



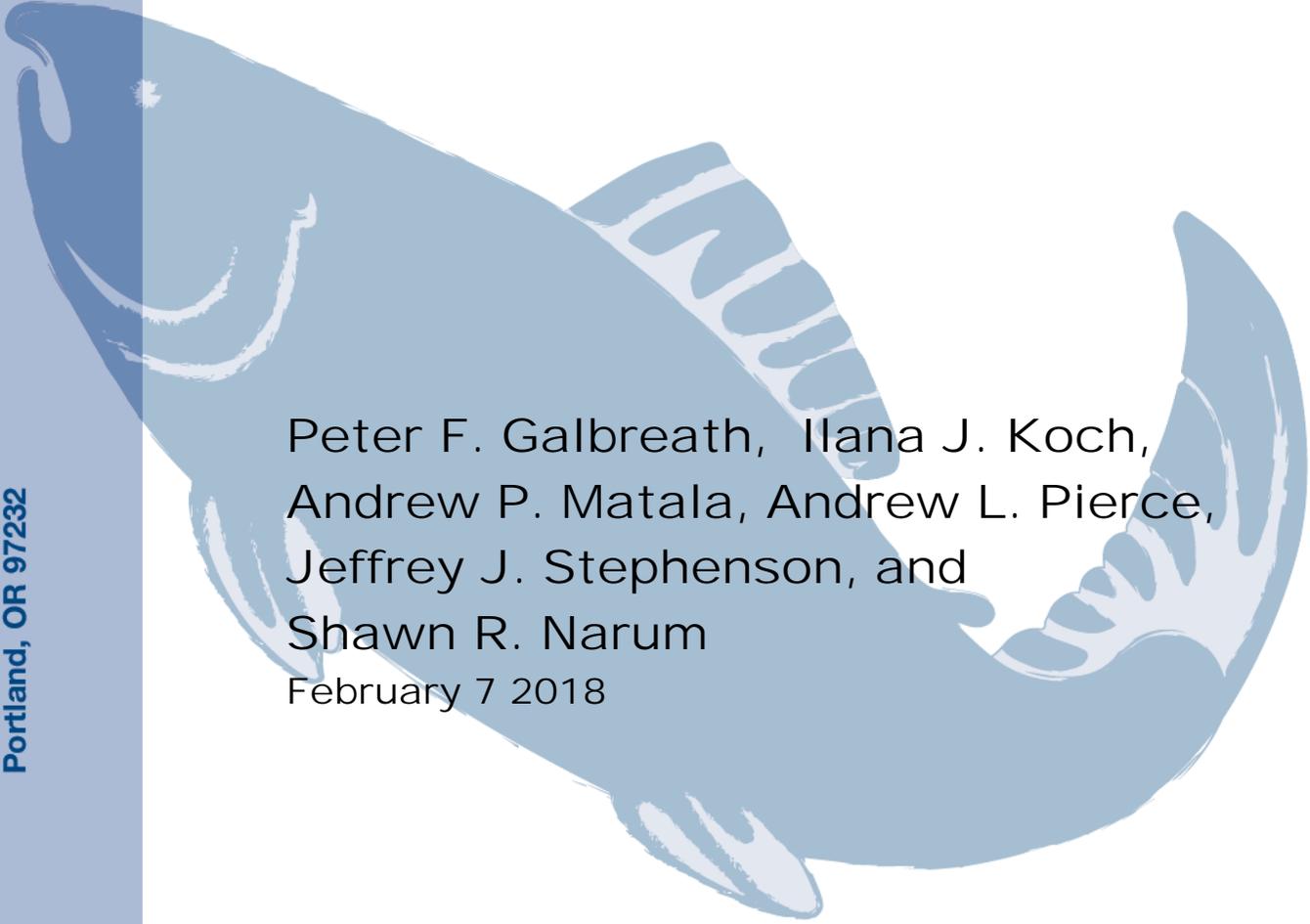
CRITFC

TECHNICAL REPORT 18-08

Columbia River Inter-Tribal Fish Commission
503.238.0667
www.critfc.org

700 NE Multnomah, Suite 1200
Portland, OR 97232

Basinwide Supplementation Evaluation Project: 2017 Annual Progress Report



Peter F. Galbreath, Ilana J. Koch,
Andrew P. Matala, Andrew L. Pierce,
Jeffrey J. Stephenson, and
Shawn R. Narum
February 7 2018



2017 Annual Progress Report
Basinwide Supplementation Evaluation

BPA Project # 2009-009-00

Report covers work performed under BPA contracts # 72525 and 76019

Report was completed under BPA contract # 76019

1/1/2017 - 12/31/2017

**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

Report created 02/07/2018

This report was funded by the Bonneville Power Administration (BPA), U.S. Department of Energy, as part of BPA's program to protect, mitigate, and enhance fish and wildlife affected by the development and operation of hydroelectric facilities on the Columbia River and its tributaries. The views in this report are the authors' and do not necessarily represent the views of BPA.

This report should be cited as follows:

Galbreath, P. F., I. J. Koch, A. P. Matala, A. L. Pierce, J. J. Stephenson, and S. R. Narum. 2017 Annual Progress Report - Basinwide Supplementation Evaluation, 1/1/2017 - 12/31/2017 Annual Report, BPA Project No. 2009-009-00, Contract No. 76019.

Table of Contents

Table of Contents	i
Acknowledgements	ii
I. Executive Project Summary	1
II. Introduction	3
III. Work Elements / Tasks	
A. Project Administration	4
B. Project Objective #1: Support RRS studies of supplemented spring Chinook Salmon	
B.1 Johnson Creek spring/summer Chinook Salmon	4
B.2 Upper Yakima River spring Chinook Salmon	5
C. Project Objective #2: Support RRS studies of reintroduced salmon populations	9
C.1 Lookingglass Creek (Grande Ronde River) spring Chinook Salmon	10
D. Project Objective #3: Support genetics studies of reintroduced Sockeye Salmon	
D.1 Cle Elum Lake (Cle Elum/Yakima Rivers) Sockeye Salmon	13
D.2 Suttle Lake/Lake Billy Chinook Salmon(Metolius/Deschutes Rivers) sockeye salmon/kokanee	18
E. Project Objective #4: Project Objective #6: Evaluate effect of hatchery broodstock age on minijack production	21
F. Project Objective #5: Assess productivity and capacity parameters associated with tribal supplementation programs	25
G. Project Objective #6: Coordinate inter-tribal workshops and genetics training programs	26
H. Project Objective #7: Participate in regional forums for review of hatchery effects on natural populations	26
I. Project Objective #8: Prepare manuscripts for publication in scientific journals	27
IV. Synthesis of Findings: Discussion/Conclusions	28
V. References	31

Acknowledgements

Funding of the Project was provided by the Bonneville Power Administration under agreements reached within the Columbia Basin Fish Accords (2008). We appreciate the BPA administrative support supplied by Barbara Shields and Dorothy Welch, Contracting Officer Technical Representatives, Kristi Van Leuven and Karen Wolfe, Contracting Office Representatives, and Israel Duran, Environmental Compliance Lead. Additional administrative support to the Project was provided by Zachary Penney, Douglas Hatch, Robert Lessard, Jeremiah Newell, Melissa Holland and Chrissy Bynum (CRITFC Fish Science Department), Christine Golightly (CRITFC Policy Department), and by the CRITFC Finance Department. Technical support for the Project was provided by the following fisheries biologists and managers: Jeff Hogle (Confederated Tribes of the Warm Springs Reservation of Oregon - CTWSRO); Brian Saluskin, Mark Johnston, Charlie Strom, Bill Bosch and David Fast (Yakama Nation - YN); Gene Shippentower, Les Naylor and Carrie Crump (Confederated Tribes of the Umatilla Reservation - CTUIR); Sherman Sprague, Justin Bretz, Craig Rabe and Jay Hesse (Nez Perce Tribe - NPT), Lea Medeiros and Nick Hoffman (University of Idaho), Curtis Knudsen (Oncorh Consulting), and Ian Courter (Mt Hood Environmental).

I. Executive Project Summary

This report summarizes activities for calendar year 2017 under BPA Contracts # 72525 and 76019, 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 within the Statement of Work for the contracts as described in PISCES. 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 fish of out-of-basin origin (both of hatchery origin or natural origin), in subbasins where the indigenous population had been extirpated. A relative reproductive success (RRS) study of supplemented Johnson Creek spring/summer Chinook Salmon *Oncorhynchus tshawytscha*, financed jointly by the Nez Perce Tribe and the Project, is ongoing (Project Objective #1). Results for six consecutive brood years (BYs) indicate that supplementation is indeed providing a demographic boost to the depressed population with little or no difference in natural productivity between natural origin and hatchery origin adults. A parallel RRS study based on juvenile recruits-per-spawner is underway, with results anticipated in the coming year. A similar though larger scale RRS study under Project Objective #1 was initiated in 2014 to assess RRS of supplemented upper Yakima River spring Chinook. Preliminary results for the initial BY (2007) indicate a 2.15 greater return rate from natural origin fish brought into the hatchery for spawning relative natural origin fish left in the river to spawn naturally, and generally similar natural productivity estimates within sexes, for natural origin and hatchery origin adults spawning naturally ($RRS \approx 1$). Genotyping and productivity analyses for the remaining BYs, and a comprehensive analysis for BYs 2007-2011 should be completed in 2019. A similar RRS analysis is ongoing in Lookingglass Creek, Grande Ronde River basin. However, in this case the natal population had been extirpated and the new population under study was reintroduced with the use of hatchery stock. A preliminary analysis based on juvenile recruits-per-spawner for BYs 2008-2014 was performed, with results indicating that natural productivity estimated for both on all potential adult spawners and limited to successful spawners, was on average greater for natural origin females and males than for hatchery origin fish of the reintroduced hatchery stock. This difference was not statistically significant for females but was significant for males. These results are concordant with the expectation that the reintroduced hatchery fish would naturally spawn in the new habitat, and that natural selective forces would act on genetic and environmental traits of the new population such that the natural origin fish would demonstrate improving productivity in subsequent generations. Two genetics studies of Sockeye Salmon *O. nerka*, financed by the Project are ongoing (Project Objective #3). The first involves assessment of 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). Results indicate that the Wenatchee stock spawns on average 3 weeks earlier than Osoyoos stock, albeit with some overlap in timing. Genetics analyses of out-migrating juveniles indicate a very low proportion of inter-stock hybrids (<10%), associated with temporal and spatial segregation for spawning between stocks. 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. (Project Objective #3). 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 performed on

the adult returns confirmed >90% of them to be of Lake Billy Chinook origin. A 3-BY (2014-2016) study at the Cle Elum Supplementation Research Facility to test for an effect of spring Chinook Salmon broodstock age on survival, size and rate of precocial maturation of age 1+ smolts as minijacks is ongoing (Project Objective #4). Initial results for the first two BYs indicate that age 1 microjack males yield the highest proportions of minijacks, but unexpectedly that age 3 jack sires yield fewer minijacks than older age 4 males. Also, variation in minijack rate among families within parental age cross-types was very high, ranging in one instance from 0% to 100%. Results for BY 2016 will be available in 2017, and a comprehensive analysis and report soon thereafter. 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 is ongoing, with results due in 2018 (Project Objective #5). A 2-day "Introduction to Molecular Genetic Analyses in Tribal Fisheries Management" workshop sponsored through the Project was attended by 13 tribal fisheries biologists and technicians (Projective Objective #6). Columbia River Inter-Tribal Fish Commission (CRITFC) personnel associated with Project activities participated in a variety of inter-tribal and inter-agency meetings, workshops and symposia in which issues related to effects of hatchery management on population productivity were discussed (Project Objective #7).

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 2017, these activities included ones to:

- 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 of 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 Salmonkokanee population downstream of the Pelton-Round Butte complex (Project Objective #3)
- to build off previous research conducted at the Cle Elum Supplementation Research Facility describing incidence of precociously maturing spring Chinook Salmon smolts as age 2 minijacks, with a study to examine the effect of age (within sexes) of the natural origin hatchery broodstock on survival, size and minijack rate among their hatchery-reared smolts (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 2017 involving administration of the Project by CRITFC included: production and posting in PISCES of the annual progress report for 2016, completion of 2017 quarterly and final status reports in PISCES that record progress associated with each work element within the contract Statement of Work, and submission of 2017 monthly project expense summaries to BPA. Additional reports and associated documents summarizing activities described within Project work elements were posted under Attachments within Project 2009-009-00 Contracts #72525 and 76019 in PISCES.

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 – 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 \$60,000 annually to cover costs for these analyses - sufficient for 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 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.

RRS analyses described in Hess et al. (2012) for three consecutive BYs indicate that supplementation did indeed provide a demographic boost to the depressed spring/summer Chinook Salmon population in Johnson Creek, and that natural productivity within sexes of successfully spawning HOR fish was generally similar to that of NOR fish. These findings were confirmed by analyses for the subsequent three BYs (Hess, unpublished data; <https://afs.confex.com/afs/2015/webprogram/Paper19341.html>). Briefly, the proportion of fish identified as parents of one or more adult offspring was similar for NOR

and HOR females (age 4 and 5); 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.

Numbers of samples collected from out-migrants for the juvenile recruits-per-spawner RRS study are provided below:

Year	Spawners		Juvenile Progeny*
	NOR	HOR	
2009	197	497	
2010	465	484	2781
2011	396	310	3301
2012	447	198	3097
2013	609	301	1919
2014	1114	542	2099
2015	528	439	3571
2016	509	191	1936

* 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

** estimates

The juvenile samples collected represent progeny from BYs 2009 through 2015. Genotyping of these samples will be complete in 2018, and subsequently the data will be subjected to parentage analysis and estimation of RRS across these 7 BYs.

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 first collection of wild broodstock was in 1997, after which the fish were transported to the newly constructed Cle Elum Supplementation and Research Facility (CESRF), Cle Elum WA, for spawning and rearing of their progeny. As pre-smolts, the fish are transported to one of three acclimation sites within the upper Yakima basin, where they were held for several 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 for genotyping tissue samples from the thousands of adults returning in-basin each year. 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 Roza Adult Monitoring Facility (RAMF) adjacent to Roza Irrigation Dam (rkm 206), that were passed upstream for natural spawning in those 5 years, plus their NOR adult progeny that returned in years 2012 through 2016:

<u>Return Year</u>	<u>Adult Spawners</u>		
	<u>Natural Origin</u>	<u>Hatchery Origin</u>	<u>Unknown Origin</u>
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	500*	na	na
Sub-Totals	26,440	19,546	779

(* estimated)

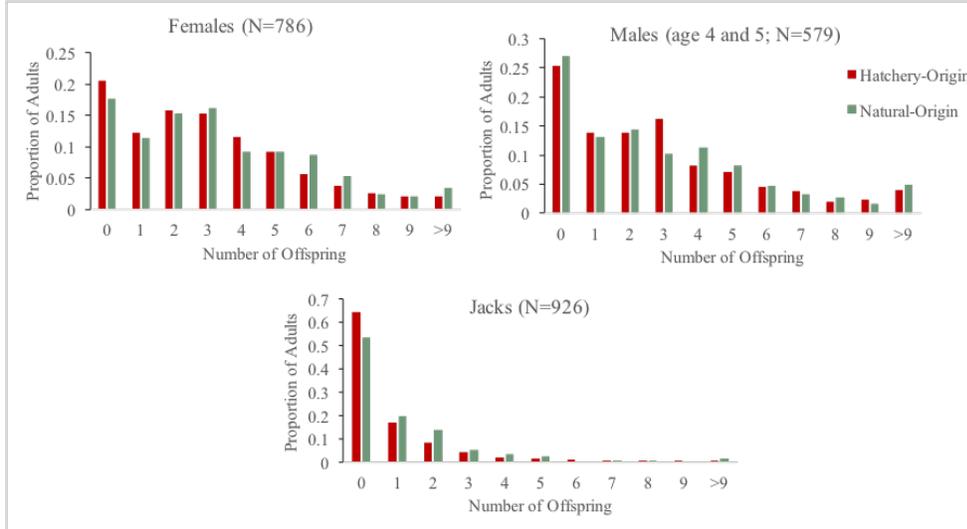
Genotyping by CRITFC using Project funds and by WDFW using YKFP funds, continued through 2017 and will be complete in 2018. Parentage and RRS analyses will be conducted within and across BYs, and results will be summarized in a technical report and in a manuscript to be submitted for publication in a scientific journal in 2019.

To provide an interim assessment, an initial RRS analysis was conducted for brood year 2007. Genotyping, parentage analyses and initial RRS comparisons were completed in 2016, and reported in the 2016 Project annual report. Subsequent refinement of these analyses were performed in 2017, and

the results presented in a CRITFC Technical Report (Galbreath et al. 2017). The primary findings are provided below:

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		Number Adult Offspring	<u>All Adults Genotyped</u>		<u>Successful Adults</u>	
		Number Adults Genotyped	(Proportion) Successful Adults		Average R/S	RRS (HOR/NOR)	Average R/S	RRS (HOR/NOR)
Females (age 4&5)	HOR	400	318 (0.80)	1,215	3.04	0.90	3.82	0.94
	NOR	386	318 (0.82)	<u>1,297</u> 2,512	3.36		4.08	
Males (age 4&5)	HOR	319	238 (0.75)	920	2.88	0.95	3.87	0.93
	NOR	260	190 (0.73)	<u>787</u> 1,707	3.03		4.14	
Jacks (age 3)	HOR	767	272 (0.35)	632	0.82	0.72	2.32	0.95
	NOR	<u>159</u> 2,291	74 (0.47)	<u>181</u> 813	1.14		2.45	

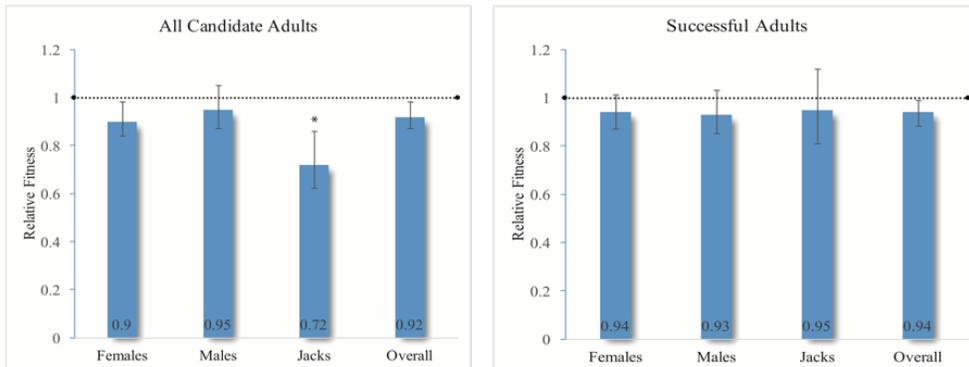


Among the adult females and males of both origins, a large majority (73% to 82%) were identified as having produced one or more adult progeny. This rate of successful adult offspring production is high relative to that observed in other studies of supplemented spring Chinook Salmon populations. For example, 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).

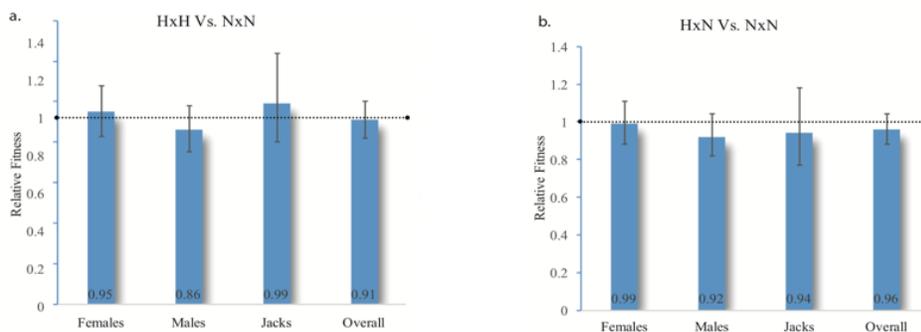
Supplementation provided a 2.15-fold demographic boost to the upper Yakima River population, i.e., a NOR fish spawned in the hatchery returned on average 2.15 more adult progeny than a NOR fish left in the river to spawn naturally:

			Progeny per	Hatchery
			<u>Parent</u>	<u>/Natural</u>
<u>HOR progeny</u>	<u>1,123</u>	=	3.08	
Broodstock	365			
				2.15
<u>NOR progeny</u>	<u>1,149</u>	=	1.43	
Natural spawners	805			

Productivity of NOR and HOR females, and NOR and HOR age 4 and 5 males spawning naturally was not significantly different, averaging approximately 3 recruits-per-spawner. Productivity of age 3 jack males was only about 1/3 that of the age 4 and 5 adults, and productivity of HOR jacks was significantly lower than for NOR jacks, though when considering only jacks for which adult progeny were identified (successful spawners), NOR and HOR jacks were similarly productive:



d) When considering average productivity HOR relative to NOR fish within sexes among identified cross-types: a) HORxHOR relative to NORxNOR, and b) HORxNOR relative to NORxNOR, no significant differences in productivity were observed.



While preliminary and based on results for only one BY, these findings nonetheless 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.

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 substantial numbers of the HOR fish released as juveniles 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 progressive phasing out stocking of juveniles produced from out-of-basin hatchery broodstock. Instead, broodfish are increasingly collected from among adults returning in-basin, to produce the juveniles with which to continue supplementation. The initial generations of "local origin" broodstock are 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 produced juveniles above the hatchery weir. 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 returning adults from the new in-basin hatchery stock, from 1998 through 2003, no adults were passed upstream of the Lookingglass weir ($\frac{1}{2}$ 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 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 to present, tissue samples have been randomly collected from out-migrating NOR juveniles (both as age 0+ parr in the summer and fall, and as age 1+ smolts in the spring) captured in a rotary screw trap located $\frac{1}{4}$ km downstream of the weir (Boe et al. 2010 and 2011).

Using financing from the Project, DNA was extracted from tissue samples for all adults intercepted at the Lookingglass weir in BYs 2008 through 2014, 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 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-Alrich, St Louis, MO), and for genotyping using the “genotyping-in-thousands by sequencing” (GT-seq) technique (Campbell et al. 2015). In 2017, the genotypic data for these BYs were entered into Cervus 3.0.7 for parentage analysis. The software uses likelihood equations to identify the most probable parent pair for each juvenile. To maximize certainty in the assignments, those which involved two or more allele mismatches, and/or those which had a threshold confidence level of <99% were deleted. The remaining data 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. The samples numbers and summary parentage results are provided in the table below:

Year	All Potential Parents*			Juvenile Progeny					
	Sex/Age	# HOR	# NOR	# Genotyped		# Assigned			
				Parr	Smolts	Parr	Smolts	Total	% Assigned
2008	Female	80	24	240	83	237	81	318	0.98
	Male	59	12						
	Jack	5	2						
2009	Female	13	36	456	145	427	141	568	0.95
	Male	14	18						
	Jack	11	6						
2010	Female	200	34	352	118	340	115	455	0.97
	Male	72	32						
	Jack	25	5						
2011	Female	209	42	423	23	412	15	427	0.96
	Male	129	34						
	Jack	35	19						
2012	Female	564	56	696	114	680	107	787	0.97
	Male	271	37						
	Jack								
2013	Female	61	16	276	193	269	188	457	0.97
	Male	63	14						
	Jack		56						
2014	Female	285	67	566	341	526	331	857	0.94
	Male	179	51						
	Jack		31						

avg: 0.96

Then, the mean estimates of recruits-per-spawner (R/S) within origin for females, males and jacks were calculated, both for all potential parents, and limited to successful adults (only those adults assigned one or more juvenile progeny). RRS (NOR/HOR) for each brood year was then calculated within females and males, presented in the tables below (sample sizes were insufficient to make reliable RRS comparisons for jacks):

Broodyear	FEMALES - ALL Potential Parents				
	HOR		NOR		RRS NOR/HOR
	#	Mean R/S	#	Mean R/S	
2008	80	3.19	24	2.54	0.80
2009	13	11.77	36	11.17	0.95
2010	200	1.85	34	2.03	1.10
2011	209	1.40	42	2.55	1.82
2012	564	1.10	56	1.68	1.52
2013	61	5.54	16	6.38	1.15
2014	285	2.06	67	3.63	1.76

avg: 1.30

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

avg: 1.12

Broodyear	MALES - ALL Potential Parents				
	HOR		NOR		RRS NOR/HOR
	#	Mean R/S	#	Mean R/S	
2008	59	3.00	12	4.92	1.64
2009	14	7.64	18	8.94	1.17
2010	72	2.22	32	3.97	1.79
2011	129	1.47	34	2.47	1.69
2012	271	1.92	37	2.54	1.33
2013	63	2.67	14	4.63	1.74
2014	179	2.48	51	3.67	1.48

avg: 1.55 ***

Broodyear	MALES - Successful Parents				
	HOR		NOR		RRS NOR/HOR
	#	Mean R/S	#	Mean R/S	
2008	41	4.32	8	7.38	1.71
2009	11	9.73	12	13.42	1.38
2010	34	4.71	21	6.05	1.29
2011	61	3.10	23	3.65	1.18
2012	127	4.09	21	4.48	1.10
2013	30	5.60	11	5.91	1.06
2014	94	4.72	25	7.48	1.58

avg: 1.33 **

These initial results indicate that natural productivity of NOR females and males (both all potential adult spawners, and limited to successful spawners) was on average greater for than for HOR fish of the reintroduced hatchery stock, the difference being statistically significant for males though not for females. These observations concord with the expectation that natural selective forces would act on the reintroduced spring Chinook Salmon to establish a new natural population that demonstrates improved productivity.

An important caveat is that the juvenile assignments combine those made to sub-yearling parr with those made to older yearling smolts. The parr are sampled at age 0+ in the summer and fall prior to overwintering in the mainstem Grande Ronde. The smolts are fish that remained upstream in Lookinglass Creek and survived that period of mortality prior to out-migrating the next spring. Pooling assignments of parr and smolts is legitimate if NOR and HOR adults produce similar proportions of each. However, our data indicated that smolts represented a larger proportion of juveniles assigned to NOR than to HOR spawners, with the difference being statistically significant for NOR females. Unfortunately, we lack a credible method to standardize the parr and smolt data as “smolt equivalents”, e.g., to estimate the number for each that would be expected to reach Lower Granite Dam during smolt out-migration. The RRS analyses, therefore, are somewhat biased against NOR fish. However, this makes the analyses conservative, and our observation of greater productivity of NOR fish additionally certain.

Parr vs Smolts - % Smolts Assigned to HOR vs NOR

Broodyear	Females			Males		
	% HOR	% NOR	%N/%H	% HOR	% NOR	%N/%H
2008	0.255	0.262	1.03	0.299	0.220	0.74
2009	0.163	0.284	1.74	0.187	0.311	1.66
2010	0.233	0.333	1.43	0.256	0.244	0.95
2011	0.046	0.056	1.22	0.037	0.024	0.65
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
			1.33 *			1.17

(low sample size for smolts)

Plans for this study in 2018 are to finish genotyping and perform parentage analyses for two additional BYs (2015 and 2016), and in 2019 to finalize a summary report and manuscript for all nine BYs (2008 to 2016).

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 populations. 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 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 study, 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 each year 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 <u>Outplants</u>	No. Adult <u>Returns</u>	<u>Total</u>
2009	1,000	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

* exceptionally high summer temperatures in 2015 and 2017 resulted in high mortality of adult Sockeye Salmon within the Columbia mainstem, and dramatically reduced returns to the RAMF relative to what was anticipated.

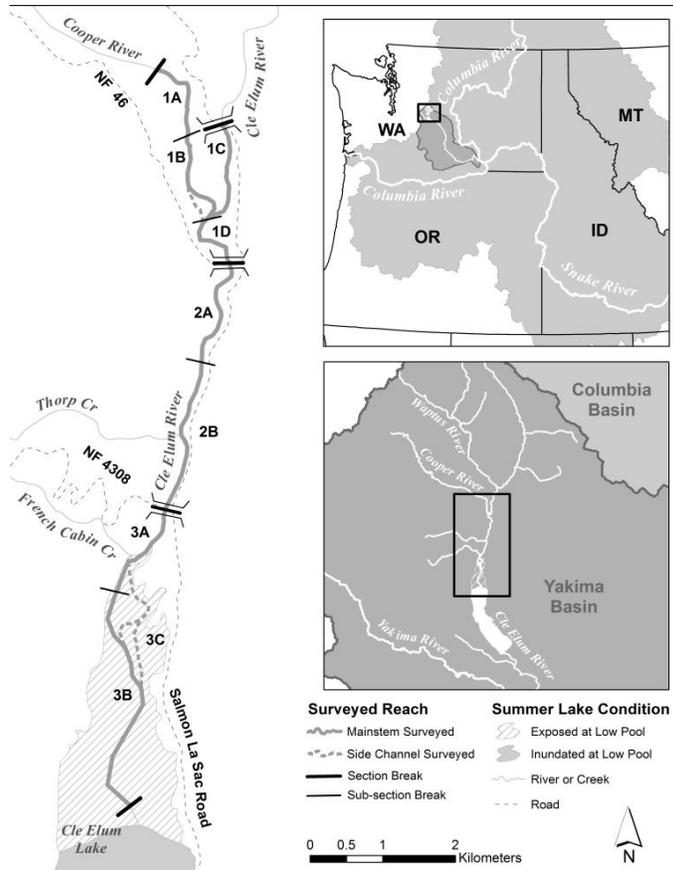
The adults at PRD represent a mix of fish originating from the two remaining Mid/Upper Columbia River stocks – Wenatchee stock from Wenatchee Lake/Wenatchee River, and Osoyoos stock from Osoyoos Lake/Okanogan River. Lake Wenatchee is substantially higher in elevation (572 m) and has colder average water 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 Wenatchee stock Sockeye Salmon is 3 to 4 weeks earlier than for the Osoyoos stock. Additionally, the two stocks exhibit differences in average age structure. Wenatchee 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 Stock	Smolt Age		Adult Age		
	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 Okanogan 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, 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 Project committed to covering costs for the genetic analyses, for tissue samples collected from: the adults sampled at the RAMF, a portion of the translocated PRD adults, a portion of out-migrating juveniles, post-spawned adults encountered during spawning ground surveys (see map below), and Sockeye Salmon caught as by-catch during gill-netting performed to remove lake trout from Cle Elum Lake.

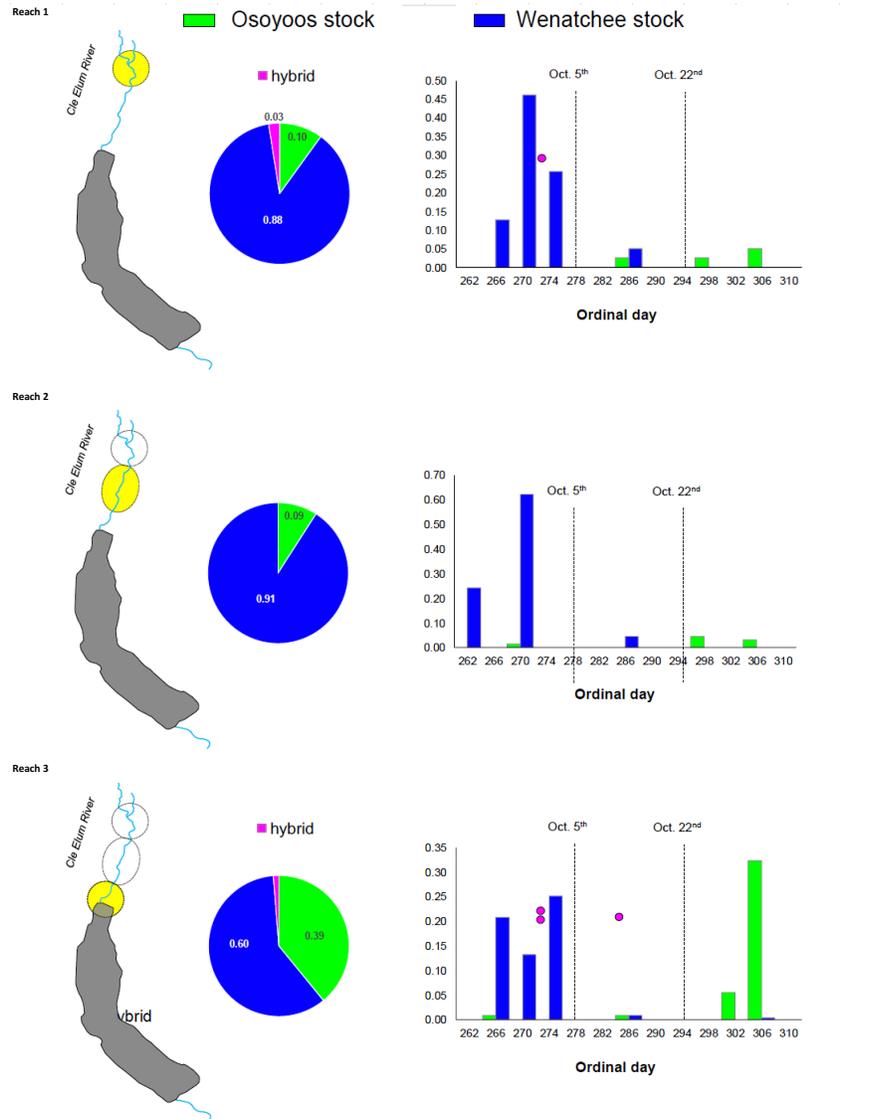
The map in the figure below identifies the different spawning ground survey reaches in the Cle Elum River upstream of the lake.



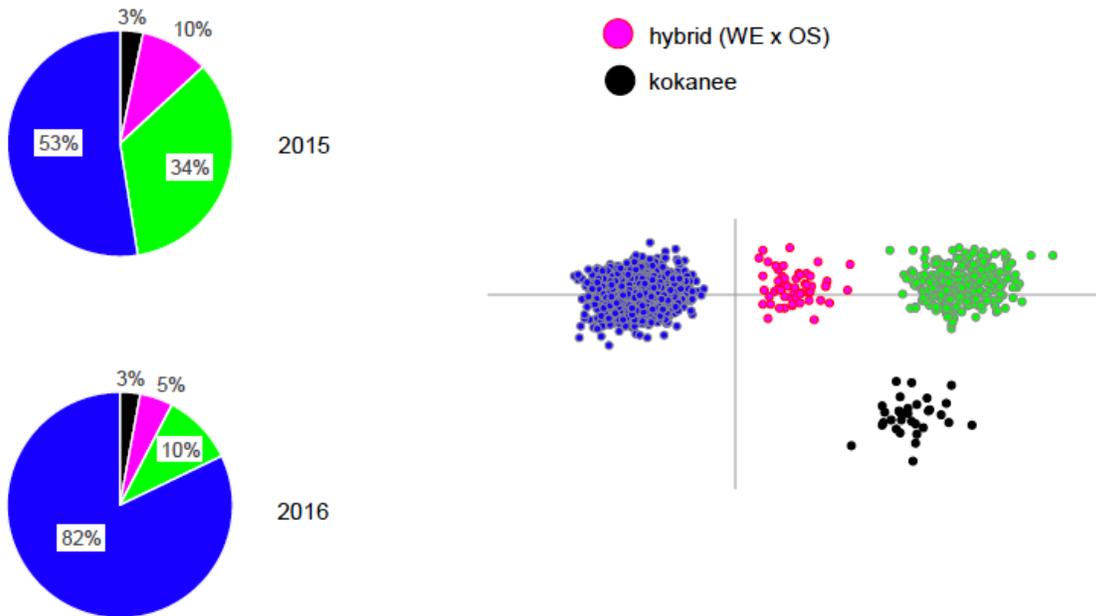
The samples were sent to CRITFC geneticists at the HFCEs where the DNA extracted and genotyped initially for a standardized suite of 94 SNP DNA markers. Sockeye Salmon of Wenatchee and Osoyoos stocks display distinctly different genetic profiles for this panel of markers (Winans et al. 1996; Campbell 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. In 2016, the SNP panel was expanded to 364 loci employing RAD sequencing (Monitoring Methods #4144, owner: Nathan Campbell) and the GT-seq protocol (Campbell et al. 2015). The larger number of SNP markers substantially increases the statistical confidence for GSI assignments and the power to perform parentage analyses. Since 2016, all samples are being genotyped with this expanded panel, and DNA previously genotyped for adults that returned to Roza in 2013-2015 were re-genotyped with the expanded panel.

As observed in previous years, both temporal and spatial differences in spawning were observed among the two stocks in 2016. Post-spawned carcasses of Wenatchee stock fish 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 in mid-October was followed by an increase of fish which were 96% Osoyoos origin, the large majority of which were found in section 3c of Reach 3. This section is an area of exposed lake bed characterized by side channels fed by hyporheic flow. The gravel in section 3c is smaller and more uniform in size compared to the larger cobble and rock typical of the mainstem in the upper reaches of the Cle Elum River. Concentration of fish in Reach 3c could be a response to higher flows associated with rain events which typically begin in October, that render the mainstem reaches

more difficult to access. However, it may also indicate a preference of Osoyoos fish for a “shore spawning” life history. It is also notable that gill netting in mid to late October to remove predatory lake trout incidentally captured a number of Sockeye Salmon which were predominantly Osoyoos stock. Among the 125 sampled bycatch in 2014 and 2016, GSI analysis identified 6 of the *O. nerka* as kokanee, while the remaining 119 were all identified as Osoyoos stock Sockeye Salmon; none were Wenatchee stock. Additionally, many of the Osoyoos bycatch fish were flowing milt or eggs, and thus appear to have been actively spawning in the lake.



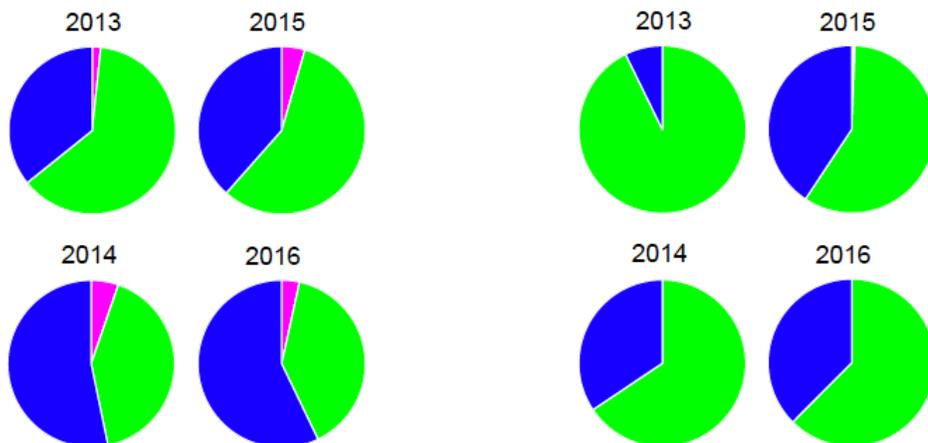
Beginning in 2011, out-migrating NOR *O. nerka* smolts have been observed in juvenile bypass facilities at Roza Dam and at the Chandler smolt collection facility. GSI analyses of smolts sampled at the Chandler facility assigned the large majority of these fish as being solely of either Wenatchee or Osoyoos stock origin; the incidence of inter-stock hybrids has been low (only 5-10% in 2015 and 2016). We presume the spatial and temporal differences in spawn timing to be responsible for this high level of reproductive isolation between stocks.



GSI analyses indicate that proportions of Osoyoos and Wenatchee stock adults captured at PRD for translocation to Cle Elum Lake (2013 to 2016; see below) have averaged approximately 75% and 25%, respectively. In contrast, the stock proportions among the out-migrating juveniles (see above) and for returning adults (see below) exhibit relatively higher proportions of Wenatchee stock fish, apparently inferring greater productivity of Wenatchee stock adults in Cle Elum Lake.

Roza returns:
2 - 5% hybrid proportion

PRD outplants:
NO hybrids observed



However, estimating relative productivity between stocks has been difficult due to an inability to reliably determine ages for Cle Elum natural-origin progeny (juvenile or adult life stages) in order to assign them

to brood year. The recent expansion of the SNP panel, will provide significantly greater accuracy in genetic parentage analyses, and we will be increasingly able to determine age of a portion of the NOR progeny. These age and parentage data will facilitate our ability to estimate relative productivity between stocks, within both translocated fish (PRD) and natural-origin fish (RAMF) returning to spawn in the Cle Elum River system.

The Project will continue to support genetic analyses for the reintroduction program, with the focus shifting to: 1) continued monitoring for inter-stock hybrids, 2) assessment of differences in the age and size at out-migration of smolts between stocks, and 3) assessment of relative productivity of new Cle Elum origin adults relative to PRD translocated fish, within and between stocks. 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.

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 it 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 population (and populations in Suttle Lake and other central Oregon lakes and reservoirs) more likely developed from repeated stocking of 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, would also exhibit a return migration behavior as mature adults that is characteristic of an anadromous Sockeye Salmon life history. A new fish transfer facility (FTF) at Round Butte Dam became operational in 2010 to fulfill the relicensing agreement. 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 an adult trap at Pelton 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 few fish identified as strays from out-of-basin sockeye 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	0
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	15	0	42	33,515

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*. Tissue samples were collected from Suttle Lake adults and out-migrating juveniles captured in Lake Creek, from LBC adults and out-migrating juveniles captured at the FTF, and from in-migrating adults captured at the Pelton adult trap. 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. Each year DNA is extracted from the samples and GSI analyses performed to assign the returning adult to their respective 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, structure 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 between LBC and Suttle Lake.

Origin	n	self-assignment			next likely assignment	
		n	%	mean likelihood	ID	%n
<u>Sockeye Salmon</u>						
Osoyoos Lake	185	185	1.00	100	---	---
Lake Wenatchee	185	185	1.00	100	---	---
Redfish Lake	81	81	1.00	100	---	---
<u>Kokanee</u>						
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 2015 (total N = 200), indicated that approximately 80% of the fish assigned with the highest likelihood to LBC, with an additional 8% assigning to Suttle Lake or other Deschutes basin lakes. GSI results for the dramatically large return in 2016 were similar (see table below). Of 524 successfully genotyped fish from 2016, greater than 91% assigned to the LBC population, with an additional 4% that assigned to Suttle Lake, or another Deschutes basin lake (see table below). For each individual the top three assigned origins (reference populations) were ranked with a corresponding likelihood score (i.e. first ranked assignment = highest likelihood). If consecutively ranked assignments were from the Deschutes River (e.g., rank1 – LBC, rank2 - Suttle Lake, rank3 - Upper Deschutes) the likelihood scores were summed to indicate a total “in-basin” assignment score.

2016 Pelton Adults			Number Assigned	% Total (n=524)	mean score	
Assigned Population	Code	Source/Origin			1st rank	in-basin
Lake Billy Chinook	LBC	kokanee smolt	484	0.91	94.3	97.2
Suttle Lake	SL	kokanee smolt	4	0.01	56.2	75.8
Upper Deschutes	UD	stocked kokanee	18	0.03	66.3	90.9
Wallowa Lake	WL	stocked kokanee	10	0.02	77.9	---
Redfish Lake	RL	stray sockeye	4	0.01	100	---
Wenatchee	WEN	stray sockeye	2	<0.01	81.2	---
Okanogan	OK	stray sockeye	2	<0.01	84.7	---

Scale analysis was also conducted on samples collected from 434 of the 2016 adults. Among these were 423 that assigned to the Deschutes basin, for which scale analysis indicated the following age structure: 14% age 3, 86% age 4 and 1% age 5. These proportions are not dissimilar to those observed for Osoyoos Lake Sockeye Salmon (Wenatchee Lake adults include essentially no age 3 fish, and relatively more age 5 fish). Unexpectedly, sex ratio was highly skewed (2:1) towards females (67% female and 33% males); sex ratio of Osoyoos Lake Sockeye Salmon interrogated at Wells Dam is estimated to be approximately 1:1, if not somewhat in favor of males.

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, although in 2017 returns numbered only 57. The 2016 return, as it was made up primarily of age 4 fish (BY2012), and therefore largely derived from the high 155,031 smolt release from Round Butte Dam in 2014. Additionally, there was a record

high return of Sockeye Salmon to the Columbia Basin in 2016 (N= 614,179 at Bonneville Dam), indicative of good ocean conditions for BY2012 Sockeye Salmon. The low adult return to the Deschutes River in 2017 was likely a reflection of the relatively low juvenile release number in 2015 (N= 38,702), as well as the low adult Sockeye Salmon return to the Columbia River in general (N=87,693 at Bonneville Dam). Discussion is ongoing among co-managers of the Deschutes Sockeye Salmon reintroduction programs 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 effect of hatchery broodstock age on 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. Maturation in male juvenile spring Chinook Salmon males can 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 are believed to be very low, e.g., less than 5% for minijacks (Larsen et al. 2013), and reproductive success of these small young males is also thought to also be low (e.g., Schroder et al. 2010 and 2011). In hatchery reared stocks, however, rates of precocious maturation can be dramatically elevated, with rates as high as 80% having been observed. Research conducted on Yakima River spring Chinook Salmon in the supplementation program operated at the CESRF indicates minijack rates average approximately 40% (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).

The 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 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).

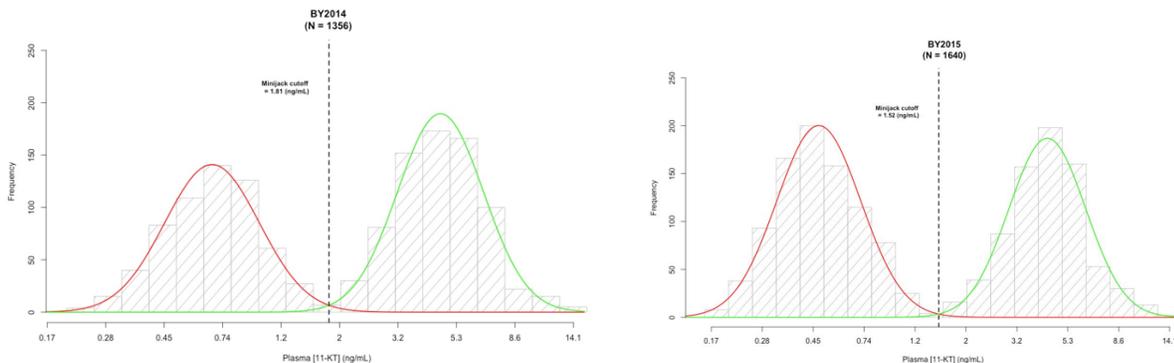
To assess the extent to which age of hatchery broodstock might have a genetics-based effect on the rate of precocial minijack production, we designed a study in which gametes from broodstock of known ages would be subdivided and factorially crossed to produce matings of all possible parental age combinations. An agreement was reached with YN in 2014 to perform this study at the CESRF for three BYs, beginning in fall 2014. Over four to five consecutive weeks in September 2014, 2015 and 2016, samples of gametes from a subset of the CESRF broodfish were factorially crossed with respect to broodfish age. The initial 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, only three age 5 males were available in 2014, and no age 5 fish were available in 2015 and 2016. As such, analyses of the data were limited to differences in minijack rate associated with sire age in crosses to age 4 females. In 2015 and 2016, we also incorporated age 1 microjack males 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 within each mating), samples of 50 swim-up fry per cross were pooled into a raceway for rearing to the smolt stage (age 1+). The fish were fed at rates similar to standard CESRF practices. In April at age 1+, the smolts from BYs 2014 and 2015

were sacrificed, dissected and identified to phenotypic sex, and the males were measured (length and weight), and blood and tissue sampled. (Juveniles from brood year 2016 juveniles will be sacrificed, measured and sampled in April 2018.) A biochemical assay of 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). 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.

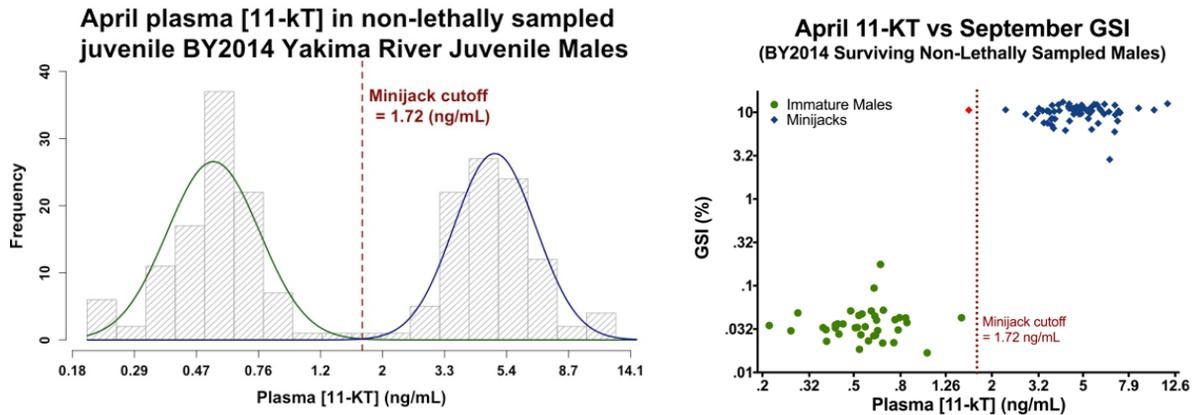
In 2016 we performed an additional series of seven factorial crosses each involving a single age 4 supplementation hatchery origin (SH) female crossed to three different age 4 males – one each of natural origin (WN), supplementation hatchery origin (SH) and hatchery control line origin (HC). The rationale to add these crosses to the study design stemmed from observations of Harstad et al. (2014) in a review of minijack rates across several regional Chinook Salmon hatchery program. Their data indicated that segregated programs (in which 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). Segregated programs totally exclude contribution from microjack and minijack males each generation, whereas NOR broodfish in integrated programs are susceptible of having precocial males in their (grand)parentage. Presuming that male maturation as minijacks has a heritable genetic component, Harstad et al. (2014) hypothesized that segregated programs select against this trait. 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.

In both 2014 and 2015 BYs, the frequency distributions for 11-KT as measured in April were significantly bimodal, with similar cutoff values between low and high 11-KT for the purposes of identifying the juveniles as maturing versus non-maturing: 2014 = 1.81 ng/mL and 2015 = 1.52 ng/mL.



Numerous studies have demonstrated that Chinook Salmon juveniles separate into two groups relative to springtime 11-KT (Larsen et al. 2004, 2006, 2010, 2013 and 2014; Harstad et al. 2014). However, though it seemed unlikely that it would occur at a substantial rate, it remained unproven that a male fish with high springtime 11-KT level, couldn't arrest the maturation process and revert to an immature status by the coming spawning season. It is also conceivable that an individual with low springtime 11-KT might belatedly initiate maturation and develop into a mature minijack by the subsequent spawning

season. Therefore, prior to characterizing the study smolts as maturing minijacks versus non-maturing smolts based on springtime 11-KT analyses, we non-lethally sampled a group of brood year individually tagged 2014 smolts and reared them until the following spawning season, to confirm their maturation status. Among the 103 surviving males, 102 of them were correctly identified based on their April 11-KT measure (see figures below).

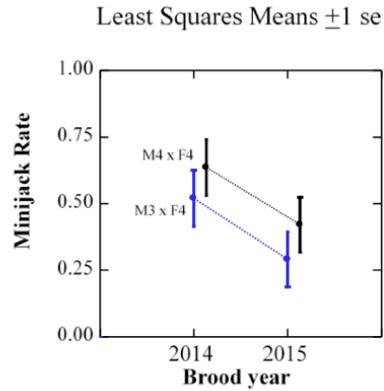
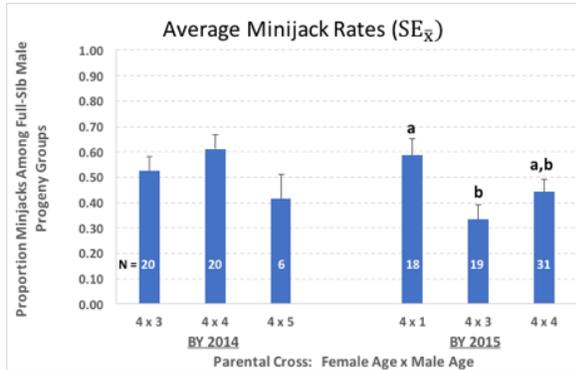


With confirmation of the reliability of the April 11-KT measures to characterize maturation status, we proceeded to calculate minijack rates within male full siblings for BYs 2014 and 2015, as identified via genetic parentage analyses:

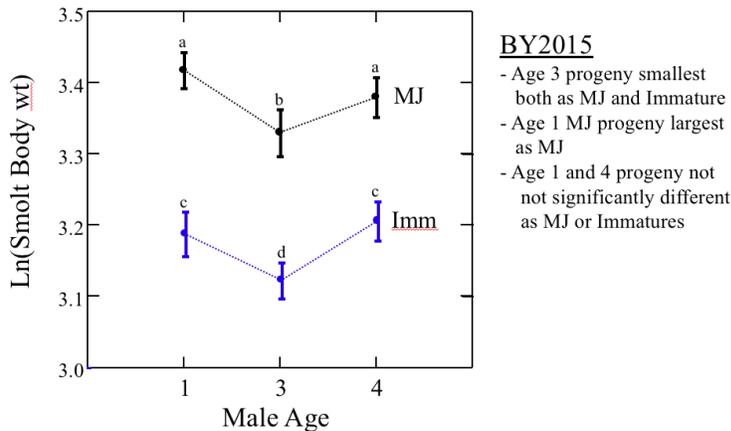
	BY 2014 minijack rates			BY 2015 minijack rates			
	4x3	4x4	4x5	4x1	4x3	4x4	
	0.13	0.17	0.04	0.00	0.00	0.00	0.52
	0.18	0.17	0.25	0.19	0.04	0.04	0.53
	0.22	0.23	0.41	0.26	0.05	0.05	0.55
	0.25	0.27	0.55	0.39	0.08	0.06	0.56
	0.30	0.45	0.56	0.48	0.13	0.14	0.61
	0.32	0.53	0.68	0.48	0.15	0.17	0.64
	0.38	0.57		0.48	0.19	0.17	0.65
	0.44	0.59		0.55	0.23	0.18	0.73
	0.50	0.62		0.55	0.32	0.25	0.74
	0.52	0.64		0.56	0.35	0.27	0.78
	0.58	0.65		0.60	0.35	0.27	0.84
	0.60	0.68		0.70	0.40	0.29	0.85
	0.60	0.69		0.83	0.41	0.31	0.85
	0.61	0.75		0.87	0.43	0.36	1.00
	0.67	0.76		0.88	0.44	0.40	
	0.77	0.79		0.91	0.57	0.43	
	0.79	0.86		0.91	0.70	0.50	
	0.81	0.91		0.95	0.71		
	0.85	0.94			0.80		
	1.00	0.95					
Average	0.50	0.59	0.42	0.59	0.33	0.44	
StDev	0.25	0.25	0.23	0.27	0.24	0.28	
Number	20	20	6	18	19	31	

When compared within BYs, differences in minijack rate among cross-types were not significant in 2014. (Note: proportion data were arsine-square root transformed prior to analysis.) However, significant differences were apparent in 2015. As hypothesized, microjack males producing the highest proportion of minijacks when crossed with age 4 females (4x1). Additionally, paired data for age 4 females crossed to an age 3 jack (4x3) and an age 4 male (4x4), the two most common cross-types, when compared for

the two BYs combined in a two-way ANOVA, indicated differences to be significant. However, the differences were in the opposite direction relative to what was hypothesized: the jack sires produced fewer, not more, minijacks than the older age 4 sires.



In addition to the unexpected lower minijack rate for jack sires was the very high variation in minijack rate among families within cross-types. ANOVAs performed for cross-type within BYs indicated significant differences for each (with the exception of 2014 4x4 crosses), indicative of strong individual effects among sires. While it is unclear what specific characteristics of the fish are responsible, they are no doubt associated with factors affecting growth rate. When size (body weight) measured in April was compared among brood year 2015 progeny for the different cross-types, progeny of jack sires were the smallest, both among maturing minijacks and among the immature juveniles – in keeping with their lower minijack rate, as illustrated below.



- BY2015
- Age 3 progeny smallest both as MJ and Immature
 - Age 1 MJ progeny largest as MJ
 - Age 1 and 4 progeny not significantly different as MJ or Immatures

Smolts from brood year 2016 will be sampled April 2018, with the 11-KT and genetic parentage analyses to be performed in the summer. After the brood year 2016 data is available, it will be combined with that for 2014 and 2015, for summary analysis. Drafting of a final report and a manuscript for submission to a scientific journal will follow.

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

A primary focus of the Project activities described above is on 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 supplementation can be managed to minimize any negative effects, is the need to assess whether a supplementation program is capable of rebuilding and maintaining abundance and productivity of a natural population, while also providing fish for some level of in-basin harvest. Dramatic increases in abundance of a few supplemented populations have been achieved, however, in many other cases the increase has been relatively small, resulting in criticism of supplementation as a “restoration” action. Environmental constraints to use of supplementation to rebuild populations were highlighted in the recent ISAB report, “Density Dependence and Its Implications for Fish Management and Restoration in the Columbia River Basin” (ISAB 2015).

Recently, Ian Courter, Mount Hood Environmental LLC, provided a presentation to CRITFC of results from a re-evaluation of a time-series of abundance data for native winter-run steelhead in the Clackamas River relative to data for out-of-basin summer-run steelhead from a hatchery program designed to augment the sport fishery. The hatchery program was initiated in 1970, but terminated in 1997, as it was felt that addition of the summer run fish was having a depressant effect on productivity of the native steelhead. An analysis by Kostow and Zhou (2006) that included data from 6 BYs following cessation of the hatchery program reported this to have been the case, with competition for spawning and juvenile rearing resources as the primary cause for reduced productivity of the winter-run stock. This study is widely cited as providing evidence that hatchery programs in general will have a negative effect of on productivity of native populations. However, Mr. Courter questioned various aspects of the Kostow and Zhou analysis, and with funding from Portland General Electric and the North Coast Salmon & Steelhead Enhancement Fund initiated a re-examination of the data used by Kostow and Zhou (2006).

In contrast with Kostow and Zhou (2006), Mr. Courter’s 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 variation in the Clackamas population correlated well with fluctuations of other winter-run populations, and that variation in productivity of the Clackamas winter-run steelhead was a response to regional (in particular ocean) environmental effects.

Mr. Courter explained, however, that the analytical model he used required refinement, and he has sought out input from biometricians with related expertise. In 2017, Mr. Courter was contracted by CRITFC under the Project to pursue re-examination of the Clackamas data set, in collaboration with Robert Lessard, CRITFC biometrician with expertise in life cycle modeling. Refinement of the analytical models is near finalization, and a report on the study is in draft form. The analysis and report will be finalized in 2018, as will be a manuscript for submission to a scientific journal.

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.

In coordination with CRITFC geneticists Andrew Matala, Jeff Stephenson and Ilana Koch, another 2-day “Introduction to Molecular Genetics for Tribal Fisheries Management” training program was held in 2017 (December 12-13). Participants in this workshop included 13 tribal biologists and technicians from the four tribes: CTWSRO (3), CTUIR (3), YN (6), and NPT (1). Since 2011 we have conducted a total of 13 workshops with 111 participants, distributed as follows: CTWSRO (18), CTUIR (25), YN (27), NPT (23), and CRITFC (15). Copies of the December 2017 workshop agenda and PowerPoint presentations are available at: <http://www.critfc.org/fish-and-watersheds/fishery-science/hagerman-genetics-laboratory/genetics-training/>.

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, Pierce, Medeiros and Koch) participated in various inter-tribal and inter-agency meetings, workshops and symposia in 2017, 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 hatcheries 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: Attendance and oral presentation at the Sockeye Salmon Enhancement Workshop, Harrison Hot Springs BC ("Genetic monitoring of Sockeye Salmon reintroductions: Informed opportunities and adaptive management", A. P. Matala, S. R. Narum, P. F. Galbreath, B. Saluskin, M. Johnston, and J. Hogle).

Feb 28-Mar 3: Attendance and oral presentation at the 53rd Annual Meeting of the Oregon Chapter of the American Fisheries Society, Bend OR ("Genetic monitoring of Sockeye Salmon reintroductions: Informed opportunities and adaptive management", A. P. Matala, S. R. Narum, P. F. Galbreath, B. Saluskin, M. Johnston, and J. Hogle).

May 16-17: Attendance at the Snake River Fall Chinook Salmon Symposium, Clarkston WA.

May 22-25: Attendance and oral presentation at the annual meeting of the Western Division of the American Fisheries Society, Missoula MT ("Evaluating minijack rates in spring Chinook: Comparing minijack rates based on spring plasma 11-ketotestosterone levels with rates based on fall gonadosomatic index" L. R. Medeiros, A. L. Pierce, C. M. Knudsen, C. A. Stockton, and P. F. Galbreath).

June 14-15: Attendance and oral presentation at the annual Yakima Basin Science & Management Conference, Ellensburg WA ("Stuff about genetic monitoring of Sockeye Salmon in Cle Elum Reservoir" A. P. Matala, S. R. Narum, P. F. Galbreath, B. Saluskin, and M. Johnston), and ("Initial relative reproductive success results for brood year 2007 Yakima River spring Chinook Salmon supplementation program" I. J. Koch).

June 27-28: Attendance and oral presentation at the 2017 Pelton Round Butte Project Fisheries Workshop ("Fate and Origin: Genetic monitoring of *O. nerka* in the Deschutes River Basin", A. P. Matala, S. R. Narum, P. F. Galbreath, and J. Hogle).

December 6-8: Attendance and oral presentation at the 68th Annual Northwest Fish Culture Conference, Redding, CA Dec 5-7, 2017. ("Effect of Broodstock Age on Precocial Maturation of Male Chinook Salmon Smolts – Minijacks" P. F. Galbreath, C. M. Knudsen, C. A. Stockton, L. R. Medeiros, A. L. Pierce, and I. J. Koch).

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

Medeiros, L. R., P. F. Galbreath, C. M. Knudsen, C. A. Stockton, I. J. Koch, W. J. Bosch, S. R. Narum, J. J. Nagler, and A. L. Pierce. *In preparation*. Plasma 11-ketotestosterone in individual age 1+ spring Chinook Salmon juvenile males accurately predicts age 2 maturation status. Transactions of the American Fisheries Society.

Courter, I. I., G. Wyatt, R. Perry, J. Plumb, N. Ackerman, P. Galbreath, and R. Lessard. *In preparation*. Retrospective analysis of a natural-origin steelhead population's response to exclusion of hatchery fish. Transactions of the American Fisheries Society.

IV. Synthesis of Findings: Discussion/Conclusions

Project activities are centered on the theme of supplementation hatchery RM&E, with particular focus on assessing effects of hatchery rearing on life history and natural productivity of supplementation HOR fish relative to NOR fish.

Two Project efforts involve NOR vs HOR RRS studies of tribal programs to supplement depressed natural spring Chinook Salmon populations in Johnson Creek and in the upper Yakima River. Results for these studies are of particular interest, as they are the only two supplementation programs within the Columbia basin that apply the recommendation for fully integrated broodstock management - broodstock is 100% NOR. Maximizing integration of NOR fish in the broodstock was among the recommendations made on behalf of the tribes by Cuenco et al. (1993), a manuscript which provided the first detailed description of the principles for this new management approach to hatchery production - supplementation. By not “recycling” HOR fish into the broodstock in successive generations (segregated broodstock management), integrated management provides natural selective forces the opportunity to reverse any genetically-based domestication effects associated with hatchery rearing - effects that under segregated management could accrue and progressively reduce fitness of a hatchery stock. Results from the Johnson Creek spring Chinook Salmon supplementation program indicate that NOR and HOR fish that successfully contributed adult progeny to the next generation had similar productivity. Moreover, productivity for HOR x NOR matings was similar to that for NOR x NOR matings, suggesting a lack of a significant negative fitness impact on the NOR population. Project funding is being used to perform additional genotyping of juvenile progeny from a series of 6 BYs (2009 to 2015). Given the 3-4 fold greater number of juvenile progeny relative to adult progeny per brood year, the juvenile recruits-per-spawner RRS analysis will have increased power to test not only for effects of origin, but also for interacting effects of parent sex, age, size and return time to the Johnson Creek weir. As adult progeny from the latter of these BYs return (complete as of 2019), RRS analyses will be conducted based on adult recruits-per-spawner and compared to those for juvenile recruits within corresponding BYs.

The second RRS study, initiated in 2014, involves five BYs (2007-2011) of the spring Chinook Salmon supplementation program in the upper Yakima River. The genetics analyses are being performed collaboratively by CRITFC (with Project funding) and WDFW (with YKFP funding). This study involves a number of samples that is several fold greater than the Johnson Creek adult-to-adult study, and thus should provide an even stronger assessment of the supplementation approach. Results for a preliminary analysis of data from the initial brood year (2007) resemble those observed in Johnson Creek – generally similar productivity of NOR and HOR adults. Genotyping continues and should be completed for fish involved in all five BYs in 2018. Parentage and RRS analyses will follow, involving tests for effects of origin within sexes, as well as for interacting effects of parent age, size and return time, and with summarization in a final report and manuscript for submission to a scientific journal in 2019.

The Project also supports RRS studies of a reintroduced population of spring Chinook Salmon in Lookingglass Creek (Project Objective #2). Reintroduction programs typically begin with stocking of fish from out-of-basin hatcheries. These out-of-basin fish are anticipated to initially exhibit a decreased level of natural productivity relative to the extirpated indigenous stock, due to habitat differences relative to the natal river system of the hatchery stock, and to negative genetic (domestication) effects that may have accrued in the stock over repeated generations of hatchery rearing. As adults return in-basin following the initial years of reintroduction, the programs are designed to transition to use these

returning adults as broodstock, and eventually to incorporate increasing proportions of NOR fish into the broodstock. Presuming that the source stock for a reintroduction program retains the genotypic and phenotypic potential to respond to supplementation management strategies and to natural selective forces, it is expected that over successive generations the natural populations created will progressively adapt to the new environment. RRS analyses in these studies are therefore calculated in an inverse manner to that for studies of supplemented native populations (which compare average productivity of HOR fish standardized against NOR: $RRS = \text{HOR}/\text{NOR}$). Adaptation of reintroduced fish in these programs would be assessed by NOR productivity standardized against HOR within BYs: $RRS = \text{NOR}/\text{HOR}$, with RRS ratios that are greater than 1.0 indicative of increased productivity of fish which have spent a generation of more within the natural environment relative to fish that are the direct product of hatchery rearing.

Spring Chinook Salmon were reintroduced upstream of the hatchery weir in Lookingglass Creek in 2004, via passage of returning HOR adults from a new Catherine Creek hatchery stock (a nearby Grande Ronde River tributary). Since that time, returning HOR fish of this stock, plus NOR adults beginning with age 3 fish in 2007 have been passed upstream to reestablish a new Lookingglass Creek spring Chinook Salmon population. Beginning in 2004, tissue samples were collected from all adults intercepted at the weir, as well as samples from out-migrating juveniles since 2007. Genotyping at HFCES and initial parentage analyses based on juvenile recruits-per-spawner were completed for BYs 2008 through 2014. Results indicate that natural productivity of NOR females and males was on average greater than for HOR fish of the reintroduced hatchery stock, the difference being statistically significant for males though not for females. These observations concord with the expectation that natural selective forces would act on the returning HOR adults and their NOR progeny, resulting in improved productivity over the source hatchery stock. A final collection of out-migrating smolts in spring 2018 will complete sampling for an additional two BYs (2015 and 2016). Genotyping for these samples will be completed in 2018 and followed by parentage analyses and estimation of RRS. A full analysis for the combined BY2008-BY2016 dataset will be performed, with additional consideration of interacting effects of parent sex, age and size.

The Project is supporting genetics studies associated with two other reintroduction projects, both involving Sockeye Salmon (Project Objective #3). The YN program for translocating Sockeye Salmon adults collected at PRD (approximately $\frac{3}{4}$ Okanogan River and $\frac{1}{4}$ Wenatchee River stock) into Cle Elum Lake began in 2009. Genetic analyses of post-spawned carcasses indicate very strong temporal and spatial segregation for spawning between stocks – Wenatchee fish spawn early in the season and upstream in the Cle Elum River above the lake, while Okanogan fish spawn later in the season and almost exclusively in areas near the head of the lake. GSI analyses of out-migrating juveniles indicates <10% inter-stock hybrids, confirming relatively strong reproductive isolation between stocks. Comparison of stock proportions among out-migrating juveniles with those of the PRD plus Yakima river returning adults, appear to indicate higher productivity of Wenatchee stock fish. However, annual variation in these proportions and differences between stocks in age of their respective progeny (smolts and returning adults) confounds this comparison. The Project will continue to finance genetic analyses, with focus turning to a) use of parentage analyses to better quantify smolt age structure within stocks, b) to assess relative productivity of PRD versus Yakima River returns within stocks, and c) to monitor the frequency of inter-stock hybrids and determine whether the new Cle Elum population begins evolving a genetically homogenized stock unique to Cle Elum Lake.

The second study is associated with a CTWSRO/ODFW/PGE program to create a new run of Sockeye Salmon in the Deschutes River. The program was initiated in 2009 following construction of a facility at Round Butte Dam that permits downstream passage of out-migrating LBC juvenile kokanee. GSI

analyses performed on the adults returns from 2010 through 2016 assigned >90% of these fish to LBC with an additional few percent assigning to Suttle Lake or other Upper Deschutes populations. However, with the exception of 2016 when 536 adults were collected in the Pelton adult trap. Returns have been disappointingly low, ranging from 10 to 98. CRITFC has committed to continue use of Project funds to support GSI analyses of the fish returning to the Deschutes River.

Work on a three brood year (2014-2016) study to assess the effect of parent age on the rate of minijack production in spring Chinook Salmon produced with NOR broodstock at the CESRF is ongoing. In concordance with our hypothesis that minijack rate will decline as parent age increases, results for BYs 2014 and 2015 indicate that the highest proportion of minijacks were observed in the progeny of age 1 microjack males. However, contrary to our hypothesis, age 3 jack parents produced fewer minijacks than older age 4 males. Additionally, variation in minijack rate among families within parental age cross-types was unexpectedly high, ranging in one instance from 0% to 100%. Results for BY 2016 will be obtained in 2018, and a full analysis and report on the study should be ready by the end of the year.

In 2017, Ian Courter, Mount Hood Environmental, was sub-contracted to lead a study to re-evaluate the effects that a summer-run steelhead hatchery augmentation program in the Clackamas river had on productivity of the native winter-run population. Kostow and Zhou (2006) reported that competition with hatchery stock fish for spawning and juvenile rearing habitat significantly diminished productivity of the winter-run population. However, an initial re-evaluation by Courter of the dataset, which included additional post-hatchery BYs, implicated instead out-of-basin (ocean effects) as the driver for variation in winter-run productivity. Robert Lessard, CRITFC biometrician with expertise in life cycle modeling, worked with Courter to refine the analytical models and analyses. These analyses will be finalized in 2018, and a report and manuscript for submission to a scientific journal will follow.

In 2017, the Project funded another 2-day "Introduction to Molecular Genetics for Tribal Fisheries Management" workshops involving 13 tribal biologists and technicians. This brings the total number of workshops supported by the Project to 13 and the number of participants to 111. The agenda and presentations from the most recent workshop are available at: <http://www.critfc.org/fish-and-watersheds/fishery-science/hagerman-genetics-laboratory/genetics-training/>.

In 2015 the Project financed attendance and oral presentations by CRITFC personnel at several different inter-agency meetings that included discussion of hatchery management approaches associated with management of regional fisheries (Project Objective #5).

Studies financed by the Project are at different stages of analysis. As each study is brought to completion, results will be summarized in a technical report, and several will be further developed into manuscripts for submission for publication in peer-reviewed scientific journals (Project Objective #7).

V. References

- Ad Hoc Supplementation Monitoring and Evaluation Workgroup (AHSWG). 2008. Recommendations for broad scale monitoring to evaluate the effects of hatchery supplementation on the fitness of natural salmon and steelhead populations. Final Draft Report of the Ad Hoc Supplementation Monitoring and Evaluation Workgroup. (<http://www.cbfwa.org/csmep/web/content.cfm?ContextID=11>)
- Boe, S. J., C. A. Crump, R. L. Weldert, and J. Wolf. 2010. Reintroduction of spring Chinook Salmon in Lookingglass Creek: Analysis of three stocks over time. (<http://www.fws.gov/lsnakecomplan/Meetings/2010SpringChinookHatcheryReviewSymposium.html>)
- Boe, S. J., C. A. Crump, R. L. Weldert, and J. Wolf. 2011. Lower Snake River Compensation Plan Confederated Tribes of the Umatilla Indian Reservation Evaluation Studies for 1 January 2008 to 31 December 2008. Confederated Tribes of the Umatilla Indian Reservation, La Grande OR. (<http://www.fws.gov/lsnakecomplan/Reports/CTUIR/2008%20LSRCP%20Annual.pdf>)
- Bosch, W. J., T. H. Newsome, J. L. Dunnigan, J. D. Hubble, D. Neeley, D. T. Lind, D. E. Fast, L. L. Lamebull, and J. W. Blodgett. 2007. Evaluating the feasibility of reestablishing a coho salmon population in the Yakima River, Washington. *North American Journal of Fisheries Management* 27:198-214.
- Bowles, E., and E. Leitzinger, 1991. Salmon Supplementation Studies in Idaho Rivers; Idaho Supplementation Studies", 1991 Technical Report, Project No. 198909800, 204 electronic pages,(BPA Report DOE/BP-01466-1) (<http://pisces.bpa.gov/release/documents/documentviewer.aspx?pub=A01466-1.pdf>)
- Bureau of Reclamation (BOR). 2007. Coho Salmon Production Potential in the Cle Elum River Basin, Storage Dam Fish Passage Study, Yakima Project, Washington, Technical Series No. PN-YDFP-007, Bureau of Reclamation, Boise, Idaho, March 2007.
- Burck, W. A. 1994. Life history of spring Chinook Salmon in Lookingglass Creek, Oregon. Information Reports Number 94-1. Oregon Department of Fish and Wildlife, Fish Division. Portland, Oregon.
- Campbell, N., and S. R. Narum. 2011. Development of 54 novel single-nucleotide polymorphism (SNP) assays for Sockeye Salmon and coho salmon and assessment of available SNPs to differentiate stocks within the Columbia River. *Molecular Ecology Resources* 11(Suppl. 1): 20–30.
- Campbell, N., S. A. Harmon and S. R. Narum. 2015. Genotyping-in-Thousands by sequencing (GT-seq): A cost effective SNP genotyping method based on custom amplicon sequencing. *Molecular Ecology Resources*. 15:855-867.
- Columbia Basin Fish Accords. 2008. Memorandum of Agreement between the Three Treaty Tribes (Confederated Tribes of the Umatilla Reservation, Confederated Tribes of the Warm Springs Reservation of Oregon, Yakama Nation, and Columbia River Inter-Tribal Fish Commission) and FCRPS Action Agencies (Bonneville Power Administration, U.S. Army Corps of Engineers, and U.S. Bureau of Reclamation). Signed May 2, 2008. (<http://www.critfc.org/cbp/moa.html>)
- Fast, D.E., C.M. Knudsen, W.J. Bosch, A.L. Fritts, G.M. Temple, M.V. Johnston, T. N. Pearsons, D.A. Larsen, A.H. Dittman, D. May, and C.R. Strom. 2015. A Synthesis of Findings from an Integrated Hatchery Program after Three Generations of Spawning in the Natural Environment. *North American Journal of Aquaculture* 77:377–395.

- Fraser, D. J. 2008. How well can captive breeding programs conserve biodiversity? A review of salmonids. *Evolutionary Applications* 1: 2009-009-00535-586.
- Fulton, L. A. 1968. Spawning areas and abundance of Chinook Salmon, *Oncorhynchus tshawytscha*, in the Columbia River Basin--Past and present. U.S. Fish and Wildlife Service, Special scientific report, fisheries (U.S. Bureau of Commercial Fisheries) vol. no. 571.
- Galbreath, P. F. M. A. Bisbee Jr., D. W. Dompier, C. M. Kamphaus, and T. H. Newsome. 2014. Extirpation and Tribal Reintroduction of Coho Salmon to the Interior Columbia River Basin. *Fisheries* 39(2):77-87.
- Galbreath, Peter F., I. J. Koch, S. R. Narum, K. I. Warheit, T. R. Seamons, T. W. Kassler, D. E. Fast, W. J. Bosch, M. V. Johnston, and C. R. Strom. 2017. Relative reproductive success of supplemented upper Yakima River spring Chinook Salmon— Preliminary report for brood year 2007. CRITFC Technical Report 17-08.
- Gustafson, R. G., T. C. Wainwright, G. A. Winans, F. W. Waknitz, L. T. Parker, and R. S. Waples. 1997. Status review of Sockeye Salmon from Washington and Oregon. U. S. Department of Commerce, NOAA Technical Memorandum NMFS-NWFSC-33, 282 p. (http://www.nwfsc.noaa.gov/assets/25/4242_06172004_120234_sockeye.pdf)
- Harstad, D. L., D. A. Larsen, and B. R. Beckman. 2014. Variation in minijack rate among hatchery populations of Columbia River basin Chinook Salmon. *Transactions of the American Fisheries Society* 143:768-778.
- Hess, M. A., C. D. Rabe, J. L. Vogel, J. J. Stephenson, D. D. Nelson, and S. R. Narum. 2012. Supportive breeding boosts natural population abundance with minimal negative impacts on fitness of a wild population of Chinook Salmon. *Molecular Ecology* 21: 5236–5250.
- Hess, J. E., N. R. Campbell, A. P. Matala, D. J. Hasselman, S. R. Narum. 2015. Genetic assessment of Columbia River stocks, 4/1/2014-3/31/2015 Annual Report, BPA Project 2008-907-00.
- ISRP and ISAB. 2005. Monitoring and Evaluation of Supplementation Projects. ISRP&ISAB Report 2005-15. Northwest Power and Conservation Council, Portland, Oregon. (<http://www.nwcouncil.org/library/isrp/isrpisab2005-15.pdf>)
- ISAB. 2015. Density Dependence and Its Implications for Fish Management and Restoration in the Columbia River Basin. ISRP&ISAB Report 2015-1. Northwest Power and Conservation Council, Portland, Oregon. (<http://www.nwcouncil.org/fw/isab/isab2015-1/>)
- Larsen et al
- Larsen, D.A., B. R. Beckman, K. A. Cooper, D. Barrett, M. Johnston, P. Swanson, and W. W. Dickhoff. 2004. Assessment of high rates of precocious male maturation in a spring Chinook Salmon supplementation hatchery program. *Transactions of the American Fisheries Society* 133:98-120.
- Larsen, D.A., Beckman, B.R., Strom, C.R., Parkins, P.J., Cooper, K.A., Fast, D.E., and Dickhoff, W.W. 2006. Growth modulation alters the incidence of early male maturation and physiological development of hatchery reared spring Chinook Salmon: a comparison with wild fish. *Transactions of the American Fisheries Society* 135:1017-1032.

Larsen D. A., Brian R. Beckman & Kathleen A. Cooper (2010). Examining the conflict between smolting and precocious male maturation in spring (Stream-Type) Chinook Salmon. *Transactions of the American Fisheries Society* 139:564-578.

Larsen, D. A., D. Harstad, R. Strom, M. V. Johnston, C. M. Knudsen, D. E. Fast, T. N. Pearsons, and B. R. Beckman. 2013. Early life history variation in hatchery- and natural-origin spring Chinook Salmon in the Yakima River, Washington. *Transactions of the American Fisheries Society* 142(2): 540-555.

Larsen, D. A., B. R. Beckman, D. Spangenberg, P. Swanson, M. Middleton, J. Dickey, R. Gerstenberger, C. Brun and G. Young. 2014. Parkdale NOAA Comparative Hatchery Study, 2014 Annual report. Prepared for the Bonneville Power Administration, Project Number 1988-053-03, Contract Number 58847, Portland Oregon. (<https://pisces.bpa.gov/release/documents/DocumentViewer.aspx?doc=P141616>)

Lutch, J., J. Lockhart, C. Beasley, K. Steinhorst, and D. Venditti. 2005. An updated study design and statistical analysis of Idaho Supplementation Studies. Technical Report, Project No. 198909800, 101 electronic pages, (BPA Report DOE/BP-00020863-1). (<http://pisces.bpa.gov/release/documents/documentviewer.aspx?doc=00020863-1>, or <http://www.nezperce.org/~dfm/documents/ISS%20Study%20Design%20%20Final%20Statistical%20Analysis%20of%20ISS.pdf>)

Matala, A. P., J. E. Hess, and S. R. Narum. 2011. Resolving adaptive and demographic divergence among Chinook Salmon populations in the Columbia River Basin. *Transactions of the American Fisheries Society* 140:783-807.

McElhany, P., M. H. Ruckelshaus, M. J. Ford, T. C. Wainwright, and E. P. Bjorkstedt. 2000. Viable salmonid populations and the recovery of evolutionarily significant units. U.S. Department of Commerce, NOAA Technical Memorandum NMFS-NWFSC-42, 156 p. (<http://www.nwfsc.noaa.gov/publications/techmemos/tm42/tm42.pdf>)

Mullan, J. W. 1983. Overview of Artificial and Natural Propagation of Coho Salmon (*Oncorhynchus kisutch*) on the mid-Columbia River. Fisheries Assistance Office, U.S. Fish and Wildlife Service, Leavenworth, Washington. December 1983.

Murdoch, K., C. Kamphaus, S. Prevatte, and C. Strickwerda. 2006. Mid-Columbia coho reintroduction feasibility study", 2005-2006 Annual Report, Project No. 199604000, 107 electronic pages, (BPA Report DOE/BP-00022180-1). (<http://pisces.bpa.gov/release/documents/documentviewer.aspx?doc=00022180-1>)

Narum, S. R., W. D. Arnsberg, A. J. Talbot, and M. S. Powell. 2007. Reproductive isolation following reintroduction of Chinook Salmon with alternative life histories. *Conservation Genetics* 8:1123-1132.

Nehlson, W., J. E. Williams, and J. A. Lichatowich. 1991. Pacific salmon at the crossroads: stocks at risk from California, Oregon, Idaho and Washington. *Fisheries* 16:4-21.

Nehlson, W. 1995. Historical salmon and steelhead runs of the upper Deschutes River basin and their environments. Portland General Electric Company, Hydro Licensing Department, Portland OR.

Nielson, R. S. 1950. Survey of the Columbia River and its Tributaries. Part V. U.S. Fish and Wildlife Service Special Scientific Report: Fisheries No. 38.

- Olsen, E. A., P. M. P. Beamesderfer, M. L. McLean, and E. S. Tinus. 1994. Salmon and steelhead stock summaries for the Deschutes River Basin: An interim report. Oregon Department of Fish and Wildlife, Portland, 136 p.
- O'Toole, P., J. Newton, R. Carmichael, S. Cramer, and K. Kostow. 1991. Hood River Production Master Plan, Project No. 1988-05300, 102 electronic pages, (BPA Report DOE/BP-00631-1). (<http://pisces.bpa.gov/release/documents/documentviewer.aspx?doc=00631-1>)
- Phillips, J. L., J. Ory, and A. Talbot. 2000. Anadromous salmonid recovery in the Umatilla River basin, Oregon: A case study. *Journal of the American Water Resources Association* 36:1287-1308.
- Rabe, C. D., and D. D. Nelson. 2010. Status and monitoring of natural and supplemented Chinook Salmon in Johnson Creek, Idaho - Annual Progress Report: 2008 to 2009. Nez Perce Tribe Department of Fisheries Resources Management, McCall, ID. (<https://pisces.bpa.gov/release/documents/documentviewer.aspx?doc=P124099>)
- Schroder, S.L., C. M. Knudsen, T. N. Pearsons, T. W. Kassler, S. F. Young, E. P. Beall, and D. E. Fast. 2010. Behavior and breeding success of wild and first generation hatchery male spring Chinook Salmon spawning in an artificial stream. *Transactions of the American Fisheries Society* 139:989–1003.
- Schroder, S.L., C. M. Knudsen, T. N. Pearsons, T. W. Kassler, E. P. Beall, S. F. Young, and D. E. Fast. 2011. Breeding success of four male life history types of spring Chinook Salmon spawning in an artificial stream. *Environmental Biology of Fishes* 139:989–1003.
- Steele, C., McCane, J., Ackerman, M., Vu, N., M. Campbell, M. Hess, and S. Narum. 2016. Parentage based tagging of Snake River steelhead and Chinook Salmon, Annual Progress Report January 1, 2015 – December 31, 2015. Project Number 2010-031-00, Contract Number 65500. IDFG Report Number 16-02.
- Underwood, K., C. Chapman, N. Ackerman, K. Witty, S. Cramer, and M. Hughes. 2003. Hood River Production Program review', Project No. 1988-05314, 501 electronic pages, (BPA Report DOE/BP-00010153-1). (<http://pisces.bpa.gov/release/documents/documentviewer.aspx?doc=00010153-1>)
- Waples, R. S., P. B. Aebersold, and G. A. Winans. 2011 Population genetic structure and life history variability in *Oncorhynchus nerka* from the Snake River Basin. *Transactions of the American Fisheries Society* 140:716-733.
- Winans, G. A., P. B. Aebersold and R. S. Waples. 1996. Allozyme variability of *Oncorhynchus nerka* in the Pacific Northwest, with special consideration to populations of Redfish Lake, Idaho. *Transactions of the American Fisheries Society* 125:645-663.
- Yakama Nation. 2011. Yakima/Klickitat Fisheries Project monitoring and evaluation, Project Number 1995-063-25, Contract Number 00042445, Final report for the performance period May 1, 2010 through April 30, 2011. Prepared for Bonneville Power Administration, Portland, Oregon. (<https://pisces.bpa.gov/release/documents/documentviewer.aspx?doc=P122475>)
- Zimmerman, C. E., R. W. Stonecypher Jr., and M. C. Hayes. 2003. Migration of precocious male hatchery Chinook Salmon in the Umatilla River, Oregon. *North American Journal of Fisheries Management* 23:1006-1014.