

RFS# 014

**Proposal to Evaluate Reproductive Success of Natural-Origin,
Hatchery-Origin, and Kelt Steelhead in the Columbia River Basin
(FCRPS BiOp Action #184)**

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Project / Program Summary

This evaluation program is designed to investigate the reproductive success of hatchery-reared, natural-origin, and reconditioned kelt steelhead *Oncorhynchus mykiss* in three different evolutionary significant units (Upper Columbia, Mid Columbia, and Snake River) under natural conditions. The two major goals are 1) directly examine reproductive success in several streams; and, 2) replicate and evaluate kelt reconditioning procedures and protocols at a variety of locations. This project is a collaborative effort among five tribes (Nez Perce, Warm Springs, Yakama Nation, Umatilla, and Colville), the University of Idaho, and the Columbia River Inter-Tribal Fish Commission¹.

We will apply kelt reconditioning methods developed by project 2000-017 with geographic replication. Direct examination of reproductive success will be accomplished using pedigree analysis. At all of our study sites (anticipate 5 different streams) returning adults of all variants (hatchery-origin identified by missing adipose fin, natural-origin identified by intact adipose fin, and reconditioned kelt collected from the previous spawning migration) will be DNA-typed in order to establish parentage of juveniles sampled from rearing areas above the weir. A total of 10 to 12 microsatellite loci will be assayed using nonlethal tissue sampling methods. Additionally, we will also collect and DNA-type approximately 200 adult resident rainbow trout (*O. mykiss*) at each stream in an attempt to identify parentage from resident forms. We also plan to implant PIT tags in each fish sampled to track their migration through the hydrosystem.

If reproductive success is high, reconditioning kelt steelhead could provide a means for maintaining this natural life history characteristic and potentially aid in the recovery of listed stocks. This study will provide resolution on uncertainty and genetic risk associated with the use of artificial propagation and reconditioning kelts in recovery of listed populations.

¹ Because of the speed in which this proposal came together, and the deadlines we adhered to, not all the collaborating agencies had the opportunity to have this proposal reviewed by their respective administrative processes, which could, after review, affect the scope of the project.

Project / Program Description

Introduction

This proposal details a collaborative approach to directly evaluate reproductive success of three variants of steelhead *Oncorhynchus mykiss* (natural-origin, hatchery-origin, and reconditioned kelt) across all ESUs of interest in the Request for Studies. Additionally, the project would evaluate kelt steelhead reconditioning and rematuration rates spatially and temporally. This project would provide evaluation of 5 replicates that would enhance the power of analyses but hold costs relatively low by collaboration with tribes and other groups that own and operate much of the necessary equipment. Through pioneering studies in kelt steelhead reconditioning, we have developed the experience, knowledge and expertise to enhance the survival and rematuration of post-spawn steelhead.

BACKGROUND

Iteroparity rates for *O. mykiss* were estimated to be as high as 79% for 1994-96 in the Utkholok River of Kamchatka (MSU undated; M. Powell UI and R. Williams, ISRP personal communication). Reported iteroparity rates for Columbia basin steelhead were considerably lower, due largely to high mortality of downstream migrating kelts at hydropower dams (Evans and Beaty 2001; Evans 2002), and to inherent differences in iteroparity rate based on geography (e.g. latitudinal effect, inland distance effect; Withler 1966; Bell 1980; Fleming 1998). Outmigrating steelhead averaged 58% of annual upstream runs in the Clackamas river from 1956 to 1964 (Gunsolus and Eicher 1970). Recent estimates of repeat spawners in the Kalama River (tributary of the unimpounded lower Columbia River) have exceeded 17% (NMFS 1996), which is the highest published iteroparity rate we found from the Columbia River Basin. Farther upstream, 4.6% of the summer run in the Hood River (above only one mainstem dam) are repeat spawners (J. Newton, ODFW, pers. comm.). Iteroparity for Klickitat River steelhead was reported at 3.3% from 1979 to 1981 (Howell et al. 1985). Summer steelhead in the South Fork Walla Walla River exhibited estimated 2% to 9% iteroparity rates (J. Gourmand, ODFW, pers. comm.), whereas repeat spawners composed only 1.6% of the Yakima River wild run (from data in Hockersmith et al. 1995) and 1.5% of the Columbia River run upstream from Priest Rapids Dam (L. Brown, WDFW, unpubl. data).

KELT RECONDITIONING IN THE YAKIMA RIVER (BPA Project 200001700)

The recent ESA listing of many Columbia Basin steelhead populations has prompted interest in developing reconditioning methods for wild steelhead populations within the Basin. To address recovery and reinstate this valuable life history trait, the Yakima/Klickitat Fisheries Project (YKFP) in cooperation with the University of Idaho and the Columbia River Inter-Tribal Fish Commission (CRITFC) began capturing wild emigrating steelhead kelts from the Yakima River in 1999 to test reconditioning and the

effects of several diet formulations on its success at Prosser Hatchery on the Yakima River (BPA Project 200001700).

The kelts reconditioned during that project substantially bolstered the number of repeat spawners in the study streams. Valuable knowledge regarding kelt husbandry, food type preferences, condition, and rearing environments are being obtained during the research endeavor. Since this project's inception in 1999, 20-30% of the kelts collected annually have been successfully reconditioned, and radio telemetry provided the ability to track some of these fish to the spawning grounds and to obtain documentation of successful redd construction. In terms of numbers of fish, this means that an additional 100-200 steelhead females were available to spawn a second time (total potential of approximately 300,000-600,000 eggs at an estimated 3,000 eggs per female) in the Yakima Basin that, without this program, would likely not have been able to do so. Total egg production estimates are impressive, however, the reproductive success of these reconditioned kelt steelhead is unknown and the basis for this proposal.

In the Yakima River kelt study, passive Integrated Transponder (PIT) tags are also being used to track captured steelhead kelts through the reconditioning process. An example from the PIT tag history of two such female kelts demonstrates the promise of this program to substantially increase the number of repeat-spawning steelhead in the Yakima River Basin (Table 1). Data from the second female demonstrate that the reconditioning process apparently did not affect this kelt's ability to home to that portion of the Yakima River Basin from which it originated.

Description	Location	Date	Post-eye-to-hyperal length (cm)	Weight (kg)
3D9.1BF11A2C6A				
Captured / tagged / reconditioned	Prosser	10-May-01	45	1.3
Released	Prosser	15-Nov-01	52	3.0
Recaptured / reconditioned	Prosser	30-Apr-02	51	1.9
Released	Prosser	10-Dec-02	53	2.3
3D9.1BF1456C7D				
Captured / tagged	Roza	2-Apr-02	47	2.1
Recaptured / reconditioned	Prosser	25-Apr-02	48	1.7
Released	Prosser	10-Dec-02	52	3.7
Recaptured / released	Roza	7-Mar-03	53	3.5

Information collected during this feasibility study has been significantly incorporated into the experimental design for upcoming years of research, and is expected to continue to increase survival and successful expression of repeat spawning. For a detailed report on BPA project 200001700 see Hatch et al. (2002).

KELT STEELHEAD ENUMERATION IN THE SNAKE RIVER

The CRITFC through funding from the U.S. Army Corps of Engineers has also been enumerating kelt steelhead in the juvenile by pass facility in Lower Granite Dam on the Snake River. From this work we reported (Evans and Beaty 2000; Evans and Beaty 2001; Evans 2002) that a substantial number of kelt steelhead were passing Lower Granite Dam (Table 2.)

Year	Kelt Est. / associated ladder count	% of upstream migration later enumerated as kelts in the by-pass
2000	2,780 / 13,238	21%
2001	3,348 / 49,470	7%
2002	4,695 / 22,362	23%

Note that these estimates of kelt steelhead abundance are based only on passage through the juvenile bypass facility and don't include other passage routes. Additionally, the lower kelt estimate in the bypass in 2001 is probably due to greater spill in that year resulting in fewer kelts using the bypass and instead presumably opting for passage through the spillway. Unfortunately, kelt steelhead mortality through the hydrosystem is very high and radio telemetry and PIT tag data show that about 3% or less of the kelt steelhead at Lower Granite are detected below Bonneville Dam. Innovative restoration approaches that take advantage of opportunities to use kelt steelhead or enhance kelt rematuration like the Yakima River may be vital not only in maintaining this natural life history trait but also aid in the recovery of listed populations and should be tested at other locations.

TOWARD EVALUATING MANAGEMENT OPTIONS

Beyond enumeration of kelt steelhead at Lower Granite Dam and investigating migration routes we have also begun evaluating various management actions that could enhance iteroparity expression by kelt steelhead. At Lower Granite in 2002, we PIT tagged and released 1,411 kelt steelhead with 660 individuals released in the tailrace and assigned to an in-river treatment group and 751 individuals were assigned to a transport group placed on barges and released below Bonneville Dam. In 2002, we conducted a short-term reconditioning experiment on approximately 400 kelt steelhead. These fish were PIT tagged and fed for 4 to 8 weeks and then trucked and released below Bonneville Dam. Lastly, we PIT tagged, held and reconditioned several hundred kelt steelhead in a long-term treatment and then released them back into the Yakima River. Figure (1) provides preliminary return data on these various treatments. We have proposed to expand this work through proposal 200001700 submitted in the Mainstem/Systemwide Province.

Figure 1. Preliminary evaluation of various approaches to enhance iteroparity in kelt steelhead.

However, as suggested by action 184 of the NOAA Fisheries 2000 Biological Opinion on the Operation of the Federal Columbia River Power System and BPA's request for study proposals, there is a need to expand iteroparity studies such as the one summarized above and to evaluate the reproductive success and potential genetic consequences of kelt reconditioning programs. This is a proposal to do exactly that.

PROBLEM STATEMENT

In the Columbia River Basin over 100 anadromous fish hatcheries release approximately 185 million juveniles per year that accounts for 75% of the anadromous fish production in the basin (NPPC 1999). The hatchery system has grown from mitigation for hydro development, harvest augmentation programs, and most recently conservation programs with goals of rebuilding depleted populations. Topics involving the use of surplus hatchery fish, the reproductive contribution of hatchery-origin fish in the wild, and supplementation have been hotly debated (ISAB 2002). Much of the debate has centered on the reproductive success of hatchery-origin fish on natural spawning grounds. The ISAB (2002) framed this debate with three questions: 1.) do the hatchery-origin salmon spawn; 2.) do the hatchery-origin salmon produce off-spring equally as well as wild salmon; and, 3.) does interbreeding between hatchery-origin and wild salmon affect the fitness of the wild population? These questions have been addressed in varying degrees by the literature. Hatchery-origin coho *O. kisutch*, chinook *O. tshawytscha*, and Atlantic salmon *Salmo salar* can spawn naturally (Fleming and Gross 1993; Chevanov and Riddell 1998; Fleming et al. 2000) and we have observed reconditioned steelhead spawning in the wild.

The literature evaluating the last two questions asked by ISAB is less convincing primarily due to constraints of field studies, using inappropriate hatchery stocks, and difficulties associated with measuring fitness. Many of the studies that have been designed to compare reproductive performance of hatchery-origin and wild-origin steelhead have used non-locally derived hatchery stocks and then reported that hatchery-origin fish were disadvantaged (Chilcote et al. 1986; Leider et al. 1990). Other researchers have used observations of spawning position and reproductive behavior to conclude that hatchery-origin salmon had lower reproductive abilities in natural-like settings (Fleming and Gross 1989; Fleming and Gross 1993). 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 (Fleming and Petersson 2001). This led Fleming and Gross (1993) to conclude 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 collected individuals are female (Evans 2002; Hatch et al. 2002). Field study observations of reproductive success of natural-origin, and hatchery-origin chinook salmon under joint spawning conditions inferred that wild spawners were more successful in spawning (Chebanov and Riddell 1998), although direct measures by

Marshall et al. (2000) found that hatchery origin chinook salmon can reproduce successfully under natural conditions. Technological advances in DNA-typing make direct measurement of reproductive success using pedigree analysis practical. Employing these new techniques, our proposed study will directly 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.

A consistent finding in our previous steelhead kelt reconditioning work is that the large majority of all kelts available for reconditioning are female (approx. 88% during 2000 and 2001 at Prosser Dam) which may be indicative of the evolutionary advantage of female iteroparity. Naturally occurring female iteroparity essentially acts in analogous ways as cryopreserving males in iteroparous salmon populations in the Columbia Basin. In addition, the fact that females are naturally able to reproduce with males during different years increases the probability of increased gene flow between and among cohorts or year classes. This has a direct theoretical benefit in the form of increasing the number of breeders (N_b), and the effective population size (N_e) during each spawning season, thus contributing to increased population viability and persistence, crucial to threatened and endangered fish restoration. Rather than a genetic hazard, experimental reconditioning should be viewed as a potential demographic and population genetic enhancement measure, aimed at restoring a recently jeopardized, but naturally occurring evolutionarily stable life history strategy.

This proposed project will have significant cost sharing through the utilization of existing weirs, tanks, and combining equipment purchases with existing projects. We are proposing a phased approach for this project because of contract timing. Steelhead sampling won't be possible in 2003, so instead we will conduct detailed planning, coordination, purchase equipment and sample adult resident *O. mykiss*.

The objectives of this collaborative proposal are to:

1. Identify specific streams where reproductive success of natural-origin, hatchery-origin, and reconditioned kelt steelhead can be tested.
2. Evaluate reproductive success of natural-origin, hatchery-origin, and reconditioned kelt steelhead and adult resident *O. mykiss* at a variety of streams in the Upper Columbia, Mid-Columbia, and Snake River ESUs using pedigree analysis.
3. Apply kelt steelhead reconditioning techniques at selected streams to post-spawners for release back into study streams.

Approach

Objective 1. Identify specific streams where reproductive success of natural-origin, hatchery-origin, and reconditioned kelt steelhead can be tested.

The downstream migration of post-spawn steelhead in the Columbia River generally occurs between 1 April and 30 June (Hatch et al. 2002) and implementation of proposals by successful applicants under this request for studies is to begin 20 June 2003. Thus due to timing of these two events, it is impossible to begin sampling adult steelhead (pre- or post-spawn) in 2003. Additionally, selecting streams to include in a study design for this work is difficult and time consuming. Difficulties are primarily related to physical stream features such as discharge and flow that add challenges to maintaining and operating weirs during the steelhead spawning run. Other challenges include access to stream reaches during times when weirs would be operated due to winter conditions. Additional stream selection issues include steelhead abundance, presence of hatchery fish, juvenile fish trapping facilities, and abundance of resident rainbow trout.

In the brief amount of time that was available to prepare this proposal we developed a list of potential streams to include in this study (Table 3). We intend to include 5 streams in our study for replication purposes, as well as to achieve dispersal among ESUs of interest. We have developed some selection criteria (Table 4) to apply to potential streams and allow us to determine individual streams to use for this study. This selection process would be initiated immediately after the contract was in place and be concluded no later than 31 July 2003. The ideal study stream would have approximately 200 prespawn adult steelhead comprised of both hatchery and natural-origin individuals. All of these fish would have tissues sampled at a weir for DNA profiling and then passed upstream. After spawning, a large proportion of the run would be collected at the weir in the kelt stage where they would be transported to a reconditioning facility. Juvenile steelhead smolts would be collected in a screw trap and tissue samples would be collected. The ideal stream would have low abundance of resident rainbow trout that would also be sampled and genotyped. Maximum effort will be made to include in the study streams that contain all three variants but it may not be possible since many potential areas do not contain hatchery populations above weirs collection facilities.

Stream	Adult Collection	Juvenile Collection	Reconditioning location?	Comments
Shitike Ck	Weir	Screw trap	Warms Spring National Hatchery & Round Butte Hatchery nearby. Probably need tanks.	All 3 variants could be available. Equip in place. Potential problem with large resident population.
Omak Ck	Weir	Screw trap	Cassimer Bar facility with tanks available	All 3 variants could be available. Equip in place

Upper Grande Ronde	Floating weir	Screw trap	Need circular tank	Problem - lack of hatchery fish above weir.
Lookingglass Ck	Picket weir / falls	Screw trap	Need circular tank	Problem - lack of hatchery fish above weir.
Catherine Ck	Hydraulic weir	Screw trap	Need circular tank	Problem - lack of hatchery fish above weir.
Little Sheep Ck (Imnaha)	Weir	NA	Need circular tank	Link with NMFS pedigree study. Adult steelhead abundance is high could go to 1000.
Lightning Ck (Imnaha)	Weir. Weir efficiency ranges between 54 and 97%.	NA	Need circular tank	Problems - Hatchery composition is low (2-28%), population sizes have generally been below 200. No comanager agreement on use of kelts. No juvenile emigration trapping to date and it would be difficult.
Rapid River (Little Salmon River)	Weir	NA	Need circular tank	Problems - no hatchery fish passed above weir, no juvenile trapping.
Newsome Ck (South Fork Clearwater River)	Weir. Some logistic issues with operating weir for steelhead.	Screw trap	Need circular tank	Potential to link with NPTH M&E.
Crooked River (South Fork Clearwater River)	Weir	Screw trap	Need circular tank	Problem - part of SSS study design.
Red River (South Fork Clearwater River)	Weir		Need circular tank	Weir may not be operational during spring?
Clear Ck (Middle Fork Clearwater River)	Weir	Screw trap	Need circular tank	Hatchery fish are not currently passed above the weir.

Lolo Creek	NA	Screw trap	Need circular tank	Would require substantial weir investment. Worth discussing due to link and associated needs of NPTH M&E.
Willie Dick Ck (Yakima R)			Prosser Hatchery facility available	
Harrah Drain (Yakima R)			Prosser Hatchery facility available	
Mule Dry Ck (Yakima R)			Prosser Hatchery facility available	
Simcoe Ck (Yakima R)			Prosser Hatchery facility available	
Wahtum Ck (Yakima R)			Prosser Hatchery facility available	

Table 4. Criteria used to select streams for inclusion in the study.		
Criteria	Target	
Adult steelhead abundance	100 to 300	
Existing weir	yes	
Existing juvenile trapping	yes	
Presence of hatchery fish	100 from a local stock	
Resident rainbow population	Low abundance	
Location for kelt reconditioning equipment	Relatively close.	
ESU of interest	Upper Columbia, Mid-Columbia, Snake River	
Natural and hatchery-origin steelhead present	Ideally both.	

Objective 2. Evaluate reproductive success of natural-origin, hatchery-origin, and reconditioned kelt steelhead and adult resident *O. mykiss* at a variety of streams in the Upper Columbia, Mid-Columbia, and Snake River ESUs using pedigree analysis.

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

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). Utilizing microsatellite loci optimized for steelhead studies (Narum et al. in review), we plan to determine the reproductive success of wild, hatchery, and kelt steelhead from five replicate sites in the Columbia Basin. The process will be comprised of four steps: 1) collect year 2004 samples from each of five study sites (all adult returns over five selected weirs, smolt progeny from screwtraps, and adult resident rainbow trout), 2) generate microsatellite genotypes from all samples taken in 2004 and perform parentage assignments, 3) collect adult returns of brood year 2004 steelhead (annually in 2006, 2007, and 2008), 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) back to adult collections of wild, hatchery, or kelt steelhead. This method will allow us to quantify not only the reproductive contribution of individual fish, but also quantify the adult returns related to each parental category of steelhead.

GENETIC METHODS

Sampling and Laboratory Techniques

All adult fish passing the five selected weirs will be sampled for an approximate total of 1000 adult samples in 2004 (sample size will depend on size of adult runs). Further, brood year 2004 smolts (n=300-400) will be collected from screwtraps from each of the five streams (total n=1500-2000). Starting in year 2006 and continuing through 2008, annual adult returns of brood year 2004 will be collected at each site (total estimated n=2000). In order to potentially identify the spawning contribution of resident rainbow trout, 200 resident fish will also be sampled from each sample site (total n=1000).

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 manufacturer'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 10-12 microsatellite loci

designed from *O. mykiss*. PCR amplifications will be performed using the AmpliTaq Reagent System (Applied Biosystems®) in an MJ Research® PTC-100 thermal cycler following manufacturer's protocols. Forward PCR primers will be fluorescently labeled (Applied Biosystems®), and PCR products genotyped using manufacture's protocols with an Applied Biosystems® model 3100 or 3730 genetic analyzer.

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 exclude possible crosses and parents (parental exclusion analysis). The software program FaMoz (Gerber et al. 2003) will be used for this analysis.

With sampling resident fish, we anticipate collecting and processing 4,000 individual fish samples. The direct cost for laboratory work including technician and scientist labor, consumables, and minor equipment is \$240,000.

Equipment costs for the first year would include partial cost of an ABI 3730 with the 6x upgrade. The ABI 3730 costs \$250,000 and the 6x upgrade adds an additional \$70,000. CRITFC already has \$130,000 budgeted for a sequencer in the accrual estimate for the CCAFS project (2001-046). This money could be used to cover part of the cost for an ABI 3730 and this proposed project to pay the remainder (approximately \$190,000). This instrument is required to assay the very large number of fish that this project will provide.

	Adult steelhead	Smolts	Adult resident	BY 2004 Adult returns
Each site	200	400	200	200
# sites	5	5	5	5
Total	1000	2000	1000	1000

Objective 3. 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.

COLLECTION

At each study site, kelt steelhead would be collected as they accumulate on the upstream side of each picket weir. These fish will be removed with dip nets and placed in an anesthetic tank. Anesthetized steelhead will be visually examined to classify each fish as a kelt or prespaw individual. Methods for visual classification are available (Hatch et al. 2002) and primarily involve keying specimens based on an imploded abdomen. This visual technique was highly precise (>95 %) when compared with the use of ultrasound analysis (Evans 2002). If a specimen is suspected to be a pre-spawner the fish will be released on the downstream side of the weir. Following collection anaesthetized kelts will be “in-processed”, where they are scanned for a PIT tags, measured, weighed, fish color and condition noted, injected with Ivomec intubate (parasite treatment), and injected with a PIT tag if not present in the specimen. The kelts are then released into large circular holding tanks for reconditioning. 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 IvermectinTM – a treatment often used to control parasites in swine and cattle – can 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. 2000), IvermectinTM will be diluted with saline (1:30) and injected into the posterior end of the fish’s esophagus using a small (10cc) plastic syringe.

HOLDING

One of our criteria for study stream selection is having a location suitable for kelt reconditioning. All of the streams in Table (1) have fish culture facilities located relatively close to the weirs. Some of these hatcheries have 20-foot diameter circular tanks available for reconditioning. At other hatcheries, this project would have to purchase new circular tanks for reconditioning. From our experience with reconditioning kelt steelhead the preferred container is 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. Formalin will be administered five times weekly at 1:6,000 for 1 hour in all reconditioning tanks to minimize fungal outbreaks. During the first year of the project we would purchase necessary tanks and plumbing equipment to insure that each study stream had a kelt reconditioning facility.

FEEDING / RECONDITIONING

Initially, a diet of frozen krill will be fed to the kelt steelhead followed by a maintenance diet of Moore-Clarke salmon pedigree diet. Experience at Prosser Hatchery has demonstrated that frozen krill was superior to starter paste diets in eliciting feeding behavior (Hatch et al. 2002). Kelts that received krill as a starter diet had an average

survival rate of 45% compared to only 28% survival of kelts not exposed to krill in 2001 experiments. Despite the apparent advantages of krill, a maintenance feed was necessary to augment rematuration rates. In the absence of a maintenance diet, re-maturation rate was only 10% compared to a 27% rematuration rate with a maintenance diet. In general, results indicated that frozen krill followed by Moore-Clarke salmon pedigree diet provided the best overall survival and rematuration rates in 2001.

EVALUATION / RELEASE

Surviving reconditioned kelt steelhead will be released above weir sites during the fall of the year. Prior to release each kelt will be anesthetized and examined with ultrasound equipment to determine maturation. Ultra-sound image captures of each fish will be stored electronically and later individual egg size will be determined. Such data may be usually 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.

Records of mortalities will be kept during the reconditioning process. 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.

Collaboration and Cost Sharing

Because of collaboration among tribes, the University of Idaho, and CRITFC, this project is possible on the scale that is proposed. Field collections will be dovetailed, to greatest extent possible, with existing projects so that equipment costs and operation can be minimized (Table 5).

Yakama Nation staff at Prosser Hatchery headed by Joe Blodgett has been quite successful developing practical solutions to kelt steelhead reconditioning while working on Project 2000-017-00. This knowledge of kelt husbandry will be shared among collaborators through site visits, workshops, or other coordination tools. Disseminating information from this uniquely experienced staff will be a key component of insuring successful operations at other sites.

The CRITFC through the Collaborative Center for Applied Fish Science has the resources necessary to successfully implement this project. This project also links with Project 2001-046-00 (Collaborative Center for Applied Fish Science CCAFS) that includes improvements to the University of Idaho's Hagerman Fish Culture Experiment

Station by providing construction and equipment funds. The equipment budget (and identified in the 2003 BPA Accrual Estimate) for the Collaborative Center includes an ABI 3100 DNA sequencer. Equipment costs for the first year of this proposed project would include partial cost of an ABI 3730 DNA sequencer with the 6x upgrade. The ABI 3730 costs \$250,000 and the 6x upgrade adds an additional \$70,000. CRITFC already has \$130,000 budgeted for the ABI 3100 sequencer in the accrual estimate for the CCAFS project (2001-046). This money could be used to cover part of the cost for an ABI 3730 and this proposed project could pay the remainder (approximately \$190,000).

Table 5. List of project collaborators and their primary role.	
<i>Collaborator</i>	<i>Primary Role</i>
Confederated Tribes of the Umatilla Indian Reservation	Field Operations
Nez Perce Tribe	Field Operations
Confederated Tribes of the Warm Springs Reservation of Oregon	Field Operations
Yakama Nation	Field Operations / Sharing Kelt culturing techniques
Confederated Tribes of the Colville Reservation	Field Operations
University of Idaho	Laboratory Analysis
Columbia River Inter-Tribal Fish Commission	Coordination / Laboratory Analysis / Reporting

Statement of Work

Objective 1. Identify specific streams where reproductive success of natural-origin, hatchery-origin, and reconditioned kelt steelhead can be tested.

Task 1.1: Hold three meetings with collaborators to determine, final criteria for stream selection, streams of interest for the study, and ultimately streams where we will conduct the work and reconditioning sites.

Timeline: 30 to 90 days after contracting.

Task 1.2: Determine equipment needs for each study stream, and procure, and install equipment.

Timeline: 45 to 90 days after contracting.

Task 1.3: Procure genetic analysis equipment and complete installation and setup.

Timeline: 60 to 90 days after contracting.

Task 1.4: Project coordination and collaboration. Includes technology transfer for reconditioning, planning and permitting if necessary.

Timeline: Over the entire duration of the project.

Objective 2. Evaluate reproductive success of natural-origin, hatchery-origin, and reconditioned kelt steelhead and adult resident *O. mykiss* at a variety of streams in the Upper Columbia, Mid-Columbia, and Snake River ESUs using pedigree analysis.

Task 2.1: Collect tissue samples for DNA analysis from resident rainbow trout populations located upstream of each weir. Target sample size is 200 individuals per year.

Timeline: Beginning September 2003.

Task 2.2: Install and operate weirs in any study streams that don't have them. Anesthetize individual adult steelhead trapped at the weir and insert a PIT in the fish and collect a tissue sample for DNA analysis.

Timeline: March through June 2004.

Task 2.3: Collect kelt steelhead at each weir, anesthetize, each fish, check for PIT tags and insert a tag if not present, measure for fork length, weight, note marks, and collect a tissue sample for DNA analysis and retain the fish for reconditioning.

Timeline: March through June 2004.

Task 2.4: Collect tissue samples from Age 0 *O. mykiss* in areas above weirs using electrofishing or seining techniques.

Timeline: August-September 2004 through 2006.

Task 2.5: Install and operate screw traps in streams that don't already have them in place. Collect tissue samples from 300 to 400 steelhead smolts from the 2004 brood for pedigree analysis.

Timeline: March through June 2006 on.

Task 2.6: Extract DNA from all 2004 collections. 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.

Timeline: Beginning April 2004

Task 2.7: Genotype adult collections at 10-12 microsatellite loci. The polymerase chain reaction (PCR) will be used to amplify 10-12 microsatellite loci designed from *O. mykiss*. PCR amplifications will be performed using the AmpliTaq Reagent System (Applied Biosystems®) in an MJ Research® PTC-100 thermal cycler following manufacturer's protocols. Forward PCR primers will be fluorescently labeled (Applied Biosystems®), and PCR products genotyped using manufacture's protocols with an Applied Biosystems® model 3100 or 3730 genetic analyzer.

Timeline: Beginning August through December 2004

Task 2.8: Assign parentage to individuals based on co-dominant microsatellite genotypes. Exclusion probabilities of parentage will be calculated to identify individual pedigrees. The software program FaMoz (Gerber et al. 2003) will be used for this analysis.

Timeline: Beginning December 2004 through February 2005

Objective 3. Apply kelt steelhead reconditioning techniques at selected streams to post-spawners for release back into study streams.

Task 3.1: Collection of post-spawn steelhead at each weir site. Collect biological information on each individual and check for tags (insert PIT tag if the fish untagged), intubate with Ivomec and transport to reconditioning station.

Timeline: March through June beginning 2004.

Task 3.2: Routine maintenance of tanks and culturing captive kelts. Several times a day technicians, to insure that the kelts have the best possible chance for reconditioning, monitor the tanks. This monitoring includes checking water levels, and flow as well as removal and data collection (individual tag number, length, and weight) of mortalities. Fish are fed the appropriate diet and observations of fish behavior (to detect sickness) are noted.

Timeline: March through November beginning 2004.

Task 3.3: Process surviving kelt steelhead for release. Late in the calendar year beginning in late 2004 all kelts will be processed again for PIT tag number, length, weight, and examined with ultrasound to determine maturation status.
Timeline: November beginning 2004.

Task 3.4: Release kelts above the weir where they were collected.
Timeline: November beginning 2004.

Timeline

The timeline for this proposal would span six years. This time period is equivalent to the life span of the oldest age-class (3 freshwater and 3 saltwater) steelhead that we anticipate to sample in the study. Timelines for each task are listed by month in Table (6) for first two years and Tables (7 and 8) for the following 4 years. Tasks are detailed in the statement of work section above.

This timeframe should yield answers regarding the spawning to smolt phase over a generation but it will not address spawning to adult return questions.

Table 6. Timeline for task completion by month during the first two years of the project starting June 2003 through May 2005.																								
Task	2003							2004											2005					
	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M
1.1	X	X	X	X																				
1.2	X	X	X	X	X																			
1.3		X	X	X																				
1.4	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2.1				X	X											X	X							
2.2										X	X	X	X									X	X	X
2.3										X	X	X	X									X	X	X
2.4															X	X								
2.5																								
2.6													X	X	X									
2.7															X	X	X	X	X					
2.8																			X	X	X			
3.1											X	X	X	X								X	X	X
3.2											X	X	X	X	X	X	X	X				X	X	X
3.3																			X					
3.4																		X						

Table 7. Timeline for task completion by month during the first two years of the project starting June 2005 through May 2007.																								
Task	2005							2006												2007				
	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M
1.1																								
1.2				X																				
1.3				X																				
1.4	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2.1				X	X											X	X							
2.2										X	X	X	X									X	X	X
2.3										X	X	X	X									X	X	X
2.4															X	X								
2.5										X	X	X	X									X	X	X
2.6													X	X	X									
2.7															X	X	X	X	X					
2.8																			X	X	X			
3.1										X	X	X	X									X	X	X
3.2										X	X	X	X	X	X	X	X	X				X	X	X
3.3																			X					
3.4																			X					

Table 8. Timeline for task completion by month during the first two years of the project starting June 2007 through May 2009.																								
Task	2007							2008												2009				
	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M
1.1																								
1.2				X																				
1.3				X																				
1.4	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2.1				X	X											X	X							
2.2										X	X	X	X									X	X	X
2.3										X	X	X	X									X	X	X
2.4															X	X								
2.5	X									X	X	X	X									X	X	X
2.6													X	X	X									
2.7															X	X	X	X	X					
2.8																			X	X	X			
3.1										X	X	X	X									X	X	X
3.2										X	X	X	X	X	X	X	X	X				X	X	X
3.3																			X					
3.4																			X					

Qualifications of Participants

The Production and Restoration Research Group (PRRG) from the Columbia River Inter-Tribal Fish Commission coordinated this proposal and Individuals from this group will be responsible for implementation of the project. Key staff includes Dr. André Talbot, Douglas Hatch, and Shawn Narum.

CRITFC

Dr. André Talbot is the leader of the PRRG at CRITFC and will act as the Principle Investigator of this study. He has broad expertise in conservation biology, including the important elements of biostatistics, population dynamics and quantitative/population genetics in an international development framework. He has a particularly strong background understanding variability in reproductive success and habitat-based predictive models of fish production. His research, education, and experience provide the necessary skills for a critical appraisal of methodologies employed in sampling designs, monitoring systems, research programs and resource management. In addition, he has extensive experience in analytical and statistical fisheries methods. From the design of experiments to data collection to statistical analysis, he has assisted tropical and temperate research teams to develop research programs and management plans for integrated aquaculture as well as traditional and industrial fisheries operations. Dr. Talbot also has been active in teaching, through workshops and university courses. Dr. Talbot began working in International Development Biological Research in 1983 at Dalhousie University as a research associate and project coordinator for the Aquaculture Genetics Network in Asia (AGNA). While holding an Associate Scientist position at the University of Québec at Chicoutimi, he directed a firm specialising in international development from 1989-1994, with particular interest in fisheries resource monitoring, population dynamics, aquaculture and genetics. Within this framework, he managed projects, supervised graduate students, professional and technical staff in Canada and in tropical countries and collaborated on research in four continents. From 1994 to 1997, he accepted an overseas post as a Regional Unit manager and Scientific Advisor - Biostatistician for the Caribbean Fisheries Resource Assessment and Management Programme, a regional project financed by the Canadian International Development Agency. Most recently, Dr. Talbot has been employed as a conservation scientist since 1997 at the Columbia River Inter-Tribal Fish Commission, in Portland, Oregon (USA).

Douglas Hatch will be the Project Leader and responsible for coordination of group and implementation of the project. Mr. Hatch received a Masters of Science Degree in Fisheries Resources from the University of Idaho in 1991 and a Bachelor of Science Degree in Fisheries Resources from the University of Idaho in 1986. He has been employed as a Fisheries Scientist at CRITFC since 1990. Mr. Hatch has been the Project Manager on the BPA Kelt Steelhead Reconditioning Project (2000-017) and the Kelt Enumeration Study at Lower Granite Dam (COE funded) since 2001. During the thirteen

years that Mr. Hatch has been with CRITFC he has led projects on developing escapement estimation techniques where he gained an extensive knowledge of weir construction, placement, and utilization. He also has managed all aspects of numerous contracts (with BPA 92-055; 2000-017; 2001-049), and subcontracts (with all CRITFC member tribes) including budgeting, contracting, and procurement.

Shawn Narum will be the lead Geneticist on the project. Shawn Narum received a Masters of Science Degree in Marine Science from the University of San Diego in 2000 and a Bachelor of Science Degree in Fishery Biology from Colorado State University in 1996. Mr. Narum has been employed by CRITFC (stationed at the Collaborative Center for Applied Fish Science Laboratory in Hagerman, ID) as Fisheries Scientist / Conservation Geneticist since 2002. Prior to coming to CRITFC, Mr. Narum was a Senior Research Associate for Chugai Biopharmaceuticals in San Diego and a Contract Geneticist for the National Marine Fisheries Service Southwest Fisheries Science Center. Mr. Narum is the lead geneticist on several projects related to conservation biology of coho salmon, chinook salmon, and steelhead in the Pacific Northwest. Research has focused on employing microsatellite markers to infer genetic relation of populations and races, as well as introgression of life history types and hatchery/wild interactions.

Dr. Madison Powell received his Ph.D. in the Systematics & Evolutionary Biology program at Texas Tech University in 1995 and is currently an Assistant Professor in the Department of Fish and Wildlife Resources and Department of Animal and Veterinary Sciences at the University of Idaho. Dr Powell is also the director of the Center for Salmonid & Freshwater Species at Risk at the University of Idaho. He supervises UofI molecular genetic laboratories at the Aquaculture Research Institute in Moscow, ID and at the Hagerman Fish Culture Experiment Station in Hagerman, Idaho. The laboratories' primary goals are to provide timely genetic information to applied conservation genetic questions, and provide genetic advice and consultation to state, federal, and tribal agencies regarding endangered fishes and fisheries management. Dr. Powell is currently the Principal investigator of several genetic projects examining reproductive success of hatchery and wild fish using microsatellite DNA analyses including (sockeye BPA project, Chinook captive broodstock project). Dr. Powell will assist in the development of the research study design, supervise genetic lab work, analyze data and report results.

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Education

University of Washington, Seattle, Washington
Doctor of Philosophy in Fisheries Science, 1987.

University of Puerto Rico, Mayaguez, Puerto Rico
Master of Science in Marine Sciences, 1974.

St. John's University, Collegeville, Minnesota
Bachelor of Science in Zoology, 1969.

Research Experience

1988-Present: Research Manager. Fisheries Resource Management Program, Yakama Nation. Responsible for the design, development, and implementation of a major supplementation and research facility to test the concept of using artificial production to rebuild natural spawning populations of spring chinook salmon in the Yakima Basin. Develop research programs to reintroduce extirpated coho salmon populations and to recondition ESA listed steelhead kelts for multiple spawning. Write detailed project plans, develop short- and long-term project goals and objectives, and supervise professional and technical staff.

1985-1988: Project Leader. Spring Chinook Enhancement Study. Responsible for research project designed to determine the best methods of enhancing the spring chinook salmon population in the Yakima Basin. Evaluate survival through various life stages and total production of naturally producing salmon. Determine methods of supplementation with hatchery-reared fish while minimizing adverse genetic impacts.

1989-1984: Various research and teaching positions at the College of Fisheries, UofW. Instructor and Teaching Assistant positions for various fisheries courses. Research assistant on project to determine the impacts of hydropower generation fluctuations in river flows on selected stages of salmon development.

1974-1977: Research Biologist, Department of Marine Sciences, University of North Carolina. Conduct field studies of fishes and invertebrates affected by diversion of water from Cape Fear River to cool nuclear power plant.

Education Experience

1991-Present: Faculty, Heritage College, Toppenish, WA. Developed and Implemented Associate of Arts Degree in Fisheries at Heritage College. Developed Curriculum and taught classes in various aspects of fisheries and aquaculture with goal of training fisheries technicians and hatchery culturists for Yakama Nation Fisheries programs.

1991-Present: Adjunct Professor, Central Washington University, Ellensburg WA Serve as faculty advisor on graduate student committees.

**1988-1992: Instructor. Bahamian Field Station, San Salvador Island Bahamas.
Co-sponsor: Oklahoma State University.**

Taught “Introduction to Tropical Marine Biology” course on San Salvador Island. Course included intensive classroom and field studies of various ecosystems associated with tropical islands.

1982-1984: Instructor. University of Washington, College of Fisheries.

Taught Fisheries Course in “Methods and Techniques of Fisheries Sampling” with classroom and weekly field trips.

Selected Reports and Publications:

Fast, D.E. 1987. The behavior of salmonid alevins in response to light, velocity and dissolved oxygen during incubation. Pages 84-92 in Salmonid Migration and Distribution Symposium (E.L. Brannon, ed.), School of Fisheries, University of Washington, and Directorate for Nature Management, Norway, Trondheim, Norway.

Fast, D.E. 1989. Supplementation Strategies for the Yakima/Klickitat Production Facility. Pages 143-147 in Northwest Fish Culture Conference Proceedings (R.Z. Smith, ed.).

Fast, D.E., J.D. Hubble, M.S.Kohn, and B.D.Watson. 1991. Yakima River Spring Chinook Enhancement Study. Project Completion Report to Bonneville Power Administration. Project 82-16. Volume 1 - 345 pp. and Volume 2 (Appendices) 133 pp.

Hager, R. C. and R. J. Costello (eds.). 1999. Optimal Conventional and Semi-Natural Treatments for the Upper Yakima Spring Chinook Salmon Supplementation Project: Treatment Definitions and Descriptions and Biological Specifications for Facility Design. Final Report to BPA. Project 95-06404. BPA Report DOE/BP-64878-2. 84 pp.

Sampson, M. and D. E. Fast. 2001. Monitoring and Evaluation: Yakima/Klickitat Fisheries Project. 2000 Report to BPA. Project 199506325. BPA Report DOE/BP-00000650-1. 265 electronic pp.

References

Bell, G. 1980. The costs of reproduction and their consequences. *The American Naturalist* 116(1):45-76.

Bernatchez, L. and P. Duchesne. 2000. Individual-based genotype analysis in studies of parentage and population assignment: How many loci, how many alleles? *Canadian Journal of Fisheries and Aquatic Sciences* 57:1-12.

- Chebanov, N. A., and B. E. Riddell. 1998. The spawning behavior, selection of mates, and reproductive success of chinook salmon (*Oncorhynchus tshawytscha*) spawners of natural and hatchery origins under conditions of joint spawning. *Journal of Ichthyology* 38:517-526.
- Chilcote, M. W., S. A. Leider, and J. J. Loch. 1986. Differential reproductive success of hatchery and wild summer-run steelhead under natural conditions. *Transactions of the American Fisheries Society* 115:726-735.
- Eldridge, W. H., M. D. Bacigalupi, I. R. Adelman, L. M. Miller, and A. R. Kapuscinski. 2002. Determination of relative survival of two stocked walleye populations and resident natural-origin fish by microsatellite DNA parentage assignment. *Canadian Journal of Fisheries and Aquatic Sciences* 59:282-290.
- Estoup, A., K. Gharbi, M. SanChristobal, C. Chevalet, P. Haffray, and R. Guyomard. 1998. parentage assignment using microsatellites in turbot (*Scophthalmus maximus*) and rainbow trout (*Oncorhynchus mykiss*) hatchery populations. *Canadian Journal of Fisheries and Aquatic Sciences* 55:715-725.
- Evans, A. F., and R. E. Beaty. 2000. Identification and Enumeration of Steelhead (*Oncorhynchus mykiss*) Kelts at Little Goose Dam Juvenile Bypass Separator, 1999 Ann. Rep. To US Army Corps of Engineers, Walla Walla District, for Contract No. DACW68-99-M-3102. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland OR.
- Evans, A. F., and R. E. Beaty. 2001. Identification and enumeration of steelhead (*Oncorhynchus mykiss*) Kelts in the juvenile collection systems of Lower Granite and Little Goose dams, 2000. Ann. Rep. To US army Corps of Engineers, Walla Walla District, for Contract No. DACW-00-R-0016. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland, OR.
- Evans, A. F. 2002. Steelhead (*Oncorhynchus mykiss*) kelt outmigration from Lower Granite Dam to Bonneville Dam: Abundance, downstream conversion rates, routes of passage, and travel times. Ann. Rep. To US army Corps of Engineers, Walla Walla District, for Contract No. DACW68-01-0016. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland, OR.
- Fleming, I. A., and M. R. Gross. 1993. Breeding success of hatchery and wild coho salmon (*Oncorhynchus kisutch*) in competition. *Ecological Applications* 3:230-245.
- Fleming, I. A. 1998. Pattern and variability in the breeding systems of Atlantic salmon (*Salmo Salar*), with comparisons to other salmonids. *Canadian Journal of Fisheries and Aquatic Sciences* 55:59-76.

- Fleming, I. A., K. Hindar, I. B. Mjolnerod, B. Jonsson, T. Balstad, and A. Lamberg. 2000. Lifetime success and interactions of farm salmon invading a native population. *Proceedings of the Royal Society of London* 267:1517-1523.
- Fleming, I. A., and E. Petersson. 2001. The ability of released, hatchery salmonids to breed and contribute to the natural productivity of wild populations. *Nordic Journal of Freshwater Research* 75:71-98.
- Gerber S., Chabrier P., Kremer A. (2003) FaMoz: a software for parentage analysis using dominant, codominant and uniparentally inherited markers, *Molecular Ecology Notes*, in press.
- Gunsolus, R.T. and G. J. Eicher. 1970. Evaluation of fish-passage facilities at the North Fork project on the Clackamas River in Oregon. Research report to the Fish Commission of Oregon, Oregon Game Commission, United States Bureau of Commercial Fisheries, United States Bureau of Sport Fisheries and Wildlife, and Portland general Electric.
- Hatch, D. R., P. J. Anders, A. F. Evans, J. Blodgett, B. Bosch, D. Fast, and T. Newsome. 2002. Kelt reconditioning: A research project to enhance iteroparity in Columbia Basin steelhead (*Oncorhynchus mykiss*). Project 2000-017-00, Annual Report the Bonneville Power Administration, Portland, OR.
- Heath, D. D., J. W. Heath, C. A. Bryden, R. M. Johnson, and C. W. Fox. 2003. Rapid evolution of egg size in captive salmon. *Science* 299:1738-1740.
- Hockersmith, E., J.Vella, L. Stuehrenberg, R. N. Iwamoto, and G. Swan. 1995. Yakima

- River radio-telemetry study: Steelhead, 1989-93. Report to US Dept. Energy, Bonneville Power Administration, for Proj. No. 89-089, Contract No. DE-AI79-89BP00276, by Northwest Fisheries Science Center, National Marine Fisheries Service, Seattle, WA.
- Howell, P., K. Jones, D. Scarnecchia, L. LaVoy, W. Kendra, and D. Ortmann. 1985. Stock assessment of Columbia River anadromous salmonids Volume II: steelhead stock summaries stock transfer guidelines - information needs. Final Report to U.S. Department of Energy, Bonneville Power Administration, Project No. 83-335.
- ISAB. 2002. Hatchery surpluses in the Pacific Northwest. *Fisheries* 27(12):16-28.
- Lange, K. 1997. *Mathematical and Statistical Methods for Genetic Analysis*. Springer-Verlag, New York, 265p.
- Leider, S. A., P. A. Hulett, J. J. Loch, and M. W. Chilcote. 1990. Electrophoretic comparison of the reproductive success of naturally spawning transplanted and wild steelhead trout through the returning adult stage. *Aquaculture* 88:239-252.
- Letcher, B. H. and T. L. King 2001. Parentage and grandparentage assignment with known and unknown matings: application to Connecticut River Atlantic salmon restoration. *Canadian Journal of Fisheries and Aquatic Sciences* 58:1812-1821.
- Lewis, P. O., and D. Zaykin. 1999. GDA: Genetic Data Analysis (version 1.2) free program distributed by the authors at the GDA homepage: <http://chee.unm.edu/gda/>
- Marshall, A. R., H. L. Blankenship, and W. P. Connor. 2000. Genetic characterization of naturally spawned Snake River fall-run chinook salmon. *Transactions of the American Fisheries Society* 129:680-698.
- Marshall, T. C. J. Slate, L. Kruuk, and J.M. Pemberton. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* 7:639-655.
- Narum, S. R., C. Contor, A. Talbot and M. Powell. *Submitted*. Genetic divergence of sympatric resident and anadromous forms of *Oncorhynchus mykiss* in the Walla Walla River and Columbia River Basin, USA. *Journal of Fish Biology*.
- Neff, B. D., J. Repka, and M. R. Gross. 2001. A Bayesian framework for parentage analysis: The value of genetic and other biological data. *Theoretical Population Biology* 59:315-331.

- NMFS (National Marine Fisheries Service). 1996. Status review of west coast steelhead from Washington, Idaho, Oregon, and California. Seattle, WA.
- Norris, A. T., D. G. Bradley and E. P. Cunningham. 2000. Parentage and relatedness determination in farmed Atlantic salmon (*Salmo salar*) using microsatellite markers. *Aquaculture* 182:73-83.
- NPPC 1999. Artificial Production Review: Report and recommendations of the Northwest Power Planning Council. Document 99-15.
- O'Reilly, P.T., C. Herbinger and J.M. Wright. 1998. Analysis of parentage determination in Atlantic salmon (*Salmo salar*) using microsatellites. *Animal Genetics* 29:363-370.
- Withler I. L. 1966. Variability in life history characteristics of steelhead trout (*Salmo gairdneri*) along the Pacific Coast of North America. *Journal of the Fisheries Research Board of Canada* 23: 365-393.

