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CRITFC

TECHNICAL REPORT 06-07

2005 Annual Report

An Evaluation of the Reproductive Success of Natural-origin, Hatchery-origin, and Kelt Steelhead in the Columbia Basin

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16 March 2006

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Abstract

We initiated a field study to investigate the relative reproductive success of artificially reconditioned kelt steelhead. Prior to an implementation of a large-scale steelhead kelt reconditioning program, it is important to evaluate the reproductive success of reconditioned kelts relative to hatchery and natural first time spawners. To assess kelt reproductive success, three sites (Satus Creek,WA; Omak Creek, WA: and Shitike Creek, OR) have been chosen to replicate studies of relative reproductive success of each variant of steelhead. Technological advances in DNA-typing make direct measurement of reproductive success using pedigree analysis practical. Employing these new techniques, our study will measure the reproductive success of natural-origin, hatchery-origin, and reconditioned kelt steelhead in natural stream settings. This will yield quantitative data replicated geographically and temporally that will add resolution to the issue.

The 2005 field season was the initial year of capturing, reconditioning, and analyzing kelts in the three subbasins. While we were successful in capturing and reconditioning kelts, the numbers and condition of kelts was unusually poor presumably due to lower than normal precipitation and flows. Physical evaluation and the collection of genetic material from first time spawning adults resulted in the capture of 113 adults at Omak Creek, 57 in Shitike Creek, and 68 from Satus Creek (retained steelhead destined for Section Corner and South Fork Logy Creeks). We detected 34 kelts attempting to outmigrate from Omak Creek, 39 kelts from Shitike, and 3 from Section Corner Creek. Kelts collected at Omak Creek did not survive to be reconditioned so post spawn steelhead from the localized broostock program were used and three were released in October of 2005. Ten kelts were collected for reconditioning from Shitike Creek and one survived to release in February 2006. None of the post spawn steelhead from Section Corner Creek survived to be reconditioned.

In 2005 we were able to collect parentage data in Section Corner Creek, because reconditioned kelt steelhead were available and the experiment site is analogous to a field laboratory. We released 16 steelhead into Section Corner Creek on March 9, 2005. This release was composed of five reconditioned female kelts, six female first time spawners, and five male first time spawners. Parentage analysis of 159 juveniles indicated that production was from first-time spawning females only. We believe that the reconditioned kelts were over ripe and did not spawn. These fish were held on well water with water temperatures that exceeded the river's, likely accelerating their maturation. We are implementing procedures to more closely synchronize maturation levels from the two groups for future experiments. Parentage analysis for Omak and Shitike Creek will begin in 2006.

ACKNOWLEDGEMENTS

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We also thank, Michael (Sonny) Fiander, Carrie Skahan, Chuck Carl Bill Fiander and other Yakama Nation Fisheries Program staff for providing fish husbandry and telemetry expertise. Additionally we would like to thank, Brad Houslet, Jens Lovetang, and the Warm Springs Fisheries staff as well to the Colville Fisheries staff for all of their support. We appreciate the input of Mike Paiya of U.S. Fish and Wildlife Service. This work would not have been possible without their assistance. We thank Phil Roger and Bobby Begay from the Columbia River Inter-Tribal Fish Commission for providing comments on the project and reviews of the annual report.

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INTRODUCTION

Oncorhynchus mykiss are considered to have one of the most diverse life histories in Salmoninae (Behnke 1992) with variants that include resident, estuarine, and anadromous ecotypes, widely ranging ages and timing of juvenile and adult migrations, and various reproductive strategies including precocity, semelparity, and iteroparity. This complex array of life history variation is possibly a compensating or bet hedging device for life in stochastic environments (Taborsky 2001). Overlapping generations provide resources in the event of failure of any brood year due to brief catastrophic events. While fluctuating populations and overlapping generations may reduce the effective size (N_e; Waples 2002), persistence of the species may be favored due to these compensating life histories (Narum et al. in review).

Steelhead are unusual among anadromous Pacific salmonids in that they are iteroparous (can spawn multiple times). Iteroparity rates for *0. mykiss* outside the Columbia River basin are estimated to be as high as 30% in neighboring British Columbia (Withler 1966), 21% in the Rogue River (Busby et al. 1996) with extremely high rates in Russia at 79% (Savvaitova et al. 1996). Rates for the Columbia River are considerably lower ranging from 1.5-17% with rates generally decreasing with the distance traveled upstream. The reduced iteroparity rates may be influenced by latitude (Fleming 1998) and inland distance effects (Withler 1966; Bell 1980; Meehan and Bjornn 1991), but the hydrosystem also directly selects against this life history strategy as evidenced by high mortality (Hatch et al. 2003a; Evans et al. 2004; Wertheimer and Evans 2005) resulting in a potential loss of this important evolutionary legacy.

Observations in the Columbia River indicate that emigrating kelts are abundant and the sex ratio is highly skewed toward females (>85%) and wild origin fish (Evans 2002; Hatch et al. 2003a). Utilizing this resource in a recovery program would take advantage of a relatively abundant group of primarily wild-origin

females that would perish under standard river operating conditions. Even hatchery origin kelt steelhead may be important for recovery. Fleming and Petersson (2001) reported that hatchery-origin females generally showed greater reproductive abilities than hatchery-origin males and in most cases there are few differences in reproductive abilities and performance between hatchery-origin and natural-origin. Additionally, Fleming and Gross (1993) concluded that introducing hatchery-origin females rather than males may be an important technique for rebuilding wild populations using hatchery fish. This could have important implications with kelt reconditioning since greater than 85% of the post spawn steelhead collected are female (Evans 2002; Hatch et al. 2003a).

Endangered Species Act (ESA) listing of Columbia River Basin steelhead populations (NMFS 1997; NMFS 1999) has prompted interest in developing artificial kelt reconditioning to help bolster diminishing natural populations. Reviews on this subject (Hatch et al. 2002, 2003a, 2004) provide strong support of the benefits of kelt reconditioning as a novel strategy for steelhead recovery. To evaluate the feasibility of kelt reconditioning the Yakima/Klickitat Fisheries Project (YKFP) in collaboration with the Columbia River Inter-Tribal Fish Commission (CRITFC) have been capturing wild emigrating kelt steelhead from the Yakima River and experimenting with several artificial kelt reconditioning methods since 1999 at Prosser Hatchery (BPA Project 200001700). The success of the reconditioning project has generated the need for this study. Specific questions regarding the success of artificially reconditioning kelt steelhead include: do reconditioned kelts produce viable offspring that contribute to recruitment, how does kelt reproductive success compare with natural first time spawners, and how does kelt reproductive success compare with hatchery origin spawners? Answers to these questions will be important in determining if kelt reconditioning is a viable restoration tool that will aid in the recovery of ESA listed steelhead populations in the Columbia River Basin. We have established that kelt reconditioning is possible and have demonstrated successful spawning migrations and redd construction (BPA Project 200001700). However, the

reproductive success of reconditioned kelts needs to be explored to assess the net benefit of this program. In addition, comparisons to natural origin first time spawners can be used to evaluate reconditioned kelt contributions relative to a first time spawner baseline, and comparisons with hatchery origin steelhead is important to evaluate if kelt reconditioning is a viable alternative to more traditional hatchery supplementation.

Technological advances in DNA-typing make direct measurements of reproductive success using pedigree analysis practical. Employing these new techniques, this study directly measures the reproductive success of natural-origin, hatchery-origin, and reconditioned kelt steelhead in natural stream settings. This will yield quantitative data replicated geographically and temporally that will add resolution to the issue.

The objectives and hypotheses of this study are to:

1) Apply kelt steelhead reconditioning techniques at selected streams to post-spawners for release back into study streams.

Ho: Kelt steelhead reconditioning rates are similar spatially and temporally.

Ho: Kelt steelhead rematuration rates are similar spatially and temporally.

2) Evaluate reproductive success of natural-origin, hatchery-origin, and reconditioned kelt steelhead and adult resident *O. mykiss* at Shitike, Omak, Section Corner, and South Fork Logy creeks using pedigree analysis.

Ho: Reproductive success among natural-origin, hatchery-origin, and reconditioned kelt steelhead is equal within and among streams.

METHODS

Pedigree analysis will be used to identify parents of juvenile steelhead to quantify the reconditioned kelts contribution towards natural production. To accomplish this, the study stream must have a weir to collect and sample upstream migrating adults and downstream migrating kelts. Ideally, 100% of the upstream migrating adults would be sampled for DNA. Incidentally this project will provide us with data on kelt reconditioning at multiple locations which should provide insights into the use of the technique for restoration purposes.

Study Area

Field collections were made on Shitike Creek, a tributary to the Deschutes River (Figure 1); Omak Creek, a tributary to the Okanagon River; and Satus Creek a tributary to the Yakima River (Figure 2). Reconditioning was conducted at Warm Springs National Fish Hatchery (Shitike fish), Cassimer Bar Fish Hatchery (Omak fish), and Prosser Fish Hatchery (Satus fish). Section Corner and South Fork Logy creeks, tributaries to Satus Creek, were used as “natural laboratories” with each receiving reconditioned kelt steelhead and first time spawners.

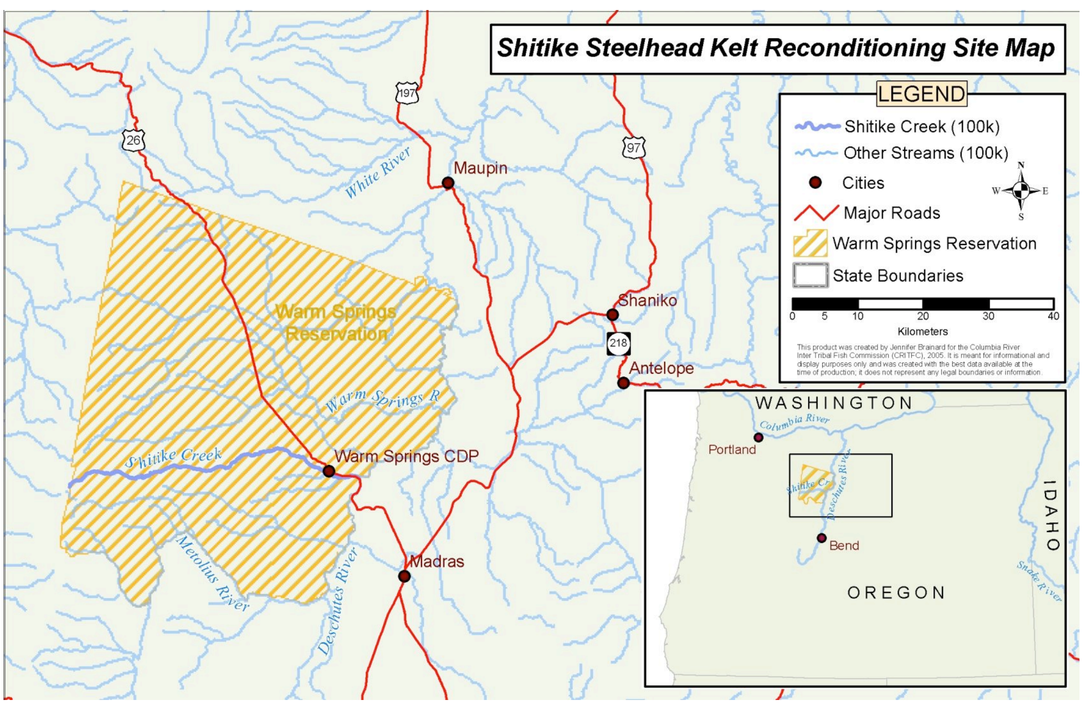

Figure 1. Map showing the location of Shitike Creek.

Figure 2. Map showing the locations of Omak, Satus, Section Corner, and South Fork Logy creeks.

Weir Operation

Weirs were installed and operated for adult and kelt steelhead collections at Shitike, Omak, and Satus creeks. Biological data was taken for each adult and kelt steelhead collected. Fork length was recorded; each fish was scanned for PIT tags and tagged if necessary. Observations about condition and color and marks were recorded. Tissue samples were collected and placed in ethanol for later genotyping. After data collection adult steelhead in Shitike and Omak creeks were released upstream of the weir to spawn naturally. Adults from Satus Creek were relocated to either Section Corner or South Fork Logy creeks. All

kelt steelhead collected were transported to the reconditioning facility for additional reconditioning.

The Shitike Creek picket weir was installed at the scale bridge (1.1 rkm) upstream of the creek's confluence with the Deschutes River on March 14, 2005. Operation of weir began on March 18, 2005 and continued through May 27, 2005. On the evening of March 27th, 2005, a high flow event decommissioned the weir until March 31st, 2005. The trap was checked twice a day for 70 days. A camera box was installed on May 28th, 2005 and due to ambient water temperatures, operation switched to video only from 6/4/05-10/3/05. Security, accessibility, and feasibility determined the location of the weir. The design of the weir incorporated two trap boxes for both upstream and downstream migration (Figure 3). The location of the downstream trap was situated at the edge of the thalweg to minimize the amount of flow inside and was designed to reduce any potential for crowding (10'l x 4'w x 3'h). The angle of approach on both the upstream and downstream picket fence was set at approximately 30 degrees.

Figure 3. Photograph of the picket weir operating on Shitike Creek in 2005.

Trapping at the semi-permanent weir on Omak Creek started on March 16, 2005 and continued through May 13, 2005 (Figure 4). In addition to our reproductive success project, this weir was also used for localized broodstock collection project. Broodstock collection started on March 21, 2005, when fish began showing up in the trap. The last broodstock collection was on April 21, 2005, with the majority of fish collected by April 1. Those fish that weren't selected for broodstock were sampled for DNA, marked with a fin clip, and passed above the trap. Trap operation ceased for first-time spawners on April 8, 2005 at which point the trap was moved back to capture kelts exiting the system.

Figure 4. Photograph of the resistance board weir located on Omak Creek.

To collect first time spawner adults for use in Section Corner and South Fork Logy creeks a removable picket type structure was installed on Satus Creek to direct adult steelhead into the old Satus dam fish ladder where they could be removed with a dip net (Figure 5). On February 22, 2005 at 12:00 noon the picket structure was installed and fished 24 hours a day until March 8, 2005. The weir was operated continuously during this time span, except for a few periods of high flow.

Figure 5. Photograph of the trapping facility located at the old dam site on Satus Creek.

Picket weirs were installed in South Fork Logy (Figure 6) and Section Corner (Figure 7) creeks to prevent adults and kelts from leaving and for fish collection. The South Fork Logy weir was operated shortly from March 9, 2005 to March 28, 2005 due to loss of our weir and specimens during a high water event. Section Corner Creek's weir was in operation from March 9, 2005 to January, 2006.

Figure 6. Photograph of the South Fork Logy Weir: Taken directly after release of kelts and first-time spawners.

Figure 7. Section Corner Creek Weir: Taken directly after release of kelts and first-time spawners.

Reconditioning

Steelhead Kelt Collection

Kelt steelhead captured at the weir in Omak Creek were reconditioned at the Cassimer Bar facility which is currently rearing steelhead for the Omak Creek Localized Broodstock Project. The initial Yakima Basin kelts were collected at the Chandler Juvenile Evaluation Facility (CJEF) on the Yakima River and reconditioned at the neighboring Prosser Hatchery. These fish were placed into Section Corner Creek and South Fork Logy Creek (tributaries of Satus Creek) to spawn and were to be reclaimed for another reconditioning effort. Steelhead kelts at Shitike Creek were captured using a picket weir and transported to the Warm Springs National Fish Hatchery for reconditioning.

At each study site, kelt steelhead were collected in traps or as they accumulated on the upstream side of the picket weir. They were removed with dip nets and placed in an anesthetic tank. Anesthetized steelhead were visually examined to classify each fish as a kelt or pre-spawn individual. Methods for visual classification are available (Hatch et al. 2003a) and primarily involve keying specimens based on an imploded abdomen. This visual technique was highly precise when compared with the use of ultrasound analysis (Evans 2002). If a specimen is suspected to be a pre-spawner that fish was released on the downstream side of the weir.

Holding and Transport

After processing, captured kelts were then transferred to a small truck holding tank that circulated (during capture) or recirculated (during transport) river water to keep the water cool and well oxygenated. Field Crews determined if it was necessary for kelt steelhead to be transported in standard fish tankers with injected oxygen or smaller tanks hauled by truck. Steelhead were then transported to reconditioning facilities within 12 hours of capture.

Feeding / Reconditioning

Upon delivery to reconditioning facilities steelhead were anaesthetized, for "in-processing", where they were scanned for a PIT tag, measured, weighed, fish color and condition noted, treated for parasites, and injected with a PIT tag if not present in the specimen (Hatch et al. 2002; and 2003b). The kelts were then released into large circular holding tanks. In closed aquatic environments, such as kelt reconditioning tanks, severe infestation of parasites can be lethal to cultured fishes, which may be especially susceptible to *Salmincola* in such environments. *Salmincola* is a genus of parasitic copepods that can inhibit oxygen uptake and gas exchange at the gill lamellae/water surface interface by attachment to the lamellae. Recent research by Johnson and Heindel (2000), suggested that Ivermectin™ – a treatment often used to control parasites in swine and cattle – can potentially increase the survivorship of cultured fish by killing the adult morph of the parasite. Due to its successful use in treating *Salmonicola* at the Prosser Hatchery Facility reconditioning experiments during 2000 (Evans et al. 2002), Ivermectin™ was diluted with saline (1:30) and injected into the posterior end of the fish's esophagus using a small (10cc) plastic syringe. Formalin was administered up to five times weekly at 1:6,000 for 1 hour in all reconditioning tanks to minimize fungal outbreaks. Steelhead kelts were reconditioned for a 5-9 month time span before being released to insure rematuration. Fish in the experiments will be released to coincide with natural spawn timing. Growth measurement data and rematuration status was also recorded on all released individuals. Success indicators in this phase of the experiment are based on the proportion of fish that survive the reconditioning process and the number of fish that successfully remature (based on ultrasound examinations).

From our experience with reconditioning kelt steelhead the preferred containers are 20 ft (diameter) large circular tanks (Hatch et al. 2002). Individual tank carrying capacity has been estimated at 200 fish based on the aquaculture experience of YN hatchery staff, and the project goal of maximizing kelt survival

in captivity. Initially, a diet of frozen krill will be fed to the kelt steelhead followed by a maintenance diet of Moore-Clarke salmon pedigree diet (Hatch et al. 2002).

Reconditioning Facilities

Cassimer Bar Hatchery (Figure 8) is located at the confluence of the Okanogan River below Chief Joseph Dam. Currently the Colville Confederated Tribes operate the Cassimer Bar Hatchery.   The facility was originally constructed in 1994, as a sockeye production facility in an attempt to supplement sockeye salmon (*O. nerka*) in Lake Osoyoos. The facility is currently utilized for the production of locally-adapted stock to supplement natural production of steelhead in Omak Creek. One 22' circular tank was used to recondition Omak Creek steelhead kelts. Water was circulated at 120 gallons/minute at an average temperature of 13.3^0C or 56^0F.

Warm Springs National Hatchery is located on the Warm Springs River, a tributary of the Deschutes River, which flows into the Columbia. It is operated by the U.S. Fish and Wildlife Service on lands leased from the Confederated Tribes of Warm Springs to propagate and raise wild and hatchery spring chinook. It was necessary to construct a circular kelt reconditioning tank, 15' (w) x 15' (l) x 5' (h), at Warm Springs National Fish Hatchery Isolation Rearing Facility (IRF). The tank was installed (Figure 9) to insure successful kelt reconditioning. The tank was fed with Warm Springs River water. Before induction into the reconditioning tank water was filtered and treated with ultraviolet light to reduce pathogens. Water was circulated at 100 gallons/minute and temperatures were maintained by mixing in chilled water to maintain a steady 59^0F or 15^0C through the critical high temperature months (June-September). Artificial lighting was installed to replicate the photoperiod.

Figure 8. Photograph of the steelhead kelt reconditioning facilities at Cassimer Bar Hatchery.

Figure 9. Photograph of the steelhead kelt reconditioning facilities at Warm Springs National Fish Hatchery.

The Satus Creek kelt reconditioning research was conducted at the Prosser Fish Hatchery in Prosser, Washington. Kelts from Satus Creek were retained in one of the four 20'(l) x 20'(w) x 4'(h) circular tanks. Tanks were fed oxygenated well

water (57^{0}F or 13.8^{0}C) at 200 gallons/minute. Prosser Hatchery is located on the Yakima River (Rkm 75.6), downstream from Prosser Dam, and adjacent to the Chandler Juvenile Evaluation Facility (CJEF) (Figure 10). The Yakama Nation (YN) operates Prosser Hatchery, with a primary function of rearing, acclimation, and release of fall chinook salmon (*O. tshawytscha)*. The facility is also used for coho salmon (*O. kisutch)* rearing prior to acclimation and release in the upper Yakima River Basin.

Figure 10. Photograph of the steelhead kelt reconditioning facilities at Prosser, WA.

Evaluation / Release

Reconditioned kelt steelhead at Shitike Creek were released above weir sites during the following fall migration. Steelhead kelts from Omak were released into the Okanogon due to high water temperatures at the Cassimer Bar facility in early Fall of 2005. except for the mixed stock kelts that were captured at Prosser, WA in 2004 and then placed into Section Corner and South Fork Logy creeks in 2005. Prior to release each kelt was anesthetized and examined with ultrasound equipment to determine maturation stage (Evans 2002). Sonogram captures of each fish was stored electronically and later individual egg size will later be determined. Such data may be used for comparison between hatchery-origin and natural-origin individual since it has been reported that hatchery-origin fish tend to produce smaller eggs (Heath et al. 2003). Data such as PIT tag number, length, weight, marks, and condition by individual will be recorded.

Mortalities were externally examined by hatchery personnel to record the suspected time of death, general condition (good, fair, poor), fish color (bright, intermediate, dark), color of the gill arches (red, pink, white), size of the abdomen (fat, thin), presence of any scars or obvious lesions, and any other anomalies. An internal examination was then conducted to record color of muscle tissue (red, pink, white), type of gonads (ovaries, testes), size of gametes (small, large), and presence of any internal anomalies. Any PIT, acoustic and/or radio tags were removed from mortalities and identification numbers entered into a computer database along with the growth measurement data.

Reconditioning rates will be determined by the ratio of the number of fish released alive to the number of kelts initially stocked in the tank. Rematuration rates will be calculated by the number of mature fish at release divided by the total number of fish released. We will use two-way analysis of variance to test for differences in reconditioning and rematuration among study sites.

Reproductive Success

Reproductive Success will be determined by the relative genetic contribution of natural-origin, hatchery origin and reconditioned kelt steelhead to the juvenile progeny. This is accomplished using microsatelite DNA markers and pedigree analysis. We are conducting this work in complete field settings (Shitike and Omak creeks) and in field laboratory settings (Section Corner and South Fork Logy creeks). We are terming these streams as field laboratories because we are planting adult spawners into the streams that are located above barrier falls and not accessible to anadromous steelhead. These sites do have the benefit of providing results sooner than the natural stream settings because juveniles can be collected at the parr stage instead of having to wait 2-3 years for smolts to naturally migrate. The disadvantage to the field laboratory streams is that we are unable to measure adult returns or F2 effects.

Juvenile *O. mykiss* Collection

Juvenile collections in 2005 were conducted in Section Corner and South Fork Logy creeks. No juveniles were present in South Fork Logy Creek, as there was no successful redd completion. Parr were collected in Section Corner Creek from the V-weir trap and by seining.

Resident *O. mykiss* Population Collection

Resident trout can contribute to the anadromous cohort from a stream and therefore it is important to account for juveniles with undetermined linage by surveying resident populations. Collections of resident trout were made using traps and/or seines. Tissue samples were collected from Shitike and Omak creeks and genotyped, there were no collections that were obtained from Section Corner Creek. All of the resident adults were collected during our collection of migrating adult steelhead.

Statistical Analysis

Data will be analyzed with two specific goals: 1) to quantify gene flow between adult wild, hatchery and kelt steelhead within and between sites, using traditional population genetics tests (Hardy-Weinberg equilibrium, F statistics, assignment tests) [Genepop (Raymond and Rousset 1995); GDA (Lewis and Zaykin 1999)], and 2) to assign parentage of individuals based upon genotypes from 10-12 microsatellite loci. Maximum likelihood (Marshall et al. 1998) and Bayesian (Neff et al. 2001; Lange 1997) procedures will be used to assign parentage. The software program CERVUS v 2.0 (Marshall et al. 1998) will be used for this analysis.

Prior to statistical analysis, all juveniles sampled in Section Corner Creek were compared to known brook trout sampled in Section Corner Creek. Samples with genotypes matching those of brook trout were removed (one juvenile sample was identified as a brook trout).

Deviation from Hardy-Weinberg equilibrium was evaluated using exact tests (Haldane 1954, Weir 1990, Guo and Thompson 1992) implemented in GENEPOP v3.4 (Raymond and Rousset 1995). Genotypic linkage disequilibrium between all pairs of loci for each group was tested using exact tests in GENEPOP. Pairwise F_{ST} values (Cockerham 1973, Weir and Cockerham 1984) and genic differentiation values were also calculated using GENEPOP. Significance levels for multiple tests were adjusted using Bonferronni corrections (Rice 1989). Expected and Observed heterozygosity were calculated using the Excel Microsatellite Toolkit (Park 2001). Number of alleles and allelic richness were calculated using FSTAT (Goudet 2001). Pairwise genetic distances (Cavalli-Sforza and Edwards 1967) were calculated between all populations using PHYLIP v. 3.5 (Felsenstein 1993) and the resulting genetic chord distances

were then used to construct a neighbor joining tree with NEIGHBOR. Parentage analysis was performed using CERVUS v 2.0 (Marshall et al. 1998).

Parentage Assignment

Highly polymorphic microsatellite loci have become the marker of choice for parentage and population studies due to the potential for differentiating closely related populations and accurate parentage assignment (Bernatchez and Duchesne 2000; Eldridge et al. 2002; Estoup et al. 1998; Letcher and King 2001; Norris et al. 1999; O'Reilly et al 1998). This process will be comprised of four steps: 1) collect year 2005 samples from each of three study sites (all adult returns over three selected weirs, juvenile progeny, and adult resident rainbow trout), 2) generate microsatellite genotypes from all samples taken in 2005 and perform parentage assignments, 3) collect adult returns of brood year 2005 steelhead (annually in 2007, 2008, and 2009), and 4) generate microsatellite genotypes of annual adult returns and assign parentage. Specifically, we will attempt to assign the parentage of juvenile progeny (and subsequent adults) to adult collections of wild, hatchery, or kelt steelhead. This method will allow us to quantify of each parental category of steelhead.

In 2005, parentage assignments from Section Corner Creek were the only stream with potential production from artificially reconditioned kelt steelhead. A limited quantify of samples were also collected from the fall migration of *O. mykiss* in Shitike Creek, however these are likely all progeny of resident rainbow trout, and not emigrating steelhead smolts.

Generalized Sampling Scheme

The sampling scheme that has been devised includes one pretreatment / control and 3 temporal replicates per site for a 17-year study period. This aspect of the study clarifies a concern from the ISRP regarding temporal and spatial replication in our study and also illustrates a general concern expressed by the ISRP that

studies designed to address the RFS require a long-term commitment. Based on collection data from the Umatilla River (pers. comm. Craig Contor) in order to fully analyze kelt contribution to the population it is necessary to sample to the F3 generation. We have estimated the number of samples that would be genotyped to follow three year-classes (Table 1).

Steelhead in the Columbia River Basin exhibit a variety of life-history strategies that require a number of sampling approaches. We will concentrate our sampling effort on collecting 3, 4, and 5 year-old fish with 2 to 4 year freshwater residency. We have ignored Age 1.x fish in this example because they make up a very small fraction of the run. Age 4.x fish also make up a small fraction of the run, but we anticipate that this age may comprise a larger component in our study streams. Additionally, allocating samples to the Age 4.x component does not have much effect on the total.

Below is a table that indicates which samples collected at weirs and rotary screw traps will be genotyped. Please note that the weirs will be operated during each of the 17 years of the study and tissue samples will be collected and archived from all fish that pass. In the table below we also include estimated sample sizes by collection year and life stage per sample stream.

Table 1: Samples that will be genotyped for pedigree analysis. Ages will be determined using scale pattern analysis and/or length frequency prior to parentage analysis. This scheme will be followed at each study stream (Shitike and Omak), except in the Satus Creek Basin where only adults and age 0 year-classes will be sampled due to the nature of the study site. Sample size allocations were calculated based on age structure data from the Umatilla River.

Year	Sample Adults (Replicate:Generation)	Sample Juveniles (Age)	Sample Residents	# of Adults genotyped	# of juveniles genotyped
2005	Preliminary Capture		XX		
2006	R1-F0	0	XX	600	100
2007	R2-F0	0,1	XX	600	100, 100
2008	R3-F0	0,1,2	XX	600	100, 100, 400
2009	R1-F1	0,1,2,3	XX	600	100, 100, 400, 200
2010	R1-F1, R2-F1	0,1,2,3	XX	600	100, 100, 400, 200

2011	R1-F1, R2-F1, R3- F1	0,1,2,3	XX	600	100, 100, 400, 200
2012	R1-F2, R2-F1, R3- F1	0,1,2,3	XX	600	100, 100, 400, 200
2013	R1-F2, R2- F2, R3- F1	0,1,2,3	XX	600	100, 100, 400, 200
2014	R1-F2, R2- F2, R3- F2	0,1,2,3	XX	600	100, 100, 400, 200
2015	R1-F2, R2- F2, R3- F2	0,1,2,3	XX	600	100, 100, 400, 200
2016	R1-F2, R2- F2, R3- F2	0,1,2,3	XX	600	100, 100, 400, 200
2017	R2- F2, R3- F2	0,1,2,3	XX	600	100, 100, 400, 200
2018	R3- F2	0,1,2,3	XX	600	100, 100, 400, 200
2019		1,2,3	XX		100, 400, 200
2020		2,3	XX		400, 200
2021		3	XX		200
Total				7,800	18,200

Laboratory Techniques

We initially planned 10-12 loci but instead sampled 17 loci. Microsatellite markers used were Ogo 4 (Olsen et al. 1998), Oki 23 (GenBank Accession #AF272822), Omm 1036 (GenBank Accession #AF346686), Omm 1046 (GenBank Accession #AF346693), Oke 4 (Buchholz et al. 1999), Oki 10 (Smith et al. 1998), Omy 1001, Omy 1011 (P. Bentzen pers. comm.), Omy 7 (K.Gharbi, pers. comm.), One 102 (Olsen et al. 2000), One u14 (Scribner et al. 1996), Ots 100 (Nelson and Beacham 1999), Ots 3M (Greig and Banks 1999), Ots 4 (Banks et al. 1999), Ssa 289 (McConnell et al. 1995), Ssa 407 and Ssa 408 (Cairney et al. 2000) Data was collected on an ABI 3730, and scored using Genemapper v3.7 Software.

Samples will be collected and stored in ethanol or lysis buffer for preservation of DNA. Samples will be shipped to the Hagerman Fish Culture Experiment Station in Hagerman, ID. DNA will be extracted from tissue samples using standard manufacture's protocols from Qiagen® DNeasy™ in conjunction with a Qiagen® 3000 robot. Genomic DNA will be quantified and arrayed into 96 well plates for high throughput genotyping. The polymerase chain reaction (PCR) will be used to amplify up to 17 microsatellite loci. Forward PCR primers will be fluorescently labeled (Applied Biosystems®), and PCR products genotyped using

manufacture's protocols with an Applied Biosystems® model 3130 or 3730 genetic analyzer.

RESULTS and DISCUSSION

Reconditioning

Steelhead Kelt Collection

Shitike Creek

A total of 57 (43 wild and 14 hatchery) adult steelhead were collected at the weir in Shitike Creek. Genetic samples were collected and all fish were passed above the weir to spawn naturally. A total of 32 wild- and 7 hatchery-origin kelts were collected at the weir during their migration out of the system. Hatchery-origin kelts were released downstream of the weir. Warm Springs fisheries staff determined that 10 of the kelts were in adequate condition for reconditioning; the remaining kelts were released below the trap.

Omak Creek

A total of 113 adult steelhead were captured at the weir on Omak Creek. Genetic samples were collected and the fish were passed above the weir to spawn naturally. Thirty-four kelt steelhead were collected at the weir, attempting to migrate out of the system. Twelve were assessed to be in poor condition and passed below the weir, 20 kelts were passed downstream as mortalities (2 predator related, 4 died prior to transport, 14 were DOA), and two were transported to Cassimer Bar Hatchery for reconditioning but died during transport. Low water conditions in Omak Creek likely contributed to the poor condition and mortality of these fish. An additional 17 kelt steelhead were obtained from the localized broodstock study after they were air spawned and were retained for reconditioning.

Prosser Hatchery

The existing BPA project (20001700) captures and reconditions mixed origin kelts from the Yakima River at the Prosser Hatchery. A total of 10 female reconditioned kelts of mixed origin were used for the release into Section Corner and South Fork Logy Creek.

Feeding / Reconditioning

Shitike Creek

The 10 wild kelts that were considered in good condition were taken to Warm Springs NFH for reconditioning and one survived to re-maturation. Steelhead kelts were given an initial diet of krill but were gradually switched to Skretting Pellets that were dyed red and combined with krill. This diet was administered twice per day for seven days a week.

Omak Creek

Since there were no surviving kelt steelhead collected from Omak Cr, 17 post-spawned steelhead from the naturalized broodstock program at Cassimer Bar Hatchery (collected from Omak Cr.) were retained for reconditioning, and 3 of these were successfully reconditioned (1 hatchery female, 1 wild female, 1 wild male) and released on October 18, 2005. Kelts were fed an initial diet of krill and then were gradually switched to a size 6.0 Bio-Vita feed.

Prosser Collected Kelts

A total of 10 kelts were obtained from BPA project 20001700 for use in this project. These kelts were fed an initial diet of krill for just over 2 months and then gradually switched to a diet of Moore-Clark pellets.

Evaluation / Release

Shitike Creek

The steelhead kelt held at Warm Springs National Fish Hatchery was successfully released into Shitike Creek on February 16, 2006.

Omak Creek

Three surviving reconditioned kelts were released into the Okanogan River on October 18, 2005. This early release was done to prevent potential overipening due to increasing well water temperatures approaching unacceptable temperatures that were >60 0 F. We released the steelhead kelts near the mouth of the Okanagon River. This was our best option considering that Omak Creek was almost dewatered due to an extremely poor water year followed by high summer temperatures. We anticipate collection and analysis by spring of 2006.

Satus Creek Tributaries

We captured a total of 68 first-time spawning steelhead at the Satus Creek weir with 22 of these sent to either Section Corner or South Fork Logy. The remaining fish were either retained for the gamete and progeny study (BPA project 20001700) or were released back into Satus Creek. We placed 6 female first time spawners, 5 male first time spawners, and 5 female kelts into Section Corner Creek and South Fork Logy Creeks for a total of 32 fish partitioned into both tributaries on March 9, 2005. Shortly after release, a total of 11 redds were counted in Section Corner Creek. From this group we recaptured three post-spawned steelhead but none of these survived the reconditioning process. Shortly after release there was a large precipitation event in South Fork Logy Creek, and the weir was blown out. Crews were sent to determine if any more steelhead were still in the tributary but could not detect any fish left in the system.

Reproductive Success

Statistical Analysis

Of 161 tests for Hardy Weinberg equilibrium, 16 were significant. Of these, 12 were found in Section Corner Creek juveniles, three in Omak Creek residents and one in Yakima River kelt steelhead. Of 1,168 pairwise tests of linkage disequilibrium, 250 were significant. Of these, 128 were found in Section Corner

Creek Juveniles, 83 in Omak Creek residents above Mission Falls, and 39 in Omak Creek adult steelhead. Significant results in Section Corner Creek juveniles were expected as they were derived from a small number of parents.

Pairwise F_{ST} values (Table 4) were significant in all but one comparison. Insignificant results (P = 0.00204) and the corresponding low F_{ST} value (.0021) between Shitike Creek residents and Shitike Creek juveniles is indicative that the juveniles are predominately progeny of residents and not anadromous steelhead.

Table 2. Pairwise F_{ST} values. See Table 4 for population abbreviations.

	Omak Adult	Omak Res1	Omak Res2	Shitike Adult	Shitike Res.	Shitike Juv.	Satus Adu.	Yakim a Kelt	Section Juv.
Omak Adu	---								
Omak Res1	0.0256	---							
Omak Res2	0.0294	0.0077	---						
Shitike Adu	0.0158	0.0275	0.0313	---					
Shitike Res	0.0817	0.1060	0.1046	0.0497	---				
Shitike Juv	0.0849	0.1083	0.1054	0.0555	0.0021*	---			
Satus Adu	0.0225	0.0348	0.0410	0.0176	0.0781	0.0820	---		
Yakima Kelt	0.0276	0.0162	0.0283	0.0114	0.0957	0.1020	0.0168	---	
Section Juv	0.0501	0.0691	0.0754	0.0432	0.0953	0.1017	0.0127	0.0518	---

* NOT significant at adjusted critical level =.05 / 36 tests = 0.0014

The dendrogram depicted in Figure (11) shows the relative relationship of the study populations. As expected, resident populations of rainbow trout grouped most tightly with steelhead from the same drainage. Section Corner Creek juveniles are shown to group closest to Satus Creek adult steelhead. Shitike Creek juveniles group closest to Shitike Creek resident rainbow trout. Neighbor joining dendrogram of Cavalli-Sforza Edwards genetic distance among studied populations. Numbers at nodes represent bootstrap percentage from 1000 replicates (only those greater than 50 percent shown).

Parentage Assignment

A total of 759 samples were received and genetically analyzed. Since species identification (in the field) of young-of-year juveniles was not certain between brook trout and steelhead, a reference collection of 32 samples from known

brook trout were included to genetically identify any brook trout juveniles and remove them from the steelhead parentage analysis. Brook trout (32 adult + 1 par = 33), unknown source samples (n=17) and samples with matching duplicates (N=7) were removed from the dataset prior to statistical analysis. In addition, 43 samples were removed after a second unsuccessful attempt at extraction and amplification. The remaining 659 samples were included in statistical tests. Sample numbers per population (n) can be seen in Table 2.

Table 3. Each population is reported in terms of sample size (n), expected heterozygosity (H_E), observed heterozygosity (H_O), average number of alleles per locus (A) and allelic richness (AR).

Population	Abbreviated	n	H_E	H_O	A	AR
Omak Adult Steelhead	Omak Adu	95	0.8155	0.7970	13.12	8.22
Omak Residents Above MF	Omak Res1	21	0.8326	0.7785	9.94	8.29
Omak Residents Below MF	Omak Res2	78	0.8277	0.7996	12.18	8.05
Shitike Adult Steelhead	Shitike Adu	55	0.8242	0.8099	14.18	9.17
Shitike Resident RBT	Shitike Res	146	0.7027	0.7006	13.29	7.25
Shitike Juveniles	Shitike Juv	61	0.6973	0.7044	11.65	7.17
Satus Creek Adult Steelhead	Satus Adu	31	0.7864	0.7779	9.94	7.47
Yakima River Kelts	Yakima Kelt	13	0.8210	0.7670	8.41	8.27
Section Corner Creek Juveniles	Section Juv	159	0.7411	0.7903	7.06	5.85

Shitike Creek

Parentage analysis of juveniles sampled in Shitike Creek was not successful in determining parental matches. This is likely the result of the juveniles sampled being progeny of resident rainbow trout that were not collected in our sampling efforts. Relationships in the neighbor joining dendogram (Figure 11) support this conclusion since Shitike Creek juveniles clustered tightly with Shitike Creek residents and not Shitike Creek adult steelhead. This grouping is also supported by lower F_{ST} values between juveniles and residents (0.0021) than between juveniles and adult steelhead (0.0555) (Table 2). Resident rainbow trout are less likely to be sampled because of their high numbers and smaller size. Adult resident rainbow trout in Shitike Creek also migrate later than adult steelhead (Zimmerman and Reeves 2000) and the majority of residents may migrate after weir operation and genetic sampling has ended. Additional analysis should be

available by either late 2006 or 2007 and will be reported in that years corresponding annual reports.

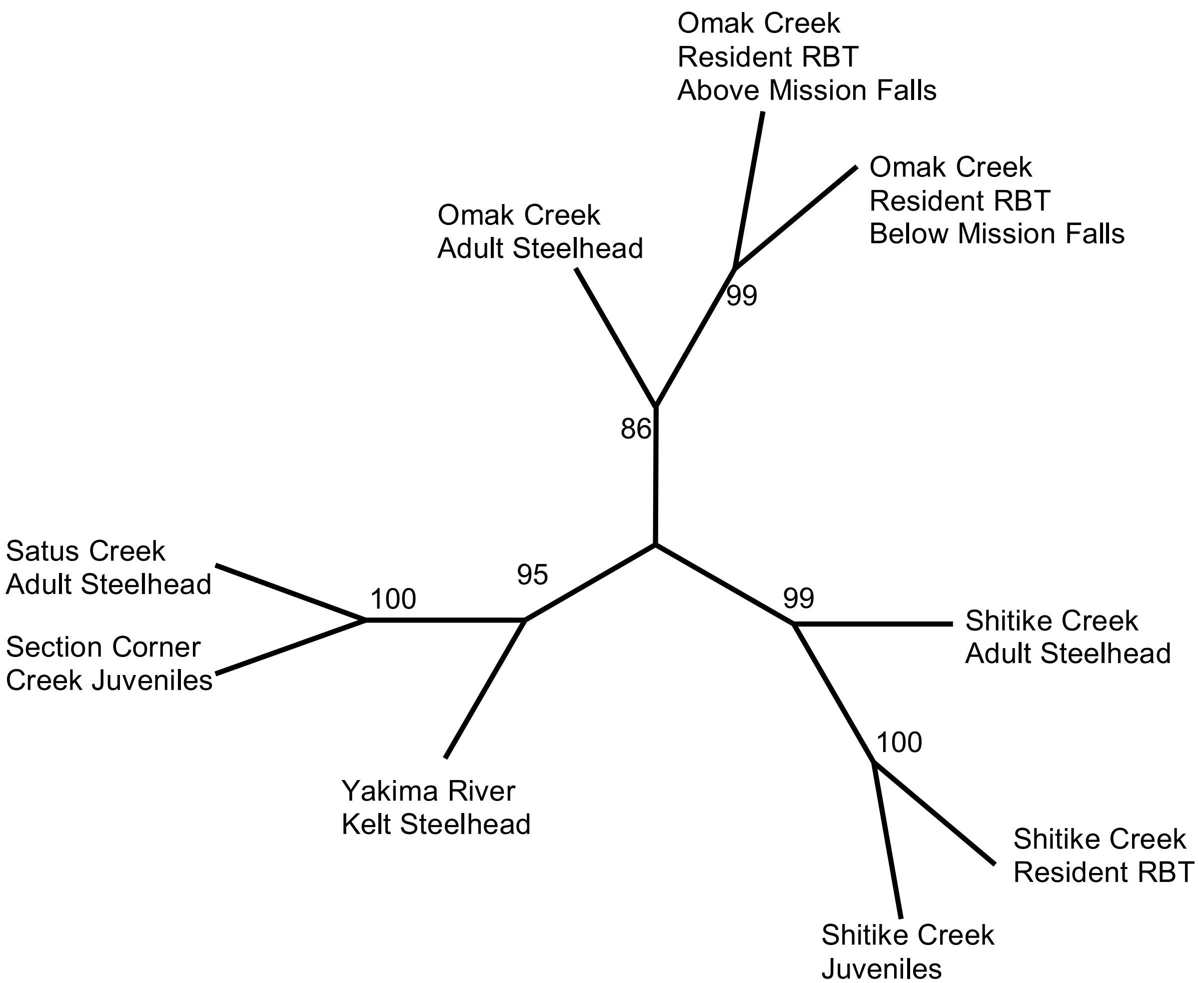

Figure 11. Neighbor joining dendrogram of Cavalli-Sforza Edwards genetic distance among studied populations. Numbers at nodes represent bootstrap percentage from 1000 replicates (only those greater than 50 percent shown).

Omak Creek

No parentage analysis was conducted for Omak Creek steelhead. Baseline data was collected for first time spawning adults and was analyzed to determine genetic distance between study populations (figure 11).

Satus Creek Tributaries

Parentage analysis successfully assigned each (n=159) juvenile samples sampled in Section Corner creek to a single cross in the candidate parents (Satus Creek adult Steelhead and Yakima River Kelts) (Table 3). However, no juveniles were assigned to the Yakima River kelts (Table 3). These results were unexpected and may be due to the reconditioned kelts being overripe. Reconditioned kelts were held on well water which is significantly warmer than river water during winter months and may have contributed to these fish maturing faster. Over-ripening has been shown to negatively affect spawning behavior and the capacity to spawn under natural conditions (De Gaudemar and Beall 1998), and also has been shown to effect gamete viability in rainbow trout (Lahnsteiner 2000). Additionally, the first-time spawners were apparently ripe for spawning when they were first planted in the creeks because redds were constructed within a day of release. These apparently ripe first-time spawners had been living in much cooler river water while the reconditioned kelts were held in warmer well water. It also should be noted that some of these fish were observed to have damaged eyes that occasionally happens during reconditioning.

It was assumed that there were no resident rainbow trout in Section Corner creek above the weir. Despite the potential problems with timing maturation between reconditioned kelts and first time spawners, results from this stream demonstrate that the genotyping methodology along with parentage analysis can produce reliable reproductive success results.

Table 4. Parentage assignment matrix. Males are listed across the top, females across the left side. Sums for each individual are also listed as number of juveniles and number of crosses in parentheses.

	Male 1	Male 2	Male 3	Male 4	Male 5	Sum
Female 1	41					41 (1)
Female 2		6		7		13 (2)
Female 3	9	15	6	1	2	33 (6)
Female 4	1	3		2	7	13 (4)
Female 5		27			11	38 (3)
Female 6		10		1	10	21 (3)
Kelt 1						
Kelt 2						
Kelt 3						
Kelt 4						
Kelt 5						
Sum	51 (3)	61 (5)	6 (1)	11 (3)	30 (4)	

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