Columbia River Inter-Tribal Fish Commission
700 NE Multnomah, Suite 1200
Portland, OR 97232
503.238.0667
www.critfc.org

CRITFC

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Basinwide Supplementation Evaluation Project: 2018 Annual Progress Report

Peter F. Galbreath, Ilana J. Koch, Andrew P. Matala, Andrew L. Pierce, Jeffrey J. Stephenson, and Shawn R. Narum

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2018 Annual Progress Report

Basinwide Supplementation Evaluation

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Peter F. Galbreath, Ilana J. Koch, Andrew P. Matala, Andrew L. Pierce, Jeffrey J. Stephenson, and Shawn R. Narum

Columbia River Inter-Tribal Fish Commission (CRITFC), Portland, OR, 97232

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I. Executive Project Summary

This report summarizes activities for calendar year 2018 under BPA Contracts # 76019 and 73354, performed as part of the multi-year Basinwide Supplementation Evaluation project 2009-009-00 (hereafter the Project). The report is organized under the eight Project Objectives identified in the contract Statement of Work: https://www.cbfish.org/Contract.mvc/Summary/73354%20REL%205. The primary focus of the Project involves monitoring and evaluation of tribal hatchery programs to assess: a) critical uncertainties related to effects of hatchery supplementation on productivity of depressed natural anadromous fish populations, and b) productivity trends in new natural populations established through reintroduction of out-of-basin origin fish (both of hatchery origin or natural origin) in subbasins where the indigenous population had been extirpated.

Project Objective #1:

- A relative reproductive success (RRS; HOR/NOR) study of supplemented Johnson Creek spring/summer Chinook Salmon *Oncorhynchus tshawytscha*, based on juvenile recruits-per-spawner, is ongoing. Results will be compared to recently published findings obtained for RRS based on adult recruits-per-spawner.
- A larger scale adult recruits-per-spawner RRS study was initiated in 2014 to assess RRS of supplemented upper Yakima River spring Chinook. A preliminary analysis for the first of five BYs in this study (BY 2007) performed in 2017 and was limited to data for identified parent pairs, was updated in 2018 using software that identifies parent pairs and single parent assignments. The updated analysis indicated that overall RRS for females was approximately 0.85, which was significantly lower than 1.0. RRS for adult males was approximately 0.90 and for age 3 jack males was 0.95, neither of which was significantly different from 1.0. Despite that slightly lower average productivity of the HOR adults, the analysis showed that hatchery supplementation provided a significant demographic boost to the population, i.e., a natural origin fish taken into the hatchery for spawning returned 5.7 times more adult progeny relative to a natural origin fish left in the river to spawn naturally. Genotyping for the remaining four BYs is complete, and a comprehensive analysis and report for BYs 2007-2011 should be completed in 2019.
- Genetic analysis of genotypes for natural origin spring Chinook juveniles out-migrating from the upper Warm Springs River indicate that the effective number of breeders (Nb; the proportion of fish that parented one or more of the outmigrants) was only 21% for BY 2015 and 12% for BY 2016 Additionally, the redd count for each broodyear was similar to the value of the Nb estimate divided by 4 – the historic fish per redd ratio. The data support that a large proportion of the adult escapement failed to successfully spawn.

Project Objective #2:

- A RRS analysis based on juvenile recruits-per-spawner is ongoing for spring Chinook Salmon in Lookingglass Creek, Grande Ronde River basin. In this case, the natal population had been extirpated and the study examines relative productivity of a new population that was reintroduced with the use of a hatchery stock. An initial analysis 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 stock would create a naturalized population that demonstrates improving reproductive success. Productivity data for two additional BYs was recently acquired and a full analysis for the nine BYs will be performed in 2019.

Project Objective #3:

- 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 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, with genetic analyses of juvenile and adult progeny indicating very low (<5%) proportions of inter-stock hybrids (Matala et al. 2018). Subsequent years' work will include RRS analyses within stock type, for Cle Elum origin versus translocated adults.
- The second *O. nerka* study is being conducted in conjunction with a program to reintroduce an anadromous run of Sockeye Salmon in the Deschutes River, for which the Warm Springs Tribe is a co-manager. Since 2009, tissue samples of juveniles from the resident kokanee population in Lake Billy Chinook have been released downstream of Round Butte Dam, and adults (presumptive Sockeye Salmon) that return to the Pelton Dam adult trap are being enumerated and genetically analyzed. While tens to hundreds of 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. Genetic stock identification analyses confirmed that >90% of the adult returns are indeed of Lake Billy Chinook origin.

Project Objective #4

- 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 analyses. Initial results do not indicate a consistent effect of parent age, possibly masked by an overwhelming effect of high variation in minijack rate among families within parental age cross-types, ranging in one instance fully from 0% to 100%. Reporting of results from a comprehensive analysis is anticipated in 2019.
- A one-year study was conducted to test the effects of diet supplementation with tetradecylthioacetic acid (TTA), previously reported to have been effective in reducing early maturation of juvenile male Atlantic salmon. While TTA-supplemented fish demonstrated reduced growth during the treatment period, there was no reduction in minijack rate relative to controls.
- Two additional studies are underway, one to examine individual growth through the juvenile rearing period for maturing versus non-maturing fish, and the second to compare minijack rate and age structure of returning adults for fish produced with an age 3 jack versus and age 4 adult male parent.

Project Objective #5

- A re-examination of effects of a past summer steelhead harvest augmentation hatchery program on the indigenous winter steelhead population in the Clackamas River, performed in collaboration with a consultant with Mt. Hood Environmental, LLC, was completed and published in 2018. Results were contrary to those described in a prior publication which found that competition for spawning and juvenile rearing habitat reduced productivity of the native winter steelhead. Our reanalysis indicated instead that variation in winter steelhead abundance was driven instead by variation in ocean and other regional environmental factors, not by presence of the hatchery summer steelhead.

Projective Objective #6

- Unlike prior years, we did not conduct an "Introduction to Molecular Genetic Analyses in Tribal Fisheries Management" workshop in 2018.

Project Objective #7

- 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 #8 Project Objective #8

- Three different manuscripts describing Project-funded studies were published in 2018.

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 2018 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 Chinook Salmon *Oncorhynchus tshawytscha* in Johnson Creek (Salmon River basin) and 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)
- 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 for adult *O. nerka* returning to the Pelton trap on the Deschutes River, in response to a program for capture and release juveniles from the Lake Billy Chinook kokanee population downstream of the Pelton-Round Butte complex (Project Objective #3)
- to build off previous research conducted at the Cle Elum Supplementation Research Facility examining factors associated with precocious maturation of male spring Chinook Salmon smolts as age 2 minijacks, with a series of studies to: 1) 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 reduction in minijack rate associated with supplementing feed with tetradecylthioacetic acid (TTA), 3) to compare individual juvenile growth rates of maturing versus non-maturing fish, and 4) to compare minijack rate and age structure of returning adults for progeny of fish parented by age-3 jacks versus age-4 adult males (Project Objective #4)
- to collaborate on a re-examination of data presented in a previously published study of the effects of a summer steelhead harvest augmentation hatchery program on productivity of the indigenous winter steelhead population in the Clackamas River (Project Objective #5)

- to continue support for training of tribal personnel in use of molecular genetics analyses to address questions in fisheries management (Project Objectives #6), for participation in regional forums involving review of hatchery management and supplementation efforts (Project Objectives #7), and for reporting of Project results in scientific journals (Project #8).

III. Work Elements / Tasks

A. Project Administration

Activities in 2018 involving administration of the Project by CRITFC included: production and posting in PISCES of the annual progress report for 2017, completion of 2018 quarterly and final status reports in PISCES that record progress associated with each work element within the contract Statement of Work, and submission of 2018 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 76019 and 73354 in www.cbfish.org.

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

B.1 Johnson Creek spring/summer Chinook Salmon

CRITFC collaborates with the Nez Perce Tribe (NPT) on a study to assess RRS of supplemented spring/summer Chinook Salmon as part of the Johnson Creek Artificial Propagation Enhancement Project (JCAPE; Project No. 199604300; Rabe and Nelson 2010). The population of spring/summer Chinook Salmon in Johnson Creek - a tributary of the East Fork of the South Fork of the Salmon River, Idaho – was reduced to very low abundance in the 1990s. In 1998, NPT initiated the JCAPE project. As part of an associated monitoring program, NPT biologists have collected tissue samples and biodata on all returning adults intercepted at a weir at river kilometer (rkm) 8, as well as tissues from a limited number of out-migrating NOR juveniles collected at a rotary screw trap operated directly downstream of the weir. The tissues have been sent to CRITFC geneticists at the Hagerman Fish Culture Experiment Station (HFCES) for genetic analysis, initially involving genotyping for a suite of microsatellite DNA markers and later for a standardized panel of single nucleotide polymorphism (SNP) markers, followed by parentage analyses. The NPT commits funds sufficient for analysis of approximately 1,500 samples per year. Following initiation of the productivity study, supplemental funding has provided by the Project to cover costs for genotyping of samples in excess of that which could be covered under the NPT contract. Supplemental funding was also needed to respond to a request made to NPT in 2009, to increase in the number of juvenile out-migrant samples collected each year, so that an additional Project-funded RRS study based on juvenile recruits-per-spawner could be performed. A juvenile RRS study has the advantage that the relatively larger number of juvenile progeny that can be collected per BY increases the power of the parentage analyses to differentiate productivity relative to origin, as well as to other parental characteristics, e.g., return time, sex and age. Additionally, the RRS results based on juveniles and on adult progeny will be compared to see how closely they concord.

Results of RRS analyses described in Hess et al. (2012) for the initial four BYs (2002-2005) were recently updated with data for an additional six BYs (2006-2011; Janowitz-Koch et al. 2018). They indicate that

supplementation provided a demographic boost to the depressed spring/summer Chinook Salmon population, on average 4.56X in the 1st generation, and a 2.52X in the 2nd generation. While the number of fish identified as parents of one or more adult offspring was somewhat smaller for HOR fish relative to NOR fish, productivity of successfully spawning fish was generally similar for HOR and NOR fish – RRS for females (age 4 and 5) was ≈ 0.9, although HOR adult males (age 4 and 5) and HOR jack (age 3) males were somewhat less successful than NOR counterparts. However, within all three sex/age categories, relative reproductive success (HOR/NOR) among successful spawners (i.e., those that produced at least one returning adult offspring) was not significantly different from 1.0.

Genotyping for the juvenile recruits-per-spawner RRS study (BYs 2010-2016) is complete. Productivity analyses and a report of results will be completed in 2019. The number of adults passed above the weir for natural spawning and the corresponding number of juvenile out-migrants per BY is provided below:

	Spawners		Juvenile
Year	NOR	HOR	Progeny*
2010	465	484	2,781
2011	396	310	3,301
2012	447	198	3,097
2013	609	301	1,919
2014	1114	542	2,099
2015	528	439	3,571
2016	509	191	1,936

* the large majority of juveniles collected each year are age 0+ parr – progeny of adults that returned the previous calendar year, although a small proportion (approx. 5%) are age 1+ smolts from the brood year two years previous

B.2 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 spring Chinook Salmon population 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 are 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 are 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 – similar to the JCAPE program) was designed to test whether artificial propagation can increase natural production and harvest opportunities while keeping ecological and genetic impacts within acceptable limits. An unsupplemented population in the adjacent Naches River (tributary to 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 an adult-to-adult RRS analysis of the supplemented population. However, funding has been insufficient to take on the expense to genotype samples from the thousands of adults returning in-basin each year. However, with the development of a large array of SNP markers for Chinook Salmon and new high throughput genotyping techniques (Campbell et al. 2015), the per-sample genotyping cost has dramatically diminished and a large scale RRS study became feasible. In discussions between YN, WDFW, and CRITFC, an agreement was reached to perform a RRS study of naturally spawning NOR and HOR fish in the upper Yakima River, covering five consecutive BYs (2007-2011). The study involves genotyping of tissue samples collected from in-migrating NOR and HOR adults interrogated in the RAMF, that were passed upstream for natural spawning in those 5 years, plus their NOR adult progeny that returned in years 2012 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 by CRITFC using Project funds and by WDFW using YKFP funds, was completed in 2018. Parentage and RRS analyses will be conducted within and across BYs, and results will be summarized in a technical report and in a manuscript to be submitted for publication in a scientific journal in 2019.

To provide an interim assessment, an initial RRS analysis 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 from the analysis indicate

somewhat lower RRS ratios. In the case 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.

Numbers of BY 2007 spawners within sex and origin, and the number of assigned adult progeny are provided in the following table, along with average productivity and RRS estimates.

Sex/Age Origin	Number Adults Genotyped	Number (Proportion) Successful Adults	RS HOR	RS NOR	RRS (HOR/NOR)	p value
Females (age 4&5)	758	657 (0.87)	4.70	5.41	0.87	0.01
Males (age 4&5)	559	422 (0.75)	3.54	3.95	0.90	0.15
Jacks (age 3)	925	432 (0.47)	2.29	2.37	0.97	0.77

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.

The average productivity of HOR females that crossed with HOR or NOR males was compared to those NOR females that crossed with NOR males (all fish age 4 or age 5 adults). Overall, there were no significant differences in productivity observed for a) HORxHOR relative to NORxNOR, or b) HORxNOR relative to NORxNOR. When considering the average productivity of HORxHOR males relative to NORxNOR males and HORxNOR males relative to NORxNOR males, significant differences in productivity were observed ($p = 0.02$).

For one BY at least, these findings indicate that the supplementation program did indeed provide a demographic boost as intended, and that natural productivity of adults returning from the hatchery program was generally similar to that of natural origin fish. However, average productivity tended to be lower for fish that crossed with a hatchery origin fish compared to those that crossed with a natural origin fish. A comprehensive report for the five BYs will be produced in 2019.

B.3 Warm Springs River spring Chinook Salmon effective breeder number (Nb)

Spring Chinook Salmon smolts are produced in the Warm Spring National Fish Hatchery (WSNFH), co-managed by the US Fish and Wildlife Service and the Confederated Tribes of the Warm Springs Reservation of Oregon (CTWSRO), to supplement the fishery downstream of the hatchery. The spring Chinook Salmon population upstream of the hatchery, however, has remained unsupplemented. All hatchery smolts are released below the impassable weir at the WSNFH, and only NOR adults are passed upstream of the weir for natural spawning – the number and species of which are recorded annually. In addition to this adult escapement information, since the 1980s the CTWSRO has conducted spawning ground surveys in the upper basin to obtain an annual redd count. While the escapement and redd counts have fluctuated over the years, they have done so relatively synchronously, such that the ratio of the adult escapement to total number of redds has ranged from 2 to 6, with an average of 4. Since 2010, however, this ratio has increased dramatically, averaging approximately 12, and with the ratio being as high as 19 in 2015 and 24 in 2016. These high adults per redd ratios infer that a substantial proportion of the adults failed to successfully spawn.

The reduction in spawning success might be indicative of a recent increase in pre-spawn mortality (e.g., due to disease, or physiological stress associated with high temperature or other hydrologic conditions, etc.). Alternatively, the possibility exists that the spring Chinook Salmon have recently expanded their spawning into areas of the basin outside the standardized spawning ground survey reaches, such that the number of redds is underestimated and the actual fish per redd ratio is closer to 4, as observed in previous years. To assess this latter possibility of redd undercounting we performed a genetic analysis of a samples of juvenile outmigrants from BYs 2015 and 2016 to estimate the number of adults that parented the juveniles each year (effective number of breeders; Nb).

Fin clips and biodata from BY 2015 and 2016 spring Chinook juveniles were taken from fish captured in a rotary screw trap located 0.5 km upstream of the WSNFH weir. The samples were sent to CRITFC geneticists at the HFCES, DNA was extracted from each, and the DNA genotyped for a panel of 298 SNP markers using the "genotyping-in-thousands by sequencing" (GT-seq) technique (Campbell et al. 2015). Genotypes for juveniles confirmed to be Chinook Salmon (i.e., some of the samples were erroneously taken from Coho Salmon) were analyzed with the software program Colony (https://www.zsl.org/science/software/colony; Jones and Wang 2010) which has the option, in the absence of samples from the potential parents, to derive an estimate of Nb.

Adult escapement, redd count, and fish per redd Nb estimates and Nb/4 ratios for BYs 2015 and 2016 are provided below:

Broodyear	Adult Escapement	Redd Count	Fish per Redd	Nb Estimate	Nb/4
2015	1,300	70	19	277	69
2016	400	15	24	49	12

Nb estimates represented only 21% and 12% of the adult escapement for BYs 2015 and 2016, respectively. Additionally, the redd count for each broodyear was similar to the value of the Nb estimate divided by 4 – the historic fish per redd ratio. As such, the data do not support the possibility of significant undercounting of redds, but instead confirm the original high fish per redd values and the inference that a large proportion of the adult escapement failed to successfully spawn. This analysis was summarized in a CRITFC Technical Report (Koch et al. 2018).

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, however, the effects have been even more dramatic, leading to the extinction of affected populations. This included extirpation of all populations whose natal streams were above the impassable mainstem Chief Joseph and Grand Coulee dams (Columbia River) and Hells Canyon Dam Complex (Snake River). However, many populations downstream 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). Generally, these reintroduction programs have seen a portion of the HOR smolts return 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; Yakama Nation Fisheries Resource Management 2012; Galbreath et al. 2014).

The broodstock management protocol for reintroduction programs recommends that use of out-of-basin hatchery broodstock be progressively in favor 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, and NOR fish would to be increasingly incorporated into the 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 recent meta-analysis, Fraser (2008) reviewed published 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 have 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 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 generation or more of natural selection), should on average produce more recruits-per-spawner than HOR fish (fish that lack this generation of natural selection), and the relative reproductive success ratio (NOR/HOR) should be greater than 1.0. To test this hypothesis, the Project is performing an 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, the native population of spring Chinook Salmon in Lookingglass Creek had already been extirpated. Efforts to reintroduce spring Chinook Salmon were implemented over the following two decades through annual stocking of hatchery-reared juveniles. Different hatchery stocks were successively used for the reintroduction, initially Carson National Fish Hatchery, then Wind River, Imnaha River, and Rapid River hatcheries. Despite these efforts, a naturally spawning population never established itself 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 for supplementing the Grande Ronde and Imnaha basins. A captive broodstock program was therefore initiated using juveniles captured from Catherine Creek (a Grande Ronde River tributary upstream of Lookingglass Creek). In anticipation of adults returning from smolt releases of the new in-basin hatchery stock, from 1998 through 2003 no adults whatsoever were passed upstream of the Lookingglass weir (½ km upstream of the hatchery). This effectively extirpated any remnant spring Chinook Salmon derived from the prior out-of-basin hatchery stocks. In 2004, adults from the initial 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, and the remaining HOR fish passed upstream for natural spawning. Beginning in 2007, with return of the first NOR adults (age 3 jack males) from the new reintroduction program, a portion of the NOR and of the HOR adults captured at the weir are integrated into the hatchery broodstock while the remainder are passed upstream for natural spawning (Boe et al. 2010 and 2011).

Beginning in 2004, CTUIR biologists collected tissue samples 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, in anticipation of 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 year following the broodyear, and as age 1+ smolts in January through May the 2nd 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 in BYs 2008 through 2016, plus all samples from NOR juvenile out-migrants corresponding to these BYs. Extraction was performed initially at the HFCES using a Qiagen DNeasy Blood & Tissue Kit (Qiagen; www.qiagen.com) according to the manufacturer's instructions, and the DNA was then genotyped for a standardized suite of 95 (BYs 2008-2014) or 188 (BYs 2015-2016) 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, the protocol for DNA extraction has switched to the Chelex 100 method (Sigma-Aldrich, St Louis, MO), and for genotyping using the "genotyping-in-thousands by sequencing" (GT-seq) technique (Campbell et

al. 2015). The genotypic data for these BYs 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 single parent assignments, and , and occasionally deletion of both parental assignments. The remaining high-certainty assignments were then examined to calculate the total number of juveniles assigned to each adult female and male (age 4 and 5), and jack (age 3 male) passed above weir for natural spawning.

Results from a preliminary analysis for BYs 2008-2014 were provided in the 2017 Annual Progress Report for the Project. Analysis of data for BYs 2015 and 2016 was recently completed, and a summary of productivity data for all nine BYs are provided below.

		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						

On average, genetic parentage for BYs 2008-2016 resulted in successful assignment of 97% (see table below) of the juveniles to a parent pair, or to a single parent. Among these juveniles, 73% were assigned to a female + male parent pair, 4% to a male parent only, and 23% to a female parent only. For the 4% with a missing female parent, the female was likely among the adults sampled at the hatchery weir, but because of occasional loss of tissue samples, tissue degradation, or errors in the genotyping process, the female parent was not identified. In contrast, 23% of the juveniles lacked an assigned male parent. The above reasons for sample loss and genotyping errors also apply to adult males passed above the weir, but do not explain the much higher percent of unidentified male relative to female

parents. It is highly probable that many of the missing male parents were resident males – most likely precociously matured parr (microjacks). Indeed, precocious parr are occasionally observed among the juveniles sampled in the rotary screw trap or during electrofishing surveys, and there were a few genetic parentage assignments made to precocious parr that were captured, tissue sampled and their genotypes included among those of the adult considered in the parentage analyses.

The percent of adults per BY that were successfully assigned one or more progeny averaged 60% and 61% for NOR females and males, respectively, and 51% for both HOR females and males; the %NOR/%HOR ratios were 1.23 for females and 1.25 for males. Note, these calculations were not performed for age 3 jack males as jack numbers (especially for NOR jacks) were typically low, and in several years no HOR jacks were passed upstream.

The number of successful adults, and the geometric mean number of progeny per adult, within sexes and BYs, are provided in the table below. Note – the geometric as opposed to arithmetic mean was calculated in light of the highly skewed frequency distribution of the individual recruits-per-spawner data (similar to a negative binomial). Average productivity was higher for both NOR females and males, with the RRS ratio approximately 1.2 for both sexes. Analyzed with a t-test for paired means the difference was highly significant for males. On the other hand, the variability in the female data was much greater, and while average productivity was numerically higher for NOR females (of a magnitude similar to that for males) the NOR versus HOR difference was not statistically significant.

	FEMALES - Successful Parents				
	HOR		NOR		RRS
Broodyear	#	Geomean R/S	#	Geomean R/S	NOR/HOR
2008	51	3.29	18	2.46	0.75
2009	12	10.39	30	9.12	0.88
2010	91	2.87	14	3.99	1.39
2011	100	2.14	28	2.61	1.22
2012	209	2.32	29	2.45	1.06
2013	32	7.08	11	5.81	0.82
2014	131	3.04	35	4.92	1.62
2015	78	3.03	39	3.65	1.20
2016	52	2.25	85	4.46	1.99
				avg:	1.21

	MALES - Successful Parents				
	HOR		NOR		RRS
Broodyear	#	Geomean R/S	#	Geomean R/S	NOR/HOR
2008	41	2.77	8	3.39	1.22
2009	11	6.31	12	7.37	1.17
2010	34	3.05	21	3.98	1.31
2011	61	2.25	23	2.56	1.14
2012	127	2.86	21	3.25	1.14
2013	30	3.25	11	4.25	1.31
2014	94	3.07	25	3.46	1.13
2015	78	2.48	36	3.65	1.59
2016	52	2.98	66	3.93	1.32
				avg:	1.26 ***

A caveat regarding these analyses concerns calculation of the total number of progeny per parent as the sum of assigned parr + smolts. Mortality of embryos increases over time during juvenile rearing, and

especially during overwintering, when a juvenile's age category changes from parr to smolt. If the proportion of progeny of NOR adults that out-migrate as smolts (and are captured for tissue sampling) is similar to that for progeny of HOR adults, the RRS analyses will be unbiased. This is the case for males – the proportion of assigned progeny that were smolts for NOR versus HOR adults was similar, and the differences non-significant. 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, possibly to the level of statistical significance. However, the data do not exist to estimate these survival factors.

	Females			Males		
Broodyear	% 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
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 finalization of statistical analyses in 2019, the findings from this study will be summarized in a report and in a manuscript to be submitted for publication in a scientific journal. Pending confirmation from the statistical analyses, the findings indicate support for the presumption of the reintroduction program, that NOR progeny of the reintroduced HOR stock did indeed exhibit improved natural productivity, as would be expected if natural selection were indeed acting to increase adaptation of the population to the new environment.

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 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

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

* 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 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 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, 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).

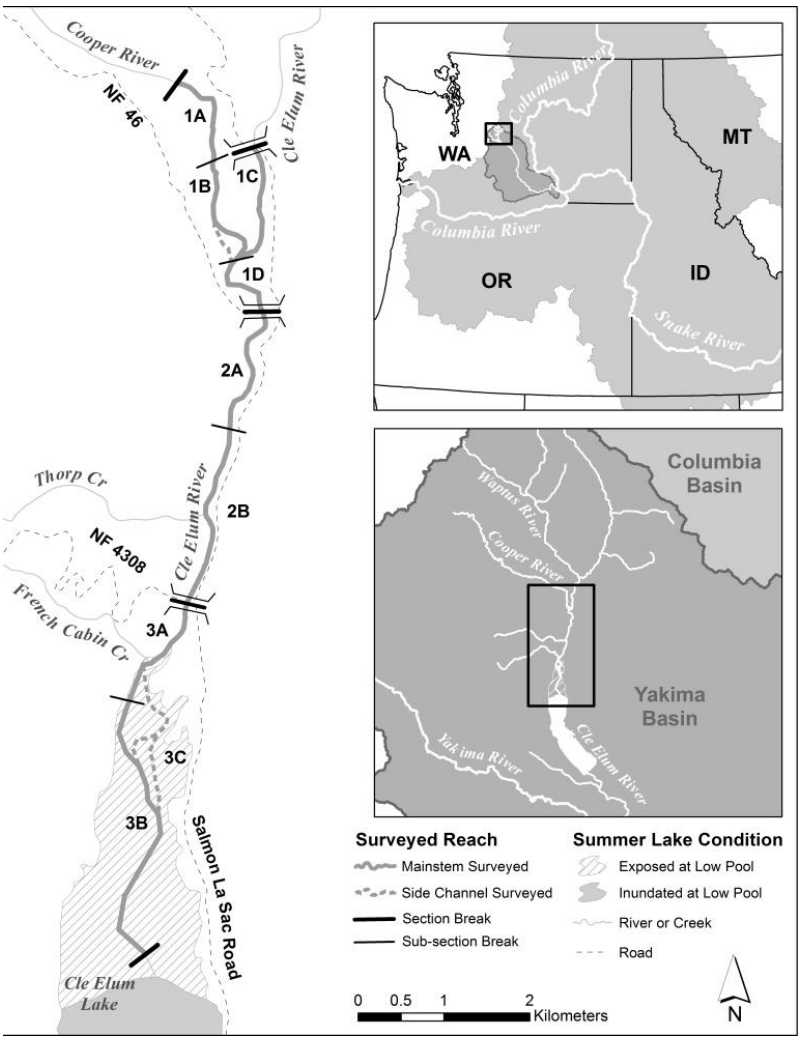
Sockeye	Smolt Age		Adult Age		
Stock	1+	2+	3	4	5
Osoyoos	0.91	0.09	0.11	0.73	0.16
Wenatchee	0.82	0.18	0.00	0.59	0.41

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, 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

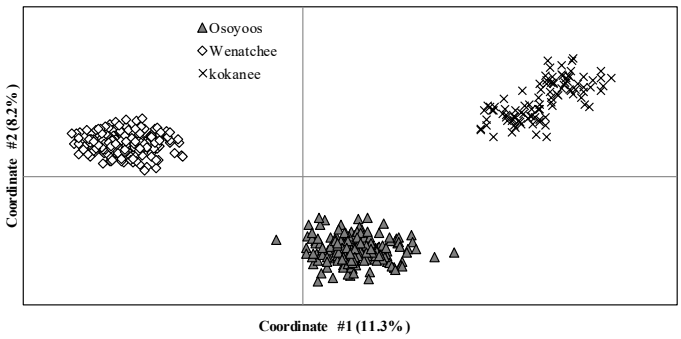
productivity of the reintroduced fish, in 2011 the Project committed to financing genetic analyses of tissue samples collected from: a portion of the translocated PRD adults, all returning adults sampled at the RAMF, post-spawned adults encountered during spawning ground surveys (see map below), Sockeye Salmon caught as by-catch during gill-netting performed to remove 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.

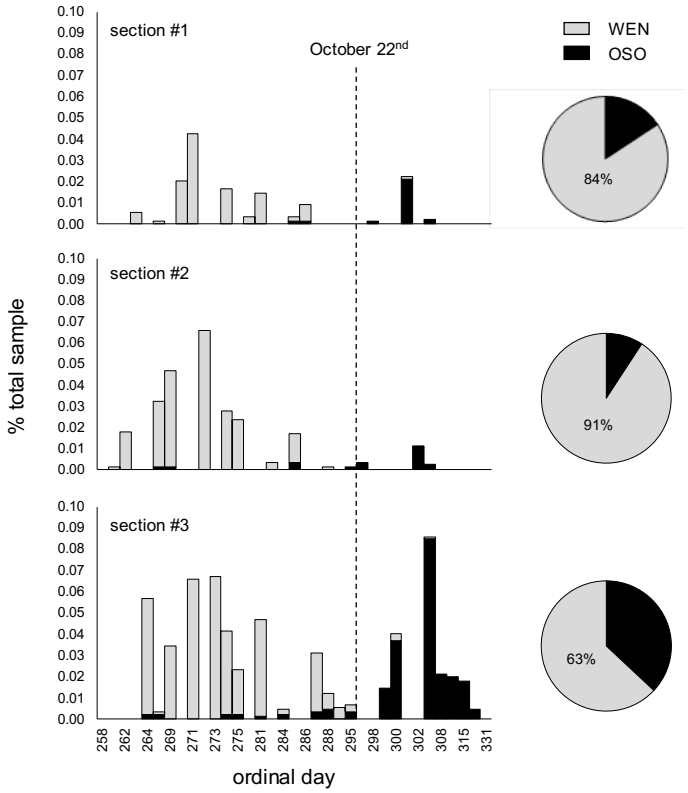


The tissue samples were sent to CRITFC geneticists at the HFCES where DNA was extracted and genotyped initially for a standardized panel of 94 SNP DNA markers. In 2016, the SNP panel was expanded to 364 loci employing RAD sequencing and the GT-seq protocol (Campbell et al. 2015), and DNA from adults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

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



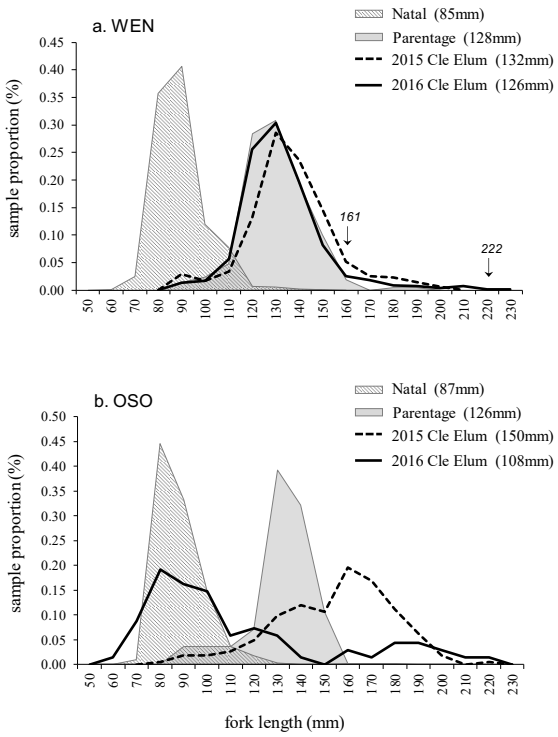
Temporal and spatial differences in spawning between stocks have been consistently observed among the two stocks. Post-spawned WEN carcasses were encountered from late September through early to mid-October, and were found across all three reaches (1, 2 and 3; see figure below). A lull in carcass recovery occurs in mid-October followed by an increase of fish which were 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 assign 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. The kokanee are no doubt 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; we presume the spatial and temporal differences in spawn timing, observed in the carcass GSI analyses, to be 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 were possible for 33 OSO juveniles, and all 33 were determined 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. 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 likely 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 markedly (Nielson 1950, Olsen et al. 1994, Nehlson 1995, Gustafson et al. 1997). Nonetheless, a limited amount of spawning of Sockeye Salmon persisted in the Metolius River downstream of these obstructions, with some juveniles apparently rearing in the lower Deschutes or the Columbia River (Gustafson et al. 1997). Then from 1958 through 1964 the Pelton-Round Butte Hydroelectric Project was created, involving construction on the Deschutes River 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 anadromous populations above the complex were functionally extirpated.

Round Butte Dam created a reservoir, Lake Billy Chinook Salmon (LBC), in which a large non-anadromous *O. nerka* (kokanee) population developed. Mature kokanee in LBC migrate upstream into the 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 a remnant of the now landlocked Sockeye Salmon population, the LBC kokanee (and current populations in Suttle Lake and other central Oregon lakes and reservoirs) more likely developed from repeated stocking with out-of-basin kokanee smolts from various hatcheries through the mid-1900s (Nehlson 1995, Gustafson et al. 1997).

In recent negotiations for relicensing of the Pelton-Round Butte Hydroelectric Complex, an agreement was reached to re-establish passage of anadromous fish, including Sockeye Salmon. Co-managers presumed that some portion of juvenile kokanee that exhibited out-migration behavior from LBC and/or Suttle Lake, might exhibit anadromy and return as mature adults characteristic of a Sockeye Salmon life history. A new fish transfer facility (FTF) at Round Butte Dam became operational in 2010. Each year since the new facility began operation, *O. nerka* juveniles that volunteer into the FTF have been passed downstream, though the number has varied widely between years. Returning adults from these releases are captured at a trap at Pelton Regulation Dam. These adults have either been transported upstream for release into LBC, or held for use as hatchery broodstock at Pelton Hatchery, located immediately below Round Butte Dam. Among the trapped adults, a few fish identifiable as strays from out-of-basin Sockeye Salmon populations were released back downstream of the complex. Annual numbers of out-migrating juveniles, of adults trapped at Pelton Dam, and of released hatchery-reared parr from adults retained for use as broodstock are illustrated below.

Year	Juveniles at FTF	Total Adults at Pelton Trap	Adults Released into LBC	Adults Released Downstream	Adults Kept for Broodstock	Hatchery Parr released into LBC
2010	49,734	10	10	0	0	0
2011	225,761	23	0	4	19	0
2012	5,126	98	86	12	0	3,870
2013	25,265	33	25	8	0	0
2014	155,031	27	20	7	0	0
2015	38,702	36	0	0	36	0
2016	49,497	536	463	0	73	13,122
2017	439,458	57	18	0	39	33,515
2018	47,161	49	27	0	22	22,000

The primary question of interest to the co-managers (ODFW, CTWSRO, and Portland General Electric - PGE) is whether the *O. nerka* captured at the adult trap are indeed LBC origin kokanee that have demonstrated reversion to an anadromous life history, and thus constitute a new run of Deschutes River

Sockeye Salmon. To address this question, an agreement was reached with CTWSRO in 2011 for the Project to finance a genetic study of the Deschutes River *O. nerka*. To provide a baseline of populations that might be represented among the returning adults, tissue samples were collected 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). These samples were genotyped for the standardized panel of 94 SNP markers that have been used to characterize *O. nerka* reference populations from across the Columbia River basin, and from two hatchery populations (Meadow Creek and Lake Whatcom). Tissue samples collected annually from in-migrating adults captured at the Pelton adult trap have been genotyped for the same SNP panel and genetic stock identification (GSI) analyses performed to assign each to the population of highest probability among the reference baseline populations.

GSI analyses readily distinguish Sockeye Salmon from kokanee from any of the Columbia Basin populations in the baseline. Additionally, the analyses will confidently identify a Sockeye Salmon to its source population – Osoyoos Lake, Lake Wenatchee or Red Fish Lake. Among the Columbia Basin kokanee populations, analyses reveal evidence of past kokanee stocking from some common out-of-basin hatchery sources, in particular kokanee stocks from Lake Whatcom, Washington, and the Meadow Creek spawning channel, Kootenai Lake British Columbia. These two stocks were widely used through the 1900s for stocking in lakes across the Pacific Northwest. Nonetheless, sufficient variation exists such that GSI analyses can generally distinguish fish from among the baseline kokanee populations, including between LBC, Suttle Lake, and other upper Deschutes kokanee populations, and between the Deschutes populations and other Columbia Basin kokanee populations.

		self-assignment			next likely assignment	
Origin	n	n	%	mean likelihood	ID	%n
Sockeye Salmon						
Osoyoos Lake	185	185	1.00	100	---	---
Lake Wenatchee	185	185	1.00	100	---	---
Redfish Lake	81	81	1.00	100	---	---
Kokanee						
Lake Billy Chinook	49	46	0.94	99.4	Wizard Falls	0.06
Suttle Lake	45	36	0.80	98.3	Wizard Falls	0.13
Wizard Falls	50	45	0.90	99.1	Suttle Lake	0.08
Odell Lake	40	40	1.00	99.9	---	---
Meadow Cr.	49	18	0.37	82.2	Dworshak Res.	0.57
Lake Whatcom	49	49	1.00	100	---	---

Results from GSI analyses for in-migrating adult *O. nerka* captured at the Pelton trap that returned between 2010 and 2017 (total N = 777), indicated that approximately 91% of the fish assigned with highest likelihood to LBC, with an additional 4% assigning to Suttle Lake or other Deschutes basin lakes.

	Total	Upper Deschutes Stocks			kokanee	Sockeye Salmon Stocks		
Run Year	Number Assigned	Lake Billy Chinook	Suttle Lake	Upper Deschutes	Wallowa Lake	Redfish Lake	Wenatchee Lake	Okanogan Lake
2010	10	8		1				1
2011	22	20		2				
2012	98	94		2				2
2013	30	26		1		1		2
2014	0							
2015	36	17		1	1	6	2	9
2016	524	484	4	18	10	4	2	2
2017	57	56		1				
2018								
Total	777	705	4	26	11	11	4	16
Percent		0.91	0.01	0.03	0.01	0.01	0.01	0.02

Sex was identified and scales were sampled for most adults collected at the Pelton Regulation Dam trap each year. Sex and scale age analyses for 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). On the other hand, 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

The large return of Deschutes Sockeye Salmon in 2016 (greater than 10 fold any previous year) raised program co-managers' hopes for a trend of increasing returns. However, adult returns in 2017 and 2018 were once again well below 100. Discussion is ongoing among co-managers of the Deschutes Sockeye Salmon reintroduction program as to possible changes in management approach that would encourage increased returns. In the meantime, the Project will continue to provide the GSI and scale age assessments to aid in their decision-making processes.

E. Project Objective #4: Evaluate factors affecting minijack production

In Columbia River spring (stream-type) Chinook Salmon populations, male maturation typically occurs at the end of their 3rd (jacks), 4th or 5th year of age. However, maturation may also occur precociously at age 1 (precocious parr, or "microjacks"), or age 2 ("minijacks"; Zimmerman et al. 2003; Larsen et al. 2013). Natural rates of precocious maturation of males as microjacks or minijacks are believed to be very low, e.g., less than 5% (Larsen et al. 2004), and reproductive success of these small young males relative to larger adults is also thought to generally be low (Schroder et al. 2010 and 2011). In hatchery reared stocks, however, the incidence of precocious maturation can be dramatically elevated, with rates as high as 80% having been observed (Harstad et al. 2014). Research conducted on Yakima River spring Chinook Salmon in the supplementation program operated at the CESRF, which uses strictly natural origin broodstock, indicates minijack rates averaging approximately 40% among their hatchery-reared male progeny (Harstad et al. 2014). In addition to their minimal contribution to natural spawning, minijacks do not survive to reach a size to provide fishery benefits. High incidence of minijacks thus represents a substantial biological and economic loss to a supplementation hatchery program (Larsen et al. 2004).

Research demonstrates that the rate of minijack production is strongly influenced by environmental factors associated with hatchery rearing conditions, principally high feeding rates which lead to increased growth rate, body size, and lipid levels relative to wild juveniles. However, studies also demonstrate an additional genetic component to age at male maturation, including evidence for differences between stocks and a positive correlation between parental and progeny age at maturation, including minijack rate (Larsen et al. 2006, 2010, 2013 and 2014; Harstad et al. 2014; Spanenberg et al. 2015).

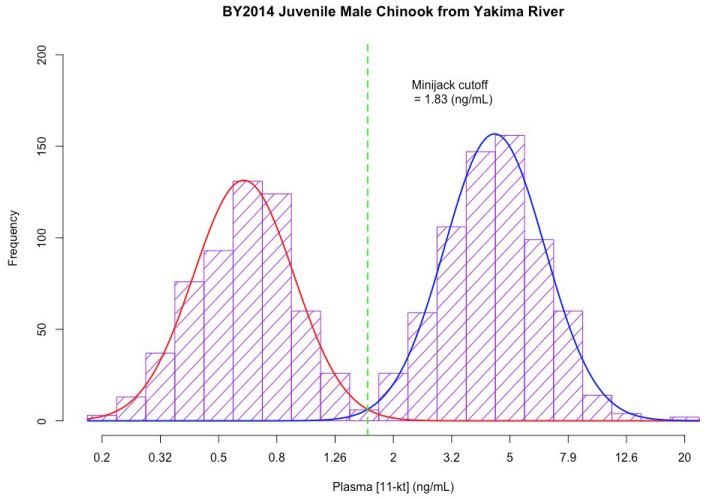
E.1 Evaluate effect of hatchery broodstock age on minijack production

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 3 by 3 factorials involving crosses of one each of age 3, age 4 and age 5 males with two age 4 and one age 5 females. However, few age 5 fish were available in any year. However, 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 spawning surveys, 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, at age 1+, the smolts were sacrificed, dissected, and identified to phenotypic sex, and the males were measured (length and weight), and blood and 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 (low 11-KT; Larsen et al. 2004; Medeiros et al. 2018). DNA was extracted from the tissue samples and genotyped for a standardized panel of SNP DNA markers, and parentage analysis used to assign each individual to its family. The proportion of minijacks within each male progeny group was analyzed for an effect of sire age on minijack rate within and across parental cross-types.

In 2016 we performed an additional series of seven factorial crosses each involving a single age 4 supplementation hatchery origin (SH) female crossed to three different age 4 males – one each of natural origin (WN), supplementation hatchery origin (SH) and hatchery control line origin (HC). The rationale to add these crosses to the study design stemmed from observations of Harstad et al. (2014) that indicated that segregated programs (in which spring Chinook Salmon broodstock is comprised entirely of HOR adults) demonstrated substantially lower minijack rates than integrated hatchery programs (in which broodstock is comprised predominantly, or wholly, of NOR fish). Natural populations are influenced to varying extent by successful spawning of microjack and minijack males each generation, and thus NOR broodstock used in integrated programs are susceptible of having precocial males in their (grand)parentage. In contrast, segregated programs use exclusively adult fish, and as male maturation as minijacks is presumed to have a heritable genetic component, Harstad et al. (2014) hypothesized that segregated programs will progressively select against this trait with each successive generation. We designed our study to test this hypothesis, with the expectation that minijack rates within females should be highest for crosses to the WN males (0 generations of hatchery rearing) and lowest for crosses to the HC males (3-4 generations of segregated hatchery rearing), with minijack rate for crosses to the SH males (1 generation of hatchery rearing) being intermediate.

The frequency distributions for plasma 11-KT in the male smolts was significantly bimodal in each of the three years, with the following cutoff values between low and high 11-KT for the purposes of identifying the juveniles as maturing versus non-maturing: BY 2014 = 1.83 ng/mL, BY 2015 = 1.52 ng/mL, and BY 2016 = 1.27 ng/mL, as illustrated below for BY 2104.



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:

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 female broodstock available did not permit assessment of female age effect). However, results of our study do 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, although ANOVAs within BYs, for cross-types indicate no significant differences associated with male broodstock age. It is possible that male age did have an effect on minijack rate, but that significance is masked by 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%.

Results for the second set of crosses performed in 2016 between eleven age 4 SH females and different WN, SH and HC age 4 males are provided in the table below. While the average minijack rate among females was numerically highest for crosses to WN males, this rate was not significantly different from the average value for crosses to SH males, nor from the average value for crosses to HC males. Again, the very high variation among families within cross types possibly masked what stock effects may have been present in the males. An additional observation was that the females could have a substantial effect on minijack rate. For example, all three crosses with female 141 showed less than 5% minijacks, and with female 210 less than 15%. In contrast, minijack rates for female 137 ranged between 62% and 86%. Minijack rates within the other four females were more variable.

	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	

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 2019. Results from these analyses will be summarized in a final report and a manuscript for submission to a scientific journal will be prepared.

E.2 Effects of feed supplementation with TTA on minijack production

In a previous study conducted on hatchery-reared juvenile Chinook Salmon at CESRF, feeding rate was reduced for some 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 (Larsen et al. 2006). However, reduced feeding also reduced final smolt size. Decrease in smolt size at release is generally known to be associated with increased mortality during out-migration and lower adult return rates. Several recent studies in Atlantic salmon 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. 2009a; Arge, et al. 2014).

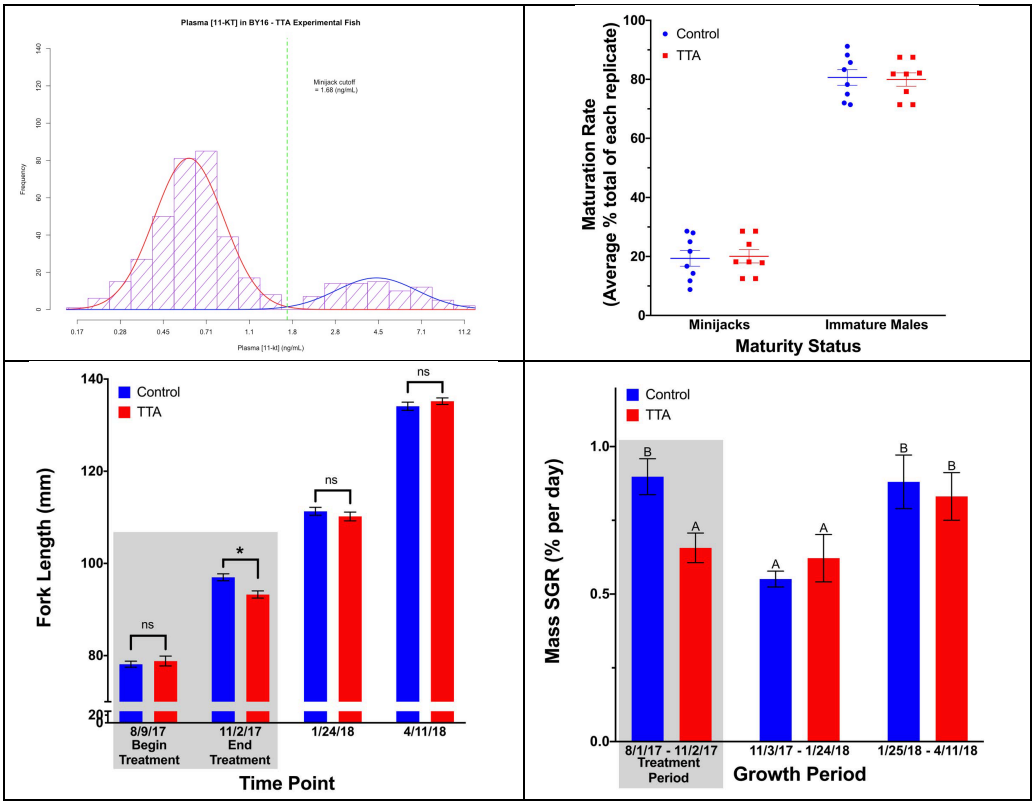
TTA, a commercially available nutritional supplement, is a 3-thia saturated fatty acid which upregulates cellular fatty acid oxidation, causing an increase in liver and muscle lipid metabolism similar to fasting. TTA as a supplement in salmonid diets, stimulates fatty acid oxidation and reduces lipid stores (Moya-Falcon et al. 2004; Gjoen et al. 2007; Alne et al. 2009a; Arge et al. 2014;) and leads to reduced sexual maturation in male post-smolt age 1+ Atlantic Salmon (Alne et al. 2009a). 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 fish oil minus the TTA.

Prior to initiation of the TTA treatment, a random sample of 12 fish from each tank was over-dosed in anesthetic, lengths and weights recorded, and the carcasses were frozen for subsequent analysis of whole body lipid content. 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 measured then frozen. 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 frozen.

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, although final size and growth of the fish at the smolt stage was similar. Results for the lipid analyses are anticipated in the first half of 2019. Afterwards, a technical report and a manuscript for publication in a scientific journal will be produced.



E.3 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 this period, juvenile male spring Chinook Salmon that exceed a predetermined threshold for size, growth, and lipid content will initiate gonadal development and mature as minijacks the following Sept-Oct spawning season (Thorpe 2007, Mangel and Satterthwaite 2008). And while this theory is widely accepted, it still remains unclear which physiological factors will exhibit measurable

differences to distinguish between maturing and non-maturing males during juvenile rearing or exactly when the timing of the maturation decision occurs. A better understanding of how and when this decision occurs will be necessary if managers wish to consider modification of rearing practices (e.g., reduction in feed lipid content and/or feeding rates; use of feed additives that accelerate lipid metabolism, etc.) in an effort to reduce the incidence of precocious maturation.

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 single-pair mating test crosses among CESRF spring Chinook broodfish 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 female crossed to an age 4 male were identified. When the embryos reached the swim-up stage in late February 2018, 70 fry per family were pooled into a common tank for rearing. On June 27, 2018 each fish (n=739) was tagged with a 9 mm PIT tag, and a tissue sample (fin clip) collected for genotyping – to identify genetic sex of each individual and their respective family – then returned to the rearing tank. Beginning July 11, and repeated every three weeks, all fish were anesthesized, measured for length and weight, and randomly redistributed among two rearing tanks at equal density per tank.

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 measurement. Afterwards, these fish were sacrificed in an overdose of anesthetic, blood sampled, dissected, the liver removed and weighed, and the pituitary removed. Samples of liver tissue, blood, and pituitaries were placed in pre-labeled vials, frozen over dry ice, transported to the University of Idaho, and stored at -80^{o}C until processing. These samples will be used to determine the hepatosomatic index (HSI), total body lipid, liver IGF-I mRNA, and pituitary FSH mRNA levels for each fish.

The final size sampling for fish kept for rearing to the smolt stage occurred on December 12, after which the fish were repooled in CESRF raceway #19 for rearing over winter. In April 2019, the fish will be sacrificed in an overdose of anesthetic, measured for size, and blood sampled for determination of plasma 11-KT concentration and maturation status determination. Size (FL and BW) and instantaneous growth rate (measured over certain time periods) will be compared within and across families, among smolts identified as minijacks, non-maturing males or non-maturing females, to determine when divergence for increased size is evident among the minijacks. Likewise, measures for HSI, and whole body lipid, liver IGF-I, and pituitary FSH mRNA levels will be compared. Results will be subjected to statistical analysis, and a technical report and a manuscript for publication in a scientific journal will be produced.

E.4 Minijack Rate and Adult Age structure

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 are related. That is, how might management modifications to decrease the magnitude of minijack production affect subsequent age structure of the returning adult progeny?

In 2018, we initiated a study involving a series of 2x2 factorial matings of CESRF production broodstock - two age 4 females crossed with an age 3 and an age 4 male – to be produced over three consecutive broodyears (2018, 2019 and 2020). At swim-up, progeny from these crosses will be combined for rearing to the pre-smolt stage in four raceways at CESRF, after which the fish will be transferred to four raceways at the Easton Acclimation site, for acclimation and final rearing to the smolt stage. Prior to release, 250 smolts from each raceway will be sacrificed, measured, and fin tissue sampled (for sex and parentage analysis) and blood sampled (for 11-KT analysis). In subsequent years (ending in 2025 for age 5 BY 2020 progeny), adults identified via their VIE tag, as Easton releases will be measured, and tissue and scale sampled. As data accumulate over the coming years, 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.

F. Project Objective #5: Assess productivity and capacity parameters associated with supplementation hatchery programs

A primary focus of the Project activities described above is use of genetic tools to assess effects that hatchery rearing may have on the life history and productivity characteristics of fish that are used to reintroduce a natural population where the indigenous population was extirpated, or to supplement an extant depressed natural population. Beyond assessment of how hatchery supplementation can be managed to minimize any negative effects on a supplemented population, is the need to assess whether a hatchery program has deleterious effects of the productivity for a non-target natural population.

In 2006, Kostow and Zhou published results from an analysis that indicates a significant repressive effect of an out-of-basin summer-run steelhead hatchery program on productivity of an indigenous winter-run steelhead population in the upper Clackamas River, Oregon. The study analyses a time-series of data that begins several years prior to initiation of the hatchery program in 1970, through 2003, six years following termination of the program in 1997. Kostow and Zhou (2006) concluded that competition for spawning and juvenile rearing resources significantly reduced productivity of the native winter-run fish.

The Kostow and Zhou (2006) study has been frequently cited in scientific and public reports that caution against the use of hatchery programs, due to negative effects such programs may have on wild salmonid populations. However, for various reasons, the conclusions of Kostow and Zhou (2006) were questioned by some biologists familiar with fisheries management in the Clackamas River basin. In 2016, Mr. Ian Courter, Mount Hood Environmental, LLC initiated a re-examination of the data on which was based the Kostow and Zhou (2006) study, complemented with an additional six years of post-hatchery adult return information. Preliminary findings did not indicate competition with summer-run fish as having a significant causal relationship with downward fluctuations in productivity of the winter run fish. Instead, he observed that winter-run steelhead abundance did not increase significantly following termination of the hatchery program, and that variation in abundance of Clackamas River winter-run steelhead correlated well with fluctuations in other winter-run populations, inferring that productivity of the Clackamas population was a response, not to presence of the hatchery summer run fish but to regional (in particular ocean) environmental effects. However, the analytical approach that Mr. Courter used was in need of refinement, and to this end in 2017, he was contracted by CRITFC under the Project to pursue further re-examination in collaboration with additional biometricians, including Robert Lessard, CRITFC biometrician with expertise in life cycle modeling.

Reanalysis of the Clackamas River steelhead data fit to a Bayesian state-space stock-recruitment model, as well as data sets for other regional winter steelhead populations, was completed in 2018. Results confirmed those of the preliminary analyses. Abundance of hatchery summer steelhead spawners (1972-2001) did not have a negative effect on winter steelhead recruitment in the upper Clackamas River, and winter steelhead abundance in the upper river did not rebound to pre-hatchery program levels following termination of the program. Instead, significant effects were observed for both spill at North Fork Dam, the gateway to the upper Clackamas Basin, and Pacific Decadal Oscillation (an index of ocean conditions). Additionally, variation in abundance of Clackamas River was correlated with variation for other regional winter steelhead stocks. In summary, the analyses did not support reduction in abundance of Clackamas River winter steelhead due to competition with of hatchery origin summer steelhead for spawning and juvenile rearing resources, but instead the analyses indicated that winter run abundance was driven primarily by regionwide (freshwater and ocean) environmental factors (Courter et al. 2018; see Section I Project Objective #8.)

G. Project Objective #6: Coordinate inter-tribal workshops and genetics training programs

Tribal fisheries personnel are involved in monitoring and evaluation programs of essentially all anadromous fish populations within their reservations and ceded territories. Tissue sampling of fish (at weirs and ladders, in smolt traps, and during carcass surveys) is often included as part of standard monitoring activities. Samples are also being collected from all broodstock at tribally managed salmon and steelhead hatcheries, as part of a basinwide parental based tagging (PBT) program to genetically "tag" all hatchery releases in the basin (Steele et al. 2016). Similarly, all adult Pacific salmon translocated by the tribes to restore interior lamprey production are tissue sampled. Samples collected by the tribes are sent to CRITFC geneticists at the HFCES for molecular genetic analyses, and the resulting data are analyzed to inform a variety of management questions. However, the tribal field personnel involved have little formal training in the principles of molecular and quantitative genetics, and limited knowledge of how the information can be applied to guide management. Conversely, the CRITFC genetics laboratory personnel have limited exposure in the field to the tribes' monitoring activities which limits their understanding of the logistical constraints under which field crews operate that affect sample collection.

With the objective of providing the field personnel a better understanding of basic genetic principles and of how the tissue samples are processed and the genotypic data analyzed, and to improve understanding and communication between the tribal field personnel and the HFCES geneticists and laboratory technicians, we developed a curriculum for a 2-day "Introduction to Molecular Genetic Analyses in Tribal Fisheries Management" workshop, held at the HFCES laboratory. The program consists of a series of oral/slide presentations, videos, and demonstrations by CRITFC staff on basic principles of genetics and inheritance, types of molecular DNA markers, and analyses using these markers applicable to fisheries management questions. Emphasis is placed on use of SNP DNA markers for GSI and for parentage/productivity analysis. Workshop presentations are interspersed with "hands-on" exercises to provide greater familiarity with genetics principles and laboratory techniques. Additionally, the entire HFCES staff is invited to attend a noontime slide presentation on each of the two days, by one of the participants who reviews a tribal project on which he/she works.

As indicated in the 2017 Annual Progress Report, since 2011 a total of 13 workshops with involving 111 participants have been conducted. In 2018, specific requests were received for participation of only 1-2 additional tribal personnel, and it was decided to forego conducting a workshop in 2018. Funds will

nonetheless be earmarked in the budget for the upcoming contract, should there be interest in conducting a workshop in 2019.

H. Project Objective #7: 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 2018, 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 persons with 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, and the nature of CRITFC's participation at each:

- Feb 21-23: Annual Meeting of the Washington/British Columbia Chapter of the American Fisheries Society, Kelowna BC, March 19-22, 2018. Presentation: Matala, A. P., S. R. Narum, P. F. Galbreath, B. P. Saluskin, M. V. Johnston, and J. Hogle. Sockeye Salmon reintroduction strategies in the CRB: pros, cons and surprises.
- April 17: Columbia Gorge Fisheries and Watershed Science Conference, The Dalles OR.
- June 19-21: Coastwide Salmonid Genetics Meeting, Seattle WA,. Presentation: A. Matala. Reintroduction of Sockeye Salmon stocks to Cle Elum Lake.
- June 13-14: Yakima Basin Science and Management Conference, Ellensburg WA. Presentations: Medeiros L. R., et al. Evaluating minijack rates in spring Chinook Salmon. Knudsen, C., et al. Assessing effects of parental traits on production of spring Chinook minijacks. Matala, A. P., et al. Genetic monitoring of Sockeye Salmon reintroduction in the Cle Elum Reservoir.
- August 27-28: Northwest Power and Conservation Council Research Project Status Review, Portland OR. Presentation: Galbreath, P. Basinwide Supplementation Evaluation, Project 2009-009-00.

I. Project Objective #8: Prepare manuscripts for publication in scientific journals

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