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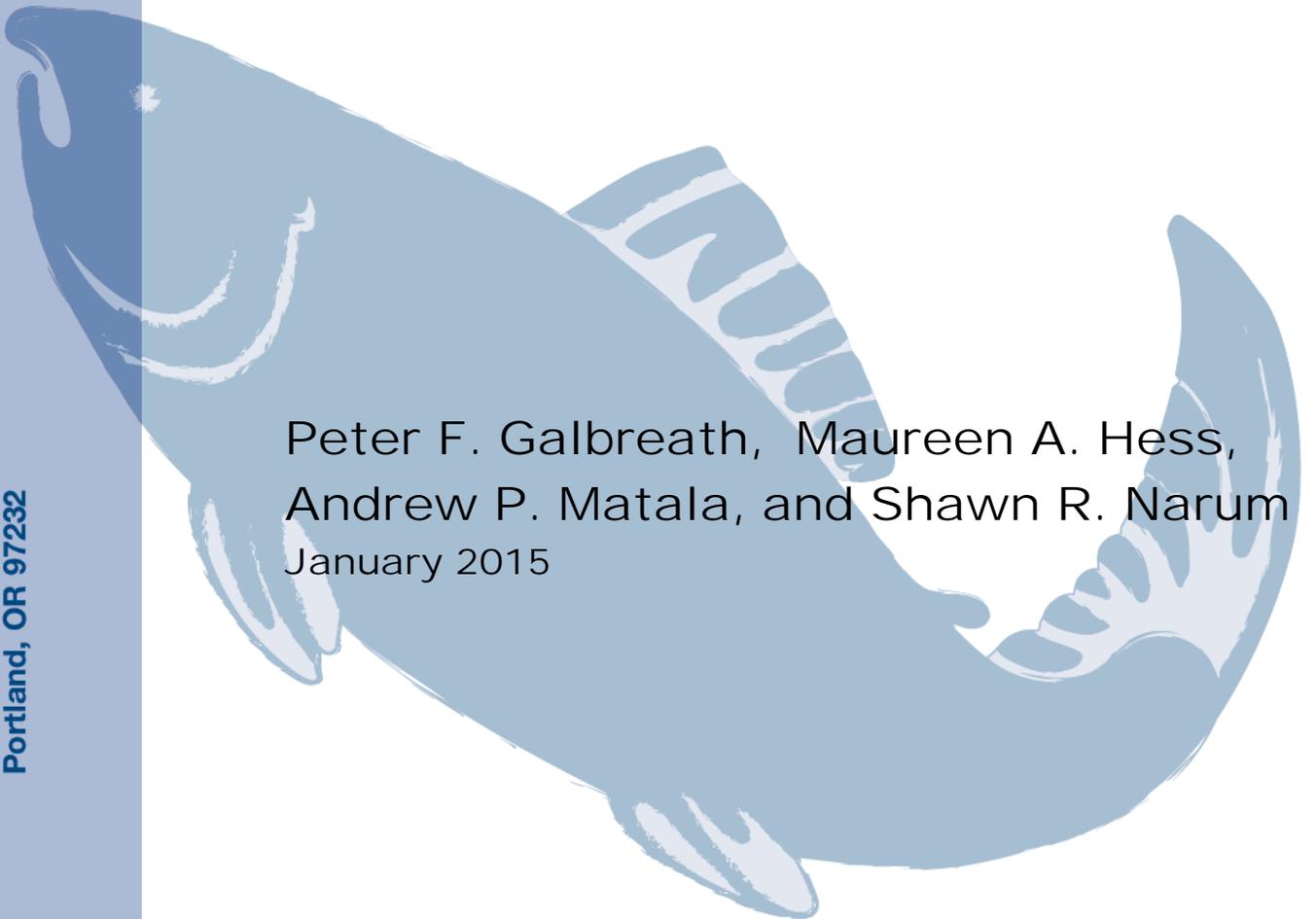
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Basinwide Supplementation Evaluation Project: 2014 Annual Progress Report

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I. Executive Project Summary

This report summarizes activities for the 2014 calendar year, performed as part of the multi-year Basinwide Supplementation Evaluation project 2009-009-00 (hereafter the Project), Contract #65188. The report is organized under the seven 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 salmon populations, as well as on programs to establish new natural populations, through stocking of fish of out-of-basin fish hatchery origin or of natural 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 results indicate that the program is indeed providing a demographic boost to the depressed population with little or no apparent deleterious effect on population productivity (Hess et al. 2012). A similar though larger scale RRS study was initiated during this contract year to assess RRS of supplemented upper Yakima River spring Chinook. A RRS study of reintroduced spring Chinook in Hood River is complete (Project Objective #2) and a manuscript submitted for publication in a scientific journal summarizing results is under revision (Project Objective #7). Results indicate that natural origin fish of the reintroduced stock generally demonstrate improved productivity, suggestive of adaptation of the reintroduced fish to the new environment. Additional RRS studies of reintroduced spring Chinook are ongoing in Lookingglass Creek and Newsome Creek (Project Objective #2). Genetic analyses financed by the Project are also being conducted to assess relative spawning and rearing success of reintroduced sockeye salmon in the Cle Elum Lake/upper Yakima River system, and to perform genetic stock identification to examine relationships among *O. nerka* (kokanee) in Lake Billy Chinook on the Deschutes River, in the Metolius River, and in Suttle Lake, as well as 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). Two 2-day "Introduction to Molecular Genetic Analyses in Tribal Fisheries Management" training programs sponsored through the Project were attended by 15 tribal fisheries biologists and technicians (Projective Objective #4). 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 #5). A 3-broodyear study to test for an effect of spring Chinook broodstock on survival, size and minijack production among their progeny was initiated in 2014 at the Cle Elum Supplementation Research Facility. As results accumulate from each of the various studies supported by the Project, they are summarized in study-specific technical reports and oral presentations, and in manuscripts submitted for publication in scientific journals (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 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 2014, these activities included ones to:

- use genetic analyses to derive productivity information with which to assess RRS of natural 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 spring Chinook that were reintroduced in the Hood River, Newsome Creek (Clearwater River basin) and in Lookingglass Creek (Grande Ronde River basin) (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 on the Deschutes River, in the Metolius River, and in Suttle Lake, and 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 initiate a new study, conducted with spring Chinook at the Cle Elum Supplementation Research Facility, to examine the effect of hatchery broodstock age on survival, size and minijack rate among their juvenile progeny (Project Objective #6)
- 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 project reporting (Project Objectives #4, #5 and #7, respectively).

III. Work Elements / Tasks

A. Project Administration

Activities in 2014 involving administration of the Project by CRITFC included: production and posting in PISCES of the annual progress report for 2013, completion of quarterly and final status reports in PISCES that record progress associated with each work element within the contract Statement of Work, and submission of 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 # 65188 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, and as part of an associated monitoring program NPT biologists have collected tissue samples and biodata on all returning adults intercepted at a weir (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. Funding from the Project was therefore used to produce SNP genotypes for samples from a limited number of prior broodyears to cover the transitional period (beginning with return year 2008).

Results from RRS analyses for the initial two broodyears described in Hess et al. (2012) and confirmed in analyses for an additional three broodyears (Hess, unpublished data), indicated 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. 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 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 effects of parent sex, age, size and return time to the Johnson Creek weir. Increased juvenile sampling began the same year, as well as supplemental funding from the Project to cover the additional laboratory costs in 2010. In 2014, approximately 3,900 additional adult and juvenile tissue samples collected by the NPT were sent to HFCES for genotyping. Following collection of a sample of yearling juvenile out-migrants in spring 2015, sampling will be complete for five consecutive broodyears. Genotyping of all samples will be completed in the 2015-2016 Contract Year, after which the data will be entered into a juvenile recruits-per-spawner RRS analysis for the five broodyears (2009 through 2013) to test for effects of the above mentioned factors on natural productivity. Results will be subsequently compared to those based on adult recruits-per-spawner for the same broodyears, as the associated adult progeny return over the coming years.

<u>Broodyear</u>	<u>Juvenile Out-migrants</u>	
	<u>fall (age 0+)</u>	<u>spring (age 1+)</u>
2008		244
2009	2537	754
2010	2547	754
2011	2343	734
2012	1185	750*
2013	1500*	na

(* estimates)

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 annual 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, where they were held for several weeks prior to release. The first age-4 adults returned to the Yakima River in 2001. 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., in press). 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 fish 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.

The intention for this program has been to complement the initial spawning channel RRS study with similar analyses for the population as a whole. However, funding to present has been insufficient to take on the full expense for genotyping the thousands of adults returning in-basin each year. With the development of SNP markers for Chinook salmon, and reduction in per-sample genotyping costs, such a large scale RRS study can now be envisaged. Discussions between YN, WDFW and CRITFC were held in 2014, in which an accord was reached to perform a joint RRS study of naturally spawning NO and HO fish in the upper Yakima, covering three consecutive broodyears (2007-2009) and their respective adult progeny (returning between 2010 and 2014); the total number of samples involved is approximately 25,000:

<u>Adult Spawners</u>		
	Natural	Hatchery
<u>Broodyear</u>	<u>Origin</u>	<u>Origin</u>
2007	1285	1504
2008	1220	3114
2009	2544	4494
<u>Adult Progeny</u>		
2010	433	BY 07 jacks (analyses jacks only)
2011	4395	BY07 4 yr olds + BY08 jacks
2012	2704	BY07 5 yr olds + BY08 4 yr olds + BY09 jacks
2013	3000*	BY08 5 yr olds + BY09 4 yr olds (exclude jacks)
2014	500*	BY09 5 yr olds (exclude jacks)
TOTAL	25193*	(* estimates)

Genotyping of some samples is to be performed by WDFW using YKFP funds, with the other samples to be genotyped by CRITFC at the HFCES using Project funds. Tissue samples from all returning adults, collected annually by the YN at the Roza Dam Adult Collection Facility, are currently archived at the WDFW Molecular Genetics Laboratory, Olympia WA. Genotyping for this study was initiated in 2014 by CRITFC for samples collected in 2007 (n = 2,789) and a portion of samples collected in 2011 (n= 2,915). Genotyping by CRITFC and WDFW will continue through 2017, after which the data will be analyzed to assess relative productivity, and results will be summarized 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 (Columbia River) and Hells Canyon (Snake River) dams. 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 or more of strictly natural production (Phillips et al. 2000; Underwood et al. 2003; Lutch 2005; Murdoch et al. 2006; Bosch et al 2007; Narum et al. 2007; Yakama Nation 2011; Yakama Nation Fisheries Resource Management 2012, Galbreath et al. 2014).

The broodstock management protocol for these reintroduction programs involves the progressive phasing out of juveniles from out-of-basin hatcheries. Instead, broodfish are increasingly being 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 with improved ability to adapt 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 relative reproductive success (NO/HO) should be greater than 1.0. To test this hypothesis, the Project has 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 to be 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 Carson National Fish Hatchery (NFH) juveniles. Managers then switched to use of Deschutes River stock from Round Butte Hatchery, with occasional input from the nearby Warm Springs NFH as needed. Stocking of age 1+ smolts resumed in 1995 and has continued annually since. (Underwood et al. 2003, Reagan 2011). Beginning in 1992, scales and ancillary biodata (sex, size, date, etc.) were collected for nearly 100% of in-migrating adult Chinook salmon intercepted in 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 have been stored in individual envelopes at 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 suite of microsatellite DNA loci, parentage analysis, and estimation of RRS by sex and broodyear. Approximately 8,300 individuals collected from 1992 through June 2010 (when Powerdale Dam was decommissioned) were sampled and genotyped. Parentage and statistical analyses were completed in 2013. Results indicate that the reintroduced stock of spring Chinook is establishing a natural population, and that as hypothesized, the NO fish generally demonstrate improved productivity relative to HO fish of the same stock within broodyears. Interestingly, the data also indicates that some of the fish are derived from an alternative lower Columbia River genetic lineage which appears to have colonized the Hood River. The lower Columbia 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). The Carson and Deschutes stocks chosen for the reintroduction effort belong to a group of interior Columbia stream-type populations. Fish (both NO and HO) of the lower Columbia 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 lower Columbia lineage is insufficient to determine the source population(s) of these Lower Columbia colonizers. There are 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. 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 for adults collected over the 19 run years. Relative productivity for NO and HO fish (RRS; natural-origin/hatchery-origin) was then assessed within sexes and within all broodyears where a minimum of 10 adults per origin were available; which due to low return rates in many years, limited the number of possible comparisons. The NO showed statistically higher productivity in two of five broodyears for females and in one of four broodyears for males, although overall average RRS did not statistically differ from 1.0 in either sex:

	<u>Broodyear</u>	<u>RRS (NO/HO)</u>
Females	2000	1.46
	2001	0.79
	2002	2.60 *
	2003	5.75 *
	2005	<u>1.26</u>
	Overall	2.11
Males (age 4 and 5 only)	1997	0.98
	2002	0.63
	2003	3.62 *
	2005	<u>1.89</u>
	Overall	0.82

(*indicates significant differences, $p < 0.05$ or 95% CI did not include 1.0)

While origin had the largest effect on fitness, later return-time and larger body length were also associated with fitness; traits which were more common in NO fish. Analyses also indicated that fish of the Lower Columbia genetic lineage (apparent natural colonizers) demonstrated significantly higher productivity than the NO fish of the reintroduced interior Columbia stock. A manuscript summarizing these results is currently under revision and will be resubmitted for publication in a scientific journal in 2015.

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). The Lookingglass Hatchery program has been used to supplement tributary populations within the subbasins. 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 source stocks were successively used for hatchery production; initially from Carson NFH, then Wind River stock, Imnaha River, and finally Rapid River Hatchery stock propagated in the Little Salmon River subbasin of Idaho. Despite these efforts, a naturally spawning population never fully established itself in Lookingglass Creek. Co-managers (CTUIR and ODFW) therefore decided to cease use of an out-of-basin stock, and instead collect adult broodstock from the Catherine Creek population. Catherine Creek is a tributary to the Grande Ronde River, located downstream from Lookingglass Creek. 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 remnant Rapid River origin fish. A new attempt to reintroduce spring Chinook salmon began with hatchery spawning in 2000 of adults collected from Catherine Creek, and release their juveniles from the hatchery in 2001 and 2002 (Boe et al. 2010). In 2004, the first adults from these hatchery releases returned to the weir. Then and each year since, a portion of the fish were selected for use as broodstock, and the remainder were passed upstream for natural spawning. In 2007, the first naturally spawned adults (age 3) from the new reintroduction program returned in-basin. Beginning in 2004, CTUIR biologists collected tissue samples from all these adults and archived the samples in anticipation of eventual genetics monitoring to assess return rates and productivity.

Additionally, systematic sampling of out-migrating NO juveniles, captured in a rotary screw trap located ¼ km downstream of the weir, began in 2008. Juveniles have been captured both as age 0+ parr in the summer and fall, and as age 1+ smolts in the spring (Boe et al. 2011).

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, beginning with collection year 2014 the decision was made to switch to a panel of ~300 SNP markers. Recent advances in laboratory techniques have substantially lowered the per-samples costs for genotyping, and the increased number of markers will increase the resolving power of the parentage analyses. Since the agreement was reached tissue samples have been collected and sent to HFCES for genotyping, including: all adults intercepted at the Lookingglass weir, adult carcasses, out-migrating juveniles (age 0+ parr and age 1+ smolts), including 1,356 samples from 2014. The number of samples collected and genotyped per calendar year is presented in the table below.

Run Year	Adults – Natural Spawners, Juveniles - Parr	
	<u>Carcasses and Broodstock</u>	<u>and Smolts</u>
2004	22	0
2005	49	0
2006	305	250
2007	139	0
2008	368	250
2009	222	249
2010	561	542
2011	955	500
2012	1139	544
2013	388	740
2014	787	316
2015	na	na

Out-migrating smolts from the 2013 broodyear will be collected in spring 2015, and genotyped in the 2015-2016 Contract Year. At this time, data for six consecutive broodyears (2008 through 2013) of adults released for natural spawning and their respective juvenile progeny will be available. These data will be used to assess the effect of origin in RRS analyses based on juvenile recruits-per-spawner. Additionally the effects of broodyear, and parent sex and age on productivity will be evaluated. Results will be summarized in a technical report and 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 basin beginning in the 1960s, primarily through stocking of Rapid River (Little Salmon River) hatchery juveniles in 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 stocking of variable numbers of pre-smolts, smolts or adults of spring/summer Chinook produced at the Clearwater Anadromous Fish Hatchery. 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, 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 been consistently 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 a 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. 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 SNPs. 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 genetic RRS analyses.

The number of samples from in-migrating adults and out-migrating parr and smolts that have been collected and genotyped as of the end of 2014 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 sex and origin of the returning adults, revealed via the genetics and parentage analyses. Evaluations of return time and productivity relative to parental origin could therefore not be considered reliable until broodyear 2010. Genetically assigned parent and progeny trios were used to correct for errors in parental origin ID beginning with the application of BY2010 juvenile genotypes. Reliable genetic sex ID was available from SNP genotypes beginning with the 2008 adult collection.

Run Year	Adults			Parr	Juveniles	
	Natural Origin	Hatchery Origin	Carcasses		Smolts	Precocial
2008	34	23				
2009	24	8		50		
2010	22	127		313	48	
2011	44	69		496	22	97
2012	53	86		856	292	

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 were equivocal. In 3 of the 6 comparisons HO fish were on average numerically less productive, and in the other 3 comparisons they were more productive (Matala and Narum 2012 and 2014).

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

D.1 Cle Elum 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 (Bureau of Reclamation 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) on 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 - Lake Wenatchee/Wenatchee River stock, and Osoyoos Lake/Okanogan River stock. 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 adaptive potentials between donor stocks 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; the latter involves lake bottom areas that are exposed during the low water period in the late summer-fall. This area is characterized by numerous braided water channels fed by hyporheic flow. Initial results indicate that spawning in these two areas appears to be temporally and spatially bimodal. Spawning during the early pulse occurs to a greater extent in the river upstream of the lake. A later pulse of spawning appears to occur predominantly in the braided channels in the exposed area at the head of the lake. Beginning in 2011, out-migrating *O. nerka* smolts 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).

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. 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 assignment accuracy to stock-of-origin, as well as for identification of inter-stock hybrids. Since that time, YN has collected tissue samples 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), out-migrating juveniles sampled at the Chandler and Roza facilities, and from in-migrating adults collected in the fish ladder at Roza Dam. All collections are sampled before transport and release into Cle Elum Lake. Number of samples per fish type and year that have been genotyped (or will be genotyped by the end of the current contract year) are illustrated below:

<u>Year</u>	<u>PRD</u>	<u>Adults</u>			<u>Juveniles</u>
		<u>Roza</u>	<u>Carcasses</u>	<u>Gillnet</u>	
2009	0	18	0	0	0
2010	0	41	0	0	196
2011	275	###	31	0	450
2012	849	148	90	0	108
2013	250	688	110	0	446
2014	350	2575	377	63	108

Results of analyses through May 2014 indicate the following: 1) the 2009 to 2012 Roza returns were identified as likely “fall-backs” from out-planted fish that entrained down river from the lake after translocation, and nearly all were assigned as Okanogan stock; 2) fewer than 1% of NO out-migrating juveniles were identified as inter-stock hybrids, indicating limited interbreeding between Okanogan and Wenatchee out-plants; 3) carcasses collected in 2011 and 2013 in the river upstream of the lake were dominated (83 of 84) by Wenatchee stock, while carcasses collected in the exposed area at the head of the lake (26 of 26) were all of Okanogan stock, indicating spatial spawning segregation; 4) the majority of out-migrating smolts collected in 2012 and 2013 were Wenatchee stock (which comprised approximately 25% of out-planted adults), indicating successful production by both donor stocks but favoring the Wenatchee stock; 5) Okanogan stock represented a larger proportion of the first returning adult progeny of naturally spawning out-plants (2013), although this result may be biased by “fall-backs” from among the 4,000 sockeye out-planted from PRD in 2013 (see Matala et al. presentation “Genetic monitoring of sockeye salmon reintroduction into Cle Elum Lake” presentation in Attachments in the Project site in PISCES). More intensive carcass sampling was conducted in 2014, and more intensive juvenile sampling is planned in 2015, to help clarify these assessments.

D.2 Deschutes River sockeye salmon/kokanee

Suttle Lake was a nursery lake for sockeye salmon, located in the headwaters of the Metolius River, a tributary to the Deschutes River. 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. Another barrier was constructed downstream in Lake Creek (the stream which

connects Suttle Lake to the Metolius River), and the sockeye population subsequently went extinct. However, a limited amount of spawning of sockeye persisted in the Metolius River below these obstructions, with the 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 contribution from fish of the remnant *O. nerka* population in the Metolius River, versus kokanee from out-of basin. 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. 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. Importantly, these lakes were also both repeatedly stocked through the mid-1900s, with kokanee juveniles from various out-of-basin hatchery sources (Nehlsen 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* emanating from LBC and/or Suttle Lake retain the capacity to exhibit 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 out-migrating *O. nerka* smolts captured at the FTF have been passed downstream annually. CTWSRO is leading the monitoring program of upper Deschutes *O. nerka* (kokanee and sockeye). The primary question of interest to the co-managers (ODFW, CTWSRO, and Portland General Electric - PGE) do the *O. nerka* in the basin possess sufficient sockeye salmon genetic heritage from the historical local stock with which to demonstrate an anadromous life history? Secondly, is there a greater propensity for anadromy within one stock or the other (LBC versus Suttle Lake)? What are the relative stock proportions of the two stocks among the juveniles passed downstream at the FTF? What are the relative stock proportions among returning mature adults? Do fish from the two stocks exhibit differences in age, size or timing at migration? How best might this information be used to adjust management of the reintroduction program?

In 2011, an agreement was reached with CTWSRO for the Project to finance a genetics study of the *O. nerka* program, involving genotyping for a standardized panel of 96 SNP markers. Genetic analyses are performed on tissue samples collected from both juveniles and adults from the two (albeit interconnected) systems, and from in-migrating adults captured at the Pelton adult trap (including some samples collected and archived prior to the agreement). The numbers genotyped to present are illustrated below:

Year	Adults			Juveniles	
	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

Genetic analyses indicate multiple origins in both the LBC and Suttle Lake stocks, presumably associated with past kokanee stocking from out-of-basin hatchery sources, and some likely stocked in both

Deschutes Basin locations. Nonetheless, differences in the genetic profiles between stocks are apparent and of sufficient magnitude to be generally useful for differentiating LBC versus Suttle Lake origin fish. Genotyping of returning adults captured at the Pelton trap showed that the majority assigned to the LBC population, as might be expected given the much larger size of the LBC population, with only four assignments to Suttle Lake/Link Creek among the 174 fish collected between 2009 and 2013. The analyses also revealed a few high probability genetic assignments to Okanogan River and Wenatchee River stocks, and a single fish in 2013 to the endangered Redfish Lake stock, all representing apparent strays into the Deschutes River (see Matala et al. presentation “Genetic Characterization of Deschutes River *O. nerka*” in Attachments for the previous Project contract #61294).

E Project Objective #4: Coordinate inter-tribal workshops and genetics training programs

Tribal fisheries personnel are involved in monitoring and evaluation programs of essentially all 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 used to guide management. Conversely, the CRITFC 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” training 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 made each of the two days by one of the participants, who reviews a tribal project on which he/she works.

In 2014 two more of these workshops were conducted (November 17-18, and December 9-10). Participants included 15 biologists and technicians working for tribal fisheries agencies - CTUIR (4), YN (4), NPT (2), and CRITFC (5). Lodging and per diem for each of the participants was financed through the Project. Copies of the 2013 program schedule and presentations are available at: <http://www.critfc.org/fish-and-watersheds/fishery-science/hagerman-genetics-laboratory/genetics-training/>.

F. Project Objective #5: 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:

- Feb 4-6: Attendance at the Willamette Basin Science Review meeting (Portland OR)
- March 11-12: Attendance and presentation ("Genetic Characterization of Deschutes River *O. nerka*"; A. Matala, P. Galbreath and J. Hogle) at the 2014 Pelton-Round Butte Project Fisheries Workshop (Madras OR)
- March 18-20: Attendance at the 2014 Pacific Coast Steelhead Management Meeting, (Skamania WA)
- April 15: Attendance at the Klickitat and White Salmon Rivers (Columbia Gorge) Fisheries and Watershed Conference (The Dalles OR)
- April 23-24: Attendance at the Future of Our Salmon 2014 Conference (Portland OR)
- April 29-30: Attendance at the Peer Review Workshop Okanagan Sockeye Re-introduction Experiment: Lessons & Future Direction" conference (West Kelowna, BC Canada)
- June 18-19: Attendance and presentation ("Genetic Monitoring of Sockeye Salmon: Evaluating Relative Productivity Among Two Donor Stocks"; A. Matala, P. Galbreath, B. Saluskin and M. Johnston) at the annual Yakima Basin Science and Management Conference (Ellensburg WA)
- August 17-21: Attendance at the 144th Annual Meeting of the American Fisheries Society (Quebec City, Quebec Canada)
- December 2-4: Attendance at the 65th Annual Northwest Fish Culture Conference (Pendleton OR)

G. Project Objective #6: 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 affect the rate of 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, a biochemical assay (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 from each pre-smolt will also be genotyped, and parentage analysis used to identify each individual to its parental mating. The proportion of minijacks within each 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:

	<u>Male Age-3</u>	<u>Male Age-4</u>	<u>Male Age-5</u>
Female Age-4	25	25	19
Female Age-5	5	11	2

Survival of the fertilized eggs was estimated at the eyed stage (December 2014), and survival and length and weight of a sample of fry from each cross will be measured in March 2015. A sample of 50 fry per cross will then be pooled and transferred to an outdoor raceway for a year of rearing. In early 2015, blood samples will be collected from approximately 300 BY 2013 pre-smolts (the juveniles that are sampled by US FWS for disease analysis). These samples will be used for initial testing of the hormone assay procedures by CRITFC personnel at the University of Idaho.

In March-April 2016 the BY 2014 juveniles will be sacrificed, measured for length and weight, fin-clipped and blood sampled, then dissected to identify phenotypic sex. The fin clips from all male progeny will be genotyped by CRITFC geneticists at HFCES and assigned to full-sib family (brother) groups following parentage analysis. Blood samples from the males will be assayed for 11-ketotestosterone for determination of their status as maturing (minijack) versus non-maturing (Larsen et al. 2004). A minijack rate will then be estimated for each family 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 broodyears then be subjected to a comprehensive statistical analysis to test for an effect parent age on minijack rate, results from which will be summarized in a manuscript to be prepared for publication in a scientific journal.

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

A manuscript entitled “Tribal reintroduction of extirpated coho salmon to the interior Columbia River basin”, submitted in 2013 to Fisheries (American Fisheries Society), was published in February 2014. The manuscript provides an overview of methodologies and results for tribal programs to reintroduce coho salmon into the Yakima, Wenatchee, Methow and Clearwater rivers.

A manuscript summarizing results of the RRS study of reintroduced Hood River spring Chinook is undergoing revision, and will be resubmitted for publication in a scientific journal in 2015.

IV. Synthesis of Findings: Discussion/Conclusions

Project activities are centered around the theme of Hatchery RM&E, with particular focus on assessing effects of supplementation hatchery rearing on life history and natural productivity of HO 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 within the Columbia basin that use only NO fish as broodstock. As such, these programs are the only ones that apply the recommendation to maximize (100% as possible) the percent of NO hatchery broodstock, made by Cuenco et al. (1993), who on behalf of the tribes, provided the first detailed description of the principles for a new management approach to hatchery production - supplementation. Initial results from the Johnson Creek study (genetic analyses financed primarily by the NPT, with a minor contribution from the Project), based on adult-to-adult return rates, indicate that NO and HO fish have very similar productivity. Additional genetic analyses, financed through the Project, for juvenile progeny collected in Johnson Creek over 5 broodyears will be complete in the coming contract year. These data will be subjected to an in-depth RRS assessment, based on juvenile recruits-per-spawner, to test for effects of origin concurrently with effects for 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 these broodyears. A three broodyear RRS study (2007-2009) of upper Yakima River spring Chinook, to be performed collaboratively by CRITFC (with Project funding) and by WDFW (with YKFP funding) was initiated in 2014. The study involves a sample number that is approximately 10-fold greater than the Johnson Creek adult-to-adult. 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 Project also supports RRS studies of three reintroduced populations, 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. Initially, these fish are anticipated to initially exhibit relatively low natural productivity, due to negative genetic effects associated with repeated generations of hatchery rearing. However, presuming that these stocks retain the potential to respond to 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 would be suggested by RRS ratios for NO/HO 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 Hood River study, involving analysis of DNA extracted from archived scale samples collected from returning adults from 1992 to 2010, are complete. Results have been summarized in a manuscript that is currently undergoing revision, after which it will be resubmitted for publication. While low sample number limited power of the statistical analyses, results nonetheless indicated that, as hypothesized, NO fish of the reintroduced stock (an interior Columbia River lineage) generally exhibited greater productivity than first generation hatchery origin fish. Interestingly, the genetic analyses also indicated the unexpected presence of fish from a second stock of spring Chinook - a lower Columbia River lineage. These fish apparently colonized the Hood River on their own, and have persisted over the years since introduction of the interior Columbia stock. In addition, the lower Columbia stock demonstrated greater productivity than NO fish of the introduced interior stock.

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, 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 juveniles fall within one of three categories: non-maturing age 0+ parr, age 0+ precocial parr, and age 1+ smolts. 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 Catherine Creek (a nearby Grande Ronde River tributary) stock fish were reintroduced into Lookingglass Creek above the hatchery weir in 2004, and the first NO adults returned in 2008. Adult progeny (age 3, 4 and 5) from the 2004 broodyear were complete all returned by 2013. As such we have only begun to accumulate data with which to perform RRS analyses based on adult-to-adult returns. On the other hand, as of spring 2015, we will have data available with which to conduct a RRS assessment based on juvenile recruits-per-spawner for six consecutive broodyears (2008 to 2013). This analysis will be performed over the coming contract year, involving testing for an effect of origin and parent sex, age and size. Similar to the Johnson Creek study, as additional adult samples are collected each year, RRS analyses will be conducted based on adult recruits-per-spawner and compared to those for juveniles within the 2008 to 2013 broodyears.

The Project is supporting genetics studies associated with two other reintroduction projects, both involving sockeye salmon (Project Objective #3). One study is of sockeye salmon that are being reintroduced to Cle Elum Lake, through annual out-planting of adults collected at Priest Rapids Dam, which began in 2009. These adults are a mix of approximately $\frac{3}{4}$ Okanogan River and $\frac{1}{4}$ Wenatchee River stock. Initial genetic analyses of carcasses indicated spatial segregation of spawning fish between stocks – Wenatchee fish spawning in the Cle Elum River upstream of the lake, and Okanogan fish spawning in exposed areas at the head of the lake. Analyses of out-migrating juveniles indicated them to be predominantly of Wenatchee stock, although the returning adults were predominantly Okanogan stock. In both cases very few fish appeared to be inter-stock hybrids. Of note, all of these observations are as yet preliminary due to limited sample sizes. Sampling rate was increased this past year and future analyses should clarify questions regarding spatial, and possibly temporal, segregation in spawning between the two stocks, and regarding relative productivity of the stocks.

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 kokanee juveniles from Lake Billy Chinook, plus other juveniles that may have migrated downstream from Suttle Lake into 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 fish from out-of-basin hatcheries, although the two stocks do exhibit some level of genetic differentiability. Initial sockeye salmon returns to the Pelton trap from the kokanee juveniles passed downstream from the FTF have been very few in number. Additionally, significant passage constraints persist in the creek linking Suttle Lake to the Metolius River. Sampling will continue over the coming years, although a comparison of the relative propensity for anadromy between fish from the two lakes will not be possible until higher out-migration success of Suttle Lake juveniles is established, and/or until a translocation program for juveniles from Suttle Lake to the Deschutes River below the Pelton-Round Butte complex is initiated.

In 2014 the Project supported participation of CRITFC personnel at several different forums that involved discussion of hatchery management approaches associated with management of regional fisheries (Project Objective #5). One manuscript was published in a science journal, and another is under revision. Other studies are at different stages of analysis, with results from each to be summarized 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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