WASHINGTON DEPARTMENT OF FISH AND WILDLIFE

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Evaluating the Reproductive Success of Natural- and Hatchery-Origin Columbia River Chum Salmon

(FCRPS BiOp Action #182)

Proposal Contacts:

Steve Schroder
600 Capitol Way North
Olympia, WA 98501-1091
(360) 902 – 2751
schrosls@dfw.wa.gov

Howard Fuss
600 Capitol Way North
Olympia, WA 98501-1091
(360) 902 – 2664
fusshjf@dfw.wa.gov

Project Summary

Beginning in 1998 the Washington Department of Fish and Wildlife began a chum salmon supplementation program in the Grays River, a Washington State stream that enters the Columbia River at RK 50. Chum salmon native to Grays River are captured, artificially spawned, and one hundred percent of their offspring are thermally marked during the incubation period. Upon completion of the incubation period, juveniles are reared for several months prior to release into the Grays River. The fish are spawned using a factorial-mating scheme, and biological data, including length, weight, egg size, fecundity, age, and reproductive effort are collected on each adult fish used in the program. In addition, survival data from fertilization to eyeing and from eyeing to emergence are collected on the offspring produced by each female. Since its inception, the program has steadily grown from an initial release of 110,000 fry in 1999 to over 400,000 fry in 2003. The first hatchery-origin adults produced from the program returned to the Grays River in 2001 as three-year-old adults. Last year, both three- and four-year-old adult fish returned and in 2003 all three-age classes of chum (3-, 4- and 5-year-olds) will return to the stream. Before the inception of the supplementation program, one thousand or fewer chum typically returned to the Grays River. Last year the greatest number of chum ever recorded (10,200 individuals) returned. Extensive stream surveys were conducted and otoliths were collected from 1500 fish that had spawned in the river. These otoliths are currently being examined for thermal marks to ascertain the contribution rate and distribution patterns of hatchery-origin chum salmon in the Grays River. Because of the highly dynamic nature of the Grays River watershed, the chum program will continue until substantial habitat improvements can be made.

Many of the fish produced by this program spawn naturally in the Grays River. A key assumption associated with this type of recovery effort is that adults produced via hatchery intervention reproduce successfully in the wild. However, as stated in the Request For Studies Narrative, there is concern that adult salmon produced by artificial culture will not be as reproductively successful as natural origin fish. A growing body of literature suggests a number of trends. First, within a generation, the longer individual fish are held in culture the greater the likelihood they will experience genetic change (e.g., domestication), or have their individual behaviors altered by the hatchery environment. Thus, salmonids like steelhead, coho, and spring chinook that typically experience prolonged rearing periods in hatcheries are expected to be more susceptible to domestication and more likely to express mal-adaptive behaviors than species that have shorter tenures in a hatchery environment. Second, the more generations a population is subjected to a hatchery environment the greater the risk that domestication effects will accumulate and manifest themselves. Third, the impact of hatchery life on naturally spawning hatchery fish is not equivalent across sexes. Naturally spawning hatchery-origin males typically express a greater deficit in breeding success than females. Further, artificial breeding programs substantially alter traditional life-history patterns. For example, mate selection does not occur and energy that would have been allocated to territory acquisition, nest construction, redd shaping, and guarding (for females) and

searching, courting, and defense of potential mates (for males) is not expended. Instead, it becomes available for other attributes that may increase fitness under hatchery regimes and reduce it among a domesticated individual's offspring that must spawn under natural conditions.

The Grays River chum population represents the only one that we are aware of in the Columbia River that is comprised of both hatchery- and wild-origin chum salmon. Performing an evaluation of the relative reproductive success of these two types of chum salmon entirely within the Grays River watershed is however, problematic. The watershed is relatively large and thousands of adult fish are now returning to the basin each year. Sampling these fish would require significant expenditures to first collect DNA samples from the fish and next to analyze and perform pedigree analyses based on microsatellite DNA to determine parental origins. To avoid these problems, we propose to perform a direct comparison of the reproductive success of Grays River hatchery- and wild-origin chum salmon in the Elochoman River, an adjacent basin that offers many logistical advantages. It has a very small remant population of chum (5 to 30 adults/year) and thus is a logical target for a re-introduction program. It can be intensively sampled for adult carcasses, and WDFW owns land that can be used to create a semi-controlled stream where chum can spawn under observation with rigorous and efficient sampling of progeny.

Briefly our experimental design is as follows: An artificial observation stream and weir will be built on the Beaver Creek Hatchery grounds in the Elochoman watershed. For three consecutive years (2003, 2004, and 2005) wild- and hatchery-origin chum salmon from the Grays River will be captured and transported from the Grays River and released into the observation stream and allowed to reproduce naturally. A suite of behavioral and physiological measurements will be made on the adults prior to, during, and after spawning. Each fish placed into the stream will receive a Petersen Disk tag so that individual behavior can be linked to treatment origin (hatchery or wild) and, ultimately, to breeding and reproductive success. Moreover, DNA samples will be collected on each adult fish prior to being placed into the observation stream. Traps will be established in the observation stream so that the total number of fry produced by these fish can be enumerated. A representative sub-sample of the fry will be collected throughout the emergence period. Returning adults will similarly be sampled as they return. DNA samples from fry and adult specimens will be analyzed via established microsatellite DNA (msDNA) protocols to determine the parental types of each offspring. The analyses will allow us to directly establish the reproductive competence of hatchery and wild chum salmon as they spawn among themselves and with each other. We will be able to relate the reproductive performance of the parents to their hatchery or wild origins, to their sex, and to their expression of innate reproductive behaviors. In subsequent years, we will introduce a representative sample of the naturally produced returning adults into the observation stream to assess the capacity of these fish to produce F_2 offspring.

Project Description

This project is designed to address the information needs expressed in **Action 182** (FCRPS BiOp Action 182). That is, "to determine [the] relative reproductive success of natural-origin and wild-spawning hatchery-origin anadromous salmonids in the Columbia Basin". Specifically we will examine the reproductive success of Lower Columbia River chum salmon spawning in a controlled flow observation stream located in the Elochoman River basin. Our experimental design, briefly described in the above summary, allows us to 1) Directly compare the capacity of hatchery- and wild-origin chum salmon to produce offspring via natural spawning, 2) Compare the fry-to-adult survival of first generation (F_1) individuals from H x H, H x W, W x H, and W x W matings, 3) Ascertain whether hatchery- or wild-origin adults differ in their capacity to create second generation (F_2) or 'grand progeny', 4) Determine whether significant physiological and behavioral differences exist between hatchery- and wild-origin adults during the spawning and incubation periods, and 5) Assess via a microsatellite DNA pedigree approach the relationships between behavioral or physiological traits in hatchery- and wild-origin chum salmon and overall fitness as measured after one and two generations.

The study is designed so that it can be extended indefinitely beyond the F_2 generation if desired. As alluded to above, chum salmon, fall chinook, pink, and sockeye salmon may face a decreased risk of domestication because they are typically held in hatchery environments for shorter periods of time than other cultured salmonids. Nonetheless, the results of this program can certainly be applied to other salmon restoration programs throughout the basin. If significant reductions in reproductive success are found in hatchery-origin chum salmon this would suggest that similar effects are probably occurring in other species that have a more prolonged exposure period to hatchery conditions. Some previous studies that have assessed the relative reproductive success of hatchery- and wild-origin salmonids have imported hatchery-origin individuals into a watershed and then compared their reproductive success with native fish. Clear differences have been demonstrated, yet two factors -- exposure to hatchery conditions and a transplantation effect -- may have been responsible for the differences seen. Our design avoids this difficulty because both hatchery- and wild-origin chum salmon from the same population are being introduced at the same time into a new environment.

Statement Of Work

Attempting to decipher differences in the reproductive success of hatchery- and wild-origin salmonids is daunting. For example, consider the logistical challenges associated with the capture of hatchery- and wild-adults and the creation of a suitable location for the fish to spawn that permits observations on the adults and also allows us to capture their offspring. Moreover, laboratory time and costs associated with sampling and analyzing DNA samples and performing pedigree analyses will be time consumptive and expensive. The approach we present below represents the combined thoughts of agency geneticists, regional biologists, and research scientists that are either currently involved with chum recovery in the Lower Columbia River or who are engaged in other evaluations of hatchery- and wild-origin reproductive success projects. What follows is a

brief step-by-step description of a project that we believe most efficiently addresses critical questions with a design conferring the greatest likelihood of success.

1) Project Location -- why the Elochoman basin was chosen:

The most direct way to compare the reproductive success of fish representing two treatments (hatchery and wild) is to create a situation where almost all the fish returning to a stream have originated from your study. This clearly would not be the case if we established a spawning area in the Grays River. Currently, many thousands of chum return to this watershed. Thus, to find adult fish produced from an in-basin study would require an extensive field sampling effort and costly laboratory evaluations of microsatellite DNA on the sampled adults. Consequently, we opted to create a situation where almost all the chum adults returning to a stream would be from our study. The Elochoman River was chosen because it historically supported chum salmon, is close to the Grays River, has similar geological and hydrographic features, and therefore should be readily colonized by Grays River chum. Also, WDFW owns property in the lower river where a controlled-flow observation stream and adult weir can be constructed. Furthermore the Elochoman is a smaller basin than the Grays and thus can be sampled more efficiently.

2) The need for an observation stream

There are three reasons why an observation stream is needed to perform this evaluation. First, a sequestered location where hatchery- and wild-origin adults can reproduce is obviously important if microsatellite pedigree analyses will be used to track parentage. The capacity to successfully determine which parents produced an offspring depends upon having a complete genetic census of the parents that could have potentially produced that individual. If large numbers of unknown parents could have contributed offspring to those being sampled, then pedigree assignments become less certain, and more expensive to perform because of the need to include additional loci in the analysis. Second, to link behavioral or physiological traits in adult fish with their breeding success (i.e., the capacity to produce fry) individual fish need to be observed and examined. The observation stream provides us with the means to track individual fish and document their spawning behavior. Moreover, post-mortem evaluations can be performed on every fish placed into the channel to determine egg retention rates in females and gonad depletion in males. In aggregate, these types of observations will be used to correlate adult attributes with breeding success. Furthermore, the expression of these attributes by hatchery- and wild-origin fish will be statistically compared. Lastly, the observation stream will be an important protected spawning area in the Elochoman River basin and therefore will become another asset in our efforts to restore this ESA-listed species in the Lower Columbia River.

3) How many adult fish representing each treatment are needed and the physical parameters that will be present in the observation stream

The size of the observation stream is directly linked to the number of adult fish from each treatment (hatchery and wild) needed to detect pre-established differences in their breeding and reproductive success. Some of us are currently involved with a study that is examining the reproductive success of hatchery- and wild-origin spring chinook. That study also relies on microsatellite DNA pedigree analyses to determine the breeding success of adult male and female hatchery- and wild-origin fish. We have used the data collected on these fish in a series of power analyses to help size this present project. Male reproductive success was defined as the capacity to father fry. Female reproductive success was split into two components. One measured how successful a female had been at converting her eggs into offspring; while the other measured the survival of eggs actually spawned by a female. The latter measurement is used to appraise the ability of a female to produce an adequate incubation environment for her eggs regardless of the number she may have deposited.

As might be expected there is a great deal of variation in the breeding success of males, some individuals are highly successful, spawning with multiple females and producing thousands of offspring while others apparently never spawned. Variation in female success was significantly lower, as most females were able to spawn and produce fry. Plainly to detect differences in male success due to treatment origin the inherent "noise" in the ability of individuals representing the same treatment to produce offspring has to be overcome by using a large number of individuals from each treatment. The same concern also exists, to a lesser extent, for females. Our initial power analyses suggest that at a minimum, 100 wild females, 100 hatchery females, 100 wild males, and 100 hatchery males should be evaluated each year. Therefore the observation stream should be designed to accommodate the spatial needs of 200 or more females to be accompanied by an equal number of males.

Previous work that examined the effects of instantaneous female densities on the capacity of female chum salmon to spawn showed that each female required 2.5 square meters of space to successfully bury her eggs. Not all areas in a stream are likely to be equally attractive to females for egg burial. For example, chum are known to prefer upwelling locations or those with accelerating flows. Given their spatial and hydrological requirements and likely heterogeneity in our stream, we plan to build a 3.05 m wide by 245 m long observation stream (10 ft wide by 800 ft long). The stream will be lined with geotextite material to reduce water loss caused by percolation. It will be filled with gravel having a Fredle Index value $\geq$ 5 to a depth of 50 cm. Water velocities will range between 22 cm to 37 cm per second, and depth will average 30 cm. The structure will be split into 4 equal sections by cross weirs. The capacity to subdivide the stream into sections is critical. It will allow us to load the stream with adults in a sequential fashion and will also enhance our capacity to perform accurate pedigree analyses and perform detailed behavioral observations. Traps will be installed at the end of these sections so that the fry produced from them can be captured, counted, and subsampled.

4) Capture of adult fish in the Grays and verification of treatment origin

Every hatchery-origin chum salmon returning to the Grays River possesses a thermal mark in its otoliths. These marks, which are similar to bar codes, indicate that an individual was produced by parents that were artificially spawned. Thermal marks, however, are not visible until otoliths have been extracted and processed, so mark identification must occur after a fish has died. However, we can still regulate the relative numbers of hatchery- and wild-origin adults in the observation stream by collecting fish from locations where they are known to be predominately of one type or another. Work done in the Grays River drainage has shown that hatchery-origin chum will most likely migrate into the West Fork of the Grays River. Prior to our supplementation effort, a dozen or fewer chum were seen in this part of the watershed. During the last two years, thousands of fish were observed there. Otoliths collected from these fish indicate that the vast majority are hatchery-origin fish. Conversely, another tributary to the Grays River, Crazy Johnson Creek, has few hatchery-origin individuals. These and other locations within the Grays River basin will be used to capture putative hatchery- and wild- origin chum salmon. The fish will be captured by using large seines. Females that appear to have spawned and males with worn fins or wounds will not be used. The fish will be placed into a truck and transferred to the observation stream. Prior to being released into the stream, each fish will be sexed, DNA-sampled, weighed, measured, and tagged with a unique Petersen disk and then released into one of the channel sections. Each section will be filled with fish over a 48-hour period. After the fish die, otoliths will be extracted and decoded to verify whether the fish was a wild- or hatchery-produced individual.

The observation stream will be filled with adults originating from the Grays River for three consecutive years beginning in 2003 and ending in 2005. In 2006 we expect to see the first returns of adults back from the fry liberated into the Elochoman during the spring of 2004. Only 3-year-old project fish will be available in 2006. Depending upon their abundance all or a randomly selected sample of these fish will be placed into the observation stream and allowed to reproduce. As briefly mentioned elsewhere, a weir will be built at the mouth of Beaver Creek to help capture adult chum produced from the observation stream. This structure will also be used to regulate the abundance of chum salmon into Beaver Creek, a location we believe will become an increasingly important natural spawning area for this species in the Elochoman watershed.

5) *Types of observations that will be made on the adult fish while they are alive in the observation stream*

Once adult chum salmon have been liberated into the observation stream scan observations (visual surveys) will be made on the fish during daylight hours on the days that the females are preparing nests and spawning. Each scan observation will last for approximately five minutes and the following information will be recorded: fish tag number, sex, location, reproductive status, nuptial color pattern, and all courting and agonistic interactions experienced by the fish during the observation period. Chum have two basic nuptial color patterns: a stripe pattern characteristically present on territorial females and subdominate males and a bar pattern present on

dominate males. They can be used to assess the social status of the fish. The scan observations will be made vocally by using tape recorders. These vocal records will be transcribed to create permanent records of the reproductive activities of each individual. For example, from them it will be possible to determine the percentage of time a male dominated potential rivals, how often females attacked him, what his prevalent color pattern was, and how much he moved throughout the stream. To facilitate these observations a grid system will be placed over the stream to pinpoint fish locations and also aid in the mapping of female redd locations. Step-wise multiple regressions will be used to determine the relative importance of these behavioral traits on breeding success. Some of the traits measured will have a high degree of multicollinearity. When that occurs these highly correlated traits will be combined or one or more will be dropped when final analyses are performed.

6) *Post-mortem observations that will be made on the adult fish placed into the observation stream*

The number of hours (longevity) each fish lives in the observation stream will be recorded by noting when each fish dies. This will be accomplished by examining the observation stream for mortalities three to four times every day. Non-parametric tests (most likely a Kruskal-Wallace Analysis of variance by ranks) will be used to ascertain if differences exist in how long hatchery- or wild-origin males and females live. In addition, every female will be inspected to determine if she retained any eggs. The ability of hatchery and wild females to deposit their eggs will be tested using Mann-Whitney U tests or ANOVA methods. To make such an evaluation the fecundity of each fish has to be estimated because we need to know the percentage of eggs that are retained not the absolute number. Data that we have collected on Grays River chum salmon has shown that a multiple regression analyses using female body weight, egg weight, and Fulton's condition factor can explain about 70% or more of the variation in female fecundity. All of these parameters will be obtained when possible and used to generate fecundity estimates on each female placed into the observation stream. The number of eggs retained by each female will be divided by her estimated fecundity or PED (potential egg deposition) value. The resulting quotient provides an estimate of how successful each female was in depositing her eggs. These percent egg deposition values are the ones that will be statistically compared with one another to see if hatchery and wild females differ in their ability to deposit their eggs. Egg retention data and fecundity data will also be used to produce an actual egg deposition (AED) value for each female. AEDs are calculated by subtracting retained eggs from each female's fecundity or PED estimate. As mentioned previously, the PED and AED values will be used to measure how successfully each female converted her eggs into fry and how well deposited eggs survived to the fry stage. ANOVA or Mann Whitney U tests will used to make these comparisons.

Our spring chinook study has shown that gonad depletion in males is related to reproductive success. As might be expected, males with depleted testes fathered more fry than those with less depleted gonads. Consequently, during the post-mortem, testes will be carefully removed from each male and weighed to the nearest

0.1 gram. Previous work on chum salmon has shown that a linear relationship exists between male body weight at maturity and testes weight. Therefore, it will be possible to estimate the pre-spawning testes weights of each male salmon placed into our observation stream. The testes weight obtained at death will be divided by a male's expected testes weight to produce an estimate of how much gonad weight was lost during the spawning period. These gonad depletion values will be compared by using Mann Whitney U tests or ANOVA methods to see if differences exist between hatchery- and wild-origin males, and to determine the relationship between gonad depletion and the numbers of fry fathered in this study.

7) Capture and analysis of fry produced from the observation stream

Given the physical conditions that should be present in the observation stream we would expect a 30 to 50% egg-to-fry survival rate. As mentioned above, fry traps (most likely modified fyke nets with floating live boxes) will be placed in the cross weirs that subdivide the channel. All the fry produced from each section will be counted and then randomly sub-sampled throughout the entire fry migration period. We anticipate that 50 females will be placed into each subsection of the observation stream and that a total of forty-five to seventy-five thousand fry will be produced from each of these areas. A power analysis suggested that approximately 1,500 fry should be sampled from each of these sections to detect differences in breeding success. Therefore, we will randomly remove 3% of the fry captured each day and preserve those in 100% ethanol for later DNA analyses.

The procedures used to develop DNA profiles on the fry are the same that will be used to characterize the adults placed into the channel. We will use microsatellite DNA to genetically identify hatchery- and wild-origin adults, and their offspring. As mentioned previously, tissue samples will be obtained from the adults prior to their introduction into the observation stream. Adult tissue samples will consist of operculum punches that will be preserved in 100% ethanol. Fry samples will be obtained from whole individuals. Each individual (adults and fry) will be genotyped at approximately 20 microsatellite loci using standard protocols: template DNA will be extracted from tissue using chelex resin, microsatellite DNA loci will be selectively amplified from template DNA by using the polymerase chain reaction (PCR), microsatellite alleles shall be run on an automated sequencer (ABI 3730), and genotypes will be assessed using GENEMAPPER software, The 20-locus microsatellite suite will definitively identify broodstock adults, their juveniles and returning adult offspring. Lifetime reproductive success of different crosses (H x H, H x W, W x H, and W x W) will be estimated by assigning juveniles and returning adults to parents using PARENTE, CERVUS or similar software. Overall success of broodstock individuals and mating types will be determined by genotyping adults returning throughout three generations and identifying production from various pedigrees. We are reviewing statistical approaches to comparing these types of data for reproductive success experiments. For now, we propose to test against the null hypothesis of equal reproductive competence across origins of spawners using the chi-square or log likelihood ratio test. Essentially, if hatchery and wild spawners differ in reproductive competence, the numbers of each variant offspring type should

depart from binomially distributed abundances driven by abundances of the original spawner types.

8) Fry release into the Elochoman

The fry that are not sampled for pedigree analyses will be placed into tanks lined with netting and supplied with running water. After darkness, the fry will be released into the Elochoman River.

9) *Number of replicate founding populations that will be introduced into the observation stream*

The observation stream will be loaded with 400 adults originating from the Grays River for three consecutive years beginning in 2003. Starting in 2006, the first adults produced from these fish will return to the Elochoman River. From this point on, no additional adults from Grays River will be imported into the observation stream. If possible, all of the fish returning in 2006 will be allowed to spawn in the observation stream. However, if numerous fish return, a random sample will be obtained and placed into the stream. Excess fish will be allowed to spawn either in Beaver Creek or in the Elochoman itself. An extensive sampling effort will be made to collect as many DNA samples from these returning fish as possible to allow for robust estimates of progeny production to the adult stage from our founding populations.

10) How F_1 and F_2 adults will be utilized and sampled

Beginning in 2007, both 4- and 3-year-old chum salmon originating from our founding populations will return to the Elochoman River. If adult abundance is low, every returning fish (up to 200 females) will be allowed to spawn in the observation stream. If abundance is high then a random sample of the adults returning to the river will be placed into the observation stream. Again, as in 2006, DNA samples will be obtained from as many adults as possible. This approach will continue until the project is terminated. We will explore the opportunities (or requirements) for processing DNA samples from F_2 adults in real time to allow for more controlled abundances of spawners of varying hatchery or wild ancestry.

Timeline

The timelines for the major tasks that must be completed to perform the work described above are identified in Table 1.

Table 1. The tasks associated with comparing the reproductive success of hatchery- and wild origin chum salmon and their planned completion dates.

Task Description:	**Completion Date**
Obtain necessary construction permits for the observation stream and weir at Beaver Creek. Obtain ESA permits that will allow the transfer of Grays River chum to the Elochoman River	July 2003
Design and develop plans for the observation stream	Aug 2003

Table 1. The tasks associated with comparing the reproductive success of hatchery- and wild origin chum salmon and their planned completion dates continued. . .

Task Description	Completion Date
Design and develop plans for the weir on Beaver Creek	Aug 2003
Build Observation stream and sample gravel prior to placement to ensure it meets project specifications	Aug 2003-Oct 2003
Capture and transport adult chum salmon to new observation stream (collect tissues for later DNA analyses, measure and tag adults)	Late Oct – Early Dec 2003
Perform behavioral observations on adult chum placed into observation stream	Late Oct-Early Dec 2003
Perform post-mortem evaluations on chum placed into observation stream (includes otolith collection from adults to confirm their treatment origins)	Late Oct-Mid Dec 2003
Provide adult DNA samples to WDFW's Genetics Laboratory for genotyping	Dec 2003
Transcribe and analyze scan observations made on spawning adults observed in the observation stream	Jan 04 – Jun 04
Decode otoliths collected on adults spawning in the observation stream	Jan 04 – Jun 04
Build fry traps	Jan 04
Establish tanks where fry produced from the observation stream can be sampled, counted, and held prior to being released into the Elochoman River	Feb 04
Install fry traps and count, sub-sample, and release chum salmon fry produced from the stream	Feb 04
After fry emergence has been completed, provide fry DNA samples to WDFW's Genetics Laboratory for pedigree analyses	Jun 04
Perform pedigree analyses	Jun 04 – Feb 05
Take gravel samples from the observation stream and determine Fredle index	Jun 04 – Jul 04
Clean and level gravel in the observation stream as needed and prepare the channel for fish introductions in 2004.	Aug 04 –Sep 04
Prepare report to BPA describing results obtained during the first year of the project	Jun 04 – Sep 04 (depends on due date)
Begin Second Year of Experiment: No construction will take place from this point on, the following major steps are chronologically arranged	
Capture and transport adults from the Grays River to the observation stream	Late Oct – Early Dec 2004
Weigh, measure, tag, and release adults into observation stream	Oct – Dec 2004

Table 1. The tasks associated with comparing the reproductive success of hatchery- and wild origin chum salmon and their planned completion dates continued. . .

Task Description	Completion Date
Perform scan observations on fish placed into the observation stream	Late Oct – Early Dec 2004
Perform Post-mortem examinations on adults placed into observation stream to determine egg retention and gonad depletion values. Collect otoliths from each adult to establish treatment origin.	Late Oct – Early Dec 2004
Analyze adult DNA	Jan – Jun 2005
Transcribe and analyze scan observations	Jan – Jun 2005
Decode otoliths collected from adults to confirm origin	Jan – Jun 2005
Install fry traps, count, sub-sample, and release chum salmon produced by the observation stream	Feb 2005
Provide DNA samples to WDFW's Genetics Laboratory	Jun 2005
Perform Pedigree analysis	Jun 05 – Feb 06
Take gravel samples from the observation stream and determine Fredle Index value	Jun – Jul 05
Clean and level gravel in the observation stream	Jul – Aug 05
Prepare annual report to BPA	Jun – Sep 05
Begin Third Year of Experiment	
Repeat 13 steps performed during second year of experiment	Oct 05 – Feb 07
Begin Fourth Year of Experiment	
This is the first year that F_1 adults produced from the project are expected to return to the Elochoman River. No further transfers of Grays River chum salmon will occur. In addition, this will be the first year that comprehensive stream surveys in the Elochoman River will take place. The objective of these surveys is to collect DNA samples from the returning adults to determine which parents produced them. Otherwise, the tasks outlined for year two, and three will be performed. This work will take place from Oct 06 – Feb 08	
Begin Fifth Year of Experiment	
During this year, both 3- and 4-year-old F_1 adult fish will return to the Grays River. These fish will be placed into the observation stream and allowed to spawn naturally. If returns are high, a random selection process will be used to determine which individuals are placed into the observation stream. All other fish will either spawn in Beaver Creek or the Elochoman River. As in year four, extensive stream surveys will be conducted to collect DNA from as many returning adults as possible. The same tasks outlined for years 2, 3, and 4 will be conducted. This work will take place from Oct 07 – Feb 10	

Table 1. The tasks associated with comparing the reproductive success of hatchery- and wild origin chum salmon and their planned completion dates continued. . .

Task Description	Completion Date
Years 6 – 13 of Experiment	
In order to obtain a complete F-2 generation this project will have to be continued until 2015 when the last F-2, 5-yr-old fish are expected to return. The tasks outlined in years 4 through 5 will be carried out each year. Potentially some F-4 fish may return to the stream in 2015 along with many F-3 fish as well. The parental origins of these fish will be determined.	

A final report describing and summarizing the results of this comprehensive study will be produced in 2016. Table 2 shows when F_1, F_2, adults produced from the project are expected to enter and spawn in the Elochoman River.

Table 2. The expected arrival date of F_1 and F_2 chum salmon produced from the observation stream located on Beaver Creek.

Return Year	Origin Of The Adults Returning To The Observation Stream					
	3-Yr-Olds		4-Yr-Olds		5-Yr-Olds	
	Brood Yr From	Generation[5]	Brood Yr From	Generation[5]	Brood Yr From	Generation[5]
2003	Grays River	Founder Pop.	Grays River	Founder Pop.	Grays River	Founder Pop.
2004	Grays River	Founder Pop.	Grays River	Founder Pop.	Grays River	Founder Pop.
2005	Grays River	Founder Pop.	Grays River	Founder Pop.	Grays River	Founder Pop.
2006	2003	F-1	-	-	-	-
2007	2004	F-1	2003	F-1	-	-
2008	2005	F-1	2004	F-1	2003	F-1
2009	2006	F-2	2005	F-1	2004	F-1
2010	2007	F-2	2006	F-2	2005	F-1
2011	2008	F-2	2007	F-2	2006	F-2
2012	2009	F-2[1], F-3[2]	2008	F-2	2007	F-2
2013	2010	F-2[4], F-3[3]	2009	F-2[1], F-3[2]	2008	F-2
2014	2011	F-3	2010	F-2[4], F-3[3]	2009	F-2[1], F-3[2]
2015	2012	F-4[2], F-3[3]	2011	F-3	2010	F-2[4], F-3[3]

1 = If adult originated from 4- & 5-yr-old parents
2= If adult was produced by 3-yr-old parents
3= If adult was produced by 3- & 4-yr-old parents
4= If adult was produced by 5-yr-old parents
5= Obviously in some instances adults will have been produced by parents from different generations, therefore half of their genes will be in one generation and the half will be in another generation. This possibility was not considered when this table was produced as its purpose is to simply indicate the length of time needed to procure fish that are various generations removed from the original founding populations. It should be clear however, that as we move forward in time that greater admixtures of genes from our original founding populations would occur.

Qualifications Of Participants

This project requires expertise in three areas: 1) The capture and handling of adult broodstock from natural rivers, 2) Experience in observing and comparing reproductive behavior in salmonids, and 3) The ability to perform pedigree analyses that are based on microsatellite DNA. Washington Department of Fish and Wildlife Region Five staff, Dan Rawding, Todd Hillson, and Joe Hymer (Pacific States Marine Fisheries Commission) will be leading the effort to capture and transport chum salmon from the Grays River to the Elochoman location. All three are professional biologists that are currently working on other Lower Columbia River chum salmon recovery efforts (e.g. the reintroduction of Chum salmon into Duncan Creek, assessing chum salmon abundance throughout the Lower Columbia, and determining the abundance and distribution patterns of chum salmon returning to the Grays River). Four other staff members, Dr. Steve Schroder, Howard Fuss, Pat Hulett, and Cameron Sharpe will lead the work associated with comparing the reproductive behavior of hatchery- and wild-origin chum salmon. Steve Schroder is currently leading an effort to compare the reproductive success of hatchery- and wild Yakima River spring chinook. He has also examined the reproductive ecology of chum, fall chinook, coho, and Atlantic salmon. Moreover, Howard Fuss is an experienced salmon ecologist who is performing a study with NOAA-Fisheries scientists that examines the reproductive success of hatchery- and wild-coho salmon. Pat Hulett and Cameron Sharpe lead a number of investigations that appraise the reproductive success of hatchery- and wild-origin steelhead. In combination these staff members have the expertise to determine if quantitative differences exist in the reproductive behavior of hatchery- and wild-origin chum salmon.

Sewall Young, Drs. Jim Shaklee, Maureen Small, Janet Loxterman and support staff in WDFW's genetics laboratory will conduct all the microsatellite DNA analyses. DNA data collection and processing (microsatellite analysis and sequencing) are done using computer-controlled, semi-automated DNA sequencers. The laboratory is equipped with two capillary-based electrophoresis genetic analyzers, an ABI-3100 (16-capillary) and an ABI-3730 (48-capillary) and equipment necessary for extracting DNA from tissue samples and amplifying target segments of DNA (microsatellite loci and others) via the polymaerase chain reaction (PCR) as well as other common pieces of laboratory equipment. We use ABI Collection and Genemapper software and Sequencher (Gene Codes Corp.) software for data collection and processing. The ABI-3730's sample throughput capability is approximately 96 samples / hour for a suite of 3 to 6 microsatellite loci. We have four Win-NT PCs dedicated to DNA data collection, processing and analysis in the laboratory as well as four networked PCs at biologists desks outside the laboratory for statistical analysis and other computer tasks. DNA laboratory staff dedicated to genetic analysis include three biologists, and 2-2/3 scientific technician FTEs.

Analyses and interpretation of the behavioral and genetic results will be a collaborative effort among the professional staff associated with this project.

Budget Estimates

The cost estimate for Year One includes expenses associated with the permitting, design, and construction of the observation stream. Additional one-time costs for equipment, fry traps, and other items are also included. Expenses shown for Year Two and beyond (Table 3.) reflect the actual cost of performing the work outlined above. In 2006 a temporary weir will be built and installed in Beaver Creek. This expense is added to the Year Four budget. A three percent inflation factor was used to forecast the costs shown for years Three through Thirteen.

Project Year (Calendar Year)	Estimated Cost (Includes a 25% Indirect Cost value)
1 (2003-4)	$955,000
2 (2004-5)	$520,000
3 (2005-6)	$535,000
4 (2006-7)	$561,000
5 (2007-8)	$568,000
6 (2008-9)	$585,000
7 (2009-10)	$603,000
8 (2010-11)	$621,000
9 (2011-12)	$639,000
10 (2012-13)	$658,000
11 (2013-14)	$678,000
12 (2014-15)	$699,000
13 (2015-16)	$719,000
TOTAL	$8,341,000