Supplementation, Monitoring, and Evaluation Program

Annual Report

October 2007 – September 2008

Prepared by:

Lytle P. Denny and Kurt A. Tardy

Shoshone-Bannock Tribes
Fish and Wildlife Department
3rd and B Avenue
Fort Hall, Idaho
(208) 239-4555

Prepared for:

United States Fish and Wildlife Service
Lower Snake River Compensation Plan
1387 S. Vinnell Way, Suite 343
Boise, Idaho 83709

Cooperative Agreement 141108J014

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EXECUTIVE SUMMARY

Chinook salmon (*Oncorhynchus tshawytscha*) and steelhead (*Oncorhynchus mykiss*) are cultural and social symbols for the Shoshone-Bannock Tribes (Tribes) and historically provided annual subsistence fisheries in the Columbia River basin. Despite the significance of these fish to the Tribes, there has been widespread and dramatic decline in abundance, distribution, genetic diversity, and productivity of Chinook salmon and steelhead in the Salmon River sub-basin, resulting in both species being listed as threatened under the Endangered Species Act (ESA) in 1992 and 1997, respectively. In response to declining anadromous fish runs, the Tribes initiated several artificial propagation (i.e. supplementation) programs designed to improve runs, re-distribute fish, and improve natural populations so that Tribal fisheries can be maintained at higher than existing levels.

The Tribes acquired funding from the Lower Snake River Compensation Plan (LSRCP) in fiscal year 2008 for participation in planning activities associated with anadromous fish, utilization of supplementation as an artificial propagation strategy, monitoring and evaluation of supplementation activities, and development of future programs. Under Cooperative Agreement #141108J014, the Tribes achieved multiple goals by completing numerous objectives, tasks, and activities from October 1, 2007 through September 30, 2008.

Continuous coordination and planning between the Tribes and LSCRP is necessary to maximize efforts for Chinook salmon and steelhead artificial propagation, fish health, harvest, and monitoring and evaluation. Cooperative efforts between the Tribes, LSRCP, and other agencies will ultimately promote the existence and recovery of ESA listed species in the Snake River Basin.

The Tribes maintain that supplementation can increase abundance, distribution, genetic diversity, and productivity of anadromous salmonids. Specific programs established to increase the aforementioned performance measures include: Dollar Creek Eggbox Program; Steelhead Smolt Supplementation Program; and Steelhead Streamside Incubation Program.

Supplementation projects were designed to utilize adaptive management to improve and guide future restoration activities. This report provides detailed information for each project funded by the LSRCP in FY 2008; the Yankee Fork Chinook Salmon Program is covered in the 2008 Annual Run Report.

Authors:

Lytle P. Denny
Anadromous Fish Manager

Kurt A. Tardy
Anadromous Fish Biologist

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CHAPTER 1. DOLLAR CREEK CHINOOK SALMON SUPPLEMENTATION PROGRAM

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ABSTRACT

The Tribes initiated an in-stream Chinook salmon supplementation program (DCCSS) in 1997 to utilize excess or surplus summer Chinook salmon production from McCall Fish Hatchery (MFH) to help maintain, rehabilitate, and enhance summer Chinook in tributary habitats of the South Fork Salmon River (SFSR). This report covers accomplishments resulting from broodyear 2008.

In 2008, IDFG spawned 78 pairs of Chinook salmon at the South Fork Satellite Facility for the Tribes. There were a total of 323,015 eyed salmon eggs outplanted into five in-stream egg incubators in Dollar Creek, a tributary to the SFSR. Of the five total incubators, the Tribes believe all successfully produced fry that volitionally emigrated into the stream. Due to limited funding, staff was unable to enumerate dead eggs to determine an approximate hatch success for 2008. However, the Tribes applied the 85% success historical average of the program to the five effective incubators and estimated that approximately 274,563 fry were seeded in Dollar Creek.

Through minimal previous monitor and evaluation activities, in-stream egg incubation appear most effective in glide habitats with specified ranges for temperature, dissolved oxygen, conductivity, pH, flow, and sediment accumulation.

ACKNOWLEDGEMENTS

The Tribes thank Scott Marshall and staff at the LSRCP-Office for providing funding to further support this program. Special thanks to IDFG personnel including Sharon Kiefer, Sam Sharr, Tom Rogers, Donald McPherson, Joel Patterson, Steven Kammeyer, and Doug Munson for their support. We especially thank Mike DeLarm for providing technical expertise and commitment to this program.

The Tribes provided the administrative framework for this project to be successful. We would like to thank the Fish and Wildlife Department personnel Scott Brandt, Carlos Lopez, Alex Graves, and Josh Mendez for project operations and monitoring and evaluation. Thanks to Hal Hayball of the Shoshone-Bannock Tribes for furnishing maps.

INTRODUCTION

As part of the Tribes effort to maintain, rehabilitate, and enhance Chinook salmon populations, an in-stream egg incubation (eggbox) supplementation program was initiated in the SFSR in 1997 to increase abundance of Chinook salmon. Since there is already a successful hatchery program in the SFSR, the Tribes are focused on hatchery reform since natural-origin Chinook salmon are currently listed under the ESA and warrant further attention.

Prior to 1997, the Tribes position was to utilize hatchery fish to rebuild wild fish populations. The clear imbalance of excessive hatchery fish and depleted natural-origin Chinook in the SFSR led the Tribes to analyze alternatives for increasing abundance of natural-origin fish, where the problem was exemplified. The MFH Chinook salmon smolt program was previously capped by the addition of the Johnson Creek Artificial Propagation Enhancement (JCAPE) Program at 1.1 million smolts (AOP 2007); however, the Tribes identified additional egg incubation space at MFH and excess adult production.

The eggbox program is designed to utilize surplus hatchery production (i.e. adults and eggs) in an effort to increase abundance of natural origin fish under the assumption that hatchery fish can rebuild natural origin fish populations contingent that hatchery eggs are incubated in stream water and hatched fry enter the stream in a manner and time as fry produced naturally. Surprisingly, there is little research on the variation in adult reproductive success as a function of spawning date, and the relationship between fry emergence and survival. Therefore, gametes for this program are collected from the entire salmon run to maximize genetic diversity and adaptability to the natural environment and further identify factors that limit production.

Under Cooperative Agreement 141108J014, the Tribes monitored and evaluated in-stream eggboxes containing broodyear 2008 eyed summer run Chinook salmon eggs and released Chinook salmon fry into Dollar Creek to test the following hypotheses:

Hypothesis 1: The eggbox program produces F1 juvenile parr and smolt offspring.

Hypothesis 2: F1 eggbox individuals survive and successfully produce F2 progeny.

Hypothesis 3: Emergence timing of juvenile Chinook salmon originating from eggboxes is the same as naturally spawned Chinook.

Hypothesis 4: The size of emigrating parr and smolts originating from eggboxes is the same as those from naturally spawned adults.

Hypothesis 5: The relative productivity from eggboxes compared to naturally deposited eggs is the same at various program sizes and natural deposition rates.

Goal and Objectives

The goal of the DCCSS program is to investigate whether in-stream incubation can increase adult returns of summer Chinook in the South Fork Salmon River.

The Tribes are seeking to accomplish three primary objectives with summer Chinook in Dollar Creek (Denny et al. 2006):

1) Increase viability and production of the SFSR Chinook population.

2) Increase harvest of summer Chinook for members of the Tribes.

3) Increase knowledge of fishery management techniques to accomplish the first two objectives in the timeliest, cost-effective and least intrusive manner.

Study Area

Dollar Creek, a tributary of the South Fork Salmon River, is located in the Boise National Forest (Appendix A; Figure 9). The dendritic flow drainage is a single 10,590 acre sixth field subwatershed approximately four miles north of Warm Lake, Idaho. Elevations range from 7,900 feet at the northern boundary to 4,900 feet at the confluence with the South Fork Salmon River. Mean stream length is 16.5 miles annually and average precipitation is roughly 33.2 inches. Base flows in Dollar Creek at the confluence with the South Fork Salmon River are approximately eight cubic feet per second and mean flows (Qa) are 28 cubic feet per second.

METHODS

Eyed eggs for the Tribes eggbox project are from Chinook broodstock collected for normal program purposes at MFH (see MFH HGMP for a description of adult collection, holding, mating and incubation procedures). During spawning, caudal fin tissue samples and scale samples will be taken from parents of fertilized eggs destined for the Tribes eggbox project. All tissue samples will be stored in 95% ethanol. Scales will be secured in standard scale envelopes. Standard phenotypic information will be collected from each spawner and its origin, hatchery or wild, noted.

Tissue and scale samples from broodstock will be shipped to Abernathy Fish Technology Center, 1440 Abernathy Creek Road, Longview, Washington 98632 for potential analysis. Tissue samples will be processed, DNA amplified using polymerase chain reaction (PCR), and microsatellite loci identified following standard laboratory procedures. Scales will be used if problems arise with any given tissue sample.

Eggs destined for the Tribes eggbox program will be incubated separately, but following regular hatchery procedures. Temperature units of incubating eggs will be recorded. Upon eyeing, eggs will be supplied to the Tribes for transport and planting. Eggboxes will be distributed throughout incubation areas. Eggboxes will be planted following standard Tribal protocols. At least one recording thermograph will be installed at the location of the middle eggbox site. Temperature information from the thermograph will be used to ascertain time of hatching and emigration from the eggboxes.

When fry are estimated to have vacated the eggboxes in substantial numbers, eggboxes will be examined and number of remaining dead eggs enumerated to estimate hatch success rate.

In late March or early April, after eggbox and natural fry emergence (check for timing), a field crew will sample for age-0^+ Chinook fingerlings in the habitat immediately adjoining the location of the eggboxes. The sampling objective will be to collect ≥ 100 tissue samples from fingerlings arising from the Tribes eggbox program. Total tissue collection will depend on the estimated proportion of fry produced from the eggboxes compared to fry produced from the egg deposition of naturally spawning Chinook the previous year.

In the first year of study, egg deposition from naturally spawning Chinook should be estimated from detailed spawning ground surveys. Numbers and locations of redds relative to eggbox sites need to be recorded. Average fecundity of summer Chinook from MFH broodstock will be used to estimate the naturally spawning population each year in Dollar Creek. In later years, when returning adults from the program are expected, natural egg deposition will also be estimated based on the number of adults passed through the established adult sampling station.

RESULTS

Parental Selection and Mating

In 2008, IDFG staff randomly spawned a total of 78 pairs on 8/19/08, 8/22/08, and 8/26/08. During spawning, staff collected genetic tissue samples and fork lengths from all 156 individuals. After spawning, eggs were transferred to MFH and incubated following standard MFH protocols to the eyed stage, separately from general production gametes.

Egg Planting

Five incubation sites with pool habitats were located and utilized in Dollar Creek (Appendix A; Figure 9). Outplanting occurred on two separate occasions: 10/14/09 and 10/15/09 with totals of 162,856 and 160,159, respectively. Of the five boxes, four contained approximately 54,400 eggs and one reared 107,770 eggs. Overall, staff outplanted a total of 323,015 eggs into Dollar Creek.

For each outplanting date, eggs were initially randomized upon collection and loaded based on volumetric calculations (obtained at MFH) into Rubbermaid in-stream boxes standardized with 1/8 inch mesh sides for flow and 1/4 inch mesh tops for volitional emigration. In-stream boxes were anchored to the stream bed using a combination of rebar and tie wire.

Research, Monitoring, and Evaluation

In-stream incubators were unmonitored due to funding restricted to only outplant the eyed eggs. During planting, staff recorded temperature, dissolved oxygen, conductivity, pH, flow, and possible sediment accumulation. Due to funding limitations, staff was unable to accurately determine hatch success, conduct juvenile sampling, or collect juvenile tissue samples for future parentage analysis with Abernathy Fish Technology Center.

Little results are available due to the constraints of funding allocated to the in-stream Chinook salmon supplementation program. However, through previous monitor and evaluation, boxes located in glides objectively performed much higher than boxes placed in pools due to sediment accumulation. Historically, sediment accumulation averaged greater than 63% in boxes within pool habitats compared to less than 21% accumulation in those boxes located in glides. In addition, areas of greater flow indices (i.e. glides) showed greater fry development due to the inability of sediment to readily drop-out of the water column. Temperature, dissolved oxygen, conductivity, and pH varied insignificantly within and between sites.

From previous data collected, it is apparent that in-stream boxes placed in glide habitats are more efficient than those in pool habitats most likely due to increased flows and less sedimentation. However, due to the large volumetric size of the incubation boxes, pools were randomly selected over glide habitats.

Since staff was unable to enumerate dead egg totals in 2008 to estimate hatch success, the historical average (85%) of the program was applied to each egg box to determine the approximate amount of fry seeded in Dollar Creek. There was an estimated 274,563 fry produced from outplanting 323,015 eggs in Dollar Creek.

DISCUSSION

In 2008, IDFG spawned adult Chinook salmon at the South Fork Salmon River and Tribal staff outplanted a total of 323,015 eyed eggs in Dollar Creek. As a result of limited funding in 2008, staff determined, by applying the historical average, a total of 274,563 fry were seeded in the Dollar Creek tributary. Although research, monitor, and evaluation was extremely minimal, given that these eggs were “surplus,” any level of fry production is an accomplishment toward maintaining, rehabilitating, and enhancing salmon populations in the Salmon River basin and supplementing natural production in SFSR tributaries where limited numbers of naturally spawning Chinook are known to occur.

Even though staff assumes fry were produced in Dollar Creek, the SBT acknowledges, in conjunction with scientific literature that suggests hatchery offspring experience lower survival to the adult stage, that life stages beyond that of fry may not be produced. However, juveniles from the eggbox program will experience natural selection and this natural process is thought to produce highly fit fish (Denny et al. 2006).

With additional funding, the SBT proposes that future evaluations will include pairing Dollar Creek with a control stream to document changes in fish densities resulting from in-stream egg incubation. However, the program will need to pair multiple treatment and control streams to detect significant relationships. Fish densities will be evaluated by baiting fry traps, electro-fishing, and/or snorkeling and data will be compared to control streams to determine significant differences. If fry densities indicate supplementation is increasing the population, this will warrant further cooperation between the parties to justify future investigations.

CITATIONS

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Denny, L. P., K. Witty, and S. Smith. 2006. A monitoring and evaluation plan for the Shoshone-Bannock Tribes: Hatchery supplementation activities Yankee Fork; Salmon River sub-basin. Draft Review Shoshone-Bannock Tribes, Department of Fisheries Resources Management.

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ABSRACT

To maintain, rehabilitate, and enhance steelhead populations, the Tribes, under Cooperative Agreement 141108J014, initiated a steelhead supplementation program in Yankee Fork, Valley Creek, and Slate Creek.

The main objective of the program is to release 330,000 in Yankee Fork, 50,000 in Valley Creek, and 100,000 in Slate Creek for an approximate total release of 480,000 smolts. The goal is to return > 2,000 adults; a level to help rebuild the populations, collect broodstock, and sustain harvest. In 2008, with the help of Idaho Fish and Game and US Fish and Wildlife, SBT staff released at total of 482,090 smolts (100.4% of overall objective) collectively in the three tributaries. Staff met or exceeded the release values for each tributary within the program.

Initial estimates of juvenile survival and migration timing through the hydrosystem will be conducted by monitoring PIT tag evaluations using the SURPH model. The Tribes propose to install a weir, screw trap, and/or PIT tag reader in the Yankee Fork to effectively estimate population size, collect genetic samples from returning adults, and determine the efficacy of the steelhead smolt supplementation program.

ACKNOWLEDGEMENTS

The Tribes thank Scott Marshall and staff at the LSRCP-Office for providing funding to further support this program. Special thanks to IDFG personnel including Sharon Kiefer, Sam Sharr, Tom Rogers, and Richard Lowell as well as USFWS personnel Bryan Kenworthy and Nathan Wiese. We especially thank Mike DeLarm for providing technical expertise and commitment to this program.

The Tribes provided the administrative framework for this project to be successful. We would like to thank the Fish and Wildlife Department personnel Carlos Lopez, Dean Hicks, and Willie Hicks for project operations and monitoring and evaluation. Thanks to Hal Hayball of the Shoshone-Bannock Tribes for furnishing maps.

INTRODUCTION

The Tribes initiated a smolt supplementation program in Yankee Fork, Valley Creek, and Slate Creek to increase the viability and production of the steelhead populations, increase harvest of steelhead for members of the Tribe, and increase knowledge of fishery management techniques to accomplish the first two goals in a timely, cost-effective, and least intrusive manner.

The objectives of the steelhead smolt supplementation program, under the agreement in *U.S. v Oregon*, are to release approximately 330,000 in Yankee Fork, 50,000 in Valley Creek and 100,000 in Slate Creek for a total of 480,000 smolts with a goal of returning > 2,000 adults.

In cooperation with LSRCP, the current focus of monitor and evaluation for smolt supplementation research is structured in the Yankee Fork. This focus allows the Tribes to evaluate the efficacy of multiple programs in one location, i.e. smolt supplementation compared to streamside incubation compared to natural production.

STUDY AREA

Salmon River Sub-basin

Physical and biological characteristics of the Salmon River sub-basin influence the potential to enhance anadromous salmonid populations. Generally, streams have high gradient that causes them to be dynamic environments for fish. In addition to natural factors limiting fish production, humans have taken water for irrigation, reduced riparian vegetation, mined, developed rural residential areas, built and maintained roads, grazed domestic livestock, and logged (Kutchins et al. 2001). However, the Salmon River sub-basin has potential to rear large numbers of salmon and steelhead.

The Salmon River sub-basin is located in central Idaho (Appendix A; Figure 8). The total drainage area of the Salmon River watershed is over 14,000 square miles (36,260 square kilometers). The Salmon River flows 410 miles (650 kilometers) in a large arch from northeast to northwest to join the Snake River at River Mile 188.2. The Salmon River is the second largest sub-basin in the Columbia River drainage with the Snake River drainage being the largest (Kutchins et al. 2001). Major tributaries of the Salmon River include the Little Salmon River, South Fork Salmon River, Middle Fork Salmon River, Panther Creek, Lemhi River, Pahsimeroi River, East Fork Salmon River, Valley Creek, and Yankee Fork Salmon River (Appendix A; Figure 8) (Kutchins et al. 2001).

Yankee Fork Salmon River

Yankee Fork, located in the Salmon-Challis National Forest in Custer County, Idaho, is a major tributary of the upper Salmon River (Appendix A; Figure 8). The Yankee Fork flows through narrow canyons and moderately wide valleys with forest of lodgepole pine (*Pinus contorta)* (Richards and Cernera 1989). The West Fork of the Yankee Fork is the largest tributary. Other notable tributaries to the Yankee Fork include Jordan, Lightning, Greylock, Cearly, and Eightmile Creeks (Appendix A; Figure 13). Most of the system is characterized by highly erosive sandy and clay-loam soils. Yankee Fork is an important spawning and rearing stream for Chinook salmon and steelhead. Utilization by Chinook salmon and steelhead has declined since the mid-1960's. Other fish species present in the Yankee Fork system include bull trout (*Salvelinus confluentus*), cutthroat trout (*Oncorhynchus clarki*), mountain whitefish (*Prosopium williamsoni*), and short head sculpin (*Cottus confuses*).

The drainage is composed of 190 square miles of river. Elevations range from 8,204 feet at the northern boundary to 6,171 feet at the confluence with the Salmon River. Mean stream length varies annually and average precipitation is roughly 27 inches. Base flows in Yankee Fork are approximately 40 cubic feet per second and mean flows (Qa) are 247 cubic feet per second.

Historic mining activities in the Yankee Fork further aggravated the tenuous status of natural stocks, resulting in further decline. Mining activities have resulted in the complete re-channeling of lower portions of the Yankee Fork and the deposition of extensive unconsolidated dredge piles. Such activities have eliminated or degraded much of the rearing and spawning habitat in the lower Yankee Fork. As a result, the Yankee Fork drainage is grossly underutilized with respect to salmon and steelhead production (Reiser and Ramey 1987).

METHODS

Steelhead for this program are collected, spawned, and sampled by SBT and IDFG staff at the Sawtooth Fish Hatchery. Smolts destined for Yankee Fork supplementation will be incubated and reared separately from all other hatchery production at the Magic Valley and Hagerman National Fish Hatcheries. Upon transport to Yankee Fork, the 330,000 smolts will be scatter planted at several pre-determined habitat areas, with approximately 110,000 smolts at each location.

Upon return to Yankee Fork, adult, F1 steelhead will be sampled in the SBT harvest. Tissue samples, scales, and phenotypic information will be collected. In the summer, following spawning, age-0^+ parr will be collected and sampled. DNA typing will be used to differentiate steelhead produced from the smolt supplementation program from all other steelhead produced either naturally or planted in egg incubators in the study watershed. Each steelhead (P1, parent) used in brood-stock mating to produce the supplementation smolts (F1) are genotyped, allowing for all progeny to later be identifiable when captured and sampled as F1 adults or later, as F2 parr or F2 adults.

A parental exclusion, pedigree analysis (Letcher and King 2001) will be performed to determine the relative reproductive success of hatchery origin steelhead compared to natural origin steelhead in producing F2 juveniles. The number of naturally spawning steelhead in Yankee Fork will be determined by the number of unique genotypes that will be assessed in sampling of age-0^+ juvenile parr.

Monitor and evaluation activities will focus on recording juvenile smolt out-migration and estimating adult steelhead escapement resulting from smolt releases. Staff will estimate juvenile survival and timing through the hydrosystem using the SURPH model and searching for PIT tags. Implanted tags will be used in IDFG hatchery evaluations and data will be shared with staff for evaluation purposes. Information will be applied to estimate adult escapement by assuming similar survival of SFH general production steelhead.

RESULTS

Parental Selection and Mating

SBT staff, in coordination with Idaho Department of Fish and Game (IDFG) at the Sawtooth Fish Hatchery (SFH), randomly spawned 32 pairs, 26 pairs, 20 pairs, and 18 pairs for a total of 96 pairs on 4/17/08, 4/21/08, 4/24/2008, and 5/1/2008, respectively.

During spawning, staff collected genetic tissue samples and fork lengths from all 192 individuals. All other adults utilized for Valley Creek and Slate Creek smolt production were spawned by SFH personnel. After incubation at SFH, eggs were transferred to Magic Valley and Hagerman Hatcheries where Yankee Fork, Valley Creek, and Slate Creek fish were reared separately from general production fish.

Smolt Release

Smolts were released into Pond Series 1 and 4 and also at the confluence of Jordan Creek in Yankee Fork (Appendix A; Figure 10), in Valley Creek at the Stanley Lake Creek confluence (Appendix A; Figure 11), and into an upper and lower site in Slate Creek (Appendix A; Figure 12).

With the help of IDFG and USFWS, staff was present to release 327,546 smolts into Yankee Fork (99.3% of our objective), 92,059 smolts into Slate Creek (92.1% of our objective), and 62,485 smolts into Valley Creek (125% of our objective). Overall, IDFG, USFWS, and SBT staff released a total of 482,090 smolts in YF, Valley Creek, and Slate Creek (100.4% of our overall objective). Locations, dates, release numbers, collaborating hatchery, and mark values are presented below in Table 1.

Table 1. 2008 steelhead smolt releases in Yankee Fork, Valley Creek, and Slate Creek.

Date	Location	Hatchery	Ad-clipped	Ad + CWT	Pit-Tag	No-mark	Total
4/22/2008	Lower Slate Creek	Magic Valley	0	31,975	593	0	31,975
4/30/2008	Yankee Fork P1	Magic Valley	30,709	30,722	1,591	30,695	92,126
4/29/2008	Valley Creek	Magic Valley	0	0	996	62,485	62,485
5/1/2008	Lower Slate Creek	Magic Valley	0	0	1,287	60,084	60,084
5/12/2008	Yankee Fork P4	Hagerman	17,380	0	1490	31,809	49,189
5/13/2008	Yankee Fork P4	Hagerman	35,467	0	983	18,105	53,572
5/14/2008	Strata 4 JC	Hagerman	18,249	0	0	34,480	52,729
5/15/2008	Yankee Fork P1	Hagerman	29,783	0	0	24,946	54,729
5/16/2008	Yankee Fork P1	Hagerman	0	0	0	25,201	25,201
	Yankee Fork	MVH & HFH	131,588	30,722	4,064	165,236	327,546
	Valley Creek	Magic Valley	0	0	996	62,485	62,485
	Slate Creek	Magic Valley	0	31,975	1,880	60,084	92,059
	Total Release		**131,588**	**62,697**	**6,940**	**287,805**	**482,090**

Of the 482,090 smolts released, 246,670 were reared at the Magic Valley Fish Hatchery (51% of total) and 235,420 were reared at the Hagerman National Fish Hatchery (49% of total). In summary, there were 131,588 ad-clipped, 62,697 ad-clipped plus coded-wire tagged, 6,940 pit-tagged, and 287,805 smolts with no marks released in Yankee Fork, Valley Creek, and Slate Creek, collectively.

Juvenile Survival

Staff conducted initial queries for PIT-tagged juveniles in PITAGIS and Fish Passage. Estimates of juvenile survival and migration through the hydrosystem will be conducted by continued monitoring and evaluation of PIT tags and utilizing the SURPH model. Staff will be collecting information on PIT tagged juvenile steelhead detected in the hydrosystem to complete a comprehensive survival estimate for 2006-2008 along with additional PIT-tag data obtained from operation of a rotary screw trap in the Yankee Fork.

Furthermore, additional evaluations will be conducted, including trapping adults at a weir located in Yankee Fork, sampling tissue for genetic analysis, and determining whether adults successfully reproduce.

DISCUSSION

Of the major objectives and tasks under the SBT steelhead smolt supplementation program, staff completed the spawning of 192 individuals and the release of 482,090 smolts in Yankee Fork, Valley Creek, and Slate Creek, collectively. Furthermore, the final tasks, estimating juvenile survival and adult escapement, will be complete for 2009 reporting with additional information from tags applied during screw trap operation.

Genetic samples taken from spawning all 192 individuals were transferred to Abernathy Fish Technology Center (AFTC) for future analysis. With additional funding, AFTC will conduct parentage exclusion analysis to estimate adult production and smolt-to-adult survival. Under this study design, staff will be able to determine the efficacy and cost-effectiveness of the steelhead smolt program compared to natural production and/or the steelhead streamside incubation program. To increase the efficiency of monitoring and evaluation, the SBT propose to install a weir in the Yankee Fork to trap returning adults to collect additional genetic samples beyond just creel surveys for conclusive parentage analysis studies. In addition, the SBT propose to install a screw trap at the mouth of the Yankee Fork in 2009 to effectively determine population and relative breeding size of steelhead.

In the past, steelhead smolts have been released on an irregular schedule in the Lemhi River, Yankee Fork, Valley Creek, or Slate Creek. To date, evaluation of this program has been limited to observation of adult steelhead and minimal redd counts. However, with the completion of a sound monitor and evaluation plan, the SBT can evaluate, using DNA, survival from the steelhead smolt release program in Yankee Fork. The plan is also designed to estimate capacity of the natural environment to support additional hatchery steelhead, give early warning of adverse impacts caused by the projects, and track trends in environmental quality.

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CHAPTER 3. STEELHEAD STREAMSIDE INCUBATION (SSI) PROGRAM

Annual Report

October 1, 2007 – September 30, 2008

Prepared by:

Lytle P. Denny and Kurt A. Tardy

Shoshone-Bannock Tribes
Fish and Wildlife Department
3rd and B Avenue
Fort Hall, Idaho
(208) 239-4555

Prepared for:

United States Fish and Wildlife Service
Lower Snake River Compensation Plan
1387 S. Vinnell Way, Suite 343
Boise, Idaho 83709

Cooperative Agreement 141108J014

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ABSTRACT

The Tribes initiated a steelhead streamside supplementation program in 1995 to help maintain, rehabilitate, and enhance steelhead populations. The objective is to release approximately 1.0 million eggs into five Upper Salmon River tributaries to meet a return goal of > 2,000 adults.

Under Cooperative Agreement 141108J014 with the LSRCP, the focus of study evaluations is confined to the Yankee Fork Salmon River (Yankee Fork). The Yankee Fork, a major tributary to the upper Salmon River, is an important spawning and rearing system for anadromous salmonids, as well an important traditional use area for the Shoshone-Bannock Tribes. Based on the population delineations and viability criteria from the Interior Columbia Basin Technical Recovery Team, the Yankee Fork is underutilized by anadromous species.

The Tribes operate the steelhead streamside incubation (SSI) program in the Yankee Fork Salmon River, Idaho. An objective is to determine if targets for hatchery contributions are being achieved and can be improved using DNA parentage analysis. Approximately 1,135,510 eggs were planted into five Upper Salmon River tributaries with 1,044,319 estimated fry seeded. Steelhead are spawned and reared at both the Sawtooth and Pahsimeroi Fish Hatcheries. Eggs taken from Sawtooth are outplanted in Basin Creek and Yankee Fork where as Pahsimeroi eggs are transferred to incubators in Panther, Morgan, and Indian Creeks.

There were 500,575 total eggs planted in Panther, Morgan, and Indian combined. Overall survival equaled 99.1% with an estimated 496,255 fry seeded in the three tributaries. There were 634,935 total eggs planted in Yankee Fork and Basin Creek combined. Overall survival equaled 86.7% with an estimated 548,094 fry seeded in the two tributaries. Due to funding allocation, monitor and evaluation is restricted to the Yankee Fork where the Tribes have developed a comprehensive M&E plan.

Staff completed three-pass depletion electrofishing at 25 total randomly stratified reaches for density, distribution, parentage analysis, and genetic structure in the Yankee Fork in 2008. Observed sample densities for all fish was 0.070 fish/m^2, for steelhead 0.06 fish/m^2, and for Chinook 0.01 fish/m^2. Steelhead progeny evaluations conducted by Abernathy Fish Technology Center revealed juvenile SSI survival to both the age-0^+ and 1^+ life stage.

ACKNOWLEDGEMENTS

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The Tribes provided the administrative framework for this project to be successful. We would like to thank the Fish and Wildlife Department personnel Chad Colter, Claudeo Broncho, Scott Brandt, Carlos Lopez, and Tyron Broncho for project operations and monitoring and evaluation. Special thanks to the Tribal ISS crew (Andy Kohler, Duane Dupris, Angelo Teton, and John Fred) for collecting tissue samples from the West Fork screw trap. Thanks to Hal Hayball of the Shoshone-Bannock Tribes for furnishing maps.

INTRODUCTION

The objectives of the SSI program, under the agreement in *U.S. v Oregon*, are to outplant 1.0 million eyed steelhead eggs into five Upper Salmon River tributaries; 375,000 in Yankee Fork, 250,000 in Panther Creek, 125,000 in Basin Creek, 125,000 in Morgan Creek, and 125,000 in Indian Creek.

Current monitor and evaluation focus for the SSI program is structured in the Yankee Fork. The purpose of monitor and evaluation activities is designed to evaluate the efficacy of a steelhead eyed egg incubator program in the Yankee Fork as compared to naturally spawning steelhead and/or the on-going smolt supplementation program. The Tribes primary purposes under the SSI program are to: 1) increase the viability and production of the Yankee Fork steelhead population; 2) increase harvest of steelhead for members of the Shoshone-Bannock Tribes; and, 3) increase knowledge of fishery management techniques to accomplish the first two objectives in a timely, cost-effective and least intrusive manner.

The Yankee Fork, a major tributary of the Salmon River, is a spawning and rearing system for steelhead trout (*Onchorhychus mykiss*) and Chinook salmon (*O. tshawytscha*). Historically, there were large spawning populations of steelhead and Chinook in Yankee Fork which are a cultural, social and subsistence based resource of historical significance for the Tribes. Factors including hydroelectric dam construction, reduced riparian habitat, irrigation, river and ocean harvest, and fish passage have caused a decline in salmon and trout populations.

Reiser and Ramey (1987) determined Yankee Fork could produce an estimated 740,064 Chinook and 295,499 steelhead smolts. Based on information from the Interior Columbia Basin Technical Recovery Team (TRT 2005) and Reiser and Ramey (1987), Yankee Fork is underutilized by anadromous fish.

The Tribes developed supplementation activities to enhance the viability of natural steelhead populations. Without changing downstream harvest and hydrosystem management, supplementation may be necessary to maintain elevated populations to support harvest and improve abundance, productivity, structure, and genetic diversity (Denny et al. 2006). Effective management of steelhead stocks can be determined by increases in abundance and distribution through a combination of electroshocking and DNA genotyping.

The tasks, as identified within monitor and evaluation objectives, were to: (1) collect genetic samples for parentage analysis, (2) document salmonid species, (3) estimate relative abundances for wild origin and SSI progeny, (4) determine condition of wild origin and SSI progeny, (5) determine if program objectives for rate of contribution by hatchery fish is being achieved or can be improved, (6) determine natural production increase resulting from supplementation of steelhead in Yankee Fork and relate this information to possible limiting factors, (7) identify adaptive management to increase sampling resolution, and (8) communicate monitoring and evaluation findings to resource managers.

STUDY AREA

Salmon River Sub-basin

Physical and biological characteristics of the Salmon River sub-basin influence the potential to enhance anadromous salmonid populations. Generally, streams have high gradient that causes them to be dynamic environments for fish. In addition to natural factors limiting fish production, humans have taken water for irrigation, reduced riparian vegetation, mined, developed rural residential areas, built and maintained roads, grazed domestic livestock, and logged (Kutchins et al. 2001). However, the Salmon River sub-basin has potential to rear large numbers of salmon and steelhead.

The Salmon River sub-basin is located in central Idaho (Appendix A; Figure 8). The total drainage area of the Salmon River watershed is over 14,000 square miles (36,260 square kilometers). The Salmon River flows 410 miles (650 kilometers) in a large arch from northeast to northwest to join the Snake River at River Mile 188.2. The Salmon River is the second largest sub-basin in the Columbia River drainage with the Snake River drainage being the largest (Kutchins et al. 2001). Major tributaries of the Salmon River include the Little Salmon River, South Fork Salmon River, Middle Fork Salmon River, Panther Creek, Lemhi River, Pahsimeroi River, East Fork Salmon River, Valley Creek, and Yankee Fork Salmon River (Appendix A; Figure 8) (Kutchins et al. 2001).

Yankee Fork Salmon River

Yankee Fork, located in the Salmon-Challis National Forest in Custer County, Idaho, is a major tributary of the upper Salmon River (Appendix A; Figure 8). The Yankee Fork flows through narrow canyons and moderately wide valleys with forest of lodgepole pine (*Pinus contorta)* (Richards and Cernera 1989). The West Fork of the Yankee Fork is the largest tributary. Other notable tributaries to the Yankee Fork include Jordan, Lightning, Greylock, Cearly, and Eightmile Creeks (Appendix A; Figure 13). Most of the system is characterized by highly erosive sandy and clay-loam soils. Yankee Fork is an important spawning and rearing stream for Chinook salmon and steelhead. Utilization by Chinook salmon and steelhead has declined since the mid-1960's. Other fish species present in the Yankee Fork system include bull trout (*Salvelinus confluentus*), cutthroat trout (*Oncorhynchus clarki*), mountain whitefish (*Prosopium williamsoni*), and short head sculpin (*Cottus confuses*).

The drainage is composed of 190 square miles of river. Elevations range from 8,204 feet at the northern boundary to 6,171 feet at the confluence with the Salmon River. Mean stream length varies annually and average precipitation is roughly 27 inches. Base flows in Yankee Fork are approximately 40 cubic feet per second and mean flows (Qa) are 247 cubic feet per second.

Historic mining activities in the Yankee Fork further aggravated the tenuous status of natural stocks, resulting in further decline. Mining activities have resulted in the complete re-channeling of lower portions of the Yankee Fork and the deposition of extensive unconsolidated dredge piles. Such activities have eliminated or degraded much of the rearing and spawning habitat in the lower Yankee Fork. As a result, the Yankee Fork drainage is grossly underutilized with respect to salmon and steelhead production (Reiser and Ramey 1987).

METHODS

Parental Selection and Mating

Spawning occurs at Sawtooth Fish Hatchery (SFH), where staff actively participates in spawning returning pairs of steelhead adults. Staff collects genetic samples, gametes, and fork length for each parent fish isolated for Yankee Fork and Basin Creek. Mating is conducted as 1:1 female to male and eggs are incubated separately from general production gametes. All other adults utilized for Panther Creek, Morgan Creek, and Indian Creek are spawned and fork length recorded by IDFG personnel at the Pahsimeroi Fish Hatchery (PFH).

Egg Planting

Staff constructed two incubators located in Morgan Creek, two in Indian Creek, two in Panther Creek, two in Basin Creek, and six in Yankee Fork.

Incubators were standardized with 2-inch PVC pipe with a 3-inch head pipe to collect additional flow from the stream. Each head pipe was fitted with ¼ inch mesh screen to minimize sediment and debris collection. Each incubator consisted of a 50-gallon polyurethane cylinder with a sediment tray, gravel, saddles, six egg trays, and one cover tray to contain eggs until hatching occurred. Each catch tank was a 30-gallon Rubbermaid polyurethane tub with a custom fit cover.

Steelhead eyed eggs from PFH were collected and transferred to SSI incubators in Panther Creek (Appendix A; Figure 14), Morgan Creek (Appendix A; Figure 15), and Indian Creek (Appendix A; Figure 16). Eyed eggs from SFH, destined for Yankee Fork, were initially randomized (to mix family units) prior to transport and then loaded proportionately into six incubators on three outplanting dates. Steelhead eggs for Basin Creek were only transported and outplanted, not randomized due to no further juvenile assessment.

Incubation and Hatching Success

Incubators were monitored twice weekly from 5/05/2008 through 7/30/2008. Staff recorded water condition, temperature, dissolved oxygen, conductivity, pH, and embryo stage as well as cleaned and removed debris from head pipe screens.

Upon full volitional emigration, hatch success was estimated from enumerating dead eggs in the incubator and dead fry in the catch tank. Fry seeded is estimated as the number of eggs planted minus the number of dead eggs enumerated.

Juvenile Assessment

Juvenile sampling was conducted on the Yankee Fork drainage during September 16 – 26, 2008.

Konopacky et al. (1985, 1986) divided the drainage into seven distinct strata (Appendix A; Figure 17); three reaches were selected within each stratum including Pond Series 1 and 3 except for stratum five which contained four reaches. The 25 total sites were selected for a variety of habitats (pools, glides, riffles) and ease of accessibility for an upper, middle, and lower location within each stratum. Appendix A; Figure 17 shows the center position for all sampling locations throughout Yankee Fork. Sites were generally rectangular in shape, aligned with the shoreline, and divided into transects for habitat measurements.

Multiple-pass electrofishing requires closed populations to minimize emigration and immigration; hence the use of block nets. Sites were predominately 100 m in length, but did reach above 100 m due to habitat inclusion and accessibility for block net placement. Upstream and downstream ends of the sampling reach were blocked using 7-mm-mesh nets secured to the streambed with tri-pods and rebar, generally at habitat unit separations. Sites were electrofished in an upstream direction between 20 – 30 minutes with one crew member electroshocking (Smith-Root, Inc. Pulsed DC LR-24 Backpack Electrofisher) and two to three others utilizing dip nets to capture fish drifting downstream under electronarcosis. Voltage and frequency were adjusted and monitored to maximize capture, but limit fish injury (voltage: 350-450, frequency: 30-50 Hz, duty cycle: 10-12%). Fish were transferred immediately to a bucket and then to a holding tub for further analysis.

Fish were anesthetized in a 10 p.p.m. solution of clove oil. Prior to mixing solution, clove oil was first dissolved in 95% ethanol (1:10 ratio clove oil-ethanol) since clove oil is insoluble at water temperatures below 15 ^{o}C (Cho and Heath 2000). Trout and salmon were measured for fork length to the nearest 1 mm and weight to the nearest 0.01 g. Fin clips were taken from the ventral caudal lobe and scales were taken anterior of the caudal fin for parentage analysis and aging, respectively. Post-sampling, fish were transferred to a tub of fresh water to recover. A minimum of 20 minutes between passes was given to allow the return of normal fish activity and visual clarity. Fish were released after full recovery once sampling was finished.

Population estimates and probability of capture was calculated using model $M_{(b)}$ (Zippen removal population estimator, Zippen 1956) by the program CAPTURE. CAPTURE computes estimates of capture probability and population size for all electrofishing passes based on a stationary population, equal probability of capture for each animal, and constant probability of capture.

Habitat measurements included reach length, mean width (wetted), mean depth, maximum depth, start temperature, end temperature, woody debris, and dominant substrate. Mean width was obtained by taking width measurements on every twenty-meter transect along the stream section length. Mean depth was estimated from five equally spaced measurements on each width transect. Maximum depth was observed as the deepest point along each width transect. Temperature was recorded at the start and finish of each three-pass electrofishing.

Both woody debris and dominant substrate composition were visually graded on a scale. Woody debris was classified as absent (1), slight (2), dominant (3), or ubiquitous (4). Dominant substrate was coded as sand (1), gravel (2), cobble (3), or boulders (4). In sites with multiple habitat types, a visual estimate of stream section area (%) was recorded as well as each habitat was coded separately for woody debris and dominant substrate.

Total reach area sampled was determined as the product of stream section length and mean width. Width: depth ratio was calculated by dividing mean width by mean depth. Catch-per-unit-effort (CPUE) for each site, strata, and Yankee Fork was calculated as fish per meter squared (fish/m^2).

A parental exclusion, pedigree analysis (Letcher and King 2001) was/will be performed to determine the relative reproductive success of hatchery origin steelhead compared to natural origin steelhead in producing F2 juveniles. The number of naturally spawning steelhead in YF will be determined by the number of unique genotypes that will be assessed in sampling of age-0$^+$ and 1$^+$ juvenile parr.

RESULTS

Egg Planting, Hatch Success, and Fry Seeded

Overall, staff gathered 532,527 and 738,297 green eggs at PFH and SFH, respectively, for a total of 1,270,824 green eggs. Green eggs were incubated on pathogen free well water for approximately 45 days at 43°C to achieve 500,575 (94% green egg to eyed egg survival) and 634,935 (86% green egg to eyed egg survival) eyed eggs, respectively, for a total of 1,135,510 eyed eggs received.

Upon full volitional emigration from incubators and catch tanks, hatch success and total fry seeded were estimated from enumerating dead eggs. Average hatch success for all fourteen incubators equaled 92.00% (0 – 99.60%) with a total of 1,044,319 fry seeded in the five Upper Salmon River tributaries (Table 2) from 1,135,510 outplanted eggs. Hatch success from the six incubators in Yankee Fork averaged 82.60% (0 – 99.60%) with a total of 398,528 fry seeded from outplanting 483,904 eyed eggs (Table 2).

Table 2. SSI program tributary incubator characteristics.

SSI LOCATION	*RIVER*	*STOCK*	*EGGS PLANTED*	*TOTAL DEAD*	*% SURVIVAL*	*FRY SEEDED*
Hay Creek	Basin	SFH	73,837	949	0.987	72,888
East Basin Creek	Basin	SFH	77,194	516	0.993	76,678
Cearly Creek #1	Yankee	SFH	78,657	313	0.996	78,344
Cearly Creek #2	Yankee	SFH	82,397	358	0.996	82,039
Jordan Creek	Yankee	SFH	83,983	1,564	0.981	82,419
Greylock Creek	Yankee	SFH	85,000	357	0.996	84,643
WF Yankee Fork	Yankee	SFH	71,978	895	0.988	71,083
12 Mile Main	Yankee	SFH	81,889	81,889	0.000	0
WF Morgan Creek	Morgan	PFH	83,862	527	0.994	83,335
Lick Creek	Morgan	PFH	85,319	566	0.993	84,753
Indian Creek #1	Indian	PFH	81,859	656	0.992	81,203
Indian Creek #2	Indian	PFH	83,040	831	0.990	82,209
Beaver Creek #1	Panther	PFH	82,777	983	0.988	81,794
Beaver Creek #2	Panther	PFH	83,718	787	0.991	82,931
TOTAL			**1,135,510**	**91,191**	**0.920**	**1,044,319**

Habitat Characterization

Average area sampled was 1000.4 m^2 with length, wetted width, and depth averaging 102 m, 9.42 m, and 0.28 m, respectively. Mean start temperature was 7.3 ^{o}C, but ranged from 3.0 ^{o}C to 11.0 ^{o}C. End of sampling temperature averaged 7.9 ^{o}C among all sites with a range of 3.6 ^{o}C to 12.5 ^{o}C.

Sampling area was dominated by glide and riffle, 53.7% and 38.1%, respectively, for all reaches sampled. Pool percentage accounted for only 8.2% of the reaches sampled. Cobble was the most common substrate and the categorical designation, absent to slight, was the most commonly used to describe abundance of woody debris.

Total Salmonid Density and Relative Abundance

Area sampled and abundance, density, and biomass for all captured species (per strata and total) are displayed below in Table 3. Total salmonid density was highest in stratum 7 (0.240 fish/m^2), and was approximately 3.0x that of the next highest density, 0.085 fish/m^2, in stratum 3. Strata 5 and Pond Series 1 had the lowest fish densities of all seven strata sampled. Biomass/m^2 was highest in stratum 2, due to the presence of large mountain whitefish, and lowest in Pond Series 3, an area of limited natural production. Density for the entire sampling area (25,010 m^2) was 0.070 fish/m^2, well below the suggested carrying capacity of 1.0 fish/m^2, while overall biomass equaled 1.966 g/m^2 (Table 3).

Steelhead were the most ubiquitous species in Yankee Fork, equaling richness of 70% or more in eight of the nine strata sampled (including both Pond Series) (Figure 1). Upper Yankee Fork (stratum 5) was almost entirely bull trout with very few steelhead and several Chinook and cutthroat individuals. Chinook and cutthroat abundances were highest in stratum four and seven, respectively. Multiple species were present in each stratum except Pond Series 1; strata 1 had the highest species richness containing all five salmonid species (steelhead, Chinook, bull trout, cutthroat, and whitefish).

Table 3. Abundance, density, and biomass presented per strata and total for all captured species in Yankee Fork, Idaho 2008.

Strata	Area (m^2)	Abundance	95% CI	Density (fish/m^2)	95% CI	Biomass (g/m^2)
PS1	900	31	16	0.034	0.016	0.525
PS3	900	47	19	0.052	0.021	0.309
1	3633	226	137	0.062	0.102	0.872
2	4828	300	85	0.062	0.061	3.694
3	3416	291	99	0.085	0.080	3.518
4	3990	261	85	0.065	0.073	1.854
5	2930	113	35	0.039	0.046	0.639
6	3494	255	39	0.073	0.040	1.107
7	919	221	160	0.240	0.507	2.469
Total	**25010**	**1745**	**103**	**0.070**	**0.062**	**1.966**

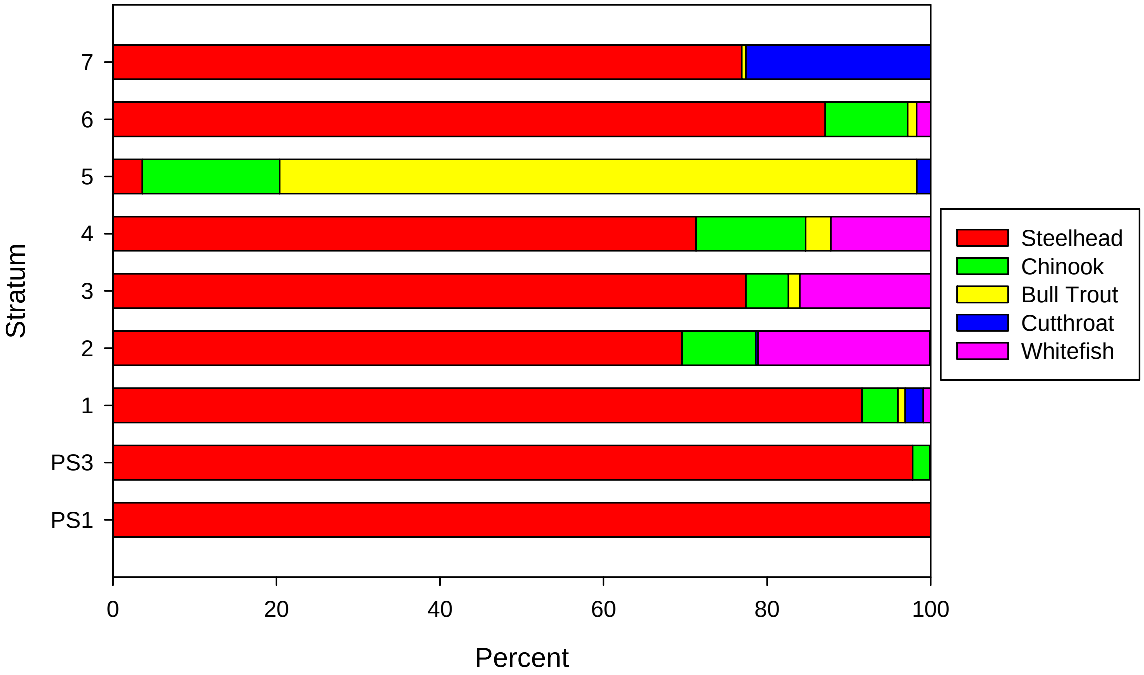

Figure 1. Relative abundance of fish species by strata, Yankee Fork, Idaho 2008.

Onchorhychus mykiss

Steelhead were present in all strata sampled in 2008. Total estimated steelhead abundance was greatest in strata 3 (n = 279) and strata 4 (n = 261). However, these densities were significantly lower compared to Jordan Creek (0.238 fish/m^2) due to greater stream widths and, subsequently, larger coverage area (Figure 2). Biomass was highest in strata 6 (0.877 g/m^2) and lowest in strata 5 (0.008 g/m^2), although individuals in strata 1 were observably larger with average fish length and mass being 90 mm and 17.3 g. Total estimated steelhead density (Figure 2), and biomass for the entire sampling area in 2008 were 1,500 individuals, 0.060 fish/m^2, and 0.296 g/m^2, respectively. The following figure graphically displays *O. mykiss* estimated density from 2006 – 2008. Density has continued to gradually increase since 2006 potentially from supplementation activities; however, until a permanent structure is constructed to trap returning adults full efficacy of supplementation is undetermined.

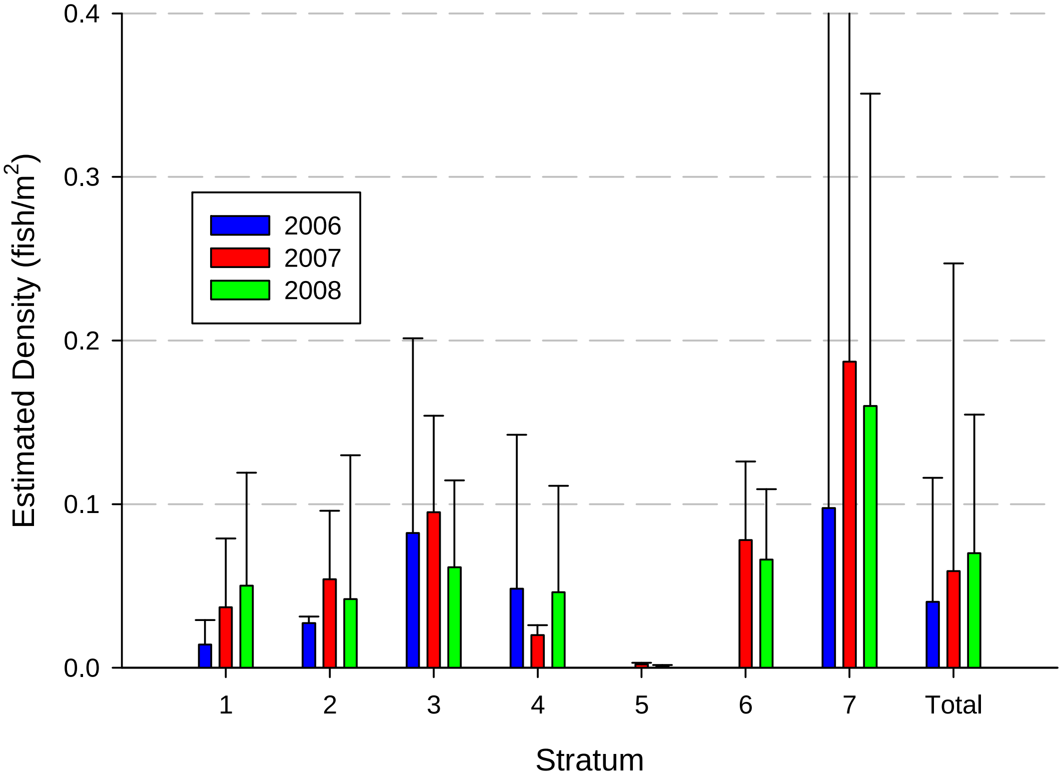

Figure 2. *O. Mykiss* density by strata (n=3) and total for Yankee Fork, Idaho 2006-2008. Error bars represent 95% confidence interval of mean.

From literature review, the Tribes classified age-0$^+$ individuals as ≤ 80 mm and age-1$^+$ or older as > 80 mm. Future scale analyses, collected during sampling, will indicate any variation in age at length and be applied to our genetic parentage analysis. Of the 1,500 individuals, 1,242 (83%) were classified as age-0$^+$ and 258 (17%) as age-1$^+$. Highest age-0^+ abundance was found in strata 3 (n = 190) while age-1^+ was greatest in strata 6 (n = 91) due to additional samples collected at the West Fork screw trap.

Due to greater stream widths and, subsequently, larger coverage area in the mainstem sampling reaches, both age-0^+ and age-1^+ densities (0.129; 0.030 fish/m^2, respectively) were the highest in stratum 7. Overall density for age-0^+ and 1^+ individuals for the entire sampling area was 0.041 and 0.008 fish/m^2, respectively (Figure 3).

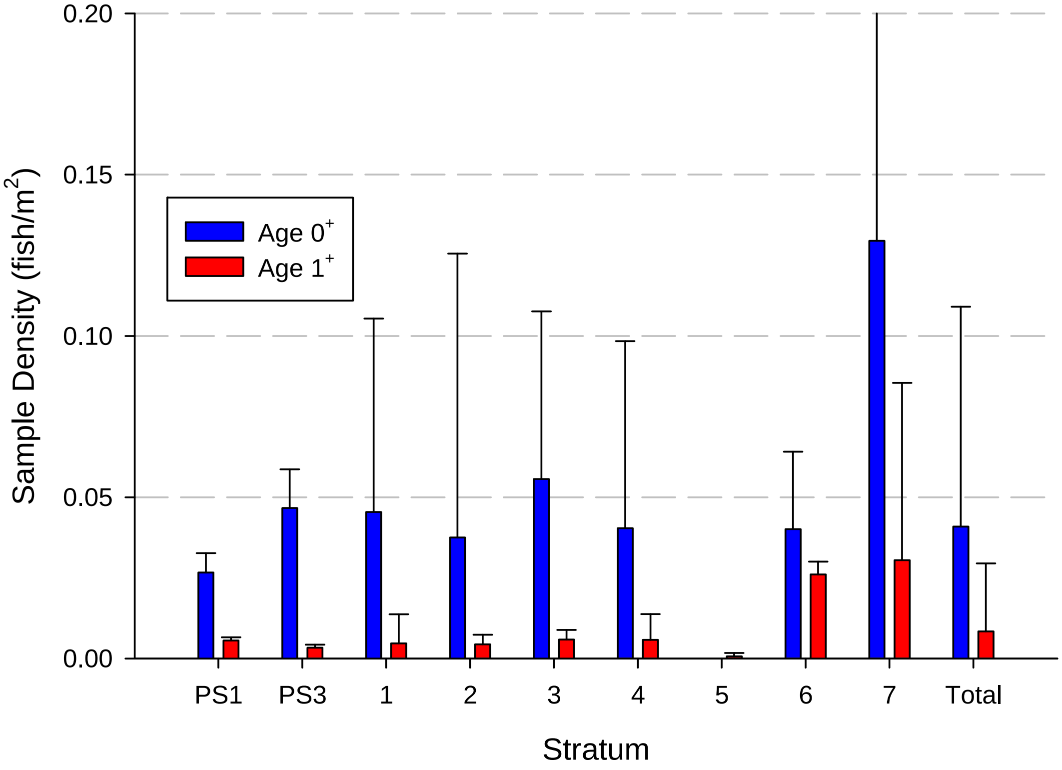

Figure 3. Age-0^+ and 1^+ *O. mykiss* density by strata (n=3) and total for Yankee Fork, Idaho 2008. Error bars represent 95% confidence interval of mean.

Mean steelhead length ranged from 53 mm in stratum 7 to 96 mm in stratum 6. Average length equaled 62 mm in Pond Series 1 & 3, 62 mm in lower YF (strata 1 – 3), 97 mm in West Fork Yankee Fork (inclusion of smolts from screw trap), and 71 mm in upper Yankee Fork (strata 4 and 5) and Jordan Creek. Length frequency distribution consisted predominately of age-0^+ individuals with a median of 53 mm and mode 55 mm. Majority of the individuals (n = 376) were between 50 – 59 mm (Figure 4). The following figure graphically displays length frequency of *O. mykiss* between 2006 and 2008. Dominant sampled size class over the past three years has been between 50 and 59 mm fork length.

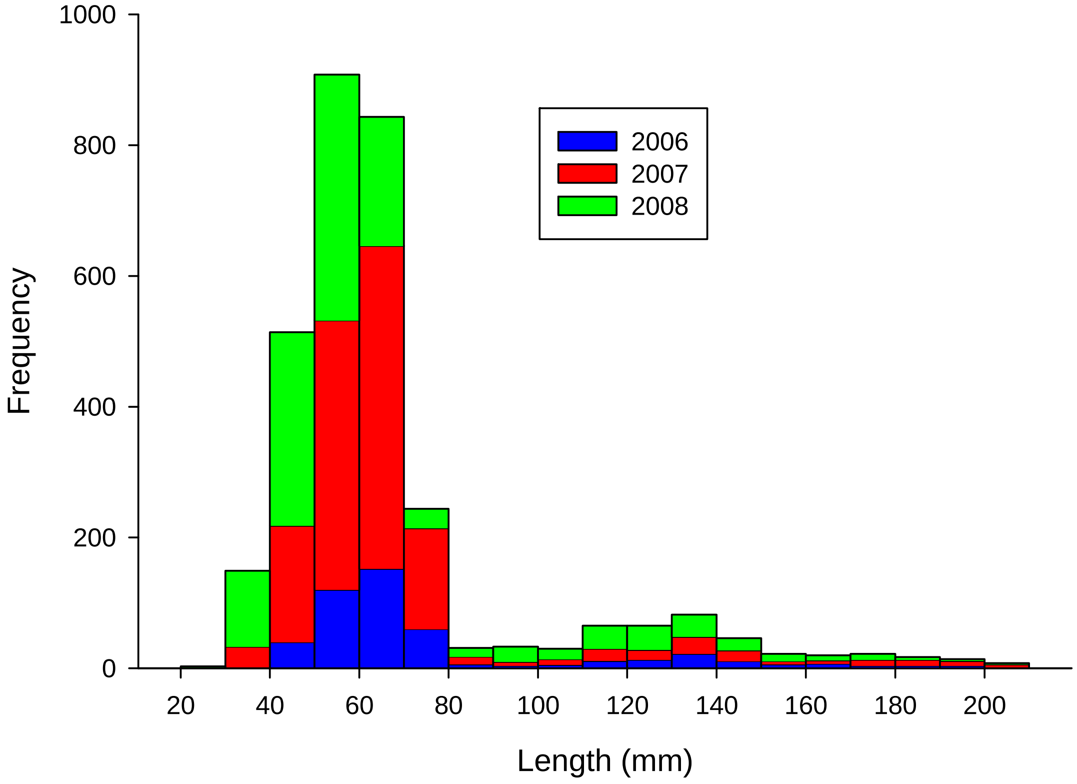

Figure 4. Frequency distribution of *O. mykiss* for all strata Yankee Fork, Idaho 2006-2008.

Genetic Parentage Analysis

Staff collected 743 BY07 age-0^+ (2007) and 187 BY07 age-1^+ (2008; 120 mainstem and 67 WF RST) juvenile genetic samples that were analyzed by Abernathy Fish Technology Center for parentage analyses. In order to minimize potential bias from differential numbers of juveniles sampled per stratum, 0^+ samples were randomly selected based on equal percentages per stratum, and therefore, only a total of 464 age-0 + juveniles were genotyped. The percentage of juvenile samples per stratum to use (0.248) was calculated as the total number of samples to be genotyped (400), divided by the point estimate of the sum of all sampled reaches (1615).

All incubators for BY07 SSI incubation in Yankee Fork were successful unlike previous years. In comparison to BY06 1^+ juveniles (n = 2), there were 15 individuals assigned to BY07 SSI progeny. Thirteen of the fifteen identified 1+ progeny were sampled in Jordan Creek (stratum 7); strata 5 and 6 (WFYF) each produced one identified juvenile. Primary age-0^+ juvenile migration, as seen by increased densities below upweller sites, is restricted downstream due to poorly developed functional morphology. All identified 0^+ progeny were sampled downstream of incubator locations. Stratum four, site two, located below the Greylock Creek upweller, once again showed 100% genetic assignment (n = 1) as in 2006 (n = 38). Stratum 7, site 3, located directly below the Jordan Creek upweller, showed 97% and 100% assignment for BY07 0^+ and 1^+ juveniles, respectively. This may indicate that SSI progeny may be overwintering in the same location and potentially emigrating as two year old smolts. No progeny were identified in either Pond Series or from the West Fork screw trap. Total percent assignment equaled 13.50% (87 of 646) slightly higher than 2006 which was 13.14% (62 of 472). There were a total of 72 age-0^+ (15.7%) and 15 age-1^+ (8.0%) assignments from broodyear 2007 outplants (Figure 5) (Williamson et al. 2009; Appendix B).

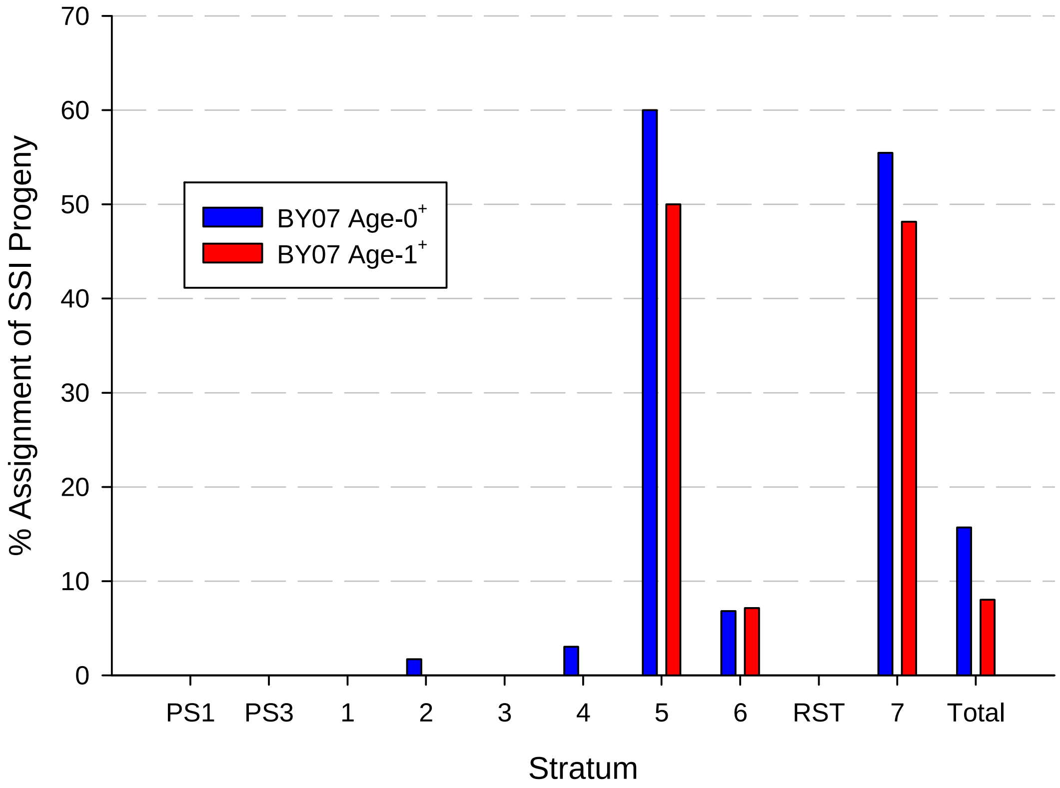

Figure 5. Parental assignment percentages per stratum and total. RST indicates samples collected by the SBT ISS crew at the West Fork Yankee Fork screw trap. Note: upper scale max is 70%.

There was no significant difference in mean condition factor or mean fork length between SSI progeny (genetically identified) and wild steelhead (unidentified) sampled in the Yankee Fork. Slightly shorter streamside progeny length may be the result of earlier emigration as compared to natural steelhead.

Staff calculated the area by strata and for the entire Yankee Fork from length and width data collected by SBT field crews, Konopakcy et al. (1986), and Ray et al. (SBT unpublished). Using densities collected during 2007 sampling, the Tribes estimated total abundance by strata and for the overall drainage. Percent parental assignment by strata was applied to the estimated abundance of *O. mykiss* to determine the percent of SSI progeny. There were 90 estimated SSI progeny in strata two, 71 in strata four, 91 in strata five, 787 in strata six, and 4,681 in strata seven.

Overall estimated age-0^+ *O. mykiss* equaled 32,276 juveniles in 2007 with 5,720 individuals being from streamside origin (Figure 6). Therefore, we estimate that 16.7% of the Yankee Fork age-0^+ steelhead population originates from streamside incubator supplementation. Stratum seven shows the highest abundance of incubator progeny at 82% of the sample. Survival of hatchery-origin fry to age-0^+ parr is estimated at 1.7%; however, fish maybe leaving the system before sampling or low sample size underestimates abundances. Operation of a rotary screw trap in 2009 will answer migration timing questions as well as help solidify population abundance estimates.

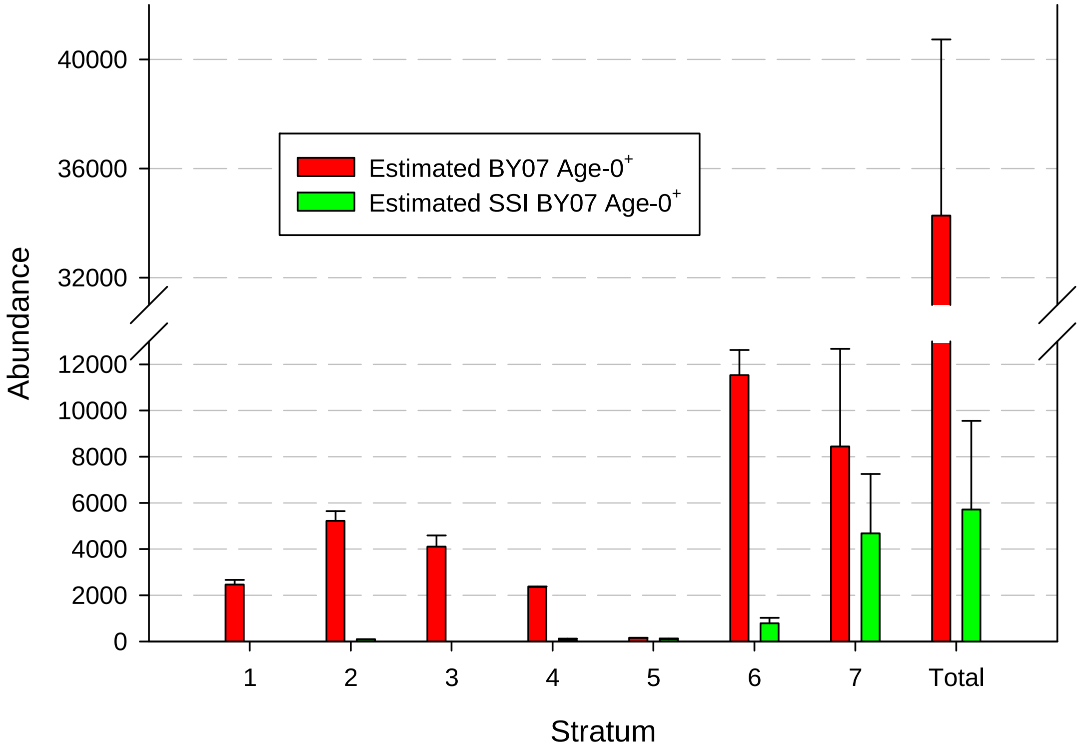

Figure 6. Age-0^+ abundance estimates for SSI progeny and total *O. mykiss* in 2007. Error bars represent 95% confidence interval of mean.

Using densities collected during 2008 sampling, the Tribes estimated total age-1^+ abundance by strata and for the overall drainage. Percent parental assignment by strata was applied to the estimated abundance of *O. mykiss* to determine the percent of SSI progeny. There were 26 estimated SSI progeny in strata five, 274 in strata six, and 431 in strata seven.

Overall estimated age-1^+ *O. mykiss* equaled 6,464 juveniles in 2008 with 730 individuals being from streamside origin (Figure 7). Therefore, we estimate that 11.3% of the Yankee Fork age-1^+ steelhead population originates from streamside incubator supplementation. Stratum seven shows the highest abundance of incubator progeny at 59% of the sample. Survival of hatchery-origin fry to age-1^+ smolt is estimated at 0.2%; parr to smolt is estimated at 12.8%; however, fish maybe leaving the system before sampling or low sample size underestimates abundances. Operation of a rotary screw trap in 2009 will answer migration timing questions as well as help solidify population abundance estimates.

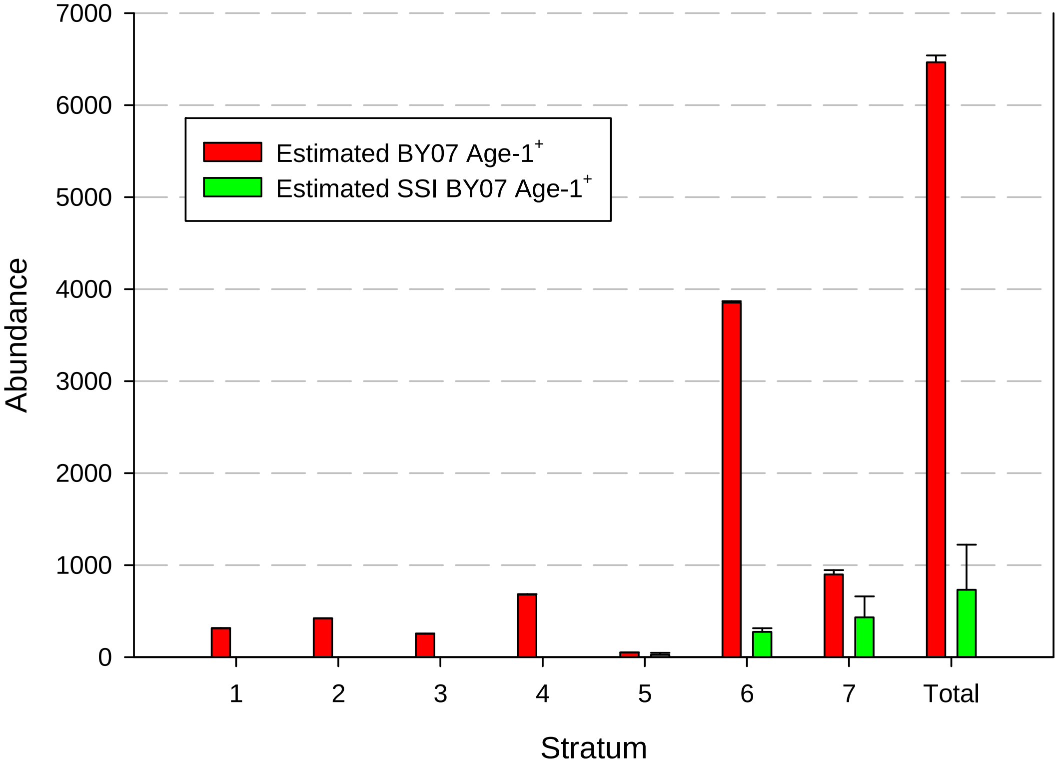

Figure 7. Age-1^+ abundance estimates for SSI progeny and total *O. mykiss* in 2008. Error bars represent 95% confidence interval of mean.

Survival estimates between life stages were equated from the SSI progeny estimates determined from the parentage analysis. For SSI supplementation activities in broodyear 2007, egg to fry survival was estimated at 92.9%, fry to parr survival at 1.7%; and parr to smolt survival was 12.8%. Smolt to adult survival can only be determined with access to handling returning adult steelhead. Currently, with no permanent trapping facilities, the SBT must potentially rely on obtaining adult tissue samples from Tribal harvest.

DISCUSSION

Overall, staff collected 479 BY08 age-0^+ (2008) and 187 BY07 age-1^+ (2008; 120 mainstem and 67 WF RST) juvenile genetic samples. Abernathy Fish Technology Center analyzed a total of 459 BY07 age-0^+ and 187 BY07 age-1^+ juvenile samples for parentage analyses in the fall of 2008. There were sixty-five total mortalities or 3.7% of all fish handled. In addition, scale samples were randomly taken from a total of 18 steelhead individuals for analysis at or just above 80 mm fork length.

Salmonid species observed in Yankee Fork included steelhead, Chinook salmon, bull trout, cutthroat trout, and mountain whitefish. Steelhead were the most ubiquous species in Yankee Fork, although, bull trout was found dominating upper Yankee Fork (strata 5). On average, strata 5 water temperature was cooler than other strata and may restrict the presence of other salmonid species. Highest percentage of cutthroat trout was found in strata 7, an area characterized as a large resident population. Chinook salmon densities remain low, although should increase in the near future under Yankee Fork Chinook Salmon supplementation activities. Overall density was extremely low (0.070 fish/m^2), not nearly close to a predicted carrying capacity of 1.0 fish/m^2. Densities of stream salmonids may be lower in the fall due to lower temperature and emigration. Peery and Bjornn (2000) reported seeing lower fall salmonid densities at or below 10 ^{o}C. At low temperatures, salmonids may seek cover in the bottom substrate and be less susceptible to electrofishing. In addition, emigration to locate over wintering habitat may have also contributed to low salmonid densities (Peery and Bjornn 2000).

The use of DNA, especially the parental exclusion method, has improved the ability to discriminate stocks and progeny of parental crosses without harming fish in the collection process. The success of the upweller program and contributions to overall abundance of steelhead in the Yankee Fork has been difficult to evaluate beyond documenting changes in overall density. Survival of hatchery origin progeny has not been well documented, however, parentage assignments observed from the 2007 analysis provides evidence that upweller origin juveniles successfully emerge and survive in-stream through the first year of life (Matala et al. 2008). Broodyear 2007 evaluations indicate that age-1$^+$ juveniles do survive in stream (Williamson et al. 2009) although production may be limited to poor survival from the fry to parr life stage.

Migration time and age at migration is unknown; however, parentage analysis provides an initial foundation for understanding movement of juvenile steelhead in the Yankee Fork. Migratory behavior of age-0$^+$ juveniles appears to be limited and individuals remain in areas directly adjacent to and below incubators. Likewise, Richards and Cernera (1989), Close and Anderson (1992), and Peery and Bjornn (2000) determined upstream steelhead fry densities were insignificant and movement was exclusively downstream from release sites. Greater movement was observed with age-1$^+$ juveniles in 2007 as two SSI progeny were identified in strata five and seven, areas with no incubator influence. Staff predetermined that age-1$^+$ juveniles may be migrating from the Yankee Fork before sampling in the second fall. However, genetic results from strata seven site three (Williamson et al. 2009) may indicate that juveniles continue to overwinter in the same location adjacent to upwellers until potentially migrating as age-2$^+$ smolts. That said, migration as age-1$^+$ smolts does occur as an identified BY07 age-0$^+$ (2007) parr in strata 7 was subsequently sampled as a BY07 age-1$^+$ smolt downstream in strata 1 in 2008 (Williamson et al. 2009).

Acknowledging there is combination of SSI and natural progeny in the Yankee Fork, differences in length and condition factor should be observable. Irvine and Bailey (1992) and Perry and Bjornn (2000) determined hatchery-origin fish are typically larger in length and mass. Irvine and Bailey (1992) have also reported higher mean condition factors for hatchery-origin fish than wild fry in supplemented regions. After genetic analysis, staff was able to compare fork length and condition factor of identified SSI progeny and wild juveniles and found no statistical difference. Countering the above studies, through hatchery domestication, progeny from outplanted eggs may migrate earlier and, therefore, be slightly smaller in size (i.e. length and condition) compared to the natural origin counterpart (Matala et al. 2008)

Estimated overall productivity in the Yankee Fork is extremely low. Staff estimated 34,276 (± 3,228) broodyear 2007 *O. mykiss* age-0^+ and 6,464 (± 38) age-1^+ juveniles in Yankee Fork. Of the 34,276 age-0^+ juveniles, 5,720 (± 1,917) or 16.7% of the population were estimated as SSI progeny. Likewise, 11.3% or 730 (± 245) age-1^+ juveniles were identified as SSI progeny.

Full broodyear survival percentages were estimated for supplementation activities in 2007. Egg to fry survival remains extremely high as in previous years at approximately 93%. Fry to parr survival (1.7%) is low and may be the indicating factor why few age-1^+ smolts are encountered in the following fall sampling season. Although limited information is available on migration timing, the low survival rate to the parr stage indicates that SSI progeny are likely not surviving through the conditions in the Yankee Fork. Parr to smolt survival was estimated at 12.8%. Given the higher survival at this life stage, supplementation efficacy may benefit from other release strategies besides streamside egg incubation.

Continued genetic evaluation is critical to determine the long-term efficacy of steelhead streamside supplementation activities. Limited information on numbers of returning adults, redd counts, size of the natural origin population, and migration timing restricts our ability to fully estimate the relative productivity of upweller supplementation. The Tribes propose that the addition of a weir and continued screw trap operation would greatly increase the ability to document the natural spawning population and estimate the efficacy of streamside supplementation to increase population abundance in Yankee Fork.

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APPENDIX A. MAPS

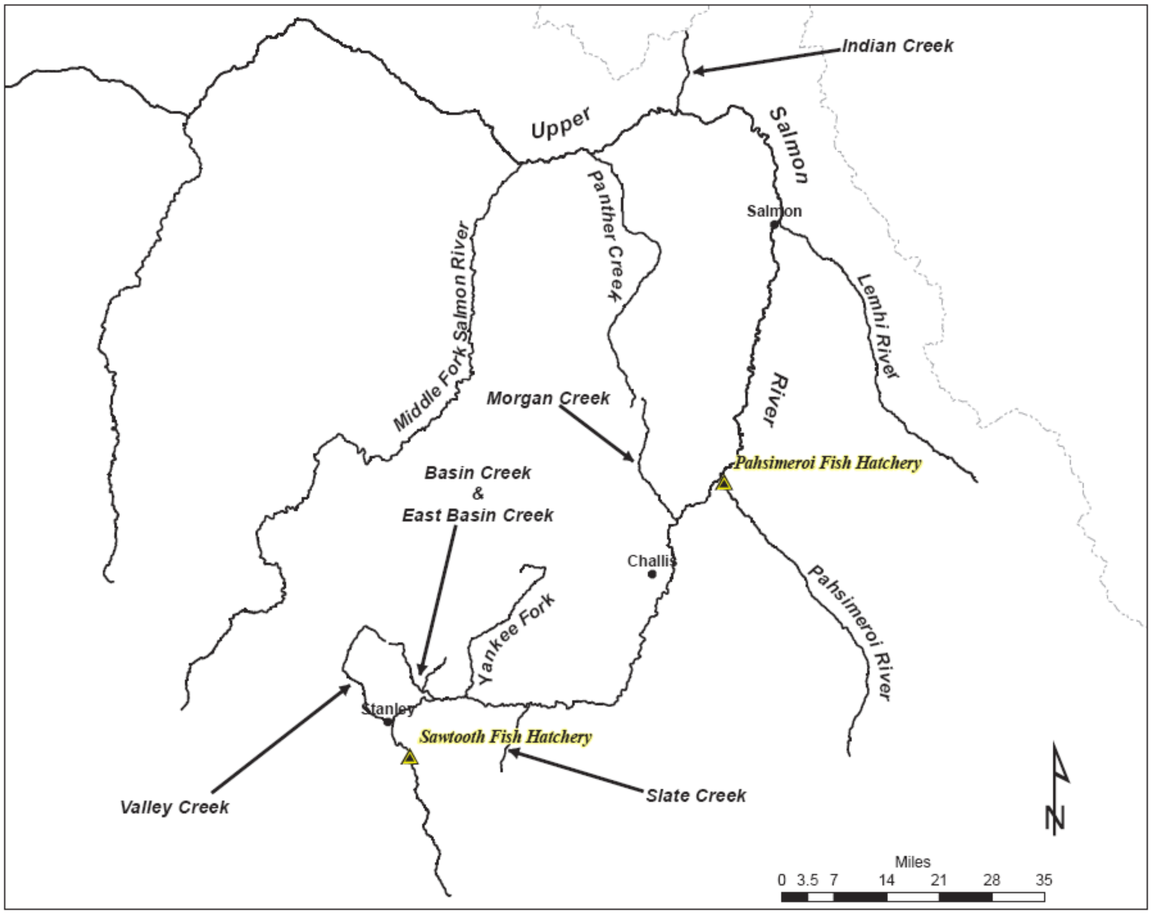

Figure 8. Upper Salmon River Basin.

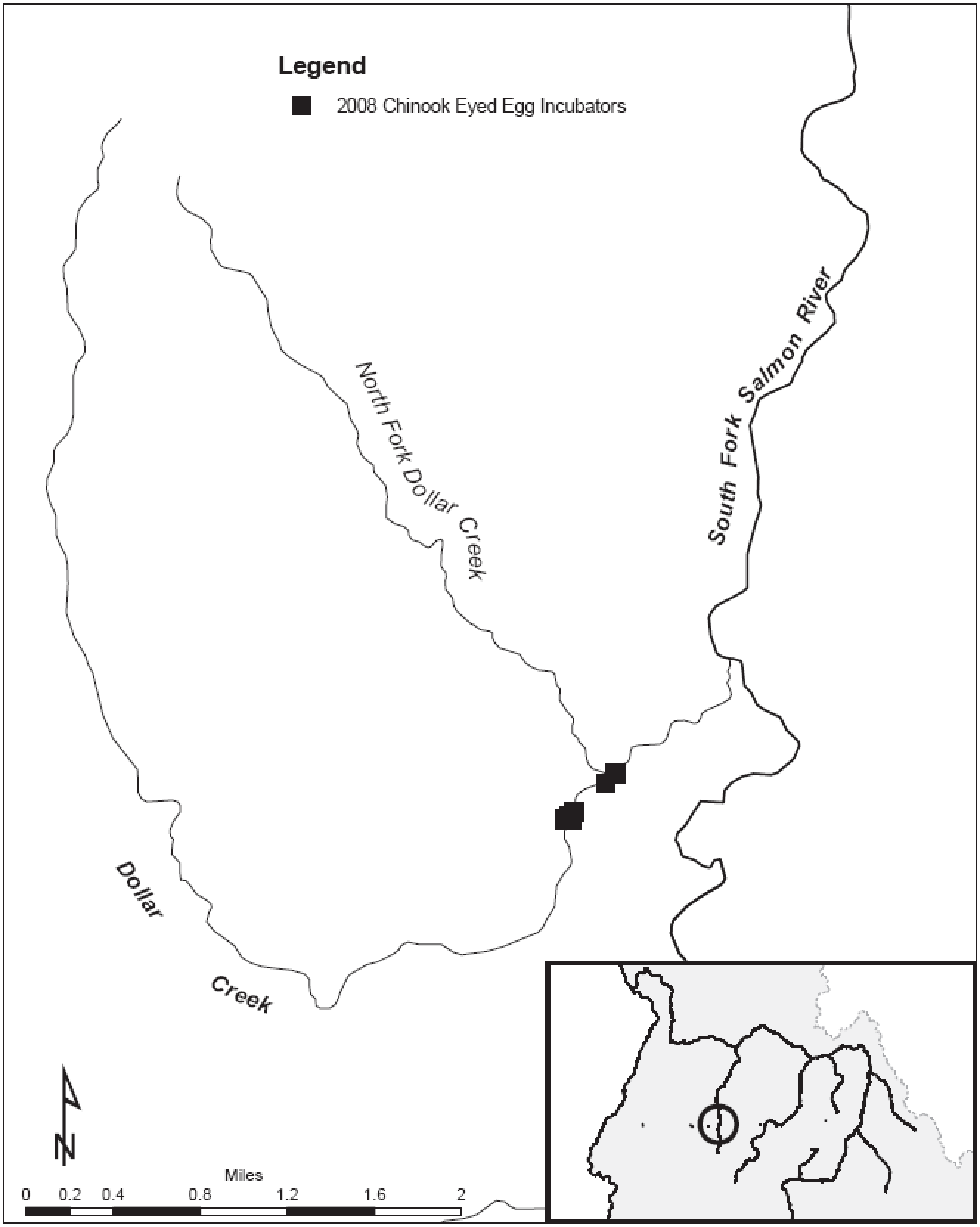

Figure 9. South Fork Salmon River with respect to Dollar Creek incubators.

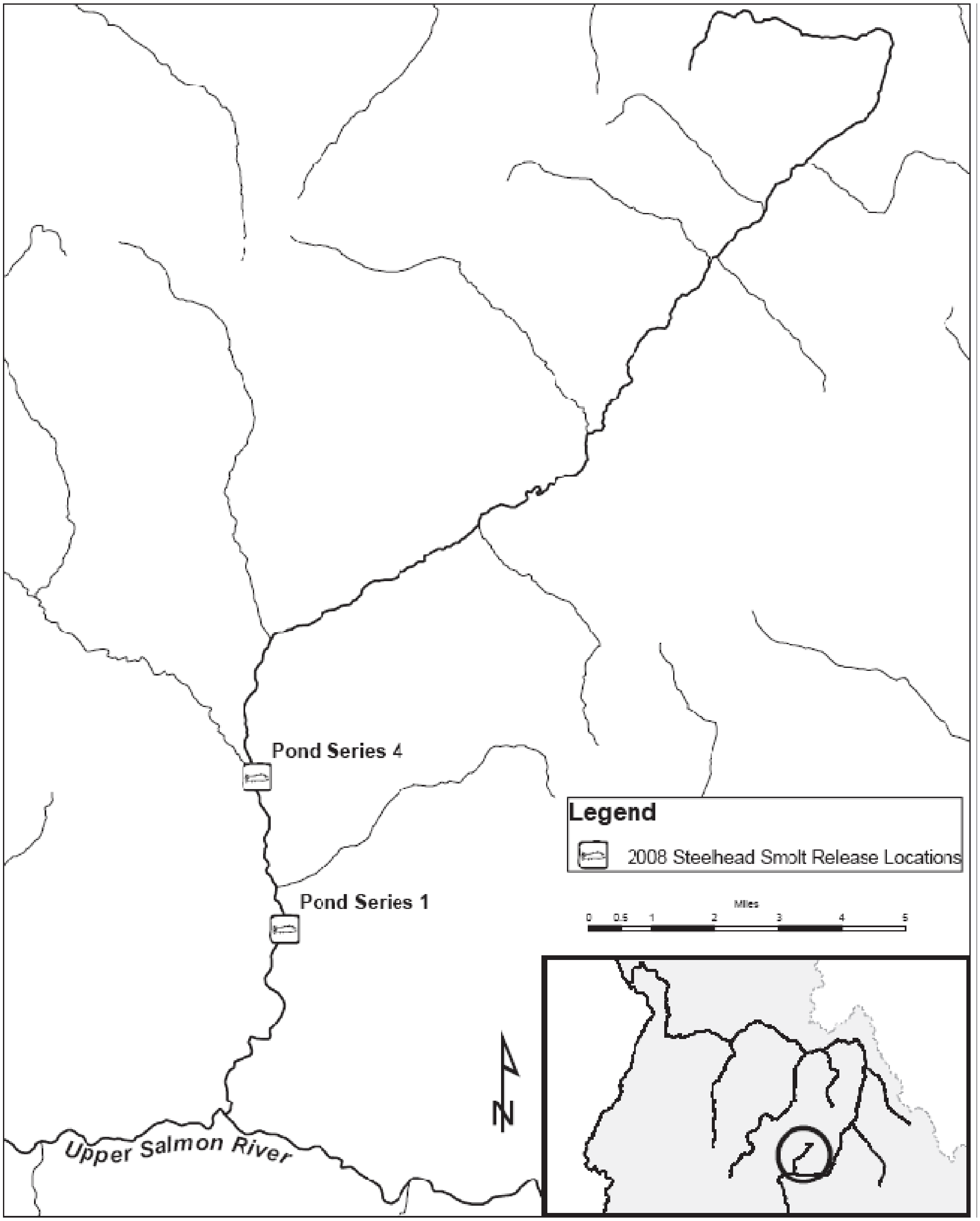

Figure 10. Yankee Fork Salmon River with smolt release locations.

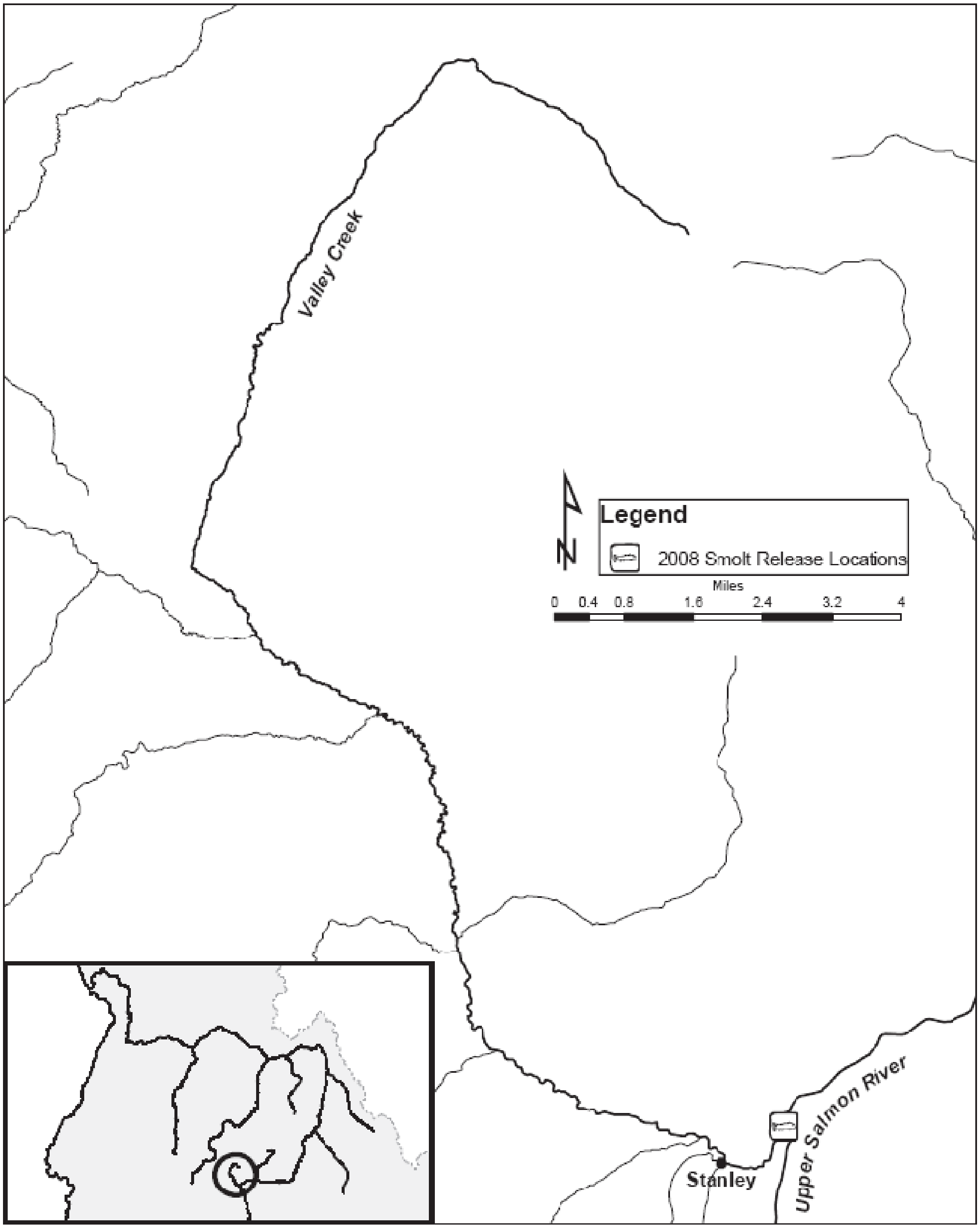

Figure 11. Valley Creek with smolt release locations.

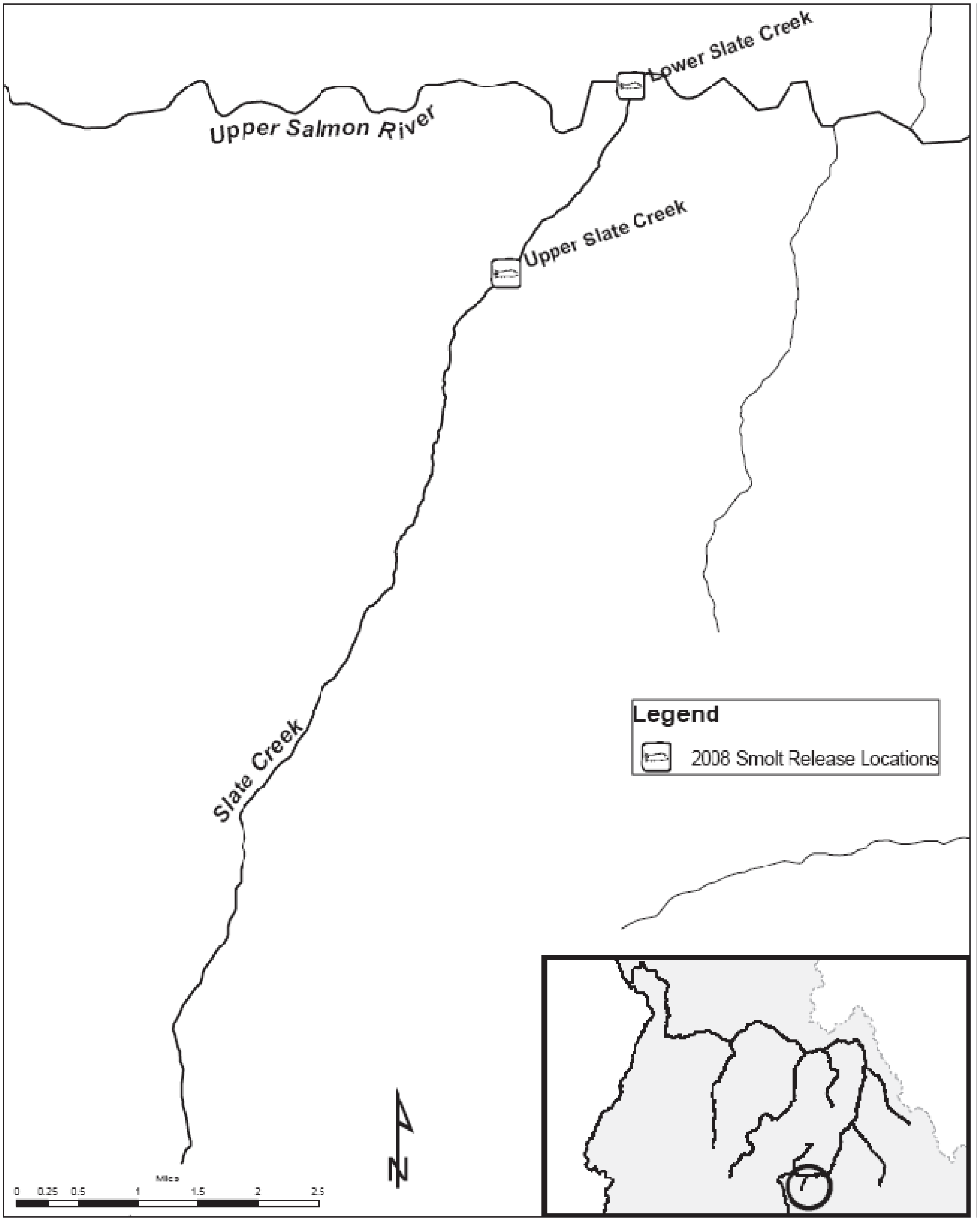

Figure 12. Slate Creek with smolt release locations.

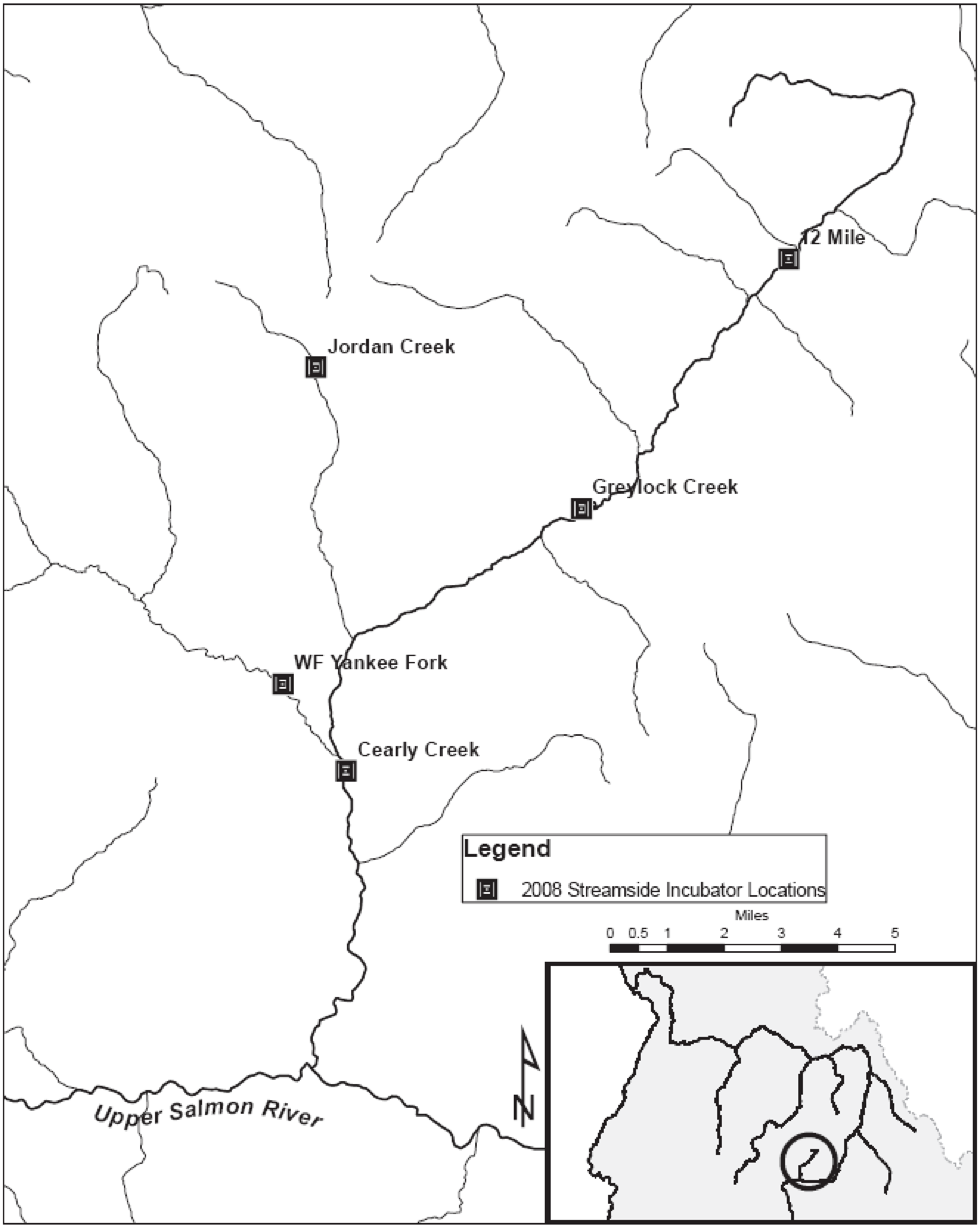

Figure 13. Yankee Fork Salmon River with egg incubation locations.

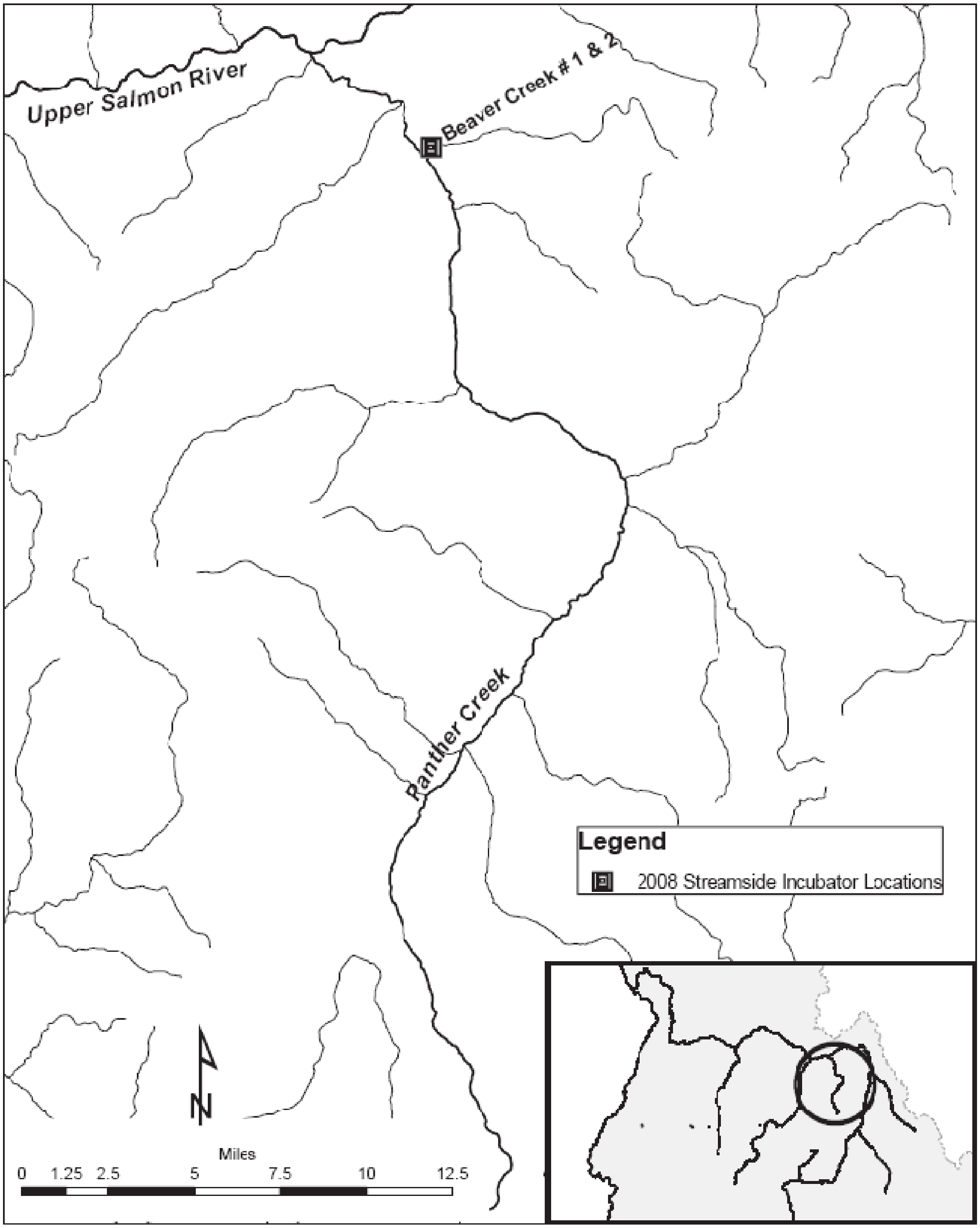

Figure 14. Panther Creek with egg incubation locations.

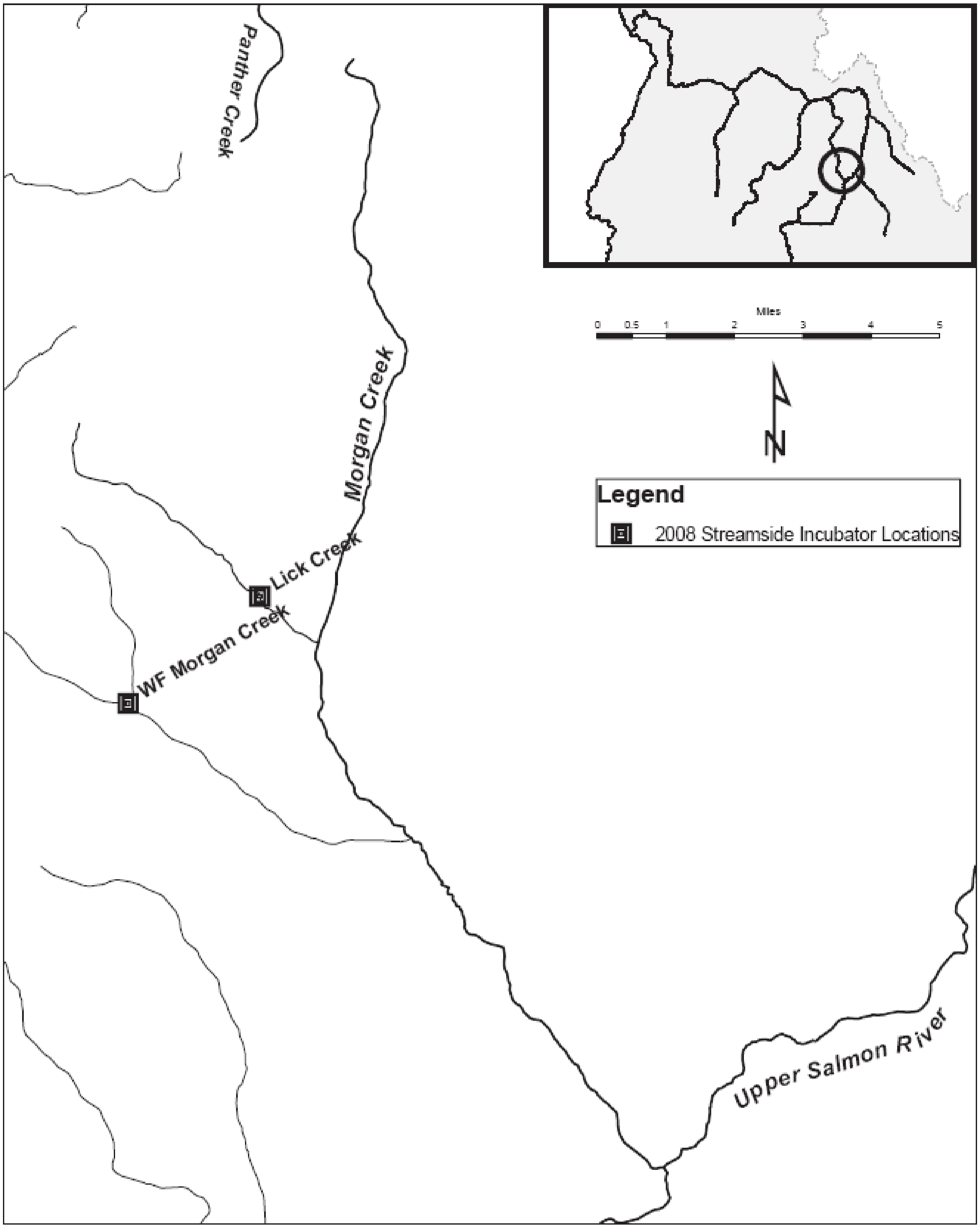

Figure 15. Morgan Creek with egg incubation locations.

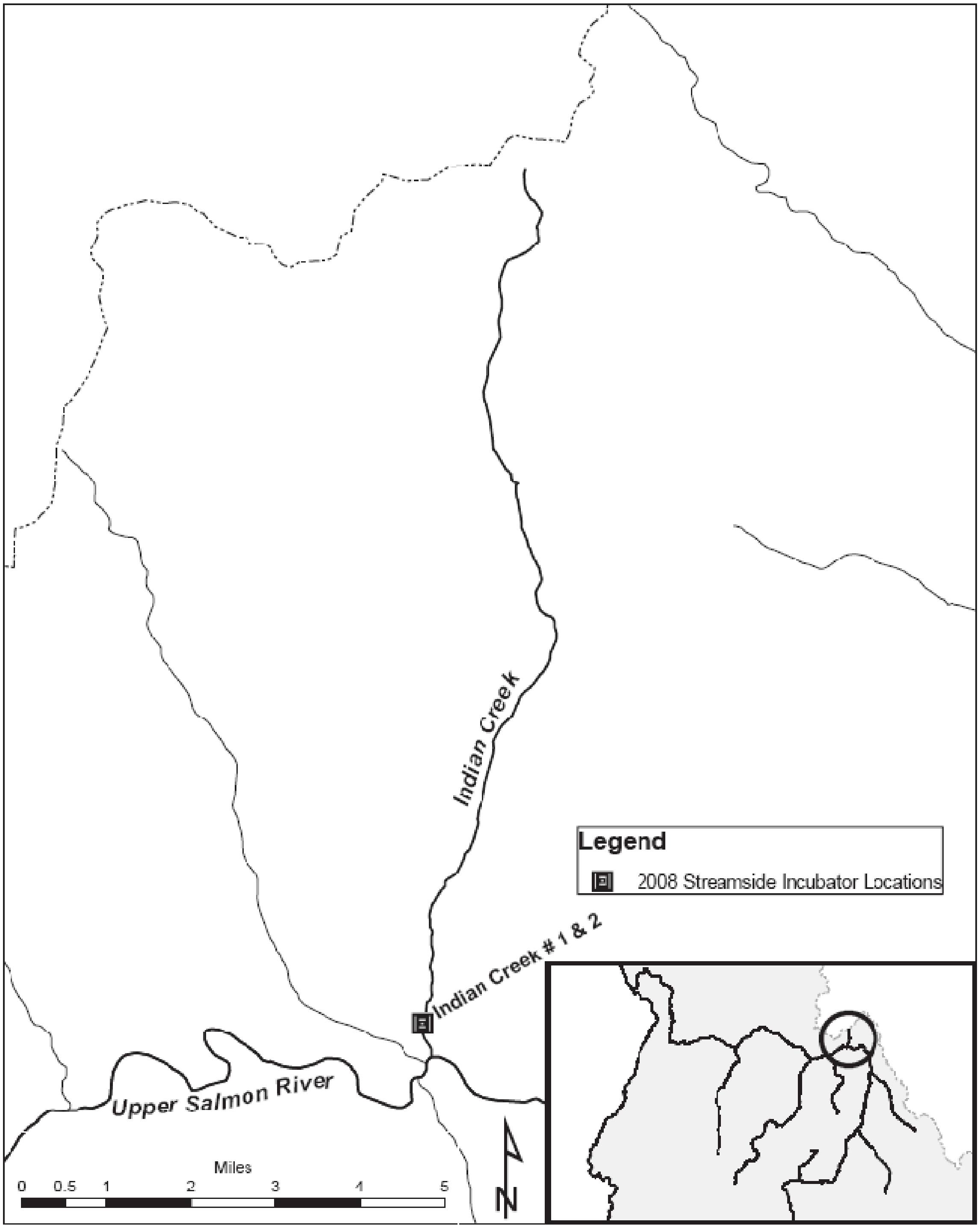

Figure 16. Indian Creek with egg incubation locations.

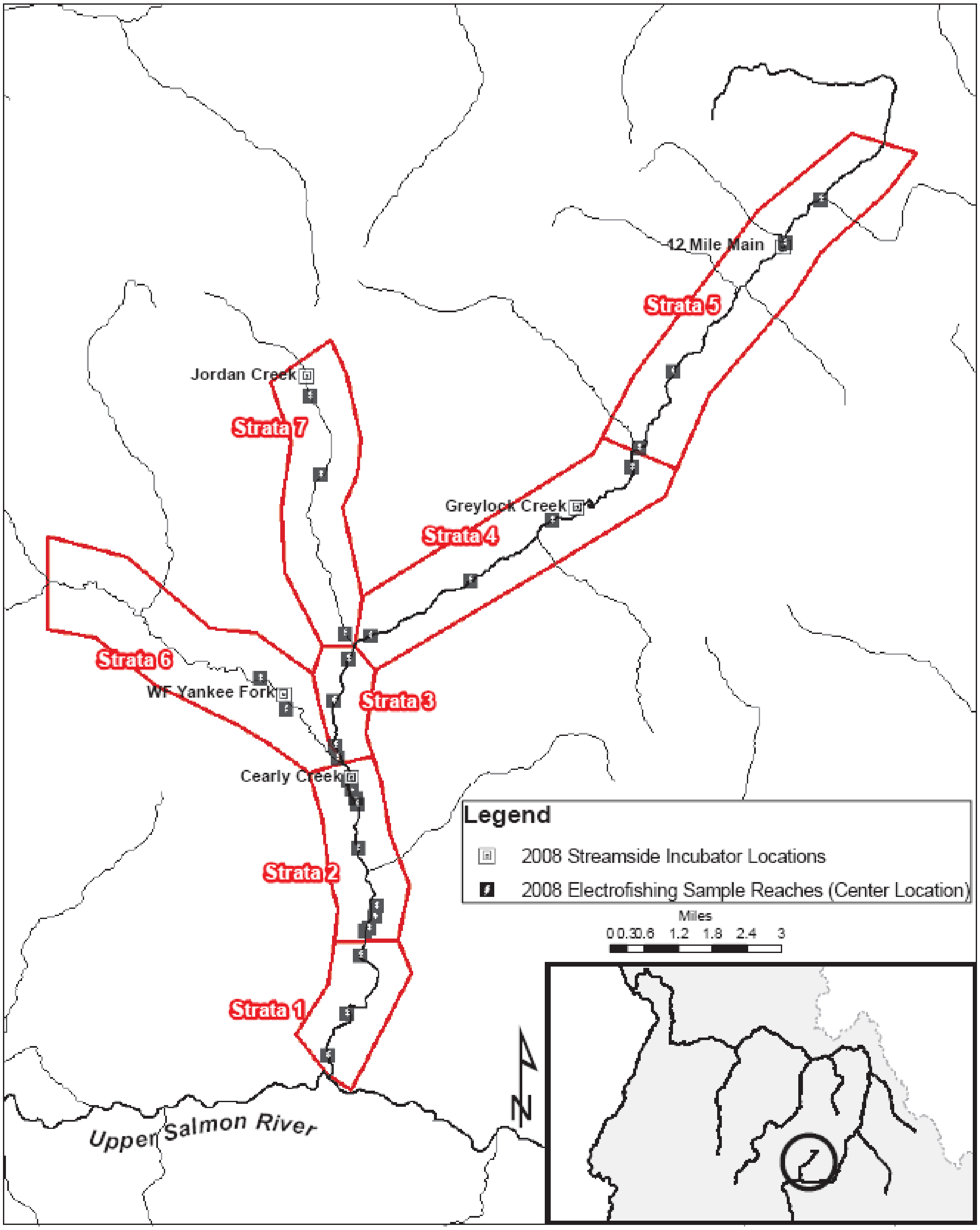

Figure 17. Yankee Fork stratified by Konopacky et al. (1985, 1986) displaying 2008 sampling locations.

APPENDIX B. ABERNATHY FISH TECHNOLOGY CENTER FY2008 STREAMSIDE INCUBATOR PROJECT REPORT

Pedigree analysis reveals relative survival and abundance of juvenile hatchery steelhead outplanted as eyed eggs in the Yankee Fork Salmon River, Idaho

United States Fish & Wildlife Service Abernathy Fish Technology Center Report

October 15, 2009

Prepared by:

Kevin S. Williamson[1], Ph.D. and Andrew P. Matala[2]
[1]Fish4Genes Consulting
Seattle, WA 98107
TEL: 206-795-4568
fish4genes@gmail.com

[2]U.S. Fish and Wildlife Service
Abernathy Fish Technology Center
1440 Abernathy Creek Road
Longview, WA 98632
TEL: 360-425-6072
FAX: 360-636-1855

Prepared for:

Christian Smith, Ph.D. and Denise Hawkins, Ph.D.
U.S. Fish and Wildlife Service
Abernathy Fish Technology Center
1440 Abernathy Creek Road
Longview, WA 98632
TEL: 360-425-6072
FAX: 360-636-1855
Christian_Smith@fws.gov, Denise_Hawkins@fws.gov

In cooperation with:

Lytle Denny and Kurt Tardy
Shoshone-Bannock Tribes
Fish and Wildlife Department
3rd and B Avenue
Fort Hall, ID 83203
ldenny@shoshonebannocktribes.com
ktardy@shoshonebannocktribes.com

Summary

The Shoshone-Bannock Tribes (Tribes) are currently conducting monitoring and evaluation (M&E) of steelhead trout (*Oncorhynchus mykiss*) supplementation activities in the Yankee Fork, which includes the use of streamside incubators (upwellers) for rearing hatchery origin (HOR) fish. Juvenile HOR fish that hatch from eggs transplanted to upwellers have no physical mark whereby they may be distinguished from natural origin (NOR) juveniles. Consequently, the success of the upweller program and its contributions to overall abundance of steelhead in the Yankee Fork has been difficult to evaluate. This report presents the results of a DNA-based parentage analysis performed in 2008 that provides the second year's estimate of the contribution from the upweller program to total abundance of steelhead in the Yankee Fork. Full parental genotypes (FPG) were generated for all 2007 Sawtooth Fish Hatchery brood stock (N=174) that supplied gametes for artificial spawning. Eyed eggs produced by the artificial spawnings were transplanted to streamside upwellers at five locations within the Yankee Fork in 2007. Multilocus genotypes were generated for age-0^+ and age-1^+ juveniles (N = 464 and 120, respectively) collected throughout the Yankee Fork during 2007 and 2008, respectively. In addition, outmigrating (smolt) juveniles (N = 67) collected in a rotary trap in 2008 were genotyped. Parentage assignment was performed to determine the proportion of juveniles that originated from upwellers. Juveniles that were not assigned to known parents were assumed to be NOR. Parentage assignment identified 72 age-0^+ and 15 age-1^+ juveniles produced from upwellers, with an overall relative proportion of 0.135 HOR juveniles produced from the 2007 spawning efforts among all juveniles sampled (N = 651). Under this study design, Shoshone-Bannock Tribal staff will be able to determine the efficacy and cost-effectiveness of the steelhead trout streamside incubation program.

Introduction

Within the Salmon River, the Yankee Fork sub-basin (Figure 1) has historically supported productive populations of steelhead trout that constitute a significant cultural, social, and subsistence based resource for the Tribes. Several steel head trout and Chinook salmon (*O. tshawytscha*) populations have experienced significant declines coincident with construction of hydroelectric dams on the lower Snake and Columbia Rivers (Raymond 1988; Williams 1989). Among these populations smolt-to-adult return rates decreased from above 4% during the 1960's, when only four dams existed, to less than 2% on average during the 1970's after eight dams were in place. Another potential limiting factor to production in the Salmon River sub-basin is the declining availability of rearing habitat. Efforts to restore habitat have been implemented in Yankee Fork including installation of dredge ponds and connecting channels (Richards et al. 1992). These types of restoration efforts may br beneficial to both NOR and supplemented HOR juvenile rearing.

The Tribes have established and implemented an M&E plan to determine the degree to which steelhead trout supplementation in the Yankee Fork contributes to adult-to-adult survival and recruitment (Denny et al. 2007). Initial monitoring of HOR juvenile survival and their ability to reproduce in the natural environment as adults will be important in evaluating the efficacy of the supplementation program. As part of the supplementation activities implemented by the Tribes streamside upwellers are utilized to provide natural incubation and permit volitional release of HOR juveniles. Both Solazzi et al. (1999) and Denny and Tardy (2008) have demonstrated high egg-to-fry survival in upwellers. However, the ecological processes affecting post-release survival of HOR juveniles (fry and parr) are unknown. For instance, it is possible that NOR juveniles are able to utilize winter concealment and shelter habitat more effectively than HOR juveniles (Nickleson et al. 1992; Orpwood et al. 2004). It is also possible that artificial spawning carried out in the supplementation program itself presents a modified selection regime that imparts genetic and phenotypic differences between HOR and NOR fish, and consequently results in HOR steelhead experiencing decreased survival in the natural environment (Kostow 2003 and 2004; Miller et al. 2004; Berejikian and Ford 2004), or decreased reproductive fitness relative to NOR fish (Araki et al. 2007; Chilcote et al. 1986; Chilcote 2003; Matala et al. 2005). Relative survival and recruitment of HOR juveniles originating from upwellers and that rear in the natural environment is not well documented.

In this report we provide results from the second year of a proposed four year study to determine the efficacy of upwellers used as a supplementation tool for steelhead trout in the Yankee Fork. To assess the ability of the upweller program to contribute to production or abundance relative to NOR fish, post-release survival of HOR juveniles was investigated using DNA-based parentage assignment. This molecular genetic method of determining the origin of juveniles sampled throughout Yankee Fork involves matching multilocus genotypes (based on the rules of Mendelian inheritance) between juveniles and known parental pairs used to supply the upwellers. Jones and Ardren (2003) provide a review of DNA-based parentage assignment methods. The likelihood of correctly matching progeny to a parental pair increases as a function of the number and degree of polymorphism of the loci evaluated (Bernatchez and Duchesne 2000). Parentage assignment has been used to make inferences regarding mating systems (Seamons et al. 2004), factors influencing relative fitness and productivity among HOR and NOR stocks (Blouin and Araki 2004; Williamson et al. 2009), dispersal and migration (Paetkua et al. 2004), and grand parentage (Letcher and King 2001).

Methods

Broodstock and Field Sampling

Fin clips were taken to supply DNA for genetic analysis from all 87 mated pairs of Sawtooth Hatchery broodstock (P2; represents the 2nd generation parental evaluated) used for the broodyear (BY) 2007 Yankee Fork upweller program. Artificial spawning was conducted on April 12, 19, and 26 in 2007. Each family was produced using only one male and one female parent. Fertilized eggs intended for outplanting were incubated separately from general production within the Sawtooth Hatchery. Family identity was preserved during egg outplant by recording which broodstock pairs were represented in each streamside incubator, however, families were randomly assigned to incubators. Linking family identity to streamside upwellers provides a means to identify the initial stratum into which subsequently captured juveniles had been placed and to trace their movement within the Yankee Fork watershed. Eyed eggs were transferred in numbers ranging from 71,177 to 73,179 per upweller. One upweller was maintained at each of the following sites in the Yankee Fork in 2007: Cearly Creek, Greylock Creek, Jordan Creek, West Fork Yankee Fork, and 12 Mile Creek (Figure 2). The upwellers were used to incubate 358,353 steelhead eyed-eggs, in total, from the 2007 brood year. Eggs were planted on June 6 and 8 in 2007, and fry volitionally emigrated throughout the season. Following outplant, egg survival was quantified by removal and inspection of incubator trays to enumerate dead eggs (N= 25,159). The hatch success rate was 93%, resulting in an estimated release of 333,194 seeded fry.

Non-lethal genetic sampling of juvenile *O. mykiss* (F1; putative steelhead) was performed throughout the Yankee Fork. Juvenile fish were collected in a three-pass procedure using block nets for crowding and electroshocking. To maximize the number of family groups represented in the sample of juveniles, thereby preventing family group or kinship bias, no more than 20 juveniles were collected per each 100 meters of stream surveyed during any one sampling event. Age-0^+ juveniles (N=464) and age-1^+ juveniles (N=120) were sampled in 2007 and 2008, respectively. In addition, migrating (smolt) juveniles (N = 67) were collected in a rotary trap located on the West Fork Yankee Fork in 2008. In order to minimize potential bias due to variability in the number of juveniles sampled per stratum, samples were randomly selected such that the same percentage of juveniles from each stratum was included in the pedigree analysis. The percentage of juvenile samples per stratum to use (0.248) was calculated as the total number of samples to be genotyped (400), divided by the point estimate of the sum of all sampled reaches (1615).

Juvenile sampling was performed within the Yankee Fork sub-basin during September 12-13, 18-19 and October 2-5, 10-12 in 2007, and Sept. 16-17, 22-26, Oct. 1, 8, 10, 21, 23, and Nov. 2 in 2008. The Tribes utilized Konopacky et al. (1985, 1986) to sub-divide the Yankee Fork drainage into seven strata (Figure 2). Three reaches were selected for sampling within each stratum except for stratum four which contained four reaches in 2008. Sampling sites were randomly selected within the mainstem flow for a variety of habitats (pools, glides, riffles) and ease of accessibility for an upper, middle, and lower location within each stratum, but did not include any dredge pond habitat until sampling in 2008. Sites were, in general, rectangular in shape, aligned with the shoreline, and divided into transects for habitat measurements. Sites were electrofished in an upstream direction between 20-30 minutes with a crew member electroshocking (Smith-Root, Inc. Pulsed DC LR-24 Backpack Electrofisher) and two or three other members handling dip nets to capture fish drifting downstream under electronarcosis. Up- and downstream ends of the sampling site were blocked using 7mm mesh nets secured to the streambed with tripods and rebar, generally at habitat unit separations. Morphological measurements (fork length, weight) were recorded and scale samples to verify age were taken for each juvenile sampled. A small section (~3mm^2) of rayed fin tissue was taken from each juvenile and placed into a vial containing 100% non-denatured ethanol and labeled with a unique individual identification number.

Microsatellite genotyping

Genomic DNA was extracted from fin clips according to the method of Miller and Kapuscinski (1996). Fourteen nuclear microsatellite loci were amplified by polymerase chain reaction (PCR) assays in all adult and juvenile samples. The 14 microsatellite locus primers included: *Omy1011UW* (Spies et al. 2005), *Ssa*407 and 408 (Cairney et al. 2000), *Ocl*1 (Condrey and Bentzen 1998), *Ogo*3 and 4 (Olsen et al. 1998), *Ots*3, 4, and 100 (Banks et al. 1999), *Oki*23 (Smith et al. 1998), *Omy*7iNRA (K. Gharbi and R. Guyomard, Unpublished), *Omy*77 (Morris et al. 1996), and *Ssa*289 (McConnell et al. 1995). An ABI-3130xl automated DNA sequencer and GeneMapper software (Applied Biosystems, Inc.) were used to resolve and evaluate the size of PCR amplified microsatellite alleles, respectively.

Data quality assurance and control

Genotyping errors may arise from a variety of sources (Hoffman and Amos 2005; DeWoody et al. 2006). Genotyping error rate was determined through systematic re-sampling individuals and duplicating the genotypic analysis. DNA from every 10th individual from among all broodstock and juveniles sampled was subsequently re-extracted from tissue, re-amplified and genotyped at the same 14 microsatellite loci. The original genotypic scores were aligned with the scores obtained from the subsequent duplicate analysis and a genotyping error rate for each locus was calculated from the number of mismatches observed. Original data collection and collection of QA/QC data were performed independently by separate laboratory personnel. Over all 14 loci the genotyping error rate was calculated as 0.0054.

Evaluating dataset for temporal replicate sampling

The entire dataset of genotypic information was first screened for duplicate multilocus genotypes, which indicate either temporal replicate sampling, or low power of the loci to differentiate individuals and exclude non-parents. CERVUS version 3.0 (Marshall et al. 1998; Kalinowski et al. 2006) was used to screen the entire genotypic dataset for duplicate multilocus genotypes. CERVUS was also used to calculate non-exclusion probabilities, across all 14 loci, for identity, the average probability that the set of loci will fail to differentiate between two randomly selected individuals; and for parent pair, the average probability that the set of loci will not exclude a pair of unrelated candidate parents from parentage of an arbitrarily chosen offspring. Assumptions of calculations for non-exclusion include: all loci are in Hardy-Weinberg equilibrium, and all individuals are completely genotyped. The true probability of non-exclusion may be substantially higher if there are missing genotypes.

Parentage assignment simulation

Parentage analysis was simulated using CERVUS to estimate the resolving power of loci given their allele frequencies and the critical values of log-likelihood statistics, delta Log of Odds (ΔLOD) score, so that the confidence of parentage assignments for real data could be evaluated statistically. CERVUS calculates the parentage likelihood (measured as a LOD score) of each of the simulated true and unrelated candidate parent pairs for a large number of simulated offspring. The ΔLOD score is defined as the difference between the LOD scores of the first and second most likely parent pairs to which a given offspring has been assigned. In brief, the allele frequencies for all 14 loci were calculated using the 2007 broodstock genotype data. Allele frequencies (simulation input) were used to generate pairs of parental genotypes and a series of random genotypes representing unrelated candidate parents. For the simulated parentage analysis, 87 true candidate parent pairs were simulated. The proportion of spawning escapement consisting of the true candidate parents was set at 0.378. A total of 7,569 parent pairs (true and unrelated) were simulated. In turn, 10,000 offspring genotypes were generated using the simulated parents following the rules of Mendelian inheritance, and the genotype data were manipulated according to a series of parameters, so that the genetic data were as realistic as possible. Genotyping and likelihood calculation error rates were set at 0.0054 and 0.010, respectively. The stringency level for parentage analysis was set to a minimum of 13 loci compared and allow 0-2 mismatches between parent pair/progeny genotypes (e.g., data from no fewer than 13 of 14 loci must be available and no more than two mismatches occur with respect to alleles inherited from each parent).

During parentage analysis with real data the critical ΔLOD value determined through simulation allows the confidence level of the parentage assignment to be evaluated. Once the parentage analysis simulation is complete, CERVUS generates distribution statistics of ΔLOD scores for each class of parentage assignment outcome produced: (1) assignment to the true parent pair, (2) assignment to a non-parent pair (where either the true mother or father was unsampled), and (3) assignment to an unrelated parent pair (where neither true parent was sampled). The critical ΔLOD score for the simulated parentage analysis is defined as the minimum difference between the first and second most likely parent pair/juvenile LOD scores that contains 95% of the true parent pair/juvenile assignments. During parentage analysis with real data parent pair/juvenile assignment ΔLOD scores that meet or exceed the simulated critical ΔLOD value are accepted as correct.

Parentage assignment with real data

Parentage assignments were performed using CERVUS. The same parameters used in the parentage simulations, genotyping and likelihood error rates, proportion of spawning escapement comprised of candidate parents, and stringency level for mismatched loci were used to perform parentage assignment. A juvenile fish was considered HOR if genetic data confirmed that adults used to supply streamside upwellers had parented it. Juveniles with no identified parental match within the broodstock were assumed to be NOR.

Descriptive statistics

Eight sample groups were evaluated in population structure analysis: broodstock (N=174), assigned HOR progeny (N=87), stratum #1 (N=55), stratum #2 (N=78), stratum #3 (N=137), stratum #4 and #5 (N=56), stratum #6 (N=95), and stratum #7 (N=63). Allele frequencies were generated using the program CONVERT (Glaubitz 2004). Numbers of alleles, private alleles (i.e. an allele that is observed in only a single group), expected and observed heterozygosities, and index of inbreeding (F_{IS}; indicating heterozygote deficiency) were calculated using the software package Genetic Data Analysis Version 1.1 (Lewis and Zaykin 2001). Hardy-Weinberg Equilibrium (HWE) probability and linkage disequilibrium tests were conducted on a locus by locus basis using GENEPOP (Raymond and Rousset 1995). Statistical significance (α) was adjusted for the number of simultaneous tests k (α/k for $\alpha = 0.05$, where k = number of loci) by the sequential Bonferroni correction (Rice 1989). The program FSTAT v2.9.3.2 (Goudet 1995) was used to calculate allelic richness between the HOR and putative NOR groups, where allelic richness is a weighted estimate of the number of alleles per locus, scaled to the smallest sample size (52 individuals).

Population differentiation

Significance testing of pair-wise population F_{ST} values (θ; Weir and Cockerham 1984) was performed using ARLEQUIN Version 3.1 (Excoffier et al. 2005). The pair-wise F_{ST} statistic indicates the proportion of total variation attributed to differences between the two groups being compared. The SEQBOOT sub-routine of the software program PHYLIP version 3.68 (Felsenstein 1992) was used to generate 1000 randomized replicates (bootstrap resampling) of the dataset. For each of the 1000 replicates a pair-wise genetic distance matrix of Cavalli-Sforza and Edwards (1967) cord distance measures (D_{CE}) was generated and an unrooted Neighbor-Joining phenogram of genetic distance was calculated from the matrix using the sub-routines GENDIST and NEIGHBOR, respectively. The sub-routine CONSENSE was used to calculate the most parsimonious phenogram topology. Confidence in the nodes of the consensus phenogram was based on the percentage of replicate phenograms that displayed the same topology. The program MEGA2 (Mukhopadhyay et al. 2005) was used to graphically display the consensus phenogram.

Results

Temporal replicate sampling of juveniles

Only a single case of temporal replicate sampling was observed for juvenile fish born in 2007. Two putative NOR juvenile fish samples (ID# 986-086 and 1318-064) had identical genotypes at 14 loci. No identical multilocus genotypes were observed within the 2007 broodstock sample. Overall 14 loci, non-exclusion probabilities for identity and parent pair were calculated as $1.02X10^{-18}$ and $5.28X10^{-12}$, respectively.

Simulated parentage assignment

In the simulated parentage analysis 1462 (~15%) offspring were assigned to either true or unrelated candidate parent pairs. The most likely candidate parent pair/offspring assignments were broken down as follows: 1390 (95.08%) offspring were assigned to the true parent pair, 71 (4.86%) were incorrectly assigned to a non-parent pair (where either the true mother or father was unsampled), and 1 (0.06%) offspring was incorrectly assigned to a non-parent pair (where neither true parent was sampled). The distribution of ΔLOD scores for offspring assigned to the correct candidate parent pair (mean ΔLOD = 27.32; SD = 5.52) was much higher than the distributions obtained for the case of offspring incorrectly assigned to non-parent pairs where either one parent was unsampled (mean ΔLOD = 3.17; SD = 2.89), or both parents were unsampled (mean ΔLOD = 3.40; SD = 2.28) (Figure 3). The simulated parentage analysis suggests that the resolving power of the loci is sufficient to assign offspring to the correct candidate parent pair.

Simulation results indicated that parent pair/juvenile assignment critical ΔLOD score = 9.1. This critical ΔLOD was applied to parentage assignments with real data, and all assignments with a ΔLOD ≥ 9.1 were accepted.

Parentage assignment with real data

Parentage assignments produced 100 parent-pair/juvenile trios. Out of 87 trios that met or exceeded both the stringency level of loci used for assignment and critical ΔLOD score we observed the following production from parent pairs: one offspring – 19 pairs, two offspring – 15 pairs, three offspring – 7 pairs, four offspring – 1 pair, six offspring – 1 pair, and seven offspring – 1 pair (Table 1). No offspring were observed that had been produced by the remaining 43 known mated broodstock pairs in 2007. Out of the 87 parent-pair/juvenile trios that meet both the stringency level of loci and the critical ΔLOD score, 19 (22%) did not match the pairs of adults recorded as having been mated in 2007. Inspection of the 2007 mating records revealed that all 19 mismatched parent pairs were due to 12 instances where the recorded female parent was listed immediately adjacent to the genetically assigned female parent. Since these 19 trios meet the criteria for correct assignment, and in some cases multiple offspring had been assigned to the same parent pair, the 19 juveniles were included in the dataset of HOR juveniles produced by the 2007 broodstock. The remaining 13 parent-pair/juvenile trios were classified as incorrectly assigned. These trios were considered incorrect since the assigned parents had been spawned on different dates (3 cases) and/or did not meet the critical ΔLOD score (10 cases).

It is possible that some of the incorrectly assigned parent-pair/juvenile trios were due to full sibling relatives of either parent, and of both genders among the candidate parents present within the 2007 Sawtooth Fish Hatchery broodstock. The web-based program PEDIGREE version 2.2 (Smith et al. 2001; Herbinger 2005) was used to evaluate whether putative full-sibling groups were present within the candidate parent pool used to supply the streamside upweller program in 2007 (data not shown). Ten of the incorrect parent pair/offspring assignments were not attributed to incompatible spawning date record. In 7 out the 10 incorrect assignments one of the parents was a member of a putative full-sibling group.

Progeny distribution

We observed a total of 87 HOR offspring throughout the Yankee Fork (Table 2). Parental assignments for age-0^+ juveniles were distributed among five sites in strata #2 and #4-7, and ranged from 6-100% within a site. The majority of age-0^+ HOR juveniles (n=61) were detected in stratum #7, site 3. Parental assignments for age-1^+ juveniles were distributed among four sites in strata #5-7, and ranged from 13-100% within a site. Similarly, most of the age-1^+ HOR juveniles (n=13) were detected in sites 2 and 3 of stratum #7. No parental assignments were identified among the age-0^+ juveniles (n=165) captured in any of the six sites of Strata #1 and #3. Likewise, no parental assignments were identified among the age-1^+ juveniles (n=144) captured in the 13 sites of strata #1-4, both pond sampling sites, or the rotary screw trap. The overall assignment proportion across strata and sites, indicating HOR was 11.1% and 2.3% among age-0^+ and age-1^+ juveniles, respectively.

Descriptive statistics

A high level of polymorphism was observed across all 14 microsatellite loci evaluated in the 2008 analysis (Table 3). Observed numbers of alleles ranged from 4 at *Ssa289* within the combined strata #4 and #5 juvenile sample to 20 at *Ssa407* within the 2007 broodstock (mean = 11, over all loci and sample groups). Observed heterozygosity (H_o) ranged from 0.429 for *Ssa289* to 0.955 for *Ssa408* both within the stratum #3 sample group (mean H_o=0.772, over all loci and sample groups). There were 15 departures from expected genotypic proportions among 112 total HWE tests. Departures (heterozygote deficits) from HWE primarily occurred at *Omy77* (n=6) and *Ots1* (n=8) across most sample groups. Statistically significant (α=0.00357) linkage disequilibrium was observed for 14 and 12 (out of 91) pair-wise comparisons in the 2007 broodstock and putative NOR group from stratum #7 (data not shown). Few (0-7) significant pair-wise linkage disequilibrium tests were observed for the remaining putative NOR groups. While in general there were few private alleles in most sample groups, both combined strata #4 and #5 sample group had two private alleles at both *Ocl*1 and *Ssa*408, and Sawtooth Hatchery broodstock had two private alleles at *Ogo*4. Mean allelic richness ranged from 10.0 (stratum #7) to 10.6 (Stratum #2). There was no significant difference in allelic richness between HOR (broodstock and assigned offspring) groups and NOR (strata #1-7) juvenile groups (*P*=0.91).

Population differentiation

Among the 8 groups of steelhead evaluated, we observed pair-wise F_{ST} values ranging from -0.0001 to 0.0097 (Table 4). The overall estimate of 0.0030 (99% C.I. = 0.002-0.004) indicates low, but statistically significant genetic variability among groups. Among group variation was detected between the HOR groups (broodstock and HOR assigned juveniles) and all putative NOR groups. Significantly different pair-wise F_{ST} values between geographically proximate, putative NOR groups was not detected. Consistent clustering of HOR samples (broodstock and HOR assigned juveniles) was observed in unrooted Neighbor-Joining phenograms for pair-wise D_{CE} among putative NOR juvenile samples grouped either spatially (Figure 4), or by both collection site and age class (Figure 5). When NOR juveniles were grouped by collection site alone (Figure 4), individuals in strata #7 and #3 were the most genetically distant (overall) from the HOR groups. When putative NOR samples were also grouped by age class, cluster analysis indicated the largest genetic distance occurred between age-1^+ juveniles from stratum #1 (n=7) and the HOR fish (Figure 5). Age-0^+ juveniles collected from both stratum #1 and #2 clustered the closest to the HOR groups in the phenogram (Figure 5).

Discussion

Parentage assignment with real data

Parentage assignments observed in the 2007 (Matala and Ardren 2008) and 2008 (this study) Yankee Fork pedigree analyses provide evidence that juvenile steelhead trout produced by the streamside upweller program successfully emerge and survive in-stream through the first year of life. The 2008 parentage assignment identified 72 age-0^+ and 15 age-1^+ juveniles produced from upwellers, with an overall assignment proportion of 0.135 HOR juveniles produced from the 2007 spawning efforts among all juveniles genotyped (N = 651). The overall proportion of HOR assigned juveniles in the 2008 analysis was similar to that observed in the 2007 analysis (0.131; Matala and Ardren 2008). The estimated abundance of age-0^+ *O. mykiss* in Yankee Fork for 2007 was 34,276 juveniles, with an estimated 5,720 individuals produced by the streamside incubators (Tardy and Denny 2009).

The presence of full sibling relatives of either parent within the pool of candidate parents may reduce the power to correctly assign offspring (Olsen et al. 2001). This is because full sibling candidate parents are genetically more similar to one another compared to individuals that are unrelated. Accordingly, an offspring may have similar likelihoods of being assigned to either multiple single, or multiple pairs of candidate parents if those parents are closely related to one another. Such ambiguous assignments are more likely to occur as the number of full sibling relatives of each true parent increases within the pool of candidate parents.

It is possible that a few, small number full-sib groups exist within the set of adults taken for broodstock in 2007. Since 1996 the Yankee Fork Steelhead Supplementation program has produced four generations of artificially spawned fish in response to low spawning escapement. In addition, steelhead trout exhibit large variance in family size (e.g., Chilcote et al. 1998; Ardren and Kapuscinski 2003). Sampling vicariance, especially when the source population is small may lead to over representation of a few families within the group of individuals selected for use as broodstock (Herbinger et al. 2006). All of these factors may contribute to the possibility that related individuals occur within the sample of broodstock. It is conceivable that as the proportion of spawning escapement taken as broodstock is increased, so would the possibility of related individuals being present in the broodstock. Broodstock taken for the 2007 streamside upweller program comprised ~38% of the total spawning escapement that year (Denny and Tardy 2008). The ability to correctly assign offspring to the true parent pair would begin to deteriorate as the number of closely related candidate parents increased. The presence of full-sibs within the pool of candidate parents (2007 broodstock) may explain several of the incorrect parent pair/juvenile assignments.

Progeny distribution

Juveniles assigned to broodstock were not evenly distributed throughout the Yankee Fork drainage system in 2007 (Table 2). The majority of age-0^+ and age-1^+ HOR assigned offspring were encountered in stratum #7. Comparison between the BY2006 and BY2007 age-0^+ HOR progeny distributions may be made for only strata #1-4. Between 2006 and 2007, no significant differences were observed in the percentage of age-0^+ HOR assigned juveniles encountered in strata #1, #3, and #4. In 2006, however, a significantly higher percentage of HOR assigned juveniles were encountered in stratum #2 (t-Test=3.148, p-value=0.0346) compared to that in 2007. In 2007, no age-0^+ juveniles were encountered in strata #5-7 (Matala and Ardren 2008), thereby precluding any comparison between 2006 and 2007 for those strata. In 2006 (Matala and Ardren 2008) and 2007 (Table 2, this study), interestingly, all juveniles sampled in stratum #2, site-2 were HOR assigned. Between 2007 and 2008, no significant differences were observed in the percentage of age-1^+ HOR assigned juveniles encountered in strata #1-7. Since family identity was preserved during egg outplant by recording which broodstock pairs were represented in each streamside incubator, data describing the migratory behavior, habitat preference, and successful incubator site selection for all HOR assigned juvenile steelhead trout during their first year of life in the Yankee Fork watershed may be documented. For instance, comparison of the locations where juveniles had been outplanted into an upweller and where they were subsequently captured could provide information regarding juvenile movement, or whether (or not) age-0^+ juveniles are only encountered in the same stratum into which they had been outplanted.

Putative NOR juveniles (ID# 986-086 and 1318-064) whose genotypes were an exact match at 14 loci are likely the same individual collected on two separate occasions. Field data (e.g., sample date, FKL, weight) collected and calculated non-exclusion probability for identity corroborates the idea that a temporally replicate sample of the same individual occurred. The age-0^+ juvenile (ID# 986-086) was originally sampled in Stratum #7 (site1), and was subsequently sampled as an age-1^+ fish (ID# 1318-064) in stratum #1 (site2). This observation coincides with those of other studies showing that movement of juvenile Chinook salmon and steelhead trout during the first year of life will be relatively small, and in a predominantly downstream direction (Richards and Cernera 1989; Close and Anderson 1992; Peery and Bjornn 2000).

Putative NOR, age-1^+ juveniles were captured throughout all strata and in the rotary screw trap. However, 13 out of 15 age-1^+ HOR juveniles were captured in Stratum #7 where the majority of HOR assigned fish were encountered (Tables 1 and 2). The remaining two age-1^+ HOR juveniles were captured in strata #5 and #6 (Table 2). As suggested earlier by Matala and Ardren (2008), interpretation of the results and inferences about relative survival [and distribution] of HOR progeny requires a cautious approach. The Sawtooth Hatchery also has a program to release age-1^+ juveniles into the Yankee Fork (Denny and Tardy 2008). Migratory behaviors perpetuated and inherited through a history of domestication selection (Lynch and O'Hely 2001; Ford 2002) in the hatchery may be conveyed to progeny produced from outplanted HOR eggs. For instance, HOR progeny of upweller origin may have a tendency to migrate earlier relative to their NOR counterparts, thus fewer age-1^+ HOR fish were captured, in general, throughout Yankee Fork. An alternative explanation may be that age-0+ juveniles migrate to more hospitable over-wintering habitat in the mainstem Salmon River.

Descriptive statistics

Patterns of observed allelic diversity (number of alleles/locus, H_o; Table 3) for the 2007 parentage analysis were similar to those observed during the previous analysis for 2006 (Denny and Tardy 2008). Similarly, departures from HWE expectations occurred at *Omy77* and *Ots1* as had occurred in the 2006 analysis. However, unlike the 2006 analysis, only one other locus *Oki23*, departed from HWE expectations. The observed number of private alleles was lower in 2007 (range: 0-2/locus per sample group) compared to 2006 (range: 0-11/locus per sample group). For all groups sampled in 2007 allelic richness was higher than observed in 2006. In part, both the lower number of private alleles and higher allelic richness observed in 2007 can be explained by the larger sample size obtained in 2007 (n=649; sample location data was not available for all juveniles) compared to that obtained in 2006 (n=472).

Population differentiation

Population admixture does not appear to be evident in the putative NOR juvenile samples since few deviations from HWE expectations (Table 3) and few instances of locus by locus pair-wise linkage disequilibrium (data not shown) were observed. Pair-wise linkage disequilibrium (LD), however, was higher in both the 2007 broodstock and the NOR juveniles from stratum #7. Linkage disequilibrium observed within the broodstock may be due to family structure owing to the broodstock sample containing a higher proportion of HOR fish. Linkage disequilibrium observed for putative NOR juveniles from stratum #7 may indicate sample admixture between the offspring of recognized resident rainbow trout and naturally spawning HOR.

Steelhead supplementation within Yankee Fork also includes the release of smolts from the Sawtooth Fish Hatchery which may contribute to natural production as returning adults (Denny and tardy 2008). Therefore, the genetic similarity of age-0^+ putative NOR juveniles captured in Strata #1 and #2 may indicate a HOR influence from a source other than the streamside upweller program (Figure 5). A similar possible trend was noted in the analysis of the first year of DNA-based parentage monitoring data (Matala and Ardren 2008).

Future analyses

1. Determine if including the other three loci (Oke4, Omy1001, and Oneμ14 (Olsen et al. 1998, Spies et al. 2005, and Scribner et al. 1996, respectively) used to identify hybrids/cutthroats in the parentage analysis would decrease the number of incorrect parent pair/offspring assignments due to putative full-sibs present in the broodstock. This could be simulated for the 2007 broodstock and compared to the simulation results using the original 14 loci to see what, if any, incorrect assignments are corrected.
2. Compare upweller and subsequent capture locations for HOR assigned juveniles by linking family ID (parental pairs) to upweller info within a Microsoft Access database.

3. Including all captured juveniles in the initial monitoring of HOR fish survival and their ability to reproduce in the natural environment will increase the likelihood of providing a comprehensive assessment of the efficacy of the Yankee Fork streamside upweller program as a supplementation tool. In 2007-2008, 942 juveniles (743 age-0^+ in 2007; 199 age-1^+ in 2008) were sampled, but only 651 were genotyped. By increasing the number of juveniles genotyped for parentage analysis, greater resolution may be obtained regarding the spatial distribution and habitat preference of HOR assigned juveniles and the estimated proportion of juveniles produced through the upweller program. Juvenile HOR and NOR fish initially sampled represent potential future natural (or artificial) spawners. By increasing the sample size of juveniles genotyped for parentage analysis, subsequent analysis would be more likely to identify HOR fish that have spawned naturally, potential full-sibs taken as broodstock, and the means to compare relative fitness between HOR and NOR adult steelhead trout that spawn naturally within Yankee Fork.
4. Continue to compare genetic data obtained between years to determine if there are any trends (e.g., change in genetic diversity) over time, or if there is year to year differences in genetic diversity. Does the Neighbor -joining tree (Spatial x Age) suggest temporal differences? (e.g., age-1 fish cluster separate (>50%?) from age-0 juveniles?)

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Figures and Tables

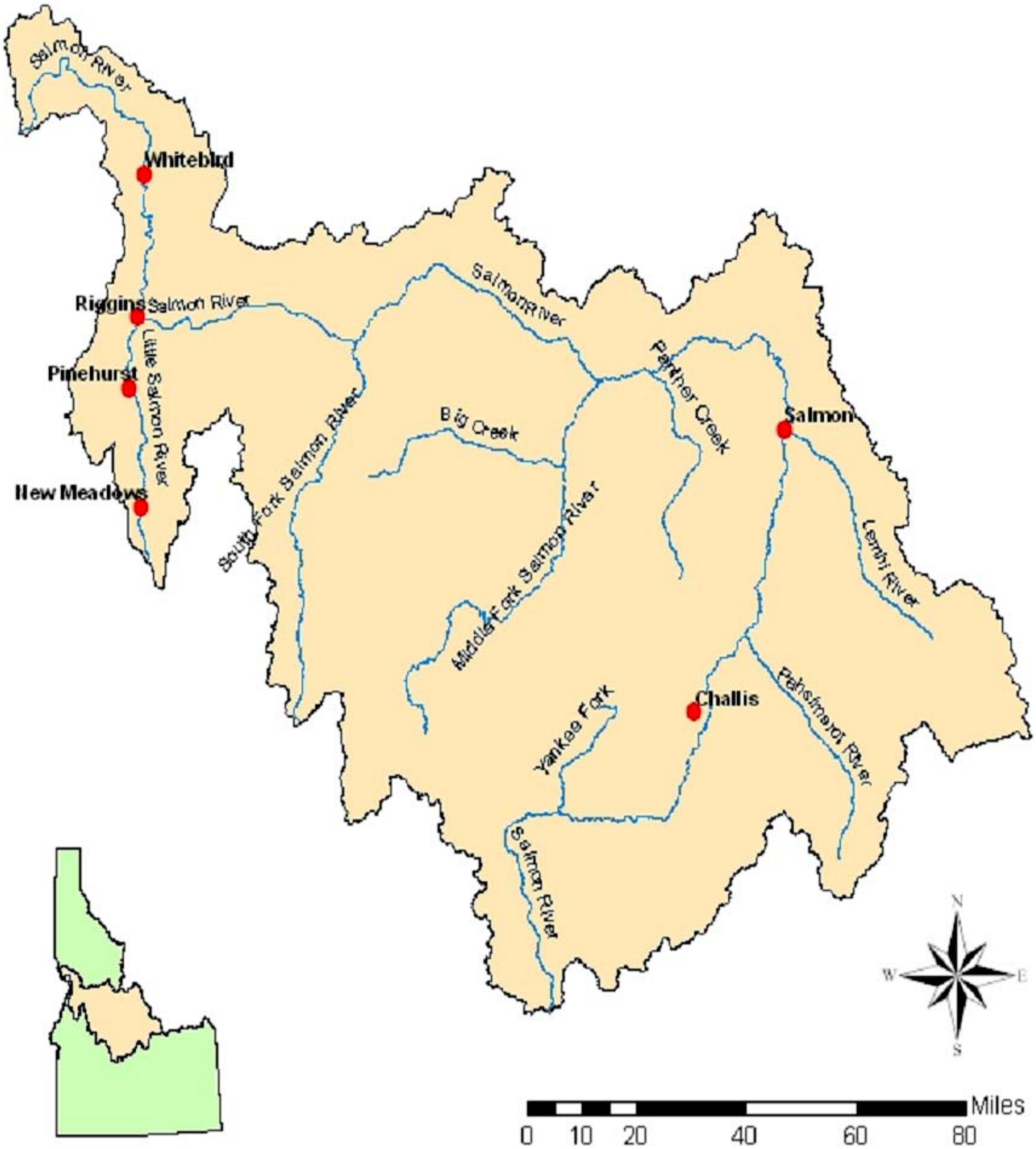

Figure 1. Salmon River Basin

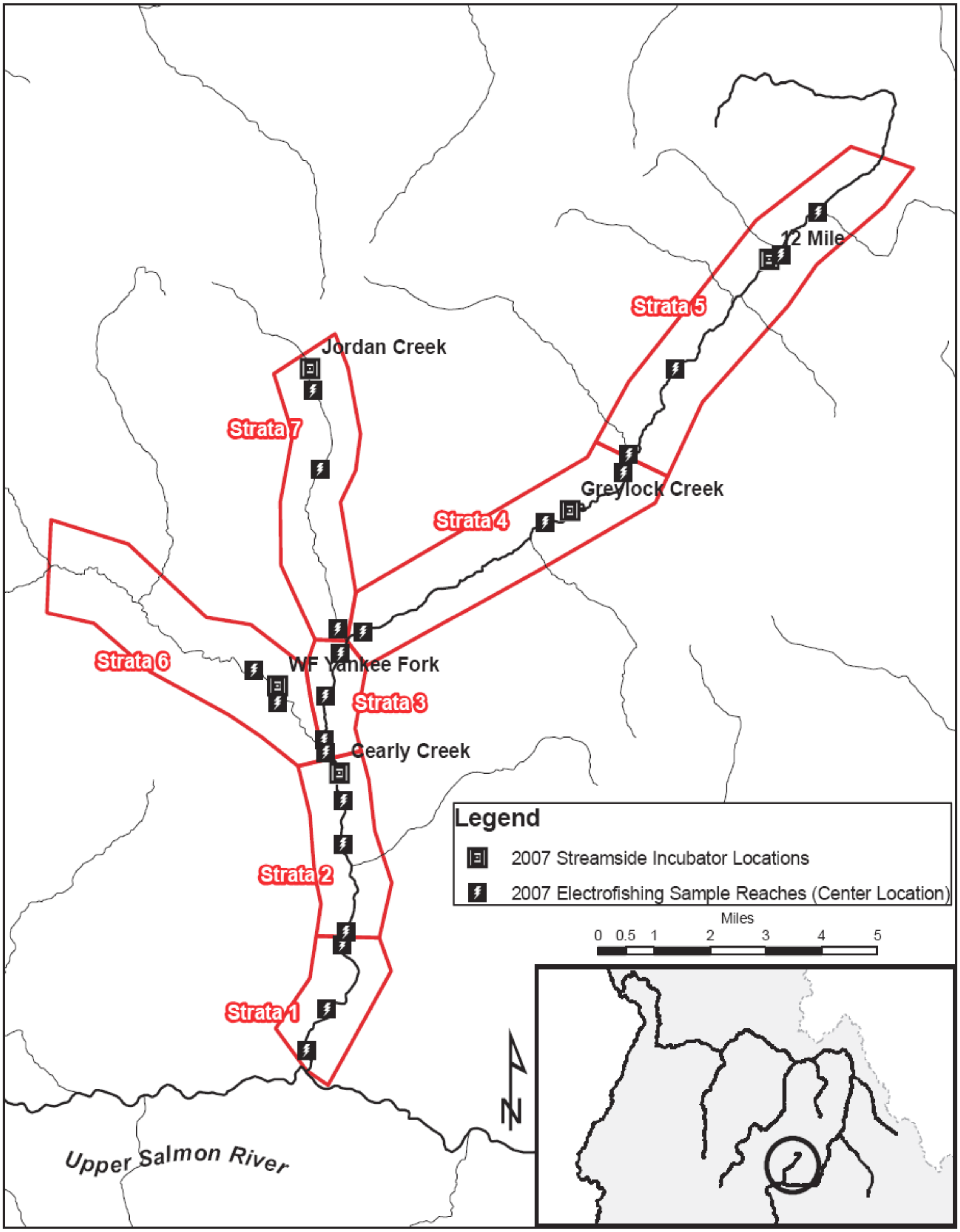

Figure 2. 2007 incubator and juvenile sampling sites in Yankee Fork, Idaho.

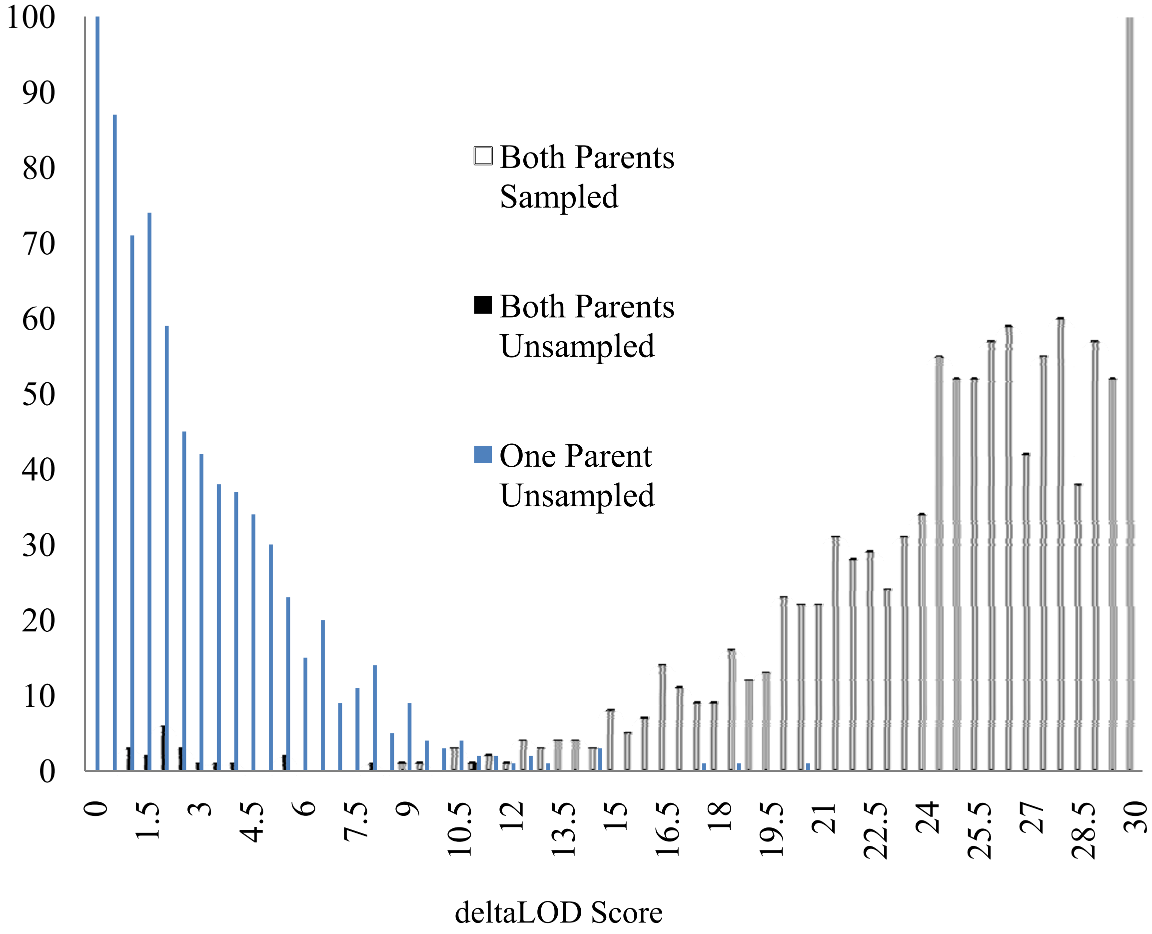

Figure 3. Histogram of simulated deltaLOD scores of most likely parent pair/offspring assignments for various classes of parentage assignment outcomes.

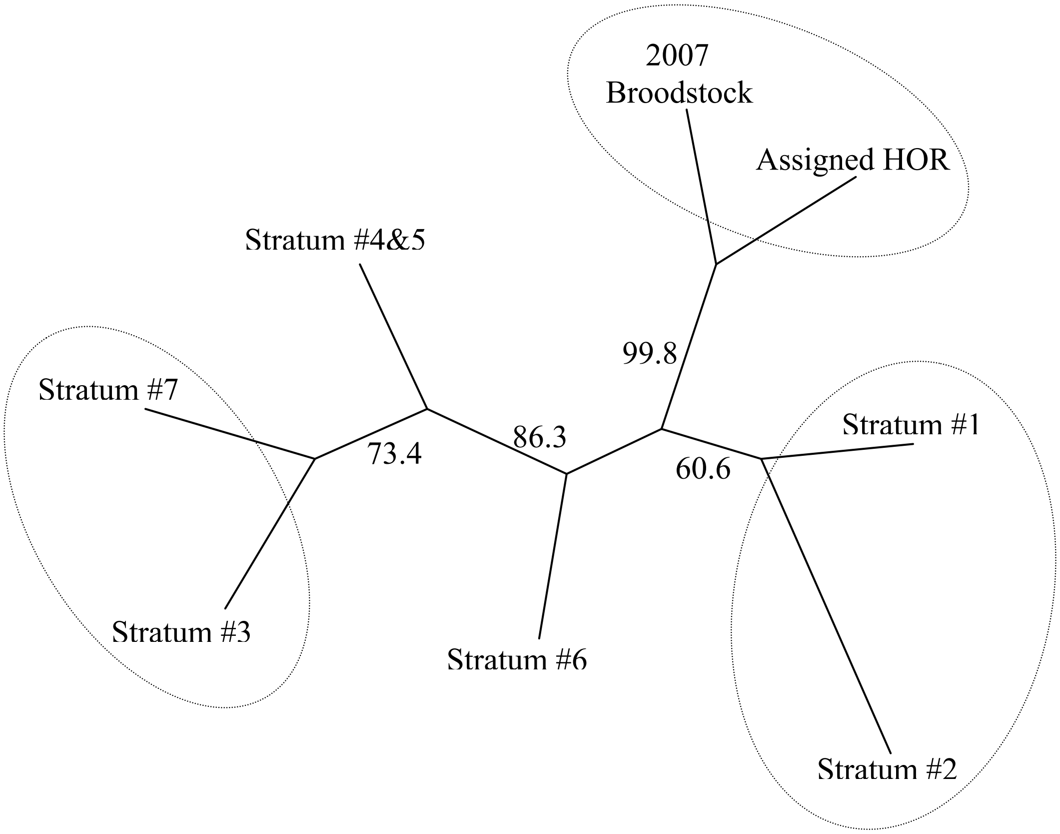

Figure 4. Unrooted Neighbor-joining phenogram based on Cavalli-Sforza and Edwards (1967) cord distance units among the 2007 Yankee Fork steelhead trout. The phenogram was constructed using data from 14 microsatellite loci with PHYLIP (Felsenstein 1989). For 1000 boot-strap replicates, node values of 50% and greater are given. Clusters of samples are circled. Based on parent pair assignment with adults used to supply streamside upwellers, juvenile fish were classified as hatchery origin (HOR). Remaining putative natural origin (NOR) individuals were grouped according to sampling strata within Yankee Fork (Figure 2).

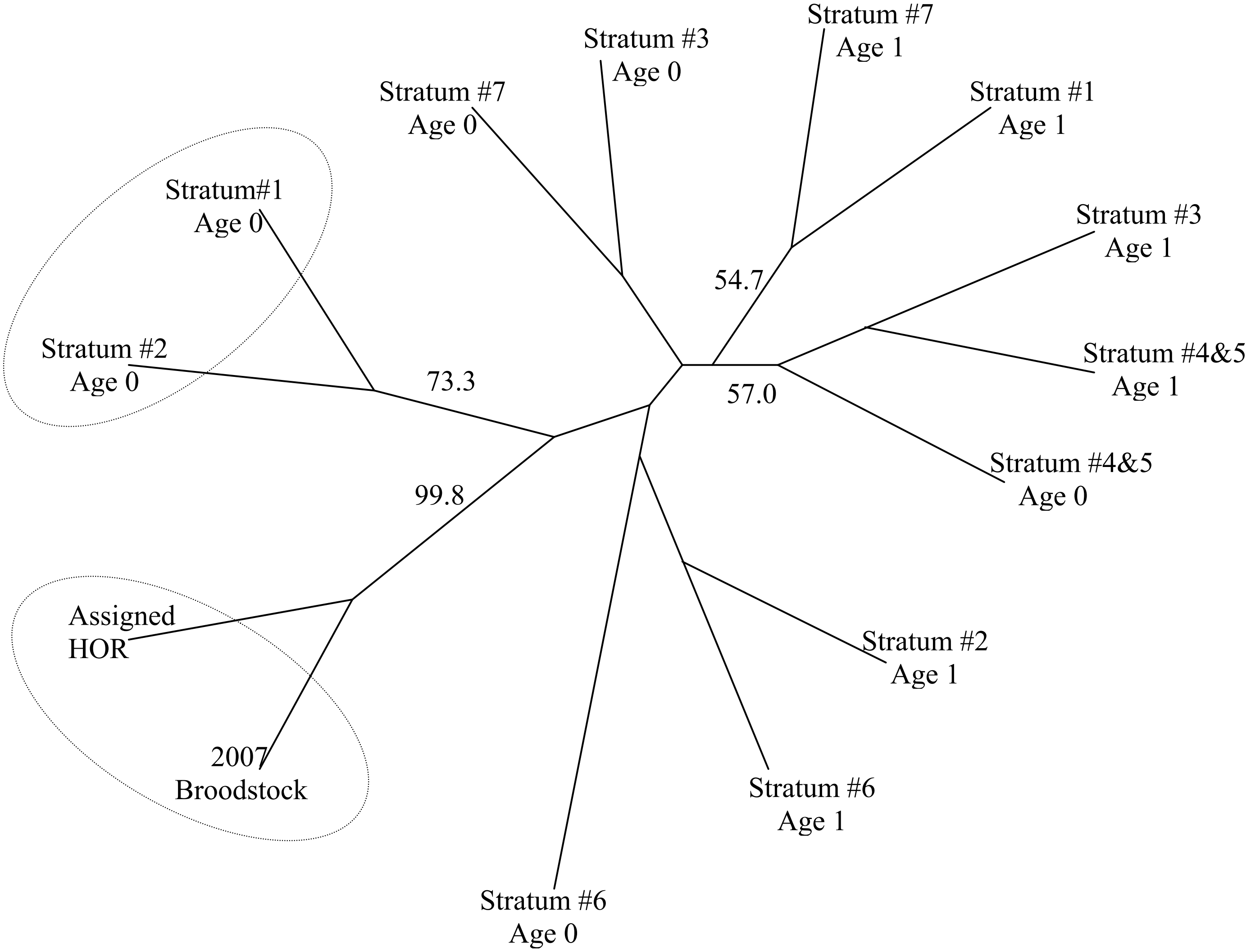

Figure 5. Unrooted Neighbor-joining phenogram based on Cavalli-Sforza and Edwards (1967) cord distance units among the 2007 Yankee Fork steelhead trout juveniles grouped by age and strata location where they were sampled. The phenogram was constructed using data from 14 microsatellite loci with PHYLIP (Felsenstein 1989). For 1000 boot-strap replicates, node values of 50% and greater are given. Clusters of samples for which there is strong bootstrap support are circled. Based on parent pair assignment with adults used to supply streamside upwellers, juvenile fish were classified as hatchery origin (HOR). Remaining putative natural origin (NOR) individuals were grouped according to sampling strata within Yankee Fork (Figure 2).

Table 1. Parentage assignment results reported from parent perspective. The pool of potential parents was composed of all Sawtooth Hatchery broodstock pairs used to supply eggs outplanted into Yankee Fork upwellers in 2007.

Broodstock		Assigned offspring					
Father	**Mother**	**ID**	**Stratum**	**Site**	**Age**	**Length (mm)**	**Mass (g)**
889-018	889-010	991-024	7	3	0	54	1.67
		991-032	7	3	0	42	0.64
		987-038	7	3	0	53	0.59
		1308-036	7	3	1	92	7.77
		1308-056	7	3	1	83	5.73
		1308-040	7	3	1	84	6.93
		987-029	7	3	0	50	0.14
889-051	889-044	991-030	7	3	0	50	1.25
		991-021	7	3	0	48	1.12
		987-068	7	3	0	47	1.05
		1308-037	7	3	1	93	9.24
		987-054	7	3	0	52	1.51
		995-095	6	3	0	65	2.92
889-036	889-028	987-062	7	3	0	48	1.08
		987-066	7	3	0	45	0.86
		987-060	7	3	0	54	1.60
		987-036	7	3	0	42	0.66
711-013	711-005	987-025	7	3	0	37	0.55
		1308-055	7	3	1	87	9.18
		994-089	2	2	0	51	1.87
711-060	711-052	987-072	7	3	0	47	0.82
		987-040	7	3	0	50	1.39
		986-006	5	12M	0	45	1.02
889-001	888-093	987-048	7	3	0	45	0.94
		1308-013	7	2	1	84	7.36
		995-045	6	2	0	57	2.22
889-021	889-013	987-081	7	3	0	49	1.26
		987-074	7	3	0	51	1.78
		987-058	7	3	0	47	1.34
889-022	889-014	987-021	7	3	0	45	0.76
		991-012	7	3	0	49	1.00
		991-015	7	3	0	52	1.24
889-053	889-047	1308-011	7	2	1	104	19.36
		987-077	7	3	0	49	1.04
		987-093	7	3	0	46	0.70
889-097	889-088	987-079	7	3	0	44	0.65
		987-050	7	3	0	45	0.92
		987-035	7	3	0	39	0.50
711-002	889-094	1317-075	6	1	1	125	18.85
		1308-043	7	3	1	85	7.52
711-044	711-036	1308-015	7	2	1	103	15.78
		987-089	7	3	0	49	1.16
888-088	888-079	991-038	7	3	0	47	1.31
		991-040	7	3	0	45	0.92

Table 1. (*continued*)

Broodstock		Assigned offspring					
Father	**Mother**	**ID**	**Stratum**	**Site**	**Age**	**Length (mm)**	**Mass (g)**
888-089	888-081	1308-032	7	3	1	94	11.00
		987-056	7	3	0	45	1.11
888-091	888-083	991-034	7	3	0	34	0.35
		1308-061	5	12M	1	93	8.56
888-092	888-084	986-003	5	12M	0	49	1.33
		991-014	7	3	0	45	0.76
889-002	888-094	991-008	7	3	0	42	0.77
		995-060	6	2	0	61	3.03
889-005	888-098	987-091	7	3	0	53	1.41
		987-087	7	3	0	49	1.04
889-020	889-012	987-098	7	3	0	50	1.06
		987-075	7	3	0	49	1.09
889-023	889-015	1308-053	7	3	1	93	10.25
		987-046	7	3	0	49	1.46
889-025	889-017	991-002	7	3	0	43	0.72
		987-070	7	3	0	44	0.70
889-038	889-030	995-041	6	2	0	60	2.76
		995-053	6	2	0	60	2.74
889-039	889-031	991-043	4	2	0	61	2.93
		986-005	5	12M	0	44	0.80
889-050	889-043	987-083	7	3	0	51	1.30
		991-017	7	3	0	49	1.24
889-099	889-090	991-026	7	3	0	46	1.10
		991-028	7	3	0	40	0.64
711-001	889-092	987-064	7	3	0	46	0.93
711-029	711-020	987-033	7	3	0	48	1.12
711-047	711-004	995-057	6	2	0	56	2.00
711-048	711-041	991-042	7	3	0	40	0.57
711-064	711-056	991-019	7	3	0	43	0.77
711-067	711-059	1308-030	7	3	1	90	8.83
711-085	711-086	991-006	7	3	0	44	0.81
888-085	888-077	991-004	7	3	0	46	0.46
888-086	888-078	987-051	7	3	0	44	0.80
889-004	888-096	987-043	7	3	0	45	0.82
889-009	889-008	991-010	7	3	0	50	1.38
889-034	889-026	987-085	7	3	0	50	1.27
889-035	889-027	987-095	7	3	0	45	0.91
889-037	889-029	987-100	7	3	0	53	1.41
889-041	889-033	987-031	7	3	0	53	1.49
889-052	889-045	987-023	7	3	0	47	0.97
889-054	889-046	1308-038	7	3	1	90	9.10
889-096	889-089	987-042	7	3	0	44	0.77
889-098	889-091	987-027	7	3	0	43	0.66

Table 2. Parentage assignment results from the progeny perspective. The numbers of parent-progeny matches are reported for each juvenile collection location (e.g., stratum and site).

		Brood Year 2007					
		Age-0$^+$ juvniles			Age-1$^+$ juvniles		
Location		**n sampled**	**# assigned**	**% assigned**	**n sampled**	**# assigned**	**% assigned**
Stratum 1	Site 1	8	0	0	2	0	0
	Site 2	13	0	0	2	0	0
	Site 3	27	0	0	3	0	0
Stratum 2	Site 1	28	0	0	5	0	0
	Site 2	16	1	6	4	0	0
	Site 3	14	0	0	12	0	0
Stratum 3	Site 1	19	0	0	6	0	0
	Site 2	45	0	0	8	0	0
	Site 3	53	0	0	6	0	0
Stratum 4	Site 1	32	0	0	13	0	0
	Site 2	1	1	100	1	0	0
	Site 3	0	--	--	1	0	0
	Site 4	0	--	--	6	0	0
Stratum 5	Site 1	0	--	--	1	0	0
	Site 12M	5	3	60	1	1	100
Stratum 6	Site 1	51	0	0	8	1	13
	Site 2	18	5	28	1	0	0
	Site 3	19	1	5	5	0	0
Stratum 7	Site 1	47	0	0	3	0	0
	Site 2	0	--	--	14	3	21
	Site 3	63	61	97	10	10	100
Pond	Series 1	0	--	--	5	0	0
Pond	Series 3	0	--	--	3	0	0
Screw Trap		0	--	--	67	0	0
Overall		459	72	16	187	15	8

Table 3. Descriptive genetic statistics for broodstock and juvenile samples collected during 2007-08. Column headings are defined as follows: **n** and **A** are the number of individuals and observed alleles, respectively, **AP** is the number of private alleles, **AR** is the allelic richness, **H_E** is Nei's (1978) unbiased estimate of expected heterozygosity, **H_O** is observed heterozygosity, and **F_{IS}** is the index of inbreeding (Weir and Cockerham 1984). Significant deviation (Bonferroni corrected; α=0.00357) from HWE is indicated by an asterisk.

	Sawtooth Hatchery Broodstock (n=174)							Assigned HOR progeny (n=87)						
Locus	n	A	AP	AR	H_E	H_O	F_{IS}	n	A	AP	AR	H_E	H_O	F_{IS}
Ocl1	174	12	0	10	0.881	0.897	-0.018	87	11	0	11	0.882	0.954	-0.083
Ogo3	174	8	1	5	0.683	0.655	0.030	87	6	0	6	0.704	0.724	-0.029
Ogo4	174	11	2	9	0.828	0.828	0.001	87	9	0	9	0.797	0.736	0.078
Oki23	173	14	0	11	0.798	0.809	-0.014	87	12	0	11	0.781	0.678	0.133
Omy1011	173	14	0	13	0.882	0.890	-0.009	87	13	0	13	0.878	0.943	-0.074
Omy77	167	17	0	14	0.884	0.617	*0.303	86	16	0	15	0.887	0.698	*0.214
Omy7i	174	12	0	11	0.816	0.787	0.036	87	12	0	11	0.831	0.839	-0.010
Ots1	172	12	0	11	0.860	0.622	*0.277	84	12	0	11	0.858	0.655	*0.238
Ots100	174	12	0	10	0.829	0.822	0.009	87	11	0	10	0.820	0.805	0.019
Ots3	174	8	1	6	0.712	0.730	-0.025	87	6	0	6	0.702	0.747	-0.064
Ots4	174	7	1	6	0.780	0.770	0.013	87	6	0	6	0.770	0.770	0.000
Ssa289	174	8	1	6	0.554	0.592	-0.067	87	6	0	6	0.574	0.586	-0.021
Ssa407	173	20	1	15	0.872	0.896	-0.028	87	17	0	16	0.881	0.943	-0.070
Ssa408	173	18	0	15	0.913	0.931	-0.020	87	16	0	15	0.896	0.885	0.012

	Stratum #1 -Juveniles (n=55)							Stratum #2 -Juveniles (n=78)						
Locus	n	A	AP	AR	H_E	H_O	F_{IS}	n	A	AP	AR	H_E	H_O	F_{IS}
Ocl1	55	11	0	11	0.865	0.891	-0.030	78	12	0	12	0.884	0.923	-0.045
Ogo3	55	6	0	6	0.681	0.582	0.146	78	6	0	5	0.717	0.628	0.124
Ogo4	55	9	0	9	0.809	0.836	-0.034	78	9	0	9	0.825	0.859	-0.042
Oki23	55	12	0	12	0.819	0.800	0.023	75	14	1	13	0.831	0.840	-0.011
Omy1011	55	12	0	12	0.869	0.927	-0.068	78	14	0	13	0.882	0.897	-0.017
Omy77	55	13	0	13	0.879	0.582	*0.340	75	17	0	15	0.881	0.720	*0.184
Omy7i	55	10	0	10	0.782	0.745	0.047	78	11	0	11	0.796	0.795	0.001
Ots1	52	11	0	11	0.833	0.615	*0.264	76	13	1	12	0.850	0.671	*0.211
Ots100	55	12	0	12	0.845	0.873	-0.033	77	11	0	11	0.803	0.766	0.046
Ots3	55	7	1	7	0.723	0.764	-0.057	78	6	0	6	0.724	0.679	0.062
Ots4	55	6	0	6	0.759	0.727	0.042	78	6	0	6	0.729	0.821	-0.126
Ssa289	55	6	0	6	0.509	0.527	-0.035	77	6	0	6	0.506	0.506	-0.002
Ssa407	55	16	0	16	0.891	0.927	-0.042	78	16	0	14	0.866	0.872	-0.007
Ssa408	55	17	0	17	0.907	0.909	-0.002	78	17	0	16	0.917	0.910	0.008

Table 3. (*continued*)

	Stratum #3 -Juveniles (n=137)							Stratum #4 & 5 -Juveniles (n=56)						
Locus	n	A	AP	AR	H_E	H_O	F_{IS}	n	A	AP	AR	H_E	H_O	F_{IS}
Ocl1	137	13	0	12	0.877	0.920	-0.049	56	13	2	12	0.878	0.857	0.024
Ogo3	137	6	0	6	0.679	0.591	0.129	56	5	0	5	0.668	0.661	0.011
Ogo4	137	10	0	10	0.835	0.832	0.003	56	11	1	11	0.845	0.893	-0.057
Oki23	129	12	0	11	0.819	0.814	0.006	56	11	0	11	0.858	0.857	0.001
Omy1011	137	15	1	14	0.875	0.891	-0.017	56	13	0	13	0.866	0.875	-0.010
Omy77	131	15	0	14	0.878	0.672	*0.236	56	16	1	14	0.900	0.786	0.128
Omy7i	137	12	0	11	0.785	0.796	-0.014	56	11	0	10	0.716	0.661	0.078
Ots1	134	12	0	11	0.854	0.560	*0.345	55	12	0	11	0.861	0.673	*0.221
Ots100	134	11	0	11	0.840	0.806	0.040	56	12	0	11	0.863	0.857	0.007
Ots3	135	7	0	7	0.716	0.793	-0.107	56	7	0	7	0.750	0.696	0.072
Ots4	137	6	0	6	0.732	0.737	-0.007	56	5	0	5	0.717	0.839	-0.172
Ssa289	136	5	0	4	0.434	0.426	0.017	56	4	0	4	0.490	0.500	-0.020
Ssa407	137	18	1	14	0.853	0.891	-0.044	56	16	0	15	0.878	0.875	0.003
Ssa408	137	17	0	16	0.897	0.956	-0.066	56	16	2	15	0.873	0.929	-0.064

	Stratum #6 -Juveniles (n=95)							Stratum #7 -Juveniles (n=63)						
Locus	n	A	AP	AR	H_E	H_O	F_{IS}	n	A	AP	AR	H_E	H_O	F_{IS}
Ocl1	95	13	1	11	0.862	0.821	0.048	63	11	0	10	0.851	0.889	-0.045
Ogo3	95	8	0	7	0.725	0.747	-0.032	62	6	0	5	0.668	0.694	-0.038
Ogo4	95	9	0	9	0.828	0.811	0.021	63	9	1	8	0.809	0.778	0.039
Oki23	95	14	0	13	0.847	0.821	0.030	62	11	0	10	0.792	0.758	*0.043
Omy1011	95	13	0	13	0.878	0.842	0.041	62	13	0	13	0.863	0.935	-0.084
Omy77	94	16	0	14	0.889	0.660	*0.259	59	15	0	14	0.858	0.763	0.111
Omy7i	95	12	0	11	0.794	0.811	-0.022	63	12	0	11	0.807	0.810	-0.003
Ots1	92	11	0	11	0.855	0.609	*0.289	61	13	0	11	0.875	0.557	*0.365
Ots100	94	11	0	10	0.837	0.830	0.009	62	11	0	10	0.855	0.806	0.058
Ots3	95	7	0	7	0.739	0.747	-0.012	63	7	0	6	0.778	0.746	0.042
Ots4	95	5	0	5	0.748	0.642	0.142	63	7	0	5	0.773	0.810	-0.048
Ssa289	95	5	0	5	0.516	0.526	-0.020	63	6	1	6	0.570	0.571	-0.002
Ssa407	95	16	0	14	0.869	0.905	-0.042	62	13	0	15	0.804	0.806	-0.003
Ssa408	95	15	0	15	0.913	0.937	-0.027	62	17	1	15	0.912	0.806	0.117

Table 4. Pair-wise F_{ST} comparisons among 2007 Yankee Fork steelhead trout sample collections. The observed F_{ST} -values are recorded in the top half-matrix, and Bonferroni adjusted (Rice 1989) significance is shown in the lower half-matrix.

F_{ST}	Broodstock	Stratum #1	Stratum #2	Stratum #3	Stratum #4&5	Stratum #6	Stratum #7
Stratum #1	0.0041	--					
Stratum #2	0.0022	-0.0001	--				
Stratum #3	0.0023	0.0050	0.0009	--			
Stratum #4&5	0.0042	0.0043	0.0028	-0.0001	--		
Stratum #6	0.0031	0.0029	0.0018	0.0015	0.0022	--	
Stratum #7	0.0048	0.0097	0.0047	0.0023	0.0066	0.0049	--
Assigned HOR	0.0002	0.0053	0.0048	0.0054	0.0050	0.0050	0.0080

P -value							
Stratum #1	***	--					
Stratum #2	***	0.2266	--				
Stratum #3	***	***	0.0089	--			
Stratum #4&5	***	***	***	0.0220	--		
Stratum #6	***	***	0.0043	***	***	--	
Stratum #7	***	***	***	0.0202	***	***	--
Assigned HOR	0.8769	***	***	***	***	***	***