2006 Annual Report

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

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Abstract

We conducted a field study to investigate the reproductive success of hatchery, wild, and artificially reconditioned kelt steelhead. Three sites (Omak Creek, WA; Section Corner Creek, WA; and Shitike Creek, OR) were chosen to provide replicates to evaluate reproductive success of *Oncorhynchus mykiss*. Potential adults and progeny were sampled in each drainage and genotyped with 16 microsatellite loci to determine parentage.

In Omak Creek, two of three reconditioned kelts released in fall of 2005 returned in 2006. If they successfully reproduced, their progeny should be detectable in 2007 and 2008. Genetic evidence supports the presence of distinct resident and anadromous populations in Omak Creek. The combination of population and parentage assignment tests showed that Mission Falls may be a genetic barrier limiting the passage of adult steelhead. Twelve of the 21 juveniles sampled above Mission falls in 2005 were assigned to the anadromous population whereas no fish sampled above the falls in 2006 were assigned to the anadromous population. This may be the result of a velocity barrier at Mission Falls resulting from high water flows in 2006 but not in 2005.

In Section Corner Creek, all juveniles sampled were assigned to a single parental cross based on genetic identity and parentage analysis. Brood year 2005 juveniles were sampled as both fry in 2005 and parr in 2006 and matched to adults released in Section Corner Creek in 2005. Six female and five male parents contributed to the progeny sampled in each year. While the same fish contributed each year, there was a significant difference between the yearly distribution of progeny assigned to each individual. This difference was largely due to the differential survival or migration of progeny between females. Fewer adults contributed to reproduction in 2006 with only two females and two males contributing to the fry sampled. None of the kelt females were identified as parents of juveniles collected in either year, likely due to problems with rematuration timing during reconditioning. Further research is being completed to determine environmental cues and dietary needs of reconditioned kelts.

In Shitike Creek, juveniles collected at the screwtrap were not successfully assigned to any of the potential anadromous parents. It is apparent that the sampled juveniles were not progeny of the anadromous population. Analysis of allele frequencies showed that the juveniles that were sampled in 2006 are more similar to and likely progeny of, the resident population in Shitike Creek. However, parentage analysis was not possible as the resident population is large and the majority of resident adults were not sampled. Juvenile sampling protocols will be expanded in 2007 to help identify anadromous progeny.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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 (>75%) and includes wild origin fish (57%) (Evans 2002, Evans et al. 2004, 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.

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. 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. 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.

METHODS

Reproductive success will be determined by genetic contribution of adult steelhead to the juvenile progeny. This is accomplished using microsatellite DNA markers and pedigree analysis. We conducted this work in complete field settings (Omak and Shitike Creeks) and in a field laboratory setting (Section Corner Creek). We describe Section Corner Creek as a field laboratory because we planted adult spawners (both kelts and first time spawners) above barrier falls. This area is not naturally accessible to anadromous steelhead and does not contain resident rainbow trout.

Generalized Sampling Scheme

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. The sampling scheme that has been devised includes one pretreatment / control and three 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 shows the general sampling scheme for samples to be genotyped. Please note that the weirs and traps will be operated during all years of the study and tissue samples will be collected and archived from all fish that pass.

Table 1. Generalized sampling scheme

<table>
<tr><td colspan="6">Ages will be determined using scale pattern analysis and/or length frequency prior to parentage analysis. This scheme will be followed at Omak and Shitike Creek. Section Corner Creek will have only fry and parr juveniles sampled due to the nature of the study site. Sample size allocations were calculated based on age structure data from the Umatilla River.</td></tr>
<tr><td>Year</td><td>Sample Adults (Replicate Generation)</td><td>Sample Juveniles (Age)</td><td>Sample Residents</td><td># of Adults genotyped</td><td># of juveniles genotyped</td></tr>
<tr><td><i>2005</i></td><td>Preliminary Capture</td><td></td><td>100-200</td><td></td><td></td></tr>
<tr><td>2006</td><td>R1-F0</td><td>0</td><td>100-200</td><td>600</td><td>100</td></tr>
<tr><td>2007</td><td>R2-F0</td><td>0,1</td><td>100-200</td><td>600</td><td>100, 100</td></tr>
<tr><td>2008</td><td>R3-F0</td><td>0,1,2</td><td>100-200</td><td>600</td><td>100, 100, 400</td></tr>
<tr><td>2009</td><td>R1-F1</td><td>0,1,2,3</td><td>100-200</td><td>600</td><td>100, 100, 400, 200</td></tr>
<tr><td>2010</td><td>R1-F1, R2-F1</td><td>0,1,2,3</td><td>100-200</td><td>600</td><td>100, 100, 400, 200</td></tr>
<tr><td>2011</td><td>R1-F1, R2-F1, R3- F1</td><td>0,1,2,3</td><td>100-200</td><td>600</td><td>100, 100, 400, 200</td></tr>
<tr><td>2012</td><td>R1-F2, R2-F1, R3- F1</td><td>0,1,2,3</td><td>100-200</td><td>600</td><td>100, 100, 400, 200</td></tr>
<tr><td>2013</td><td>R1-F2, R2- F2, R3- F1</td><td>0,1,2,3</td><td>100-200</td><td>600</td><td>100, 100, 400, 200</td></tr>
<tr><td>2014</td><td>R1-F2, R2- F2, R3- F2</td><td>0,1,2,3</td><td>100-200</td><td>600</td><td>100, 100, 400, 200</td></tr>
<tr><td>2015</td><td>R1-F2, R2- F2, R3- F2</td><td>0,1,2,3</td><td>100-200</td><td>600</td><td>100, 100, 400, 200</td></tr>
<tr><td>2016</td><td>R1-F2, R2- F2, R3- F2</td><td>0,1,2,3</td><td>100-200</td><td>600</td><td>100, 100, 400, 200</td></tr>
<tr><td>2017</td><td>R2- F2, R3- F2</td><td>0,1,2,3</td><td>100-200</td><td>600</td><td>100, 100, 400, 200</td></tr>
<tr><td>2018</td><td>R3- F2</td><td>0,1,2,3</td><td>100-200</td><td>600</td><td>100, 100, 400, 200</td></tr>
<tr><td>2019</td><td></td><td>1,2,3</td><td></td><td></td><td>100, 400, 200</td></tr>
<tr><td>2020</td><td></td><td>2,3</td><td></td><td></td><td>400, 200</td></tr>
<tr><td>2021</td><td></td><td>3</td><td></td><td></td><td>200</td></tr>
<tr><td>Total</td><td></td><td></td><td></td><td>7,800</td><td>18,200</td></tr>
</table>

Adult Steelhead Collection

Research was conducted at Omak Creek (Figure 1), a tributary to the Okanagon River; Section Corner Creek, a tributary to the Yakima River; and Shitike Creek (Figure 2), a tributary to the Deschutes River. Weirs and traps were installed and operated for adult steelhead collections. Biological data was taken for each steelhead collected. Fork length was recorded; each fish was scanned for PIT tags and tagged if necessary. Observations about condition, color and marks were recorded. Tissue samples were collected and placed in ethanol for later genotyping.

Figure 1. Map showing the locations of Omak, and Section Corner creeks as well as the Colville and Yakama reservations.

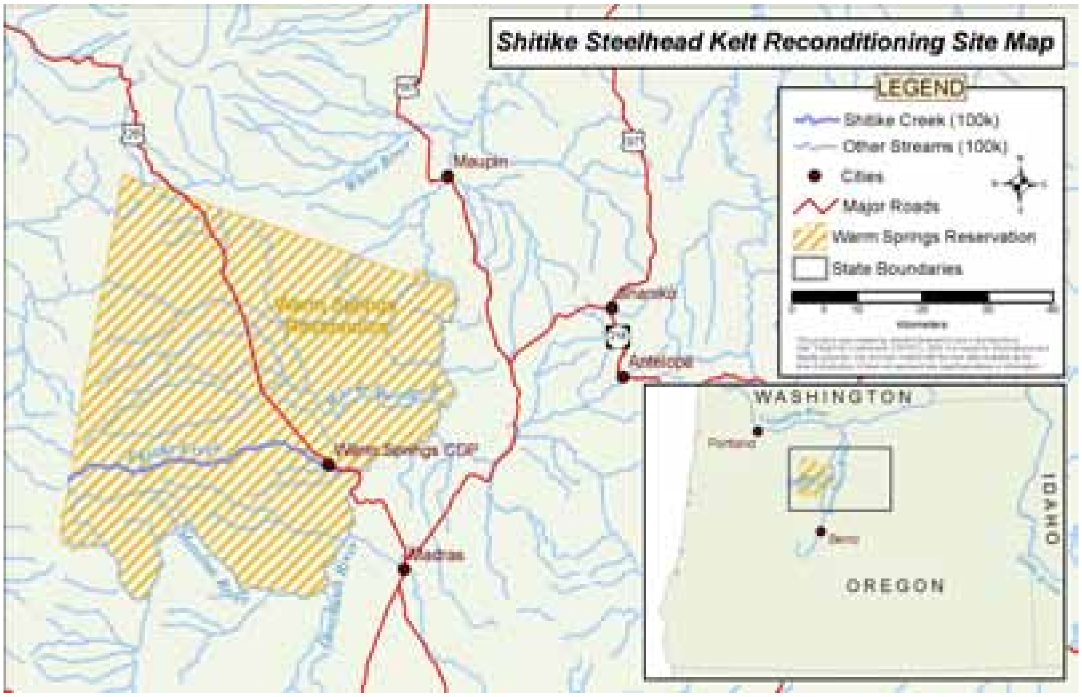

Figure 2. Map showing the location of Shitike Creek and the Warms Springs Reservation.

Trapping at the semi-permanent weir (Figure 3) located at approximately RKM 1.2 on Omak Creek, started on March 17, 2006 and continued through July 17, 2006. This weir was also used for a localized broodstock collection project. Fish not 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 May 8, 2006 at which point the trap was moved to capture kelts exiting the system. A total of 104 DNA samples were taken between March 25, 2006 and May 19, 1006. Two of the samples were from returning reconditioned kelts released in the fall of 2005. Additional fish are likely to have passed the weir unsampled due to weir panels being compromised by high flows. Eleven steelhead collected at neighboring Bonaparte Creek were also included in genetic analysis.

The Colville Tribe also operates a Crump weir antenna located approximately ½ mile downstream of the semi-permanent weir. The Crump weir antenna is a system that does not impede migration and detects PIT tags contained in fish that pass over the structure.

Figure 3. Photograph of the resistance board weir located on Omak Creek

Adults for stocking into Section Corner Creek were collected in Satus Creek using a removable picket type structure that directed adult steelhead into the old Satus Dam fish ladder (RKM 13.5) (Figure 4) where they could be removed with a dip net. The picket structure was installed and fished 24 hours a day from Feb 6 2006 until March 13 2006. The weir was operated continuously during this time span, except for a few periods of high flow. Five males and eight maiden females were stocked into Section Corner Creek between Feb 7 2006 and March 2 2006. Thirteen kelts reconditioned at the Prosser Kelt facility were stocked between Feb 7 2006 and March 13 2006. A weir was installed in Section Corner Creek (Figure 5) to keep adult steelhead from leaving.

Figure 4. Photograph of the trapping facility located at the old dam site on Satus Creek.

Figure 5. Section Corner Creek Weir: Taken directly after release of kelts and maiden spawners in 2005.

The Shitike Creek picket weir was installed at the scale bridge (RKM 1.1) upstream of the creek's confluence with the Deschutes River on March 6, 2006. Spring runoff forced the deactivation of the weir from May 18 to May 22. The design of the weir incorporated separate trap boxes for upstream and downstream migrants. The location of the downstream trap was situated at the edge of the thalweg to minimize the amount of flow inside the trap box and to reduce any potential for crowding (10'l x 4'w x 3'h). In 2006 the weir fence was placed perpendicular to the flow to reduce potential holding of downstream migrating fish (Figures 6 and 7). The weir was operated as a trap until June 20 when operation switched to video monitoring only. Fish 508 mm or greater in length (20 inch ODFW standard) were treated as anadromous adult steelhead for which a total of 41 DNA samples were collected.

Figure 6. Photograph of the picket weir operating on Shitike Creek in 2005.

Figure 7. Photograph of the picket weir operating on Shitike Creek in 2006

Juvenile *O. mykiss* Collection

Emigrating smolts in Omak Creek were collected using a screwtrap and additional fish were collected in Omak Creek by electrofishing. The screwtrap was installed on April 1, 2006 and operated for two days before flows came up and the cone was pulled. The cone was put back down on May 4, 2006 and the trap was fished until June 2, 2006. The average length of smolts sampled was 187 mm. Electrofishing occurred both above and below Mission Falls, and included possible resident rainbow trout. Fish under 85mm in length were treated as young of the year. Fish over 180 mm in length that were captured after the spring migration were considered to be resident rainbow trout. Fish falling in between the sizes were considered unknowns.

Juveniles in Section Corner Creek were collected using a V-weir trap and by electrofishing. The 2006 sample collection for juveniles in Section Corner included both parr from brood year 2005, and fry from brood year 2006. Parr collected in 2006 were of the same brood year as fry collected in 2005.

Shitike Creek juveniles were collected using a screw trap located downstream of the adult trap at RKM 0.9. The screw trap was operated in both fall of 2005 and spring of 2006. While collection of anadromous steelhead progeny was the goal, it was expected that resident rainbow trout progeny would also be captured.

Resident *O. mykiss* Population Collection

Collections of resident trout were made using traps, electrofishing and/or seines. Residents in Omak Creek were collected above and below Mission Falls. Fish that were greater than or equal to180 mm in length and captured after the spring migration in were treated as resident rainbow trout.

It is believed that no resident rainbow trout population exists in Section Corner Creek. Future sampling efforts will, however, have to include the possibility of parental contribution from residualized or precocial offspring of stocked anadromous steelhead.

Adult residents in Shitike Creek were collected in the upstream migration that overlapped the upstream migration of anadromous steelhead. Fish under 508 mm in length (20 inch ODFW standard) were considered resident rainbow trout.

Genetic Analysis

Samples were collected and stored in ethanol for preservation of DNA. Genetic analysis was conducted at the Hagerman Fish Culture Experiment Station in Hagerman, ID. DNA was extracted from tissue samples using standard manufacture's protocols from Qiagen® DNeasy™ in conjunction with a Qiagen® 3000 robot. The polymerase chain reaction (PCR) was used to amplify 16 microsatellite loci; 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), 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). PCR products were genotyped using manufacture's protocols with an Applied Biosystems® model 3730 genetic analyzer and scored using Genemapper v3.7 Software.

Expected and observed heterozygosity were calculated using Excel Microsatellite Toolkit (Park 2001). The number of alleles and allelic richness were calculated using FSTAT (Goudet 2001). 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 genetic differentiation values were also calculated using GENEPOP. Significance levels were adjusted for multiple tests with a modified version of the False Discovery Rate referred to as the B-Y FDR (Benjamini and Yuketieli 2001; Narum 2006). Pairwise genetic distances (Cavalli-Sforza and Edwards 1967) were calculated between all populations using POPULATIONS1.2.30beta (Langella 2002) and the resulting genetic chord distances were then used to construct a neighbor joining tree with TreeView (Page 1996) .

Parentage analysis was performed using CERVUS v 3.0 (Marshall et al. 1998, Kalinowski et al. 2006). Information on fish gender was not included in the analysis. For Section Corner Creek, the proportion of progeny per male and female parent was compared between fry sampled in 2005 and parr sampled in 2006. Significance was tested using chi squared distribution tests.

To help infer population structure in Omak Creek, the program STRUCTURE v.2.0 (Pritchard et al. 2000, Falush et al. 2003) was used. Aside from the adult anadromous steelhead, samples in Omak Creek were expected to be mixed collections of the anadromous and resident populations. In an attempt to separate the two expected population groups, K=2 was used with the population containing the majority of anadromous adult steelhead referred to as the anadromous population. The alternative population was labeled the resident population. Samples with assignment probabilities lower than 0.70 were treated as unassigned.

To discriminate between anadromous and residents in Shitike Creek, the program GENECLASS v2.0 (Piry et al. 2004) was used. Reference populations of putatively anadromous and resident populations were created using length based assignments of the upstream migrating adults. Self assignment testing of each population was performed, and juveniles collected in the screwtrap were treated as unknowns and assigned to either the anadromous or resident reference population.

RESULTS

Kelt Detection

Two kelts were detected returning to Omak Creek in the spring of 2006. One female was detected at the crump weir and again at the adult trap during the downstream migration. This female was not, however, detected at the adult trap during the upstream migration. A male kelt arrived at the adult trap one week after the female kelt was detected at the crump weir. The male, however, bypassed detection at the crump weir. While the third kelt was not detected, the possibility that it returned cannot be excluded since trapping efficiency was not 100%. High water flows in 2006 compromised both the adult trap and crump weir efficiency.

Thirteen kelts from the Prosser Kelt Facility were placed into Section Corner Creek in Spring 2006. Kelts were subject to high predation rates prior to spawning activity, and none were believed to survive to spawning.

Genetic Analysis

A total of 1,727 samples were processed for genetic analysis. After genotyping was completed, 118 samples were removed from the dataset due to incorrect species (brook trout-32), confirmed duplicate samples (19), and incomplete genotypes (67). The remaining 1,609 samples were included in statistical tests. Sample numbers (n) per sample collection are reported in Table 2.

Table 2. Population Statistics. 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), allelic richness (AR), private allelic richness (PAR), number of loci out of Hardy-Weinberg equilibrium (HW), and number of pairwise loci comparisons in linkage disequilibrium (LD).

Population	Abbr	n	H_E	H_O	A	AR	PAR	HW	LD
Bonaparte Adult Steelhead	BO06AS	11	0.813	0.844	7.9	7.7	0.195	0	0
Omak Adult Steelhead	OM56AS	196	0.817	0.802	14.7	7.8	0.125	2	27
Omak 2005 Above Mission Falls	OM05MF	21	0.833	0.780	10.0	7.7	0.068	0	1
Omak 2005 Below Mission Falls	OM05CR	78	0.827	0.800	12.3	7.5	0.034	7	60
Omak 2006 Above Mission Falls	OM06MF	45	0.768	0.790	9.7	6.4	0.137	2	11
Omak 2006 Young of Year	OM06YO	29	0.836	0.839	11.0	8.0	0.069	0	5
Omak 2006 Unknown Age	OM06UN	53	0.846	0.817	13.1	8.3	0.144	1	7
Omak 2006 Below Mission Falls	OM06CR	11	0.771	0.697	6.8	6.6	0.079	0	0
Omak 2006 Screw Trap	OM06ST	96	0.839	0.797	13.9	8.0	0.099	6	25
Section Corner 2006 Adult Steelhead	SE06AS	25	0.793	0.740	10.6	7.5	0.182	0	2
Section Corner 2005 Fry (BY2005)	SE05FR	279	0.744	0.788	7.2	5.7	0.010	15	120
Section Corner 2006 Parr (BY2005)	SE06PA	165	0.740	0.793	7.3	5.5	0.009	15	120
Section Corner 2006 Fry (BY2006)	SE06FR	91	0.661	0.832	4.7	4.0	0.040	15	57
Yakima River 2005 Kelts	SE05YA	31	0.790	0.778	10.1	7.1	0.067	0	0
Satus 2005 Adult steelhead	SE05SA	13	0.822	0.768	8.5	7.8	0.200	1	0
Shitike Adult Steelhead	SH56AS	107	0.820	0.806	15.9	8.4	0.287	0	6
Shitike Adult Residents	SH56RS	203	0.713	0.711	14.2	6.8	0.235	0	3
Shitike Screw Trap	SH56ST	155	0.706	0.704	13.9	6.8	0.249	0	0

Departure from Hardy-Weinberg equilibrium and linkage disequilibrium were common (See Table 2). At the extreme, Section Corner 2005 fry and Section Corner 2006 parr were out of equilibrium at 15 of 16 loci, and showed linkage disequilibrium at 120 of 120 pairwise locus comparisons. All three juvenile collections for Section Corner Creek show an excess of heterozygotes. Omak Creek collections had a deficiency of heterozygotes in five of the seven non-adult steelhead collections, with up to seven loci out of Hardy-Weinberg equilibrium. These results are consistent with population admixture resulting in the Wahlund effect.

Pairwise F_{ST} values (Table 3) were significant in all but seven comparisons after B-Y FDR adjustment (0.05/5.61 = 0.0089). Nonsignificant results were seen at SE06AS and SE05SA (P= 0.7397, F_{ST}=-0.0041), OM06MF and OM06CR (P=0.0999, F_{ST}=0.0050), OM06UNK and OM06CR (P=0.0677, F_{ST}=0.0187), BO06AS and OM56AS (P=0.0591

F_{ST}=0.0022), BO06AS and SH56AS (P=.0311 F_{ST}=0.0091), SE06AS and SE05YA (P=0.0157 F_{ST}=0.0082) and OM05MF and OM06UN (P=0.0114 F_{ST}=0.0037). These sample collections were not condensed for analysis to allow additional analysis as discrete collections. Low F_{ST} value (0.0021) between Shitike Creek residents and Shitike Creek juveniles is indicative that the juveniles are predominately progeny of residents and not anadromous steelhead.

Table 3. Pairwise F_{ST} values. (* not significant at adjusted critical level =0.0089) . Population abbreviations follow those in Table 2.

	BO06AS	OM56AS	OM05MF	OM05CR	OM06MF	OM06YO	OM06UN	OM06CR	OM06ST	SE06AS	SE05FR	SE06PA	SE06FR	SE05SA	SE05YA	SH56AS	SH56RS
OM56AS	0.0022*																
OM05MF	0.0212	0.0238															
OM05CR	0.0190	0.0289	0.0086														
OM06MF	0.1099	0.1129	0.0565	0.0515													
OM06YO	0.0105	0.0121	0.0164	0.0224	0.0894												
OM06UN	0.0255	0.0311	0.0037*	0.0077	0.0371	0.0121											
OM06CR	0.0859	0.0946	0.0303	0.0283	0.0050*	0.0646	0.0187*										
OM06ST	0.0184	0.0181	0.0050	0.0096	0.0596	0.0140	0.0082	0.0411									
SE06AS	0.0135	0.0195	0.0306	0.0336	0.1236	0.0272	0.0397	0.0959	0.0290								
SE05FR	0.0471	0.0444	0.0702	0.0776	0.1621	0.0564	0.0779	0.1461	0.0643	0.0265							
SE06PA	0.0550	0.0521	0.0783	0.0839	0.1638	0.0645	0.0832	0.1502	0.0689	0.0319	0.0029						
SE06FR	0.1069	0.0958	0.1132	0.1170	0.2090	0.1098	0.1173	0.1948	0.1004	0.0725	0.1008	0.1083					
SE05SA	0.0148	0.0184	0.0362	0.0418	0.1270	0.0276	0.0422	0.1036	0.0322	-0.0041*	0.0133	0.0207	0.0805				
SE05YA	0.0182	0.0244	0.0182	0.0308	0.1055	0.0164	0.0245	0.0773	0.0251	0.0082*	0.0515	0.0573	0.1026	0.0159			
SH56AS	0.0091*	0.0137	0.0307	0.0354	0.1143	0.0154	0.0328	0.0941	0.0255	0.0145	0.0404	0.0458	0.0871	0.0158	0.0151		
SH56RS	0.0700	0.0699	0.1037	0.1015	0.1911	0.0749	0.0998	0.1727	0.0873	0.0781	0.0936	0.0999	0.1176	0.0763	0.0896	0.0414	
SH56ST	0.0785	0.0760	0.1100	0.1084	0.1950	0.0824	0.1053	0.1775	0.0933	0.0881	0.1015	0.1085	0.1232	0.0832	0.0984	0.0481	0.0017

The neighbor-joining dendrogram (Figure 8) grouped all sample collections within their respective drainages with the exception of Bonaparte adult steelhead (BO06AS). The bootstrap value connecting Bonaparte Creek with the Section Corner Creek fish was the weakest value (41) observed in the dendrogram. Further clustering patterns can be seen within the Section Corner and Shitike Creek samples. Section Corner Creek fry sampled in 2006 (SE06FR) group with Section Corner Creek adults from 2006 (SE06AS). Fry sampled in 2005 (SE05FR), parr sampled in 2006 (SE06PA) and adults collected in Satus Creek and stocked into Section Corner Creek in 2005 (SE05SA) all group together. In Shitike Creek, juveniles sampled at the screw trap (SH56ST) grouped closer to resident rainbow trout (SH56RS) than the anadromous steelhead (SH56AS).

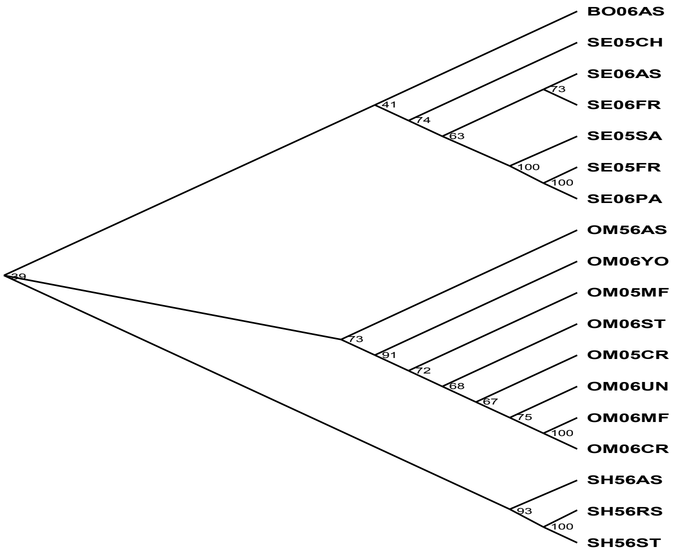

Figure 8. Neighbor joining dendrogram of Cavalli-Sforza Edwards genetic distance among studied populations. Numbers at nodes represent bootstrap percentage from 1000 replicates.

Population tests for samples collected in Omak and Bonaparte Creeks were performed using STRUCTURE. Results are reported for k=2 with the populations labeled as either anadromous or resident (Table 4). A third column is included to account for samples not assigning strongly to either population (scores between 0.3 and 0.7). The population labeled anadromous included all the adult anadromous steelhead sampled. Fish sampled above Mission Falls in 2006 were all assigned to the resident grouping. All other Omak Creek sample collections had individuals that assigned to both populations.

Table 4. Population assignments using STRUCTURE for Omak Creek drainage. Results are reported in proportional assignments with k=2.

Collection	n	Anadromous	Resident	Unassigned
BO06AS	11	1.000	0.000	0.000
OM56AS	196	1.000	0.000	0.000
OM05MF	21	0.571	0.381	0.048
OM06MF	45	0.000	0.867	0.133
OM05CR	78	0.564	0.308	0.128
OM06CR	11	0.182	0.818	0.000
OM06UN	53	0.509	0.377	0.113
OM06YO	29	0.759	0.069	0.172
OM06ST	96	0.677	0.208	0.115

Self assignment rates in Shitike Creek using GENECLASS were high for both anadromous steelhead (102 of 107) and resident rainbow trout (197 of 203). Of 155 juveniles assigned, 150 assigned to the resident population and 5 assigned to the anadromous population.

Parentage assignment in Omak Creek had limited success. Parentage assignment used only known adults (anadromous steelhead) as parents and as such accounts only for contribution of anadromous adults sampled. The percent of progeny assigned to at least one anadromous parent for each collection are 42.7% for Omak Creek 2005 below Mission Falls, 28.6% for Omak Creek 2005 above Mission Falls, 0.0% for Omak Creek 2006 above Mission Falls, 30.2% for Omak Creek 2006 screwtrap, 37.7% for Omak Creek 2006 unknown age, and 58.6% for Omak Creek 2006 young of the year. Since few juveniles were identified as progeny of adult steelhead, unsampled fish (either resident or anadromous) likely contributed to reproduction of many of the juvenile samples.

Parentage assignment for Section Corner Creek was comprehensive with all progeny tested assigned to a single parental cross. Results are reported in Table 5 for brood year 2005 (05 Fry and 06 Parr) and Table 6 for brood year 2006 (06 Fry). Parentage assignment for Brood year 2005 is reported in terms of both fry sampled in 2005 and parr sampled in 2006. Overall relative parental success, and female parental success were both significantly different between years (overall $p<0.0001$, female $p<0.0001$). Relative male parental success between years was not significant (p=0.0805). As previously seen, no progeny of reconditioned kelts were detected from either brood year 2005 or 2006.

Table 5. Section Corner parentage for brood year 2005. Number (ratio) of progeny for each parental cross. Total for each male and female appears at the end of each row and column

05 Fry	Male 1	Male 2	Male 3	Male 4	Male 5	*Female sum*
Female 1	76 (0.27)					76 (0.27)
Female 2	1 (0)	7 (0.03)		11 (0.04)		19 (0.07)
Female 3	16 (0.06)	26 (0.09)	12 (0.04)	2 (0.01)	6 (0.02)	62 (0.22)
Female 4	2 (0.01)	6 (0.02)		7 (0.03)	8 (0.03)	23 (0.08)
Female 5		43 (0.15)			18 (0.06)	61 (0.22)
Female 6		20 (0.07)			18 (0.06)	38 (0.14)
Male sum	95 (0.34)	102 (0.37)	12 (0.04)	20 (0.07)	50 (0.18)	

06 Parr	Male 1	Male 2	Male 3	Male 4	Male 5	*Female sum*
Female 1	58 (0.35)					58 (0.35)
Female 2	2 (0.01)	4 (0.02)		3 (0.02)		9 (0.05)
Female 3	5 (0.03)	3 (0.02)	2 (0.01)	2 (0.01)	2 (0.01)	14 (0.08)
Female 4	1 (0.01)	3 (0.02)		2 (0.01)	1 (0.01)	7 (0.04)
Female 5		34 (0.21)			20 (0.12)	54 (0.33)
Female 6		12 (0.07)			11 (0.07)	23 (0.14)
Male sum	66 (0.4)	56 (0.34)	2 (0.01)	7 (0.04)	34 (0.21)	

Table 6. Section Corner parentage for brood year 2006 (Fry only). Number (ratio) of progeny for each parental cross. Total for each male and female appears at the end of each row and column

	Male 1	Male 2	*Female sum*
Female 1	77 (0.85)	2 (0.02)	79 (0.87)
Female 2	3 (0.03)	9 (0.10)	12 (0.13)
Male sum	80 (0.88)	11 (0.12)	

Parentage Assignment in Shitike Creek was not useful and is not reported. See the discussion on population assignment for further explanation.

DISCUSSION

Omak Creek

Results for Omak Creek were not easily interpreted because sample collections did not represent discrete populations. Although an attempt was made to separate 2006 juvenile collections by length, tests for Hardy-Weinberg equilibrium and linkage disequilibrium still show evidence of population mixtures (Table 2). As a result, clustering relationships in the dendrogram were unclear in Omak Creek. More informative results are seen when using the program STRUCTURE in conjunction with the limited parentage data that was available. If the primary population subdivision in Omak Creek is between anadromous and resident life histories, running STRUCTURE with k=2 should partition individuals into each group. This was seen with the assignment of all adult anadromous steelhead to a single population that was then labeled as anadromous. With the exception of fish sampled above Mission Falls in 2006, all other sample collections include individuals assigned to both the population labeled anadromous and the alternative resident population.

Parentage results for Omak Creek support the population assignment results. Whenever parentage assignment was successful, the juvenile was also assigned to the anadromous population. This pattern is not surprising as only adult anadromous steelhead were included as potential parents. Incomplete parentage assignment of many of the individuals is reasonable given the incomplete sampling of adults. Adult anadromous steelhead are known to have passed the weir undetected and complete sampling of all potential resident parents is unfeasible. Still, the ability to use population assignment in conjunction with parentage assignment can provide a tool for quantification of relative success of the sampled adult anadromous steelhead.

Of 21 fish sampled above Mission Falls in 2005, 12 were assigned to the anadromous population. Six of these individuals were also matched to at least one of the adult anadromous steelhead captured at the weir in 2005, showing that Mission Falls was passable to adult steelhead in 2005. Passage in 2006 may have been blocked by high flows at the falls, however, samples collected above Mission Falls in 2006 should have also included brood year 2005 fish. A possible explanation for this is a change in location in 2006 when samples may have been collected from higher in the drainage.

A limited number of juveniles (7) sampled in 2006 were assigned to two adults that were captured for reconditioning in spring 2005 and subsequently returned as reconditioned kelts in 2006. These juveniles detected were emigrating smolts and too large to be young of the year from brood year 2006. Therefore, it is apparent these fish are progeny from the first time spawning event (air spawn in hatchery) and not post reconditioning. This supports the ability to detect parental contribution, but underscores the need for adequate age calculations. Unless the age of each juvenile can be determined, kelt contribution will be indistinguishable from first time spawning events. Consistency and accuracy in length measurements are essential if ages are to be calculated.

Section Corner Creek

All sample collections for Section Corner Creek grouped together on the tree, and additional patterns can be seen. Fry sampled in 2005 (SE05FR) grouped strongly with parr sampled in 2006 (SE06PA). These two sample collections represent the Brood year 2005 spawning event, with the parental group consisting of 2005 Satus Creek adult steelhead (SE05SA) planted as first time spawners in Section Corner Creek. As expected, fry collected in 2006 (SE06FR) cluster in the dendrogram with adult spawners in Section Corner (SE06AS).

Parentage analysis correctly assigned each (n=535) juvenile sample in Section Corner Creek to a single cross in the candidate pool of parents. However, no juveniles were assigned to kelts. The kelts may be suffering from rematuration timing issues associated with missing environmental cues or dietary deficiencies. Reconditioned kelts were held on well water that is significantly warmer than river water during winter months and this may have caused an acceleration of maturation. 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). It also should be noted that kelts released in 2005 were observed to have damaged eyes and may have been blind. Alterations to holding tanks have greatly reduced problems associated with eye damage, and further research is being conducted on environmental cues and dietary needs.

Parentage of brood year 2005 fish was performed for both juveniles sampled as fry in 2005 and parr in 2006. Relative reproductive success between the two sampling events was significant ($p<0.0001$). The significant value of the females ($p<0.0001$) contrasted with the insignificant value of the males ($p=0.0805$) shows that there is a female effect of reproductive success with respect to sampling time. Either there was a female effect on migration and hence sampling time of juveniles, or juveniles from different females had different survival rates between the sampling dates.

Despite the potential problems with timing maturation and condition between reconditioned kelts and first time spawners, results from Section Corner Creek demonstrate that the genotyping methodology along with parentage analysis can produce reliable reproductive success results. Moreover, continued analysis at this site may provide additional insight into mating patterns and contribution of residualized steelhead.

Shitike Creek

Parentage analysis in Shitike Creek was not accomplished, the result of only the resident population being sampled in the juvenile migration. This is supported by the low F_{ST} value (0.0017) and the dendrogramatic relationship between juveniles (SH56ST) and the resident population (SH56RS). Furthermore, population assignment using GENECLASS assigned 150 of 155 juveniles to the resident population. 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.

Without the ability to exclude residents from genetic testing, it may be unpractical to perform parentage analysis for emigrating juveniles. If a high proportion of juveniles are expected to be progeny of residents, the number of juvenile samples that need to be sampled will be cost prohibitive. Alternatively, the downstream migration of anadromous progeny may be occurring earlier than the screw trap is presently operated. This presents logistical issues of its own, as high water flows coinciding with emigration would limit the ability to operate the screwtrap. Juvenile sampling in spring of 2007 should include an effort to identify the migration pattern of these individuals.

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APPENDIX 1: Steelhead Kelt Collection Reconditioning

METHODS

Reconditioning Facilities

Omak Creek kelts were reconditioned at Cassimer Bar Hatchery (Figure 9), located at the confluence of the Okanogan River below Chief Joseph Dam. urrently the Colville Confederated Tribes operate the Cassimer Bar Hatchery. The facility was originally constructed in 1994, as a sockeye salmon (O. nerka) production facility in an attempt to supplement 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 13.3C (56.0F).

Figure 9. Photograph of the steelhead kelt reconditioning facilities at Cassimer Bar Hatchery.

Shitike Creek kelts were reconditioned at Warm Springs National Hatchery, located on the Warm Springs River, approximately 10 miles north (downstream on the Deschutes River) of Shitike Creek. The hatchery 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 salmon (O. tshawytscha). It was necessary to construct a circular kelt reconditioning tank, 16’ (d) x 4’ (h), at Warm Springs National Fish Hatchery Isolation Rearing Facility (IRF). The tank was installed (Figure 10) exclusively for 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 15.0C (59.0F) through the critical high temperature months (June-September). Artificial lighting was operated to replicate the photoperiod.

Figure 10. Photograph of the steelhead kelt reconditioning facilities at Warm Springs National Fish Hatchery.

The Section Corner Creek kelt reconditioning was conducted at the Prosser Fish Hatchery in Prosser, Washington. Kelts from Satus Creek were retained in one of the four 20’(dl) x 4’(h) circular tanks. Tanks were fed oxygenated 13.8C (57.0F) well water 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 11). The Yakama Nation (YN) operates Prosser Hatchery, with a primary function of rearing, acclimation, and release of fall chinook salmon. The facility is also used for coho salmon (O. kisutch) rearing prior to acclimation and release in the upper Yakima River Basin.

Figure 11. Photograph of the steelhead kelt reconditioning facilities at Prosser, WA.

Steelhead Kelt Collection

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 pre-spawn it was released on the downstream side of the weir.

Captured kelts were 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 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. Steelhead kelts were reconditioned for a 5-9 month time span before being released to promote rematuration. Growth measurement data and rematuration status was also recorded on all released individuals.

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 was fed to the kelt steelhead followed by a maintenance diet of Moore-Clarke salmon pedigree diet (Hatch et al. 2002).

Due to its successful use in treating Salmonicola at the Prosser Hatchery Facility reconditioning experiments during 2000 (Evans et al. 2002), IvermectinTM was diluted with saline (1:30) and injected into the posterior end of the fish's esophagus using a small (10cc) plastic syringe. 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. Research by Johnson and Heindel (2000), suggested that IvermectinTM – 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. Formalin was administered up to five times weekly at 1:6,000 for 1 hour in all reconditioning tanks to minimize fungal outbreaks.

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.

Evaluation / Release

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.

Reconditioned steelhead kelts from Omak were released into the Okanogon River instead of Omak Creek to avoid low flows and potentially lethal temperatures in Omak Creek. Kelts in Section Corner were released above the Section Corner Creek weir along with first time spawners and males.

RESULTS and DISCUSSION

Steelhead Kelt Collection

During the 2006 season, 45 live kelts were captured at the Omak Creek weir. Of these, 27 kelts were in adequate condition and were transported to the hatchery for reconditioning. The remaining 18 kelts were released downstream. An additional 22 kelt mortalities were counted at the weir.

The existing BPA project (20001700) captures and reconditions mixed origin kelts from the Yakima River at the Prosser Hatchery. See the 2006 Annual report for details on kelt reconditioning.

During the downstream migration 12 live kelts were captured at the Shitike Creek weir. Warm Springs Fisheries staff determined that five female kelts (1hatchery and 4 wild) were in adequate condition for reconditioning. Kelts not suitable for reconditioning and the hatchery female were released below the trap. The remaining 4 wild female kelts were transported to WSNFH for reconditioning.

Feeding / Reconditioning

While feeding was initiated, Omak Creek kelt mortalities began within the first week of being on station. Of 27 kelts captured, only 2 survived reconditioning. Necropsies showed that fish were suffering from deep tissue injury with internal bleeding and were not digesting food in the form of pellets. High water flows in 2006 coupled with abnormally high sediment and debris loads are suspected to be the cause of the causing internal bleeding. While it appears that fish were able to digest the krill easily, pellets were likely introduced too quickly. Undigested high protein pellets may suggest that the digestive flora needed was not yet developed. Necropsies were performed by a Washington State Department of Fish and Wildlife Pathologist. Based on discussions about environmental conditions in Omak Creek, he recommended changes including; limiting handling of fish, transportation in .8% salt water, continued formalin treatments, and a prophylactic treatment with Chloramine T for bacterial coldwater.

Of the four wild Shitike Creek kelts in good condition, all were eventual mortalities. One mortality occurred during transport to the holding facility, one was due to an accidental overdose of MS-222 during the in-processing phase and the final two occurred within a few days after induction into the reconditioning tank.

Release

2 female kelts from Omak Creek were released into the Okanogon River on October 18, 2006. Thirteen kelts were released into Section Corner Creek between November 15, 2006 and December 6, 2006 to overwinter and spawn in the spring of 2007. Predation in Section Corner Creek was high based on remains. Recent observations corroborate a high level of predation as no kelts appear to remain in this system. No steelhead survived to be released into Shitike Creek.