2009

STEELHEAD STREAMSIDE INCUBATION (SSI) PROGRAM

Shoshone Bannock Tribes

Fish and Wildlife Department

2009 Steelhead Streamside Incubation Program

Annual Report

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TABLE OF CONTENTS

LIST OF FIGURES **3**

LIST OF TABLES **4**

ABSTRACT **5**

ACKNOWLEDGEMENTS **5**

INTRODUCTION **5**

BACKGROUND 6

STUDY AREA **8**

SALMON RIVER BASIN 8

Yankee Fork Salmon River *9*

Panther Creek *10*

Indian Creek *10*

METHODS **10**

PARENTAL SELECTION AND MATING 10

EGG PLANTING 10

INCUBATION AND HATCHING SUCCESS 11

YANKEE FORK JUVENILE ASSESSMENT 14

BY2008 Age-0+ Sampling *14*

BY2008 Age-1+ Sampling *15*

GENETIC PARENTAGE ANALYSIS 16

RESULTS **16**

PARENTAL SELECTION AND MATING 16

EGG PLANTING, HATCH SUCCESS, AND FRY SEEDED 16

JUVENILE TRAPPING 17

GENETIC PARENTAGE ANALYSIS 18

DISCUSSION **19**

CITATIONS **21**

APPENDIX **23**

LIST OF FIGURES

Figure 1. Upper Salmon River Basin 9
Figure 2. Four Beaver Creek incubators and catch tank located in Panther Creek, 2009. 11
Figure 3. Two Cearly Creek incubators and catch tank located in Yankee Fork, 2009. 11
Figure 4. Panther Creek with egg incubation locations, 2009. 12
Figure 5. Indian Creek with egg incubation locations, 2009. 13
Figure 6. Yankee Fork with egg incubation locations and screw trap, 2009. 14
Figure 7. Yankee Fork hydrograph, screw trap efficiency, and migration timing of steelhead juveniles from 2009 trapping operations. 18

LIST OF TABLES

Table 1. SSI program tributary incubator characteristics, 2009. 17

ABSTRACT

The Shoshone-Bannock Tribes initiated the Steelhead Streamside Incubation Program in 1995 to help maintain, rehabilitate, and enhance summer steelhead (*Oncorhynchus mykiss*) populations in the upper Salmon River. The 2009 production objective is to incubate approximately 1.0 million eyed-eggs in three Upper Salmon River tributaries (i.e. Yankee Fork Salmon River, Panther Creek, and Indian Creek) and release unfed fry. Adult broodstock were collected for the program at Sawtooth and Pahsimeroi fish hatcheries. Approximately 1,010,461 eyed-eggs were outplanted into three upper Salmon River tributaries with 900,217 estimated fry seeded. Panther and Indian creeks received 397,535 and 99,514 eyed-eggs from Pahsimeroi Fish Hatchery, respectively. Four incubators were used in Panther Creek and one used in Indian Creek. Panther Creek hatch success averaged 99% and the Indian Creek incubator achieved 98.9% hatch success. We estimate 393,348 fry were seeded in Panther Creek and 98,419 fry in Indian Creek. Approximately 513,412 eyed-eggs were transferred from Sawtooth Fish Hatchery to six streamside incubators located in the Yankee Fork Salmon River. Hatch success averaged 77.7% with an estimated 408,450 fry seeded. Four Yankee Fork incubators exceeded 98.6% hatch success, one achieved 70% hatch success, and one incubator failed (Twelve Mile Creek), resulting in a complete loss. Monitoring and evaluation beyond hatching success is conducted in the Yankee Fork Salmon River. The objective is to determine if targets for hatchery contributions are being achieved and can be improved using DNA parentage analysis. The Tribes installed a rotary screw trap in the Yankee Fork Salmon River in 2009 to estimate juvenile migrants from program operations. We estimate a total of 97,504 (SE 7,326) juveniles migrated passed the screw trap from July 3 through November 13, 2009.

ACKNOWLEDGEMENTS

The Shoshone-Bannock Tribes (Tribes) provided the administrative framework for this project to be successful. We would like to thank the Fish and Wildlife Department personnel including Carlos Lopez, Scott Brandt, Paul Dann, Tyron Broncho, Cory Bradley, and Willie Hicks for project operations and monitoring and evaluation. Thanks to Hal Hayball for furnishing maps.

The Tribes would also like to thank Scott Marshall and staff at the Lower Snake River Compensation Plan (LSRCP)-Office for providing funding to further support this program. Special thanks to Idaho Department of Fish and Game (IDFG) personnel including Brent Snider, Mel Hughes, Jocelyn Hatch, Danielle Dorsch, and Phil Stone at Sawtooth Fish Hatchery and Todd Garlie and Lars Alsager at Pahsimeroi Fish Hatchery.

INTRODUCTION

The Salmon River historically supported a robust steelhead fishery for the Shoshone and Bannock peoples and as many as 60,000 fish were annually harvested by them in the Salmon River (Walker 1993). Estimates indicate 10-16 million adult salmon (*Oncorhynchus sp.*) and steelhead annually returned to the Columbia River during the 1800s (NPCC 2009). Mallet (1974) estimated 55% of all steelhead originated from the Snake River basin.

Tribal fisherman observed a significant decline in the number of steelhead returning to the Salmon River and this prompted the Tribes to develop management options that could increase the number of steelhead. The Tribes initiated the Steelhead Streamside Incubation (SSI) Program in 1995 to help maintain, rehabilitate, and enhance steelhead populations in the upper Salmon River. Shortly thereafter, in 1997, NOAA-Fisheries formally listed the Snake River Steelhead Distinct Population Segment (DPS) as threatened under the Endangered Species Act (ESA) (62 FR 43937).

The objectives of the SSI program, under the agreement in *U.S. v Oregon*, are to outplant 1.0 million eyed steelhead eggs into three Upper Salmon River tributaries; 500,000 in Yankee Fork, 400,000 in Panther Creek, and 100,000 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 field sub-sampling and DNA genotyping.

Background

Over the last fifteen years, the SSI project has evolved due to cooperator fall-out, funding challenges, and the ESA. The original project goal was to, "*develop an experimental project for assessing the potential for using artificial production to increase early life survival of steelhead and salmon populations* (Kutchins 1995)." An experimental project has been developed;

however, juvenile and adult production (i.e., smolts and adults) resulting from the project must be quantified.

Cooperators in this project once included the Upper Salmon Basin Watershed Project (USBWP), Forest Service (FS), Shoshone-Bannock High School, Salmon Corporation, private landowners, and numerous volunteers. The USBWPs focus transitioned to restoring impaired habitat and they quickly lost site of the importance of the SSI. The FS was intimately involved in the SSI Program through 2004, but eventually had disagreements with IDFG. Eventually the FS's involvement in the project became political and key staff could no longer participate in the SSI Program. The Shoshone-Bannock High School was also intimately involved in the project until 2004. In fact, they actually had their own program called "Dance of the Salmon." However, when key staff departed from the high school, funding commitments fell through and eventually so did their participation. The Salmon Corps project allowed Tribal youth to become involved in fish restoration activities such as the SSI. In 2001, funding was not renewed and support from the Salmon Corps was lost. The majority of landowners and/or volunteers that participated in the project began to dwindle because a number of sites were eliminated from the project.

This SSI Program cooperators currently include the LSRCP, Idaho Power Company, IDFG, and the Tribes' Fish and Wildlife Department. IDFG's responsibility continues to be broodstock collection and green egg to eyed-egg incubation. Adult broodstock are collected at Pahsimeroi and Sawtooth fish hatcheries from hatchery returns. The IDFG operates on funding provided by the LSRCP and Idaho Power Company; these funding sources have remained constant since the onset of the project. The Tribes are responsible for setting up remote incubation sites, transporting eyed eggs, operating and maintaining incubators, and conducting monitoring and evaluation.

Due to major funding constraints and lack of adequate monitoring and evaluation to assess the program, the Tribes, IDFG, and NOAA Fisheries eventually scaled the project sites to three upper Salmon River tributaries including Yankee Fork, Indian Creek, and Panther Creek. The fish production objectives for the project for 2008 – 2017, consistent with the *US v Oregon* Agreement, are to incubate and release one million eyed eggs (US v Oregon 2008). The production objectives are as follows: 1) 500,000 in Yankee Fork, 2) 400,000 in Panther Creek and 3) 100,000 in Indian Creek. The operational and maintenance objectives include: (1) testing the streamside incubator technology for successful hatching; (2) increasing egg to fry survival; (3) determining optimum hatchbox densities and configurations; (4) providing incentives for habitat improvements; (5) minimizing cost; (6) minimizing process; (7) minimizing fish handling; (8) increasing community education, involvement, and caring; (9) fulfilling the requirements of *US v Oregon*; (10) fulfilling the requirements of the LSRCP; and (11) Idaho Power Company mitigation (Kutchins 1995).

Several types of incubation units have been used and evaluated, including streamside incubators constructed from discarded refrigerators, commercial upwelling incubators of various sizes, and in-stream incubation systems (e.g., wooden boxes, Jordan-Scotty, and Haddix boxes) (Haddix 2000). Upwelling incubators have become the sole unit used to incubate eyed eggs. A properly functioning incubation unit may exceed 95% hatch success.

From 1995 – 2009, the SSI project outplanted 13,836,563 eyed eggs and seeded 11,496,066 fry in twenty eight different locations. The number of eggs incubated ranges from 201,600 in 1995 to 1,135,510 in 2008. Over this period, survival from eyed egg to hatching averaged 82.3%, with the lowest hatch success of 56.8% in 2004 and highest hatch success of 99.3% in 2005. The number of sites has decreased from 23 in 2003 to 12 in 2007. We currently seed one million eggs in the three tributaries using twelve upwellers.

Monitoring and evaluation activities are designed to evaluate the efficacy of the SSI Program in the Yankee Fork (Denny et al. 2006). Effective management of steelhead stocks can be determined by increases in abundance and distribution through a combination of field sub-sampling and DNA genotyping. The tasks, as identified within monitoring and evaluation objectives, are to: (1) collect genetic samples for parentage analysis, (2) document salmonid species, (3) estimate relative abundances for wild origin and SSI progeny, (4) determine natural production increase resulting from supplementation of steelhead in Yankee Fork and relate this information to possible limiting factors, and (5) communicate monitoring and evaluation findings to resource managers.

STUDY AREA

Salmon River Basin

Physical and biological characteristics of the Salmon River 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 basin has potential to rear large numbers of salmon and steelhead.

The Salmon River basin is located in central Idaho and has a total drainage area of 36,260 km^2. The Salmon River flows 650 km in a large arch from northeast to northwest to join the Snake River at river kilometer 302.9. 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 (Figure 1) (Kutchins et al. 2001).

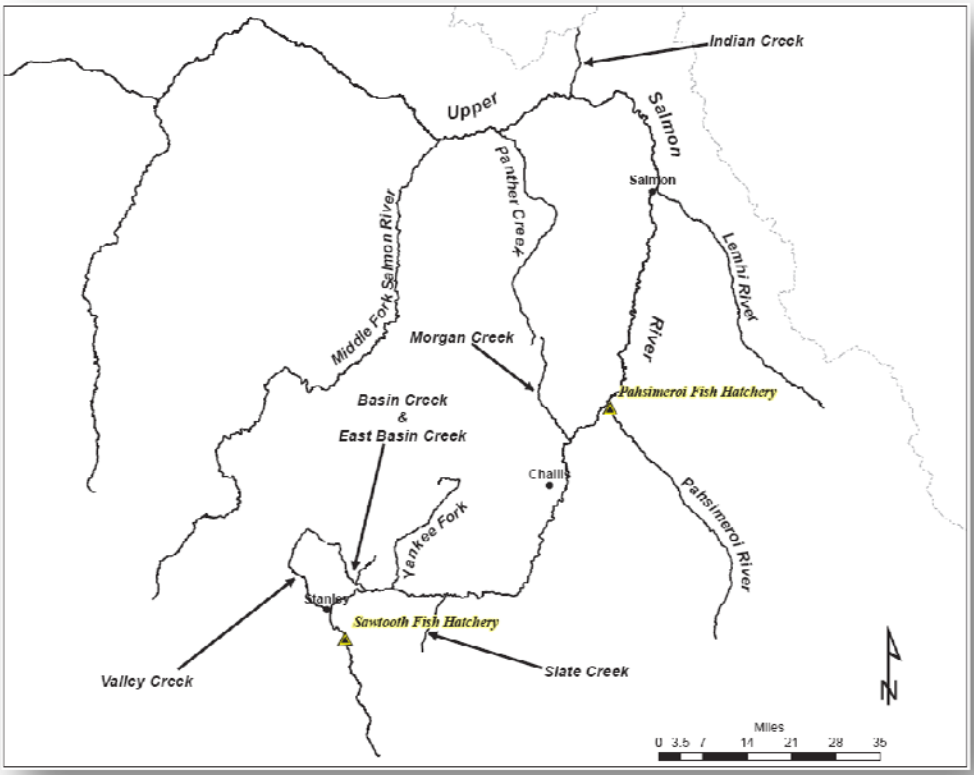

Figure 1. Upper Salmon River Basin

Yankee Fork Salmon River

Yankee Fork is located in the Salmon–Challis National Forest near Stanley, Idaho (Figure 6). The Yankee Fork flows through narrow canyons and moderately wide valleys with forest of lodgepole pine (*Pinus contorta*) (Richards and Cernera 1989). The Yankee Fork flows 41.8 kilometers (km) from north to south and enters the upper Salmon River at river rkm 590.6. The Yankee Fork headwaters originate at an elevation of 2,500 m and the watershed enters the upper Salmon River at an elevation of 1,880 m. The drainage is composed of 313.8 km^2 and includes Yankee Fork proper, West Fork Yankee Fork (largest tributary), followed by other notable tributaries including Ramey, Cearly, Lightning, Cabin, Jordan, Five Mile, Greylock, and Eight Mile creeks. Average precipitation is roughly 68.6 cm, base flows are approximately 1.13 cubic meters per second (m^3/s), and mean flows are 6.99 m^3/s. 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 (*O. tshawytscha*) 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 (*O. clarki lewisii*), mountain whitefish (*Prosopium williamsoni*), and short head sculpin (*Cottus confusus*).

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).

Panther Creek

Panther Creek, a tributary to the upper Salmon River is located in Lemhi County near the town of Salmon, Idaho (Figure 4). Panther Creek flows approximately 69 km from south to north and includes 644 km of perennial streams (IDEQ 2001). Major tributaries include Clear, Beaver, Trail, Big Deer, Napias, Deep, Blackbird, Woodtick, Moyer, and Musgrove creeks. Average annual discharge is 8.5 m^3/s, with highest flows generally occurring between April and June due to snowmelt (IDEQ 2001).

Anadromous fish populations declined rapidly following the development of Blackbird Mine in 1949 (Platts 1972). Mining began in the Blackbird area in the 1890s for copper and cobalt and most of Panther Creek from Blackbird Creek to the Salmon River (32 km) is polluted by heavy metals including copper, arsenic, cobalt, and iron. Fish species present in Panther Creek include Chinook salmon, summer steelhead, bull trout, cutthroat trout, mountain whitefish, short head sculpin, and brook trout (*S. fontinalis*).

Indian Creek

Indian Creek is a tributary of the upper Salmon River near Indianola, Idaho (Figure 5). Indian Creek flows over 21 km from north to south, originating on the south side of the continental divide.

METHODS

Parental Selection and Mating

Hatchery broodstock for the SSI Program are collected at Sawtooth and Pahsimeroi fish hatcheries, located in the upper Salmon Rivers (Figure 1). Adult A-run summer steelhead are collected from early March through mid-May, with spawning occurring over the same time period. Adult fish collected for the SSI program are spawned following typical spawning protocols. Hatchery staff collects tissue samples, gametes, and fork length for each parent fish isolated for the SSI Program. SSI eggs are incubated separately from general production gametes.

Egg Planting

Prior to receiving eyed-eggs, Tribal staff constructed four incubators in Panther Creek, one in Indian Creek, and six in Yankee Fork. All Panther Creek incubators are located on Beaver Creek, using diverted water from a private landowner (Figure 4). The Indian Creek incubator is plumbed into spring water obtained from a Salmon-Challis National Forest water source (Figure 5). Of the six incubators installed in Yankee Fork, two were plumbed into Cearly Creek, one in Jordan Creek, one in Greylock Creek, and one in Twelvemile Creek, and the last was installed in West Fork Yankee Fork (Figure 6).

Incubators were standardized with 5.1 cm polyvinyl chloride (PVC) pipe with a 7.6 cm head pipe to collect additional flow from the stream (Figure 2). Each head pipe was fitted with 0.6 cm mesh screen to minimize sediment and debris collection. Each incubator consisted of a 189.3 L polyurethane cylinder with a sediment tray, gravel, saddles, six egg trays, and one cover tray to contain eggs until hatching occurred. A catch tank consisting of a 113.6 L Rubbermaid polyurethane tub with a custom fit cover (Figures 2 and 3).

Figure 2. Four Beaver Creek incubators and catch tank located in Panther Creek, 2009.

Figure 3. Two Cearly Creek incubators and catch tank located in Yankee Fork, 2009.

Eyed-eggs from Pahsimeroi Fish Hatchery were collected and transferred to SSI incubators in Panther (Figure 2 & 4) and Indian creeks (Figure 5), respectively. Eyed-eggs from Sawtooth Fish Hatchery were transported to Yankee Fork and loaded proportionately into six incubators (Figure 3 & 6) based on parent pair matings on seven outplanting dates.

Incubation and Hatching Success

Incubators were monitored twice weekly after installation from April 29 until removal on August 2, 2009. 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 by enumerating dead eggs in the incubator and counting all dead fry in the catch tank and/or incubator. Fry seeded is estimated as the number of eggs planted minus the number of dead eggs and fry enumerated.

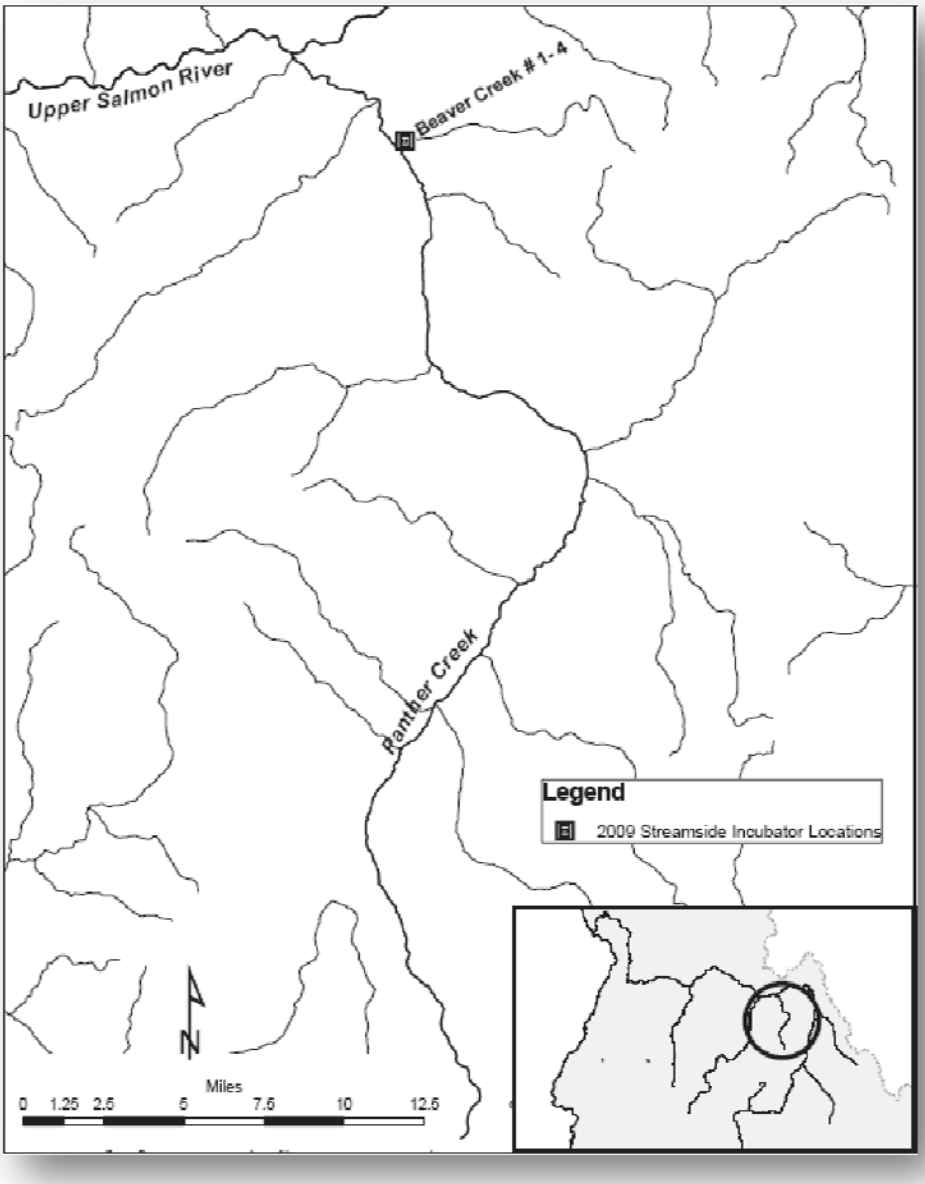

Figure 4. Panther Creek with egg incubation locations, 2009.

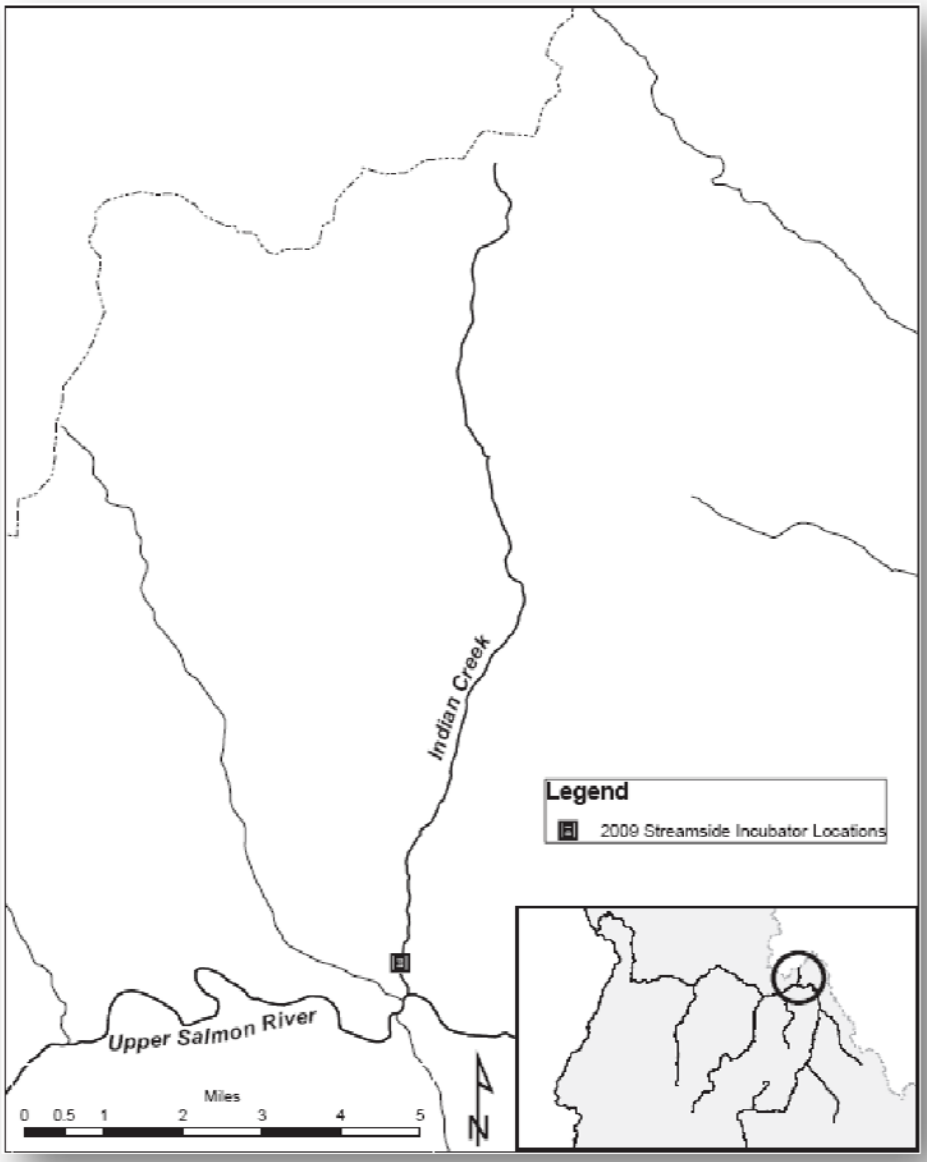

Figure 5. Indian Creek with egg incubation locations, 2009.

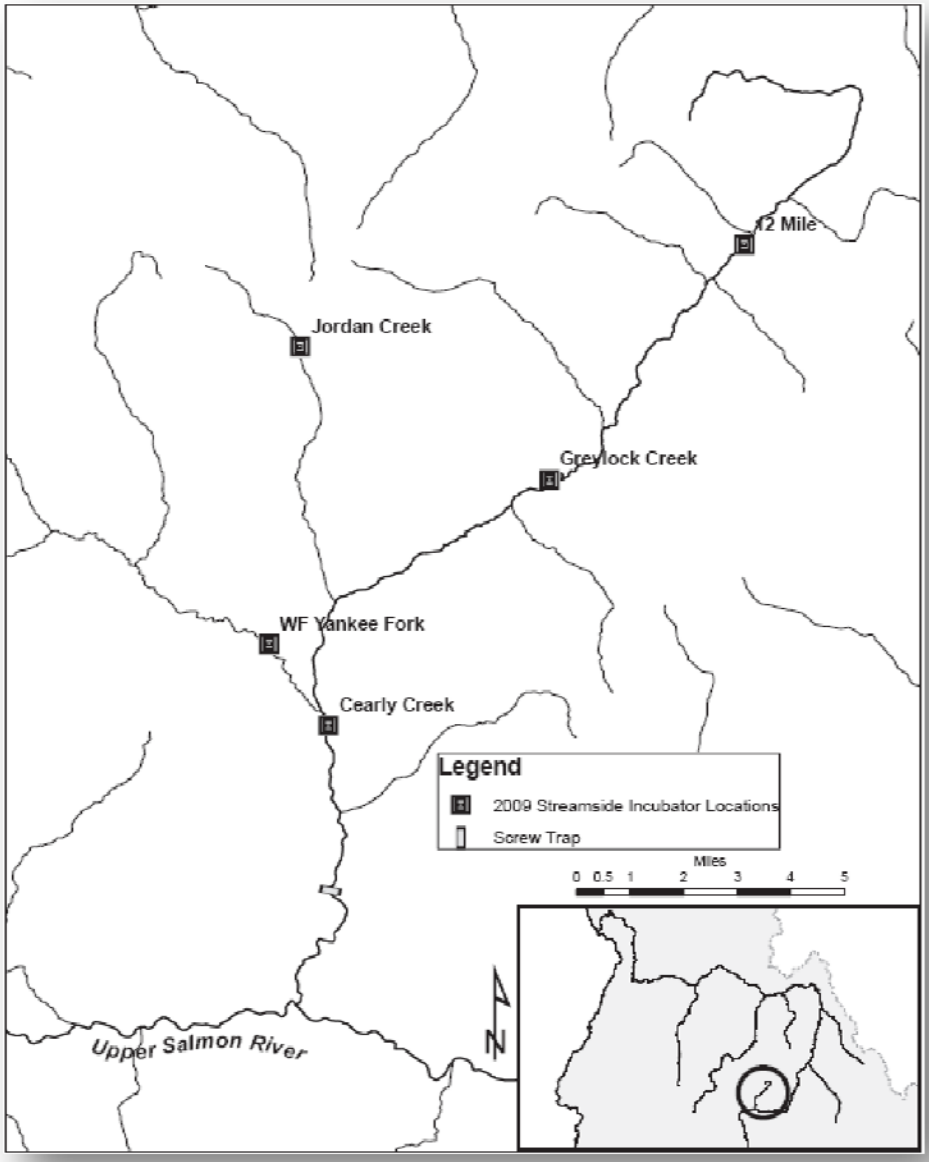

Figure 6. Yankee Fork with egg incubation locations and screw trap, 2009.

Yankee Fork Juvenile Assessment

BY2008 Age-0+ Sampling

Juvenile age-0^+ sampling was conducted in the Yankee Fork drainage during September 16 – 26, 2008. Following Konopacky et al. (1985, 1986), the Yankee Fork drainage was divided into seven distinct strata; 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. 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°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.

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).

BY2008 Age-1+ Sampling

The Tribes installed a rotary screw trap in the Yankee Fork in 2009 for the purposes of enumerating, tagging, and genetic tissue sampling migrating juveniles for research, monitoring, and evaluation. The screw trap was installed on July 2 downstream of Pole Flat Campground approximately 5.0 rkm upstream from the confluence with the Salmon River. The trap was located within the Salmon–Challis National Forest, and authorized under a U.S. Department of Agriculture Forest Service Temporary Special – Use Permit YFK66.

The screw trap is a temporary structure consisting of two floating pontoons, a rotating cylindrical, corkscrew cone, and a live box. Two inch braided wire cable attached to each pontoon islet was connected to a pulley hook on the main cable spanning approximately 20 m across the river allowing the trap to operate in the channel thalweg. Due to low flows later in the season, the trap was relocated on August 19, downstream above the canyon confluence at approximately 4.8 rkm upstream from the confluence with the Salmon River.

On a daily basis, the live box on the screw trap was emptied at approximately 11:00 a.m. into one large cooler; evident non-target species were enumerated, recorded, and released directly downstream of the trap. Temperature and staff gauge measurements were recorded prior to transporting fish and additional coolers to the working station.

Juveniles ≤ 69 mm were stained using Bismark Brown (1.5 L to 15.1 L water) for a minimum of 20 minutes and maximum of 40 minutes. Juveniles ≥ 70 mm were injected with a Passive Integrated Transponder (PIT) tag after being anesthetized in a clove oil solution. Daily, a maximum of 25 individuals per size criteria were marked. All marked fish were measured to the nearest 1 mm, weighed to the nearest 0.01 g, and tissue sampled. Stained and PIT tagged juveniles were released upstream of the trap at Maternity Hole for mark-recapture analysis to obtain trap efficiency. Recaptures and remaining fish by species were enumerated, recorded, and released downstream of the trap near Pole Camp Creek. Mortalities were recorded as either the result of trapping or handling. If the mortality was a PIT tagged individual, the tag was recollected prior to disposing of the mortality downstream of the trap.

Genetic Parentage Analysis

A parental exclusion, pedigree analysis (Letcher and King 2001) was performed to determine the relative reproductive success of hatchery origin steelhead compared to natural origin steelhead in producing F_1 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^+ and 1^+ juvenile parr.

RESULTS

Parental Selection and Mating

IDFG staff spawned 119 and 168 adult steelhead pairs at Sawtooth and Pahsimeroi fish hatcheries, respectively.

Egg Planting, Hatch Success, and Fry Seeded

Green eggs were incubated on pathogen free well water for approximately 45 days at 43°C to achieve 513,412 and 497,049 eyed eggs, respectively, for a total of 1,010,461 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 eleven incubators equaled 87.4% (0 – 99.5%) with a total of 900,217 fry seeded in the three upper Salmon River tributaries (Table 1) from 1,010,461 outplanted eggs. Panther and Indian creeks received 397,535 and 99,514 eyed-eggs from Pahsimeroi Fish Hatchery, respectively. Four incubators were used in Panther Creek and one used in Indian Creek. Panther Creek hatch success averaged 99% and the Indian Creek incubator achieved 98.9% hatch success. We estimate 393,348 fry were seeded in Panther Creek and 98,419 fry in Indian Creek. Approximately 513,412 eyed-eggs were transferred from Sawtooth Fish Hatchery to six

streamside incubators located in the Yankee Fork Salmon River. Hatch success averaged 77.7% (0 – 99.4%) with an estimated 408,450 fry seeded. Four Yankee Fork incubators exceeded 98.6% hatch success, one achieved 70% hatch success, and one incubator failed (Twelve Mile Creek), resulting in a complete loss.

Table 1. SSI program tributary incubator characteristics, 2009.

SSI LOCATION	RIVER	STOCK	EGGS PLANTED	TOTAL DEAD	% SURVIVAL	FRY SEEDED
Cearly Creek #1	Yankee Fork	SFH	96,540	704	0.993	95,836
Cearly Creek #2	Yankee Fork	SFH	89,936	537	0.994	89,399
Jordan Creek	Yankee Fork	SFH	91,563	1,022	0.989	90,541
Greylock Creek	Yankee Fork	SFH	93,655	28,097	0.700	65,558
WF Yankee Fork	Yankee Fork	SFH	68,069	953	0.986	67,116
12 Mile Main	Yankee Fork	SFH	73,649	73,649	0.000	0
Indian Creek	Indian Creek	PFH	99,514	1095	0.989	98,419
Beaver Creek #1	Panther Creek	PFH	100,061	1001	0.990	99,060
Beaver Creek #2	Panther Creek	PFH	99,498	1194	0.988	98,304
Beaver Creek #3	Panther Creek	PFH	100,295	1504	0.985	98,791
Beaver Creek #4	Panther Creek	PFH	97,681	488	0.995	97,193
TOTAL			**1,010,461**	**110,244**	**0.874**	**900,217**

Juvenile Trapping

The Yankee Fork screw trap was installed on July 2 and operated for 133 days until removal on November 13. Staff trapped and handled a total of 54,292 juvenile individuals. Non-target species including bull trout (640), cutthroat trout (249), rainbow trout (25), and mountain whitefish (990) resulted in 1,904 captures. There were 47,182 juvenile Chinook salmon, a focal species for the Yankee Fork Chinook Salmon Supplementation Program, captured in screw trap operations with 557 (1.2%) mortalities recorded. Staff marked a total of 12,227 juveniles (11,306 stained parr; 921 PIT tagged pre-smolt) and collected 497 genetic samples for parentage analysis under monitor and evaluation activities. There were a total of 949 recaptures for an overall trap efficiency of 0.078 ± 0.002.

Steelhead trout, a focal species for the Streamside Incubation and Smolt Release Programs, enumerated as 5,206 captures with 50 (0.96%) mortalities recorded. Staff marked a total of 2,672 steelhead juveniles of which 2,155 were stained and 517 were PIT tagged and collected 247 genetic samples for parentage analysis under monitor and evaluation activities. There were a total of 142 recaptures for an overall trap efficiency of 0.053 ± 0.004.

Using the methods and data collected by the Idaho Supplementation Studies (ISS), staff stratified time periods by significant changes in the hydrograph and trap re-location to calculate a Gauss population estimate (Figure 7). Juveniles were stratified into three periods due to significant changes in the hydrograph and trap re-location on August 19.

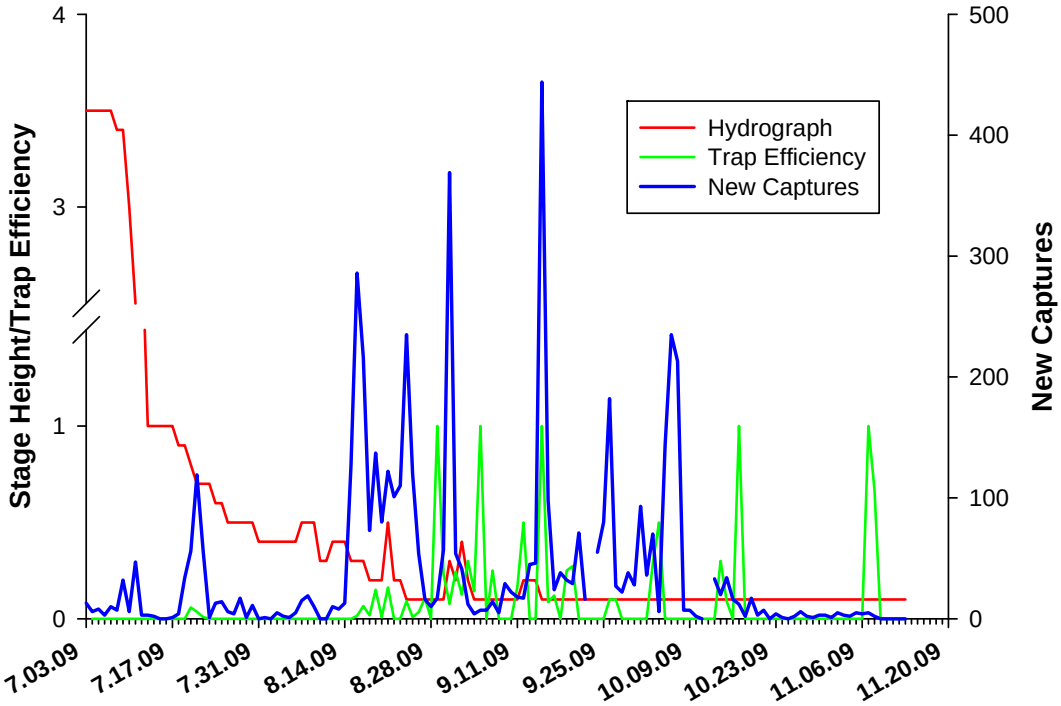

Figure 7. Yankee Fork hydrograph, screw trap efficiency, and migration timing of steelhead juveniles from 2009 trapping operations.

During the period of July 3 through November 13, staff estimates 97,504 (SE 7,326) steelhead juveniles migrated downstream past the screw trap. Due to late acquisition and installation of the screw trap, estimates for BY07 migrants could not be calculated.

Genetic Parentage Analysis

Staff collected 474 BY08 age-0^+ (2008; electroshocking) and 228 BY08 age-1^+ (2009; screw trap) 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 386 age-0^+ juveniles were genotyped. The percentage of juvenile samples per stratum to use (0.263) was calculated as the total number of samples to be genotyped (394), divided by the point estimate of the sum of all sampled reaches (1500).

All incubators for BY08 SSI incubation in Yankee Fork were successful except for the 12 Mile Main location. In comparison to BY07 1^+ juveniles (n = 15), there were only 6 individuals assigned to BY08 SSI progeny. All identified 1^+ progeny were sampled downstream in the Yankee Fork mainstem screw trap. Five of the six assigned 1^+ smolts captured in the screw trap in 2009 had originally incubated and entered the natural environment from the Jordan Creek SSI site. Primary age-0^+ juvenile migration, as seen by increased densities below upweller sites, is restricted downstream due to poorly developed functional morphology and migration direction. All identified 0^+ progeny were sampled downstream of incubator locations. Juveniles hatched in

both the Jordan and Greylock Creek incubators were subsequently sampled downstream in Stratum 4. Eggs outplanted in the two Cearly Creek upwellers were identified as 0^+ hatchery progeny downstream in Pond Series 3, Stratum 2.

Stratum four, site two, located below the Greylock Creek upweller, showed 88% genetic assignment (n = 22). Stratum 7, site 3, located directly below the Jordan Creek upweller, showed 50% assignment for BY08 0^+ juveniles. This may indicate that SSI progeny may be overwintering in the same location and potentially emigrating as two year old smolts. Further comprehensive parentage analysis supports this hypothesis as 16 additional hatchery progeny were captured migrating past the screw trap as age-2^+ smolts. Total percent assignment equaled 11.40% (70 of 614) lower than 2006 (13.10%) and 2007 (13.50%). There were a total of 64 age-0^+ (17.0%) and 6 age-1^+ (3.0%) assignments from broodyear 2008 outplants (Williamson et al. 2010).

Staff calculated the area by strata and for the entire Yankee Fork from length and width data collected by SBT field crews. Using densities collected during 2008 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.

Overall estimated age-0^+ *O. mykiss* equaled 37,647 (± 6,414) juveniles in 2008 with 7,786 (± 4,619) individuals being from streamside origin. Therefore, we estimate that 20.7% of the Yankee Fork age-0^+ steelhead population originates from streamside incubator supplementation. Stratum four shows the highest abundance of incubator progeny at 41.4% of the sample. Survival of hatchery-origin fry to age-0^+ parr is estimated at 2.0%; however, fish maybe leaving the system before sampling or low sample size underestimates abundances.

Using densities collected during 2009 screw trap operations, the Tribes estimated total age-1^+ abundance by strata and for the overall drainage. Overall estimated age-1^+ *O. mykiss* equaled 19,403 (± 213) juveniles from BY2008 with 956 (± 401) individuals being from streamside origin. Therefore, we estimate that 4.9% of the Yankee Fork age-1^+ steelhead population originates from streamside incubator supplementation. Survival of hatchery-origin fry to age-1^+ smolt is estimated at 0.2%; parr to smolt is estimated at 12.3%; however, fish maybe leaving the system before sampling or low sample size underestimates abundances.

Survival estimates between life stages were equated from the SSI progeny estimates determined from the parentage analysis. For SSI supplementation activities in broodyear 2008, egg to fry survival was estimated at 82.4%, fry to parr survival at 2.0%; and parr to smolt survival was 12.3%. 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

Salmonid species observed in Yankee Fork included steelhead, Chinook salmon, bull trout, cutthroat trout, and mountain whitefish. Prior to Chinook salmon supplementation activities in

2008, 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 remained low in 2008, although increased dramatically in 2009 with adult outplanting activities in 2008. Overall density was extremely low in 2008 electrofishing surveys (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° 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). Screw trap operations provide tighter estimates of juvenile density as compared to point estimates generated from electroshocking in the fall. Operations in 2009 indicate an estimate of 97,504 (SE 7,326) *O. mykiss* juveniles between July 3 and November 13, 2010 as compared to point estimates in 2006 (17,850) and 2007 (40,740).

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 SSI 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 2006 - 2008 analyses provides evidence that upweller origin juveniles successfully emerge and survive in-stream through the first year of life (Matala et al. 2008). Broodyear 2008 evaluations indicate detection of age-2^+ smolts provides evidence that juvenile steelhead trout produced by the streamside upweller program continue to survive in-stream through the second year of life and that the age of seaward migration may vary for juvenile steelhead produced from the SSI Program (Williamson et al. 2010). Estimated overall productivity in the Yankee Fork is extremely low. Staff estimated 37,647 (± 6,414) broodyear 2008 *O. mykiss* age-0^+ and 19,403 (± 213) age-1^+ juveniles in Yankee Fork. Of the 37,647 age-0^+ juveniles, 7,786 (± 4,619) or 20.7% of the population were estimated as SSI progeny. Likewise, 4.9% or 956 (± 401) age-1^+ juveniles were identified as SSI progeny.

Full broodyear survival percentages were estimated for supplementation activities in 2008. Egg to fry survival remains high as in previous years at approximately 82.4%, but lower in 2008 due to the loss of one incubation unit (Williamson et al. 2009). Fry to parr survival (2.0%) is low and may be the indicating factor why few age-1^+ and older smolts are encountered in the following 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. However, that said, age-2^+ smolts were identified in the comprehensive analyses indicating a life history strategy not previously documented in the Yankee Fork. Parr to smolt survival was estimated at 12.3%. 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 in 2011 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

Pedigree analysis reveals relative 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

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Summary

The Shoshone-Bannock Tribes (Tribes) are currently conducting monitoring and evaluation of steelhead trout (*Oncorhynchus mykiss*) supplementation activities in the Yankee Fork, which includes the use of streamside incubators (upwellers) for rearing hatchery origin (HAT) steelhead. Juvenile HAT steelhead 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 that provides the third of three consecutive yearly estimates of the contribution from the upweller program to total abundance of steelhead in the Yankee Fork. In addition, a comprehensive parentage analysis encompassing brood years 2006 to 2008 was performed. Full parental genotypes were generated for all 2008 Sawtooth Fish Hatchery brood stock (N=214) that supplied gametes for artificial spawning. Eyed eggs produced by the artificial spawnings were transplanted to six streamside upwellers at five locations within the Yankee Fork in 2008. Multilocus genotypes were generated for age-0^+ juvenile steelhead (N= 386) collected throughout the Yankee Fork during 2008 and 2009 and age-1^+ steelhead smolts (N = 228) collected in a rotary trap in 2009. Parentage assignment was performed to determine the proportion of juveniles that originated from upwellers in 2008. Juveniles that were not assigned to known parents were assumed to be NOR. Parentage assignment identified 64 age-0^+ and 6 age-1^+ juveniles produced from upwellers, with an overall relative proportion of 0.114 HAT juveniles produced from the 2008 spawning efforts among all steelhead juveniles evaluated (N = 614). The comprehensive parentage analysis identified additional age-1^+ (n=5) and age-2^+ (n=16) HAT steelhead offspring produced during brood years 2006 and 2007. 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) had historically supported productive populations of steelhead trout that represented a significant cultural, social, and subsistence based resource for the Tribes. Several steelhead trout and Chinook salmon (*O. tshawytscha*) populations have experienced significant decline 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 juvenile 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 be beneficial to both NOR and supplemented HAT juvenile rearing.

The Tribes have established and implemented a monitoring and evaluation 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. 2006). Initial monitoring of HAT 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 HAT juveniles. Both Solazzi et al. (1999) and Denny and Tardy (2008) have demonstrated high (>93%) egg-to-fry survival in upwellers. However, the ecological processes affecting post-release survival of HAT 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 HAT 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 HAT and NOR fish, and consequently results in HAT steelhead experiencing decreased survival in the natural environment (Kostow et al. 2003 and 2004; Miller et al. 2004; Berejikian and Ford 2004), or decreased reproductive fitness relative to NOR fish (Chilcote et al. 1986; Chilcote 2003; Matala et al. 2005; Araki et al. 2007). Relative survival and recruitment of HAT juveniles originating from upwellers and that rear in the natural environment is not well documented.

In this report we provide results from the third year of a DNA-based parentage analysis implemented to evaluate the efficacy of upwellers used as a supplementation tool for steelhead trout in the Yankee Fork. In addition, the results of a comprehensive parentage analysis encompassing brood years 2006 to 2008 are evaluated. The purpose of the comprehensive parentage analysis is to identify age-2^+ HAT smolts encountered during screw trap operations and field sampling, thereby further refining estimates of relative abundance of HAT offspring produced by streamside incubator supplementation activity in the Yankee Fork in 2006 and 2007. The age-2^+ HAT smolts would have been classified as presumed NOR individuals since the brood stock that produced them would not have been included in earlier single year pedigree analyses. In 2009, a rotary screw trap was placed on the mainstem Yankee Fork to collect older HAT smolts as they migrated out of Yankee Fork. To assess the ability of the streamside upweller program to contribute to production or abundance relative to NOR fish, post-release survival of HAT 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 HAT and NOR stocks (Blouin and Araki 2004; Williamson et al. 2009), and dispersal and migration (Paetkua et al. 2004).

Methods

Brood stock and Field Sampling

Fin clips were taken to supply DNA for genetic analysis from all 107 mated pairs of Sawtooth Fish Hatchery brood stock (P3; represents the 3rd parental generation evaluated) used

for the brood year 2008 Yankee Fork upweller program. Artificial spawning was conducted on April 17 and 24, and May 1 in 2008. 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 brood stock 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. Records of which brood stock pairs were represented in each streamside upweller were available for only brood year 2008. Eyed eggs were transferred in numbers ranging from 71,978 – 85,000 per upweller. One upweller was maintained at each of the following sites in the Yankee Fork in 2008: Greylock Creek, Jordan Creek, West Fork Yankee Fork, and 12 Mile Creek (Figure 2). Two adjacent upwellers were maintained on Cearly Creek. The upwellers were used to incubate 483,904 steelhead eyed-eggs, in total, from the 2008 brood year. Eggs were planted on June 5, 13, and 20 in 2008, and fry volitionally emigrated in July and August, 2008. Following volitional release, egg survival was quantified by removal and inspection of incubator trays to enumerate dead eggs (N= 85,376). The hatch success rate was 82.6%, resulting in an estimated release of 398,528 seeded fry.

Non-lethal genetic sampling of juvenile *O. mykiss* (F1; presumed steelhead) was performed throughout the Yankee Fork. Juvenile fish were collected in a three-pass removal procedure using block nets to facilitate 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=474) were sampled in 2008. Migrating (smolt) juveniles (N = 246) were collected in a rotary trap located on the main stem Yankee Fork in 2009. 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 age-0^+ juvenile samples per stratum to use (0.263) was calculated as the total number of samples to be genotyped (394), divided by the point estimate of the sum of all sampled reaches (1500).

Juvenile sampling was performed within the Yankee Fork sub-basin during September 16-26, 2008. The Tribes utilized the sampling design of Konopacky et al. (1985, 1986) to sub-divide the Yankee Fork drainage into seven strata (Figure 2). Sampling sites were randomly selected within the main stem flow for a variety of habitats (pools, glides, riffles) and ease of accessibility for an upper, middle, and lower location within each stratum, and included dredge pond habitat (Pond Series #1 and #3; located in stratum #2) 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 from fin clips was extracted with a DNA extraction kit (QIAGEN, Inc., Valencia, California). Seventeen nuclear microsatellite loci were amplified by polymerase chain reaction (PCR) assays in all adult and juvenile samples collected in 2008-2009. The 17 microsatellite locus primers included: *Omy*1001UW *and* 1011UW (Spies et al. 2005), *Ssa*407 and *Ssa*408 (Cairney et al. 2000), *Ocl*1 (Condrey and Bentzen 1998), *Ogo*3 and *Ogo*4 (Olsen et al. 1998), *Oke*4 (Buchholtz et al. 2001), *Ots*1, *Ots*3, *Ots*4, and *Ots*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), *One*μ14 (Scribner et al. 1996), and *Ssa*289 (McConnell et al. 1995). Three of the loci (*Omy*1001UW, *Oke*4, and *One*μ14) amplified by PCR were evaluated to detect cutthroat trout (*O. clarki clarki*) and *O. clarki* x *O. mykiss* hybrids in juvenile trout collections. An ABI-3130xl automated DNA Genetic Analyzer and GeneMapper Version 4.0 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 brood stock and juveniles sampled was subsequently re-extracted from tissue, re-amplified and genotyped at the same 17 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 17 loci the genotyping error rate was calculated as 0.0043.

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 (e.g., age-0^+ juvenile sampled later on as an age-1^+ smolt), 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 17 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- brood year 2008

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 17 loci were calculated using the 2008 brood stock 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, 106 true candidate parent pairs were simulated. Since two females with duplicate genotypes were detected in the 2008 brood stock collection, one of the duplicate individuals (ID # 1250-067) along with its recorded mate was excluded from the parentage assignment simulations. The proportion of the 2008 spawning escapement consisting of the true candidate parents was set at 0.074 (K. Tardy, personal communication). A total of 11,236 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 simulated offspring genotype data and parentage likelihood calculations were manipulated according to specified error rate parameters, so that the simulation output was as realistic as possible. Genotyping and likelihood calculation error rates were set at 0.0043 and 0.010, respectively. Genotyping error rate was quantified during quality control for data collection, and the likelihood error rate used was that suggested by Meagher and Thompson (1986). The stringency level for parentage analysis was set to a minimum of 16 loci compared and allow 0-2 mismatches between parent pair/progeny genotypes (e.g., data from no fewer than 16 of 17 loci must be available and no more than two mismatches occur with respect to alleles inherited from each parent**).**

Comprehensive parentage assignment simulation- brood years 2006 to 2008

A comprehensive parentage analysis was simulated using CERVUS to estimate the ΔLOD score so that the confidence of parentage assignments for real data could be evaluated statistically. Since the 2006 brood stock had been genotyped at all loci except *Oke*4, *Omy*1001, and *One*μ14 (Matala and Ardren 2008), the comprehensive parentage analysis included only the remaining 14 microsatellite loci genotyped for the 2007 and 2008 brood stocks. Allele frequencies for 14 loci were calculated using the 2006, 2007, and 2008 brood stock genotype data (52, 87, and 106 parent pairs, respectively). 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, 245 true candidate parent pairs were simulated. The proportion of spawning escapement consisting of the true candidate parents was set at 0.071 (K. Tardy, personal communication). A total of 60,025 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 simulated offspring genotype data and parentage likelihood calculations were manipulated according to specified error rate parameters, so that the simulation output was as realistic as possible. Genotyping and likelihood calculation error rates were set at 0.0088 and 0.010, respectively. Genotyping error rate was quantified during quality control for data collection, and the likelihood error rate used was that suggested by Meagher and Thompson (1986). 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 using either the brood year 2008 data alone, or the comprehensive (brood years 2006 to 2008) dataset were performed using CERVUS. Age-0^+ juvenile steelhead collected during the 2008 fall electrofishing survey of Yankee Fork and age-1^+ smolt steelhead collected at the mainstem Yankee Fork screw trap were included in the brood year 2008 parentage analysis. All juvenile and smolt steelhead collected from 2006 to 2009 were included in the comprehensive parentage analysis. The same parameters used in the respective 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 with real data. A juvenile fish was considered HAT if genetic data confirmed that adults used to supply streamside upwellers had parented it. Juveniles with no identified parental match within the brood stock were assumed to be NOR.

Descriptive statistics

Eight brood year 2008 sample collections were evaluated in genetic diversity and population genetic structure analyses: 2008 brood stock (N=213; only duplicated female removed), assigned HAT progeny (N=70), and 2008 presumed NOR juveniles collected in stratum #1 (N=41), stratum #2 (N=54), stratum #3 (N=74), stratum #4 (N=43), stratum #6 (N=72), and dredge ponds (N=25; Series #1 and #3 combined). In 2008, no trout were collected in stratum #5. The sample number of 2008 presumed NOR juveniles per stratum are those remaining after HAT origin individuals were identified in the comprehensive (brood years 2006 to 2008) parentage analysis. The 2008 presumed NOR stratum #7 collection contained only five individuals after removal of HAT assigned juveniles. Since the remaining five juveniles do not provide a representative sample, the stratum #7 collection was excluded from genetic diversity and structure analyses. 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 collection), expected and observed heterozygosities, and index of inbreeding (F_{IS}) were calculated using the software package Genetic Data Analysis Version 1.1 (Lewis and Zaykin 2001). Hardy-Weinberg Equilibrium (HWE) probability and linkage disequilibrium (LD) tests were conducted on a locus by locus basis using GENEPOP Version 3.4 (Raymond and Rousset 1995). Statistical significance was adjusted for the number of simultaneous tests k (α/k for α = 0.05, where k = number of loci) by Bonferroni correction (Rice 1989). The program FSTAT Version 2.9.3.2 (Goudet 1995) was used to calculate allelic richness between the HAT and

presumed NOR groups, where allelic richness is a weighted estimate of the number of alleles per locus, scaled to the smallest sample size (24 individuals).

Population differentiation

GENETIX Version 4.05 (Belkhir et al. 2004) was used to generate pair-wise F_{ST} estimates among brood year 2008 collections, and test for significant departure from 1000 permuted F_{ST} estimates calculated from randomly mating populations given the allele frequencies in the study. The significance of individual pair-wise F_{ST} comparisons was evaluated after applying a Bonferroni correction based on the number of pair-wise tests (P=0.05/28; 0.0018; Rice 1989). 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 2002) was used to generate 1000 randomized replicates (bootstrap resampling) of the brood year 2008 dataset. For each of the 1000 replicates a pair-wise genetic distance matrix of Cavalli-Sforza and Edwards (1967) chord distance measures (D_{CE}) was generated and an unrooted Neighbor-Joining phenogram (Saitou and Nei 1987) 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 consensus 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. Cluster analysis was similarly performed for all brood stock, HAT assigned juveniles (identified by the comprehensive parentage analysis), and age-0^+ presumed NOR juvenile collections (grouped by stratum) sampled in brood years 2006 through 2008.

Juvenile movement

Movement patterns of brood year 2008 HAT assigned age-0^+ juvenile steelhead within Yankee Fork and among its tributaries were evaluated by comparing location where juveniles were encountered during electrofishing (e.g., strata in Figure 2) with the location of streamside upwellers into which the juveniles had been originally incubated. The streamside upweller origin for HAT assigned juveniles was tracked using streamside upweller outplant records for the brood year 2008 parent pairs. The hypothesis that age-0^+ HAT steelhead are subsequently sampled within the same stratum where they were incubated in a streamside upweller (e.g., juveniles placed into the upweller at 12 mile Creek are later sampled within stratum #5) was tested by using the Wilcoxon sign-rank test (Wilcoxon and Wilcox 1964) as implemented by the statistical software package R Version 2.9.10 (R Development Core Team 2009). Specifically, the Wilcoxon sign-rank test was applied to evaluate whether (or not) there exists a statistically significant difference in the distributions of upweller and subsequent sampling locations by stratum for brood year 2008 HAT assigned juveniles.

Results

Identification of species and replicate samples

Genetic analysis for species specific alleles detected one cutthroat trout and 12 F1 hybrids (cutthroat x rainbow trout) in the brood year 2008 age-0^+ juvenile, and 17 F1 hybrids in

the age-1^+ smolt collections. All 30 fish were removed from the data set prior to further genetic data analysis.

No temporally replicate samples were detected for juvenile steelhead hatched in 2008. However, two presumed NOR age-0^+ juvenile fish samples (ID# 1309-011 and 1309-019), two age-1^+ smolt samples (ID# 1681-038 and 1681-039), and two 2008 brood stock samples (1250-066 and 1250-067) had identical genotypes at 17 loci. Individuals 1309-019, 1681-039, and 1250-067 were removed from dataset prior to performing analyses. Overall 17 loci, non-exclusion probabilities for identity and parent pair were calculated as $3.34X10^{-22}$ and $3.78X10^{-14}$, respectively.

Simulated parentage assignment – brood year 2008

In the simulated parentage analysis for the 2008 brood year, 928 (~9.3%) offspring were assigned to either true or unrelated candidate parent pairs. The most likely candidate parent pair/offspring assignments were broken down as follows: 379 (40.8%) offspring were assigned to the true parent pair, 545 (58.7%) were incorrectly assigned to a non-parent pair (where either the true mother or father was unsampled), and 4 (0.4%) offspring were 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 = 34.65; SD = 5.76) 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 = 3.36; SD = 3.03), or both parents were unsampled (mean ΔLOD = 2.06; SD = 1.98) (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.

Parentage assignment simulation results for brood year 2008 indicated that parent pair/juvenile assignment critical ΔLOD score = 10.5. This critical ΔLOD was applied to parentage assignments with real data, and all assignments with a ΔLOD ≥ 10.5 were accepted.

Parentage assignment with real data – brood year 2008

Parentage assignments produced 74 parent-pair/juvenile trios that met or exceeded both the stringency level of loci used for assignment and critical ΔLOD score. Seventy of the 74 trios were produced by 40 recorded parent pairs: one offspring – 24 pairs, two offspring – 9 pairs, three offspring – 4 pairs, four offspring – 1 pair, five offspring – 1 pair, and seven offspring – 1 pair (Table 1). No offspring were observed that had been produced by the remaining 66 recorded 2008 brood stock mated pairs. Out of the 74 parent-pair/juvenile trios that met both the stringency level of loci and the critical ΔLOD score, 8 (11%) did not match the pairs of adults recorded as having been mated in 2008. Inspection of the 2008 mating records revealed that 4 mismatched parent pairs were due to instances where the recorded male parent was listed immediately adjacent to the genetically assigned male parent. Since these 4 trios met the criteria for correct assignment, and multiple offspring were assigned to the same parent pairs, the 4 juveniles were included in the dataset of HAT juveniles produced by the 2008 brood stock. The remaining 4 parent-pair/juvenile trios were classified as incorrectly assigned. These trios were considered incorrect since the assigned parents had been spawned on different dates.

Simulated parentage assignment – brood years 2006 to 2008

In the simulated parentage analysis for the combined 2006 to 2008 brood years 1786 (~17.9%) offspring were assigned to either true or unrelated candidate parent pairs. The most likely candidate parent pair/offspring assignments were broken down as follows: 400 (22.4%) offspring were assigned to the true parent pair, 1318 (73.8%) were incorrectly assigned to a non-parent pair (where either the true mother or father was unsampled), and 68 (3.8%) offspring were 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 = 26.39; SD = 5.87) 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 = 2.98), or both parents were unsampled (mean ΔLOD = 2.05; SD = 1.84) (Figure 3). The simulated parentage analysis suggests that the resolving power of the set of loci is sufficient to assign offspring to the correct candidate parent pair.

Parentage assignment simulation results for the combined brood year 2006 to 2008 dataset indicated that parent pair/juvenile assignment critical ΔLOD score=12.0. This critical ΔLOD was applied to parentage assignments with real data, and all assignments with a ΔLOD ≥ 12.0 were accepted.

Comprehensive parentage assignment with real data – brood years 2006 to 2008

The comprehensive genetic pedigree analysis produced 258 parent-pair/juvenile trios that met or exceeded both the stringency level of loci used for assignment and critical ΔLOD score. Two hundred and ten trios out of 258 trios were produced by 118 recorded parent pairs: one offspring – 52 pairs, two offspring – 39 pairs, three offspring – 15 pairs, four offspring – 7 pairs, five offspring – 3 pairs, seven offspring – 1 pair, and nine offspring- 1pair (Appendix 1). No offspring were observed that had been produced by the remaining 127 recorded 2006 to 2008 brood stock mated pairs. Out of the 258 parent-pair/juvenile trios that met both the stringency level of loci and the critical ΔLOD score, 48 (~19%) did not match the pairs of adults recorded as having been mated from 2006 to 2008. Inspection of the 2006 to 2008 mating records revealed that 30 mismatched parent pairs were due to instances where either the recorded male (2008 artificial spawning records), or female (2007 artificial spawning records) parent was listed immediately adjacent to the genetically assigned parent. Since these 30 trios met the criteria for correct assignment, and multiple offspring were assigned to parent pairs, the 30 juveniles were included in the dataset of HAT juveniles produced by the 2006 to 2008 brood stock. The remaining 18 parent-pair/juvenile trios were classified as incorrectly assigned. These trios were considered incorrect since the assigned parents had been spawned on different dates.

Relative to parentage analyses performed for single brood year datasets, the comprehensive parentage analysis identified additional age-1^+ (n=5) and age-2^+ (n=16) HAT steelhead offspring produced during brood years 2006 and 2007 (Table 2). Four of the 16 age-2^+ HAT steelhead identified were produced in brood year 2006; one individual was identified emigrating from West Fork Yankee Fork during the 2008 screw trap operation, and three individuals in stratum #4 during the fall 2008 Yankee Fork electrofishing survey. In addition, five age-1^+ HAT steelhead produced in 2007 were identified in stratum #7 during the fall 2008 electrofishing survey in Yankee Fork. Another 12 brood year 2007 age-2^+ HAT individuals were identified emigrating from Yankee Fork during the 2009 screw trap operation. In general,

the additional HAT offspring identified by the comprehensive parentage analysis augmented the family size of parent-pair/offspring trios that had already been identified in previous parentage analyses. Six of the 21 age-1^+ and age-2^+ HAT steelhead identified by the comprehensive parentage analysis revealed five new parent-pair/offspring trios that had not been previously identified by previous parentage analyses. The updated estimates of overall HAT production of steelhead in Yankee Fork for brood year 2006 and 2007 are 0.140 and 0.161, respectively (Table 2).

Progeny distribution –brood year 2008

The 64 age-0^+ HAT steelhead identified as having been produced during 2008 were collected throughout Yankee Fork (Table 3). Parental assignments for age-0^+ steelhead were distributed among seven sites in strata #3, #4, #6, #7, and in one of the dredge ponds (Pond Series #3) and ranged from 4-88% HAT assigned juveniles per site. The majority of age-0^+ HAT juveniles (n=52) identified were encountered in stratum #4, sites 2 and 4, and Pond Series #3. All age-1^+ HAT juveniles (n=6; 3% of smolts collected in 2009) were collected at the screw trap on the main stem Yankee Fork. No parental assignments were identified among the age-0^+ juveniles (n=146) captured in any of the six sites of strata #1 and #2. The overall percentage of HAT assigned individuals across all strata and sites sampled in 2008 and 2009 was 10.42% and 0.98% among age-0^+ juvenile and smolt steelhead, respectively.

Descriptive statistics

A high level of polymorphism was observed across all 17 microsatellite loci evaluated in the analysis of brood year 2008 steelhead collections (Table 4). Observed numbers of alleles ranged from 3 at *Ssa*289 within the assigned HAT progeny and at *Ogo*3 within the Pond (Series #1 and #3 combined) NOR juvenile collection, to 21 at *Omy*1001 within the 2008 brood stock (mean = 10, over all loci and collections). Observed heterozygosity (H_o) ranged from 0.320 for *Ssa*289 to 0.986 for *Omy*1001 in the Pond and stratum #6 NOR collections, respectively (mean H_o=0.757, over all loci and collections). There were 12 departures from expected genotypic proportions among 136 total HWE tests. Departures (heterozygote deficits) from HWE primarily occurred at *Omy*77 (n=6) and *Ots*1 (n=3) across most sample groups. Statistically significant (P=0.0004) linkage disequilibrium was observed for 25 and 13 (out of 136) locus by locus pair-wise comparisons in the 2008 HAT assigned juveniles and brood stock, respectively (data not shown). Few (0-7) significant pair-wise linkage disequilibrium tests were observed for presumed NOR age-0^+ juvenile collections from strata #1 and #2, and the combined (Series #1 and #3) dredge pond collections. Collections of presumed NOR age-0^+ juveniles from strata #3, #4, and #6 had slightly higher numbers (10-18) of significant pair-wise linkage disequilibrium tests. While in general there were few (0-4) private alleles in the presumed NOR and HAT assigned juvenile collections, 10 private alleles were observed in the 2008 SFH brood stock collection. Mean allelic richness (AR) ranged from 8.1 (stratum #4 NOR collection) to 9.0 (2008 brood stock). There was no significant difference in allelic richness between the hatchery origin (brood stock and assigned offspring) group and the group of combined presumed NOR juvenile collections (AR = 8.9 and 8.6, respectively; *P*=0.32).

Genetic diversity was also compared between the brood year 2007 and 2008 steelhead collections. Relative to all brood year 2007 NOR collections, the 2008 NOR collections had significantly less allelic richness (AR=9.02 and 8.54, respectively; P=0.002) and observed

heterozygosity (H_o=0.769 and 0.753, respectively; P=0.041). In contrast, no significant differences were detected when brood year 2007 and 2008 NOR collections were compared by either fixation index (F_{IS}=0.038 and 0.045, respectively; P=0.357), or variation among collections (F_{ST}=0.003 and 0.008, respectively; P=0.053). Comparison between the combined 2007-2008 brood stock collections and all 2007 and 2008 presumed NOR collections by allelic richness, fixation index, observed heterozygosity, and variation among collections indicated no significant differences (data not shown). Likewise, comparison of allelic richness, fixation index, and observed heterozygosity between the 2007 and 2008 brood stocks indicated no significant differences (data not shown).

Population differentiation

Among the 8 groups of 2008-2009 steelhead evaluated, we observed pair-wise F_{ST} estimates ranging from 0.000 to 0.018 (Table 5). The overall F_{ST} estimate of 0.008 (99% C.I. = 0.006-0.011) indicates low, but statistically significant genetic variability among collections. Among collection variation was detected between the HAT collections (brood stock and HAT assigned juveniles) and all presumed NOR collections, except the combined (Series #1 and #3) Pond collections. Pair-wise F_{ST} estimates for the combined Pond collections with each of the other presumed NOR collections detected statistically significant differences between the Pond and both stratum #3 and #6 collections. Likewise, pair-wise F_{ST} estimates for juveniles sampled in stratum #1 and all other presumed NOR juveniles sampled indicated a statistically significant difference between the strata #1 and #3 collections. Consistent clustering (100% bootstrap support) was observed for only the HAT collections (brood stock and HAT assigned juveniles) in the unrooted Neighbor-Joining phenogram of pair-wise D_{CE} among the 2008 Yankee Fork steelhead (Figure 4).

Cluster analysis of all HAT assigned and age-0^+ juvenile NOR collections, and adults used as brood stock from 2006 to 2008 also indicated consistent clustering of HAT collections by brood year (i.e., high ≥85% bootstrap support for clusters of HAT assigned juveniles and the respective brood stock that produced them) (Figure 5). However, the groups of HAT collections were dispersed from one another on the Neighbor-Joining phenogram. Two groups of 2007 NOR collections, strata #3 and #7 (87% bootstrap support), and strata #1 and #2 (86% bootstrap support), consistently clustered together. Weak clustering (66% bootstrap support) was observed for the 2006 NOR strata #1 and #7 collections. All other remaining NOR collections in the Neighbor-Joining phenogram were, in general, dispersed (<50% bootstrap support) in regard to either sample stratum or brood year.

Juvenile movement

Out of the 64 HAT assigned age-0+ steelhead detected in Yankee Fork during 2008, the majority had been incubated in streamside upwellers located in Cearly Creek (n=20; ~31%), Greylock Creek (n=13; ~20%), and Jordan Creek (n=27; ~42%). Five of the six age-1^+ HAT steelhead smolts hatched in 2008 and detected at the main stem Yankee Fork rotary screw trap in 2009 had been originally planted and incubated in the streamside upweller located in Jordan Creek (stratum #7; Figure 2). A significant difference (V=475.5; P<0.001) was detected between the distributions of egg outplant and subsequent juvenile sampling locations by stratum for the brood year 2008 HAT assigned age-0^+ juvenile steelhead. Where differences between streamside upweller and juvenile sampling location occurred, the majority (n=25) of the

individuals had been originally outplanted into the Jordan Creek, stratum #7 upweller and subsequently sampled within stratum #4. In contrast, juvenile steelhead (n=20) originally outplanted into the two streamside upwellers on Cearly Creek were subsequently sampled in the series #3 dredge pond (both located in stratum #2). Juvenile steelhead (n=6) originally outplanted into the Greylock Creek (stratum #4) streamside upweller were subsequently sampled in stratum #4.

Discussion

Parentage assignment with real data

Parentage assignments observed in the brood year 2006 (Matala and Ardren 2008), 2007 (Williamson and Matala 2009), 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 64 age-0^+ and 6 age-1^+ juveniles produced from upwellers, with an overall assignment proportion of 0.114 HAT juveniles produced from the 2008 spawning efforts among all steelhead juveniles genotyped (N = 614). The overall proportion of HAT assigned juveniles in the 2008 analysis was similar to that observed in the brood year 2006 (0.131; Matala and Ardren 2008) and 2007 (0.135; Williamson and Matala 2009) analyses. Not surprisingly, the comprehensive parentage analysis that utilized the combined 2006 to 2008 datasets detected additional age-1^+ and age-2^+ HAT assigned steelhead in Yankee Fork. The brood year 2007 age-2^+ individuals were primarily detected at the rotary screw trap as they emigrated from Yankee Fork in 2009. Detection of these age-2^+ smolts provides evidence that juvenile steelhead trout produced by the streamside upweller program continue to survive in-stream through the second year of life and that the age of seaward migration may vary for juvenile steelhead produced from the streamside upweller program. Comparison of the proportions of HAT and NOR age-2^+ steelhead smolts that emigrate from Yankee Fork could not be performed since age-specific data (i.e., scale or otolith samples) were not taken from migrating NOR steelhead smolts. The estimated abundance of age-0^+ *O. mykiss* in Yankee Fork for 2008 was 37,647 juveniles, with an estimated 7,786 individuals produced by the streamside incubators (Tardy and Denny 2010). The estimated abundance of age-1^+ *O. mykiss* produced by the streamside incubators in Yankee Fork for 2009 was 956 juveniles (Tardy and Denny 2010). Therefore, there were an estimated total of 8,742 juvenile individuals produced from brood year 2008 streamside incubators (Tardy and Denny 2010).

Progeny distribution and movement

Similar to the brood year 2007 analysis (Williamson and Matala 2009), brood year 2008 HAT juvenile steelhead were not evenly distributed throughout the Yankee Fork drainage system (Table 3). The majority of the brood year 2008 age-0^+ and age-1^+ HAT assigned offspring were encountered in stratum #4 and one of the dredge ponds in stratum #2. In contrast, the majority of brood year 2007 HAT assigned juveniles offspring were encountered in stratum #7 (Williamson and Matala 2009). Comparison between the brood year 2007 and 2008 age-0^+ HAT progeny distributions may be made for only strata #2, #3, #4, #6, #7. Between 2007 and 2008, no significant differences were observed in the percentage of age-0^+ HAT assigned juveniles encountered in the five strata compared (data not shown). Many (83%) of the age-0+ steelhead

encountered within dredge pond (series #3; located in stratum #2) were HAT assigned individuals. None of the brood year 2008 HAT steelhead were detected elsewhere in stratum #2. Interestingly, all juveniles sampled in stratum #2, site-2 in previous parentage analyses of brood year 2006 (Matala and Ardren 2008) and 2007 (Williamson and Matala 2009) were HAT assigned. Since HAT progeny have been consistently encountered from year to year within stratum #2, this region of Yankee Fork may represent favorable or preferred rearing habitat for HAT juvenile steelhead produced by the streamside upweller program in Yankee Fork. Brood year 2008 age-1^+ HAT assigned offspring were encountered only at the rotary screw trap on the mainstem Yankee Fork. Since age-1^+ steelhead were not captured in the Yankee Fork tributaries during the fall 2008 electrofishing surveys, comparison of the distributions of brood year 2007 and 2008 HAT age-1^+ steelhead within Yankee Fork could not be performed.

Since family identity was preserved during egg outplant and the identity of the 2008 brood stock pairs represented in each streamside upweller was recorded, data describing the migratory behavior, habitat preference, and successful incubator site selection for all HAT assigned juvenile steelhead trout during their first year of life in the Yankee Fork watershed may be documented. Comparison of the locations where brood year 2008 age-0^+ HAT juvenile steelhead had been outplanted into an upweller and where they were subsequently captured indicated that juveniles are often not encountered in the same stratum into which they had been outplanted. In fact, 25 of the brood year 2008 HAT assigned steelhead initially placed into and incubated in the streamside upweller on Jordan Creek (stratum #7) were subsequently encountered downstream within stratum #4. Likewise, temporally replicate field sampling of a brood year 2007 presumed NOR juvenile was observed, first as an age-0^+ individual in stratum #7 and subsequently as an age-1^+ individual in stratum #1 (Williamson and Matala 2009). These observations coincide 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).

The comprehensive parentage analysis permitted an expanded assessment of HAT production in Yankee Fork for brood years 2006 and 2007 and provided evidence that at least some HAT juveniles migrate seaward as age-2^+ individuals. Interpretation of the results and inferences about changes in the overall distribution of HAT steelhead in Yankee Fork as well as the age of seaward migration of HAT progeny requires a cautious approach. First, the Yankee Fork sub-basin experiences periods of high water flow (K. Tardy, personal communication) which may create a dynamic environment wherein the accessibility, location, and quality of suitable rearing habitat may change from year to year. Second, 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 HAT progeny outplanted into streamside upwellers. For instance, HAT origin trout may have a tendency to migrate at a younger age relative to their NOR counterparts, thus fewer age-1^+ HAT steelhead were detected, compared to age-0^+ HAT and age-1^+ NOR steelhead, throughout Yankee Fork. Alternative explanations may be that age-0^+ juveniles migrate to more hospitable over-wintering habitat in the main stem Salmon River, and/or HAT juvenile steelhead have lower survival relative to NOR juveniles.

Descriptive statistics

Patterns of observed allelic diversity (number of alleles/locus, H_o; Table 4) for the brood year 2008 genetic analysis were similar to those observed during the previous analysis for 2007 (Williamson and Matala 2009). Similar to previous analyses of genetic diversity of brood year 2006 (Matala and Ardren 2008) and 2007 (Williamson and Matala 2009) Yankee Fork steelhead, departures from HWE expectations primarily occurred at *Omy77* and *Ots1*. The observed number of private alleles was higher (10 over all 17 loci) in the 2008 brood stock compared to all presumed NOR and the HAT assigned collections (range: 0-4 over all 17 loci). A similar pattern was observed in the 2007 brood year analysis (Williamson and Matala 2009). Likewise, higher linkage disequilibrium, was detected in both the brood year 2007 (Williamson and Matala 2009) and 2008 brood stocks compared to all presumed NOR and the HAT assigned collections. This evidence suggests that steelhead obtained from the Sawtooth Hatchery and used as brood stock in the 2007 and 2008 Yankee Fork streamside upweller program may represent admixed samples, or have family structure owing to the brood stock collections containing a higher proportion of HAT individuals. However, unlike the brood year 2007 analysis, moderate levels of LD were also detected in multiple 2008 NOR juvenile collections (strata #3, #4, and #6). Linkage disequilibrium observed for presumed NOR juvenile collections may indicate sample admixture between the offspring of recognized resident rainbow trout and naturally spawning HAT steelhead.

Population differentiation

Clear differences exist between the 2008 HAT collections (brood stock and assigned juveniles) and the remaining presumed NOR collections (Figure 4, Table 5). This pattern is similar to that observed for earlier analyses [brood year 2006 (Matala and Ardren 2008); brood year 2007 (Williamson and Matala 2009)], and is illustrated in Figure 5. Each of the HAT groups are dispersed (rather than all HAT collections being clustered together) in the topology of the phenogram (Figure 5). This arrangement indicates that year to year variation in allele frequencies occurs within the brood stock collections taken for the Yankee Fork streamside upweller program. Two possible explanations follow. First, logistical limits on the numbers of adults selected as brood stock for the Yankee Fork upweller program may preclude a representative sample being taken each year from the Sawtooth Hatchery. Numbers of brood stock for 2007 and 2008 were 174 and 213 individuals, respectively. Given these numbers of adults, it seems unlikely that the brood stock collections do not constitute representative samples on the sole basis of low numbers. Second, adult steelhead taken at the Sawtooth Hatchery may represent either an admixed sample, or contain family structure. It is conceivable that yearly differences in the degree of sample admixture and/or family structure may lead to significant allele frequency differences in the collections of adults taken as brood stock for the upweller program from year to year. Elevated linkage disequilibrium and higher numbers of private alleles observed in the 2007 and 2008 brood stock collections suggest that family structure may be a more plausible explanation. In addition, a limited number (3-4) of artificial spawning events were performed over a fairly short period (2-3 weeks) for brood years 2007 and 2008. It is possible that the limited number of spawning events performed over a relatively short period do not necessarily capture a representative sample of the genetic diversity of steelhead that return to Sawtooth Hatchery. Comparison of a collection of Sawtooth Hatchery steelhead that were sampled over the entire duration of the Sawtooth Hatchery spawning run with the 2007 and 2008 collections of adults selected as brood stock for the Yankee Fork upweller program would

provide a way to resolve the explanations (i.e., admixture or family structure) of the observed genetic signals (i.e., higher LD detected and number of private alleles observed) in the brood stock used to seed the streamside upwellers.

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Figures and Tables

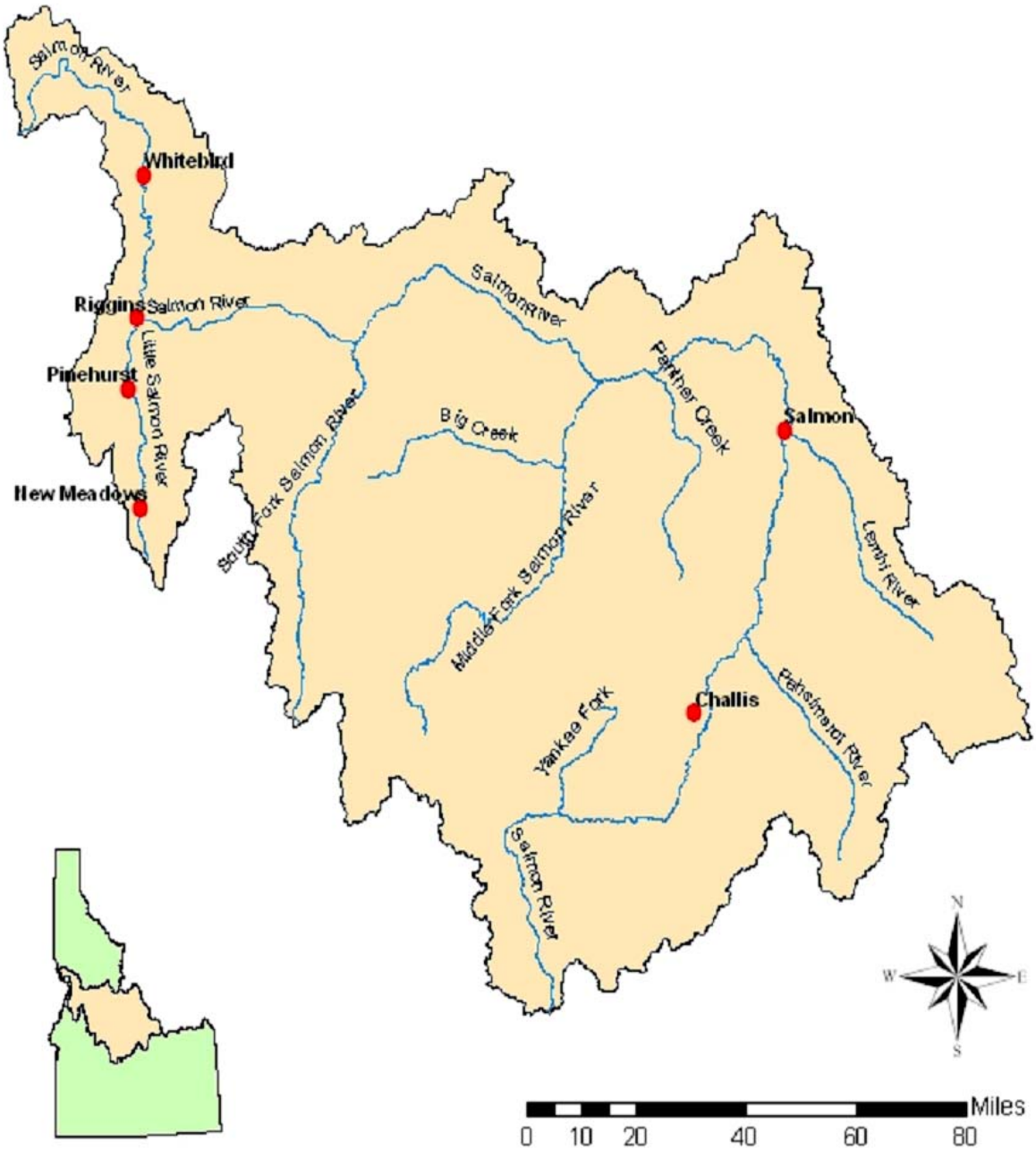

Figure 1. Salmon River basin

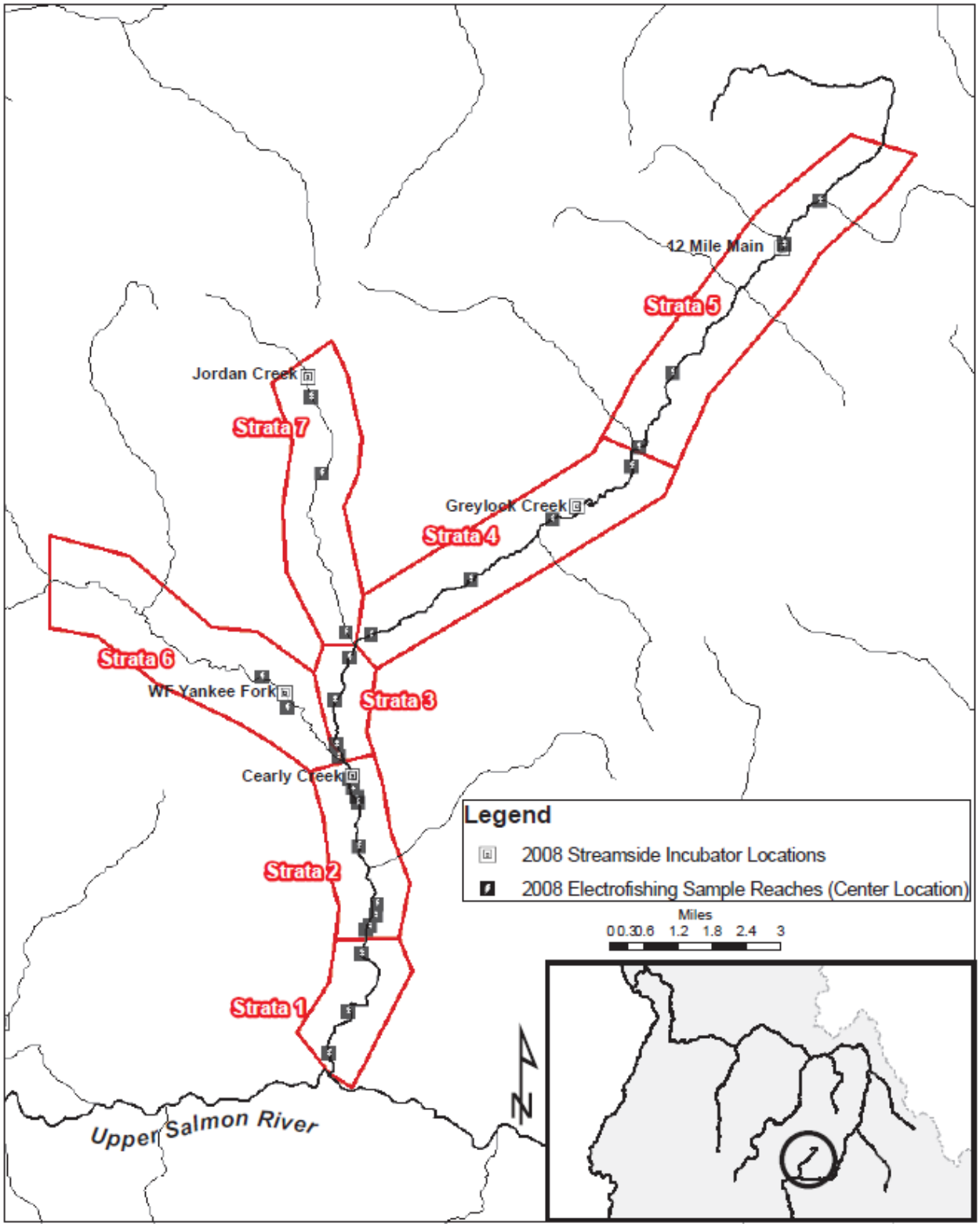

Figure 2. 2008 incubator and juvenile sampling sites in Yankee Fork, Idaho.

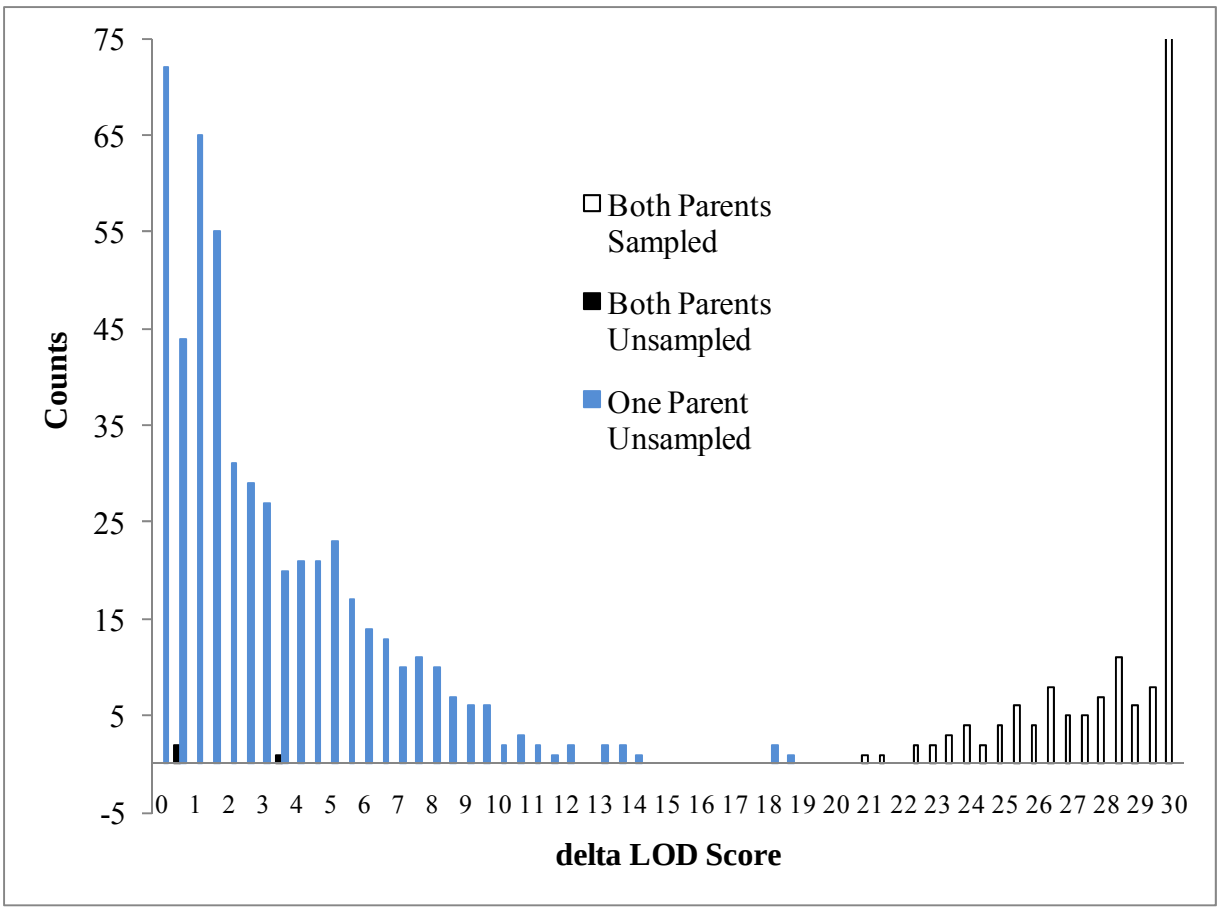

Figure 3. Histogram of simulated delta LOD scores of most likely parent pair/offspring assignments for various classes of the brood year 2008 parentage assignment outcomes.

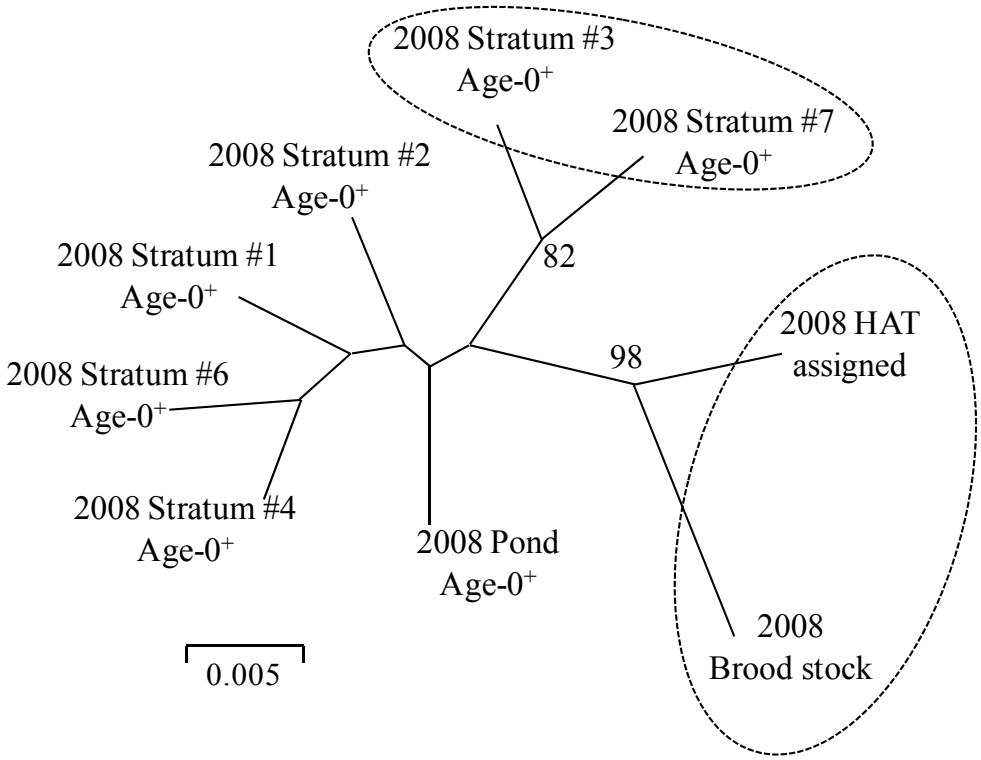

Figure 4. Unrooted Neighbor-joining phenogram based on Cavalli-Sforza and Edwards (1967) chord distance units among the 2008 Yankee Fork steelhead trout. The phenogram was constructed with PHYLIP (Felsenstein 1989) using data from 17 microsatellite loci. For 1000 boot-strap replicates, node values of 50% and greater are given. Clusters of samples with strong boot strap support are circled. Based on parent pair assignment with adults used to supply streamside upwellers in 2008, juvenile fish were classified as hatchery origin (HAT). Remaining presumed natural origin (NOR) individuals were grouped according to sampling strata within Yankee Fork (Figure 2).

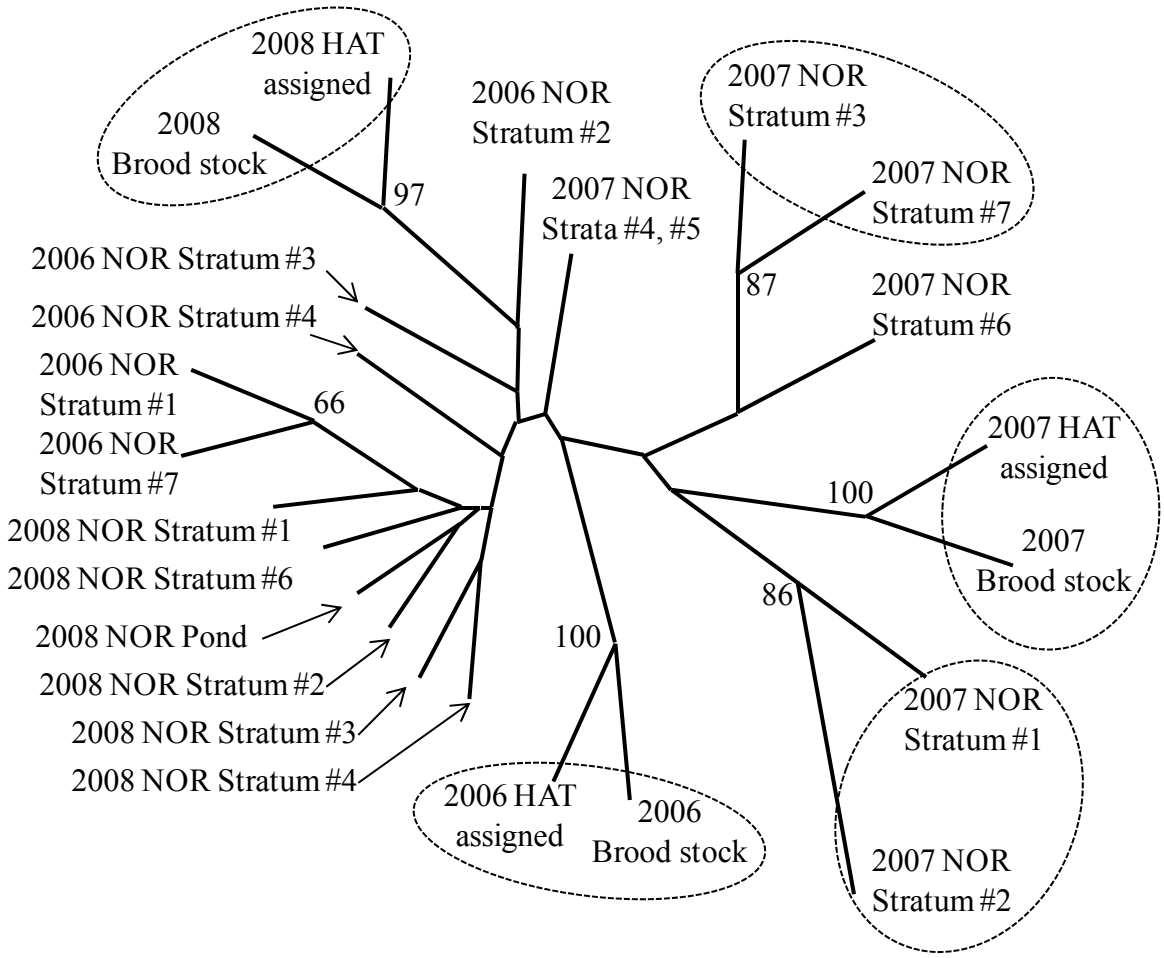

Figure 5. Unrooted Neighbor-joining phenogram based on Cavalli-Sforza and Edwards (1967) chord distance units among the 2006 to 2008 Yankee Fork age-0^+ steelhead trout juveniles grouped by stratum where they were sampled. The phenogram was constructed with PHYLIP (Felsenstein 1989) using data from 14 microsatellite loci. 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 parentage assignment to adults used to supply streamside upwellers from 2006 to 2008, juvenile fish were classified as hatchery origin (HAT). Remaining presumed natural origin (NOR) individuals were grouped according to year and 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 brood stock pairs used to supply eggs outplanted into Yankee Fork upwellers in 2008. See Figure 2 for strata locations.

Brood stock		Assigned offspring					
Father	Mother	ID	Stratum	Site	Age	Length (mm)	Mass (g)
1250-055	1250-045	1308-067	4	2	0	58	2.25
		1308-069	4	2	0	62	2.56
		1308-071	4	2	0	55	1.88
		1308-095	4	2	0	65	2.83
		1309-017	4	4	0	59	2.33
		1309-022	4	4	0	61	2.53
		1681-021	ScrewTrap	--	1	154	39.42
1250-087	1250-081	1308-079	4	2	0	52	1.50
		1308-086	4	2	0	60	2.51
		1308-087	4	2	0	55	1.85
		1309-018	4	4	0	50	1.35
		1679-054	ScrewTrap	--	1	139	35.89
1250-074	1250-064	1308-002	7	1	0	53	2.48
		1308-068	4	2	0	51	1.44
		1309-016	4	4	0	55	1.63
		1309-026	4	4	0	54	1.67
1250-058	1250-048	1308-080	4	2	0	61	2.17
		1308-084	4	2	0	55	2.05
		1679-023	ScrewTrap	--	1	108	13.79
1250-069	1250-059	1308-094	4	2	0	55	1.05
		1309-089	3	3	0	53	2.02
		1679-072	ScrewTrap	--	1	131	21.22
1250-073	1250-063	1308-078	4	2	0	53	1.77
		1308-082	4	2	0	55	2.11
		1308-085	4	2	0	48	1.41
1250-086	1250-080	1308-088	4	2	0	50	1.51
		1309-009	4	4	0	53	1.83
		1680-047	ScrewTrap	--	1	120	15.58
1250-075	1250-065	1308-073	4	2	0	55	2.11
		1308-077	4	2	0	48	1.30
1151-084	1151-078	1310-100	Pond	Series3	0	55	1.86
		1311-004	Pond	Series3	0	50	1.03
1151-092	1151-086	1309-036	Pond	Series3	0	62	2.97
		1311-007	Pond	Series3	0	55	2.19
1151-092	1151-087	1311-003	Pond	Series3	0	61	2.82
		1311-013	Pond	Series3	0	56	1.92

Table 1. (*continued*)

Brood stock		Assigned offspring					
Father	Mother	ID	Stratum	Site	Age	Length (mm)	Mass (g)
1151-095	1151-089	1311-005	Pond	Series3	0	55	1.73
		1311-006	Pond	Series3	0	50	1.40
1157-005	1151-099	1311-011	Pond	Series3	0	55	2.13
		1311-015	Pond	Series3	0	50	1.71
1250-049	1250-039	1308-048	7	3	0	42	0.76
		1308-052	7	3	0	40	0.60
1250-056	1250-046	1309-008	4	4	0	50	1.32
		1681-031	ScrewTrap	--	1	91	6.81
1250-076	1250-066	1308-093	4	2	0	47	1.04
		1309-021	4	4	0	51	1.62
1151-081	1151-075	1311-020	Pond	Series3	0	48	1.27
1151-082	1151-076	1309-034	Pond	Series3	0	55	1.88
1151-083	1151-077	1311-008	Pond	Series3	0	57	2.03
1151-093	1151-087	1311-001	Pond	Series3	0	63	2.55
1151-096	1151-090	1311-009	Pond	Series3	0	51	1.53
1157-004	1151-098	1311-016	Pond	Series3	0	53	0.73
1157-006	1151-100	1311-019	Pond	Series3	0	51	1.56
1157-008	1157-001	1311-021	Pond	Series3	0	41	0.78
1151-096	1157-002	1311-009	Pond	Series3	0	51	1.53
1157-008	1157-002	1311-021	Pond	Series3	0	41	0.78
1250-017	1250-007	1308-045	7	3	0	36	0.33
1250-031	1250-021	1308-049	7	3	0	40	0.54
1250-032	1250-022	1308-039	7	3	0	45	0.97
1250-049	1250-040	1308-058	7	3	0	37	0.48
1250-050	1250-040	1308-033	7	3	0	45	0.74
1250-070	1250-060	1308-076	4	2	0	47	1.11
1250-071	1250-061	1309-028	4	4	0	55	1.90
1250-078	1250-068	1308-074	4	2	0	56	2.09
1250-089	1250-083	1308-066	4	2	0	51	1.65
1250-090	1250-091	1308-092	4	2	0	56	1.99
1251-068	1251-058	1317-049	6	1	0	57	1.95
1251-071	1251-061	1318-027	6	2	0	63	3.45
1251-083	1251-073	1318-041	6	2	0	69	3.47
1250-086	1251-081	1309-009	4	4	0	53	1.83

Table 2. Comparison of the number of parent pair/offspring assignments detected by either single year parentage analyses (i.e., assignment of juveniles collected during 2007 electrofishing and 2008 screw trap sampling to 2007 brood stock), or comprehensive parentage analysis (i.e., assignment of all juveniles collected between 2006-2009 to combined 2006-2008 brood stock). Note: Age-2^+ HAT individuals that hatched during 2008 were not detected since they would not have been potentially sampled until the 2010 screw trap operations.

	No. of steelhead genotyped				**Single year analysis**			**Comprehensive analysis**			
		Electrofishing		**Trap**	**No. of ssignments**			**No. of assignments**			
Year	**Brood stock**	**Age-0^+ Juveniles**	**Age-1^+ Smolts**	**Age-1^+ Smolts**	**Age-0^+ Juveniles**	**Age-1^+ Smolts**	**Overall HAT**	**Age-0^+ Juveniles**	**Age-1^+ Smolts**	**Age-2^+ Smolts**	**Overall HAT**
2006	104	349	123	--	57	5	0.131	57	5	4	0.140
2007	174	459	120	67	72	15	0.135	72	20	12	0.161
2008	213[a]	386[b]	0	228	64	6	0.114	64	6	--	0.114
totals:	491	1194	243	295	193	26		193	31	16	

[a] One duplicate individual detected in 2008 brood stock was excluded from parentage analyses.

[b] Includes four age-0^+ steelhead sampled at the West Fork Yankee Fork screw trap in 2008.

Table 3. 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).

		Age-0+ juveniles			Age-1+ juveniles		
Location		**n sampled**	**# HAT assigned**	**% HAT assigned**	**n sampled**	**# HAT assigned**	**% HAT assigned**
Stratum 1	Site 1	14	0	0	0	0	0
	Site 2	14	0	0	0	0	0
	Site 3	13	0	0	0	0	0
Stratum 2	Site 1	24	0	0	0	0	0
	Site 2	9	0	0	0	0	0
	Site 3	21	0	0	0	0	0
Stratum 3	Site 1	23	0	0	0	0	0
	Site 2	28	0	0	0	0	0
	Site 3	24	1	4	0	0	0
Stratum 4	Site 1	26	0	0	0	0	0
	Site 2	25	22	88	0	0	0
	Site 3	--	--	--	0	0	0
	Site 4	25	10	40	0	0	0
Stratum 5	Site 1	--	--	--	--	--	--
	Site 12M	--	--	--	--	--	--
Stratum 6	Site 1	25	1	4	0	0	0
	Site 2	25	2	8	0	0	0
	Site 3	25	0	0	0	0	0
Stratum 7	Site 1	4	1	25	0	0	0
	Site 2	--	--	--	--	--	--
	Site 3	14	7	50	0	0	0
Pond	Series 1	19	0	0	0	0	0
Pond	Series 3	24	20	83	0	0	0
Screw Trap		4[a]	0	0	228	6	3
Overall by age-class		386	64	17[b]	228	6	3[b]

[a] Age-0+ steelhead sampled at the West Fork Yankee Fork screw trap in 2008.

[b] These values are the % HAT assigned by age-class.

Table 4. Descriptive genetic statistics for brood stock and juvenile samples collected during 2008-09. 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, $\mathbf{H_E}$ is Nei's (1978) unbiased estimate of expected heterozygosity, $\mathbf{H_O}$ is observed heterozygosity, and $\mathbf{F_{IS}}$ is the index of inbreeding (Weir and Cockerham 1984). Significant deviation (Bonferroni corrected; α=0.00294) from HWE is indicated by an asterisk.

	Sawtooth Hatchery Brood stock (n=213)							Assigned HAT progeny (n=70)						
Locus	n	A	AP	AR	H_E	H_O	F_{IS}	n	A	AP	AR	H_E	H_O	F_{IS}
Ocl1	213	14	1	11	0.883	0.873	0.011	70	12	0	11	0.885	0.871	0.016
Ogo3	213	6	0	4	0.712	0.718	-0.008	70	4	0	4	0.700	0.629	0.103
Ogo4	213	9	0	8	0.828	0.808	0.025	70	9	0	9	0.833	0.857	-0.03
Oke4	213	10	0	8	0.831	0.850	-0.023	70	9	0	8	0.843	0.829	0.017
Oki23	213	15	1	10	0.818	0.850	-0.038	70	12	0	9	0.792	0.686	0.135
Omy1001	213	21	1	15	0.915	0.920	-0.006	70	18	0	14	0.909	0.857	0.057
Omy1011	213	16	2	12	0.889	0.892	-0.003	70	12	0	11	0.901	0.900	0.001
Omy77	211	18	0	12	0.865	0.616	*0.288	69	17	0	13	0.847	0.551	*0.351
Omy7i	213	12	1	10	0.802	0.817	-0.018	70	10	0	9	0.778	0.700	0.101
One14	213	9	0	6	0.675	0.559	0.172	70	6	0	5	0.703	0.629	0.107
Ots1	210	12	1	9	0.848	0.667	*0.214	68	11	0	10	0.862	0.706	*0.182
Ots100	213	12	0	9	0.790	0.826	-0.045	70	11	0	9	0.826	0.757	0.083
Ots3	213	9	2	5	0.688	0.662	0.037	70	6	0	5	0.681	0.700	-0.028
Ots4	213	6	0	5	0.775	0.784	-0.011	70	5	0	5	0.753	0.814	-0.082
Ssa289	213	7	0	4	0.423	0.432	-0.022	70	3	0	3	0.494	0.571	-0.159
Ssa407	212	20	1	13	0.873	0.882	-0.010	70	15	0	12	0.896	0.914	-0.021
Ssa408	212	17	0	13	0.904	0.901	0.003	70	16	0	12	0.885	0.900	-0.017

Table 4. (*continued*)

	Stratum #1 -Juveniles (n=41)							Stratum #2 -Juveniles (n=54)						
Locus	n	A	AP	AR	H_E	H_O	F_{IS}	n	A	AP	AR	H_E	H_O	F_{IS}
Ocl1	41	12	0	11	0.863	0.854	0.011	54	12	0	11	0.884	0.907	-0.026
Ogo3	41	4	0	4	0.644	0.732	-0.138	54	4	0	4	0.629	0.537	0.148
Ogo4	41	10	0	9	0.865	0.854	0.014	54	9	0	8	0.807	0.889	-0.102
Oke4	41	9	0	8	0.829	0.854	-0.030	54	9	0	8	0.831	0.889	-0.071
Oki23	41	11	0	10	0.810	0.780	0.037	54	13	1	10	0.798	0.833	-0.044
Omy1001	41	17	0	15	0.922	0.854	0.075	54	16	0	13	0.907	0.926	-0.022
Omy1011	41	11	0	10	0.875	0.927	-0.060	54	13	1	11	0.882	0.870	0.013
Omy77	40	13	0	11	0.872	0.675	0.228	54	14	0	12	0.890	0.519	*0.420
Omy7i	41	10	0	9	0.771	0.829	-0.076	54	10	0	9	0.749	0.704	0.062
One14	41	6	0	6	0.678	0.659	0.029	53	7	0	6	0.647	0.604	0.068
Ots1	41	8	0	8	0.862	0.610	*0.295	54	10	0	8	0.822	0.648	0.213
Ots100	41	9	0	9	0.824	0.732	0.113	54	11	1	9	0.819	0.907	-0.109
Ots3	41	6	0	5	0.679	0.463	*0.320	54	4	0	4	0.736	0.704	0.044
Ots4	41	5	0	5	0.757	0.902	-0.195	54	6	0	5	0.762	0.667	0.126
Ssa289	41	5	0	4	0.476	0.488	-0.024	54	6	0	5	0.614	0.519	0.157
Ssa407	41	13	0	11	0.854	0.854	0.000	54	16	0	13	0.865	0.852	0.015
Ssa408	41	17	0	15	0.925	0.902	0.024	54	15	0	13	0.908	0.907	0.001

	Stratum #3 -Juveniles (n=74)							Stratum #4 -Juveniles (n=43)						
Locus	n	A	AP	AR	H_E	H_O	F_{IS}	n	A	AP	AR	H_E	H_O	F_{IS}
Ocl1	74	12	0	11	0.878	0.811	0.077	43	9	0	8	0.728	0.767	-0.055
Ogo3	74	6	0	5	0.682	0.716	-0.050	43	4	0	4	0.734	0.698	0.050
Ogo4	74	10	0	9	0.816	0.770	0.056	43	10	0	9	0.838	0.884	-0.055
Oke4	74	10	0	9	0.807	0.757	0.063	43	8	0	8	0.843	0.744	0.118
Oki23	74	10	0	9	0.786	0.797	-0.015	43	10	0	8	0.783	0.791	-0.010
Omy1001	74	16	2	13	0.908	0.932	-0.028	43	14	0	13	0.912	0.953	-0.046
Omy1011	74	12	0	11	0.890	0.851	0.043	43	13	0	12	0.886	0.930	-0.051
Omy77	74	13	0	10	0.866	0.784	0.096	43	13	0	11	0.862	0.628	*0.274
Omy7i	74	11	0	9	0.740	0.730	0.013	43	8	0	7	0.708	0.628	0.114
One14	74	7	0	6	0.743	0.527	*0.292	43	6	0	6	0.734	0.605	0.177
Ots1	74	11	0	10	0.864	0.797	0.078	43	10	0	9	0.841	0.698	0.172
Ots100	74	12	2	10	0.858	0.865	-0.008	41	10	1	8	0.797	0.854	-0.072
Ots3	74	5	0	4	0.655	0.649	0.009	43	5	0	5	0.683	0.767	-0.125
Ots4	74	6	0	5	0.758	0.811	-0.071	43	5	0	5	0.750	0.605	*0.195
Ssa289	74	6	0	5	0.603	0.514	0.150	43	4	0	4	0.413	0.395	0.043
Ssa407	74	15	0	12	0.887	0.959	-0.082	43	11	0	9	0.803	0.837	-0.043
Ssa408	74	15	0	12	0.902	0.973	-0.079	43	14	0	13	0.900	0.860	0.044

Table 4. (*continued*)

	Stratum #6 -Juveniles (n=72)							Pond, Series 1 and 3 combined -Juveniles (n=25)						
Locus	n	A	AP	AR	H_E	H_O	F_{IS}	n	A	AP	AR	H_E	H_O	F_{IS}
Ocl1	72	11	0	9	0.862	0.875	-0.015	25	10	1	10	0.865	0.720	0.171
Ogo3	72	5	0	5	0.677	0.625	0.078	25	3	0	3	0.631	0.480	0.243
Ogo4	72	9	0	9	0.849	0.833	0.018	25	9	0	9	0.843	0.840	0.004
Oke4	72	9	0	7	0.751	0.764	-0.017	25	8	0	8	0.826	0.840	-0.017
Oki23	72	12	0	9	0.799	0.764	0.044	24	8	0	8	0.816	0.750	0.082
Omy1001	72	17	0	13	0.913	0.986	-0.081	25	13	0	13	0.880	0.920	-0.046
Omy1011	72	12	0	10	0.837	0.847	-0.013	25	12	0	12	0.860	0.720	0.166
Omy77	72	13	0	10	0.873	0.597	*0.317	25	12	0	12	0.873	0.720	*0.178
Omy7i	72	11	0	9	0.780	0.778	0.003	25	10	0	10	0.742	0.760	-0.025
One14	72	7	0	7	0.771	0.736	0.046	25	5	0	5	0.629	0.680	-0.082
Ots1	72	10	0	9	0.838	0.722	0.139	24	9	0	9	0.874	0.750	0.145
Ots100	72	10	0	9	0.756	0.708	0.064	25	11	0	11	0.868	0.720	0.173
Ots3	72	6	0	6	0.711	0.722	-0.016	25	5	0	5	0.717	0.720	-0.005
Ots4	72	6	0	6	0.720	0.653	0.094	25	6	0	6	0.748	0.640	0.147
Ssa289	72	4	0	4	0.445	0.514	-0.156	25	5	0	5	0.513	0.320	0.382
Ssa407	72	13	0	11	0.837	0.861	-0.029	25	10	0	10	0.841	0.920	-0.096
Ssa408	72	17	0	14	0.902	0.875	0.030	25	12	0	12	0.903	0.880	0.026

Table 5. Pair-wise F_{ST} comparisons among 2008 Yankee Fork steelhead trout sample collections. Pair-wise F_{ST} estimates are recorded in the top half-matrix, and Bonferroni adjusted (α=0.0018; Rice 1989) significance is shown in the lower half-matrix.

		Putative NOR juveniles sampled in strata					
F_{ST}	Brood stock	#1	#2	#3	#4	#6	Pond
Stratum #1	0.006	--					
Stratum #2	0.006	0.002	--				
Stratum #3	0.009	0.007	0.007	--			
Stratum #4	0.011	0.008	0.015	0.011	--		
Stratum #6	0.012	0.005	0.011	0.014	0.014	--	
Pond	0.004	0.000	0.000	0.007	0.014	0.012	--
Assigned HAT	0.002	0.011	0.007	0.009	0.018	0.016	0.004
P -value							
Stratum #1	***	--					
Stratum #2	***	0.118	--				
Stratum #3	***	***	***	--			
Stratum #4	***	0.002	***	***	--		
Stratum #6	***	0.010	***	***	***	--	
Pond	0.014	0.374	0.388	***	0.004	***	--
Assigned HAT	0.012	***	***	***	***	***	0.052

Appendix 1. Results of comprehensive parentage assignment reported from parent perspective. The pool of potential parents was composed of all Sawtooth Hatchery brood stock pairs used to supply eggs outplanted into Yankee Fork upwellers from 2006 to 2008. An asterisk (*) under the column "Comp" denotes individuals that were subsequently sampled in later brood years.

Brood stock		Assigned offspring							Brood
Father	Mother	ID	Stratum	Site	Age	Length (mm)	Mass (g)	Comp	Year
643-032	643-067	001-079	2	3	0	64	2.39		2006
		1309-027	4	4	1	180	78.40	*	2006
		649-094	4	2	0	53	2.13		2006
		712-015	4	2	0	54	1.83		2006
645-054	645-096	001-025	1	2	0	55	1.58		2006
		649-092	4	2	0	40	0.70		2006
		649-096	4	2	0	45	0.84		2006
		712-052	4	2	0	52	1.21		2006
645-026	645-098	001-054	2	1	0	57	1.71		2006
		001-088	2	3	0	57	1.79		2006
		710-001	2	2	0	56	2.04		2006
		710-005	2	2	0	56	1.89		2006
643-016	643-071	649-098	4	2	0	45	0.95		2006
		712-020	4	2	0	54	1.41		2006
		712-036	4	2	0	50	1.10		2006
643-005	643-072	712-027	4	2	0	55	1.67		2006
		712-055	4	2	0	51	1.28		2006
		987-020	7	2	1	120	17.09		2006
643-012	643-086	001-064	2	1	0	61	2.17		2006
		649-089	4	2	0	57	2.20		2006
		712-072	4	2	0	54	1.84		2006
645-035	645-087	002-081	3	3	0	63	2.20		2006
		649-093	4	2	0	50	1.24		2006
		712-054	4	2	0	47	1.13		2006
643-020	643-059	001-059	2	1	0	58	1.89		2006
		712-053	4	2	0	53	1.68		2006
643-021	643-074	1308-090	4	2	1	205	130.30	*	2006
		994-026	2	3	1	130	19.91		2006
643-009	643-080	649-095	4	2	0	55	1.73		2006
		712-019	4	2	0	50	1.29		2006
643-003	643-081	712-011	4	2	0	49	1.22		2006
		712-063	4	2	0	58	2.16		2006
645-039	645-071	001-090	2	3	0	53	1.58		2006
		991-044	4	2	1	115	15.77		2006
645-021	645-072	001-096	2	3	0	40	0.64		2006
		712-066	4	2	0	52	1.81		2006
645-022	645-073	001-042	1	2	0	55	1.54		2006
		712-014	4	2	0	46	0.92		2006
645-023	645-078	712-039	4	2	0	55	1.50		2006
		712-046	4	2	0	44	0.98		2006
645-061	645-086	001-072	2	3	0	58	1.83		2006
		712-071	4	2	0	59	2.12		2006
645-033	645-094	001-016	1	2	0	59	2.38		2006
		649-100	4	2	0	49	0.87		2006

Appendix 1. (continued)

Brood stock		Assigned offspring							Brood
Father	Mother	ID	Stratum	Site	Age	Length (mm)	Mass (g)	Comp	Year
645-043	645-099	712-029	4	2	0	50	1.31		2006
		712-056	4	2	0	51	1.22		2006
645-030	646-002	001-065	2	1	0	59	1.95		2006
		986-001	5	2	1	122	18.20		2006
645-027	646-007	1070-057	1	3	1	108	14.29		2006
		649-090	4	2	0	42	0.75		2006
643-006	643-061	002-019	3	1	0	64	3.02		2006
643-024	643-064	712-070	4	2	0	57	2.36		2006
643-008	643-085	712-031	4	2	0	57	2.08		2006
643-018	645-016	649-099	4	2	0	55	1.96		2006
645-040	645-067	712-042	4	2	0	53	1.81		2006
645-064	645-069	001-062	2	1	0	62	2.01		2006
645-044	645-074	001-092	2	3	0	57	1.80		2006
645-059	645-075	001-089	2	3	0	55	1.63		2006
645-049	645-080	712-033	4	2	0	58	1.93		2006
645-056	645-081	1309-023	4	4	1	181	61.99	*	2006
645-019	645-088	712-048	4	2	0	47	1.14		2006
645-057	645-095	WFST.08-063	ScrewTrap	--	2	179	68.85	*	2006
645-036	645-100	712-017	4	2	0	47	0.93		2006
645-025	646-005	712-012	4	2	0	50	1.21		2006
645-050	646-012	649-097	4	2	0	43	0.75		2006
889-018	889-010	1308-036	7	3	1	92	7.77		2007
		1308-040	7	3	1	84	6.93		2007
		1308-056	7	3	1	83	5.73		2007
		1680-027	ScrewTrap	--	2	191	79.22	*	2007
		1680-034	ScrewTrap	--	2	163	48.21	*	2007
		987-029	7	3	0	50	0.14		2007
		987-038	7	3	0	53	0.59		2007
		991-024	7	3	0	54	1.67		2007
		991-032	7	3	0	42	0.64		2007
889-051	889-044	1308-037	7	3	1	93	9.24		2007
		987-054	7	3	0	52	1.51		2007
		987-068	7	3	0	47	1.05		2007
		991-021	7	3	0	48	1.12		2007
		991-030	7	3	0	50	1.25		2007
		995-095	6	3	0	65	2.92		2007
889-036	889-028	1681-008	ScrewTrap	--	2	170	42.82	*	2007
		987-036	7	3	0	42	0.66		2007
		987-060	7	3	0	54	1.60		2007
		987-062	7	3	0	48	1.08		2007
		987-066	7	3	0	45	0.86		2007
889-053	889-047	1308-011	7	2	1	104	19.36		2007
		1308-057	7	3	1	80	6.58	*	2007
		1681-033	ScrewTrap	--	2	155	33.52	*	2007
		987-077	7	3	0	49	1.04		2007
		987-093	7	3	0	46	0.70		2007
889-021	889-013	1308-060	7	3	1	75	5.10	*	2007
		987-058	7	3	0	47	1.34		2007
		987-074	7	3	0	51	1.78		2007
		987-081	7	3	0	49	1.26		2007

Appendix 1. (continued)

Brood stock		Assigned offspring							Brood
Father	Mother	ID	Stratum	Site	Age	Length (mm)	Mass (g)	Comp	Year
889-097	889-088	1318-035	6	2	1	104	12.32		2007
		987-035	7	3	0	39	0.50		2007
		987-050	7	3	0	45	0.92		2007
		987-079	7	3	0	44	0.65		2007
711-013	711-005	1308-055	7	3	1	87	9.18		2007
		987-025	7	3	0	37	0.55		2007
		994-089	2	2	0	51	1.87		2007
711-060	711-052	986-006	5	12M	0	45	1.02		2007
		987-040	7	3	0	50	1.39		2007
		987-072	7	3	0	47	0.82		2007
888-089	888-081	1308-032	7	3	1	94	11.00		2007
		1308-041	7	3	1	67	3.10	*	2007
		987-056	7	3	0	45	1.11		2007
889-001	888-093	1308-013	7	2	1	84	7.36		2007
		987-048	7	3	0	45	0.94		2007
		995-045	6	2	0	57	2.22		2007
889-002	888-094	1679-094	ScrewTrap	--	2	183	75.02	*	2007
		991-008	7	3	0	42	0.77		2007
		995-060	6	2	0	61	3.03		2007
889-020	889-012	1679-100	ScrewTrap	--	2	173	58.94	*	2007
		987-075	7	3	0	49	1.09		2007
		987-098	7	3	0	50	1.06		2007
889-022	889-014	987-021	7	3	0	45	0.76		2007
		991-012	7	3	0	49	1.00		2007
		991-015	7	3	0	52	1.24		2007
711-029	711-020	1681-015	ScrewTrap	--	2	166	45.43	*	2007
		987-033	7	3	0	48	1.12		2007
711-044	711-036	1308-015	7	2	1	103	15.78		2007
		987-089	7	3	0	49	1.16		2007
711-049	711-040	1679-052	ScrewTrap	--	2	169	59.56	*	2007
		1680-058	ScrewTrap	--	1	185	62.20	*	2007
888-088	888-079	991-038	7	3	0	47	1.31		2007
		991-040	7	3	0	45	0.92		2007
888-091	888-083	1308-061	5	12M	1	93	8.56		2007
		991-034	7	3	0	34	0.35		2007
888-092	888-084	986-003	5	12M	0	49	1.33		2007
		991-014	7	3	0	45	0.76		2007
889-005	888-098	987-087	7	3	0	49	1.04		2007
		987-091	7	3	0	53	1.41		2007
889-023	889-015	1308-053	7	3	1	93	10.25		2007
		987-046	7	3	0	49	1.46		2007
889-025	889-017	987-070	7	3	0	44	0.70		2007
		991-002	7	3	0	43	0.72		2007
889-037	889-029	1681-027	ScrewTrap	--	2	152	35.32	*	2007
		987-100	7	3	0	53	1.41		2007
889-038	889-030	995-041	6	2	0	60	2.76		2007
		995-053	6	2	0	60	2.74		2007
889-039	889-031	986-005	5	12M	0	44	0.80		2007
		991-043	4	2	0	61	2.93		2007

Appendix 1. (continued)

Brood stock		Assigned offspring							Brood
Father	Mother	ID	Stratum	Site	Age	Length (mm)	Mass (g)	Comp	Year
889-041	889-033	1308-054	7	3	1	78	7.04	*	2007
		987-031	7	3	0	53	1.49		2007
889-050	889-043	987-083	7	3	0	51	1.30		2007
		991-017	7	3	0	49	1.24		2007
889-054	889-046	1308-038	7	3	1	90	9.10		2007
		1679-014	ScrewTrap	--	2	130	24.27	*	2007
889-099	889-090	991-026	7	3	0	46	1.10		2007
		991-028	7	3	0	40	0.64		2007
711-002	889-094	1308-043	7	3	1	85	7.52		2007
		1317-075	6	1	1	125	18.85		2007
711-047	711-004	995-057	6	2	0	56	2.00		2007
711-048	711-041	991-042	7	3	0	40	0.57		2007
711-064	711-056	991-019	7	3	0	43	0.77		2007
711-067	711-059	1308-030	7	3	1	90	8.83		2007
711-067	711-059	1680-028	ScrewTrap	--	2	195	85.25	*	2007
711-085	711-086	991-006	7	3	0	44	0.81		2007
888-085	888-077	991-004	7	3	0	46	0.46		2007
888-086	888-078	987-051	7	3	0	44	0.80		2007
889-004	888-096	987-043	7	3	0	45	0.82		2007
889-007	888-099	1308-046	7	3	1	75	4.70	*	2007
889-009	889-008	991-010	7	3	0	50	1.38		2007
889-034	889-026	987-085	7	3	0	50	1.27		2007
889-035	889-027	987-095	7	3	0	45	0.91		2007
889-052	889-045	987-023	7	3	0	47	0.97		2007
889-096	889-089	987-042	7	3	0	44	0.77		2007
889-098	889-091	987-027	7	3	0	43	0.66		2007
711-001	889-092	987-064	7	3	0	46	0.93		2007
1250-055	1250-045	1308-067	4	2	0	58	2.25		2008
		1308-069	4	2	0	62	2.56		2008
		1308-071	4	2	0	55	1.88		2008
		1308-095	4	2	0	65	2.83		2008
		1309-017	4	4	0	59	2.33		2008
		1309-022	4	4	0	61	2.53		2008
		1681-021	ScrewTrap	--	1	154	39.42		2008
1250-087	1250-081	1308-079	4	2	0	52	1.50		2008
		1308-086	4	2	0	60	2.51		2008
		1308-087	4	2	0	55	1.85		2008
		1309-018	4	4	0	50	1.35		2008
		1679-054	ScrewTrap	--	1	139	35.89		2008
1250-074	1250-064	1308-002	7	1	0	53	2.48		2008
		1308-068	4	2	0	51	1.44		2008
		1309-016	4	4	0	55	1.63		2008
		1309-026	4	4	0	54	1.67		2008
1250-075	1250-065	1308-073	4	2	0	55	2.11		2008
		1308-077	4	2	0	48	1.30		2008
		1309-014	4	4	0	58	2.32		2008
		1680-086	ScrewTrap	--	1	130	24.26		2008

Appendix 1. (continued)

Brood stock		Assigned offspring							Brood
Father	Mother	ID	Stratum	Site	Age	Length (mm)	Mass (g)	Comp	Year
1250-058	1250-048	1308-080	4	2	0	61	2.17		2008
		1308-084	4	2	0	55	2.05		2008
		1679-023	ScrewTrap	--	1	108	13.79		2008
1250-069	1250-059	1308-094	4	2	0	55	1.05		2008
		1309-089	3	3	0	53	2.02		2008
		1679-072	ScrewTrap	--	1	131	21.22		2008
1250-073	1250-063	1308-078	4	2	0	53	1.77		2008
		1308-082	4	2	0	55	2.11		2008
		1308-085	4	2	0	48	1.41		2008
1250-086	1250-080	1308-088	4	2	0	50	1.51		2008
		1309-009	4	4	0	53	1.83		2008
		1680-047	ScrewTrap	--	1	120	15.58		2008
1151-084	1151-078	1310-100	Pond	Series3	0	55	1.86		2008
		1311-004	Pond	Series3	0	50	1.03		2008
1151-092	1151-086	1309-036	Pond	Series3	0	62	2.97		2008
		1311-007	Pond	Series3	0	55	2.19		2008
1151-092	1151-087	1311-003	Pond	Series3	0	61	2.82		2008
		1311-013	Pond	Series3	0	56	1.92		2008
1151-095	1151-089	1311-005	Pond	Series3	0	55	1.73		2008
		1311-006	Pond	Series3	0	50	1.40		2008
1157-005	1151-099	1311-011	Pond	Series3	0	55	2.13		2008
		1311-015	Pond	Series3	0	50	1.71		2008
1250-049	1250-039	1308-048	7	3	0	42	0.76		2008
		1308-052	7	3	0	40	0.60		2008
1250-049	1250-040	1308-051	7	3	0	45	0.55		2008
		1308-058	7	3	0	37	0.48		2008
1250-056	1250-046	1309-008	4	4	0	50	1.32		2008
		1681-031	ScrewTrap	--	1	91	6.81		2008
1250-076	1250-066	1308-093	4	2	0	47	1.04		2008
		1309-021	4	4	0	51	1.62		2008
1151-081	1151-075	1311-020	Pond	Series3	0	48	1.27		2008
1151-082	1151-076	1309-034	Pond	Series3	0	55	1.88		2008
1151-083	1151-077	1311-008	Pond	Series3	0	57	2.03		2008
1151-093	1151-087	1311-001	Pond	Series3	0	63	2.55		2008
1151-096	1151-090	1311-009	Pond	Series3	0	51	1.53		2008
1157-004	1151-098	1311-016	Pond	Series3	0	53	0.73		2008
1157-006	1151-100	1311-019	Pond	Series3	0	51	1.56		2008
1157-008	1157-002	1311-021	Pond	Series3	0	41	0.78		2008
1250-017	1250-007	1308-045	7	3	0	36	0.33		2008
1250-031	1250-021	1308-049	7	3	0	40	0.54		2008
1250-032	1250-022	1308-039	7	3	0	45	0.97		2008
1250-050	1250-040	1308-033	7	3	0	45	0.74		2008
1250-070	1250-060	1308-076	4	2	0	47	1.11		2008
1250-071	1250-061	1309-028	4	4	0	55	1.90		2008
1250-078	1250-068	1308-074	4	2	0	56	2.09		2008
1250-089	1250-083	1308-066	4	2	0	51	1.65		2008
1250-090	1250-091	1308-092	4	2	0	56	1.99		2008
1251-068	1251-058	1317-049	6	1	0	57	1.95		2008
1251-071	1251-061	1318-027	6	2	0	63	3.45		2008
1251-083	1251-073	1318-041	6	2	0	69	3.47		2008