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Basinwide Supplementation Evaluation Project: 2016 Annual Progress Report

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2016 Annual Progress Report

Basinwide Supplementation Evaluation

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I. Executive Project Summary

This report summarizes activities for the 2016 calendar year under BPA Contracts #69191 and 72525, 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 contracts' Statements of Work in PISCES, not counting the associated administrative activities. 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 salmon populations, and b) productivity trends in new natural populations established through reintroduction of fish of out-of-basin hatchery origin in subbasins where the indigenous population had been extirpated. A relative reproductive success (RRS) study of supplemented Johnson Creek spring/summer Chinook (*Oncorhynchus tshawytscha*), financed jointly by the Nez Perce Tribe and the Project, is ongoing (Project Objective #1). Results for six consecutive broodyears 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 for multiple broodyears 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 broodyear (2007) indicate a 2.8 greater return rate for fish brought into the hatchery, and generally similar natural productivity estimates for natural origin and hatchery origin adults (RRS≈1). Analyses for broodyear 2007 will be finalized in 2017. Genotyping and productivity analyses for the additional four broodyears (2008-2011) will accumulate over the 2017 to 2018 period. A RRS study of reintroduced spring Chinook in Lookingglass Creek is ongoing (Project Objective #2), with results based on juvenile recruits-per-spawner for 7 consecutive broodyears anticipated in 2017. Two genetics studies of sockeye salmon/kokanee (*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 distinct stocks (Wenatchee River and Okanogan River), and results indicate that while the introduced fish showed some overlap in timing, the Wenatchee stock spawned 3-4 weeks earlier than Okanogan stock. Genetics analyses of out-migrating juveniles indicate a near total absence of inter-stock hybrids, due at leats in part to the temporal segregation for spawn timing between stocks. With returns of Cle Elum origin fish to the Yakima River now numbering in the thousands, 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, co-managed by the Warm Springs Tribe. 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). Annual adult return numbers have been low (ranging from 10 to 98), and of these only a limited number were confirmed to be of Lake Billy Chinook origin and of a size and age typically seen in Columbia River basin sockeye salmon. By contrast, the adult return in 2016 was 536, of which >90% were of Lake Billy Chinook origin and of sizes and ages indicative of anadromy. A 3-broodyear (2014-2016) study at the Cle Elum Supplementation Research Facility to test for an effect of spring Chinook broodstock age on survival, size and minijack rate among their progeny at the smolt stage is ongoing (Project Objective #4). Validity for use of springtime 11-ketotestosterone level to characterize maturation status (non-maturing versus maturing minijack) of male juveniles was confirmed based on results for fish sampled in April and held until spawning season the following September. Parentage analyses for the 2014 broodyear were completed at the end of 2016. Initial

analyses did not reveal obvious effects for parent age on minijack rate, however more refined analyses that will incorporate parent size, and egg/fry size as factors are underway. A study of population productivity and capacity characteristics of the native (since extirpated) spring Chinook population in Lookingglass Creek relative to those of the new population following reintroduction was postponed (Project Objective #5). In lieu of this study, one to re-examine effects of a hatchery summer steelhead on the indigenous winter steelhead population in the Clackamas River was initiated. Two 2-day "Introduction to Molecular Genetic Analyses in Tribal Fisheries Management" training programs sponsored through the Project were attended by a total of 16 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 salmonid populations. 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 broodyears 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 associated with a tribally managed program. In 2016, 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 in Newsome Creek (Clearwater River basin) and in Lookingglass Creek (Grande Ronde River basin) – streams where the natural populations had been extirpated, and the species reintroduced through stocking of juveniles from out-of-basin hatchery stocks (Project Objective #2)
- to assess relative spawning success of sockeye salmon reintroduced into the Cle Elum Lake/Yakima River system, and to examine genetic stock relationships among *O. nerka* (kokanee) in Lake Billy Chinook/Deschutes River versus Suttle Lake/Metolius River, as well as among juveniles (presumptive smolts) that are passed downstream of the Pelton Round Butte complex, and adults (presumptive sockeye salmon) that return to the Pelton trap (Project Objective #3)
- to build off previous research conducted at the Cle Elum Supplementation Research Facility describing incidence of precocial maturation of spring Chinook smolts as minijacks, to examine the effect of age (within sexes) of the natural origin hatchery broodstock on survival, size and minijack rate within their progeny (Project Objective #4)
- to initiate a retrospective study of productivity and capacity characteristics of spring Chinook salmon in Lookingglass Creek, based on historical data (1960s-1970s) and data collected following reintroduction in 2004, as well as to collaborate on a re-examination of a previously published study on the effects of a hatchery summer steelhead on 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 studies 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 2016 involving administration of the Project by CRITFC included: production and posting in PISCES of the annual progress report for 2015, completion of 2016 quarterly and final status reports in PISCES that record progress associated with each work element within the contract Statement of Work, and submission of 2016 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 Contract #72525 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 levels 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), to be genotyped, initially for a suite of microsatellite DNA 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. This has generally been sufficient to genotype samples collected at the weir and during spawning surveys. However, in a few years returns were exceptionally high and NPT funds were insufficient, therefore the Project provided supplemental funding to complete the genotyping. Also, a decision was made in 2013 to switch from microsatellite to single nucleotide polymorphism (SNP) DNA markers, given the higher reliability and lower laboratory costs for genotyping. Supplemental funding was provided by the Project to re-genotype samples from a limited number of prior broodyears with the SNP marker panel, to cover this transition in marker types (beginning with return year 2008).

RRS analyses described in Hess et al. (2012) for three consecutive broodyears 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 broodyears (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 (HO/NO) among successful spawners (i.e., those that produced at least one returning adult offspring) was not significantly different from 1.0.

In 2009, CRITFC recommended to NPT biologists that the number of juvenile samples collected annually be increased significantly. The greater number of juvenile progeny that can be sampled, relative to the number of returning adult progeny per broodyear, would permit a RRS analysis based on juvenile recruits-per-spawner with greater power to quantify effects of parent origin, as well as interacting effects of parent sex, age, size and return time to the Johnson Creek weir. Increased juvenile sampling began the same year, with funding from the Project to cover the associated genotyping costs. As of spring 2016, sampling of juvenile progeny was complete for five consecutive broodyears (2009 through 2014). Genotyping of all of these samples will be completed in early in 2017. Following parentage assignment, RRS analyses will be performed, including consideration for interacting effects of spawner sex, size/age and return time. Results will be summarized in oral presentations at fisheries management and scientific meetings, and in a manuscript for publication in a scientific journal. The number of juvenile samples collected for this period is illustrated below:

	Spawners		Juvenile Progeny	
Year	NO	HO	Year	number*
2009	197	497	2010	2781
2010	465	484	2011	3301
2011	396	310	2012	3097
2012	447	198	2013	1919
2013	609	301	2014	2099
2014	1114	542	2015	3571
2015	528	439	2016	1936
2016**	509	191	2017	N/A

* the large majority of juveniles age 0+ parr and pre-smolts that are progeny of adults from the previous year; a small proportion (approx. 5%) are age 1+ smolts from the broodyear two years previous

** estimates

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 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, 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. 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 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), however, 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 broodyears (2007-2011). The study involves genotyping a total of approximately 47,000 samples, involving the NOR and HOR adults passed upstream of Roza Dam (rkm 206) for natural spawning in those 5 years, plus their NOR adult progeny that returned in years 2012 through 2016:

	Adult Spawners		
	Natural	Hatchery	Unknown
Return Year	Origin	Origin	Origin
2007	1,284	1,504	
2008	1,677	3,240	191
2009	2,543	4,476	173
2010	3,186	5,514	157
2011	4,392	4,812	244
2012	2,927	na	160
2013	2,784	na	na
2014	3,761	na	na
2015	3,386	na	14
2016	500*	na	na
Sub-Totals	26,440	19,546	779

(* estimated)

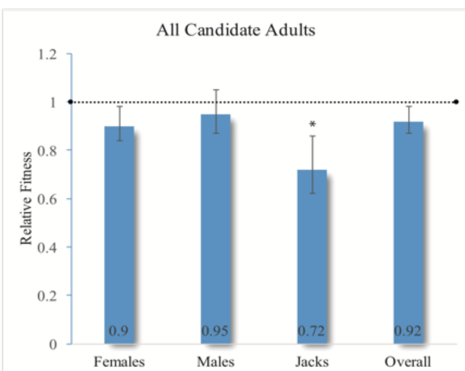
Genotyping by CRITFC using Project funds and by WDFW using YKFP funds, continued through 2016 and will be complete in 2018. Parentage and RRS analyses will be conducted within and across broodyears, and results will be summarized in a technical report and in a manuscript to be submitted for publication

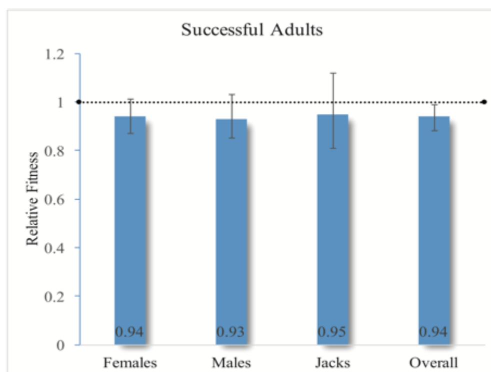
in a scientific journal. Genotyping was completed in late 2016 for the parents and progeny associated with the first broodyear (2007). Initial parentage and relative productivity analyses indicate that:

a) supplementation provided a 2.8-fold demographic boost to the population, i.e., NOR fish spawned in the hatchery returned 2.8 more adult progeny than NOR + HOR fish left in the river to spawn naturally:

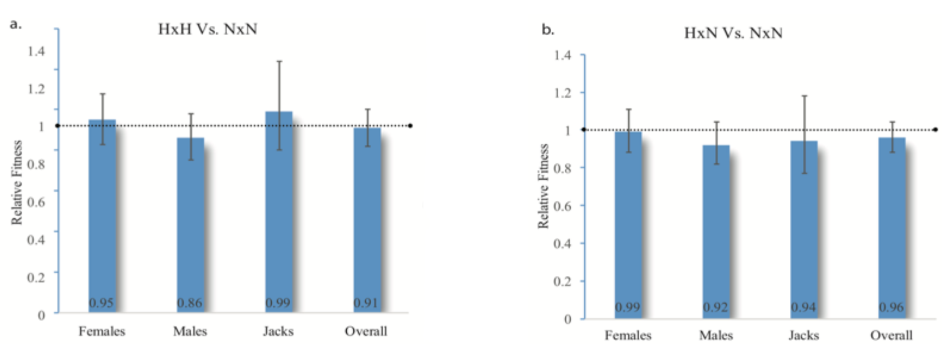
			Progeny per Parent	Hatchery /Natural
HOR progeny / Broodstock	1,123 / 365	=	3.08	
				2.8
NOR progeny / Natural spawners	2,520 / 2,291	=	1.10	

b) productivity of NOR and HOR females, and NOR and HOR age-4&5 males spawning naturally was not significantly different; productivity of HOR age-3 jack males was significantly lower, though when considering only jacks for which adult progeny were identified (successful spawners), NOR and HOR jacks were similarly productive:





and, c) average productivity between NOxNO, NOxHOR and HOxHOR crosses was not significantly different:



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 obviously 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 of these dams were also lost, e.g., spring Chinook in the Hood, Umatilla, Okanogan and Clearwater rivers, and 100% of the coho salmon populations native to the Columbia basin 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 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 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). Results from the reintroduction programs have been encouraging. Substantial numbers of the HOR fish released as juveniles returned as mature adults and engaged in natural spawning, and increasing numbers of NOR juveniles have been observed. Additionally, observation of NOR adults in subsequent return years indicates these to be fish that 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 recommended broodstock management protocol for the reintroduction programs involves progressive phasing out of 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 an increasing 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 will be increasing 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 sufficiently reversed, new naturally reproducing populations appear to be re-establishing themselves. However, these programs are relatively recent and hatchery supplementation continues. Uncertainty therefore remains as to whether the observed production is being supported by spawning of a progressively better adapted naturalized population, or simply by natural production of returning adults from the ongoing annual stocking of supplementation juveniles.

If adaptation is occurring, increased productivity is expected. As such, 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 (NO/HO) should be greater than 1.0. To test this hypothesis, the Project is currently performing RRS studies in two tribal reintroduction programs, each involving spring Chinook salmon –

Lookingglass Creek (Grande Ronde River) and Newsome Creek (South Fork Clearwater River). We also continue efforts to investigate possibilities for RRS studies in additional reintroduction programs.

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

Spring Chinook 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, spring Chinook in Lookingglass Creek had already been extirpated. Efforts to reintroduce spring Chinook into Lookingglass Creek were implemented over the following two decades by annual stocking of hatchery produced juveniles above the hatchery weir. Different hatchery stocks were successively used for the reintroduction, initially Carson NFH, then Wind River stock, Imnaha River, and finally Rapid River Hatchery stock (located in the Little Salmon River subbasin of Idaho). Despite these efforts, a naturally spawning population never fully established itself in Lookinglass Creek. For this and other reasons, co-managers (CTUIR and ODFW) decided to iniitate use of a stock of local Grande Ronde River origin. From 1998 through 2003, no returning adults were passed upstream of the Lookingglass weir (½ km upstream of the hatchery) - effectively extirpating any remnant fish of the reintroduced Rapid River stock. In the meantime, adults from Catherine Creek, a tributary to the Grande Ronde River located downstream from Lookingglass Creek, were collected and transported to Lookingglass Hatchery for use as broodstock. 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 the adults are selected for use as broodstock, and the remaining HOR fish, and subsequently NOR fish, have been passed upstream for natural spawning. The first NOR adults (age-3 "jack" males) from the new reintroduction program returned in-basin in 2007.

Beginning in 2004, CTUIR biologists collected tissue samples from all adults encountered at the weir (both those passed above the weir for natural spawning, and those taken for broodstock). The samples were archived in anticipation of eventual genetics studies to assess return rates and productivity. Tissue samples from carcasses have also been opportunistically collected during spawning ground surveys. Additionally beginning in 2008, samples were 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 ¼ km downstream of the weir (Boe et al. 2011). Numbers of samples collected to date and sent to HFCES for genotyping are provided in the table below:

Brood	NOR + HOR Adults				NOR Progeny	
Year	Spawners	Broodstock	Carcasses	Unknown	Parr	Smolts
2004	22					
2005	49					
2006	47		10			
2007	73	66	0			
2008	189	149	31		250	80
2009	105	65	49		463	146
2010	382	151	28		354	134
2011	554	148	253		395	5
2012	926	152	61	9	675	116
2013	223	170	46	6	285	184
2014	634	159	0	6	613	304
2015	769	0	83		543	
2016	632	0	39			

In 2010, an agreement was reached with the Confederated Tribes of the Umatilla Reservation (CTUIR), which manages the monitoring program in Lookingglass Creek, for the Project to finance genotyping of the adult and juvenile tissue samples, including those that had been collected previously and archived. Initially the samples were genotyped for a standardized panel of 96 SNP markers, which a few years later was expanded to two separate panels. More recently a new "Genotyping-in-Thousands" (GT-seq) methodology (Campbell et al. 2015) was developed that provides substantial cost-per-sample savings while also permitting expansion to ~300 SNP loci per individual. Samples from earlier years were re-genotyped with GT-seq for the ~300 SNP panel, and genotyping was for the entire sample set of adults and juvenile progeny for broodyears 2004 through 2013 was nearing completion at the end of 2016. In 2017, genotyping will be completed, the data quality controlled, parentage analysis performed, and a RRS analysis conducted to test for the effects of origin based on juvenile recruits-per-spawner, considering effects of broodyear, and parent sex and age. Results will be subsequently summarized in a technical report and in a manuscript to be submitted for publication in a scientific journal.

C.2 Newsome Creek (South Fork of the Clearwater River) spring Chinook salmon

Spring Chinook were functionally extirpated from the entire Clearwater River subbasin following construction of Lewiston Dam (rkm 6) in 1927 (Fulton 1968). Renovation of the defective fish ladder in 1940 permitted limited upstream movement, but it was not until removal of the dam in 1973 that full access to the subbasin for anadromous fish was once again re-established. Spring Chinook were reintroduced to the subbasin beginning in the 1960s, primarily through stocking of Rapid River hatchery juveniles into various tributary streams. However, Newsome Creek, a tributary to the South Fork of the Clearwater River (rkm 84), was not stocked and surveys conducted between 1987 and 1992 indicated that no fish had volunteered into the stream. IDFG initiated a reintroduction/supplementation program shortly thereafter, involving annual stocking of variable numbers of pre-smolts, smolts or adults of spring/summer Chinook produced at the Clearwater Anadromous Fish Hatchery (Ahsahka ID). In the early 2000s, management of the program was taken over by the NPT and juvenile production was shifted to the Nez Perce Tribal Hatchery (NPTH, Juliaetta ID), from which 75,000 age 1+ smolts were planned for stocking into Newsome Creek each year (Bradley et al. 2009). The program also included collection of returning adults at a weir in Newsome Creek for transport to NPTH and use as broodstock with which to produce these smolts. As part of the monitoring program for the project, NPT began collecting tissue samples from the returning adults, for use in a NPT-funded genetics study to assess productivity and RRS of HOR and NOR adults (Backman et al. 2009; Bradley et al. 2009). Subsequently, an agreement was reached for the Project to take over funding of the genetics study, and for NPT to also begin tissue sampling juvenile out-migrants collected in a rotary screw trap the tribe located just upstream from the confluence of Newsome Reek and the South Fork of the Clearwater River.

The primary objective of the study was to evaluate RRS of NOR and HOR adults passed upstream of the Newsome Creek weir within and across broodyears, based on both juvenile recruits-per-spawner and adult recruits-per-spawner. Results of these evaluations as described below, however, must be interpreted with some caution considering various management changes that arose over the course of the study. Adult returns to Newsome Creek have remained low, limiting sample size and thus power to observe differences. Additionally, fish collected for hatchery broodstock in the early years experienced high levels of pre-spawn mortality, and managers were unable to produce the target number of smolts to stock into Newsome Creek using in-basin spawners. Therefore, NPT managers decided that begining in 2011, no more adults would be collected in-basin for use as broodstock. Instead, all returning adults were passed upstream for natural spawning, and smolts used to supplement Newsome Creek would be of NPTH stock (Sherman Sprague, personal communication). The Newsome Creek weir and the rotary

screw trap are both located only 50-100 m from the creek's confluence with the South Fork of the Clearwater River. Over the course of the study, we determined that a substantial number of juveniles collected in the screw trap were not of Newsome Creek origin, but instead were fish that were spawned in locations upstream that strayed into Newsome Creek during out-migration. This included fish determined to be summer-run Chinook salmon smolts stocked by IDFG in nearby Crooked River for a 3-4 year period. Also, strays from this summer-run program represented a measureable proportion of adults subsequently captured at the Newsome Creek weir. Also of note, as returning adults are interrogated at the weir, sex and origin is identified for each prior to the fish being released upstream. Among the SNO markers with which the fish are genotyped, is one that is sex-specific. Results for genetic sex identification, however, was found to contradict the sex identifies in the field. The data provided below includes correction for the field data errors. Additionally, beginning with BY 2013 all HOR fish could be identified to hatchery of origin via assignment to individual hatchery broodfish using the recently developed Parental Based Tagging (PBT) database (Steele 2016). Since then, all adults were tested against the PBT database, and the origin of several fish identified as NOR at the weir was corrected to HOR following their assignment to hatchery broodfish via PBT. Of note, it was via PBT assignment to hatcheries producing summer-run Chinook stocks that identified the stray summer-run juveniles and adults mentioned above.

In the initial years of the study, samples were genotyped for a standardized suite of 13 microsatellite DNA markers. Beginning with BY 2010, the genotyping was switched to a panel of 192 SNP markers, which provided higher resolving power and lower per-sample laboratory costs.

The number of samples from in-migrating adults categorized by sex and origin, and of these the proportion that were assigned as parents of juvenile progeny is provided below:

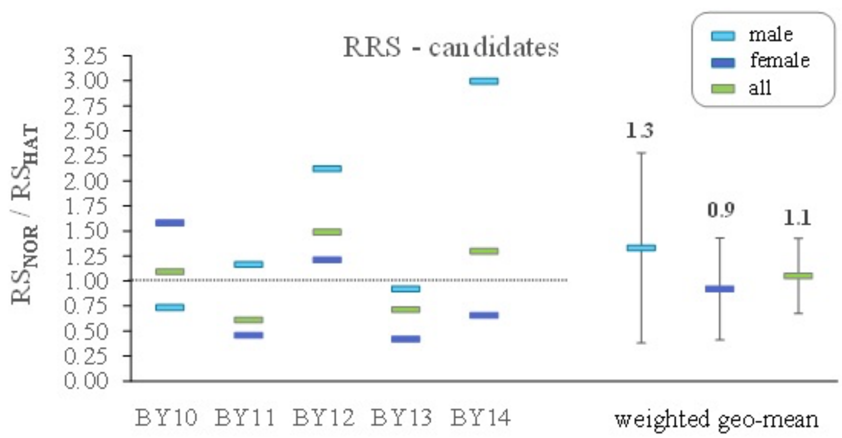
	HOR			NOR			unknown (U)		total /
adult sample	(M)	(F)	(U)	(M)	(F)	(U)	(M)	(F)	mean
Weir/carcass									
BY2010	71	60	0	13	9	0	0	0	153
BY2011	31	40	0	17	18	1	3	3	113
BY2012	34	33	2	38	30	2	1	0	140
BY2013	25	9	0	18	20	0	2	5	79
BY2014	23	23	0	16	18	0	2	2	84
total sample	184	165	2	102	95	3	8	10	569
genotyped	***181***	***158***	***2***	***101***	***92***	***3***	***2***	***2***	***541***
% assigned									
BY2010	0.58	0.64	---	0.54	0.86	---	---	---	0.61
BY2011	0.58	0.83	---	0.63	0.56	0.00	---	---	0.67
BY2012	0.67	0.52	0.00	0.79	0.53	0.50	0.00	---	0.62
BY2013	0.40	0.67	---	0.50	0.40	---	---	0.00	0.45
BY2014	0.43	0.57	---	0.69	0.41	---	0.00	0.00	0.51

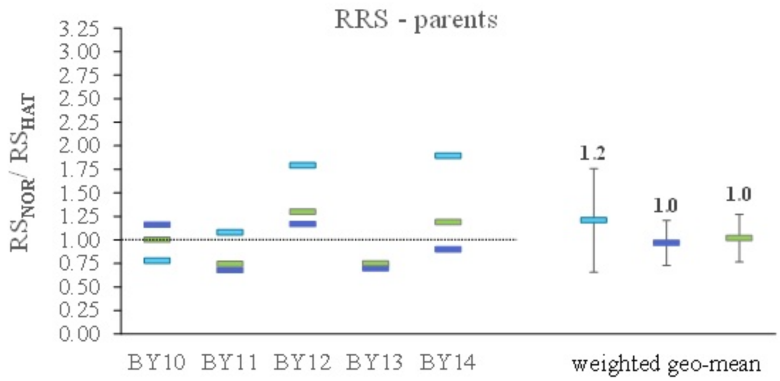
The number of samples from out-migrating juveniles categorized as pre-smolts (age 0+ parr), smolts (age 1+) or precocial parr, and of the number and proportion that were assigned as progeny to sampled adults is provided below:

sampled outmigration

screw trap sample		2011	2012	2013	2014	2015	total
pre-smolts (n)		496	848	1028	191	93	2656
	# assigned	378	558	856	135	46	1973
	% assigned	***0.76***	***0.66***	***0.83***	***0.71***	***0.49***	***0.74***
smolts (n)		0	291	164	123	2	580
	# assigned	---	226	116	81	1	424
	% assigned	---	***0.78***	***0.71***	***0.66***	***0.50***	***0.73***
precocial (n)		0	136	0	0	0	136
	# assigned	---	66	---	---	---	66
	% assigned	---	***0.49***	---	---	---	***0.49***
total (n)		496	1275	1192	314	95	3372
	average # assigned	378	850	972	216	47	2463
	average % assigned	***0.76***	***0.67***	***0.82***	***0.69***	***0.49***	***0.73***

RRS estimates within sexes and broodyears, based on juvenile recruits-per-spawner are illustrated below for all adults passed upstream for natural spawning ("candidates"), and for just those adults to which juvenile progeny were assigned by parentage analysis (successful "parents"). The overall weighted geometric mean indicated no difference in productivity within genders between NOR and HOR. However, among the HOR adults were identified a number of summer-run strays, and in BYs 2012-2015 the summer-run adults exhibited higher average (across broodyears) productivity than spring-run HOR adults - 2.88 versus 1.30 recruits-per-spawner (Matala 2016). Presuming that all NOR fish are spring-run (there had been insufficient time since stocking of summer-run smolts began for them to have returned as adults and produced returning NOR adult progeny), the RRS analyses may be somewhat biased against the NOR fish.



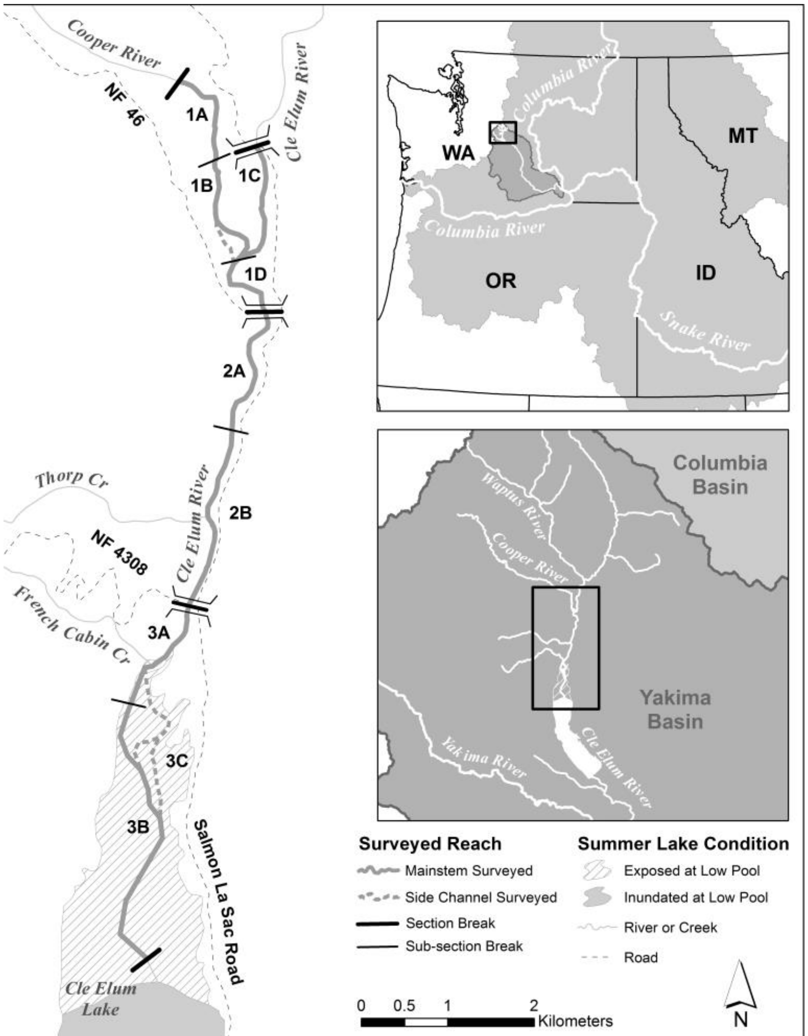


Additional observations of interest include: the proportion of adults each broodyear to which zero juvenile offspring were assigned ranged from 34% to 48%, and the number of offspring for adults which were assigned progeny ranged from 1 to 72; mean size of NOR and HOR pre-smolts was similar within years, as was mean size of NOR and HOR smolts, although smolts were a significantly larger proportion of total assigned HOR offspring compared to NOR (Matala 2016).

D. Project Objective #3: Support genetics studies of reintroduced sockeye salmon

D.1 Cle Elum Lake (Cle Elum/Yakima Rivers) sockeye salmon

Cle Elum Lake in the upper Yakima River basin once supported a native population of sockeye salmon. However, construction of an impassable dam at the lake outlet in the early 1900s resulted in extirpation of the population. This dam was later enlarged by the Bureau of Reclamation (BOR) to provide increased water storage. As a first step toward investigating the feasibility of a YN proposal to reintroduce sockeye 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 coho salmon smolts that had been released into the lake (BOR 2007). Results of the tests were positive, and in 2009 the YN began an annual program to out-plant adult sockeye salmon onto the lake, using fish collected from among adult sockeye salmon migrating upstream through the Priest Rapids Dam (PRD) fish ladder. The fish are transported by truck and released in the upper portion of the lake. The adults at PRD represent a mix of fish originating from the two extant upstream Mid-Columbia stocks – Wenatchee stock from Wenatchee Lake/Wenatchee River, and Okanogan stock from Osoyoos Lake/Okanogan River. The two lake/river systems have very different thermal regimes, and the two stocks exhibit variation for run and spawn timing, and in other life history characteristics. It was believed that these life history differences might affect the adaptive potential of each stock in the novel Cle Elum environment, and influence relative reproduction and rearing success.



Each year since the reintroduction program began, spawning ground surveys have indicated that spawning activity of the translocated adults occurs from mid-September to mid-November, although in an apparently temporally and spatially bimodal manner. In the early half of the season, spawning occurs predominantly upstream of the lake in the Cle Elum River and the lower portion of the Cooper River (Sections 1ABC&D, 2A&B and 3A). In the latter half of the season, spawning occurs predominantly in river channel and in the braided side-channels in the portion of the upper lake bed exposed in the late summer and fall (Sections 3B&C). The spatial difference is likely influenced to some extent by rain events that typically occur beginning mid-October which are followed by spikes in river flow creating conditions that render migration and spawning upstream in the mainstem of the river more difficult. Also of note, section 3C is an area of exposed lake bed which differs from the other mainstem sections, characterized by more uniform and smaller sized gravel, through which a braided network of streams is fed by relatively stable hyporheic flow.

Beginning in 2011, out-migrating *O. ne*rka have been observed in the juvenile bypass facilities at Roza Dam (rkm 206 on the Yakima River) and at the Chandler smolt collection facility adjacent to Prosser Dam (rkm 76). These fish are presumed to be predominantly age 1+ sockeye smolts, but including some that are age-2+. Also, counts of adult sockeye at Roza Dam - presumed fish that were the product of natural

spawning by previously translocated adults (and presumed to be predominantly age-4) - have increased dramatically in recent years:

Year	No. Adult Outplants	No. Adult Returns
2009	1,000	17
2010	2,500	40
2011	4,000	13
2012	10,000	154
2013	4,500	691
2014	10,000	2,576
2015	10,000	95*
2016	10,000	3,677

* exceptionally high summer temperatures in 2015 induced high mortality of migrating adult sockeye within the Columbia mainstem for all sockeye stocks

Questions of interest to the tribe regarding management of the Cle Elum sockeye reintroduction program include: 1) Does the bi-modal spawning activity of the out-planted adults in Cle Elum Lake correspond to the differences in spawn timing observed between Wenatchee and Okanogan stocks in their natal rivers? 2) What is the relative natural productivity of the two stocks in Cle Elum Lake - measured as number of out-migrant smolts and returning adults? 3) Do fish from the two stocks interbreed, and at what rate? 4) Do NOR juveniles from matings within and between stocks demonstrate differences in age, size and timing at out-migration, age and size at return, and in smolt-to-adult return rates?

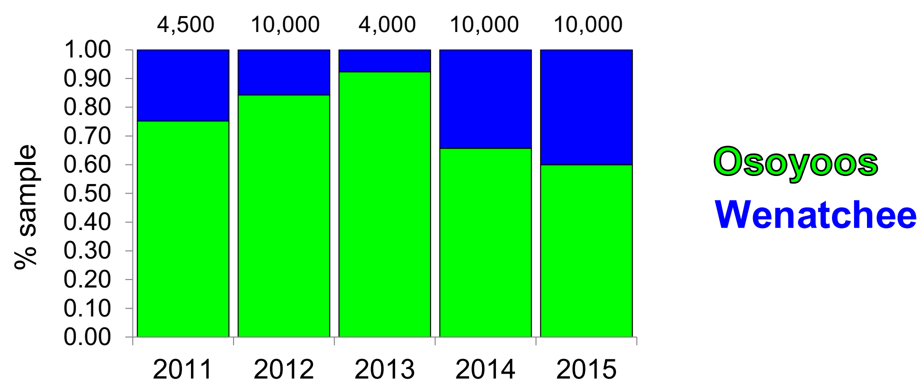
An agreement was reached in 2011 between YN and CRITFC, for personnel from the tribe and from CRITFC to collect tissue samples (fin clips) from the reintroduced sockeye salmon and send them to CRITFC geneticists at HFCES (accompanied with associated biodata) for genetic analysis financed through the Project. Since that time, tissue samples have been collected each year from: a) a temporally stratified portion of the out-planted adults captured at PRD, b) a sample of post-spawned carcasses observed during spawning ground surveys, c) adults coincidentally captured in gill-nets set in the lake for the purpose of removing exotic lake trout (a period concurrent with the latter portion of the sockeye spawning season), d) a sample of out-migrating juveniles intercepted at the Chandler Juvenile Bypass facility, Prosser WA, and e) from all in-migrating adults collected in the fish ladder at Roza Dam.

Genotyping of these samples was initially performed for a standardized suite of 94 SNP DNA markers. Results reflected findings from previous genetics studies, that sockeye from the Wenatchee and Okanogan stocks display distinctly different genetic profiles (Winans et al. 1996; Campbell and Narum 2011; Waples et al. 2011; Matala, unpublished data). This level of difference permits a very high degree of certainty for genetic stock identification (GSI) assignment to stock-of-origin, as well as for identification of any inter-stock hybrids among the Cle Elum juveniles. In 2016, the SNP panel was expanded to over 400 loci employing the GT-seq protocol (Campbell et al. 2015), and all 2016 samples are being genotyped with this expanded panel. Also, samples for juveniles collected in 2014 and 2015 (potential matches with individuals among the 2016 returning adults) and for adults from 2012 through 2015 (potential parents of adults returning in 2016 and beyond), are being re-genotyped with the expanded panel. The increased number of markers will provide high certainty for more in-depth parentage analyses, including cases where one parent is missing from the database.

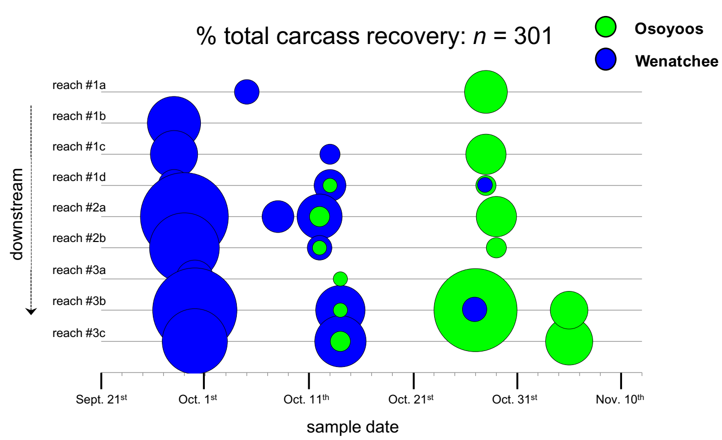
Number of samples per group of fish that were collected for genetics analysis are illustrated below:

	Adults				
Year	PRD	Roza	Carcasses	Gillnet	Juveniles
2009	0	18	0	0	0
2010	0	41	0	0	196
2011	275	95	31	0	450
2012	849	148	90	0	108
2013	250	688	110	0	446
2014	350	2575	377	63	108
2015	200	95	380	0	768
2016	450	3673	480	0	734

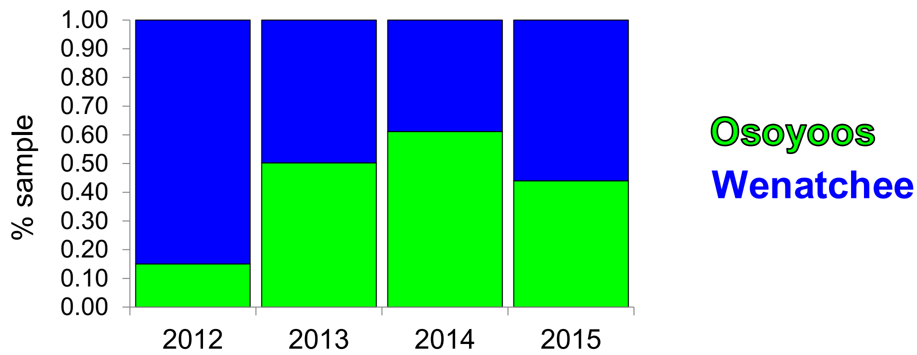
GSI analyses indicate that proportions of Okanogan and Wenatchee stock adults captured at PRD and translocated to Cle Elum Lake (2011 to 2015) have averaged approximately 75% and 25%, respectively (numbers above the columns represent the number of fish translocated each year):



While surveys of the spawning grounds to collect carcass tissue samples were conducted opportunistically in the early years, in 2015 the surveys were performed in a relatively systematic manner (temporally and spatially). Genetics analysis of the carcass samples confirmed there to be a distinct temporal separation in spawn timing between stocks, with fish spawning early in the season identified as the Wenatchee stock, and fish spawning later in the season predominantly Okanogan stock (see figure below). A spatial segregation between stocks, however, was not evident, with fish of both stocks collected across all reaches.



While Wenatchee stock fish have averaged approximately 25% of the adults translocated to Cle Elum Lake, the proportion of Wenatchee stock fish among out-migrating juveniles each year (2012 to 2015), though highly variable, has been consistently higher than 25% - apparently inferring greater productivity of Wenatchee stock fish (see below).



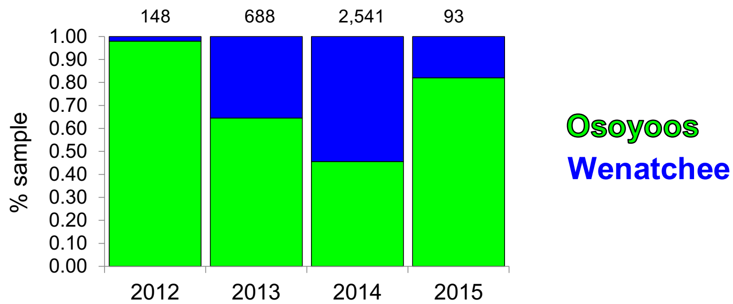
However, this interpretation is potentially confounded by possible stock-specific differences in proportion of smolts that out-migrate at age 1+ versus age 2+. Unfortunately, we lack scale age data with which to confirm individual out-migrant age and to assess annual inter-stock differences. We do know, however, that the two stocks exhibit differences in age composition in their native lake/river systems. Average age composition of juveniles at out-migration (smolts) for the years 1988 through 2011, and total age of returning adults at spawning based on scale reading of adults sampled at Tumwater Dam (Wenatchee stock) and Wells Dam (Okanogan stock), are illustrated 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):

Smolt Age	Okanogan	Wenatchee
Age-1+	91	82%
Age-2+	9%	18%
Adult Age		
Age-3	11%	0%
Age-4	73%	59%
Age-5	15%	39%
Age-6	<1%	2%

The most striking observation from analyses of the juvenile samples, however, remains the near complete lack of fish that represent inter-stock hybrids. Supported by our observation of a temporal difference in spawn timing, it is apparent that to present, fish from the two stocks have remained strongly reproductively segregated from each other.

Stock proportions among the returning adults for 2012 through 2015 are illustrated below (numbers above the bars represent total returns enumerated at Roza Dam). The Cle Elum origin adults in 2012 are age-3 fish (largely jack males), progeny from the initial 2009 translocation. That these fish were almost entirely Okanogan stock concords with the information presented in the table above indicating age-3

adults returning to the Okanogan river averaging approximately 11% of the return, while age-3 adults returning to the Wenatchee River are almost non-existent.



Again, because we lack information on age composition with which to attribute fish to their corresponding broodyear, we are as yet unable to make a controlled assessment of relative productivity to the adult stage of Wenatchee versus Okanogan stocks in the new environment of Cle Elum Lake/Yakima River. Whether one stock is more productive than the other, however, may be less important than the observation that both stocks have been reproductively successful and are likely occupying somewhat different ecological niches, such that overall production from the lake may have been increased by introduction of a mix of the two stocks as opposed to just one or the other. Also, of greater interest than a comparison of productivity between stocks of the translocated fish, will be comparisons of productivity within stocks between translocated adults versus Cle Elum origin returning adults. Presuming the Cle Elum origin adults inherited characteristics associated with successful reproduction of their translocated parents, then we might expect the Cle Elum origin fish to be relatively more successful ("adapted"), and will demonstrate increased average juvenile and adult recruits-per-spawner measures relative to translocated adults within broodyears. This kind of RRS assessment will be possible beginning with BY2013 adults, the first year that a substantial number of adults returned to Roza Dam (n=688). These fish were tissue sampled and genotyped, and parentage analyses will be performed for their Age-3, 4 and 5 progeny among adults captured at Roza Dam in 2016-2018. Adults that are unassigned will be presumed to be progeny of PRD translocated parents and to broodyear based on average size distribution. These data will permit assessment of relative productivity within broodyears.

D.2 Suttle Lake/Lake Billy Chinook (Metolius/Deschutes Rivers) sockeye salmon/kokanee

Suttle Lake, located in the headwaters of the Metolius River, a tributary to the Deschutes River, Oregon, was historically a nursery lake for a native sockeye salmon population. Suttle Lake and Wallowa Lake (located in the Wallowa River in the Grande Ronde River basin) 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 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 small fish ladder; however, the ladder was undersized and presence of the dam further hindered upstream passage, or totally blocked passage depending on water flows. In addition, screens installed in the inlets to the turbines prevented downstream escapement of juveniles. Over subsequent years, sockeye numbers diminished markedly (Nielson 1950, Olsen et al. 1994, Nehlson 1995, Gustafson et al. 1997). Nonetheless, a limited amount of spawning of sockeye 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 the construction of Pelton Dam (rkm 160) the Reregulating Dam (rkm 164) and Round Butte Dam (rkm 176) on the Deschutes River. While constructed with systems to provide both upstream and downstream passage for anadromous fish, the system for downstream passage proved ineffective, and anadromous populations above the complex were functionally extirpated.

Round Butte Dam created a reservoir, Lake Billy Chinook (LBC), in which a large non-anadromous *O. nerka* (kokanee) population developed. Mature kokanee migrate upstream from LBC 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 the remnant of the now landlocked sockeye population, LBC and Suttle Lake were repeatedly stocked with out-of-basin hatchery kokanee (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. Co-managers presumed that some portion of juvenile *O. nerka* that exhibited out-migration behavior from LBC and/or Suttle Lake, would also exhibit a return migration behavior at adult maturity, in accordance with an anadromous sockeye salmon life history. In 2010, the new fish transfer facility (FTF) at Round Butte Dam, constructed as part of the relicensing agreement, became operational. Since that time, *O. nerka* juveniles that volunteer into the FTF (following which until 2013 they received a maxillary clip) have been passed downstream annually. Returning adults from these releases would subsequently be captured at an adult trap at Pelton Dam (rkm 161). These fish have either been transported upstream for release into LBC, or used as hatchery broodstock. From 2010 until 2014, those lacking a maxillary clip were considered strays and were returned downstream most years. Numbers of out-migrating juveniles and adults trapped at Pelton Dam are illustrated below.

Year	Juveniles at FTF	Total Adults at Pelton Trap	Number for Broodstock	Released into LBC	Released Downstream
2010	49,734	10	0	10	0
2011	225,761	23	19	0	4
2012	5,126	98	0	86	12
2013	25,265	33	0	25	8
2014	155,031	27	0	20	7
2015	38,702	36	36	0	0
2016	49,497	536	73	463	0

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 of LBC origin and represent kokanee that have demonstrated an anadromous life history, and thus constitute a new run of Deschutes River sockeye salmon. To help address this question, an agreement was reached with CTWSRO in 2011 for the Project to finance a genetics 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 96 SNP markers that have been used to characterize reference collections from *O. nerka* populations across the Columbia basin. GSI analyses

assigned each adult captured at the Pelton trap to its population of highest probability among the reference baseline populations.

Genetics analyses of samples collected from LBC, Suttle Lake and a few other lakes in the upper Deschutes basin indicated there to be a high degree of variation within populations and strong evidence of the past kokanee stocking from out-of-basin hatchery sources. There was a strong signal in particular for identity with Whatcom Lake and Meadow Creek kokanee stocks – two hatchery populations that were widely used in the 1900s for stocking in lakes across the Pacific Northwest. There was a level of similarity between the genetic profiles for LBC and Suttle Lake fish, although differences were nonetheless of sufficient magnitude to be generally useful for differentiating fish of LBC versus Suttle Lake origin. Whether these differences might, in part at least, be due to a residual signature from the indigenous Suttle Lake sockeye salmon population is unknown.

Genotyping of in-migrating adult *O. nerka* captured at the Pelton trap between 2010 and 2015 indicated that approximately 80% assigned to the LBC population, and that almost no fish assigned to the Suttle Lake population. Among the remaining fish were several that assigned to known sockeye salmon populations (Wenatchee, Okanogan or Red Fish Lake stocks) and they were also among the larger fish – apparently fish that strayed into the Deschutes River. While the majority of fish captured at the adult trap were indeed of LBC origin, many of them were small relative to the size of a typical age-4 anadromous sockeye salmon (age-4 being the predominant age at return for sockeye salmon in the Columbia basin). Indeed, age estimates of the fish via scale analysis performed by CRITFC, indicated many of these smaller fish to be age-3. Given their small size and young age, it is possible that these fish had only been partially anadromous, i.e., had migrated maybe no further than them lower river or estuary for overwintering, before returning to the Deschutes. This observation, and more importantly the low number of returning adults provided minimal evidence that the project was advancing towards its objective to reestablish an anadromous run of sockeye salmon.

Results for 2016, however, contrast with those from the previous years. First, the return number to the Pelton trap was 536, more than an order of magnitude greater than the average for prior years. Second, the fish were "sockeye-like" in appearance and size, and scale analysis indicated them to be almost all age-4. Third, GSI analysis indicated the adults to be 92 % of LBC origin, with the proportion increasing to 96% when including Suttle Lake and Upper Deschutes Lakes:

Population of Assignment	Number	Percent
LBC	484	92%
Upper Deschutes*	18	3%
Suttle Lake	4	<1%
Wallowa Lake**	10	2%
Redfish Lake	4	<1%
Wenatchee	2	<1%
Okanogan	2	<1%

* a composite of samples from Paulina Lake/Wizard Falls Hatchery, Odell Lake and Wickiup Reservoir
** the actual origin of these fish may or may not be Wallowa Lake; similar to many Columbia basin kokanee lakes, Wallowa Lake was stocked multiple times with some of the same out-of-basin hatchery stocks, which reduces the certainty of these assignments

Sex and age composition of the 2016 LBC adults was: 64% females and 36% males; 13% age 1.1 (age-3 at time of spawning) and 87% age 1.2 (age-4 at time of spawning). Predominance of age 1.2 fish is expected for Columbia basin sockeye salmon, e.g., the proportion of age 1.2 sockeye sampled at Bonneville Dam in 2014 was 84% (Jones et al., 2015). The magnitude of the skew in sex ratio towards females was unexpected; sex ratio of sockeye interrogated at Wells Dam is estimated to be approximately 1:1, if not somewhat in favor of males. Somewhat in contrast, however, sex ratio observed among Cle Elum origin sockeye that returned to Roza Dam in the Yakima River was skewed towards females, though not as strongly: 59% females among 2,575 adults in 2014, and 54% females among 3,674 adults in 2016.

The Deschutes sockeye program co-managers are hopeful that the increase in abundance of returning sockeye salmon observed in 2016 will continue to grow over the coming years. CRITFC, through the Project, will continue to provide annual GSI analyses. Additionally, genotypes for returning adults will be subjected to parentage analyses, to identify any fish among them that assign as progeny of adults that were hatchery spawned in prior years.

E. **Project Objective #4:** Evaluate effect of hatchery broodstock age on minijack production

In wild Columbia River spring (stream-type) Chinook salmon populations, male maturation typically occurs in their 3rd (jacks), 4th or 5th year of age. Maturation in wild spring Chinook males can also occur precocially at age-1 (precocious parr, or "microjacks"), or age-2 ("minijacks"; Larsen et al. 2013). Natural rates of precocial maturation are believed to be very low, e.g., less than 5% for minijacks (Larsen et al. 2013), as might be expected that reproductive success of these small young males is also thought to be low (e.g., Schroder et al. 2010 and 2011). In hatchery reared stocks, however, rates of precocial maturation can be dramatically elevated. Research conducted on Yakima River spring Chinook salmon in the supplementation program operated at the Cle Elum Supplementation Research Facility (CESRF; Cle Elum WA) 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 conducted at CESRF, and elsewhere, demonstrates that the rate of minijack production is strongly influenced by environmental factors associated with hatchery rearing conditions, principally high feeding rates (relative to wild juveniles) which lead to increased growth rate, body size, and lipid levels. However, studies also demonstrate an additional genetic component of age at male maturation, including evidence for a positive correlation between parental and progeny age at maturation (Larsen et al. 2006, 2010, 2013 and 2014; Harstad et al. 2014).

To assess the extent to which broodstock age might also affect the rate of precocial minijack production in their hatchery-reared progeny, we designed a study in which gametes from broodstock of known ages were to be subdivided and factorially crossed to produce matings of all possible parental age combinations. Following incubation (and measurement of fry survival and growth within each mating), a sample of 50 swim-up fry per cross would be pooled into a raceway for rearing to the smolt stage (age-1+). Feeding rate during this period would be at least as high as that for the normal production, to assure maturation of a measureable proportion of the juveniles as minijacks. At this stage, the fish would be sacrificed, dissected and identified to phenotypic sex, and males measured (length and weight), and blood and tissue sampled. A biochemical assay of the blood plasma (11-ketotestosterone,

11-KT; Larsen et al. 2004) would be used to characterize the male progeny as maturing (destined to become minijacks) versus non-maturing. Tissue samples would be genotyped, and parentage analysis used to assign each individual to its parental pair. The proportion of minijacks within each male progeny group would then be analyzed for an effect of parent age on minijack rate within and across parental crosstypes.

An agreement was reached with YN in 2014 to perform this study at the CESRF for three broodyears, 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 mated with respect to broodfish age. The initial target design was for multiple 3x3 factorials within years, involving crosses of one each of age-3, age-4 and age-5 males with two age-4 and one age-5 females. Because only a limited number of jacks were included among the broodstock, and with age-5 fish being even more rare, the actual make-up of the matrices varied. In 2015 and 2016, we also incorporated age-1 microjack males (precocial parr) that were captured by WDFW field crews during snorkel spawning surveys, then transported and held at the hatchery.

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 was derived from observations of Halstad 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 of 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 minijack 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.

The overall number and type of factorial matings performed within each of the three broodyears are illustrated below:

BY 2014

	Male					
Female	jack Age 3	Age 4	jack Age 3	Age 4	Age 5	Total:
Age 4			30	36	12	
Age 5			0	6	3	87

BY 2015

Age 4	17	3	20	32	4	
Age 5	1	0	2	2	0	81

BY 2016

Age 4	14		17	18	14	
Age 5	4		5	5	2	79

	Male			
Female	WN	SH	HC	
SH	7	7	7	21

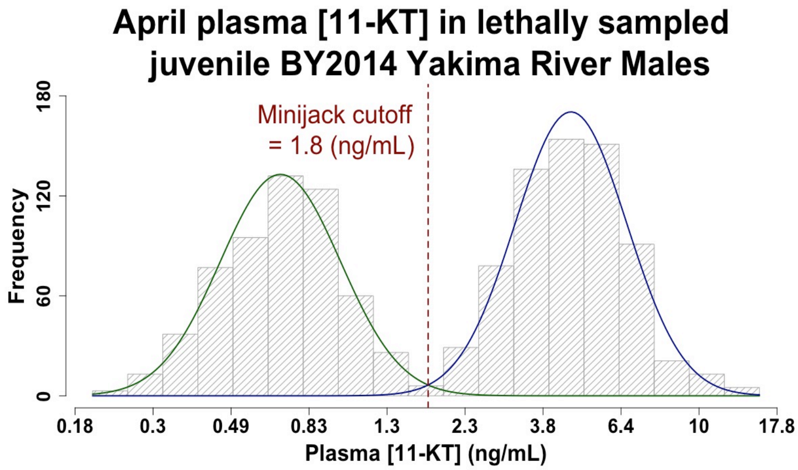
BY 2014 - When the BY 2014 fry reached the swim-up (initiation of feeding) stage in March 2015, a sample of fry from each cross was measured for individual length and weight, then 50 fry per cross were transferred to an outdoor raceway (#19) for rearing to the smolt stage. In April 2016, 2,452 of the 2,911 juveniles were sacrificed (over-dosed in anesthetic), measured for length and weight, dissected and identified to phenotypic sex, and a blood and tissue sample was retained for all male progeny. Assays for 11-KT were subsequently run on all samples, as was genotyping and parentage analysis.

Findings of Larsen et al. (2004) that springtime 11-KT measures in a sample of juvenile male Chinook salmon can be used to estimate the proportion among a population of smolts that would mature as minijacks the following fall has been used in several additional studies (Larsen et al. 2006, 2010, 2013 and 2014; Harstad et al. 2014). In our study, however, we needed to make these characterizations on an individual basis, something that has not been demonstrated to present. We felt it possible some individuals might initiate the maturation process, thus showing a springtime 11-KT level above the threshold for fish that have initiated maturation, but then arrest the process and revert to an immature status by the coming spawning season. Conversely, it is possible that an individual would initiate maturation relatively late, such that its springtime 11-KT level would be below the threshold, but increase over the summer and cause the fish to develop into a mature minijack by spawning season. To confirm rate at which a low versus high springtime 11-KT corresponds to an immature versus mature status during the following spawning season, we did not sacrifice 459 of the BY 2014 smolts. Instead, the fish were anaesthetized, measured and sampled as above, then individually PIT tagged and returned to the outdoor tanks for recovery and continued rearing. While survival rate for the latter fish was poor, due to mortality associated directly with handling and to subsequent fungal and/or bacterial infection, 190 were successfully reared until September 2016 (the beginning of the spring Chinook salmon spawning season). At this time, the fish were sacrificed, identified by their individual PIT tags, measured, dissected to identify phenotypic maturation status, and blood sampled and 11-KT assays performed.

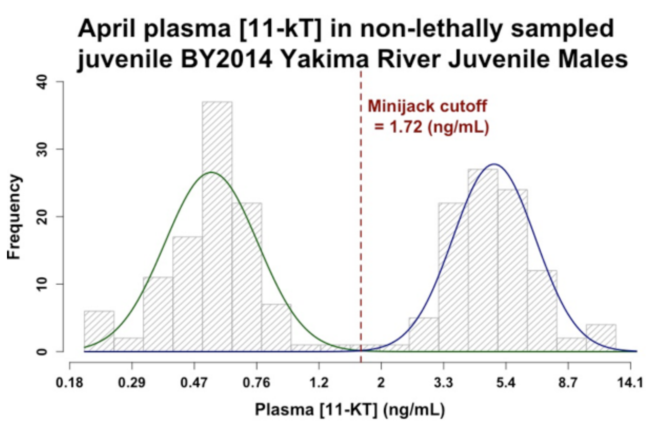
BY 2015 – In March 2016 the BY 2015 juveniles were sampled and measured for length and weight, and 50 fry per cross were transferred to outdoor raceway (#19) for rearing to the smolt stage. In April 2017, the fish will all be sacrificed, measured, and sampled as described above.

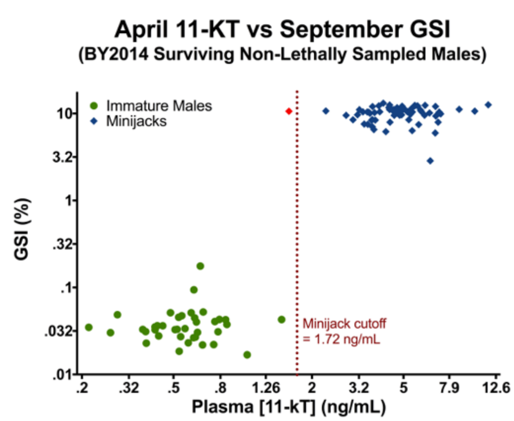
BY 2016 – Fry from the BY 2016 crosses reached swim-up in March 2017, at which time they were measured for length and weight, and 50 fry per cross transferred to outdoor tanks above raceway #18, then following sacrifice of the BY 2015 smolts and cleaning of raceway #19, the fry will be transferred to the raceway for rearing to the smolt stage (April 2018).

Results BY 2014 - The 11-KT assays run on blood samples from the 1,224 lethally sampled smolts that were identified as males, showed a clearly bimodal distribution in values, with a threshold value of 1.8 ng/mL separating the two distributions. Fish with values < 1.8 ng/mL were classified as non-maturing, and fish with values > 1.8 ng/mL were classified as maturing minijacks. The overall minijack rate for juvenile males was 54.3%, which is within the high end of the range previously reported for this population (Harstad et al. 2014).



Among the 190 smolts that were non-lethally blood sampled in April 2016 and survived until September, 105 were males. The frequency distribution for April 11-KT for these males shows a threshold between the non-maturing and maturing modes of 1.72 ng/mL, statistically similar to that for the lethally sampled fish illustrated above. Among these males, 37 (35%) exhibited low (<1.72 ng/mL) April 11-KT measures and had small immature testes in September (average gonadsomatic index = 0.04%). Sixty-seven (65%) males exhibited high (>1.72 ng/mL) April 11-KT measures and large mature testes in September (including flowing milt in many cases; average gonadsomatic index = 10.0%). There was a single exception to this pattern – a fish (red diamond in the figure below) that was mature in September (GSI=10.6%) but whose April 11-KT measure (1.59 ng/mL) was slightly below the 1.72 threshold. Results, therefore, indicate that springtime measures of 11-KT exhibit a strongly bimodal frequency distribution that provides relatively high certainty for categorization of individual Chinook salmon smolts as non-maturing (low 11-KT) or maturing minijacks (high 11-KT).





Parentage analysis for BY 2014 juveniles identified 67 full sibling male progeny groups, among which 57 involved parents of confirmed age and concordance with the 2014 mating records. These progeny groups included a total of 1,170 male progeny, with an average of 21 fish per progeny group. This subset of the BY 2014 factorial matings is illustrated below with their respective minijack rates:

Female Age	Male Age		
	3	4	5
4	0.52	0.91	0.41
4	0.58	0.75	0.25

	3	4	5
4	0.70	0.76	0.04
4	0.83	0.95	0.56

	3	4
4	1.00	0.62
4	0.50	0.17
4	0.81	0.64

	3	4	4
4	0.18	0.17	0.23
4	0.77	0.45	0.94

	3	4	5
4	0.61	0.57	0.68
5	N/A	0.05	0.35
5	0.46	0.26	0.57

Female Age	Male Age
	3
4	0.67
4	0.60
4	0.79

	3	3	4
4	0.25	0.13	0.69
4	0.44	0.32	0.86
4	0.38	0.22	0.79

	3	4	4
4		1.00	
4	0.60	0.65	0.53
4	0.30	0.27	0.68

	3	4	5
4	0.85	0.59	0.55
5	0.57	0.70	0.20

Minijack rates listed by cross type is illustrated below. ANOVAs for all cross types, and for crosses with age-4 females only (ignoring nesting for crosses within females), were performed on the arcsine transformed data. No significant differences were observed (p>0.05). An unexpected observation, however, was of the very high variation in minijack rate within cross types – ranging generally from <20% to >90% - suggesting possible genetic effects, albeit related to factors other than parent age at maturity.

Female Age x Male Age

	4 x 3	**4 x 4**	**4 x 5**	**5 x 3**	**5 x 4**	**5 x 5**
	0.13	0.17	0.04	0.46	0.05	0.20*
	0.18	0.17	0.25	0.57	0.26	0.35
	0.22	0.23	0.41		0.70	0.57*
	0.25	0.27	0.55			
	0.30	0.45	0.56			* (n < 10)
	0.32	0.53	0.68			
	0.38	0.57				
	0.44	0.59				
	0.50	0.62				
	0.52	0.64				
	0.58	0.65				
	0.60	0.68				
	0.60	0.69				
	0.61	0.75				
	0.67	0.76				
	0.77	0.79				
	0.79	0.86				
	0.81	0.91				
	0.85	0.94				
	1.00	0.95				
	4 x 3	**4 x 4**	**4 x 5**	**5 x 3**	**5 x 4**	**5 x 5**
Avg:	0.52	0.61	0.42	0.51	0.34	0.37
StDev:	0.246	0.245	0.234	0.076	0.334	0.187
	(4,5) x 3	**(4,5) x (4,5)**				
Avg:	0.52	0.53				
StDev:	0.235	0.261				

A more refined logistic regression analysis of the data for the BY 2014 fish will be performed in 2017 – an analysis that will incorporate other potentially interacting factors, e.g.:

> Probability(Mature as MJ) = constant + Sire age + Dam age + Sire FL+ Sire BdWt + Dam FL + Dam BdWt + Dam Egg wt + Fry Condition Factor at emergence + Cross
>
> (where MJ = minijack; FL = fork length; BdWt = body weight)

F. **Project Objective #5:** Assess productivity and capacity parameters associated with tribal supplementation 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 behavioral characteristics and productivity 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).

CRITFC quantitative fishery scientist with expertise in life cycle modeling, Robert Lessard, was solicited to examine data from tribal supplementation and reintroduction programs relative to trends in abundance associated with supplementation, and what factors may be constraining population growth. The Project Statement of Work for 2016 proposed an examination of a CTUIR monitored program to reintroduce spring Chinook into Lookingglass Creek, Grande Ronde River basin, Oregon. A pair of approximately 10-year data sets (adult escapement, redd counts, juvenile production, etc.) are available – one for the 1960-1970s prior to extirpation of the indigenous population, and the second beginning in 2004 when the new reintroduction program began (Burck 1994; Boe et al. 2010). The objective of the study will be to use these data in combination with additional information on freshwater environmental variables as available and on mainstem passage, on ocean survival, and on harvest rates for spring Chinook in the associated years, and to develop stock-recruitment relationships and freshwater capacity estimates that can then be compared between the pre- and post-reintroduction periods.

Lessard, however, is called upon to serve as biometrician for several other projects and he was unable to initiate the Lookingglass study within 2016. Additionally, interest has since arisen for an examination that is viewed as higher priority - a re-evaluation of effects of a summer run steelhead hatchery program in the Clackamas River on a native winter run steelhead population. This hatchery program was terminated after 20+ years of operation, as it was felt that addition of the summer run fish was having a depressant effect on productivity of the native winter run steelhead. A retrospective analysis by Kostow and Zhou (2006) claimed to have confirmed such an effect. Recently, Ian Courter, Mount Hood Environmental LLC, received funds from Portland General Electric and the North Coast Salmon & Steelhead Enhancement Fund to re-examine the data used by Kostow and Zhou (2006). This effort included correction of what he observed was an error in one of the year’s escapement numbers and inclusion of data for additional post-hatchery years. Preliminary findings from his reanalysis inferred

that the hatchery program did not have a causal relationship with the 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 unaffected by hatchery programs, i.e., the variation was more likely a response to more widespread (in particular ocean) environmental effects.

The Kostow and Zhou (2006) study has been widely referenced, erroneously according to Courter's initial findings, as proof of the deleterious effects that a supplementation program may have on a natural population. Mr. Courter explained, however, that the analytical model he used requires refinement, and he has requested input from biometricians with related expertise, such as Lessard. We (CRITFC) therefore took steps to contract with Courter to collaborate with Lessard to make the needed adjustments to his analytical model, and to finalize a re-examination of the Clackamas steelhead data. A contract with Courter to initiate this collaboration was submitted early in 2017, to be financed under the current BPA contract 72525. Presuming work goes satisfactorily, a second contract with Courter in the upcoming Project BPA contract (May 1, 2017 to April 30, 2018) will be proposed to summarize the findings of the retrospective analysis in both a technical report and in 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 for essentially all anadromous salmon and steelhead 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 hatcheries, as part of a basinwide parental based tagging (PBT) program to genetically "tag" all hatchery releases in the basin (Steele et al. 2016). Tissue samples collected by the tribes are sent to CRITFC geneticists at 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 the tribes' monitoring programs and to the logistical and working 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, the practicalities of how the tissue samples are processed, how the genotypic data are analyzed at the HFCES, 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 familiarity with 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 principally with CRITFC geneticists Ilana Koch, Andrew Matala and Jeff Stephenson, another pair of 2-day "Introduction to Molecular Genetics for Tribal Fisheries Management" training programs were held in 2016 (August 2-3, and December 13-14). Participants in these workshops included 15 tribal biologists and technicians from the four tribes: CTWSRO (9), CTUIR (1), YN (3), and NPT (2). Since 2011 we have conducted a total of 12 workshops for 98 participants, distributed as follows: YN (21), CTWSRO (18), CTUIR (22), NPT (22), and CRITFC (15). Copies of the December 2016 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 or University of Idaho personnel (Matala, Pierce, Medeiros) participated in various inter-tribal and inter-agency meetings, workshops and symposia in 2016, in which Project-related issues were discussed. Depending on the meeting, participation also included oral and/or poster presentations of findings from Project-funded studies. The objective of our attendance was to exchange information acquired during these studies with persons working on similar issues with other agencies, as well as to develop and articulate the tribal perspective on how hatcheries can be appropriately managed to minimize possible negative effects on productivity and to benefit from positive effects on the other three viable salmonid population (VSPs) parameters - abundance, spatial structure and diversity (McElhany et al. 2000). The following is a list of the workshops and symposia, and the nature of CRITFC's participation at each:

- March 6-10: Attendance at the 2016 Annual Meeting of the Idaho Chapter of the American Fisheries Society, Coeur d'Alene ID.
- March 28-31: Attendance and oral presentation at the 2016 Annual Meeting of the Washington and British Columbia Chapter of the American Fisheries Society, Lake Chelan WA, ("Sockeye salmon reintroduction in Cle Elum Lake: Monitoring reproductive success and behavior of two distinct upper Columbia River donor stocks", A. Matala, P. Galbreath, B. Saluskin and M. Johnston).
- April 19: Attendance at the Columbia Gorge - Fisheries and Watershed Conference, The Dalles OR.
- June 15-16: Attendance and presentation at the annual Yakima Basin Science and Management Conference. Yakama Nation, Ellensburg WA ("Genetic monitoring of sockeye salmon: Evaluating relative productivity among two donor stocks", A. Matala, P. Galbreath, B. Saluskin and M. Johnston).
- June 22-23: Attendance at the 2016 Pelton-Round Butte Project Fisheries Workshop, Madras OR.
- December 6-8: Attendance and poster presentation at the 67th Annual Northwest Fish Culture Conference, Grand Mound, WA Dec 6-8, 2016. ("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. Pierce, C. Knudsen, C. Stockton, B. Bosch, and P. Galbreath).

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

The manuscript "Evaluating Minijack Rates in Spring Chinook: Comparing minijack rates based on spring plasma 11-ketotestosterone levels with rates based on fall gonadosomatic index" is currently being drafted.

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 populations, in Johnson Creek and in the upper Yakima River. Results for these two 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 supplementation program indicate that NOR and HOR fish that 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 that no negative fitness impacts to the NOR population were apparent. Project funding is being used to perform additional genotyping of juvenile progeny from a series of 6 broodyears (2009 to 2014). Genotyping will be completed in 2017, to be followed by parentage and RRS analyses. Given the 3-4 fold greater number of juvenile progeny relative to adult progeny per broodyear, the RRS analyses will have increased power to test not just 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 broodyears 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 broodyears.

The second RRS study, initiated in 2014, involves five broodyears (2007-2011) of the spring Chinook supplementation program in the upper Yakima River. The genetics analyses are being performed collaboratively by CRITFC (with Project funding) and by WDFW (with YKFP funding). This study involves a number of fish that is several fold greater than the Johnson Creek adult-to-adult study, and thus should constitute an even stronger assessment of the supplementation approach. While still preliminary, results for the initial broodyear (2007) resemble those observed in Johnson Creek – generally similar productivity of NOR and HOR adults. Genotyping continues and should be near complete for fish involved in all five broodyears over the coming contract year. Parentage and RRS analyses will follow in 2018, involving tests for effects of origin within sexes, as well as for interacting effects of parent age, size and return time.

The Project also supports RRS studies of two reintroduced populations of spring Chinook salmon in Newsome Creek and 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 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 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 stocking, the programs are designed to transition to use returning adults for broodstock, and eventually incorporation of increasing proportions of NOR fish as

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 measures for HOR/NOR fish). Adaptation of reintroduced fish in these programs would be suggested by NOR/HOR 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.

Relative productivity calculated for juvenile recruits-per-spawner within genders of naturally spawning NOR and HOR adults in Newsome Creek varied widely among years (BYs 2010-2014), and the overall weighted geometric mean for RRS was not statistically different from 1.0. The comparison, however, is confounded somewhat by the presence of HOR summer-run adults from a stocking program in nearby Crooked River – these later returning summer-run HOR fish appeared to demonstrate greater productivity that HOR fish for the spring-run that has been used in the Newsome Creek program (2.88 versus 1.30 overall recruits-per-spawner, respectively). Adult returns to Newsome Creek continue to remain very low, which has precluded an effective RRS assessment based on adult recruits-per-spawner.

In 1998, the Lookinglass Creek weir was closed to upstream passage of adult spring Chinook, in order to extirpate previously reintroduced fish from the out-of-basin Rapid River stock. Then in 2004, HOR adults from a new Catherine Creek spring Chinook hatchery stock (a nearby Grande Ronde River tributary) were again permitted upstream passage. 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 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 of samples collected through 2016 will be completed in early 2017. This includes re-genotyping of some earlier samples with an expanded panel of ~300 SNP markers. Parentage and a RRS analysis based on juvenile recruits-per-spawner for 7 consecutive broodyears (2008-2014) will be performed in 2017. Besides an effect of origin (NOR versus HOR), the analysis will also test for interacting effects of parent sex, age and size.

The Project is supporting genetics studies associated with two other reintroduction projects, involving sockeye salmon (Project Objective #3). One study is of a YN program involving annual out-planting into Cle Elum Lake of sockeye adults collected at Priest Rapids Dam. These adults are a mix of approximately ¾ Okanogan River and ¼ Wenatchee River stock. Genetic analyses of carcasses collected during spawning ground surveys upstream of Cle Elum Lake indicate very strong temporal segregation for spawning between stocks – Wenatchee fish spawn early in the season while Okanogan fish do so later in the season. Additionally, GSI analyses of out-migrating juveniles confirm a near complete lack of inter-stock hybrids. Stock proportions among out-migrating juveniles have been variable relative to the proportions among the translocated adults, although comparisons to assess relative productivity of are confounded by apparent differences between stocks in the proportion of age 1 and age 2 fish among the out-migrants. Over 3,500 Cle Elum origin sockeye salmon returned to Roza Dam in 2016. All of these fish were tissue sampled and are currently being genotyped. Genotypes for juvenile out-migrants to be sampled in 2018 and 2019 will be subjected to parentage analysis against the 2016 adults, to assess relative productivity within stocks of Cle Elum origin versus translocated PRD adults. Additionally, plans are to continue annual tissue collection and genotyping of a sample of juvenile out-migrants and of all adult returns. GSI analyses will be performed to detect stock proportions and presence of inter-stock

hybrid fish, and to determine whether the new Cle Elum population remains of two distinct origins, or whether it begins evolving towards a “hybrid” 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, plus other juveniles that may have migrated downstream to LBC from Suttle Lake in the Metolius River basin. Annual return numbers of adults collected at the Pelton adult trap from 2010 through 2015 have been low (ranging between 10 and 98), but in 2016 the number jumped to 536. GSI analysis of the 2016 fish confirmed that 92% assigned to LBC with an additional 4 % assigning to Suttle Lake or other Upper Deschutes populations. The predominant size range and age 1.2 (age-4) of these fish was typical for fish of known Columbia basin sockeye salmon populations (Wenatchee and Okanogan stocks). CRITFC has committed to continue to provide genetics analyses of the fish returning to the Deschutes River, to aid the co-managers monitor this sockeye salmon reintroduction effort.

Work on a three broodyear study to assess the effect of parent age on the rate of minijack production in spring Chinook produced with NOR broodstock at the Cle Elum Supplementation Research Facility is on-going. Results of 11-KT assays for a portion of the BY 2014 smolts non-lethally blood sampled in April and retained for rearing until the spawning season (September) provided individual confirmation that a spring-time assay for 11-KT can be used to reliably categorize the juveniles as non-maturing versus maturing minijacks. The spring-time 11-KT measures were then used to categorize all of the BY 2014 juveniles as to maturation status, and genetic parentage analyses were used to organize the fish into full sibling male progeny groups. Initial analyses of average proportion of minijacks per progeny group did not reveal a significant effect of parent age; due in part to the unexpectedly high variation in minijack rate between progeny groups within parental age cross types. More refined analyses of the data that incorporate potentially interacting effects of broodfish size, egg size and fry size, will be completed in 2017.

A planned assessment of population productivity of the reintroduced Lookingglass spring Chinook was not performed in 2016, due to unavailability of CRITFC biometrician Robert Lessard. While he will be available in early 2017, we have decided to further postpone the Lookingglass study. Instead, Lessard has been asked to collaborate with contractor Ian Courter, Mount Hood Environmental, on a re-evaluation of Clackamas River winter run steelhead productivity. This population was reported to have been negatively affected during the period that a summer steelhead harvest augmentation program was conducted in the basin (Kostow and Zhou 2006). However, an initial re-evaluation of the data by Courter implicates environmental factors, not the hatchery program, as having been the driver of changes in productivity of the winter run fish. Lessard will work with Courter in 2017 to refine the analytical model and to finalize this re-evaluation.

In 2016, the Project funded another pair of 2-day “Introduction to Molecular Genetics for Tribal Fisheries Management” workshops involving 15 participants. This brings the total number of workshops supported by the Project to 12 and the number of tribal biologists and technicians participating to 98. 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 participation of 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, although results from each will be summarized in a technical report, and among them several will be further developed into manuscripts for submission for publication in peer-reviewed scientific journals (Project Objective #7).

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