information needs. Final Report to Bonneville Power Administration, Contract DE-AI79–84BP12737, Project 83-335.
Huang YS, Rousseau K, Le Belle N, Vidal B, Burzawa-Gerard E, Marchelidon J & Dufour S 1998 Insulin-like growth factor-I stimulates gonadotrophin production from eel pituitary cells: a possible metabolic signal for induction of puberty. Journal of Endocrinology 159 43-52.
Hutchings JA 2011 Old wine in new bottles: reaction norms in salmonid fishes. Heredity 106 421-437.
ISRP 2011 Retrospective Report 2011. Independent Scientific Review Panel.
ISRP. 2014. Review of 2014 Progress Report for the Yakama Nation's Upper Columbia Kelt Reconditioning Program, #2008-458-00, an Accord Proposal. Independent Scientific Review Panel, 2014-9.
Iwama G.K., McGeer J.C. & Pawluk M.P. (1989). The effects of five fish anesthetics on acid-base balance, hematocrit, cortisol and adrenaline in rainbow trout. Canadian Journal of Zoology 67:2065-2073.
Jeffries KM, Hinch SG, Donaldson MR, Gale MK, Burt JM, Thompson LA, Farrell AP, Patterson DA & Miller KM 2011 Temporal changes in blood variables during final maturation and senescence in male sockeye salmon Oncorhynchus nerka: reduced osmoregulatory ability can predict mortality. Journal of Fish Biology 79 449-465.
Jenkins LE, Pierce AL, Graham N, Branstetter R, Hatch DR & Nagler JJ. 2018. Reproductive performance and energy balance in consecutive and repeat spawning female steelhead reconditioned in captivity. Transactions of the American Fisheries Society 147 959-971.
Jenkins LE, Pierce AL, Graham ND, Medeiros LR, Hatch DR & Nagler JJ 2019 Elevated plasma triglycerides and growth rate are early indicators of reproductive status in post-spawning female steelhead trout (Oncorhynchus mykiss). Conservation Physiology 7.
Jenkins RC & Ross RJM 1996 Acquired growth hormone resistance in catabolic states. Baillieres Clinical Endocrinology and Metabolism 10 411-419.
Johnston CE, Gray RW, Mclennan A & Paterson A 1987 Effects of photoperiod, temperature, and diet on the reconditioning response, blood chemistry, and gonad maturation of Atlantic salmon kelts (Salmo salar) held in fresh water. Canadian Journal of Fisheries and Aquatic Sciences 44 702-711.
Jorgensen C, Ernande B, Fiksen O & Dieckmann U 2006 The logic of skipped spawning in fish. Canadian Journal of Fisheries and Aquatic Sciences 63 200-211.
Jensen, D.W., E. Ashley Steel, A.H. Fullerton, and G.R. Pess. 2009. Impact of Fine Sediment on Egg-To-Fry Survival of Pacific Salmon: A Meta-Analysis of Published Studies. Reviews in Fisheries Science, 17(3):348-359.
Justice, C., S. White, and D. McCullough. 2012. Spawning Gravel Composition Survey Methods. A Component of Monitoring Recovery Trends in Key Spring Chinook Habitat Variables and Validation of Population Viability Indicators BPA Annual Report 2012.
Kadri S, Mitchell DF, Metcalfe NB, Huntingford FA & Thorpe JE 1996 Differential patterns of feeding and resource accumulation in maturing and immature Atlantic salmon, Salmo salar. Aquaculture 142 245-257.
Kalinowski S.T., M.L. Taper, and T.C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases confidence in paternity. Molecular Ecology 16:1099-1106.

Kato Y, Murakami Y, Sohmiya M & Nishiki M 2002 Regulation of human growth hormone secretion and its disorders. Internal Medicine 41 7-13.

Keefer ML, Wertheimer RH, Evans AF, Boggs CT & Peery CA. 2008. Iteroparity in Columbia river summer-run steelhead (Oncorhynchus mykiss): implications for conservation. Canadian Journal of Fisheries and Aquatic Sciences 65 2592-2605.

Kelley KM, Desai P, Roth JT, Haigwood JT, Arope SA, Flores RM, Schmidt KE, Perez M, Nicholson GS & Song WW 2000 Evolution of endocrine growth regulation: the insulin like growth factors (IGFs), their regulatory binding proteins (IGFBPs), and IGF receptors in fishes and other ectothermic vertebrates. In Recent Advances in Marine Biotechnology, pp 189-228. Eds M Fingerman, M-F Thompson & R Nagabhushanam. New Delhi: Oxford and IBH Publishing.

Kendall NW, McMillan JR, Sloat MR, Buehrens TW, Quinn TP, Pess GR, Kuzishchin KV, McClure MM & Zabel RW 2015 Anadromy and residency in steelhead and rainbow trout (Oncorhynchus mykiss): a review of the processes and patterns. Canadian Journal of Fisheries and Aquatic Sciences 72 319-342.

Kennedy J, Witthames PR, Nash RDM & Fox CJ 2008 Is fecundity in plaice (Pleuronectes platessa L.) down-regulated in response to reduced food intake during autumn? Journal of Fish Biology 72 78-92.

Krogdahl A & Bakke-McKellep AM 2005 Fasting and refeeding cause rapid changes in intestinal tissue mass and digestive enzyme capacities of Atlantic salmon (Salmo salar L.). Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology 141 450-460.

Lahood, E. S., J. J. Miller, C. Apland, and M. J. Ford. 2008. A rapid, ethanol-free fish tissue collection method for molecular genetic analyses. Transactions of the American Fisheries Society 137(4):1104–1107.

Larsen DA, Beckman BR & Dickhoff WW 2001 The effect of low temperature and fasting during the winter on growth and smoltification of coho salmon, Oncorhynchus kisutch. North American Journal of Aquaculture in press.

Larsen DA, Beckman BR, Strom CR, Parkins PJ, Cooper KA, Fast DE & Dickhoff WW 2006 Growth modulation alters the incidence of early male maturation and physiological development of hatchery-reared Spring Chinook Salmon: A comparison with wild fish. Transactions of the American Fisheries Society 135 1017-1032.

Le Bail PY, Sumpter JP, Carragher JF, Mourot B, Niu PD & Weil C 1991 Development and validation of a highly sensitive radioimmunoassay for chinook salmon (Oncorhynchus tshawytscha) growth hormone. Gen Comp Endocrinol 83 75-85.

Leitritz, E., and R.C. Lewis. 1976. Trout and Salmon Culture (Hatchery Methods). State of California The Resources Agency Department of Fish and Game Fish Bulletin 164.

Lotspeich, F.B., and F.H. Everest. 1981. A New Method for Reporting and Interpreting Textural Composition of Spawning Gravel. U.S. Department of Agriculture: Forest Service; Pacific Northwest Forest and Range Experiment Station; Research Note PNW-369.

Lubzens E, Young G, Bobe J & Cerda J 2010 Oogenesis in teleosts: how eggs are formed. General and Comparative Endocrinology 165 367-389.

Luckenbach JA, Dickey JT & Swanson P 2010 Regulation of pituitary GnRH receptor and gonadotropin subunits by IGF1 and GnRH in prepubertal male coho salmon. General and Comparative Endocrinology 167 387-396.

Lupu F, Terwilliger JD, Lee K, Segre GV & Efstratiadis A 2001 Roles of growth hormone and insulin-like growth factor 1 in mouse postnatal growth. Developmental Biology 229 141-162.

Marshall, T.C.J. Slate, L. Kruuk, and J.M. Pemberton. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Molecular Ecology 7:639-655.

Matala, A. P., M. W. Ackerman, M. R. Campbell, S. R. Narum (2014) Relative contributions of neutral and non-neutral genetic differentiation to inform conservation of steelhead trout across highly variable landscapes. Evolutionary Applications, 7, 682–701.

McBride RS, Somarakis S, Fitzhugh GR, Albert A, Yaragina NA, Wuenschel MJ, Alonso-Fernandez A & Basilone G 2015 Energy acquisition and allocation to egg production in relation to fish reproductive strategies. Fish and Fisheries 16 23-57.

McCormick SD 2012 Smolt Physiology and Endocrinology. In Fish Physiology, pp 200-253. Eds SD McCormick, AP Farrell & CJ Brauner.

McGlauflin, M. T., D. E. Schindler , L.W. Seeb , C. T. Smith , C. Habicht and J. E. Seeb. 2011. Spawning Habitat and Geography Influence Population Structure and Juvenile Migration Timing of Sockeye Salmon in the Wood River Lakes, Alaska, Transactions of the American Fisheries Society, 140:3, 763-782, DOI: 10.1080/00028487.2011.584495

Meador JP, Sommers FC, Ylitalo GM & Sloan CA 2006 Altered growth and related physiological responses in juvenile Chinook salmon (Oncorhynchus tshawytscha) from dietary exposure to polycyclic aromatic hydrocarbons (PAHs). Canadian Journal of Fisheries and Aquatic Sciences 63 2364-2376.

Medeiros LR, Elliott M & Nagler JJ 2016 Stressor timing, not cortisol, is an important embryo viability determinant in female rainbow trout Oncorhynchus mykiss. Journal of Fish Biology 88 557-566.

Medeiros LR, Galbreath PF, Knudsen CM, Stockton CA, Koch IJ, Bosch WJ, Narum SR, Nagler JJ & Pierce AL 2018 Plasma 11-Ketotestosterone in Individual Age-1 Spring Chinook Salmon Males Accurately Predicts Age-2 Maturation Status. Transactions of the American Fisheries Society 147 1042-1051.

Moffett IJJ, Kennedy GJA & Crozier WW 1996 Freshwater reconditioning and ranching of Atlantic salmon, Salmo salar l., kelts: growth and reproductive performance. Fisheries Management and Ecology 3 35-44.

Mommsen TP 1998 Growth and metabolism. In Physiology of Fishes, pp 65-97. Ed D Evans. Boca Raton, Florida: CRC Press.

Mommsen TP, French CJ & Hochachka PW 1980 Sites and patterns of protein and amino acid utilization during the spawning migration of salmon. Can J Zool 58 1785-1799.

Moore DS, Chaput G & Pickard R 1995 The effect of fisheries on the biological characteristics and survival of mature Atlantic salmon (Salmo salar) from the Miramichi River. In Water, Science, and the Public: the Miramichi Ecosystem, pp 229-247. Ed EMP Chadwick.

Moore, J.W., J.D. Yeakel, D. Peard, J. Lough, and M. Beere. 2014. Life-history diversity and its importance to population stability and persistence of a migratory fish: steelhead in two large North American watersheds. Journal of Animal Ecology 83, 1035-1046.

Moriyama S, Ayson FG & Kawauchi H 2000 Growth regulation by insulin-like growth factor-I in fish. Bioscience Biotechnology and Biochemistry 64 1553-1562.

Moriyama S, Swanson P, Nishii M, Takahashi A, Kawauchi H, Dickhoff WW & Plisetskaya EM 1994 Development of a Homologous Radioimmunoassay for Coho Salmon Insulin-Like Growth-Factor-I. General and Comparative Endocrinology 96 149-161.

Nagahama Y 1994 Endocrine regulation of gametogenesis in fish. International Journal of Developmental Biology 38 217-229.

Nagler JJ, Cavileer TD, Verducci JS, Schultz IR, Hook SE & Hayton WL 2012 Estrogen receptor mRNA expression patterns in the liver and ovary of female rainbow trout over a complete reproductive cycle. General and Comparative Endocrinology 178 556-561.

Narum, S. R., D. Hatch, A. J. Talbot, P. Moran and M. S. Powell. 2008. Iteroparity in complex mating systems of steelhead Oncorhynchus mykiss (Walbaum). Journal of Fish Biology 72:1-16.

Navarro I & Gutierrez J 1995 Fasting and starvation. In Biochemistry and Molecular Biology of Fishes, pp 393-434. Eds PW Hochachka & TP Mommsen. Amsterdam: Elsevier Science.

NMFS (National Marine Fisheries Service). 1996. Status review of west coast steelhead from Washington, Idaho, Oregon, and California. Seattle, WA.

NMFS (National Marine Fisheries Service). 2008. Endangered Species Act Section 7(a) (2) Consultation Biological Opinion and Magnuson-Stevens Fishery Conservation and Management Act Essential Fish Habitat Consultation on Remand for Operation of the Federal Columbia River Power System, 11 Bureau of Reclamation Projects in the Columbia Basin and ESA Section 10(a) (I) (A) Permit for Juvenile Fish Transportation Program (Revised and reissued pursuant to court order, NWF v. NMFS, Civ. No. CV 01-640-RE (D. Oregon))

NMFS (National Marine Fisheries Service). 2010. Endangered Species Act Section 7(a) (2) Consultation Supplemental Biological Opinion Supplemental Consultation on Remand for Operation of the Federal Columbia River Power System, 11 Bureau of Reclamation Projects in the Columbia Basin and ESA Section 10(a) (I) (A) Permit for Juvenile Fish Transportation Program

NMFS (National Marine Fisheries Service). 2014. Endangered Species Act Section 7(a) (2) Supplemental Biological Opinion Supplemental Consultation on Remand for Operation of the Federal Columbia River Power System

NMFS (National Marine Fisheries Service). 2019. Endangered Species Act Section 7(a) (2) Consultation Biological Opinion and Magnuson-Stevens Fishery Conservation and Management Act Essential Fish Habitat Response Continued Operation and Maintenance of the Columbia River System. NMFS Consultation Number: WCRO-2018-00152. March 29, 2019.

Norbeck LA, Kittilson JD & Sheridan MA 2007 Resolving the growth-promoting and metabolic effects of growth hormone: Differential regulation of GH-IGF-I system components. General and Comparative Endocrinology 151 332-341.

Nielsen JL, Turner SM & Zimmerman CE 2011 Electronic tags and genetics explore variation in migrating steelhead kelts (Oncorhynchus mykiss), Ninilchik River, Alaska. Canadian Journal of Fisheries and Aquatic Sciences 68 1-16.

Niemelä E, Erkinaro J, Julkunen M, Hassinen E, Lansman M & Brors S 2006a Temporal variation in abundance, return rate and life histories of previously spawned Atlantic salmon in a large subarctic river. Journal of Fish Biology 68 1222-1240.

Niemelä E, Orell P, Erkinaro J, Dempson JB, Brors S, Svenning MA & Hassinen E 2006b Previously spawned Atlantic salmon ascend a large subarctic river earlier than their maiden counterparts. Journal of Fish Biology 69 1151-1163.

Norberg B & Haux C 1985 Induction, isolation and a characterization of the lipid content of plasma vitellogenin from 2 salmo species - rainbow trout (Salmo gairdneri) and sea trout (Salmo trutta). Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology 81 869-876.

Oguri M & Ooshima Y 1977 Early changes in plasma osmolality and ionic concentrations of rainbow trout and goldfish following direct transfer from fresh-water to sea water. Bulletin of the Japanese Society of Scientific Fisheries 43 1253-1257.

Olsen, J.B., P. Bentzen, M.A. Banks, J.B. Shaklee, & S.Young. 2000. Microsatellites reveal population identity of individual pink salmon to allow supportive breeding of a population at risk of extinction. Transactions of the American Fisheries Society 129:232-242.

Osborne, V. (Ed.). (2015, April 1). Anglers Help Catch Steelhead Brood Stock. Retrieved January 14, 2016, from https://fishgame.idaho.gov/content/article/anglers-help-catch-steelhead-broodstock.

Palstra AP, Crespo D, van den Thillart GE & Planas JV 2010 Saving energy to fuel exercise: swimming suppresses oocyte development and downregulates ovarian transcriptomic response of rainbow trout Oncorhynchus mykiss. American Journal of Physiology: Regulatory, Integrative and Comparative Physiology 299 R486-499.

Pankhurst NW, King HR, Anderson K, Elizur A, Pankhurst PM & Ruff N 2011 Thermal impairment of reproduction is differentially expressed in maiden and repeat spawning Atlantic salmon. Aquaculture 316 77-87.

Penney ZL & Moffitt CM 2014a Histological assessment of organs in sexually mature and post-spawning steelhead trout and insights into iteroparity. Reviews in Fish Biology and Fisheries 24 781-801.

Penney ZL & Moffitt CM 2014b Proximate composition and energy density of stream-maturing adult steelhead during upstream migration, sexual maturity, and kelt emigration. Transactions of the American Fisheries Society 143 399-413.

Penney ZL & Moffitt CM 2015 Fatty-acid profiles of white muscle and liver in stream-maturing steelhead trout Oncorhynchus mykiss from early migration to kelt emigration. Journal of Fish Biology 86 105-120.

Perez-Sanchez J & Le Bail PY 1999 Growth hormone axis as marker of nutritional status and growth performance in fish. Aquaculture 177 117-128.

Perez-Sanchez J, Marti-Palanca H & Kaushik SJ 1995 Ration size and protein intake affect circulating growth hormone concentration, hepatic growth hormone binding and plasma insulin-like growth factor-I immunoreactivity in a marine teleost, the gilthead sea bream (Sparus aurata). J Nutr 125 546-552.

Perez-Sanchez J, Simo-Mirabet P, Naya-Catala F, Martos-Sitcha JA, Perera E, Bermejo-Nogales A, Benedito-Palos L & Calduch-Giner JA 2018 Somatotropic Axis Regulation Unravels the

Differential Effects of Nutritional and Environmental Factors in Growth Performance of Marine Farmed Fishes. Frontiers in Endocrinology 9.
Perez-Sanchez J, Weil C & Le Bail PY 1992 Effects of human insulin-like growth factor-I on release of growth hormone by rainbow trout (Oncorhynchus mykiss) pituitary cells. J Exp Zool 262 287-290.
Picha ME, Turano MJ, Beckman BR & Borski RJ 2008a Endocrine biomarkers of growth and applications to aquaculture: A minireview of growth hormone, insulin-like growth factor (IGF)-I, and IGF-Binding proteins as potential growth indicators in fish. North American Journal of Aquaculture 70 196-211.
Picha ME, Turano MJ, Tipsmark CK & Borski RJ 2008b Regulation of endocrine and paracrine sources of Igfs and Gh receptor during compensatory growth in hybrid striped bass (Morone chrysops X Morone saxatilis). Journal of Endocrinology 199 81-94.
Pierce AL, Beckman BR, Shearer KD, Larsen DA & Dickhoff WW 2001 Effects of ration on somatotropic hormones and growth in coho salmon. Comp Biochem Physiol B Biochem Mol Biol 128 255-264.
Pierce AL, Blodgett JW, Cavileer TD, Medeiros LR, Boyce J, Caldwell LK, Bosch WJ, Branstetter R, Fast DE, Hatch DR, et al. 2017 Reproductive development in captive reconditioned female steelhead kelts: evidence for consecutive and skip spawning life histories. Canadian Journal of Fisheries and Aquatic Sciences 74 1049-1060.
Pierce AL, Breves JP, Moriyama S, Hirano T & Grau EG 2011 Differential regulation of Igf1 and Igf2 mRNA levels in tilapia hepatocytes: effects of insulin and cortisol on GH sensitivity. Journal of Endocrinology 211 201-210.
Pierce AL, Fukada H & Dickhoff WW 2005a Metabolic hormones modulate the effect of growth hormone (GH) on insulin-like growth factor-I (IGF-I) mRNA level in primary culture of salmon hepatocytes. Journal of Endocrinology 184 341-349.
Pierce AL, Shimizu M, Beckman BR, Baker DM & Dickhoff WW 2005b Time course of the GH/IGF axis response to fasting and increased ration in chinook salmon (Oncorhynchus tshawytscha). Gen Comp Endocrinol 140 192-202.
Piry, S., A. Alapetite, J. M. Cornuet, D. Paetkau, L. Baudouin and A. Estoup. 2004. GeneClass2: A Software for Genetic Assignment and First-Generation Migrant Detection. Journal of Heredity 95:536-539.
Plummer, M. 2003. JAGS: A program for analysis of Bayesian graphical models using Gibbs sampling. Proceedings of the 3rd International Workshop on Distributed Statistical Computing. Vienna, Austria.
Prat F, Sumpter JP & Tyler CR 1996 Validation of radioimmunoassays for two salmon gonadotropins (GTH I and GTH II) and their plasma concentrations throughout the reproductive cycle in male and female rainbow trout (Oncorhynchus mykiss). Biology of Reproduction 54 1375-1382.
Quinn TP 2005 The Behavior and Ecology of Pacific Salmon and Trout. Seattle: University of Washington Press.
Quinn TP, McGinnity P & Reed TE 2016 The paradox of "premature migration" by adult anadromous salmonid fishes: patterns and hypotheses. Canadian Journal of Fisheries and Aquatic Sciences 73 1015-1030.

Quinn TP & Myers KW 2004 Anadromy and the marine migrations of Pacific salmon and trout: Rounsefell revisited. Reviews in Fish Biology and Fisheries 14 421-442.
Quinn TP, Seamons TR, Vøllestad LA & Duffy E 2011 Effects of Growth and Reproductive History on the Egg Size-Fecundity Trade-off in Steelhead. Transactions of the American Fisheries Society 140 45-51.
Rannala, B., and J. L. Mountain. 1997. Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Sciences of the United States of America 94(17):9197-9201.
Reid JE & Chaput G 2012 Spawning history influence on fecundity, egg size, and egg survival of Atlantic salmon (Salmo salar) from the Miramichi River, New Brunswick, Canada. ICES Journal of Marine Science 69 1678-1685.
Ricker, W.E. 1954. Stock and recruitment. J. Fish. Res. Board Can. 11: 559–623.
Reindl KM & Sheridan MA 2012 Peripheral regulation of the growth hormone-insulin-like growth factor system in fish and other vertebrates. Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology 163 231-245.
Rideout RM, Rose GA & Burton MPM 2005 Skipped spawning in female iteroparous fishes. Fish and Fisheries 6 50-72.
Rideout RM & Tomkiewicz J 2011 Skipped spawning in fishes: more common than you might think. Marine and Costal Fisheries: Dynamics, Management, and Ecosystem Science 3 176-189.
Reiser, D.W. 1999. Sediment in gravel bed rivers: ecological and biological consideration. In Gravel-bed rivers in the environment, 199-225. Edited by P.C. Klingeman, R.L. Beschta, P.D. Komar, and J.B. Bradely. Highlands Ranch, Colorado: Water Resources Publications.
Reiser, D. W., and R. G. White. Effects of two sediment size classes on survival of steelhead and Chinook salmon eggs. N. Am. J. Fish. Management, 8: 432–437 (1988).
Roberts RJ, Johnson KA & Kasten MT 2004 Control of Salmincola californiensis (Copepoda: Lernaeapodidae) in rainbow trout, Oncorhynchus mykiss (Walbaum): a clinical and histopathological study. Journal of Fish Diseases 27 73-79.
Rousseau K, Huang YS, Le Belle N, Vidal B, Marchelidon J, Epelbaum J & Dufour S 1998 Long-term inhibitory effects of somatostatin and insulin-like growth factor 1 on growth hormone release by serum-free primary culture of pituitary cells from European eel (Anguilla anguilla). Neuroendocrinology 67 301-309.
Rowe DK & Thorpe JE 1990 Differences in growth between maturing and nonmaturing male Atlantic salmon, Salmo salar L., parr. Journal of Fish Biology 36 643-658.
Sadoul B & Vijayan MM 2016 Stress and Growth. In Fish Physiology, pp 167-205.
Satterthwaite WH, Beakes MP, Collins EM, Swank DR, Merz JE, Titus RG, Sogard SM & Mangel M 2009 Steelhead life history on California's central coast: insights from a state dependent model. Transactions of the American Fisheries Society 138 532-548.
Seamons, TR, TP Quinn. 2010. Sex-specific patterns of lifetime reproductive success in single and repeat breeding steelhead trout (Oncorhynchus mykiss). Behav. Ecol. Sociobiol. 64:505–513. DOI 10.1007/s00265-009-0866-7.
Schindler DE, Hilborn R, Chasco B, Boatright CP, Quinn TP, Rogers LA & Webster MS 2010 Population diversity and the portfolio effect in an exploited species. Nature 465 609-612.

Shearer K, Parkins P, Gadberry B, Beckman B & Swanson P 2006 Effects of growth rate/body size and a low lipid diet on the incidence of early sexual maturation in juvenile male spring Chinook salmon (Oncorhynchus tshawytscha). Aquaculture 252 545-556.

Shearer KD & Swanson P 2000 The effect of whole body lipid on early sexual maturation of 1+ age male Chinook salmon (Oncorhynchus tshawytscha). Aquaculture 190 343-367.

Sheridan MA 1994 Regulation of lipid metabolism in poikilothermic vertebrates. Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology 107 495-508.

Shimizu M, Swanson P, Fukada H, Hara A & Dickhoff WW 2000 Comparison of extraction methods and assay validation for salmon insulin-like growth factor-I using commercially available components. General and Comparative Endocrinology 119 26-36.

Schreck CB, Contreras-Sanchez W & Fitzpatrick MS 2001 Effects of stress on fish reproduction, gamete quality, and progeny. Aquaculture 197 3-24.

Schroder, S.L., C.M. Knudsen, T.N. Pearsons, T.W. Kassler, S.F. Young, C.A. Busack, & D.E. Fast. 2008. Breeding Success of Wild and First-Generation Hatchery Female Spring Chinook Salmon spawning in an Artificial Stream. Transactions of the American Fisheries Society 137:1475-1489.

Schroder, S. L., C. M. Knudsen, T. N. Pearsons, T. W. Kassler, S. F. Young, E.P. Beall, & D. E. Fast. 2010. Behavior and Breeding Success of Wild and First-Generation Hatchery Male Spring Chinook Salmon Spawning in an Artificial Stream. Transactions of the American Fisheries Society, 139:989-1003.

Silverstein JT, Shearer KD, Dickhoff WW & Plisetskaya EM 1998 Effects of growth and fatness on sexual development of chinook salmon (Oncorhynchus tshawytscha) parr. Canadian Journal of Fisheries and Aquatic Sciences 55 2376-2382.

Simpkins DG, Hubert WA, Del Rio CM & Rule DC 2003 Physiological responses of juvenile rainbow trout to fasting and swimming activity: effects on body composition and condition indices. Transactions of the American Fisheries Society 132 576-589.

Small BC & Peterson BC 2005 Establishment of a time-resolved fluoroimmunoassay for measuring plasma insulin-like growth factor I (IGF-I) in fish: effect of fasting on plasma concentrations and tissue mRNA expression of IGF-I and growth hormone (GH) in channel catfish (Ictalurus punctatus). Domestic Animal Endocrinology 28 202-215.

Stead SM, Houlihan DF, McLay HA & Johnstone R 1999 Food consumption and growth in maturing Atlantic salmon (Salmo salar). Canadian Journal of Fisheries and Aquatic Sciences 56 2019-2028.

Stearns SC 1992 The Evolution of Life Histories. New York: Oxford University Press.

Steele, C., M. Ackerman, J. McCane, M. Campbell, M. Hess and S. Narum. 2012. Parentage based tagging of Snake River hatchery-origin steelhead and Chinook salmon. 2011 Annual Report. Submitted to: U.S. Department of Energy, Bonneville Power Administration, Division of Fish and Wildlife, Project Number 2010-031-00; Contract Number 53238. Available at: https://pisces.bpa.gov/release/documents/documentviewer.aspx?doc=P127156

Steele, C. A., E. C. Anderson, M. W. Ackerman, M. A. Hess, N. R. Campbell, S. R. Narum, and M. R. Campbell. 2013. A validation of parentage-based tagging using hatchery steelhead in the Snake River basin. Canadian Journal of Fisheries and Aquatic Sciences 70:1046–1054.

Stephenson, J., M. Campbell, J., Hess et al. (2009). A centralized model for creating shared, standardized, microsatellite data that simplifies inter-laboratory collaboration. Conservation Genetics, 10, 1145–1149.
Stoddard JW, Parsons JE & Nagler JJ 2005 Early onset of embryonic mortality in sub-fertile families of rainbow trout (Oncorhynchus mykiss). Reproduction Fertility and Development 17 785-790.
Sumpter JP, Lebail PY, Pickering AD, Pottinger TG & Carragher JF 1991 The Effect of Starvation on Growth and Plasma Growth-Hormone Concentrations of Rainbow-Trout, Oncorhynchus-Mykiss. General and Comparative Endocrinology 83 94-102.
Sutton SG, Bult TP & Haedrich RL 2000 Relationships among fat weight, body weight, water weight, and condition factors in wild Atlantic salmon parr. Transactions of the American Fisheries Society 129 527-538.
Takei Y & McCormick SD 2012 Hormonal Control of Fish Euryhalinity. In Fish Physiology, pp 70-125. Eds SD McCormick, AP Farrell & CJ Brauner: Academic Press.
Tannenbaum GS 1993 Genesis of Episodic Growth-Hormone Secretion. Journal of Pediatric Endocrinology 6 273-282.
Tappel, P.D. and T.C. Bjornn. 1983. A New Method of Relating Size of Spawning Gravel to Salmonid Embryo Survival. Idaho Cooperative Fishery Research Unit. University of Idaho, Moscow, Idaho 83843.
Taranger GL, Carrillo M, Schulz RW, Fontaine P, Zanuy S, Felip A, Weltzien FA, Dufour S, Karlsen O, Norberg B, et al. 2010 Control of puberty in farmed fish. General and Comparative Endocrinology 165 483-515.
Taylor JF, Migaud H, Porter MJR & Bromage NR 2005 Photoperiod influences growth rate and plasma insulin-like growth factor-I levels in juvenile rainbow trout, Oncorhynchus mykiss. General and Comparative Endocrinology 142 169-185.
Taylor JF, Porter MJR, Bromage NR & Migaud H 2008 Relationships between environmental changes, maturity, growth rate and plasma insulin-like growth factor-I (IGF-I) in female rainbow trout. General and Comparative Endocrinology 155 257-270.
Thissen JP, Ketelslegers JM & Underwood LE 1994 Nutritional Regulation of the Insulin-Like Growth-Factors. Endocrine Reviews 15 80-101.
Thissen JP, Underwood LE & Ketelslegers JM 1999 Regulation of insulin-like growth factor-I in starvation and injury. Nutr Rev 57 167-176.
Thorpe JE 1994 Reproductive strategies in Atlantic salmon, Salmo salar L. Aquaculture and Fisheries Management 25 77-87.
Thorpe JE 2007 Maturation responses of salmonids to changing developmental opportunities. Marine Ecology Progress Series 335 285-288.
Thorpe JE, Mangel M, Metcalfe NB & Huntingford FA 1998 Modelling the proximate basis of salmonid life-history variation, with application to Atlantic salmon, Salmo salar L. Evolutionary Ecology 12 581-599.
Trammell JLJ, Fast DE, Hatch DR, Bosch WJ, Branstetter R, Pierce AL, Blodgett JW & Frederiksen CR 2016 Evaluating steelhead kelt treatments to increase iteroparous spawners in the Yakima River Basin. North American Journal of Fisheries Management 36 876-887.
Tyler CR & Sumpter JP 1996 Oocyte growth and development in teleosts. Reviews in Fish Biology and Fisheries 6 287-318.

Tyler CR, Sumpter JP & Witthames PR 1990 The dynamics of oocyte growth during vitellogenesis in the rainbow trout (Oncorhynchus mykiss). Biology of Reproduction 43 202-209.

Uchida K, Kajimura S, Riley LG, Hirano T, Aida K & Grau EG 2003 Effects of fasting on growth hormone/insulin-like growth factor I axis in the tilapia, Oreochromis mossambicus. Comp Biochem Physiol A Mol Integr Physiol 134 429-439.

Whitehead C, Bromage NR & Breton B 1983 Changes in serum levels of gonadotropin, estradiol 17-beta and vitellogenin during the 1st and subsequent reproductive cycles of female rainbow trout. Aquaculture 34 317-326.

Wilkinson RJ, Longland R, Woolcott H & Porter MJR 2010 Effect of elevated winter-spring water temperature on sexual maturation in photoperiod manipulated stocks of rainbow trout (Oncorhynchus mykiss). Aquaculture 309 236-244.

Wilkinson RJ, Porter M, Woolcott H, Longland R & Carragher JF 2006 Effects of aquaculture related stressors and nutritional restriction on circulating growth factors (GH, IGF-I and IGF-II) in Atlantic salmon and rainbow trout. Comparative Biochemistry and Physiology 145 214-224.

Winans, G. A., M. M. Paquin, D. M. Van Doornik, B. M. Baker, P. Thornton, D. Rawding, A. Marshall, P. Moran, and S. Kalinowski. 2004. Genetic stock identification of steelhead in the Columbia River Basin: an evaluation of different molecular markers. North American Journal of Fisheries Management 24:672–685. doi:10.1577/M03-052.1.

Withler, I. L. 1966. Variability in life history characteristics of steelhead trout (Salmo gairdneri) along the Pacific coast of North America. Journal of the Fisheries Research Board of Canada 23, 365–393.

Wood AW, Duan C & Bern HA 2005a Insulin-like growth factor signaling in fish. International Review of Cytology 243 215-285.

Wood AW, Duan C & Bern HA 2005b Insulin-like growth factor signaling in fish. Int Rev Cytol 243 215-285.

Wootton RJ & Smith C 2015 Reproductive Biology of Teleost Fishes. West Sussex, UK: John Wiley & Sons, Ltd.

Xu Y, Wang B, Liu X, Shi B & Zang K 2017 Evidences for involvement of growth hormone and insulin-like growth factor in ovarian development of starry flounder (Platichthys stellatus). Fish Physiol Biochem 43 527-537.

Yamamoto Y, Adam Luckenbach J, Goetz FW, Young G & Swanson P 2011 Disruption of the salmon reproductive endocrine axis through prolonged nutritional stress: changes in circulating hormone levels and transcripts for ovarian genes involved in steroidogenesis and apoptosis. General and Comparative Endocrinology 172 331-343.

Yamashita S & Melmed S 1986 Insulin regulation of rat growth hormone gene transcription. J Clin Invest 78 1008-1014.

Zaldua N & Naya DE 2014 Digestive flexibility during fasting in fish: a review. Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology 169 7-14.

Appendices

A1.a Master Kelt Tracking Table

Strategy	Year	Location	# Collected	# released	# @ Bonneville	Return Rate to Bonneville (%)	# @ Prosser or Lower Granite Dam	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	4.6%			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	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
Total and weighted mean			***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%	4.32	0.68	1.96
In-river	2010	Lower Granite	1410	1399	5	0.4%	4	0.3%	1.36	0.21	0.42
In-river	2011	Lower Granite	1633	1613	3	0.2%	3	0.2%	0.71	0.11	0.10
In-river	2012	Lower Granite	2098	2098	4	0.2%	3	0.1%	0.73	0.11	0.10
In-river	2013	Lower Granite	840	827	3	0.4%	2	0.2%	1.37	0.22	0.37
In-river	2014	Lower Granite	2584	2571	11	0.4%	9	0.4%	1.64	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%	0.84	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%	1.33	0.21	0.31
In-river	2019	Lower Granite	935	934	TBD 2020	TBD 2020	TBD 2020	TBD 2020	TBD 2020	TBD 2020	TBD 2020
Total and weighted mean			***14406***	***14332***	**35**	***0.3%***	**26**	***0.2%***	***1.23***	***0.16***	***0.30***

Strategy	Year	Location	# Collected	# released
Transported: Immature (Fall Release @ Hamilton Is.)	2019	Prosser	***103***	***103***

Strategy	Year	Location	# Collected	# released	S @ release (%)	# remature	Retained	skip remature	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	6	15.62	27.33	67.76
Long-term	2019	Prosser	351	248	70.66	145	0	0	24.32	42.56	193.12
Total and weighted mean			***10375***	***4467***	***43.1***	***1823***	***363***	***92***	***14.82***	***25.94***	***83.52***

Strategy	Year	Location	# Collected	# *Survived*	S @ release (%)	Released	# consecutive. remature	Retained	skip remature	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	-	-	-	9.80	1.08	3.58
Long-term	2012	Lower Granite	124	10	8.06	10	3	0	-	169.19	4.86	7.49
Long-term	2013	Lower Granite	110	57	51.82	57	3	0	-	217.64	31.22	47.30
Long-term	2014	Lower Granite	110	34	30.91	34	0	0	-	99.84	18.62	53.41
Long-term	2015	Lower Granite	22	11	50.00	8	8	3	3	170.23	30.12	96.99
Long-term	2016	Lower Granite	227	120	52.86	19	19	101	77	243.30	31.85	851.10
Long-term	2017	Lower Granite	269	79	29.37	21	21	58	29	99.99	17.69	56.97
Long-term	2018	Lower Granite	259	177	68.34	50	50	99	1	197.73	41.17	102.05
Long-term	2019	Lower Granite	288	121	42.01	39	39	85	***TBD 2020***	143.04	25.31	114.84
Total and weighted mean			***1520***	***611***	***40.2***	***240***	***143***	***346***	***110***	***136.86***	***24.22***	***77.98***

Strategy	Year	Location	# Collected	# @ 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
Total			22890	118	***0.52***

[redacted]

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.

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

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

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

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

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

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

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

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

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

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

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

Trammell, J.L.J., D.E. Fast, D.R. Hatch, W.J. Bosch, R. Branstetter, J.W. Blodgett, A.L. Pierce, and C.R. Frederiksen. 2016. Evaluating steelhead management scenarios to increase iteroparous spawners in the Yakima River Basin. North American Journal of Fisheries Management.

Published in 2019

Jenkins LE, Pierce AL, Graham ND, Medeiros LR, Hatch DR, and Nagler JJ. 2019. Elevated plasma triglycerides and growth are early indicators of reproductive status in post-spawning female steelhead trout Oncorhynchus mykiss. Conservation Physiology.

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.

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.

A2.A: Elevated plasma triglycerides and growth rate are early indicators of reproductive status in post-spawning female steelhead trout (*Oncorhynchus mykiss*)

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Many iteroparous fishes spawn after skipping one or more yearly cycles, which impacts recruitment estimates used for fisheries management and conservation. The physiological mechanisms underlying the development of consecutive and skip spawning life histories in fishes are not well understood. In salmonids, lipid energy reserves and/or growth are thought to regulate the initiation of reproductive maturation during a critical period ~1 year prior to spawning. The fasting spawning migration of summer-run steelhead trout (*Oncorhynchus mykiss*) results in significant depletion of energy reserves during the proposed critical period for repeat spawning. To determine whether and when lipid energy reserves and growth influence repeat spawning, measures of lipid energy reserves, growth rate and reproductive development were tracked in female steelhead trout from first to second spawning as a consecutive or skip spawner in captivity. Plasma triglyceride (TG) levels and growth rate were elevated by 10 weeks after spawning in reproductive (i.e. consecutive spawning) versus non-reproductive (i.e. skip spawning) individuals. Muscle lipid (ML) levels, condition factor and plasma estradiol levels increased at later time points. The early differences in plasma TG levels and increases in growth rate are attributable to differential rates of feeding and assimilation between the groups following spawning. A year after spawning, plasma TG levels, MLs and growth rate decreased in consecutive spawners, attributable to transfer of lipid reserves into the ovary. During the year prior to second spawning, energy reserves and plasma estradiol levels were higher in reproductive skip spawners versus consecutive spawners, reflecting the energy deficit after first spawning. These results suggest that the decision to initiate ovarian recrudescence occurs by 10 weeks after first spawning and are consistent with the differences in energy reserves acquired following spawning being a consequence of that decision. This information will increase the success of conservation projects reconditioning post-spawning summer-run steelhead trout.

Key words: Estradiol-17β, growth, life history, reproduction, skip spawning, triglycerides

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Introduction

Skipped spawning is common in seasonally breeding iteroparous fishes (Rideout and Tomkiewicz, 2011). After the first spawning event, individuals may spawn again after a 1-year interval (consecutive spawners) or after skipping 1 or more years (skip spawners). Many fish populations of conservation concern exhibit consecutive and skip spawning, which significantly impacts management, particularly of exploited stocks (Rideout *et al*., 2005). Reproductive sched- ules are phenotypically plastic and respond to environmental conditions (Thorpe *et al*., 1998; Rideout *et al*., 2005; Chaput and Benoit, 2012), suggesting that reproductive decisions and subsequent life history diversity will be sensitive to climate change, for example. Energetic status is thought to be the main determinant of the decision to engage in reproductive activity (i.e. initiate ovarian recrudescence) as a consecutive spawner or to defer reproductive activity for a future year leading to skip spawning (Thorpe, 1994; Rideout *et al*., 2005). Many of the fish species that exhibit skip spawning are capital breeders that fund reproduction from energy stores acquired prior to the majority of reproductive investment (McBride *et al*., 2015). This has resulted in the idea of a threshold level of energy reserves required to successfully complete gonadal development, spawning and associated activities such as migration. In this reaction norm framework, an individual's condition or level of energy reserves interacts with a genetically determined threshold to generate a decision to either engage in reproductive activity or to remain reproductively inactive for the given reproductive cycle (Hutchings, 2011). However, the proximate physiological mechanisms involved in the decision to initiate or defer reproductive activity as a post-spawning adult are not fully understood.

The critical period hypothesis of salmonid maturation was developed for Atlantic salmon (Salmo salar) and proposes that the maturation decision takes place during a seasonally defined critical period ~1 year before spawning and that it is permissively gated by stores of metabolic fuels (Thorpe *et al*., 1998; Thorpe, 2007). The aspect of metabolic fuel storage that gates entry into a reproductive cycle, which is thought to occur at the transition to secondary oocyte growth in salmonids (Campbell *et al*., 2006; Lubzens *et al*., 2010), is not known but is functionally defined as either the absolute level or the rate of change of body size (growth) and/or lipid reserves (Thorpe, 2007; Taranger *et al*., 2010). Triglycerides (TGs) are the primary form in which lipids are stored for energy in fishes (Sheridan, 1994) and are a rea- sonable representation of lipid energy reserves. Reproductive decisions in salmonids have been most studied in the context of maturation (i.e. puberty). It is reasonable to assume that similar mechanisms operate in repeat spawning as in first time spawning, although energy depletion from the initial spawning event will also play a role. Early gametogenesis was found to be under energetic control during a critical period approximately a year before repeat spawning in winter flounder (*Pleuronectes americanus*) (Burton, 1994). Although considerable support for such critical periods exists, to our knowledge, the timing of the maturation decision window and the relative importance of lipid reserves and growth rate in the initiation of recrudescence have not been precisely delineated in any species.

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

Reconditioning of post-spawning steelhead trout (kelts) has been developed as a conservation measure for declining populations of interior summer-run CRB steelhead trout listed as threatened under the US Endangered Species Act (ESA) (Hatch *et al*., 2013; Trammell *et al*., 2016). Kelt reconditioning projects are being implemented at several locations in the interior CRB and aim to increase the productivity of steelhead trout populations by allowing wild- origin fish the opportunity to spawn again. Kelts are captured after spawning, held and fed in freshwater and then released to migrate upstream and spawn again. Kelts collected for reconditioning are predominantly female, and some projects only recondition female fish (Hatch *et al*., 2013; Hatch *et al*., 2016). The consecutive and skip spawning life histories are observed in reconditioned female kelts and vary significantly in proportion by location and year (Hatch *et al*., 2016; Pierce *et al*., 2017). Individuals determined to be reproductive after reconditioning over the summer are released to spawn as consecutive spawners, whereas fish determined to be non- reproductive after a summer of reconditioning must be held for an additional year until they can be released as reproductive skip spawners. This adds complexity to the man- agement of kelt reconditioning projects. Thus, understanding

the timing and basis of reproductive decisions in repeat spawning female steelhead trout will directly assist in the management of CRB steelhead kelt reconditioning projects. More generally, advances in understanding of the physiology of reproductive life history decisions in repeat spawning steelhead trout would be expected to lead to improvements in the management of other

fish populations displaying consecutive and skip spawning.

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

Materials and methods

Fish

Female steelhead trout O. mykiss were captured after return- ing on their first spawning migration and ascending the adult ladder trap at DNFH, on the Clearwater River in Ahsahka, ID, USA. Fish were collected for up to several weeks prior to spawning and maintained in holding ponds supplied with North Fork Clearwater River water. Females were selected for spawning based on a minimum criterion of 70 cm fork length (FL). Fish in good and fair condition with no visible wounds were selected for this study (Hatch et al., 2013, 2016). Nearly all females included in the study became sexually mature for the first time at age 4; age was confirmed for a subset of the study fish (L. Jenkins et al., unpublished data).

Spawning

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

Sampling

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

Fish husbandry

After spawning fish were placed in 4.6 m diameter outdoor tanks, with a water height of 1.5 m located at DNFH. Tanks were supplied with water at a flow of ∼200 L min^{-1} drawn from the North Fork Clearwater River, with a seasonally varying temperature profile (4.9–11.0°C). Fish were fed *ad libitum* a mixture of boiled krill (*Euphausia superba*, Atlantic Pacific Products Inc., Kingston, RI) and pellets (Bio- brood 6 mm pellet size, BioOregon Inc., Longview, WA) top coated with menhaden oil (Argent Aquaculture LLC, Redmond, WA) and freeze-dried decapsulated *Artemia* cysts (American Brine Shrimp, Ogden, UT) for increased palata- bility. At spawning, fish were prophylactically treated for bacterial infection with oxytetracycline (Durvet, Blue Springs, Missouri; 20 mg kg^{-1} body mass) and for parasitic gill copepods (*Salmincola californiensis*) with emamectin (Sigma- Aldrich, St. Louis, Missouri; 200 µg kg^{-1} body mass), both via intraperitoneal injection. Oxytetracycline injections con- tinued at 10-week intervals during sampling, with emamectin injections applied only when copepods were visible on the gills. Tanks were treated with formalin (Syndel USA, Portland, OR; flow through treatment, 1:6000 for 1 h daily) to control *Saprolegnia*.

Survival

Mortality occurred during reconditioning as expected for steelhead trout kelts (Hatch et al., 2013). On average, 55% of the mortality occurred within 10 weeks of spawning (47% and 62% for 2015 and 2016, respectively). Survival to 30 weeks after spawning was 29% in 2015 (43/150 fish) and 18% in 2016 (30/163). Survival for fish that did not spawn 1 year after first spawning was 17% in 2015 (25/150) and 14% (21/150) to 1 year plus 30 weeks after first spawning. In November 2016, ~35 weeks after first spawning for the 2016 fish, 1 year and 35 weeks after first spawning for the 2015 fish and ~15 weeks prior to second spawning for the 2015 skip spawners and the 2016 consecutive spawners, all fish died due to an equipment malfunction. A necropsy was performed on all mortalities.

Assays

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

Morphometric analysis

Fulton's condition factor (K) was calculated as

$K = 100 * \text{body mass}\,(\text{g}) * (\text{fork length}\,(\text{cm}))^{-3}$. Mass specific growth rate (MSGR) was calculated as:

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

Length specific growth rate (LSGR) was calculated in the same manner as that for mass.

Determination of reproductive status

Reproductive status was assigned in early autumn, 30 weeks after spawning, based on complete separation of fish into two E2 concentration groups (high levels = reproductive, low levels = non-reproductive) and confirmed by spawning of sur- vivors (Jenkins et al., 2018) or at necropsy for pre-spawn mortalities by examining developing ovaries for large oocytes. In Year 1 following first spawning, consecutive spawners were reproductive and skip spawners were non-reproductive. In the year prior to second spawning, both consecutive and skip spawners were reproductive. Reproductive skip spawners had to survive Year 1 as non-reproductive and be assigned as reproductive in Year 2.

Statistical analysis

Fish were first compared based on reproductive status in Year 1. Reproductive ($n = 13$, 2015; $n = 12$, 2016) and non-reproductive fish ($n = 30$, 2015; $n = 18$, 2016) were compared at 10-week intervals in a time series starting at spawning. Fish of the 2015 spawn year were then compared based on repro- ductive interval: consecutive spawners (Year 1, $n = 13$) and reproductive skip spawners (Year 2, $n = 18$) were compared at the same relative time points during the year prior to second spawning.

Two-way repeated measures analysis of variance (ANOVA) was employed to test for time, group, interaction and subject effects on TG levels, ML levels, K, MSGR, LSGR and E2 levels. E2 levels were $\log_{10}$-transformed, and ML levels were arcsine square root transformed prior to analysis to conform to assumptions of normality. Where significant effects were found, one-way repeated measures ANOVA was used to assess the effects of time and reproductive status or interval on individual fish, followed by Tukey's honestly significant difference (HSD) test or a T-test. T-tests were used to assess differences in individual trajectories for reproductive and non-reproductive fish from 0 to 10 weeks (TG) and from 10 to 20 weeks (E2) in the year following first spawning, assessed as a percentage of the level at the previous time period.

Of the 2015 consecutive spawners ($n = 13$) in 2015, $n = 12$ were included in the analysis during the year following first spawning, as one fish was excluded due to a missing sampling point as required by repeated measures ANOVA. Of the 2015 non-reproductive skip spawners ($n = 30$), $n = 20$ were analysed, as nine fish had missing sampling points and one fish was excluded due to a distinctly non-representative negative TG, growth rate, ML and K trajectory starting in early summer (20 weeks post-spawn), followed by death 1 year after spawning. Of the 2016 consecutive spawners ($n = 12$), $n = 10$ consecutive spawners and $n = 18$ non- reproductive skip spawners were analysed, as two consecutive spawners had missing sampling points. Additional individuals were excluded from the TG analysis due to missing sampling points (one consecutive

spawner 2015, one non-reproductive skip spawner 2016 and five reproductive skip spawners 2015). The Rout Outlier Test was used to detect and remove outliers (0.3% average number of outliers per group). Unless otherwise indicated, all statistical analyses were conducted with PRISM software version 7.0 (GraphPad Inc., La Jolla, CA). Results are reported as significant when $P < 0.05$.

Ethics

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

Table 1: Two-way repeated measures ANOVA test statistics for each dependent variable tracked over the year after first spawning (2015, 2016) for reproductive and non-reproductive groups of female steelhead trout.

Measure	Source of variation	2015		2016	
		F (DF_n, DF_d)	P-value	F (DF_n, DF_d)	P-value
TG	Time	F (4.2, 122.8) = 33.5	$P < 0.001$	F (2.3, 57.4) = 14.7	$P < 0.0001$
	Group	F (1, 29) = 10.08	$P = 0.0035$	F (1, 25) = 8.275	$P = 0.0081$
	Interaction	F (5, 145) = 8.783	$P < 0.001$	F (3, 75) = 2.286	**$P = 0.0856$**
	Subject	F (29, 145) = 2.923	$P < 0.001$	F (25, 75) = 1.837	$P = 0.0232$
ML	Time	F (2.3, 67.7) = 97.9	$P < 0.001$	F (1.3, 34.9) = 71.6	$P < 0.0001$
	Group	F (1, 30) = 1.652	**$P = 0.2085$**	F (1, 26) = 6.446	$P = 0.0174$
	Interaction	F (5, 150) = 26.65	$P < 0.001$	F (3, 78) = 5.307	$P = 0.0022$
	Subject	F (30, 150) = 5.127	$P < 0.001$	F (26, 78) = 2.248	$P = 0.0033$
K	Time	F (2.2, 64.9) = 127.6	$P < 0.001$	F (1.5, 39.2) = 78.6	$P < 0.0001$
	Group	F (1, 30) = 5.480	$P = 0.0261$	F (1, 26) = 10.46	$P = 0.0033$
	Interaction	F (5, 150) = 7.868	$P < 0.001$	F (3, 78) = 9.740	$P < 0.0001$
	Subject	F (30, 150) = 10.09	$P < 0.001$	F (26, 78) = 3.669	$P < 0.0001$
MSGR	Time	F (3.3, 96.9) = 42.9	$P < 0.0001$	F (1.9, 47.3) = 42.5	$P < 0.0001$
	Group	F (1, 29) = 16.33	$P = 0.0004$	F (1, 25) = 10.10	$P = 0.0039$
	Interaction	F (4, 116) = 1.786	**$P = 0.1364$**	F (2, 50) = 0.07	**$P = 0.9351$**
	Subject	F (29, 116) = 1.358	**$P = 0.1294$**	F (25, 50) = 1.7	**$P = 0.0554$**
LSGR	Time	F (3.4, 93.9) = 45.31	$P < 0.0001$	F (1.7, 43.4) = 72.8	$P < 0.0001$
	Group	F (1, 28) = 4.908	$P = 0.0350$	F (1, 26) = 2.895	**$P = 0.1008$**
	Interaction	F (4, 112) = 9.267	$P < 0.0001$	F (2, 52) = 1.909	**$P = 0.1584$**
	Subject	F (28, 112) = 1.329	**$P = 0.1505$**	F (26, 52) = 1.402	**$P = 0.1486$**
E2	Time	F (2.8, 78.0) = 49.7	$P < 0.0001$	F (2.1, 51.8) = 22.8	$P < 0.0001$
	Group	F (1, 28) = 208.2	$P < 0.0001$	F (1, 25) = 105.4	$P < 0.0001$
	Interaction	F (5, 140) = 51.77	$P < 0.0001$	F (3, 75) = 40.46	$P < 0.0001$
	Subject	F (28, 140) = 2.096	$P = 0.0027$	F (25, 75) = 1.257	**$P = 0.2223$**

Bolded P-values indicate non-significance.

Results

Post-spawning reproductive status

Of fish that survived to 30 weeks after first spawning, 30% (13/43) and 40% (12/30) became reproductively active as consecutive spawners in 2015 and 2016, respectively. Of the 30 non-reproductive skip spawners from 2015, 70% survived to 1 year plus 30 weeks after spawning (21/30), and 86% became reproductively active skip spawners in 2016 (18/21). No evidence of arrested reproductive development after 20 weeks post-spawning (i.e. premature decreases in plasma E2 level) was detected in any individual.

10 20 30 40 50

0

Time course following first spawning

Two-way repeated measures ANOVA found significant effects of group, time, group∗time interactions and subject (Table 1).TG levels were greater in reproductive than in non- reproductive fish at 10 weeks after first spawning in both years, remaining that way except for Week 20 in 2016 and Week 50 in 2015 (Fig. 1). At the individual level, from Week 0 to Week 10, TG decreased in non-reproductive fish (19/20, 15/16 decreased; to 56% and 58% of Week 0 in 2015 and 2016, respectively) and stayed the same or decreased to a significantly lesser extent in reproductive fish (8/11, 5/10

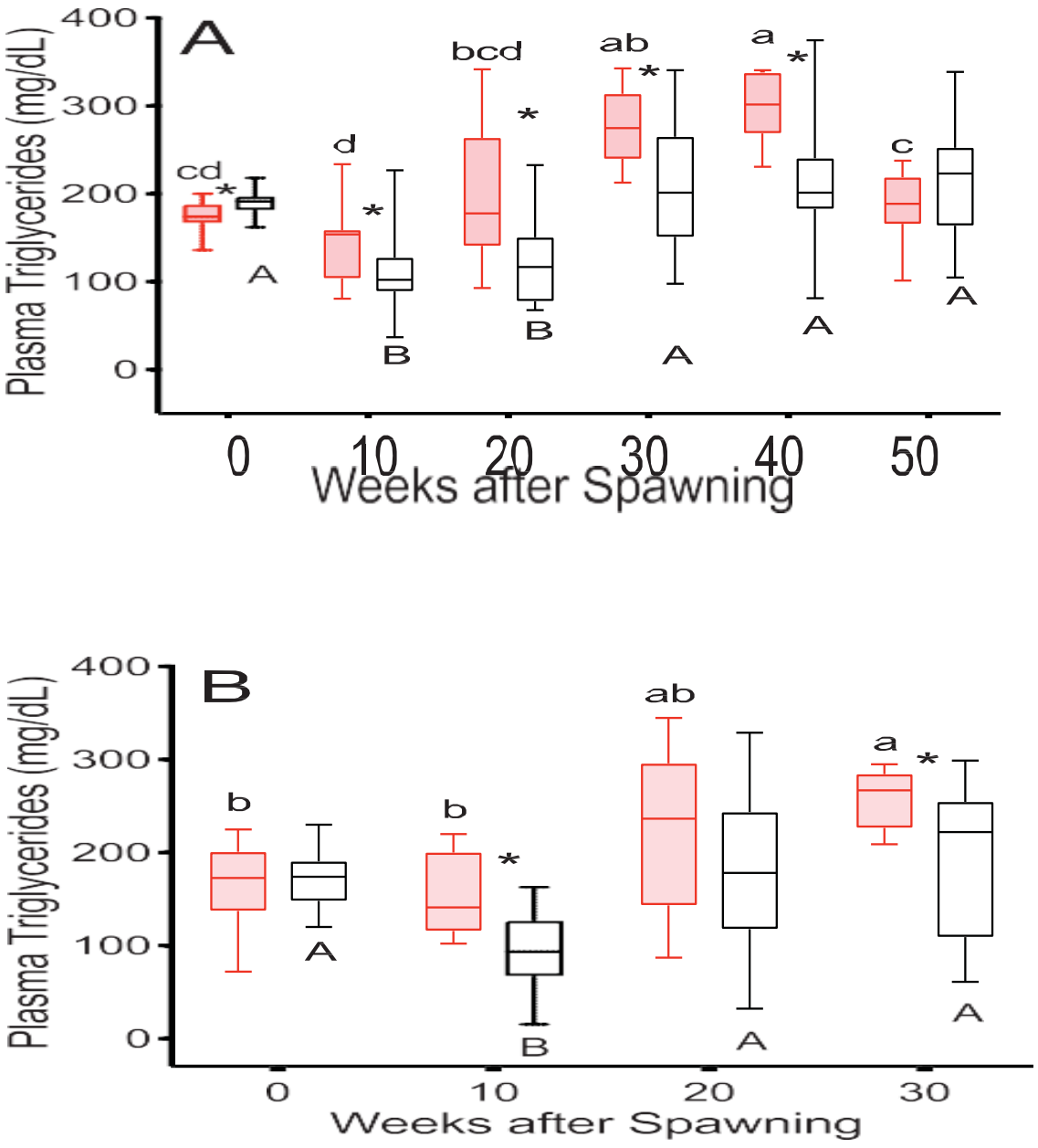


Figure 1: Plasma TG concentrations in female steelhead trout from the Clearwater River, Idaho, sampled in 2015 (A) and 2016 (B) at 10-week intervals following spawning such that analysis included reproductive (red, shaded boxes, $n = 11, 10$; 2015 and 2016, respectively) and non-reproductive fish (black, $n = 20, 17$; 2015 and 2016, respectively), box heights indicate interquartile range, horizontal lines within indicate the median and whiskers show the data range, time points within a group sharing the same letter do not differ significantly (repeated measures one-way ANOVA followed by Tukey's HSD Test, $P < 0.05$), and asterisks indicate significant differences between groups at each time point (T-test, $P < 0.05$)

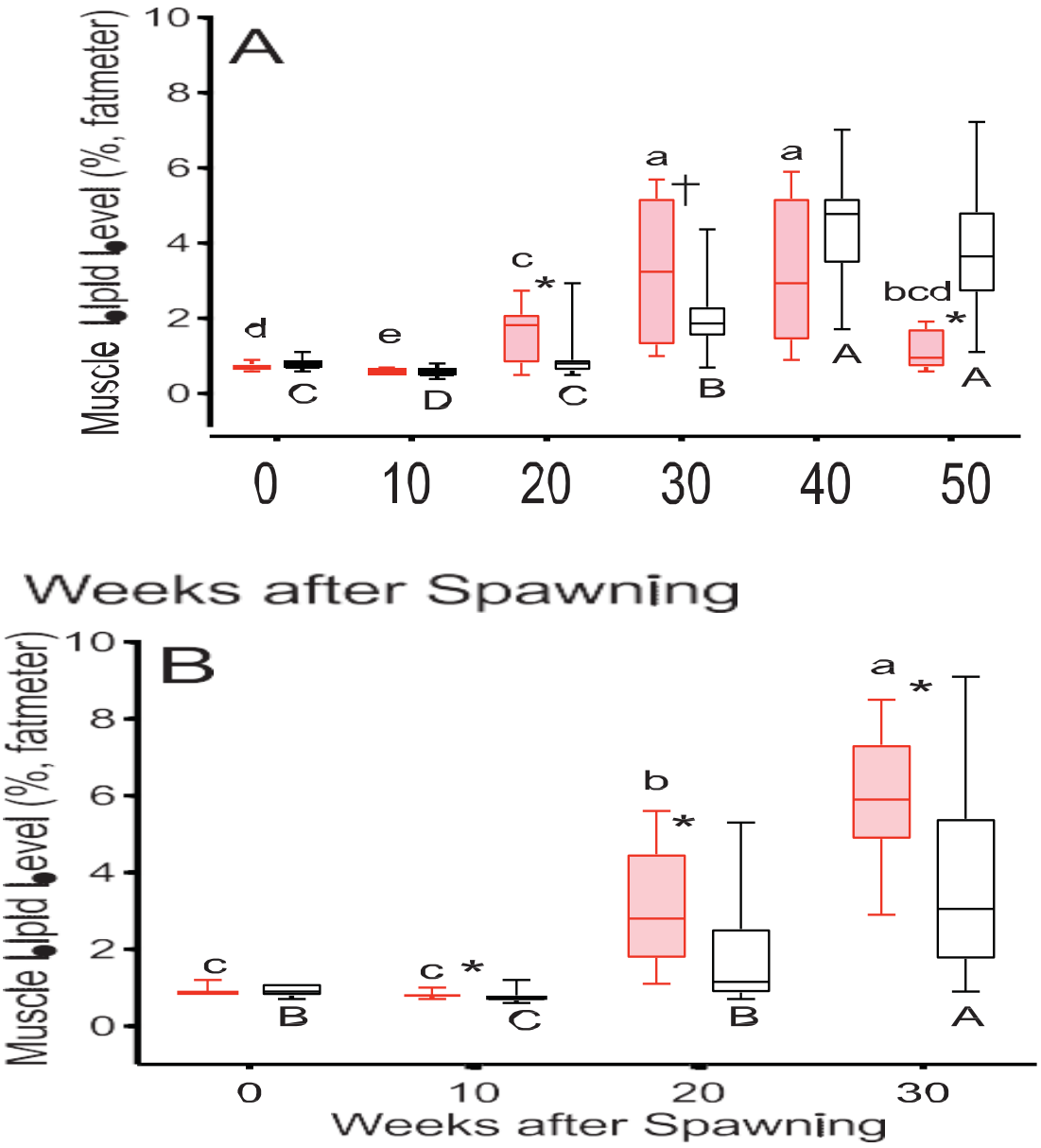


Figure 2: ML levels in female steelhead trout from the Clearwater River, Idaho, sampled in 2015 (A) and 2016 (B) at 10-week intervals following spawning, such that groups, box and whisker plots and significance indication are as in Fig. 1, and analysis included reproductive (n = 12, 10; 2015 and 2016, respectively) and non-reproductive fish (n = 20, 18; 2015 and 2016, respectively)

decreased; to 84%, 100% of Week 0 in 2015 and 2016, respectively; T-test, $P = 0.0102$, 0.0036 in 2015 and 2016, respectively). After the 10-week time point, TG in non- reproductive fish returned to first-spawning levels at 20 (2016) or 30 (2015) weeks and for all following time points. TG increased over spawning levels in all reproductive fish by 30 weeks in both years. In 2015, TG levels were slightly but significantly greater in non-reproductive than in reproductive fish at the time of first spawning.

ML level was greater in reproductive than non-reproductive fish at Weeks 20 and 30 in 2015 and 2016 (Fig. 2). At Week 50 in 2015 ML level was greater in non-reproductive than in reproductive fish. ML level increased progressively from Week 10 to 30 in both groups and years.K was greater in reproductive than non-reproductive fish at Weeks 20–40 in 2015 and at Weeks 10–30 in 2016 (Fig. 3). K increased progressively from Week 10 to Week 30 in reproductive fish in both years. K increased progressively from 10 to 40 weeks (2015) and 10 to 30 weeks (2016) in non-reproductive fish.

MSGR was greater in reproductive than non-reproductive fish during Weeks 0–10 after first spawning in both years (Fig. 4), continuing for Weeks 10–20 and Weeks 20–30 in 2015. MSGR was positive for reproductive and negative for non-reproductive fish during Weeks 0–10 in both years (Fig. 4). MSGR increased strongly from Weeks 0–10 to Weeks 10–20 in both reproductive and non-reproductive fish in both years and remained high through Weeks 20–30. In 2015, MSGR declined from Weeks 20–30 to Weeks 30–40 in reproductive fish, reaching levels below that of Weeks 0–10 during Weeks 40–50. MSGR also declined from

Weeks 30–40 to Weeks 40–50 in non-reproductive fish, returning

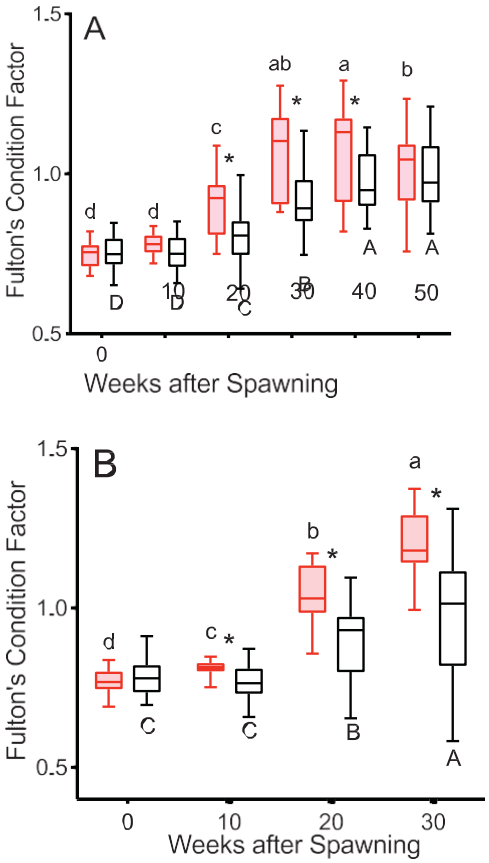


Figure 3: Fulton's condition factor in female steelhead trout from the Clearwater River, Idaho, sampled in 2015 (A) and 2016 (B) at 10-week intervals following spawning, such that groups, box and whisker plots, significance indication and statistical analyses are as in Fig. 1, and analysis included reproductive (n = 12, 10; 2015 and 2016, respectively) and non-reproductive fish (n = 20, 18; 2015 and 2016, respectively).

to levels similar to Weeks 0–10. MSGR was greater in non- reproductive fish than reproductive fish over Weeks 40–50 in 2015.LSGR was negative during Weeks 0–10 and increased dur- ing Weeks 10–20 in both reproductive and non-reproductive fish in both years (Fig. 5). LSGR subsequently decreased from Weeks 30–40 to 40–50 in non-reproductive fish in 2015. LSGR was greater in reproductive than non-reproductive fish at Weeks 10–20, 20–30 and 40–50 in 2015 and Weeks 20–30 in 2016.

E2 levels were greater in reproductive than non-reproductive fish starting at 20 weeks after first spawning, remaining that way for the study duration in both years (Fig. 6). E2 levels decreased from spawning to Week 10 in both groups and years, except in the 2016 reproductive group (P = 0.1354). At the individual level, in reproductive fish in both years, log E2 levels increased from Week 10 to Week 20 (9/11, 10/10

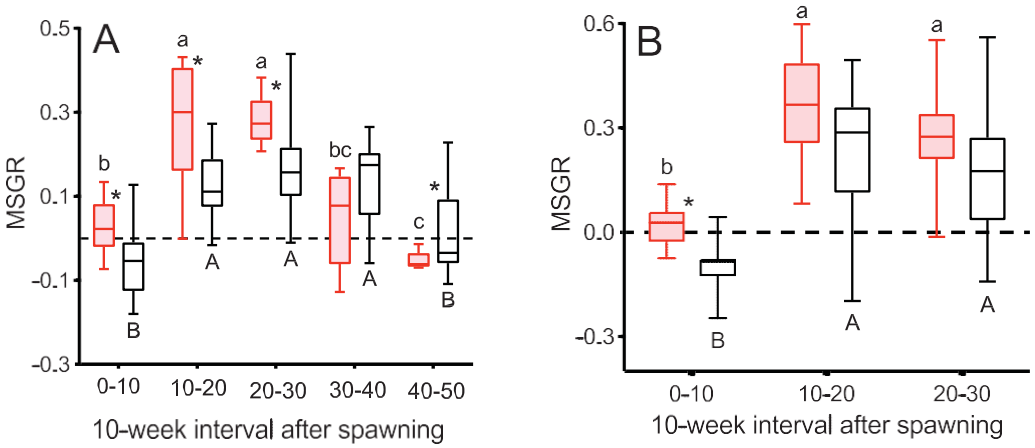
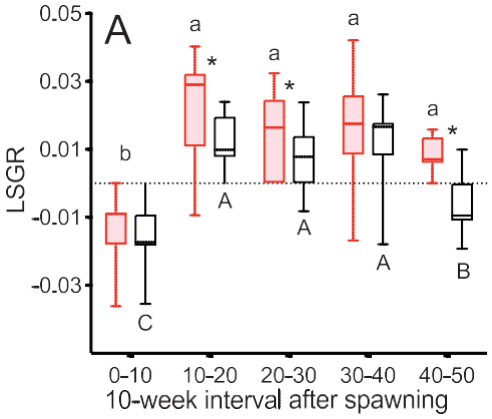


Figure 4: MSGR as % change in body weight per day in female steelhead trout from the Clearwater River, Idaho, sampled in 2015 (A) and 2016 (B) calculated over 10-week intervals following spawning, such that groups, box and whisker plots, significance indication and statistical analyses are as in Fig. 1, and analysis included reproductive (n = 10, 10; 2015 and 2016, respectively) and non-reproductive fish (n = 20, 17; 2015 and 2016, respectively).

increased; to 145%, 163% of Week 10 in 2015 and 2016, respectively) and decreased in non-reproductive fish (12/19, 15/17 decreased; to 97%, 81% of Week 10 in 2015 and 2016, respectively). Individual level changes were significantly different between groups (T-test, $P = 0.0029$, $P < 0.0001$ for 2015 and 2016, respectively). E2 levels increased again from Week 20 to Week 30 in reproductive fish, then decreased from Week 40 to Week 50 to levels similar to Week 0 in 2015. In non-reproductive fish in both years, E2 remained below first spawning levels, despite increasing from Weeks 20–30 (30–40 in 2015).

Time course prior to second spawning

Two-way repeated measures ANOVA found significant effects of maturation group, time, group∗time interactions and sub- ject (Table 2).



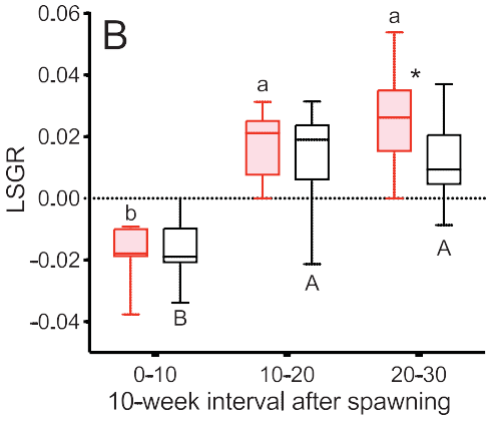


Figure 5: LSGR as % change in FL per day in female steelhead trout from the Clearwater River, Idaho, sampled in 2015 (A) and 2016 (B) at 10-week intervals following spawning, such that groups, box and whisker plots, significance indication and statistical analyses are as in Fig. 1, and analysis included reproductive (n = 11, 10; 2015 and 2016,respectively) and non-reproductive fish (n = 20, 18; 2015 and 2016, respectively).

TG levels were greater in reproductive skip spawners than in consecutive spawners at Weeks 0 and 10 in the year prior to second spawning (Fig. 7). TG levels decreased in reproductive skip spawners from Week 10 to Week 20 and then increased in both groups from Week 20 to Week 30. ML levels were greater in reproductive skip spawners than in consecutive spawners at all time points (Fig. 8). ML levels increased at Week 20 in both groups. K was greater in reproductive skip spawners than in con- secutive spawners at all time points (Fig. 9). K increased progressively in consecutive spawners from Week 10 to 30 and in reproductive skip spawners from Week 0 to 20.MSGR was greater in reproductive skip spawners during Weeks 0–10, but greater in consecutive spawners during Weeks 20–30 (Fig. 10). MSGR increased from Weeks 0–10 to Weeks 10–20 in both groups. During Weeks 20–30 MSGR in reproductive skip spawners decreased to levels similar to Weeks 0–10.

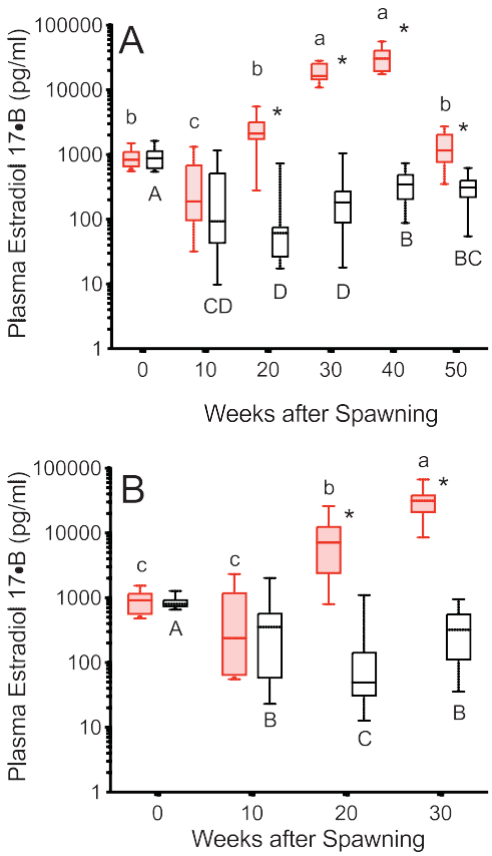


Figure 6: Plasma estradiol-17β concentrations in female steelhead trout from the Clearwater River, Idaho, sampled at 10-week intervals following first spawning in 2015 (A) and 2016 (B), such that groups, box and whisker plots, significance indication and statistical analyses are as in Fig. 1, and analysis included reproductive (n = 11, 10; 2015 and 2016, respectively) and non-reproductive fish (n = 20, 17; 2015 and 2016, respectively).

LSGR was greater in reproductive skip spawners than consecutive spawners (which had negative LSGR) during Weeks 0–10 (Fig. 11). LSGR increased from Weeks 0–10 to Weeks 10–20 in both groups. E2 levels were greater in consecutive spawners than repro- ductive skip spawners at Week 0, but greater in reproductive skip spawners from 10 to 30 weeks (Fig. 12). From 0 to 10 weeks E2 levels increased in reproductive skip spawners and decreased in consecutive spawners. Thereafter, E2 levels increased for both groups.

Discussion

The sequence of events over the year after first spawning tracked in this study (Figs 1–6) illustrates the timing of repro- ductive decisions and differences in energy acquisition and allocation between consecutive and skip spawning female steelhead trout. TG levels and growth rate in mass were signif- icantly greater 10 weeks after first spawning in reproductive

Table 2: Two-way repeated measures ANOVA test statistics for each dependent variable in female steelhead trout tracked over the year prior to second spawning for consecutive spawners and reproductive skip spawners first spawned in 2015.

Measure	Source of variation	F (DF_n, DF_d)	P-value
TG	Time	F (2.3, 51.0) = 19.28	$P < 0.0001$
	Group	F (1, 22) = 23.74	$P < 0.0001$
	Interaction	F (3, 66) = 8.519	$P < 0.0001$
	Subject	F (22, 66) = 1.528	**$P = 0.0954$**
ML	Time	F (1.3, 38.9) = 66.37	$P < 0.0001$
	Group	F (1, 29) = 120.7	$P < 0.0001$
	Interaction	F (3, 87) = 9.114	$P < 0.0001$
	Subject	F (29, 87) = 6.639	$P < 0.0001$
K	Time	F (1.5, 45) = 131.1	$P < 0.0001$
	Group	F (1, 29) = 54.40	$P < 0.0001$
	Interaction	F (3, 87) = 12.16	$P < 0.0001$
	Subject	F (29, 87) = 12.62	$P < 0.0001$
MSGR	Time	F (2.0, 54) = 33.12	$P < 0.0001$
	Group	F (1, 27) = 11.95	$P = 0.0018$
	Interaction	F (2, 54) = 23.30	$P < 0.0001$
	Subject	F (27, 54) = 1.361	**$P = 0.1658$**
LSGR	Time	F (2.0, 58) = 42.72	$P < 0.0001$
	Group	F (1, 29) = 12.76	$P = 0.0013$
	Interaction	F (2, 58) = 12.31	$P < 0.0001$
	Subject	F (29, 58) = 1.642	**$P = 0.0543$**
E2	Time	F (2.5, 71) = 229.6	$P < 0.0001$
	Group	F (1, 28) = 39.62	$P < 0.0001$
	Interaction	F (3, 84) = 31.18	$P < 0.0001$
	Subject	F (28, 84) = 1.342	**$P = 0.1536$**

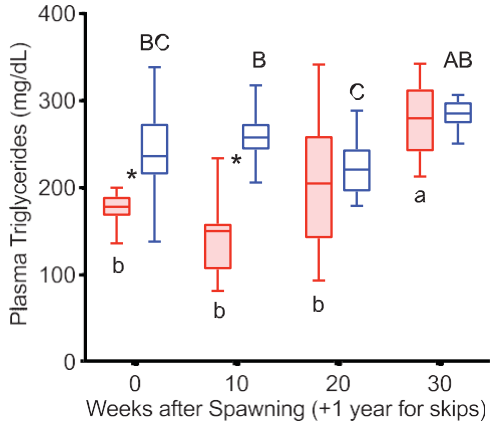


Figure 7: Plasma TG concentrations tracked over the year prior to second spawning in female steelhead trout from the Clearwater River, Idaho, where fish first spawned in 2015 were sampled at 10-week intervals in 2015 or 2016, and analysis included consecutive spawners (n = 12, red, shaded boxes) and reproductive skip spawners (n = 12, blue).

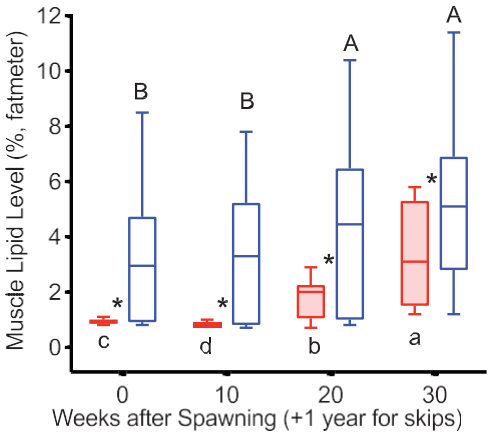


Figure 8: ML levels tracked over the year prior to second spawning in female steelhead trout from the Clearwater River, Idaho, such that groups are as in Fig. 7, box and whisker plots and significance indication are as in Fig. 1, and analysis included consecutive spawners (n = 13) and reproductive skip spawners (n = 18). Bolded P-values indicate non-significance.

versus non-reproductive fish. This implies greater feeding in reproductive fish over this time period and is consistent with the decision to enter the next reproductive cycle having occurred by 10 weeks after first spawning. Reproductive fish accumulated greater energy reserves and grew faster than non-reproductive fish over the summer growing season in the year following first spawning, consistent with the decision to enter the next reproductive cycle having stimulated feeding, without precluding the opposite scenario. The sequence of events over the year prior to repeat spawning (Figs 7–12) illustrates the effect of recovery from first spawning on energy reserves, growth and reproductive decisions. The increase in E2 occurred at relatively earlier time points in the year prior to second spawning in reproductively active skip spawners than in fish recrudescing in the year immediately following first spawning (consecutive spawners), suggesting that in con- secutive spawners reproductive development was delayed by the energetic or physiological demands of first spawning. Reproductive skip spawners had substantially greater energy reserves (i.e. ML and K) and E2 levels during oogenesis for second spawning. This likely allowed for greater reproductive investment in reproductive skip spawners versus consecutive spawners at the time of second spawning, as was found in our companion study (Jenkins *et al.*, 2018). This study provides the first mechanistic look at the timing and physiological factors involved in reproductive decisions in repeat spawning female steelhead trout. These results will directly inform the management of kelt reconditioning conservation programs and advance knowledge about the underlying physiology of consecutive and skip spawning, an important issue in the management of many fish populations.

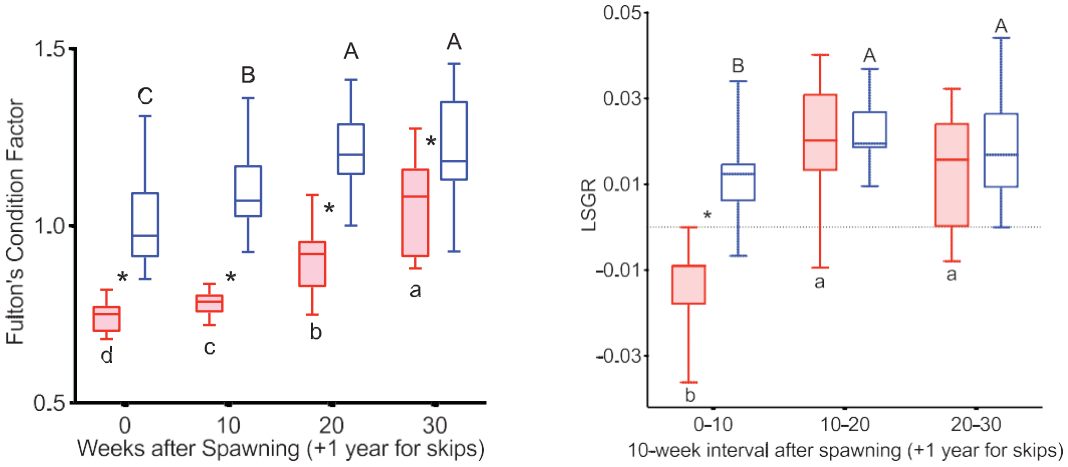


Figure 9: Fulton's condition factor tracked over the year prior to second spawning in female steelhead trout from the Clearwater River, Idaho, such that groups are as in Fig. 7, box and whisker plots, significance indication and statistical analyses are as in Fig. 1, and analysis included consecutive spawners (*n* = 13) and reproductive skip spawners (*n* = 18).

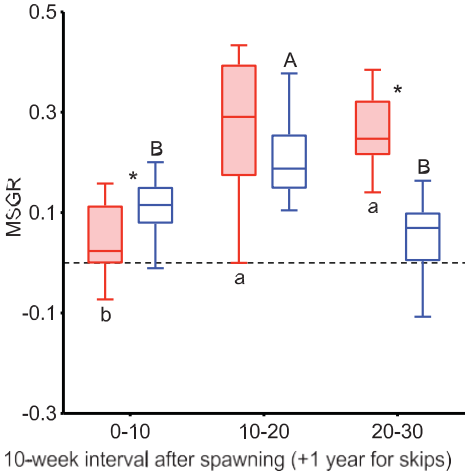


Figure 10: MSGR as % change in body weight per day tracked over the year prior to second spawning in female steelhead trout from the Clearwater River, Idaho, such that groups are as in Fig. 7, box and whisker plots, significance indication and statistical analyses are as in Fig. 1 and analysis includes consecutive spawners (*n* = 12) and reproductive skip spawners (*n* = 17).

Energy reserves

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

Figure 11: LSGR as % change in FL per day tracked over the year prior to second spawning in female steelhead trout from the Clearwater River, Idaho. Groups are as in Fig. 7, such that box and whisker plots, significance indication and statistical analyses are as in Fig. 1, and analysis included consecutive spawners (n = 13) and reproductive skip spawners (n = 18).

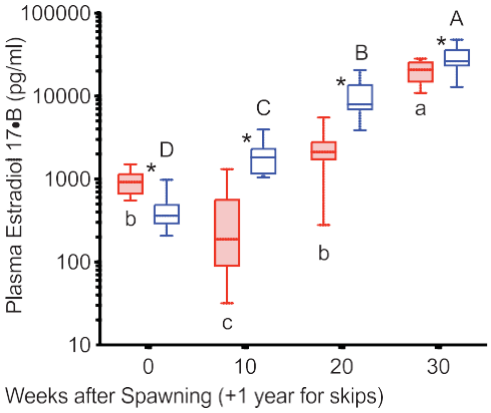


Figure 12: Plasma estradiol-17β concentrations tracked over the year prior to repeat spawning in female steelhead trout from the Clearwater River, Idaho, such that groups are as in Fig. 7, box and whisker plots, significance indication and statistical analyses are as in Fig. 1, and analysis includes consecutive spawners (n = 12) and reproductive skip spawners (n = 18).

Year after first spawning

After spawning, TG levels were greater in reproductive fish from 10 to 40 weeks, with minor variation in significance between years and then decreased immediately before spawn- ing. The divergence in circulating TG levels at 10 weeks was due to a significantly lesser decrease from Week 0 in indi- vidual fish reproductive in the year following first spawning. This is most likely because of greater food intake, assim- ilation and more rapid somatic recovery in reproductively active fish. However, we cannot exclude the possibility that lipid metabolism differed between reproductive and non- reproductive female steelhead trout during this period. Our interpretation that the increase in TG in reproductive fish was due to greater food intake is supported by the MSGR results discussed below. If our interpretation is correct, then physiological differences manifesting in the form of greater feeding motivation occurred between reproductive and non- reproductive fish by 10 weeks after spawning, implying that the decision to enter a reproductive cycle is linked to the difference in feeding motivation. This suggests two possi- ble mechanisms: (i) the decision occurs late during the first 10 weeks or afterward, with increased feeding early on after spawning causing improved energetic status that leads to the initiation of ovarian recrudescence or (ii) the decision occurs prior to or early during the first 10 weeks, with the initiation of ovarian recrudescence stimulating increased feeding and nutrient assimilation. The second possibility is supported in part by a study in winter flounder, which linked recrudescence to high condition in the period immediately following first spawning, regardless of access to food during that period (Burton, 1994). Further, studies in Atlantic salmon and rainbow trout showed increased feed intake, growth and plasma levels of insulin-like growth factor-1 in maturing fish during early stages of maturation ~1 year before spawning (Kadri et al., 1996; Stead et al., 1999; Wilkinson et al., 2010). In addition, the presence of small ovarian follicles in rainbow trout and steelhead trout kelt ovaries immediately after ovulation (De Mones et al., 1989; Penney and Moffitt, 2014a) suggest that secondary oocyte growth (and hence a decision to initiate recrudescence) begins prior to ovulation of the mature oocyte cohort.

Elevations in TG level in reproductive fish in this study at time points after 10 weeks post-spawning, as well as the decrease immediately before spawning, are consistent with both greater feeding and mobilization of lipid reserves during vitellogenesis. The major fates of circulating TGs are expected to be storage in muscle, mesenteric and liver lipid depots (Sheridan, 1994) and incorporation into the developing ovary during exogenous vitellogenesis (Norberg and Haux, 1985). This is supported by studies that have reconditioned repeat spawning Atlantic salmon (Johnston et al., 1987) and prior to peak vitellogenesis in rainbow trout (Bon et al.,1997). In female brown trout (Salmo trutta), plasma TG levels decreased ~50% after spawning (Gauthey et al., 2015), sim- ilar to the post-spawning decrease found in non-reproductive fish in the present study. In non-reproductive fish, TG levels increased to 100–300 mg dL−1 by Week 30, remained in this range through the following winter, then increased to 200–300 mg dL−1 by the spring for skip spawners (now reproductively active, 1 year + 10–30 weeks after first spawning) remaining in this range throughout the summer. This suggests that plasma TG levels are maintained by homeostatic processes in actively feeding post-spawning female steelhead trout and that increased levels reflect both seasonality and reproductive status.

ML levels and K increased more rapidly in reproductive than in non-reproductive fish diverging at 20 weeks following the increase in TG levels. The greater ML levels and K in reproductive fish in the year following first spawning is consistent with reconditioned wild female steelhead trout ~6 months after spawning (Pierce et al., 2017) and fully fed versus feed restricted adult female rainbow trout over the first 20 weeks after spawning (Caldwell et al., 2013). Similarly, K was greater in reproductive reconditioned repeat spawning female Atlantic salmon from 18 to 34 weeks after spawn- ing (Johnston et al., 1987). As spawning approached for reproductive fish, ML levels decreased and ultimately became significantly lower than ML levels in non-reproductive fish at Week 50. This likely reflects mobilization of MLs for incorporation into the ovary and reduced appetite.

K also decreased in reproductive fish over the period immediately before spawning, but this decrease was not nearly as dramatic as in plasma TG and ML levels. This was likely due to the presence of fully developed ovaries in the body cavity of the reproductive fish. The much higher K levels at second versus first spawning in reproductive fish is both because the mass of the eggs was not included in somatic mass at first spawning and because feeding and somatic growth continued through the fall prior to spawning in reproductive fish, unlike in first-time spawners. Similar results were observed in consecutive spawning reconditioned Atlantic salmon kelts; these fish experienced a minor decline in K following spawning but remained well above first spawning levels (Johnston et al., 1987).

At first spawning, no biologically significant differences in lipid reserve metrics were detected between subsequently reproductive and non-reproductive fish, providing no evidence for a determinative role of lipid reserves at spawning in the decision to initiate recrudescence. This does not nec- essarily imply that the critical period hypothesis of salmonid maturation does not hold for reproductive decisions in repeat spawners. The critical period during which reproductive decisions are sensitive to energy reserves may simply occur earlier, before first spawning for consecutive spawners, or greater than 1 year prior to second spawning. However, it is also possible that the lipid reserve metrics employed in this study did not capture the relevant physiological signals. Signalling factors associated with energy reserves, rather than energy reserves themselves, presumably directly interact with neuroendocrine mechanisms underlying this decision (Wootton and Smith, 2015). Future study of signalling factors associated with energy reserves and growth is required to elucidate mechanisms underlying reproductive decisions in salmonids.

Year prior to second spawning

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

Over the summer before repeat spawning, ML levels con- tinued to increase in reproductive skip spawners and thus remained much higher than those in consecutive spawners, indicating greater energy reserves in the reproductive skip spawners were due to the much longer time for recovery from first spawning. K increased in both consecutive spawners and reproductive skip spawners. Although the increase was steeper in consecutive spawners, this was not sufficient to sur- pass the much higher K levels in reproductive skip spawners. As K is a measure of body shape, this likely reflects increased size and greater energy stores in a variety of tissues and organs in the skip spawners, including muscle tissue, visceral lipids and the developing ovaries.

Growth

Year after first spawning

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

Growth in mass decreased compared to earlier time periods in both reproductive and non-reproductive fish as annual spawn timing approached. This is likely at least in part due to seasonal growth patterns dictated by water temperature and photoperiod (Burgner *et al*., 1992). Decreases in growth as spawn timing approached were also reported in both reproductive and non-reproductive female Atlantic salmon. Moreover, similar to what was observed in the present study, decreases were more dramatic in maturing fish (Kadri *et al*., 1996). Additionally, Stead *et al*. (1999) found a correlation between decreased growth, increased levels of plasma sex

steroids and decreased food consumption during later stages of maturation in Atlantic salmon. Negative mass growth (e.g. weight loss) in reproductive fish during the 10 weeks before spawning may reflect both reduced food consumption and the energetic cost of ovarian growth.

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

Year prior to second spawning

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

Estradiol-17β

Year after first spawning

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

Premature decreases in E2, which would indicate arrested reproductive development after the start of exogenous vitel- logenesis, were not observed in this study. Similarly, low E2 levels used to categorize non-reproductive status, as was done in the present study, coincided with low vitellogenin in skipping steelhead trout (Pierce et al., 2017) and low gona- dosomatic index (GSI) tracked over time in skipping rain- bow trout (Caldwell et al., 2013). Additionally, vitellogenin levels were elevated by early summer in reproductive post- spawning summer-run steelhead trout (Pierce et al., 2017), indicating that exogenous vitellogenesis occurs roughly dur- ing the time at which summer-run steelhead must leave ocean feeding areas to begin their spawning migration (Burgner et al., 1992). Thus, skipping after this time would involve a seemingly maladaptive migration pattern. Finally, salmonids are thought to commit to a reproductive cycle when oocytes begin to accumulate cortical alveoli, further narrowing the transition to that of the beginning of secondary oocyte devel- opment (Campbell *et al*., 2006; Taranger *et al*., 2010). Taken together with the present results, we interpret these find-

ings as suggesting that post-spawning summer-run steelhead likely halt reproductive development in the perinucleolar stage of oocyte development, coinciding with the 'spent- recovery' stage of ovarian development, representing the 'rest- ing' form of skipped spawning as defined by Rideout and Tomkiewicz (2011).

Year prior to second spawning

E2 was low through the winter in non-reproductive fish but increased in Year 2 from Week 0 (+1 year) to Week 10 to ~10-fold higher in skip spawners than consecutive spawners and comparable to levels in consecutive spawn-
ers at Week 20. This indicates that the decision to initiate reproductive activity occurred by Week 10 in skip spawners and suggests that reproductive development was accelerated in reproductive skip spawners versus consecutive spawners. Consistent with this idea, spawning was later in consecutive versus skip spawning steelhead trout (Jenkins *et al*., 2018), as in consecutive versus first-time spawning Atlantic salmon (Pankhurst *et al*., 2011). Plasma E2 levels remained greater in reproductive skip spawners versus consecutive spawners as oogenesis proceeded through the summer growing sea- son and levels increased. Similarly, plasma E2 levels were
lower in consecutive spawning Atlantic salmon versus first- time spawners at sampling time points ~10–20 weeks after spawning (Pankhurst *et al*., 2011). The higher E2 levels in reproductive skip spawners versus consecutive spawners may have resulted in the 14% greater size-adjusted total egg mass found in these fish at the time of second spawning (Jenkins *et al*., 2018). Both the delay in initiation of maturation in consecutive spawners and the greater reproductive invest-
ment observed in skip spawners at second spawning can be attributed to the effects of recovery from first-time spawning on consecutive spawners. These effects were likely largely mediated by energetic status, as discussed above. However, in addition, reproductive development in consecutive spawn- ers may have been directly affected by recovery from first spawning due to time required for clearance of steroids and other gonadal factors, continued steroid production by post- ovulatory follicles and tissue resorption and remodelling of the post-ovulatory ovary (De Mones *et al*., 1989; Chyb *et al*., 1999; Caldwell *et al*., 2014). The impact of first spawning on reproductive development in consecutive spawners illustrates the benefits of having time to recover from first spawning that occur before the early stages of oogenesis.

Conclusions

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

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References

Andersson E, Schulz RW, Male R, Bogerd J, Patina D, Benedet S, Norberg B, Taranger GL (2013) Pituitary gonadotropin and ovarian gonadotropin receptor transcript levels: seasonal and photoperiod- induced changes in the reproductive physiology of female Atlantic salmon (*Salmo salar*). *Gen Comp Endocrinol* 191: 247–258.

Bobe J, Montfort J, Nguyen T, Fostier A (2006) Identification of new participants in the rainbow trout (Oncorhynchus mykiss) oocyte mat- uration and ovulation processes using cDNA microarrays. Reprod Biol Endocrinol 4: 39.

Bon E, Corraze G, Kaushik S, LeMenn F (1997) Effects of accelerated photoperiod regimes on the reproductive cycle of the female rainbow trout: I—seasonal variations of plasma lipids correlated with vitellogenesis. *Comp Biochem Phys A Physiol* 118: 183–190.

Breton B, Govoroun M, Mikolajczyk T (1998) GTH I and GTH II secre- tion profiles during the reproductive cycle in female rainbow trout: relationship with pituitary responsiveness to GnRH-A stimulation. Gen Comp Endocrinol 111: 38–50.

Burgner RL, Light JT, Margolis L, Okazaki T, Tautz A, Ito S (1992) Distribu- tion and Origins of Steelhead Trout (Oncorhynchus mykiss) in Offshore Waters of the North Pacific Ocean. International North Pacific Fisheries Commission, p. 51, Vancouver, BC.

Burton MPM (1994) A critical period for nutritional control of early gametogenesis in female winter flounder, Pleuronectes americanus (Pisces, Teleostei). J Zool 233: 405–415.

Caldwell LK, Pierce AL, Nagler JJ (2013) Metabolic endocrine factors involved in spawning recovery and rematuration of iteroparous female rainbow trout (Oncorhynchus mykiss). Gen Comp Endocrinol 194: 124–132.

Caldwell LK, Pierce AL, Riley LG, Duncan CA, Nagler JJ (2014) Plasma nesfatin-1 is not affected by long-term food restriction and does not predict rematuration among iteroparous female rainbow trout (Oncorhynchus mykiss). PLoS One 9: e85700.

Campbell B, Dickey J, Beckman B, Young G, Pierce A, Fukada H, Swanson P (2006) Previtellogenic oocyte growth in salmon: relationships among body growth, plasma insulin-like growth factor-1, estradiol-17beta, follicle-stimulating hormone and expression of ovarian genes for insulin-like growth factors, steroidogenic- acute regulatory protein and receptors for gonadotropins, growth hormone, and somatolactin. Biol Reprod 75: 34–44.

Chaput G, Benoit HP (2012) Evidence for bottom-up trophic effects on return rates to a second spawning for Atlantic salmon (Salmo salar) from the Miramichi River, Canada. ICES J Mar Sci 69: 1656–1667.

Chyb J, Mikolajczyk T, Breton B (1999) Post-ovulatory secretion of pituitary gonadotropins GtH I and GtH II in the rainbow trout (Oncorhynchus mykiss): regulation by steroids and possible role of non-steroidal gonadal factors. J Endocrinol 163: 87–97.

Courter II, Child DB, Hobbs JA, Garrison TM, Glessner JJG, Duery S (2013) Resident rainbow trout produce anadromous offspring in a large interior watershed. Can J Fish Aquat Sci 70: 701–710.

De Mones A, Fostier A, Cauty C, Jalabert B (1989) Ovarian early postovulatory development and estrogen production in rainbow trout (Salmo gairdneri R.) from a spring-spawning strain. Gen Comp Endocrinol 74: 431–441.

Fostier A, Weil C, Terqui M, Breton B, Jalabert B (1978) Plasma estradiol- 17-beta and gonadotropin during ovulation in rainbow trout (Salmo gairdneri R). Ann Biol Anim Biochim Biophys 18: 929–936.

Gauthey Z, Freychet M, Manicki A, Herman A, Lepais O, Panserat S, Elosegi A, Tentelier C, Labonne J (2015) The concentration of plasma metabolites varies throughout reproduction and affects offspring number in wild brown trout (Salmo trutta). Comp Biochem Physiol A Mol Integr Physiol 184: 90–96.

Hanson KC, Ostrand KG, Gannam AL, Ostrand SL (2010) Comparison and validation of nonlethal techniques for estimating condition in juvenile salmonids. Trans Am Fish Soc 139: 1733–1741.

Hatch DR, Branstetter R, Stephenson J, Pierce AL, Matala A, Lessard R, Bosch B, Everett S, Newell J, Graham N et al. (2016) Kelt Reconditioning and Reproductive Success Evaluation Research, 1/1/2015–12/31/2015 Annual Report. Columbia River Inter-Tribal Fish Commission, pp. 2007–401–00, Portland, OR.

Hatch DR, Fast DE, Bosch WJ, Blodgett JW, Whiteaker JM, Branstetter R, Pierce AL (2013) Survival and traits of reconditioned kelt steelhead Oncorhynchus mykiss in the Yakima River, Washington. N Am J Fish Manag 33: 615–625.

Hutchings JA (2011) Old wine in new bottles: reaction norms in salmonid fishes. Heredity 106: 421–437.

Jenkins LE, Pierce AL, Graham N, Branstetter R, Hatch DR, Nagler JJ (2018) Reproductive performance and energy balance in consecutive and repeat spawning female steelhead reconditioned in captivity. Trans Am Fish Soc 147: 959–971.

Johnston CE, Gray RW, Mclennan A, Paterson A (1987) Effects of pho- toperiod, temperature, and diet on the reconditioning response, blood chemistry, and gonad maturation of Atlantic salmon kelts (Salmo salar) held in fresh water. Can J Fish Aquat Sci 44: 702–711.

Kadri S, Mitchell DF, Metcalfe NB, Huntingford FA, Thorpe JE (1996) Differential patterns of feeding and resource accumulation in matur- ing and immature Atlantic salmon, Salmo salar. Aquaculture 142: 245–257.

Keefer ML, Wertheimer RH, Evans AF, Boggs CT, Peery CA (2008) Iteropar- ity in Columbia River summer-run steelhead (Oncorhynchus mykiss): implications for conservation. Can J Fish Aquat Sci 65: 2592–2605.

Krogdahl A, Bakke-McKellep AM (2005) Fasting and refeeding cause rapid changes in intestinal tissue mass and digestive enzyme capac- ities of Atlantic salmon (Salmo salar L.). Comp Biochem Physiol A Mol Integr Physiol 141: 450–460.

Leitritz E, Lewis RC (1976) Trout and salmon culture (hatchery methods).Fish Bull 164: 1–197.

Lubzens E, Young G, Bobe J, Cerda J (2010) Oogenesis in teleosts: how eggs are formed. Gen Comp Endocrinol 165: 367–389.

McBride RS, Somarakis S, Fitzhugh GR, Albert A, Yaragina NA, Wuenschel MJ, Alonso-Fernandez A, Basilone G (2015) Energy acquisition and allocation to egg production in relation to fish reproductive strate- gies. Fish Fish (Oxf) 16: 23–57.

Meador JP, Sommers FC, Ylitalo GM, Sloan CA (2006) Altered growth and related physiological responses in juvenile Chinook salmon (Oncorhynchus tshawytscha) from dietary exposure to polycyclic aro- matic hydrocarbons (PAHs). Can J Fish Aquat Sci 63: 2364–2376.

Moore JW, Yeakel JD, Peard D, Lough J, Beere M (2014) Life-history diver- sity and its importance to population stability and persistence of a migratory fish: steelhead in two large north American watersheds. J Anim Ecol 83: 1035–1046.

Nagahama Y (1994) Endocrine regulation of gametogenesis in fish. Int J Dev Biol 38: 217–229.

Nagler JJ, Cavileer TD, Verducci JS, Schultz IR, Hook SE, Hayton WL (2012) Estrogen receptor mRNA expression patterns in the liver and ovary of female rainbow trout over a complete reproductive cycle. Gen Comp Endocrinol 178: 556–561.

Nielsen JL, Jonsson B, Turner SM, Zimmerman CE (2011) Electronic tags and genetics explore variation in migrating steelhead kelts (Oncorhynchus mykiss), Ninilchik River. Alaska Can J Fish Aquat Sci 68: 1–16.

Norberg B, Haux C (1985) Induction, isolation and a characterization of the lipid content of plasma vitellogenin from 2 salmo species— rainbow trout (Salmo gairdneri) and sea trout (Salmo trutta). Comp Biochem Physiol B 81: 869–876.

Pankhurst NW, King HR, Anderson K, Elizur A, Pankhurst PM, Ruff N (2011) Thermal impairment of reproduction is differentially expressed in maiden and repeat spawning Atlantic salmon. Aquacul- ture 316: 77–87.

Penney ZL, Moffitt CM (2014a) Histological assessment of organs in sexually mature and post-spawning steelhead trout and insights into iteroparity. Rev Fish Biol Fisher 24: 781–801.

Penney ZL, Moffitt CM (2014b) Proximate composition and energy den- sity of stream-maturing adult steelhead during upstream migration, sexual maturity, and kelt emigration. Trans Am Fish Soc 143: 399–413.

Pierce AL, Blodgett JW, Cavileer TD, Medeiros LR, Boyce J, Caldwell LK, Bosch WJ, Branstetter R, Fast DE, Hatch RD et al. (2017) Reproduc- tive development in captive reconditioned female steelhead kelts: evidence for consecutive and skip spawning life histories. Can J Fish Aquat Sci 74: 1049–1060.

Quinn TP (2005) The Behavior and Ecology of Pacific Salmon and Trout.American Fisheries Society, Bethesda, MD, pp. 5–6.

Quinn TP, McGinnity P, Reed TE (2016) The paradox of “premature migra- tion”by adult anadromous salmonid fishes: patterns and hypotheses. Can J Fish Aquat Sci 73: 1015–1030.

Rideout RM, Rose GA, Burton MPM (2005) Skipped spawning in female iteroparous fishes. Fish Fish (Oxf) 6: 50–72.

Rideout RM, Tomkiewicz J, Carrillo M, Schulz RW, Fontaine P, Zanuy S, Felip A, Weltzien FA, Dufour S, Karlsen 0, Norberg B (2011) Skipped spawning in fishes, more common than you might think. Mar Coast Fish 3: 176–189.

Sheridan MA (1994) Regulation of lipid metabolism in poikilothermic vertebrates. Comp Biochem Physiol B 107: 495–508.

Simpkins DG, Hubert WA, Del Rio CM, Rule DC (2003) Physiological responses of juvenile rainbow trout to fasting and swimming activity: effects on body composition and condition indices. Trans Am Fish Soc 132: 576–589.

Stead SM, Houlihan DF, McLay HA, Johnstone R (1999) Food consump- tion and growth in maturing Atlantic salmon (Salmo salar). Can J Fish Aquat Sci 56: 2019–2028.Sutton SG, Bult TP, Haedrich RL (2000) Relationships among fat weight, body weight, water weight, and condition factors in wild Atlantic salmon parr. *Trans Am Fish Soc* 129: 527–538.

Taranger GL *et al.* (2010) Control of puberty in farmed fish. *Gen Comp Endocrinol* 165: 483–515.

Thorpe JE (1994) Reproductive strategies in Atlantic salmon, Salmo salar

L. Aquacult Fish Manage 25: 77–87.

Thorpe JE (2007) Maturation responses of salmonids to changing devel- opmental opportunities. Mar Ecol Prog Ser 335: 285–288.

Thorpe JE, Mangel M, Metcalfe NB, Huntingford FA (1998) Modelling the proximate basis of salmonid life-history variation, with application to Atlantic salmon, Salmo salar L. Evol Ecol 12: 581–599.

Trammell JLJ, Fast DE, Hatch DR, Bosch WJ, Branstetter R, Pierce AL, Blod- gett JW, Frederiksen CR (2016) Evaluating steelhead kelt treatments to increase iteroparous spawners in the Yakima River Basin. N Am J Fish Manag 36: 876–887.

Vandenberghe EP, Gross MR (1989) Natural selection resulting from female breeding competition in a Pacific salmon (coho, Oncorhynchus kisutch). Evolution 43: 125–140.

Whitehead C, Bromage NR, Breton B (1983) Changes in serum levels of gonadotropin, estradiol 17-beta and vitellogenin during the 1st and subsequent reproductive cycles of female rainbow trout. Aquaculture 34: 317–326.

Wilkinson RJ, Longland R, Woolcott H, Porter MJR (2010) Effect of elevated winter-spring water temperature on sexual maturation in photoperiod manipulated stocks of rainbow trout (Oncorhynchus mykiss). Aquaculture 309: 236–244.

Wootton RJ, Smith C (2015) Reproductive Biology of Teleost Fishes. John Wiley & Sons, Ltd., West Sussex, UK, pp. 113–114.

Zaldua N, Naya DE (2014) Digestive flexibility during fasting in fish: a review. *Comp Biochem Physiol A Mol Integr Physiol* 169: 7–14.

A2.B: Effects of physiological condition on aspects of repeat spawning in female Steelhead Trout 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 impact of first spawning on condition could result in trade-offs with future reproduction, mediated by post-spawning 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 Trout (*Oncorhynchus mykiss*) undergoing post-spawning reconditioning in captivity. Measures of condition and reproductive characteristics (i.e. fecundity, egg size, and total egg mass (TEM)) at first spawning were examined for effects on post-spawning survival and future spawning schedules, and condition was tracked during reconditioning to examine effects on reproductive characteristics at repeat spawning. 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 a tradeoff 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 timepoints. 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 impacts survival, repeat spawning schedules, and reproductive characteristics in female Steelhead Trout up to a year or more before repeat spawning. If more broadly applicable, these relationships could provide mechanisms linking environmental conditions with reproductive characteristics in salmonids and other species.

Introduction

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; Campbell et al. 2006; Thorpe 2007; 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 can be arrested at a second critical period approximately 6 months later should energy reserves become insufficient (Thorpe 1994; Thorpe 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 post-spawning 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). Mechanisms linking post-spawning condition with reproductive decisions in repeat spawners are likely to be similar to those acting during maturation, with the additional influence of the energetic cost of first spawning (McBride et al. 2015). In addition, condition impacts 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 impacts of any trade-offs. A study on winter-run Steelhead Trout found reduced reproductive success at initial spawning in successfully iteroparous individuals, consistent with a tradeoff between energy allocation to reproduction and post-spawning 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 employed in fisheries studies are structural indices intended to measure energy reserves. Fork length, Fulton's condition factor, and muscle lipid levels are structural condition measures frequently utilized to estimate energy reserves, i.e. energetic condition (Koops et al. 2004; Quinn et al. 2011). However, these measures may not adequately capture aspects of condition relevant to survival, spawning schedules, or reproduction. Additional rapid, non-lethal measures that reflect the functioning of physiological systems (i.e., functional condition) 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 Trout, returning to Dworshak National Fish Hatchery (DNFH) in Ahsahka, Idaho, were used to examine relationships between first-time spawning reproductive effort, condition, post-spawning survival, spawning schedules, and reproductive characteristics at repeat spawning. Fish were captured upon return to the hatchery following an approximately 800km 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 Trout are iteroparous, approximately 50% of the study population survives beyond 10 weeks after spawning when reconditioned in captivity, and fewer than half initiate ovarian recrudescence in consecutive years (Jenkins et al. 2018; Jenkins et al. 2019). This provides a unique opportunity to investigate condition- dependent outcomes and potential trade-offs. The objectives of this study were to determine whether post-spawning survival and spawning schedules were (1) condition-dependent and (2) traded off 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 Trout, originating from DNFH, were captured upon their return after ascending the DNFH adult ladder on the North Fork Clearwater River in Ahsahka, ID. Fish were captured beginning in February-April in 2015 and 2016, and were held unfed in holding ponds supplied with river water. During February through April in 2015 and 2016 DNFH staff selected fully mature fish >70 cm fork length (FL) for use as brood stock. Of these, fish in good or fair external condition, i.e. lacking morphological damage, lesions, or fungal infection (Evans et al. 2004; Hatch et al. 2013), n = 150 in 2015 and n = 164 in 2016, were selected for this study and individually marked with passive integrated transponder tags inserted into the pelvic girdle.

Spawning.

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

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, IEM, TEM, and fecundity were standardized based on fish mass (Jenkins et al. 2018).

Sampling.

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

Reconditioning husbandry.

Fish were held at DNFH in 4.6m diameter outdoor tanks, supplied with North Fork Clearwater River water at approximately 200 L/min, and maintained at a water height of 1.5m, with a seasonally varying temperature profile (4.9 – 11.0^{o}C). Tanks were treated with Formalin (Syndel USA, Portland, OR) as a flow-through treatment (1:6000 dilution) for one hour daily to control *Saprolegnia*. 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:

$$(1) K = 100 * \text{body mass (g)} * (\text{fork length (cm)})^{-3} \#$$

$$(2) \text{MSGR} = \% \text{ body mass gain} * \text{day}^{-1} = 100 * (\ln(\text{body mass final}) - \ln(\text{body mass initial})) * \text{days}^{-1} \#$$

LSGR was calculated in the same manner as was MSGR. Before calculation of K and MSGR, body mass was adjusted to account for any eggs retained from first spawning in the body cavity as follows: the mass of any eggs 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 SGR (Jenkins et al. 2018).

Assays.

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

Statistical analysis.

Survival in post-spawning female Steelhead Trout over time in each year (2015 and 2016) was compared using a Log-rank test conducted in PRISM (version 8, GraphPad).

The probability of fish surviving after first spawning was assessed using multiple logistic regression. Of the fish spawned (n = 314), n = 291 were included in the analysis due to missing

values (n = 2) and 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 days post-spawning, assigned as "1") versus mortalities (*i.e.,* fish that died prior to 70 days post-spawning, assigned as "0").

Predictor variables (n = 7) measured at first spawning included K, ML (arc-sine square-root transformed), TG, OS, PL, TEM, and E2 ($\log_{10}$-transformed). 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). Data from 2015 and 2016 were examined for a year effect. Because preliminary univariate tests saw a Year effect (in the survival analysis only, P = 0.00393), we tested for first order interactions with Year. Interaction effects were non-significant (P < 0.29), so the interactions were dropped from the survival model. The multiple logistic regression equation was as follows:

$$\text{Survival} = \text{Intercept} + \text{Year} + \text{TG} + \text{ML} + \text{K} + \text{OS} + \text{PL} + \text{TEM} + \text{E2} + \text{error\#} \quad (3)$$

Points representing the predicted probability of survival were plotted in 3D space 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 days following first spawning (mortalities, n = 172). Mortalities (n = 156) were included in the analysis due to missing values (n = 2) and the removal of outliers (n = 14, Q = 1%). Analysis utilized the same standardized predictor variables as the multiple logistic regression on survival. TG and OS were found to interact with year in preliminary models and thus were included in the final model as follows:

$$\text{Survival70} = \text{Intercept} + \text{Year} + \text{TG} + \text{ML} + \text{K} + \text{OS} + \text{PL} + \text{TEM} + \text{E2} + \text{Year} * \text{OS} + \text{Year} * \text{TG} + \text{error\#} \quad (4)$$

Factors associated with reproductive status in the year following first spawning were assessed in a multiple logistic regression utilizing the same standardized predictor variables as the multiple logistic regression on survival. Reproductive status was determined in fish that survived to 30 weeks post-spawning (n = 73) using E2 concentrations. Previous studies indicate that E2 levels in females at this time point have diverged completely between reproductive (consecutive spawning schedule, E2 range 8,000-70,000 pg ml-1) and non-reproductive (skip spawning schedule, E2 range 10-1,000 pg ml-1) individuals, including the fish used in this study (Pierce et al. 2017; Jenkins et al. 2019). Fish with high E2 concentration were classified as

reproductive and assigned as "1" and fish with low E2 concentration were classified as non-reproductive 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 were found in preliminary models and interactions were dropped. The final model was:

$$(5)\text{Status} = \text{Intercept} + \text{Year} + \text{TG} + \text{ML} + \text{K} + \text{OS} + \text{PL} + \text{TEM} + \text{E2} + \text{error\#}$$

2007).

Data were plotted in R using the popbio package (Smart et al. 2004; Stubben and Milligan

To identify potential sensitive periods for condition-dependent regulation of reproductive characteristics in consecutive spawners, simple linear regression was used to describe relationships between size-standardized reproductive metrics (IEM, TEM, fecundity) at consecutive spawning and 6 independent variables, measured at 6 time points (first spawning and at 10-week intervals thereafter out to 50 weeks). Independent variables were MSGR, LSGR, TG, E2, ML, and K. Consecutive spawners (n = 12) from the 2015 spawn year were included in the analysis as the most complete dataset was available for these fish. Coefficients of determination (R) and an associated P-value were calculated for each predictor at each time period (spawning, and at each 10-week sampling period or interval thereafter). Unless otherwise indicated, 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 reviewed and approved by the University of Idaho Animal Care and Use Committee.

Results

Survival

Survival declined steeply during the first 70 days following first spawning and remained relatively constant between weeks 10-30 (Figure 1). Survival was significantly lower in 2016 than in 2015 (Log-rank test, $\chi^2 = 9.335$, $P = 0.0022$). Survival at 70 days post-spawning was 53% (80/150) in 2015 and 38% (62/164) in 2016, averaging 45% (142/314) in the two years combined (Table 2). Of all the measures examined, OS and TG were the only ones strongly and significantly positively related to survival (Figure 2; Equation 3: χ^2= 10.94, 7.20; P = 0.0009 and 0.0073, respectively; Figure 3). The

95% confidence intervals of all other standardized coefficients overlapped zero and were thus without significant effect. Survival_{70} was significantly related to TG, OS, TG*Year, and OS*Year (Equation

4) (adjusted model R^2 = 17.3%, Table 3).

Spawning Schedule

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

Reproductive Characteristics

Consecutive spawners spawned for the first time in 2015 (*n* = 12) included in this analysis had an average TEM of 721.9g (SD = 132.8), which can be broken down into an average IEM of 96.1mg (SD = 13.5), and an average fecundity of 7,505 eggs (SD = 1,456) at second spawning. Reproductive characteristics at the second spawning were related to condition early in the interval between spawning events (10 to 20 weeks post-spawning) (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 time period coinciding 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

Post-spawning survival was condition-dependent based on functional measures of condition in female Steelhead Trout in this study. 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 reflecting 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.

IEM 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 tradeoff 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 tradeoff 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 time points prior to first spawning.

Survival and Spawning Schedule

Survival in the present study was in the range reported for other studies of Steelhead Trout and Atlantic Salmon kelts reconditioned in freshwater (Moffett et al. 1996; Hatch et al. 2013), and was much higher than in-river survival to repeat spawning for Snake River Steelhead Trout (Keefer et al. 2008; Keefer et al. 2017). Mortality profiles were similar in both years, with post-spawning mortality leveling off by 10 weeks after spawning. Heavy mortality during the initial reconditioning period is typical in Steelhead Trout 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 fish surviving to 30 weeks post-spawning. Similarly, 38% of wild Snake River Steelhead Trout tagged as out-migrating adults at Lower Granite Dam in 2002-2004 returned as consecutive spawners (Keefer et al. 2008).

Predictors of Survival

Energy Reserves.

Survival to 70 days after spawning and survival duration for fish that died during the first 70 days 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 post-spawning (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 to access stored energy than on energy acquisition through feeding. Plasma TG level at spawning integrates both body levels of stored lipids and the functioning of physiological systems enabling 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 vitellogenin synthesis (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 Yakima River Steelhead Trout kelts (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.

OS 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 below approximately 290 mmol/kg were highly subject to mortality soon after first spawning. 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 pre-spawning 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 Trout 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 allocated to reproduction at first spawning (measured as TEM) and survival post-spawning, thought to be a classic life history trade-off. Post-spawning survival was not significantly negatively correlated with TEM in the whole model, so a 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 Trout from the Hood River, Oregon (Christie et al. 2018). However, survival percentages were much lower in the wild fish study, consistent with stronger selection on post-spawning energy reserves for fish 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 Trout.

Plasma Estradiol and Parasite Load.

E2 at spawning did not predict subsequent survival. E2 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 impact survival in adult salmonids (Jia et al. 2019).

First Spawning Reproductive Investment and Spawning Schedule

Consecutive reproductive status was positively related to first spawn TEM in female Steelhead Trout 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 trade-off between current and future reproduction proposed in life history theory (Stearns 1992), but instead suggests that both first spawn reproductive effort and consecutive reproduction are positively regulated by condition at a time 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 potential trade-offs (Hendry et al. 2000). The strong positive relationships 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 Trout (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 measures of condition at first spawning and consecutive reproductive status (the consecutive spawning schedule), suggesting that the reproductive decision is not condition-dependent at 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 the fish were sampled during ovarian development. Sea-run Brown Trout spawn in the fall and most overwinter in freshwater before returning to the ocean. Thus, ovarian development would be underway in 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

Reproductive characteristics at consecutive spawning were sensitive to post-spawning condition measures early in ovarian development up to approximately 9.5 months prior to second spawning. IEM was strongly positively correlated with growth rates (mass, FL) and TG at 10 weeks after first spawning. TEM 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, when compared to 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 Trout or apply more broadly. The physiological mechanisms involved, and whether and how determination of egg size and fecundity by these mechanisms is adaptive, are not clear at present.

Conflict of Interest

The authors declare no conflict of interest.

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References

Boeuf, G., and P. Payan. 2001. How should salinity influence fish growth? Comparative Biochemistry and Physiology C-Toxicology & Pharmacology 130:411-423.

Bon, E., G. Corraze, S. Kaushik, and F. LeMenn. 1997. Effects of accelerated photoperiod regimes on the reproductive cycle of the female rainbow trout: I—Seasonal variations of plasma lipids correlated with vitellogenesis. Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology 118:183-190.

Bull, J. J., and R. Shine. 1979. Iteroparous animals that skip opportunities for reproduction. American Naturalist 114:296-303.

Burton, M. P. M. 1994. A critical period for nutritional control of early gametogenesis in female winter flounder, Pleuronectes americanus (Pisces, Teleostei). Journal of Zoology 233:405- 415.

Campbell, B., J. Dickey, B. Beckman, G. Young, A. Pierce, H. Fukada, and P. Swanson. 2006. Previtellogenic oocyte growth in salmon: Relationships among body growth, plasma insulin- like growth factor-1, estradiol-17beta, follicle-stimulating hormone and expression of ovarian genes for insulin-like growth factors, steroidogenic-acute regulatory protein and receptors for gonadotropins, growth hormone, and somatolactin. Biology of Reproduction 75:34-44.

Christie, M. R., G. G. McNickle, R. A. French, and M. S. Blouin. 2018. Life history variation is maintained by fitness trade-offs and negative frequency-dependent selection. Proceedings of the National Academy of Sciences of the United States of America 115:4441-4446.

Congleton, J. L., and T. Wagner. 2006. Blood-chemistry indicators of nutritional status in juvenile salmonids. Journal of Fish Biology 69:473-490.

Crim, L. W., C. E. Wilson, Y. P. So, D. R. Idler, and C. E. Johnston. 1992. Feeding, reconditioning, and rematuration responses of captive Atlantic salmon (Salmo salar) kelt. Canadian Journal of Fisheries and Aquatic Sciences 49:1835-1842.

De Mones, A., A. Fostier, C. Cauty, and B. Jalabert. 1989. Ovarian early postovulatory development and estrogen production in rainbow trout (Salmo gairdneri R.) from a spring-spawning strain. General and Comparative Endocrinology 74:431-441.

Evans, A. F., R. E. Beaty, M. S. Fitzpatrick, and K. Collis. 2004. Identification and enumeration of steelhead kelts at a Snake River hydroelectric dam. Transactions of the American Fisheries Society 133:1089-1099.

Fleming, I. A., and S. Ng. 1987. Evaluation of Techniques for Fixing, Preserving, and Measuring Salmon Eggs. Canadian Journal of Fisheries and Aquatic Sciences 44:1957-1962.

Gauthey, Z., M. Freychet, A. Manicki, A. Herman, O. Lepais, S. Panserat, A. Elosegi, C. Tentelier, and J. Labonne. 2015. The concentration of plasma metabolites varies throughout reproduction and affects offspring number in wild brown trout (Salmo trutta). Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology 184:90-96.

Haraldstad, T., E. Hoglund, F. Kroglund, A. Lamberg, E. M. Olsen, and T. O. Haugen. 2018. Condition-dependent skipped spawning in anadromous brown trout (Salmo trutta). Canadian Journal of Fisheries and Aquatic Sciences 75:2313-2319.

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 33:615-625.

Henderson, B. A., and J. L. Wong. 1998. Control of lake trout reproduction: role of lipids. Journal of Fish Biology 52:1078-1082.

Hendry, A. P., A. H. Dittman, and R. W. Hardy. 2000. Proximate composition, reproductive development, and a test for trade-offs in captive sockeye salmon. Transactions of the American Fisheries Society 129:1082-1095.

Hill, G. E. 2011. Condition-dependent traits as signals of the functionality of vital cellular processes. Ecology Letters 14:625-634.

Jeffries, K. M., S. G. Hinch, M. R. Donaldson, M. K. Gale, J. M. Burt, L. A. Thompson, A. P. Farrell, D. A. Patterson, and K. M. Miller. 2011. Temporal changes in blood variables during final maturation and senescence in male sockeye salmon Oncorhynchus nerka: reduced osmoregulatory ability can predict mortality. Journal of Fish Biology 79:449-465.

Jenkins, L. E., A. L. Pierce, N. Graham, R. Branstetter, D. R. Hatch, and J. J. Nagler. 2018. Reproductive performance and energy balance in consecutive and repeat spawning female steelhead reconditioned in captivity. Transactions of the American Fisheries Society 147:959-971.

Jenkins, L. E., A. L. Pierce, N. D. Graham, L. R. Medeiros, D. R. Hatch, and J. J. Nagler. 2019. Elevated plasma triglycerides and growth rate are early indicators of reproductive status in post-spawning female steelhead trout (Oncorhynchus mykiss). Conservation Physiology 7:coz038.

Jia, B. B., M. K. V. C. Delphino, B. Awosile, T. Hewison, P. Whittaker, D. Morrison, M. Kamaitis, A. Siah, B. Milligan, S. C. Johnson, and I. A. Gardner. 2019. Review of infectious agent occurrence in wild salmonids in British Columbia, Canada. Journal of Fish Diseases

Keefer, M. L., M. A. Jepson, G. P. Naughton, T. J. Blubaugh, T. S. Clabough, and C. C. Caudill. 2017. Condition-dependent en route migration mortality of adult chinook salmon in the Willamette River main stem. North American Journal of Fisheries Management 37:370-379.

Keefer, M. L., R. H. Wertheimer, A. F. Evans, C. T. Boggs, and C. A. Peery. 2008. Iteroparity in Columbia River summer-run steelhead (Oncorhynchus mykiss): Implications for conservation. Canadian Journal of Fisheries and Aquatic Sciences 65:2592-2605.

Koops, M. A., J. A. Hutchings, and T. M. McIntyre. 2004. Testing hypotheses about fecundity, body size and maternal condition in fishes. Fish and Fisheries 5:120-130.

Leitritz, E., and R. C. Lewis. 1976. Trout and salmon culture (hatchery methods). State of California, The Resources Agency, Department of Fish and Game, Fish Bulletin 164.

McBride, R. S., S. Somarakis, G. R. Fitzhugh, A. Albert, N. A. Yaragina, M. J. Wuenschel, A. Alonso-Fernandez, and G. Basilone. 2015. Energy acquisition and allocation to egg production in relation to fish reproductive strategies. Fish and Fisheries 16:23-57.

Meador, J. P., F. C. Sommers, G. M. Ylitalo, and C. A. Sloan. 2006. Altered growth and related physiological responses in juvenile Chinook salmon (Oncorhynchus tshawytscha) from dietary exposure to polycyclic aromatic hydrocarbons (PAHs). Canadian Journal of Fisheries and Aquatic Sciences 63:2364-2376.

Moffett, I. J. J., G. J. A. Kennedy, and W. W. Crozier. 1996. Freshwater reconditioning and ranching of Atlantic salmon, Salmo salar l., kelts: Growth and reproductive performance. Fisheries Management and Ecology 3:35-44.

Oguri, M., and Y. Ooshima. 1977. Early changes in plasma osmolality and ionic concentrations of rainbow trout and goldfish following direct transfer from fresh-water to sea water. Bulletin of the Japanese Society of Scientific Fisheries 43:1253-1257.

Penney, Z. L., and C. M. Moffitt. 2014a. Histological assessment of organs in sexually mature and post-spawning steelhead trout and insights into iteroparity. Reviews in Fish Biology and Fisheries 24:781-801.

Penney, Z. L., and C. M. Moffitt. 2014b. Proximate composition and energy density of stream-maturing adult steelhead during upstream migration, sexual maturity, and kelt emigration. Transactions of the American Fisheries Society 143:399-413.
Quinn, T. P., T. R. Seamons, L. A. Vøllestad, and E. Duffy. 2011. Effects of growth and reproductive history on the egg size–fecundity trade-off in steelhead. Transactions of the American Fisheries Society 140:45-51.
Reid, J. E., and G. Chaput. 2012. Spawning history influence on fecundity, egg size, and egg survival of Atlantic salmon (Salmo salar) from the Miramichi River, New Brunswick, Canada. ICES Journal of Marine Science 69:1678-1685.
Rideout, R. M., G. A. Rose, and M. P. M. Burton. 2005. Skipped spawning in female iteroparous fishes. Fish and Fisheries 6:50-72.
Rideout, R. M., and J. Tomkiewicz. 2011. Skipped spawning in fishes: more common than you might think. Marine and Coastal Fisheries 3:176-189.
Sheridan, M. A. 1988. Lipid Dynamics in Fish - Aspects of Absorption, Transportation, Deposition and Mobilization. Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology 90:679-690.
Sievert, C., C. Parmer, T. Hocking, S. Chamberlain, K. Ram, M. Corvellec, and P. Despouy. 2018. plotly: Create Interactive Web Graphics via'plotly. js'. 2016. R package version 3.6.0.
Simpkins, D. G., W. A. Hubert, C. M. Del Rio, and D. C. Rule. 2003. Physiological responses of juvenile rainbow trout to fasting and swimming activity: effects on body composition and condition indices. Transactions of the American Fisheries Society 132:576-589.
Smart, J., W. J. Sutherland, A. R. Watkinson, and J. A. Gill. 2004. A new means of presenting the results of logistic regression. Bulletin of the Ecological Society of America 85:100–102.
Stearns, S. C. 1992. The Evolution of Life Histories. Oxford University Press, New York.
Stubben, C., and B. Milligan. 2007. Estimating and analyzing demographic models using the popbio package in R. Journal of Statistical Software 22:1-23.
Sutton, S. G., T. P. Bult, and R. L. Haedrich. 2000. Relationships among fat weight, body weight, water weight, and condition factors in wild Atlantic salmon parr. Transactions of the American Fisheries Society 129:527-538.
Taranger, G. L., M. Carrillo, R. W. Schulz, P. Fontaine, S. Zanuy, A. Felip, F. A. Weltzien, S. Dufour, O. Karlsen, B. Norberg, E. Andersson, and T. Hausen. 2010. Control of puberty in farmed fish. General and Comparative Endocrinology 165:483-515.
Thorpe, J. E. 1994. Reproductive strategies in Atlantic salmon, Salmo salar L. Aquaculture and Fisheries Management 25:77-87.
Thorpe, J. E. 2007. Maturation responses of salmonids to changing developmental opportunities. Marine Ecology Progress Series 335:285-288.
Trudel, M., S. Tucker, J. F. T. Morris, D. A. Higgs, and D. W. Welch. 2005. Indicators of energetic status in juvenile coho salmon and Chinook salmon. North American Journal of Fisheries Management 25:374-390.

Table 1. Correlation coefficients between parameters measured at spawning in female steelhead trout collected in 2015 and 2016 (n = 314). P-values for correlations are above and correlation coefficients below the diagonal.

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

Bolded R-values indicate significance ($P < 0.05$).

[a]Correlations were based on transformed data (ML: arcsine-square root; Plasma E2: $\log_{10}$).

Table 2. Number (*n* (%)) by outcome category of female steelhead trout collected in 2015 and 2016 with major condition and reproductive metrics (mean (SD)). Survived: alive at 70 days after spawning; Mortality: died before 70 days after spawning; Consecutive: consecutive spawner; Skip: skip spawner.

		n (%)	Muscle Lipids (%)	Condition K	Triglycerides	Plasma Osmolality (mmol/kg)	Parasite Load (#copepods)	Total Egg Mass (g)	Plasma Estradiol-17b (ng/mL)
	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)
2015	Survived	80 (53)	0.96 (0.12)	0.758 (0.05)	189.6 (28.4)	303.1 (5.7)	4.0 (2.7)	688.8 (97)	984.8 (327.6)
	Mortality	70 (47)	0.95 (0.13)	0.763 (0.05)	180.8 (33.4)	301.0 (6.1)	4.1 (2.7)	698.0 (116)	958.0 (292.6)
	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)
	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)
2016	Survived	62 (38)	0.93 (0.14)	0.779 (0.05)	167.7 (36.1)	300.3 (5.6)	3.9 (2.8)	776 (129)	956.4 (382.5)
	Mortality	102 (62)	0.90 (0.11)	0.763 (0.05)	143.5 (40.2)	294.6 (9.2)	4.3 (2.5)	811.3 (127)	851.1 (307.5)

	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)
	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)
All	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)

Mass (g)

Table 3. Standardized parameter estimates resulting from multiple linear regression analysis of post-spawning survival duration within the first 70 days after first spawning in female steelhead trout (n = 156) collected in 2015 and 2016.

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**

Bolded R-values indicate significance ($P < 0.05$).

Table 4. Correlation coefficients (R) of relationships between condition measures over time starting at first spawning and reproductive characteristics measured at consecutive spawning in n = 12 female steelhead trout first spawned in 2015.

Repeat Reproductive Performance	Sampling Point	MSGR[a]	LSGR[a]	Plasma Triglycerides	Plasma E2	Muscle Lipids %	Condition K
Individual Egg	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

Bolded R-values indicate significance ($P < 0.05$).

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

Figure Captions

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

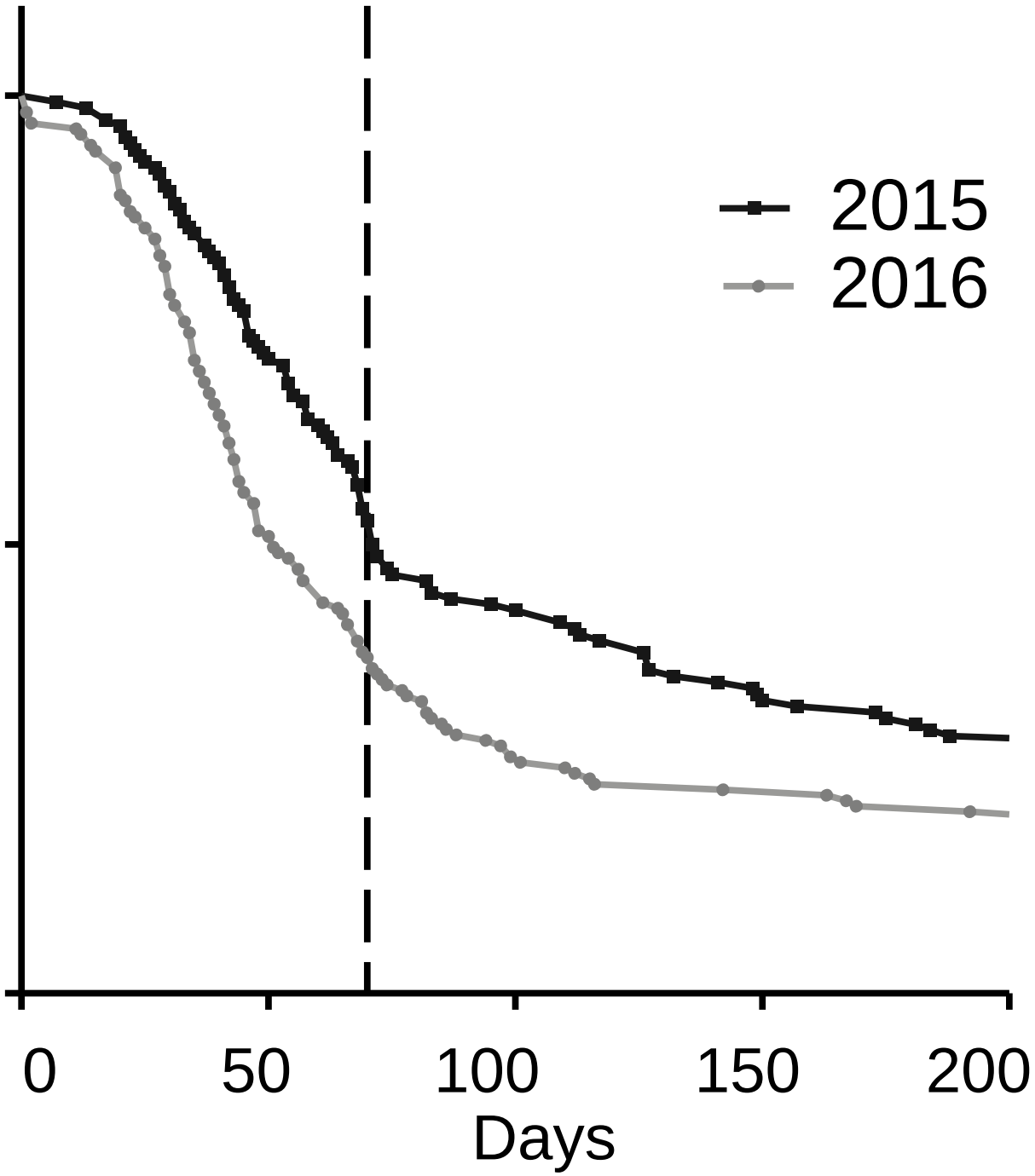
Figure 2. Standardized coefficients ([x-mean]/SD) with 95% confidence intervals of potential predictors in a multiple logistic regression model of survival of female steelhead trout to 70 days after first spawning in 2015 and 2016 (n = 291). Positive coefficients indicate a greater probability of post-spawning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

Figure 3. Post-spawn survival probabilities (PSurv) to 70 days for female steelhead trout, as predicted by plasma triglcyerides (mg/dL) (TG) and plasma osmolality (mmol/kg) (OS) measured at first spawning in 2015 and 2016 (n = 291)

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 trout (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.

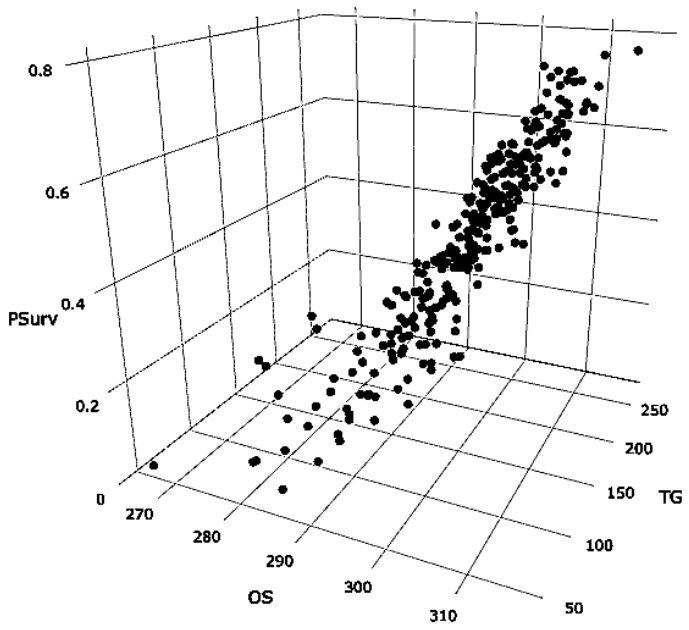
Figure 5. The probability of consecutive spawning reproductive status in female steelhead trout, in relation to total egg mass at first spawning (g, mass-standardized, n = 60). The bars at the top and bottom of the graph represent the number of fish that became reproductively active (top) or remained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

Figure 6. Relationships between reproductive characteristics at consecutive spawning (individual egg mass, total egg mass, and fecundity) with plasma triglycerides or mass specific growth rate (MSGR) during ovarian recrudescence in female steelhead trout.


2015
2016
0
50
100
150
200
Days

Standardized survival coefficient

tafs_10224_f2.eps



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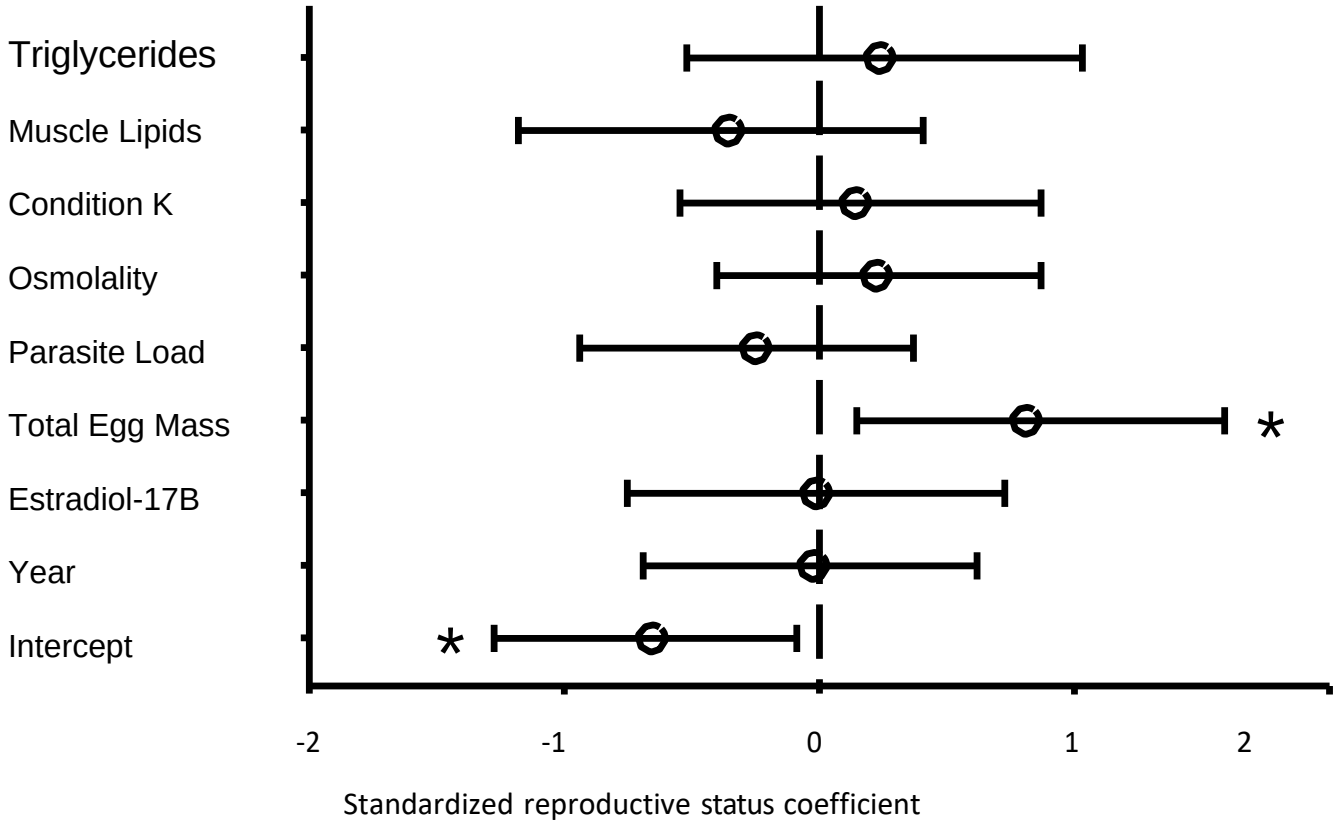

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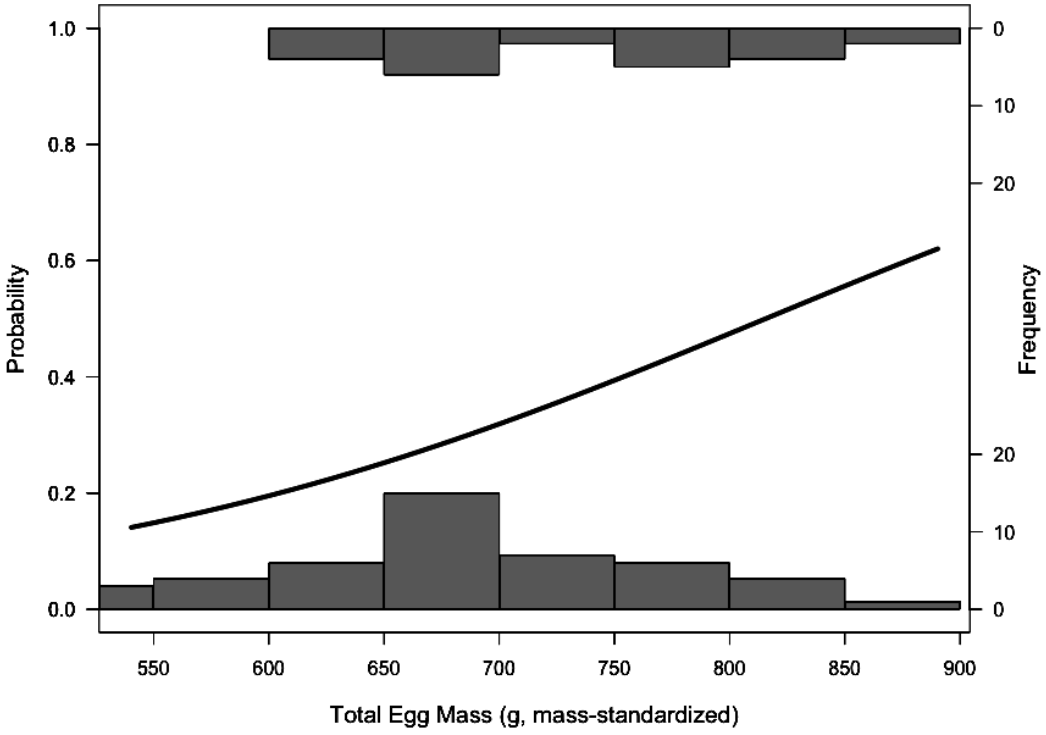
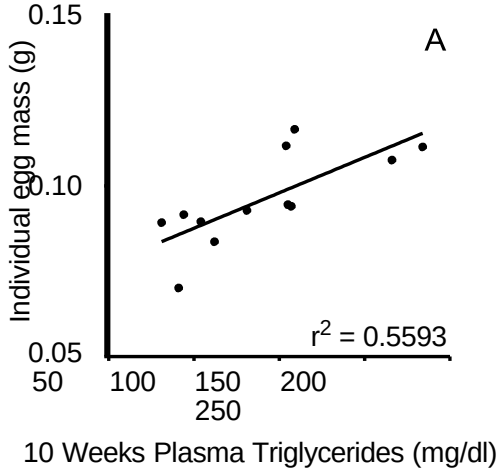
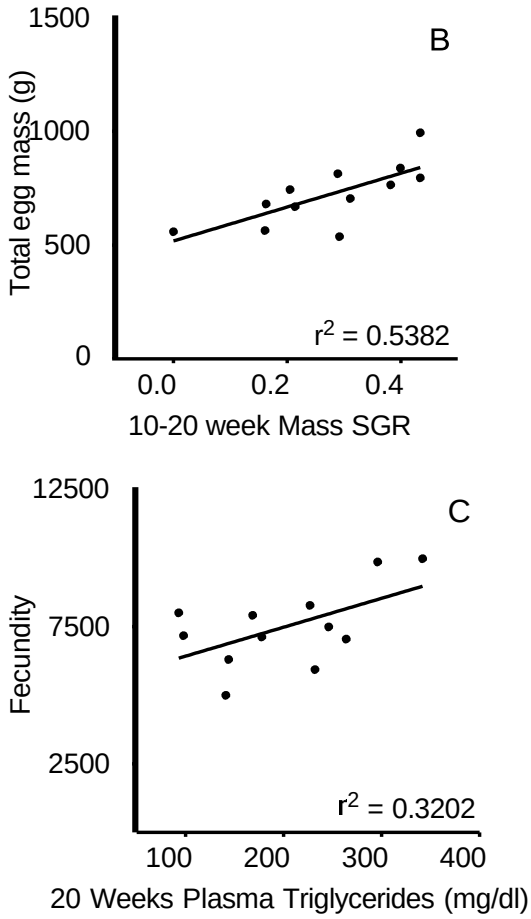

Probability
Frequency
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
20
10
0
550
600
650
700
750
800
850
900
Total Egg Mass (g, mass-standardized)


A
Individual egg mass (g)
0.15
0.10
0.05
r^2 = 0.5593
50
100
150
200
250
10 Weeks Plasma Triglycerides (mg/dl)


B
1500
1000
500
0
Total egg mass (g)
r2 = 0.5382
0.0
0.2
0.4
10-20 week Mass SGR
C
12500
7500
2500
Fecundity
r2 = 0.3202
100
200
300
400
20 Weeks Plasma Triglycerides (mg/dl)

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

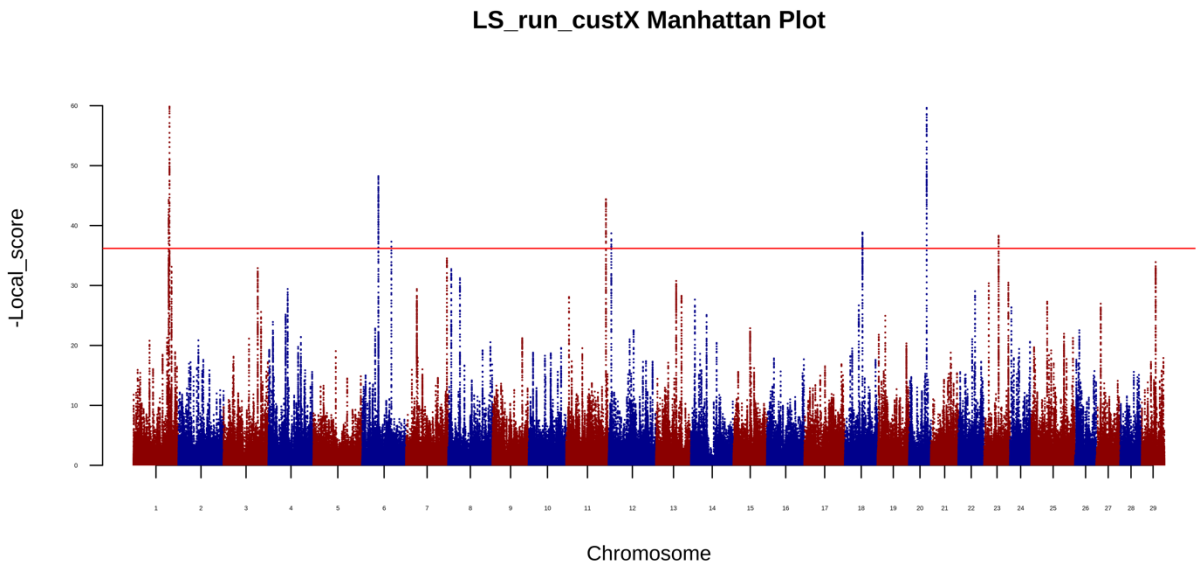
"Kelt homing"	Fish	Distribution of Fish Species (ID: 62)	Fish Life Stage: RANGE: Adult to Adult	NA
"Fecundity"	Fish	Fecundity: Fish (ID: 68)	NA	NA
"Growth rate"	Fish	Growth Rate: Fish (ID: 73)	Fish Life Stage: RANGE: Juvenile to Adult	NA
"Kelt length"	Fish	Length: Fish Species (ID: 75)	Fish Life Stage: 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

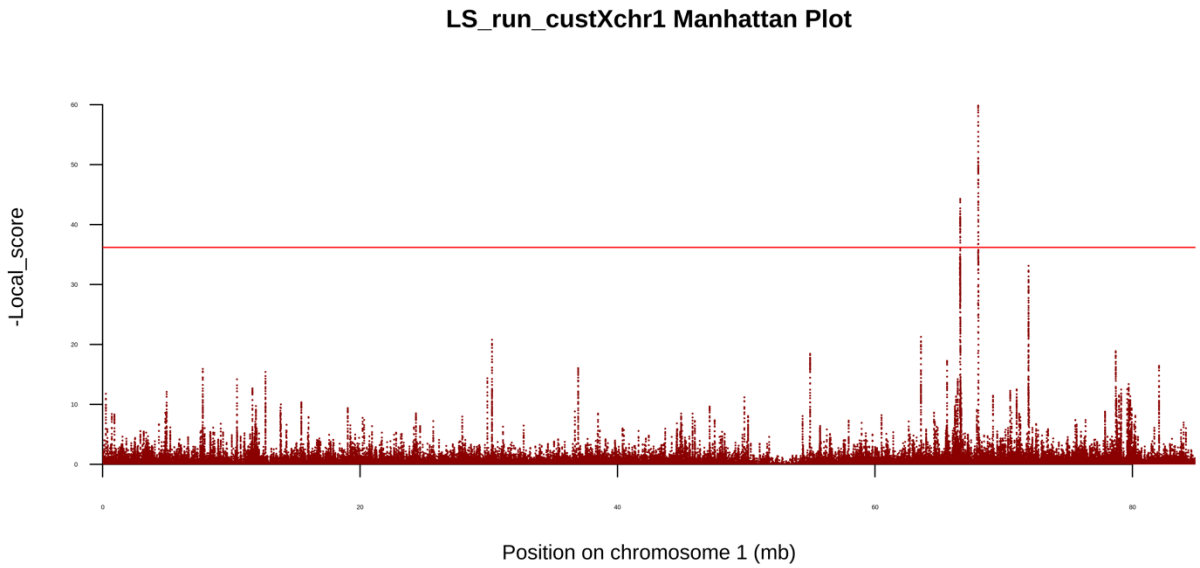
Title	Category	Subcategory	Subcategory Focus 1
"Relative reproductive success of artifically reconditioned kelt steelhead"	Fish	Relative Reproductive Success (RRS) (ID: 88)	Fish Origin: Natural

Appendix A.4: Searching for Genetic Basis for Consecutive and Skip Spawner Life Histories: Genome re-sequencing of 3 years of kelts from Dworshak Hatchery (Manhattan Plots).

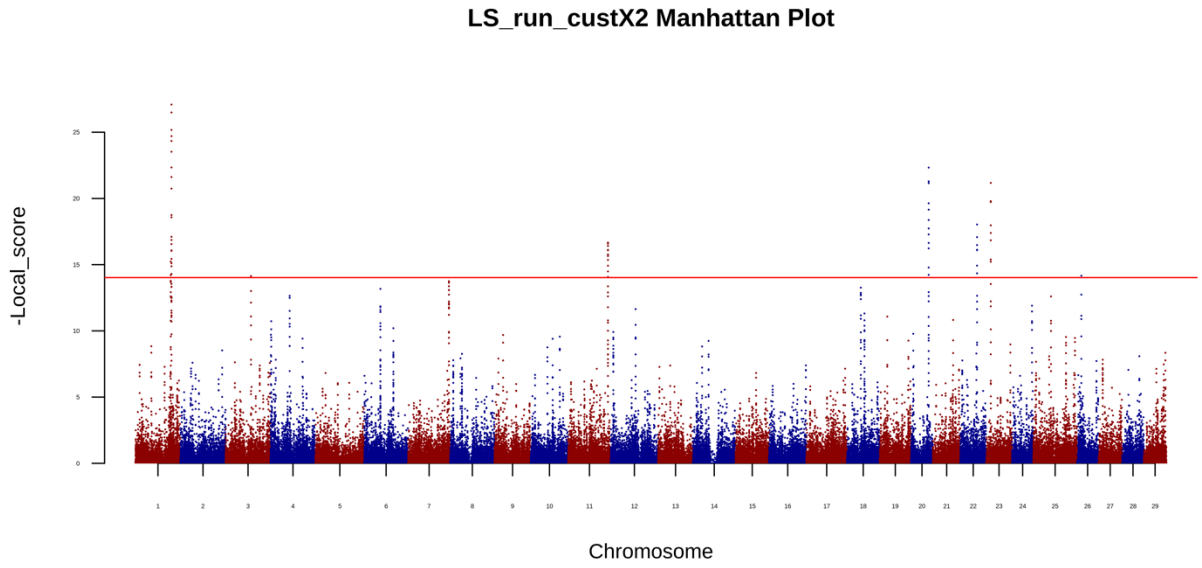
Local score (arbitrary ξ of 1) pooled by year



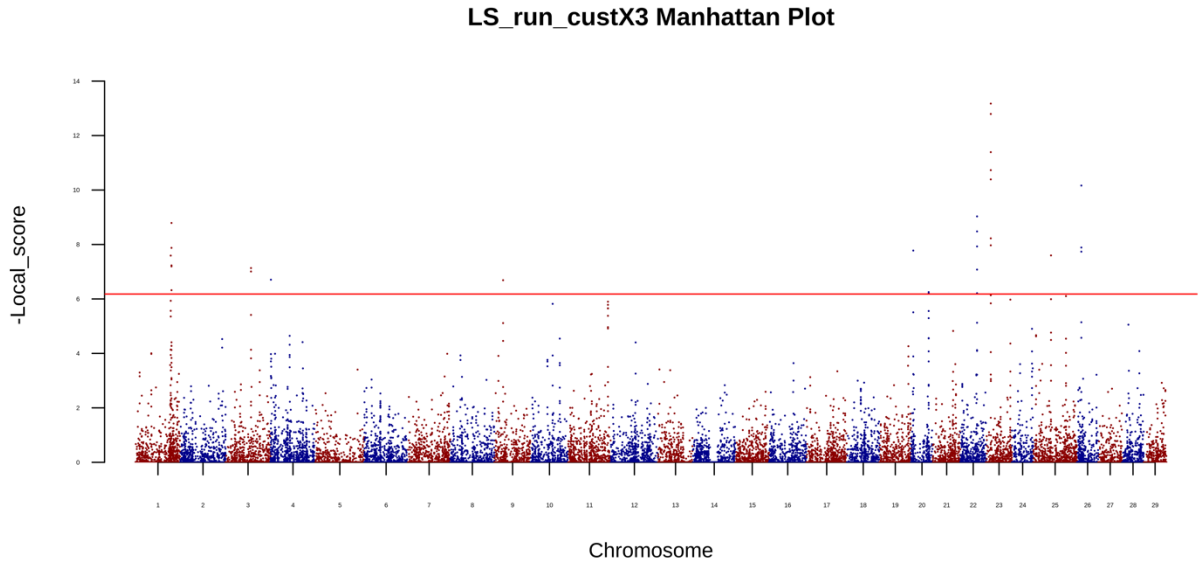
Local score (arbitrary ξ of 1) pooled by year, **chromosome 1**



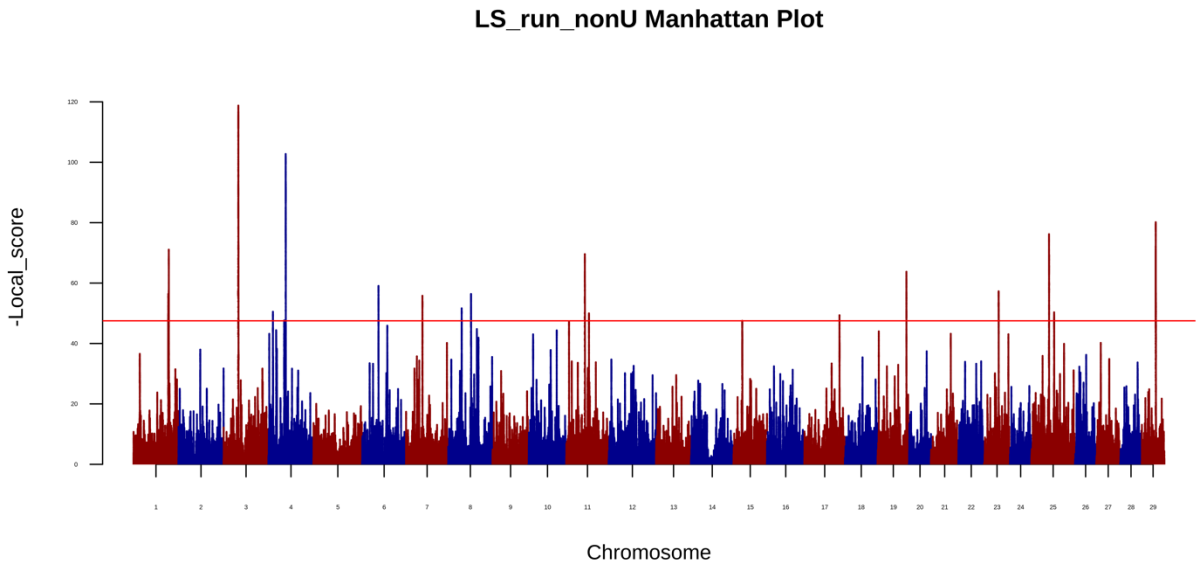
Local score (arbitrary ξ **of 2**) pooled by year



Local score (arbitrary ξ **of 3**) pooled by year



Local score (optimized ξ **of 0.308** [85th percentile] for non-uniform data) by **reproductive status by year**



Local score (arbitrary ξ **of 1**) by **reproductive status by year**