STATE OF WASHINGTON June 2007

Lyons Ferry Complex Hatchery Evaluation: Summer Steelhead Annual Report 2005 Run Year

by Joseph D. Bumgarner, and Jerry Dedloff

Washington Department of
FISH AND WILDLIFE
Fish Program
Science Division

FPA 07-08

Lyons Ferry Complex Hatchery Evaluation:
Summer Steelhead Annual Report 2005 Run Year

by

Joseph D. Bumgarner
Jerry Dedloff

Including a supplemental genetics report by
Scott M. Blankenship, Maureen P. Small, Joseph D. Bumgarner, Mark Schuck, and Glen Mendel

Washington Department of Fish and Wildlife
Fish Program / Science Division
Hatchery & Wild Interactions Sub-Unit
600 Capital Way North
Olympia, Washington 98501-1091

to

U.S. Fish and Wildlife Service
Lower Snake River Compensation Plan Office
1387 Vinnell Way, Suite 343
Boise, Idaho 83709
Cooperative Agreements #'s 141105J056, 141106J013

June 2007

Acknowledgments

The ongoing success of the steelhead and trout program is the result of the coordinated and dedicated efforts of many Washington Department of Fish and Wildlife (WDFW) employees, as well as employees from other State and Federal Agencies. We especially thank Steve Rodgers, Doug Maxey, Brandon Kilmer, and the Lyons Ferry/Tucannon staff for their hard work, insight, and assistance of summer steelhead activities conducted at Lyons Ferry Complex for the last year. We also thank other Snake River Lab permanent and temporary staff that helped out with steelhead evaluation activities throughout the year.

We thank Jon Hansen, and Larry Barrett, of Idaho Fish and Game for their assistance in conducting joint Snake River creel surveys and providing CWT recoveries from Idaho fisheries. Thanks to Rich Carmichael's crew from Oregon Department of Fish and Wildlife, especially Mike Flesher, for their leadership in conducting the Grande Ronde River creel survey and providing the CWT recoveries. Dave Marvin with Pacific States Marine Fisheries Commission provided valuable assistance with our PIT tag files. The accuracy and timeliness of all the data provided by the above individuals is always appreciated.

We thank additional WDFW personnel (John Sneva, Jim Shaklee, Scott Blankenship, Susan Markey, and Lynn Anderson) for their assistance with portions of the project.

We thank Mark Schuck, Glen Mendel, Scott Marshall, Todd Pearsons, and Jim Scott for their critical review of the draft annual report.

Finally we thank the entire staff of the Lower Snake River Compensation Plan Office for their firm support in funding these monitoring and evaluation studies.

Table of Contents

List of Figures

List of Tables

List of Appendices

Executive Summary

The 2005 run year annual report is one in a continuing series describing Washington Department of Fish and Wildlife's progress toward meeting summer steelhead and rainbow trout mitigation goals established in the Lower Snake River Compensation Plan. The reporting period covers 1 July 2005 through 30 June 2006.

The LSRCP mitigation trout program has focused on providing recreational fishing opportunities in southeast Washington. Currently, the LFC goal is to produce 237,500 trout for release into southeast Washington area lakes to provide for the 67,500 angler days of recreation. During the report period, stocking of LSRCP produced rainbow trout within Washington and transfers to the State of Idaho were less than planned.

The LFC raises four summer steelhead stocks for the mitigation program (LFH, Wallowa, Tucannon and Touchet). Program releases range from 50,000-160,000 depending on location, with all groups programmed for a release size of 4.5 fish/lb. The numbers of steelhead released in 2006 were slightly above program goals, but size goals were not met for the Wallowa, Tucannon or Touchet stocks. Groups of hatchery steelhead released into the Tucannon and Touchet rivers were also PIT tagged for estimation of smolt-to-adult return rates since they are currently not marked for harvest.

We operated a rotary screw trap in the Tucannon River to estimate the number of migrating natural steelhead smolts and other salmonids. We estimated that 16,209, and 10,080 natural steelhead smolts emigrated from the Tucannon River from the 2004/2005 and 2005/2006 trapping years, respectively. Age compositions and characteristics of smolts captured from all years are presented.

As part of our annual broodstock collection and research activities, WDFW hatchery and evaluation staffs operate a series of adult steelhead traps in SE Washington. At LFH, a total of 1,674 adult steelhead were trapped, with 120 females and 241 males spawned, producing 430,667 eyed eggs to satisfy program goals. At Cottonwood Creek Trap, 2,006 adult steelhead were trapped with 120 females and 115 males spawned producing 316,059 fertilized eggs for the program. At Tucannon Hatchery, staff trapped 20 natural, 18 Tucannon River endemic hatchery stock, and one LFH stock hatchery-origin steelhead for the season; none of these were collected for broodstock purposes. At the lower Tucannon Trap, staff trapped 90 natural fish, 23 Tucannon River endemic hatchery stock, and 48 LFH stock hatchery fish. Thirty-five of the natural fish were collected for broodstock, of which 13 females and 17 males were spawned for a total eggtake of 72,520. At the Touchet River Trap, staff trapped 164 Touchet River natural, 14 LFH stock hatchery, and 35 Touchet River endemic hatchery steelhead. Thirty-nine natural origin fish were collected, of which 18 females and 18 males were spawned for a total eggtake of 88,668 eggs. For the second year in a row, three of the Touchet River females tested positive for IHNV; the progeny from these fish were planted into the Touchet River as fry (14,276 total).

WDFW personnel surveyed steelhead sport anglers within the LSRCP area of Washington to recover CWTs from tagged steelhead. During the 2005/2006 steelhead sport fishery we surveyed 10,181 anglers that caught 3,236 steelhead, of which 1,165 were natural origin fish

(36.0% of the total catch). In addition, we cooperate with ODFW in conducting a joint survey of anglers on the lower Grande Ronde River of Washington and Oregon. Angler effort, catch rates, and harvest that were estimated by ODFW staff are presented.

During 2006, evaluation staff surveyed spawning grounds in the Touchet River, Asotin Creek, and Cumming Creek (Tucannon River Basin). High, turbid stream flows much of season prevented us from conducting surveys in the Tucannon River, and hampered our success in other areas.

The LFC summer steelhead program (LFH and Wallowa stocks) continues to exceed the original hatchery mitigation goal to the Snake River project area by supplying hatchery fish for sport harvest. Based on creel surveys and adult traps, we estimated that a minimum of 5,099 (3,156 goal/run year) LFH stock and 2,339 (1,500 goal/run year) Wallowa stock fish returned from the 2002 brood year. That represents 162% and 156% of the Washington mitigation goal for each of these stocks, respectively. However, original goals of the LSRCP also assumed that about ¾ of the annual return would be captured in downriver fisheries. To date, the downriver harvest has not approached that rate of harvest, mainly due to curtailment of fisheries in recent years due to ESA listings.

As in previous years, WDFW electrofished index sites to estimate natural juvenile steelhead densities, derive population estimates for specific river reaches, and to estimate residual hatchery steelhead. In addition, we conducted mark/recapture tests to compare with our standard electrofishing methods to examine bias in the estimates. In 2005 we found that the multiple pass estimates of Age 0 and Age 1+ summer steelhead were 26% and 22% lower, respectively, than the mark/recapture estimates. The relationships were highly correlated so the possibility of determining a correction factor to previous years' data looks promising.

Since 1998, the Snake River Lab and WDFW's Fish Management staff have periodically collected samples from SE Washington summer steelhead populations (adult and juvenile) for genetic stock analysis. Samples have been collected from the Walla Walla, Touchet and Tucannon River basins, the LFH stock, and portions of the Grande Ronde. During the fall of 2006, WDFW genetics staff, in cooperation with the Snake River Lab and WDFW Fish Management for SE Washington assembled a summer steelhead genetics summary that includes most samples collected through 2005. The genetics report is provided as an Appendix to this report.

Introduction

This annual report is one in a continuing series describing Washington Department of Fish and Wildlife's (WDFW) progress toward meeting summer steelhead (*Oncorhynchus mykiss)* and rainbow trout mitigation goals established in the Lower Snake River Compensation Plan (LSRCP). The reporting period covers 1 July 2005 through 30 June 2006. Coded wire tag recoveries and expansions from the summer steelhead sport fishery in the Columbia and Snake River basins will also be presented in a future report.

The LSRCP program in Washington State began in 1981 with construction of Lyons Ferry Hatchery (LFH). Refurbishing of the Tucannon Fish Hatchery (TFH) followed in 1984-1985. In addition to the hatchery construction and modifications, three remote acclimation ponds (AP) were built along the Tucannon (Curl Lake AP), Touchet (Dayton AP), and Grande Ronde (Cottonwood AP) rivers to acclimate juvenile summer steelhead before release. All of these facilities make up WDFW's Lyons Ferry Complex (LFC) (Figure 1).

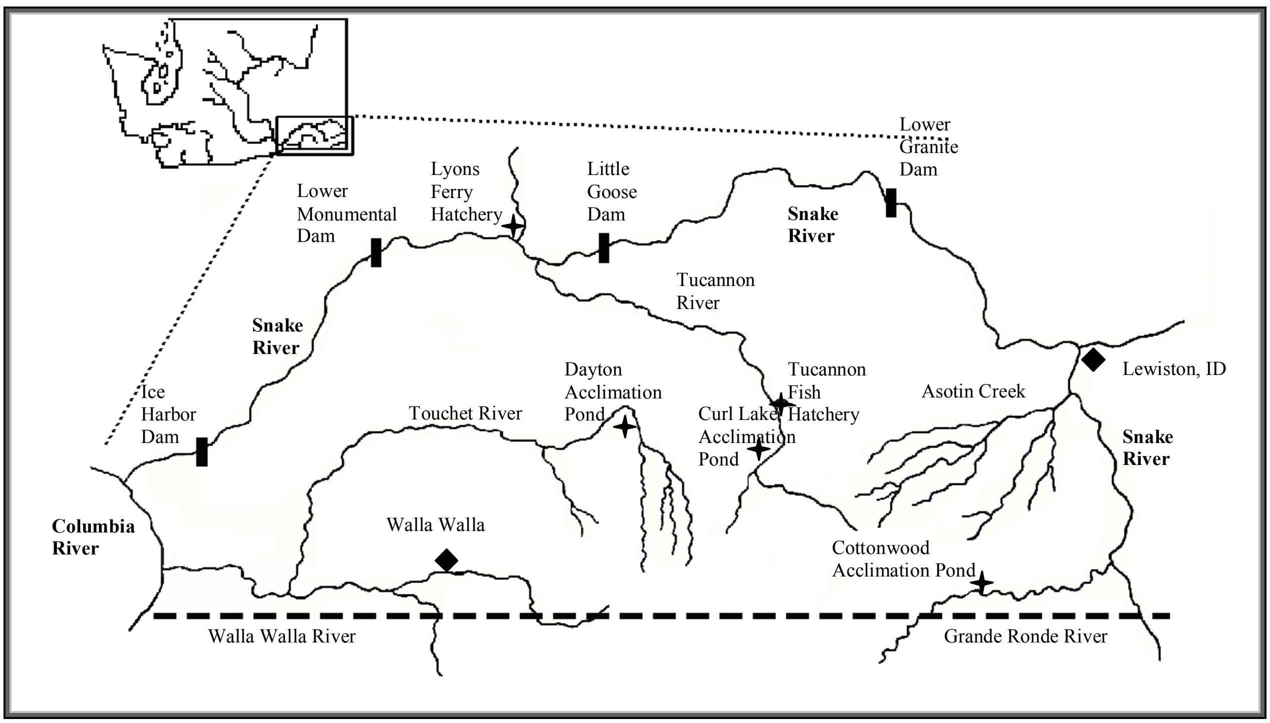

Figure 1. Map of major rivers and streams in Southeast Washington, and Lyons Ferry Complex facilities.

Production Goals of Steelhead and Rainbow Trout Stocks

The LFC currently uses four summer steelhead stocks to produce smolts for release into the Snake (60,000 smolts of LFH stock), Tucannon (100,000 smolts of LFH stock, 50,000 smolts of Tucannon Endemic stock), Grande Ronde (160,000 smolts of Wallowa stock), Walla Walla (100,000 smolts of LFH stock), and Touchet rivers (85,000 smolts of LFH stock, 50,000 smolts of Touchet Endemic stock) to enhance recreational opportunities for steelhead anglers and for recovery purposes. All steelhead smolts for the program are planned for a release size of 4.5 fish/lb (about 100 g/fish). Current releases of summer steelhead smolts are lower than originally specified by the LSRCP program. Releases have periodically been reduced through the years (in 2001 the LFH and Wallowa stock programs were reduced by 37%) in partial response to Endangered Species Act (ESA) concerns and documented smolt-to-adult (SAR) survival rates far exceeding the original SAR goal of 0.5% (USACE 1975).

The LSRCP mitigation trout program has focused on providing recreational fishing opportunities in southeast Washington. Currently, the LFC goal is to produce 237,500 trout for release into southeast Washington area lakes to provide for the 67,500 angler days of recreation (USACE 1975). The LFC also produces another 150,000 (3,000 lbs) fry (Spokane stock), and 50,000 (3,333 lbs) fingerlings (Kamloops stock) for Idaho Fish and Game's (IDFG) LSRCP program. The ESA listings of Chinook (*O. tshawytscha*), steelhead, and bull trout (*Salvelinus confluentus*) caused the stocking of rainbow trout from LFC into Washington State area waters to be shifted exclusively to small lakes and ponds to reduce the potential negative effects on listed species. During the report period, stocking of LSRCP produced rainbow trout within Washington (Table 1 – catchable size only), and transfers to the State of Idaho were less than planned. WDFW also produces larger sized (1.5-2.5 lbs/fish or 181.4-302.4 g/fish) rainbow trout at TFH for stocking into area lakes (Table 1) as part of a State program.

In-Hatchery Survival

Survival rates of steelhead at LFC remain highly variable among stocks and among years. Fish health problems (e.g., cold water disease), presence of pathogens such as Infectious Hematopoetic Necrosis virus (IHNV), and spawning conditions at LFC and at remote spawning sites have all affected in-hatchery survival over the years (Table 2). Despite extra measures taken by both hatchery and science staffs to obtain accurate estimates of eggs or newly hatched steelhead fry, there continues to be errors discovered when more than 100% of the estimated fish on hand are counted during the marking phase. Within hatchery survival estimates are reasonably accurate, but imprecise (we often have fry-smolt survival in excess of 100%). This imprecision is not absolutely critical to program evaluations or determining program success and tends to be within +/- 5% of actual as determined during marking/tagging fish prior to release.

Table 1. Summary of rainbow trout plants (catchable size) from Lyons Ferry Complex, 2006 (Represents both LSRCP and State funded programs).

County	Location	Number of Plants	LSRCP lbs of fish planted	LSRCP # of fish planted	State lbs of fish planted	State # of fish planted
Adams	Sprague Lake	1	1,579	4,500		
	Total	***1***	***1,579***	***4,500***		
Asotin	Golf Course Pond	8	5,687	17,965	677	400
	Headgate Pond	1	426	200		
	Silcott Pond	1	417	1,000		
	West Evans Pond	8	5,637	17,998	677	400
	Total	***18***	***12,167***	***37,164***	***1,354***	***800***
Columbia	Beaver Lake	2	273	1,000		
	Big Four Lake	2	1,200	3,000	450	300
	Blue Lake	10	5,849	18,071	491	300
	Curl Lake	5	2,175	10,055	490	213
	Dam Pond	1	370	1,000		
	Dayton Jv. Pond	5	525	2,004	245	100
	Deer Lake	3	889	3,594		
	Donnie Lake	2	128	665		
	Orchard Pond	1	741	2,000		
	Rainbow Lake	9	3,951	12,984	501	300
	Spring Lake	7	2,707	16,082	506	300
	Watson Lake	8	4,047	15,395	512	300
	Total	***55***	***22,855***	***85,850***	***3,195***	***1,813***
Franklin	Dalton Lake	6	7,490	24,497	600	300
	Marmes Pond	2	801	2,000		
	Total	***8***	***8,291***	***26,497***	***600***	***300***
Garfield	Casey Pond	1	107	503		
	Total	***1***	***107***	***503***		
Walla Walla	Bennington Lake	5	5,725	18,528	476	200
	Fishhook Pk. Pond	3	1,465	5,064	357	150
	Jefferson Pk Pond	4	422	1,502	200	100
	Lions Park Pond	5	452	1,650	206	100
	Quarry Pond	6	7,327	23,999	600	300
	Total	***23***	***15,391***	***50,743***	***1,839***	***850***
Whitman	Garfield Pond	2	416	1,997	60	25
	Gilcrest Pond	2	313	1,502	60	25
	Pampa Pond	4	1,606	6,089	400	200
	Riparia Pond	1	769	2,000		
	Union Flat	1	429	1,501		
	Total	***10***	***3,533***	***13,089***	***520***	***250***
Totals		**98**	**63,923**	**218,346**	**7,508**	**4,013**

Table 2. Number spawned, average fecundity, and survival by life state of LFH stock steelhead spawned at LFH, 2005 and 2006 brood years.

BY	Spawned Female	Spawned male	Average eggs/ female	Eggs taken	Eggs retained [a]	Percent retained	Fry	Egg-fry survival	Smolts	Fry-smolt survival
Wallowa Stock										
2005	60	70	4,711	282,675	274,586	97.1	273,608	96.8	169,390	61.9
2006	120	115	NA	316,059	290,903	92.0	289,647	92.0		
Lyons Ferry Stock										
2005	133	263	4,428	571,185	452,011	79.1	439,803	77.0	350,028	79.6
2006	120	241	4,411	529,379	430,667	81.4	423,397	80.0		
Tucannon Stock										
2005	14	25	5,509	77,131	71,933	93.3	70,254	91.1	65,245	92.9
2006	13	17	5,578	72,520	67,341	92.9	66,169	91.2		
Touchet Stock										
2005	18	17	4,147	79,540	50,629	63.6	49,870	62.7	52,476	100.0 [b]
2006	18	18	4,926	88,668	71,453	80.6	75,417	85.0		

[a] The number of eggs retained includes all losses from green egg to eye up (mortality and eggs destroyed due to IHNV).
[b] The impression of hatchery methods at times measures survival between life stages as >100%, where that occurs 100% is reported as a maximum.

Marking

All steelhead from the LFH and Wallowa stocks were marked with an adipose fin clip prior to release for the mark-selective fishery in the Columbia and Snake rivers, and to distinguish hatchery and natural-origin adults at counting sites in natural spawning areas. In January 2006, study groups within the LFH and Wallowa stocks were also marked with a left ventral (LV) fin clip and given a coded-wire tag (CWT) for specific contribution studies and/or to document straying (Table 3). The Tucannon and Touchet rivers endemic steelhead stocks, which are conservation programs, were marked with a red Visual Implant Elastomer (VIE) tag behind the eye rather than an adipose clip (Table 3). This provides a means to identify adults returning from these programs without promoting retention in mark-selective fisheries. Evaluation staff conducted quality control tag/mark checks on all release groups. In addition, about 9,000 passive integrated transponder (PIT) tags were inserted into the following 2006 release groups: Tucannon Endemic stock, Touchet Endemic stock, and LFH stock release in the Tucannon River. Since the endemic stock releases are not marked for sport harvest (see above), we will rely solely on adult PIT tag detections at the mainstem dams and other locations to determine smolt-to-adult survival rates for these groups. This is also being done for the LFH stock release in the Tucannon as we dropped the LV clip/CWT due to cost and space issues at LFH. We assume that PIT tag loss and differential mortality is negligible on these groups as they are tagged at a relatively large size (90 g, 200 mm). An assessment of these programs' success and recommendations about the endemic program will be presented in future reports.

Table 3. Summer steelhead smolt releases from Lyons Ferry Complex, 2006.

Location (Stock)	Rkm	Date	Total release	Marked release [a]	CWT code	Marks/ Brand/ VIE	Lbs	Size #/lb	CWT %Loss	VIE %Loss
Grande Ronde @ Cottonwood AP (Wallowa)	45.9	4/24	169,390	20,233	63-32-91	AD ADLV	35,199	4.8	1.2048	NA
Snake River @ LFH (LFH)	92.8	4/17-4/20	61,431	20,547	63-31-91	AD ADLV	14,300	4.3	1.1838	NA
Tucannon River ~200m ↓ Pataha Creek (LFH)	18.5	4/17-4/20	101,724	None	None	AD ONLY	24,062	4.2	NA	NA
Touchet River @ Dayton AP (LFH)	86.4	4/17-4/24	86,528	20,898	63-32-93	AD ADLV	20,122	4.3	1.5414	NA
Walla Walla River (LFH)	56.0	4/17-4/20	100,345	20,225	63-32-92	AD ADLV	23,899	4.2	2.6650	NA
Tucannon River @ Camp Wooten Br. (Tucannon)	67.0	4/12-4/15	65,245	65,245	None	RR[b] VIE ONLY	13,716	4.8	NA	3.2224
Touchet River @ NF Touchet Bridge (Touchet)	91.5	4/13, 5/03	52,476	52,476	None	LR[b] VIE ONLY	10,932	4.8	NA	5.7779

[a] The number shown as marked released has not been adjusted for tag/mark loss. Endemic stock releases are not externally marked, therefore the unmarked release is equal to the total release number.

[b] LR (Left Red) and RR (Right Red) denote side and color of the Visual Implant Tag placed in the adipose tissue behind the eye.

Juvenile Releases

Evaluation staff collected pre-release samples for all LFC release locations (Table 4) to assess the consistency of length, weight, condition factor, and other characteristics with program goals. All LFH stock release groups were at or above program goals in 2006. The Wallowa stock (cooler final rearing water) and both Endemic stocks (later spawn timing) were slightly below the program goal size at release, but release size in both endemic stocks has improved compared to previous years through some excellent, and modified, fish culture practices. As an example, for the third consecutive year, hatchery staff size graded both endemic stocks in an effort to prevent a bi-modal size distribution in the release groups. This effort was somewhat successful for the Tucannon stock, though the hatchery was forced to release the smaller fish earlier than desired due to conflicts with other programs. The strategy was more successful with the Touchet endemic stock as we were able to delay their release date until early May which allowed them to nearly attain the size goal of 4.5 fish/lb. Additional measures to eliminate these size differences, which have been a continual problem in the endemic stock programs from the beginning, continue to be investigated. The addition of small, intermediate rearing vessels installed at LFH during the summer of 2006 may help LFH staff manage the bi-modal size problem in the endemic stock groups in the future.

Table 4. Mean fork lengths, weights, condition factor (K), co-efficient of variation (CV), fish per pound (FPP), and the percent of each release visually documented as precociously mature males from LFC steelhead prior to release, 2006.

Location (Stock)	Date	N	Avg LN (mm)	Avg WT (g)	K	CV	FPP	Percent precocious
Cottonwood (Wallowa)	4/04	290	204.0	94.0	1.05	12.8	4.8	0.34%
Tucannon (LFH)	4/17	335	214.0	101.2	1.01	8.4	4.5	0.30%
Tucannon (Endemic-Large)	4/10	301	212.9	105.6	1.06	10.0	4.3	0.67%
Tucannon (Endemic-Small)	4/14	320	198.2	84.7	1.04	11.7	5.4	0.60%
Touchet (LFH)	4/14	318	208.1	104.6	1.13	9.9	4.3	0.00%
Touchet (Endemic-Large)	4/07	300	205.4	94.0	1.04	11.0	4.8	4.33%
Touchet (Endemic-Small)	5/02	312	198.5	92.1	1.09	15.2	4.9	4.20%
Walla Walla (LFH)	4/17	307	217.5	107.2	1.01	10.0	4.2	0.00%
Lyons Ferry (LFH)	4/17	301	213.3	100.2	1.00	9.7	4.5	0.00%
Lake #1 [a] (LFH)	4/17	365	228.6	115.4	0.95	6.7	3.9	0.00%
	4/18	355	225.8	109.4	0.94	6.4	4.1	0.30%
	4/19	306	225.5	111.3	0.96	6.9	4.1	0.00%
	4/20	290	216.6	101.9	0.98	10.2	4.5	1.40%

[a] Fish removed from Lake#1 during April were released in the Tucannon and Walla Walla rivers, and on-station at Lyons Ferry.

Smolt Migration

In the past we have calculated relative smolt passage (migration success) during down river migration in the Snake River (Cottonwood, Tucannon and Lyons Ferry releases) and the Columbia River (Touchet Endemic stock releases) from PIT tags, freeze brands, and VIE tags sampled at the juvenile bypass facilities located at dams (Fish Passage Center unpublished data). We no longer use freeze brands to identify our fish, and a tally of VIE tags is no longer being kept at the dams. Therefore, the only indication of smolt migration success we have is from PIT tag detections. The standard default “action” of a PIT tag detected at a mainstem dam is for that fish to be bypassed around the facility and put back in the river for natural migration. This “action” can then be used to estimate survival between dams, and if sample sizes are large enough, can be used to estimate total mortality to the last dam in the system. However, depending on the river flow and spill, a large percentage of our annual hatchery releases are captured at bypass facilities and loaded into barges or trucks for the trip downstream. Hence, PIT tags with the default action may not represent the majority of the release group in question.

The main purpose of the Tucannon and Touchet endemic stock PIT tag groups in the last two years has been to estimate smolt-to-adult survival since these are not marked for the mark-selective fishery (see above). If the PIT tagged fish do not represent the entire release group, then our evaluations about smolt-to-adult survival for these two endemic programs will be incorrect. Fortunately, the PIT tag system has what's termed the "Separation by Code" (SbyC) action. The SbyC system allows the manager/researcher to have zero, to a multitude of actions taken on each specified PIT tagged fish when detected. For our purposes, "no action" is taken on PIT tagged fish (i.e. they are treated like they have no PIT tag, so many of the PIT tagged fish are collected at bypass facilities and are being barged downriver by the transportation program). Using the SbyC system as described above will allow us to estimate smolt-to-adult survivals on these unmarked groups of fish, but does not allow us to use the SURPH model to estimate juvenile emigration survival. Hence we present only unique detections to each facility (Table 5), which provides a relative, minimum survival to the first detection facility only.

During the spring of 2005 and 2006, we PIT tagged groups of natural steelhead at the Tucannon River smolt trap, each of the endemic stocks released, and the release of LFH stock fish into the Tucannon River (2006 only). Cumulative unique PIT tag detections were summarized (Table 5).

Table 5. Unique detections of PIT tags from natural or endemic stock steelhead tagged and released in the Tucannon and Touchet rivers, 2005 and 2006.

Release group	Number tagged	Detection Facility [a] LMO	ICH	MCN	JDA	BONN	TWX	Total	%
2005 Release Groups									
Tucannon River (Endemic Hatchery Origin)	9,968	2,178	83	173	85	11	10	2,541	25.5
Tucannon R. @ smolt trap (Natural Origin)	1,835	937	65	125	69	4	13	1,213	66.1
Touchet River (Endemic Hatchery Origin)	9,993	- - -	- - -	211	104	11	3	329	3.3
2006 Release Groups									
Tucannon River (Endemic Hatchery Origin)	8,953	2,286	304	153	285	50	7	3,085	34.5
Tucannon R. @ smolt trap (Natural Origin)	1,417	696	97	47	64	23	1	927	65.5
Tucannon River (LFH Hatchery Origin)	8,997	3,705	941	453	458	109	5	5,670	63.0
Touchet River (Endemic Hatchery Origin)	8,987			120	175	22	4	321	3.6

[a] Detection Facilities: LMO – Lower Monumental Dam, ICH – Ice Harbor Dam, MCN – McNary Dam, JDA – John Day Dam, BONN – Bonneville Dam., TWX – Traveling Array Experiment.

Tucannon River Natural Smolt Production

We operated a 1.5 m rotary screw trap at rkm 3.0 on the Tucannon River between fall of 2005 and spring 2006 to estimate the number of migrating natural steelhead smolts. Methods to estimate smolt production have been previously described (Gallinat and Ross 2007 - in review).

In the 2004/2005 trapping season, 2,134 natural steelhead smolts were captured for an estimated 16,209 (+/- 1,405) total smolt out-migration. During the season, five mark/recapture trials were conducted with a mean trapping efficiency of 13.2% (range: 2.6%-18.9%). About 89% of the migrant smolts were captured between 15 March and 15 June. Age composition based on scale readings was 20.3% Age 1, 59.0% Age 2, 20.6% Age 3, and 0.1% Age 4. During the main out-migration period (March-early June) mean length, weight, and K-factor for natural fish was 179.0 mm, 57.6 g and 1.00, respectively. The mean size of smolts captured was similar to previous years, but remains highly variable within years (Table 6.) Peak of migration for natural steelhead was 9 May, with an estimated 1,512 (9%) summer steelhead smolts migrating past the trap on that day.

In the 2005/2006 trapping season, 1,751 natural steelhead smolts were captured for an estimated 10,080 (+/- 621) total smolt out-migration. During the season, seven mark/recapture trials were conducted with a mean trapping efficiency of 17.4% (range: 5.6%-26.7%). About 82% of the migrant smolts were captured between 15 March and 15 June. Age composition based on scale readings was 16.4% Age 1, 72.3% Age 2, 11.2% Age 3, and 0.1% Age 4. During the main out-migration period (March-early June) mean length, weight, and K-factor for natural fish was 182.3 mm, 61.1 g and 0.98, respectively. The mean size of smolts captured was similar, but highly variable, as in previous years (Table 6.) Peak of migration for natural steelhead was 18 May, with an estimated 990 (9.8%) summer steelhead smolts migrating past the trap on that day.

Table 6. Estimated smolt emigration, percent composition by age class, and mean length of natural-origin steelhead smolts from the Tucannon River by brood year (1996-2005).

Brood	Age 1 # of smolts	Age 1 % of brood	Age 1 Avg ln (mm)	Age 2 # of smolts	Age 2 % of brood	Age 2 Avg ln (mm)	Age 3 # of smolts	Age 3 % of brood	Age 3 Avg ln (mm)	Estimated Total
1993	- - -	- - -	- - -	- - -	- - -	- - -	835	- - -	NA	- - -
1994	- - -	- - -	- - -	8,249	- - -	NA	908	- - -	NA	- - -
1995	5,583	36.3	NA	8,967	58.3	NA	834	5.4	190.5	15,384
1996	6,069	32.3	NA	11,584	61.7	187.0	1,133	6.0	189.2	18,786
1997	16,684	47.6	184.1	14,095	40.2	186.5	4,272	12.2	196.5	35,051
1998	9,000	25.9	173.1	24,822	71.4	189.3	960	2.8	197.3	34,782
1999	14,081	40.6	182.3	20,262	58.3	186.4	386	1.1	202.6	34,729
2000	5,332	30.8	177.6	10,998	63.5	176.2	981	5.7	186.0	17,311
2001	8,071	40.5	166.7	9,695	48.7	176.9	2,146	10.8	191.0	19,912
2002	9,243	39.7	163.1	10,723	46.0	185.9	3,324	14.3	183.7	23,290
2003	2,602	19.6	167.7	9,515	71.8	179.9	1,128	8.5	187.5	13,245
2004	3,269	- - -	174.3	7,282	- - -	183.9	- - -	- - -	- - -	- - -
2005	1,651	- - -	169.7	- - -	- - -	- - -	- - -	- - -	- - -	- - -
Average	- - -	34.8	173.3	- - -	57.8	183.5	- - -	7.4	191.6	

Note: Some length data by age not available because scales were not collected. Also, Age 4 smolts (generally <0.5 of 1%) have not been included due to their low frequency each year and to simplify the table.

Broodstock Collections / Adult Returns

As part of our annual broodstock collection and research activities, WDFW hatchery and evaluation staffs operate a series of adult steelhead traps in SE Washington. Lyons Ferry hatchery staff operates the LFH and Cottonwood Creek adult traps. The TFH staff operates the upper Tucannon adult trap, and evaluation staff operates an adult trap on the lower Tucannon River and the Touchet River trap in Dayton. Traps in the Touchet and Tucannon rivers are being used for endemic broodstock development and evaluation. Returns from endemic stocks have been low and difficult to obtain. Hence, WDFW and the co-managers agreed to extend the evaluation a few more years before a decision is reached on the fate/direction of these two endemic stock programs.

Lyons Ferry Hatchery Trap

Adult steelhead were trapped from 7 September through 15 November 2005. A total of 1,674 adult steelhead (983 female (58.7%) and 691 male (41.3%)) were trapped. Fish to be retained for broodstock were sorted on 30 November. All fish not needed for broodstock or retained to recover CWTs were returned to the Snake River to contribute to the sport fishery (1,014). Of all the fish trapped, one was wild origin (unmarked). We recovered 333 fish with CWTs (Table 7). Age composition based on CWT recoveries was 73.3% one-ocean, and 26.7% two-ocean. Mortality during trapping, holding, and spawning was 176 fish (10.5% of all fish trapped). Pre-spawning mortality rate in 2006 was within the range of those observed in previous years (1999 – 28.8%, 2000 – 10.3%, 2001 – 25.3, 2002 – 10.3%, 2003 – 10.1%, 2004 - 7.0%, 2005 - 8.0%). During January and February of 2006, 120 females were spawned with 241 males (two males were generally combined into one bag and used on a single female), producing 430,667 eyed eggs (Table 2) for the LFH stock program. Eggs from one female were destroyed due to a high titer level of IHNV in the ovarian fluid. Fecundities of one-ocean and two-ocean females were 4,304 and 5,380 eggs, respectively.

Cottonwood Creek Trap

At the Cottonwood Creek Trap, 2,006 adult steelhead (1,376 female, 630 male) were trapped from 10 March to 25 April 2006. Thirty-five natural origin fish (15 male, 20 female) were captured during the season. Age composition based on CWT recoveries and fork lengths of sampled fish was 48.1% one-ocean and 51.9% two-ocean. For the season, 120 females were spawned with 115 males producing 316,059 fertilized eggs. Two females tested positive for IHNV in 2006. Average fecundity by age class could not be determined in 2006 as all females that were taken for broodstock were partially spawned (~50%) based on size. Fish that did not contain CWTs or were not spawned were passed above the trap to spawn naturally. Any carcasses from spawning and fish that were killed outright to retrieve the CWTs were distributed in upper Cottonwood Creek for nutrient enhancement, or donated to Walla Walla Community College for science lab dissections. We recovered 232 fish that had, or should have had CWTs (Table 8); all recovered CWTs were originally released on-site at Cottonwood AP.

Table 7. Summary of tagged adult summer steelhead trapped at LFH for the 2005 run year / 2006 brood year.

Brood Year	Freeze Brand	CWT code	Stock	Release site		Number of tags
2002	LA-IC-1	63 / 15 / 23	Wallowa	Grande Ronde @Cottonwood AP		1
	LA-2-2	63 / 15 / 16	Lyons Ferry	Snake River – On Station		38
	RA-2-2	63 / 15 / 79	Lyons Ferry	Tucannon River		5
	NONE	63 / 15 / 80	Lyons Ferry	Touchet River @ Dayton AP		25
	NONE	63 / 15 / 81	Lyons Ferry	Walla Walla River		20
					Total	89
2003	LA-S-1	63 / 15 / 28	Wallowa	Grande Ronde @Cottonwood AP		2
	LA-IJ-1	63 / 21 / 70	Lyons Ferry	Snake River – On Station		59
	RA-IJ-1	63 / 21 / 87	Lyons Ferry	Tucannon River		23
	NONE	63 / 21 / 88	Lyons Ferry	Touchet River @ Dayton AP		53
	NONE	63 / 21 / 89	Lyons Ferry	Walla Walla River		107
					Total	244
				Lost tags, Unreadable tags, No Wire	Total	8
				Grand Total		**341**

Table 8. Summary of tagged adult summer steelhead trapped at Cottonwood Trap for the 2005 run year / 2006 BY.

Brood Year	Freeze Brand	CWT code	Stock	Release site	CWT	Number of tags
2002	LA-IC-1	63 / 15 / 23	Wallowa	Cottonwood AP	Recovered	61
2003	LA-S-1	63 / 15 / 28	Wallowa	Cottonwood AP	Recovered	160
					Lost	2
					No Tag	9
				Grand Total		**232**

Tucannon FH Trap

A permanent adult steelhead and salmon trap was installed in 1998 at the TFH water intake diversion dam. Natural and Tucannon River endemic stock origin steelhead are enumerated, sampled and passed upstream to spawn, while LFH stock fish are returned to below the trap. In 2006 hatchery staff trapped 20 natural, 18 Tucannon River endemic stock, and one LFH stock hatchery-origin steelhead.

Lower Tucannon Adult Trap

Evaluation staff deployed and operated a temporary trap at rkm 17.7 in the lower Tucannon River during the fall to early spring of 2005/2006, with the primary focus to collect natural-origin fish for a new hatchery broodstock (Bumgarner et al. 2002). A secondary objective of the trap is to enumerate and collect biological samples from natural-origin steelhead in the Tucannon River. The trap was operated between 12 September and 5 April. Nearly continuous operation was accomplished due to a floating weir that dramatically reduced debris loads and scouring of gravel around the trap. The trap was generally opened up, and a section of the weir panels were pushed down on the weekends to allow unrestricted passage of salmonids (upstream or downstream). In all, 90 natural fish (48 males and 42 females), 23 Tucannon River endemic stock, and 48 LFH hatchery fish were trapped. We collected and hauled 35 natural fish (15 females and 20 males) to LFH for broodstock. Natural origin fish not collected for broodstock were passed upstream after length and sex were determined and scales samples were collected.

During 2005/2006, pre-spawning loss was three females and one male. Pre-spawning loss in recent years has been low because of aggressive fungus control treatments once fish are captured and held. During February and March 2006, 13 adult females were spawned with 17 males at LFH. Only 16 of the 20 males collected were spawned (three fish died in the pond, one fish never matured), and one additional male was live spawned at the trap and released. One female was not spawned and was returned to the river to spawn. Total eggtake was estimated at 72,520 (Table 2). Natural fish trapped from the lower Tucannon Trap or the Tucannon Hatchery Trap consisted of 51.4% one-ocean and 48.6% two-ocean age fish (Table 9). In addition to the summer steelhead captured in the lower trap, we captured or found on the floating weir panels three fall Chinook, 11 Coho salmon (*O. kisutch*), three bull trout, and 27 suckers (*Catostomus columbianus or C. macrocheilus*).

Table 9. Summary of fresh and salt-water age composition of natural origin adult steelhead from the Tucannon River, 2000-2006 brood years.

Year	Age [a] 1.1		Age 1.2		Age 2.1		Age 2.2		Age 3.1		Age 3.2		Repeat spawners
	N	%	N	%	N	%	N	%	N	%	N	%	
2000	18	25.0	6	8.3	36	50.0	7	9.7	5	6.9	0	0.0	NONE
2001	0	0	13	27.1	13	27.1	19	39.6	0	0.0	3	6.3	NONE
2002	5	8.8	10	17.5	29	50.9	10	17.5	3	5.3	0	0.0	NONE
2003	0	0	4	3.9	29	28.2	56	54.4	5	4.9	6	5.8	YES [b]
2004	0	0	0	0.0	42	68.9	13	21.3	5	4.9	0	0.0	YES [c]
2005	15	4.8	32	10.3	99	31.9	141	45.5	14	4.5	7	2.3	YES [d]
2006	5	4.6	7	6.5	44	40.7	44	40.7	6	5.6	1	0.9	YES [e]
Combined	43	5.7	72	9.5	292	38.5	290	38.2	38	5.0	17	2.2	0.9%

a Age reporting protocol is F.S, where F=freshwater years and S=saltwater years of age.
b Three fish sampled in 2003 were repeat spawners, one fish was 1.1S, two were 2.1S for 3.6% of the run.
c One fish sampled in 2004 was a repeat spawner (2.1S1).
d Two fish sampled in 2005 were repeat spawners, one fish was 1.1S, the other was 2.1S for 0.6% of the run.
e One fish sampled in 2006 was a repeat spawner (1.1S) for 0.9% of the run.

Touchet River Adult Trap

Evaluation staff operated the adult trap in the Touchet River from 25 January to 7 July 2006. We trapped 164 (77.0%) Touchet River natural, 14 (6.6%) LFH hatchery origin, and 35 (16.4%) Touchet River endemic hatchery origin steelhead during the 2005 run year. In addition, we trapped two (11.7%) natural and 15 (88.3%) LFH hatchery origin from the 2006 run year. Natural fish trapped in 2005 consisted of 64.4% one-ocean and 35.6% two-ocean age (Table 10). Sex ratio of natural and hatchery steelhead was skewed toward females (63.7%). We collected 39 natural origin fish (19 females and 20 males) for broodstock. Pre-spawning mortality was low in 2006 with one fish dying (2.6%). For the season, 18 females were spawned with 18 males yielding 88,668 eggs. However, for the second year in a row, three of the females spawned tested positive for IHNV. WDFW again consulted with NOAA Fisheries and the Umatilla Tribe to determine the fate of these fish. After consultation, it was decided that to reduce the risk of contamination to the Touchet Endemic stock and other steelhead at LFH, the progeny from these fish would be planted into the Touchet River as fry (14,276). After the fry plants, the program was left with an estimated 71,453 eyed eggs.

We also captured 60 bull trout, 54 bridgelip suckers (*C. columbianus*), 31 brown trout (*Salmo trutta*), and 33 whitefish in the Touchet adult trap during the season. Data collected from bull trout, brown trout and whitefish are provided in Appendix A.

We operated a Logie 2100C Resistivity Fish Counter irregularly at the Touchet River trap in 2006. For the season we captured video of 75 steelhead crossing the counter ramp (most were wild or endemic stock origin based on fin clips that could clearly be seen on the video). However, of those fish observed, only 23 actually passed the counter ramp and proceeded upstream. For unknown reasons, when the fish reached the top of the counter ramp, they appeared to hesitate, and were then swept back down the counter. This fall back behavior was recorded over three separate weekends when the counter was being operated in late March and early April. We attempted to correct the problem, but high stream flows following that made it impossible for staff to safely make changes until later in the season when most of the steelhead run was past. As such, the counter was not operated the rest of the season. We will attempt to correct the problem for spring 2007.

Table 10. Summary of fresh and salt-water age composition of natural origin adults from the Touchet River, 1994-1995 and 1999-2006 brood years.

BY	Age 1.1		Age 1.2		Age 2.1		Age 2.2		Age 3.1		Age 3.2		Age 4.1		Age 4.2		Repeat spawners
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	
1994	0	0.0	0	0.0	6	28.6	8	38.1	3	14.3	3	14.3	0	0.0	0	0.0	Yes [a]
1995	0	0.0	0	0.0	0	0.0	6	85.7	0	0.0	0	0.0	0	0.0	1	14.3	None
1999	0	0.0	1	3.2	18	58.1	9	29.0	2	6.5	0	0.0	0	0.0	0	0.0	Yes [b]
2000	1	3.2	1	3.2	17	54.8	8	25.8	3	9.7	1	3.2	0	0.0	0	0.0	None
2001	1	0.6	14	8.0	84	48.3	40	23.0	15	8.6	9	5.2	1	0.6	0	0.0	Yes [c]
2002	6	4.8	3	2.4	84	67.7	20	16.1	6	4.8	3	2.4	0	0.0	0	0.0	Yes [d]
2003	0	0.0	8	6.7	20	16.7	73	60.8	2	1.7	10	8.3	0	0.0	0	0.0	Yes [e]
2004	0	0.0	1	0.8	47	39.2	18	15.0	18	15.0	2	1.7	1	0.8	0	0.0	Yes [f]
2005	0	0.0	0	0.0	37	44.0	21	25.0	15	17.9	8	9.5	0	0.0	0	0.0	Yes [g]
2006	2	1.3	7	4.5	85	54.8	38	24.5	7	4.5	11	7.1	0	0.0	0	0.0	Yes [h]
Totals	10	1.2	35	4.1	398	47.2	241	28.6	71	8.4	47	5.6	2	0.2	1	0.1	4.9%

[a] One fish sampled in 1994 was a repeat spawner, 2.1S for 4.8% of the run.
[b] One fish sampled in 1999 was a repeat spawner, 2.1S for 3.2% of the run.
[c] Ten fish sampled in 2001 were repeat spawners, eight fish were 2.1S, and two were 2.1S1 for a total of 5.7% of the run.
[d] Two fish sampled in 2002 were repeat spawners, one fish was 2.1S, and one was 2.1S for a total of 1.6% of the run.
[e] Six fish sampled in 2003 were repeat spawners, one fish was 1.1S, four were 2.1S, and one was 3.1S for a total of 5.8% of the run.
[f] Ten fish sampled in 2004 were repeat spawners, four were 2.1S, one was 3.1S, five were 2.1S1, and one was 2.1SS for a total of 8.1%.
[g] Three fish sampled in 2005 were repeat spawners, one was 2.1S, one was 2.2S, and one was 2.1S1S for a total of 3.6% of the total run.
[h] Five fish sampled in 2006 were repeat spawners, one was 2.1S, and four were 2.1S1 for a total of 3.2% of the total run.

Creel Surveys

WDFW personnel surveyed steelhead sport anglers within the LSRCP area of Washington (see Schuck et al. 1990 for methods) to recover CWTs from tagged steelhead. We then estimate the number of LFC steelhead in the Washington sport catch in SE Washington using WDFW sport harvest estimates from Washington catch record cards. Also, data from each week's surveys are summarized in-season and provided to the local news media to assist anglers. During the 2005/2006 steelhead sport fishery season we surveyed 10,181 anglers that caught 3,226 steelhead within the LSRCP area of Washington (Table 11). A total of 1,165 natural origin fish (36.2% of the total catch documented from creel surveys) were caught and released during the 2005/2006 season. We suspect that some of the natural-origin fish may have been caught and released more than once during the 2005-2006 season. This may be especially true in the tributary fisheries (Walla Walla, Tucannon, and Touchet rivers). All CWTs collected during the fishery were extracted and sent to Olympia for eventual inclusion in the Pacific States Marine Fisheries Commission / CWT database (Regional Mark Information System – RMIS) maintained in Portland, OR. Individual tag recoveries and fishery expansions for each CWT recovered from the steelhead fishery have been calculated and provide the basis for Figure 2 (LSRCP Contribution). Further presentation of CWT fisheries and trap recoveries and their locations will be presented in the next summer steelhead annual report.

The CWTs recovered from creel surveys, and those recovered from hatchery traps make up a substantial portion of the total CWT recoveries from each brood year, and provide the basis to calculate smolt-to-adult survival rates and to estimate total adult contribution of summer steelhead back to the Lower Snake River project area. Computer spreadsheets with the data and appropriate expansions are maintained at the Snake River Lab office. Full presentation of all CWT recoveries (downriver and within project area) from all previous years tag groups will be presented in the 2006 run year report.

Table 11. Steelhead angler interview results for fall/winter/spring of the 2005 run year from Washington State licensed anglers.

River Basin River section description [a]	River section number	Anglers Surveyed	Total hours fished	Natural fish released [b]	Hatchery fish kept	Hatchery fish released	Catch rate (hr/fish)
Columbia River Basin							
McNary Dam to Pasco	533	1,690	5,072.3	107	240	5	14.4
Walla Walla Subbasin							
Walla Walla River	659	443	1,058.3	63	90	6	6.7
Touchet River	657	220	475.0	94	47	10	3.2
Snake River Basin							
Mouth to IHR	640	32	75.8	1	2	0	25.3
IHR to LMD	642	3,631	12,160.0	181	416	10	20.0
LMD to LGD	644	1,902	9,058.0	280	532	21	14.4
LGD to LGR	646	999	3,689.0	57	92	2	24.4
LGR to Hwy 12 Br.	648	272	1,351.0	33	62	3	13.8
Hwy 12 Br. upstream	650	704	4,135.0	247	395	38	6.1
Tucannon River	653	284	963.8	102	74	16	5.0
Totals		10,181	38,073.4	1,165	1,950	111	11.8

[a] Abbreviations as follows: IHR=Ice Harbor Dam, LMD=Lower Monumental Dam, LGD=Little Goose Dam, LGR=Lower Granite Dam, Hwy=Interstate Highway. Creel information from sections 648 and 650 include data collected by IDFG.

[b] The number of natural fish presented does not reflect individual fish.. We suspect that some of these fish have been hooked and released several times during the season.

In addition, we cooperate with ODFW in conducting a joint survey of anglers on the lower Grande Ronde River of Washington and Oregon. Angler effort, catch rates, and harvest were estimated by ODFW staff as described in Carmichael et al. (1988). The total number of fish sampled during the fishery and estimated harvest by the joint surveys (2004 and 2005 run years) from the Grande Ronde fishery in the Washington portion are supplied by ODFW for these annual reports (Tables 12 and 13).

Table 12. Estimated angler effort, catch rates, and harvest for steelhead anglers on a portion of the Grande Ronde River in Washington, run year 2004 (Mike Flesher, ODFW).

	2004				2005				Total
	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	
Effort Hours	1,078.9	4,491.8	2,861.1	2,042.7	3,198.5	5,616.3	6,866.4	1,109.0	27,264.7
Catch Rate a	0.0486	0.1414	0.1215	0.1147	0.1731	0.1583	0.2800	0.1140	0.1440
Total Catch b	52	635	348	234	554	889	1,922	126	4,760
Fish Kept	0	108	170	125	339	465	810	51	2,068
Hatchery Released	40	339	66	60	79	191	662	63	1,500
Natural Released	12	188	112	49	136	233	451	12	1,193

[a] Catch rate here is defined as the estimated fish captured divided by the hours fished.
[b] Estimated fish captured have been rounded to whole numbers, so total of fish kept and released may not always add up to total catch.

Table 13. Estimated angler effort, catch rates, and harvest for steelhead anglers on a portion of the Grande Ronde River in Washington, run year 2005 (Mike Flesher, ODFW).

	2005				2006				Total
	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	
Effort Hours	515.2	5,237.9	3,555.8	865.9	3,287.3	6,104.5	6,062.0	659.8	26,288.4
Catch Rate a	0.0122	0.0734	0.1485	0.2347	0.2091	0.2971	0.4042	0.2909	0.2088
Total Catch b	6	384	528	203	687	1,814	2,450	192	6,264
Fish Kept	0	98	236	64	408	970	1,011	84	2,871
Hatchery Released	0	116	98	85	156	547	1,214	94	2,310
Natural Released	6	170	194	55	123	296	225	14	1,083

[a] Catch rate here is defined as the estimated fish captured divided by the hours fished.
[b] Estimated fish captured have been rounded to whole numbers, so total of fish kept and released may not always add up to total catch.

Spawning Ground Surveys

During spring 2006, evaluation staff attempted to survey spawning grounds in select reaches (Appendix B) of the Tucannon and Touchet rivers and Asotin Creek for steelhead redds. From these surveys we estimated the total number of redds in each (Table 14). High, turbid stream flows hampered spawning surveys during 2006, resulting in complete redd counts for some index sections and preventing any estimate for other river sections.

Contributions to LSRCP Mitigation Goals

The LFC summer steelhead program (LFH and Wallowa stocks) continues to meet and/or exceed their original hatchery mitigation goals (USACE 1975 - 4,656 total steelhead/year by WDFW) to the Snake River project area by supplying hatchery fish for sport harvest. Based on creel surveys and adult traps, we estimated that a minimum of 5,099 (3,156 goal/run year) LFH stock and 2,339 (1,500 goal/run year) Wallowa stock fish returned from the 2002 brood year. That represents 162% and 156% of the Washington mitigation goal for each of these stocks, respectively (Figure 2). While the goals of the LSRCP were set by run year returns, we have presented the following graph by brood year for ease in calculations. Fish escaping to the spawning grounds have not been accounted for in these calculations. Since program inception, LFH stock releases have averaged roughly 283% of the mitigation goal (back to the project area), while the Wallowa stock releases have averaged 279% (back to the project area - Figure 2). Program reductions of ~40% for both the LFH and Wallowa stocks in 2001 have brought these two programs more in line with mitigation goals back to the project area. Further, these actions have protected ESA listed fish by decreasing excess hatchery fish of inappropriate stocks on the spawning grounds. However, original goals of the LSRCP (USACE 1975) also assumed that about ¾ of the annual return would be captured in downriver fisheries. To date, the downriver harvest has not approached that rate of harvest, mainly due to curtailment of fisheries because of ESA listings. Further, although hatchery return goals to the project area have been met, natural stocks within the basin (which both hatchery stocks were assumed to be supplementing for under the original program design) have continued to remain at a depressed level, or have declined further.

Table 14. Results of summer steelhead index redd surveys in 2006.

Stream Section surveyed	Est. Rkm	Dates Surveyed	Redds counted	Total redds	Expanded # of redds	% of total index reach surveyed	Total est. redds for reach
Tucannon River Basin (Index)	73.6	- - - - - -	76	NA	NA	39%	NA
Index 3- Hatchery Intake to Br 14	8.0	4/28	7	7	NA	100%	
Index 4 – Bridge 12 to Silt Basin	10.2	3/24, 4/27	6, 22	28	NA	100%	
Cummings Creek (Old Mine to Mouth)	10.6	5/9	41	41	41	100%	41
Touchet River Basin (Index)	69.0	- - - - - -	106	NA	NA	43.6	NA
North Fork Touchet Reach – MP 13 to Mouth	19.2	- - - - - -	44	44	47	53.6	88
Index 2 - LE of Frames to Wolf Fork Bridge	4.0	3/30, 4/25, 5/15	6, 9, 14	29	29	100%	
Final Walk 1 - Bridge at MP 13 to Dedloff's House	6.3	5/15	15	15	18	100%	
South Fork Touchet Reach – Griffen Fork to Mouth	24.5	- - - - - -	16	16	NA	21.6	NA
Index 1 – 1.6 rd miles above Bridge 2	2.4	3/30, 5/3	4, 3	7	NA	100%	
Index 2 - Camp Nancy Lee down 1.8 miles	2.9	3/30, 5/3	3, 6	9	NA	100%	
Wolf Fork Touchet Reach – Newby Cabin to Mouth	16.5	- - - - - -	19	19	22	49.1	44
Index 2 – 0.3 miles below Nelson's to Robinson Fork bridge	2.0	4/10,4/24,5/3,5/12	0,4,1,1	6	6	100%	
Final Walk 1 – Newby Cabin to Steinhoff House	6.1	5/12	13	13	16	100%	
Robinson Fork Touchet Reach – 5.0 miles to Mouth	8.8	- - - - - -	27	27	27	72.7	37
Index 1 – 4.0 mi. above BLC Gate, back to gate.	6.4	4/24, 5/3	27	27	27	100%	
Asotin Creek Basin (Index)	55.0		150	NA	NA	75.6	NA
Asotin Creek Reach – NF/SF to George Cr Mouth	20.5		62	62	NA	77.0	NA
Index 1 - NF/SF confluence ↓ 2.4 road miles	4.0	3/22, 3/30, 4/21	4, 6, 5	15	NA	100%	
Index 2 - 2 miles above Headgate Park to Headgate Park	8.0	3/08, 3/22, 3/30	8, 15, 10	33	NA	100%	
Index 3 – bridge above Hendrickson's to George Creek	3.8	3/22	14	14	NA	100%	
Charley Creek Reach – Old Corral to Mouth	10.3		31	31	33	94.2	35
Index 1 – 4.0 miles above Koch Gate down 3.0 miles	4.9	3/30, 4/13, 4/21, 5/10	7, 2, 7, 5	21	21	100%	
Final Walk 1 - Old Corral to top of index	3.2	5/10	4	4	5	100%	
Final Walk 2 – Bottom of index down to Koch Gate	1.6	5/10	6	6	7	100%	
South Fork Asotin Creek Reach – Old Ruins to Mouth	11.4		5	5	NA	28.9	NA
Index 1 – 2 rd miles above mouth, down to mouth	3.3	3/23, 4/14	4, 1	5	NA	100%	
North Fork Asotin Creek Reach – 2nd FS Fence to Mouth	12.8		52	52	53	80.0	66
Index 1 - End of old rd down to Lick Creek	6.8	4/13, 4/21, 5/05	11, 10, 10	31	31	100%	
Index 2 - Lick Creek to confluence	1.7	3/22,3/30,4/13,4/21,5/05	5, 3, 5, 2, 0	15	15	100%	
Final Walk 1 - Second FS Fence to top of index	4.3	5/05	6	6	7	100%	

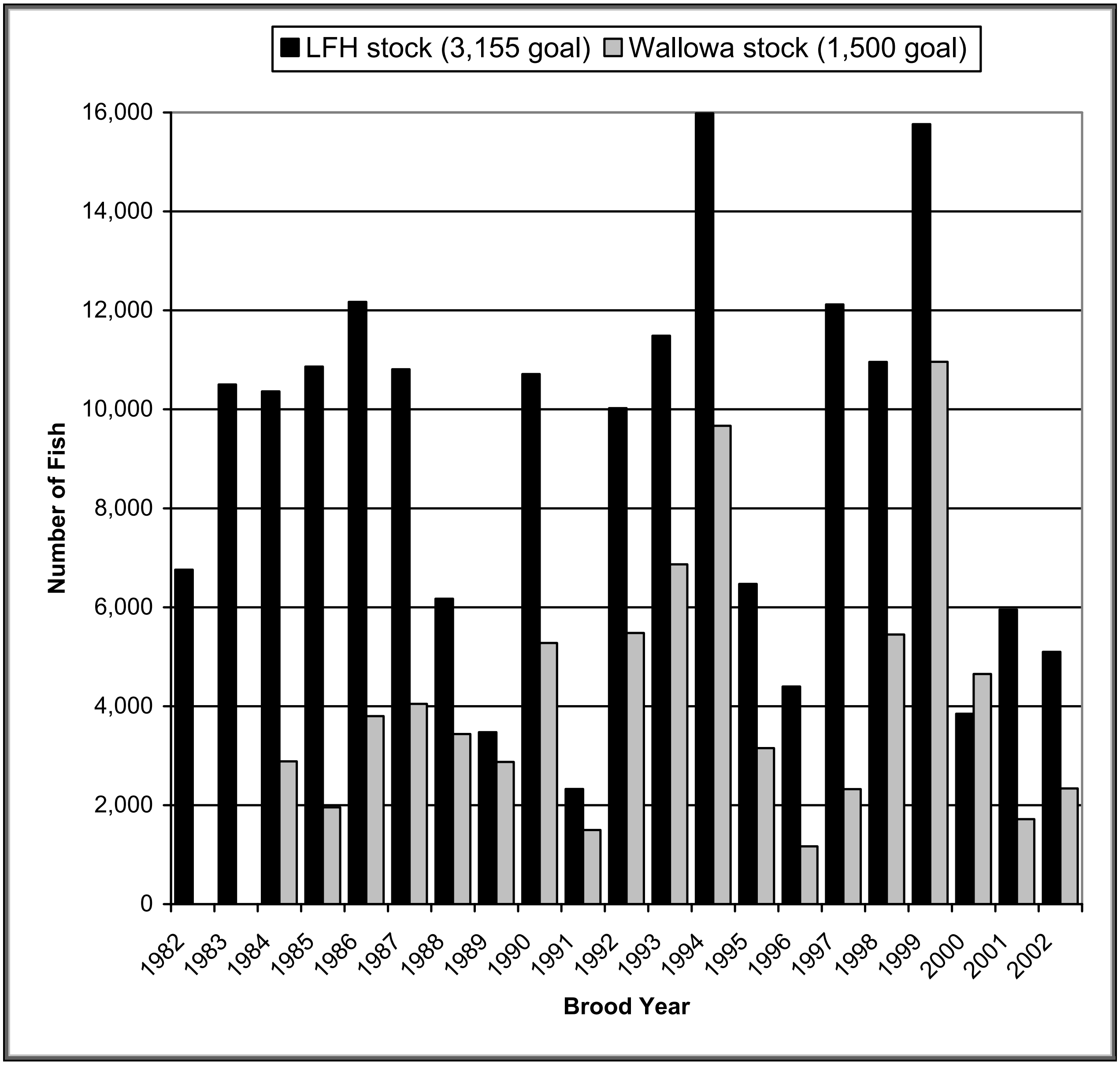

Figure 2. Contributions by brood year of LFH and Wallowa stock summer steelhead to the LSRCP mitigation area (The Snake River above Ice Harbor and its tributaries, and the Walla Walla and Touchet rivers in the Walla Walla River basin).

Natural Juvenile Production in Area Rivers

As in previous years, WDFW electrofished using either a multiple pass removal method (Zippin 1958) or a single pass method at index sites to estimate Age 0 and Age 1+ juvenile steelhead densities and derive population estimates for specific river reaches (Tables 15 and 16). Summer steelhead Age 0 and Age 1+ mean densities by river reach, densities per site, site descriptions, and data for other sensitive species captured during electrofishing surveys are provided in Appendix B.

The potential for residual hatchery steelhead to negatively affect natural salmonid populations through competition, displacement, or predation was identified as a concern by NOAA Fisheries after Chinook salmon were listed as threatened under the ESA. In the early 1990's, WDFW began a series of experiments to examine methods to reduce residualism. Results from the Tucannon, Touchet, and Grande Ronde rivers have been provided in the past (Viola and Schuck 1995, Schuck et al. 1998, Martin et al. 2000).

Table 15. Summary of mean fish density (Fish/100 m^2) and population estimates of Age 0 summer steelhead in index areas of Asotin Creek, and Touchet and Tucannon rivers for specific tributaries/reaches in 2005.

Basin	Reach/Strata	Sites	Mean Density	Population Estimate	95% C.I.
Asotin Creek	Mainstem	6	41.2	70,345	+/- 8,051
	North Fork [a]	4	31.1	18,718	+/- 4,987
	South Fork	8	15.3	7,050	+/- 7,044
	Charley Cr.	6	12.0	4,062	+/- 1,970
	Total			*105,065*	
Touchet River	Mainstem	4	24.9	42,369	+/- 33,684
	North Fork	5	33.3	46,871	+/- 15,209
	Wolf Fork	8	24.9	27,548	+/- 9,108
	South Fork	5	15.0	18,430	+/- 9,154
	Robinson Fork	5	18.4	3,972	+/- 1,562
	Total			*135,760*	
Tucannon River	Lower	3	9.4	20,084	+/- 13,653
	Marengo	6	4.5	8,956	+/- 4,276
	Hartsock	3	5.7	10,989	+/- 2,201
	HMA	6	5.5	11,547	+/- 11,353
	Wilderness	0	NA	NA	NA
	Cummings Cr.	4	19.7	4,747	+/- 3,116
	Total			*51,576*	

[a] Does not include about ½ of the typical survey area. We were unable to sample the upper reaches due to fire danger.

Table 16. Summary of mean fish density (Fish/100 m^2) and population estimates of Age 1+ summer steelhead in index areas of Asotin Creek, and Touchet and Tucannon rivers for specific tributaries/reaches in 2005.

Basin	Reach/Strata	Sites	Mean Density	Population Estimate	95% C.I.
Asotin Creek	Mainstem	6	23.3	39,708	+/- 17,129
	North Fork [a]	4	17.5	10,542	+/- 3,311
	South Fork	8	13.9	6,523	+/- 843
	Charley Cr.	6	33.4	11,329	+/- 2,559
	Total			*70,448*	
Touchet River	Mainstem	4	5.8	9,831	+/- 10,543
	North Fork	5	16.4	23,093	+/- 7,550
	Wolf Fork	8	13.5	14,935	+/- 4,972
	South Fork	5	17.3	21,267	+/- 13,833
	Robinson Fork	5	11.6	2,505	+/- 2,375
	Total			*71,556*	
Tucannon River	Lower	3	0.05	98	+/- 195
	Marengo	6	1.5	2,889	+/- 3,117
	Hartsock	3	2.4	4,609	+/- 4,830
	HMA	6	5.3	10,994	+/- 4,987
	Wilderness	0	NA	NA	NA
	Cummings Cr.	4	11.9	2,864	+/- 2,476
	Total			*18,590*	

[a] Does not include about ½ of the typical survey area. We were unable to sample the upper reaches due to fire danger.

During 2005, we estimated residual hatchery steelhead (LFH stock and Endemic stocks) present in the Tucannon and Touchet rivers in July and August through the use of electrofishing surveys (Tables 17 and 18). Estimated residualism is therefore a minimum as natural mortality and harvest from trout fisherman would have occurred between the time of release (April) and before electrofishing surveys were complete. In addition, we believe our residual estimates are biased. Bias in our electrofishing occurs because we consistently underestimate larger sized fish within a site, as they are not as easily captured, yet our methodology assumes a constant catchability for all sizes of fish during the survey. Bias can also occur if fish are able to enter or escape the site while the surveys are taking place. We have assumed that our block nets were "fish tight" during the relatively short time for the electrofishing surveys (2-3 hours), however in tests we conducted during 2005 (and again in 2006 which will be presented in the next annual report), this was not the case. Our results were similar to those of Peterson et al (2004) and Temple and Pearsons (2006). A minimum estimate of residualism for the Tucannon River in 2005 was 1.9% of the endemic stock release (65,245) and 1.3% of the LFH stock release (100,345). Estimated residualism for the Touchet River in 2005 was 6.0% of the endemic stock release (52,476) and 0.7% of the LFH stock release (86,258).

Table 17. Summary of mean fish density (Fish/100 m^2) and population estimates of hatchery endemic stock summer steelhead residuals in index areas of the Touchet and Tucannon rivers for specific tributaries or reaches in 2005.

Basin	Reach/Strata	Sites	Mean Density	Population Estimate	95% C.I.
Touchet					
	Mainstem	4	0.77	1,229	+/- 2,440
	North Fork	5	0.90	1,271	+/- 1,837
	Wolf Fork	8	0.30	255	+/- 271
	South Fork	5	0.33	406	+/- 634
	Robinson Fork	5	0.00	0	+/- 0
	Total			*3,150*	
Tucannon	Lower	3	0.00	0	+/- 0
	Marengo	6	0.00	0	+/- 0
	Hartsock	3	0.07	134	+/- 267
	HMA	6	0.96	1,091	+/- 676
	Wilderness	0	NA	NA	+/- NA
	Total			*1,225*	

Table 18. Summary of mean fish density (Fish/100 m2) and population estimates of LFH hatchery stock summer steelhead residuals in index areas of the Touchet and Tucannon rivers for specific tributaries or reaches in 2005.

Basin	Reach/Strata	Sites	Mean Density	Population Estimate	95% C.I.
Touchet					
	Mainstem	4	0.33	530	+/- 622
	North Fork	5	0.00	0	+/- 0
	Wolf Fork	8	0.00	0	+/- 0
	South Fork	5	0.05	60	+/- 120
	Robinson Fork	5	0.00	0	+/- 0
				590	
Tucannon	Lower	3	0.55	1,177	+/- 2,341
	Marengo	6	0.05	92	+/- 166
	Hartsock	3	0.00	0	+/- 0
	HMA	6	0.00	0	+/- 0
	Wilderness	0	NA	NA	+/- NA
				1,269	

Mark/Recapture Studies to Determine Bias in Electrofishing Survey Estimates

Accurate, precise juvenile population abundance estimates are crucial for describing survival trends of populations over time, and to measure response to management actions such as hatchery supplementation and habitat manipulation/restoration. A recent study by Peterson et al. (2004) identified bias, and resulting error, associated with traditional multiple pass removal methodologies for backback electrofishing. Correctly, the study called for researchers to carefully evaluate bias and error associated with their study data by conducting separate population estimates with methods having demonstrated accuracy and precision. In this case (Peterson et al. 2004), suggested that mark/recapture methods were likely less biased, and further strongly suggested that researchers test all the assumptions of population estimators being used. Important assumptions of both multiple removal and mark/recapture estimators are: 1) the population size does not fluctuate from immigration or emigration during the time of sampling [for mark/recapture this is less critical as long as marked and unmarked fish are fluctuating at the same rate]; 2) marked and/or unmarked fish are equally catchable during recapture or standard sampling; and 3) marked fish do not lose their marks and are identified and reported correctly.

While the evidence for estimator bias and error seem consistent in the literature, our methods differ somewhat from those described in the literature, and thus needed to be tested to estimate the level of error, and confirm compliance of our methods with underlying assumptions. Moreover, we possess significant long-term data sets for juvenile populations in southeast Washington streams. If bias in our methods is consistent over the term of the data, it could be adjusted as appropriate once bias was measured, thus improving the accuracy of the data. These corrections could be important in understanding ecological and population response relationships that might be masked by error resulting from methodology bias.

Most authors have recommended a minimum 24-h recovery period between mark and recapture electrofishing passes. The recovery period has been cited as necessary for fish to resume normal behavior (Schreck 1976, Mesa and Schreck 1989, Peterson et al. 2004) after being exposed to electrofishing. However, it was unclear whether shorter recovery periods could be sufficient fish recovery, and thus still produce an unbiased population estimate. This is a critical point in our evaluations, as it is not possible in most of the places we conduct electrofishing surveys to maintain site blocking nets in place for 24 hours. Debris loads accumulate even with low summer stream flows that would require frequent net cleaning. Temple and Pearsons (2006) investigated the use of shorter recovery periods and commented:

> "The use of long recovery periods may help satisfy the catchability assumption but also provides an opportunity for failure of the movement assumption, particularly in stream that contain heterogeneous habitats (e.g., large, deep, complex), fast flows, or substantial debris. Short recovery periods may help satisfy the movement assumption, but they may create an opportunity for failure of the catchability assumption if marked fish do not mix randomly with the unmarked population or the marked and unmarked fish do not exhibit equal catchability."

During the summer of 2005, we tested estimator bias (multiple pass estimate vs mark/recapture estimate) at 44 sites following methods similar to those used by Temple and Pearsons (2006). Due to crew size and locations, we determined that a 3-4 hour wait between the initial marking period and the recapture event would suffice. However, a comparison between 3 hour and 24 hour wait periods needed to be conducted, and we needed to determine if we contained all fish in the site between sampling periods. Thus when the surveys were started in 2005, we were unsure of compliance with two of our assumptions.

It wasn't long before we realized that both of our pre-season assumptions were in error. It became obvious that the number of fish caught during the recapture event was generally much less than what had been caught during pass one of the multiple pass removal method (i.e. catchability was different), and we had evidence that fish were moving out of the site. Even so, we continued with the original plan and gathered all the data to test the differences between the two estimator types. After the season was complete, the data were analyzed, and population estimates were derived from the methodologies and compared.

For the 2005 surveys, we found that the multiple pass estimates of Age 0 and Age 1+ summer steelhead were 26% and 22% lower, respectively, than the mark/recapture estimates. There was variability around the estimates, but the two appear to be highly correlated (Figure 3a, 3b), and the possibility of determining a correction factor to previous years data looked very promising. These correlations will be explored further in future analysis. Because we documented some fish movement out of the site (i.e. marked fish only), and it appeared we had unequal catch during the recapture events, we believed the mark/recapture estimates are potentially biased high, and the true population estimate likely falls between the two estimators. For the 2006 field season, we will address issues of catchability and make a better attempt to determine movement in/out of the site to better describe this potential bias in the mark/recapture estimate.

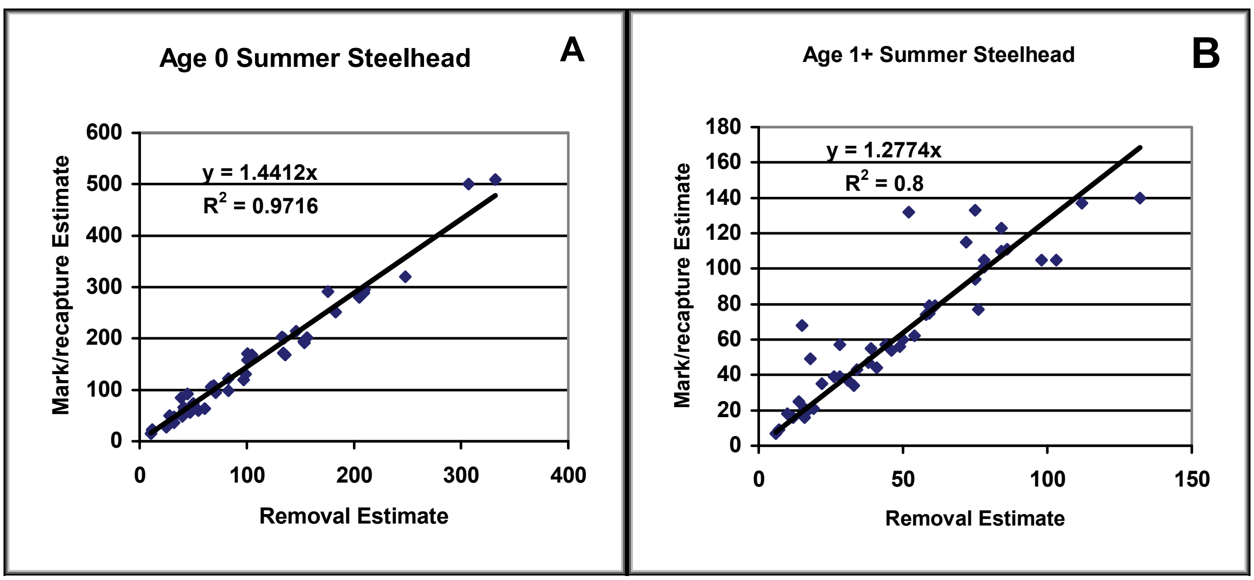

Figure 3. Relationship between the estimated number of Age 0 or Age 1+ summer steelhead determined by a multipass removal method vs. a mark-recapture method during 2005 from index sites on the Tucannnon and Touchet rivers, and Asotin Creek.

Genetic Analysis

Since 1998, the Snake River Lab and WDFW's Fish Management staff have periodically collected samples from SE Washington summer steelhead populations (adult and juvenile) for genetic stock analysis. Samples have been collected from the Walla Walla, Touchet and Tucannon River basins, the LFH stock, and portions of the Grande Ronde. During the fall of 2006, WDFW genetics staff, in cooperation with the Snake River Lab and WDFW Fish Management for SE Washington assembled a summer steelhead genetics summary that includes most samples collected through 2005. This report has been provided as an attachment (Appendix D).

WDFW Snake River Lab staff believes we have answered the key critical questions regarding genetic introgression (or lack thereof) from Lyons Ferry stock fish into the Tucannon and Touchet rivers. These genetic results, in combination with results from the endemic broodstock developments, will be used to determine the fate of the endemic stock programs, and future planning efforts. At this time, no more genetic tissue samples (fin clips or opercle punches) are being collected from natural or hatchery origin steelhead associated with the Lower Snake River Compensation Program in Washington. However, scale samples from natural origin summer steelhead continue to be collected annually at both the Tucannon and Touchet river traps, and could be used for future genetic analysis if deemed necessary.

Conclusions and Recommendations

In an effort to maintain successful mitigation in an ESA environment, we offer the following conclusions/recommendations from our monitoring and evaluation work, and suggest additional critical questions that should be pursued in the future:

1) The NOAA Fisheries ruled that LSRCP hatchery steelhead jeopardized listed steelhead populations within the Snake and Columbia river basins (NMFS 1999), and called for the development of new endemic broodstocks for the hatchery steelhead program. Initial efforts in the Tucannon and Touchet rivers appear to be somewhat successful, but more data about the demonstration of acceptable adult returns are needed before concluding that the program is successful and should be expanded.

The numbers of fish used to develop these endemic broodstocks are very low, raising genetic concerns (potential lack of genetic diversity within the broodstock (low N_e), and escapement of potentially large numbers of these hatchery fish onto the spawning grounds) for the future. At present, none of the adult fish that return will be used as broodstock in the hatchery because of their low founding population size. If the program should expand, it will require collecting more unmarked (natural) fish from each river, potentially causing further genetic and demographic damage to these listed populations. These programs should proceed with caution.

Adult traps are utilized to collect hatchery and new endemic steelhead stocks, or to assess stock/population potential in other areas. In addition, they provide an opportunity to collect tagged (ADLV+CWT) hatchery steelhead from the LRSCP program that allows us to determine program contribution and assess stray rates from other programs throughout the region.

Recommendations: Continue evaluation of endemic broodstocks in the Tucannon and Touchet rivers. Continue PIT tagging representative groups of endemic stock smolts for program evaluation. Investigate other broodstock collection methods or change mating protocols (i.e. factoral mating) in a way that would increase the effective population size of these stocks in the hatchery. Evaluate the effect of partially spawning females on completing their spawning in the wild (Wallowa Stock in Cottonwood Creek). Determine if a similar strategy would be appropriate to increase effective population size of endemic stock programs. At all trapping locations, sacrifice tagged adult hatchery production steelhead (AD clipped or ADLV clipped) to determine release points and assess straying.

2) Juvenile population abundance estimates were conducted as part of our evaluations for the last 20 years, describing survival trends of populations over time, and attempting to measure response to management actions such as hatchery supplementation and habitat manipulation/restoration. Recent studies (Hillman et al. 1992, Peterson et al. 2004) and our work conducted in 2005 on the Touchet and Tucannon rivers, and Asotin Creek, identified bias and resulting error associated with our traditional sampling methodologies. The evidence gathered in 2005 for estimator bias and error were consistent with what was documented in the literature. It appears that we may be able to correct the bias, but further investigations are needed.

Recommendation: For each survey method that we use to estimate populations, critically evaluate the assumptions that need to be followed to obtain unbiased estimates. If assumptions appear to be violated, examine/implement additional surveys to test or validate assumptions, and use resulting data for comparison or correction of past surveys results.

3) Creel Surveys have been an important tool for recovering CWTs, and ultimately used in estimating total contribution of LFC summer steelhead to commercial and sport fisheries in the Columbia and Snake river basins. Creel surveys require considerable time and resources, yet standard commercial fishery sampling target goals (20% sample rate) are seldom met for the river sections where we survey. Decreasing budgets and other ESA issues (i.e. development of local broodstocks) have limited our ability to maintain past creel efforts in the lower Snake River in recent years. Yet, mandates have come forth in various forums to CWT all hatchery steelhead release groups to monitor straying within and outside of the Snake River Basin. While CWT

tagging all groups may eventually lead to a better understanding of straying, the inability to conduct adequate creel surveys locally could bias the tag recovery data, and make it look as if our summer steelhead are straying at higher rates than they really are. Further, estimates of total contribution and SAR's will be less, causing it to appear that the program is not working, when we have shown for many years that adult returns to the project area are well above the mitigation goals set forth by LSRCP.

The use of PIT tags within the Columbia and Snake river basins continues to expand. More adult detection sites at the mainstem dams are operational, providing valuable return information that, under the right circumstances, may also be used to estimate total adult returns from PIT tagged groups. While the PIT tags and tagging operations can be expensive, little time is required to retrieve adult PIT tag detections to determine survival rates. Hence, PIT tags may be an alternative and/or supplement to creel surveys for accurately estimating adult returns.

Recommendation: For the 2007 release, implant PIT tags (along with CWTs) in selected LFC summer steelhead release groups (tributaries where it is difficult to conduct adequate creel surveys – Tucannon, Touchet, and Walla Walla rivers). During 2007, evaluate the need for PIT tagging groups that are released on-station at LFH and in the Grande Ronde River at Cottonwood AP. Also in 2007, evaluate the relationships of return rate to LFH of CWT fish from all release groups, to their total return rate based on creel surveys and traps combined. Determine if a significant relationship exists, and can be used as a surrogate to estimate total returns of LFC summer steelhead to the Snake River Basin.

Literature Cited

Bumgarner, J., M. Schuck, S. Martin, J. Dedloff and L. Ross. 2002. Lyons Ferry Complex Hatchery Evaluation: Summer Steelhead and Trout Report 1998, 1999 and 2000 Run Years to USFWS Lower Snake River Compensation Plan Office. Report # FPA02-09.

Carmichael, R.W., R. T. Messmer and B.A. Miller. 1988. Summer Steelhead Creel Surveys in the Grande Ronde, Wallowa and Imnaha rivers for the 1987-88 Run Year. Progress Report, 1988. Oregon Department of Fish and Wildlife, Portland, Oregon.

Gallinat, M. P., and L. A. Ross. 2007. Tucannon River Spring Chinook Salmon Hatchery Evaluation Program: 2006 Annual Report to USFWS Lower Snake River Compensation Plan Office. (In Review).

Hillman, T. W., J. W. Mullan, and J. S. Griffith. 1992. Accuracy of Underwater Counts of Juvenile Chinook Salmon, Coho Salmon, and Steelhead. North American Journal of Fisheries Management. Volume 12 (3) 598-603.

Martin, S., M. Schuck, J. Bumgarner, J. Dedloff and A. Viola. 2000. Lyons Ferry Hatchery Evaluation, Trout Report: 1997-98. Washington Department of Wildlife Report to the USFWS. Report No. FPA00-11.

Mesa, M. G., and C. B. Schreck. 1989. Electrofishing mark-recapture and depletion methodologies evoke behavioral and physiological changes in cutthroat trout. Transactions of the American Fisheries Society. 118:644-658.

National Marine Fisheries Service. 1999. Biological Opinion on Artificial Propagation in the Columbia Basin – Section 7 Consultation. NOAA/NMFS, March 29, 1999. 175 pp.

Peterson, J. T., R. F. Thurow, and J. W. Guzevich. 2004. An evaluation of multipass electrofishing for estimating the abundance of stream-dwelling salmonids. Transactions of the American Fisheries Society 133:462-475.

Schreck, C. B., R. A. Whaley, M. L. Bass, O. E. Maughan, and M. Solazzi. 1976. Physiological responses of rainbow trout (*Salmo gairdneri*) to electroshock. Journal of the Fisheries Research Board of Canada. 33:76-84.

Schuck, M., A. Viola and S. Nostrant. 1990. Lyons Ferry Evaluation Study: Annual Report 1988-89 . Washington Department of Wildlife Report to the USFWS. Report No. AFF1/LSR-90-04.

Schuck, M., A. Viola, J. Bumgarner and J. Dedloff. 1998. Lyons Ferry Trout Evaluation Study: 1996-97 Annual Report. Washington Department of Fish and Wildlife Report to the USFWS. Report No. H98-10.

Temple, G. M., and T. N. Pearsons 2006. Evaluation of the recovery period in mark-recapture population estimates of rainbow trout in small streams. North American Journal of Fisheries Management. 26:941-948.

USACE (U. S. Army Corps of Engineers). 1975. Special Report: Lower Snake River Fish and Wildlife Compensation Plan. Walla Walla, Washington.

Viola, A. E., and M. L. Schuck. 1995. A method to reduce the Abundance of residual hatchery steelhead in rivers. North American Journal of Fisheries Management. 15:488-493.

Zippin, C. 1958. The Removal Method of Population Estimation. Journal of Wildlife Management. 22(1):82-90.

Appendix A: Bull Trout, Whitefish, and Brown Trout Capture Data from the Touchet River Adult Trap, 2006

Appendix A: Table 1. Bull trout captured at the Dayton Adult Trap on the Touchet River, 2006. Data shown represents first time captures that were then PIT tagged, or fish that were recaptures from previous years.

Year	Date	Ln (cm)	Wt (g)	Age [a]	PIT Tag Code	Recap	Year	Date	Ln (cm)	Wt (g)	Age [a]	PIT Tag Code	Recap
2006	3/7	31.5	370.0	4	3D9.1BF1CF327B		2006	5/16	34.0	430.0	4	3D9.1BF204AB64	
2006	3/28	35.0	490.1	4			2006	5/18	35.0	480.0	5	3D9.1BF204D711	
2006	3/30	44.0	980.0	5	3D9.1BF205070B		2006	5/18	55.0	2350.0	6	3D9.1BF1C4E8B0	3 Year
2006	4/21	49.0	1386.1	6	3D9.1BF1F8AF72		2006	5/18	43.0	1125.0	6	3D9.1BF1A2F561	2 Year
2006	4/22	31.0	336.8	3	3D9.1BF204E54B		2006	5/19	40.0	640.0	5	3D9.1BF1B60D58	
2006	4/25	31.0	336.8	3	3D9.1BF204E54B		2006	5/20	30.5	360.0	3	3D9.1BF1F85FA4	
2006	4/27	31.0	290.0	4	3D9.1BF1A05A14		2006	5/24	40.0	830.0	R	3D9.1BF2054F58	
2006	4/29	48.5	1342.8	R	3D9.1BF1B751AA		2006	5/24	46.0	1300.0	5	3D9.1BF205685B	2 Year
2006	5/1	48.5	1420.0	6	3D9.1BF1E7A0A8	2 Year	2006	5/25	39.0	710.0	R	3D9.1BF1B60815	
2006	5/1	33.0	500.0	4	3D9.1BF204AC46		2006	5/25	36.0	780.0	4	3D9.1BF1CF1E2B	
2006	5/1	38.5	690.0	4	3D9.1BF1F9E971		2006	5/30	42.0	925.0	5	3D9.1BF1F926DD	2 Year
2006	5/2	38.0	631.8	4	3D9.1BF1A29C17		2006	5/30	43.0	875.0	5	3D9.1BF1CD71E4	2 Year
2006	5/5	49.0	1610.0	5	3D9.1BF1B02C2D		2006	5/30	41.0	820.0	4	3D9.1BF204B0DB	
2006	5/5	35.0	625.0	5	3D9.1BF1B6D9D1		2006	5/31	56.5	2150.0	7	3D9.1BF123A317	5 Year
2006	5/5	41.0	660.0	4	3D9.1BF20542CD		2006	5/31	48.0	1325.0	5	3D9.1BF1936C4A	2 Year
2006	5/5	33.5	420.0	4	3D9.1BF1B848B0		2006	6/1	40.0	780.0	4	3D9.1BF1F6A29B	
2006	5/5	38.0	625.0	4	3D9.1BF1AE40E1		2006	6/2	55.0	2350.0	6	3D9.1BF1C4E8B0	3 Year
2006	5/8	35.0	510.0	4	3D9.1BF1B6ECC6		2006	6/2	42.0	925.0	5	3D9.1BF1F926DD	2 Year
2006	5/15	34.5	490.0	4	3D9.1BF1B75913		2006	6/3	35.0	640.0	3	3D9.1BF1A2ECEC	
2006	5/15	35.0	450.0	4	3D9.1BF204D320		2006	6/5	47.0	1275.0	5	3D9.1BF1F8E509	2 Year
2006	5/15	43.0	1125.0	6	3D9.1BF1A2F561	2 Year	2006	6/5	30.5	310.0	5	3D9.1BF1A2B53F	
2006	5/15	54.0	2075.0	6	3D9.1BF1C71ED4	3 Year	2006	6/5	36.5	580.0	4	3D9.1BF1CF31E5	
2006	5/15	51.0	1775.0	6	3D9.1BF1B70048	3 Year	2006	6/5	43.0	875.0	5	3D9.1BF1CD71E4	2 Year
2006	5/15	35.0	425.0	4	3D9.1BF204E856		2006	6/5	33.0	340.0		3D9.1BF1A2EC2C	
2006	5/15	36.0	500.0	4	3D9.1BF1937793		2006	6/8	31.0	325.0	4	3D9.1BF1A7824A	
2006	5/15	46.0	1300.0	5	3D9.1BF205685B	2 Year	2006	6/8	33.5	450.0	4	3D9.1BF1CF0563	
2006	5/15	43.0	875.0	5	3D9.1BF1CD71E4	2 Year	2006	6/15	31.0	336.8			
2006	5/16	47.0	1275.0	5	3D9.1BF1F8E509	2 Year	2006	6/17	41.0	799.1	5	3D9.1BF1E8F128	2 Year
2006	5/16	48.0	1180.0	R	3D9.1BF1CF1A7E		2006	6/29	25.0	173.3	3	3D9.1BF1A7752D	
2006	5/16	42.0	875.0	5	3D9.1BF1A7385F		2006	7/5	21.0	101.1	2	3D9.1BF1CF1E97	

[a] Age determined from scale samples. Missing ages are due to unreadable scale samples.

Appendix A: Table 2. Whitefish captured at the Dayton Adult Trap on the Touchet River, 2006.

Year	Date	LN (cm)	Age [a]	Year	Date	LN (cm)	Age [a]	Year	Date	LN (cm)	Age [a]
2006	3/29	27	2	2006	5/17	29	3	2006	6/7	27.5	3
2006	4/23	32	4	2006	5/17	26.5	3	2006	6/7	26.5	2
2006	4/24	34	4	2006	5/19	27.5	3	2006	6/8	27.5	3
2006	4/28	29	3	2006	5/25	27	3	2006	6/8	26	3
2006	5/5	26	2	2006	5/25	27	3	2006	6/9	30	3
2006	5/8	33	4	2006	5/31	27.5	3	2006	6/9	28	2
2006	5/10	26	2	2006	6/1	29.5	4	2006	6/12	26	2
2006	5/12	30	4	2006	6/5	27	3	2006	6/14	28.5	2
2006	5/15	31	- - -	2006	6/5	28	4	2006	6/20	30	3
2006	5/15	35	4	2006	6/5	26	3	2006	6/27	35	6
2006	5/16	27	4	2006	6/5	28	3	2006	7/6	28	3

[a] Age determined from scale samples. Missing ages are due to unreadable scale samples.

Appendix A: Table 3. Brown trout captured at the Dayton Adult Trap on the Touchet River, 2006.

Year	Date	LN (cm)	Age [a]	Year	Date	LN (cm)	Age [a]	Year	Date	LN (cm)	Age [a]
2006	2/28	26	- - -	2006	6/12	53	5	2006	6/29	43	3
2006	4/28	29	2	2006	6/13	38	3	2006	6/30	44	3
2006	5/17	48	4	2006	6/15	35	3	2006	7/3	33	2
2006	5/20	31	2	2006	6/22	22	2	2006	7/3	31	2
2006	6/2	41	3	2006	6/28	32	3	2006	7/3	38	3
2006	6/2	27	2	2006	6/28	32.5	3	2006	7/3	40	3
2006	6/3	53	4	2006	6/28	31	- - -	2006	7/3	31.5	2
2006	6/5	54.5	5	2006	6/28	42.5	3	2006	7/5	37	3
2006	6/5	40.5	3	2006	6/28	43	4	2006	7/5	34	3
2006	6/8	60.5	5	2006	6/28	38	3	2006	7/6	19	1

[a] Age determined from scale samples. Missing ages are due to unreadable scale samples.

Appendix B: Summer Steelhead Index Areas for Spawning Ground Surveys in 2006

Appendix B: Table 1. Start and stop coordinates (latitude and longitude) for stream reaches, index sections, and final walks for summer steelhead spawning ground surveys in the Tucannon and Touchet rivers, and Asotin Creek, 2006. (Note: Reference coordinates were determined from Maptech® Terrain Navigator Pro Software – Decimal Format – WGS 84). Locations provided are in a downstream to upstream progression.

Stream – Surveyed Section	Upstream coordinates (Start)	Downstream coordinates (Stop)
Tucannon River		
Reach 0	*46 29' 20.29" N, 117 57' 37.79" W*	*46 32' 52.18" N, 118 10' 31.82" W*
Index 1	46 29' 20.29" N, 117 57' 37.79" W	46 30' 22.18" N, 118 00' 37.87" W
Index 2	46 30' 22.18" N, 118 00' 37.87" W	46 30' 17.47" N, 118 03' 50.71" W
Index 3	46 30' 17.47" N, 118 03' 50.71" W	46 30' 47.21" N, 118 07' 03.28" W
Index 4	46 31' 12.43" N, 118 07' 47.80" W	46 32' 52.18" N, 118 10' 31.82" W
Reach 1	*46 18' 35.87" N, 117 39' 22.73" W*	*46 29' 20.29" N, 117 57' 37.79" W*
Index 1	46 27' 41.64" N, 117 51' 31.33" W	46 27' 56.64" N, 117 53' 50.34" W
Index 2	46 23' 49.00" N, 117 43' 00.89" W	46 26' 42.47" N, 117 46' 44.27" W
Index 3	46 18' 36.18" N, 117 39' 22.90" W	46 22' 07.00" N, 117 41' 25.91" W
Reach 2	*46 11' 18.29" N, 117 37' 25.95" W*	*46 18' 35.87" N, 117 39' 22.73" W*
Index 1	46 12' 24.04" N, 117 42' 21.77" W	46 18' 35.87" N, 117 39' 22.73" W
Reach 3		
Final Walk 1	*46 15' 49.62" N, 117 36' 55.61" W*	*46 19' 57.76" N, 117 40' 25.73" W*
Touchet River		
North Fork Touchet Reach	*46 11' 21.53" N, 117 49' 19.79" W*	*46 18' 05.41" N, 117 57' 30.80" W*
Index 1	46 17' 16.61" N, 117 55' 13.14" W	46 18' 05.41" N, 117 57' 30.80" W
Index 2	46 14' 28.74" N, 117 51' 58.07" W	46 16' 16.33" N, 117 53' 20.71" W
Final Walk 1	46 11' 21.53" N, 117 49' 19.79" W	46 13' 56.00" N, 117 51' 07.10" W
South Fork Touchet Reach	*46 07' 15.30" N, 117 58' 22.92" W*	*46 18' 05.41" N, 117 57' 30.80" W*
Index 1	46 14' 39.84" N, 117 55' 54.94" W	46 15' 48.66" N, 117 56' 19.34" W
Index 2	46 11' 58.60" N, 117 57' 17.18" W	46 13' 20.02" N, 117 56' 48.71" W
Final Walk 1	46 09' 09.19" N, 117 58' 24.01" W	46 11' 58.60" N, 117 57' 17.18" W
Wolf Fork Touchet Reach	*46 08' 56.71" N, 117 52' 29.14" W*	*46 16' 27.10" N, 117 53' 42.41" W*
Index 1	46 13' 41.11" N, 117 52' 25.01" W	46 15' 20.67" N, 117 53' 09.43" W
Index 2	46 12' 10.85" N, 117 52' 03.80" W	46 13' 18.79" N, 117 52' 25.72" W
Final Walk 1	46 08' 56.71" N, 117 52' 29.14" W	46 11' 20.01" N, 117 51' 54.54" W
Robinson Fork Touchet Reach	*46 10' 14.62" N, 117 55' 10.44" W*	*46 14' 16.42" N, 117 53' 41.60" W*
Index 1	46 10' 14.62" N, 117 55' 10.44" W	46 13' 58.45" N, 117 53' 32.33" W
Asotin Creek		
Main Asotin Creek Reach	*46 16' 21.42" N, 117 17' 27.79" W*	*46 19' 34.44" N, 117 06' 18.82" W*
Index 1	46 16' 21.42" N, 117 17' 27.79" W	46 17' 57.12" N, 117 15' 15.54" W
Index 2	46 19' 02.37" N, 117 14' 12.30" W	46 19' 45.51" N, 117 09' 13.14" W
Index 3	46 19' 30.89" N, 117 08' 51.82" W	46 19' 32.63" N, 117 06' 27.63" W
Final Walk 1	46 17' 57.12" N, 117 15' 15.54" W	46 19' 02.37" N, 117 14' 12.30" W
NF Asotin Creek Reach	*46 11' 48.87" N, 117 26' 03.08" W*	*46 16' 21.42" N, 117 17' 27.79" W*
Index 1	46 15' 44.23" N, 117 17' 45.12" W	46 16' 21.42" N, 117 17' 27.79" W
Index 2	46 14' 11.53" N, 117 21' 26.31" W	46 15' 44.23" N, 117 17' 45.12" W
Final Walk 1	46 13' 01.76" N, 117 23' 45.40" W	46 14' 11.53" N, 117 21' 26.31" W
Final Walk 2	46 11' 48.87" N, 117 26' 03.08" W	46 13' 01.76" N, 117 23' 45.40" W
SF Asotin Creek Reach	*46 11' 32.61" N, 117 19' 14.57" W*	*46 16' 21.42" N, 117 17' 27.79" W*
Index 1	46 14' 27.46" N, 117 17' 01.43" W	46 16' 21.42" N, 117 17' 27.79" W
Final Walk 1	46 11' 32.61" N, 117 19' 14.57" W	46 14' 27.46" N, 117 17' 01.43" W
Charley Creek Reach	*46 16' 58.50" N, 117 23' 49.12" W*	*46 17' 18.92" N, 117 16' 38.71" W*
Index 1	46 16' 57.73" N, 117 21' 18.00" W	46 17' 20.14" N, 117 18' 01.38" W
Final Walk 1	46 16' 58.50" N, 117 23' 49.12" W	46 16' 57.73" N, 117 21' 18.00" W
Final Walk 2	46 17' 20.14" N, 117 18' 01.38" W	46 17' 17.80" N, 117 17' 05.28" W

Appendix C: Estimates of Juvenile Summer Steelhead Densities in SE Washington Rivers that are part of the LSRCP Program – Summer of 2005.

Appendix C: Table 1. Summary of natural origin juvenile summer steelhead / rainbow trout mean densities (fish/100 m^2) by age class for SE Washington rivers that are a part of the LSRCP Program.

Stream Name	Asotin Creek				Touchet River				Tucannon River	Cummings Creek
Year	Main	North Fork	South Fork	Charley Creek	North Fork	South Fork	Wolf Fork	Robinson Fork	Main	Main
Age 0 Steelhead / Rainbow Trout										
1983	- - -	23.7	44.3	- - -	- - -	- - -	- - -		- - -	- - -
1984	- - -	6.6	39.0	- - -	- - -	- - -	- - -		16.0	- - -
1985	- - -	- - -	- - -	73.0	- - -	- - -	- - -		- - -	- - -
1986	- - -	29.7	- - -	- - -	- - -	- - -	- - -		18.4	- - -
1987	- - -	- - -	- - -	- - -	- - -	- - -	- - -		20.6	- - -
1988	- - -	45.8	- - -	- - -	- - -	- - -	- - -		- - -	- - -
1989	- - -	22.8	6.0	- - -	- - -	- - -	- - -		18.1	- - -
1990	- - -	- - -	- - -	- - -	- - -	- - -	- - -		19.1	- - -
1991	- - -	22.1	1.8	- - -	- - -	- - -	- - -		13.0	- - -
1992	- - -	56.9	50.0	- - -	35.5	42.8	41.1		17.4	- - -
1993	49.1	36.8	78.7	- - -	26.0	8.7	21.8		14.6	43.2
1994	36.8	20.4	0.8	19.0	20.8	16.2	20.2		- - -	42.9
1995	47.7	23.4	34.5	- - -	42.5	31.1	25.0		11.0	32.4
1996	62.8	13.0	2.0	64.4	4.9	1.9	2.3		15.8	47.8
1997	33.4	24.0	32.5	- - -	28.5	11.6	21.1		16.5	- - -
1998	52.2	44.6	32.9	18.3	15.4	16.7	23.6		17.2	12.5
1999	20.9	11.0	27.4	12.7	24.5	9.4	15.6		5.2	31.3
2000	26.6	41.9	21.8	43.0	15.6	10.9	15.3	20.4	19.3	40.3
2001	35.6	33.9	68.8	38.5	23.6	13.8	13.6	25.0	17.8	14.8
2002	37.1	40.4	84.7	65.8	48.0	52.1	43.4	41.7	27.2	54.9
2003	51.9	36.9	83.6	57.7	54.2	32.8	42.9	39.6	21.7	48.9
2004	41.4	23.6	15.0	48.0	33.5	33.8	35.0	16.4	5.3	17.7
2005	41.2	31.1	15.3	12.0	33.3	15.0	24.9	18.4	7.4	19.7
Age 1+ Steelhead / Rainbow Trout										
1983	- - -	8.7	25.3	- - -	- - -	- - -	- - -		- - -	- - -
1984	- - -	7.5	30.6	- - -	- - -	- - -	- - -		2.5	- - -
1985	- - -	- - -	- - -	37.6	- - -	- - -	- - -		- - -	- - -
1986	- - -	37.6	- - -	- - -	- - -	- - -	- - -		13.7	- - -
1987	- - -	- - -	- - -	- - -	- - -	- - -	- - -		8.5	- - -
1988	- - -	8.1	- - -	- - -	- - -	- - -	- - -		- - -	- - -
1989	- - -	18.1	34.0	- - -	- - -	- - -	- - -		10.6	- - -
1990	- - -	- - -	- - -	- - -	- - -	- - -	- - -		9.8	- - -
1991	- - -	14.2	13.9	- - -	- - -	- - -	- - -		6.5	- - -
1992	- - -	22.2	10.4	- - -	19.0	15.5	8.7		4.8	- - -
1993	22.1	28.1	42.5	- - -	19.3	15.0	10.5		7.0	26.3
1994	39.6	34.9	16.4	20.0	18.9	5.8	11.5		- - -	20.4
1995	13.1	11.2	21.7	- - -	8.9	9.5	6.4		4.0	29.6
1996	12.2	17.4	11.2	15.3	3.6	10.2	5.3		3.2	16.6
1997	6.9	6.7	4.6	- - -	2.3	2.8	7.4		4.6	- - -
1998	10.2	25.5	22.8	49.0	4.9	16.2	13.4		6.4	12.7
1999	14.4	13.9	17.3	22.9	3.4	8.4	13.0		4.2	16.1
2000	9.7	16.6	22.3	17.9	11.2	13.3	8.9	11.1	4.9	17.3
2001	19.7	30.4	29.8	23.6	13.7	13.6	11.6	13.6	6.9	8.6
2002	12.0	19.7	24.7	19.4	12.1	10.7	6.6	14.3	4.3	27.4
2003	15.5	18.7	36.2	38.3	16.7	17.2	16.2	27.4	7.20	28.3
2004	20.1	23.6	21.1	27.2	21.1	13.9	16.1	15.9	8.5	25.1
2005	23.3	17.5	13.9	33.2	16.4	17.3	13.5	11.6	2.7	11.9

Appendix C: Table 2. Densities of natural origin juvenile steelhead/rainbow trout (fish/100 m^2) from single (S) or multiple pass (MP) electrofishing sites in the Tucannon River basin, 2005.

Stream Site Name	Est. rkm	Site length (m)	Mean width (m)	Area (m^2)	Fish/100m^2 Age 0	Fish/100m^2 Age 1+	Fish/100m^2 Legal (>200mm)
Tucannon River							
TUCA-05 (S)	2.7	82.5	15.1	1251.9	5.51	2.24	0.00
TUCB-05 (S)	11.8	50	9.6	478.0	4.81	0.21	0.21
TUCC-05 (S)	17.7	50	12.4	620.0	6.77	4.35	0.16
TUC1-00 (MP, MR)	22	75	10	750.0	33.07	2.13	0.00
TUC2-00 (MP, MR)	28	75	11.6	867.9	4.49	0.81	0.00
TUC2a-05 (S)	28	75	11.1	832.5	0.48	0.36	0.12
TUC3-00 (S)	31.9	75	12.6	943.9	4.24	0.64	0.00
TUC5-00 (MP, MR)	36.7	50	10.5	522.5	7.85	4.78	0.00
TUC5a-05 (S)	36.7	50	11.1	554.0	5.42	0.54	0.00
TUC8-00 (MP, MR)	49.1	82.8	15.1	1251.9	5.51	2.24	0.00
TUC8a-05 (S)	49.1	50	9.6	478.0	4.81	0.21	0.21
TUC9a-05 (MP, MR)	55.6	50	12.4	620.0	6.77	4.35	0.16
TUC12-00 (MP, MR)	64.4	50	10	498.0	13.45	2.81	0.00
TUC12a-05 (MP, MR)	64.4	45	13.5	605.7	11.72	3.63	0.00
TUC13-00 (MP, MR)	68.4	50	12.8	641.0	1.87	7.49	0.16
TUC13a-05 (MP, MR)	68.4	50	9.5	473.0	2.33	7.19	0.21
TUC14-00 (MP, MR)	72.9	52	10.3	533.5	2.25	7.12	0.00
TUC14a-00 (S)	72.9	50	9.8	492.0	1.63	2.85	0.00
Cummings Creek							
CC1-01	0.0	50	3.0	148.3	19.55	3.37	0.00
CC2-02	1.8	49	3.2	156.8	24.23	24.23	0.00
CC3-02	3.8	50	2.8	140.0	35.00	19.29	0.00
CC4-02	5.8	50	3.1	154.2	0.00	0.65	0.00

Appendix C: Table 3. Densities of natural origin juvenile steelhead/rainbow trout (fish/100 m^2) from single (S) or multiple pass (MP) electrofishing sites in Asotin Creek, 2005.

Stream Site Name	Est. rkm	Site length (m)	Mean width (m)	Area (m^2)	Fish/100m^2 Age 0	Fish/100m^2 Age 1+	Fish/100m^2 Legal (>200mm)
Asotin Creek							
AC1-01 (MP)	4.4	50	7.5	374.2	36.08	11.76	0.00
AC1a-05 (MP)	4.4	50	7.2	360.0	48.89	16.94	0.00
AC3-01 (MP)	11.5	50	8.8	438.0	34.93	19.18	0.00
AC3a-05 (MP)	11.5	50	9.1	456.0	40.13	16.45	0.00
AC4-01 (MP)	15.2	50	8.5	426.0	47.65	20.42	0.00
AC5-01 (MP)	19.0	50	6.7	337.0	39.76	24.93	0.00
North Fork							
NF0-04 (MP)	0.8	50	7.2	360.0	23.06	16.11	0.00
NF1-01 (MP)	1.6	75	7.1	534.6	33.11	11.78	0.00
NF2-01 (MP)	3.8	57	7.5	425.6	42.06	24.91	0.23
NF2a-05 (MP)	3.8	46	8.3	380.9	26.25	17.07	0.00
South Fork							
SF1a-05 (MP)	0.1	75	4.4	331.9	47.01	17.78	0.00
SF1b-05 (S)	0.1	75	3.2	240.0	32.92	22.92	0.42
SF1c-05 (MP)	0.4	75	4.2	311.3	46.91	17.35	0.64
SF1d-05 (MP)	0.8	75	4.2	313.1	43.43	23.31	0.64
SF1e-05 (S)	0.8	75	2.9	217.5	58.85	17.01	0.00
SF2-00 (S)	3.0	75	3.8	288.2	0.35	12.84	0.35
SF3-00 (S)	5.4	75	3.2	237.9	0.00	11.77	0.42
SF4-00 (S)	8.2	75	3.7	277.5	13.69	13.33	0.00
Charley Creek							
CC2-02 (MP)	3.7	75	3.1	232.5	13.76	25.38	0.00
CC2a-05 (S)	3.7	75	3.2	238.9	6.70	25.53	0.42
CC3-02 (MP)	6.4	75	3.6	270.8	14.77	36.20	0.00
CC3a-05 (MP)	6.4	75	4.2	317.8	17.31	40.90	0.63
CC4-02 (S)	9.1	75	2.8	207.9	19.24	25.02	0.00
CC5-02 (S)	11.8	75	2.9	143.8	0.00	45.91	0.00

Appendix C: Table 4. Densities of natural origin juvenile steelhead/rainbow trout (fish/100 m^2) from single (S) or multiple pass (MP) electrofishing sites in the Touchet River basin, 2005.

Stream Site Name	Est. Rkm	Site length (m)	Mean width (m)	Area (m^2)	Fish/100m^2 Age 0	Fish/100m^2 Age 1+	Fish/100m^2 Legal (>200mm)
Mainstem							
MT01-01 (MP)	70.5	75	12.7	955.7	9.42	1.05	0.10
MT04-01 (MP)	79.2	75	11.6	867.9	11.64	1.84	0.00
MT05-01 (MP)	81.6	74	8.5	630.1	33.17	7.14	0.95
MT07-01 (MP)	87.0	75	9.8	735.0	45.17	11.70	0.27
North Fork							
NFT1-01 (MP)	0.1	85	9.4	801.4	33.31	10.73	0.25
NFT3-01 (MP)	6.8	50	6.3	317	41.96	24.61	0.00
NFT3a-05 (MP)	6.8	65	7.9	513.5	40.90	19.67	0.58
NFT5-01 (MP)	12.4	75	8.3	622.5	32.93	12.21	0.32
NFT7-01 (MP)	17.7	75	5.3	395.4	12.39	13.66	0.00
South Fork							
SFT1-01 (MP)	0.1	67	3.4	299.7	20.35	5.67	0.00
SFT3-02 (MP)	7.0	70	5.5	381.5	12.32	10.75	0.26
SFT3a-05 (MP)	7.0	75	5.5	409.7	10.95	9352	0.00
SFT5-02 (MP,)	13.4	75	4.2	315.9	26.27	35.45	0.00
SFT7-02 (S)	19.8	75	5.6	418.9	5.01	24.83	0.00
Wolf Fork							
WF1-01 (MP,)	0.2	50	7.4	369.0	13.55	7.05	0.00
WF1a-05 (MP)	0.2	50	8.2	410.0	25.61	4.39	0.00
WF3-01 (MP)	4.3	50	7.6	379.0	26.12	13.46	0.00
WF3a-05 (MP)	4.3	50	7.1	354.1	28.53	14.69	0.00
WF5-01 (MP)	8.6	50	6.2	309.0	15.21	24.60	0.00
WF5a-05 (MP)	8.6	50	5.1	255.8	10.94	19.15	0.00
WF7-01 (MP)	12.6	51	5.8	296.8	32.68	10.44	0.39
WF7a-05 (MP)	12.6	50	6.2	328.6	46.87	14.00	0.00
Robinson Fork							
RF1-01 (S)	0.8	50	3.5	174.3	12.05	24.67	0.00
RF2-01 (MP)	2.4	50	2.7	135.0	8.89	8.89	0.00
RF3-01 (MP)	3.8	50	2.6	130.8	19.0	0.00	0.00
RF4-01 (MP)	5.6	50	2.8	139.3	22.97	24.41	0.00
RF5-01 (S)	7.2	50	2.8	138.0	29.00	0.00	0.00

Appendix C: Table 5. Estimated number of other sensitive species present from electrofishing sites in the Tucannon River basin, 2005. Sites were surveyed using single (S) or multiple pass (MP) and/or mark/recapture (MR) surveys. Estimates shown below are from MP surveys estimates.

Stream Site Name	Bull Trout Age 0	Bull Trout Age 1+	Bull Trout legal (>200 mm)	Whitefish [a]	Spring Chinook	Hatchery Steelhead	Endemic Hatchery Steelhead
Tucannon River							
TUCA-05 (S)	0	0	0	0	0	0	0
TUCB-05 (S)	0	0	0	0	0	0	0
TUCC-05 (S)	0	0	0	1 (0)	0	12	0
TUC1-00 (MP, MR)	0	0	0	8 (Legal)	4	4	0
TUC2-00 (MP, MR)	0	0	0	1 (Legal)	2	2	0
TUC2a-05 (S)	0	0	0	0	0	0	1
TUC3-00 (S)	0	0	0	0	0	0	0
TUC5-00 (MP, MR)	0	0	0	0	0	0	0
TUC5a-05 (S)	0	0	0	1 (0)	7	0	0
TUC8-00 (MP, MR)	0	0	0	0	82	0	0
TUC8a-05 (S)	0	0	0	0	9	0	0
TUC9a-05 (MP, MR)	0	0	0	1 (legal)	45	0	1
TUC12-00 (MP, MR)	0	0	0	0	34	0	4
TUC12a-05 (MP, MR)	0	0	1	1 (legal)	55	0	18
TUC13-00 (MP, MR)	0	0	0	0	77	0	10
TUC13a-05 (MP, MR)	0	0	0	0	113	0	13
TUC14-00 (MP, MR)	0	1	1	0	72	0	9
TUC14a-00 (S)	0	0	4	0	55	0	5
Cummings Creek							
CC1-01	0	0	0	0	5	0	0
CC2-02	0	1	0	0	0	0	0
CC3-02	0	0	0	0	0	0	0
CC4-02	0	0	0	0	0	0	0

[a] Whitefish have been observed as Age 0 or legal based on size.

Appendix C: Table 6. Estimated number of other sensitive species present from electrofishing sites in Asotin Creek, 2005. Sites were surveyed using single (S) or multiple pass (MP) and/or mark/recapture (MR) surveys. Estimates shown below are from MP surveys estimates.

Stream Site Name	Bull Trout Age 0	Bull Trout Age 1+	Bull Trout legal (>200 mm)	Whitefish [a]	Spring Chinook	Hatchery Steelhead	Endemic Hatchery Steelhead
Asotin Creek							
AC1-01 (MP, MR)	0	0	0	0	1	0	0
AC1a-05 (MP, MR)	0	0	0	0	0	0	0
AC3-01 (MP, MR)	0	0	0	0	0	0	0
AC3a-05 (MP, MR)	0	0	0	0	0	0	0
AC4-01 (MP)	0	0	0	0	6	0	0
AC5-01 (MP, MR)	0	0	0	0	44	0	0
North Fork							
NF0-04 (MP, MR)	0	0	0	0	7	0	0
NF1-01 (MP)	0	0	0	0	26	0	0
NF2-01 (MP)	0	0	0	0	22	0	0
NF2a-05 (MP)	0	0	0	0	29	0	0
South Fork							
SF1a-05 (MP, MR)	0	0	0	0	2	0	0
SF1b-05 (S)	0	0	0	0	2	0	0
SF1c-05 (MP, MR)	0	0	0	0	0	0	0
SF1d-05 (MP, MR)	0	0	0	0	2	0	0
SF1e-05 (S)	0	0	0	0	0	0	0
SF2-00 (S)	0	0	0	0	0	0	0
SF3-00 (S)	0	0	0	0	0	0	0
SF4-00 (S)	0	0	0	0	0	0	0
Charley Creek							
CC2-02 (MP, MR)	0	0	0	0	0	0	0
CC2a-05 (S)	0	0	0	0	0	0	0
CC3-02 (MP, MR)	0	0	0	0	1	0	0
CC3a-05 (MP, MR)	0	0	0	0	0	0	0
CC4-02 (S)	0	0	0	0	0	0	0
CC5-02 (S)	0	0	0	0	0	0	0

[a] Whitefish have been observed as Age 0 or legal based on size.

Appendix C: Table 7. Estimated number of other sensitive species present from electrofishing sites in the Touchet River basin, 2005. Sites were surveyed using single (S) or multiple pass (MP) and/or mark/recapture (MR) surveys. Estimates shown below are from MP surveys estimates.

Stream Site Name	Bull Trout Age 0	Bull Trout Age 1+	Bull Trout legal (>200 mm)	Whitefish [a]	Brown Trout [b]	Spring Chinook	Hatchery Steelhead	Endemic Hatchery Steelhead
Mainstem								
MT01-01 (MP, MR)	0	0	0	0	0	0	0	0
MT04-01 (MP, MR)	0	0	0	0	1 (1+)	0	2	1
MT05-01 (MP, MR)	0	0	0	0	0	0	0	0
MT07-01 (MP, MR)	0	0	0	0	6 (0), 2 (1+)	5	5	17
North Fork								
NFT1-01 (MP, MR)	0	0	0	0	15 (0), 1 (1+)	4	0	28
NFT3-01 (MP, MR)	0	0	0	0	2(0)	0	0	2
NFT3a-05 (MP, MR)	0	0	0	0	1(0)	0	0	2
NFT5-01 (MP, MR)	1	0	0	0	1(0)	0	0	0
NFT7-01 (MP)	0	0	0	0	0	0	0	0
South Fork								
SFT1-01 (MP, MR)	0	0	0	0	2 (legal)	0	0	4
SFT3-02 (MP, MR)	0	0	0	0	0	0	0	0
SFT3a-05 (MP, MR)	0	0	0	0	0	0	1	0
SFT5-02 (MP, MR)	0	0	0	0	0	0	0	1
SFT7-02 (S)	0	0	0	0	0	0	0	0
Wolf Fork								
WF1-01 (MP, MR)	0	0	0	0	0	0	0	3
WF1a-05 (MP, MR)	0	0	0	0	5(0), 1(legal)	0	0	0
WF3-01 (MP, MR)	0	0	0	0	0	0	0	0
WF3a-05 (MP, MR)	0	0	0	0	1(0)	0	0	0
WF5-01 (MP, MR)	0	1	0	0	0	0	0	0
WF5a-05 (MP, MR)	0	0	0	0	0	0	0	0
WF7-01 (MP, MR)	0	0	2	0	0	0	0	1
WF7a-05 (MP, MR)	0	1	0	0	0	0	0	1
Robinson Fork								
RF1-01 (S)	0	0	0	0	0	0	0	0
RF2-01 (MP, MR)	0	0	0	0	0	0	0	0
RF3-01 (MP, MR)	0	0	0	0	0	0	0	0
RF4-01 (MP, MR)	0	0	0	0	0	0	0	0
RF5-01 (S)	0	0	0	0	0	0	0	0

[a] Whitefish have been observed as Age 0 or legal based on size.

[b] Brown Trout have been observed to have at least three age classes in the Touchet River. We have designated age based on length at time of capture.

Appendix C: Table 8. 2005 electofishing site locations for the Tucannon River, Cummings Creek, and Asotin Creek.

Stream / Site name	Approximate site location/description
Tucannon River	
TUCA-05	Above HWY261 Bridge (below smolt trap location) (Road Mile 1.7)
TUCB-05	Above Smith Hollow Bridge (Road Mile 7.4)
TUCC-05	Across from Dick Ducharmes House (Road Mile 11.0)
TUC1-00	100m below HWY 12 Bridge (Road Mile 13.5)
TUC2-00	100 m above Enrich Bridge (Road Mile 17.1)
TUC2a-05	100 m above Enrich Bridge (Road Mile 17.1) (50m above TUC2-00)
TUC3-00	MP6 on Tucannon Road (Road Mile 19.5)
TUC5-00	Hovrud's Silt Basin, (RM 23.2)
TUC5a-05	Hovrud's Silt Basin, (RM 23.2) (50m above TUC5-00)
TUC8-00	Directly under Bridge 13 (Road Mile 30.6) going upstream
TUC8a-05	~100m above Bridge 13 (Road Mile 30.6)
TUC9a-05	10m below Old Cummings Creek Bridge
TUC12-00	Across from Big 4 Lake, top is at the overflow from lake (Road Mile 40.0)
TUC12a-05	Across from Big 4 Lake (50m above TUC12)
TUC13-00	Across from Camp Wooten, old HMA 15 (Road Mile 42.3)
TUC13a-05	Across from Camp Wooten (50m above TUC13)
TUC14-00	100m above Cow Camp Bridge (Road Mile 44.5)
TUC14a-00	100m above Cow Camp Bridge (50m above TUC14)
Cummings Creek	
CC1-01	~50 m above mouth of Cummings Creek
CC2-02	1.2 miles above the Gate along the Cummings Creek Trail Road
CC3-02	2.4 miles above the Gate along the Cummings Creek Trail Road
CC4-02	3.6 miles above the Gate along the Cummings Creek Trail Road
Asotin Creek	
AC1-01	~200m above bridge at George Creek mouth, behind Joe Curl's house
AC1a-05	~250m above bridge at George Creek mouth, behind Joe Curl's house
AC3-01	~100m upstream of Headgate Park Dam
AC3a-05	~150m upstream of Headgate Park Dam
AC4-01	~2.5 miles below confluence bridge, public fishing access area
AC5-01	Upper end of 1998 meander reconstruction (Frank Koch's property)
North Fork Asotin	
NF0-04	~20m above mouth of South Fork Asotin
NF1-01	Just Above Lick Creek
NF2-01	1.4 miles above Lick Creek Crossing
NF2a-05	1.4 miles above Lick Creek Crossing
South Fork Asotin	
SF1a-05	~20m above South Fork mouth
SF1b-05	~75m above SF1a-05
SF1c-05	~0.4 road miles above the SF mouth
SF1d-05	~0.8 road miles above the SF mouth (below beaver dam complex)
SF1e-05	~0.8 road miles above the SF mouth (below beaver dam complex)
SF2-00	2 miles above mouth of South Fork
SF3-00	~50 m downstream from Schlee Bridge
SF4-00	1.7 miles above Schlee Bridge
Charley Creek (Asotin)	
CC2-02	1.7 miles above main Gate at Koch's house
CC2a-05	1.7 miles above main Gate at Koch's house (75m above CC2-02)
CC3-02	2.9 miles above main Gate at Koch's house
CC3a-05	2.9 miles above main Gate at Koch's house (75m above CC3a-05)
CC4-02	4.4 miles above main Gate at Koch's house
CC5-02	5.9 miles above main Gate at Koch's house

Appendix C: Table 9. 2005 electofishing site locations for the Touchet River.

Site name	Approximate site location/description
Mainstem	
MT01-01	Upstream from Waitsburg City Park Bridge (Road Mile 44.3)
MT04-01	Behind Lewis and Clark State Park (Road Mile 48.5)
MT05-01	~100m above Rose Gulch Bridge (Road Mile 49.9)
MT07-01	½ mile below mouth of Patit Creek (Road Mile 53.5)
North Fork	
NFT1-01	~50m above the mouth of the South Touchet (Road Mile 0.1)
NFT3-01	~50m above Wolf Fork Bridge (Road Mile 4.2)
NFT3a-05	~100m above Wolf Fork Bridge (Road Mile 4.2)
NFT5-01	Behind Jerry Dedloff's House (Road Mile 7.6)
NFT7-01	~20m above last bridge on North Touchet Rd. at MP 13 (Road Mile 11.0)
South Fork	
SFT1-01	~20m up from mouth (Road Mile 0.0)
SFT3-02	2 miles above Pettyjohn Bridge (Road Mile 4.4)
SFT3a-05	2 miles above Pettyjohn Bridge (Road Mile 4.4) (50m above WF3-02)
SFT5-02	~100m above Camp Nancy Lee Bridge (Road Mile 8.4)
SFT7-02	4 miles above Camp Nancy Lee Bridge (Road Mile 12.4)
Wolf Fork	
WF1-01	~100m above mouth of the Wolf Fork, behind Fairchild's house
WF1a-05	~150m above mouth of the Wolf Fork, behind Fairchild's house
WF3-01	2.4 miles above Wolf Fork Bridge
WF3a-05	2.4 miles above Wolf Fork Bridge (50 m above WF3-01)
WF5-01	Donnelly's Bridge (Road Mile 5.2)
WF5a-05	Donnelly's Bridge (Road Mile 5.2) (50m above WF5-01)
WF7-01	Mouth of Coates Creek (Road Mile 7.8)
WF7a-05	Mouth of Coates Creek (Road Mile 7.8) (50m above WF7-01)
Robinson	
RF1-01	½ Mile upstream from bridge at mouth
RF2-01	1.5 miles upstream from bridge at mouth
RF3-01	2.4 miles upstream from bridge at mouth
RF4-01	3.5 miles upstream from bridge at mouth
RF5-01	4.5 miles upstream from bridge at mouth

Appendix D: Genetic Relationships Among Tucannon, Touchet, and Walla Walla River Summer Steelhead (*Oncorhynchus mykiss*) Receiving Mitigation Hatchery Fish From Lyons Ferry Hatchery

A Supplemental Report By

Scott M. Blankenship*, Maureen P. Small*, Joseph D. Bumgarner†, Mark Schuck†, and Glen Mendel‡

* Washington Department Fish and Wildlife, Genetics Section, 600 Capitol Way N, Olympia, WA 98501-1091, USA

† Washington Department Fish and Wildlife, Snake River Laboratory, 401 S. Cottonwood St., Dayton, WA 99328, USA

‡ Washington Department of Fish and Wildlife, Fish Management, 529 W Main St., Dayton, WA 99328, USA

Summary

Limited information is available on the temporal stability of population allele frequencies. In salmonids, recent empirical studies provide conflicting results regarding the consistency of genetic variation over time within populations. Additionally, since many salmonid populations are of conservation concern and reduced in size, knowledge about effective population size (N_e) and the degree of temporal stability in gene frequencies becomes particularly important as a device for assessing the potential effects of genetic drift. We conduct a temporal analysis of allele frequencies at 14 microsatellite loci for sample collections replicated over a period of eight brood years. We compare the triad of two natural-origin summer steelhead (*Oncorhynchus mykiss*) populations (Tucannon and Touchet rivers) with a single hatchery population (Lyons Ferry Hatchery (LFH) stock) that is used for harvest augmentation within both rivers. We report that allele frequencies for the two natural summer steelhead populations are stable over seven brood years, and the phylogenetic relationships are constant for temporally stratified samples from a single location. In contrast, yearly allele frequency estimates from LFH samples are generally divergent from each other. Evidence suggests that LFH samples may have a lower N_e, as compared to the natural population samples. We also report on several management specific questions, 1) are steelhead caught in the lower and upper Tucannon River trap genetically different, 2) are steelhead that migrate after 1 year in freshwater divergent from those that chose to migrate after 2 or more years in freshwater, and 3) is there evidence for LFH introgression into the Tucannon, Touchet, and Walla Walla Rivers? We find no evidence that steelhead trapped in the lower or upper trap are different genetically. We find no evidence that freshwater age 1 individuals are more related to LFH steelhead, or are genetically different from freshwater age 2-3 steelhead. Based on phylogenetic data and individual assignment analysis we find evidence for LFH introgression into the Tucannon River, but not the Touchet or Walla Walla Rivers. Additionally, there was specific concern for introgression of LFH steelhead into Coppei Creek (Touchet tributary). We found no evidence for LFH introgression to this population. This report also incorporates genetic data from other steelhead studies, which results in the first comparison of lower Columbia River, Walla Walla River, Snake River, and Grand Ronde River steelhead. We report that Kalama River steelhead are approximately twice as differentiated from Tucannon, Touchet, and Walla Walla Rivers (between region $F_{ST} \sim 0.04$) than they are to themselves (Within region $F_{ST} \sim 0.02$). We report that Cougar Creek steelhead are quite differentiated from Tucannon, Touchet, and Walla Walla Rivers (between region $F_{ST} \sim 0.05$), though Cougar Creek samples were highly suspect due to the low number of juvenile fish and that there may have only been a few adult steelhead spawning in the stream. The amount of genetic variance partitioned among groups is similarly different comparing either Rattlesnake Creek or Wallowa stock to the Tucannon, Touchet, and Walla Walla Rivers (between region $F_{ST} \sim 0.02$).

Introduction

Temporal variation in the genetic composition of a population has long been of fundamental interest to evolutionary biologists, since changes in gene frequency over time are the signal of microevolutionary processes and may elucidate the agents responsible for genetic changes in populations (Lessios et al. 1994 and references therein). Although until recently there was a lack of empirical studies reporting genetic diversity estimates based on temporally replicated sampling, there is now an increasing trend in the literature of studies using samples collected over multiple years. This trend is being driven by two factors, 1) concern that biased estimates of population differentiation are being inferred from "snapshot" genetic heterogeneity studies, where samples are collected at single time-points (Waples 1998), and 2) interest in using temporal data to estimate the effective population size (N_e)(Waples 1989), a key parameter in conservation and population biology (Hedgecock et al. 1992). For both these analyses, knowledge about the amount of temporal stability is essential to understanding population trends.

In salmonids, recent empirical studies have provided conflicting results regarding the consistency of within-population genetic variation over time, with both long-term temporal stability and temporal variability observed. There is evidence suggesting that allele frequencies are stable over time, with reports concluding that the temporal variation within a population is minor compared to differences among populations (Banks et al. 2000, Carlsson and Nilsson 2000, Estoup et al. 1998, Hansen et al. 2002, Nielsen et al. 1999, Tessier and Bernatchez 1999). Yet, several recent studies have reported inconsistencies in allele frequency estimates taken from a single population at multiple time periods, with some temporal variation high enough in magnitude to cause erroneous conclusions about population differentiation (Jensen et al. 2005, Laikre et al. 2002, Østergaard et al. 2003, Palm et al. 2003). Analyzing collections from multiple generations to reliably estimate genetic diversities is especially important within a conservation setting, where critical population management decisions are made using population genetic information and population sizes are usually reduced.

Populations with small effective population size (N_e) are more prone to temporal instabilities in gene frequencies and genetic erosions than populations with large N_e (Frankham et al. 2002), since N_e is the main factor mediating any changes in neutral genetic diversity over time caused by genetic drift. Even though temporal variation in gene frequency may not have direct biological significance, stochastic fluctuations in allele frequencies may signify a small N_e, which is a legitimate concern for imperiled populations being impacted by environmental or anthropogenic factors. There are two observations suggesting a potential for reduced N_e in endemic populations of summer steelhead (*Oncorhynchus mykiss*) in western North America. One factor is that census sizes are drastically reduced from historic levels for many steelhead populations (Busby et al. 1997), and N_e is thought to be between 0.10 and 0.33 of the estimated census size (Bartley et al. 1992, Waples pers. comm.). Another factor pertains to the common practice in salmonids for hatchery supplementation programs. Hatchery programs have the potential to alter the N_e of small populations by over-representing certain segments of the population in a subsequent generation, thereby stochastically altering the genetic constitution of the total population (Busack et al. 1997, Ryman and Laikre 1991). A static census size coupled with hatchery supplementation has potential to lower N_e, which then increases the influence of genetic drift and thus temporal fluctuations in allele frequencies. This temporal instability may undermine efforts to document the genetic characteristics of populations and lower the accuracy of inferred genetic relationships between populations. Moreover, the common assumptions in surveys of genetic diversity that allele frequency estimates are stable over time and do not require temporal study become dubious. N_e depends on a variety of demographic factors and is a difficult quantity to estimate. For salmonids, which exhibit a life history strategy for differential age-at-maturity, each generation of juveniles is produced from multiple cohorts of adults from several previous years. As a result, calculation of N_e in salmonids is complex, and is often reduced to estimating the effective number of breeders (N_b) contributing to a cohort.

Here we describe a genetic analysis of allele frequencies at 14 microsatellite loci for Tucannon, Touchet, and Walla Walla River population samples of summer steelhead collected from 1998 – 2005. We compared the natural summer steelhead populations with Lyons Ferry Hatchery population samples, the source of hatchery mitigation fish. There are no previous genetic studies available comparing these populations, although Waples et al. (1993) found Tucannon River and LFH summer steelhead were differentiated based on allozyme data. Our main objective was to assess the genetic relationships among the natural steelhead samples with that of Lyons Ferry Hatchery. Additionally, we wanted to investigate the genetic relationships of these populations within the broader geographic context of the Snake and Columbia River steelhead populations. There were several secondary objectives of the study due to the complex nature of steelhead, regional reporting requirements, and specific management needs. Box 1 lists a series of questions developed by WDFW Science and Fish Management personnel covered in this report.

Box 1. Questions developed by WDFW Science and Fish Management personnel covered in this report.

Question #1: Are there significant genetic differences between Tucannon, Touchet, or Walla Walla River endemic steelhead stocks?

Question #2: Are there significant genetic differences from any natural steelhead stocks to the Lyons Ferry Hatchery stock?

Question #3: How similar are freshwater Age 1 wild Tucannon River adults to Lyons Ferry Hatchery Stock? Are freshwater Age 2 and Age 3 wild adults different in genetic makeup from freshwater Age 1 fish?

Question #4: How do wild fish collected from lower Tucannon River adult trap compare to wild fish collected at the Tucannon Fish Hatchery trap? Are the lower river collections more similar to Lyons Ferry stock fish?

Question #5: How do the results from this study compare with results and conclusions from Narum et al. (2004)? Is there strong evidence for hatchery introgression into either Walla Walla or Touchet steelhead? Is there evidence of hatchery introgression into Coppei Creek steelhead?

Supplemental Question #1: How do the endemic stocks from the Tucannon, Touchet, and Walla Walla Rivers compare to other steelhead stocks in the Snake or Columbia River basins?

Supplemental Question #2: Given the close similarity between these stocks, how confidently does the data allow us to assign individual fish to the correct location?

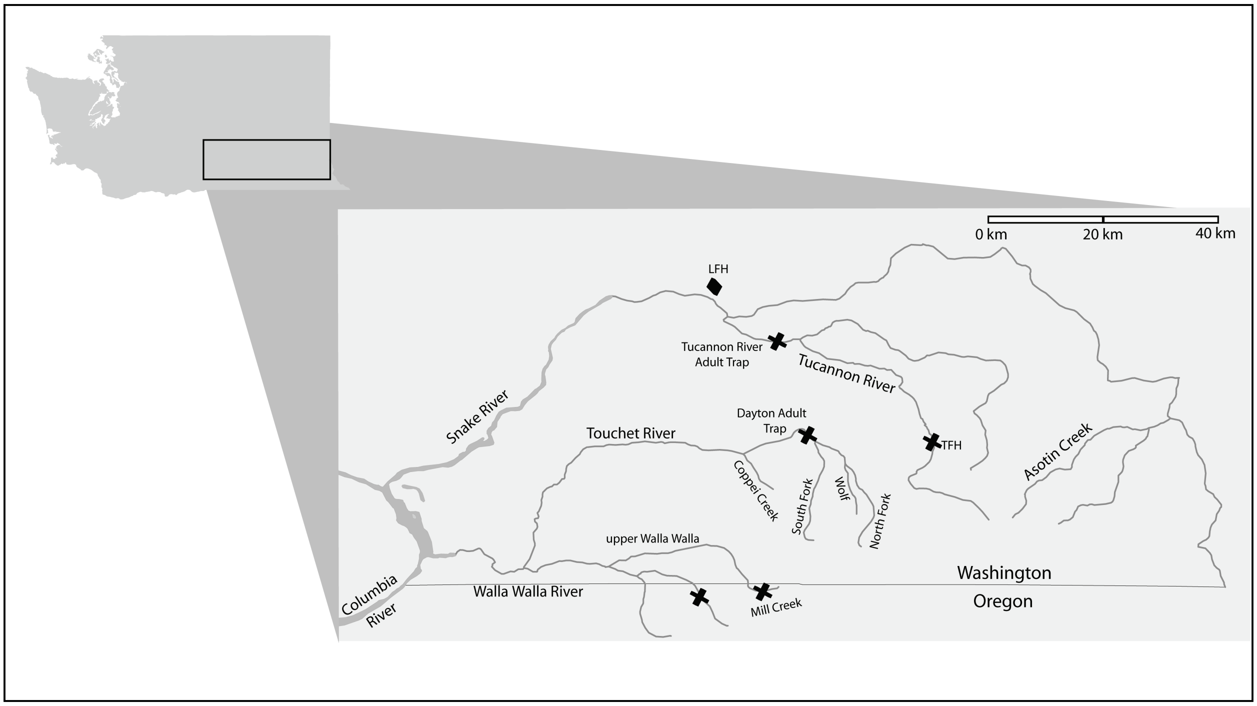

Figure 1. Collection locations for natural Tucannon, Touchet, and Walla Walla River summer steelhead, and the hatchery Lyons Ferry stock. Diamond symbol identifies LFH, X symbols identify trap locations.

Methods and Materials

Tissue collection and DNA Extraction

Natural summer steelhead (*O. mykiss*) individuals used to analyze the temporal stability of allele frequencies were collected 1998 to 2005 from two localities, Tucannon River, a tributary of the Snake River, and Touchet River, a tributary of the Walla Walla River (Figure 1, Table 1). Steelhead adults from the Tucannon River (n=458) were collected at either the lower Tucannon River Adult Trap at river kilometer (rkm) 17.7 or from the Tucannon Fish Hatchery (TFH) Adult Trap at rkm 36 (Figure 1). Steelhead adults from the Touchet River (n=508) were collected at the Dayton Adult Trap, located within the city of Dayton, WA (rkm 87.4). The hatchery sample included in the temporal analysis was from LFH (Figure 1; Table 1). The LFH stock was developed primarily from Wells Hatchery Stock (upper Columbia River) and the Wallowa stock. The Wallowa stock is a composite A and B-run stock that was developed from trapping adult summer steelhead at the lower Snake River dams and reared at Wallowa Hatchery by Oregon Department of Fish and Wildlife. Wells and Wallowa stock fish that returned to LFH during the 1980's and used for broodstock were eventually termed the "LFH stock". Forty-eight adults and 45 juveniles were collected in 1998/1999, and 100 adults were collected each year from 2003-2005, for a total of 393 individuals. Additional samples from the Walla Walla River drainage are shown in Table 1. Although these samples were not included in the temporal analysis, they are listed because they were included in the phylogenetic and N_b analyses. Juvenile samples were collected from five upper Touchet River tributaries in 1999 and 2000: 179 individuals from North Fork Touchet River, 94 individuals from South Fork Touchet River, 100 individuals from

Wolf Fork, 60 individuals from Coppei Creek (a lower Touchet R. tributary), and 59 individuals from Robinson Fork. Fish that escape past the Dayton Adult Trap populate the upper Touchet River, so Touchet River and upper Touchet River samples should be genetically similar. Adult steelhead collections were made in 1998 and 1999 from the Walla Walla River (n=137) and in 1998 from Mill Creek (n=49), a Walla Walla tributary upstream of the Touchet River confluence. Tissue collections were either fin clips or operculum punches, stored immediately in ethanol after collection. DNA was extracted from stored tissue using Nucleospin 96 Tissue following the manufacturer's standard protocol (Macherey-Nagel, Easton, PA, U.S.A.).

Table 1 Within population genetic data analysis summary. N is the number of sampled individuals, A/J is the adult or juvenile life stage, Hz is heterozygosity, LD is linkage disequilibrium.

Sample Collection	N	A/J	Unbiased Hz	Obs. Hz	Allele Richness	F_{IS}	Proportion LD	Bottleneck
98/99 Tucannon River………	36	A	0.809	0.764	13.65	0.056***	0.05	0.30
2000 Tucannon River ………	45	A	0.817	0.757	14.56	0.074***	0.10	0.81
2001 Tucannon River ………	51	A	0.817	0.780	13.99	0.045***	0.03	0.22
2002 Tucannon River ………	45	A	0.826	0.786	14.58	0.049***	0.07	0.39
2003 Tucannon River ………	85	A	0.811	0.727	14.07	0.048***	0.03	0.36
2004 Tucannon River ………	69	A	0.813	0.767	14.39	0.056***	0.08	0.86
2005 Tucannon River ………	127	A	0.815	0.774	14.68	0.049***	0.15	0.54
98/99 Lyons Ferry Hatchery	45	A	0.824	0.796	14.31	0.034**	0.07	0.05*
1999 Lyons Ferry Hatchery…	48	J	0.752	0.702	11.58	0.068***	0.10	0.50
2003 Lyons Ferry Hatchery…	100	A	0.803	0.753	12.53	0.063***	0.23	0.22
2004 Lyons Ferry Hatchery…	100	A	0.806	0.774	13.05	0.040***	0.34	0.24
2005 Lyons Ferry Hatchery…	100	A	0.814	0.793	12.78	0.026***	0.19	0.02*
1999 Touchet River…………	33	A	0.819	0.765	14.32	0.067***	0.04	0.24
2000 Touchet River…………	30	A	0.812	0.770	13.88	0.052***	0.08	0.90
2001 Touchet River…………	116	A	0.811	0.769	13.45	0.052***	0.05	0.33
2002 Touchet River…………	85	A	0.811	0.778	13.39	0.042***	0.05	0.24
2003 Touchet River…………	73	A	0.803	0.748	13.54	0.069***	0.09	0.15
2004 Touchet River…………	96	A	0.816	0.779	13.69	0.046***	0.11	0.17
2005 Touchet River…………	75	A	0.823	0.790	13.95	0.040***	0.11	0.58
1999 N.Fork Touchet River…	100	J	0.801	0.774	13.15	0.034***	0.33	0.33
2000 N.Fork Touchet River…	79	J	0.817	0.768	13.21	0.061***	0.15	0.10
1999 S.Fork Touchet River…	94	J	0.811	0.785	13.00	0.033***	0.15	0.30
1999 W.Fork Touchet River	100	J	0.814	0.785	12.82	0.036***	0.35	0.50
2000 Coppei Creek…………	60	J	0.793	0.752	12.38	0.052***	0.20	0.24
2000 Robinson Creek………	59	J	0.791	0.769	11.77	0.028***	0.13	0.14
1998 Walla Walla River……	77	A	0.795	0.750	13.40	0.057***	0.03	0.67
1999 Walla Walla River……	60	A	0.810	0.742	13.81	0.084***	0.15	0.22
1998 Mill Creek ……………	49	J	0.828	0.759	14.08	0.084***	0.19	0.63

Note. – The α-levels for statistical significance are coded * = 0.05, ** = 0.01, *** = 0.001

Laboratory Analysis

Polymerase chain reaction (PCR) amplification was performed using 14 fluorescently end-labeled microsatellite marker loci, *One* 101, 102, 108 and 114 (Olsen et al. 2000), *Omy* 77 (Morris et al. 1996), *Omy* 1001 and 1011 (Spies et al. 2005), *Omm* 1070, 1128, and 1130 (Rexroad et al. 2001), *Ots* 1 and 3M (Banks et al. 1999), *Ots* 100 (Nelson and Beacham 1999), and *Ots* 103 (Small et al. 1998). PCR reaction volumes were 10 µL, and contained 1 µL 10x PCR buffer (Promega), 1.0 µL $MgCl_2$ (1.5 mM final) (Promega), 0.2 µL 10 mM dNTP mix (Promega), and 0.1 units/µL *Taq* DNA polymerase (Promega). Loci were amplified as part of multiplexed sets, so primer molarities and annealing temperatures varied. Multiplex one had an annealing temperature of 55°C, and used 0.08 Molar (M) *One* 102, 0.07 M *One* 114, and 0.04 M *Ots* 100. Multiplex two had an annealing temperature of 62°C, and used 0.06 M *Omm* 1130, 0.03 M *Omm* 1070, and 0.04 M *Omy* 1011. Multiplex three had an annealing temperature of 55°C, and used 0.04 M *One* 108, 0.011 M *Ots* 103, and 0.021 M *One* 101. Multiplex four had an annealing temperature of 52°C, and used 0.03 M *Omy* 1001, and 0.025 M *Omm* 1128. Multiplex five had an annealing temperature of 49°C, and used 0.035 M *Ots* 1, 0.03 M *Omy* 77, and 0.02 M *Ots* 3M. All thermal cycling was conducted on a PTC200 thermal cycler (MJ Research) as follows: 95°C (2 min); 30 cycles of 95°C for 30 sec., 30 sec. annealing, and 72°C for 30 sec.; a final 72°C extension and then a 10°C hold. PCR products were visualized by denaturing polyacrylamide gel electrophoresis on an ABI 3730 automated capillary analyzer (Applied Biosystems). Fragment analysis was completed using GeneMapper 3.0 (Applied Biosystems).

Genetic Data Analysis

Assessing within population genetic diversity - Heterozygosity measurements are reported using Nei's (1987) unbiased heterozygosity formula and Hedrick's (1983) formula for observed heterozygosity. Both tests are implemented using the microsatellite toolkit (Park 2001). Allelic richness was calculated using FSTAT version 2.9.3.2 (Goudet 1995). GENEPOP version 3.4 (Raymond and Rousset 1995) was used to assess Hardy-Weinberg equilibrium, where deviations from the neutral expectation of random associations among alleles are calculated using a Markov chain method (5000 iterations in this study) to obtain unbiased estimates of Fisher's exact test. Global estimates of F_{IS} according to Weir and Cockerham (1984) were calculated using FSTAT version 2.9.3.2 (Goudet 1995). Statistical significance at $\alpha = 0.05$ of F_{IS} was adjusted for multiple comparisons. Genotypic linkage disequilibrium was calculated following Weir (1979) using GENETIX version 4.05 (Belkhir et al. 1996). Linkage results are reported as the proportion of pairwise (locus by locus) tests that are significant based on a permutation procedure implemented in GENETIX. Linkage disequilibrium is considered statistically significant if more than 5% of the pairwise tests based on permutation are significant for a sample. To assess if historic changes in population size have caused deviations from mutation-drift equilibrium, we compared observed heterozygosity to that expected under mutation-drift equilibrium, given the observed allele diversity. Excess heterozygosity is expected in populations that have experienced recent size reductions, as rare alleles are lost more rapidly. This test was implemented in the program BOTTLENECK version 1.2.02 (Piry et al. 1999), and statistical significance of the BOTTLENECK result is reported as a two-tailed Wilcoxon test for heterozygosity excess or deficit, given a two-phase microsatellite mutation model. P-value significance was not adjusted for multiple tests.

Temporal analysis of allele frequencies - Within a location, temporal samples were compared using Friedman's method for randomized blocks (Sokal and Rohlf 3rd edition pg. 440), a non-parametric analysis of variance (ANOVA) without replication. The total number of alleles, observed heterozygosity, and unbiased heterozygosity were used as blocks, with the collection year as treatment effect (Appendix 1). The null hypothesis for this test is that there is no year effect. The temporal stability of allele frequencies was assessed by the genetic differentiation randomization chi-square test implemented in FSTAT version 2.9.3.2 (Goudet 1995). Alleles were randomized between samples (i.e. genic test).
Effective population size (N_e) – Estimates of the effective population size were obtained using two methods, a multi-sample temporal method (Waples 1990) on consecutive cohorts of steelhead and a single sample linkage disequilibrium method on upper Touchet River juvenile samples. Combining population samples with age data from scale analysis generated cohort samples. Only cohorts samples with greater than 20 individuals were used in the temporal method analysis. For the temporal method, $\hat{F}$ (standardized variance in allele frequency) is calculated according to Pollack (1983). The parameter b is calculated analytically from age structure information and the number of years between samples (Tajima 1992). The age-at-maturity information required to calculate b was obtained from the cohort data. Waples (1990) developed a method to estimate the effective number of breeder (N_b) from $\hat{F}$ that incorporates the Pacific salmon life history:

$$\hat{N}_{b(i,j)} = \frac{b}{2\,(\hat{F} - 1/\hat{S}_{i,j})}$$

The Waples (1990) temporal method has been updated by Waples et al. (2007). Consecutive cohort samples are analyzed to estimate the pairwise N_b ($\hat{N}_{b(i,\,j)}$). Various $\hat{N}_{b(i,\,j)}$ will not have the same information content (sample size, alleles), so pairwise estimates are weighted by the reciprocal variance of the global estimate of N_b ($\tilde{N}_b$) (i.e. harmonic mean of all $\hat{N}_{b(i,\,j)}$). $\tilde{N}_b$ is the estimate of the effective population size (N_e). SALMONNb (Waples et al. 2007) was used to calculate ($\tilde{N}_b$) (i.e. N_e). A single sample method was used to estimate N_b from the Touchet River juvenile samples. Juvenile samples may be used for N_b calculations, although estimates are not directly comparable to estimates made using temporal methods, as juvenile samples estimate N_b for the parental generation only (Waples 2005). The linkage disequilibrium method was used to estimate N_b for each juvenile sample from the upper Touchet River. This method uses the mean squared correlation of allele frequencies at different gene loci (Bartley et al. 1992, Campton 1987, Waples 1991). Estimates of the linkage disequilibrium method were calculated using the software NEESTIMATOR (Peel et al. 2004).

Among population genetic differentiation - Population structure was investigated using pairwise estimates of F_{ST} and within an analysis of molecular variance framework (AMOVA). Multi-locus estimates of pairwise F_{ST}, estimated by a "weighted" analysis of variance (Weir and Cockerham, 1984), were calculated using GENEPOP version 3.4 (Raymond and Rousset 1995). To determine if the F_{ST} estimates were statistically different from zero, 1000 permutations were implemented in GENETIX version 4.05 (Belkhir et al.1996). The hierarchical AMOVA partitioned the total variance into covariance components due to intra-individual, inter-individual, inter-population, and inter-group differences (Weir and Cockerham, 1984). The covariance components are used to compute fixation indices (i.e. probability of identity by descent) in terms of coalescent times. The significance of the fixation indices was tested using a non-parametric permutation approach described in Excoffier et al. (1992). After each permutation round, 20,000 in total, all variance statistics are recomputed to get their null distribution. ARLEQUIN 3.01 (Excoffier et al. 2005) was used to conduct the AMOVA. The structure of the AMOVA was the same as the temporal analysis of allele frequencies, and defined three groups, Tucannon River, Touchet River, and LFH. All temporally replicated samples were analyzed within each group.

Genetic distances were calculated using the program GENDIST (Phylip 3.6, Felsenstein 2005), using the Cavalli-Sforza's chord measure (Cavalli-Sforza and Edwards, 1967). The neighbor-joining algorithm was used to construct trees (Phylip 3.6, Felsenstein 2005). The robustness of the population tree topology was assessed using 1000 bootstrap datasets of the above genetic distances and the program CONSENSE (Phylip 3.6, Felsenstein 2005).

Individual assignment – A population baseline file containing 1,948 individuals was constructed, with samples subdivided based on genetic similarity into four population categories, Tucannon, LFH, Touchet, and Walla Walla. All individuals in baseline had data for 10 or more loci. Individual steelhead were assigned to their most likely population of origin based on the partial Bayesian criteria of Rannala and Mountian (1997), using a "jack-knife" procedure, where each individual to be assigned was removed from the baseline prior to the calculation of population likelihoods. All tests were implemented using GENECLASS2 software (Piry et al. 2004). A LOD score assessed the quality of each assignment. The LOD statistic was manually constructed using the likelihood rank scores from each GENECLASS2 assignment, with the odds ratio having the form of "most likely" divided by "second most likely". An individual was classified as unassigned if the assignment LOD < 1. In addition to requiring a minimum LOD score for assignment, the probability of each assignment was assessed using the simulation procedure of Paetkau et al. (2004). The simulation was used to exclude a population from consideration for the assignment of an individual. If the probability of any assignment did not fall within the expected 95% confidence interval derived by the simulation, the rank-based assignment was not allowed to that population irrespective of LOD score. The results reported are the proportions of individuals assigned to each population category, given that the assignment LOD was greater than one and that the individual's likelihood resided within the 95% confidence interval for the estimated population of origin.

Results

Microsatellite diversity within populations - Substantial genetic diversity was observed within populations, with unbiased heterozygosity estimates, over all loci, ranging from a low of 0.752 (1999 LFH) to 0.828 (1998 Mill Creek) (Table 1). Mean allele richness over all populations and loci was 13.50, with allele richness ranging from a low of 11.58 (1999 LFH) to a high of 14.68 (2005 Tucannon) (Table 1). The number of alleles sampled per locus was standardized to the smallest sample size of complete genotypes (N=28, 56 alleles) using a rarefaction method, although the mean sample size was much larger (N=73). Departures from expected random mating genotypic proportions, quantified as statistically significant heterozygote deficiencies (F_{IS}), were observed for all populations (Table 1). Values ranged from a high of 7.4% deviation from expectation for the 2000 Tucannon River sample, to a low of 2.6% deviation for the 2005 LFH sample (Table 1). Significant linkage disequilibrium was detected for 21 of the 28 samples (Table 1). Results for tests of mutation-drift equilibrium (BOTTLENECK) are shown in Table 1. The BOTTLENECK results reported are the p-values for the null hypothesis of equilibrium, with significant deviations from the null expectation observed for the 98/99 LFH (p= 0.05) and the 2005 LFH (p= 0.02) samples. All other population samples were consistent with mutation-drift equilibrium based on the comparison between observed allelic diversity and expected heterozygosity.

Temporal analysis of allele frequencies - The null hypothesis of no year effect was rejected for five out of 42 ANOVA tests using Friedman's method for randomized blocks. A significant year effect was seen at *Ots* 100 for Tucannon River, *Ots* 1 and *Omy* 1001 for LFH, and *Ots* 1 and *Ots 103* for Touchet River samples. P-values for genic differentiation tests (within region) are shown in Table 2, with pairwise comparisons of allele frequencies conducted separately for collections where stratified temporal samples were available. (A p-value of 0.0001 is significant at alpha=0.05 after correction for multiple tests). Allele frequencies for all Tucannon River samples, except one pairwise comparison, were statistically equivalent (Table 2). The comparison between 2003 and 2005 Tucannon samples were differentiated based on the chi-square test. Regarding LFH samples, all samples were largely differentiated. The 98/99 and 2004 sample comparison was the only pairwise test that was statistically equivalent (Table 2). For the Touchet River samples, 2001 and 2002 samples were differentiated from the 2004 sample. The allele frequencies for the 2000 Touchet sample are not equivalent to all other Touchet River samples. Regarding the upper Touchet River tributary samples, all samples are statistically different (Table 2). Both Walla Walla samples are statistically equivalent, but the p-value is low. The Walla Walla samples are statistically different from the Mill Creek sample.

P-values for genic differentiation tests (between region) are shown in Table 3, with pairwise comparisons of allele frequencies conducted separately for collections where stratified temporal samples were available. In general, between region allele frequency comparisons are statistically different. The 98/99 LFH sample was statistically equivalent to all the Tucannon River samples. There was also some similarity between the 2000 Touchet sample and the Tucannon samples. The 1999 Touchet sample was similar to the upper Touchet juvenile samples, and all Walla Walla River samples.

Table 2 Genetic differentiation. Values for within population pairwise tests are shown for Tucannon, Touchet, Lyons Ferry Hatchery, and Walla Walla collections. Above the diagonal are p-values for pairwise tests of allelic differentiation. Below the diagonal are pairwise estimates of F_{ST}. Statistically significant pairwise F_{ST} estimates are bolded.

	98/99Tuc	00Tuc	01Tuc	02Tuc	03Tuc	04Tuc	05Tuc
98/99Tucannon	-	0.4082	0.5291	0.1607	0.2357	0.0433	0.0024
00 Tucannon…	0.001	-	0.2341	0.7335	0.1665	0.0597	0.0126
01 Tucannon…	0.000	0.001	-	0.4376	0.0448	0.2612	0.0373
02 Tucannon…	0.003	-0.001	0.000	-	0.5320	0.6660	0.0217
03 Tucannon…	0.001	0.001	0.000	-0.001	-	0.0315	0.0001
04 Tucannon…	**0.004**	0.001	0.001	-0.001	0.001	-	0.0851
05 Tucannon…	**0.004**	**0.004**	0.001	**0.003**	**0.002**	**0.002**	-

	98/99LFH	99LFH	02LFH	04LFH	05LFH
98/99LFH	-	0.0001	0.0001	0.0004	0.0001
99LFH …	**0.037**	-	0.0001	0.0001	0.0001
02LFH …	**0.004**	**0.040**	-	0.0001	0.0001
04LFH …	0.002	**0.039**	**0.006**	-	0.0001
05LFH …	0.002	**0.036**	**0.008**	**0.007**	-

	99Tou	00Tou	01Tou	02Tou	03Tou	04Tou	05Tou
99 Touchet	-	0.0020	0.0050	0.0003	0.0152	0.0052	0.0045
00 Touchet	**0.026**	-	0.0001	0.0001	0.0001	0.0001	0.0001
01 Touchet	0.001	**0.027**	-	0.0114	0.1470	0.0001	0.0049
02 Touchet	0.001	**0.032**	**0.002**	-	0.5279	0.0001	0.0217
03 Touchet	0.003	**0.029**	**0.002**	0.000	-	0.0004	0.0163
04 Touchet	0.001	**0.029**	0.002	**0.002**	**0.003**	-	0.0023
05 Touchet	0.002	**0.030**	0.001	**0.003**	**0.004**	**0.002**	-

	99NFTou	00NFTou	99SFTou	99WFTou	00Copp	00RobTou
99NFTou	-	0.0001	0.0001	0.0001	0.0001	0.0001
00NFTou	**0.010**	-	0.0001	0.0001	0.0001	0.0001
99SFTou	**0.011**	**0.011**	-	0.0001	0.0001	0.0001
99WFTou	**0.009**	**0.011**	**0.009**	- 0.0001	0.0001	
00Coppei	**0.015**	**0.021**	**0.016**	**0.018**	-0.0001	
00Robinson	**0.016**	**0.014**	**0.013**	**0.011**	**0.021**	-

	98 Walla	99 Walla	98 Mill
98 Walla	-	0.0008	0.0001
99 Walla	0.002	-	0.0001
98 Mill	**0.007**	**0.006**	-

Table 3 Genetic differentiation. P-values for between population pairwise tests of allelic differentiation are shown for Tucannon, Touchet, Lyons Ferry Hatchery, and Walla Walla collection.

	98/99 LFH	99 LFH	03 LFH	04 LFH	05 LFH	99 Tou	00 Tou	01 Tou	02 Tou	03 Tou	04 Tou	05 Tou	98 Walla	99 Walla	98 Mill
98/99 Tuc	0.1978	*	*	*	*	*	*	*	0.0004	*	*	*	*	*	*
2000 Tuc	0.6952	*	*	*	*	0.0216	*	*	*	*	*	*	*	*	*
2001 Tuc	0.1947	*	*	*	*	*	*	*	*	*	*	*	*	*	*
2002 Tuc	0.6204	*	*	*	*	0.0034	*	*	*	*	*	*	*	*	*
2003 Tuc	0.4730	*	*	*	*	0.0003	*	*	*	*	*	*	*	*	*
2004 Tuc	0.4855	*	*	*	*	*	*	*	*	*	*	*	*	*	*
2005 Tuc	0.0009	*	*	*	*	0.0003	*	*	*	*	*	*	*	*	*

	99 Tou	00 Tou	01 Tou	02 Tou	03 Tou	04 Tou	05 Tou	98 Walla	99 Walla	98 Mill
98/99 LFH	*	*	*	*	*	*	*	*	*	*
1999 LFH	*	*	*	*	*	*	*	*	*	*
2003 LFH	*	*	*	*	*	*	*	*	*	*
2004 LFH	*	*	*	*	*	*	*	*	*	*
2005 LFH	*	*	*	*	*	*	*	*	*	*

	98 Walla	99 Walla	98 Mill
99TouA	0.0012	0.0492	0.0036
00TouA	*	0.0004	*
01TouA	*	*	*
02TouA	*	*	*
03TouA	*	*	*
04TouA	*	*	*
05TouA	*	*	*

	98 - 05 Tuc	98 - 05 LFH	99 Tou	00 Tou	01 Tou	02 Tou	03 Tou	04 Tou	05 Tou	98 Walla	99 Walla	98 Mill
99NFTouJ	*	*	0.0075	*	*	*	*	*	*	*	*	*
00NFTouJ	*	*	0.0774	0.0003	*	*	*	*	*	*	*	*
99SFTouJ	*	*	0.0489	*	*	*	*	*	*	*	*	*
99WFTouJ	*	*	0.1852	*	*	*	*	*	*	*	*	*
00CoppJ	*	*	*	*	*	*	*	*	*	*	*	*
00RobTouJ	*	*	0.0004	*	*	*	*	*	*	*	*	*

Note - * denotes a p-value of 0.0001 or less. Tuc = Tucannon, LFH = Lyons Ferry Hatchery, Tou = Touchet, Walla = Walla Walla

Microsatellite diversity among populations - Significant heterogeneity in allele frequencies was observed among populations, although the variance attributed to population subdivision was small. The global F_{ST} value was 0.013 (+/- 0.004). Between watersheds (termed group in AMOVA), the mean pairwise F_{ST} estimates were, 0.010 Tucannon River v. Touchet River (0.006 when 2000 Touchet sample excluded), 0.011 Tucannon River v. LFH (0.006 when 1999 LFH juvenile sample excluded), and 0.023 LFH v. Touchet River (0.012 excluding both 1999 LFH and 2000 Touchet). The proportion of variation attributed to the among-group AMOVA component was 0.44% (Table 4). Additionally, the proportion of variance attributed to among-population differences within groups, 0.86% in Table 4, was similar to that observed for among-group differences. Correspondingly, the mean pairwise F_{ST} estimates from temporally replicated samples were 0.001 for Tucannon River, 0.010 for Touchet River (0.002 with 2000 Touchet sample excluded), and 0.018 for LFH (0.005 when 1999 LFH juvenile sample excluded).

Table 4 Global AMOVA results as a weighted average over loci.

Source of variation	Variance components	Percentage variation
Among groups	0.0258	0.44
Among populations within groups	0.0500	0.86
Among individuals within populations	0.4922	8.47
Within individuals	5.2436	90.23

Effective population size – Estimates of effective number of breeder (N_b) derived from Waples (1990) temporal method are shown in Table 5-7. For the Tucannon River samples, cohorts from 1997 – 2002 were used (Table 5). From scale analysis for all Tucannon samples, 6% of individuals were age 2, 48% were age 3, 43% were age 4, and 3% were age 5. Those percentages were used as the population age-at-maturity information to calculate b. The harmonic mean of all pairwise estimates of N_b ($\tilde{N}_b$) was 222.7. This estimate is the contemporary N_e for Tucannon River. For the Touchet River samples, cohorts from 1996 – 2002 were used (Table 6). From scale analysis for all Touchet samples, 2% of individuals were age 2, 53% were age 3, 39% were age 4, and 6% were age 5. Those percentages were used as the population age-at-maturity information to calculate b. The harmonic mean of all pairwise estimates of N_b ($\tilde{N}_b$) was 173.8. This estimate is the contemporary N_e for Touchet River. For the LFH samples, cohorts from 2000 – 2002 were used (Table 7). From scale analysis for all Touchet samples, 85% were age 3, 15% were age 4. Those percentages were used as the population age-at-maturity information to calculate b. The harmonic mean of all pairwise estimates of N_b ($\tilde{N}_b$) was 144.4. This estimate is the contemporary N_e for LFH stock.
The linkage disequilibrium method was used to estimate N_b for each juvenile sample from the upper Touchet River. The estimates were of similar magnitude as the pairwise estimates derived from the temporal method. The N_b estimates ranged from a low of 81.2 for 2000 Coppei Creek to a value of 206.2 for 2000 NF Touchet.

Table 5 Summary of output from program SALMONNb and data for six consecutive years of summer steelhead samples from Tucannon River. For each pairwise comparison of samples *i* and *j*, $\tilde{S}$ is the harmonic mean sample size, *n* is the number of independent alleles used in the comparison, $\hat{N}_{b(i,j)}$ are the pairwise estimates of N_b, and Var [$\hat{N}_{b(i,j)}$] is the variance of $\hat{N}_{b(i,j)}$. $\tilde{N}_b$ is the harmonic mean of the $N_{b(i,j)}$. Alleles with a frequency below 0.05 were excluded from the analysis to reduce potential bias.

Year	1997	1998	1999	2000	2001	2002
Pairwise $\tilde{S}$ (above diagonal) and *n* (below diagonal):						
1997		31.6	39.7	35.4	43.4	32.5
1998	87		47.1	41.2	52.5	37.4
1999	87	85		56.1	79.5	49.3
2000	84	82	87		63.9	42.9
2001	90	85	87	86		55.3
2002	84	89	87	90	85	
Pairwise $\hat{N}_{b(i,j)}$ (above diagonal) and Var [$\hat{N}_{b(i,j)}$] (below diagonal):						
1997		475.3	152.3	256.5	98.2	155
1998	53225		infinity	430.7	437.3	100.7
1999	31638	28082		228.6	1307.8	92.5
2000	97677	31602	20881		970	253.4
2001	55514	48584	11164	17370		302.8
2002	43823	72659	52731	27012	21901	
$\tilde{N}_b$	= 222.7					

Table 6 Summary of output from program SALMONNb and data for seven consecutive years of summer steelhead samples from Touchet River. For each pairwise comparison of samples *i* and *j*, $\tilde{S}$ is the harmonic mean sample size, *n* is the number of independent alleles used in the comparison, $\hat{N}_{b(i,j)}$ are the pairwise estimates of N_b, and Var [$\hat{N}_{b(i,j)}$] is the variance of $\hat{N}_{b(i,j)}$. $\tilde{N}_b$ is the harmonic mean of the $N_{b(i,j)}$. Alleles with a frequency below 0.05 were excluded from the analysis to reduce potential bias.

Year	1996	1997	1998	1999	2000	2001	2002
Pairwise $\tilde{S}$ (above diagonal) and *n* (below diagonal):							
1996		31.2	38.7	40.5	32.2	32.7	26.1
1997	82		50.3	53.4	39.9	40.7	30.9
1998	77	81		80.2	53.1	54.6	38.3
1999	79	81	77		56.6	58.3	40.1
2000	76	80	86	82		42.5	32
2001	83	82	85	79	88		32.5
2002	75	81	83	85	87	86	
Pairwise $\hat{N}_{b(i,j)}$ (above diagonal) and Var [$\hat{N}_{b(i,j)}$] (below diagonal):							
1996		infinity	298.7	146.8	infinity	509.6	176.2
1997	21986		83.6	89.1	82.9	63.3	101.7
1998	13996	10557		632.8	1052.1	124.4	166.1
1999	35422	8289	5864		410.9	256.9	58.4
2000	38274	35865	7868	8804		infinity	185.6
2001	17009	23905	19989	7538	12509		212.1
2002	35984	19100	26129	33484	16690	19587	
$\tilde{N}_b$	= 173.8						

Table 7 Summary of output from program SALMONNb and data for three consecutive years of summer steelhead samples from Lyons Ferry Hatchery. For each pairwise comparison of samples *i* and *j*, $\tilde{S}$ is the harmonic mean sample size, *n* is the number of independent alleles used in the comparison, $\hat{N}_{b(i,j)}$ are the pairwise estimates of N_b, and Var [$\hat{N}_{b(i,j)}$] is the variance of $\hat{N}_{b(i,j)}$. $\tilde{N}_b$ is the harmonic mean of the $N_{b(i,j)}$. Alleles with a frequency below 0.05 were excluded from the analysis to reduce potential bias.

Year	2000	2001	2002
Pairwise $\tilde{S}$ (above diagonal) and *n* (below diagonal):			
2000		96.3	84.2
2001	85		92.2
2002	91	90	
Pairwise $\hat{N}_{b(i,j)}$ (above diagonal) and Var [$\hat{N}_{b(i,j)}$] (below diagonal):			
2000		225.2	114.1
2001	1194.2		132.1
2002	1172.3	1164.3	
$\tilde{N}_b$	= 144.4		

Table 8 Estimates of the effective number of breeders (N_b) for the parental cohorts contributing to juvenile steelhead samples from the Upper Touchet River. Single samples were analyzed using the linkage disequilibrium method (Bartley et al. 1992, Waples 1991).

Sample	N_b	Confidence Interval
1999 NF Touchet	118.1	(107.6 – 130.4)
2000 NF Touchet	206.2	(172.3 – 254.9)
1999 SF Touchet	157.7	(139.0 – 181.2)
1999 Wolf F Touchet	100.8	(92.7 – 110.1)
2000 Coppei Creek	81.2	(71.5 – 93.3)
2000 Robinson Creek	93.8	(81.0 – 110.6)

Genetic distance analysis - Considering the Tucannon and Touchet rivers samples and LFH, analysis of genetic distances among populations revealed two distinct clusters of population samples (Figure 2) with strong bootstrap support (98.5%) for a division between Touchet River and a grouping containing Tucannon River and LFH. Within the Tucannon group, the branch containing LFH had lower bootstrap support (66%) (Figure 2), and contained all but the 98/99 collection.

The analysis was extended to include samples from the upper tributaries of the Touchet River and samples from the Walla Walla River to give a wider geographic perspective. When additional samples were included in the analysis, the same basic genetic relationships remained (Figure 3). The Tucannon River and LFH were distinct from all Walla Walla River populations. Within the Walla Walla River, the Touchet River samples from 2001-2005 formed a distinct cluster within the tree, separated from the remaining samples from the Walla Walla River (Figure 3). In the population tree, the 1999 Touchet sample is placed within the upper Touchet tributary samples, and the 2000 Touchet River sample pairs with the 2000 North Fork Touchet River sample. Additionally, there was bootstrap support for a population cluster containing the Upper Walla Walla River and Mill Creek samples.

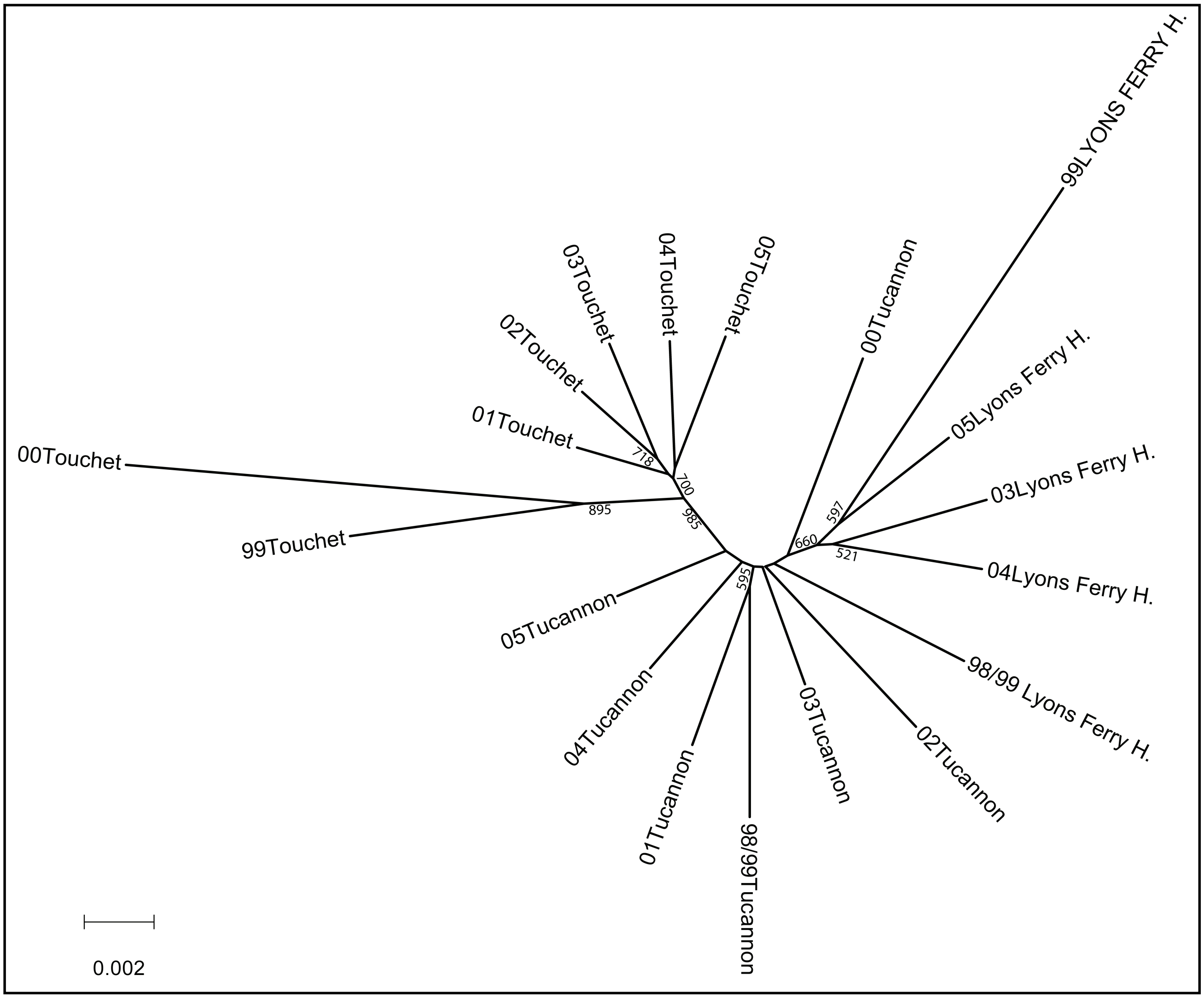

Figure 2. Chord-distance tree for temporally stratified adult samples. Node support numbers are values from bootstrap analysis (1000 bootstraps). Note: only 1999 LFH samples were from juveniles.

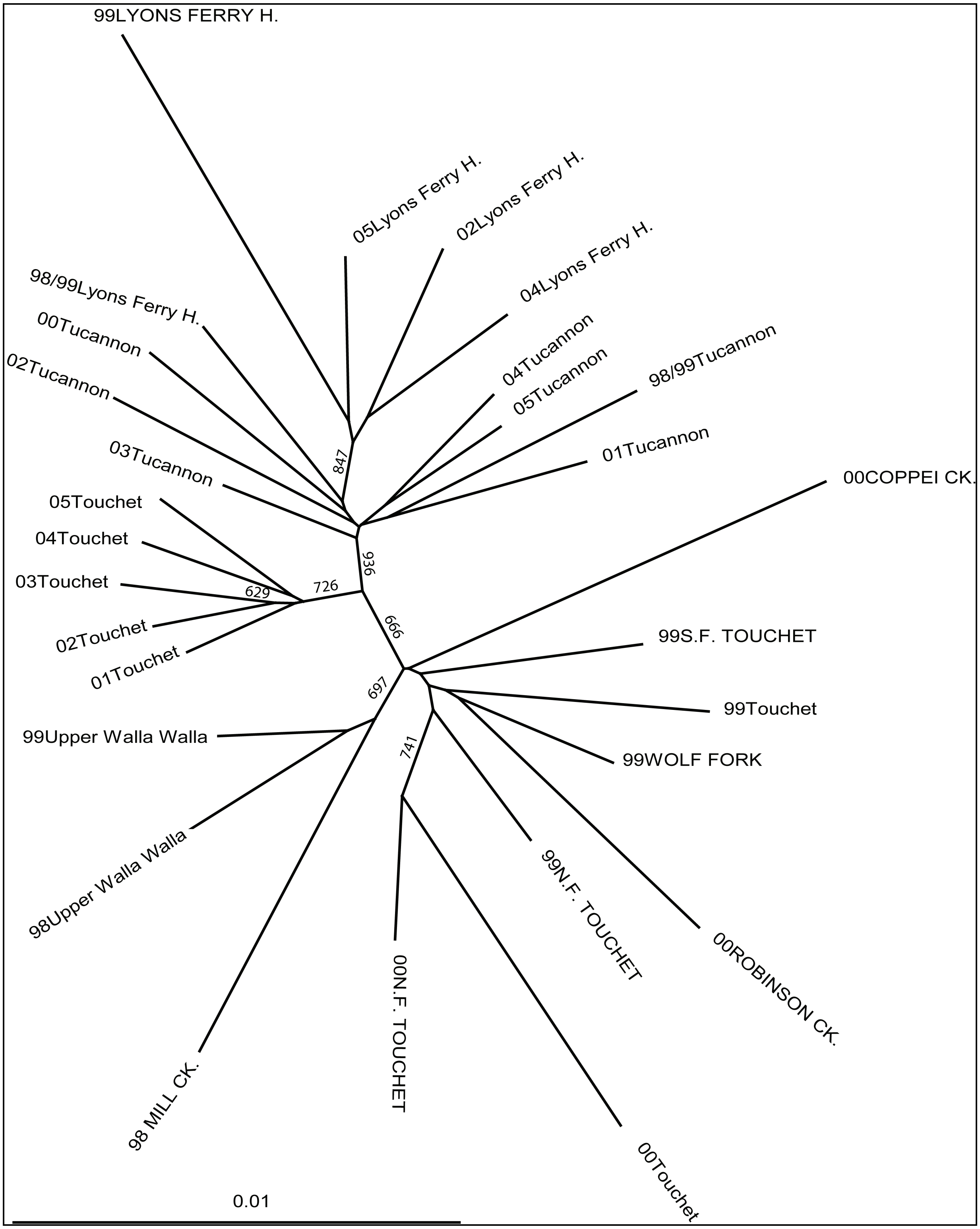

Figure 3. Chord distance tree that includes temporally stratified samples (from Figure 2), plus samples from Touchet River tributaries, Mill Creek, and Walla Walla River. Sample labels with all letters capitalized are juvenile samples. Node support numbers are values from bootstrap analysis (1000 bootstraps).

Individual assignment – Assignment proportions are shown in Table 9. The Tucannon steelhead sample had the lowest self-assignment proportion, 29%, and the highest number of unassigned individuals, 43%. Additionally, 14% assigned to LFH, 9% assigned to the Touchet and 5% assigned to the Upper Walla Walla. The LFH had a 46% self-assignment rate, approximately 10% assignment to Tucannon and Touchet, and 1% assignment to Walla Walla. The Touchet sample had 53% self-assignment, 6% assignment to Tucannon, 5% assignment to LFH, and 5% assignment to Walla Walla. The Walla Walla sample had the highest self-assignment rate, 56%, the fewest number of individuals assigning to LFH, 1%, and the lowest number of unassigned fish, 27%.

Table 9. Individual assignment results reported are the proportions of individuals assigned to each population category, given the assignment LOD was greater than one and the individual's likelihood resided within the 95% confidence interval for the estimated population of origin.

	N	Tucannon	LFH	Touchet	Walla Walla	Unassigned
Tucannon River ……	451	0.29	0.14	0.09	0.05	0.43
Lyons Ferry Hatchery	333	0.10	0.46	0.13	0.01	0.31
Touchet River………	987	0.06	0.05	0.53	0.05	0.30
Walla Walla ………	177	0.04	0.01	0.12	0.56	0.27

Discussion

Results interpreted related to questions from Box 1

Question #1 - Are there significant genetic differences between Tucannon, Touchet, or Walla Walla River endemic steelhead stocks?

Data from both the genic differentiation tests (Table 2 and Table 3) and genetic distance analysis (Figures 2, 3, and 4) address this question. We report that allele frequencies for two natural summer steelhead populations (Tucannon and Touchet Rivers) were stable over seven brood years (Table 2). Therefore, allele frequencies for population samples from a single location (e.g. Tucannon River samples) are statistically equivalent from year to year. With the exception of the pairwise comparison between the 2003 and the 2005 samples, allele frequency estimates from eight consecutive years of Tucannon River collections were statistically equivalent (Table 2). For the Touchet River collections, six of the seven sample years were statistically equivalent and the 2000 sample year (N=30 adults) appears to be anomalous (Table 2). This same observation holds for the Walla Walla samples, where the 1998 Walla Walla sample is not statistically differentiated from the 1999 Walla Walla, although the p-value is low (Table 2). In contrast, most of the between population genic differentiation tests are statistically different. Therefore, the Tucannon, Touchet, and Walla Walla Rivers are genetically distinct (Table 3), although the divergence is slight (Table 4). The genetic distance dendrograms (Figures 2 – 4) also support the conclusion that the Tucannon, Touchet and Walla Walla Rivers are genetically distinct, since samples from the same population cluster together on the tree.

Question #2 - Are there significant genetic differences from any natural steelhead stocks to the Lyons Ferry Hatchery stock?

Contrary to the results for natural population samples, the allele frequency estimates for LFH samples were temporally divergent (Table 2). The only pairwise allele frequency comparison from LFH that was statistically equivalent was between the 98/99 and 2004 samples, although the p-value was low; all other sample comparisons were divergent. Additionally, the 98/99 LFH sample was indistinguishable from the Tucannon River natural samples (Table 3). This observation implies that the 98/99 LFH adult sample, comprised of marked hatchery fish collected at the TFH trap, either had allele frequencies similar to the natural Tucannon River sample by chance, or the natural sample contained many steelhead with hatchery ancestry that year. The later explanation is most likely, since LFH stock hatchery fish were the dominant returns to the area above the hatchery in the years from which the natural origin fish originated. In addition to LFH samples having statistically different allele frequencies by year, Table 3 shows that LFH (with the exception of the 98/99 LFH collection) is divergent from Tucannon, Touchet, and Walla Walla River samples.

Question #3 - How similar are freshwater Age 1 wild Tucannon River adults to Lyons Ferry Hatchery Stock? Are freshwater Age 2 and Age 3 wild adults different in genetic makeup than freshwater Age 1 fish?

During 2000 – 2005, 47 natural origin steelhead were sampled that were freshwater age 1 and N=288 were freshwater age 2 or 3. We tested equivalency of allele frequencies between the two sample sets using FSTAT (i.e. allelic differentiation). The allele frequencies were statistically equivalent (p-value 0.55). Additionally the estimated pairwise F_{ST} was negligible between the two sample groups ($F_{ST} = 0.0008$). These results suggest there is no difference between the steelhead choosing to emigrate after one year in freshwater versus two or more.

Question #4 - How do wild fish collected from lower Tucannon River adult trap compare to wild fish collected at the Tucannon Fish Hatchery trap? Are the lower river collections more similar to Lyons Ferry stock fish?

There were only two sample years that steelhead were collected from both upper and lower Tucannon River traps. For the 2003 sample year, 16 steelhead were sampled at the TFH trap and 65 steelhead were sampled at the lower Tucannon River adult trap. For the 2004 sample year, 8 steelhead were sampled at the TFH trap and 47 steelhead were sampled at the lower Tucannon River adult trap. The substantially different sample sizes for the trapping locations limits the statistical power of comparisons. We subdivided genetic data by trapping location, combining all Tucannon River samples (years 1998/1999 – 2005) collected from the lower Tucannon River adult trap (N=347) and combining steelhead samples from 2003 and 2004 collected from the TFH trap (N=24), and tested equivalency of allele frequencies between the two sample sets using FSTAT (i.e. allelic differentiation). The allele frequencies were statistically equivalent (p-value 0.20). Additionally the pairwise F_{ST} estimated was negligible between the two sample groups. These results suggest there is no difference between the steelhead trapped in the upper or lower Tucannon River traps.

Question #5 - How do the results from this study compare with results and conclusions from Narum et al. 2004? Is there strong evidence for hatchery introgression into either Walla Walla or Touchet steelhead? Is there evidence of hatchery introgression into Coppei Creek steelhead?

We found that Walla Walla River samples were significantly different genetically from Touchet River samples. Narum et al. (2004) reported this result as well. Narum et al. (2004) largely focuses on differentiation between resident and anadromous forms of steelhead within the same stream. Our study did not include resident rainbow populations, so it is difficult to comment on Narum's results regarding resident rainbow; however one sample from our study has mixed ancestry (1998 Mill Creek). That sample likely includes both juvenile steelhead and large resident rainbows from Mill Creek. The 1998 Mill Creek sample is statistically different from both Walla Walla River steelhead samples. Narum et al. (2004) reported heterozygote deficits in the Touchet River. Heterozygote deficit could be the result of population admixture within a sample or an artifact resulting from variable age-at-maturity. We observed heterozygote deficit in the Tucannon, Touchet, and Walla Walla Rivers. The deviations were approximately 5%, and this amount of deficit is typical for salmon populations. Due to low levels of linkage disequilibrium observed in the Tucannon and Touchet River samples, admixture in these population samples is unlikely. The 1999 Walla Walla sample shows elevated levels of linkage disequilibrium compared to the 1998 sample. It is possible the 1999 Walla Walla sample contains resident rainbow (note: samples were all adult steelhead >20 inches in length), but the genetic distance analysis shows the Walla Walla River samples cluster together, and as expected, cluster regionally with the Touchet River samples. Due to general genetic similarity among steelhead sample groups and the absence of resident trout samples in our study, we are unable to test for the presence of rainbow trout in the Walla Walla River samples.

We report results relevant to concerns about introgression of Lyons Ferry Hatchery fish into the Walla Walla River. First are analyses of Molecular Variance (AMOVA) results and second are individual assignment results. The AMOVA results show that 98.70% of genetic variation observed is present within populations, 0.86% is present among population samples within rivers, and 0.44% is present among rivers. In other words, the Tucannon, Touchet, and Walla Walla Rivers are, in general, closely related, so it is difficult to document the migration of alleles (i.e. introgression). It is unlikely that a complete absence of gene flow exists among these groups. Therefore, the hypothesis to test involves the magnitude of gene flow relative to the time of divergence among these populations. We are unable to distinguish between the competing hypotheses of 1) low gene flow over a long time period or 2) high gene flow over a short time period, due to current genetic similarities among these populations. The assignment of individual steelhead to most-likely population of origin elucidates this issue.

The individual assignment results are shown in Table 9. The Walla Walla sample, which contains mainstem and Mill Creek samples, had the highest self-assignment rate: 56%, the fewest number of individuals assigning to LFH: 1%, and the lowest number of unassigned fish: 27%. This result suggests Walla Walla River samples are more distinct from LFH than other population samples in this study (i.e. Touchet or Tucannon Rivers). While the assignment results do not quantify introgression (or migration), the results suggest that there is not a large amount of gene flow between Walla Walla River and LFH. If there were a high migration rate from LFH to Walla Walla River, the expectation would be many misassigned individuals from the Walla Walla to the LFH population. Individual assignment results show that the Touchet population sample has a slightly higher misassignment rate to LFH than the Walla Walla sample (Table 9). Regarding Coppei Creek, a lower Touchet River tributary, 86.7% of individuals assigned correctly back to the Touchet population sample (0 to Tucannon, 3 to LFH, 52 to Touchet, and 5 to Walla Walla), which is a higher assignment rate than the overall Touchet sample. There is not strong evidence for hatchery introgression in the Touchet, Coppei, or Walla Walla from LFH based on the individual assignment results.

There is evidence for hatchery introgression in the Tucannon from LFH based on the individual assignment results. The Tucannon sample has a higher proportion of steelhead misassigned to LFH, as compared to Touchet River misassignments to LFH (Table 9). Additionally, Tucannon had the lowest self-assignment rate, and highest proportion of unassigned fish, so there may be demographic factors affecting genetic diversity in the Tucannon River, such as increased numbers of migrants. This is perhaps not surprising given that in the Tucannon River, LFH stock hatchery fish are essentially allowed access all the way to TFH, which results in a large overlap of spawning area. In the Touchet River, hatchery fish tend to come back to the acclimation pond area, so there is less overlap with the majority of the natural spawning area. Ongoing genetic monitoring of natural steelhead populations would be required to document the introgression of LFH steelhead. Introgression can be inferred by observing specific changes in population allele frequencies. The most superior sampling scheme for a genetic monitoring plan would be to collect population samples (approximately N=50 randomly chosen individuals) every year. All samples would not necessarily be genotyped, but all cohorts would be available if needed. A minimum sampling effort for genetic monitoring would be to collect population samples for three consecutive years every ten years. If more detailed monitoring were required, such as following parentage or the calculation of effective population size, more intensive sampling would be required.

Supplemental Question #1 - How do the endemic stocks from the Tucannon, Touchet, and Walla Walla Rivers compare to other steelhead stocks in the Snake or Columbia River basins?

The genetic distance analysis provides results relevant to this question. For multiple samples collected within rivers, the general conclusion is that the genetic relationships among locations remain consistent across sample years (Figures 2 and 3). When the temporally stratified samples were analyzed (Figure 2), there was strong bootstrap support for the Touchet River sample cluster separate from the Tucannon River and LFH samples on the population tree. Additionally, since all the Tucannon River samples cluster together, and all but one of the LFH samples cluster together, there was support for the conclusion that the genetic relationships among populations was consistent from year-to-year. Stated differently, there was a greater genetic affinity among multiple samples from a single location, than among samples collected the same year from different localities. Yet, there were some observations that conflicted with the general conclusion of phylogenetic consistency.

In Figure 2, a well-supported sub-branch within the Touchet River contains the 1999 and 2000 Touchet River samples. This divergent branch may be a case of long-branch-attraction (Hendy and Penny 1989); since those two samples cluster with upper Touchet River tributary samples when additional groups were included in the analysis (Figure 3). It is possible that the small sample sizes collected in 1999 and 2000 imprecisely estimate the actual allele frequencies for these Touchet River samples, which contributed to their placement outside the Touchet River cluster on the trees. Yet, evidence suggests sample size is not the only issue. First, levels of allelic diversity were not atypical for the 1999 and 2000 samples (Table 1), so the sample sizes capture genetic information similar to the other sample collections. Second, when using individual assignment, a higher proportion of fish from the 1999 and 2000 Touchet samples assign to upper Touchet samples and a lower proportion of these same fish assign to Tucannon, when compared to the remaining Touchet River samples (data not shown). Thus, the genetic constitution appears slightly different between the early and late Touchet samples. This slight difference is probably the result of both small sample sizes and the presence of upper Touchet River (north, south and Wolf forks; Table 1) juvenile samples from those years in the dataset. In general, steelhead reproducing in the upper Touchet River are individuals not collected for broodstock that have been allowed to escape trapping in Dayton, so juveniles produced in the upper Touchet would be genetically related to the Touchet River adult samples. Specifically for the 1999 sample year, all Touchet River adults sampled at the Touchet trap were allowed to escape upriver and spawn naturally, and could even be the parents of the juveniles sampled. This relationship is corroborated by results in Table 2, where the 1999 Touchet sample is largely undifferentiated from the juvenile samples. This sampling effect likely altered the relative genetic distances within the phylogenetic tree. Another complicating factor from a different sampling effect is the possible presence of related individuals within juvenile samples, which may have altered allele frequencies from the contributing parental generation. We did not attempt to remove highly related individuals from the juvenile collections and redo the analysis since there was not strong evidence for increased relatedness within the juvenile samples. Relatedness was surveyed by calculating the pairwise relatedness (Queller and Goodnight 1989) for all individuals in the dataset, calculating the arithmetic mean relatedness, then comparing the actual mean with the mean calculated from the null distribution of unrelated individuals. 1000 datasets of N randomly selected genotypes (without replacement) was used to generate the null distribution of relatedness. The p-values for the actual mean pairwise relatedness values by juvenile population are 0.449 for 1999 NFTouJ, 0.958 for 2000 NFTouJ, 0.974 for 1999 SFTouJ, 0.314 for 1999 WFTouJ, 0.108 for 2000 CoppJ, and 0.856 for 2000 RobTouJ. A p-value of 0.95 is significant at alpha=0.05, and a p-value of 0.99 is significant at alpha=0.01. The p-values for the 2000 NFTouJ and 1999 SFTouJ samples suggest some increased relatedness. A non-significant result indicates that individuals are not more related than expected under the null hypothesis. Additionally, whether the juvenile samples were absent (Figure 2) or present (Figure 3), the 1999 and 2000 Touchet River samples were slightly different from the remaining Touchet River samples. This result would not likely be altered by removing possibly related individuals from the juvenile samples, since the expected result would be the shortening of branch lengths on the dendrogram, not a different topology.

To place the genetic distance results into a broader geographic context, we obtained genotype data for a lower Columbia River steelhead population (2000, 2001 Kalama River), and three Grande Ronde juvenile steelhead samples (2000 Rattlesnake Creek, 2000 Cougar Creek, and 2000 Wallowa stock). Moran and Waples (2004) have published population structure information for Snake River summer steelhead. The 2000 Rattlesnake and 2000 Cougar Creek samples from their study are included in our study. Moran and Waples (2004) also included

Tucannon River steelhead in their study (1991, 1992, and 1995), but the sample years are different from our study. Moran and Waples (2004) did not include any populations from the Columbia or Walla Walla Rivers. Our inclusion of the Kalama and Walla Walla River samples provides a wide geographic scope in our study for comparison with the extensive Snake River survey conducted by Moran and Waples (2004). To enhance comparability between this study and Moran and Waples (2004), we present the genetic distance results as a rectangular dendrogram as in Moran and Waples (2004) (Figure 4); however, note that the genetic distance metric used to construct the dendrograms differs between studies. The five replicated samples from both the Tucannon and Touchet Rivers were combined into single samples, as were the two samples from the Kalama River. Our results corroborate the findings of Moran and Waples (2004), with genetic differentiation observed among Tucannon River steelhead and Grande Ronde samples from Rattlesnake Creek, Cougar Creek, and Wallowa stock. We report substantial differentiation among Kalama River, Tucannon River, Touchet River, Walla Walla River, Grande Ronde River, and LFH. The branching structure of the dendrogram (Figure 4) is well supported by bootstrap analysis.

While the F_{ST} metric should not be interpreted as a genetic distance, documenting the amount of total genetic variance attributed to population subdivision is informative (Table 10). The F_{ST} estimate between Kalama and Tucannon samples is 0.038 and the F_{ST} estimate between Kalama and Touchet samples is 0.037. The mean F_{ST} estimate between Kalama and Walla Walla samples is 0.040. These data suggest that the magnitude of divergence among Kalama River steelhead and more interior steelhead populations are similar. The Grande Ronde samples from Rattlesnake Creek and Wallowa stock had a mean pairwise F_{ST} estimate of 0.02 when compared to Tucannon, Touchet, or Walla Walla samples. The Cougar Creek sample was more divergent, with pairwise F_{ST} estimated as 0.043 for Tucannon River, 0.045 for Touchet River, and 0.050 for Walla Walla River. These data suggest substantial differentiation between the Columbia River and Snake River steelhead, and substantial genetic differentiation between Snake River and Walla Walla River steelhead.

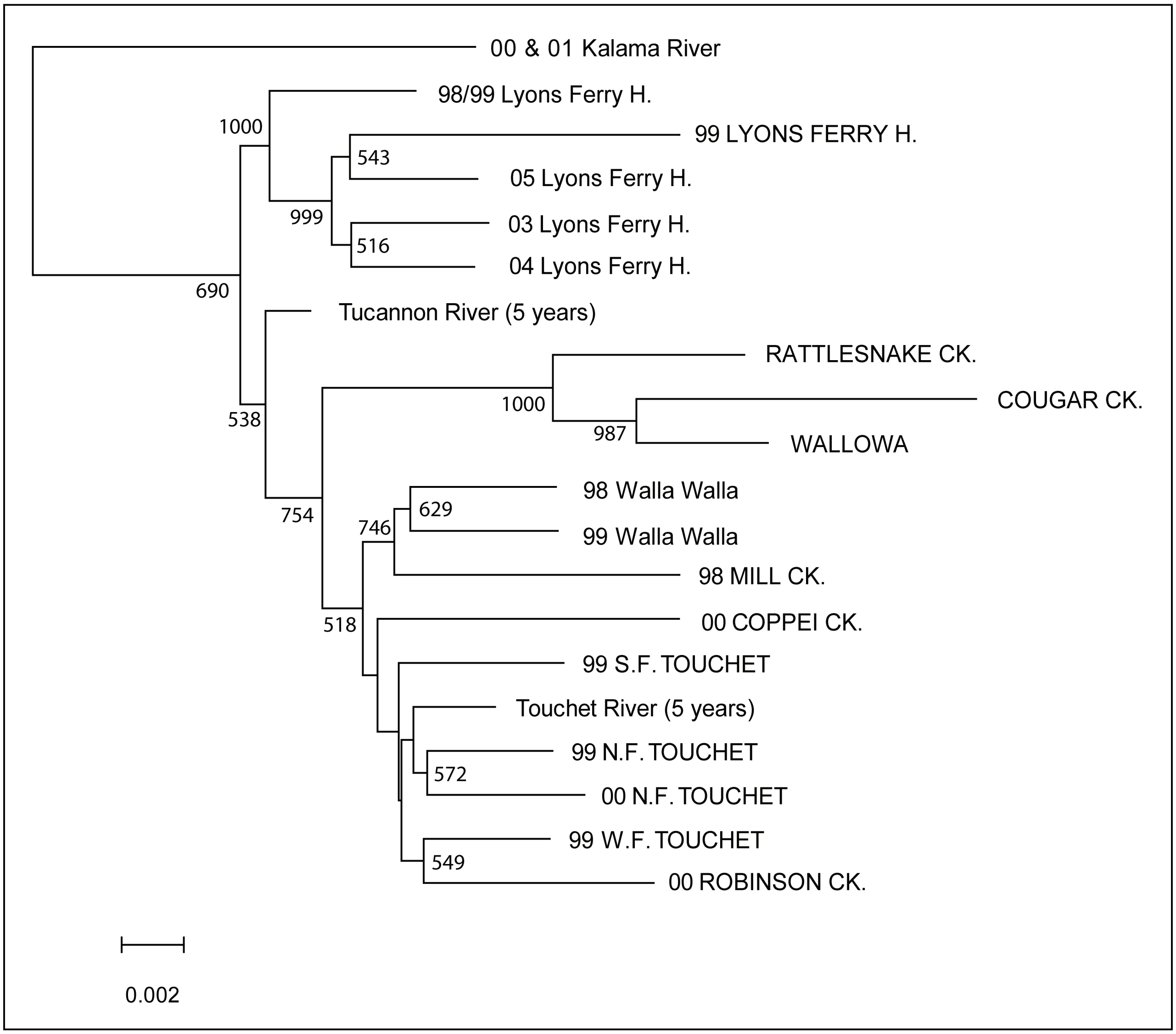

Figure 4. Chord distance tree from steelhead samples from Columbia River, Walla Walla River, and Snake River. Sample labels with all letters capitalized are juvenile samples. Node support numbers are values from bootstrap analysis (1000 bootstraps).

Table 10 Pairwise estimates of F_{ST} for Tucannon, Touchet, LFH, and Walla Walla steelhead compared to Kalama River, Rattlesnake Creek, Cougar Creek, and Wallowa stock.

	Kalama	Rattle	Cougar	Wallowa
Tucannon	0.038	0.017	0.043	0.019
98/99LFH	0.033	0.017	0.039	0.018
99LFH	0.074	0.060	0.087	0.062
03LFH	0.045	0.024	0.054	0.028
04LFH	0.045	0.024	0.055	0.026
05LFH	0.048	0.027	0.054	0.028
Touchet	0.037	0.022	0.045	0.024
99NFTouc	0.040	0.026	0.049	0.027
00NFTouc	0.042	0.027	0.053	0.032
99SFTouc	0.047	0.023	0.049	0.027
99WFTouc	0.040	0.028	0.041	0.028
00Coppei	0.048	0.030	0.052	0.032
00Robins	0.053	0.028	0.050	0.028
98Walla	0.043	0.020	0.056	0.024
99Walla	0.040	0.020	0.048	0.023
98Mill	0.040	0.022	0.052	0.027

Supplemental Question #2 - Given the close similarity between these stocks, how confidently or surely does the data allow us to assign individual fish to the correct location?

For any individual chosen at random, the probability of its genotype belonging to a specific population is based on that population's allele frequencies. Since the steelhead populations in this study are genetically similar, an individual's genotype may have a high likelihood of originating from multiple populations. Of the steelhead sampled from the Tucannon River (N=451) that were assigned based on the LOD > 1 criteria (57%), 29% were correctly assigned back to Tucannon River (Table 7). Of the steelhead sampled from LFH (N=333) that were assigned based on the LOD > 1 criteria (69%), 46% were correctly assigned back to LFH (Table 9). Of the steelhead sampled from the Touchet River (N=987) that were assigned based on the LOD > 1 criteria (70%), 53% were correctly assigned back to Touchet River (Table 9). Of the steelhead sampled from the Walla Walla River (N=177) that were assigned based on the LOD > 1 criteria (73%), 56% were correctly assigned back to Walla Walla River (Table 9). These results suggest that the power to correctly identify an individual steelhead to stock of origin is generally low based on these data; however Walla Walla River exhibits the greatest power and Tucannon River the lowest.

The individual assignment results can be used as a formal power analysis (Table 11). When determining type-1 and type-2 error based on individual assignment, all individuals are assigned (i.e. there is no unassigned fraction based on a LOD criteria). The type-1 error is quantified by observing the number of individuals from a population that misassign to another population. The type-2 error is quantified by observing the number of individuals that are falsely included in a population sample. The power is by definition 1 – type-2 error.

Table 11 Power analysis based on assignment of individual steelhead to stock of origin.

LFH	All samples			Excluding 98/99 LFH and 1999		
	Type-1 Power	Type-2	Power	Type-1	Type-2	
Tucannon River ……	0.670	0.453	0.547	0.615	0.421	0.579
Lyons Ferry Hatchery	0.181	0.423	0.577	0.180	0.420	0.580
Touchet River………	0.175	0.178	0.822	0.168	0.192	0.808
Walla Walla ………	0.376	0.423	0.577	0.370	0.380	0.620

Conservation concerns

The observation of temporal stability of allele frequencies for natural populations and temporal instability at the hatchery suggests that a smaller N_e may exist for the hatchery samples. The BOTTLENECK results corroborate this idea to some degree, as two LFH samples showed heterozygosities in excess of expectations under mutation-drift equilibrium, suggesting a recent reduction in population size. In contrast, when census data is considered for natural and LFH populations, a comparable N_e is expected for natural steelhead and the LFH stock. For brood years between 1998-2006 the harmonic mean of census size was 326.9 for Tucannon River, 336.0 for Touchet River, and 410.0 for LFH (J.D. Bumgarner unpublished data). The census estimate for Tucannon River is likely an underestimate, because for three brood years (1998, 2000, and 2003) the census size was estimated from trapping data not spawning ground surveys. Nevertheless, estimates of N_e calculated for the LFH samples were lower than the natural population samples (Table 5-7). Ratios of the N_e estimated by the temporal method and the harmonic mean of census size are 0.68 for Tucannon River, 0.52 for Touchet River, and 0.35 for LFH. These numbers are consistent with the general thought that N_e is between 0.10 and 0.33 of the estimated census size (Bartley et al. 1992, Waples pers. comm.). Arden and Kapuscinki (2003) found that for 18 brood years of Snow Creek steelhead surveyed, the N_e to N ratio ranged from 0.41 to 0.67, and had an overall harmonic mean of 0.61. We have not yet investigated the possible causes of lower N_e observed for LFH. In general N_e is lower than the N (census size) because of fluctuations in population size, unequal sex ratios, and variance in reproductive success (i.e. the number of offspring produced per individual). The census size of LFH is similar to the natural populations from year to year. Therefore, unequal sex ratios or variance in reproductive success are possible explanations for the slightly lower N_e of LFH.

Since the Tucannon, Touchet, and Walla Walla River summer steelhead are populations of conservation concern, there are management implications to the observations reported. Even though population differentiation was low, in general, the Tucannon, Touchet, and Walla Walla River populations were significantly differentiated, and all groups were differentiated from LFH (Figure 2; Tables 3). Although, the Tucannon River samples were more closely related to the LFH samples, this is likely from 20 years of interbreeding between hatchery and natural fish and not shared ancestry. The LFH stock was developed primarily from Wells Hatchery Stock (upper Columbia River) and the Wallowa stock (Snake River composite). As such, the LFH stock was not historically similar to either Tucannon River or Touchet River fish. Since that is the case, the Tucannon River steelhead and LFH fish have become similar more rapidly then Touchet River steelhead have, given the genetic distance data (Figures 2 and 3). The difference in convergence rates observed between Tucannon River steelhead and LFH steelhead, as compared to Touchet River and LFH, is likely due to differences in the magnitude of recent gene flow (i.e. hatchery introgression). Ecological and genetic data support this supposition. First, juveniles from LFH are released into the Tucannon, Touchet, and Walla Walla Rivers. Yet, historically there was more opportunity for gene flow in the Tucannon River, since hatchery juveniles were released in the vicinity of spawning habitat for natural Tucannon River steelhead and thus may have returned to the spawning area of natural steelhead (Bumgarner et al. 2003). Furthermore, hatchery origin adults that are not brought into the hatchery for spawning are left in the stream to increase sport-fishing opportunities within the Tucannon River. In contrast, LFH stock juveniles are released at the lower end of spawning areas for natural steelhead in both the Touchet and Walla Walla River (Bumgarner et al. 2003). Second, genetic distance and individual assignment results were consistent with differential gene flow between all three natural steelhead populations studied and LFH stock (Figure 3; Table 9). The Tucannon River natural adults were similar to LFH and the Touchet River natural steelhead were divergent from Tucannon River, LFH, and other Walla Walla River samples included in the study. Narum et al. (2004) also observed differentiation between Touchet River and Walla Walla River populations. Figure 3 shows the Touchet River samples as a distinct branch and reliably places that branch between the samples from the Snake River and Walla Walla River. Individual assignment results show that Touchet samples were more distinct from LFH than Tucannon samples, as a higher proportion of Tucannon River fish were misassigned to LFH, as compared to Touchet River misassignments to LFH (Table 9). Additionally, Tucannon had the lowest self-assignment rate, and highest proportion of unassigned fish. While the Tucannon, Touchet and Walla Walla Rivers are all distinct from each other and LFH, we conclude that the collective data provides evidence for hatchery introgression in the Tucannon River, but not the Touchet or Walla Walla rivers. If LFH releases continue in the Tucannon, Touchet, and Walla Walla Rivers, it will be important to continue monitoring the populations for changes in genetic composition, effective population sizes, and estimates of gene flow. The most superior sampling scheme for a genetic monitoring plan would be to collect population samples (approximately N=50 randomly chosen individuals) every year. The age data and tissue should be archived for future analysis.

Acknowledgements

Samples: We would like to thank Jon Germond and Tim Bailey, Oregon Department of Fish and Wildlife, and Larry Boe, U.S. Forest Service, for collecting steelhead from the Upper Walla Walla River and upper Mill Creek, respectively. We would like to thank Dave Karl, Jeremy Trump, Mike Gembala, Jerry Dedloff, and Lance Ross, Washington Department of Fish and Wildlife.

Laboratory: We would like to thank Cherril Bowman, Norm Switzler, and Judy Morgan for laboratory analysis

Reviews: We would like to thank Denise Hawkins and Ken Warheit for helpful reviews.

Funding: Funding for this project was provided by the Lower Snake River Compensation Plan Program, Bonneville Power Administration, and WDFW through the Washington State General Fund.

Literature Cited

Arden WR and Kapuscinski AR (2003) Demographic and genetic estimates of effective population size (N_e) reveals genetic compensation in steelhead trout. Molecular Ecology 12: 35-49

Banks MA, Blouin MS, Baldwin BA et al (1999) Isolation and inheritance of novel microsatellites in chinook salmon (Oncorhynchus tschawytscha). Journal of Heredity 90:281-288.

Banks MA, Rashbrook VK, Calavetta MJ et al (2000) Analysis of microsatellite DNA resolves genetic structure and diversity of chinook salmon (Oncorhynchus tshawytscha) in California's Central Valley. Canadian Journal of Fisheries and Aquatic Sciences 57:915-927.

Bartley D, Bentley B, Brodziak J et al (1992) Geographic variation in population genetic structure of chinook salmon from California and Oregon. Fishery Bulletin, U.S. 90:77-100.

Belkhir K, Borsa P, Chikhi L et al (2001) 1996-2001 GENETIX 4.02, logiciel sous Windows TM pour la Génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier (France).

Busack C, Pearsons T, Knudsen C et al (1997) Yakima Fisheries Project Spring Chinook Supplementation Monitoring Plan. U. S. Department of Energy, Bonneville Power Administration, BPA Report DOE/BP-64878-1, Pp. 176.

Busby PJ, Wainwright TC, Bryant GJ et al (1997) Status Review of West Coast Steelhead from Washington, Idaho, Oregon, and California. U.S. Dept. of Commerce, NMFS-NWFSC-27, Pp. 275.

Bumgarner J, Small MP, Ross L et al (2003) Lyons Ferry Complex Hatchery Evaluation: summer steelhead and trout report 2001 and 2002 run years. Washington Department Fish and Wildlife, Annual Report Number: FPA03-15.

Bumgarner JD, Dedloff J, Herr M (2006) Lyons Ferry Complex Hatchery Evaluation: Summer Steelhead Annual Report 2004 Run Year to USFWS Lower Snake River Compensation Plan Office, Boise, ID. FPA 06-01. 46pp.

Campton, DE (1987) Natural hybridisation and introgression in fishes: methods of detection and genetic interpretations. In: Ryman, N and Utter, F (Eds.) Population genetics and fisheries management. Washington Sea Grant Program, University of Washington Press, Seattle, USA.

Carlsson J, Nilsson J (2000) Population genetic structure of brown trout (Salmo trutta L.) within a northern boreal forest stream. Hereditas 132:173–181.

Cavalli-Sforza LL, Edwards AWF (1967) Phylogenetic analysis: models and estimation procedures. Evolution 32:550-570.

Estoup A, Rousset F, Michalakis Y et al (1998) Comparative analysis of microsatellite and allozyme markers: a case study investigating microgeographic differentiation in brown trout (Salmo trutta). Molecular Ecology 7:339-353.

Excoffier L, Smouse P, and Quattro J (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131:479-491.

Excoffier L, Laval G, and Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47-50.

Felsenstein J (2005) PHYLIP (Phylogeny Inference Package). Department of Genome Sciences, University of Washington, Seattle.

Frankham R, Ballou JD, Briscoe DA (2002). Introduction to Conservation Genetics, Cambridge University Press, Cambridge, UK.

Goudet J (1995) FSTAT (Version 1.2): A computer program to calculate F-statistics. Journal of Heredity 86: 485-486.

Hansen MM, Ruzzante DE, Nielsen E et al (2002) Long-term effective population sizes, temporal stability of genetic composition and potential for local adaptation in anadromous brown trout (Salmo trutta) populations. Molecular Ecology 11:2523-2535.

Hedgecock D, Chow V, Waples RS (1992) Effective population numbers of shellfish broodstocks estimated from temporal variance in allelic frequencies. Aquaculture 108:215–232.

Hedrick PW, (1983) Genetics of Populations, Science Books International, Boston

Hendy, MD and Penny, D. (1989) A framework for the quantitative study of evolutionary trees. Systematic Zoology 38: 297–309.

Jensen LF, Hansen MM, Carlsson J et al (2005) Spatial and temporal genetic differentiation and effective population size of brown trout (Salmo trutta, L.) in small Danish rivers. Conservation Genetics 6:615-621.

Laikre L, Jarvi T, Johansson L et al (2002) Spatial and temporal population structure of sea trout at the Island of Gotland, Sweden, delineated from mitochondrial DNA. Journal of Fish Biology 60:49–71.

Lessios HA, Weinberg JR, Starczak VR (1994) Temporal Variation in Populations of the Marine Isopod Excirolana: How Stable are Gene Frequencies and Morphology? Evolution 48:549-563.

Moran P, Waples RS (2004) Monitor and Evaluate the Genetic Characteristics of Supplemented Salmon and Steelhead, 1999-2003 Progress Report, Project No. 198909600. U. S. Department of Energy, Bonneville Power Administration, BPA Report DOE/BP-00015834-1.

Morris DB, Richard KR, Wright JM (1996) Microsatellites from rainbow trout (Oncorhynchus mykiss) and their use for genetic study of salmonids. Canadian Journal of Fisheries & Aquatic Sciences 53:120-126.

Narum SR, Contor C, Talbot A, et al (2004) Genetic divergence of sympatric resident and anadromous forms of Oncorhynchus mykiss in the Walla Walla River, U.S.A. Journal of Fish Biology 65: 471-488.

Nei M (1987) Molecular Evolutionary Genetics, Columbia University Press, New York.

Nei M, Tajima F (1981) Genetic drift and estimation of effective population size. Genetics 98: 625–640.

Nelson RJ, Beacham TD (1999) Isolation and cross species amplification of microsatellite loci useful for study of Pacific salmon. Animal Genetics 30:228-229.

Nielsen EE, Hansen MM, Loeschcke V (1999) Genetic variation in time and space: microsatellite analysis of extinct and extant populations of Atlantic salmon. Evolution 53:261–268.

Olsen JB, Wilson SL, Kretschmer EJ et al (2000) Characterization of 14 tetranucleotide microsatellite loci derived from sockeye salmon. Molecular Ecology 9:2185-2187.

Østergaard S, Hansen MM, Loeschcke V et al (2003) Long-term temporal changes of genetic composition in brown trout (Salmo trutta L.) populations inhabiting an unstable environment. Molecular Ecology 12:3123–3135.

Paetkau D, Slade R, Burden M et al (2004) Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. Molecular Ecology 13:55-65.

Palm S, Laikre L, Jorde PE, et al (2003) Effective population size and temporal genetic change in stream resident brown trout (Salmo trutta, L.). Conservation Genetics 4:249-264.

Park SDE (2001) Trypanotolerance in West African Cattle and the Population Genetic Effects of Selection. University of Dublin.

Peel D, Ovenden JR, Peel SL (2004) NeEstimator: software for estimating effective population size, Version 1.3. Queensland Government, Department of Primary Industries and Fisheries

Piry S, Alapetite A, Cornuet JM, et al (2004) GENECLASS2: A software for genetic assignment and first-generation migrant detection. Journal of Heredity 95:536-539.

Piry S, Luikart G, Cornuet JM (1998) BOTTLENECK, a program for detecting recent population effective population size reductions from allele frequency data, Available from http://www.ensam.inra.fr.URLB.

Pollack E (1983) A new method for estimating the effective population size from allele frequency changes. Genetics 104:531-548

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution 43(2):258-275.

Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Sciences 94:9197-9201.

Raymond M, Rousset F (1995) GENEPOP (Version-1.2) - Population-Genetics Software for Exact Tests and Ecumenicism. Journal of Heredity 86:248-249.

Rexroad CE, Coleman RL, Martin AM et al (2001) Thirty-five polymorphic microsatellite markers for rainbow trout (Oncorhynchus mykiss). Animal Genetics 32:317-319.

Ryman N, Laikre L (1991) Effects of supportive breeding on the genetically effective population size. Conservation Biology 5:325-329.

Small MP, Beacham TD, Withler RE et al (1998) Discriminating coho salmon (Oncorhynchus kisutch) populations within the Fraser River, British Columbia. Molecular Ecology 7:141-155.

Sokal RR, Rohlf FJ (2003) Biometry, W. H. Freeman and Company, New York.

Spies IB, Brasier DJ, O'Reilly PT et al (2005) Development and characterization of novel tetra-, tri-, and dinucleotide microsatellite markers in rainbow trout (Oncorhynchus mykiss). Molecular Ecology 5:278-281.

Tajima F (1992) Statistical method for estimating the effective population size in Pacific salmon. Journal of Heredity 83: 309-311

Tessier N, Bernatchez L (1999) Stability of population structure and genetic diversity across generations assessed by microsatellites among sympatric populations of landlocked Atlantic salmon (Salmo salar L.). Molecular Ecology 8:169-179.

Waples RS (1990) Conservation genetics of Pacific salmon: III. estimating effective population size. Journal of Heredity, 81: 277-289

Waples R (1991) Genetic methods for estimating the effective size of cetacean populations. Report of the International Whaling Commission, Special issue 13, Pp. 279-300.

Waples RS, Johnson OW, Aebersold PB et al (1993) A genetic monitoring and evaluation program for supplemented populations of salmon and steelhead in the Snake River Basin. Annual report. Bonneville Power Administration. DE-A179-89BP0091. Pp. 41

Waples RS (1998) Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. Heredity 89:438-450.

Waples R (2005) Genetic estimates of contemporary effective population size: to what time periods do the estimates apply? Molecular Ecology 14:3335-3352.

Waples RS, Masuda M, and Pella J (2007) SALMONNb: a program for computing cohort-specific effective population sizes (N_b) in Pacific salmon and other semelparous species using the temporal method. Molecular Ecology Notes 7: 21-24

Weir BS (1979) Inferences about linkage disequilibrium. Biometrics 35:235-254.

Weir BS, Cockerham CC (1984) Estimating F-Statistics for the Analysis of Population-Structure. Evolution 38:1358-1370.

Weir B, Hill W (1980) Effect of mating structure on variation in linkage disequilibrium. Genetics 95:477-488.

APPENDIX 1

Allele size range (S), Total number of alleles (A_T), sample size (N), observed heterozygosity (H_O),
unbiased heterozygosity (H_E) by locus for multi-year samples of summer steelhead from Tucannon River, Lyons Ferry Hatchery, and Touchet River

	Tucannon River						
Locus	98/99	2000	2001	2002	2003	2004	2005
One-102							
S	188-285	188-289	188-261	188-285	188-290	188-285	188-285
A_T	17	21	19	20	22	22	23
N	36	45	51	45	85	69	127
H_O	0.778	0.909	0.843	0.842	0.941	0.971	0.921
H_E	0.901	0.919	0.919	0.924	0.912	0.927	0.917
Ots-100							
S	168-203	168-215	168-215	168-219	168-215	168-215	160-215
A_T	10	15	13	19	12	16	17
N	36	45	51	45	85	69	127
H_O	0.778	0.844	0.824	0.864	0.795	0.826	0.787
H_E	0.808	0.833	0.825	0.848	0.816	0.843	0.848
One-114							
S	189-260	181-272	189-280	181-280	189-280	189-281	189-345
A_T	17	21	17	22	21	19	22
N	36	45	51	45	85	69	127
H_O	0.917	0.864	0.961	0.932	0.940	0.891	0.960
H_E	0.928	0.927	0.922	0.947	0.927	0.932	0.929
One-101							
S	119-222	119-222	119-166	119-198	119-235	119-230	119-254
A_T	3	6	4	4	6	9	8
N	36	45	51	45	85	69	127
H_O	0.314	0.405	0.392	0.432	0.482	0.448	0.405
H_E	0.312	0.449	0.393	0.514	0.421	0.461	0.374
One-108							
S	169-265	169-261	169-269	169-257	169-261	169-249	169-261
A_T	17	17	17	18	18	19	21
N	36	45	51	45	85	69	127
H_O	0.771	0.833	0.804	0.762	0.777	0.833	0.873
H_E	0.918	0.926	0.932	0.923	0.923	0.907	0.921

Ots-103								
	S	74-90	56-90	60-90	74-94	74-90	60-90	60-90
	A_T	4	6	6	5	4	5	6
	N	36	45	51	45	85	69	127
	H_O	0.314	0.262	0.300	0.273	0.294	0.169	0.238
	H_E	0.347	0.260	0.306	0.251	0.275	0.161	0.240
Ots-1								
	S	162-245	164-247	162-245	162-247	164-245	158-247	162-249
	A_T	13	12	11	11	13	16	17
	N	36	45	51	45	85	69	127
	H_O	0.639	0.591	0.740	0.861	0.747	0.725	0.646
	H_E	0.829	0.826	0.811	0.841	0.810	0.836	0.836
Omy-77								
	S	99-134	101-134	99-134	97-140	99-147	99-147	99-147
	A_T	14	16	16	19	17	18	19
	N	36	45	51	45	85	69	127
	H_O	0.889	0.711	0.776	0.886	0.747	0.818	0.819
	H_E	0.894	0.894	0.912	0.899	0.891	0.922	0.908
Ots-3M								
	S	134-145	134-147	128-147	132-156	132-156	128-145	134-156
	A_T	6	7	8	8	8	8	7
	N	36	45	51	45	85	69	127
	H_O	0.667	0.756	0.766	0.636	0.750	0.696	0.701
	H_E	0.735	0.723	0.746	0.741	0.713	0.717	0.728
Omy-1001								
	S	181-224	167-224	175-224	175-224	167-224	162-224	167-228
	A_T	14	20	18	16	20	19	26
	N	36	45	51	45	85	69	127
	H_O	0.889	0.889	0.922	1.000	0.868	0.925	0.929
	H_E	0.905	0.917	0.918	0.929	0.921	0.922	0.932
Omm-1128								
	S	206-337	211-345	211-365	227-357	223-388	207-357	207-373
	A_T	28	20	26	24	29	30	34
	N	36	45	51	45	85	69	127
	H_O	0.857	0.775	0.896	0.906	0.817	0.853	0.889
	H_E	0.959	0.940	0.953	0.950	0.955	0.949	0.948
Omm-1130								
	S	200-372	197-387	197-379	200-341	197-379	197-368	197-379
	A_T	23	31	30	26	36	29	44

N	36	45	51	45	85	69	127
H_O	0.944	0.978	0.922	0.884	0.964	0.927	0.969
H_E	0.946	0.961	0.956	0.958	0.955	0.953	0.961
Omm-1070							
S	164-369	164-384	164-354	164-330	172-369	164-358	164-384
A_T	25	30	25	22	34	30	37
N	36	45	51	45	85	69	127
H_O	0.944	0.933	0.902	0.886	0.817	0.809	0.832
H_E	0.956	0.963	0.947	0.948	0.957	0.945	0.955
Omy-1011							
S	151-203	138-210	138-249	138-206	138-206	138-214	138-206
A_T	14	16	19	13	16	16	17
N	36	45	51	45	85	69	127
H_O	1.000	0.844	0.880	0.839	0.868	0.853	0.873
H_E	0.885	0.897	0.900	0.890	0.874	0.902	0.908

	Lyons Ferry Hatchery				
Locus	98/99	1999	2003	2004	2005
One-102					
S	188-285	188-289	188-285	188-290	188-290
A_T	19	18	20	22	20
N	45	48	100	100	100
H_O	0.884	0.875	0.860	0.940	0.910
H_E	0.924	0.926	0.916	0.919	0.907
Ots-100					
S	168-215	168-203	168-215	168-203	168-215
A_T	14	12	12	11	14
N	45	48	100	100	100
H_O	0.829	0.830	0.727	0.800	0.830
H_E	0.854	0.846	0.768	0.828	0.844
One-114					
S	181-280	189-280	177-280	189-276	189-281
A_T	20	18	22	18	21
N	45	48	100	100	100
H_O	0.905	0.875	0.878	0.869	0.960
H_E	0.938	0.931	0.931	0.919	0.933
One-101					

S	119-131	119-178	119-230	119-254	119-230
A_T	3	3	5	7	4
N	45	48	100	100	100
H_O	0.415	0.319	0.392	0.340	0.290
H_E	0.397	0.394	0.405	0.363	0.324
One-108					
S	169-245	177-245	169-245	169-245	169-244
A_T	18	15	17	16	17
N	45	48	100	100	100
H_O	0.886	0.830	0.792	0.788	0.880
H_E	0.930	0.889	0.892	0.908	0.906
Ots-103					
S	74-94	65-90	60-90	78-90	78-90
A_T	5	4	6	4	4
N	45	48	100	100	100
H_O	0.326	0.174	0.250	0.181	0.380
H_E	0.290	0.165	0.231	0.207	0.336
Ots-1					
S	122-247	120-247	162-247	162-247	162-247
A_T	12	13	12	14	12
N	45	48	100	100	100
H_O	0.857	0.702	0.687	0.776	0.680
H_E	0.850	0.860	0.852	0.849	0.833
Omy-77					
S	99-138	101-134	99-134	103-147	103-134
A_T	16	12	15	16	12
N	45	48	100	100	100
H_O	0.844	0.766	0.849	0.814	0.730
H_E	0.915	0.873	0.895	0.917	0.887
Ots-3M					
S	132-145	132-145	132-156	132-156	132-156
A_T	7	7	8	8	8
N	45	48	100	100	100
H_O	0.698	0.617	0.753	0.794	0.830
H_E	0.750	0.748	0.758	0.758	0.799
Omy-1001					
S	167-224	167-224	167-224	175-224	175-224
A_T	19	17	17	18	18
N	45	48	100	100	100
H_O	0.875	0.936	0.901	0.950	0.940

	H_E	0.928	0.884	0.911	0.914	0.914	
Omm-1128							
	S	211-341	231-300	211-350	211-365	211-350	
	A_T	24	7	25	29	27	
	N	45	48	100	100	100	
	H_O	0.974	0.174	0.935	0.930	0.900	
	H_E	0.942	0.243	0.928	0.935	0.935	
Omm-1130							
	S	197-383	197-368	197-304	197-383	197-379	
	A_T	27	25	25	27	30	
	N	45	48	100	100	100	
	H_O	0.900	0.938	0.794	0.939	0.930	
	H_E	0.953	0.932	0.933	0.943	0.938	
Omm-1070							
	S	164-369	164-334	164-322	164-384	164-384	
	A_T	26	20	22	31	29	
	N	45	48	100	100	100	
	H_O	0.889	0.875	0.798	0.849	0.930	
	H_E	0.949	0.924	0.927	0.932	0.944	
Omy-1011							
	S	138-199	138-230	138-203	138-199	138-203	
	A_T	14	15	15	14	15	
	N	45	48	100	100	100	
	H_O	0.857	0.915	0.923	0.869	0.910	
	H_E	0.912	0.920	0.895	0.894	0.899	

		Touchet River						
Locus		1999	2000	2001	2002	2003	2004	2005
One-102								
	S	192-253	188-277	188-285	188-285	188-277	188-277	188-285
	A_T	14	15	23	22	21	21	22
	N	33	30	116	85	73	96	75
	H_O	0.893	0.900	0.887	0.868	0.940	0.925	0.880
	H_E	0.897	0.911	0.911	0.911	0.929	0.913	0.897
Ots-100								
	S	168-211	168-205	160-209	168-215	168-215	168-211	160-224
	A_T	11	11	13	15	13	13	16
	N	33	30	116	85	73	96	75

H_O	0.857	0.862	0.868	0.840	0.868	0.813	0.867
H_E	0.871	0.860	0.854	0.856	0.860	0.822	0.868
One-114							
S	189-280	189-256	185-272	181-260	189-260	185-281	189-272
A_T	20	16	21	19	18	22	19
N	33	30	116	85	73	96	75
H_O	0.893	0.931	0.876	0.904	0.846	0.883	0.946
H_E	0.923	0.904	0.907	0.910	0.897	0.916	0.918
One-101							
S	119-127	116-127	119-239	119-235	119-239	119-254	119-262
A_T	2	4	8	5	5	6	9
N	33	30	116	85	73	96	75
H_O	0.394	0.300	0.489	0.381	0.386	0.458	0.514
H_E	0.416	0.606	0.521	0.420	0.457	0.494	0.562
One-108							
S	169-269	181-257	169-269	169-269	169-261	169-317	169-267
A_T	15	13	21	22	17	19	20
N	33	30	116	85	73	96	75
H_O	0.849	0.800	0.770	0.918	0.843	0.819	0.800
H_E	0.895	0.874	0.883	0.917	0.891	0.881	0.905
Ots-103							
S	56-90	82-90	60-90	60-90	60-90	60-90	60-90
A_T	7	3	5	5	5	5	5
N	3	30	116	85	73	96	75
H_O	0.333	0.200	0.228	0.262	0.167	0.263	0.247
H_E	0.303	0.188	0.243	0.258	0.158	0.266	0.248
Ots-1							
S	164-247	158-245	164-247	158-245	164-247	164-256	158-247
A_T	10	10	11	14	11	13	12
N	3	30	116	85	73	96	75
H_O	0.594	0.767	0.711	0.812	0.753	0.821	0.773
H_E	0.844	0.834	0.848	0.869	0.859	0.858	0.853
Omy-77							
S	99-134	99-147	103-134	99-147	99-134	97-134	99-147
A_T	14	15	15	15	14	18	16
N	3	30	116	85	73	96	75
H_O	0.700	0.828	0.830	0.777	0.781	0.844	0.853
H_E	0.909	0.900	0.877	0.887	0.882	0.902	0.897
Ots-3M							

S	134-156	132-145	136-147	132-147	134-147	134-145	134-145
A_T	6	7	6	8	7	6	6
N	3	30	116	85	73	96	75
H_O	0.719	0.571	0.705	0.729	0.603	0.635	0.667
H_E	0.756	0.655	0.659	0.702	0.668	0.702	0.702
Omy-1001							
S	167-216	167-216	167-228	167-228	167-228	167-224	167-228
A_T	14	15	20	17	20	18	18
N	3	30	116	85	73	96	75
H_O	0.906	0.897	0.948	0.916	0.932	0.874	0.947
H_E	0.908	0.910	0.921	0.907	0.917	0.919	0.918
Omm-1128							
S	223-357	223-329	206-337	215-365	215-373	207-388	207-369
A_T	27	25	31	25	27	33	32
N	3	30	116	85	73	96	75
H_O	0.879	0.931	0.904	0.847	0.836	0.915	0.878
H_E	0.962	0.967	0.946	0.936	0.947	0.950	0.947
Omm-1130							
S	197-376	197-376	197-376	197-379	197-379	197-379	197-383
A_T	30	25	35	35	32	34	35
N	3	30	116	85	73	96	75
H_O	0.970	0.933	0.917	0.940	0.890	0.926	0.920
H_E	0.968	0.949	0.958	0.955	0.949	0.964	0.963
Omm-1070							
S	164-334	164-334	164-322	164-354	164-330	164-354	164-354
A_T	26	24	27	27	29	29	28
N	3	30	116	85	73	96	75
H_O	0.788	1.000	0.759	0.732	0.729	0.830	0.867
H_E	0.931	0.949	0.937	0.940	0.946	0.943	0.943
Omy-1011							
S	147-230	138-203	138-210	138-203	138-206	134-210	138-210
A_T	14	14	17	16	16	19	17
N	3	30	116	85	73	96	75
H_O	0.939	0.867	0.872	0.963	0.900	0.895	0.901
H_E	0.890	0.864	0.889	0.889	0.881	0.899	0.900