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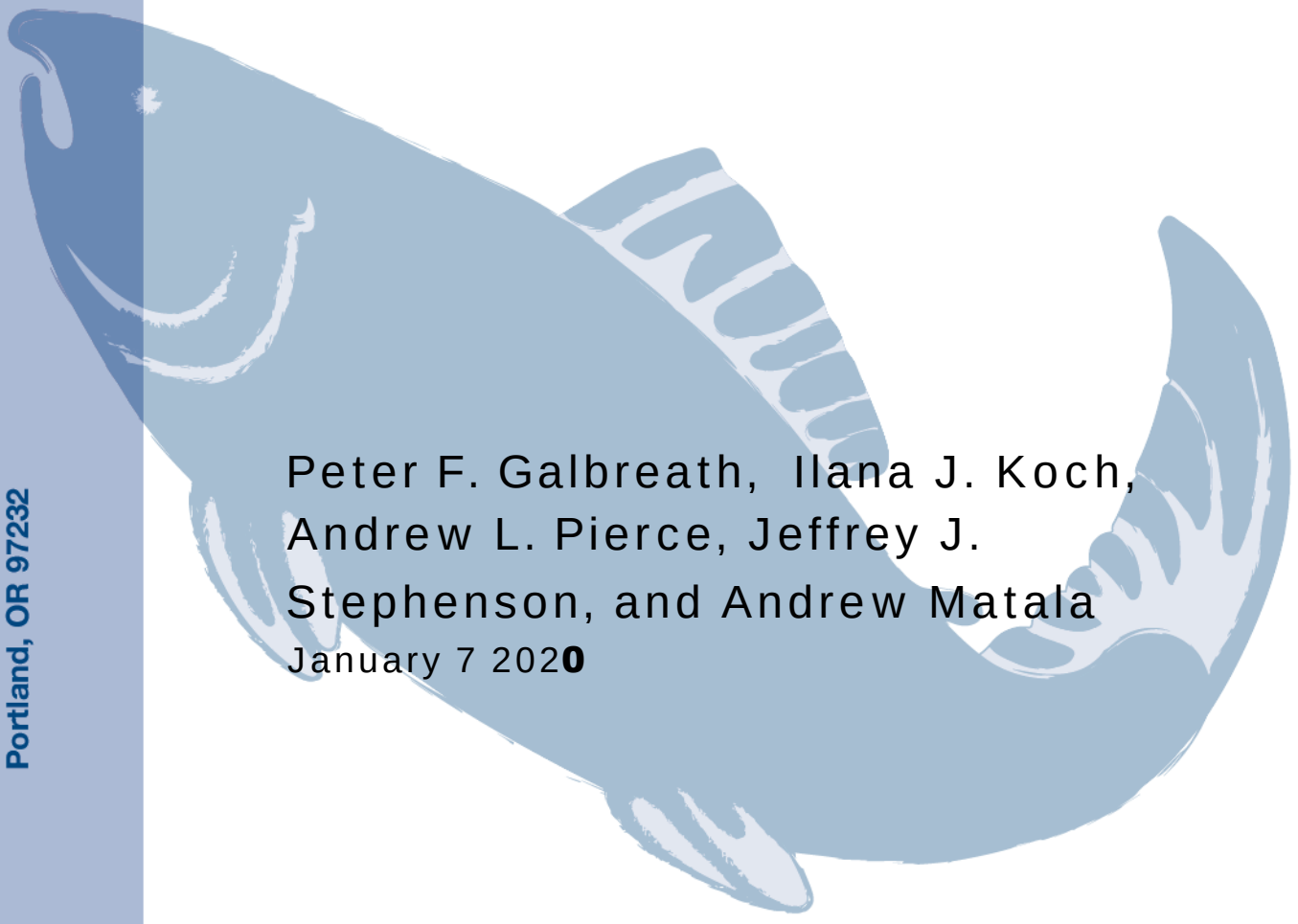
CRITFC

TECHNICAL REPORT 20-07

Basinwide Supplementation Evaluation Project: 2019 Annual Progress Report



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2019 Annual Progress Report

Basinwide Supplementation Evaluation

BPA Project # 2009-009-00

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I. Executive Project Summary

This report summarizes activities for calendar year 2019 under BPA Contracts No.s 73354 REL 5 and 73354 REL 25, performed as part of the multi-year Basinwide Supplementation Evaluation project 2009-009-00 (hereafter the Project). The report is organized under the seven Project Objectives identified in the contract Statement of Work: https://www.cbfish.org/Contract.mvc/ContractActionsWorkStatementVersion/73354%20REL%2025~49766. The primary objective of the Project is to facilitate studies associated with monitoring and evaluation of tribal programs to assess: a) critical uncertainties related to effects of hatchery management and supplementation on abundance and productivity of depressed natural anadromous fish populations, and b) abundance and productivity trends in new natural populations established through reintroduction of out-of-basin origin fish (of hatchery origin and/or natural origin) in subbasins where the indigenous population had been extirpated.

Project Objective #1 - Support RRS studies of supplemented spring Chinook Salmon:

- Genotyping of fish involved in a 5-brood year (BY 2007-2011) adult recruits-per-spawner relative reproductive success (RRS) study of supplemented upper Yakima River spring Chinook Salmon is complete. A preliminary analysis of BY 2007 data was performed in 2018. The analysis indicated that overall RRS for adult (age-4 and 5) females was approximately 0.85, and significantly lower than 1.0. RRS for adult males was approximately 0.90 and for age-3 jack males was 0.95, neither significantly different from 1.0. While productivity of the HOR adults was to generally slightly lower than for NOR adults, the analysis showed that hatchery supplementation provided a significant demographic boost to the population, i.e., a NOR fish taken into the hatchery for spawning returned 5.7 times more adult progeny relative to a NOR fish left in the river to spawn naturally. Comprehensive analyses for the five brood years is underway, results from which will be summarized in a report and manuscript in 2020.

Project Objective #2 - Support RRS studies of reintroduced spring Chinook Salmon:

- A RRS analysis (BYs 2008-2016) based both on juvenile and on adult recruits-per-spawner is ongoing for spring Chinook Salmon in Lookingglass Creek, Grande Ronde River basin. As the indigenous population had been extirpated, the study examines relative productivity of a new population that was reintroduced with the use of a hatchery stock. An initial analysis for juvenile RRS for BYs 2008-2014 indicated higher productivity of natural origin females and males relative to hatchery origin fish of the reintroduced hatchery stock. This is concordant with the expectation that natural selective forces acting on the reintroduced hatchery origin fish would create a naturalized population that demonstrates improving reproductive success. A parallel RRS analysis based on adult recruits-per-spawner for the same broodyears is underway, and a comprehensive analysis and report will be produced in 2020.

Project Objective #3 - Support genetics studies of reintroduced Sockeye Salmon:

- A genetics study is ongoing to assess relative spawning and rearing success of Sockeye Salmon reintroduced by the Yakama Nation into Cle Elum Lake, WA. The reintroduced adults are captured at Priest Rapids Dam (PRD) and consist of a mix of two genetically distinct stocks (Wenatchee Lake/River and Osoyoos Lake/Okanogan River). Recently published results indicate that the Wenatchee stock spawns on average-3 weeks earlier than Osoyoos stock, albeit with some overlap in timing, and genetic analyses of juvenile and adult progeny indicate a very low (<5%) incidence of inter-stock hybridization (Matala et al. 2018). Only 201 adults returned in-basin and were captured at the Roza Adult Monitoring Facility in 2019, from where they were translocated to the lake. The Sockeye Salmon run to Bonneville Dam in 2019 did not reach the minimum required to permit

collection and translocation of migrating adults from PRD. The Project will to continue to support genetic stock identification and productivity analyses over the coming years.

- The Project supports an ongoing genetic study of a program to reintroduce an anadromous run of Sockeye Salmon in the Deschutes River, for which the Warm Springs Tribe is a co-manager. Beginning in 2009, juveniles of the resident kokanee population in Lake Billy Chinook have been captured at Round Butte Dam and released downstream of the dam complex. Since then, all adults (presumptive anadromous Sockeye Salmon) that return to the Pelton Dam adult trap have been tissue sampled, prior to release of the fish upstream of into Lake Bully Chinook or held for use as hatchery broodstock. Genetic stock identification analyses of the returning adults confirmed them to be >90% of Lake Billy Chinook origin. However, while many thousands of juvenile *O. nerka* have been released each year, annual adult return numbers have been low (ranging from 10 to 98), with the exception of 2016 when 536 adults returned. In light of these results, co-managers are discussing possible modification of the reintroduction approach.

Project Objective #5 - Evaluate factors affecting minijack production:

- The Project is also financing a series of studies examining factors associated with precocious maturation of hatchery-reared male spring Chinook Salmon smolts – minijacks. The first, a three BY (2014-2016) study conducted at the Cle Elum Supplementation Research Facility to test for an effect of broodstock age, is undergoing final data analyses. Initial results do not indicate a consistent effect of parent age, possibly masked by the very high variation in minijack rate among families within parental age cross-types, ranging in one instance from 0% to 100%. Reporting of results from a comprehensive analysis is anticipated in 2020.
- A one-year study was conducted to test the effects of diet supplementation with tetradecylthioacetic acid (TTA). Published reports on the use of TTA-treated feeds with juvenile male Atlantic salmon indicated that whole body lipid content was reduced with no effect on growth rate. However, our treated spring Chinook Salmon exhibited slower growth during the treatment period, and there was no reduction in minijack rate relative to controls.
- Another study was designed to compare relative growth rate through the juvenile rearing period of non-maturing smolts versus maturing minijacks, measured among full-sibling groups from 11 different families. Interestingly, size and condition factor at the first sample date (July 2019) was already generally greater for fish destined to become minijacks, and these differences remaind throughout juvenile rearing. Further analyses to parse out an effect of family are underway.
- A study to compare minijack rate and age structure of returning mature adults for fish produced with an age-3 jack versus and age-4 adult male parent was initiated in 2018. A similar set of crosses was performed in 2019, with a third set planned in 2020. The juveniles for each broodyear will be sampled just prior to smolt release to assess minijack rate. Sampling of adult returns will begin in 2021 with return of age-3 BY 2018 jacks, and finish in 2025 with BY 2020 age-5 adult returns.
- Two more studies are underway using BY 2018 juveniles. The first will test the effect of two different 5-week periods of feed deprivation on minijack rate, and the second (conducted at the University of Idaho, Moscow ID) will assess of two 24 h light photoperiod treatments on minijack rate. The juveniles in these studies will be sacrificed and sampled when they reach the smolt stage in April 2020.

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

- As in prior years, Columbia River Inter-Tribal Fish Commission (CRITFC) personnel associated with Project activities participated in a variety of inter-tribal and inter-agency meetings, workshops and symposia in which issues related to effects of hatchery management on population productivity were discussed.

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 prepared for publication in scientific journals.

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

- Through meetings, attendance at regional conferences and while pursuing ongoing Project activities opportunities where financing from the Project could be profitably used to support supplemental genetic and physiological studies associated with tribal M&E of hatchery supplementation and/or reintroduction projects have been identified. Studies have been developed, which may be proposed within the Project Statement of Work in the coming years may include: a) assessment of effective breeder number and relative individual productivity of spring Chinook Salmon in a wild population in the upper Warm Springs River (Deschutes River basin); b) assessment of effective breeder number and relative individual productivity of Coho Salmon that have recently reintroduced themselves to the upper Warm Springs River (Deschutes River basin), c) initiation of a study to assess productivity and RRS of Coho Salmon associated with a new supplementation program in the upper Yakima River basin, d) assessment of return rate and productivity of Sockeye Salmon in Wallowa Lake associated with a proposed reintroduction program.

II. Introduction

In their 2005 report submitted to the Northwest Power and Conservation Council (NPCC) entitled "Monitoring and Evaluation of Supplementation Projects" (ISRP and ISAB 2005), the Independent Scientific Review Panel (ISRP) and Independent Scientific Advisory Board (ISAB) recommended that an interagency workgroup be formed to design a monitoring and evaluation approach to obtain a basinwide understanding of the critical uncertainties associated with use of hatchery supplementation for rebuilding depressed anadromous fish populations (focused on salmonids). In response, the Ad Hoc Supplementation Workgroup (AHSWG) was formed – a group of volunteer scientists and managers working in tribal, state and federal fisheries agencies, power companies, and other non-governmental agencies. Following a series of workshops and ancillary discussions, the AHSWG recommended a three-pronged approach: 1) conduct treatment/reference (T/R) comparisons of long-term trends in the abundance and productivity of multiple supplemented (treatment) populations relative to un-supplemented (reference) populations, 2) conduct a series of relative reproductive success (RRS) studies to quantify short-term impacts through comparisons of productivity within brood years (BYs) of hatchery origin (HOR) and natural origin (NOR) fish observed in programs to supplement depressed natural populations, and in programs where an extirpated stock has been reintroduced and supplemented with hatchery-reared fish, and 3) develop a request for proposals to fund several intensive small-scale studies designed to elucidate various biological mechanisms by which introduction of hatchery-produced fish may influence natural population productivity (AHSWG 2008).

The Basinwide Supplementation Evaluation project was submitted by CRITFC as part of the Columbia Basin Fish Accords (2008). The Project was designed to implement a variety of actions in support of the AHSWG recommendations, each directly or indirectly associated with a tribally managed program. In the 2019 Statement of Work, the following Project activities were planned:

- Use genetic analyses to derive productivity information with which to assess RRS of NOR and supplementation HOR spring (stream-type) Chinook Salmon *Oncorhynchus tshawytscha* in the upper Yakima River (Project Objective #1), and to assess RRS of reintroduced spring Chinook Salmon in Lookingglass Creek (Grande Ronde River basin), where the natural population had been extirpated and the species reintroduced through stocking of returning adults from a new hatchery program (Project Objective #2).
- Use genetic analyses to assess relative spawning success of Sockeye Salmon *O. nerka* reintroduced into the Cle Elum Lake/Yakima River system, and to provide annual genetic stock identification analyses of adult *O. nerka* returning to the Pelton trap on the Deschutes River, in response to a program to restore a Sockeye Salmon run via capture and release juveniles from the Lake Billy Chinook kokanee population downstream of the Pelton-Round Butte complex (Project Objective #3).
- Build off previous research conducted at the Cle Elum Supplementation Research Facility to examine factors affecting precocious maturation of hatchery-reared male spring Chinook Salmon smolts as age-2 minijacks, with a series of studies: 1) to examine the effect of age (within sexes) of the natural origin hatchery broodstock on survival, size and minijack rate among their hatchery-reared smolts, 2) to test for a progressive effect on minijack rate from successive generations (0, 1 or 4) of hatchery rearing in the broodstock, 3) to test for a reduction in minijack rate associated with supplementing feed with tetradecylthioacetic acid (TTA), 4) to compare individual juvenile growth rates of maturing versus non-maturing fish, and 5) to compare minijack rate and age structure of returning hatchery-origin adults parented by age-3 jacks versus age-4 adult male broodfish, 6) to assess the effect of feed deprivation on minijack

rate and juvenile growth, and 6) to assess the effect of photoperiod manipulation on minijack rate and juvenile growth (Project Objective #5).

- to continue participation in regional forums involving review of hatchery management, supplementation and reintroduction efforts (Project Objectives #6), and for reporting of Project results in scientific journals (Project #7), and identification of potential new studies of tribal programs apt for financing through the Project (Project Objective #8).

III. Work Elements / Tasks

A. Project Administration

Activities in 2019 involving administration of the Project by CRITFC included: production and posting online in CBFISH the annual progress report for 2018, completion of 2019 quarterly and final status reports in CBFISH that record progress associated with each work element within the contract Statement of Work, and submission of 2019 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.s 73354 REL 5 and 73354 REL 25.

B. **Project Objective #1:** Support RRS studies of supplemented spring Chinook Salmon

B.1 Upper Yakima River spring Chinook Salmon

The Yakama Nation (YN), in collaboration with the Washington Department of Fish and Wildlife (WDFW), initiated a hatchery program to supplement the depressed population of spring Chinook Salmon in the upper Yakima River under the BPA-funded Yakima/Klickitat Fisheries Project (YKFP; http://www.ykfp.org/). The program began in 1997 with collection of wild broodstock at the Roza Adult Monitoring Facility (RAMF) adjacent to Roza Irrigation Dam (rkm 206). The adults were transported to the newly constructed Cle Elum Supplementation and Research Facility (CESRF), Cle Elum WA, where they were spawned and their progeny reared to the pre-smolt stage. The juveniles were then transported to one of three acclimation sites within the upper Yakima basin, where they are held for an additional 6-8 weeks prior to release. The first age-4 adults (the dominant age at return for this population) from the supplementation program returned to the Yakima River in 2001. Hatchery production and supplementation has continued annually since 1997. This fully integrated program (100% of fish chosen for broodstock are NOR) was designed to test whether artificial propagation can be used to increase natural production and harvest opportunities while keeping ecological and genetic impacts on the native population within acceptable limits. An unsupplemented population in the adjacent Naches River (tributary of the Yakima River) provides a reference for evaluating environmental influences. The program has been comprehensively monitored, and data analyses indicate that while HOR fish show some small differences in morphometric and life history traits, supplementation has increased harvest, redd counts, and spatial distribution of spawners (Fast et al. 2015). Additionally, NOR abundance has been maintained, and straying to non-target systems has been negligible. Lastly, an RRS study (based on fry recruits-per-spawner) for adults stocked in an artificial spawning channel indicated

that productivity of NOR females was slightly higher than HOR females, while productivity of NOR and HOR males was comparable.

Since its inception, there has been a desire to perform a direct comparison of relative productivity of naturally spawning NOR and HOR adults in the supplemented population. However, funding for an adult-to-adult RRS study has been insufficient. This situation changed with the recent development of a large array of SNP markers for Chinook Salmon and of new high throughput genotyping techniques (Campbell et al. 2015). The per-sample genotyping cost has dramatically diminished, bringing a large scale RRS study within the realm of feasibility. In discussions between YN, WDFW, and CRITFC, an agreement was reached to perform a collaborative RRS study of naturally spawning NOR and HOR fish in the upper Yakima River, financed with Project funds and YKFP funds allocated to the WDFW genetics laboratory. The five brood year study (BYs 2007-2011) involves genotyping of tissue samples collected from in-migrating NOR and HOR adults interrogated in the RAMF, that were passed upstream for natural spawning, plus their NOR adult progeny that returned in years 2010 through 2016:

	Adult Spawners		
	Natural	Hatchery	Unknown
Return Year	Origin	Origin	Origin
2007	1,284	1,504	
2008	1,677	3,240	191
2009	2,543	4,476	173
2010	3,186	5,514	157
2011	4,392	4,812	244
2012	2,927	na	160
2013	2,784	na	na
2014	3,761	na	na
2015	3,386	na	14
2016	1,856	na	na
Sub-Totals	26,512	19,546	779

Genotyping was completed in 2019, and parentage and RRS analyses are underway. Results, expected in 2020, will be summarized in a manuscript to be submitted for publication in a scientific journal.

As reported previously, an interim RRS assessment was conducted for brood year 2007 and described in a CRITFC Technical Report (Galbreath et al. 2017). The progeny assignments were conducted with the software program SNPPIT which limits assignments to parent pairs. The analysis for BY 2007 was rerun in 2018 using a second program, Colony, which provides progeny assignments to both parent pairs and to single parents (data for the other parent is missing from the dataset, or is present but unassignable with sufficient certainty). While further study and confirmation of the computer script associated with the Colony analyses is required, initial results indicate somewhat lower RRS ratios. For females, RRS was approximately 0.85, which was significantly lower than 1.0. RRS was approximately 0.91 for adult males and 0.95 for age-3 jack males, both of which were not significantly different from 1.0.

Among the adult females and males of both origins, a large majority (87% and 75%, respectively) were identified as having produced one or more adult progeny. This rate of successful adult offspring production is high relative to that observed in other studies of supplemented spring Chinook Salmon populations. For example, spawning success averaged across multiple BYs was approximately 40% in both the Wenatchee River (Williamson et al. 2010) and in Johnson Creek (Hess et al. 2012, Supplemental Data). However, 2007 was a relatively low return year, and reduced competition for spawning and juvenile rearing habitat is likely associated with the high success rate.

Despite the somewhat lower average productivity of the HOR fish, at least for the females, supplementation provided a substantial demographic boost to the spawning population. For each NOR adult collected for use as a hatchery broodfish, there was 5.7 fold increase in the average number of returning adult progeny relative to a natural origin fish that had been left in the river to spawn naturally.

In discussions between CRITFC, WDFW and YN, it was decided to extend the initial 5 broodyear RRS study (2007-2011) for an additional 5 broodyears (2012-2016). To do so will permit estimation of productivity based on grand-offspring of fish from the initial broodyears. Differences observed among first generation progeny of naturally spawning NOR and HOR will represent the combined effects of heritable and non-heritable (genetic and environmental) factors. Extending the study for an additional 5 years, will permit examination of differences among grand-offspring that will be strictly heritable (genetic in basis), and provide inference as to what might be long-term effects of hatchery supplementation on population productivity.

C. Project Objective #2: Support RRS studies of reintroduced salmon populations

Freshwater habitat loss and degradation, and increased mortality during migration within the hydrosystem are the primary factors responsible for the current depressed state of natural salmon and steelhead populations in the Columbia basin. In some cases, the effects have led to the extinction of affected populations. This included extirpation of all populations whose natal streams were upstream of the impassable mainstem Chief Joseph and Grand Coulee dams (Columbia River) and the Hells Canyon Dam Complex (Snake River). However, additional populations downstream of these dam complexes were also lost, e.g., spring Chinook Salmon in the Hood, Umatilla, Okanogan and Clearwater rivers, and 100% of native Coho Salmon populations upstream of The Dalles Dam, etc. (Fulton 1968; Mullan 1983; Nehlson et al. 1991; O'Toole et al. 1991).

Tribal fisheries management agencies have initiated programs to re-establish naturally spawning salmon populations in some of these Columbia Basin rivers. Reintroduction efforts generally involve stocking of juveniles produced from out-of-basin hatchery stocks, on the presumption that these stocks possess the phenotypic and genotypic capacity to adapt to the new natural environment (e.g., Bowles and Leitzinger 1991; Phillips et al. 2000; Underwood et al. 2003; Lutch et al. 2005; Murdoch et al. 2006; Bosch et al 2007; Narum et al. 2007). These reintroduction programs have seen a portion of the HOR smolts return in-basin as mature adults, many of these fish have engaged in natural spawning, and increasing numbers of NOR juveniles have been observed. Additionally, observation of NOR adults in subsequent return years indicates that these fish underwent a full generation or more of strictly natural production (Phillips et al. 2000; Underwood et al. 2003; Lutch 2005; Murdoch et al. 2006; Bosch et al 2007; Narum et al. 2007; Yakama Nation 2011; Galbreath et al. 2014).

The broodstock management protocol for reintroduction programs recommends that use of out-of-basin hatchery broodstock be progressively diminished while increasing the proportion of adults

returning in-basin. The initial generations of "local origin" broodstock would be comprised largely of mature HOR adults. However, in subsequent generations, NOR adults should make up a growing proportion of the escapement, as well as of the hatchery broodstock. This management approach is expected to create a new natural population, and associated hatchery stock, that will be increasingly adapted to local conditions.

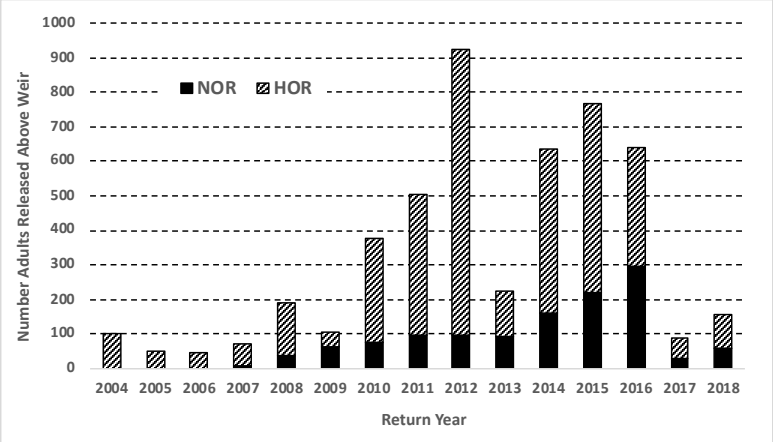
In a meta-analysis of published information, Fraser (2008) reviewed reports for 31 different salmonid reintroduction programs, including several within the Columbia basin. For programs where effects of hydrosystem blockages and habitat degradation that contributed to the extirpation of the original populations had been reversed, natural production by returning adults from the reintroduced HOR smolts does appear to be re-establishing new natural populations. However, these programs are relatively recent and with few exceptions hatchery supplementation continues. Uncertainty therefore remains as to whether these populations are becoming progressively better adapted (and eventually self-sustaining), or that the populations are simply being maintained by the ongoing annual stocking of supplementation juveniles.

If adaptation is occurring, NOR fish (fish that have been exposed to a full generation or more of natural selection), should on average produce more recruits-per-spawner than HOR fish (fish that lack this generation of exposure to local natural selective forces), and the relative reproductive success ratio for a reintroduction program (NOR/HOR) should be greater than 1.0. To test this hypothesis, the Project is performing a RRS study of the spring Chinook Salmon reintroduction program in Lookingglass Creek (Grande Ronde River) which is being monitored by the Confederated Tribes of the Warm Springs Reservation of Oregon (CTUIR).

C.1 Lookingglass Creek (Grande Ronde River) spring Chinook Salmon

Spring Chinook Salmon populations within the Grande Ronde and Imnaha River subbasins declined dramatically in abundance by the 1980s. As part of the Lower Snake River Compensation Plan (LSRCP), a hatchery was constructed at rkm 3 along Lookingglass Creek (a tributary to the Grande Ronde at rkm 136). Juveniles produced at the Lookingglass Hatchery were used to supplement tributary populations within these basins. However, use for broodstock of many of the remaining adults from the native population of spring Chinook Salmon in Lookingglass Creek, further reduced the abundance of naturally spawning fish in the basin above the hatchery weir, located½ km upstream of the hatchery. Then, managers brought in fish from out-of-basin hatchery stocks of spring Chinook Salmon to rebuild abundance in the basin, which assured the functional extinction of the native Lookingglass Creek stock. Different hatchery stocks were successively introduced, initially from Carson National Fish Hatchery, then Wind River, Imnaha River, and Rapid River hatcheries. Despite their numbers, a naturally spawning population of these out-of-basin fish never became well established in Lookingglass Creek (Burck 1994; Boe et al. 2010 and 2011). Then in 1995, NOAA mandated that the hatchery switch to use of an in-basin stock of spring Chinook Salmon for supplementing the Grande Ronde and Imnaha basins. Captive broodstock programs were therefore initiated using juveniles captured from Catherine Creek, the Lostine River and the Imnaha River (a Grande Ronde River tributary upstream of Lookingglass Creek) – all fish having been transported for rearing in Lookingglass Hatchery. In anticipation of adults returning from smolt releases of the new in-basin hatchery stocks to their respective streams. As the Lookingglass Creek population had been extirpated it was decided to reintroduce spring Chinook Salmon to the subbasin above the hatchery weir using returning adults from a portion of the Catherine Creek stock juveniles that were released in Lookingglass Creek. While awaiting the in-basin returns from the new Catherine Creek stock, from 1998 through 2003 no adults whatsoever were passed upstream of the

Lookingglass Creek weir. This effectively extirpated any remnant spring Chinook Salmon derived from the prior out-of-basin hatchery stocks. In 2004, adults from the Catherine Creek stock releases returned to the Lookingglass Creek weir. In this year, and each year since, a portion of these adults are selected for use as broodstock, creating the new Lookingglass Creek hatchery stock, and the remaining HOR fish are passed upstream of the hatchery weir for natural spawning. Prior to release, all fish are measured for fork length and opercule punched with the tissue retained for eventual genetic analysis. Beginning in 2007, with return of the first NOR adults (age-3 jack males) from the new reintroduction program, a portion of the NOR adults captured at the weir have been included among the HOR returns to form the hatchery broodstock, with the remainder of the adults (NOR and HOR) passed upstream for natural spawning (Boe et al. 2010 and 2011), as illustrated below:



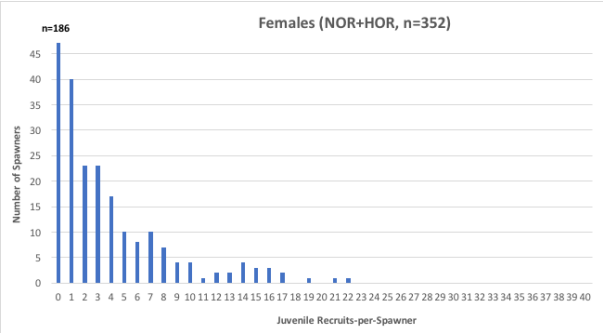
In 2004, CTUIR biologists began collecting tissue samples annually from all adults encountered at the weir (both those passed above the weir for spawning, and those taken for broodstock). The samples were archived at the CRITFC molecular genetics laboratory at the HFCES, for eventual genetic studies to assess return rates and productivity. Tissue samples from carcasses have also been opportunistically collected during spawning ground surveys. Additionally, beginning in 2008 and continuing through spring 2018, tissue samples have been collected from out-migrating NOR juveniles (both as age 0+ parr in June through December the calendar year following the broodyear, and as age-1+ smolts in January through May the second calendar year following the broodyear) captured in a rotary screw trap located ¼ km downstream of the weir (Boe et al. 2010 and 2011).

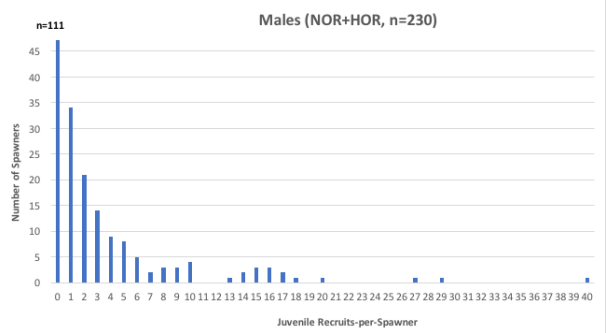
Using Project financing, DNA was extracted from tissue samples for all adults intercepted at the Lookingglass weir from 2008 through 2019, plus all samples from NOR juvenile out-migrants from 2009 through 2018. Extraction of DNA from these samples was originally performed at the HFCES using a Qiagen DNeasy Blood & Tissue Kit (Qiagen; www.qiagen.com) according to the manufacturer's instructions, and the DNA was genotyped for a standardized suite of 95 (BYs 2008-2014) SNP markers using Taqman Assays (Applied Biosystems) and PCR amplification and imaging using a Fluidigm™ IFC controller and EP1 imager, as described in detail by Matala et al. (2011). More recently, DNA extraction switched to use of the Chelex 100 method (Sigma-Aldrich, St Louis, MO), the SNP panel was expanded to 188 markers (BYs 2015-2016), and genotyping was performed using the "genotyping-in-thousands by sequencing" (GT-seq) technique (Campbell et al. 2015). The genotypic data were entered into Cervus 3.0.7 for parentage analysis. The software uses likelihood algorithms to identify the most probable parent pair for each juvenile. To maximize certainty in the parent assignments, those which involved two or more allele mismatches (for the 95 marker panel) or three or more (for the 188 marker panel),

and/or those which had a threshold confidence level of assignment for the logarithm of the odds (LOD) score of <99% were deleted, resulting in rejection of one of the parents among some of the parent pair assignments, and occasionally deletion of both parents. The remaining high-certainty assignments were then examined to calculate the total number of juveniles assigned to each adult (age-4 and 5) female and male, and jack (age-3) male passed above weir for natural spawning.

On average for BYs 2008-2014, 97% of the juveniles were successfully assigned as follows: 73% were assigned to a female+male parent pair, 4% to a male parent only, and 23% to a female parent only. Reasons that some juveniles can be assigned only a single parent include a rare loss of the tissue sample for the missing parent, tissue degradation, errors in the genotyping process, or instances of the adult fish having fallen back downstream below the weir prior to spawning. These reasons, however, do not explain the much higher percent of juveniles with an unidentified male parent (23%) relative to female parent (4%). It is probable that many of the missing male parents were precociously mature parr (microjacks); female juveniles are not known to mature precociously. Indeed, precocious male parr are occasionally observed among the juveniles sampled in the rotary screw trap or during electrofishing surveys, and when samples from some of these fish were collected and included in the genetic parentage analysis as potential parents, a few of them were assigned as a male parent.

The number of juvenile progeny genetically assigned to fish passed upstream of the weir for natural spawning across origins and across sexes generally followed a negative binomial frequency distribution, with a large number assigned zero juvenile progeny, and the frequency of adults assigned 1, 2, 3 etc. progeny diminishing rapidly, as illustrated below for BY 2014:





The percent of adults per BY that were successfully assigned one or more juvenile progeny (successful adults) averaged 60% and 61% for NOR females and males, respectively, and 51% for both HOR females and males. Note, success rate, and subsequent relative reproductive success, of age-3 jack males was not estimated; the number of jacks passed upstream was typically low, and in years of relatively high returns HOR jacks were purposely not released upstream. Importantly, the higher success rate of NOR adults might does not necessarily represent genetic adaptation. It is possible that the difference is due to non-heritable environmental effects associated with hatchery rearing to the smolt stage. Reproductive success of the adults in this study require that they remain above the weir following release, and that they find appropriate spawning habitat and exhibit appropriate spawning behaviors. If, for example, HOR adults exhibited a greater tendency to fall back below the weir than NOR adults (e.g., a homing response for proximity of the hatchery), this would reduce their relative success rate. However, this appears not to have been the case. Spawning surveys are conducted annually below the

weir, and while sample sizes are limited, among the carcasses that bore an opercule punch (indicative of fish that fell back below the weir), the proportions of NOR versus HOR fish were generally similar to the proportions of NOR and HOR fish released above the weir, i.e., the fish of both origins fell back at comparable rates (C. Crump, personal communication). Similarly, if HOR adults tend to remain lower in the system (in closer proximity to the hatchery), at higher densities, and/or in poorer quality habitat, this could bias the analysis against HOR fish (e.g., see Williamson et al. 2010). Again, however, this does not appear to be the case. No obvious difference in spatial distribution between NOR and HOR carcasses was noted during spawning ground surveys over the years in Lookingglass Creek (C. Crump, personal communication).

	All Potential Parents								Juveniles					
		Number		% Successful					# Genotyped		Number Assigned to Parent(s)			
Broodyear	Sex/Age	HOR	NOR	# HOR	% HOR	# NOR	% NOR	NOR/HOR	Parr	Smolts	Parr	Smolts	Total	% Assigned
2008	Female	80	24	51	0.64	18	0.75	1.17	240	83	237	81	318	0.98
	Male	59	12	41	0.69	8	0.67	0.96						
	Jack	5	2	3		2								
2009	Female	13	36	12	0.92	30	0.83	0.90	456	145	427	141	568	0.95
	Male	14	18	11	0.79	12	0.67	0.85						
	Jack	11	6	11		6								
2010	Female	200	34	91	0.46	14	0.41	0.90	352	118	340	115	455	0.97
	Male	72	32	34	0.47	21	0.66	1.39						
	Jack	25	5	8		2								
2011	Female	209	42	100	0.48	28	0.67	1.39	423	23	412	15	427	0.96
	Male	129	34	61	0.47	23	0.68	1.43						
	Jack	35	19	10		7								
2012	Female	564	56	209	0.37	29	0.52	1.40	696	114	680	107	787	0.97
	Male	271	37	127	0.47	21	0.57	1.21						
	Jack													
2013	Female	61	16	32	0.52	11	0.69	1.31	276	193	269	188	457	0.97
	Male	63	14	30	0.48	11	0.79	1.65						
	Jack		56			21	0.38							
2014	Female	285	67	131	0.46	35	0.52	1.14	566	341	526	331	857	0.94
	Male	179	51	94	0.53	25	0.49	0.93						
	Jack		31			14	0.45							
2015	Female	286	88	92	0.32	39	0.44	1.38	498	115	492	113	605	0.99
	Male	230	80	78	0.34	36	0.45	1.33						
	Jack		20			12	0.60							
2016	Female	173	153	65	0.38	85	0.56	1.48	527	277	522	277	799	0.99
	Male	158	133	52	0.33	66	0.50	1.51						
	Jack		5			1	0.20							
Female					0.51		0.60	1.23					avg	0.97
Male					0.51		0.61	1.25						

In addition to NOR adults of both sexes having been more successful at producing one or more juvenile progeny relative to HOR adults, NOR adults also produced on average a greater number of juvenile recruits-per-spawner (parr + smolts combined), with the overall average RRS for females being 1.23 and for males 1.35.

Broodyear	FEMALES - Successful Parents				
	HOR		NOR		RRS
	#	Mean R/S	#	Mean R/S	NOR/HOR
2008	51	5.00	18	3.39	0.68
2009	12	12.75	30	13.40	1.05
2010	91	4.06	14	4.93	1.22
2011	100	2.84	28	3.82	1.35
2012	209	2.98	29	3.24	1.09
2013	32	10.56	11	9.27	0.88
2014	131	4.47	35	6.94	1.55
2015	92	4.16	39	5.08	1.22
2016	65	2.25	85	4.46	1.99
				Avg:	1.22

Broodyear	MALES - Successful Parents				
	HOR		NOR		RRS
	#	Mean R/S	#	Mean R/S	NOR/HOR
2008	41	4.32	8	7.38	1.71
2009	11	9.73	12	13.42	1.38
2010	34	4.71	21	6.05	1.29
2011	61	3.10	23	3.65	1.18
2012	127	4.09	21	4.48	1.10
2013	30	5.60	11	5.91	1.06
2014	94	4.72	25	7.48	1.58
2015	92	3.77	36	5.72	1.52
2016	65	4.39	66	5.92	1.35
				Avg:	1.35

A caveat regarding these analyses is that they involve calculation of the total number of juvenile progeny per parent as the sum of assigned parr + smolts. In the Grand Ronde basin, juvenile spring Chinook Salmon that out-migrate from the tributaries as age 0+ parr will overwinter in the mainstem Grande Ronde, then finish out-migration to the Snake and Columbia rivers the following spring. Juveniles out-migrating from the tributaries as age-1 smolts will not delay in the mainstem Grande Ronde, and parr and smolt out-migrants from the same broodyear will arrive at Lower Granite Dam on the Snake River and continue their migration to the ocean concurrently.

Mortality of juvenile salmon accrues over juvenile rearing, and can be especially high during overwintering. As such, the proportion of (pre-overwintering) parr out-migrants from Lookingglass Creek that reach Lower Granite Dam (and that successfully complete out-migration, and return as mature adults) will be reduced relative to the number of (post-overwintering) smolt out-migrants. If the proportion of progeny of NOR adults that out-migrate as smolts is similar to that for progeny of HOR adults, the RRS analyses will be unbiased. This appears to be the case for males – the proportion of assigned progeny per BY that were smolts for NOR versus HOR adults were generally similar, and the differences non-significant (see table below). In contrast, NOR females were assigned a significantly greater proportion of smolt progeny than HOR females. Therefore, the RRS analyses will be biased against NOR females. Were it possible to remove this bias by, for example, multiplying the parr and smolt numbers by an associated survival factor to obtain estimates of survivors ("smolt equivalents") to Lower Granite Dam, the relative productivity for NOR females would increase. However, the data do not exist to estimate these survival factors. Recognizing this bias, the RRS results can be considered conservative – that the true value for a productivity advantage would be even larger than the observed value.

Proportion of smolts among progeny assigined to HOR versus NOR adults within sexes.

Broodyear	Females			Males			
	% HOR	% NOR	%N/%H	% HOR	% NOR	%N/%H	
2008	0.255	0.262	1.03	0.299	0.220	0.74	
2009	0.163	0.284	1.74	0.187	0.311	1.66	
2010	0.233	0.333	1.43	0.256	0.244	0.95	
2011	0.046	0.056	1.22	0.037	0.024	0.65	(low sample size for smolts)
2012	0.235	0.372	1.58	0.233	0.351	1.51	
2013	0.627	0.618	0.99	0.649	0.615	0.95	
2014	0.381	0.461	1.21	0.356	0.428	1.20	
2015	0.170	0.217	1.28	0.163	0.209	1.28	
2016	0.240	0.349	1.46	0.377	0.368	0.98	
avg:			1.326	*		1.101	ns

Note - data for BY 2011 were excluded due to low sample size for BY 2011 smolts.

Following genotyping of the NOR adults that returned in 2019, a similar set of RRS analyses will be performed based on adult recruits-per-spawner, followed by comparison of results based on juvenile versus adult recruits. Additionally, the data will be analyzed with inclusion of adult size and return date to the weir as potential factors affecting productivity. These analyses should be complete during the first half of 2020, after which the findings from this study will be summarized in a report and in a manuscript to be submitted for publication in a scientific journal.

D. Project Objective #3: Support genetic monitoring of reintroduced Sockeye Salmon

D.1 Cle Elum Lake (Cle Elum/Yakima Rivers) Sockeye Salmon

Cle Elum Lake in the upper Yakima River basin was a natural lake that once supported a native population of Sockeye Salmon. However, construction of an impassable timber crib-dam at the lake outlet in the early 1900s resulted in extirpation of the Sockeye Salmon and other anadromous fish. In 1933, the Bureau of Reclamation (BOR) replaced the crib-dam with a 165 foot high earthen dam, to further increase water storage of the impoundment for irrigation purposes. As a first step toward investigating the feasibility of a YN proposal to reintroduce Sockeye Salmon to the lake, a plywood flume was constructed by the BOR on the dam spillway and tested to see if it would work effectively as a route for out-migration of anadromous smolts. The flume was initially tested with hatchery reared Coho Salmon smolts that were held in a net-pen in the lake for a period of time, then released just prior to the time of normal smolt out-migration (BOR 2007). Given the positive results from this trial, in 2009 the YN initiated their Sockeye Salmon reintroduction program, involving annual out-planting of adult Sockeye Salmon collected from the Priest Rapids Dam (PRD) fish ladder as they migrate upstream through the Columbia River. The fish are transported from PRD by truck and released in the upper portion of the Cle Elum Lake. Additionally, returning adults to the Yakima River are collected at the RAMF and translocated to the lake.

Year	No. Adult Outplant	No. Adult Returns		Total
2009	1,00	17		1,017
2010	2,500	40		2,540
2011	4,000	13		4,013
2012	10,000	154		10,154
2013	4,500	691		5,191
2014	10,000	2,576		12,576
2015	10,000	95	*	10,095
2016	10,000	3,677		13,677
2017	1,000	372	*	1,032
2018	4,700	455	*	5,155
2019	0**	174		174

* exceptionally high summer temperatures in 2015, 2017 and 2018 resulted delays in upstream migration and increased mortality during migration of adult Sockeye Salmon, and dramatically reduced returns to the RAMF relative to what was anticipated

** the Sockeye Salmon run to Bonneville Dam did not reach the minimum threshold of 80,000 required for take of fish at PRD for the translocation to Cle Elum

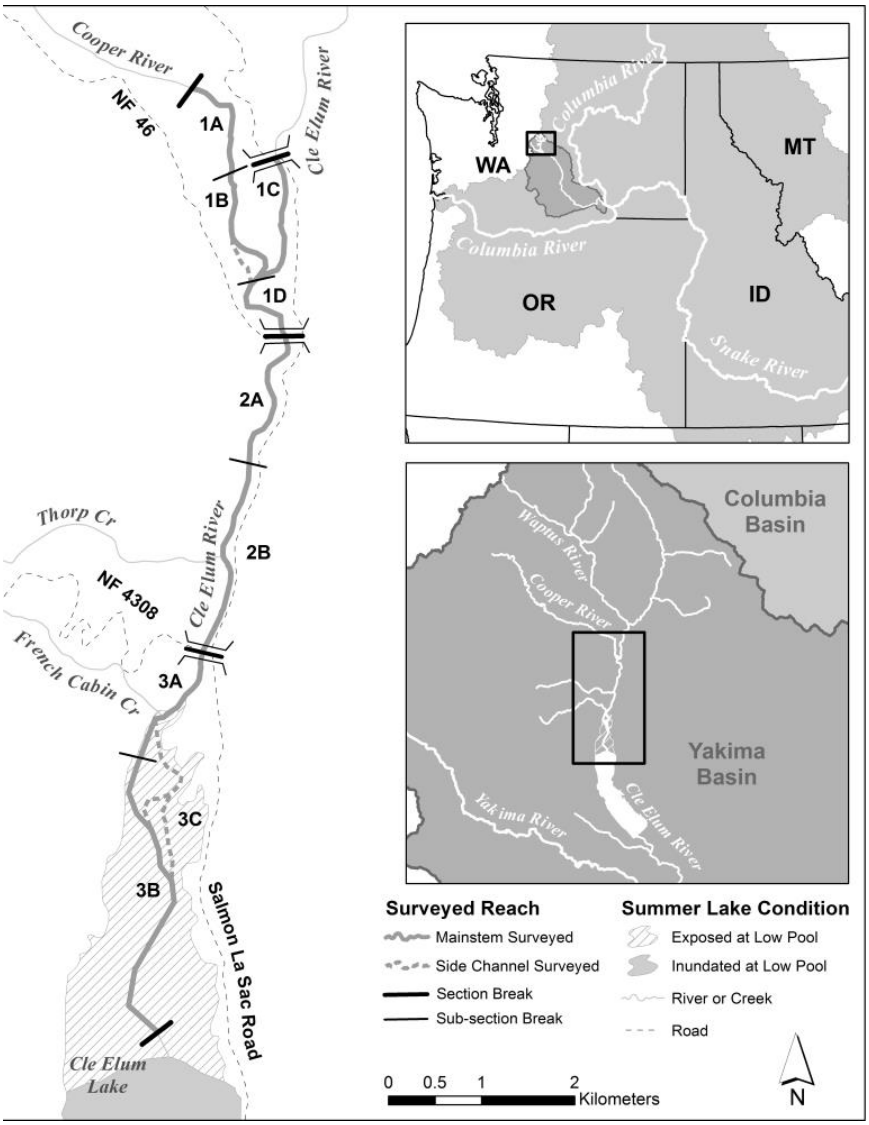
The adults at PRD represent a mix of fish originating from the two remaining Mid/Upper Columbia River Sockeye Salmon populations – Wenatchee stock (WEN) from Wenatchee Lake/Wenatchee River, and Osoyoos stock (OSO) from Osoyoos Lake/Okanogan River. Lake Wenatchee is substantially higher in elevation (572 m) and has colder average water temperatures (https://waterdata.usgs.gov/wa/nwis/) than Osoyoos Lake (278 m). While timing of the return migration through the Columbia River mainstem of adults from the two stocks is similar, spawn timing for WEN Sockeye Salmon is 3 to 4 weeks earlier than for OSO Sockeye Salmon. Additionally, the two stocks exhibit differences in average age structure. WEN stock exhibit a somewhat higher incidence of age-2 smolts than does the OSO stock, and among adult returns, a near total absence of age-3 jacks and a higher incidence age-5 fish (see below; "Identification of Columbia Basin Sockeye Salmon Stocks" annual reports http://www.critfc.org/fish-and-watersheds/fishery-science/scientific-reports/search/?r_keyword=IDENTIFICATION+OF+COLUMBIA+BASIN+SOCKEYE++SALMON+STOCKS; and Jeffrey Fryer, personal communication).

Sockeye	Smolt Age		Adult Age		
Stock	1+	2+	3	4	5
Osoyoos	0.91	0.09	0.11	0.73	0.16
Wenatchee	0.82	0.18	0.00	0.59	0.41

While Cle Elum Lake is more similar to Wenatchee Lake in elevation and water temperature profile, river flow and water temperature in the lower Yakima River, particularly during the summer months when the adults return in-basin, more closely resemble conditions observed in the Okanagan River. Upon initiation of the Cle Elum reintroduction program, it was therefore unclear how adaptive differences between stocks might affect their productivity in the novel Cle Elum Lake/Yakima River environment.

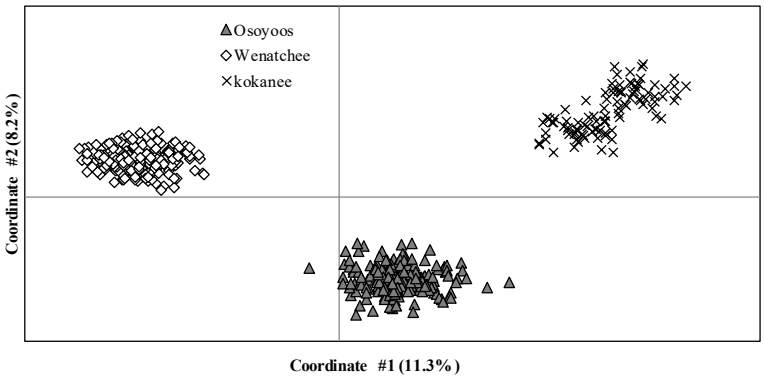
As part of normal YN monitoring for migratory salmonids in the Yakima River, tissue samples (fin clips) are collected from all adult salmon, including Sockeye Salmon, migrating upstream through the RAMF, as well as from a sample of out-migrating juveniles at the Chandler smolt collection facility adjacent to Prosser Dam (rkm 76), Prosser WA. However, the Sockeye Salmon reintroduction program has had only minimal funds and personnel available to support monitoring activities, and lacked any means to finance genetic analyses of the collected tissues. In order to provide the needed information with which to assess relative productivity of the reintroduced fish, in 2011 the Project committed to financing genetic analyses of tissue samples collected from: a portion of the translocated PRD adults, all returning adults sampled at the RAMF, post-spawned adults encountered during spawning ground surveys (see map below), Sockeye Salmon caught as by-catch during gill-netting performed to remove exotic Lake Trout from Cle Elum Lake, and a sample of out-migrating juveniles.

The map in the figure below identifies the different spawning ground survey reaches in the Cle Elum River upstream of the lake.

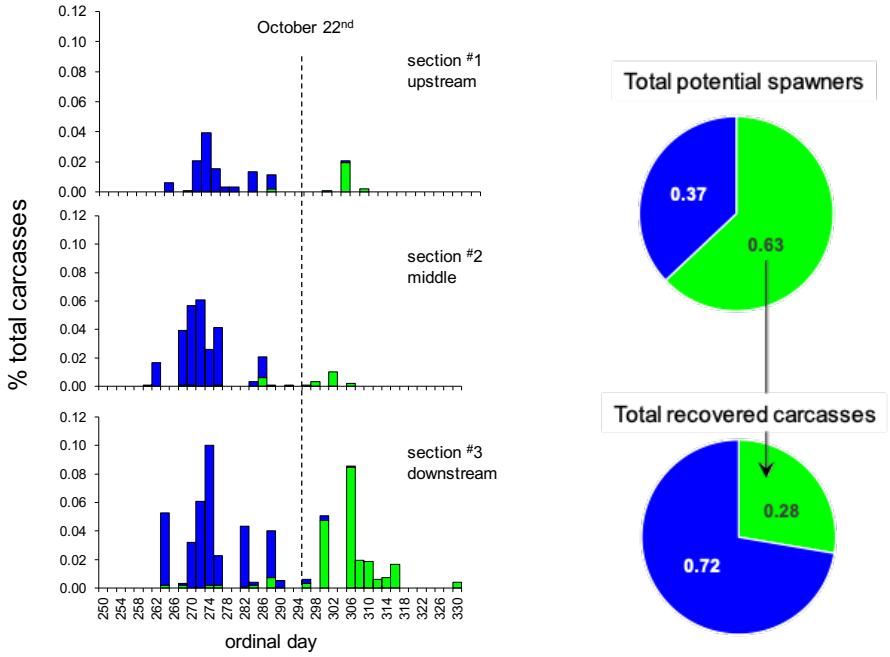


The tissue samples collected annually have been sent to CRITFC geneticists at the HFCES, where DNA was extracted and genotyped initially for a standardized panel of 94 SNP DNA markers. In 2016, the SNP panel was expanded to 364 loci employing RAD sequencing and the GT-seq protocol (Campbell et al. 2015), and DNA from adults that returned to Roza in 2013 through 2015 was re-genotyped with the expanded panel. The larger number of SNP markers substantially increases statistical confidence in the GSI assignments, and in parentage analyses.

Sockeye Salmon of the WEN and OSO stocks display distinctly different genetic profiles (Winans et al. 1996; Campbell and Narum 2011; Waples et al. 2011; Campbell et al. 2015; Hess et al. 2015), allowing accurate assignment of individuals to stock-of-origin using genetic stock identification (GSI) analysis, as well as of any inter-stock hybrids, as illustrated in the Principal Components analysis (PCA) plot:

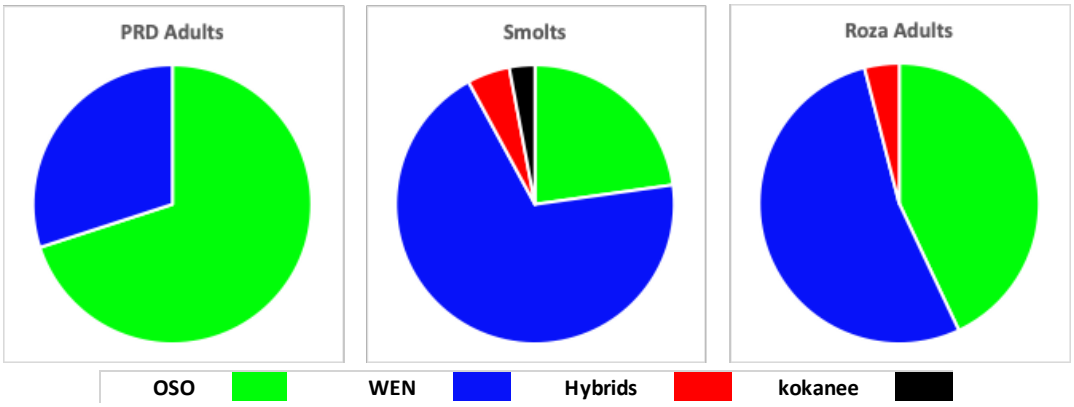


Temporal and spatial differences in spawning between stocks have been consistently observed among the two stocks. Post-spawned WEN carcasses are encountered from late September through early to mid-October, and are found across all three reaches (1, 2 and 3; see figure below). A lull in the number of carcass recoveries occurs in mid-October followed by an increase of fish which are 96% OSO, the large majority of which were found in Reach 3. It is also notable that gill netting conducted in Cle Elum Lake in mid to late October to remove predatory exotic Lake Trout incidentally captured a number of Sockeye Salmon, 100% of which were OSO. Many of these fish were flowing milt or eggs, and thus appear to have been actively spawning in the lake.



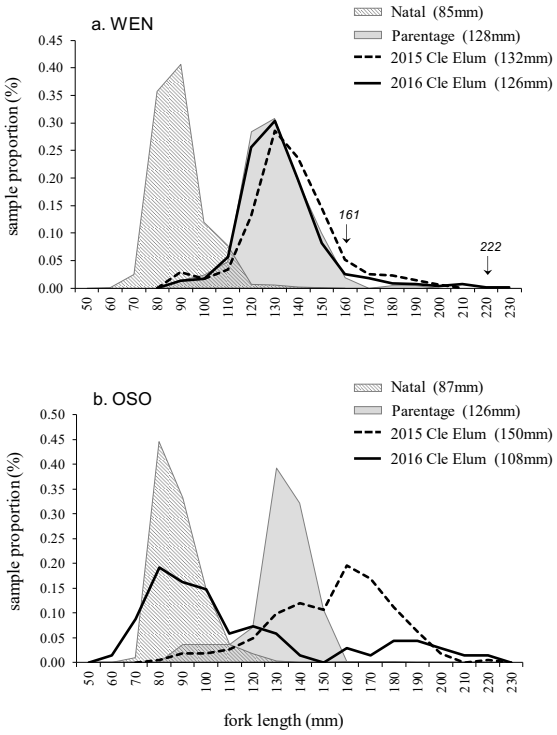
GSI analyses indicate that the average proportions of PRD Sockeye Salmon translocated to Cle Elum Lake have been 70% OSO and 30% WEN. In contrast, returning adults sampled at Roza Dam then transported to the lake have averaged 43% OSO and 53% WEN, plus 4% that assigned as inter-stock hybrids. Together, stock proportions of these potential spawners released in the lake have been 66% OSO and 34% WEN (plus an insignificant number of hybrids). GSI analyses of juvenile out-migrants sampled

principally at the Chandler smolt collection facility have been 23% OSO, 70% WEN, 5% inter-stock hybrids, and 3 % kokanee (most likely outmigrants from kokanee populations in other Yakima Basin lakes upstream of Chandler). The proportion of hybrids (5%) is far below that which would be expected presuming random mating of the fish. The spatial and temporal differences in spawn timing observed in the carcass GSI analyses, are no doubt largely responsible for this high level of reproductive isolation between stocks. The disproportionately higher percentage of juveniles that are WEN stock attest to an apparent higher rate of reproductive success of WEN adults relative to OSO adults. However, it is also notable that to present, the proportion of returning adults that assign to OSO is higher than for the juveniles – possibly indicative of a higher smolt to adult return rate. However, to assess whether this might be the case will require further years of monitoring.



Observations on the size and age composition of the juvenile out-migrants have been somewhat unexpected, and also complicate assessment of the relative productivity of adults of OSO versus WEN stock. Fork lengths of the smolts measured in 2015 and 2016 for WEN and for OSO adults are illustrated below. In both years the size frequency distributions for WEN juveniles were normally distributed with an average of approximately 130 mm. As indicated previously, however, smolts out-migrating from Lake Wenatchee average only 85 mm in fork length. Given their larger size, one might presume the Cle Elum smolts to be age-2 (18% of Lake Wenatchee smolts out-migrate as age-2 fish). However, genetic parentage assignments obtained for 202 juveniles showed them to all be age-1. As such, one might presume that food resources per individual must have been much more abundant relative to those in Lake Wenatchee, leading to the larger size at age-1.

In contrast to the WEN juveniles, OSO juveniles showed highly skewed size frequency distributions, which contrasted between years. In 2015 it was skewed to the right, with a modal value of 160 mm, while in 2016 it was skewed to the left with a modal value of 80 mm. One might expect a preponderance of age-2 fish in 2015 and of age-1 fish in 2016. However, parentage assignments for OSO juveniles, though limited to only 33 fish, determined them all to be age-1. Why there was such a strong difference in growth and size at outmigration between years for OSO juveniles and not WEN juveniles is unknown.



The Project will continue to support genetic analyses for the reintroduction program over the coming years, with the focus shifting to: 1) monitoring for inter-stock hybrids, 2) assessment of differences in age and size at out-migration of smolts between stocks, 3) assessment of relative productivity of new Cle Elum origin adults relative to PRD translocated fish, within and between stocks, and 4) investigation of stock differences in movement and spawning behavior of a portion of the translocated PRD adults that are released in Cooper Lake - a small lake located several miles upstream of Cle Elum Lake - for the purposes of increasing juvenile production. Information obtained will be important to YN, not just for evaluating success of the current reintroduction program, but to help guide future reintroduction efforts in the Yakima River basin that have been proposed for Keechelus, Kachess, Bumping and Rimrock lakes. Similarly, findings can inform how Sockeye Salmon reintroduction might be considered in other basins, e.g., Wallowa Lake, Palmer Lake, and Lake Billy Chinook and Suttle Lake (see below).

D.2 Suttle Lake/Lake Billy Chinook Salmon (Metolius/Deschutes Rivers) Sockeye Salmon/kokanee

Suttle Lake is located in the headwaters of the Metolius River, a tributary to the Deschutes River, Oregon. Suttle Lake and Wallowa Lake (Grande Ronde River subbasin) were the only two locations in Oregon where Sockeye Salmon were indigenous, and both populations were similarly extirpated. In approximately 1925, a small dam was constructed near the outlet of Suttle Lake to Lake Creek (which flows approximately 8 km from Suttle Lake to the Metolius River) to create a swimming area for the nearby Lake Creek Lodge. While not a total blockage, the dam impaired upstream migration of Sockeye Salmon adults. A few years later a larger (1.2 m) concrete dam associated with a small hydroelectric facility was constructed just downstream in Lake Creek. The dam was constructed with a fish ladder, however, the ladder was undersized and upstream passage was hindered or totally blocked depending on water flows. In addition, screens installed in the inlets to the turbines prevented downstream

escapement of juveniles. Over subsequent years, Sockeye Salmon numbers diminished further, and the population in Suttle Lake soon became functionally extirpated (Nielson 1950; Olsen et al. 1994; Nehlson 1995; Gustafson et al. 1997). Nonetheless, a limited number of Sockeye Salmon continued to return to the Deschutes basin and migrated to the upper Metolius River (downstream of Suttle Lake) where they spawned. The juveniles produced apparently reared in the lower Deschutes or the Columbia River, as a few Sockeye Salmon continued to return to the Deschutes Basin each year (Olsen et al. 1994; Gustafson et al. 1997). Then from 1958 through 1964 the Pelton-Round Butte Hydroelectric Project was created on the Deschutes River, involving construction of Pelton Dam (rkm 160), the Reregulating Dam (rkm 164) and Round Butte Dam (rkm 176). While the complex was constructed with facilities to provide both upstream and downstream passage of anadromous fish, the system for downstream passage proved ineffective, and within a generation or two, the small remnant run of Sockeye Salmon became functionally extirpated from the Deschutes Basin (Olsen et al. 1994; Gustafson et al. 1997).

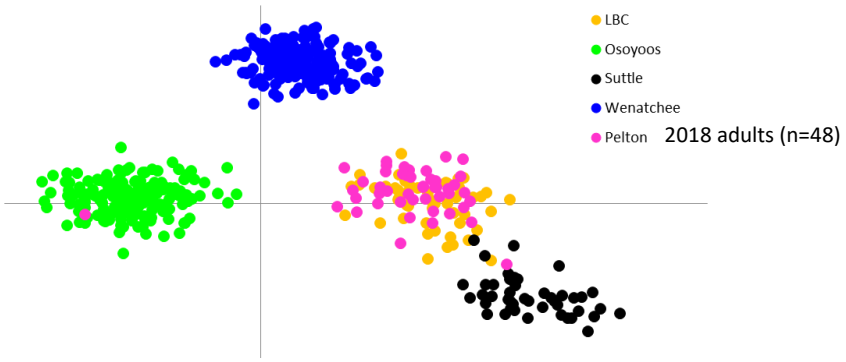
However, in Lake Billy Chinook Salmon (LBC), the reservoir created by Round Butte Dam, a large non-anadromous *O. nerka* (kokanee) population developed. Mature kokanee in LBC migrate upstream into the upper Metolius River for spawning each year, with the newly emerged juveniles migrating back down to the lake for rearing (Nehlson 1995, Gustafson et al. 1997). While this kokanee population may have derived initially from the remnant Sockeye Salmon population, the LBC kokanee, as well as a kokanee population that developed in Suttle Lake, more likely developed from repeated stocking with smolts, both Sockeye Salmon and kokanee, from various out-of-basin hatcheries through the mid-1900s (Olsen et al. 1994; Nehlson 1995; Gustafson et al. 1997).

In negotiations finalized in 2005 for relicensing of the Pelton-Round Butte Hydroelectric Complex, an agreement was reached to re-establish passage for Sockeye Salmon (as well as for steelhead, spring Chinook Salmon and bull trout). Co-managers presumed that some portion of juvenile kokanee that exhibited out-migration behavior from LBC and/or Suttle Lake, might also exhibit anadromy and return as mature adults - characteristic of a Sockeye Salmon life history. A fish transfer facility (FTF) on the new surface water withdrawal (SWW) structure at Round Butte Dam, constructed as part of the relicensing agreement, became operational in 2010. Each year since, *O. nerka* juveniles (silvering, and of a size characteristic of an out-migrating Sockeye Salmon smolt) that volunteer into the FTF have been passed downstream. Notably, the number has varied widely between years, from a few thousands to a few hundreds of thousands. Returning adults from these releases are captured at a trap at Pelton Regulation Dam. These adults have generally been transported upstream for release into LBC. In some years, some of these adults were for use as hatchery broodstock at Pelton Hatchery, located immediately below Round Butte Dam. There the fish were spawned, the eggs incubated, and the juveniles reared for a few months before release into LBC as small parr. Number of out-migrating juveniles released downstream of the dam complex annually, adults trapped at Pelton Dam, the number of these adults retained as hatchery broodstock, and the number of hatchery-reared progeny from these broodstock are illustrated below. The table also provides a smolt-to-adult (SAR) estimate, which presumes that smolts released from the FTF are age-1+, and that adults corresponding to each release year return two years later at age-3+ (although see subsequent table providing sex ratio and age structure data).

Year	Juveniles Released Downstream of SWW	Total Adults at Pelton Trap	% "SAR" (presuming All Age 1+ Smolts Return as Age 3+ Adults*)	Adults Released into LBC	Adults Recycled Downstream	Adults Kept for Broodstock	Hatchery Parr Released into LBC
2010	49,734	10	0.197%	10	0	0	-
2011	225,761	23	0.015%	0	4	19	-
2012	5,126	98	0.527%	86	12	0	3,870
2013	25,265	33	0.142%	25	8	0	-
2014	155,031	27	0.346%	20	7	0	-
2015	38,702	36	0.147%	0	0	36	-
2016	49,497	536	0.099%	463	0	73	13,122
2017	439,458	57	0.016%	18	0	39	35,515
2018	47,161	49		27	0	22	22,000
2019	82,146	69			0		

Success of the reintroduction program depends on two factors: 1) that the anadromous adults captured at the Pelton trap are indeed of LBC origin, and 2) that the number of returning adults will be sufficient to restore an eventually self-sustaining population of anadromous Sockeye Salmon. To answer the first question, an agreement was reached with CTWSRO in 2011 for the Project to finance a genetic stock identification (GSI) study. Tissue samples were collected annually from in-migrating adults captured at the Pelton adult trap. The samples were sent to CRITFC geneticists at the HCFES where the DNA was extracted and genotyped for a standardized panel of 94 SNP markers. Genotypes for these individuals were then compared to the genotypic profiles for a collection of reference/baseline Sockeye Salmon and kokanee populations from across the Columbia Basin, that also included samples collected within the Deschutes Basin from Suttle Lake adults and out-migrating juveniles captured in Lake Creek, LBC adults and out-migrating juveniles captured at the FTF, and kokanee at other upper Deschutes River locations (Paulina Lake, Wizard Falls Hatchery, and Odell Lake), and from two hatchery populations (Meadow Creek and Lake Whatcom) stocks widely used for stocking of lakes and reservoirs across the Columbia Basin. The GSI analyses assigned each returning adult to the population of highest probability among these reference populations.

As an illustration of the GSI analyses, results for the 2018 adults (n=48) are presented graphically below, limited to comparison to Osoyoos Lake and Wenatchee Lake Sockeye Salmon (the only two remaining Columbia Basin populations with significant abundance), and to LBC and Suttle Lake kokanee. Of the 48 samples (pink dots), all but one cluster together with the reference sample of LBC kokanee, assigning with 100% likelihood to being of LBC origin. A single individual (pink dot) clusters with the Osoyoos Lake reference population (green dots), indicating that this individual was an Osoyoos Lake Sockeye Salmon that strayed into the Deschutes River. Of interest, the Suttle Lake and LBC reference populations form two nearly distinct clusters, indicative of differences in their genetic profiles of sufficient magnitude to distinguish LBC origin fish from Suttle Lake origin fish with relatively high certainty. These differences in profile relate, no doubt, to differences in the number and magnitude of stocking events with the various out-of-basin kokanee and Sockeye Salmon stocks that occurred in Suttle Lake and LBC during the 1900s, and the extent to which fish from these stocking events successfully spawned and their progeny became integrated into the natural population.



Of further interest are results from an exercise in which samples within the *O. nerka* baseline were pooled within kokanee and Sockeye Salmon populations separately, then individuals from Suttle Lake and LBC were tested for assignment to one or the other composite population. The rate at which Suttle Lake kokanee individuals assigned to the kokanee composite was 100%. In contrast, assignment of the LBC individuals to the kokanee composite was 90%, with 10% of the individuals assigning with greatest likelihood to the Sockeye Salmon composite. This would appear to indicate a larger remnant signal of Sockeye Salmon genetic heritage in the LBC population.

Overall GSI results for in-migrating adults captured at the Pelton Trap in 2010 through 2018, that were successfully genotyped (total N = 825) are illustrated below. Over 91% of the fish were indeed assigned with highest likelihood to LBC, with an additional 4% assigning to Suttle Lake or other upper Deschutes Basin lakes.

Reference Population	% Assignment
Lake Billy Chinook	91.2%
Suttle Lake	0.5%
Other Upper Deschutes Lakes	3.2%
Kokanee	
Wallowa Lake	1.3%
Sockeye Salmon	
Redfish Lake	1.3%
Wenatchee Lake	0.5%
Osoyoos Lake	2.1%

In addition to collecting tissue samples for genetic analysis, sex was identified for most adults captured in the adult trap, and a scale sample was collected. Sex and scale age analyses, limited to those adults that assigned to the Deschutes Basin (LBC, Suttle Lake or the upper Deschutes) for 2012 through 2018 (less 2017 for which age data are not yet available) is summarized in the table below. Unexpectedly, sex ratio was highly skewed (2:1) towards females (overall - 65% females and 35% males). This contrasts markedly with the sex ratio of Osoyoos Lake Sockeye Salmon sampled at Wells Dam which for the years 2014 through 2017 averaged 47% females and 53% males (Fryer et al. 2016, 2017 and 2018). The age structure for the adults captured at the Pelton trap which assigned to the Deschutes Basin was: 21% age-3, 75% age-4 and 4% age-5, which is not dissimilar to those observed for Osoyoos Lake Sockeye Salmon, especially if the proportion of age-3 fish is overestimated (due to excessive resorption at the scale margins for the fish that occurs by the time they arrive at the Pelton trap). Wenatchee Lake adults include essentially no age-3 fish, and relatively more age-5 fish).

	Females		Males		Age 3 1.1		Age 4 1.2 (2.1)		Age 5 2.2 (1.3)	
Year	#	%	#	%	#	%	#	%	#	%
2012	48	0.62	30	0.38	22	0.27	61	0.73		
2013	14	0.58	10	0.42	1	0.04	23	0.96		
2014	14	0.70	6	0.30	13	0.72	5	0.28		
2015	5	0.31	11	0.69	9	0.64	3	0.21	2	0.14
2016	285	0.67	138	0.33	57	0.16	285	0.82	6	0.02
2017	32	0.56	25	0.44						
2018	34	0.71	14	0.29	11	0.23	26	0.54	11	0.23
Overall	432	0.65	234	0.35	113	0.21	403	0.75	19	0.04

Results from the GSI analyses provide confirmation that juvenile progeny released downstream from the FTF have indeed (re)expressed an anadromous life history. However, relative to the return number being sufficient for restoring a self-sustaining natural population of Sockeye Salmon above Round Butte Dam, results have been discouraging. The overall SAR for the juveniles released downstream of the FTF has only been approximately 0.1%, with less than 100 fish have returned annually. The sole exception is 2016 when over 500 fish returned to the basin, for an approximate SAR of 0.35%. Program co-managers are currently examining possible modifications to management of the reintroduction program, including: increased hatchery spawning of returning adults; rearing of hatchery juveniles to the smolt stage; and/or bringing in fish from out-of-basin Sockeye Salmon populations – as smolts, or mature adults for release into LBC or for hatchery spawning. In the meantime, the Project will continue to provide GSI analyses of the returning adults.

E. Project Objective #5: Evaluate factors affecting minijack production

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 initiate maturation in freshwater prior to out-migration to the ocean, maturing at age-1 (precocial parr, or microjacks) or at age-2 (minijacks; Larsen et al. 2013). Microjacks have been observed among both NOR and HOR cohorts, though their proportion within a broodyear's progeny is low and presumed of minimal concern to fisheries and hatchery managers (Gebhards 1960; Mullan et al. 1992; Larsen et al. 2013). Likewise, the rate at which NOR juvenile males mature precociously as minijacks is very low, though not so for HOR males. In a recent series of studies using a physiological assay of blood plasma (Larsen et al. 2004, 2006, and 2010; Beckman and Larsen 2005), it has been observed that among male spring Chinook Salmon smolts in regional hatchery programs, significant proportions (as high as 70%) are maturing precociously as minijacks. Because hatchery smolts that have initiated maturation are not externally distinguishable from their non-maturing counterparts at the time of release in the spring, the magnitude of minijack production within and across hatchery programs has generally been overlooked (Beckman and Larsen 2005). However, because minijacks do not migrate to the ocean to return one or more years later as adults, their incidence proportionally reduces the ability of regional hatchery programs objectives to return large adult sized fish for fisheries and/or for natural population supplementation (Taylor 1988; Clarke and Blackburn 1994; Shearer and Swanson 2000; Zimmerman et al. 2003; Beckman and Larsen 2005).

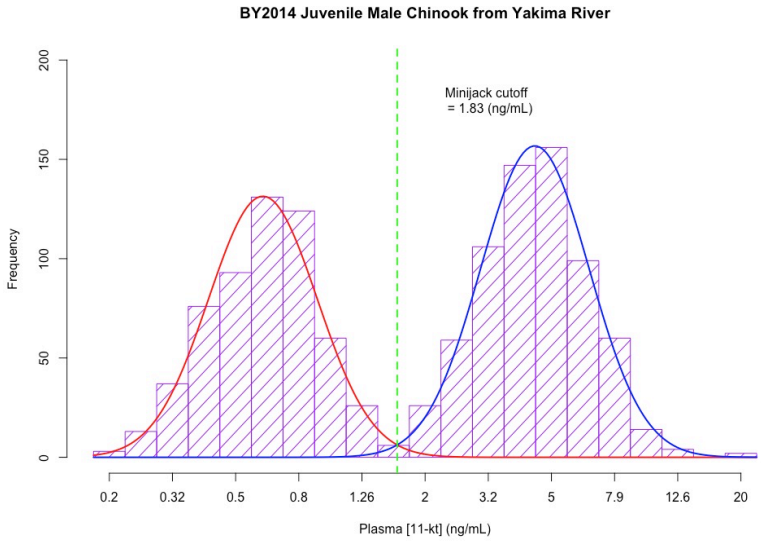
High rates of precocious male maturation of hatchery Chinook Salmon juveniles appears to be largely an environmental response to the rearing conditions they experience. High feeding rates and in many cases elevated water temperatures relative to natural conditions, lead to accelerated growth and high adiposity levels, which in turn affect physiological and endocrinological processes that can trigger precocious initiation of gonadal maturation among juvenile males (Rowe and Thorpe 1990; Clarke and Blackburn 1994; Saunders et al. 1982 and 1986; Rowe et al. 1991; Silverstein et al. 1997 and 1998; Shearer and Swanson 2000; Shearer et al. 2006; Thorpe 2004). However, there is also ample evidence for a heritable genetic component to the trait, both for adult age at maturity (e.g., Gjerde 1984; Iwamoto et al. 1984; Herbinger and Newkirk 1990; Wild et al. 1994; Garant et al. 2002; Carlson and Seamons 2008; Hankin et al. 2009), as well as for precocious male maturation rate in freshwater (e.g., Thorpe et al. 1983; Myers and Hutchings 1986; Herbinger and Newkirk 1990; Silverstein and Hershberger 1992; Duston et al. 2005; Piche et al 2008; Easton et al. 2011). To look further into the factors associated with precocious minijack production, and to test possible approaches 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 Project-funded studies:

E.1 Evaluate effect of hatchery broodstock age on minijack production

To assess the extent to which age of hatchery broodstock might have a genetically based effect on the rate of precocious minijack production, we designed a study in which samples of gametes from broodstock of known ages would be subdivided (approximately 200 eggs per mating) and factorially crossed to produce test matings of all possible parental age combinations. An agreement was reached with YN in 2014 to perform this study at the CESRF for three BYs - 2014, 2015 and 2016. Initially, the target design was for multiple 3x3 factorials involving crosses of one each of age-3, age-4 and age-5 males with two age-4 and one age-5 females, although as it turned out, very few age-5 fish were available in any year. Additionally, in BYs 2015 and 2016 the design was expanded to include age-1 microjacks as sires – fish that were captured by WDFW field crews during snorkel surveys of the spawning grounds, then transported and held at the hatchery.

Following incubation (and measurement of fry survival and growth for each mating), samples of 50 swim-up fry per cross were pooled into a raceway for rearing to the smolt stage. The fish were fed at standard CESRF rates. In April a year later, the age-1+ smolts were sacrificed, dissected, and identified to phenotypic sex, and the males were measured (length and weight), and blood and fin tissue sampled. A biochemical assay evaluating blood plasma 11-ketotestosterone (11-KT) concentration was used to characterize the male progeny as maturing minijacks (high 11-KT) versus non-maturing smolts (low 11-KT; Larsen et al. 2004; Medeiros et al. 2018). DNA was extracted from the fin tissue samples and genotyped for a standardized panel of SNP DNA markers, parentage analysis used to assign each individual to its family, and the proportion of minijacks was calculated for each full-sib male progeny group.

The frequency distributions for plasma 11-KT concentration in the male smolts were significantly bimodal in each of the three years, as illustrated below for BY 2014. Cutoff values between low and high 11-KT for the purposes of identifying the juveniles as non-maturing versus maturing were calculated using Hartigans' dip test (CRAN.R-project.org/package=diptest; Medeiros et al. 2018). Results for the three broodyears were: BY 2014 = 1.83 ng/mL, BY 2015 = 1.52 ng/mL, and BY 2016 = 1.27 ng/mL.



Genotyping of the juveniles for two sex-specific SNP loci was used to confirm sex identification of each smolt, and parentage analyses identified each to their respective family. The proportion of minijacks per male progeny group was then calculated, as well as average minijack rate per parental age cross type (female age x male age) within BYs. Results for the three BYs are summarized below (with the highest average rate "boxed"):

Broodyear 2014

4x3	4x4	4x5	5x3	5x4	5x5
0.13	0.17	0.04	0.46	0.05	0.20
0.18	0.17	0.25	0.57	0.26	0.35
0.22	0.23	0.41		0.70	0.57
0.25	0.27	0.55			
0.30	0.45	0.56			
0.32	0.53	0.68			
0.38	0.57				
0.44	0.59				
0.50	0.62				
0.52	0.64				
0.58	0.65				
0.60	0.68				
0.60	0.69				
0.61	0.75				
0.67	0.76				
0.77	0.79				
0.79	0.86				
0.81	0.91				
0.85	0.94				
1.00	0.95				
AVG:	**0.52**	**0.61**	**0.42**	**0.51**	**0.34**	**0.37**

Broodyear 2015

4x1	4x3	4x4
0.00	0.00	0.00
0.19	0.04	0.04
0.26	0.05	0.05
0.39	0.08	0.06
0.48	0.13	0.14
0.48	0.15	0.17
0.48	0.19	0.17
0.55	0.23	0.18
0.55	0.32	0.25
0.56	0.35	0.27
0.60	0.35	0.27
0.70	0.40	0.29
0.83	0.41	0.31
0.87	0.43	0.36
0.88	0.44	0.40
0.91	0.57	0.43
0.91	0.70	0.50
0.95	0.71	0.52
	0.80	0.53
		0.55
		0.56
		0.61
		0.64
		0.65
		0.73
		0.74
		0.78
		0.84
		0.85
		0.85
		1.00
AVG:	**0.59**	**0.33**	**0.44**

Broodyear 2016

4x1	4x3	4x4	4x5	5x1	5x3	5x4	5x5
0.00	0.11	0.04	0.11	0.04	0.20	0.00	0.27
0.05	0.19	0.09	0.17	0.38	0.23	0.04	0.40
0.06	0.20	0.20	0.27	0.52	0.36	0.16	
0.21	0.20	0.22	0.31	0.87	0.57	0.38	
0.24	0.24	0.23	0.32		0.75	0.46	
0.29	0.25	0.28	0.33		0.83	0.64	
0.29	0.27	0.29	0.38				
0.38	0.33	0.35	0.45				
0.46	0.37	0.38	0.48				
0.48	0.40	0.39	0.56				
0.55	0.41	0.41	0.62				
0.60	0.52	0.41	0.86				
0.63	0.55	0.43	0.94				
0.65	0.56	0.43	0.95				
0.68	0.57	0.50					
0.81	0.74	0.65					
0.85	0.82	0.94					
	0.84						
	0.90						
AVG:	**0.42**	**0.45**	**0.37**	**0.48**	**0.45**	**0.49**	**0.28**	**0.34**

In light of evidence in the published literature for a heritable basis to age at maturation, we hypothesized that minijack rate would decrease with an increase in parent age, in particular for male broodstock (the low numbers of age-5 broodstock among both sexes did not permit assessment of a female age effect, and limited assessment of a male effect to ages 1, 3 and 4). A simple comparison of average minijack rates within broodyears, however, does not support this hypothesis. The rank order for average minijack rate changed between broodyears, e.g., the parental age cross type with the numerically highest minijack rate (indicated by the boxed value, and limited to crosses to age-4 females (due to low sample size for age-5 female crosses) was for the 4x4 crosses in 2014, the 4x1 crosses in 2015 and the 4x5 crosses in 2016. ANOVAs for cross-types, performed within BYs, indicate no significant differences associated with male broodstock age. It is possible that male age did have an effect on minijack rate, but that the effect is masked by other factor(s) which led to the extremely high variation in minijack rates within parental age cross types. The range of values was consistently from <20% to > 80%, and in the case for BY 2015 4x4 crosses, minijacks rates ranged fully from 0% to 100%. A more in depth statistical analysis of the data is underway, which will incorporate broodyear and broodfish size as factors. We expect this analysis to be complete in 2020, after which an oral presentation and a manuscript for submission for publication will be produced.

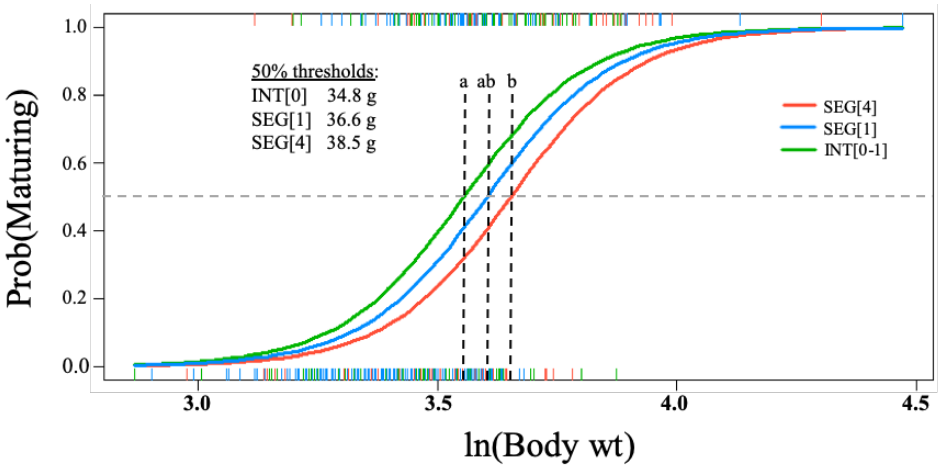
E.2 Effect of number of prior generations of hatchery rearing (domestication) on minijack production

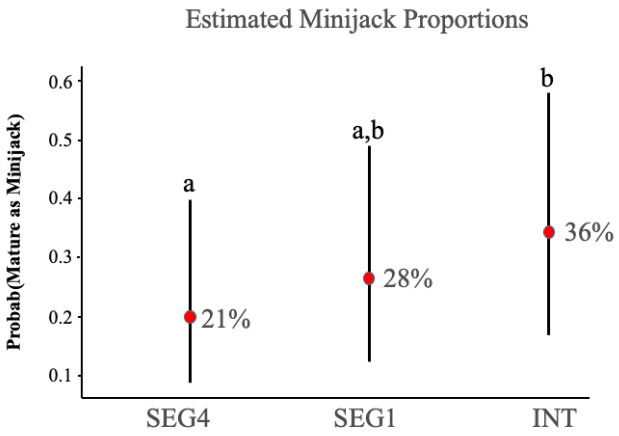
In a review of minijack rates estimated for hatchery Chinook Salmon programs across the Columbia Basin, Harstad et al. (2014) observed that segregated programs (in which broodstock is comprised entirely of HOR adults) demonstrated substantially lower minijack rates than integrated hatchery programs (in 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.

	HC	SH	WN	Avg:
F-SH-047	0.55	0.20	0.80	0.52
F-SH-137	0.62	0.84	0.86	0.77
F-SH-141	0.04	0.04	0.05	0.04
F-SH-142	0.05	0.04	0.43	0.17
F-SH-217	0.78	0.70	0.43	0.64
F-SH-209	0.08	0.28	0.24	0.20
F-SH-210	0.15	0.00	0.14	0.10
Avg:	0.32	0.30	0.42	

The data were pooled across females within sire type, and used to generate probabalistic 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 W_{50}; Larsen et al. 2019). As hypothesized, the PMRN W50 for crosses to HC sires was significantly lower than that for WN males PMRN W_{50}, with PMRN W50 for progeny of SH sires being intermediate; though given the high variation within sires, differences with the other two sires was non-significant.





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 2020. Results from these analyses will be summarized in an oral presentation and a manuscript for submission for publication will be produced.

E.3 Effects of feed supplementation with TTA on minijack production

In a previous study conducted on hatchery-reared juvenile Chinook Salmon at CESRF (Larsen et al. 2006), feeding rate was reduced different groups of fish below the standard rate, which resulted in a 15% to 60% reduction in minijack rate, 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). 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, et al. 2009; Arge, et al. 2014).

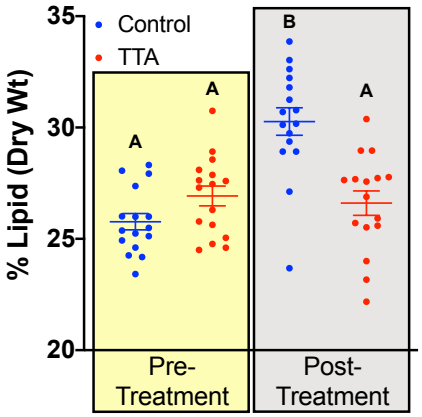
TTA, a commercially available nutritional supplement, is a 3-thia saturated fatty acid which upregulates cellular fatty acid oxidation, causing an increase in liver and muscle lipid metabolism similar to fasting. As a supplement in salmonid diets, TTA stimulates fatty acid oxidation and reduces lipid stores (Moya-Falcon et al. 2004; Gjoen et al. 2007; Alne et al. 2009; Arge et al. 2014;) and leads to reduced sexual maturation in male post-smolt age1 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, lipid metabolism, and growth regulatory hormones associated with dietary supplementation with TTA.

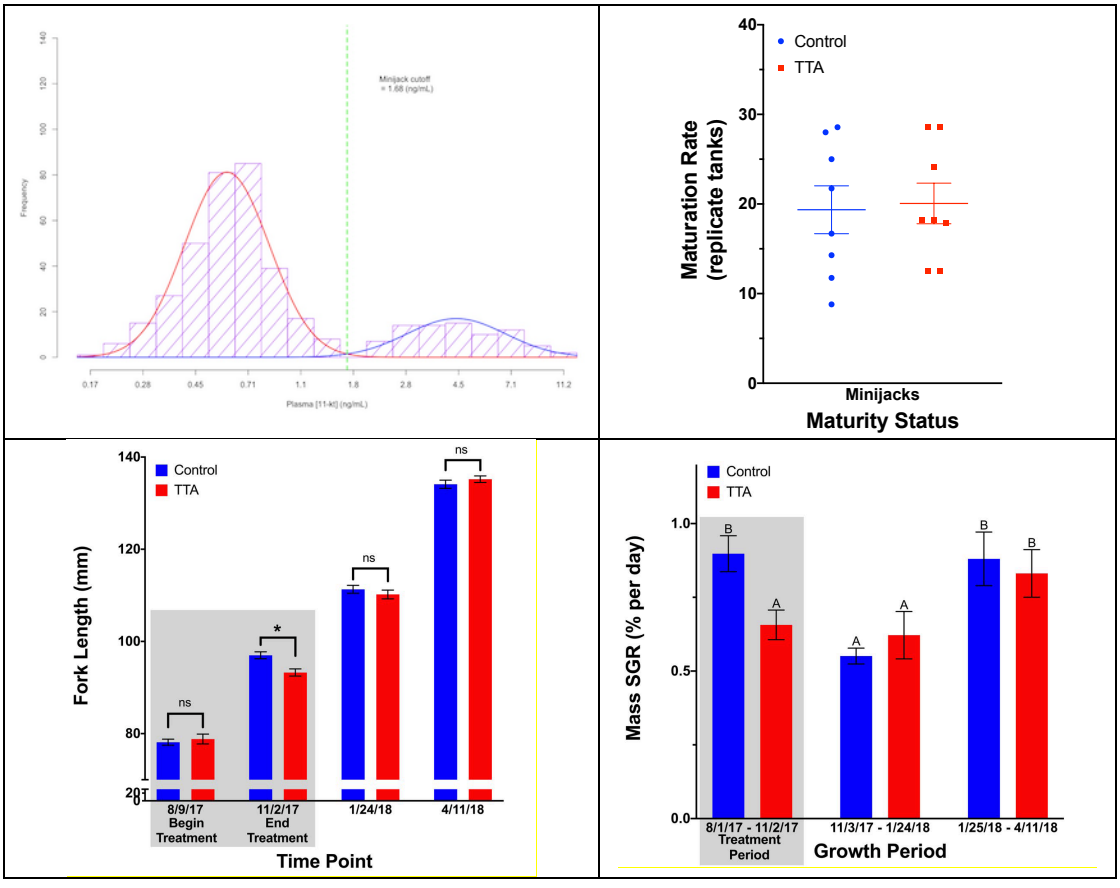
In March 2017, approximately 2,000 spring Chinook Salmon swim-up fry were transferred from CESRF to the Aquaculture Research Institute at the University of Idaho, Moscow ID. Fish were held in a common tank under simulated natural photoperiod, water temperature ranged from 12-15 ^{o}C, and the fish were 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 the fish oil top-coating 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, the livers were frozen for subsequent qPCR analysis, 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, and the livers and carcasses frozen.

Average whole body lipid content at the end of the TTA treatment was significantly lower for treated versus untreated fish.



Results for smolt plasma 11-KT concentration were strongly bimodal, with a cutoff value of 1.68 ng/mL. Below this value the fish were characterized as non-maturing, and above this level as maturing male minijacks. Unexpectedly, 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%. The treatment fish showed a reduction in size and growth relative to controls during the treatment period, though final smolt size of the treatment and control fish was similar. Given the lack of an effect of the TTA treatment on minijack rate, the logistical difficulties and expense to obtain TTA in quantities that might be required for use in a hatchery program, this line of inquiry was abandoned. Information generated in the study will be summarized in a CRITFC technical report in early 2020.



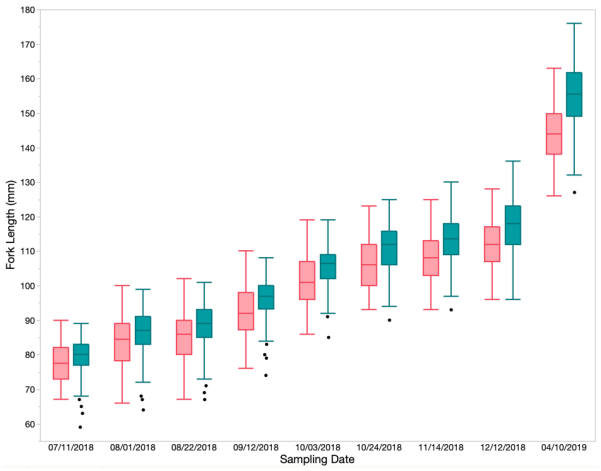
E.4 Growth of precociously maturing versus non-maturing smolts

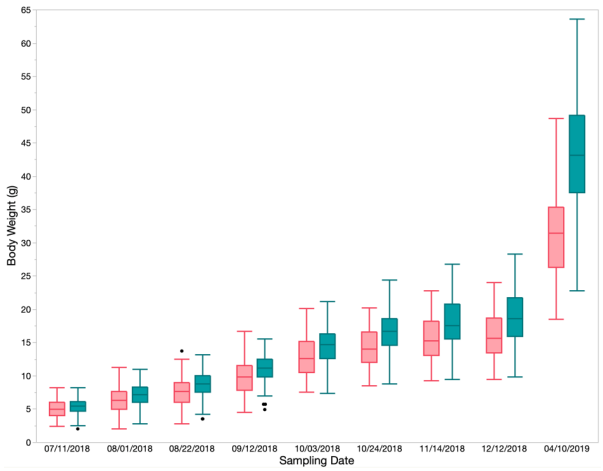
It has been proposed that the autumn months, one year following egg fertilization, constitute a critical period during which juvenile male spring Chinook Salmon make the physiological decision to initiate gonadal maturation or not, with those that do initiate the process reaching full maturation as age-2 minijacks in fall the following year. The decision is dependent whether or not the fish reaches a predetermined threshold for a combination of traits involving size, growth rate, and lipid content (Thorpe 2007, Mangel and Satterthwaite 2008). While this theory is widely accepted, it remains unclear when more precisely the decision occurs, and whether any of these and other physiological factors might exhibit measurable differences, such that one might distinguish maturing from non-maturing males at this early stage in juvenile rearing. A better understanding of these factors will be necessary if managers wish to identify modifications to rearing practices (e.g., reduction in feed lipid content and/or feeding rates; use of feed additives that accelerate lipid metabolism, etc.) in order to reduce the incidence of precocious maturation, without sacrificing juvenile smoltification, and outmigration survival and smolt-to-adult return rates.

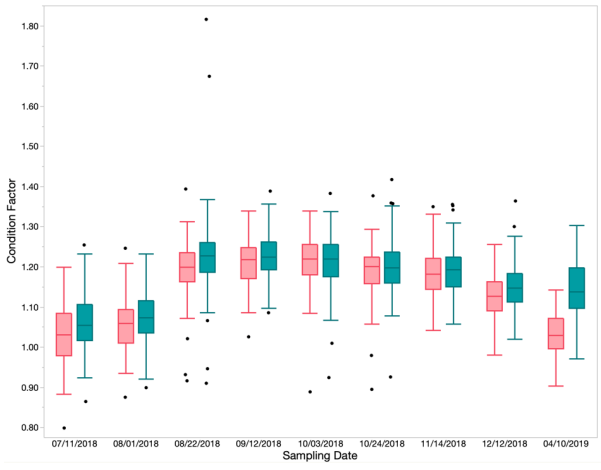
To better characterize the timing and magnitude of changes in the growth and adiposity characteristics of juveniles during the maturation decision period and how the timing and magnitude might vary among individuals, we initiated a study to follow individual growth of a cohort of spring Chinook Salmon 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 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 and maturation status determination. Additionally, the livers were weighed for estimation of hepatosomatic index (HSI) then frozen for later measurement of liver IGF-1 concentration, the pituitary glands were removed for measurement of pituitary FSH mRNA level, and the carcasses retained for measurement of whole body lipid. All collected tissues 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.

Initial data analyses for individual size and condition factor were conducted, pooling data 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 9-10, 2019) versus non-maturing smolts are illustrated below. The maturing males were significantly larger 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 4/9-10) sample dates.



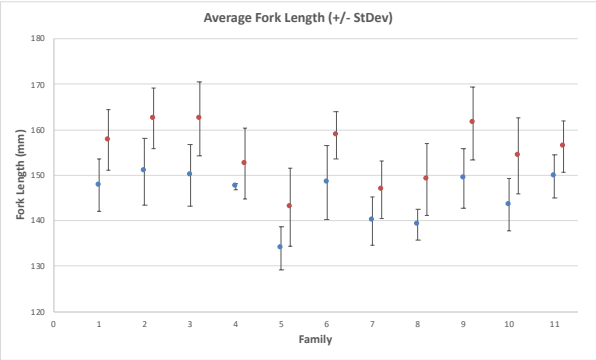


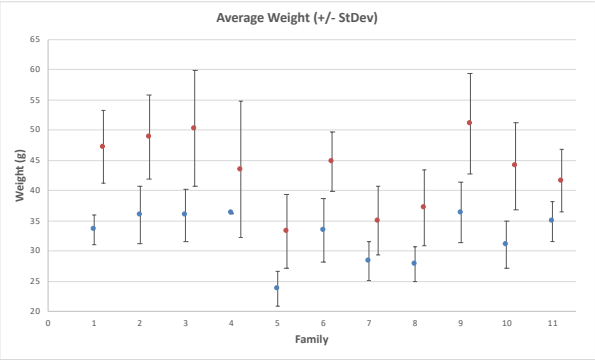


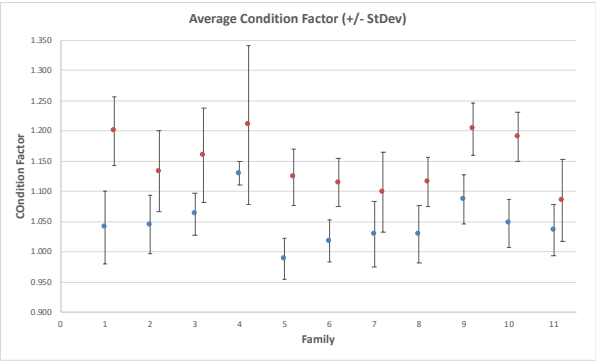
When comparisons of size, condition factor and proportion of minijacks are made between families at the final sampling in April, a substantial amount of variation was observed, as illustrated in the table and figures below. Average size and condition factors were lowest for Families #5, #7 and #8, and as might be expected Families #5 and #7 also demonstrated minijack rates well below the average rate across families (64%). In contrast, however, the minijack rate for Family #8 (73%) was well above the average, and while the male progeny in Family #3 were relatively large, the minijack rate (50%) was well below the average. The precocious maturation decision is therefore dependent on more than just surpassing a population threshold associated with size and growth, but is also strongly affected by a genetic component for additional characters that apparently varies substantially among individuals. This individual component was also observed in the very wide variation in minjack rate among families within

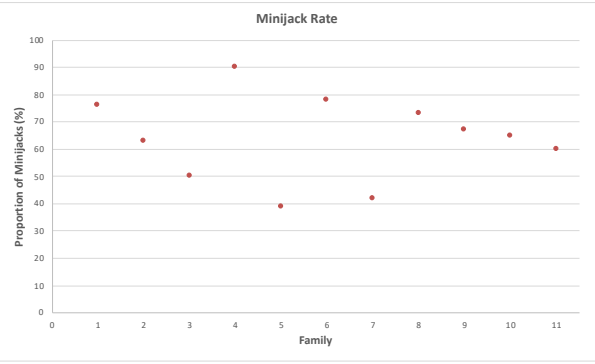
cross-types (parents of given ages) in the previously described study (E.1) to evaluate the effect of hatchery broodstock age on minijack production.

Family	Maturation	Number	Avg FL (mm)	StDev FL	Avg Wt (g)	StDev Wt	Avg Cond	StDev Cond	Minijack %
1	Non-Maturing	5	147.8	5.8	33.6	2.5	1.040	0.060	
	Minijack	16	157.7	6.7	47.2	6.0	1.200	0.057	0.76
			9.9		13.7		0.159		
2	Non-Maturing	9	150.8	7.3	36.0	4.8	1.045	0.049	
	Minijack	15	162.5	6.7	48.9	7.0	1.133	0.066	0.63
			11.7		12.9		0.088		
3	Non-Maturing	9	149.9	6.8	35.9	4.4	1.062	0.035	
	Minijack	9	162.4	8.2	50.2	9.6	1.160	0.078	0.50
			12.5		14.4		0.097		
4	Non-Maturing	2	147.5	0.7	36.2	0.1	1.130	0.020	
	Minijack	19	152.5	7.8	43.4	11.3	1.210	0.131	0.90
			5.0		7.2		0.080		
5	Non-Maturing	14	133.9	4.7	23.8	2.9	0.988	0.034	
	Minijack	9	143.0	8.5	33.2	6.1	1.124	0.046	0.39
			9.1		9.4		0.136		
6	Non-Maturing	5	148.4	8.2	33.5	5.2	1.018	0.035	
	Minijack	18	158.8	5.3	44.8	4.9	1.114	0.040	0.78
			10.4		11.3		0.097		
7	Non-Maturing	15	139.9	5.3	28.3	3.2	1.029	0.055	
	Minijack	11	146.8	6.3	35.0	5.6	1.099	0.067	0.42
			6.9		6.7		0.070		
8	Non-Maturing	6	139.2	3.4	27.8	2.9	1.029	0.047	
	Minijack	16	149.0	7.9	37.2	6.3	1.115	0.040	0.73
			9.8		9.4		0.086		
9	Non-Maturing	7	149.3	6.5	36.4	5.1	1.087	0.040	
	Minijack	14	161.4	8.0	51.1	8.3	1.203	0.043	0.67
			12.1		14.7		0.116		
10	Non-Maturing	8	143.5	5.8	31.1	3.9	1.047	0.039	
	Minijack	15	154.3	8.3	44.0	7.2	1.190	0.041	0.65
			10.8		12.9		0.143		
11	Non-Maturing	10	149.8	4.8	34.9	3.3	1.036	0.042	
	Minijack	6	156.3	5.7	41.6	5.1	1.085	0.068	0.60
			6.6		6.7		0.049		



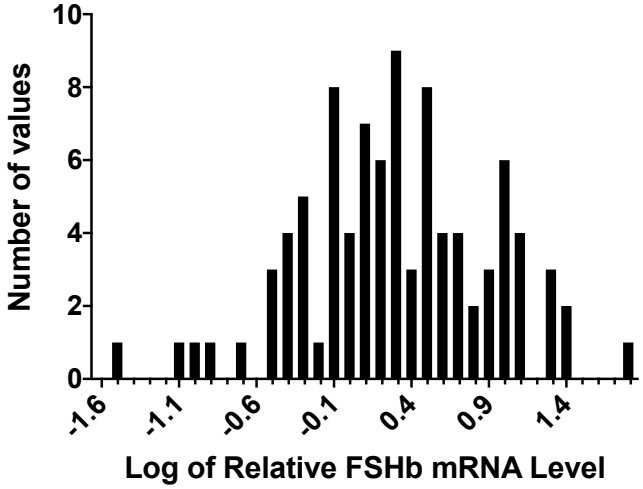






As anticipated, the juvenile males within families that initiated maturation were among those which were larger and had a higher condition factor over the course of the study. However, given the wide variation in the data, within and across families, these differences were insufficient to be reliably used to distinguish maturation status of an individual, even as late as the smolt stage. Something that we did not anticipate was observation that these size and condition factor differences were already apparent in July 2018 (4-5 months following swim-up) when tagging and sampling of the juveniles was initiated, and long before the proposed maturation decision period. However, maybe this could have been expected. Male spring Chinook Salmon juveniles may also mature as age-1 precocious parr, though it occurs at a relatively low rate. Precocious parr must make the physiological decision to mature or not based on influences very early in their development post-swim up (and possibly even during egg incubation). While factors affecting increased growth and adiposity in the initial months following swim-up did not prompt the larger fish within progeny groups to become precocious parr, larger average size 4-5 months later at the beginning of our study was associated with those individuals which later matured as minijacks.

Laboratory analyses for liver IGF-I mRNA are ongoing, both for the Sept 12, 2018 samples and the final April 9-10, 2019 samples. 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 (graph below). However, it could be that September is too early in the process and that sampling at a later date (perhaps in early winter) may provides measures that better identify maturation status. Pituitary samples collected in April of 2019 have not yet been analyzed. Following completion and analysis of all data, an oral presentation and a manuscript for submission for publication will be produced.



E.5 Effect of male broodstock age on minijack rate and progeny age-at-maturity

Both precocious maturation of juvenile hatchery-reared male juvenile 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 matings involving age-4 natural origin (WN) females crossed with an age-3 and an age-4 WN males – to be produced over three consecutive broodyears (2018, 2019 and 2020). 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 in the following February. After approximately six weeks, the outlet from the raceway to Jack Creek is opened to allow volitional release of the smolts, with forced release of any remaining juveniles in early May. In March 2020, 2021 and 2022 (for the three broodyears, respectively) prior to initiating volitional release, 100 smolts from each raceway containing the study fish will be sacrificed, measured, fin tissue sampled (for genetic sex and parentage analysis) and blood sampled (for 11-KT analysis). Returning adults from these broodyears (2021 through 2025) identified at RAMF as Jack Creek releases (via their VIE tag) will be measured, and tissue and scale sampled. As data are complete for each broodyear, they will be analyzed to test for an effect of male broodstock age both on minijack rate, and on subsequent SAR, size, sex ratio and age structure of their returning adult progeny.

E.6 Effect of feed deprivation on minijack rate

The published literature contains several studies which indicate that a reduction in the incidence of precocious male maturation can be obtained by restricting feeding during juvenile rearing. This been reported in Atlantic salmon (Rowe and Thorpe 1990; Herbinger and Friars 1992), and Chinook salmon (Hopkins and Unwin 1997). In 2002, Larsen et al. (2006) conducted a study at the CESRF to assess whether manipulation of feeding rate would alter the incidence of minijacks among the spring Chinook Salmon smolts. The study involved feeding fish either a reduced ration relative to standard CESRF feeding rates, applied in the Summer (June 1 – Aug 31) and/or the Fall (Sept 1-Nov 7), with control fish being fed normal CESRF ration throughout juvenile rearing. Results indicated that the reduced ration effectively decreased the minijack rate relative to control fish, with the greatest effect when the reduced ration was applied during the Summer relative to the Fall. However, even when applied across both periods, minijack rate decreased by only 40% relative to controls, and size of the fish at the smolt stage was also significantly reduced by approximately 40%.

In the manuscript describing their study results, Larsen et al. (2006) concluded that, “In future studies, more significant alterations in growth, perhaps through dietary lipid manipulation, may be needed to further reduce the level of precocious male maturation in hatchery-produced fish”, albeit following a protocol that does not also result in a substantial reduction in final smolt size. Following this recommendation, we designed a study that involves total feed deprivation of the fish in order to affect a more dramatic decrease in growth and adiposity, applied over a more limited time period (5-6 weeks) such that compensatory growth following the fasting treatment might permit the juveniles to catch up in size relative to the standard production fish by the time they reach the smolt stage.

The study was initiated with BY 2018 juveniles - progeny from a large number of CESRF test spawnings between SH adult returns of supplementation program smolts. In February 2019, swim-up fry from these matings were pooled into two 500-L circular fiberglass rearing tanks mounted over CESRF raceway #19 for rearing until the summer of 2019. In August 2019, a random sample of 50 fish were sacrificed, and sampled for length (mm), weight (0.1g), condition factor, fin tissue for genetic sex identification, the

livers were weighed for calculation of hepatosomatic index, and the livers analyzed to estimate lipid content. Then, a random sample of 250 fish was stocked into each of 16 of the 500-L tanks.

Six of the tanks were assigned as Controls and received feed at standard CESRF rates. The other 10 tanks were assigned (5 tanks each) to an early (8/1 to 9/11) or to a late (9/11 to 10/24) total feed deprivation treatment. Prior to the deprivation treatment, the fish were fed at the same rate as the control fish. Following deprivation, the fish were fed 5 times per day as much as they would readily consume, in the hope that compensatory growth would permit them to reach a final smolt size similar to that of the Control fish. On 9/11 and 10/24, 20 fish were randomly sampled from each tank, sacrificed and measured and tissue sampled as described previously. On 11/20, a sample of 30 fish per tank was measured for length and weight, then all fish from each tank received a tank-identifying fin clip (with or without an adipose clip, and with or without a left pelvic fin clip), and the fish were transferred to one of four sections within CESRF raceway #18 (four tanks with differing fin clips per section) for overwinter rearing. In April 2020, all fish will be sacrificed, identified to their respective rearing tank and treatment (via the fin clip), and measured, dissected for sex identification, and tissue sampled. Blood samples from male juveniles will be retained for measurement of plasma 11-KT concentration and maturation status determination. The data will be analyzed for differences in these measures between Control and Treatment fish, and between the early and late Treatment. Based on these results a follow-up study to be initiated with BY 2019 juveniles will be designed to test additional timing and duration deprivation treatment(s) in the search of one that might be most effective.

E.7 Effect of photoperiod manipulation on minijack rate

Manipulation of photoperiod is an effective tool for decreasing maturation rate among farmed salmonids, gadoids, and percids (Bromage et al 2001, Davie et al 2007, Leclercq et al 2010, Liu and Duston 2019). Studies by researchers 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 (Unwin et al 2005, Liu and Duston 2018). Furthermore, the reduction in precocious maturation occured 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 adult 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 postponed 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 (E.5), 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, which at swim-up in February 2019 were pooled into the 500-L fiberglass circular tanks for initial rearing. In March 2019, 2,500 of these fry were transferred to the Aquaculture Research Institute at the University of Idaho, Moscow ID, for continued rearing. On June 21, 2019, weight (g) and fork length (mm) was measured for a random sample of fish. Then, 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 throughout the experiment. The fish are being hand fed Bio-Oregon® feed (www.bio-oregon.com) of appropriate size based on the manufacture's recommendations. They are fed to satiation twice a day so none of the treatments will differ in food availability. All treatments receive the ambient, simulated natural photoperiod (45° N) during the light phase of the day. LL-June and LL-Sept treatments are 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 April 2020. Control treatments are subjected to a simulated natural photoperiod, adjusted twice weekly to be representative of the natural photoperiod in Moscow, ID. A portion (n=20) of the fish from each tank were lethally sampled in September 2019 when the LL-Sept treatment began. Whole body length and weight , and liver weight was recorded, and the liver was collected for possible qPCR analysis to investigate physiological parameters controlling precocious maturation. In April 2020, all remaining fish will be sacrificed, identified to sex by visual assessment, measured for length, weight and liver weight, and blood plasma will be collected from the males for assessment of maturation status via 11-KT plasma concentration. The proportion of maturing minijacks will then be compared within treatments and between treatment and control fish. Based on these results a follow-up study to be initiated with BY 2019 juveniles will be designed to test an additional photoperiod manipulation timing and duration treatments, in seach of one that might be most effective.

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

Project coordinator (Galbreath) and associated CRITFC and University of Idaho personnel (Matala, Koch, Pierce, and Medeiros) participated in various inter-tribal and inter-agency meetings, workshops and symposia in 2019, in which Project-related issues were discussed. Participation generally included an oral presentation of findings from Project-funded studies. The objective of our attendance was to exchange information acquired during these studies with biologists and managers from other agencies (tribal and non-tribal) working on similar issues, as well as to develop and articulate the tribal perspective on how hatchery supplementation and reintroduction programs can be appropriately managed to benefit viable salmonid population (VSP) parameters - abundance, spatial structure and diversity (McElhany et al. 2000), while at the same time minimizing possible negative effects on

productivity. The following is a list of the workshops and symposia attended by CRITFC and other personnel funded under the Project during 2019:

- Oregon Chapter of the American Fisheries Society (Mar 6-8, Bend OR). Presentation: Matala, A. P., S. R. Narum, J. E. Newell, P. F. Galbreath, B. P. Saluskin, M. V. Johnston. Early observations from monitoring a Sockeye Salmon reintroduction program: Who says you can't go home again?
- Yakima Basin Science and Management Conference (June 12-13, Ellensburg WA). Presentations:
    - Knudsen, C. M., P. F. Galbreath, C. Stockton, L. R. Medeiros, I. Koch, A. L. Pierce, and W. J. Bosch. Does domestication in upper Yakima River spring Chinook sires effect juvenile growth, maturation threshold or minijack production?
    - Medeiros, L. R., A. L. Pierce, C. M. Knudsen, I. Koch, S. Narum, and P. F. Galbreath. A comparison of growth trajectories in immature male and female and precociously maturing minijack male Chinook salmon.
- Pelton Round Butte Fisheries Workshop (Jul 17-18, Bend OR). Presentation: Matala, A. P., P. F. Galbreath, and J. Hogle. Evaluating reintroduction strategies & monitoring the status of the Deschutes River Sockeye program.
- Deschutes Basin Co-Manager Fish Committee meeting (Oct 17, Madras OR). Presentation: Galbreath P. F., and A. P. Matala. Sockeye Salmon Reintroduction Programs in the Pacific Northwest.

G. **Project Objective #7:** Prepare manuscripts for publication in scientific journals

Larsen D. A., D. L. Harstad, A. E. Fuhrman, C. M. Knudsen, S. L. Schroder, W. J. Bosch, P. F. Galbreath, D. E. Fast, and B. R. Beckman. 2019. Maintaining a wild phenotype in a conservation hatchery program for Chinook salmon: The effect of managed breeding on early male maturation. PLoS ONE 14(5): e0216168. https://doi.org/10.1371/journal.pone.0216168.

H. **Project Objective #8:** Identify additional studies to support tribal supplementation and reintroduction programs

Through meetings, attendance at regional conferences and while pursuing ongoing Project activities we have sought to identify opportunities and to develop study designs where support from the Project could be profitably used to finance additional studies associated with tribal M&E of hatchery supplementation and/or reintroduction projects. Studies which may be proposed within the Project statement of work in the coming years may incude: a) initiation of productivity analyses and performance, and RRS for a new Coho Salmon supplementation program in the upper Yakima River basin, b) assessment of return rate and productivity should a proposed project to reintroduce Sockeye Salmon in Wallowa Lake be enacted.

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