RFS# 004

Reproductive Success of Natural-Origin, Endemic Hatchery Origin, and Reconditioned Kelt Summer Steelhead in the Tucannon River (FCRPS BiOp RPA Action #184)

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Project Summary

We propose to quantitatively evaluate the relative reproductive success of spawning natural origin, hatchery endemic origin, and reconditioned natural origin summer steelhead in the Tucannon River. We propose to take advantage of existing hatchery facilities, monitoring programs, and recent technological advances in genetics to empirically monitor the reproductive success of these three groups of summer steelhead using a DNA-based pedigree approach. This project, if implemented will successfully answer questions for RPA 182 of the NOAA Fisheries 2000 Biological Opinion on the Operation of the Federal Columbia River Power System. Further, this study will greatly complement the current work conducted by the Columbia River Inter-Tribal Fish Commission in the Yakima River Basin

Natural origin Tucannon River summer steelhead kelts (post-spawning at Lyons Ferry Hatchery (LFH)) will be reconditioned in a circular rearing tank at LFH. Kelts will be re-conditioned for one or maybe two years, using a variety of culture techniques proven successful by the Columbia River Inter-Tribal Fish Commission in the Yakima River Basin. For the first year of the study WDFW proposed to hold 100% of the maturing kelts in the study, and attempt to spawning them again in the hatchery. This will allow evaluation of gametes from reconditioned kelts, prior to including them in the study for reproductive success in the river. WDFW is recommending this action at this time to demonstrate that viable gametes can be produced from reconditioned kelts, and will further assist in the evaluation of the pedigree study and future broodstock management. Based on the results obtained from spawning kelts in the hatchery, WDFW would then propose (second year of study) releasing the reconditioned kelts into the upper Tucannon River, above the Tucannon Fish Hatchery (TFH) adult trap at rkm 59. Potential spawning mates will include other natural origin fish, and endemic hatchery origin fish that have been passed above the TFH adult trap. All fish passed upstream of the TFH adult trap, and reconditioned kelts will be DNA tissue sampled prior to release for the microsatellite DNA analysis of the study.

The summer after spawning, WDFW proposes to conduct electrofishing surveys at index and supplemental sites in the upper Tucannon River (above the TFH adult trap) to obtain DNA tissue samples from Age 1+ natural origin summer steelhead. From the samples obtained from the juveniles, and samples collected from the adults (natural origin, hatchery endemic origin, and reconditioned kelts), a pedigree analysis will be preformed using microsatellite DNA analysis to document the relative reproductive success of the groups at the juvenile stage. The third and final part of the study will focus on trapping natural origin adults as they return from these groups and performing a pedigree analysis (adult-to-adult). Adults for this part of the analysis will be trapped and sampled at the TFH adult trap.

As part of the study, project leaders will provide data analysis (where possible) to discuss other questions posed in the RFCS about 1) increasing inbreeding by use of reconditioned kelts, 2) how this might increase domestication selection, and 3) how the reconditioning program might alter age and life history structure of the target population.

Background: Tucannon River Summer Steelhead

Summer steelhead population structure and status

The Tucannon River empties into the Snake River between Little Goose and Lower Monumental dams approximately 622 river kilometers (rkm) from the mouth of the Columbia River. Stream elevation rises from 150 m at the mouth to 1,640 m at the headwaters (Bugert et al. 1990). Total watershed area is about 1,295 km^2. Mean discharge is 174 cfs with a mean low flow of 61.5 cfs (August) and a mean high flow of 310 cfs (April/May). Over the past 100 years, the in-river habitat has been, in some locations, severely degraded by logging, road building, recreation, agriculture, and livestock grazing. These activities and harsh environmental conditions that exist in Southeast Washington, have limited the production potential of summer steelhead in the Tucannon River.

Steelhead have been documented spawning in the Tucannon River from near the mouth to as high as Sheep Creek (rkm 85), and in smaller tributaries (Cummings Creek, Panjab Creek). Spawning of both hatchery and natural origin fish typically occurs between February and May. Rearing success is dependent upon habitat and water quality, which is poor below rkm 22 and only moderate between rkm 22-39. Above rkm 39, rearing conditions are considered good for steelhead. Based on lower river smolt trapping data since 1997, juvenile steelhead will typically rear from one to three years before migrating as smolts (Bumgarner et al, 2002). Age of smoltification is likely determined by environmental factors (temperature) and genetics. The river is productive and yearling smolts have been identified emigrating from the lower reaches where spring/summer water temperatures allow for accelerated growth. Trapping data from the TFH adult trap and the lower Tucannon adult trap show the population to be made up of 2, 3 and 4 year old individuals (the majority are primarily 2 year old freshwater age and one or two year ocean age). Rarely have 5-year-old individuals been identified in the population, and to date, no known repeat spawners have been identified (Bumgarner et al 2002). Tucannon endemic steelhead are typical of "A" run summer steelhead with more fish returning as 1 salt age (55-70%) than as 2 salt (30-45%). Sex ratio varies among years and can be heavily skewed to females (70%) but is generally believed to average between 50-60% females for most years. The WDFW has estimated natural steelhead escapement into the Tucannon River since 1987. The largest escapement was seen in 1988 when an estimated 525 natural origin fish spawned. Numbers have decreased steadily since 1990, with only an estimated 31 natural origin fish spawning in 2000. Tucannon River natural origin summer steelhead are ESA listed as "threatened" under the Snake River Summer Steelhead ESU.

Hatchery steelhead programs in the Tucannon River

After completion of the four lower Snake River dams, the LSRCP was approved by the U.S. Congress to provide hatchery mitigation for spring chinook, fall chinook, and summer steelhead in the Snake River (USACE, 1975). In 1982, WDFW began the summer steelhead program at LFH, which included releases of hatchery steelhead into the Tucannon River. The hatchery steelhead used for mitigation purposes were not native to the river, and have consisted primarily of Wells (upper Columbia) and Wallowa (Snake River) Stock fish. Management of these non-endemic stocks in the Tucannon River has varied. Prior to 1997, the majority of the hatchery

steelhead releases occurred from Curl Lake Acclimation Pond (rkm 66) in the upper Tucannon River. Because of ESA concerns with listed Tucannon River spring chinook, and pending listings of natural origin summer steelhead in the Snake River Basin, further hatchery origin steelhead releases were restricted to the lower Tucannon River, thereby removing them from the prime rearing area of natural origin steelhead. Hatchery origin steelhead returning to the TFH adult trap since 2000 have been excluded from the upper basin, but continue to spawn in areas below the TFH adult trap.

Also beginning in 2000, WDFW initiated the development of an endemic steelhead broodstock for use in the Tucannon River. A temporary trap is constructed annually in the lower Tucannon River (rkm 17) to trap natural origin fish for broodstock. A portion of the trapped natural origin fish are collected, transported, spawned, and the offspring reared at LFH. The current program goal is to produce ~50,000 smolts to be released into the upper watershed. All endemic brood progeny are 100% marked with a visual implant elastomer (VIE) tag behind the eye for external identification upon adult return for program evaluation. They are also coded wire tagged for electronic sampling in case the VIE is lost or not visible. Upon adult return and capture at the TFH adult trap, these fish are sampled and released upstream to spawn naturally. The 2002 run year represents the first year that endemic brood fish will return to the system.

Reconditioning of steelhead kelts

During the first year of development for the endemic broodstock program in 2000, WDFW initiated reconditioning of kelts from the endemic program. Kelts were placed in a 20' circular rearing tank at LFH following spawning. However, our attempts at reconditioning failed. The kelts failed to eat any of the foods offered. WDFW was aware at that time that other organizations (Columbia River Inter-Tribal Fish Commission, Oregon Dept of Fish and Wildlife) were spending much more effort and research in developing techniques for reconditioning kelts. Given our poor success, reconditioning efforts were discontinued until others published successful results. Since then, considerable knowledge has been gained in the process of reconditioning kelts (CRITFC 2002), and these techniques, as well as data/information from other projects potentially funded from this RFCS, will be used in this study.

Project Description

Introduction

Estimating the relative reproductive success of naturally spawning hatchery-produced salmon and steelhead is one of the most important tasks needed to assess successful recovery efforts in the Columbia and Snake River basins. Obtaining accurate measurements of the relative reproductive success of natural and hatchery origin on the spawning ground is important both for evaluating the risks of "production-type" hatchery programs (potentially harmful to the natural population), and for evaluating the benefits of "supplementation-type" programs designed to assist natural salmon populations. In the Tucannon River system, both these types of hatchery programs are currently being managed together, with the intent that natural and hatchery endemic fish will spawn together in the wild to produce viable offspring.

Over the past several decades, several studies have suggested that salmonids produced from hatcheries have lower fitness in the natural environment than naturally produced fish. For example, Reisenbichler and McIntyre (1977) found that in the Deschutes River, in-stream egg-to-fry survival of steelhead resulting from crosses between hatchery fish survived at ~80% that of steelhead resulting from crosses between natural fish. In a similar study, Leider et al. (1990) found that the adult-to-adult reproductive success of non-native hatchery produced steelhead in the Kalama River was substantially less than that of the native natural-origin steelhead. Numerous other completed or ongoing studies suggest that either genetically or environmentally caused differences in fitness or fitness-related traits between hatchery and naturally produced fish (Fleming, et al 1996, Fleming and Gross 1992, 1993, 1994, McGinnity et al 1997, Chilcote et al 1986, Berejikian et al 1997, Berejikian 1995). Furthermore, those studies suggest that artificially propagated salmonid populations may become maladapted to the natural environment within only a few generations, and that it is reasonable to expect that fish from many existing hatchery stocks might have relatively low fitness when they spawn in nature.

Hatchery supplementation programs remain common, however, in part because salmonids are highly variable and existing studies have tended to focus only on certain species and types of hatchery. For example, most of the completed and ongoing studies that have quantified genetic reductions in fitness in hatchery stocks have focused on one species (steelhead) and on one type of hatchery ("traditional" production programs), and often focused on hatchery stocks not native to the study area. These studies have shown that non-native steelhead stocks are likely to have low reproductive success in nature, but their results may have little relevance to other species, to hatchery reared endemic stocks, or to programs specifically designed to play a conservation role. The endemic program developed for the Tucannon River was derived from the native stock, and is being used in a conservation role currently. By measuring the reproductive success of individual fish, we will obtain a more complete picture of how variation in measurable traits influences fitness than has been obtained from most studies conducted to date.

If the naturally spawning hatchery fish are part of a supplementation program (as with the Tucannon River endemic fish), then estimating their relative fitness is necessary for evaluating the effects of the hatchery program as a whole. For example, if the relative reproductive success of the endemic hatchery fish is low, the program is unlikely to be successful at increasing natural production. Further, if the gametes produced from the reconditioned kelts are not viable, or severely below normal expectation, then results obtained from the study will be skewed. Also, the amount of resources needed for large scale kelt reconditioning programs may not be worth the effort if viable gametes are not produced from reconditioned kelts in the hatchery. Evaluating relative reproductive success for both hatchery endemic supplementation program and from reconditioned kelts is therefore critical for determining if the considerable investment the region has made in hatchery supplementation programs is actually helping recover salmonid populations.

Time Period of Study

We propose that this study begin during the fall/winter of 2003 with collection of natural origin adults for the endemic program. The fish will be spawned and then dedicated as part of the kelt reconditioning program. Since kelts will not be available until the following year, WDFW

proposed to start the reproductive success study using natural origin and hatchery endemic origin adults returning to the TFH adult trap in 2004. We propose that the study run for one complete generation. Adults to cover those brood year's life histories will be sampled in the following years to cover the adult returns so the complete life cycle can be evaluated by the pedigree analysis. The full study will continue until 2013.

Specific Objective and Tasks

Objective 1: Determine the success rate of reconditioned steelhead kelts post-spawning at LFH

Task 1a. Collect steelhead kelts for reconditioning at LFH

Natural origin summer steelhead are collected each year at the WDFW lower Tucannon River adult trap (temporary weir/picket trap) for endemic broodstock development under the LSRCP program. Beginning with the 2004 brood year, all natural origin fish will be live spawned at LFH. Brood fish will be fed krill or a commercial diet pre-spawning at LFH to encourage feeding behavior in the hatchery. After spawning, fish will be PIT tagged for unique identification and transported to a 20' circular rearing tank at LFH, and the reconditioning process will begin. Based on current program levels, WDFW expects that 30-36 fish (males and females combined) will be available for reconditioning. Beginning in 2005, the endemic broodstock program is expected to increase, and as many as 80 fish may be available for reconditioning at that time. The use of PIT tags will allow tracking of individuals through the reconditioning process, and for those kept for second spawning at LFH to track gamete quality after reconditioning (first year of study).

Task 1b. Recondition steelhead kelts at LFH

All kelts for the study will be held in a single 20' circular rearing tank (operating depth of 5.5'). The tank will be covered with camouflage netting to prevent jumping, and to keep the fish sheltered from outside disturbances. The 20' circular tank is part of the BPA funded Tucannon River Spring Chinook Captive Broodstock Program. Use of a single 20' tank will not affect the captive broodstock program. Tanks have been enclosed with security fencing to keep visitors to LFH away from the area. A formalin treatment apparatus is available by each tank to provide necessary treatments to control fungus outbreaks on the kelts.

For feeding and reconditioning of the kelts, WDFW will closely coordinate with CRITFC and any other kelt reconditioning projects to determine the best diet and methods to initiate feeding in the kelts. At this point, WDFW will use the information provided by the CRTFIC 2001 Annual Report (2002) that experimented with feeding krill, Moore-Clark adult salmon pellets, Moore-Clark Kelt Diet, and a special formulated diet by the Abernathy Fish Technology Center. Results from the CRTFIC study showed that the Moore-Clark diets showed the greatest success in reconditioning kelts. Additional diets from other feed manufactures, or other kelt reconditioning trials (ODFW 2002) may also be tried depending on success and palatability observed with the kelts. In addition, WDFW will review the current literature on reconditioning that has been conducting on Atlantic Salmon. Additional diets and feeding strategies for

reconditions may be gained from other studies on Atlantic Salmon that could be applicable for this project. All kelts will be sampled (length/weight) prior to being put in the 20' circular tank. Depending on disease and fungus outbreaks, kelts will likely be sampled just prior to release in the upper Tucannon River. The PIT tags implanted in the dorsal sinus will allow for individual tracking of growth over the reconditioning phase.

The CRTFIC study showed survival to the next year was only 20%, though they appear optimistic that better success can be achieved. Further, they stated that only about ½ of the surviving kelts had mature gametes based on ultrasound observations prior to release. Second year reconditioning of kelts may be necessary for project success. Based on scales obtained by WDFW on Touchet River natural steelhead, ~25% of the documented repeat spawners require an additional year of rearing before they return to spawn again. We propose the use of ultra sound to determine if fish are ready to spawn after one year of reconditioning. The CRTFIC personnel have demonstrated the knowledge and effective use of the ultrasound equipment. We propose to share equipment and expertise with CRTFIC to reduce the cost of this study.

Task 1c. Spawn reconditioned kelts at LFH, compare fertilization success of the same fish spawning first and second years (First Year of Study).

For the first year of the study, WDFW will re-spawn the reconditioned kelts at LFH to determine the viability of gametes produced. Reconditioned kelts will be spawned with other reconditioned kelts, or from new natural origin fish trapped from the lower Tucannon River for the endemic broodstock program under the LSRCP. Since reconditioned kelts will be PIT tagged, it will allow for the evaluation of fertilization success of the re-spawned kelts. Each individual female will be incubated separately at LFH. Fecundity will be obtained from female kelts and first time spawners, as well as fertilization success to the eye-up stage (i.e. dead eggs, live eggs). Depending on the success of this test, WDFW will propose this strategy for the first year of the study only.

Task 1d. Transport desired number of kelts to spawning grounds above TFH adult trap for natural spawning (Second year of study and beyond)

At the beginning of March of the second year of the study, determine the number of remaining mature kelts on hand from the project using ultrasound equipment and techniques developed by CRTFIC. We then propose taking 100% of the mature kelts to the Tucannon River ~6 rkm upstream of the TFH adult trap and release them. Maturation rate and spawn timing may influence which individuals may be released. It is unknown at this time if water temperatures at LFH (constant 11 ^{o}C) will influence maturation rate of reconditioned kelts. In addition, some of the reconditioned kelts may require two years of reconditioning before they are mature to spawning again. Prior to release, some of the reconditioned kelts (females) will be radio tagged with Lotek ™ tags and tracked every few days after to release to track their movements and determine if they spawn in the river. Radio tagging will also allow us to track if they leave the spawning area above the TFH adult trap before spawning so these fish could be removed from the pedigree analysis.

Objective 2: Collect samples to estimate relative reproductive success and survival of natural and hatchery origin, and reconditioned kelts spawning in the Tucannon River.

Task 2a. Collect DNA tissues from all steelhead adults passed upstream of the TFH adult trap.

The TFH adult trap is used to enumerate and pass adult spring chinook, steelhead and bull trout, as well as to collect spring chinook for broodstock as part of the LSRCP program. Adult steelhead will be collected at the TFH adult trap between January and May each year. The barrier associated with the ladder/trap also serves to impound water to divert for Tucannon Fish Hatchery use. Spring chinook and steelhead have been observed jumping the barrier in past when high spring flows occur. Therefore passage will have to be more strictly controlled for the study to ensure adequate sampling of spawning fish. Erection of a temporary soft barrier or PVC pickets (such pickets are erected each year by WDFW on the Touchet River to trap adult steelhead and have proven to be effective) each year prior to the steelhead migration should effectively direct fish to the ladder/trap. The exact design (3 options currently envisioned) of this barrier remains to be finalized, but has also been proposed by Todd Pearsons (WDFW) and Mike Ford (National Marine Fisheries Service - NMFS) under their proposal to study reproductive success in spring chinook populations within the State of Washington ("Monitoring the reproductive success of naturally spawning hatchery and natural spring chinook salmon in the Wenatchee, Tucannon, and Kalama Rivers"). Funding to construct this additional barrier has been included in the estimated cost for this proposal.

The TFH adult trap will be operated 24 hours a day, 7 days a week, throughout the steelhead spawning migration. Fish will be held for a maximum of 24 hours before they are processed. Each steelhead trapped will be measured (fork length), sexed, scales collected, and will have two small pieces (~0.25 cm^2) of caudal or dorsal fin, and a small opercle punch removed for genetic analysis. This will increase the likelihood that we will be able to develop complete genotypes for all parents. Each fish will also be classified as either hatchery produced or naturally produced, based on the presence or absence of a hatchery mark (VIE tag, Coded-wire tag (electronic scanned), or intact adipose fin with eroded dorsal fin). The scales collected will be used both for aging the fish, and for confirming their hatchery or natural origin. Depending on run sizes, we may sample as many as 200 natural adults, 200 endemic hatchery adults, and expect to fully recondition as many as 50 kelts each year. Therefore, total number of genetic samples from adults each year could be as high as 450 fish.

As proposed currently, reconditioned kelts from the reconditioning program will not be available for release into the upper Tucannon River until 2006. However, natural origin and hatchery endemic origin fish will be passed above the TFH adult trap in 2004 and 2005, and could be used for pedigree analysis in this study, using just two components of natural and endemic hatchery fish. We therefore propose to initiate the pedigree analysis of the study two years prior to when kelts would be available for planting in the river. Results from the first two years can then be used to modify the study design, if necessary, prior to when reconditioned kelts would be added to the study.

Task 2b. Collect DNA tissues from a representative sample of naturally produced juveniles.

Multiple pass electrofishing surveys are conducted annually by WDFW as part of the LSCRP monitoring and evaluations in the Tucannon River. Surveys are conducted to estimate densities and derive population estimates for Age 0 and Age 1+ summer steelhead in the Tucannon River. Surveys are conducted at 16 index sites from rkm 22 (HWY 12 Bridge) to rkm 79 (Winchester Creek). Currently, eight of the index sites are in the natural production areas above the TFH adult trap. The eight sites are located approximately 2-3 miles apart, so additional sample sites will be added to collected individuals from many locations/habitats.

As an intermediate step in determining the reproductive success of natural, hatchery origin and reconditioned kelts as part of the study, WDFW is proposing to collect juveniles from the Tucannon River through electrofishing to conduct a pedigree analysis. Because of the uncertainty in the reproductive success in hatchery endemic origin or reconditioned kelts at this time, WDFW feels this step could prove vital in determining the success of these groups spawning in the wild. Waiting to obtain samples from returning adult, while obviously the most important question as it address recovery efforts, the number of returning adults from these groups could be small enough that they would not be detected by the samples obtained at the Tucannon FH, as in-river mortality or incidental harvest would remove the fish from the system before they could be sampled.

However, we also have uncertainties at this time regarding the collection of samples from electrofishing surveys to statistically determine (at the 95% confidence level) the reproductive success of each spawning group. Power analysis calculated by WDFW genetic staff determined that ~20 individuals/spawner should be sampled above the TFH adult trap, which will provide pedigree results to the 95% confidence level. With the possibility of ~450 adults spawning above the trap, it was determined that samples would have to be collected from ~9,000 individuals (age 0) on an annual basis. Unfortunately, the possibility of non-lethal sampling that many Age 0 fish is unrealistic and would not likely be approved under the ESA by NMFS. As another possibility, WDFW has roughly determined that ~30% of the Age 0 fish survive to Age 1+ in the Tucannon River. Since genetic samples from Age 1+ fish are less intrusive, we therefore propose collecting samples from these fish instead. Collecting Age 1+ fish approximately every 400 meters between the TFH adult trap and Winchester Creek, with approximately 30 Age 1+ fish collected at each location, will bring the maximum samples size to ~2,700 samples. While this sample size is considerably smaller, it still can represent a significant percent of the population above the Tucannon FH adult trap. Given the uncertainties in overall population size available above the adult trap on any given year, we propose using a sliding scale approach for DNA collections. During lower run size years, the same sample size protocol will be followed (~6 fish/adult spawner), which will reduce the samples required and reduce the overall cost of DNA analysis. (Note: Age 1+ juveniles will not be sampled until the second year of the study).

WDFW currently conducts electrofishing surveys under a Hatchery and Genetics Management Plan for the Tucannon River Endemic Summer Steelhead broodstock program. Project personnel will consult with NMFS to modify the existing HGMP as needed for collection of adult and juvenile DNA samples as part of this study, should this project be funded. Should the

juvenile sampling portion be determined by NMFS as too intrusive to the natural steelhead population as proposed in the study, WDFW will consider two options. First, WDFW would consider reducing the sample size to an agreed upon acceptable level by NMFS, where as it could potentially still be determined if all three groups of spawners were contributing to the juvenile population. However, reductions in sample size would not allow for statistical certainty of the results. Second, WDFW would drop the juvenile sampling and any associated DNA analysis from the project. With that decision, the pedigree analysis would be conducted from the adult-to-adult stage only.

Task 2c. Collect DNA tissues from a representative sample of smolts or pre-smolt from the Tucannon River.

[A smolt trap has been proposed for installation just upstream of the TFH adult trap for a genetic pedigree study on spring chinook (see BPA Basinwide Proposal by Mike Ford (NMFS) and Todd Pearson (WDFW) – "Monitoring the reproductive success of naturally spawning hatchery and natural spring chinook salmon in the Wenatchee, Tucannon, and Kalama Rivers"). ***This option is not being proposed for this study unless the other study is funded and smolt trap equipment and personnel are shared between the projects. Cost estimates for this portion have not been calculated into the current proposed budget.***

Migrants will be collected in a 5-foot screw trap at rkm 59 of the Tucannon River. WDFW operates a smolt trap at rkm 3 of the Tucannon River annually, and estimates emigration of salmon and steelhead. However the TFH adult trap lies within the spawning reach of summer steelhead and ~70-80% of natural steelhead production may come from below the trap site. To accurately complete the objectives of this study will require operation of a second rotary smolt trap (5' diameter) near the adult trapping site (rkm 59). This will allow sampling migrants that resulted from spawning adults sampled as part of the study. Moreover, extreme winter conditions in the upper Tucannon River basin can cause premature emigration of fry and fingerling into the lower river.

A systematic sample of 1,500 migrating juveniles (smolts and presmolts) will be collected at the upper screw trap throughout the entire year. The sampling rate will depend upon the estimated capture of fish throughout the entire season. Factors that will influence capture include trap efficiency, number of eggs deposited, and survival. Naturally produced fish will be distinguished from hatchery produced fish by the absence of a mark or tag. Fish will be weighed, measured, and a small portion of the distal portion of the dorsal lobe of the caudal fin (~0.1 cm^2) will be clipped for DNA. The caudal fin tissue will be placed in a vial with preservative. Fish will be collected in the screw traps annually beginning in 2006.

Task 2d. Collect DNA tissues from all natural origin returning adults at the TFH adult trap from study year fish to conduct pedigree analysis from adult to adult.

Returning natural origin steelhead adults that are part of the study are anticipated to return to the system beginning in the 2007/2008 run year. The TFH adult trap will continue to be operated by hatchery staff during January through May to sample all natural origin adults. Most of these will presumably be from fish that originated upstream of the TFH trap, but some could be from the

lower river as well. River conditions will likely influence when and what returns in a given year. The barrier on the existing dam face will continue to be installed each year to insure that nearly 100% of the fish are sampled each year. We anticipate sampling between 100-300 natural origin steelhead annually.

Objective 3: Consolidation and processing of data and DNA samples.

Task 3a. Consolidate data from adult traps and electrofishing surveys.

Large amounts of biological data will be collected in the process of the adult and juvenile sampling for this study. Staff will develop databases/spreadsheets containing all relevant data for easy data extraction when necessary. Staff will also consolidate collected biological sample data (scales and DNA tissues), and deliver to the appropriate WDFW personnel for final processing (scale reading or DNA extraction). Scales will be processed and results incorporated into the databases containing all other relevant information for each fish. The DNA data obtained will be kept on separate databases at the genetics labs by the genetics staff to use for the pedigree analysis. DNA samples will be provided to the genetics lab as samples become available throughout the year to insure quick processing and results.

Task 3b. Consolidate data from DNA extraction for pedigree analysis

After DNA analysis has been completed for each year, the genetics lab will conduct a pedigree analysis on the available data. For further information on pedigree analysis criteria, see the attached excellent discussion from the BPA Basinwide Proposal by Mike Ford (NMFS) and Todd Pearson (WDFW) – "Monitoring the reproductive success of naturally spawning hatchery and natural spring chinook salmon in the Wenatchee, Tucannon, and Kalama Rivers". Results of the analysis will be presented in annual progress reports and published in a complete report at the end of the study period, preferably in a peer reviewed journal article(s).

Results

Progress reports throughout the study time period will be completed as required by the Bonneville Power Administration (BPA). Preliminary results will be presented annually in progress reports and/or at meetings. Final results will be presented in a complete report to BPA as well as published in referred journal article(s).

Facilities and Equipment and Existing Cooperative Agreements

A major strength of this proposal is that we propose to use a great deal of existing expertise, personnel, equipment and facilities within the study area. A long-term research and monitoring crew are currently stationed in Dayton, Washington, less than 30 miles from the Tucannon River. The crew has a variety of facilities/equipment at their disposal to complete the much of the necessary work proposed (i.e electrofishing). In addition, all of the major hatchery facilities (Lyons Ferry, Tucannon FH Adult Trap, Captive Brood rearing tanks used for the reconditioning process) needed to complete the proposed project exist under the current cooperative agreement between the USFWS and BPA (LSCRP Program) and WDFW and BPA (Tucannon River Spring

Chinook Captive Broodstock Program). Evaluation and hatchery crews can be shared between the existing LSRCP program, and equipment such as an electroshocker, nets, and telemetry receivers have been purchased under existing LSRCP funding.

If possible, and if it makes the contracting for this project go easy, funding could be routed through the existing cooperative agreement with the USFWS for LSCRP funding. Budget tracking would still be possible, with assignment of a different project code for the kelt reconditioning/reproductive success activities. Reporting requirements to BPA would follow BPA protocols.

WDFW Genetics Laboratory: Existing Facilities, Equipment, & Personnel Resources

The WDFW Genetics Lab presently occupies approximately 3,100 sq. ft. of space in the Natural Resources Building in Olympia, WA. DNA data collection and processing (microsatellite analysis and sequencing) are done using computer-controlled, semiautomated DNA sequencers. The laboratory is equipped with two capillary-based 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 Sequencer (Gene Codes Corp.) software for data collection and processing. 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 4+ scientific technician FTEs.

Timline of Tasks

Year	Month(s)	Task #	Description
2004	Jan-Apr	1a	Collect/spawn adults for reconditioning
2004	Jan-May	2a	Construct barrier, sample adults at TFH and pass upstream to spawn
2004/05	Feb-Apr	1b	Recondition kelts at LFH
2004	May, Sept	3a	Consolidate samples and send in for processing
2005	Jan-Apr	1a	Collect/spawn adults for reconditioning
2005	Jan-July	3b	Pedigree Analysis and preliminary reporting
2005	Jan-May	2a	Construct barrier, sample adults at TFH and pass upstream to spawn
2005	Feb/Mar	1d	Select kelts to return to river for study, radio track fish
2005	Feb-Apr	1c	Spawn recondition adults in hatchery, determine success
2005/06	Feb-Apr	1b	Recondition kelts at LFH
2005	August	2b	Electrofish in upper Tucannon and collect samples (Age 1+)
2005	May, Sept	3a	Consolidate samples and send in for processing
2006	Jan-Apr	1a	Collect/spawn adults for reconditioning
2006	Jan-July	3b	Pedigree Analysis and preliminary reporting
2006	Jan-May	2a	Construct barrier, sample adults at TFH and pass upstream to spawn
2006	Feb/Mar	1d	Select kelts to return to river for study, radio track fish
2006/07	Feb-Apr	1b	Recondition kelts at LFH
2006	August	2b	Electrofish in upper Tucannon and collect samples (Age 1+)
2006	May, Sept	3a	Consolidate samples and send in for processing
2007	Jan-Apr	1a	Collect/spawn adults for reconditioning
2007	Jan-July	3b	Pedigree Analysis and preliminary reporting
2007	Jan-May	2a, 2d	Construct barrier, sample adults at TFH and pass upstream to spawn
2007	Feb/Mar	1d	Select kelts to return to river for study, radio track fish
2007/08	Feb-Apr	1b	Recondition kelts at LFH
2007	August	2b	Electrofish in upper Tucannon and collect samples (Age 1+)
2007	May, Sept	3a	Consolidate samples and send in for processing
2008	Jan-Apr	1a	Collect/spawn adults for reconditioning
2008	Jan-July	3b	Pedigree Analysis and preliminary reporting
2008	Jan-May	2a, 2d	Construct barrier, sample adults at TFH and pass upstream to spawn
2008	Feb/Mar	1d	Select kelts to return to river for study, radio track fish
2008/09	Feb-Apr	1b	Recondition kelts at LFH
2008	August	2b	Electrofish in upper Tucannon and collect samples (Age 1+)
2008	May, Sept	3a	Consolidate samples and send in for processing
2009	Jan-Apr	1a	Collect/spawn adults for reconditioning
2009	Jan-July	3b	Pedigree Analysis and preliminary reporting
2009	Jan-May	2a, 2d	Construct barrier, sample adults at TFH and pass upstream to spawn
2009	Feb/Mar	1d	Select kelts to return to river for study, radio track fish
2009	August	2b	Electrofish in upper Tucannon and collect samples (Age 1+)
2009	May, Sept	3a	Consolidate samples and send in for processing
2010	Jan-July	3b	Pedigree Analysis and preliminary reporting
2010	August	2b	Electrofish in upper Tucannon and collect samples (Age 1+)
2011	Jan-July	3b	Pedigree Analysis and preliminary reporting
2010	May, Sept	3a	Consolidate samples and send in for processing
2010	Jan-May	2d	Construct barrier, sample adults at TFH and pass upstream to spawn
2011	Jan-May	2d	Construct barrier, sample adults at TFH and pass upstream to spawn
2011	May	3a	Consolidate samples and send in for processing
2012	Jan-May	2d	Construct barrier, sample adults at TFH and pass upstream to spawn
2012	May	3a	Consolidate samples and send in for processing
2013	Jan-May	2d	Construct barrier, sample adults at TFH and pass upstream to spawn
2013	May	3a	Consolidate samples and send in for processing
2013	May-Dec	3b	Final Pedigree Analysis and Final Report Writing – End of Study

Outyear Budget Estimates (proposal with juvenile electrofishing sampling)

FY04	FY05	FY06	FY07	FY08
152,152	264,510	272,325	269,348	294,833

Outyear Budget Estimates (proposal with no juvenile samples)

FY04	FY05	FY06	FY07	FY08
152,152	142,546	146,702	140,046	161,338

FY2004 Proposed Budget

Item	Notes	Amount
Personnel		
Salaries	Biologist 3 (3.0 mo. @4,200/mo)	12,600
	Biologist 4 (0.5 mo. @4,428/mo)	2,214
	Genetic Bio (2.5 mo. @5,000/mo)	12,500
	Hatchery Specialist (5.0 mo. @3,215/mo)	16,075
	Scientific Tech 3 (4.0 mo. @3,200/mo)	12,800
Benefits @ 28%	Biologist 3 (2.5 mo. @4,200/mo)	3,528
	Biologist 4 (0.5 mo. @4,400/mo)	620
	Genetic Bio (2.5 mo. @5,000/mo)	3,500
	Hatchery Specialist (4.25 mo. @3,215/mo)	4,501
	Scientific Tech 3 (2.75 mo. @3,200/mo)	3,584
Personnel Total		***71,922***
Goods and Services		
Adult Tagging Supplies		400
Misc. Field Gear/Supplies		0
DNA Sampling Supplies		100
DNA Processing		12,800
Hatchery Rearing		10,000
Barrier Trap Modification		25,000
Goods and Services Total		***48,300***
Travel		1,500
Indirect Costs		30,430
Equipment		0

Summary and Totals	
Personnel	71,922
Goods and Services	48,300
Travel	1,500
Indirect	30,430
Equipment	0

Total 152,152

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Individual Qualifications of Applicants

Joseph D. Bumgarner: (Washington Department of Fish and Wildlife)
Project Responsibility: budet tracking, annual report synthesis and writing, data compilation, electrofishing samples, trap barrier installation, coordination between cooperators and other kelt reconditioning projects.

Education experience includes B.S. Degree in Fishery Science and M.S. Degree in Fishery Science from the University of Washington, School of Fisheries and Aquatic Sciences.

Responsible for WDFW's species evaluation of federal compensation/mitigation program designed to replace fish resources lost due to construction of the four lower Snake River power dams. Currently acts as assistant project leader and lead for summer steelhead evaluations under the Lower Snake River Compensation Plan – Lyons Ferry Hatchery Complex Hatchery Evaluations. I am currently involved in all aspects of hatchery production of summer steelhead in SE Washington, as well as monitoring natural summer steelhead production in the Tucannon and Touchet Rivers, and Asotin Creek (spawning ground surveys, juvenile electrofishing, smolt trapping, genetic data collection, and other biological data). Previously acted as lead spring chinook biologist for Tucannon River spring chinook salmon. Other major duties include assisting with experimental design and implementation of studies, budget tracking, and report writing. Reports are submitted in annual progress reports or referred journal articles.

Implemented Projects: Successfully implemented the BPA funded Tucannon River Spring Chinook Captive Broodstock Program in 2000(Project # 2000-019-00). Successfully implemented LSRCP funded monitoring and evaluations for WDFW Lyons Ferry Complex Hatchery Program

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RECENT RELEVANT REPORTS:

Bumgarner, J., M. Schuck, S. Martin, J. Dedloff, and L. Ross. 2002. Lyons Ferry Hatchery Evaluation Steelhead/Trout Report: 1998-2000 to 1997-98 to U.S. Fish and Wildlife Service. Lower Snake River Compensation Plan Office. Boise, ID. #FPA02-09.

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Mark L. Schuck (Washington Department of Fish and Wildlife)
Project Responsibility: budget tracking, coordination.

Educational experience includes and B.S. Degree in Fishery Biology from Colorado State University in 1974.

Project leader for WDFW's species evaluation of a federal compensation/mitigation program designed to replace fish resources lost due to construction of the four Snake River power dams. Involved in all aspects of hatchery production of summer steelhead, spring chinook, and fall chinook in SE Washington. Between 1982-1994, Mark served as the district Fish Management Biologist for Washington Dept of Wildlife in Asotin, Columbia, Garfield and Walla Walla Counties, as well as project leader for LSRCP studies on steelhead/trout (WDW). Prior to LSRCP experience, mark served as a research biologist with WDW on Lower Columbia River steelhead stock evaluation studies, and assessing effect of Mount St. Helens eruption on steelhead populations within the Toutle River drainage. Other current duties include assisting with experimental design and implementation of studies, budgeting and report writing, as well as regional planning efforts. Reports are submitted in both annual progress reports and final refereed journal articles.

BPA Implemented Projects: Currently in the process of implementing Asotin Creek summer steelhead natural production monitoring (Project # 2002-27002). Served as a Co-Project leader for BPA funded project of Bull Trout in SE Washington (Project #9005300 – Reports DOE/BP 17758-1 & DOE/BP 17758-2). Successfully implemented LSRCP funded monitoring and evaluation studies for Lyons Ferry Complex Program for 20 years.

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RECENT RELEVANT REPORTS:

Martin, S. M. Schuck, J. Bumgarner, J. Dedloff, and A. Viola. 2000. Lyons Ferry Hatchery Evaluation Trout Report: 1997-98 to U.S. Fish and Wildlife Service. Lower Snake River Compensation Plan Office. Boise, ID. # FPA00-11.

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Schuck, M., A. E. Viola, J. Bumgarner, and J. Dedloff. 1998. Lyons Ferry Trout Evaluation Study: 1996-97 Annual Report to U.S. Fish and Wildlife Service. Lower Snake River Compensation Plan Office. Boise, ID. # H98-10.

Viola, A.E., and M.L. Schuck. 1997. A Method to Reduce the Abundance of Residual Hatchery Steelhead in Rivers. North American Journal of Fisheries Management:15(2) 488-493.

James B Shaklee: (Washington Department of Fish and Wildlife)
Project Responsibility: Oversee DNA analysis, budget tracking, annual report synthesis and writing (Genetic Portions).

Educational experience includes and B.S. Degree in Zoology from Colorado State University in 1968, a M.S. Degree in Biology from Yale University in 1970, a M.S. Degree in Fishery Biology from Colorado State University in 1974, and a PhD in Biology from Yale University in 1982.

James is currently is a Research Scientist for WDFW, and oversees lab organization, budget, and operations and supervise staff conducting DNA- and allozyme-based investigations of Pacific salmon, other salmonids, marine fish, and shellfish throughout Washington. In the past, James has also served as research scientist in Australia (1981-1985), and as an Assistant Professor in the Dept of Zoology at the University of Hawaii. James has expertise in the genetic analysis of marine and freshwater fish and shellfish to address conservation, fishery management, and taxonomic issues. Major areas of current and past research include: a) genetics of population structure and zoogeographic divergence, b) applications of genetics to fishery management and conservation, c) genetic and morphological aspects of speciation and evolution, d) biochemical identification of species and processed fishery products, and e) biochemical and genetic aspects of embryonic development and physiological adaptation.

RECENT RELEVANT REPORTS:

Olsen, J.B., P. Bentzen, M.A. Banks, J.B. Shaklee, and S. Young. 2000. Microsatellites reveal population identity of individual pink salmon to allow supportive breeding of a population at risk of extinction. Trans. Amer. Fish. Soc. 129:232-242.

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Shaklee, J.B. and P. Bentzen. 1998. Genetic identification of stocks of marine fish and shellfish. Bull. Mar. Sci. 62:589-621.

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Shaklee, J.B., C. Busack, A. Marshall, M. Miller, and S.R. Phelps. 1990. The electrophoretic analysis of salmonid mixed-stock fisheries. pp.235-265 In: (Z-I. Ogita and C.L. Markert, eds.) Isozymes: Structure, Function, and Use in Biology and Medicine (Proceedings of the Sixth International Congress on Isozymes). Progress In Clinical and Biological Research. Vol. 344. Wiley-Liss, Inc., New York. 973pp.

Donald L. Peterson: (Washington Department of Fish and Wildlife)

Project Responsibility: Kelt Reconditioning and rearing/treatments/outplanting. Oversee adult trapping and genetic/biological sampling at Tucannon FH Adult Trap (hatchery staff supervision.

Educational Experience includes a B.S. Degree in Biology from Western Washington University and an Associate in Arts and Applied Science Degree from Highline Community College, as well as 26 years involved in the culture of Pacific salmon and steelhead in the Pacific Northwest.

Don is currently the Complex Manager of the Lyons Ferry Complex operated under the LSRCP mitigation program (Lyons Ferry Hatchery, Tucannon Fish Hatchery, and associated acclimation facilities). Responsible for planning, directing and coordinating the fish cultural activities, operations and maintenance of the hatchery and acclimation facilities assigned to the complex. Also serves as division representative specific to the geographic area supervised on a variety of forums. These include but are not limited to: WDFW district management teams, union management negotiations, technical committees for salmon and steelhead mitigation and recovery. Don's extensive and long term history in the fish culture arena qualify him to oversee the kelt recondition process. His experience and personnel contacts over the years within the fish culture business will prove invaluable in making this kelt reconditioning program successful.

Successfully implemented LSRCP funded hatchery operation and maintenance for WDFW's Lyons Ferry Complex Hatchery Program

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Attachment #1

Description of pedigree experimental design analysis taken from T. Pearsons (WDFW) and M. Ford (NMFS) (BPA Basin Wide Proposal) "Monitoring the reproductive success of naturally spawning hatchery and natural spring chinook salmon in the Wenatchee, Tucannon, and Kalama Rivers"

We propose to use a powerful genetic method for obtaining estimates of the number of progeny produced by individual breeding adults. Briefly, we will obtain DNA samples from potential spawners and their putative offspring and use highly polymorphic genetic markers to reconstruct pedigrees for individual fish in natural and hatchery environments. Experiments of this sort involve: a) non-lethally collecting a tissue sample and other biological information (hatchery versus natural origin, morphological characteristics, run timing, etc.) of potential spawners in a natural spawning area and a nearby hatchery, b) collecting samples of progeny resulting from these spawners at varying life-stages up to and including returning adults, c) estimating the fitness of different classes of fish by using genetic markers to assign progeny to their parents. This technique has been successfully used to estimate the relative reproductive success in a variety of species (DeWoody and Avise 2001; Morgan and Conner 2001; Neff et al. 2000a; Smouse and Meagher 1994; Smouse et al. 1999), including salmonids (Bentzen et al. 2001; Berejikian et al. 2001; Ford et al. 2002).

Fitness estimation methods

We will use a variety of methods to estimate the relative fitness of hatchery and naturally produced salmon. All of the methods are based on using DNA typing techniques to estimate how many progeny were produced by sampled adults. Some of the methods involve a two step process: determination of a pedigree that is used to explicitly count the number of offspring produced by each sampled parent, followed by statistical analyses of the counts to determine if there are significant differences between hatchery and natural salmon. These two step methods are appropriate in cases where the population pedigree can be determined unambiguously, because they treat the progeny counts as observed data. In some cases, it may be more efficient to score a larger number of progeny for a smaller number of loci. In these cases, a proportion of the progeny will probably not be assigned unambiguously to a single pair of parents. Smouse et al. (1999) and Morgan and Conner (2001) have developed a statistical method for dealing with this situation when maternity is known, and Ford et al. (2002) have modified this method for the case of two unknown parents.

Two step estimation methods

The first step of these methods is to use the genetic typing data (see specific tasks for details on laboratory methods) to determine the parentage of the sampled progeny, using either simple exclusion or one of several likelihood-based methods (e.g., Marshall et al. 1998; Sancristobal and Chevalet 1997). Any progeny that cannot be unambiguously assigned to a single pair of parents are removed from the analysis. Once parentage as been determined, the number of progeny each parent produced is determined by simple counting. The progeny counts form the

basis of all subsequent analyses. Contributions from precocious males (which will most likely not be sampled) will be tested by comparing the distribution of offspring with unassigned male parents and to the distribution with unassigned female parents.

Analyses using the progeny counts

$H_{0:1}$ -- *Random mating and equal egg-sampling survival* - In the terminology that follows, a mating between two hatchery-origin fish is labeled "HH", two wild fish "WW", a wild male and a hatchery female "WH", and a hatchery male by a wild female "HW". The null hypothesis that we are testing is that mating occurs randomly without regard to hatchery or wild origin. Under this hypothesis, if p is the proportion of wild fish passed above the weir, the expected proportions of the four possible mating types are p^2, $p(1-p)$, $p(1-p)$, and $(1-p)^2$ for the WW, WH, HW, and HH matings, respectively. If we assume for the moment that these matings could be observed directly, the null hypothesis could be tested using a χ^2 test (df = 3) comparing the observed numbers of the different mating types with their expectations under the null hypothesis. The parameters describing the degree of relative mating success and/or non-random mating would simply be the relative deviations from the expected values.

Continuing under the assumption that we could observe matings directly, we can determine *a priori* how many matings we would need to observe to have a specified power of detecting particular deviations from the null hypothesis. Power is defined as the probability of rejecting the null hypothesis when it is in fact false (1 - B, where B is the type II error rate). We explored the relationship between the number of observed matings and statistical power using a simple Monte Carlo method in which N matings were drawn randomly from a multinomial distribution with parameters p^2, $2p(1-p)(1-s)$,$(1-p)^2(1-2s)$, where s is the reduction in the probability of mating associated with a single hatchery fish. The fit of these observed matings to the null hypothesis ($s = 0$) was then determined with a χ^2 test (df = 2). For various combinations of N, s, and p, this process was repeated 1000 times each and the power to detect a deviation of magnitude s or greater was estimated as the proportion of the 1000 trials that resulted in a χ^2 test statistic greater than a specified critical value (either for $\alpha = 0.05$ or 0.15). Note that for the sake of simplicity we have lumped together the WH and HW mating categories in this analysis. Our results indicate that a fairly large number of matings (>500) must be observed to have a reasonable power to detect small ($s < 0.1$) deviations from the null hypothesis. The analysis also shows that power will be maximized when there are approximately equal numbers of hatchery and wild-origin spawners.

Testing the null hypothesis of random mating and equal mating success is actually somewhat more complicated than we have been assuming up until this point because instead of observing matings directly we will be obtaining samples of the resulting progeny. Sampling offspring instead of observing matings adds additional variability to the experiment because some matings may not be sampled due to chance. Sampling offspring will also confound any differences in mating success among groups with any differences in fecundity and egg-to-sampling survival, factors which we will control for by direct observation (see tasks 2b,c,d,e). If we assume for a moment that there are no differences in fecundity or egg-to-survival survival among groups, then

the problem of observing only a sample of 'matings' can be dealt with by sampling a sufficient number of fry that the likelihood of observing a sufficiently large number of matings is high. Note that once an offspring from a particular mating pair has been detected, additional offspring from the same pair do not provide any additional information about whether or not that mating occurred, although if there is uncertainty about parent-offspring relationships additional progeny could be used to confirm a particular mating. Under the null hypothesis of random mating and equal fertility, fecundity and egg-to-sampling survival, the probability that an observed fry came from particular mating is $q = 1/N$. The probability that a fry from any particular mating will be included in a random sample (with replacement) of size n is $1-(1-q)^n$. For example, if N is 500, then n must be ~1000 to have an ~80% probability of observing a particular mating.

The analyses above show, not surprisingly, that the power to detect differences among groups will depend dramatically on the magnitude of the differences in mating success among groups. By combining data across multiple cohorts, the total sample size will be increased considerably, providing additional power to detect smaller effects. Power can also be increased considerably if a higher probability of a type I error (probability of rejecting the null hypothesis when it is in fact true) is acceptable. In this study, where failing to reject the null hypothesis may be just as important a result as rejecting it, a careful balancing of type I and type II errors may be appropriate.

$H_{0:2}$ – Equal fecundity among groups

The hypothesis of equal fecundity among groups will be tested by counting and/or weighing the eggs of a random sample of females that spawned in the hatcheries associated with the populations of this study. If fecundity differences between hatchery and wild-origin females are found, then the null hypothesis of random mating/equal mating success (see above) can be adjusted to reflect these differences.

$H_{0:3}$ -- Equal juvenile production among groups

The hypothesis that hatchery and natural fish do not differ in their ability to produce surviving juvenile migrants will be tested using a *t*-test to compare the numbers of juveniles produced by fish from the two groups.

$H_{0:4}$ – Equal juvenile-to-adult survival among groups

This hypothesis will be tested by comparing the proportions of a sample of HH, WH, and WW juveniles with the proportions in a sample of returning adults using a 2x3 contingency table (G-test). If we arbitrarily set the relative survival of the WW fry equal to $s_1 = 0$, and the relative survival of the WH and HH fry equal to s_2 and s_3,, then s_2 and s_3 can be estimated from the deviation between the observed and expected smolt counts. We explored the relationship between juveniles and adult sample sizes and statistical power using a method similar to the Monte Carlo method we employed to estimate the power to reject $H_{0:1}$. To do this, we randomly sampled juveniles from a multinomial distribution with parameters p_1, p_2, and p_3, which are the actual proportions of WW, WH, and HH juveniles in the population, respectively. We then

randomly sampled adults from a multinomial distribution with parameters p_1, $p_2 (1-s_2)$, and $p_3 (1-s_2)$, and performed a 2x3 contingency test comparing the observed fry and smolt types. For each combination of sample sizes and parameters, this was done 1000 times, and the power to reject the null hypothesis was estimated as the proportion of the 1000 trials that resulted in a test statistic greater than the specified critical value. These results suggest that a sample of ~1000 smolts will provide reasonable power to detect moderate ($s > 0.1$) differences in relative survival among groups.

$H_{0:6}$ -- Equal adult to adult replacement rate among groups

This hypotheses will be tested with a *t*-test, comparing the observed adult counts among groups.

$H_{0:8}$ -- Variation in traits such as run timing, size, weight, and morphology have no effect on the number of offspring produced

In order to address Objective 1 (fitness of hatchery compared to natural fish), we will initially conduct our analysis using only a single trait: hatchery versus natural origin. This will provide an estimate of the relative fitness of naturally spawning hatchery fish without attempting to factor out any biological differences between the two groups. After obtaining this initial estimate, we will address Objective 2 by conducting a multiple regression analysis adding the traits such as run timing, length, weight and morphology to the analysis in order to determine to what degree variation in these traits can explain any observed difference in fitness between hatchery and natural fish. This analysis will be conducted separately for the juvenile adult samples.

One step methods

In one step methods, the process of estimating the pedigree and estimating relative fitness is combined into a single model (Smouse et al. 1999; Morgan and Conner 2000; Ford et al. 2002). The parameters of interest in this model describe the population-level relationships between an individual's traits and the number of offspring produced. The estimated numbers of offspring produced by each individual are treated as nuisance parameters and integrated out of the analysis. These models are useful in situations where parentage cannot be determined unambiguously, because they make use of parent-offspring combinations that are estimated with high, but not absolute, certainty. This situation will probably arise in one or more of the populations in our study in years of high spawning escapement. The one step methods are more efficient that the two step methods, because all of the available data is used in the analysis.

The specific model we will use is similar to that described by below:

In the model, fitness is defined by $l_i = w_i / \sum w_i$, where l_i is the relative fitness of individual *i*, and w_i is expected number of offspring produced by individual *i*. The relationship between the expected number of progeny produced by a particular parent is assumed to be a log-linear function of the parent's traits, such that

$\log w_i = \sum b_j z_{ij} + \sum g_j z_{ij}^2$, where the sums are taken over all *j* traits and z_{ij} is the value of trait *j* for individual *i*. The b_j parameters are population level parameters describing the expected linear relationship between the *j* traits and relative fitness, and the g_j parameters are the population level parameters describing the expected quadratic relationship between the traits and relative fitness. The traits can be either continuous, such as length, weight or run timing, or discrete, such as hatchery or natural origin. The likelihood of observing a specific progeny *k* is

$L_k = \sum_{ij} X_{ijk} l_i^m l_j^f$, where X_{ijk} is the genetic probability of observing offspring genotype *k* given paternal genotype *i* and maternal genotype *j*, and l_i^m is the expected relative fitness of male *i* and l_j^f is the expected relative fitness of female *j*. The likelihood of the entire sample is the product of each of the individual progeny likelihoods: $L = \prod L_k$. The parameters of interest, the b_j's and g_j's, are estimated by maximizing *L*. The statistical significance of the parameter estimates can be best determined by conducting computer simulations in which trait profiles and genotype profiles are randomized with respect to each other (Smouse et al. 1999). We have written a computer program for obtaining these estimates and used it successfully on data sets obtained from coho salmon in Minter Creek, WA (Ford et al. 2002) and steelhead in Little Sheep Creek, OR (P. Moran, personal communication).

Based on computer simulations (Morgan and Conner 2000; Ford, unpublished data), the likelihood model will require fairly large sample sizes (~1000 juveniles) to have sufficient power to detect small effects on categorical factors (e.g., hatchery compared to natural origin). Power to detect selection on continuous traits is somewhat higher. The model can produced biased results when a substantial fraction (> 40%) of potential parents are not sampled, but this bias is not large so long as selection gradients are moderate to small (e.g., $b < 0.6$). The power of the test also suffers as the fraction of potential parents sampled goes down. We have therefore chosen streams in which most potential parents can be sampled in most years. We will also explore other statistical model that may more effectively account for missing parents (e.g., Neff et al. 2000b)

In order to address Objective 1 (fitness of hatchery compared to natural fish), we will initially conduct our analysis using only a single trait: hatchery versus natural origin. This will provide an estimate of the relative fitness of naturally spawning hatchery fish without attempting to factor out any biological differences between the two groups. After obtaining this initial estimate, we will address Objective 2 by adding the traits such as run timing, length, weight and morphology to the analysis in order to determine to what degree variation in these traits can explain any observed difference in fitness between hatchery and natural fish. This analysis will be conducted separately for the juvenile and adult samples.