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Basinwide Supplementation Evaluation Project: 2015 Annual Progress Report

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2015 Annual Progress Report

Basinwide Supplementation Evaluation

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Table of Contents

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I. Executive Project Summary

This report summarizes activities for the 2015 calendar year, performed as part of the multi-year Basinwide Supplementation Evaluation project 2009-009-00 (hereafter the Project), Contract #69191. The report is organized under the eight Project Objectives identified within the contract Statement of Work in PISCES, in addition to a section regarding the requisite administrative activities. The primary focus of the Project involves Hatchery RM&E - specifically molecular genetic studies associated with tribal hatchery programs to assess critical uncertainties related to effects of hatchery supplementation on productivity of depressed natural anadromous salmon populations, as well 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). Initial published results (Hess et al. 2012) that have been confirmed with unpublished results for an additional three broodyears, indicate that the program is indeed providing a demographic boost to the depressed population with little or no apparent deleterious effect on population productivity. A parallel RRS study based on juvenile recruits-per-spawner is underway with Project financing. A similar though larger scale RRS study was initiated in 2014 to assess RRS of supplemented upper Yakima River spring Chinook. Results for the initial broodyear (2007) are expected in 2017, with results for broodyears 2008-2011 accumulating over the following two years. A RRS study of reintroduced spring Chinook in Hood River is complete (Project Objective #2) and a manuscript summarizing results was resubmitted for publication (Project Objective #8). Results indicate that natural origin fish of the reintroduced stock generally demonstrate improved productivity, suggestive of adaptation of the reintroduced hatchery origin stock to the new environment. Additional RRS studies of reintroduced spring Chinook are ongoing in Lookingglass Creek and Newsome Creek (Project Objective #2). Two genetics studies of *O. nerka* financed by the Project are ongoing (Project Objective #3). The first is assessing spawning and rearing success of reintroduced Wenatchee versus Okanogan stock sockeye salmon in the Cle Elum Lake/upper Yakima River system. The second is performing genetic stock identification of kokanee in Lake Billy Chinook/Deschutes River, and in Suttle Lake/ Metolius River, and of 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). A 3-broodyear (2014-2016) study to test for an effect of spring Chinook broodstock age on survival, size and minijack rate among their progeny is ongoing at the Cle Elum Supplementation Research Facility (Project Objective #4). Initial discussions were held with CTUIR staff regarding an assessment by a CRITFC biometrician of productivity and capacity characteristics of the spring Chinook population in Lookingglass Creek, with completion of the study anticipated later in 2016 (Project Objective #5). Two 2-day "Introduction to Molecular Genetic Analyses in Tribal Fisheries Management" training programs sponsored through the Project were attended by 20 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 age of hatchery management 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 (HO) and natural origin (NO) 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 tribal-managed program. In 2015, these activities included ones to:

- use genetic analyses to derive productivity information with which to assess RRS of NO and supplementation HO spring Chinook in Johnson Creek (Salmon River basin) and in the upper Yakima River (Project Objective #1), and to assess RRS of reintroduced spring Chinook in the Hood River, 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 continue a study being conducted at the Cle Elum Supplementation Research Facility, to examine the effect of age (within sexes) of spring Chinook hatchery broodstock on survival, size and minijack rate among their juvenile 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 (Project Objective #5)
- to continue support for training of tribal personnel in use of molecular genetics studies to address questions in fisheries management, for participation in regional forums involving review

of hatchery management and supplementation efforts, and for reporting of Project results in scientific journals (Project Objectives #6, #7 and #8, respectively).

III. Work Elements / Tasks

A. Project Administration

Activities in 2015 involving administration of the Project by CRITFC included: production and posting in PISCES of the annual progress report for 2014, completion of 2015 quarterly and final status reports in PISCES that record progress associated with each work element within the contract Statement of Work, and submission of 2015 monthly project expense summaries to BPA. Additional reports and associated documents summarizing results of activities described within the Project work elements were also posted under Attachments within Project 2009-009-00 Contract # 69191 in PISCES.

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

B.1 Johnson Creek spring/summer Chinook

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 river kilometer (rkm) 8, as well as tissues from a limited number of out-migrating NO juveniles collected at a rotary screw trap operated directly downstream. The tissues have been sent to CRITFC geneticists at the Hagerman Fish Culture Experiment Station (HFCES), to be genotyped 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, although NPT funds have been insufficient in a few years when returns were exceptionally high and supplemental funding from the Project were used 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 of successfully spawning HO fish was generally similar to that of NO fish. These findings were confirmed by analyses for the subsequent three broodyears broodyears (Hess, unpublished data; see 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 NO and HO females (age 4 and 5); although HO adult males (age 4 and 5) and HO "jack" (age 3) males were somewhat less successful than

NO 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 additional laboratory costs in 2010. As of collection of yearling juvenile out-migrants in spring 2015, sampling of juvenile prograny was complete for five consecutive broodyears (2009 through 2013). Genotyping of all of these samples will be completed in 2016, followed by RRS analyses (based on juvenile recruits-per-spawner), with 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	
Broodyear	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			2015	3571

* 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

B.2 Upper Yakima River spring Chinook

The Yakama Nation (YN), in collaboration with the Washington Department of Fish and Wildlife (WDFW), initiated a supplementation program for 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; the fish were transported to the newly constructed Cle Elum Supplementation and Research Facility in Cle Elum, Washington, for spawning and juvenile rearing. The pre-smolts were then 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 from the supplementation program returned to the Yakima River in 2001. Hatchery production and supplementation has continued annually since then. This fully integrated program (100% of fish chosen for broodstock are of natural origin – 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 watershed provides a reference for evaluating environmental influences. The program has been comprehensively monitored. Results of subsequent analyses indicate that HO fish show some differences in morphometric and life history traits, however supplementation has increased harvest, redd counts, and spatial distribution of spawners (Fast et al. 2015). Additionally, NO abundance has been maintained, and straying to non-target systems has been negligible. Lastly, a RRS study (based on fry recruits-per-spawner for adults stocked in an artificial spawning channel) indicated that productivity of NO females was slightly higher than HO females, while productivity of NO and HO males was comparable.

Since its inception, there has been a desire to perform RRS analyses on the supplemented population as a whole. However, funding to present has been insufficient to take on the expense for genotyping the thousands of adults returning in-basin each year. With the development of SNP markers for Chinook salmon and new genotyping techniques, the per-sample genotyping cost has dramatically diminished, such a large scale RRS study is now feasible. In discussions between YN, WDFW and CRITFC an agreement was reached to perform a RRS study of naturally spawning NO and HO fish in the upper Yakima, covering five consecutive broodyears (2007-2011) and their respective adult progeny (returning between 2010 and 2016), with genotyping to be shared between the WDFW (to begin in 2016 using YKFP funds) and by CRITFC (begun in 2015 using Project funds) laboratories. The total number of samples involved is approximately 48,000:

	Adult Spawners		
Return Year	Natural Origin	Hatchery Origin	Unknown Origin
2007	1,282	1,504	4
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	4,168	na	na
2015	3,962	na	na
2016	*500**	na	na
Sub-Totals	27,421	19,546	929

TOTAL = 47,896

(* *estimates*)

A total of 7,703 samples associated with broodyear 2007 adults and progeny were genotyped by CRITFC as part of the 2015-2016 Project contract. Genotyping by CRITFC and WDFW in 2016 should complete analyses for this initial broodyear, following which RRS analyses will be performed. Genotyping of the remaining samples should be complete by the end of 2018, following which results will be summarized in a technical report and in a manuscript to be submitted for publication in a scientific journal.

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 the 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 (Snake River). However, many populations downstream of these dams were also lost, e.g., spring Chinook in the Hood, Umatilla, Okanogan and Clearwater river basins, 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 engaged in efforts to re-establish naturally spawning salmon populations in some of these Columbia basin rivers. Reintroduction efforts involved stocking of juveniles produced from out-of-basin hatchery stocks, on the presumption that the stocks possess the phenotypic and genotypic capacity to readapt 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 these reintroduction programs have been encouraging. Substantial numbers of the HO fish released as juveniles returned as mature adults and engaged in natural spawning, and increasing numbers of NO juveniles (fry, parr and smolts) have been observed. Additionally, observation of NO adults in subsequent run years indicates that these fish have undergone a full generation ond more of strictly natural production (Phillips et al. 2000; Underwood et al. 2003; Lutch 2005; Murdoch et al. 2006; Bosch et al 2007; Narum et al. 2007; Yakama Nation 2011; Yakama Nation Fisheries Resource Management 2012, Galbreath et al. 2014).

The broodstock management protocol for these reintroduction programs involves the progressive phasing out of stocking of juveniles from out-of-basin hatcheries. Instead, broodfish are increasingly collected from among adults returning in-basin, to produce the juveniles with which to continue supplementation. The initial generations of such "local origin" broodstock are comprised largely of mature HO adults. However, in subsequent generations, NO adults should make up an increasing proportion of the escapement, and NO fish are to be incorporated as an increasing proportion of the hatchery broodstock. With this broodstock management approach and exposure of the fish to natural selective forces, it is expected that a new natural population will be created that 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, hatchery supplementation for these programs is ongoing, and 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 some number of returning adults from the continued annual stocking of supplementation juveniles.

If adaptation is occurring, increased productivity is expected. As such, NO fish (fish that have been exposed to a generation or more of natural selection), should on average produce more recruits-per-spawner than HO 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 initiated RRS studies in three tribal reintroduction programs, each involving spring Chinook salmon – Hood River, 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 Hood River spring Chinook

Many factors led to the extirpation of spring Chinook from the Hood River basin by the mid-1970s (O'Toole 1991). Within a decade, plans were being made to reintroduce spring Chinook as part of a program co-managed by the Confederated Tribes of the Warm Springs Reservation of Oregon (CTWSRO) and the Oregon Department of Fish and Wildlife (ODFW). Initially (1986 through 1993), the program involved annual stocking of age 1+ smolts from Carson National Fish Hatchery (NFH). In 1995, managers switched to use of Deschutes River stock juveniles from Round Butte Hatchery, with occasional input from the nearby Warm Springs NFH as needed (Underwood et al. 2003, Reagan 2011). Both of these hatchery stocks belong to a genetic lineage of interior Columbia Basin stream-type (IST) Chinook populations (Narum et al. 2010, Hess et al. 2014). Beginning in 1992, scales and ancillary biodata (sex, size, date, etc.) were collected for nearly 100% of in-migrating adult Chinook salmon intercepted in the Hood River at the Powerdale Dam fish trap (rkm 6). The fate of each fish was recorded as: 1) passed upstream for natural spawning, 2) collected for hatchery broodstock, or 3) recycled downstream to the sport fishery. In 2009, an agreement was reached with CTWSRO and ODFW to engage in an RRS study financed through the Project, involving analysis of DNA extracted from the archived scales.

The spring Chinook scales were stored in individual envelopes at the ODFW office in The Dalles, Oregon. From each envelope a few scales were sub-sampled and sent to HFCES for DNA extraction, genotyping for a standardized suite of 15 microsatellite DNA loci (Seeb et al. 2007), parentage analysis, and estimation of RRS by sex. Approximately 8,300 individuals collected from 1992 through June 2010 (when Powerdale Dam was decommissioned) were sampled and genotyped, including 5,651 NO and HO adults that were passed above the dam in the years 1992 through 2005. The genotypes for each individual were also subjected to genetic stock identification (GSI) analysis. Interestingly, while the Carson then Deschutes hatchery stocks used for reintroduction belong to the IST Chinook genetic lineage, the GSI results indicated that some of the fish are derived from an alternative lower Columbia (LC) River genetic lineage (Narum et al. 2010, Hess et al. 2014) which appears to have colonized the Hood River. The LC lineage comprises Chinook populations from tributaries to the Columbia downstream of Bonneville Dam and from the Willamette River basin, populations which may exhibit either sub-yearling or yearling juvenile out-migrant life histories (Narum et al. 2010, Hess et al. 2014). Fish (both NO and HO) of the LC lineage were observed in the initial 1992 broodyear samples, and have been present in all subsequent run years since. Genetic differentiation among populations within the LC lineage is insufficient to determine the source population(s) of these LC colonizers. There were also fish among the Hood River samples which appear intermediate in genotype, undoubtedly the result of interbreeding between stocks. Nonetheless, the genetic signature of most fish remains strongly of one lineage or the other (IST versus LC), indicative of limited inter-breeding among stocks. The extent to which this can be attributed to the continued straying of lower Columbia fish into the Hood River, and/or to assortative mating between stocks is unknown.

Genetic parentage data was used to estimate individual productivity (adult recruits-per-spawner) for fish collected over 14 broodyears (1992-2005). Relative productivity for NO and HO fish (RRS; natural origin/hatchery origin) was then assessed within both sexes and lineages. Low sample size greatly limited the number of RRS estimates that could be made within brood years. Therefore, RRS was estimated across all brood years combined (1992 through 2005), following two approaches. The first calculates a geometric mean with the RRS estimate for each brood year weighted by the size of the parent group (Christie et al. 2014); the second approach calculates 95% maximum-likelihood confidence limits, which is more influenced by the sample size of the offspring group within brood years (Kalinowski and Taper 2005).

Overall, the proportion of the adults among those released above Powerdale Dam for natural spawning in broodyears 1992 through 2005 that were identified as a parent of a returning adult was approximately 12%. Within both males and females, and in both the reintroduced IST fish and LC fish, NO fish demonstrated higher productivity than HO fish, and LC fish demonstrated higher productivity than the IST fish:

	RRS for IST		RRS for LC	
	Females	Males*	Females	Males*
Overall weighted geomean	2.00	1.39	4.99	2.59
Overall maximum likelihood	2.33	1.20	6.08	2.35

(* age 4 and age 5; excludes jacks)

Beyond assessing productivity differences relative to origin, the data were tested for interacting effects of size (length) and return time to Powerdale Dam. NO fish were on average larger and returned later than average (after mid-June) than HO fish. While general linear models illustrated that larger size and later return time (within both NO and HO fish) were associated with higher fitness, a significant advantage for NO over HO fish was maintained. Higher productivity of larger fish is intuitive – fecundity is positively correlated with female size, and larger size has been observed to offer fish of both sexes a competitive advantage during spawning. We lack, however, a physiological or behavioral explanation for higher productivity of later returning fish. One possibility would have been higher pre-spawn mortality of the early-returning fish. However, the Hood River is thought to possess relatively abundant cold water and deep pool habitat in which fish could hold over the summer, and pre-spawn mortalities observed during stream surveys conducted in the summer have been rare (R. Gerstenberger, personal communication).

A manuscript summarizing results of this study was revised and recently resubmitted for publication in the North American Journal of Fisheries Management. We anticipate acceptance and publication later in 2016.

C.2 Lookingglass Creek (Grande Ronde River) spring Chinook

Spring Chinook populations within the Grande Ronde and Imnaha River subbasins had 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 program 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 at multiple upstream locations. 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 cease stocking with an out-of-basin stock, and from 1998 through 2003, no returning adults were passed upstream of the Lookingglass weir (½ km upstream of the hatchery) for natural spawning - effectively extirpating any remnant fish of the reintroduced Rapid River stock. In the meantime, adults from the Catherine Creek, a tributary to the Grande Ronde River located downstream from Lookingglass Creek, were collected and transported to Lookingglass Hatchery to initiate a new hatchery stock local to the Grande Ronde basin. 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 were selected for use as broodstock. The remaining fish were passed upstream for natural spawning. In 2007, the first naturally spawned adults (age 3 "jack" males) from the new reintroduction program returned in-basin.

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 genetic studies to assess return rates and productivity. Tissue samples from carcasses have also been opportunistically collected when encountered during spawning ground surveys. Additionally beginning in 2008, samples were collected from out-migrating NO 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:

	Adults				NO Juveniles	
Return Year	NO + HO Spawners	Broodstock	Carcasses	Unknown	Parr	Smolts
2004	22					
2005	49					
2006	45		10			
2007	73	66	0			
2008	188	149	31		250	80
2009	108	65	49		463	146
2010	382	151	28		354	134
2011	554	148	253		406	103
2012	926	152	61	9	134	116
2013	223	119	46	6	283	198
2014	628	159		6	285	116
2015						214

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 adult and juvenile tissue samples, both archived and future collections. Initially the samples were genotyped for a standardized panel of 96 SNP markers. However, in 2014 the decision was made to switch to a panel of ~300 SNP markers, as the higher number greatly increases the resolving power of the parentage analyses, in particular for cases where only a single parent can be identified. Genotyping with the new panel of markers is performed using a recently developed "Genotyping-in-Thousands"(GT-seq) methodology (Campbell et al. 2015). This new approach substantially lowers the per-sample cost for genotyping, while also increasing number of markers that can be interrogated per run. The entire sample set (adults and juvenile progeny for broodyears 2004 through 2013) is currently being (re)genotyped with GT-seq. The laboratory results will be available during the coming contract year, after which the data will then be subjected to parentage and RRS analysis. Results will then be analyzed for effects of origin in RRS analyses based on juvenile recruits-per-spawner, taking into account 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.3 Newsome Creek (South Fork of the Clearwater River) spring Chinook

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 (Little Salmon 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 from 1987 to 1992 indicated that no fish had volunteered into the stream. IDFG initiated a reintroduction/supplementation program shortly thereafter, involving annual stocking of a 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 (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 involved collection of returning adults at a weir in Newsome Creek for use as broodstock. However, returns to Newsome Creek have remained low. Therefore, as of 2011, collection of broodstock at the weir ceased, and all returning adults have been passed upstream for natural spawning (Sherman Sprague, personal communication).

As part of the monitoring component to the Newsome Creek reintroduction/supplementation program, the NPT collects tissue samples each year from all returning adults and from a portion of out-migrating juveniles, for the purpose of assessing productivity of the naturally spawning fish (Backman et al. 2009; Bradley et al. 2009). The adults are intercepted at the weir located approximately 100 m upstream of the creek's confluence with the South Fork Clearwater River. Samples from out-migrating juveniles are collected in a rotary screw trap located directly downstream (within 50 yards) of the weir site; age 0+ parr are collected in the summer and fall, and age 1+ smolts in the spring. The samples are sent to HFCES for genotyping and data analyses. The NPT funded the laboratory analyses in the first years of the study. However, in 2010 CRITFC recommended a higher sampling rate of juveniles, and to assure coverage of the additional laboratory expenses, an agreement was reached with the NPT in 2011 for the Project to take over full responsibility for funding of the genetic study.

Initially the samples were genotyped for a suite of microsatellite DNA markers. Recently, however, the genotyping was switched to a standardized panel of 192 SNP markers, given the higher resolving power and the lower laboratory costs for the SNP panel. In 2014 the decision was made to switch yet again, to a panel of ~300 SNP markers using a recently developed "Genotyping-in-Thousands"(GT-seq) methodology (Campbell et al. 2015). This new approach increases the number of markers that can be interrogated per run and further lowers the per-sample cost for genotyping, and the higher marker number further increases the resolving power for parentage assignment.

The numbers of samples from in-migrating adults and out-migrating parr and smolts that have been collected and genotyped from 2008 to the end of the 2015-2016 contract year are presented in the table below. Of note, these data reflect corrections to the original field data. Numerous errors in these data for identification of origin of the returning adults (as well as sex, through the use of a male-specific SNP marker), revealed via the genetic and parentage analyses. Evaluations of return time and productivity relative to parental origin could therefore not be considered reliable until broodyear 2010. Genetically assigned parents + progeny "trios" were used to correct for errors in parental origin ID beginning with the application of BY2010 juvenile genotypes.

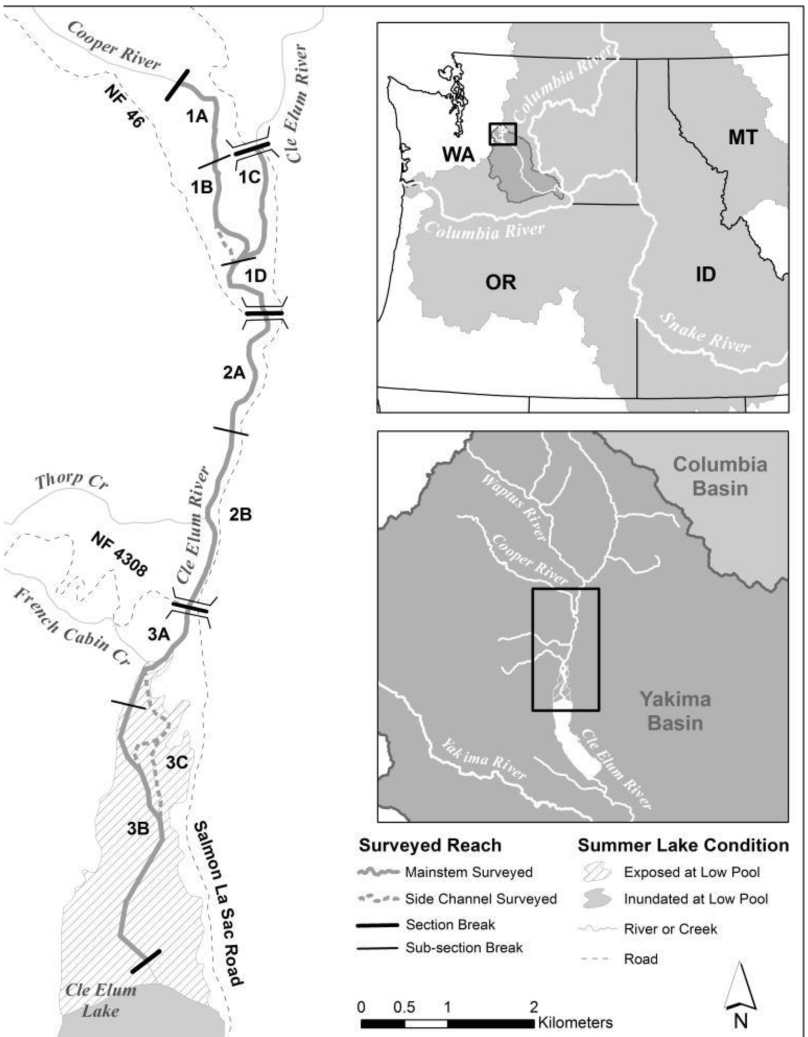
	Adults			Juveniles		
Run Year	Natural Origin	Hatchery Origin	Carcasses	Parr	Smolts	Precocial
2008	34	23				
2009	24	8		50		
2010	22	127		313	48	
2011	44	69		496	22	
2012	53	86		856	292	145
2013	81	21		1465	200	13
2014	69	19		213	216	

Analysis for NO and HO adults in broodyear 2010, based on information for their parr and smolt progeny in 2011 and 2012, are as follows. 1) The returning adults were intercepted at the Newsome Creek weir over a protracted period - from approximately ordinal day 170 to 250. However, average return date for NO fish was later than that for HO fish by 12.5 and 28.0 days for females and males respectively. 2) RRS for the 2010 adults (based on all adults passed upstream for natural spawning) was estimated within sexes for parr (non-mature) recruits-per-spawner, precocial parr recruits-per-spawner and smolt recruits-per-spawner separately. Results of relative reproductive success indicate a fairly large degree of inter-annual variation. Overall, the RRS for each individual brood year (BY2008 – BY2010) tended to favor hatchery-origin parents for pre-smolt production and natural-origin parents for smolt production. However, there is some evidence to suggest these results may be gender specific. The mean RRS for the combined three brood years is not statistically different between hatchery or natural origin parents, nor between males vs. females (see Table 3; combined geomean). Given limited data for BY2009, and somewhat conflicting results between BY2008 and BY 2010 (see Table 2a-c) it remains difficult to identify or confidently support a trend/s in reproductive success between the NPTH hatchery stock and the naturalized population in Newsome Creek. (Matala and Narum 2012 and 2014). Updated results will be available pending further genetics analyses.

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 thriving population of sockeye salmon. However, construction of a low but 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 of out-planting of adult sockeye salmon, using fish collected from among returning adult sockeye salmon captured in July at Priest Rapids Dam (PRD) during their upstream migration through the Columbia mainstem. The fish are transported by truck and released in the upper portion of the lake near its confluence with the Cle Elum River. The adults at PRD represent a mix of fish originating from two Mid-Columbia stocks – Wenatchee stock from Wenatchee Lake/Wenatchee River, and Okanogan stock fromOsoyoos Lake/Okanogan River. Waters in 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 may affect the adaptive potential of each stock in the novel Cle Elum environment, and influence relative reproduction and rearing success.



Spawning activity has been observed during September and October in each year of adult out-planting, both in the headwaters of the Cle Elum River, and in lower river and near shore areas at the head of the lake. This area of exposed lake bed in the late summer-fall is characterized by numerous braided water channels fed by hyporheic flow. Initial observations indicated that spawning was temporally and spatially bimodal. Initially, spawning occured to a greater extent in the river upstream of the lake. A later pulse of spawning, however, to occured predominantly in the braided channels in the exposed area at the head of the lake. Beginning in 2011, out-migrating *O. nerka* smolts were 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) – presumably age 1+ progeny of the initial 2009 out-planted adults.

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? 2) What is the relative natural productivity of the two stocks - measured as stock proportions among first generation smolts, and among returning adults? 3) Do fish from the two stocks interbreed, and at what rate? 4) Do NO 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 the tribe to collect samples from the reintroduced sockeye salmon and send them to CRITFC geneticists at HFCES for genotyping and genetic stock analysis, financed through the Project. Genotyping is performed for a standardized suite of 94 SNP DNA markers, employing the previously described GT-seq protocol. Prior genetics studies indicate that the Wenatchee and Okanogan sockeye stocks display distinctly different genetic profiles (Winans et al. 1996; Campbell and Narum 2011; Waples et al. 2011; Matala, unpublished data), which permits a high degree of GSI assignment accuracy to stock-of-origin, as well as for identification of inter-stock hybrids. Since that time, tissue samples have been collected each year from: a temporally stratified portion of the out-planted adults, a sample of post-spawned carcasses observed during spawning ground surveys, adults coincidentally captured in gill-nets set in the lake to capture lake trout (concurrent with the sockeye spawning season), a sample of out-migrating juveniles intercepted at the Chandler and Roza facilities, and from in-migrating adults collected in the fish ladder at Roza Dam.

Sample collection in 2015 was notable for the several reasons. 1) To better elucidate the apparent temporal and spatial bi-modality for spawning between fish of the two introduced stocks, carcass sampling was performed at a higher rate and in a more systematic manner than in prior years. 2) The number of samples collected from out-migrating juveniles was increased to over one thousand, to permit assessment of any temporal differences in migration timing between stocks. 3) Water temperature in the lower Yakima River during the adult in-migration period (July-August) was so high that it created an essentially impassable thermal block. While the original expectation was for a return of a few thousand adults, few if any successfully made the upstream migration. Only 95 adults were intercepted in the Roza trap, and of these many, or most, were likely not Cle Elum Lake-origin returning adults, but were translocation adults that had “fallen out” of the lake through the water outlet in the Cle Elum Dam, traveled downstream past Roza Dam, and were recaptured swimming up the Roza ladder.

Number of samples per group of fish and year that have been genotyped (or will be genotyped by the end of the current contract year) 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

While genotyping and analysis of samples collected in 2015 are pending, the results of analyses completed to present have provide the following information:

a) Prior to the first expected return of adult progeny of outplant parents, a small number of sockeye salmon were encountered at the Roza Dam adult trap (2009-2011). These fish were sampled for genetic analysis and later identified as likely "fall-backs". Fall-backs are PRD outplant adults that were entrained at Cle Elum Dam after translocation. Following entrainment they presumably swam downstream of Roza Dam then migrating back upstream through the ladder. Our conclusion is supported by matching genotypes observed between outplant fish and fish subsequently sampled at Roza Dam. With the exception of one fish, all were of Okanogan stock.
b) Information from carcass survey dates and locations among survey reaches (2011, 2013 and 2014) indicates both spatial and temporal spawning segregation between donor stocks. In the two upper river reaches (#1 and #2) of the Cle Elum River, carcasses were (essentially) only found early in the survey season (from late September through mid-October, and these fish were 97% Wenatchee stock. In Reach #3 which comprises the braided channels within the exposed lake bed, carcasses were observed throughout the spawning season. However, in the early half of the season, the fish were >90% Wenatchee stock, whereas the latter half of the season (mid-October through mid-November) the fish were >90% Okanogan stock. Also, sockeye salmon captured within the lake as bycatch during gill netting operations to remove exotic lake trout (*Salvelinus namaycush*), which occurs during the latter half of the sockeye spawning season were 100% Okanogan stock. Apparently some sockeye spawning activity occurs within the lake – likely associated with areas of upwelling. In addition to the carcass information, GSI analysis of out-migrating juveniles indicated that fewer than 1% assigned as inter-stock hybrids, reinforcing our hypothesis that spatial and temporal spawning segregation has to present kept the two stocks largely genetically segregated.
c) Stock proportions among out-migrating juveniles have been variable among years, coincident with apparent differences in juvenile age structure. Progeny of Wenatchee-origin spawners were generally larger in fork length, and predominantly age 2+ fish. Progeny of Okanogan-origin spawners were relatively smaller in size, and appear to be a mix of age 1+ and age 2+ fish. It remains unclear at this time whether or not there has been a significant difference in reproductive success based on stock proportions of sampled juveniles. Nonetheless, the proportion of out-migrating smolts (2012-2014) identified as Wenatchee stock has exceeded the proportion of Wenatchee stock fish among the translocated adults.
d) In possible contrast, the majority of returning adults in 2013 (age 4 progeny of naturally spawning 2009 outplants) were Okanogan stock - similar to the proportion Okanogan stock adults among the 2009 outplants. However, an inference of similar productivity between stocks may be biased due to presence among the adults captured at Roza in 2013 that were not returning adults, but instead were "fall-backs" from among the 4,000 sockeye out-planted in the lake that summer. Also, it does not account for age 5 progeny of 2009 outplants that would have returned in 2014, which may be disproportionately represented by Wenatchee stock (Lake Wenatchee generally receives a larger proportion of age 5 returning adults than does Lake Osoyoos). (see Matala et al. 2015 presentation "Genetic monitoring of sockeye salmon reintroduction into Cle Elum Lake" presentation in Attachments in the Project site in PISCES).

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, was 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 the early 1900s, adult passage into Suttle Lake was blocked by construction of a dam at the lake's outlet followed by another barrier constructed downstream in Lake Creek (the stream which connects Suttle Lake to the Metolius River), resulting in extinction of anadromous sockeye from the lake. However, a limited amount of spawning of sockeye persisted in the Metolius River below these obstructions, with some juveniles apparently rearing in the lower Deschutes or the Columbia River. Then in 1964 Round Butte Dam was constructed downstream of the Metolius on the Deschutes River at rkm 177. The dam was effectively impassable for both in-migrating adults and out-migrating juveniles. The dam did, however, create a reservoir (Lake Billy Chinook, LBC) in which a large non-anadromous *O. nerka* (kokanee) population developed, due to an unknown extent to extensive stocking of kokanee from out-of-basin hatchery stocks versus contribution from fish of the remnant *O. nerka* population in the Metolius River. Mature kokanee migrate from the lake 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). Similarly, in Suttle Lake a kokanee population developed following loss of the possibility for anadromy, with spawning occurring in Link Creek which flows into the upstream end of the lake. Like Lake Billy Chinook, Suttle Lake was also repeatedly stocked with out-of-basin hatchery fish through the mid-1900s (Nehlson 1995, Gustafson et al. 1997).

In recent negotiations for relicensing of the Pelton-Round Butte hydroelectric complex, an agreement was reached to re-establish passage of anadromous fish through the complex. It is presumed that some portion of juvenile *O. nerka* that exhibit 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, and *O. nerka* juveniles that volunteer into the FTF have been passed downstream annually. The primary question of interest to the co-managers (ODFW, CTWSRO, and Portland General Electric - PGE) is whether the *O. nerka* in the basin do indeed possess sufficient sockeye salmon genetic heritage from the historical local stock with which to demonstrate an anadromous life history, and secondarily, whether there is a greater propensity for anadromy within LBC versus Suttle Lake origin fish?

To help address these questions an agreement was reached with CTWSRO in 2011 for the Project to finance a genetics study of the *O. nerka* program. 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. Some tissue samples had been collected and archived prior to the agreement, and sampling continued through 2014. Analysis of samples from each lake was deemed sufficient to provide a "genetic signature" characteristic of the two populations. As of 2015, samples have only been collected from in-migrating adults at the Pelton trap. The numbers of samples collected and genotyped to present are illustrated below:

		Adults			Juveniles
Year	LBC	Link Cr	Pelton	LBC/FTF	Suttle
2009	97	0	15	0	0
2010	100	0	8	100	196
2011	0	0	22	100	450
2012	100	96	98	0	108
2013	100	100	31	0	446
2014	0	100	27	100	108
2015	0	0	36	0	0

Genetic analyses indicate multiple stock origins in both the LBC and Suttle Lake populations, presumably associated with past kokanee stocking from similar out-of-basin hatchery sources. Nonetheless, differences in the genetic profiles between populations are apparent and of sufficient magnitude to be generally useful for differentiating fish of LBC versus Suttle Lake origin. Genotyping of adult sockeye captured at the Pelton trap showed a dominance of fish that assigned to the LBC population, and almost no fish assigned to the Suttle Lake population (see table below). This outcome could be expected given the significantly larger size of the population in LBC compared to Suttle Lake, and the low survival of Suttle Lake juveniles as they out-migrate through Lake Creek (J. Hogle, personal communication). GSI analyses assigned a limited number of adults captured at the Pelton trap to out-of-basin stocks (e.g., Redfish Lake, Okanogan, and Wenatchee stocks) - apparent strays. (see Matala et al. presentation "Genetic Characterization of Deschutes *O. nerka* to inform sockeye reintroduction" in Attachments for the previous Project contract #65188).

Deschutes River Basin - Kokanee	
Lake Billy Chinook	161
Suttle Lake/Link Creek	4
Paulina Lake/Wizard Falls Hatchery	8
Odell Lake	2
Out-of-Basin - Sockeye	
Wenatchee Lake/River	1
Okanogan River/Osoyoos Lake	1
Redfish Lake	5
Out-of-Basin - Kokanee	
Wallowa Lake	8
Tieton River (Yakima R)	1
Gold Creek (Yakima R)	4
Speelyai Hatchery (Lewis R)	5

G. 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 at 3 (jacks), 4, or 5 years post-fertilization. Maturation in wild spring Chinook males can also occur precocially at age 1 (precocious parr, or "microjacks"), or age 2 ("minijacks"). Natural rates of precocial maturation are believed to be very low, e.g., less than 5% for minijacks, as might be expected given that reproductive success of these small young males is also thought to also be very 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 to be on the order of 40%. Minijacks provide no fishery benefits, and they are believed to contribute minimally to natural spawning. 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, and body size and lipid level. However, studies also demonstrate there to be an additional genetic component of unknown magnitude to 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 (of both males and females) 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 are 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 progeny from each cross will be pooled into a raceway for rearing to the pre-smolt stage (age 1+). At this stage, following sex identification, males will be measured (length and weight), and blood and tissue sampled. A biochemical assay of the blood plasma (11-ketotestosterone; Larsen et al. 2004) will be used to characterize the male progeny as maturing (destined to become minijacks) or non-maturing. Tissue samples will be genotyped, and parentage analysis used to assign each individual to its parental pair. The proportion of minijacks within each male progeny group will then be analyzed for an effect of parent age.

An agreement was reached with YN in 2014 to perform this study at the CESRF for three broodyears, beginning in fall 2014. Over four consecutive weeks in September 2014, samples of gametes from a subset of the CESRF broodfish were factorially mated with respect to broodfish age. The target design was a 3x3 factorial, involving crosses of one each of age-3, age-4 and age-5 males with two age-4 and one age-5 females. However, age-5 fish were rare among the broodstock, resulting in a preponderance of crosses involving age-4 fish. In the end, a total of 11 different factorials were produced, for a total of 87 individual matings distributed as illustrated below:

	Male Age-3	Male Age-4	Male Age-5
Female Age-4	25	25	19
Female Age-5	5	11	2

When the 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, approximately 400 of the BY 2014 juveniles will be transferred to a smaller tank(s) for continued rearing. All remaining fish will be sacrificed, measured for length and weight, dissected and identified to phenotypic sex, and a blood and tissue sample will be retained for all male progeny.

The fin clips from the male progeny from each brood year will be genotyped by CRITFC geneticists at HFCES and assigned to full-sib male progeny groups following parentage analysis. Blood samples from the males will be centrifuged to separate the plasma, which will be assayed for 11-ketotestosterone level, for determination of the status of each individual as maturing (minijack) versus non-maturing (Larsen et al. 2004). A minijack rate will then be estimated for each male progeny group as the proportion of maturing (minijack) male progeny. This procedure will be repeated for crosses to be made in 2015 and 2016. The data from the three brood years then be subjected to a comprehensive statistical analysis to test for an effect parent age on minijack rate, results from which will be summarized an a manuscript to be prepared for publication in a scientific journal.

In September-October 2015 a second group of factorial matings were performed (see table below) – a repetition of the same basic design as followed for the BY 2014 matings. One difference in BY 2015 was the inclusion among the male broodfish of several age 1 natural origin microjacks (and a single age 2 minijack) that were captured live on the spawning grounds and transported to the Cle Elum hatchery, where they were held until the time of spawning. Overall, a total of 89 of the individual matings will be retained for sampling of 50 fry per mating. These fry will be reared to the smolt stage (April 2017), after which blood and tissue samples will be collected for parentage analysis and maturation (minijack) status determination.

	Male Age-1	Male Age-2	Male Age-3	Male Age-4	Male Age-5
Female Age-5	15	3	18	26	12
Female Age-5	4	0	3	6	2

E **Project Objective #5:** Assess productivity and capacity parameters associated with tribal supplementation programs

A primary focus of the Project activities described above is use 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 achieving its overarching goals 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 critique of supplementation as a "restoration" action. Environmental constraints to use of supplementation to rebuild populations were highlighted in the recent ISAB/ISRP report, "Density Dependence and Its Implications for Fish Management and Restoration in the Columbia River Basin" ISAB and ISRP 2015).

In 2015, the contribution of Robert Lessard, CRITFC quantitative fishery scientist with expertise in life cycle modeling, 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. This effort recently initiated with discussions with CTUIR fisheries staff to examine of a pair of approximately 10-year data sets for spring Chinook in Lookingglass Creek (adult escapement, redd counts, juvenile production, etc.) – one from the 1960-1970s prior to extirpation of the indigenous population, and the second beginning in 2004 when the new reintroduction program began (Burke, 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 and ocean survival and 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. This assessment for Lookingglass Creek will be completed in the coming contract year. The Project anticipates funding additional assessments for supplemented populations of spring Chinook salmon in Johnson Creek and in the Upper Yakima River.

F. 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 program to genetically “tag” all hatchery releases in the basin. These samples are sent to the HFCES for molecular genetic analyses, and the resulting data are analyzed to inform a variety of management questions. However, the 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 to the logistical and working constraints under which field crews operate.

With the primary 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 also to improve understanding and communication between the tribal field personnel and the CRITFC geneticists, we developed a curriculum for a 2-day “Introduction to Molecular Genetic Analyses in Tribal Fisheries Management” workshop. The program consists of a series of presentations (lectures, videos, 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. Emphasis is placed on use of SNP DNA markers for genetic stock identification and for parentage analysis. By way of illustration, summary presentations of previous and ongoing genetics studies conducted on tribal programs at HFCES are also included. These presentations are interspersed with “hands-on” exercises to provide familiarity with the actual laboratory techniques. Additionally, the entire HFCES staff is invited to attend a noontime 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 Maureen Hess, Andrew Matala, Jeff Stephenson, another pair of 2-day “Introduction to Molecular Genetics for Tribal Fisheries Management” training programs were held in 2015 (November 17-18, and December 8-9, 2015). Participants in the November workshop included 9 tribal biologists and technicians. Participants in the December workshop included 10 biologists and technicians, each of them working specifically on tribal Pacific lamprey restoration projects. In total, the 19 participants were distributed as follows: CTWSRO (3), CTUIR (5), YN (5), and NPT (6). Copies of the 2015 workshop agenda and PowerPoint presentations are available at: http://www.critfc.org/fish-and-watersheds/fishery-science/hagerman-genetics-laboratory/genetics-training/.

G. Project Objective #7: Participate in regional forums for review of hatchery effects on natural populations

Project coordinator (Galbreath) and associated CRITFC geneticists at HFCES (Hess, Matala and Narum) participated in various inter-tribal and inter-agency meetings, workshops and symposia, in which Project-related issues were discussed – issues related to hatchery management and the nature and magnitude of effects that HO salmon and steelhead may have on the natural populations with which they interact or interbreed. The purpose was to exchange information acquired during studies conducted by CRITFC and by other participating 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:

Jan 22-23: Attendance and presentation ("Use of domesticated out-of-basin hatchery stocks to reintroduce extirpated Coho Salmon to the interior Columbia Basin" P. F. Galbreath, M. A. Bisbee, Jr., C. M. Kamphaus, and T. H. Newsome; "Supplementation with local, natural-origin broodstock may minimize negative fitness impacts in the wild" M. Hess, C. Rabe, J. Vogel, D. Nelson, and S. Narum) at the Hatchery vs. Wild Symposium – Research, Management, and Reform in the Pacific Northwest. Oregon AFS, Portland OR

Feb 10-12: Attendance and presentation ("Monitoring reproductive success of spring Chinook salmon following reintroduction in Newsome Creek"; A. Matala, S. Narum, P. Galbreath, S. Sprague, T. Backman, and J. Bretz; "Supplementation with local, natural-origin broodstock may minimize negative fitness impacts in the wild" M. Hess, C. Rabe, J. Vogel, D. Nelson, and S. Narum) at the Symposium on Salmon Supplementation. Nez Perce Tribe (Lewiston, ID)

March 18-19: Attendance and presentation ("Genetic characterization of Deschutes River *O. nerka* to inform sockeye reintroduction"; A. Matala, P. Galbreath and J. Hogle) at the 2014 Pelton-Round Butte Project Fisheries Workshop, Madras OR

April 14: Attendance at the Columbia Gorge Fisheries and Watershed Conference, Yakima Nation, The Dalles OR

June 17-18: Attendance and presentation ("Genetic monitoring of sockeye salmon reintroduction into Cle Elum Lake"; A. Matala, P. Galbreath, B. Saluskin and M. Johnston) at the annual Yakima Basin Science and Management Conference. Yakama Nation, Ellensburg WA

August 17-20: Attendance and presentation at the 145th Annual Meeting of the American Fisheries Society ("Genetic monitoring of Cle Elum Lake sockeye reintroduction" A. P. Matala, S. R. Narum, P. F. Galbreath, B. Saluskin and M. Johnston; "Supplementation with local, natural-origin broodstock may minimize negative fitness impacts in the wild", M. Hess, C. Rabe, J. Vogel, D. Nelson, and S. Narum) AFS, Portland OR

December 1-3: Attendance at the 66th Annual Northwest Fish Culture Conference. ODFW, Wilsonville OR

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

A manuscript summarizing results of the RRS study of reintroduced Hood River spring Chinook has been revised and was resubmitted for publication in the North American Journal of Fisheries Management.

I. Synthesis of Findings: Discussion/Conclusions

Project activities are centered around the theme of supplementation hatchery RM&E, with particular focus on assessing effects of hatchery rearing on life history and natural productivity of the supplementation fish.

Two Project efforts involve RRS studies of tribal programs to supplement depressed natural spring Chinook populations – Johnson Creek and upper Yakima River. Results for these two studies are of particular interest, for these are the only two supplementation programs that apply the recommendation for fully integrated broodstock management –maximization (100% in the case of these two programs) of the number of NO fish in the broodstock. By not "recycling" HO fish into the broodstock in successive generations (segregated broodstock management), this approach provides natural selective forces the opportunity to reverse genetic-based domestication effects associated with hatchery rearing – effects that that might accrue in the fish and progressively reduce fitness of a hatchery stock. Integrating the broodstock with NO fish 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. Results from the Johnson Creek study based on adult-to-adult return rates, indicate that NO and HO fish that contribute offspring to the next generation have similar productivity. Moreover, the productivity of HO x NO matings was similar to NO x NO matings, suggesting that negative fitness impacts to the NO population were minimal. Additional genotyping and RRS analyses for juvenile progeny collected in Johnson Creek over 5 broodyears will be completed in the coming contract year. Given the 3-4 fold greater number of juvenile progeny relative to adult progeny per broodyear, the RRS analyses will have greatly increased power to test not only for effects of origin, but also for interacting effects of parent sex, age, size and return time to the Johnson Creek weir. As additional returning adult progeny are collected over the coming years, RRS analyses will be conducted based on adult recruits-per-spawner and compared to those for juvenile recruits within corresponding broodyears. A five broodyear RRS study (2007-2011) of supplemented upper Yakima River spring Chinook, to be performed collaboratively by CRITFC (with Project funding) and by WDFW (with YKFP funding) was initiated in 2014. Similar to the Johnson Creek study, productivity estimates will be compared between natural and hatchery origin fish, assessed for effects of parent sex, age, size and return time. The study Yakima involves a sample number that is several fold greater than the Johnson Creek adult-to-adult study, and thus constitutes an even stronger test of the supplementation approach.

The Project also supports RRS studies of three reintroduced populations, each involving spring/summer) Chinook salmon, in the Hood River, Newsome Creek and Lookingglass Creek (Project Objective #2). Reintroduction programs typically begin with stocking of fish from out-of-basin hatchery stocks (sufficient number of natural origin fish from an appropriate population not being available). Initially, these fish are anticipated to exhibit a decreased level of natural productivity, due to negative genetic (domestication) effects associated with repeated generations of segregated hatchery rearing. In contrast, after adults return in basin following the initial years of stocking with out-of-basin juveniles, the program will transition to using HO fish for broodstock collected in-basin for continued supplementation, and eventually using NO fish as broodstock. Presuming that the source stock for a program retains the potential to respond to supplementation management strategies and to natural selective forces, it is expected that natural populations will be created that over successive generations that 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 HO/NO fish). Adaptation of reintroduced fish in these programs would be suggested by NO/HO 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.

Results for the RRS study of reintroduced spring Chinook in the Hood River (brood years 1992 to 2005) are complete. While low sample number limited power of the statistical analyses, results nonetheless indicated that as hypothesized, NO fish of the reintroduced stock (of an interior Columbia River genetic lineage) generally exhibited greater productivity than first generation hatchery origin fish. Interestingly, the genetic analyses also indicated the unanticipated presence of fish from a second stock of spring Chinook - a lower Columbia River lineage. These fish apparently naturally colonized the Hood River concurrently with the hatchery reintroduction program. NO fish of this lower Columbia stock also exhibited greater productivity than HO fish, and NO fish from this stock demonstrated even greater productivity than NO fish of the introduced interior stock. A manuscript summarizing results from this study was recently revised and resubmitted for publication, in the North American Journal of Fisheries Management.

Results for the genetic analyses of reintroduced Newsome Creek spring Chinook have been summarized in interim technical reports. To present, adult return numbers to Newsome Creek have been low, severely limiting power to make reliable estimates of adult-to-adult productivity. For this reason, juvenile sampling rate was increased in 2010, to at least permit RRS assessments based on juvenile recruits-per-spawner. The juvenile out-migrants were collected as age 0+ parr and age 1+ smolts; among the parr most were non-maturing, although a small proportion of them were exhibiting precocial maturation. Juvenile productivity analyses for broodyear 2010 adults were equivocal, with NO fish showing greater productivity in some comparisons and lower productivity in others. Of note, parentage analyses identified numerous errors in the field data for identification of sex and origin of the returning adults, and corrections to the field data were made prior to the RRS analyses.

Genotyping of samples from reintroduced Lookingglass Creek spring Chinook is ongoing. The first hatchery origin fish from a Catherine Creek (a nearby Grande Ronde River tributary) stock were reintroduced into Lookingglass Creek above the hatchery weir in 2004, and the first age 4 NO adults returned in 2008. Tissue samples have been collected from all adults intercepted at the weir since reintroduction in 2004, as well as samples from out-migrating juveniles beginning in 2008. Genotyping of the juvenile samples, representing the progeny from six consecutive broodyears (2008 to 2013), will be completed in the coming contract year, following which parentage and RRS analyses based on juvenile recruits-per-spawner will be performed. Besides an effect of origin (NO versus HO), the analyses will also test for interacting effects of parent sex, age and size. Similar to the Johnson Creek study, as additional adult samples are collected each year, RRS analyses based on adult recruits-per-spawner will be compared to those based on juvenile recruits in corresponding broodyears.

The Project is supporting genetics studies associated with two other reintroduction projects, both involving sockeye salmon (Project Objective #3). One study, which began in 2009, involves 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 indicate very strong spatial segregation and temporal segregation for spawning of fish between stocks – Wenatchee fish spawn early in the season and in areas of the Cle Elum River upstream of the lake, while Okanogan fish spawn predominantly later in the season, and in areas of exposed lake bed at the head of the lake, and to some extent within the lake itself. GSI analyses of out-migrating juveniles showed very few of them to be inter-stock hybrids. Stock proportions among out-migrating juveniles have been variable relative to the proportions of translocated adults, although comparison of these proportions is confounded by apparent differences in juvenile age at out-migration between stocks. While the rate of adult returns from the first two years of translocation were very encouraging, returns in 2015 (progeny of adults translocated in the third year of the project) were unfortunately extremely low, due to excessively high temperatures in the lower Yakima River (and across the entire Columbia basin) during the July-August migration period. Continued sampling and genetics analyses over the coming years should provide information: 1) to assess relative productivity between stocks, as well as between translocated adults and Cle Elum Lake natural origin adults of the corresponding stock, and 2) to monitor the extent to which fish of the two stocks remain genetically distinct, or whether over time there is a gradual integration of the fish into a new “hybrid” Cle Elum stock.

The second study involves a CTWSRO/ODFW/PGE project to create a sockeye run in the Deschutes River, facilitated by reestablishment of downstream passage of out-migrating Lake Billy Chinook kokanee juveniles, plus other juveniles that may have migrated downstream from Suttle Lake in the Metolius River basin. Initial genetic analyses indicate that the Lake Billy Chinook and the Suttle Lake stocks are each a composite of multiple source populations due to past stocking of kokanee from out-of-basin hatcheries, although the two stocks do exhibit some level of genetic differentiability. Initial anadromous *O. nerka* returns to the Pelton trap have been very few in number. Additionally, significant passage constraints persist in the creek linking Suttle Lake to the Metolius River, precluding their eventual contribution to an anadromous population. The Project will continue to support genetic analysis of adults returning to the Pelton trap each year. Of note, given the genetics results indicating a strong identity with the out-of-basin kokanee stocks, and poor adult returns seen to present, co-managers have begun discussion about modified approaches to the reintroduction program (e.g., adult translocation and/or hatchery supplementation). Should the program change, we will endeavor to continue to support genetic studies that might provide valuable information with which to assess and adaptively manage the new approach.

In 2015 the Project supported participation of CRITFC personnel at several different inter-agency meetings that involved discussion of hatchery management approaches associated with management of regional fisheries (Project Objective #5). One manuscript is currently under review for publication in a science journal. Other studies are at different stages of analysis, with results from each to be summarized in technical report and in a manuscript for submission for publication in peer-reviewed scientific journals (Project Objective #7). Together these efforts are helping to advance our understanding of how hatchery rearing may affect productivity characteristics of salmon, how supplementation and reintroduction/supplementation programs may affect VSP parameters of associated natural populations, and how the hatchery tool may best be used to achieve objectives for regional fisheries management.

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