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Basinwide Supplementation Evaluation Project: 2020 Annual Progress Report

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2020 Annual Progress Report

Basinwide Supplementation Evaluation Project

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I. Executive Project Summary

This report summarizes activities occurring from January 1, 2020 to December 31, 2020 under BPA Contract No. 73354 REL 25 and 73354 REL 41, all of which were performed as part of the multi-year Basinwide Supplementation Evaluation Project 2009-009-00 (hereafter the Project). The report is organized under the eight Project Objectives identified in the contract Statement of Work. The primary objective of the Project is to facilitate monitoring and evaluation of tribally managed supplementation programs to assess: a) critical uncertainties related to effects of hatchery management and supplementation on natural anadromous fish populations, and b) abundance and productivity trends within reintroduced populations.

Project Objective #1 *– Estimate individual productivity and compare reproductive success between hatchery-origin (HOR) and natural-origin (NOR) spring Chinook in the Upper Yakima River, WA*

- Genotyping of fish involved in the initially proposed 5-brood year (BY 2007-2011) study, as well as pedigree reconstruction analyses, was completed during this reporting year. Comprehensive analyses are ongoing and will primarily focus on differential reproductive success between NOR and HOR fish that spawn in Upper Yakima River. In addition to individual origin, we will evaluate several other potentially interacting factors, such as size at return, timing of return and acclimation site. This project is occurring in collaboration between the Yakama Nation (YN), Washington Department of Fish and Wildlife, and the Columbia River Inter-Tribal Fish Commission (CRITFC).

Project Objective #2 *– Estimate individual productivity and compare reproductive success between HOR and NOR spring Chinook in Lookingglass Creek, OR*

- A relative reproductive success (RRS) analysis (BYs 2008-2016) based on both juvenile and on adult recruits-per-spawner is ongoing for spring Chinook salmon in Lookingglass Creek (Grande Ronde River basin). Pedigree reconstruction analyses are ongoing and will not conclude until late-2021/early-2022, when any age-5 returns from the final study brood year (BY2016) have been genotyped. As described for Project Objective #1, several interacting effects beyond origin will be evaluated in comprehensive analyses. The project is occurring in collaboration between the Confederated Tribes of the Umatilla Indian Reservation (CTUIR) and CRITFC.

Project Objective #3 *– Perform genetic stock identification, productivity analyses and related assessments of tribally-managed sockeye salmon reintroduction efforts in the Yakima River and Deschutes River basins*

- To assess relative spawning success and productivity of sockeye salmon reintroduced to Cle Elum Lake, WA the Project has financed genotyping of individuals representative of various life stages and histories each year. In 2020, a strong sockeye return throughout the Columbia River basin allowed for the translocation of approximately 10,000 Lake Wenatchee and Osoyoos Lake origin fish intercepted at Priest Rapids Dam (PRD) to Cle Elum Lake. We obtained genetic samples from 1,125 of these translocated fish, in addition to 4,195 fish returning to the Roza Adult Monitoring Facility (RAMF), which represent naturalized, Yakima basin sockeye. We also collected 756 carcass and gillnet samples and 330 smolt samples. All samples will be genotyped by Spring 2021, and then identified to stock-of-origin to evaluate trends in productivity and spawn timing by stock of origin. This project is occurring in collaboration between the YN and CRITFC.

- A subset of the individuals intercepted at Priest Rapids Dam, and designated for translocation to Cooper Lake, WA, were acoustically tagged and monitored via an array of acoustic receivers throughout the Cooper and Cle Elum Rivers from July to November 2020. Comprehensive analysis of telemetry data is ongoing, but preliminary review suggests only two fish remained in Cooper Lake through to the end of the spawning season in late fall. This project occurred in collaboration between the YN and CRITFC.

- Starting in 2009, passage for juvenile *O. nerka* demonstrating emigration behavior from Lake Billy Chinook (Deschutes River basin) has been facilitated at the Pelton Round Butte complex of dams. Additionally, any sea-run adults returning to the Pelton adult fish trap have been transported to Lake Billy Chinook or collected for hatchery spawning since 2012. These returning adults are tissue sampled for genetic stock identification. In 2020, 65 adults returned to the Pelton adult fish trap, 63 of which were confidently identified to stock-of-origin: 58 individuals assigned to Lake Billy Chinook as stock-of-origin, five individuals assigned to Suttle Lake as stock-of-origin, and one individual appeared to be a Lake Wenatchee stray. This project occurs in collaboration between the Confederated Tribes of the Warm Springs Reservation of Oregon (CTWSRO), Oregon Fish and Wildlife, Portland General Electric and CRITFC.

Project Objective #4 *– Estimate the effective number of breeders and assess natural productivity of spring Chinook and coho salmon in the Upper Warm Springs River, OR*

- In recent years, the fish-per-redd ratio for spring Chinook in the Upper Warm Springs River has increased significantly, suggesting lower reproductive success. Simultaneously, coho salmon have returned to the Upper Warm Springs River in increasing numbers. To better understand productivity patterns for both species in this system, we plan to use pedigree reconstruction tools to directly estimate the number of effective breeders and individual reproductive success. For both species, juveniles from brood years 2015-2016, and adults and juveniles from brood years 2017-2019 will be genotyped and analyzed. Genotyping is ongoing and comprehensive analyses are expected in late-2021/early-2022. This project occurs in collaboration between the CTWSRO, US Fish and Wildlife and CRITFC.

Project Objective #5 *- Evaluate factors affecting minijack production*

- The Project is also financing several studies examining factors that may influence precocious maturation of hatchery-reared male spring Chinook salmon smolts, or "minijacks:"
    - Data analyses for a three brood year (BY2014-2016) study conducted at the Cle Elum Supplementation Research Facility (CESRF) to test for an effect of parent age on precocity among offspring are currently being finalized, and results have been drafted into a manuscript for journal publication. Results suggest no consistent effect of parent age, possibly masked by the very high variation in minijack rate among families within parental age cross-types. Reporting of results from a comprehensive analysis is anticipated in mid-2021.
    - A one-year study was conducted to test the effects of diet supplementation with tetradecylthioacetic acid (TTA), previously reported to have been effective in reducing early maturation of juvenile male Atlantic salmon. While TTA-supplemented fish demonstrated reduced whole body lipid levels during the treatment period, there was no reduction in minijack rate relative to controls. A manuscript has been submitted and is in review.
    - A study was designed to compare relative growth rate through the juvenile rearing period of non-maturing smolts versus maturing minijacks using BY2017 fish from 11

different families. Fish were PIT tagged in July of their first year and individual growth quantified through to April of their second year (in April 2019). Interestingly, size and condition factor at the first sample date (July 2018) was already generally greater for fish destined to become minijacks, and these differences remained throughout juvenile rearing period. Further analyses to parse out an effect of family are underway, and a paper is expected to be submitted in mid-2021.

- A study to investigate the effect of two different 5-week periods of feed deprivation on minijack rate was conducted using BY18 juveniles. The smolts were sacrificed and sampled in July of 2020 and the results imply that feed deprivation may indeed lower the rate of precocious maturation; however, some identification issues due to fin regrowth may have confounded results. A technical report has been written and a follow-up study (using PIT tags to identify fish) is planned for BY20 juveniles.
- Several studies investigating the effect of photoperiod manipulation on precocious maturation have been conducted at the University of Idaho in Moscow, ID. The first (conducted using BY18 juveniles) exposed treated groups to 24h light for varying lengths of time, with results indicating that 24h light treatment is a very effective means of reducing precocious maturation. Follow-up studies are exploring 1) the amount of exposure to 24h light that is necessary to result in a significant reduction in minijack rates, and 2) how the exposure to 24h light affects smoltification (using BY19 and BY20 juveniles, respectively).
- A study to compare age structure of returning mature adults for fish sired by an age-3 jack versus an age-4 adult male was initiated in 2018. A similar set of crosses was performed in 2019. Sampling of adult returns will begin in Spring 2021 with BY2018 jacks, and finish in 2024 with BY2019 age-5 returns.
- A single brood year (BY2016) study was conducted to investigate the effect of generations of hatchery rearing on minijack rate. Several factorial crosses were performed in which an age-4, SH-origin female was crossed to a natural-origin male (zero generations of hatchery rearing), a supplementation hatchery male (one generation of hatchery rearing) and a hatchery control male (four generations of hatchery rearing). The minijack rate among resulting progeny will be reviewed to test for an effect of male origin type. Data analyses are ongoing and a draft manuscript for publication in a scientific journal is anticipated in mid-2021.

Projective Objective #6 - *Participate in regional forums for review of hatchery effects on natural populations*

- Due to the COVID-19 pandemic, most workshops and conferences were either cancelled or moved to a virtual format. Columbia River Inter-Tribal Fish Commission (CRITFC) personnel and collaborators associated with Project activities participated in these virtual forums where possible.

Project Objective #7 - *Prepare manuscripts for publication in scientific journals*

- Results from analyses, when complete, for all of the above-described studies will be reported in manuscripts for publication in scientific journals.

Project Objective #8 - *Identify additional studies to support tribal supplementation and* reintroduction programs

- Through meetings and conferences, connecting with tribal fish biologist and managers, and while completing ongoing Project activities, opportunities for additional collaboration have been identified. In line with the objectives of the Project, these opportunities focus on tribally managed programs where focused monitoring and evaluation work will inform and facilitate adaptive management.

II. Introduction

The Basinwide Supplementation Evaluation (BSE) Project was submitted by the Columbia River Inter-Tribal Fish Commission (CRITFC) as part of the Columbia Basin Fish Accords (2008). The BSE Project was designed to implement various studies in support of recommendations put forth by the Ad Hoc Supplementation Workgroup (AHSWG 2008), and with each study being directly or indirectly associated with a tribally managed program. All studies supported by the BSE project aim to inform critical uncertainties associated with the use of hatchery supplementation for rebuilding depressed anadromous fish populations throughout the Columbia River basin (ISRP and ISAB, 2005). Methods employed by these studies are guided by the "three-pronged approach" recommended by the AHSWG to address these uncertainties:

I) *Compare long-term trends in abundance and productivity in supplemented relative to un-supplemented populations*

II) *Assess short-term (i.e. within brood year) differences in reproductive success between hatchery-origin (HOR) and natural-origin (NOR) fish from supplemented or reintroduced populations*

III) *Perform small-scale studies that elucidate the biological mechanisms by which the introduction of hatchery-produced fish may influence natural productivity* (AHSWG 2008).

In the 2020 BSE Project Statement of Work, the project objectives have integrated this three-pronged approach as follows:

- Approach I: Use of genetic data to perform genetic stock identification, productivity analyses and related assessments of tribally managed sockeye salmon reintroduction efforts in the Yakima River basin and the Deschutes River basin (Project Objective #3).

  In the Yakima River basin, the reintroduction effort began in 2009 and tissue samples have been collected for genetic analysis since 2011. This study therefore provides an opportunity to examine long-term trends in productivity and abundance within a population that continues to be supplemented by out-of-basin stocks to facilitate reintroduction. In the Deschutes River basin, reintroduction and genetic monitoring of this effort began in earnest in 2010. However, unlike the effort in the Yakima River basin, which uses out-of-basin adults to supplement the population, the Deschutes program focuses on facilitating downstream passage of *Oncorhynchus nerka* juveniles that demonstrate emigration behavior, with the assumption that some portion of juveniles will fully express anadromy, migrating to the ocean and returning as in-basin sockeye salmon. Hence, this provides another long-term monitoring study that can be compared to the program in the Yakima River basin to assess the implications and merits of the differing approaches to reintroduction.

  Additionally, the BSE Project is utilizing genetic data to evaluate productivity trends between species within a currently un-supplemented (i.e. reference) system (Objective #4). In the Upper Warm Springs River, spring Chinook have demonstrated downward trends in productivity in recent years, while coho salmon have simultaneously arrived in increasing numbers. The BSE Project intends to use pedigree reconstruction analyses to directly estimate individual reproductive success and effective population size for both species over five brood years. Based on these findings, the initiation of a targeted supplementation program to avoid extirpation may be deemed appropriate. The BSE Project would continue to provide monitoring and evaluation support after initiation of such a program, contributing to our collective knowledge of population productivity trends before, during, and perhaps after, a supplementation effort.
- Approach II: Use of genetic data and pedigree reconstruction analyses to evaluate individual productivity and relative reproductive success (RRS) of HOR v. NOR spring

Chinook salmon in the Upper Yakima River (Project Objective #1), as well as spring Chinook salmon that have been reintroduced to Lookingglass Creek in the Grande Ronde River basin (Project Objective #2).

Both studies focus on comparing the productivity of naturally spawning fish by origin within (Approach I) and across (Approach II) several brood years. Studies will also consider potentially interacting factors, such as spawn date or size of dam. Ultimate results from both studies can then be contextualized appropriately and integrated to better understand the effects of supplementation efforts across multiple generations and basins.

- Approach III: Development of experiments to investigate factors effecting precocial maturation amongst spring Chinook male smolts in the hatchery environment (Objective #5).

  The BSE Project continues to build off past research conducted at the Cle Elum Supplementation Research Facility (CESRF) to isolate and manipulate variables that may control the propensity of male spring Chinook salmon to precocially mature as an age-2 minijack in the hatchery environment. Ongoing experiments in 2020 included manipulations of photoperiod, feed ration and sire age. Additionally, in 2020, studies were summarized which investigated the impact of diet manipulations and parent age.

  Ultimately, these experiments seek to isolate the genetic and environmental mechanisms that significantly influence maturation trajectories so that targeted recommendations can be provided to hatchery managers. Precocial males are not believed to significantly contribute to natural production, given low expected rates of reproductive success, and also do not contribute to harvest objectives being that they never migrate to the ocean. Therefore, modifications to hatchery protocols that successfully target and reduce minijack rates may significantly improve natural production goals, with the magnitude of effect being directly related to existing rates of precocious maturation in a given hatchery.

III. Project Objectives

i. Project Administration

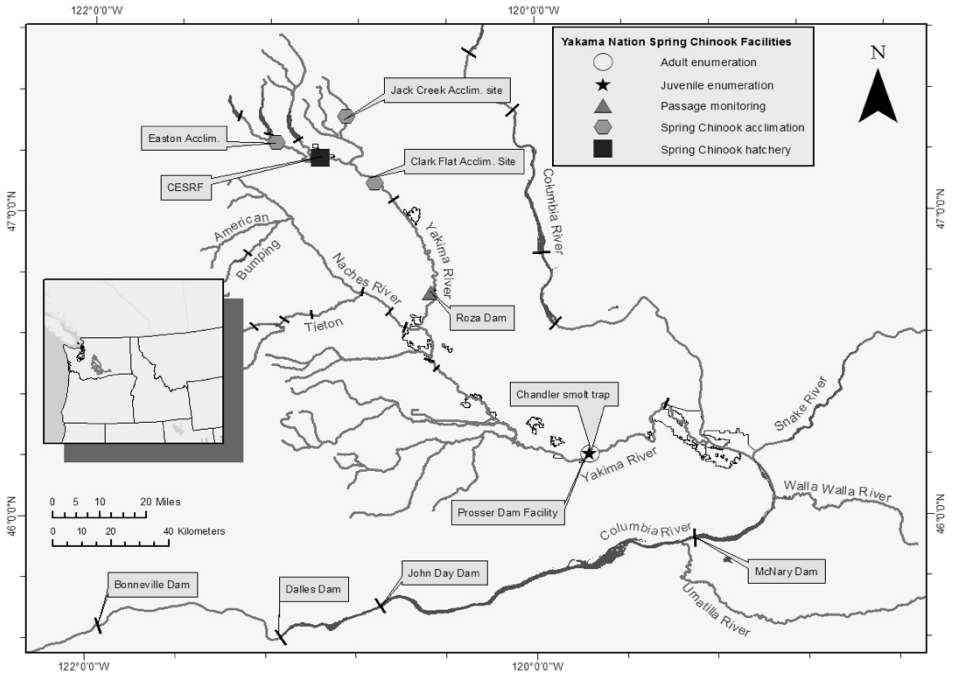
BSE Project administrative activities in 2020 included: production and posting on CBFish the annual progress report for 2019, completion of 2020 quarterly and final status reports that record progress associated with each work element within the contract Statement of Work, and submission of 2020 monthly project expense summaries to BPA. Additional reports and associated documents summarizing activities described within Project work elements were posted under Attachments within the Project 2009-009-00 web pages for Contracts No. 73354 REL 25 and 73354 REL 41.

ii. Project Objective #1 – Estimate individual productivity and compare reproductive success between hatchery-origin (HOR) and natural-origin (NOR) spring Chinook in the Upper Yakima River, WA

Introduction

In 1997, the Yakama Nation (YN), in collaboration with the Washington Department of Fish and Wildlife (WDFW), initiated a program to supplement the depressed population of spring Chinook in the Upper Yakima River, WA. Each year since, a number of natural-origin (NOR) adults in proportion to the estimated escapement, which are intercepted at the Roza Adult Monitoring Facility (RAMF) adjacent to the Roza Irrigation Dam (rkm 206), have been transferred to the Cle Elum Supplementation Research Facility (CESRF; see Fig ii.1). There, these NOR individuals are spawned and their progeny reared to the pre-smolt stage. The juvenile progeny are then transported to one of three acclimation sites where they are reared for an additional 6-8 weeks before volitional release starting in mid-March and extending through mid-May. CESRF is a fully integrated program, meaning that all supplementation hatchery broodstock are NOR. The supplementation program at CESRF is fully integrated, meaning that 100% of the supplementation hatchery broodstock is NOR; no individual has successive ancestral generations of hatchery rearing. Fully integrated programs minimize divergence between wild and hatchery lineages by allowing the natural environment to drive adaptation and fitness. As such, we would expect hatchery-origin individuals from this integrated line (hereafter referred to as SH fish) to have more comparable fitness and productivity to the natural-origin fish spawning in the river. Such supplementation programs are therefore, in theory, the best approach for minimizing ecological and genetic impacts to the native population while elevating natural production and harvest opportunities.

Figure ii.1. *Map of the study area showing the Yakima River basin, the Cle Elum Supplementation Research Facility (CESRF), the juvenile acclimation sites (Jack Creek, Easton, and Clark Flat), and the location of Roza Dam where the Adult Monitoring Facility is located (map courtesy of Paul Huffman, Yakama Nation Fisheries, retired).*



The program has been comprehensively monitored throughout its history, and analyses have suggested that while SH fish demonstrate some differences in morphometrics and life history traits (Knudsen et al., 2006), supplementation efforts have increased harvest, redd counts, and spatial distribution of spawners (Fast et al., 2015). Additionally, an investigation of genetic divergence found minimal divergence between the source population and the integrated, SH line at CESRF (Waters et al., 2015). These findings prompted the development of BSE Project Objective #1, which aims to directly estimate reproductive success and infer relative productivity between naturally spawning NOR and SH spring Chinook in the Upper Yakima River. This study addresses a gap in monitoring efforts to date, which is to *directly* assess how integrated supplementation efforts impact natural production and fitness over time.

This study is a collaborative effort between YN, WDFW and CRITFC and was initially proposed as a five-brood year study (BYs 2007-2011), but was later increased to ten-brood years (BYs 2007-2016) to permit evaluation of trends in productivity across multiple generations (i.e. parent to offspring to grand-offspring). Genotyping and primary analyses for the first five-brood year dataset (BYs 2007-2011) is now complete, while sampling (age-5 returns from BY2016) and analyses for BYs 2012-2016 is ongoing. Similarly, cumulative analyses across all ten brood years will not be complete until sampling and analyses for BYs 2012-2016 is complete (anticipated in early 2022). As such, only results from study BYs 2007-2011 are reported herein. **However, these reported results should be considered preliminary as additional comprehensive analyses for BYs 2007-2011 (i.e. demographic boost analysis, GLM modelling) are ongoing, with anticipated submission to a peer-reviewed journal in 2021.**

Methods (study BYs 2007-2011)

Sample Collection, Genotyping & Parentage Analysis

Approximately 51,000 samples have been genotyped to date, including the NOR and SH adults passed upstream of Roza Diversion Dam for natural spawning between spawn years 2007 through 2011, in addition to their potential NOR adult progeny that returned in 2009 through 2016 (Table ii.1). DNA was extracted from fin tissue using two separate methods including a standard Qiagen DNeasy protocol (Qiagen Inc., Valencia, CA) and a Chelex 100 method (Sigma-Alrich, St Louis, MO). All individuals were genotyped at a panel of 298 single nucleotide polymorphism (SNP) markers using the genotyping-in-thousands by sequencing approach (GTseq, N. R. Campbell, Harmon, & Narum, 2015; SNP markers, Janowitz-Koch et al., 2019). After filtering individual genotype data to exclude those with ≥10% missing data and duplicate samples, individuals were run through the program COLONY v2.0.6.5 (Jones & Wang, 2010), which utilizes a maximum likelihood approach to jointly assign sibship and parentage, including assignment to single parents. Assignments with a probability score <0.85 were removed from the Relative Reproductive Success analyses.

Table ii.1. *Sample numbers genotyped for each of the five study broodyears (2007-2011).*

Return Year	Natural-Origin	Integrated Hatchery-Origin (SH)	Unknown Origin	
2007	1280	1622	4	
2008	1677	3234	NA	
2009	3010	4784	4	
2010	3167	5640	NA	
2011	4346	5239	NA	
2012	2904	3429	NA	
2013	2771	NA	NA	
2014	4133	NA	27	
2015	3936	NA	14	
2016	2442	NA	NA	
Totals	*27224*	*23948*	*49*	*51221*

Relative Reproductive Success Analysis

To infer lifetime reproductive success (RS), we estimated the number of returning adult offspring for each parent. Adult females (age-4 and age-5), adult males (age-4 and age-5), and precocial and jack males (age-2 and age-3, respectively) were analyzed separately. We then compared RS between naturally spawning SH and NOR fish in BYs 2007-2011. We calculated relative reproductive success (RRS) by dividing the average RS of SH fish by the average RS of NOR fish. RRS estimates were analyzed using two approaches. For the first approach, we included all potential candidate spawners in the population, regardless of whether they were assigned as parents to returning adult offspring. For the second approach, we included only those spawners that successfully produced returning adult progeny (i.e. those that will pass on their alleles to the next generation), removing those individuals that produced zero returning adult offspring.

We then evaluated fitness effects of SH fish mating with NOR fish in nature. RS of SH fish spawning in nature with NOR fish (i.e. SH female x NOR(N) male or NOR(N) female x SH male) was compared to NxN matings. This comparison of cross types allowed us to generate a separate RRS value for the effect of the female parent and the male parent having been reared in the hatchery. Additionally, we compared SHxSH to NxN matings following similar procedures. Jacks were not included in cross estimates.

For each return year and sex, we used ANOVAs to test the null hypothesis that the mean RS was equal for NOR vs. SH fish. We then tested for differences in RS for all four types of crosses SHxSH, SHxN, NxSH, and NxN (where female is listed first in each cross type). We also used delta-method based 95% confidence intervals to test for differences in RRS (Bowerman, Keefer, & Caudill, 2016; Ford, Murdoch, & Howard, 2012). All data was organized and analyzed using R scripts from Janowitz-Koch et al. (2019).

Results (study BYs 2007-2011)

After quality filtering genotype data, 47,447 samples remained in the dataset. Parentage analyses resulted in 22,889 offspring successfully assigning to parents. When including those naturally spawned individuals that contributed zero returning adult offspring (i.e. all potential candidate spawners), RRS amongst females was significantly lower than 1.0 in four out of five compared brood years (BYs 2007, 2008, 2010, and 2011; Table ii.2; Fig. ii.2). RRS amongst males was significantly lower than 1.0 in four out of five compared brood years (BYs 2008-2011) and in three out of five compared brood years for jacks (BYs 2009-2011; Table ii.2; Fig. ii.2).

When calculating RRS estimates from only those individuals that successfully reproduced (i.e. contributed returning adult offspring), RRS amongst females was significantly lower than 1.0 in four out of five compared brood years (BYs 2007, 2008, 2010, and 2011; Table ii.3; Fig. ii.2). RRS amongst males was significantly lower than 1.0 in four out of five compared brood years (BYs 2008-2011) and in one out of five compared brood years for jacks (BY 2011; Table ii.3; Fig. ii.1).

For RRS estimates of different cross types, we found significant differences between SHxN vs. NxN crosses for females in BYs 2008-2010 (Table ii.4; Fig. ii.3) and for males in BYs 2008-2010 (Table ii.5; Fig. ii.3). We also found significant differences between SHxSH vs. NxN crosses for females in BYs 2008-2010 (Table ii.4; Fig. ii.3) and for males in BYs 2008-2011 (Table ii.5; Fig. ii.3).

Discussion (study BYs 2007-2011)

We demonstrated that SH and NOR RS was significantly different for the majority of the brood years analyzed to date, with a trend of fewer returning adult offspring produced by SH compared to NOR fish (i.e. RRS < 1.0) (Tables ii.2-3; Fig. ii.2). We also showed that crosses involving either one or two successful SH parents demonstrated RS that was significantly lower than those crosses involving two successful NOR parents (Tables ii.4-5; Fig. ii.3). These results suggest that fitness decreases in the first generation for NOR fish when mating with SH fish. However, it is still unknown whether RRS is reduced for the natural-born progeny of SHxSH and SHxN relative to NxN crosses in the wild – a question we aim to answer with the inclusion of data from brood years 2012-2016.

While many studies have reported reduced genetic risks associated with integrated supplementation programs (Fast et al., 2015; M. A. Hess et al., 2012; Janowitz-Koch et al., 2019; Schroder et al., 2008; Waters et al., 2018; 2015), factors beyond, and not exactly separate from, origin-type are likely affecting the observed fitness differences between SH and NOR fish. For example, during the study years, SH fish returning to the watershed were smaller (by fork length) than their NOR counterparts in every year except females returning in 2009. Smaller size has been linked to lower fitness and fecundity in this system and others and could explain the apparently lower productivity amongst SH fish (Knudsen et al., 2008; 2006; Williamson, Murdoch, Pearsons, Ward, & Ford, 2010). Additionally, approximately 36-43% of the returns in 2007-2011 were released from the Clark Flat acclimation site (see Fig ii.1). Individuals acclimated at Clark Flat have been shown to preferentially spawn lower in the watershed (Dittman et al., 2010). These differences in spawning distribution between SH and NOR fish may also explain observed differences in fitness (Schroder et al., 2008; Williamson et al., 2010).

We therefore have designed GLM analyses that will incorporate phenotypic and environmental data to assess the predictive strength of specific variables in estimating individual reproductive success, thereby isolating potential confounding factors. We plan to weigh several variables in these GLM analyses, including size, age, return timing, density at spawning grounds and acclimation site. In addition, there is some thought that SH broodstock might display reduced fitness due to introgression with interior ocean-type lineages strays. For example, previous work has demonstrated 5% ocean-type ancestry amongst CESRF spring Chinook broodstock, suggesting the unintentional incorporation of ocean-type fish into spawning matrices (Shawn R. Narum, Hess, & Matala, 2010). Given the strong genetic differences between interior stream-type and ocean-type Chinook, this could potentially lead to outbreeding depression, lowering individual RS, and thereby RRS. Analyses to test this hypothesis are ongoing.

As mentioned above, the results conveyed here are simply a snapshot of reproductive dynamics and productivity in the Upper Yakima River. The additional data from brood years (BYs 2012-2016), alongside the GLM and introgression analyses, will provide a more comprehensive estimate of any differential reproductive success between SH and NOR fish. Only once these data are gathered and analyzed can we develop adaptive management recommendations for the integrated supplementation program at CESRF.

Table ii.2. *Relative reproductive success (RRS) for all spawners (including those that did not assign any adult offspring); *P<0.05.*

Collection Year	Sex	n	Integrated Hatchery-origin (SH)	Natural-origin	RRS	P-value
2007	Female	765	3.74468085	4.3033419	0.87017972	0.02417783*
2008	Female	1751	1.35624476	2.21684588	0.61179028	1.32E-11*
2009	Female	2421	0.81273408	0.88121547	0.92228758	0.13074457
2010	Female	4390	0.73807854	1.03641092	0.71214855	2.02E-12*
2011	Female	3906	0.84666986	1.24518966	0.67995253	6.44E-18*
2007	Male	564	2.55298013	2.76717557	0.9225942	0.39477927
2008	Male	1367	1.04244306	1.49127182	0.69902955	0.00017708*
2009	Male	1574	0.57226107	0.79748603	0.71758131	3.95E-05*
2010	Male	2200	0.82938389	1.26417704	0.65606625	3.76E-10*
2011	Male	2236	0.84564315	1.29970902	0.65064037	2.65E-12*
2007	Jack	924	0.83246073	1.01875	0.81713937	0.21815099
2008	Jack	1122	0.46703911	0.54185022	0.86193396	0.31583781
2009	Jack	2792	0.20138568	0.25039872	0.80426001	0.03744968*
2010	Jack	1436	0.41713748	0.59090909	0.70592496	0.0014783*
2011	Jack	2344	0.33934536	0.64580874	0.52545798	1.17E-13*

Table ii.3. *Relative reproductive success (RRS) for only those spawners that assigned returning adult offspring (i.e. successful spawners); *P<0.05.*

Collection Year	Sex	n	Integrated Hatchery-origin (SH)	Natural-origin	RRS	P-value
2007	Female	631	4.58631922	5.16666667	0.88767469	0.02157374*
2008	Female	841	3.02429907	4.04248366	0.74812895	5.92E-08*
2009	Female	1197	1.70062696	1.71198569	0.99336517	0.84582673
2010	Female	1827	1.93652254	2.15405405	0.89901297	0.00106617*
2011	Female	1948	1.93537788	2.1884058	0.88437797	9.75E-05*
2007	Male	402	3.60280374	3.85638298	0.93424428	0.38394461
2008	Male	606	2.43825666	3.0984456	0.78692899	0.00096034*
2009	Male	639	1.50152905	1.83012821	0.8204502	0.00020179*
2010	Male	997	1.96945338	2.43733333	0.80803612	1.17E-05*
2011	Male	1096	2.038	2.24832215	0.90645373	0.0270681*
2007	Jack	346	2.31272727	2.29577465	1.00738427	0.95349411
2008	Jack	317	1.70612245	1.70833333	0.99870582	0.98918659
2009	Jack	473	1.24216524	1.28688525	0.96524942	0.42778081
2010	Jack	424	1.50680272	1.7	0.88635454	0.07731344
2011	Jack	669	1.45142857	1.71473354	0.84644555	0.00528887*

Table ii.4. *Relative reproductive success (RRS) for female crosses. RRS and associated P-values are scaled to NxN crosses; SHxSH, integrate hatchery-origin x integrated hatchery-origin; SHxN, integrated hatchery-origin x natural-origin; NxN, natural-origin x natural-origin; *P<0.05.*

Collection Year	SHxSH n	SHxN n	NxN n	SHxSH RRS	SHxN RRS	P-value
2007	157	310	154	0.93956201	0.96563972	0.70915218
2008	326	257	135	0.71629912	0.65164581	3.91E-08*
2009	234	318	225	0.83953056	0.85290429	4.27E-05*
2010	529	602	262	0.86302935	0.84842097	0.00021427*
2011	317	720	485	0.93986842	1.00144148	0.2792273

Table ii.5. *Relative reproductive success (RRS) for male crosses. RRS and associated P-values are scaled to NxN crosses; SHxSH, integrate hatchery-origin x integrated hatchery-origin; SHxN, integrated hatchery-origin x natural-origin; NxN, natural-origin x natural-origin; *P<0.05.*

Collection Year	SHxSH n	SHxN n	NxN n	SHxSH RRS	SHxN RRS	P-value
2007	137	271	123	0.85998114	0.88225051	0.25775724
2008	306	234	115	0.65006183	0.60966736	2.22E-08*
2009	221	284	196	0.77434348	0.83192205	0.00014749*
2010	451	520	210	0.81137702	0.7872679	3.67E-05*
2011	285	632	395	0.85140622	0.92917251	0.01288345*

Figure ii.2. *Relative reproductive success (RRS) for all spawners (top) and successful spawners (bottom). Error bars represent 95% confidence intervals; dotted line is reference point for equal RRS between hatchery- and natural-origin spawners.*

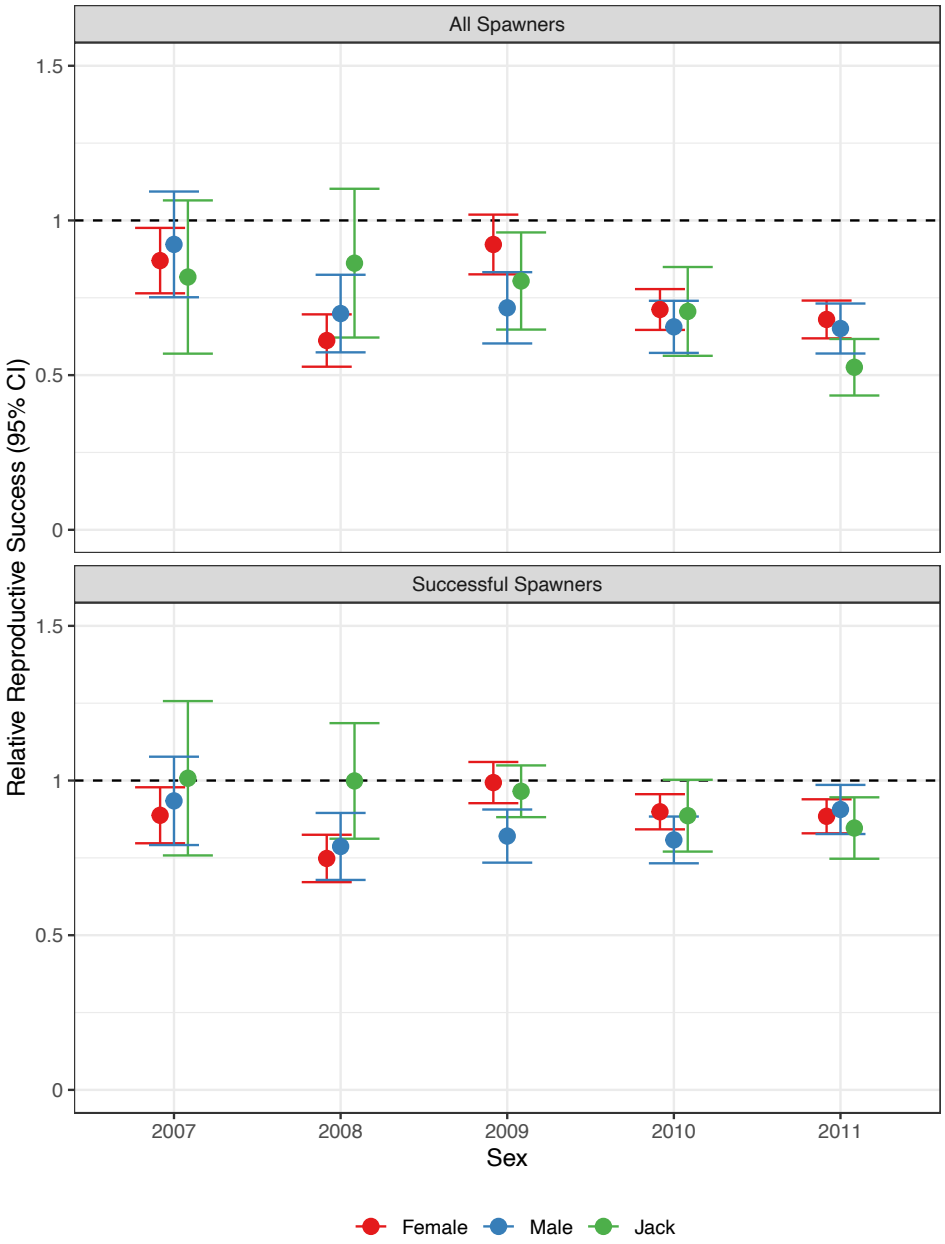
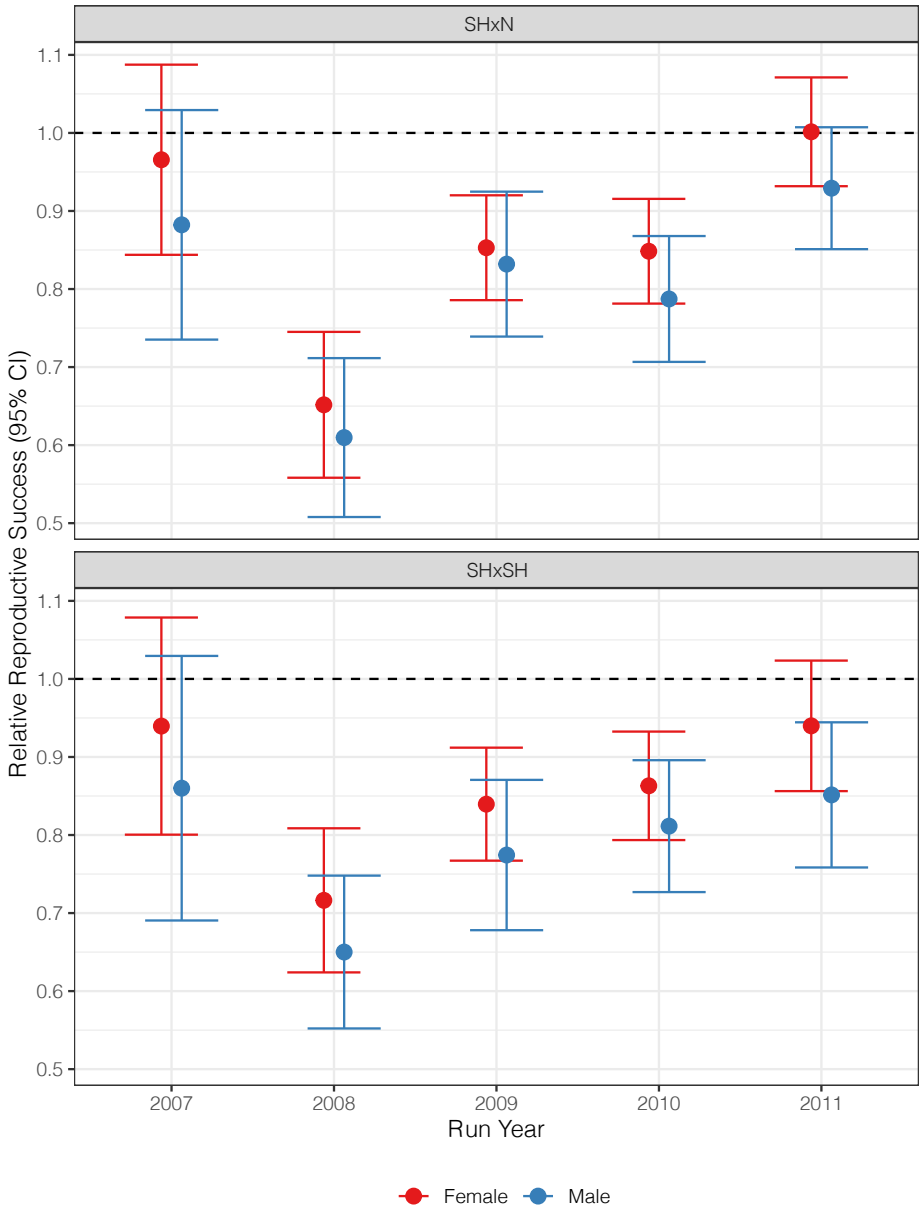


Figure ii.3. *Relative reproductive success (RRS) between SHxN (top) and SHxSH (bottom) compared to NxN crosses. Error bars represent 95% confidence intervals; dotted line is reference point for equal RRS; SHxSH, hatchery-origin x hatchery-origin; SHxN, hatchery-origin x natural-origin; NxN, natural-origin x natural-origin*



iii. Project Objective #2 – Estimate individual productivity and compare reproductive success between HOR and NOR spring Chinook in Lookingglass Creek, OR

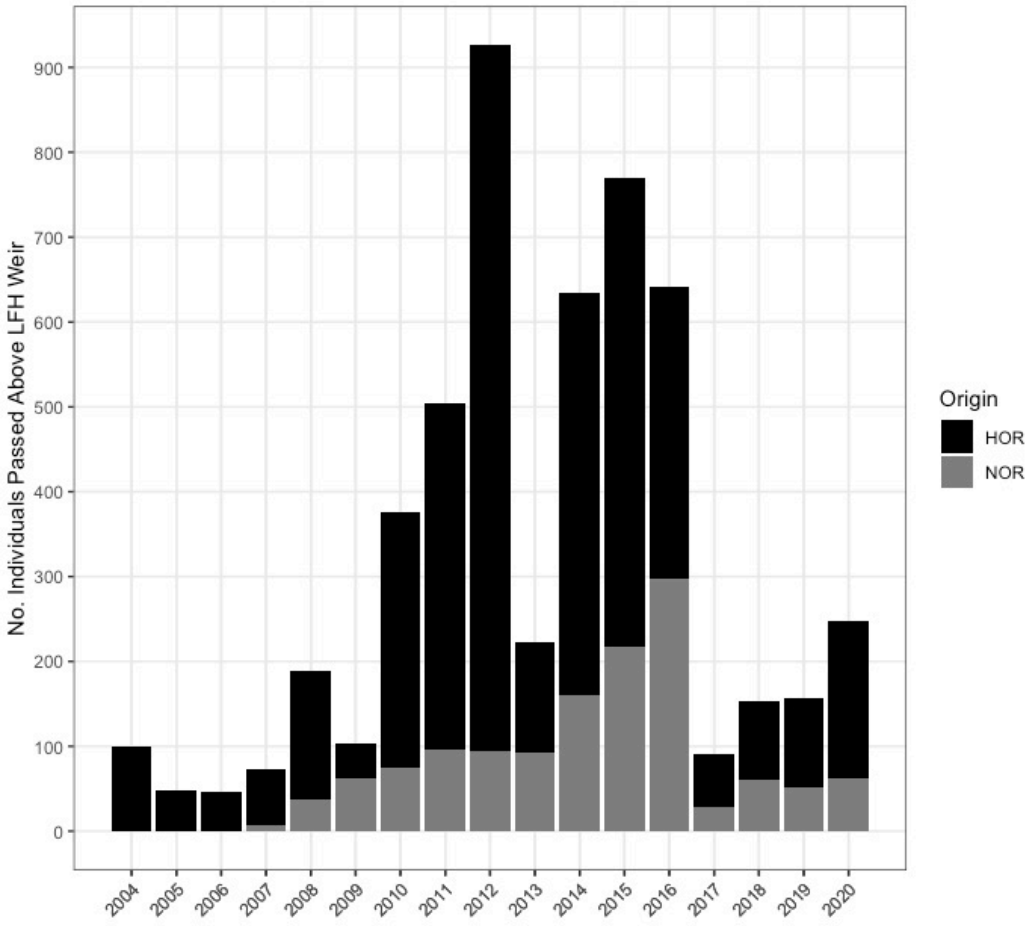
Introduction

Spring Chinook salmon populations within the Grande Ronde subbasin experienced dramatic declines in abundance beginning in the mid-1970s. Like many salmon and steelhead populations throughout the Columbia River basin, these declines are largely a product of habitat loss and degradation, paired with increased migration mortality through corridors disrupted by hydroelectric development. To mitigate for these losses in the Grande Ronde subbasin, Lookingglass Fish Hatchery (LFH) was constructed in 1982 under the Lower Snake River Compensation Plan (LSRCP; US CoE 1975; Herrig 1990; Marshall 2010). With a production goal of 1.4 million smolts, LFH was designed to serve as a central spring Chinook salmon production facility in the Grande Ronde subbasin.

To meet production goals, hatchery managers initially used remaining adults in the native population for broodstock, as well as out-of-basin stocks. The removal of the native population alongside incorporation of out-of-basin fish in spawning effectively rendered especially vulnerable populations, such as that in Lookingglass Creek, functionally extinct (McClure et al., 2008; ICTRT 2003; Boe et al. 2010; ODFW 2011; Carmichael et al. 2011) Then in 1992, NOAA listed spring Chinook populations within the Grande Ronde River systems under the Endangered Species Act (NOAA 1992; 57 FR 14653). This listing prompted LFH to change their supplementation practices and use exclusively in-basin fish as broodstock. Captive broodstock programs were therefore initiated using juveniles captured from Catherine Creek, the Lostine River and the Imnaha River.

Unfortunately, the indigenous Lookingglass Creek spring Chinook population had been effectively extirpated by the time the captive broodstock program was initiated, with out-of-basin fish dominating spawning in the upstream reaches above the hatchery weir. Therefore, starting in 2001, managers decided to use juveniles from the Catherine Creek captive broodstock program to re-introduce a within-basin population to Lookingglass Creek. Before reintroduction could take place, however, managers had to first eliminate out-of-basin production in the upstream watershed. Therefore, from 1998-2003, no adult spring Chinook returning to Lookingglass Creek were allowed passage above the weir (McLean & Lofy, 2000; Crump, Naylor, VanSickle, & Startzel-Holt, 2016). Then, in 2004, the adults from the first brood year of captively-reared Catherine Creek juveniles that had been acclimated within and released from Lookingglass Creek returned, and the reintroduction effort was officially underway (Boe, Weldert, & Crump, 2007; Crump, Naylor, VanSickle, & Startzel-Holt, 2016). A portion of the adults returning in 2004 were permitted passage upstream, while others were collected for broodstock and spawning at LFH. In 2008, the first age-4 NOR adults returned to the Lookingglass weir, and each year since, a mixture of NOR and HOR adults have been passed upstream of the weir (Figure iii.1; Boe et al., 2010).

Figure iii.1. *The number and origin (hatchery-origin, HOR; natural-origin, NOR) of adult spring Chinook salmon passed upstream of the Lookingglass Fish Hatchery (LFH) weir as part of the species' reintroduction into Lookingglass Creek. The reintroduction effort was initiated in 2004 with returning HOR adults derived from the Catherine Creek captive broodstock program. The first NOR offspring of these adults returning in 2004 are represented by the age-3 NOR returns in 2007. Data derived from CTUIR RM&E efforts.*



A review of captive breeding programs for salmonids (D. J. Fraser, 2008), found little evidence to neither affirm nor deny that captive-bred lines of salmon can successfully reintroduce self-sustaining salmon populations. Assessment can be further conflated by continued hatchery supplementation, which makes it difficult to attribute increases in population abundance or stability to actual adaptation to the environment by the reintroduced stock or simply to continued stocking with hatchery-reared juveniles. The program at Lookingglass Creek provides an opportunity to address this uncertainty. Since the reintroduction effort was initiated, biologists from the Confederated Tribes of the Umatilla Indian Reservation (CTUIR) have conducted a systematic monitoring program. This has included tissue sampling and collection of biodata on all adult spring Chinook salmon intercepted at the hatchery weir, a sample of juvenile outmigrants collected at the rotary screw trap ~0.25 km downstream of the weir, and carcasses recovered during spawning ground surveys. The collection of these data, alongside increasingly affordable genetic techniques, has allowed the BSE Project to support a study comparing

productivity between naturally spawning NOR and HOR fish. If adaptation is occurring, then one would expect the NOR fish, which have experienced at least one generation of selective forces in the natural environment, to produce more offspring on average. This might, therefore, suggest that a self-sustaining population could eventually arise from a reintroduction effort using captive broodstock.

This study focuses on naturally-spawning NOR and HOR adults returning to the Lookingglass weir from brood years 2008-2016. Reproductive success is evaluated for each individual by estimating recruits-per-spawner, where recruits include both juvenile and adult progeny. The last adult progeny of interest will return in 2021 (age-5 adults from BY2016), and as such, no conclusions can be drawn at this time. **All data presented below should be treated as preliminary. Upon study conclusion, all report sections will be updated accordingly, and results will be summarized into a manuscript for publication (anticipated 2022).**

Methods

Sample Collection and Genotyping

Approximately 12,000 samples have been genotyped and analyzed to date, including NOR and HOR adults intercepted at the LFH weir or collected during spawning ground surveys from 2008 to 2019, as well as juveniles sampled at the rotary screw trap from 2009 to 2018 (Table iii.1). DNA was extracted from fin tissue using two separate methods including a standard Qiagen DNeasy protocol (Qiagen Inc., Valencia, CA) and a Chelex 100 method (Sigma-Alrich, St Louis, MO). Genotyping methods and marker development at the Hagerman Genetics Lab within the jointly UI-CRITFC operated Hagerman Fish Culture Experiment Station (HFCES) have evolved over the course of this study. Most significantly, the standard single nucleotide polymorphism (SNP) panel at which spring Chinook salmon are genotyped has expanded from 95 markers in 2008 to 298 markers today (SNP markers, Janowitz-Koch et al., 2019). As a result, a reduced panel of 90 SNP markers, which were identified as shared and informative across study years, were used in parentage analyses. While additional SNP markers certainly lends more power to reliably estimate pedigree relationships, it has been shown that these relationships can be accurately reconstructed with as few as 60 SNPs, even amongst thousands of individuals (Anderson & Garza, 2006). Currently, all individuals are genotyped using the genotyping-in-thousands by sequencing approach (GTseq, N. R. Campbell et al., 2015). Individual genotypes were quality filtered for missing data, with a minimum threshold of 95% genotyping success. Additionally, pairwise comparisons were performed between all individual genotypes to identify potential duplicate samples. If a duplicate sample was recovered, only one representative was retained for genetic stock identification and parentage analyses.

Table iii.1. *Sample numbers genotyped and analyzed to date by sample year, life stage and origin.*

Sample Year	Life Stage	Natural-Origin (NOR)	Hatchery-Origin (HOR)	Unknown Origin	Total
2008	Adult	44	288	15	347
2009	Adult	128	63	7	198
2010	Adult	133	403	0	536
2011	Adult	169	608	32	809
2012	Adult	147	965	0	1112
2013	Adult	120	241	5	366
2014	Adult	199	562	6	767
2015	Adult	419	1043	12	1474
2016	Adult	333	336	0	669
2017	Adult	43	77	0	120
2018	Adult	74	26	1	101
2019	Adult	60	103	0	163
2009	Juvenile	246	0	0	246
2010	Juvenile	540	0	0	540
2011	Juvenile	496	0	0	496
2012	Juvenile	26	1	517	544
2013	Juvenile	115	6	596	717
2014	Juvenile	355	0	2	357
2015	Juvenile	735	2	38	775
2016	Juvenile	840	3	0	843
2017	Juvenile	3	0	522	525
2018	Juvenile	7	12	283	302
Totals		*5232*	*4739*	*2036*	*12007*

Parentage Analysis

After quality filtering individual genotype data, individuals were run through the programs SNPPIT (Anderson, 2010) and COLONY v2.0.6.6 (Jones & Wang, 2010), both of which use likelihood-based methods to assign parentage. While SNNPIT can efficiently process data from thousands of individuals and hundreds of genetic markers, it was optimized for systems in which both parents are sampled, and as such, can only reliably reconstruct parent-offspring trios. Therefore, samples were first analyzed in SNPPIT to identify parent-offspring trios, and then in COLONY v.2.0.6.6 to recover additional single-parent offspring pairs.

For both parentage analysis software, samples were divided into collections by life stage and collection year. For example, all adults collected in 2012 were analyzed as progeny against a pool of biologically plausible parents, which included adults returning in 2006-2010. Each SNPPIT run was instructed to consider parent reproductive year, such that only individuals from the same return year could be identified as parents of any given offspring. We did not supply parental sex data to SNPPIT due to significant inconsistency between genetic sex markers and field identified sex, particularly in the earliest brood years. Instead, we used the resulting trios

that passed filter, alongside genetic and field sex data to resolve individual sex identification wherever possible. All trios were filtered for False Discovery Rate score ≤0.01, MaxP.Pr.Relat = "C_Se_Se," and no more than two Mendelian Incompatible Loci.

The same collections that were run through SNPPIT, were then analyzed in COLONY v2.0.6.6. However, COLONY requires that you supply, at least, a candidate offspring group, and ideally a separate candidate dam and candidate sire group to reduce computational complexity. We, therefore, identified each potential parent as male, female or unknown as follows: 1) if an individual was identified as a parent in a SNPPIT trio passing filter, we used the sex inferred from this assignment, then 2) if an individual was not assigned as a parent, we used the genetic sex data from the most recently developed genetic sex marker, then 3) if the individual was not assigned as a parent and had no genetic sex information, we used the field identified sex, and 4) finally, if none of these data were available, the sex was left unknown. We also used the parent-offspring trios passing filter to construct known paternity and maternity matrices for each collection run in COLONY. Run parameters for each collection were as follows: both parents polygamous, medium run length and precision, and full-likelihood analysis method. Additionally, we identified probability of either dam or sire sampled as 0.90 in accordance with weir efficiency data throughout study years. All resulting single-parent offspring pairs were filtered for Probability ≥0.90.

Lastly, all filtered trio and single-parent assignments were then combined to metadata and subsequently reviewed to ensure plausibility. Assignments where the parentage strongly conflicted with recorded metadata were dropped from the analysis, which amounted to a relatively small proportion of total assignments (~0.54% of assignments). Such non-plausible assignments included those where the inferred age of the progeny given the return year of the parents does not align with recorded life stage and/or fork length, instances of precocious female spawning, and trios where the parents having non-matching dispositions. Final assignment success rates for collections genotyped to date can be seen in Table iii.2.

Relative Reproductive Success Analysis (ongoing)

From the finally processed assignments, we will estimate the number of progeny attributed to each individual, thereby inferring lifetime reproductive success (RS) for each naturally spawning fish from BYs 2008 to 2016. We will then compare RS between NOR and HOR adults amongst adult females (age-4 and age-5) and, separately, adults males (age-4 and age-5). Relative reproductive success (RRS) will be calculated by dividing the average RS amongst NOR fish by the average RS amongst HOR fish within each brood year. RRS estimates will be analyzed using two approaches. For the first approach, we will include all potential candidate spawners in the population, regardless of whether they were assigned as parents. For the second approach, we will include only those spawners that successfully produced progeny (i.e. those that will pass on their alleles to the next generation), removing those individuals that produced zero offspring.

Additionally, we intend to evaluate fitness effects of HOR fish mating with NOR fish in nature. Average RS of HOR fish spawning with NOR fish (i.e. HOR female x NOR male or NOR female x HOR male) will be compared to NOR x NOR matings, as well as HOR x HOR matings compared to NOR x NOR matings. This comparison of cross types will allow us to generate a separate RRS value for the effect of the female parent and/or the male parent having been reared in the hatchery. Lastly, the role of factors beyond origin, such as size, spawning timing, etc., in predicting reproductive success will be investigated using generalized linear models. The structure of the model will be finalized once all parentage analyses are complete.

Results

Analyses are still ongoing and not all samples relevant to this study have yet to be genotyped or collected (i.e. all adult returns in 2020 are still being processed by CTUIR field biologists, and the age-5 adults from BY2016 will return in the Spring/Summer of 2021). Therefore, at this time, we only present the proportion of individuals assigned at least one offspring within each brood year for which all potential offspring have been totally sampled and genotyped (Table iii.3), as well as family size distributions (i.e. recruits-per-spawner) (Figure iii.2).

Discussion

As described in previous sections, sample collection and analysis are ongoing for this study. Therefore, no interpretations or conclusions can be drawn at this time. We anticipate all samples and genetic data to be generated by late-2021, and aim to have a comprehensive manuscript for publication drafted by early-to-mid 2022.

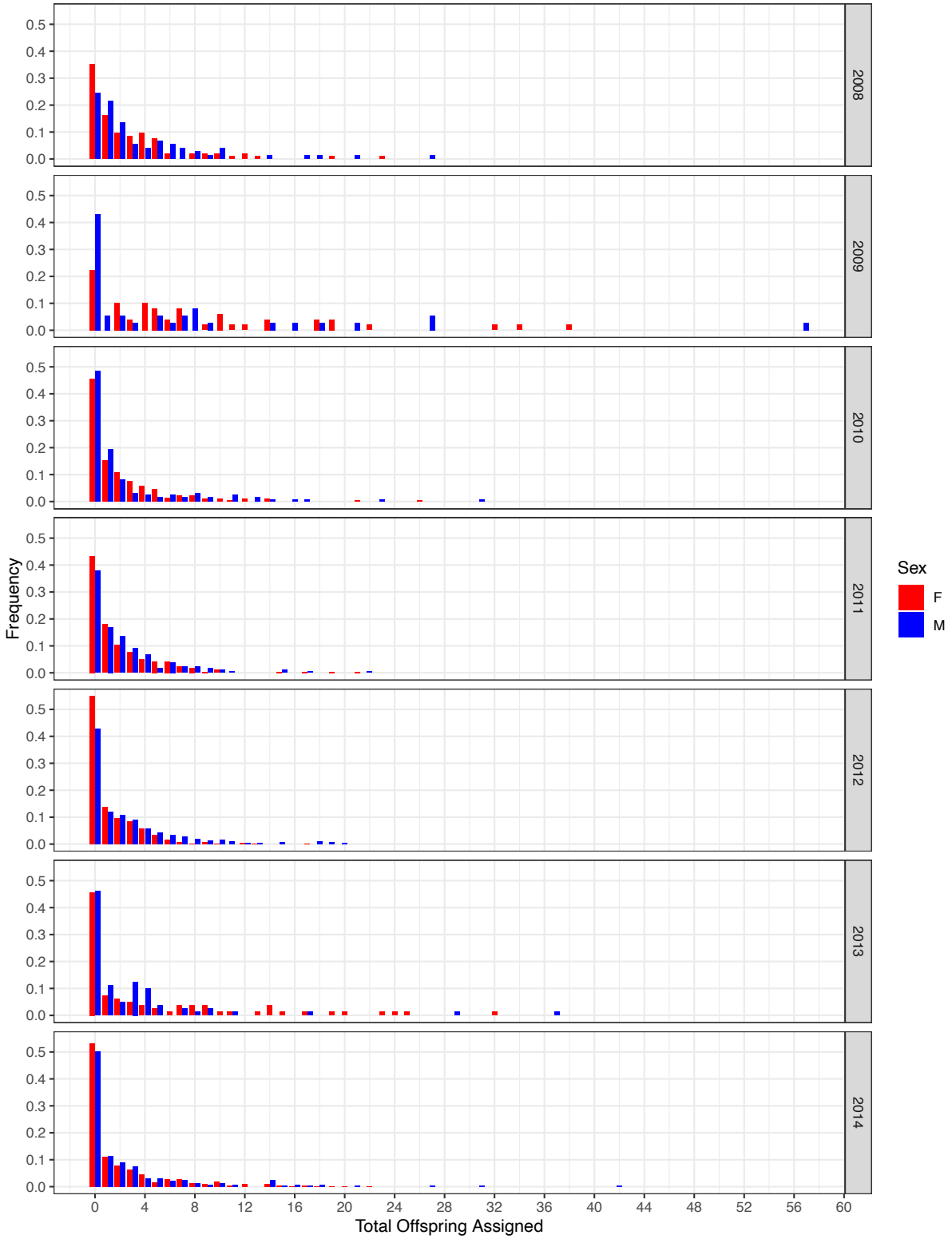
Table iii.2. Assignment success by life stage and sample year. Reflects the proportion of individuals that were assigned at least one parent by origin, and when summing across origins. Adults collected in 2012 and juveniles collected in 2009 were the first cohorts to be run as offspring for their respective life stages, given that the first brood year of interest was 2008.

Life Stage	Sample Year	No. HOR analyzed	No. HOR assigned parent(s)	% HOR assigned parent(s)	No. NOR or Unk analyzed	No. NOR or Unk assigned parent(s)	% NOR or Unk assigned parent(s)	Total No. analyzed	Total No. assigned parent(s)	% Total assigned parent(s)
Adult	2012	965	910	94.3%	147	69	46.9%	1112	979	88.0%
Adult	2013	241	209	86.7%	125	63	50.4%	366	272	74.3%
Adult	2014	562	532	94.7%	205	105	51.2%	767	637	83.1%
Adult	2015	1043	603	57.8%	431	179	41.5%	1474	782	53.1%
Adult	2016	336	288	85.7%	333	256	76.9%	669	544	81.3%
Adult	2017	77	55	71.4%	43	20	46.5%	120	75	62.5%
Adult	2018	26	22	84.6%	75	52	69.3%	101	74	73.3%
Adult	2019	103	97	94.2%	60	45	75.0%	163	142	87.1%
mean:				*83.7%*			*57.2%*			*75.3%*
Juvenile	2009	0	0	N/A	246	175	71.1%	246	175	71.1%
Juvenile	2010	0	0	N/A	540	335	62.0%	540	335	62.0%
Juvenile	2011	0	0	N/A	496	343	69.2%	496	343	69.2%
Juvenile	2012	1	1	100.0%	543	477	87.8%	544	478	87.9%
Juvenile	2013	6	2	33.3%	711	547	76.9%	717	549	76.6%
Juvenile	2014	0	0	N/A	357	285	79.8%	357	285	79.8%
Juvenile	2015	2	1	50.0%	773	663	85.8%	775	664	85.7%
Juvenile	2016	3	2	66.7%	840	752	89.5%	843	754	89.4%
Juvenile	2017	0	0	N/A	525	436	83.0%	525	436	83.0%
Juvenile	2018	12	4	33.3%	290	215	74.1%	302	219	72.5%
mean:				*56.7%*			*77.9%*			*77.7%*

Table iii.3. *Proportion of naturally spawning adults from BY2008-2014 that were assigned ≥ 1 offspring, separated by sex. Brood years 2015 and 2016 are not presented here as data collection and analysis is ongoing.*

Brood Year	Sex	No. Fish Sampled	No. Assigned ≥1 offspring	% Reproductively Successful (RS)
2008	F	105	68	64.8%
2009	F	52	39	75.0%
2010	F	225	122	54.2%
2011	F	288	156	54.2%
2012	F	625	282	45.1%
2013	F	81	44	54.3%
2014	F	362	169	46.7%
			mean:	*56.3%*
2008	M	79	61	77.2%
2009	M	61	37	60.7%
2010	M	152	76	50.0%
2011	M	264	142	53.8%
2012	M	313	179	57.2%
2013	M	142	64	45.1%
2014	M	266	132	49.6%
			mean:	*56.2%*

Figure iii.2. *Family size distribution by sex and within brood year. Brood years 2015 and 2016 are not presented here as data collection and analyses are ongoing. Across all brood years, the distribution is biased to the left, with most individuals being assigned zero offspring. The number of offspring assigned to each individual is the sum of all adult and juvenile offspring.*



iv. Project Objective #3 – Perform genetic stock identification, productivity analyses and related assessments of tribally-managed sockeye salmon reintroduction efforts in Yakima River and Deschutes River basins

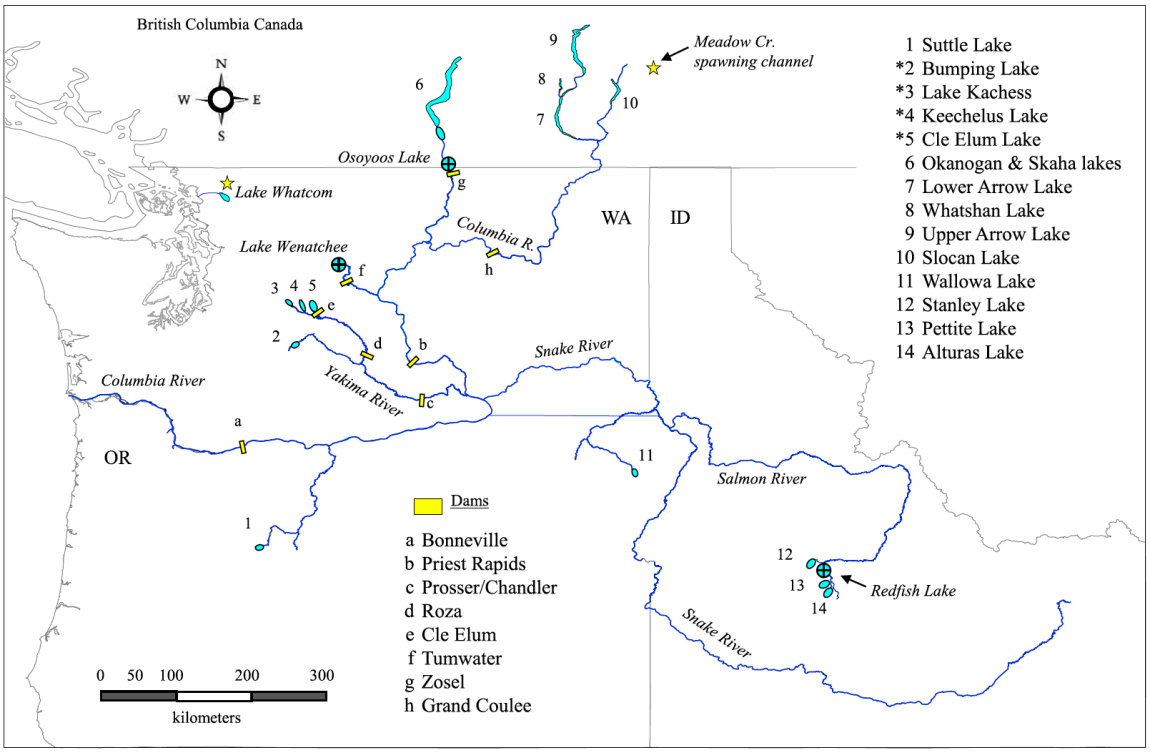
a. Cle Elum Lake Reintroduction Effort

Introduction

Sockeye salmon (*Oncorhynchus nerka*) is one of five Pacific salmon species, whose geographic range extends along the Pacific Coast from Alaska to the Columbia River basin, and which are widely recognized for their anadromous life history – requiring both freshwater and saltwater habitat to complete their life cycle. Unlike most other anadromous Pacific salmonids, however, sockeye salmon generally utilize lake habitat for spawning and rearing (Beacham & Withler, 2017). In the Columbia River basin, dams along the migration corridor, including those that have blocked access to natal rearing lakes, have significantly depleted and even extirpated native sockeye salmon populations. Currently, only three native sockeye populations remain in the Columbia River basin: Lake Wenatchee, Osoyoos Lake and Redfish Lake (Figure iv.a.1).

Cle Elum Lake, in the upper Yakima River basin, once supported an abundant native population of sockeye salmon. The total number of spawners historically returning to the Yakima River basin to rear and spawn in Cle Elum Lake, as well as three other nursery lakes in the basin, has been estimated at 150,000-200,000 spawners (CSBP, 1990; BOR, 2011). However, the construction of an impassable timber crib-dam at the lake outlet in the early 1900s, followed by its replacement with a 40-m tall earthen dam in 1933, resulted in the extirpation of sockeye salmon from the Yakima River basin. Sockeye salmon remained absent from the Yakima River basin until 2009, when the Yakama Nation (YN) began implementing a reintroduction program to Cle Elum Lake.

Figure iv.a.1. *From Matala et al., 2019. Historical and current sockeye populations within the Columbia River basin. The extent populations are marked by a ⊕ symbol, and include Redfish Lake, Lake Wenatchee and Osoyoos Lake. The extirpated lake systems are all numbered, with the four nursery lakes in the Yakima River basin numbered 2-5. Dams of relevance are marked by letters and kokanee hatcheries are highlighted with stars.*



The reintroduction program was designed in accordance with the results of several preceding feasibility studies, which sought to review the suitability of habitat in Cle Elum Lake after nearly 100 years without any anadromous fish species. These studies reviewed fish passage capabilities, carrying capacity and productivity potential within Cle Elum Lake (Flagg et al., 1988, 2000; BOR, 2007, 2011). After synthesizing these data, the Yakama Nation decided to initiate the reintroduction program by using Lake Wenatchee (WEN) and Osoyoos Lake (OSO) sockeye as "donor stocks," with the expectation that using two stocks would increase genetic diversity and life history variability, thereby maximizing adaptive potential (Braun & Reynolds, 2014; Burger, Scribner, Spearmen, Swanton, & Campton, 2000; Dylan J. Fraser, Jones, McParland, & Hutchings, 2007)

Every year since 2009, excluding 2019 (Table iv.a.1), a number of WEN and OSO-origin sockeye intercepted at Priest Rapids Dam (PRD) during their spawning migration are translocated to Cle Elum Lake. The number of translocated adults is dictated by the estimated total escapement to Bonneville Dam, ranging from 1,000 fish translocated once escapement to Bonneville reaches 80,000, and up to 10,000 fish translocated once escapement to Bonneville exceeds 300,000 fish. Lastly, any adults returning directly to the Yakima River are intercepted at the Roza Adult Monitoring Facility (RAMF), adjacent to the Roza Irrigation Dam (rkm 206), and are transported to Cle Elum Lake. The number of adults translocated from Priest Rapids Dam, as well as the number returning directly to the Yakima River, from 2009 to 2020 is represented in Table iv.a.1.

Table iv.a.1. *Escapement of sockeye salmon to Bonneville Dam, number of adults translocated from Priest Rapids Dam (PRD) to Cle Elum Lake, and escapement to the Yakima River basin via the Roza Adult Monitoring Facility (RAMF) from 2009 to 2020. Escapement data from DART (Columbia Basin Research, University of Washington).*

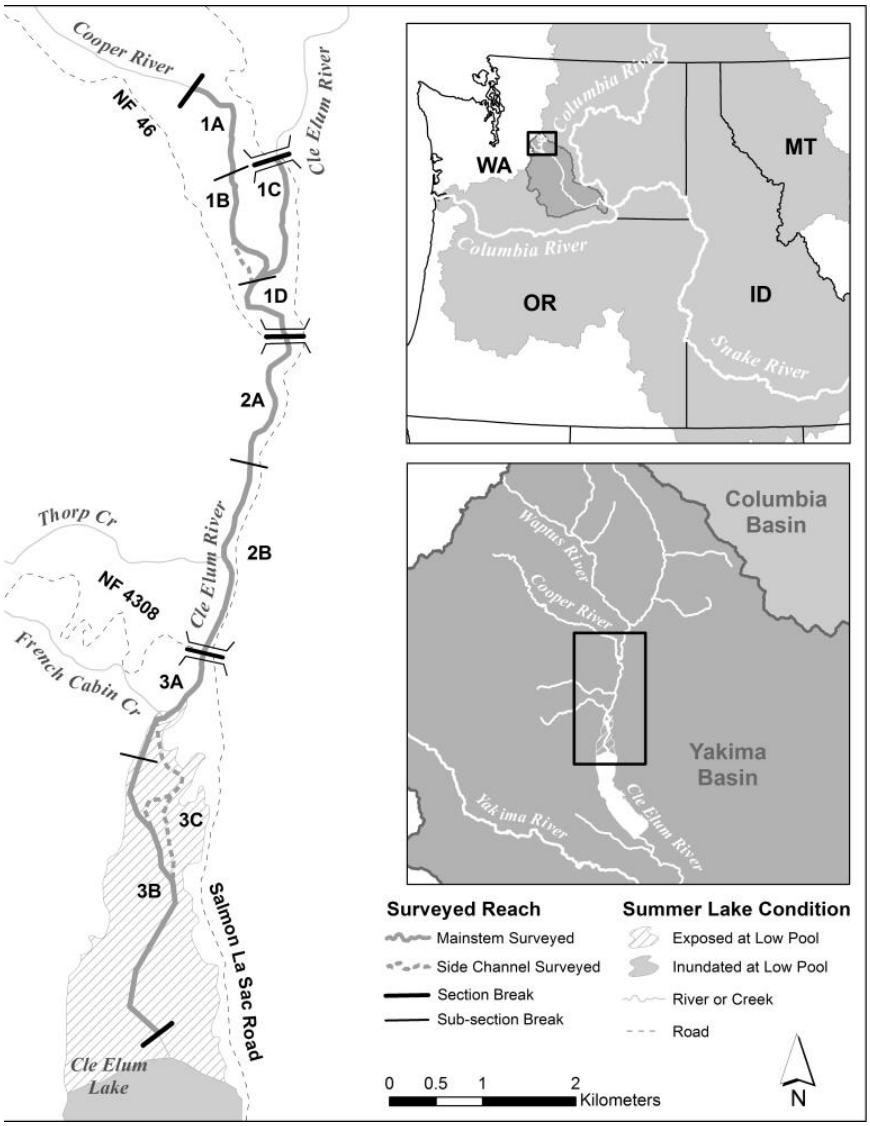
Brood Year	Bonneville Dam Count	No. Translocated from PRD	No. Intercepted at the RAMF
2009	177,823	1,000	--
2010	386,525	2,500	--
2011	185,796	4,000	--
2012	515,673	10,000	--
2013	185,505	4,500	691
2014	614,179	10,000	2576
2015	510,706	10,000	95*
2016	342,498	10,000	3949
2017	87,693	1000	137*
2018	193,816	4600	201*
2019	63,046	0**	201
2020	341,739	10,000	4,379

* Exceptionally high summer temperatures in 2015, 2017 and 2018 delayed upstream migration and increased mortality, dramatically reducing expected returns to the RAMF

**The sockeye salmon run to Bonneville Dam did not reach the minimum threshold of 80,000 required for translocation of fish from PRD to Cle Elum Lake

The Basinwide Supplementation Evaluation Project has supported genetic monitoring of the reintroduction program since 2011, which includes genotyping and analyses of tissue samples collected from: 1) a proportion of the adults translocated from PRD, 2) all adults returning to the RAMF, 3) carcasses encountered during spawning ground surveys (Figure iv.a.2), 4) sockeye caught as bycatch during non-native Lake Trout removal efforts via gillnetting, and 5) a sample of out-migrating juveniles. All samples are identified to genetic stock-of-origin to monitor for differential productivity, population demographics and inter-breeding between the two donor stocks. These results are shared with managers to inform and adapt protocols within the program as needed. Additionally, the data from these monitoring efforts will inform planned or proposed reintroduction programs elsewhere in the basin.

Figure iv.a.2. *Map of spawning ground surveys, with the numbers representing the three main reaches surveyed.*



Methods

Sample Collection and Genotyping

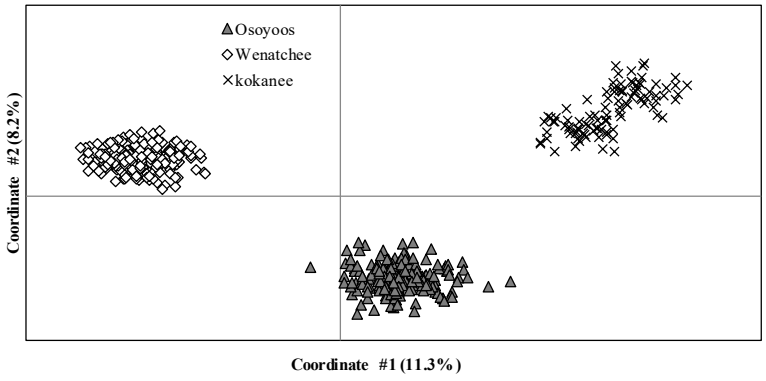
Approximately 21,000 samples have been genotyped to date. DNA has been extracted from fin tissues using either a standard Qiagen DNeasy protocol (Qiagen Inc., Valencia, CA), or more recently, a Chelex 100 method (Sigma-Alrich, St Louis, MO). Currently, all individuals are genotyped at a panel of 364 SNP markers using the genotyping-in-thousands by sequencing approach (GTseq, N. R. Campbell et al., 2015; SNP markers, Matala et al., 2019). Individual genotypes were quality filtered for missing data, with a minimum threshold of 90% genotyping success. Additionally, pairwise comparisons were performed between all individual genotypes to identify potential duplicate samples. If a duplicate sample was recovered, only one representative was retained for genetic stock identification and parentage analyses.

Genetic Stock Identification and Parentage Analysis
All individuals passing quality filters were then identified to stock-of-origin using genetic stock identification (GSI) methods implemented in GENECLASS2 (Piry et al., 2004) and STRUCTURE v.2.3.4 (Pritchard, Stephens, & Donnelly, 2000). In STRUCTURE, individuals with a *Q* value between 0.20 and 0.80 and a corresponding Likelihood Score (LS) < 0.95 were deemed putative hybrids.

Parentage analysis were performed using CERVUS v.3.0 (Kalinowski, Taper, & Marshall, 2007; Marshall, Slate, Kruuk, & Pemberton, 1998). All assignments were filtered in accordance with the confidence criteria and log-likelihood statistic thresholds determined from simulations. Assignments exceeding these thresholds were then filtered by Mendelian Incompatibilities, such that accepted single parent assignments had zero incompatibilities, while parent pair assignments were allowed two or fewer incompatibilities. From these filtered assignments, we calculated the inferred age of the offspring at time of sampling (i.e. offspring collection year – parent spawn year).

Results
First, in order to ensure accurate and reliable identification of individuals to stock-of-origin, we verified the power of the *O. nerka* genetic baseline to differentiate between stocks. A multivariate principal coordinates analysis plot from a pairwise F_{ST} matrix (GenAlEx v.6.2; Peakall & Smouse, 2006) displayed significant divergence between the WEN and OSO donor stocks, as well as relevant kokanee populations (Figure iv.a.3). This confirmed genetic differentiation between the WEN and OSO donor stocks, and thereby, the power to accurately identify stock-of-origin.

Figure iv.a.3. *From Matala et al., 2019. Principle Coordinates Analysis plot for GSI baseline populations used in assigning origin of fish sampled under the Cle Elum Lake reintroduction project. Distinct clustering represents genetic distance between populations, which allows for accurate stock-of-origin assignment amongst unknown origin individuals.*



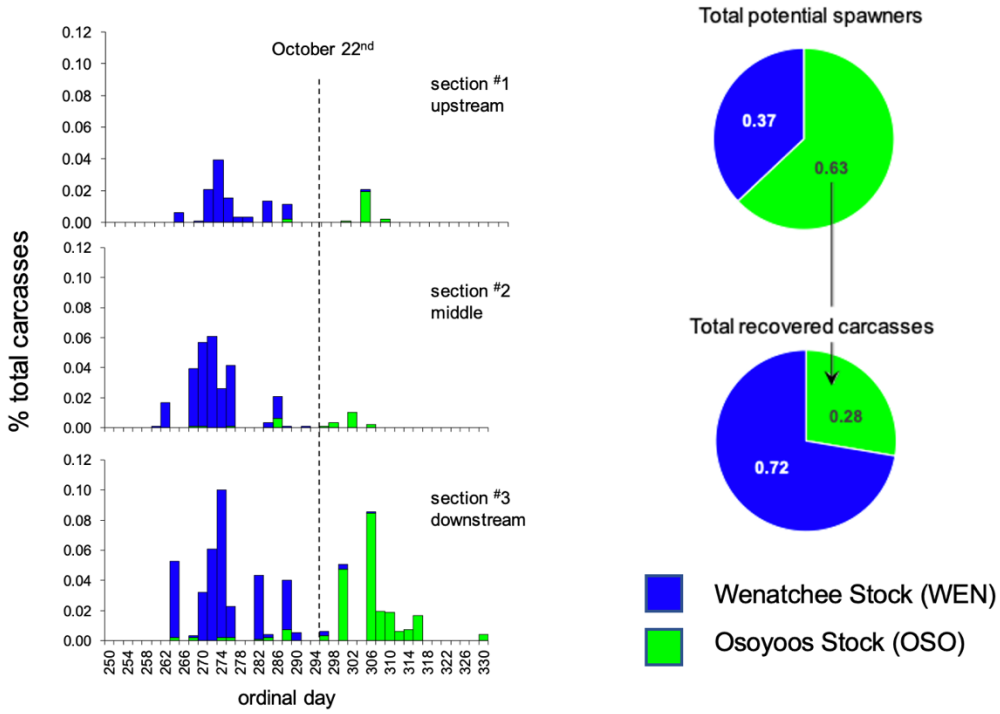
Several trends in productivity and demography by origin have been consistently observed across sampling years. First, GSI analyses indicate the majority of adults translocated from PRD each year are from the OSO population (Table iv.a.2).

Table iv.a.2. *Table reproduced from data in Matala et al., 2019. Number of Osoyoos Lake (OSO) and Lake Wenatchee (WEN) fish translocated from PRD to Cle Elum Lake during brood years 2011-2017. Inferred from GSI analysis. Across years, the majority of outplants are OSO origin, with an average of 70% OSO origin across years.*

Brood Year	OSO origin	WEN origin	Total	Proportion OSO origin	Proportion WEN origin
2011	3000	1000	4000	0.75	0.25
2012	8400	1600	10000	0.84	0.16
2013	4185	315	4500	0.93	0.07
2014	6600	3400	10000	0.66	0.34
2015	5900	4100	10000	0.59	0.41
2016	6200	3800	10000	0.62	0.38
2017	510	490	1000	0.51	0.49
			mean:	*0.70*	*0.30*

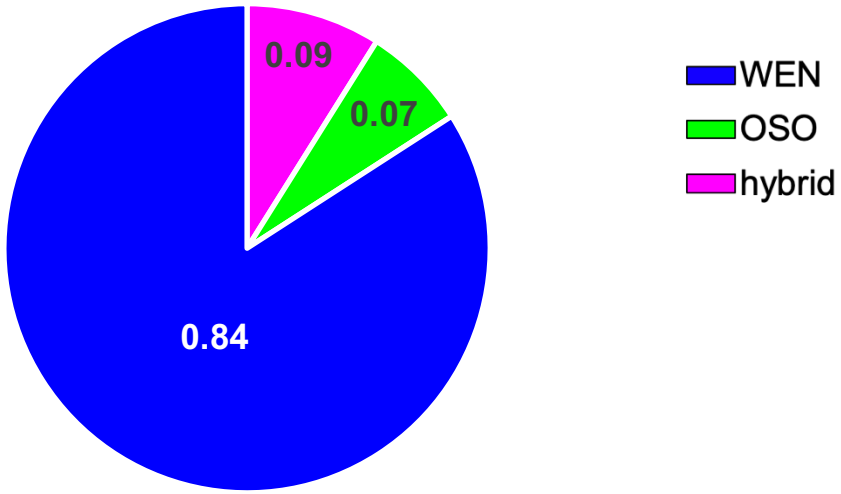
Despite the higher number of OSO fish amongst the potential spawners, out-migrating juveniles and those individuals returning directly to the Yakima River via the RAMF are predominately WEN origin. Furthermore, in brood years 2011 and 2012, the rate of replacement (%R) or adult-to-adult escapement consistently favored WEN stock, with an average %R = 0.80 compared to %R = 0.23 for the OSO stock (see Table 2 in Matala et al., 2019). Moreover, the majority of carcasses recovered during spawning ground surveys assign to WEN stock (Figure iv.a.4). The sockeye caught as bycatch during Lake Trout gillnetting efforts, which occur in mid-to-late October, have generally been exclusively OSO stock (see Matala et al., 2019). Many of the individuals caught as bycatch have been spilling gametes, suggesting they are actively spawning in that area of the lake.

Figure iv.a.4. *Genetic stock assignment for carcasses sampled from 2013-2016. Overall, the majority of carcasses assign to WEN stock. Carcasses assigning to WEN stock are recovered earlier in the spawning season (mid-Sept to early-Oct), whereas those assigning to OSO stock are recovered in late-Oct to mid-Nov. Additionally, WEN origin fish are found spawning in all three sections, whereas OSO carcasses are predominately found in section 3, closest to Cle Elum Lake.*



Analyses of early brood years have recovered a small proportion of hybrids amongst both the naturalized adult returns and the juvenile progeny (~5% amongst smolts and ~4% amongst naturalized adults; Matala et al., 2019). While this trend has generally continued in recent years, amongst the sampled 2018 adults returning to the RAMF, hybrids actually comprised a larger proportion of returning adults than OSO stock. Nonetheless, both represented a much lower proportion overall than WEN stock (Figure iv.a.5). The apparent paucity of hybrids is presumably due to the substantial spatial and temporal differences in spawning by stock as demonstrated by the carcass samples GSI results. It appears that the spawn timing observed in their natal lake systems has been preserved upon translocation to Cle Elum Lake, facilitating assortative mating.

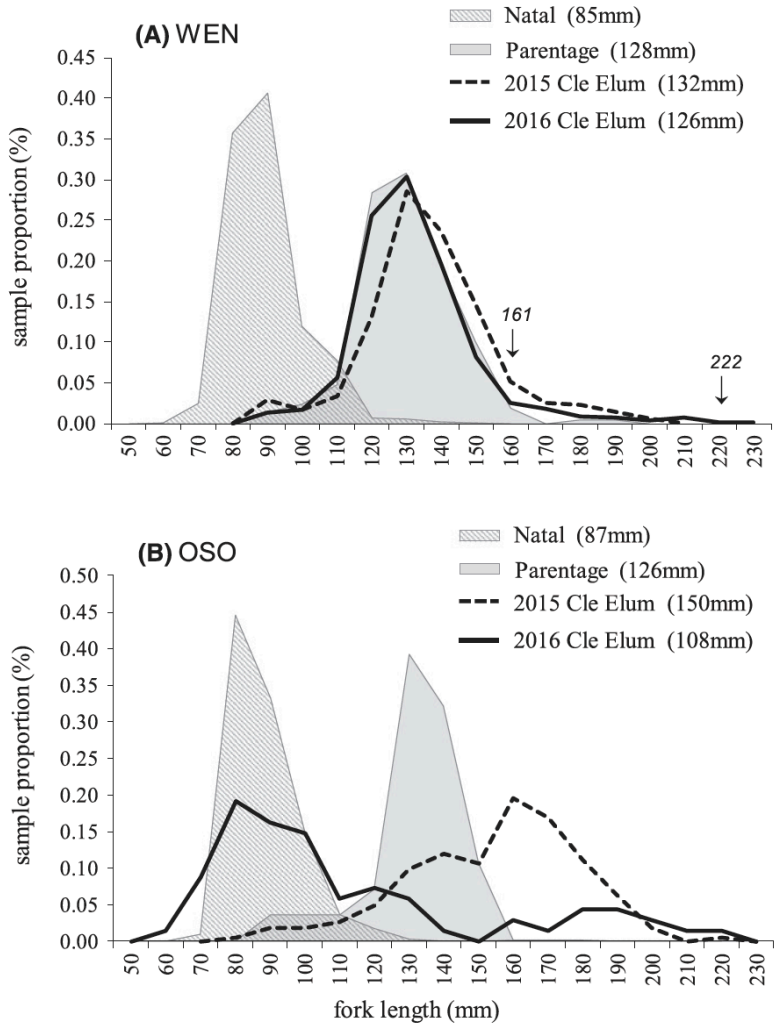
Figure iv.a.5. *Proportion of adults returning to the RAMF in 2018 that assigned to Osoyoos Lake (OSO), Lake Wenatchee (WEN) or hybrid origin.*



Lastly, we've detected significant differences in smolt size at emigration by stock-of-origin. For example, emigrating smolts in 2015 and 2016 that assigned to WEN origin, displayed a mean fork length of 128mm across both years, and with an average difference of 6mm between emigration years (see Figure iv.a.6; Matala et al., 2019). Conversely, the OSO origin smolts differed by an average of 42mm between 2015 and 2016, with a mean fork length of 150mm in 2015 and 108mm in 2016 (see Figure iv.a.6; Matala et al., 2019). In their natal environments, WEN smolts typically emigrate at 85mm, whereas OSO smolts emigrate at 87mm (Figure iv.a.6). Given the much larger size of the Cle Elum smolts, we used genetic parentage analysis to confirm that the smolts emigrating from Cle Elum Lake were, in fact, emigrating at age-1, which is the most common age at emigration observed in both natal environments. These observed differences by stock were also observed amongst the 2017 and 2018 emigrating smolts. However, the average size at emigration for OSO stock smolts in 2017 and 2018 was more comparable to that observed in 2016, suggesting that 2015 was an exceptional year and perhaps not reflective of the norm for OSO smolts emigrating from Cle Elum Lake.

In 2019, water levels in Cle Elum Lake never reached full pool, preventing access to the out-migration flume on Cle Elum Dam. As a result, no smolts were able to emigrate in 2019, and as such, no smolt tissue samples were taken in 2019. Most significantly, this means we expect zero age-4 adults, the most predominant age class, to return to the Yakima River in 2021. An additional interesting repercussion of no smolt emigration in 2019 was the observation of what appeared to be age-2 landlocked fish on the spawning grounds in 2020. Tissue samples were taken from these carcasses whenever possible, and GSI and parentage analyses will occur in 2021.

Figure iv.a.6. *From Matala et al., 2019. Fork length size distributions for age-1 emigrating smolts identified as* (A) WEN *or* (B) OSO *origin sampled in 2015 and 2016. Yearly mean sizes appear in parentheses in the respective legends. The* Parentage *distribution presents the size distribution for age-1 smolts whose age could be confirmed via genetic parentage analysis (n = 28 OSO, n = 200 WEN). Note that the* Parentage *distribution for OSO only includes 2015 emigrants as too few 2016 smolts could be aged via parentage analysis. Arrows mark the fork lengths of two confirmed age-2 WEN origin smolts. Size distributions of smolts emigrating from their natal populations in 2015 and 2016 are shown for comparisons.*



Discussion

Genetic monitoring of the sockeye reintroduction program in Cle Elum Lake to date has provided several important insights that may have remained unknown in the absence of such intensive sampling and analysis. For example, our central hypothesis that the donor stocks would interbreed, which was built on the expectation that the donor stocks would display comparable productivity and habitat use, has consistently proven false. Across monitoring years, we've observed differential smolt growth and survival, as well as differential adult-to-adult survival between stocks. Additionally, the relatively low incidence of hybrid offspring, in combination with the carcass samples GSI results, suggests assortative mating amongst the translocated adults. Spawn timing has been demonstrated to be a highly heritable trait (Abadía-

Cardoso, Anderson, Pearse, & Garza, 2013; Carlson & Seamons, 2008; J. E. Hess, Zendt, Matala, & Narum, 2016; Quinn, Peterson, Gallucci, Hershberger, & Brannon, 2002). Therefore, this strong temporal differentiation in spawn timing may be perpetuated in the naturalized adults until selective forces accumulate, and ultimately shift spawn times to align with the conditions in the Cle Elum Lake environment that optimize survival during early life stages (Fuhrman, Larsen, Steel, Young, & Beckman, 2018; Fullerton et al., 2017; Gharrett, Joyce, & Smoker, 2013; Skoglund, Einum, Forseth, & Barlaup, 2011).

Genetic monitoring efforts also revealed lake spawning behavior amongst OSO fish, which is not a characteristic behavior of OSO origin fish within their natal habitat. While the extent of lake spawning in Cle Elum Lake is currently unknown, carcass surveys suggest OSO are not prone to migrating particularly far upstream (Figure iv.a.4). This may be due to the intersection of spawn timing and river conditions, rather than strictly spawning habitat preference. For example, by the late-fall river flows have begun to increase, increasing energetic costs and mortality risks (i.e. redd scouring), creating a selective disadvantage on the later-spawning OSO fish.

This potential selective disadvantage for late spawners in the Cle Elum system may also explain the observed lower productivity amongst OSO origin fish. In each sampling year, the majority of adults translocated from PRD are OSO origin, yet the emigrating juveniles and adults returning to the Yakima River basin have been predominately WEN origin. In recent years, however, emigrating smolts have identified increasingly as OSO origin, perhaps suggesting a shift in conditions favoring OSO fish. This will become clearer as we continue to collect and analyze adults returning to the Yakima River, which are from the same brood years as these juveniles.

Nonetheless, it is important to note that assessments of productivity by stock are somewhat confounded by an inability to precisely estimate the total potential spawning population due to fallbacks and no sampling of post-spawn carcasses from the lake. Fallbacks are those fish that are released in the lake, become entrained at Cle Elum Dam and “fall back” downstream, and are re-sampled at the RAMF while trying to migrate back upstream. Given the later spawning time and longer residence time in the lake, it is possible that OSO origin fish may be more susceptible to entrainment at Cle Elum Dam. Hence, OSO fish may make up the majority of fallbacks, but we cannot accurately estimate stock-specific fallback rates and incorporate this factor into the analyses, since some number of fallbacks remain downstream and are never resampled at the RAMF.

Lastly, the finding that emigrating smolts are notably larger than the average smolt from their respective natal environment was fairly unexpected given initial assessments of abiotic and trophic conditions in Cle Elum Lake, which found low nutrient levels and low densities of macroinvertebrates and zooplankton (Flagg et al., 1988, 2000; BOR, 2007, 2011). We suspect the relatively rapid juvenile growth could simply be attributed to smolt abundances being well below the carrying capacity of Cle Elum Lake. Continued years of analysis have suggested the significant difference in average smolt size amongst OSO fish between 2015 and 2016 may be anomalous, with the 2015 average size representing an exception. We plan to continue monitoring size at emigration and integrating these data with environmental data where possible to better understand any patterns across years.

Looking Forward

The BSE Project will continue to support genetic monitoring of this reintroduction effort, with a slight shift in focus towards: 1) evaluating long-term relative fitness between the translocated adults and the naturalized population, 2) assessing changes in stock-specific spawn timing and incidences of inter-breeding, and 3) more robust assessment of fork length (size-at-age) and

relative stock abundances amongst out-migrating smolts. This project allows us to not only better understand how the reintroduced population is acclimating and adapting to the Cle Elum Lake system, but also to help managers identify what, if any, modifications to the program are likely to dramatically improve long-term success of the reintroduction program. For example, if continued monitoring suggests that productivity is limited more by postemigration factors than suitability of habitat within Cle Elum Lake, then managers may actually maximize returns by advocating for habitat restoration and water management agreements aimed at improving thermal conditions in the lower Yakima River (Singh, Faulkner, Keeley, Freudenthal, & Forshay, 2018). Notably, construction of an improved juvenile out-migration facility that will allow individuals to volitionally out-migrate is currently underway (Hanna, Higgs, Mefford & Wagner, 2016). Sampling and analysis of emigrating juveniles once construction is complete will help further clarify trends in productivity by donor stock. This project ultimately provides valuable insight as to how sockeye salmon are adapting to a novel environment and illustrates factors that may significantly determine the success of future reintroductions both in the Yakima River basin (i.e. Keechelus, Kachess, Bumping and Rimrock lakes), as well as other systems, such as Wallowa Lake in the Grande Rhonde River subbasin.

b. Cooper Lake Acoustic Tracking Study

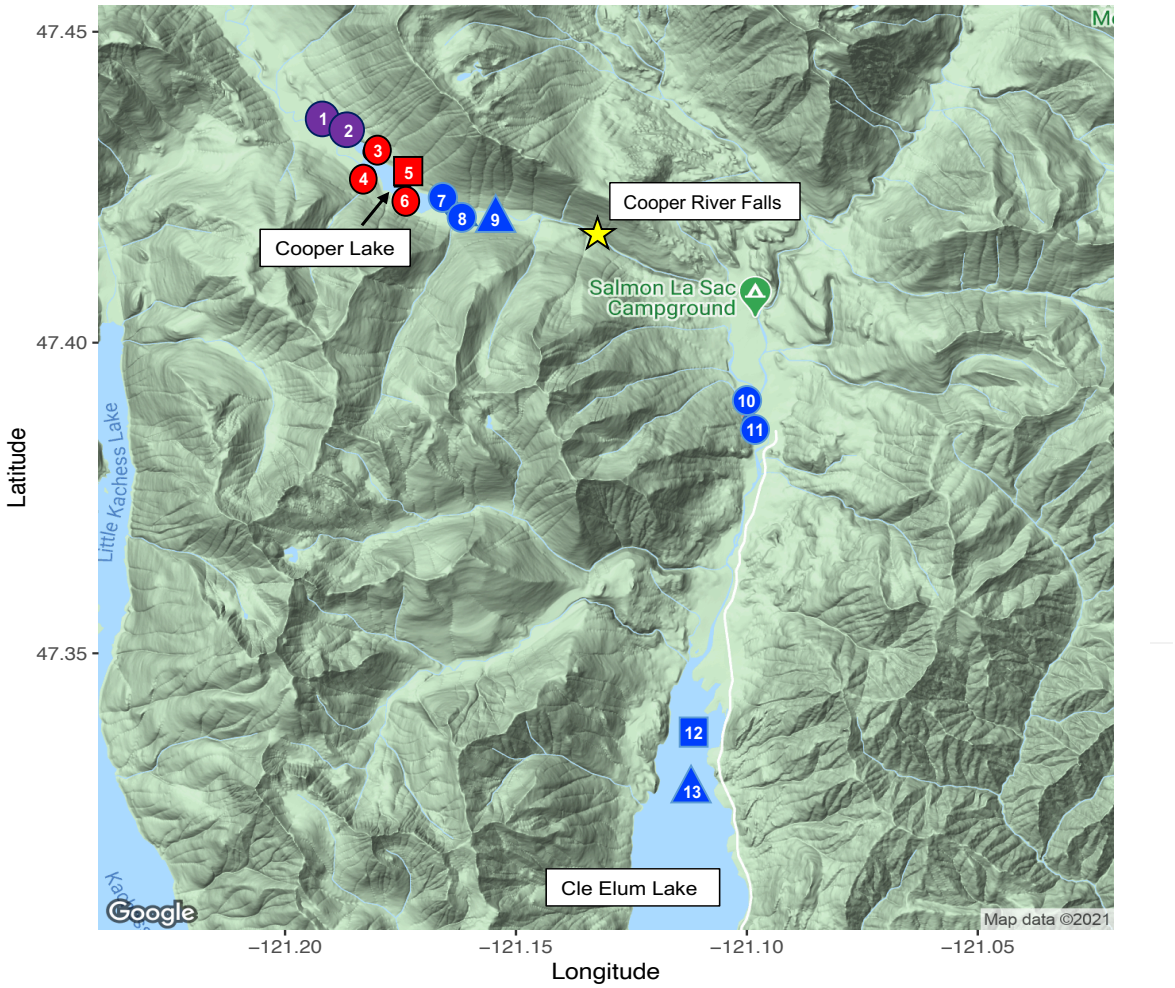
Although the majority of adult sockeye translocated from Priest Rapids Dam (PRD) each year are transplanted to Cle Elum Lake, a small proportion of these individuals have been transplanted to Cooper Lake. Cooper Lake is a 129-acre, high elevation lake (851m), that is located approximately midway between the Cooper River headwaters and its confluence with the Cle Elum River, approximately 5 km upstream of Cle Elum Lake. Cooper Lake was never known to support a native population of sockeye salmon, likely due to a significant, seemingly impassable barrier waterfall located between Cooper Lake and the confluence of the Cle Elum and Cooper Rivers (see Figure iv.b.1). However, surveys conducted along Cooper River in the late-1980s/early-1990s identified substantial spawning habitat upstream of Cooper Lake, with an estimated 10,000 m^2 of potential spawning gravel (Flagg et al., 2000). For this reason, managers felt that translocating a portion of the WEN and OSO fish from PRD to Cooper Lake each year could substantially increase juvenile production in the basin. It was expected that any juveniles that reared in Cooper Lake and survived to adulthood would then return to spawn in the lower reaches of the Cooper River, below the barrier falls.

Funding and personnel to monitor this particular aspect of the reintroduction program have not been readily available, and it wasn't until a few years ago that co-managers began performing spawning ground surveys along Cooper River above Cooper Lake. Curiously, no live fish or carcasses have been observed during any of the surveys performed to date. The apparent lack of spawning in what appeared to be optimal spawning habitat raised many questions regarding the fate of fish translocated to Cooper Lake. Therefore, in addition to our typical annual monitoring efforts under the Cle Elum Lake reintroduction program, we conducted an acoustic tagging study in 2020, from June to November, to track the movement of sockeye salmon adults translocated to Cooper Lake.

During capture of fish at Priest Rapids Dam in early July of 2020, we surgically implanted 30 fish that were designated for transport to Cooper Lake with Vemco acoustic transmitters (model V9). Just prior to the tagging event, in late-June, we placed an array of Vemco VR2W acoustic receivers upstream and downstream of Cooper Lake, and

within Cooper Lake. We positioned 11 receivers as follows: four receivers were positioned within Cooper Lake, two receivers were positioned above Cooper Lake, and five receivers were positioned downstream of Cooper Lake, extending into the Cle Elum River and down to Cle Elum Lake (Figure iv.b.1). Upon deployment, all receivers were checked to confirm recording functionality and assess detection range. Receivers remained deployed through November 2020, thereby capturing movement data for all tagged individuals from time of transplant through the end of the typical spawning season. Receivers were checked at least once a month throughout the deployment to download data and ensure proper functionality.

Figure iv.b.1. *Map of acoustic receiver positions upstream of Cooper Lake (purple shapes), within Cooper Lake (red shapes) and downstream of Cooper Lake to Cle Elum Lake (blue shapes). The Cooper Lake receiver marked by a red square (#5) was moved to the position marked by a blue square (#12) in mid-August so that we could track movement into and out of Cle Elum Lake during the spawning window. Similarly, the receiver marked with a blue triangle just downstream of Cooper Lake (#9), was moved to the position marked with a blue triangle in Cle Elum Lake (#13) in mid-August.*



All 30 tagged fish were translocated to Cooper Lake on July 2, 2020 – 26 of these individuals were detected at one or more of the four lake receivers on the day of transport, and the remaining four were detected within the lake on July 3, 2020, confirming receiver detection functionality. When reviewing total detections per individual over the season, each tagged fish can generally be grouped into one of three groups:

1) The first group includes those individuals whose last known position was detected at the receivers just downstream of Cooper Lake (receivers #7-9) from 7/4/2020 to 7/9/2020 and were never detected at any other receivers thereafter. This included 20 of the 30 tagged individuals.
2) The second group of individuals includes those that were finally detected at downstream receivers in the Cle Elum River (receivers #10-13) from 7/9/2020 to 10/29/2020. This included seven of the 30 tagged individuals. Three of these seven individuals were detected at the lowest Cle Elum receivers (nearest to Cle Elum Lake) from 9/23/2020 to 10/29/2020.
3) The third group includes those individuals who were only detected at receivers within Cooper Lake (receivers #3-6), and therefore seemingly stayed in Cooper Lake throughout the duration of the deployment. This included only 2 of the 30 tagged individuals.
4) There was one individual whose movement did not cleanly fit into any of three groups. This individual was the only tagged fish detected at the receivers upstream of Cooper Lake (receivers #1-2). This individual was detected at receivers #1 and 2 on 10/13/2020; however, later that same day, the individual was detected within the lake and just downstream of the lake (receivers #7-9). Hence, acoustic data suggests this individual did not remain upstream of Cooper Lake for more than a few hours.

While analysis of this telemetry data is ongoing, these preliminary results suggest individuals transplanted into Cooper Lake are not using this habitat for spawning. The most common movement trajectories (groups 1 & 2) indicated most fish left Cooper Lake within days of translocation, and a smaller proportion of those fish travelled downstream to Cle Elum Lake by the typical spawning window. Given that the individuals in group 1 were never detected at any of the Cle Elum River receivers (receivers #10-13), we assume that some proportion of those fish attempted to utilize the habitat along the Cooper River, between receivers 9 and 10, for spawning. While this reach of the Cooper River is not part of the standard spawning ground survey area (Figure iv.a.2), we did perform some carcass and snorkel surveys along this stretch as time allowed, and noted habitat use by sockeye salmon along this stretch of the Cooper River. Conversely, we observed no sockeye salmon during snorkel surveys upstream of Cooper Lake during the summer of 2020.

Ultimately, only two fish were detected in Cooper Lake up to the end of the typical spawning season, and apparently never swam upstream of the lake to spawn as they were never detected at receivers #1 and #2. In fact, the only fish that was detected has having ventured upstream of Cooper Lake appeared to have moved in an exploratory manner and was detected in the lake and downstream receivers (#7, 8 and 9) later that same day. The ultimate fate of the two fish residing in the lake through the duration of the study remains unknown, and while It is possible they spawned in the lake as lake-spawning behavior has been observed amongst OSO origin fish in Cle Elum Lake, it appears equally likely they died in the lake before spawning.

We took tissue samples for genetic analysis from all individuals involved in the acoustic tracking study and intend to review patterns in movement by stock-of-origin once those individuals have been genotyped. Additionally, we will compare these individual genotypes against those obtained from carcass samples in a duplicate analysis. If one of the acoustically tagged fish happened to be sampled as a carcass, and we successfully generate a genotype for that fish, the information from duplicate sampling will allow us to more accurately identify final spawning location of acoustically tagged fish.

Thus, while final conclusions and recommendations are pending the integration of these genetic data, these initial findings from telemetry data, paired with years of survey data, suggest translocated sockeye are not using the apparently pristine habitat above Cooper Lake for spawning. Initial theories center around thermal barriers – either at the inlet to Cooper Lake, where shoaling may allow for excessive heating in the summer months that disincentivizes passage upstream, or substantially cooler temperatures in the Cooper River above Cooper Lake that may alter trophic regimes and nutrient levels in that reach. Notably, at the time of deployment, the water temperature at receivers #1 and #2 was, on average, nearly 7ºC cooler than the Cle Elum River receiver stations, #7-11. Final conclusions and recommended actions are anticipated by Spring 2021.

c. Lake Billy Chinook Reintroduction Effort

Introduction

Suttle Lake, in the Deschutes River basin, and Wallowa Lake, in the Grande Ronde subbasin, were the only locations in Oregon to historically support native populations of sockeye salmon. Similar to Cle Elum Lake, however, these populations were extirpated following development along migration corridors that effectively removed access to important natal rearing and spawning habitat. In the Deschutes River basin such development first occurred in 1925, when a small dam was constructed near the outlet of Suttle Lake to Lake Creek to create a swimming area for the nearby Lake Creek Lodge. A few years later, a 1.2m concrete dam associated with a small hydroelectric facility was constructed just downstream of the first dam on Lake Creek. While neither of these structures completely blocked passage, upstream movement of adult sockeye salmon to Suttle Lake was hindered or sometimes impossible depending on water flow levels. Additionally, the installation of screens in the inlets to the turbines on the 1.2m concrete dam prevented downstream escapement of emigrating juveniles. As a result, sockeye salmon numbers steadily declined in subsequent years, and it wasn't long before the population in Suttle Lake was functionally extirpated (Gustafson et al., 1997; Nehlson 1995; Olsen et al., 1994; Nielson 1950).

Nonetheless, while only able to migrate as far as the upper Metolius River to spawn, small numbers of sockeye salmon continued to return to the Deschutes River basin. Resulting juveniles apparently reared in the lower Deschutes River or the Columbia River, perpetuating the continued, small return of adult sockeye to the Deschutes River basin each year (Gustafson et al., 1997; Olsen et al., 1994). Then, from 1958 to 1964, a series of three dams were constructed on the Deschutes River as part of the Pelton-Round Butte Hydroelectric Project – the Reregulating Dam (rkm 160), Pelton Dam (rkm 164) and Round Butte Dam (rkm 176). While this project included both upstream and downstream passage for anadromous fish, the system for downstream passage proved ineffective. Within a few generations, the small, remanent run of sockeye salmon was extirpated from the Deschutes River basin (Gustafson et al., 1997; Olsen et al., 1994).

However, in Lake Billy Chinook (LBC), the reservoir created by Round Butte Dam, a large population of non-anadromous *O. nerka* (kokanee) developed. Mature kokanee in LBC migrate upstream into the upper Metolius River to spawn each year, and newly emerged juveniles move downstream for rearing in LBC (Gustafson et al., 1997; Nehlson 1995). While this kokanee population likely shares ancestry with the remnant sockeye salmon population that was forced into a resident life history type, both the kokanee population in LBC, as well as the population that developed in Suttle Lake, were likely heavily influenced by repeated stocking events with smolts (both sockeye and kokanee) from out-of-basin hatcheries throughout the mid-1900s (Gustafson et al., 1997; Nehlson 1995; Olsen et al., 1994).

For decades, no concerted efforts were made to mitigate for the loss of sockeye salmon in the Deschutes River basin. Then, in 2005, during negotiations for relicensing of the Pelton-Round Butte Hydroelectric Complex, an agreement was reached to re-establish passage for sockeye salmon. This led to the construction of the fish transfer facility (FTF) on the new surface water withdrawal (SWW) system at Round Butte Dam. Since the FTF and SWW systems became operational in 2010, *O. nerka* juveniles that volunteer into the FTF are right-maxillary clipped, and then been passed downstream. It is assumed that these juveniles, which express emigration behavior, may return to their natal system after maturing in the ocean, thereby expressing the complete anadromous, sockeye salmon life history.

The number of juveniles passed through the FTF has varied widely throughout the years, and the number of adults captured at the trap at the Reregulating Dam has exceeded 100 fish in only a single year (Table iv.c.1). These returning adults are generally transported upstream for release in LBC; however, in some years, a portion of returning adults have been taken for hatchery broodstock at Round Butte Hatchery, immediately below Round Butte Dam (Table iv.c.1). At the hatchery, the fish are spawned, the eggs incubated, and the juveniles are reared to the parr stage, at which point they are released into LBC.

Table iv.c.1. *Total number of emigrating juveniles, returning adults, adults retained for broodstock, adults released downstream and hatchery-produced parr released each year, from 2010 to 2020. In the* No. Adults Released *column, the number in parentheses represents the portion of adults that were released directly into Lake Billy Chinook.*

Year	No. Emigrating Juveniles Released	No. Adults Returning	No. Adults Retained for Broodstock	No. Adults Released	No. parr released into LBC following spring
2010	49,734	10	0	10 (100%)	N/A
2011	225,761	23	19	4 (0.0%)	3,870
2012	5,126	98	0	98 (87.8%)	N/A
2013	25,265	33	0	33 (75.6%)	N/A
2014	155,031*	27	0	27 (74.0%)	N/A
2015	38,702*	36	36	0 (0.0%)	13,122
2016	49,497	536	73	463 (100%)	33,515
2017	439,458	57	42	15 (100%)	22,000
2018	47,392*	49	22	27 (100%)	16,558
2019	92,099*	69	0	66 (100%)	N/A
2020	32,845	65	0	65 (100%)	N/A

*No emigrating juveniles were RM-clipped after passing through the FTF in 2014, 2015, 2018 or 2019

Hence, unlike the “donor stock” approach implemented by the Cle Elum Lake reintroduction effort, the effort in the Deschutes River basin is attempting to essentially facilitate expression of the anadromous phenotype from in-basin kokanee. Success of this effort largely depends on two factors: 1) that anadromous adults captured at the Pelton adult fish trap (on the Reregulating Dam) are indeed of LBC origin, and 2) that the number of returning adults will be sufficient to restore a self-sustaining anadromous sockeye population. To address this first factor, the BSE project finances Genetic Stock Identification (GSI) of all adults returning to the Pelton adult fish trap each year, where identification to LBC-origin would suggest that some portion of the emigrating juveniles do express anadromy and successfully complete a typical sockeye salmon life cycle. Results from these analyses are provided to co-managers – Confederated Tribes of the Warm Springs Reservation of Oregon (CTWSRO), Oregon Department of Fish and Wildlife (ODFW), and Portland General Electric (PGE) – to guide future management policies and objectives.

Methods

Sample Collection and Genotyping

Approximately 1,000 samples, representing all adults returning to the Pelton adult fish trap from 2010 to 2020, have been genotyped to date. DNA has been extracted from fin tissues using either a standard Qiagen DNeasy protocol (Qiagen Inc., Valencia, CA), or more recently, a Chelex 100 method (Sigma-Alrich, St Louis, MO). Currently, all individuals are genotyped at a panel of 364 SNP markers using the genotyping-in-thousands by sequencing approach (GTseq, N. R. Campbell et al., 2015; SNP markers, Matala et al., 2019). Individual genotypes are quality filtered for missing data, with a minimum threshold of 95% genotyping success, as well as duplicate sampling.

Genetic Stock Identification and Parentage Analysis

All individuals passing quality filters are then identified to stock-of-origin using GSI methods implemented in either GENECLASS2 (Piry et al., 2004) and STRUCTURE v.2.3.4 (Pritchard et al., 2000), or, most recently, in the R package rubias (Moran & Anderson, 2019). The R package, rubias, implements a Bayesian inference for the conditional genetic stock identification model, which is better able to accommodate biased representation in the reference populations and predict accuracy. All individuals returning to the Pelton adult fish trap are compared to a reference baseline including the following populations: Lake Billy Chinook, Osoyoos Lake, Lake Wenatchee, Redfish Lake, Suttle Lake and Meadow Creek Hatchery. Resulting individual assignments are filtered by Posterior Probability ≥ 0.95.

Increasingly affordable genotyping and sufficient years of data have also allowed us to perform parentage analyses on the more recent return years. We utilize the software COLONY v2.0.6.6 (Jones & Wang, 2010), which uses likelihood-based methods, to identify parent-offspring trios, as well as single parent-offspring pairs. Samples are run by collection year, and against a biologically plausible pool of parents. For example, all adults returning in 2019 were run against a pool of parents returning in 2015-2017. Run parameters for each collection were as follows: both parents polygamous, medium run length and precision, and full-likelihood analysis method. All resulting parent assignments are filtered by Probability ≥0.90.

Results

Prior to performing GSI analyses for adults returning to the Pelton adult fish trap, we performed a Principal Coordinates Analysis on the current *O. nerka* baseline to graphically explore genetic dissimilarity between reference populations. In the resulting plot, there is clear separation between the sockeye salmon populations and kokanee populations from Idaho, while kokanee populations from Oregon and Washington generally group into a single cluster (Figure iv.8;

Table iv.c.2). However, and importantly for this analysis, the LBC reference population forms a distinct cluster (cluster #1 in Figure iv.c.1). This suggests we have sufficient power to confidently assign individuals to the following origins when all reference populations are included: Lake Wenatchee, Osoyoos Lake, Redfish Lake, Lake Billy Chinook or Idaho kokanee populations. It is much more difficult, however, to clearly identify stock-of-origin amongst kokanee populations throughout Oregon and Washington. This pattern reflects the history of stocking throughout the basin, and the resulting shared ancestry between several of the reference kokanee populations. Conversely, the anadromous populations and Idaho kokanee populations are likely highly distinguishable due to life history and geography, which has facilitated generations of reproductive isolation and genetic differentiation. It is for this reason that we only selected the six populations outlined above – Lake Wenatchee, Osoyoos Lake, Redfish Lake, Lake Billy Chinook, Suttle Lake and Meadow Creek Hatchery – to be included in the baseline for assigning stock-of-origin to the Pelton adult fish trap returns. We included Meadow Creek Hatchery as this population was historically used for stocking in the Deschutes River basin.

Figure iv.c.1. *Principal Coordinates Analysis of all* O. nerka *populations included in the reference for Genetic Stock Identification (GSI). Population codes and locations are listed in Table iv.4. Note the clear distinction between Lake Wenatchee (cluster #2), Osoyoos Lake (cluster #6), Redfish Lake (cluster #11), Idaho kokanee populations (clusters #3 &4), and Lake Billy Chinook (cluster #1).*

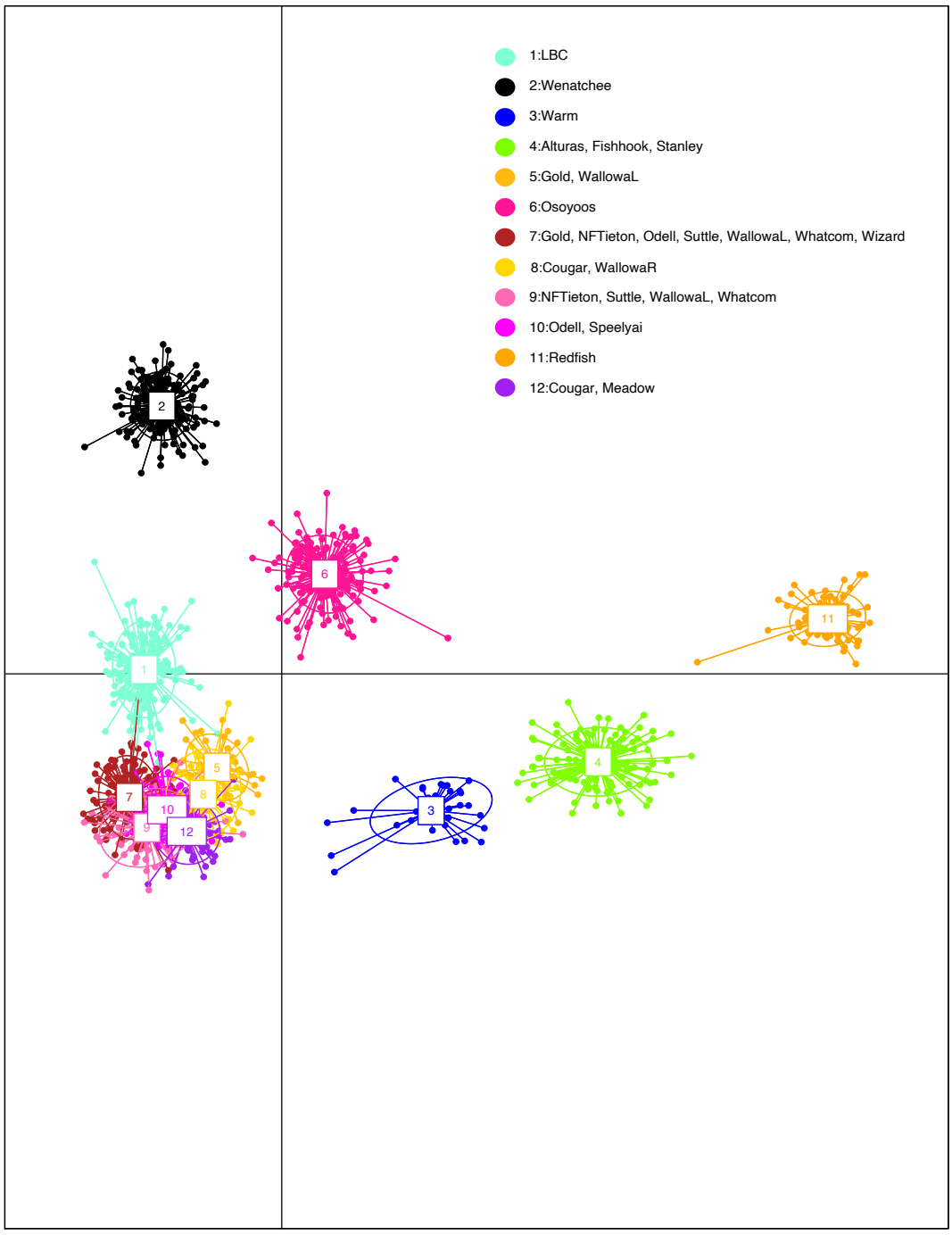


Table iv.c.2. *Geographic and life history information for each reference population. Population codes align to those used in Figure iv.8.*

Population code	Location	State	Region	Subbasin	Life History
Cougar	Cougar Creek	WA	Lower Columbia	Lewis	kokanee
Speelyai	Lewis River/Speelyai Hatchery	WA	Lower Columbia	Lewis	kokanee
LBC	Lake Billy Chinook	OR	Middle Columbia	Deschutes	kokanee
Suttle	Suttle Lake/Link Creek	OR	Middle Columbia	Deschutes	kokanee
Odell	Odell Lake	OR	Middle Columbia	Deschutes	kokanee
Wizard	Wizard Falls	OR	Middle Columbia	Deschutes	kokanee
Warm	Warm Lake	ID	Snake	S.F. Salmon	kokanee
Stanley	Stanley Lake	ID	Snake	Sawtooth	kokanee
Redfish	Redfish Lake	ID	Snake	Sawtooth	sockeye
Fishhook	Fishhook Creek	ID	Snake	Sawtooth	kokanee
Alturas	Alturas Lake	ID	Snake	Sawtooth	kokanee
WallowaL	Wallow Lake	OR	Snake	Grande Ronde	kokanee
WallowaR	Wallowa River	OR	Snake	Grande Ronde	kokanee
Meadow	Meadow Creek	Canada	Upper Columbia	B.C.	kokanee
Osoyoos	Osoyoos Lake	Canada	Upper Columbia	Okanagan	sockeye
Whatcom	Lake Whatcom	WA	Upper Columbia	Puget Sound	kokanee
Wenatchee	Wenatchee Lake	WA	Upper Columbia	Wenatchee	sockeye
Gold	Gold Creek	WA	Upper Columbia	Yakima	kokanee
NFTieton	N. F. Tieton River	WA	Upper Columbia	Yakima	kokanee

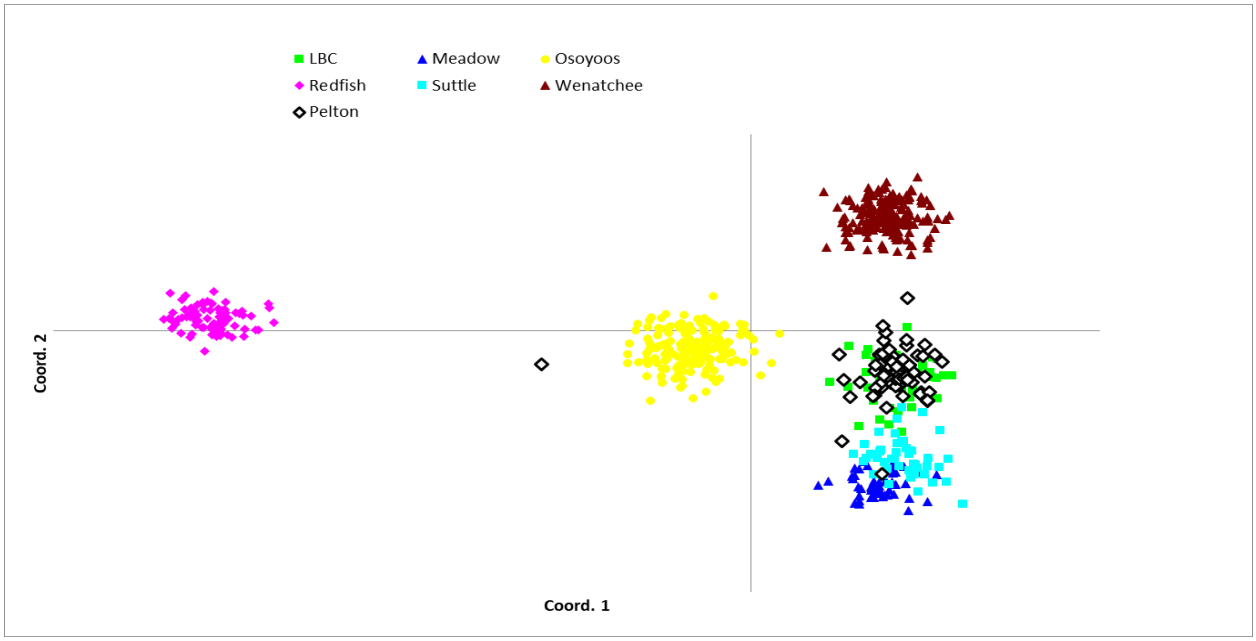
The most common single stock-of-origin for all adults returning to the Pelton adult fish trap on the Reregulating Dam, across all sampling years to date, has been Lake Billy Chinook (Table iv.c.3). A graphical representation of the GSI results is presented for the 2019 adult returns in Figure iv.c.2. Note, while there is some overlap between the Meadow Creek Hatchery and Suttle Lake populations, as seen in Figure iv.c.1., the clusters are more distinguishable when using this reduced baseline.

For the parentage analyses, only one adult from 2019 was assigned to parents at prescribed probability thresholds. This individual was marked with a left maxillary (LM) clip, indicative of having been reared at Round Butte Hatchery and released into LBC as fry. Both the assigned sire and dam returned to Pelton adult fish trap in 2015. Although only a single case, this assignment aligns with scale age data for this population, which has found age-4 to be the most common age-at-return for this population.

Table iv.c.3. *Genetic Stock Identification assignment for adults returning to Pelton adult fish trap from 2010 to 2020. The exact kokanee populations included in the reference baseline have changed throughout the study years and is therefore generalized as "kokanee stocks" for ease of representation. The anadromous sockeye populations are represented by OSO = Osoyoos Lake, WEN = Lake Wenatchee and RFL = Redfish Lake.*

Collection Year	No. Adults Assigned	No. Assigning to LBC	No. Assigning to kokanee stocks	No. Assigning to OSO	No. Assigning to WEN	No. Assigning to RFL	Proportion LBC origin
2010	10	8	1	1	0	0	0.80
2011	22	20	2	0	0	0	0.91
2012	98	94	2	2	0	0	0.96
2013	30	26	1	2	0	1	0.87
2014	0	0	0	0	0	0	N/A
2015	36	17	2	9	2	6	0.47
2016	524	484	32	2	2	4	0.92
2017	57	56	1	0	0	0	0.98
2018	48	47	0	1	0	0	0.98
2019	67	65	1	1	0	0	0.97
2020	63	57	5	0	1	0	0.90

Figure iv.c.2*. Principle Coordinates Analysis of adults returning to Pelton adult fish trap in 2019. Among the 67 genotyped samples from 2019, 65 assigned to Lake Billy Chinook (LBC), which is represented by the black diamonds overlapping with the green, LBC cluster. Two individuals had the highest probability assignment to populations other than LBC: 1) one individual assigned to Osoyoos Lake, represented by the black diamond to the left of the Osoyoos cluster, and 2) one individual assigned to Suttle Lake, represented by the black diamond overlapping with the light blue, Suttle Lake cluster.*



Discussion

In terms of addressing the two factors that will largely dictate the success of this reintroduction program, the GSI results confirm that the majority of adults returning to the Pelton adult fish trap are indeed of LBC-origin. Hence, some proportion of the juveniles that emigrate from LBC successfully express anadromy and return to their natal lake of origin (LBC) as adults. However, the second factor seems less promising, given consistently low adult returns over the past 11 years. Excluding 2016, the overall Smolt-to-Adult return rate (SAR) for juveniles released downstream is estimated at approximately 0.1%, and less than 100 adults have returned annually.

Program co-managers continue to examine possible modifications to management of the reintroduction effort, including increased hatchery spawning of returning adults, rearing of hatchery juveniles to the smolt stage, and supplementation with out-of-basin sockeye salmon smolts and/or adults. While these negotiations occur, the BSE Project will continue to support GSI and parentage analyses of the returning adults, thereby providing co-managers with data needed to advocate for and inform future management.

v. Project Objective #4 – Estimate the effective number of breeders and assess natural productivity of spring Chinook and coho salmon in the Upper Warm Springs River, OR

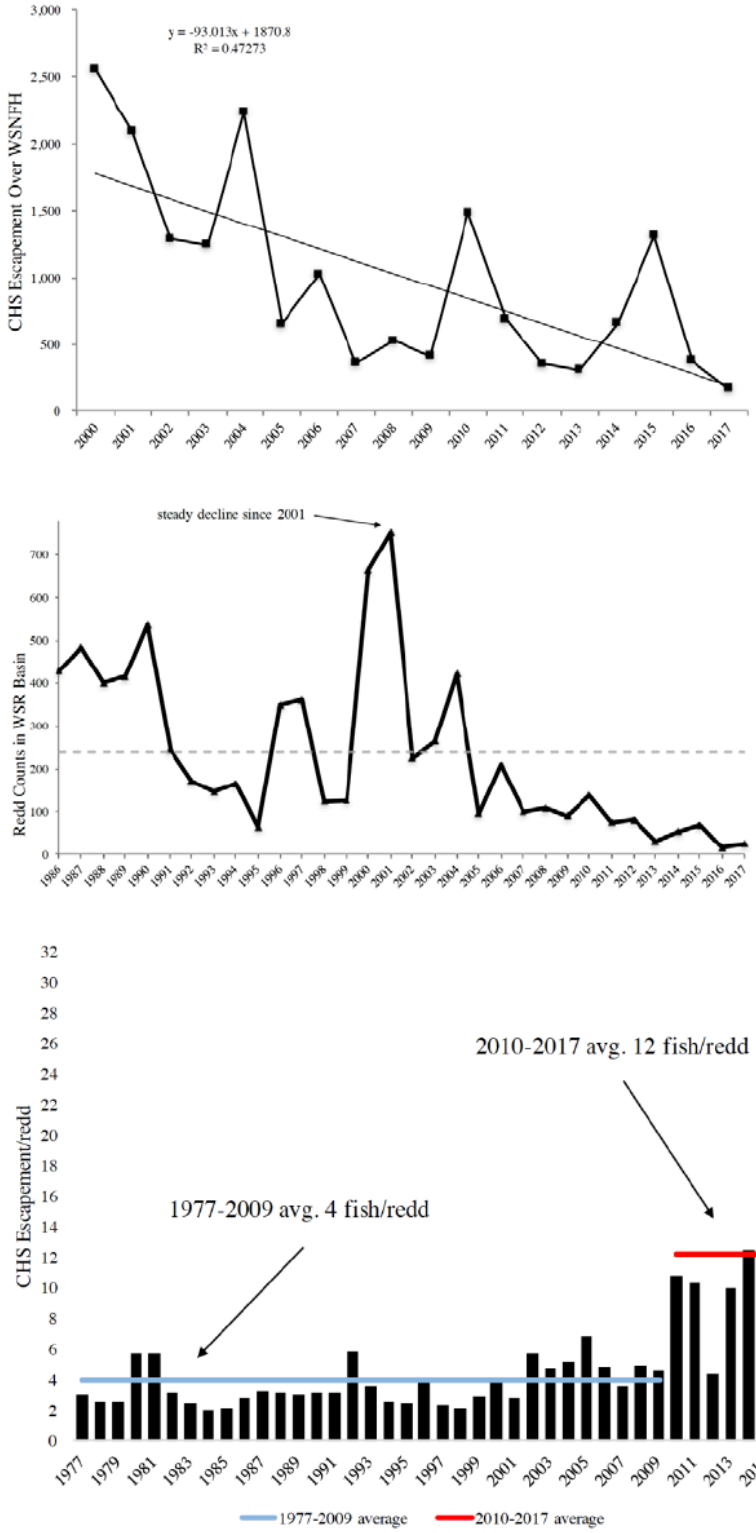
Introduction

The population of spring Chinook salmon in the upper Warm Springs River, upstream of the Warm Springs National Fish Hatchery (WSNFH; rkm 16.4), has been monitored for several decades. All returning adults are trapped at an impassable weir adjacent to the WSNFH and then passed upstream to spawn naturally if identified as a natural-origin fish. Counts and individual metadata has therefore been recorded each year since monitoring began, and more recently, all adults intercepted at the weir have been tissue sampled. Additionally, personnel associated with the Fisheries Research, Monitoring and Evaluation (RM&E) program of the Confederated Tribes of the Warm Springs Reservation of Oregon (CTWSRO) have conducted spawning ground surveys in the upper basin, providing a record of redd counts throughout the years.

These weir counts have been integrated with redd count data to monitor trends in escapement and productivity. Productivity, and particularly spawning success, has historically been estimated using the fish-per-redd ratio, which reflects the proportion of returning adults that successfully spawned. Generally, a lower fish-per-redd ratio is indicative of more equitable spawning opportunities across the total escapement. For example, in populations where pre-spawn mortality is low and spawning habitat is relatively abundant, one would expect more redd building to occur, drawing down the fish-per-redd ratio. Conversely, a population experiencing high escapement one year, but constrained spawning habitat, would result in fewer redds, driving up the fish-per-redd ratio.

From 1977 to 2000, the fish-per-redd ratio for spring Chinook in the upper Warm Springs River averaged approximately 4 +/- 2 fish-per-redd. This aligns with fish-per-redd ratios observed in other spring Chinook populations in the Columbia River basin (Kucera & Orme, 2006) and suggests a fair proportion of the population has successfully engaged in spawning. Unfortunately, in recent years, the fish-per-redd ratio has begun climbing while escapement and redd counts have steadily declined (Figure v.1). Of particular concern is the apparent three-fold increase in the average fish-per-redd ratio from 2010 to 2017, suggesting far fewer returning adults are successfully spawning. This relatively sudden and dramatic change in fish-per-redd ratio may be a reflection of changing environmental conditions, which impose greater stress and increase pre-spawn mortality. Alternatively, this change may also be due to an undercounting of redds in recent years, perhaps due to an expansion of spawning habitat not normally included in spawning ground surveys. However, recent changes to spawning ground surveys in response to increasing fish-per-redd ratios have included surveying 20% of non-index reaches annually, and thus far, no excessive spawning has been observed in these non-index reaches.

Figure v.1. *From Galbreath et al., 2018. The from top to bottom: the total escapement of spring Chinook to the Warm Springs National Fish Hatchery (WSNFH) weir from 2000 to 2017; redd counts in the upper Warm Springs River basin from 1986 to 2017; the fish-per-redd ratio for spring Chinook in the upper Warm Springs River basin from 1977 to 2017.*



Interestingly, however, while abundance and apparent productivity of spring Chinook began declining in 2010, coho salmon, which were previously extirpated, began to appear at the WSFNH weir and in generally increasing numbers. While the coho salmon that initiated this population may have been hatchery-origin, currently no individuals bear any markings and it is presumed that most are natural-origin. These dramatic shifts in spring Chinook abundance and spawning success, simultaneous to the reappearance of coho salmon, provides an opportunity to investigate species-specific productivity patterns on a dynamic riverscape, which has undoubtedly been affected by years of development and an increasingly changing climate.

While fish-per-redd ratios are informative, they only provide an indirect measure of reproductive success, and assume the reaches surveyed encompass the totality of spawning habitat in the system. Therefore, in order to verify the observed fish-per-redd trends, the BSE Project is financing pedigree reconstruction analyses of both spring Chinook and coho salmon from brood years 2015 to 2019. These analyses use genetic data to reconstruct parent and sibling relationships, thereby directly estimating effective population size and reproductive success. The resulting data will be relayed to co-managers to inform management decisions within the upper Warm Springs River system. Additionally, these data may be relatable and provide context to other systems within the Columbia River Basin that are experiencing shifting species compositions and/or notable declines in apparent spawning success.

At this time, we are cataloguing and genotyping all tissue samples collected to date. No new analyses beyond those published in CRITFC Technical Report 18-04 (Galbreath, Janowitz-Koch, Boostrom & Baker, 2018) have been completed due to pauses in funding. We therefore present below our plans for sample collection, genotyping and parentage analyses over the next 1-1.5 years.

Methods

Sample Collection and Genotyping

Tissue samples will be collected from returning spring Chinook and coho adults, interrogated at the WSNFH weir, as well as out-migrating juveniles, principally collected at a rotary screw trap located 0.5km upstream from the WSNFH weir. Sample numbers by species and brood year are presented in Table v.1.

After receipt and cataloguing at the Hagerman Genetics Lab within the Hagerman Fish Culture Experiment Station (HFCES), DNA will be extracted from fin tissue using a Chelex 100 method (Sigma-Alrich, St Louis, MO). All individuals will then be genotyped at standardized SNP panels using the genotyping-in-thousands by sequencing approach (GTseq, N. R. Campbell et al., 2015). The SNP panels are species-specific and optimized for standard genetic analyses, including parentage analysis. The spring Chinook samples will be genotyped at 298 SNP markers (Janowitz-Koch et al., 2019), whereas all coho samples will be genotyped at 342 SNP markers (Hess et al., 2020). Individual genotype data will be quality filtered for duplicate sampling, as well as missing data, such that individuals with <90% genotyping success will be dropped from the analysis. However, as was seen in the initial analyses of brood years 2015 and 2016 (Galbreath et al., 2018), it is very important to critically review juvenile samples with poor genotyping success rates as these may, in fact, be coho salmon incorrectly genotyped with the spring Chinook salmon SNP panel (or vice versa).

Table v.1. *Total number of adults and juvenile samples genotyped to date by species and brood year. Note, spring Chinook adults from brood years 2015 to 2016 were not genotyped as the initial analysis only used sibling relationship reconstruction to estimate the number of effective breeders (Nb; see Galbreath et al., 2018).*

Spring Chinook Salmon

Brood Year	NOR Adults		Juveniles Genotyped		Proposed Genetic Analysis
	Escapement	*Genotyped*	*Fall Parr*	*Spring Smolts*	
2015	1300	na	135	798	Nb
2016	400	na	171	176	Nb
2017	220	214	1,203	TBD*	Nb + parentage and individual RRS
2018	224	221	TBD*	TBD*	Nb + parentage and individual RRS
2019	196	0	TBD*	TBD	Nb + parentage and individual RRS

Coho Salmon

Brood Year	NOR Adults		Juveniles Genotyped		Proposed Genetic Analysis
	Escapement	*Genotyped*	*Fall Parr*	*Spring Smolts*	
2015	124	44	11	206	Nb + parentage and individual RRS
2016	190	188	307	528	Nb + parentage and individual RRS
2017	261	258	303	TBD*	Nb + parentage and individual RRS
2018	6	4	TBD*	TBD*	Nb + parentage and individual RRS
2019	322	0	TBD*	TBD	Nb + parentage and individual RRS

*Individuals that fall within these brood year and life stage combinations have been sampled and sent to the Hagerman Genetics Laboratory. Totals by defined categories will be available once inventory is complete.

Parentage and Reproductive Success Analysis

All individuals passing data quality thresholds will then be analyzed in COLONY v2.0.6.6 (Jones & Wang, 2010), which uses likelihood-based methods to assign parent-offspring trios, single parent-offspring pairs, and sibling relationships (sibships). For broodyears 2015-2016 only sibships will be constructed from the juvenile genotype data as no adults from the corresponding brood years (i.e. potential parents) were genotyped. Therefore, at a minimum, each brood year will undergo sibship reconstruction analysis, and then where data allows, full parentage assignment.

Once assignments are filtered by probability thresholds, we will estimate the number of unique parental genotypes for each brood year. This will translate to the number of effective breeders, or Nb, for each brood year. The inferred Nb will then be divided by four, the historic fish-per-redd ratio, to estimate the number of redds, which will be compared to the actual redd count in corresponding brood years. Additionally, for the years in which we can perform full parentage assignment, we intend to estimate individual reproductive success and compare relative reproductive success on the basis of phenotypic and environmental data, such as sex, length or return timing, where data allows.

Results & Discussion

If the resulting Nb/4 estimates from pedigree reconstruction analyses align with redd count data from spawning ground surveys, as was observed in initial analyses of brood years 2015 & 2016 (Galbreath et al., 2018), then this would suggest the increasing fish-per-redd ratios in recent

years are, in fact, a product of reduced spawning success and not undercounting of redds. This, especially when compared to the same estimates for coho salmon, may have important implications for management of spring Chinook in the Warm Springs River basin. In fact, the WSNFH is set to begin incorporation of natural-origin spring Chinook in broodstock for supplementation this upcoming brood year (BY2021). A finding of reduced spawning success across the study brood years for spring Chinook, while coho salmon display relatively stable rates of productivity, might support continued focus on more targeted supplementation efforts for spring Chinook. We anticipate providing summative results and interpretation to co-managers by mid-2022.

vi. Project Objective #5 – Evaluate factors affecting minijack production

Introduction

Female Columbia River spring Chinook salmon populations return from the ocean as mature adults almost exclusively at ages 4 and 5. Adult male spring Chinook salmon return at similar ages, though with an additional proportion maturing after only a single ocean winter at age-3, referred to as jacks (Healey, 1991; Myers et al., 1998; Quinn, 2005). In addition to early maturation as age-3 jacks, male spring Chinook salmon may precocially initiate maturation prior to out-migration to the ocean, maturing at age-1 (precocial parr or microjacks) or at age-2 (minijacks; Larsen et al., 2013). While microjacks have been observed among both natural-origin and hatchery-origin cohorts, they are presumed to compose a minimal proportion of a given brood year's progeny and are therefore of minimal concern to fisheries and hatchery managers (Gebhards, 1960; Larsen et al., 2013; Mullan, Rockhold, & Chrisman, 1992). Similarly, natural-origin juvenile males appear to precociously mature as minijacks at inconsequential rates; however, this does not appear true for hatchery-reared juvenile males.

A series of studies investigating maturation trajectories in a hatchery environment discovered significant proportions of the male juvenile progeny, sometimes as high as 70%, undergo precocious maturation as minijacks (Beckman & Larsen, 2005; Larsen, Beckman, & Cooper, 2010; Larsen et al., 2004; Larsen et al., 2006). It appears likely that the full magnitude of precocious maturation within and across hatchery programs has generally been overlooked due to the difficulty in externally distinguishing hatchery smolts that have initiated maturation from their non-maturing counterparts (Beckman & Larsen, 2005). This would have significant implications to management as minijacks do not migrate to the ocean to mature, and also likely experience reduced spawning success as sneaker males, directly impairing objectives shared across many regional hatchery programs – facilitating natural population supplementation and providing large adults for fisheries (Beckman & Larsen, 2005; Clarke & Blackburn, 1994; Shearer & Swanson, 2000; Zimmerman, Stonecypher, & Hayes, 2003).

Given the increased incidence of precocial maturation amongst hatchery-reared juveniles, it has often been assumed that environment largely influences propensity to precocially mature. For example, conditions characteristic of hatchery environments, such as high feeding rates and elevated water temperatures relative to natural conditions, are known to accelerate growth and lead to high adiposity levels, triggering endocrinological and physiological processes that activate precocious gonadal maturation among juvenile males (Clarke & Blackburn, 1994; Rowe & Thorpe, 1990; Rowe, Thorpe, & Shanks, 1991; Saunders, Henderson, & Glebe, 1982; Shearer, Parkins, Gadberry, Beckman, & Swanson, 2006; Shearer & Swanson, 2000; Silverstein, Shearer, Dickhoff, & Plisetskaya, 1998; Silverstein, Shimma, & Ogata, 1997; Thorpe, 2004). However, there is increasingly ample evidence for a heritable genetic component, both for adult age-at-maturity (Carlson & Seamons, 2008; Garant, Fontaine, Good, Dodson, & Bernatchez, 2002; Gjerde, 1984; Hankin, Fitzgibbons, & Chen, 2009; Herbinger & Newkirk, 1990; Iwamoto, Alexander, & Hershberger, 1984; Waters et al., 2020; Wild, Simianer, Gjoen, & Gjerde, 1994), as well as for precocious male maturation in freshwater (Duston, Astatkie, & MacIsaac, 2005; Easton, Moghadam, Danzmann, & Ferguson, 2011; Herbinger & Newkirk, 1990; Myers & Hutchings, 1986; Piche, Hutchings, & Blanchard, 2008; Silverstein & Hershberger, 1992; Thorpe, Morgan, Talbot, & Miles, 1983).

To look further into the factors associated with precocious minijack production, and to test possible modifications to hatchery management that might reduce minijack rate without negatively affecting juvenile post-release survival and smolt-to-adult return rates, we initiated the following series of BSE Project-funded studies:

a. Effect of sire age on minijack rate

To address the potential heritable, genetic component of precocious maturation, we designed a study to test whether the age of spring Chinook broodstock affected the rate at which their male progeny matured as age-2 minijacks. Working with the Cle Elum Supplementation Research Facility (CESRF) over three consecutive brood years (BY2014-2016) we performed factorial matings among age-4 and age-5 female broodfish crossed with age-1 precocial parr (microjacks; BY2015 and BY2016 only), age-3 jacks, age-4 adult and age-5 adult male broodfish. The composition of each matrix varied according to the availability of broodfish each spawning day, with age-5 males being relatively rare (Table vi.a.1). Unfortunately, the number of families involving age-5 female broodfish was insufficient for estimation of a female age effect, so analyses were limited to comparisons among families with age-4 dams.

Table vi.a.1. *Number of broodfish per age (in parentheses) and number of families per broodstock age cross type, by brood year. Summary only includes crosses which produced a minimum of 10 male progeny.*

		Sire			
Brood Year	**Dam**	**Age-1 microjack**	**Age-3 jack**	**Age-4**	**Age-5**
		(n = 0)	(n=8)	(n=12)	(n=3)
2014	Age-4 (n=19)	na	20	23	6
	Age-5 (n=3)	na	2	3	1
		(n=9)	(n=10)	(n=15)	(n=1)
2015	Age-4 (n=25)	18	19	31	2
	Age-5 (n=1)	1	1	1	0
		(n=8)	(n=9)	(n=10)	(n=5)
2016	Age-4 (n=22)	18	21	20	15
	Age-5 (n=5)	3	5	5	2

Progeny from these test crosses were reared following standard CESRF protocols. Then in April 2016, 2017 and 2018, approximately 19 months following egg fertilization for each brood year, smolts from test crosses were sacrificed for the collection of: (1) blood sample to quantify plasma 11-Ketotestosterone (11-KT, Medeiros et al., 2018), (2) tissue sample (fin clip) for genetic parentage analyses, (3) phenotypic sex following dissection and visual inspection of the gonads, and (4) fork length and total body weight.

Tissue samples were sent to the Hagerman Genetics Laboratory at the Hagerman Fish Culture Experiment Station for DNA extraction using a Chelex 100 method (Sigma-Alrich), followed by genotyping at a panel of 298 single nucleotide polymorphism (SNP) markers (Janowitz-Koch et al., 2019) using a genotyping-in-thousands by sequencing approach (GTseq; Campbell et al., 2015). Individual genotypes passing quality filters were then analyzed in the pedigree reconstruction program SNPPIT (Anderson 2010), whereby the individual male smolts were run against a parental pool composed of broodfish genotypes from the appropriate brood year to assign each smolt back to its parent pair.

Blood plasma samples were assayed using an 11-KT ELISA kit (Biosense, Cayman Chemical, Ann Arbor, Michigan), previously validated for *Oncorhynchus* species (Caldwell, Pierce, Riley, Duncan, & Nagler, 2014). Male fish maturation status was determined from the distribution of plasma 11-KT levels at the final sampling using a modification of the method described by Medeiros et al. (2018).

Finally, we used a binary generalized linear mixed modeling (GLMM) approach to estimate covariate effects on minijack rate among male smolts within each full-sib progeny group (Mcculloch & Neuhaus, 2014; Nelder & Wedderburn, 1972). In addition to evaluating the central hypothesis, of whether sire age significantly influences minijack rate, the modeling approach also evaluated potential effects of spawn date of the cross, dam size (POH), and average egg size of the dam.

Generally, the data analyzed to date seem to negate our initial hypothesis, such that sire age had no significant, predictive effect on minijack rate. If any limited association did exist, it seems to have been masked by very high inter-family variability for minijack rate, which ranged from ~0% to ~100% among essentially all sire ages and brood years. In fact, the only significant contrasts by sire age were found in BY2016, between age-1 sires and age-4 sires, as well as between age-4 sires and age-5 sires. However, the effects were in opposing directions in these two contrasts, such that in the age-1 v. age-4 comparison the younger sires had more minijack offspring, whereas in the age-4 v. age-5 comparison the younger sires had less minijack offspring. Additionally, none of the auxiliary variables (spawn date, dam POH, dam egg size) seemed to significantly explain minijack rate. Lastly, the GLMMs attributed a substantial amount of among-family variability to individual parents, suggesting the individual dam and sires may have intrinsic minijacks rates that are different from the average individual, but which are not apparently linked to age.

Ultimately, we believe the high variability by family, and the apparent strong random effects of both sire and dam suggest there is a heritable component to precocious maturation, but it is far more complicated than initially assumed and cannot be totally predicted by age of parent. This has important implications for guiding hatchery management, particularly with regard to the selection of fish for broodstock. We intend to further investigate the genomic architecture underlying precocious maturation by performing a genome-wide association study over the next two contract years and using study fish from our photoperiod studies (see Section III.vi.e).

Data analyses of this dataset are currently being finalized and a manuscript has been drafted for submission to a scientific journal. We anticipate the manuscript will be ready for submission for review within the next two months.

b. Effect of feed supplementation with TTA on minijack rate

In a previous study conducted on hatchery-reared juvenile Chinook Salmon at CESRF (Larsen et al., 2006; Larsen et al., 2013), feeding rate was reduced for some fish below the standard rate, which resulted in a 20% to 55% reduction in minijack rate versus production fish, with magnitude of the reduction generally increasing with increase in duration of ration reduction. However, reduced feeding also reduced final smolt size. Reduction in pre-release growth rate and size of smolts is generally known to be associated with increased mortality during out-migration and lower adult return rates (e.g. Beckman et al., 1999; Connor et al., 2004; Faulkner, Bellerud, Widener, & Zabel, 2019; Zabel & Achord, 2004). Several recent studies in Atlantic salmon (*Salmo salar*) have investigated an alternative means to repress precocious maturation in juvenile male salmonids - feeding fish a diet supplemented with tetradecylthioacetic acid (TTA). When juvenile Atlantic Salmon were provided feed treated with TTA during the spring, precocious male maturation the following fall was reduced by 30% to 60%, without reducing growth (Alne, Thomassen, Sigholt, Berge, & Rorvik, 2009; Arge et al., 2014).

TTA, a commercially available nutritional supplement, is a 3-thia saturated fatty acid which upregulates cellular fatty acid oxidation, causing an increase in liver and muscle lipid metabolism similar to fasting. As a supplement in salmonid diets, TTA stimulates fatty acid oxidation and reduces lipid stores (Alne et al., 2009; Arge et al., 2014; Gjoen et al., 2007; Moya-Falcon et al., 2004) and leads to reduced sexual maturation in male post-smolt age 1+ Atlantic Salmon (Alne et al., 2009). We designed a study involving TTA treated feed to better understand the role of lipid energy reserves in precocious maturation of hatchery-reared male spring Chinook Salmon smolts, with the objectives to:

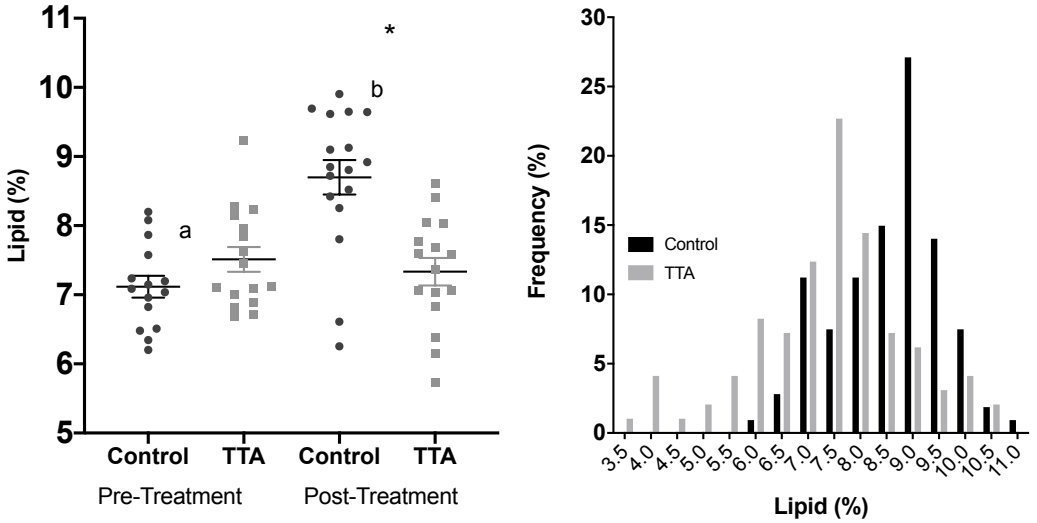
1) Determine whether dietary supplementation with TTA during the fall critical period reduces minijack maturation in male spring Chinook Salmon.
2) Assess changes in body composition and growth associated with dietary supplementation with TTA.

In March 2017, approximately 2,000 spring Chinook Salmon swim-up fry were transferred from CESRF to the Aquaculture Research Institute at the University of Idaho, Moscow ID. Fish were held in a common tank under simulated natural photoperiod, water temperature ranged from 12-15 ^{o}C, and the fish were fed a standard hatchery diet. On July 31, 2017, 1,600 fish were randomly collected and distributed among 16 identical 60 L rearing tanks (100 fish per tank), half randomly assigned as Control (n=8), and the other half as TTA treatment (n=8). Beginning Aug 1, 2017, the treatment fish were fed the standard diet top coated with 0.5% TTA dissolved in fish oil; the control fish received the standard diet with fish oil minus the TTA.

Prior to initiation of the TTA treatment, a random sample of 12 fish from each tank was over-dosed in anesthetic, lengths and weights recorded, and the carcasses were frozen for assessment of whole-body lipid levels. Following cessation of the TTA treatment on November 2, 2017, another random sample of 12 fish from each of the 16 tanks was collected and the fish were similarly sampled. Rearing of the remaining fish continued until the smolt stage (April 11, 2018), when the fish were sacrificed, measured for length and weight, blood sampled for plasma 11-KT analysis to determine maturation status.

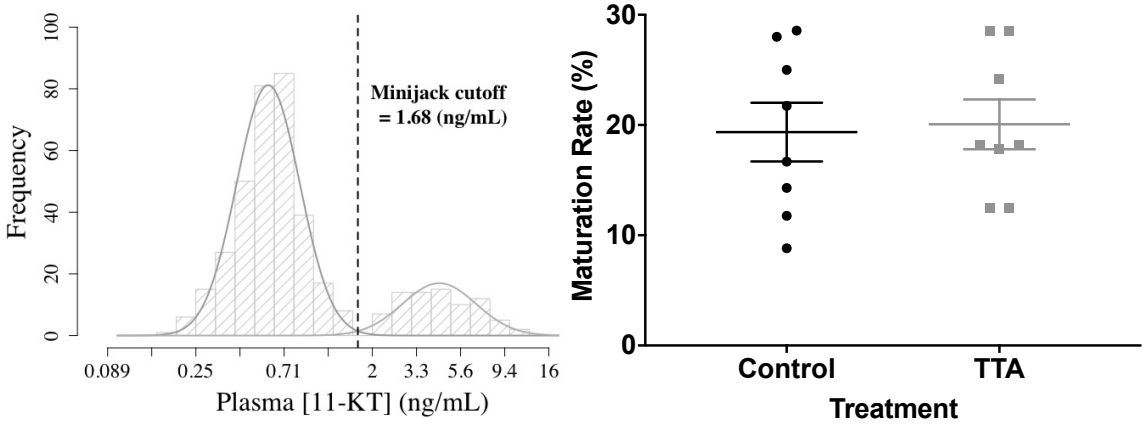
Individual carcasses were dried to constant weight, homogenized, and equal amounts pooled from each tank to determine the lipid level. Average whole body lipid content at the end of the TTA treatment was significantly lower for treated versus untreated fish. There was a strong correlation between the percentage water in samples and the lipid percentage, which allowed inference of lipid levels in individual fish carcasses. The TTA treatment shifted the distribution of lipid levels downward, from a mode of 9% in the control treatment to a mode of 7.5% in the TTA treatment (Figure vi.b.1).

Figure vi.b.1. *Lipid levels by treatment group before and after treatment, where TTA indicates feed was supplemented with tetradecylthioacetic acid.*



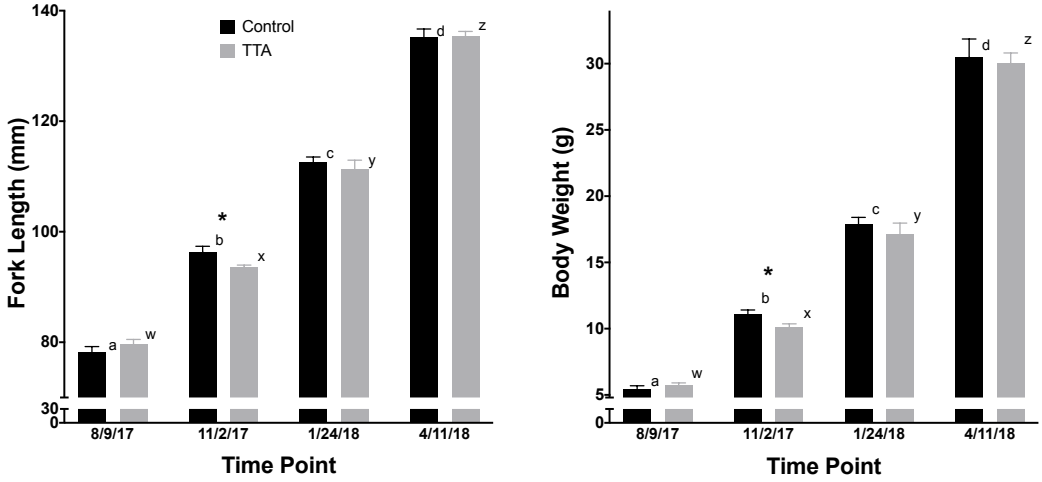
Results for male April plasma 11-KT concentration were strongly bimodal, with a cutoff value of 1.68 ng/mL (Figure vi.b.2). Below this value males were characterized as non-maturing, and above this level as maturing minijacks. However, there was no difference in minijack rate associated with the TTA treated feed; minijack rate for both control and treatment tanks averaged approximately 20% (Figure vi.b.2).

Figure vi.b.2. *Distribution of blood plasma 11-KT levels amongst study fish after final sampling in April, showing clear bimodality and an inferred cut-off of 1.68 ng/mL (left panel). Minijack rate by treatment group given blood plasma 11-KT levels at final sampling in April (right panel).*



The TTA treated fish showed a slight reduction in size relative to controls during the treatment period, but recovered to similar size by the final sampling at the smolt stage in April (Figure vi.b.3).

Figure vi.b.3. *Fork length and body weight of individuals representing each treatment group throughout the course of the study.*



The results of this study do not support a major role for lipid reserves in the regulation of precocious male maturation at the minijack stage in spring Chinook salmon over the range of lipid levels achieved in the study. The fall lipid levels in this study were comparable to levels found in previous studies on hatchery juveniles, but higher than fall lipid levels in wild fish (Beckman et al., 2000; Larsen et al., 2006; Larsen et al., 2013; Pearsons, Johnson, Ben James, & Temple, 2009; Shearer et al., 2006). The distribution of lipid levels at the end of TTA treatment showed that there was a 12.3-43.7% greater proportion of individuals in the Control vs TTA group over the range of 6.0-9.5% lipid, suggesting that, if there is a threshold lipid level for initiation of maturation, it is below this range. Although lower lipid levels can be achieved by long-term feeding of specially formulated very low lipid diets (Shearer et al., 2006; Shearer & Swanson, 2000), such an approach would not be practical under production hatchery conditions. Therefore, we do not propose to pursue this line of research further at this time. Nevertheless, the results of this study contribute to knowledge about the roles of size, growth, and lipid levels in the regulation of precocious maturation in Chinook salmon (Larsen et al., 2006; Shearer et al., 2006; Silverstein et al., 1998), and were compiled into a manuscript and submitted to a peer-reviewed journal. Initial reviews have been received indicating that the manuscript may be acceptable after revisions, and these revisions are in process.

c. Growth of precociously maturing versus non-maturing smolts

Precocious male maturation in Chinook Salmon has begun to attract notice (Harstad, Larsen, & Beckman, 2014; Larsen et al., 2004; Medeiros et al., 2018). Both subyearling (aka precocious parr) and yearling precocious males (aka minijacks) exist in some spring Chinook salmon populations (Clarke & Blackburn, 1994; Foote, Clarke, & Blackburn, 1991; Gebhards, 1960; Mullan et al., 1992), though minijacks occur at much higher rates (Larsen et al., 2004). The incidence of minijacks in wild stocks of spring Chinook salmon is poorly characterized but thought to be less than 5% (Gebhards, 1960; Larsen et al., 2004; Mullan et al., 1992). In contrast, the proportion of males maturing as minijacks in some hatchery and experimental populations of spring Chinook Salmon ranges from 11% to 80% (Foote et al., 1991; Harstad et al., 2014; Mullan et al., 1992), making it apparent that hatchery rearing is increasing the incidence of precocious maturation. This has important implications for hatchery programs and

illustrates the strong influence environmental variation (e.g., water temperature, feeding rate, food composition) can have on development in Chinook salmon.

Under favorable conditions, juvenile fish are faced with a physiological choice – to smolt and emigrate to the ocean, or to mature and stay in freshwater – and it appears that for many of the fish in the hatchery environment, the latter choice is preferred. Completion of the maturation process depends on the status of the energy stores of the individual during critical periods throughout the year and can be arrested annually (Thorpe, 1994). In general, it is believed that a fish will mature if the rate of acquisition or attainment of a particular trait (e.g., growth rate, adiposity, size) during a critical period exceeds a genetically determined threshold. Consequently, such traits are known as threshold traits, as surpassing the genetically determined value (the threshold) of a particular trait (or traits) results in adopting a different life history pattern than is typically observed. Hence, maturation (both initial and any subsequent) is regulated by inhibition, and depends on an individual's physiological status, genetic makeup, and their current environment (Piche et al., 2008; D. Spangenberg, Larsen, Gerstenberger, Brun, & Beckman, 2014; D. K. Spangenberg et al., 2015; Thorpe, 1994). During the critical window, individuals evaluate their developmental potential in terms of said threshold traits and will initiate maturation if they are above the threshold values for the necessary traits or delay maturation if they are not.

The protected, controlled environment in hatcheries permits fish to reduce the proportion of energy normally channeled into maintenance (*e.g.*, foraging, competing for resources, predator avoidance, and combating parasites and pathogens) which creates a surplus of energy, accelerates growth, and increases energy stores (Thorpe, 1991). This allows younger fish to surpass the threshold for the necessary trait(s) during an earlier critical period and mature up to several years prior to their average wild counterparts. To fully understand the age and size of onset and completion of maturation in the hatchery setting, we need to understand the underlying mechanisms triggering puberty. We know that puberty is governed by many factors such as: genetics (Larsen et al., 2013; D. K. Spangenberg et al., 2015), metabolic cues such as growth rate and energy stores at critical periods (B. Campbell et al., 2006; Rowe et al., 1991; Shearer & Swanson, 2000; Silverstein et al., 1998; Thorpe, 2007; Unwin, Poortenaar, Rowe, Boustead, & Porter, 2004), and environmental signals (Bromage, Porter, & Randall, 2001; D. Spangenberg et al., 2014). But even with all of this knowledge, the underlying mechanisms, including the exact timing of the critical periods, are not understood well enough to predictably reduce the proportion of males maturing as minijacks to rates comparable with wild males.

To better characterize the timing and magnitude of changes in the growth and adiposity characteristics of juveniles during the maturation decision period and how the timing and magnitude might vary among individuals, we initiated a study to follow individual growth of a cohort of spring Chinook Salmon juveniles at the CESRF. In fall of 2017, multiple test crosses involving single-pair matings of CESRF spring Chinook Salmon broodfish (SH; returning adults from the hatchery supplementation program smolt releases) were produced as part of the annual monitoring program to assess fry size and survival rates for CESRF broodstock. Among these crosses, 11 families, each involving an age-4 SH female crossed to an age-4 SH male were identified. When the embryos reached the swim-up stage in late February 2018, 70 fry per family were pooled into a common 500-L circular fiberglass tank for rearing. On June 27, 2018 each of the surviving fish (n=739) was tagged with a 9 mm PIT tag for individual identification, and a tissue sample (fin clip) collected for genotyping and parentage analysis (to identify genetic sex and the respective family of each individual), then the fish were returned to the rearing tank. Beginning July 11, the fish were anesthesized, measured for length and weight, then randomly redistributed among two rearing tanks at equal density per tank. Size sampling and random

redistribution of the fish between the two rearing tanks was repeated every three weeks until November 14, 2018; then the fish were sampled again after 4 more weeks on December 12, 2018. Afterwards, the fish were transferred from the fiberglass tanks to CESRF concrete raceway #18 for rearing over winter.

On April 9-10, 2019, the fish were collected from the raceway, sacrificed in an overdose of anesthetic, measured for size (fork length and weight) and calculation of condition factor and instantaneous growth rate, and blood sampled for determination of plasma 11-KT concentration. Maturation status of each individual was assigned using a modification of the method described in Medeiros et al (2018); the only change was to evaluate modality of the distribution using the excess mass statistic instead of the dip test. Livers were weighed for estimation of hepatosomatic index (HSI) and then frozen for later measurement of liver IGF-1, IGF-2, IGFbp1, and IGFbp2 concentration, and the carcasses retained for measurement of whole body lipid levels. Plasma and tissue samples were frozen over dry ice, transported to the University of Idaho, Moscow ID, and stored at −80°C until laboratory analysis. Additionally, during the September 12, 2018 size sampling, 20 fish from each family (a random selection of 10 fish per sex) were placed in an adjacent tank following size measurement. Afterwards, these fish were sacrificed in an overdose of anesthetic then tissue sampled as described above for the April 2019 sampling.

Morphological and family data were analyzed to evaluate how accurately the parameters predicted an individual's likelihood to mature precociously. First, generalized linear mixed models were constructed in RStudio using the glmmTMB package (version 1.0.2.9000) using various combinations of the available parameters (e.g., body weight, fork length, condition factor) with or without the effect of family accounted for as a random effect. When evaluating the effect of a particular morphological parameter (e.g., body weight, fork length; see Table vi.1 below) there was a strong trend for the AIC score to decrease over time; however, this is misleading as they were most likely collected after the decision to mature (or not) had already occurred. Thus, while it is undeniable that the parameters measured later on in the study are better indicators of an individual fish's maturation status as a minijack (or not), they are not biologically-relevant predictors.

Additionally, the general trend was for family to lower the AIC score of any particular model; however, neither version of a model was particularly accurate until after a decision to mature had been made. When the different versions of models (with relatively low AIC values compared to other models evaluated; see Table vi.c.1 below) were cross-validated to determine how accurately the models predicted the maturation status of actual fish, no model was more than 70% accurate until the final sampling in April; with most being between 60-70% accurate at any sampling point regardless of whether or not the random effect of family was incorporated into the model. Considering this, it was concluded that while family does appear to be important, it is not the deciding factor in determining which males will mature as minijacks. This individual component was also observed in the very wide variation in minijack rate among families within cross-types (parents of given ages) in the previously described study (Section III.vi.a) to evaluate the effect of hatchery broodstock age on minijack production. It is likely that what we are seeing as an effect of family is actually due to an inherited gene (or set of genes) that are important in deciding age at maturity in a variety of situations (e.g., developmental plasticity). Furthermore, it appears that the morphological parameters measured herein are not good predictors of maturation, at least not without knowing more about the genetic makeup of the individual than its family. Studies are being designed to investigate which genes are involved in the decision to mature, with the hope that the results will indicate which physiological factors are contributing to the initiation of maturation.

Table vi.c.1. *AIC Scores and P-values for the models predicting an individual fish's probability of maturing as a minijack (MJ) based on the body weight at each sampling event when family was not accounted for (1) and when family was accounted for as a random effect (2). Also noted are the respective model's accuracy as calculated by cross validating the model with actual fish body weights as well as the p-values for testing for non-uniformity, overdispersion, and outliers.*

AIC Scores and P-values for BY17 Growth Models

Model 1: MJ ~ Wt
Model 2: MJ ~ Wt + (1|Family)

Sampling Event	AIC		Model Accuracy		Uniformity[1]		Dispersion[1]		Outliers[1]	
	1	2	1	2	1	2	1	2	1	2
1	308.5	304.6	63.9%	68.2%	0.14	0.64	0.97	0.93	1.0	1.0
2	307.0	303.7	64.4%	65.6%	0.62	0.94	0.93	0.93	1.0	1.0
3	302.2	299.7	64.6%	67.9%	0.50	0.89	0.90	0.98	1.0	1.0
4	298.1	295.4	67.5%	70.0%	0.53	0.87	0.97	0.95	1.0	1.0
5	297.6	293.4	68.5%	72.0%	0.31	0.93	0.98	0.70	1.0	1.0
6	294.1	289.4	67.4%	71.5%	0.48	0.78	0.99	0.65	1.0	1.0
7	292.9	288.7	67.1%	71.2%	0.45	0.74	0.98	0.64	1.0	1.0
8	311.6	310.1	67.9%	71.5%	0.62	0.90	0.82	0.94	1.0	1.0
9	203.2	182.3	78.6%	84.7%	0.64	0.39	0.82	0.37	1.0	1.0

[1] P-values above 0.05 indicate that the null hypothesis for testing non-uniformity, overdispersion, and outliers in the residuals were all accepted, implying that the variability in the data agrees with that assumed by the model

As our analyses indicated that incorporating family did little to increase the predictive abilities of the models evaluated, and as models that did not account for family passed tests of variance (e.g., uniformity, dispersion and outliers; see Table vi.c.1), all further data analyses were conducted using data pooled across families. Data for fork length, body weight and condition factor of males subsequently determined to be maturing minijacks (based on 11-KT analyses of blood sampled at the smolt stage, April 10, 2019) versus non-maturing smolts are illustrated in Figure vi.c.1. When just comparing males, the maturing males were significantly larger in both body weight and fork length across all sample dates. Condition factor of maturing minijacks also tended to be larger than for non-maturing males throughout the study, although these differences were statistically significant only for the initial (7/11 through 8/22) and the final (12/12 and 4/10) sample dates. Weight specific growth rate (WSGR) was highly variable and was surprisingly similar among all of the maturation status categories; only the final sampling period (12/12 through 4/10) showed large and relatively consistent WSGRs, though the significantly larger WSGR (and body weight for that matter) for the minijacks is most likely due to the development of the gonads rather than an increase in somatic weight. As anticipated, the juvenile males within families that initiated maturation were among those which were larger and had a higher condition factor over the course of the study. However, given the wide variation in the data, within and across families, these differences were insufficient to be reliably used to distinguish maturation status of an individual, even as late as the smolt stage.

Something that we did not anticipate was that the difference in the parameters we measured were already apparent in July when tagging and sampling of the juveniles was initiated, about 2-3 months before the proposed maturation decision period. However, maybe this should have been expected. In theory, the decision to mature (or delay and continue accumulating energetic resources) occurs approximately one year prior to spawning; logic follows that the thresholds of

whatever physiological parameters that are driving the decision must be surpassed before or perhaps just after the decision window begins. Thus, it can be expected that the parameters could be significantly different for a period of time leading up to the decision window itself. However, 2-3 months does seem like a long time, especially considering that July is only 4-5 months post-swim up and it is in the middle of the summer, when fish are growing fast as a result of warmer water temperatures and abundant food sources. While much of the story lies in the interaction between the hatchery environment and the genetics, further investigations looking at growth should target earlier time points. This is made difficult by the fish being less likely to survive tagging and repeated samplings but could add important insight into the development of a minijack.

Analysis of the pituitary FSH mRNA levels at the September 12, 2018 sampling did not reveal a bimodal distribution, implying this measure to not be useful as an early indicator of maturing versus non-maturing individuals (Figure vi.c.2). However, it could be that September is too early in the process and that sampling at a later date (perhaps in early winter) may provide measures that better identify maturation status. Laboratory analyses for liver mRNA levels of IGF-1, IGF-2, IGFbp-1, and IGFbp-2 are ongoing for the Sept 12, 2018 samples.

Following completion and analysis of all data, an oral presentation and a manuscript for submission for publication will be produced.

Figure vi.c.1. *Body weight (A), fork length (B), condition factor (C), and weight-specific growth rate (D) over time in each maturation status category (females, immature males, and maturing minijacks) with the fish pooled across families. Boxes are Tukey outlier box plots; the midline represents the population median, whiskers indicate the minimum and maximum values, and points represent the suspected outliers. Within each sampling period or event, boxes sharing the same letter are not significantly different ($P < 0.05$). Significance among sampling events or periods is not noted.*

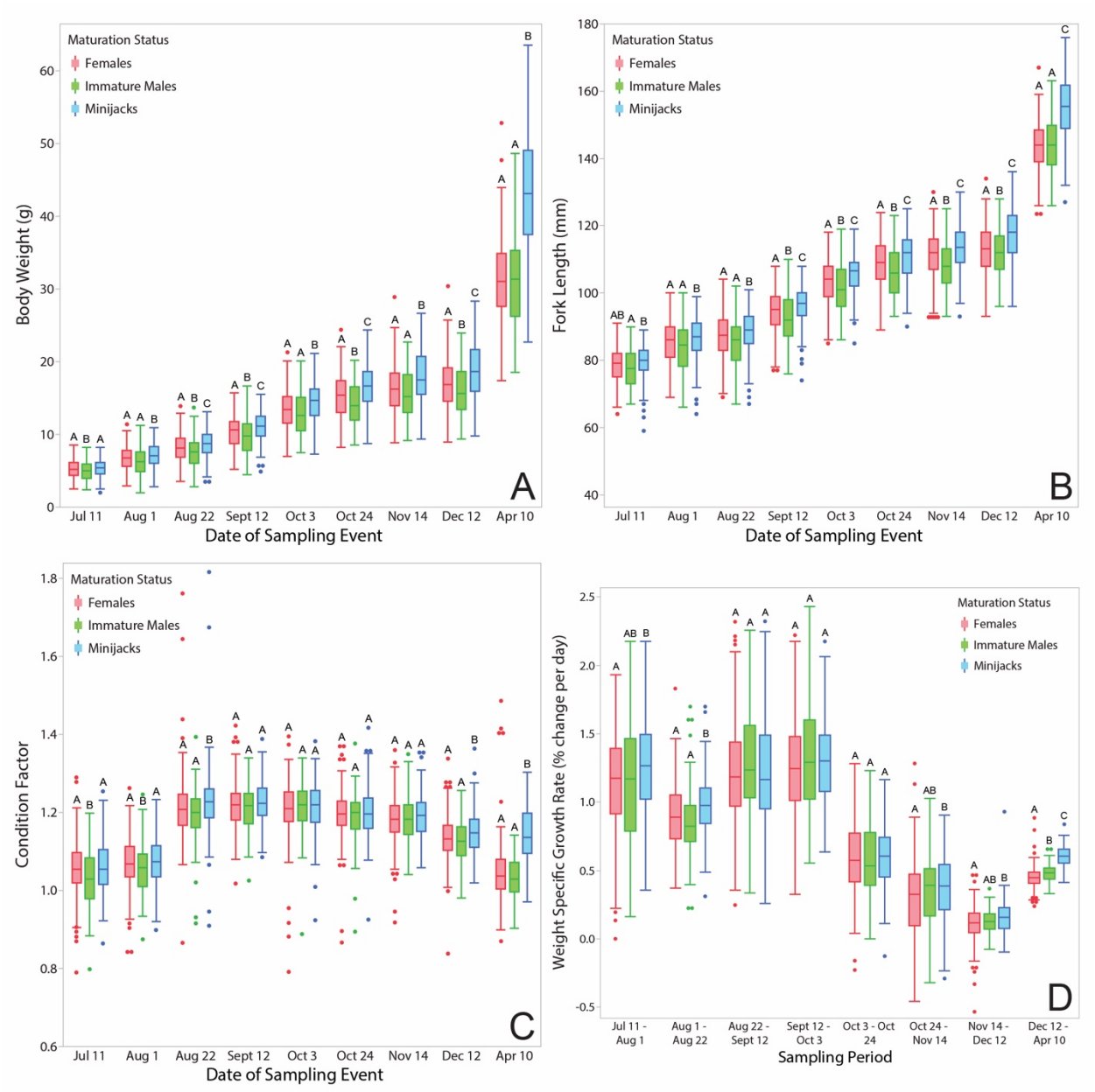
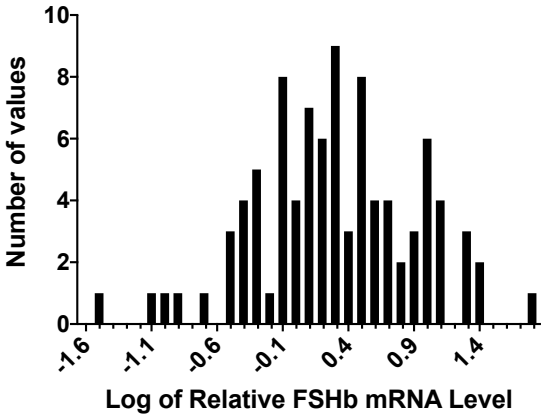


***Figure vi.c.2**. Histogram displaying relative follicle stimulating hormone beta (FSHb) mRNA levels in pituitaries collected from male juvenile spring Chinook Salmon on September 12, 2018. The distribution is unimodal and thus indicates that FSHb cannot be used to discriminate between immature and maturing individuals at this time point.*



d. Effect of targeted feed deprivation on minijack rate

While supplementation of feed with tetradecylthioacetic acid (TTA; Section III.vi.b) appeared to have little effect on propensity to initiate precocious maturation, questions surrounding the effect of food rationing on minijack rate remained. In fact, previous investigations of feed rationing in the same study system (spring Chinook at the CESRF) discovered notably lower rates of precocious maturation among juveniles that were reared under reduced rationing regimes (Larsen et al., 2006). Juveniles reared on these low feeding regimes, however, were significantly smaller than the typical production smolt. Given known relationships between size at emigration and survival, as well as smolt-to-adult return rates, (Beckman et al., 2017), it was determined that the reduction in minijack rate – while significant – did not override the potential reduction in survival. Therefore, the reducing feeding ration was not incorporated into hatchery management practices. Based on these findings, we designed a more drastic, but shorter period of total feed withholding, with the goal being to significantly affect growth and adiposity during the critical maturation time period (approximately a year prior to gonadal maturation, Berglund, 1995; Mangel, 1994; Silverstein et al., 1998; Thorpe, 1994), while still allowing enough time for compensatory growth.

Study fish were selected from the spring Chinook salmon supplementation hatchery line (SH) at CESRF in brood year 2018. Progeny from the SH line are part of an experimental line at CESRF, which is typically only reared to the fry stage to monitor for changes during the early life stages that might suggest the accumulation of domestication effects. Hence, these progeny are not part of production and are never released to the natural spawning environment. We randomly divided our study fish into treatment groups as follows: (1) ~1,250 fish (5 tanks, 250 fish per tank) designated “early feed deprivation,” in which fish were starved from Aug 8, 2019 to Sept 11, 2019, (2) ~1,250 fish (5 tanks, 250 fish per tank) designated “late feed deprivation,” in which fish were starved from Sept 11, 2019 to Oct 24, 2019, and (3) ~1,500 fish (6 tanks, 250 fish per tank) were designated “control,” in which fish received normal continuous feeding following CESRF protocols. Body size measurements (fork length and weight) were taken at the following time points: (1) Aug 6, 2019 – just before fish were distributed into one of the 16 treatment tanks, (2) Sept 11, 2019 – 20 fish per treatment tank, (3) Oct 24, 2019 – 20 fish per

treatment tank, (4) Nov 20-21, 2019 – 30 fish per treatment tank. After the November sampling, study fish were moved to single raceway for overwinter rearing. Prior to transfer, however, each fish received an identifying fin clip (with/without adipose clip and with/without pelvic fin clip) that signified which treatment it experienced. Final sampling was initially planned for April 2020, but was delayed due to COVID-19. We therefore sampled a subset of 60 fish on May 12, 2020 to determine if sex and maturation status could be visually identified. Final sampling occurred on July 8-9 and 15, 2020, at which point all fish were lethally sampled for length, weight, and dissected to visually identify sex and maturation status. Testes were removed from a random subset of non-maturing (n=161) and maturing (n=81) males to create gonadosomatic curves and confirm visual assessments of maturation status.

Generally, we found significant reductions in minijack rate among male smolts experiencing either the early or late feed deprivation period compared to male smolts from the control group. The probability of maturation for the early and late feed deprivation group was 0.242 and 0.247, respectively, compared to 0.381 for the control group (Table vi.d.1). Additionally, compensatory growth following these periods of starvation allowed the treatment smolts to reach a final smolt size, within sex and maturation status, that was similar to the control smolts. In fact, only non-maturing males from the late treatment group displayed a statistically significantly different final length from non-maturing males in the early and control group; however, body weight and condition factor were not significantly different (Table vi.d.2).

Table vi.d.1. *Estimated probability of precocious maturation of male Chinook salmon smolts as minijacks from logistic regression analyses, including standard errors of the means (SEM) and lower (LCI) and upper (UCI) 95% asymptotic confidence intervals. Multiple comparisons were performed between treatments, with differences indicated by the letters a or b.*

Treatment	Probability of Maturing	SEM	LCI	UCI
Control	0.381[a]	0.021	0.341	0.423
Early	0.242[b]	0.022	0.202	0.288
Late	0.247[b]	0.022	0.207	0.292

Table vi.d.2. *Summary treatment values, mean ± standard error (SEM), for fork length, body weight and condition factor for female, non-maturing male and maturing male smolts, averaged across mean values per replicate tank at termination of the study in July 2020. Statistical comparisons performed between sex and maturation status, with differences indicated by the letters a or b; columns not sharing a letter differ significantly.*

Treatment	Sex and Maturation Status		Fork Length (mm)	Body Weight (g)	Condition Factor (K)
Control	Females	Average	175.24[a]	59.82[a]	1.13[a]
		SEM	0.63	0.74	0.03
Early	Females	Average	175.93[a]	61.39[a]	1.12[a]
		SEM	0.66	1.11	0.02
Late	Females	Average	173.53[a]	59.12[a]	1.15[a]
		SEM	0.72	1.08	0.03
Control	Non-Maturing males	Average	177.62[a]	62.44[a]	1.10[a]
		SEM	1.15	1.43	0.01
Early	Non-Maturing males	Average	178.37[a]	64.79[a]	1.13[a]
		SEM	0.99	1.77	0.01
Late	Non-Maturing males	Average	173.38[b]	58.50[a]	1.11[a]
		SEM	0.60	1.20	0.02
Control	Minijack males	Average	183.29[a]	83.53[a]	1.36[a]
		SEM	1.52	2.26	0.02
Early	Minijack males	Average	182.68[a]	83.29[a]	1.36[a]
		SEM	1.67	3.13	0.02
Late	Minijack males	Average	178.22[a]	80.13[a]	1.40[a]
		SEM	0.86	1.04	0.01

Interpretation of these results, however, must consider several circumstances that arose during this study, not the least of which was fin regrowth that made it difficult to identify fish to treatment group at final sampling. Nonetheless, the results from this study are quite promising and have prompted the design of a follow-up study using brood year 2020 SH-line fish. In this study, we plan to focus on a single 6-week starvation window starting in August 2021. Additionally, we intend to PIT tag every individual before movement to the shared raceway in November to avoid uncertainties in the data that were created by the fin clip identifier.

For a more detailed description of the study, including thorough review of data analyses and uncertainties, please consult CRITFC Technical Report 20-05 (Galbreath et al., 2020).

e. Effect of photoperiod manipulation on minijack rate

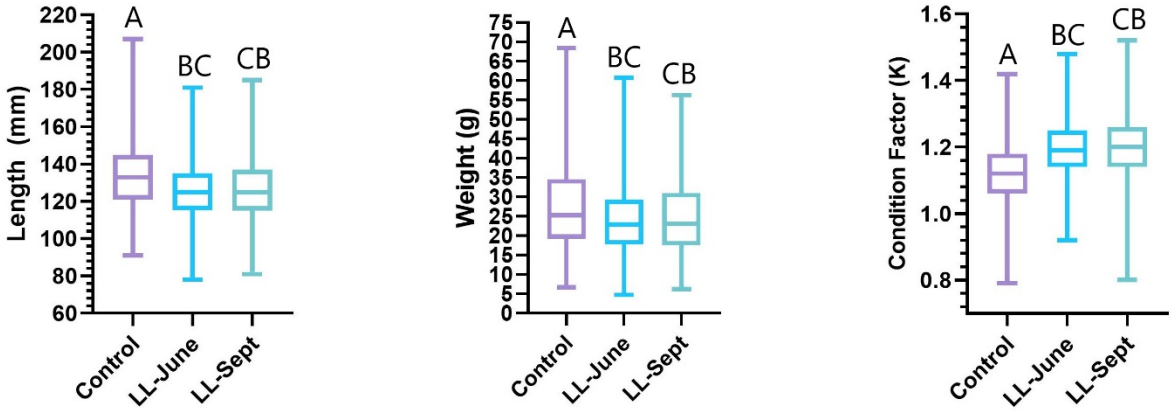
Manipulation of photoperiod is an effective tool used to decrease maturation rate among farmed salmonids, gadoids, and percids (Bromage et al., 2001; Davie, Porter, Bromage, & Migaud, 2007; Leclercq, Migaud, Taylor, & Hunter, 2010; Liu & Duston, 2019). Studies by groups in New Zealand (Chinook Salmon) and Canada (Arctic Char) have demonstrated that exposure to continuous light significantly reduces precocious maturation in both males and females (Liu & Duston, 2018; Unwin, Rowe, Poortenaar, & Boustead, 2005). Furthermore, the reduction in precocious maturation occurs without negatively affecting length, body weight, or condition

factor (when compared to control fish). While most studies have focused on preventing maturation in commercially reared fish prior to harvest, manipulating the natural photoperiod could serve as a cost-effective method of significantly reducing minijack rate in hatchery-reared age 1+ male spring Chinook Salmon smolts produced for supplementation of natural populations or for harvest mitigation, without reducing size at release.

Previous research has established that seasonal changes in day length provide the cues which trigger the initiation of maturation in many teleosts, including salmonids. Artificial manipulation of photoperiod can be used to control maturation in salmonids, either by altering its seasonal timing or by reducing its incidence. It is believed that the continuous light phase advances endogenous rhythms and reduces the incidence of sexual maturation by advancing and shortening the critical period during which puberty could be initiated. In essence, the abrupt application of a continuous light treatment tricks the fish into thinking they are "behind schedule" and have missed the window to decide to mature. As a result, maturation is inhibited, and resources are invested in somatic growth. Additionally, the continuous light treatment stimulates appetite and increases swimming behavior, both of which are known to increase growth.

As outlined for the feed deprivation study described above (Section III.vi.d), this study was initiated with the BY 2018 CESRF SH line juveniles - progeny from a large number of test spawnings between adult returns of supplementation program smolts. In February 2019, swim-up fry from these matings were pooled into fiberglass rearing tanks for initial rearing. In March 2019, 2500 fry were transferred to the Aquaculture Research Institute at the University of Idaho, Moscow ID for continued rearing. On June 21, 2019, 24 identical 60-L light-proof circular tanks were randomly stocked with 120 fish each (n = 8 replicate tanks per treatment). Each tank was fitted with a Kessil® LED aquarium light and controller, adjusted so the tanks experience no less than 300 lux at the surface of the water. Light meters were employed to ensure that the intensity is the same for all tanks and randomly checked weekly throughout the experiment. The fish were hand fed Bio-Oregon® feed (www.bio-oregon.com) of appropriate size based on the manufacture's recommendations. They were fed to satiation twice a day so none of the treatments differed in food availability. All treatments received the ambient, simulated natural photoperiod (45° N) during the light phase of the day. LL-June and LL-Sept treatments were subjected to continuous artificial light during the dark phase of the day (*i.e.*, 24 h light) beginning June 21st and September 21st, respectively, until the end of the experiment in March 2020. Control treatments were subjected to a simulated natural photoperiod, adjusted twice weekly to match the natural photoperiod in Moscow, ID. Prior to being distributed into the light-proof tanks, an initial sampling of weight (grams) and length (mm) was collected from fish to establish baselines. Subsequent samplings were conducted in September 2019 when the LL-Sept treatment began and again in March 2020 when all the fish were sacrificed. At all samplings, length and weight were recorded and a fin clip collected. Additionally, at the final sampling in March 2020, blood plasma was collected for 11-KT assay in males to assess maturation status. Fish sex was identified by visual assessment and, if necessary, through genetic assignment. On March 23, 2020 at the final sampling, length (mm) and weight (g) were collected from the remaining BY 2018 CESRF SH line fish (n = ~80 fish per tank). The tanks from each treatment were pooled and size comparisons between the control and light treatments were analyzed. Fish in the control group were larger in length and weight but had a reduced condition factor relative to the two light treatments. There was no significant difference in size parameters between LL-June and LL-Sept (Figure vi.e.1).

Figure vi.e.1. *Size parameters for control, LL-June (24-hour), and LL-Sept (24-hour) collected in March 2020 at final sampling.*



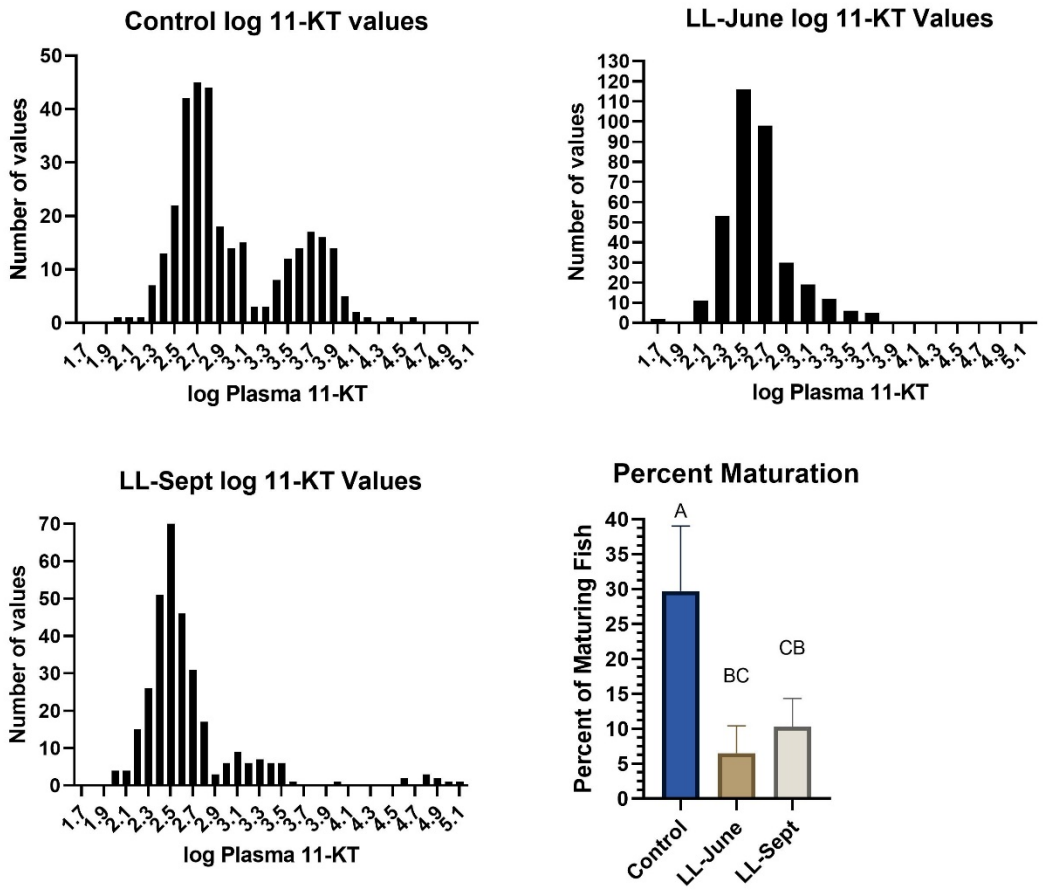
At the final sampling the two light treatment groups were slightly smaller in length and weight compared to the control. It's possible that maturation rates in each group could provide an explanation for the small differences. The control group had more maturing males, and those maturing fish were larger than immature males (Section III.vi.c.; Larsen et al., 2004; Medeiros et al., 2018). The small differences in length and weight indicates the light treatments had little or no effect on overall size at the end of the experiment. The increased condition factor in the treatment groups compared to the control warrants further investigation to test the effect of 24-hour light on smoltification, as a reduction in condition factor is associated with smoltification (Beckman et al., 1999; McCormick, 2012).

Analysis of 11-Ketotestosterone (11-KT) concentrations from plasma collected from males at the final sampling in March 2020 was conducted. Results of 11-KT concentrations from the control group showed a bimodal distribution pattern of values. Elevated 11-KT values in the upper mode represent the advancement of gonad maturation (minijacks) and fish in the lower mode represent non-maturing fish (smolts). Distributions of 11-KT values for LL-June and LL-Sept showed fewer males with elevated 11-KT indicating less fish sexually maturing (Figure vi.e.2). A minijack cutoff value of 1.62 ng/mL was calculated from the bimodal distribution of the control group and was used to assign fish from each group as being either a smolt or minijack. Fish with values below 1.62 ng/mL were assigned as smolts (immature) and fish above the value were assigned as minijacks (maturing). The percent of fish maturing was significantly higher in the control group compared to both the LL-June and LL-Sept groups (Table vi.e.1, Figure vi.e.2). LL-June showed a 76.3% reduction of maturing fish and LL-Sept showed a 65.7% reduction from the control.

Table vi.e.1. *Number of male fish from each treatment assigned as immature or mature and percent maturation in each group.*

Treatment	Total	Total Mature	Total Immature	Percent Mature (%)
Control	320	96	224	30.00
LL-June	352	23	329	6.53
LL-Sept	321	33	288	10.28
Total	993	152	841	46.81

Figure vi.e.2. *Log 11-KT distributions and percent maturation in male fish. Both 24-hour light treatments significantly reduced precocious maturation rates in BY 2018 CESRF SH line juveniles, with the LL-June treatment having the most significant effect. The low number of individuals with elevated plasma 11-KT in LL-June indicates the light treatment had a strong physiological effect that inhibited reproductive processes involved in maturation. LL-June = 24-hr light treatment starting in June 2019; LL-Sept = 24-hr light treatment starting in September 2019.*

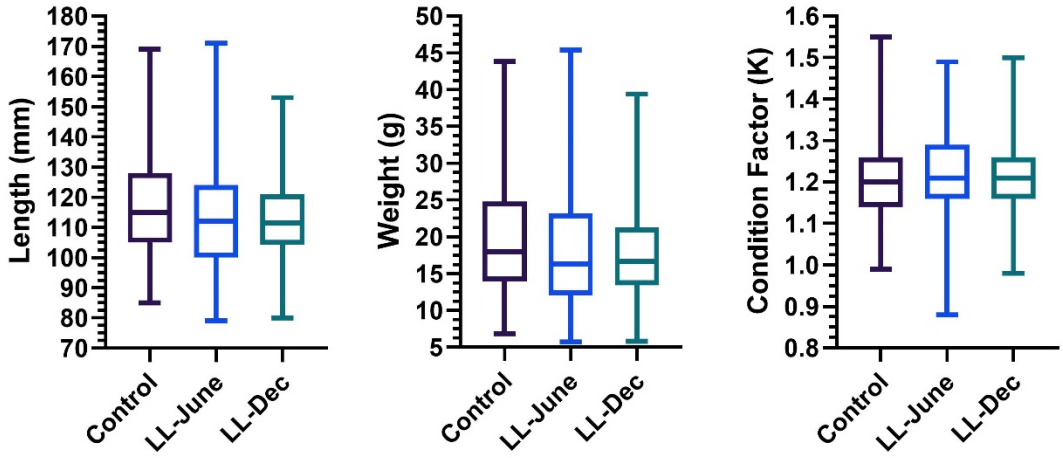


In the described study, a 24-hour light treatment beginning in June 2019 and continuing through March 2020 significantly reduced the number of male fish precociously maturing. The next step in the development of photoperiod as a method to reduce minijack rates is to determine the

minimum timeframe needed for 24-hour light to significantly reduce precocious maturation. In March 2020, 2,500 CESRF SH line fry (BY 2019) were transferred to the University of Idaho Aquaculture Research Institute and placed into fiberglass troughs. On June 21, 2020, 24 identical 60-L light-proof circular tanks were randomly stocked with 100 fish each (n = 8 replicate tanks per treatment). The tanks, rearing environments, and feeding regime were identical to that which was described for the BY2018 photoperiod study. All treatments receive the ambient, simulated natural photoperiod (45° N) during the light phase of the day. LL-June and LL-Dec treatments were subjected to continuous artificial light during the dark phase of the day (*i.e.*, 24 h light) beginning June 21st. LL-June is subjected to 24-hour light until the end of the experiment in March 2021. LL-Dec returned to a simulated natural photoperiod (45° N) on December 21, 2020. Control treatments are subjected to a simulated natural photoperiod, adjusted twice weekly to match the natural photoperiod in Moscow, ID. Sampling was conducted on December 21, 2020 when LL-Dec returned to a natural photoperiod, and length and weight were collected for 16 fish from each tank. Tanks from each treatment were pooled and analyzed to compared size parameters. No significant differences in length, weight, or condition factor were observed (Figure vi.e.3).

Final sampling is scheduled for the end of March 2021, at which point all remaining fish will be sacrificed. Length (mm), weight (g), and gill samples will be collected at each sampling. Gill samples will be collected to measure sodium-potassium ATPase activity as an initial assessment of smoltification. Additionally, at the final sampling in March 2021, blood plasma will be collected for measuring 11-KT concentrations to assess maturation status. Sex will be identified through visual gonad inspection and genetic assignment. Fin clips will also be collected for further genetic analysis.

Figure vi.e.3. *Size parameters for control, LL-June (24-hour), and LL-Dec (24-hour) collected in December 2020 for BY2019 photoperiod study.*



Genome Wide Association Study for Minijack Phenotype

Abundant production of Chinook salmon minijacks from hatcheries can be problematic for reasons described earlier. Understanding the genetic architecture underlying the minijack phenotype could prove beneficial for hatchery management practices. Previous research in closely related species have found strong genetic correlations with varying age of maturities (Gutierrez, Yanez, Fukui, Swift, & Davidson, 2015; McKinney et al., 2020; S. R. Narum, Di Genova, Micheletti, & Maass, 2018; Sinclair-Waters et al., 2020). Through much genetic work

has been done to identify genomic regions associated with age of maturity, to our knowledge this technique has not been applied to look for genomic associations with expression of the minijack phenotype in male Chinook Salmon.

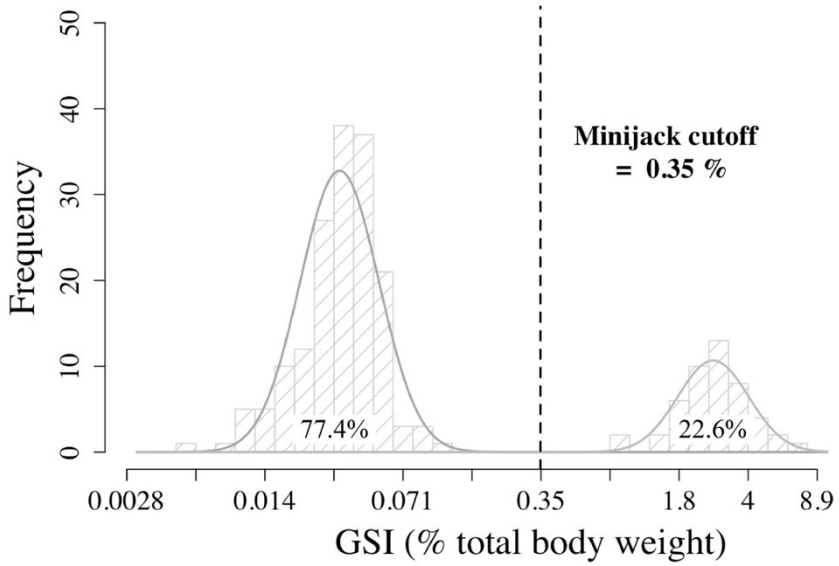
Fin clips from BY 2018/2019 CESRF SH line juveniles have been or will be collected as part of the previously described photoperiod experiments. DNA from those fin clips will be extracted, sequenced, and a genome wide association study will be conducted at the University of Idaho, Hagerman Fish Culture Experiment Station, in Hagerman, Idaho.

f. Effect of male broodstock age on minijack rate and progeny age-at-maturity

Both precocious maturation of juvenile hatchery-reared male spring Chinook Salmon as minijacks and increased incidence of hatchery-origin males returning as age 3 jacks hinder a hatchery program from achieving its objective of returning high numbers of large age 4 and 5 adult salmon to support a fishery and/or supplementation of a natural population. In addition to needing to understand what environmental and genetic factors affect early maturation of males as minijacks or jacks, there is also a need to understand how these two phenomena might be related, and what hatchery management modifications might be possible that would decrease these precocious male maturation rates.

We designed a study for production of a series of factorial matings (generally 2x2, though this varied depending on availability of jacks each spawning day) of CESRF production broodstock - two age 4 females crossed with an age 3 and an age 4 male – to be produced over two consecutive broodyears (2018 and 2019). At swim-up, progeny from these crosses are combined for rearing to the pre-smolt stage in four raceways at CESRF. In November-December (a year following spawning) all juveniles receive a coded wire tag identifying each to the CESRF brood year, and an adipose eyelid fluorescent visible elastomer implant (VIE) that will identify each to the Jack Creek acclimation site where they are transferred to the following February. Approximately six weeks after the transfer to the acclimation site, the outlet from the raceway is opened to allow volitional release of the smolts, with forced release of any remaining juveniles in early May. In early March of 2020 (prior to the opening of the gates for volitional release), 100 BY18 fish from each raceway (n = 400) were collected and held until GSI would be a sufficient measure to determine maturation status. On June 8-12, 2020, smolts were sacrificed, measured, dissected to determine sex, and gonad weight of males recorded. Analyses indicated that fish with a GSI of more than 0.35% were mature, resulting in 22.6% of the male population maturing as minijacks (Figure vi.f.1). This is a relatively low rate of precocial maturation for the hatchery; however, the maturation rate could vary between acclimation sites or could be an effect of the limited number of dams and sires (i.e., a genetic effect).

Figure vi.f.1. *Histogram depicting gonadosomatic index (GSI) of male juvenile spring Chinook Salmon sampled June 8-12, 2020. The distribution is bimodal, indicating a GSI cutoff of 0.35% whereby any individual with a GSI less than 0.35% is considered to be immature while those individuals with GSI values of greater than 0.35% will mature as minijacks. This resulted in an overall minijack rate of 22.6%.*



Returning adults from BY18 and BY19 that are identified at RAMF as Jack Creek releases (via their VIE tag) will be measured, and tissue and scale sampled. As data are completed for each brood year, they will be analyzed to test for an effect of male broodstock age on minijack rate (for BY18 only – to confirm results from the study outlined in Section III.vi.a), SAR, size, sex ratio and age structure of their returning adult progeny.

g. Effect of generations of hatchery rearing (domestication) on minijack rate

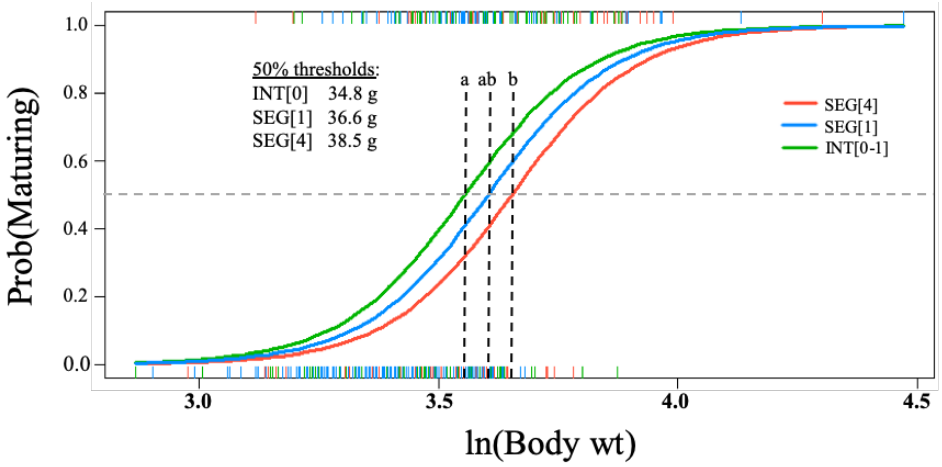
In a review of minijack rates estimated for hatchery Chinook Salmon programs across the Columbia Basin, Harstad et al. (2014) observed that segregated programs (in which broodstock is comprised entirely of HOR adults) demonstrated substantially lower minijack rates than integrated hatchery programs (in which some proportion of the broodstock is comprised of NOR fish). Natural populations are influenced to varying extents by successful spawning of microjack and minijack males each generation, and thus NOR broodstock used in integrated programs are susceptible to having precocial males in their (grand)parentage. In contrast, as segregated programs use exclusively HOR adults, Harstad et al. (2014) hypothesized that segregated programs will progressively select against the precocious maturation trait (to the extent to which it is genetically based) with each successive generation. This hypothesis was further supported by results reported by Larsen et al. (2019).

We designed a related study to test the relative proportions of minijacks produced within individual families produced by sires of varying number of prior generations of hatchery rearing, with the expectation that minijack rates would diminish as hatchery influence increased in the sires. In 2016, we performed seven factorial crosses each factorial involving a different age-4 supplementation hatchery origin (SH; 1 generation of hatchery rearing = SEG[1]) female crossed to a natural origin male from the integrated population (WN; 0 generations of hatchery

rearing = INT[0-1]), a supplementation male (SH; 1 generation of hatchery rearing = SEG[1]) and a hatchery control male (HC; 4 generations of segregated hatchery rearing = SEG[4]). Samples of juveniles from each family were pooled and reared to the smolt stage (average number of male smolts per family = 25, range 21-31), then blood and tissue sampled to assess relative 11-KT plasma concentration, genetic sex, and assignment to its respective male full-sib progeny group. Minijack rate observed for each family is presented below.

The data were pooled across females within sire type and used to generate probabilistic maturation reaction norms (PMRN) and to estimate the weight at smolt release corresponding to a 50% probability of a fish maturing precociously as a minijack (PMRN W50; Larsen et al., 2019). As hypothesized, the PMRN W50 for crosses to WN sires occurred at a significantly lower weight than the PMRN W50 for crosses to HC sires (Figure vi.g.1).

Figure vi.g.1. *Probabilistic reaction norms within sire type. Generally, we see increasing threshold weight at which smolts have a 50% probability of precocious maturation, as the number of prior hatchery generations increases. INT[0] = natural-origin male, 0 generations of hatchery rearing; SEG[1] = supplementation hatchery male, 1 generation of hatchery rearing; SEG[4] = hatchery control male, 4 generations of hatchery rearing.*



Further statistical analyses of these data to test for possible interacting effects (e.g., broodstock size, egg size, spawn date) will be finalized in early 2021. Results from these analyses will be summarized in a manuscript for publication in a scientific journal.

vii. Project Objective #6 – Participate in regional forums for review of hatchery effects on natural populations

Due to the COVID-19 pandemic, most workshops and conferences were either cancelled or moved to a virtual format. Columbia River Inter-Tribal Fish Commission (CRITFC) personnel and collaborators associated with Project activities participated in these virtual forums where possible. This included:

- Yakama Nation Fisheries IPAR meeting, 11/12/2020 – Andy Peirce (UI/CRITFC) and Nick Hoffman (UI PhD Student) presented preliminary findings from the fasting and

photoperiod studies (Project Objective #5). Ilana Koch (CRITFC) presented preliminary findings from the Upper Yakima Relative Reproductive Success study (Project Objective #1).

viii. Project Objective #7 – Prepare manuscripts for publication in scientific journals

Pierce, A. L., Medeiros, L. R., Hoffman, B. R., Koch, I., Narum, S. R., Galbreath, P. F., & Nagler, J. J. (2021). Dietary tetradecylthioacetic acid supplementation during the fall reduces body lipid levels but does not influence precocious male maturation rate in juvenile spring Chinook salmon. *Aquaculture Research, In review.*

Galbreath, P. F., Stockton, C. A., Knudsen, C. M., Medeiros, L. M., Koch, I., Staton, B. A., Nuetzel, H. M., Bosch, W. J., & Pierce, A. L. (2021) Age of male spring Chinook salmon broodstock demonstrates no significant effect on rate of precocious maturation in male juveniles as age-2 minijacks. *In prep.*

ix. Project Objective #8 – Identify additional studies to support tribal supplementation and reintroduction programs

Through meetings and conferences, connecting with tribal fish biologist and managers, and while completing ongoing Project activities, opportunities for additional collaboration have been identified. In line with the objectives of the Project, these opportunities focus on tribally-managed programs where focused monitoring and evaluation work will inform and facilitate adaptive management.

Studies that may be proposed in the Project Statement of Work in coming years include: a) initiation of a study to assess productivity and RRS of coho salmon associated with a new supplementation program in the upper Yakima River basin (Yakama Nation), b) assessment of return rate and productivity of sockeye salmon in Wallowa Lake associated with a proposed reintroduction program (Nez Perce Tribe), c) genetic monitoring of coho salmon reintroduced to the Lostine River and assessment of contribution to harvest, as well as interactions with other anadromous fish populations in the river system (Nez Perce Tribe).

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