PROGRESS REPORT

FISH RESEARCH PROJECT
OREGON

PROJECT TITLE: Lower Snake River Compensation Plan: Oregon Evaluation Studies - Steelhead Life History Characterization; Genetic Characterization; Kelt Reconditioning.

CONTRACT NUMBER: 1448-14110-97-J039 (1997-1998)
1448-14110-98-J058 (1998-1999)
141109J041 (1999-2000)
14110-0-J048 (2000-2001)

PROJECT PERIOD: 1 April 1997 to 31 March 2001

Prepared by: James R. Ruzycki
Michael W. Flesher
Richard W. Carmichael
Debra L. Eddy

Oregon Department of Fish and Wildlife
2501 SW First Street
P.O. Box 59
Portland, OR 97207

This project was financed by the U.S. Fish and Wildlife Service under the Lower Snake River Compensation Plan.

PREFACE

The purpose of this progress report is to provide summary information for aspects of the summer steelhead research, Lower Snake River Compensation Plan (LSRCP) program operated by the Oregon Department of Fish and Wildlife (ODFW) in the Grande Ronde and Imnaha river subbasins during 1997-2001. This ongoing monitoring program provides technical, logistical, and biological information to managers charged with maintaining viable steelhead populations and associated fisheries in Northeast Oregon. The information provided here is for natural production and life-history studies of summer steelhead only. This report is submitted to fulfill the reporting requirements for three objectives from four contract years (1997/98; 1998/99; 1999/2000; 2000/01). Included are objective nine from 1997/98 and 1998/99, objective eight (sub-objectives 8.1-8.3) from 1999/2000, and objective eight (sub-objectives 8.1-8.4) from 2000/01. These objectives include studies of the relationship between anadromous and resident forms of *Oncorhynchus mykiss* in NE Oregon, genetic characterization of *O. mykiss* in the Grande Ronde and Imnaha river subbasins, and kelt reconditioning for steelhead broodstock development in the Grande Ronde River subbasin. Fish culture monitoring, survival, compensation and program goals, and endangered species activities from these study years will be reported elsewhere. The report is organized into three sections (Life-History Characterization; Genetic Characterization; and Kelt Reconditioning) reflecting the aforementioned objectives.

ACKNOWLEDGMENTS

We thank Greg Davis and Mike Gribble for allowing liberal use of hatchery facilities, for rearing eggs and juveniles, and for providing much needed technical assistance. Bob Jones, Wade Bergeson, and Paul Aasrud provided additional technical assistance at hatchery facilities. Tim Whitesel was responsible for initiating much of this work. Eric Volk provided technical assistance and analytical analysis of otoliths. We thank Debra Barkow, Katheryn Frenyea, Tracy Albert, Pat Keniry, and Laura Meyers, for help in sample and data collection and Mary Edwards, Karen Reinhart, Mike Parrish, Vince Tranquilli, Chad Aschenbrenner, and Shawn Montgomery for their insight and persistence in the daily hand feeding and caring of steelhead kelts. We also thank pathologists Warren Groberg and Sam Onjukka for treatment protocols and examinations. This project was funded by the U.S. Fish and Wildlife Service under the Lower Snake River Compensation Plan, contract numbers 1448-14110-97-J039, 1448-14110-98-J058, 141109J041, and 14110-0-J048, a cooperative agreement with the Oregon Department of Fish and Wildlife.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

EXECUTIVE SUMMARY

Objectives

1. Monitor the life history characteristics of *Oncorhynchus mykiss* in NE Oregon.
    a. Determine the relationship between anadromous and resident forms of *O. mykiss* in NE Oregon.
    b. Monitor the genetic characterization of *O. mykiss* from various streams in the Grande Ronde and Imnaha River basins.
    c. Explore the possibility of using reconditioned steelhead kelts for the artificial production of anadromous juveniles.

Accomplishments and Findings

Results from our breeding experiments suggest that resident *O. mykiss* can produce progeny that smolt and emigrate from their freshwater habitat. Preliminary microchemistry analysis of otoliths from wild fish indicated the efficacy of the technique for discerning freshwater and marine growth histories. This indicates that the microchemistry technique can be successfully used to discern the maternal parentage, and aspects of the life-history strategies of *O. mykiss* in the Grande Ronde River basin. We collected 5,239 total genetic samples during the initial three years of our four-year collection period to determine the genetic characterization of steelhead in the Grande Ronde and Imnaha river subbasins. These samples were distributed throughout the subbasins and we were able to collect at least 50, age-0 *O. mykiss* for genetic samples each year in each of the 17 management units that were earlier defined. We successfully reconditioned three of eleven kelts we took into captivity during 1999. Gamete viability of reconditioned kelts was equal to or greater than that of first-year spawning adults.

Management Recommendations

The production of the anadromous life history expression from progeny of resident parents in our experimental groups indicates that managers could produce anadromous individuals from resident parental stock. This suggests that even if anadromous runs of *O. mykiss* were lost from a system, they could be regained through artificial propagation of resident progeny.

Our sampling design to assess the genetic characteristics of steelhead in the Grande Ronde and Imnaha river subbasins should be continued for a fourth year. A four-year sampling design was originally developed by a multi-agency workgroup. Funding was allocated for genetic analysis of our initial three years of samples and this funding should be extended to include the fourth sample year. This data set should then be evaluated and used as the foundation for population identification and artificial propagation management for steelhead in these subbasins.

Kelt reconditioning could be a viable option to aid in recovery efforts of steelhead in the Grande Ronde River basin. Our ability to recondition 3 of 11 kelts to repeat spawning

condition (two other kelts were reconditioned but did not have apparent mature gametes) during 1999-2000, and their high gamete viability, indicates success could be achieved using our methods. To increase success, fungus treatment and water quality attributes would need to be addressed.

STUDY AREA

These studies were conducted in the Grande Ronde and Imnaha River Basins located in Northeast Oregon. Breeding experiments of the steelhead life-history characterization studies were conducted using Wallowa and Little Sheep hatchery stocks and wild fish collected from the Wallowa River and Joseph Creek drainages. Otolith samples were collected from wild fish inhabiting various tributaries of the Grande Ronde River as well as Balm Creek, a tributary to the Powder River. Genetic characterization studies were conducted using samples collected from throughout the Grande Ronde and Imnaha River basins. Kelt reconditioning studies were conducted at the ODFW, Wallowa Fish Hatchery in Enterprise, OR using Wallowa and Little Sheep hatchery stocks as well as wild kelts collected from Deer Creek, a tributary of the Wallowa River.

SECTION I

LIFE-HISTORY CHARACTERIZATION

Introduction

Many salmonids exhibit partial migration: the phenomenon of populations partitioned into migratory and non-migratory individuals (Jonsson and Jonsson 1993). *Oncorhynchus mykiss* exhibit a complex of life-history strategies ranging from residency in small headwater streams to anadromy involving migrations of hundreds of kilometers. In the Grande Ronde River basin of Northeast Oregon, both resident and anadromous life-history forms coexist, and thus populations found there likely exhibit partial migration.

Partial migration may have important consequences for "anadromous" species listed under the Endangered Species Act (ESA). The recent decline of summer steelhead (*Oncorhynchus mykiss*) stocks in the lower Snake River has prompted their listing under the ESA. Declines in steelhead are potentially due to elevated mortality rates associated with anadromous migrations. If resident and anadromous life-history characteristics result from a phenotypically plastic trait (i.e. a genetic trait that is highly variable due to influences from environmental factors), then elevated mortality associated with the anadromous type may be shifting the populations towards residency. Further, although the anadromous expression of the trait may be declining, the trait would not necessarily be lost. Identification of the plasticity of these traits would then be important for the management of these stocks.

We investigated life history traits of *O. mykiss* with studies in both the hatchery and natural environment. We anticipated that these complimentary approaches would allow us to evaluate the relationship between the two life-history forms. They should further allow us to explore the feasibility of using hatcheries to produce anadromous progeny from resident parents if the number of anadromous life-history forms becomes severely depressed.

In the natural environment, we examined the maternal lineage (resident vs. anadromous) of juvenile and adult *O. mykiss* by examining elemental composition of their otoliths. Elemental composition of otoliths has been used to discriminate among stocks and to describe environmental or migratory history of individuals. The maternal, life-history origin (anadromy vs. residency) of individual fish has also been investigated by examining the relative strontium (Sr) to calcium (Ca) composition of various growth regions within otoliths (Kalish 1990, Reiman et al. 1994, Volk et al. 2000, Zimmerman and Reeves 2000). Environmental Sr concentrations in seawater are typically higher than those found in freshwater and Sr is commonly substituted for Ca as otoliths are formed. An individual that inhabits seawater should incorporate a higher concentration of Sr in their otoliths than a fish that resides in freshwater. Migratory fish therefore, incorporate different Sr/Ca ratios in different growth regions of their otoliths according to the chemistry experienced in their variable environment. Females residing in the ocean during egg development transfer this chemistry to their progeny through nutrients stored in eggs. Individual *O. mykiss* in the Grande Ronde River basin should therefore, be identifiable to their maternal life history through examination of their otoliths.

In the hatchery environment, we conducted various crosses among, and between anadromous and resident forms of *O. mykiss* from the Grande Ronde River basin. This work was designed to determine the frequency of production of migratory progeny from various parental crosses. We compared the propensity of emigration by progeny of various life-history crosses by rearing progeny of differing parentage under similar laboratory environments (as part of a hatchery steelhead program), PIT tagging and releasing them, and monitoring their detection at downstream sites. We also monitored progeny for physiological and morphological parameters associated with smoltification.

Our objectives were therefore, to: 1.) Determine the feasibility of using otolith microchemistry to distinguish life history strategies of *O. mykiss* for Northeast Oregon and develop baseline data on Sr/Ca ratios and water concentrations. 2.) Determine the maternal origin of individuals in the Grande Ronde sub-basin exhibiting differing life-history strategies. 3.) Determine the life-history composition of the Grande Ronde *O. mykiss* stock. 4.) Determine the plasticity of life history forms specifically, the ability of resident adults to produce anadromous progeny.

Methods

1997-1998 Otolith Collections

In 1997, we began collecting *O. mykiss* individuals for otolith microchemistry analysis. During this initial year, we collected known, life-history strategy individuals to verify the utility of the microchemistry technique for the Grande Ronde sub-basin. Known, life-history individuals included anadromous adults from hatchery-origin anadromous parents, residualized fish from hatchery-origin anadromous parents, smolts from hatchery-origin anadromous parents, and resident fish from an area blocked to anadromy.

Hatchery-origin adults and juveniles were collected at the weirs and acclimation ponds of the LSRCP collection facilities at Deer Creek (Big Canyon) and Little Sheep Creek (Figure 1). The remaining fish were collected by electrofishing. Residual fish were collected in the vicinity of the Big Canyon Facility and resident fish were collected from Balm Creek, a tributary to the Powder River. Balm Creek was chosen for resident fish sampling because this system has been blocked from anadromy since the construction of the Hell's Canyon dam complex.

Because the otolith microchemistry technique relies on a change in the strontium/calcium ratio between the freshwater and saltwater habitats, we also collected water samples throughout the basin. This sampling was to ensure that our otolith microchemistry comparisons were reliable (i.e. that we could discriminate freshwater from saltwater growth regions within the otoliths). To do this, we collected water samples from all areas where our test fish resided, from additional areas throughout the Grande Ronde River sub-basin, and at hatchery water supplies.

2000 Otolith Collections

Since the analysis of otoliths from fish collected during 1997-98 demonstrated the efficacy of the microchemistry technique, we limited our year 2000 collection efforts to the Grande Ronde River sub-basin. We attempted to represent the life-history

composition of *O. mykiss* within the sub-basin by sampling four life-history stages from four regions within the basin. The four life-history stages included age 0 juveniles, emigrating smolts, resident adults, and anadromous adults. The four primary sampling areas included the Upper Grande Ronde River, Catherine Creek, Lostine River, and Lookingglass Creek. In addition, we also collected fish from Beaver Creek (Upper Grande Ronde) that was isolated from anadromy above a barrier dam since the construction of the La Grande Reservoir (Figure 1). For each region, we attempted to represent the spatial distribution of life-history types by collecting fish throughout the drainage basin above the location where we collected smolts in rotary-screw traps (see below). Screw traps therefore, marked the downstream boundary of collection regions. By collecting fish only above our screw trap locations, we gained some assurance that the collected fish were reared within our sample regions.

We collected the four life-history groups using a variety of capture techniques. Wild, anadromous adults were collected opportunistically at weirs operated by Confederated Tribes of the Umatilla Indian Reservation (CTUIR) staff, located above rotary-screw trap sites at the Catherine Cr. and Upper Grande Ronde River locations. Emigrating smolts were captured using rotary screw traps located at the downstream boundary of each sample area. These traps are operated continuously throughout the year when ice and flow conditions permit. To represent the entire spring run of emigrating smolts, we attempted to capture smolts throughout the spring emigration period in numbers proportional to the total number of smolts migrating past the trap in any given week. Only fish that had smolt-like characteristics (slim, fusiform body morphology; deciduous scales; lack of visible parr markings; silver color; and 130-250 mm FL) were collected for otoliths. Resident adults were collected by electrofishing and angling and age 0 fish were collected by electrofishing. We generally used a minimum cut-off length of 180 mm FL for resident adults and a maximum cut-off of 80 mm FL for age 0 fish. Maturity of resident adults was verified after collection by confirming the presence of mature oocytes and spermatozoa (Crim & Glibe 1990). During 2001 we continued to collect otoliths from anadromous adults at our weir sites on Catherine Cr., Deer Cr., and the Upper Grande Ronde River and from age 0 fish in Catherine Cr.

Otolith Microchemistry Analysis

Eric Volk of the Washington Department of Fish & Wildlife (WDFW) analyzed otoliths collected in 1997 for Sr and Ca chemical composition using a wavelength-dispersive elemental, electron microprobe. Otoliths that were dissected from fish and stored in ethanol were sectioned and polished to reveal their core regions and coated with a 250 Å layer of conducting carbon. To determine maternal origin, otolith core chemistry was compared to freshwater growth regions by sampling two points within each region. Elemental chemistry samples corresponding to freshwater growth regions were obtained at a distance of 450 µm from the otolith core. On a sub-sample of otoliths, up to six replicate analyses were conducted to determine the appropriateness of using only two sub-samples. In addition on two otoliths, samples were taken every 25 µm along a transect beginning at the core and ending at a distance of 400 µm from the core region. Water samples were analyzed using inductively coupled plasma-atomic emission spectroscopy (ICP-AES) following EPA method 200.7. Additional details specific to the elemental analysis of otoliths and water chemistry can be found in Volk (1999).

Breeding Experiments

Beginning in 1998 and continuing through 2001, we collected wild resident adult *O. mykiss* from the Grande Ronde River basin and crossbred them with hatchery adults from the ODFW, Wallowa Hatchery stock. Because maturation timing of resident adults was typically later than hatchery fish we were opportunistic as to the source of resident adults for the experiments. Ripe adults of both life histories were crossbred using the following combinations:

1.) Resident or rainbow (Rb) females (F) bred with Rb males (M); RbF x RbM.
2.) RbF bred with anadromous (steelhead) males (StsM); RbF x StsM.
3.) Anadromous (steelhead) females (StsF) bred with RbM; StsF x RbM.
4.) StsF bred with StsM; StsF x StsM.

During 1999, we also bred residualized females (ResF; fish from anadromous parents that exhibited a freshwater life history) with both RbM and StsM.

Eggs and progeny from these crosses were incubated and rose using our standard steelhead production methods. Eggs were stripped from each female, placed in small cups, and fertilized using milt from 1-4 males. All eggs resulting from crosses were placed into divided trays and placed in incubators at the Wallowa Fish Hatchery. After eye-up, non-viable eggs were removed and viable eggs were counted. After approximately 450 daily temperature units (DTU) of incubation, eggs were transported to Irrigon Fish Hatchery for hatching and subsequent rearing. Each progeny type was reared separately, in circular tanks (188 cm diameter, 91 cm deep) at 11-14.5° C, to smolt at an age of one year, and a target release size of 205 mm fork length (FL; 5 fish/lb). Water depth in each tank was 76 cm until fish grew large enough to jump out, then depth was reduced to 30.5 cm. After fish were reared to approximately 60 mm FL, we equalized densities in each tank by culling a random sample of individuals. All fish that were retained were PIT-tagged for subsequent detections.

During rearing, we evaluated smolt development of progeny from each breeding group. During all years smolt morphological development was evaluated using condition factor [(K) where: K = mass (g)/ FL^3 (mm) · 10^4], and during 1998-1999 smolt physiological development was also evaluated using liver-somatic index [(LSI) where: LSI = liver mass / body mass · 100], and gill $Na^+ + K^+$ ATP-ase specific activity (moles P · hr^{-1} · mg $protein^{-1}$). Beginning the January of each release year, randomly selected fish were sacrificed at approximately two-week intervals for smolt development evaluation. Prior to and after PIT-tagging, we periodically measured (FL) individuals from a sub-sample of each breeding group to track their growth history. All fish were also measured (FL) and subsamples were weighed just prior to release.

After approximately one year of growth, PIT-tagged fish were released directly into Deer Creek (a tributary of the Wallowa River that enters at river kilometer 18) at the Big Canyon Facility in early May. We monitored the detection of these PIT-tagged fish at Snake and Columbia River dam facilities. Detections of fish at these facilities were used as indicators of smolt emigration from the Grande Ronde River system. These same dam facilities will also be monitored for the detection of released fish if they return as adults.

Results

1997 Otolith Collections

During 1997, we collected otoliths from 72 anadromous adults returning to our collection facilities. Fifteen were collected from the Big Canyon Facility, 19 from Wallowa Fish Hatchery, and 38 from the Little Sheep Cr. Facility. Of these, three males and two females from Little Sheep and 11 males and seven females from Wallowa Fish Hatchery were delivered for microchemistry analysis. During pre-release sampling, we also collected otoliths from 24 smolts from both Little Sheep and Wallowa Fish Hatchery acclimation ponds. Of these, all samples collected at Wallowa Fish Hatchery were delivered for analysis. Twenty-four residualized hatchery-origin fish were sampled from Deer Creek near the Big Canyon Facility. Otoliths from all of these fish were delivered for analysis. We also delivered otoliths from 25 fish collected in Balm Cr. Water samples collected throughout northeast Oregon during 1999 showed a range of strontium and calcium concentrations (Table 1).

2000 Otolith Collections

We collected more than 600 fish from the Grande Ronde River basin for otolith microchemistry analysis during 2000 (Table 2). In addition, 17 anadromous adults captured in Little Sheep Creek were sampled for otoliths. Samples taken in 2000 have not been analyzed for microchemistry composition. We also dissected otoliths from 52 progeny of known parentage groups from our breeding experiments during 2000. During 2001 we collected 2, 24, and 3 anadromous adults for otolith samples at our weir sites on Catherine Creek, Deer Creek, and the Upper Grande Ronde River, respectively.

Table 1. Sample site location, calcium (Ca) and strontium (Sr) concentrations in µg/l and µg/g, and relative Sr/Ca concentration (Conc., *1000) and atom ratios of water samples taken throughout northeast Oregon. Eric Volk of the Washington Department of Fish & Wildlife performed analyses.

Sample site	Ca (µg/l)	Ca (µg/g)	Sr (µg/l)	Sr (µg/g)	Sr/Ca Conc. ratio	Sr/Ca Atom ratio
Irrigon hatchery	43100	43.10	211.0	0.211	4.90	2.24
Balm Cr.	10200	10.20	142.0	0.142	13.92	6.37
Balm Cr.	4880	4.88	59.5	0.060	12.19	5.58
Balm Cr.	26400	2.64	166.0	0.166	6.29	2.88
Catherine Cr.	6480	6.48	31.2	0.031	4.81	2.20
Lostine River	13800	1.38	49.2	0.049	3.57	1.63
Meadow Cr.	11200	1.12	62.9	0.063	5.62	2.57
Little Sheep Cr.	12400	1.24	47.3	0.047	3.81	1.74
Lookingglass Cr.	6730	6.73	20.3	0.020	3.02	1.38

Table 2. Location of capture, life stage at capture, and number of samples of *O. mykiss* collected for otolith microchemistry analysis in the Grande Ronde River basin during 2000. Life stages (abbreviations) include: age 0, resident adult (res. adult), anadromous adult (anad. adult), unknown, and smolt. Fish from Beaver Creek are termed "resident" because they were captured above a migration barrier to anadromous adults. Samples from Little Sheep Cr. in the Imnaha River basin are also shown.

Subbasin	Sample site	Life stage	Number
Catherine Creek	Rkm 32 (weir)	anad. adult	1
Catherine Creek	various	age 0	60
Catherine Creek	various	res. adult	35
Catherine Creek	Rkm 32 (screw trap)	smolt	50
Upper Grande Ronde	Rkm 304 (weir)	anad. adult	6
Upper Grande Ronde	various	age 0	86
Upper Grande Ronde	various	res. adult	34
Upper Grande Ronde	rkm 299 (screw trap)	smolt	58
Upper Grande Ronde	Beaver Creek	resident	31
Lookingglass Creek	various	age 0	36
Lookingglass Creek	various	res. adult	51
Lookingglass Creek	Rkm 4.8 (screw trap)	smolt	26
Lostine River	various	age 0	45
Lostine River	various	res. adult	35
Lostine River	Rkm 3.4 (screw trap)	smolt	50
Wallowa River	Deer Creek	unknown	14
Joseph Creek	Sumac Creek	unknown	15
Imnaha River	Little Sheep Cr.	anad. adult	17

Otolith Microchemistry Analysis

Preliminary microchemistry analysis of otoliths collected during 1997 indicated the efficacy of the technique for discerning freshwater and marine growth zones for fish collected in the Grande Ronde River subbasin. Analysis of transects along the otoliths radius of two known-origin specimens raised in fresh water indicated that fairly high Sr/Ca ratios existed near the otoliths cores, reflecting anadromous parentage, followed by steep declines. The steep declines are indicative of freshwater residence (Appendix 1). Analysis of the three groups representing fish reared in freshwater but of anadromous, maternal parentage also showed distinct differences in Sr/Ca ratios between otolith core and freshwater growth regions (Appendix 1). Analysis of the group representing fish reared in freshwater blocked from anadromy showed similar Sr/Ca ratios between otolith core and freshwater growth regions, indicating continuous freshwater residence through generations. Additional details of these analyses are provided in Appendix 1 (Volk 1999).

Breeding Experiments

Until 2000 we had difficulty finding sufficient numbers of resident females each year to produce adequate numbers of progeny from these parent crosses. During 1998 we collected and spawned five female and five male resident adults from Deer Cr. with three female and five anadromous adults from the Wallowa hatchery stock. These crosses

resulted in the release of more than 200 progeny from each group except RbF x RbM which died during spawning (Table 3). During 1999 we collected and spawned two female and nine male resident adults from Deer Cr. with two female and four anadromous adults from the Wallowa hatchery stock and two residualized females that were captured at steelhead collection sites. These crosses resulted in the release of more than 350 progeny from each breeding group (Table 3). During 2000 we collected and spawned two female resident adults from Sumac Cr. (a tributary to Joseph Creek), one female resident from Deer Cr., and eight male resident adults from Deer Cr. with four female and four anadromous adults from the Wallowa hatchery stock. These crosses resulted in the release of more than 1,000 total progeny from all breeding groups (Table 3). During 2001 we switched to Prairie Cr. (tributary to the Wallowa River) as a source of resident adults and spawned three resident females and six resident males with two female and four male Wallowa-stock adults. These crosses resulted in the release of more than 3,000 total progeny from all breeding groups (Table 3).

Mean gill $Na^+ + K^+$ ATP-ase activity was more variable among 99-brood-year groups compared to those from the 1998 brood year. ATP-ase activity was generally similar and unchanged among the three groups of progeny (RbF x StsM, StsF x RbM, and StsF x StsM) during 1998 (Figure 2). However, just prior to release in May, ATP-ase activity of StsF x StsM progeny was significantly higher than ATP-ase activity of StsF x RbM progeny. ATP-ase activity of RbF x RbM progeny during 1999 initially declined during February and March but then increased through April and was significantly higher than all other groups just prior to release (Figure 3). In contrast, ATP-ase activity of RbF x StsM, StsF x RbM, and StsF x StsM progeny increased during February and March but then declined during April and early May. We did not measure ATP-ase activity for the 2000 and 2001 brood years.

Liver somatic index values for 1998 brood-year progeny generally increased before release but during 1999, these same groups were generally unchanged (Figure 4). The subset of 1998 brood-year progeny retained after the release date showed greater increases in their LSI values. Of these, StsF x StsM progeny showed the greatest increases and highest values compared to all other groups.

In general, progeny groups that had Rb mothers were more variable in length just prior to release than those from Sts mothers. These progeny groups from Rb mothers had non-normal size-frequency distributions that were strongly skewed to the left during all brood years (Figures 5-7). In contrast, size-frequency distributions of progeny groups from steelhead mothers were less skewed towards smaller individuals. In addition, progeny groups from mixed parentage had distributions that were apparently bimodal including groups of both small and large individuals. When size-frequency distributions of fish released were skewed to the left, larger fish were generally detected at a higher rate than smaller individuals from the same group at mainstem dam facilities. Compared to other release groups, RbF x RbM progeny were often significantly smaller in both length and mass just prior to release during all brood years (Tables 4-7). Except for the 1998 brood year StsF x RbM and StsF x StsM release groups, median size of fish detected at mainstem dam facilities was always greater than median size of fish released from the 1998-2000 brood years (Mann-Whitney Rank Sum tests, Figures 5-7). Condition factor at release also varied between groups with RbF x RbM progeny often having higher condition and StsF x StsM progeny often having lower condition at release (Tables 4-7). Percentage of precocious individuals was always greater for RbF x RbM progeny compared to all other groups for the 2000 and 2001 brood years.

Table 3. Number of PIT-tagged progeny released, number detected at Snake and Columbia River mainstem dam facilities, and detection rate (percentage) from six experimental release groups. Abbreviations for parental origin of release groups include rainbow (Rb, resident origin), steelhead (Sts, anadromous origin), residualized (Res.; fish from anadromous parents that have switched to a freshwater life history), female (F), and male (M). Multiple entries in any release group during a single year indicate replicate groups.

	1998 Brood Year			1999 Brood Year			2000 Brood Year			2001 Brood Year		
Release Group	Number Released	Number Detected	Detection Rate	Number Released	Number Detected	Detection Rate	Number Released	Number Detected	Detection Rate	Number Released	Number Detected	Detection Rate
RbF x RbM	0	--	--	365	24	6.6 %	206	34	16.5 %	347	--	--
										355	--	--
										409	--	--
RbF x StsM	209	79	37.8 %	379	50	13.2 %	87	22	25.3 %	482	--	--
										513	--	--
StsF x RbM	263	127	48.3 %	390	132	33.8 %	206	55	26.7 %	467	--	--
							174	49	28.2 %			
StsF x StsM	290	152	52.4 %	411	148	36.0 %	210	88	41.9 %	494	--	--
	220	112	50.9 %				241	108	44.8 %			
Res. F x RbM	--	--	--	405	75	18.5 %	--	--	--	--	--	--
Res.F x StsM	--	--	--	394	102	25.9 %	--	--	--	--	--	--

Table 4. Median fork length (mm), mass (g), and condition factor (mass • fork length^{-3} •100,000) of the progeny from three breeding groups of the 1998 brood year. All metrics shown were measured on 5 May, 1999. Significant differences among groups are also described. See methods for definitions of abbreviations for parent types.

Body measure	Breeding Group		
	RbF x StsM	StsF x RbM	StsF x StsM
Fork length (mm)	205	201	212[a]
Mass (g)	96.2	93.2	102.5[b]
Condition Factor	1.08	1.14[c]	1.07

[a] Progeny from this group were significantly longer (Kruskal-Wallis One Way ANOVA on Ranks, H = 30.1, 2 df, P < 0.05) than progeny from both other groups.
[b] Progeny from this group were significantly heavier (Kruskal-Wallis One Way ANOVA on Ranks, H = 11.6, 2 df, P < 0.05) than progeny from both other groups.
[c] Progeny from this group had a significantly higher condition factor (Kruskal-Wallis One Way ANOVA on Ranks, H = 212, 2 df, P < 0.05) than progeny from both other groups.

Table 5. Median fork length (mm), mass (g), and condition factor (mass • fork length^{-3} •100,000) of the progeny from six breeding groups of the 1999 brood year. All metrics shown were measured on 1 May, 2000. Significant differences among groups are also described. See methods for definitions of abbreviations for parent types.

Body measure	Breeding Group					
	RbF x RbM	RbF x StsM	StsF x RbM	StsF x StsM	Res. F x RbM	Res. F x StsM
Fork length (mm)	166[a]	200[b]	205	201	208	204
Mass (g)	35.8[c]	75.8	95.7	86.8	100.7	92.8
Condition Factor	1.09	1.15[d]	1.08	1.09	1.06	1.07

[a] Progeny from this group were significantly shorter (Kruskal-Wallis One Way ANOVA on Ranks, H = 289.9, 5 df, P < 0.05) than progeny from all other groups.
[b] Progeny from this group were significantly shorter (Kruskal-Wallis One Way ANOVA on Ranks, H = 289.9, 5 df, P < 0.05) than progeny from the Res. F x RbM group.
[c] Progeny from this group had significantly lower mass (Kruskal-Wallis One Way ANOVA on Ranks, H = 68.2, 5 df, P < 0.05) than progeny from all other groups.
[d] Progeny from this group had a significantly higher condition factor (Kruskal-Wallis One Way ANOVA on Ranks, H = 58.8, 5 df, P < 0.05) than progeny from all other groups.

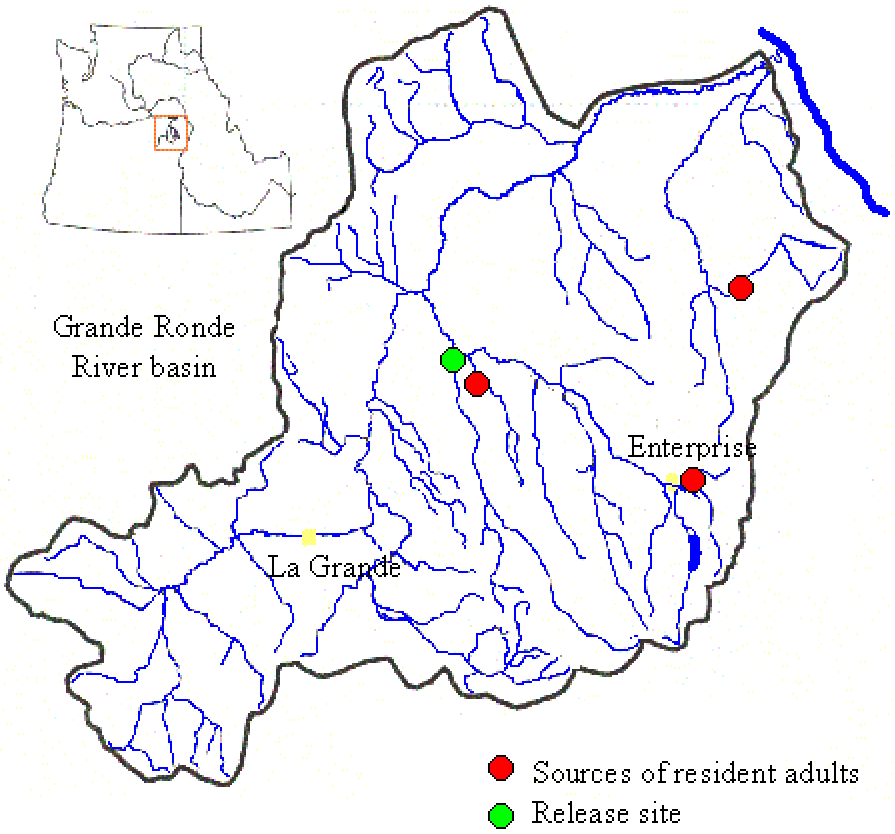

Figure 1. Map of Grande Ronde River basin showing collection locations of resident adults used in breeding experiments. Also shown is the release site of progeny for all groups.

Table 6. Median fork length (mm), mass (g), and condition factor (mass • fork length^{-3} •100,000) of the progeny from six breeding groups of the 2000 brood year. Two breeding groups, StsF x RbM and StsF x StsM, each had two replicate groups. All metrics shown were measured on 30 April, 2001. Significant differences among groups are also described. See methods for definitions of abbreviations for parent types.

Body measure	Breeding Group					
	RbF x RbM	RbF x StsM	StsF x RbM	StsF x RbM	StsF x StsM	StsF x StsM
Fork length (mm)	185[a]	198	204.5	205	208[b]	207
Mass (g)	71.4[c]	90.7	95.9	97.1	96.8	95.3
Condition Factor	1.10	1.12	1.12	1.11	1.07[d]	1.07[d]
% precocious	15.5	11.5	8.2	7.7	7.2	4.3

[a] Progeny from this group were significantly shorter (Kruskal-Wallis One Way ANOVA on Ranks, H = 112.1, 5 df, P < 0.05) than progeny from all other groups.
[b] Progeny from this group were significantly longer (Kruskal-Wallis One Way ANOVA on Ranks, H = 112.1, 5 df, P < 0.05) than progeny from the RbF x StsM group.
[c] Progeny from this group had significantly lower mass (Kruskal-Wallis One Way ANOVA on Ranks, H = 86.4, 5 df, P < 0.05) than progeny from all other groups.
[d] Progeny from these two groups had significantly lower condition factors (Kruskal-Wallis One Way ANOVA on Ranks, H = 136.3, 5 df, P < 0.05) than progeny from all other groups.

Table 7. Median fork length (mm), mass (g), and condition factor (mass • fork length^{-3} •100,000) of the progeny from six breeding groups of the 2001 brood year. Two breeding groups, RbF x RbM and RbF x StsM, had three and two replicate groups, respectively. All metrics shown were measured on 29 April, 2002. Significant differences among groups are also described. See methods for definitions of abbreviations for parent types.

Body measure	Breeding Group						
	RbF x RbM	RbF x RbM	RbF x RbM	RbF x StsM	RbF x StsM	StsF x RbM	StsF x StsM
Fork length (mm)	170	172	168	193[b]	191[b]	194[b]	202[a]
Mass (g)	59.4	63.9	54.5[d]	78.9	81.5	84.3[c]	87.5[c]
Condition Factor	1.24[f]	1.26[f]	1.18	1.14	1.14	1.15	1.08[e]
% precocious	13.3	14.8	8.3	0.2	0.4	8.1	0.4

[a] Progeny from this group were significantly longer (Kruskal-Wallis One Way ANOVA on Ranks, H = 508.2, 6 df, P < 0.05) than progeny from all other groups.
[b] Progeny from these groups were significantly longer (Kruskal-Wallis One Way ANOVA on Ranks, H = 508.2, 6 df, P < 0.05) than progeny from all RbF x RbM groups.
[c] Progeny from these groups had significantly greater mass (Kruskal-Wallis One Way ANOVA on Ranks, H = 69.2, 6 df, P < 0.05) than progeny from all RbF x RbM groups.
[d] Progeny from this group had significantly lower mass (Kruskal-Wallis One Way ANOVA on Ranks, H = 69.2, 6 df, P < 0.05) than progeny from all other groups except the first RbF x RbM group.
[e] Progeny from this group had significantly lower condition factors (Kruskal-Wallis One Way ANOVA on Ranks, H = 242.3, 6 df, P < 0.05) than progeny from all other groups.
[f] Progeny from these groups had significantly higher condition factors (Kruskal-Wallis One Way ANOVA on Ranks, H = 242.3, 6 df, P < 0.05) than progeny from all other groups.

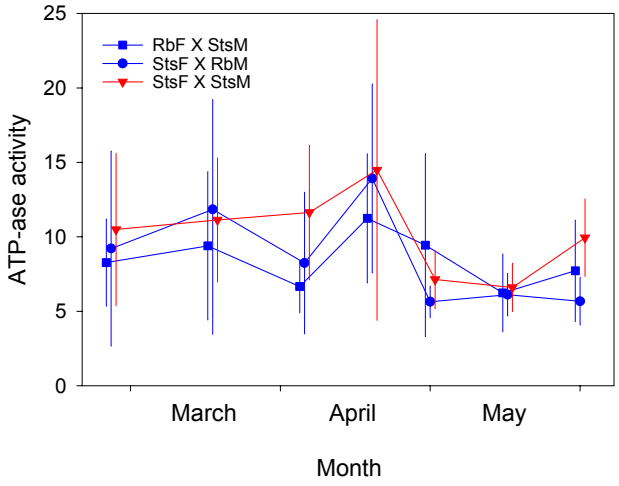

Figure 2. Mean gill $Na^+ + K^+$ ATP-ase specific activity (moles P · hr^{-1} · mg $protein^{-1}$; ± 95% CI) of progeny representing three experimental breeding groups from brood year 1998. A subset of the total progeny was held after the date when PIT-tagged fish were released for the detection evaluation.

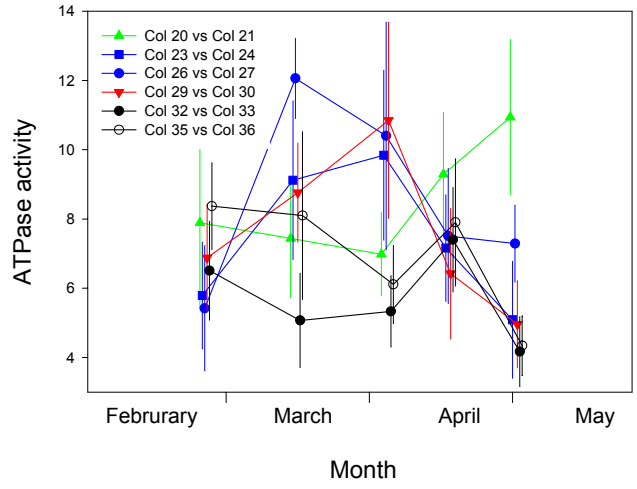

Figure 3. Mean gill $Na^+ + K^+$ ATP-ase specific activity (moles P · hr^{-1} · mg $protein^{-1}$; ± 95% CI) of progeny representing six experimental breeding groups from brood year 1999.

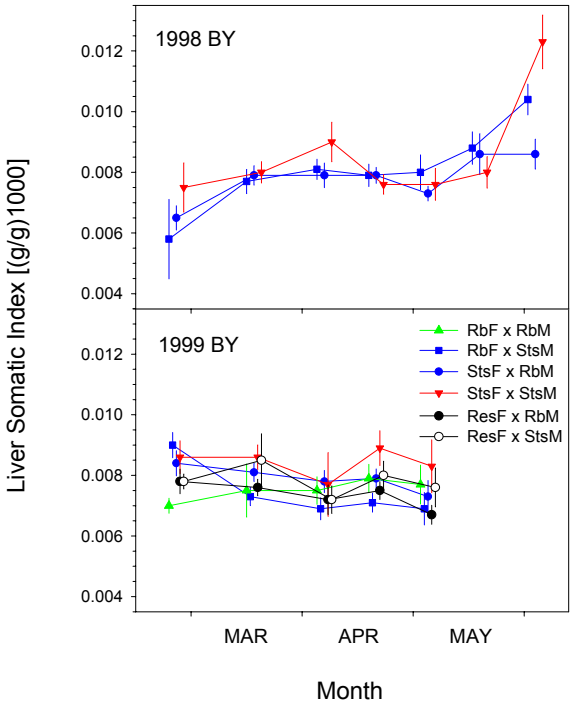

Figure 4. Mean liver somatic index [(g/g) · 1000] of progeny representing three and six experimental breeding groups from brood years 1998 and 1999, respectively. During 1998, a subset of the total progeny was held after the date when PIT-tagged fish were released for the detection evaluation.

Discussion

Results from our breeding experiments suggest that resident *O. mykiss* can produce progeny that smolt and emigrate from their freshwater habitat. Although at a reduced rate compared to StsF x StsM crosses, RbF x RbM, RbF x StsM, and StsF x RbM crosses all produced emigrating progeny that were detected at Snake and Columbia river facilities. The production of hatchery-reared emigrating progeny by RbF x RbM parents suggests that resident and anadromous life history traits are phenotypically plastic. This plasticity suggests that given suitable environments, resident fish have genetic traits allowing their progeny to exhibit the anadromous expression of this plastic life history trait. However, emigrating RbF x RbM progeny do not necessarily indicate a successful life history transition. Returning adults that reproduced successfully would indicate successful transition to anadromy. Unfortunately, our experimental group sizes during these initial years have been too small for significant returning adults. If migratory and ocean survival remain high we may obtain greater future returns from our 2001 and 2002 brood years to help address this question.

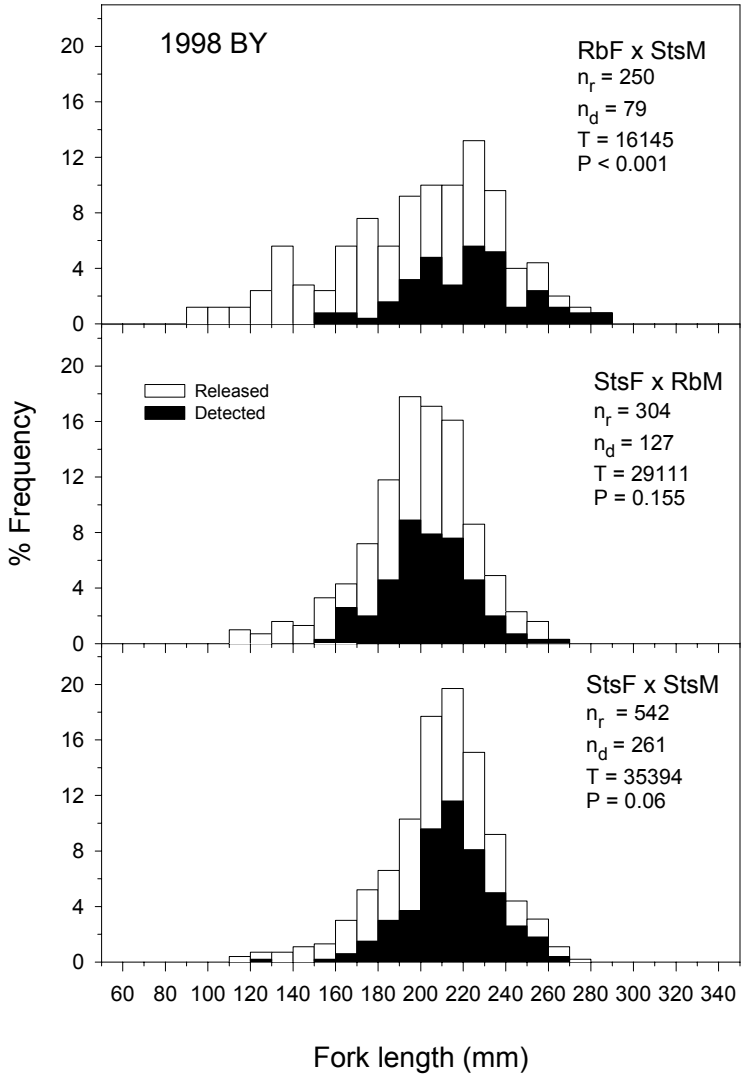

Figure 5. Size frequency distributions of groups of PIT-tagged progeny released and detected from the 1998 brood year. The three progeny groups result from three parental crossings: resident female (RbF) and anadromous male (StsM), anadromous female (StsF) and resident male (RbM), and StsF and StsM. Number of PIT-tagged progeny in each release group (n_r), number subsequently detected at mainstem dam facilities (n_d), as well as results of Mann-Whitney Rank Sum tests used to determine differences in the median fork lengths of release groups and their subsequent detections at dams are also shown.

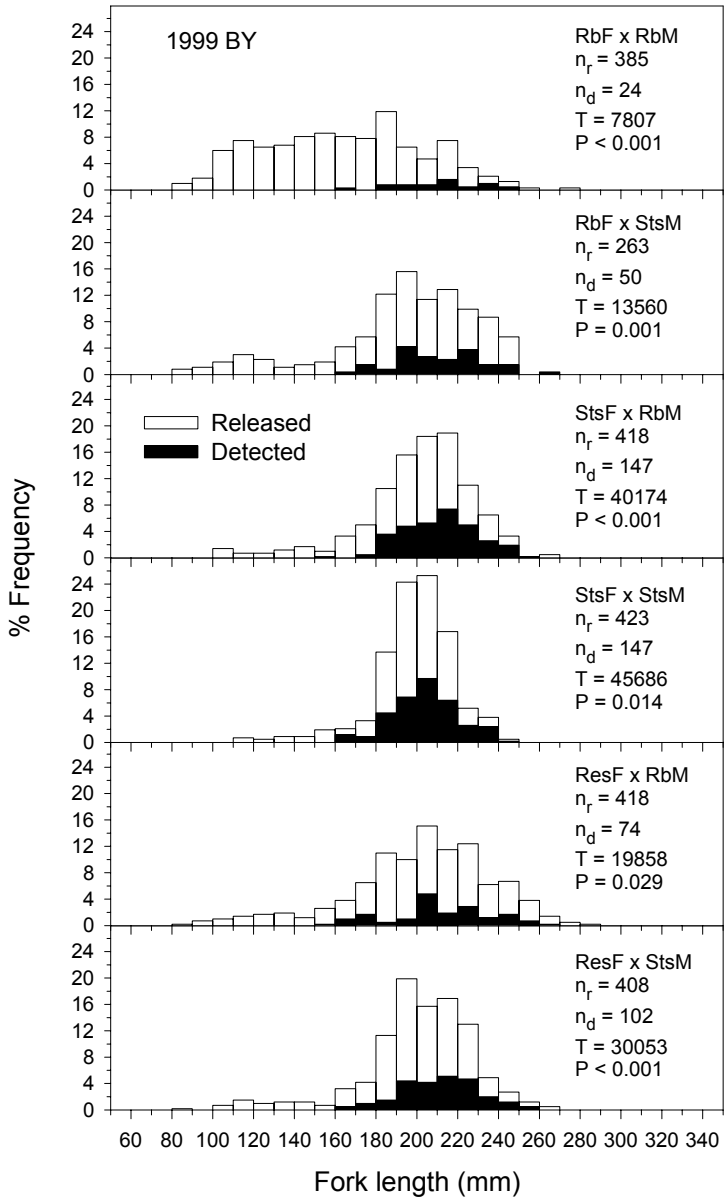

Figure 6. Size frequency distributions of groups of PIT-tagged progeny released and detected from the 1999 brood year. The six progeny groups result from six parental crossings: resident female (RbF) with anadromous male (StsM), anadromous female (StsF) with resident male (RbM), StsF with StsM, RbF with RbM, residualized female (ResF) with RbM, and ResF with StsM. Number of PIT-tagged progeny in each release group (n_r), number subsequently detected at mainstem dam facilities (n_d), as well as results of Mann-Whitney Rank Sum tests used to determine differences in the median fork lengths of release groups and their subsequent detections at dams are also shown.

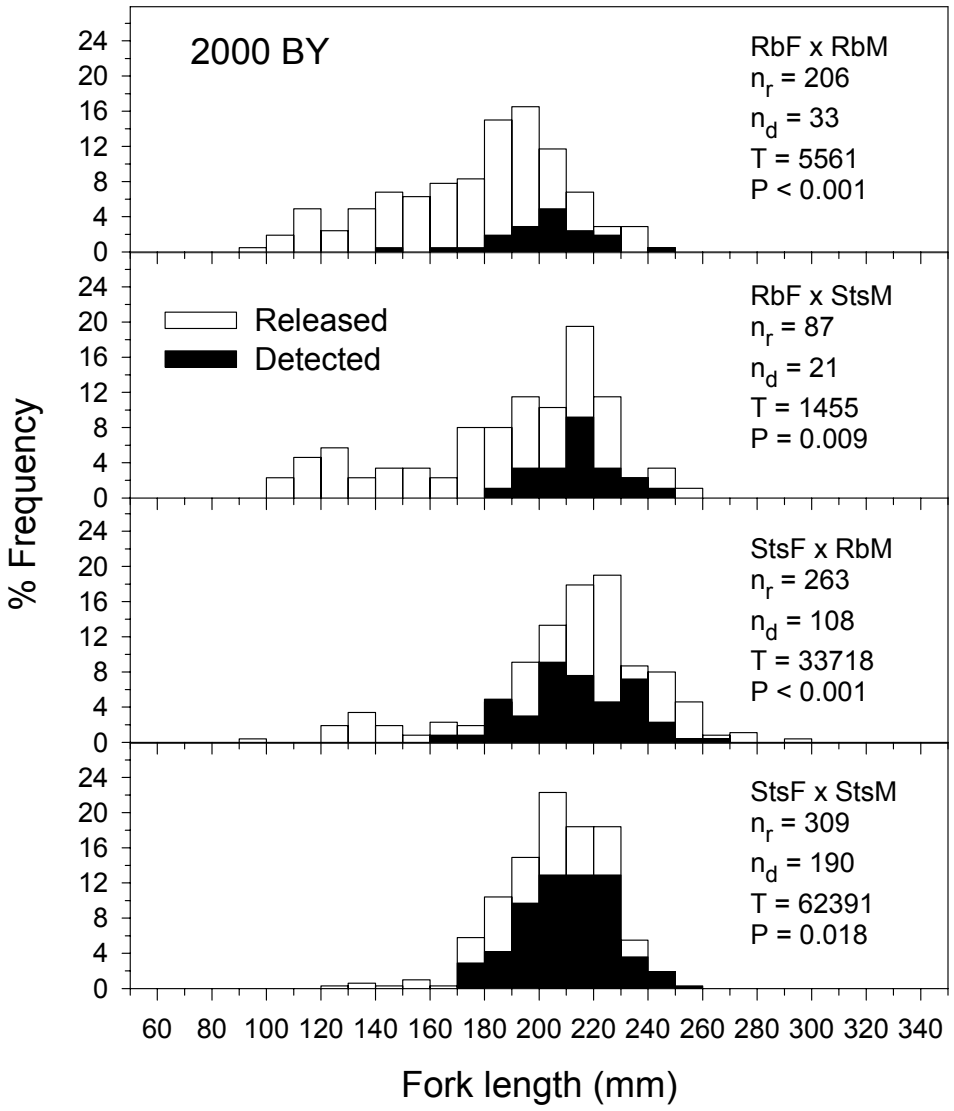

Figure 7. Size frequency distributions of groups of PIT-tagged progeny released and detected from the 2000 brood year. The four progeny groups result from four parental crossings: resident female (RbF) with anadromous male (StsM), anadromous female (StsF) with resident male (RbM), StsF with StsM, and RbF with RbM. Number of PIT-tagged progeny in each release group (n_r), number subsequently detected at mainstem dam facilities (n_d), as well as results of Mann-Whitney Rank Sum tests used to determine differences in the median fork lengths of release groups and their subsequent detections at dams are also shown.

The production of the anadromous life history expression from progeny of resident parents in our experimental groups also indicates that managers could produce anadromous individuals from resident parental stock. This suggests that even if anadromous runs of *O. mykiss* were lost from a system, they could be regained through natural or artificial propagation of resident progeny. This type of propagation has been accomplished for other salmonids (Kaeriyama et al. 1992) and rainbow trout transplanted to Argentina developed anadromous life histories after several generations as residents (Pascual 2001). Our resident fish came from riverine sources open to anadromy. Additional work would be needed to determine if the anadromous expression of the trait is lost after extended isolation from anadromy, as has occurred after the construction of many dams that lack fish passage.

Progeny from Sts parents were apparently more physiologically prepared for smoltification at an earlier date than RbF x RbM progeny. ATP-ase activity of RbF x RbM progeny was still increasing when they were released in early May whereas it was declining for Sts progeny during the same period. For all groups, LSI remained relatively unchanged prior to release dates but increased in the 1998 brood-year group subsamples that were retained after the early-May release. Increasing LSI during this period may have been an indication that these fish were experiencing physiological changes in preparation to residualize. As expected, K was often lower for StsF x StsM progeny and often higher for RbF x RbM progeny when compared to other groups. Lower K values indicate that StsF x StsM progeny likely had more smolt-like body form characteristics.

Size frequency distributions of progeny just prior to release were different among groups. RbF x RbM progeny were highly variable in length and RbF x StsM, and StsF x RbM progeny showed bimodal distributions. These variable and bimodal distributions are indicative of dual life-history strategies in these groups, which was verified by their lower and intermediate detection rates at mainstem dams. Moreover, larger individuals from these groups were predominately detected at dam facilities suggesting that larger progeny were more likely to smolt than smaller progeny from the same parental groups.

Although we were able to produce emigrating, RbF x RbM progeny in hatchery conditions, it is still unclear whether wild resident adults produce such progeny in the Grande Ronde River system. Our inability to complete the chemistry analysis of our otoliths samples at this time leaves this question unanswered. It is also unknown whether resident adults breed with anadromous adults in the Grande Ronde River. Some anecdotal evidence suggests that they do interbreed. During spawning ground surveys we have observed small resident fish near redds of actively spawning anadromous females and each year we collect hundreds of residualized hatchery males at our steelhead collection facilities. In Prairie Creek, a tributary to the Wallowa River, resident adults spawn during the same time as anadromous adults. Temporal overlap in spawn timing could allow interactions among the life history traits although it is certainly not assured. Zimmerman and Reeves (2000) found reproductive segregation among resident and anadromous adults in the Deschutes River, OR but also found both resident and anadromous progeny from alternate maternal origin in the Babine River, British Columbia at low levels. We suspect interactions in the Grande Ronde River are limited due to temporal and spatial differences in spawning and from assortative mating of different-sized adults. Future microchemistry analysis will help in determining the extent of life history interactions in the basin. Further, otolith microchemistry analysis will only allow us to determine maternal origin and thus cannot identify paternal contributions.

SECTION II

GENETIC CHARACTERIZATION

Introduction

To assess the genetic characteristics of steelhead in NE Oregon, juvenile *O. mykiss* have been sampled from various streams and hatchery programs. Most of these samples were collected for allozyme analysis while some were collected for DNA analysis. The design of this past sampling, however, was not planned to answer stock structure questions within the Grande Ronde and Imnaha river subbasins. Now that steelhead have been listed under the Endangered Species Act, the importance about questions of local adaptations and stock structure have become emphasized. NOAA Fisheries has also recommended that Wallowa stock be phased out in large part due to their straying and potential genetic influence on wild stocks. A broodstock developed from endemic fish within the Grande Ronde subbasin has been one alternative suggested as a replacement for the Wallowa stock. To develop an endemic broodstock, a more complete understanding of the genetic composition of endemic population(s) or stocks in the Grande Ronde is needed. Comanagers (ODFW, CTUIR, NPT) in consultation with NOAA Fisheries subsequently developed a genetics sampling plan for objective analysis of population structure within the Grande Ronde and Imnaha river subbasins.

Methods

Steelhead management units in the Grande Ronde and Imnaha subbasins were delineated, using limited information, to aide in the identification of population structure, status monitoring program development, and planning and development of future hatchery broodstock management (Table 8, Figure 8). Experienced fish biologists that were familiar with the regions selected management units. They hypothesized that environmental conditions influenced local adaptations and reproductive isolation, thus determining population structure. Factors that were considered when delineating the units included: proximity/connectivity, spawn timing, hydrography, water temperature, elevation, geology, and aspect. In addition, consideration was given to existing management designations that were previously identified as wild fish management units (Minam River, Wenaha River, and Joseph Creek drainages).

Spatial and temporal considerations in the sampling design were incorporated to assure adequate representation of the entire subbasins. Personnel from ODFW, CTUIR, and NPT began sampling in 1999 through 2002 to include four consecutive year classes. Representative stream reaches were selected in each management unit (Table 8, Figure 8) where a target of 80-100 fish < 100 mm TL were collected each year in each unit by electrofishing during July and August. This size range of fish was targeted to represent age-0 steelhead from each sample year. Each year we sampled two to thirteen reaches within each management unit. Additional samples were collected by NPT in the Imnaha River basin and by ODFW in the Grande Ronde River basin when fish were collected for other objectives. Fin clip samples were taken and stored in ethanol. Samples were delivered to Paul Moran (NOAA Fisheries) for microsatellite analysis.

Results and Discussion

We were able to collect at least 50 age 0 fish for genetic samples each year in each management unit (Table 9). Fifty samples are considered an adequate sample for genetic analysis (Paul Moran, personal communication). During most years we were able to collect fish samples from several locations within each management unit. Our efforts to obtain a spatial representation of fish from our sample streams were usually successful but sometimes limited by the availability of suitable habitat during low-water years (Appendix Tables A1-A4). On some streams our sample sites also varied considerably from year to year due to logistical considerations. This is especially apparent from our samples collected on the Catherine Creek drainage. During 1999 we only collected fish from the north fork of Catherine Creek. In subsequent years we collected fish from the Mainstem, North Fork, South Fork, and Little Catherine Creeks. These sampling strategies should be incorporated into any interpretation of the genetic results. Genetic analysis has not been completed for these samples.

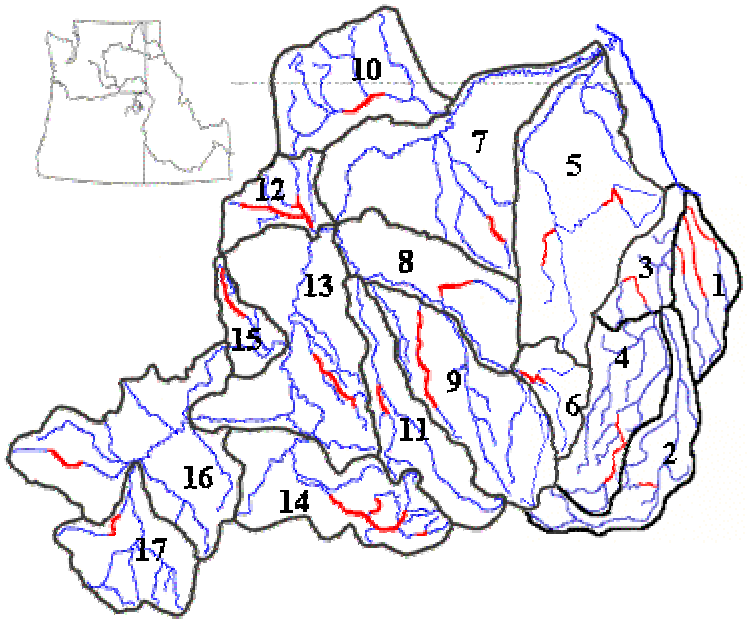

Figure 8. Map of management units used to delineate hypothesized sub-populations of wild steelhead in the Imnaha (units 1-4) and Grande Ronde (units 5-17) river subbasins. Representative genetic samples for each unit were taken from fish collected in the red highlighted reaches of streams. Descriptions of management units and sample collection sites for each unit can be found in Table 8.

Table 8. Description of management units used to delineate hypothesized sub-populations of wild steelhead in the Imnaha (units 1-4) and Grande Ronde (units 5-17) subbasins.

Management Unit	Unit Description	Sample Collection Site(s)
1. Lower Imnaha	Imnaha River downstream of Big Sheep Creek and tributaries flowing from the east.	Cow, Horse, & Lightning Cr.
2. Upper Imnaha	Imnaha River and tributaries upstream of Big Sheep Creek.	Gumboot Cr.
3. Zumwalt	Camp Cr. and Imnaha R. tributaries flowing from the west downstream of Big Sheep Cr.	Camp Cr.
4. Big Sheep	Big Sheep Cr. and tributaries	Big Sheep Cr.
5. Joseph	Joseph Cr. and tributaries	Chesnimnus & Elk Cr.
6. Prairie Creek	Prairie Cr. and tributaries	Prairie Cr.
7. Lower Grande Ronde	Grande Ronde R. and tributaries (except Wenaha R.) from mouth of Wallowa R. downstream to state line.	Mud Cr.
8. North Wallowa	Wallowa R. tributaries flowing from the north from the mouth to Prairie Cr.	Whiskey Cr.
9. South Wallowa	Wallowa River and tributaries flowing from the south (except Minam R. drainage)	Lostine R.
10. Wenaha	Wenaha R. and tributaries	Wenaha R.
11. Minam	Minam R. and tributaries	Little Minam R.
12. Lookingglass	Lookingglass Cr. and tributaries	Lookingglass Cr., Little Lookingglass Cr., Motett Cr.
13. Middle Grande Ronde	Grande Ronde R. and tributaries (except Catherine, Willow, and Lookingglass Cr. basins) from the Wallowa River to the upstream end of the Grande Ronde Valley.	Indian Cr.
14. Catherine	Catherine Cr. and tributaries including Ladd Cr.	Mainstem, South Fork, & North Fork Catherine Cr. & Little Catherine Cr.
15.Willow	Willow Cr. and tributaries	Dry Cr.
16.Upper Grande Ronde	Grande Ronde R. and tributaries from Grande Ronde valley upstream to and including Meadow Cr.	Meadow Cr.
17.South Grande Ronde	Grande Ronde R. and tributaries upstream of Meadow Cr.	Fly Cr.

Table 9. Number of genetic samples collected from juvenile O. *mykiss* each year in each management unit.

Management Unit	1999	2000	2001	2002
1. Lower Imnaha[a]	281	277	144	192
2. Upper Imnaha[a]	72	92	90	91
3. Zumwalt[a]	100	100	77	72
4. Big Sheep[a]	66	85	74	100
5. Joseph[a]	162	174	182	189
6. Prairie Creek	65	100	75	80
7. Lower Grande Ronde	100	73	67	69
8. North Wallowa	100	100	100	79
9. South Wallowa	59	111	74	77
10. Wenaha	100	99	100	80
11. Minam	64	91	86	80
12. Lookingglass	151	96	85	79
13. Middle Grande Ronde	73	100	69	76
14. Catherine	117	104	119	80
15. Willow	100	100	55	79
16. Upper Grande Ronde	100	100	51	80
17. South Grande Ronde	100	100	79	77

[a] These units were sampled by Nez Perce Tribal biologists all other units sampled by ODFW and CTUIR biologists.

SECTION III

KELT RECONDITIONING

Introduction

The NMFS has directed ODFW and comanagers to replace Wallowa stock steelhead in the Grande Ronde River basin by 2008. Current production has already been reduced by 33%. One alternative for replacing the stock is through the development of a local broodstock. Three procedures for local broodstock development that have been considered include a conventional program (through collection of upstream migrating adults), captive rearing of emigrating smolts, and reconditioning of kelts. Of these options the latter provides the least demographic risk to the existent populations because it would not significantly reduce the naturally spawning populations or their progeny. It is thought that kelts in the Snake River basin have high mortality rates and a low probability of repeat spawning. Kelt reconditioning also has the benefit of collecting only known, unmarked anadromous adults and would presumably have lower genetic selection in the hatchery environment compared to captive broodstock approaches. Kelts are also easily captured at adult collection facilities as they fall back onto weirs. For these reasons, we initiated a pilot program to investigate the feasibility of reconditioning steelhead kelts to assess the feasibility for use in the development and maintenance of a locally-adapted broodstock.

Methods

During 1998 and 1999, we attempted to recondition wild and hatchery kelts that were collected at our Big Canyon and Little Sheep Creek facilities. Some of these fish had spawned naturally, while others were live spawned by hatchery personnel. Fish were transported in 200-500 gallon aerated and oxygenated tanks. Ten grams of MS222 per 200 gallons of water was used to aide in calming fish while in transport. Fish were held at the Wallowa Fish Hatchery in small (107 cm long, 91 cm wide, and 61 cm deep) partitions in an indoor raceway covered with plastic mesh fabric. Water from the Wallowa River was supplied at 52 gallons per minute until early June when the water source was switched to spring water. During 1999, fish were injected with oxytetracycline and 4.5 ml of diluted ivermectin to control bacterial infections and parasites. During 1998, fish were allowed a two-day acclimation period with little or no human disturbance before hour-long, 1:10,000 concentration formalin treatments were administered every three days to control external fungus. When this formalin treatment failed to reduce fungus, concentrations were increased to 1:7,000 and 1:6,000 and administered five days/week. During 1999, after the two-day acclimation period, fish were given a more intensive formalin treatment beginning with 1:7000, 1:6000, and 1:5000 concentrations every other day, then continued with the 1:5000 concentration administered five consecutive days/week. In mid-June formalin treatments on the five remaining kelts were reduced to 1:6000 three days/week. When fish were moved outside to a larger circular holding tank later that summer and fungus was observed, 1:6000 formalin treatments five days/week were administered either to all fish in the circular or the fish with fungus was removed and placed back in the acclimation tank for treatment.

We implemented an intensive hand-feeding method to initiate reconditioning following methods developed for Atlantic salmon at Berkshire National Fish Hatchery in Maryland (L. Lofton, personal communication). This method entails offering small food items placed loosely on the end of wire tools to individual fish using a slow wafting motion. If the fish shows no response, food items are placed inside the fish's mouth using the wire tool. Feeding attempts

are performed daily, independent of whether the fish shows a positive response to food items. If feeding is successful, fish generally become increasingly aggressive in feeding behavior after which they are trained to take feed thrown freely into holding tanks. Once actively feeding on hand-tossed food, fish are then considered "reconditioned" and transported to larger holding tanks with other fish where they are fed daily on a diet that transitions them to standard pellet feed. At the onset of our feeding trials, various food types were offered to fish in an attempt to elicit feeding responses. Once a positive response was elicited towards a food type that food item was offered to individual fish using a hand-held tool fashioned from either a willow stick or a plastic coated coat hanger. During 1999, we also transitioned fish from beef liver to a special kelt diet developed by Ann Gannon of the USFWS Abernathy Fish Technology Center, WA (Table 10) that was adapted from the Atlantic salmon kelt diet. This diet was soft enough to form into small pieces that could be placed on the hand-held tools, and in the formulation fed to kelts, was composed of 38.4% protein, 13.0 % lipid, 7.8 % ash, and 39.1% moisture. Gross energy content of this kelt diet was 3367 cal/g.

We compared the sperm viability of the one reconditioned kelt that returned during 1998 and survived to May 25, 1999, with the sperm viability of nine newly captured, Wallowa-stock steelhead. We split the eggs of two newly captured females into 10 equal portions of approximately 800 eggs each. One egg group was fertilized with milt from the reconditioned kelt while the other nine were fertilized with one each of the nine newly captured males. Eggs were placed into trays and incubated using standard hatchery practices for steelhead at Wallowa Fish Hatchery. Viability was measured after 20 days as percent of total eggs that had visible eyes. During 2000, we also assessed the viability of gametes of reconditioned kelts that returned in 1999 by comparing them to hatchery steelhead that returned in 2000. The sperm of one male kelt was included in a 4x4 matrix with three male and four female hatchery returns. Two female kelts were included in separate 4x4 matrices, both with three other females and four males. Fertilized egg viability was evaluated as in 1998.

Warren Groberg (ODFW, NE region fish pathologist) conducted necropsies on 19 of the kelts that died in captivity during 1998. Necropsies were performed to determine the primary cause of death. Systemic bacterial infection was determined by culture of kidney smears on TYE agar and viral assays of kidney/spleen samples were also conducted. All procedures were consistent with standard adult mortality examinations conducted by ODFW pathology staff.

Table 10. Percent composition of diet fed to kelts that were successfully transitioned from beef liver during 1999 trials. Diet supplied by Ann Gannon of the USFWS Abernathy Fish Technology Center, WA.

Diet Component	Diet Percentage (%)
Starter #3 (Biodet starter)	51.49
Wet fish (Bio-Oregon)	29.01
liquid krill	7.84
beef liver	7.65
Vitamin premix (Abernathy premix #2)	0.78
Mineral premix (Abernathy premix #3)	0.52
Choline	0.52
Gelatine	1.96
Vitamin C	0.18

Results

1998

Twenty-nine fish were transported to holding facilities for reconditioning during April and May, 1998. Six of these fish had spawned naturally and 23 were live spawned by hatchery personnel (Table 11). The majority of kelts brought into the facility had some form of exterior fungal growth on scrapes and lesions of their head, fins, and/or bodies.

The first fish collected in 1998 were fed numerous food items to elicit feeding responses. Live earthworms, trout fingerlings, and krill-based pellets were introduced into the holding tank with little or no response from the kelts. Small pieces of beef liver were then hand-thrown into the tank that appeared to elicit some response by the fish. Liver was then presented to individual fish on the wire tool and some fish took them into their mouths but failed to ingest them. The majority of these fish demonstrated no response to food items with only 22% (5 of 23) showing some interest. All of these initial 23 fish died within three months of capture.

As new fish were collected, several new types of food were introduced. A combination of krill-based pellets and beef liver were mashed together to form a paste that was formed into small pieces and offered to the fish using the wire tool. Several fish tolerated insertion of these pieces into their mouths, but none were ingested. Prawns were then presented to the fish in the same manner with similar results. Pellet food was hand-tossed into the tanks each day without any apparent positive response from the fish. Other food items were tried including sardine, herring, crab, and clam pieces. None of these elicited positive responses. After 20 days, 1-cm cubes of beef liver were presented again using the wire tool and these elicited positive feeding responses. At this time two fish began ingesting liver pieces and were eventually transitioned over to pellet feed during the following months.

Twenty-seven of 29 (93%) kelts died within three months of captivity during 1998. Fish were collected from early-April through late May. Both of the long-term survivors were collected during late-May. All mortalities had visible fungus and some of the live-spawned females had retained up to 25% of their eggs. One male (originally live spawned) survived to the following spring when we performed a viability test using its gametes. Survival to eyed embryo stage for eggs fertilized by this male was 94% whereas the average embryo survival of standard hatchery production fish was 88% (n = 9, range 70-96%).

Necropsies, to identify the primary cause(s) of death, were performed on 19 kelt mortalities that died from May 14-21, 1998. The first four fish that died prior to May 14 were not sampled. All fish exhibited some level of snout and lower jaw lesions that were likely mechanically induced from repeated impacts. All 19 fish also had some level of fungus on their bodies varying from small blotches to massive coverage (Table 12). Gill fungus was not observed on any fish and gills appeared to be in generally good condition. It was determined that fungus was not the primary means of death. Cultures for systemic bacteria were positive for only one fish that had high levels of *A. salmonicida*. Aeromonad/pseudomonad (APS) bacteria were present at low to high levels in nine fish (Table 12). High levels of small colony types were isolated from 17 of 19 (90%) of these fish. Bacterial infection as a primary factor contributing to the mortality of these fish could only be identified in the one fish with *A. salmonicida*. All fish were emaciated with sunken eyes with digestive tracts devoid of contents and malnutrition was consequently deemed the primary cause of death.

Table 11. Collection location, stock origin, sex, collection date, spawning activity prior to collection (spawned naturally in wild, W; or live spawned in hatchery, H), and number of fish collected for kelt reconditioning efforts during 1998.

Location	Stock Origin	Sex	Collection Date	Spawn Activity	Quantity
Little Sheep Cr.	Little Sheep	female	April 2-May 14	H	4
Little Sheep Cr.	Little Sheep	male	April 2-May 14	H	2
Little Sheep Cr.	wild	female	April 2-May 14	H	3
Little Sheep Cr.	wild	male	April 2-May 14	H	2
Little Sheep Cr.	Little Sheep	male	May, 22-26	H	4
Little Sheep Cr.	wild	female	May, 22-26	H	2
Big Canyon	Wallowa	female	May 8	W	1
Big Canyon	Wallowa	male	May 8	W	1
Big Canyon	wild	female	May 8	W	4
Wallowa Hatchery	Wallowa	male	May 5	H	3
Wallowa Hatchery	Wallowa	female	May 5	H	3
Total					29

1999

Eleven fish were transported to holding facilities for reconditioning during April and May 1999. Five of these fish had spawned naturally and 6 were live spawned by hatchery personnel (Table 13). As in the previous year, all kelts brought to the facility had some lesions and visible fungal growth on their bodies. Formalin treatment concentrations to control fungal growth varied from 1:5000 to 1:7000 and were administered 1-5 times each week for 1-2 hr/treatment depending on severity of visual fungal growth. Parasitic copepods were also noticed on the gills and fins of most fish. Six of 11 (55%) fish died within the first three months of captivity. All fish that had long-term survival were collected after mid-May. Three fish survived until the following spring when breeding experiments were conducted to determine the viability of their gametes. Kelt gamete viability as measured at eye-up of embryos was equal to or greater than first-time spawning adults (Tables 14-15). Two fish that survived until the following spring did not develop gametes; one fish died later that summer (this fish had heavy levels of copepods and hydrogen peroxide treatments were not effective) and one was euthanized the following spring (2001).

Learning from our previous feeding trials experience we initiated feeding using only beef liver presented on wire tools during 1999. All but two of the fish ate liver pieces within two weeks of captivity. Five of the fish were successfully transitioned from liver to the special kelt diet, developed by Ann Gannon, that was hand tossed into the tanks. A typical fish would feed on liver pieces within the first week of captivity. Most fish initially fed only on liver pieces offered on wire but others initially ate liver hand tossed into their tank. After two weeks most surviving fish were eating hand tossed liver and most fish were subsequently transitioned to the special kelt diet within one additional week.

Table 12. Origin (hatchery, H; wild, W), sex (female, F; male, M), pathological notes on external inspection and systemic bacterial cultures, and date of mortality of 19 fish necropsies performed on kelt mortalities by Warren Groberg (ODFW) during 1998.

Origin	Sex	External Signs	Systemic Bacteria	Mortality Date
W	F	Severe snout/lower jaw lesions, Two small blotches of fungus	3 colonies APS, low small colony type	5-14-98
H	F	Severe snout/lower jaw lesions, Moderate density of fungus	High small colony type	5-14-98
H	M	Moderate snout/lower jaw lesions, 50% of body with fungus	Low small colony type	5-14-98
H	M	Severe snout/lower jaw lesions, Moderate density of fungus	Negative	5-14-98
H	M	Severe snout/lower jaw lesions, High density of fungus	2 colonies of APS, High small colony type	5-14-98
H	F	Moderate snout/lower jaw lesions, Low density of fungus	High small colony type	5-14-98
H	F	Severe snout/lower jaw lesions, High density of fungus	High APS, High small colony type	5-18-98
H	F	Severe snout/lower jaw lesions, Low density of fungus	High *Aeromanas salmonicida,* High small colony type	5-16-98
W	F	Moderate snout/lower jaw lesions w/fungus – Moderate density of fungus	High small colony type	5-18-98
H	M	Low snout/lower jaw lesions, High density of body fungus	4 colonies APS, High small colony type	5-19-98
W	F	Low snout/lower jaw lesions, Massive body fungus	Moderate APS, High small colony type	5-18-98
H	F	Severe snout/lower jaw lesions w/fungus – High density of fungus	High APS, High small colony types	5-18-98
H	F	Low snout/lower jaw lesions, Moderate density of fungus	Few small colony types	5-18-98
W	M	Moderate snout/lower jaw lesions, High density of fungus	12 colonies APS, High small colony type	5-18-98
W	M	Moderate snout/lower jaw lesions, High density of body fungus	Moderate small colony type	5-18-98
W	M	Severe snout/lower jaw lesions w/fungus - High body fungus	Moderate APS, High small colony type	5-18-98
H	M	Severe snout/lower jaw lesions, High density of body fungus	Low APS	5-20-98
H	F	Severe snout/lower jaw lesions, High density of fungus	11 colonies APS, High small colony type	5-21-98
W	F	Moderate snout/lower jaw lesions, High density of fungus	Low small colony type	5-18-98

Table 13. Collection location, stock origin, sex, collection date, spawning activity prior to collection (spawned naturally in wild, W; or live spawned in hatchery, H), and number of fish collected for kelt reconditioning efforts during 1999.

Location	Stock Origin	Sex	Collection Date	Spawn Activity	Quantity
Little Sheep Cr.	wild	female	April 6 – May 25	H	3
Little Sheep Cr.	wild	male	April 13 – May 25	H	3
Big Canyon	wild	male	June 7	W	1
Big Canyon	wild	female	May 17 – June 3	W	4
Total					11

Table 14. Percent viability of embryos resulting from a 4x4-spawning matrix to compare the embryo viability of a reconditioned male kelt with that of first-time spawners (A-C). Viability was measured as percent of eggs that had visible eyes. Estimated fecundity (number of eggs/female) is also shown for each female used in the matrix.

	Male				
Female	Kelt	A	B	C	Fecundity
A	91.8	69.8	81.5	51.1	5,879
B	34.2	26.9	16.0	72.2	4,445
C	1.7	7.4	9.5	7.6	4,084
D	64.9	57.3	86.6	27.4	4,310
Average	48.2	40.4	48.4	39.6	4,680

Table 15. Percent viability of embryos resulting from a 4x4-spawning matrix to compare the embryo viability of two reconditioned female kelts (K1, K2) with that of first-time spawners (A-C). Viability was measured as percent of eggs that had visible eyes. Estimated fecundity (number of eggs/female) is also shown for each female used in the matrix.

	Male					
Female	A	B	C	D	Average	Fecundity
K1	63.1	53.0	83.9	74.1	68.5	5,840
A	15.0	8.0	36.0	42.0	25.3	3,620
B	81.0	84.0	65.0	70.0	75.0	4,520
C	72.0	44.0	73.0	77.0	66.5	4,240
Average						4,555

	Male					
Female	A	B	C	D	Average	Fecundity
K2	73.4	67.7	85.2	89.5	79.0	5,140
A	85.0	83.0	76.0	78.0	80.5	3,250
B	83.0	82.0	85.0	32.0	70.5	6,560
C	84.0	73.0	64.0	72.0	73.3	4,003
Average						4,738

Discussion and Recommendations

Kelt reconditioning could be a viable option for the establishment of an endemic steelhead brood stock in the Grande Ronde River basin. Our ability to recondition 3 of 11 kelts to repeat spawning condition (two other kelts were reconditioned but did not have apparent mature gametes) during 1999-2000, and their gamete viability, indicates some success could be achieved using our methods. To increase success, fungus treatment and water quality attributes would need to be addressed. Individuals responsible for feeding kelts sometimes noticed that kelts had reduced activity and feeding aggression after formalin treatments. Holding and reconditioning kelts using spring or well water (as was requested by pathology staff) would also reduce the incidence of copepods and other parasites and pathogens.

Kelt reconditioning using the methods we adapted from Atlantic salmon efforts is labor intensive. To initiate feeding, fish need to be offered food at least once but preferably twice each day. If fish do not aggressively take food items technicians would often spend 15 min./ kelt for each feeding trial. When this effort is combined with frequent formalin treatments, the number of kelts that a single individual can care for is limited to approximately 8-10 fish. Once kelts are successfully transitioned to pellet diets labor time is significantly reduced. Despite these limitations, kelt reconditioning could become a component of an endemic brood stock development program if adequate labor and facility needs are met.

Given our results, we can provide some preliminary recommendations for future kelt reconditioning. Selecting kelts with little or no visible fungus or applying an initial topical treatment would likely aid success by avoiding the aggressive formalin treatments we administered to control fungus. Oxytetracycline and ivermectin treatments should be administered to all fish to control pathogens and parasites. Kelts show widely varying levels of aggression towards other fish. Staff need to recognize if any fish become molested by aggressors and isolate when possible. Wild kelts are prolific jumpers and covers on tanks should be designed to avoid impact and escape injuries. Kelts should be started on a liver diet first presented by hand tossing into the tank. If they do not respond to this technique then liver pieces should be presented on a hand-held tool to individuals until they can be transitioned to more efficient feeding methods. The kelt-specific diet developed by the USFWS was successful and should be used in the future.

REFERENCES

Jonsson, B., and N. Jonsson. 1993. Partial migration: niche shift versus sexual maturation in fishes. Reviews in Fish Biology and Fisheries 3:348-365.

Kalish, J.M. 1990. Use of otoliths microchemistry to distinguish the progeny of sympatric anadromous and non-anadromous salmonids. Fisheries Bulletin 88:657-666.

Kaeriyama, M., S. Urawa, and T. Suzuki. 1992. Anadromous sockeye salmon (*Oncorhynchus nerka*) derived from non-anadromous kokanees: life history in Lake Toro. Scientific Report Hokkaido Salmon Hatchery 46:157-174.

Pascual, M., P. Bentzen, C.R. Rossi, G Mackey, M.T. Kinnison, and R. Walker. 2001. First documented case of anadromy in a population of introduced rainbow trout in Patagonia, Argentina. Transactions of the American Fisheries Society 130:53-67.

Riemann, B.E., D.L. Myers, and R.L. Nielson. 1994. Use of otoliths microchemistry to discriminate *Oncorhynchus nerka* of resident and anadromous origin. Canadian Journal of Fisheries and Aquatic Sciences 51:68-77.

Volk, E.C., A Blakley, S.L. Schroder, and S.M. Kuehner. 1999. Otolith chemistry reflects migratory characteristics of Pacific salmonids: using otolith core chemistry to distinguish maternal associations with sea and freshwaters. Fisheries Research 46:251-266.

Zimmerman, C.E., and G.H. Reeves. 2000. Population structure of sympatric anadromous and nonanadromous *Oncorhynchus mykiss*: evidence from spawning surveys and otolith microchemistry. Canadian Journal of Fisheries and Aquatic Sciences 57:2152-2162.

APPENDIX A

Location and Number of Tissue Samples Collected in the Grande Ronde and Imnaha River Subbasins for Genetic Characterization of Steelhead

Table A1. Location and number of tissue samples collected at various sites during 1999 in the Grande Ronde and Imnaha River subbasins to determine the basin-wide genetic characterization of steelhead. Sampling was conducted by ODFW, CTUIR, and NPT biologists and staff.

Management Unit	Sample stream(s)	River mile	Number of samples
1. Lower Imnaha	Horse Creek	4.86	10
		4.66	9
		3.61	11
		3.07	10
		2.35	10
		1.93	9
		1.34	9
		0.52	8
	Horse (Pumpkin Creek)	5.08 + 0.05	18
	Lightning Creek	2.67	4
		3.72	4
		9.89	7
		9.4	4
		8.84	6
		8.37	9
		7.81	12
		7.33	7
		4.44	13
		3.13	5
		5.27	4
	Lightning (Sleepy Creek)	10.2 + .18	5
	Cow Creek	5.97	12
		5.77	12
		5.34	10
		4.83	10
		4.33	11
		3.62	9
		2.72	10
		1.4	3
		0.49	19
		5.97	12
2. Upper Imnaha	Gumboot Creek	0.29	12
		1	6
		1.68	12
		2.63	3
		3.14	6
		3.82	9
		4.15	5
		3.55	14
		2.29	5
3. Zumwalt	Camp Creek	1.32	10
		1.66	10
		2.03	10
		2.41	10
		2.75	10
		2.95	10

Management Unit	Sample stream(s)	River mile	Number of samples
		3.4	10
		3.73	10
		4.19	10
		4.6	10
4. Big Sheep	Big Sheep Creek	36.39	1
		26.8	16
		26.72	3
		23.85	12
		27.44	11
		28.64	12
		30.17	11
5. Joseph	Chesnimus	13.66	10
		14.67	10
		15.89	10
		16.51	10
	Chesnimus (Devil's Run Ck.)	0.04	10
		1.17	10
		2.37	10
	Elk Creek	5.49	6
		0.41	10
		1.4	10
		2.49	10
		3.63	10
		5.02	10
		6.08	10
		7.48	16
		4.43	10
6. Prairie Creek	Prairie Creek	1.0	4
		1.5	6
		2.0	2
		2.5	13
		3.2	16
		3.8	4
		4.2	4
		4.5	1
		5.5	4
		6.0	3
	Tributary at rm 4	--	8
7. Lower Grande Ronde	Mud Creek	14.0	24
		16.0	36
		17.5	40
8. North Wallowa	Whiskey Creek	2.5	31
		2.8	8
		4.5	18
		5.0	8
		5.5	8
		7.3	12
		8.0	8
		9.0	7
9. South Wallowa	Lostine River	0.5	1
		2.0	9
		4.0	11
		8.5	5
		9.0	14
		13.3	1
		14.0	2
		18.0	13
		24.0	2
		26.0	1
10. Wenaha	Wenaha River	12.2	9
		13.6	5
		13.9	5
		14.7	10
		16.1	11

Management Unit	Sample stream(s)	River mile	Number of samples
		16.6	10
		17.3	10
		17.8	12
		18.4	13
		19.0	9
		19.6	5
		--	1
11. Minam	Minam River	18.5	9
	Little Minam River	0.0	14
		0.5	12
		1.0	14
		3.3	7
		3.8	8
12. Lookingglass	Lookingglass Creek	1.0	10
		1.5	20
		2.0	15
		2.5	16
		3.5	30
		4.0	39
		7.0	11
		7.3	5
		10.2	5
13. Middle Grande Ronde	Indian Creek	5.3	19
		10.0	6
		12.0	17
		16.5	1
		17.0	12
		17.5	7
	Little Indian Creek	2.0	5
	Shaw Creek	3.5	6
14. Catherine	North Fork Catherine Ck.	0.1-0.2	1
		0.2	7
		0.7	17
		1.1	9
		1.2	15
		1.8	14
		2.0	16
		2.4	17
		2.5	5
		2.6	2
		3.0	2
		--	12
15. Willow	Dry Creek	6.5	38
		7.5	54
		10.2	8
16. Upper Grande Ronde	Meadow Creek	11.0	31
		12.5	15
		14.0	19
		14.5	16
		15.0	11
		17.5-18.5	8
17. South Grande Ronde	Fly Creek	5.6	8
		5.7	8
		5.8	1
		6.0-6.4	26
		6.4	18
		6.6	9
		7.2	1
		7.5	10
		7.7	9
		7.9	10

Table A2. Location and number of tissue samples collected at various sites during 2000 in the Grande Ronde and Imnaha River subbasins to determine the basin-wide genetic characterization of steelhead. Sampling was conducted by ODFW, CTUIR, and NPT biologists and staff.

Management Unit	Sample stream(s)	River mile	Number of samples
1. Lower Imnaha	Cow Creek	0.63	11
		1.53	8
		2.59	12
		3.1	9
		3.72	11
		4.4	10
		5.48	11
		6.2	9
		7.04	9
		7.59	10
	Horse Creek	0.63	11
		1.07	11
		1.46	11
		2.25	11
		2.78	11
		3.52	10
		3.9	15
		4.86	10
	Horse (Pumpkin Creek)	5.08 + 0.31	10
	Lightning	0.2	10
		0.8	8
		1.6	2
		3.14	6
		5.27	17
		5.67	13
	Lightning (Sleepy Creek)	10.2 + .18	15
		10.2 + .35	6
2. Upper Imnaha	Gumboot Creek	0.29	11
		0.9	10
		1.17	10
		1.87	9
		2.63	10
		3.04	12
		3.55	9
		3.94	11
		4.33	10
3. Zumwalt	Camp Creek	1.36	9
		1.82	12
		2.36	9
		2.75	12
		2.95	9
		3.21	10
		3.6	10
		3.96	11
		4.34	15
		4.93	3
4. Big Sheep	--	26.72	11
		26.8	10
		9.72	3
		9.85	16
		27.52	14
		28.29	14
		28.82	10
		29.27	7
5. Joseph	Chesnimus	13.66	13
		14.67	9
		15.89	11
		16.51	10
	Chesnimus (Devil's Run Ck.)	0.04	11

Management Unit	Sample stream(s)	River mile	Number of samples
		0.95	10
		1.84	11
		2.47	10
	Elk Creek	0.36	11
		1.4	13
		2.17	6
		2.9	13
		3.28	10
		3.98	12
		4.5	7
		5.02	10
		5.55	3
		0.91	4
6. Prairie Creek	Prairie Creek	1.0	12
		1.5	14
		2.5	13
		3.2	10
		3.8	11
		4.5	26
	Tributary at rm 4	--	14
7. Lower Grande Ronde	Mud Creek	14.0	33
		16.0	40
8. North Wallowa	Whiskey Creek	1.5	14
		2.4	13
		2.5	10
		2.7	17
		4.5	15
		5.0	16
		5.5	15
9. South Wallowa	Lostine River	2.0	13
		5.0	10
		7-9	10
		10.0	29
		10-14	26
		14.0	7
		18.0	5
		18.5	3
		20.0	2
		22.0	6
10. Wenaha	Wenaha River	13.6	10
		13.9	9
		14.7	10
		16.1	10
		16.6	10
		17.3	10
		17.8	10
		18.4	11
		19.0	9
		19.6	10
11. Minam	Minam River	18.5	10
	Little Minam River	0.0	9
		0.5	17
		1.0	21
		1.5	14
		3.3	10
		3.8	10
12. Lookingglass	Lookingglass Creek	0.8	9
		1.8	9
		2.4	8
		3.5	18
		3.7	10
		7.3	7
		9.8	17
	Little Lookingglass Ck.	0.3	14

Management Unit	Sample stream(s)	River mile	Number of samples
		2.9	2
	Motett Creek	1.5	2
13. Middle Grande Ronde	Indian Creek	5.3	19
		11.0	13
		12.0	19
		16.5	12
		17.0	18
	Little Indian Creek	2.0	5
	Shaw Creek	3.5	14
14. Catherine	Catherine Creek	20-32	25
	North Fork Catherine Ck.	0.5	11
		0.7	9
		1.0	10
		1.3	7
		1.7	7
		2.1	4
		3.0	7
		4.0	3
	Corral Creek	0.1	10
	Little Catherine Creek	0-3.0	11
	South Fork Catherine Ck.	4.0	3
15. Willow	Dry Creek	7.1	29
		8.2	10
		8.4	23
		9.7	9
		10.2	29
16. Upper Grande Ronde	Meadow Creek	11.5	8
		12.0	8
		13.0	11
		13.5	12
		14.0	10
		14.5	13
		15.0	8
		15.5	10
		16.3	10
		17.0	10
17. South Grande Ronde	Fly Creek	4.7	18
		5.1	12
		5.3	15
		5.7	14
		7.2	19
		7.7	22

Table A3. Location and number of tissue samples collected at various sites during 2001 in the Grande Ronde and Imnaha River subbasins to determine the basin-wide genetic characterization of steelhead. Sampling was conducted by ODFW, CTUIR, and NPT biologists and staff.

Management Unit	Sample stream(s)	River mile	Number of samples
1. Lower Imnaha	Lightning	9.89	13
		5.27	12
		8.37	13
		5.67	13
		3.31	4
		0.52	2
	Lightning (Sleepy Creek)	10.2 + .18	13
		10.2 + .35	11
	Horse Creek	0.63	8
		1.07	12
		1.46	10
		2.25	9
		2.78	11
		3.52	10
		3.9	10
	Cow Creek	0.35	11
		0.54	11
		1.81	11
		2.87	14
		3.4	17
		4.03	12
		4.58	17
		0.35	11
2. Upper Imnaha	Gumboot Creek	0.29	10
		0.81	10
		1.27	10
		1.67	12
		2.63	11
		3.14	12
		3.64	15
		4.15	10
3. Zumwalt	Camp Creek	1.32	10
		1.82	12
		2.36	11
		2.85	12
		3.21	13
		3.69	8
		4.19	11
5. Joseph	Elk Creek	1.61	10
		2.17	15
		3.01	12
		3.75	10
		4.71	15
		5.55	9
		5.94	11
	Chesnimus	13.66	12
		14.67	12
		15.89	12
		16.3	12
	Chesnimus (Devil's Run Ck)	0.04	13
		1.07	14
		1.9	13
		2.67	12
4. Big Sheep	Big Sheep Creek	26.72	10
		9.85	10
		38.46	6
		36.62	9
		36.14	16
		30.17	12

Management Unit	Sample stream(s)	River mile	Number of samples
		29.69	11
6. Prairie Creek	Prairie Creek	1.0	11
		1.5	10
		2.5	10
		3.2	10
		3.8	10
		4.5	7
	Tributary at rm 4	--	17
7. Lower Grande Ronde	Mud Creek	14.0	39
		15.0	13
		15.5	10
		16.0	4
8. North Wallowa	Whiskey Creek	1.5	30
		2.5	16
		2.8	16
		4.5	15
		5.5	23
9. South Wallowa	Lostine River	1.7	7
		2.3	12
		5.2	20
		10.0	15
		14.0	11
		18.0	9
10. Wenaha	Wenaha River	13.6	10
		13.9	10
		14.7	9
		16.1	10
		16.6	11
		17.3	10
		18.3	10
		18.8	10
		19.3	10
		19.6	10
11. Minam	Minam River	18.5	13
	Little Minam River	0.5	11
		1.0	12
		2.8	11
		3.3	11
		3.8	12
12. Lookingglass	Lookingglass Creek	0.5	9
		2.5	10
		3.0	9
		3.7	15
		7.3	22
		10.0	20
13. Middle Grande Ronde	Indian Creek	5.3	21
		11.0	10
		12.0	10
		16.5	10
		17.0	3
	Little Indian Creek	2.0	10
	Shaw Creek	3.5	5
14. Catherine	Catherine Creek	26.0	4
		27.5	6
		29.5	3
	Corral Creek	0.1	3
	Little Catherine Creek	0.5	3
		1.0	4
		1.9	3
	North Fork Catherine Ck.	0.3	4
		1.1	3
		2.2	4
	South Fork Catherine Ck.	0.3	4
		1.3	3

Management Unit	Sample stream(s)	River mile	Number of samples
		5.0	2
15. Willow	Dry Creek	7.1	23
		8.4	20
		10.2	12
16. Upper Grande Ronde	Meadow Creek	11.5	11
		13.0	5
		14.0	9
		14.5	2
		16.5	10
		17.0	14
17. South Grande Ronde	Fly Creek	5.2	18
		5.7	12
		6.2	13
		6.7	12
		7.2	12
		7.7	12

Table A4. Location and number of tissue samples collected at various sites during 2002 in the Grande Ronde and Imnaha River subbasins to determine the basin-wide genetic characterization of steelhead. Sampling was conducted by ODFW, CTUIR, and NPT biologists and staff.

Management Unit	Sample stream(s)	River mile	Number of samples
1. Lower Imnaha	Cow Creek	5.45	14
		0.97	15
		1.36	16
		1.6	12
		1.97	12
		3.9	10
	Horse Creek	0.63	8
		1.07	12
		1.46	10
		2.25	9
		2.78	11
		3.52	10
		3.9	10
	Lightning	3.47	15
		9.37	13
		9.89	15
		8.26	14
		7.6	14
		6.86	13
		6	11
		9.89	13
		5.27	12
2. Upper Imnaha	Gumboot Creek	0.29	11
		0.71	18
		1.37	14
		2.09	10
		2.83	12
		3.45	13
		4.73	13
3. Zumwalt	Camp Creek	1.47	11
		1.82	10
		2.36	11
		2.75	9
		3.1	12
		4.04	11
		4.34	8
		1.47	11
4. Big Sheep	Big Sheep Creek	38.88	1
		38.46	26
		36.62	17
		36.14	11
		30.17	15
		29.59	11
		26.72	12
		26.08	7
5. Joseph	Chesnimus Creek	13.66	12
		14.67	13
		15.43	14
		15.64	11
		0.04	16
	Devil's Run Creek	0.52	14
		1.28	17
		13.66	12
	Elk Creek	0.28	13
		0.91	16
		1.53	10
		2.11	11
6. Prairie Creek	Prairie Creek	1.0	10

Management Unit	Sample stream(s)	River mile	Number of samples
		1.5	12
		2.5	12
		3.2	12
		3.8	11
		4.5	11
		5.5	1
	Tributary at rm 4	--	11
7. Lower Grande Ronde	Mud Creek	14.5	10
		15.0	23
		15.5	17
		16.0	16
		17.5	3
8. North Wallowa	Whiskey Creek	1.5	20
		2.5	10
		2.8	10
		4.5	12
		5.5	13
		8.5	14
9. South Wallowa	Lostine River	0.5	11
		1.0	14
		5.2	12
		6.2	15
		9.0	11
		9.5	7
		10.0	7
10. Wenaha	Wenaha River	13.3	10
		13.8	10
		14.9	10
		16.2	10
		16.5	10
		17.3	10
		18.0	10
		18.5	10
11. Minam	Minam River	18.5	11
	Little Minam River	0.5	11
		1.0	12
		1.5	8
		2.3	11
		3.3	15
		3.8	12
12. Lookingglass	Lookingglass Creek	0.5	8
		2.5	9
		3.0	11
		3.7	10
		7.3	21
		10.0	10
	Little Lookingglass Creek	--	10
13. Middle Grande Ronde	Indian Creek	5.3	14
		11.5	13
		12.0	13
		16.5	12
	Little Indian Creek	2.0	11
	Shaw Creek	3.5	13
14. Catherine	Catherine Creek	26.0	7
		27.5	7
		29.5	6
	Corral Creek	0.1	6
	Little Catherine Creek	0.5	7
		0.6	6
		0.7	7
	North Fork Catherine Ck.	0.3	7
		1.1	7
		1.8	6
	South Fork Catherine Ck.	0.3	7

Management Unit	Sample stream(s)	River mile	Number of samples
		0.8	7
15. Willow	Dry Creek	8.0	15
		8.5	16
		9.0	13
		10.2	35
16. Upper Grande Ronde	Meadow Creek	12.0	9
		13.0	10
		14.0	13
		15.5	9
		16.0	11
		16.5	7
		17.0	11
		17.5	10
17. South Grande Ronde	Fly Creek	5.2	14
		5.7	13
		6.2	13
		6.7	11
		7.2	13
		7.7	13

APPENDIX B

Volk 1999. Otolith strontium:calcium Ratios in Four Populations of Anadromous and Resident *Oncorhynchus mykiss* from Eastern Oregon

Otolith strontium:calcium Ratios in Four Populations
of Anadromous and Resident *Oncorhynchus mykiss* from Eastern Oregon

Eric C. Volk
Washington Department of Fish and Wildlife
Otolith Laboratory

Submitted to Oregon Department of Fish and Wildlife
In fulfillment of contract # 48090861

30 August, 1999

Introduction

Nearly a decade ago, Kalish (1990) demonstrated that strontium abundance measured at the otolith core could effectively discriminate between the progeny of anadromous and freshwater resident female salmon. Disparate Sr/Ca levels characteristic of fresh and sea water (Rosenthal et al., 1970) are presumably incorporated into developing ova during yolk deposition, then deposited in the embryonic otolith, which begins growing well before hatching in salmonids. Rieman et al. (1994) found that Sr/Ca values in the otolith primordia of known anadromous sockeye salmon *(Oncorhynchus nerka)* progeny were significantly higher than those from known fresh water resident females, however, they also reported that elevated Sr/Ca values in freshwater systems may blur the distinction between progeny of resident and anadromous females. Volk et al. (in press) presented results of controlled mating experiments between anadromous and fresh water resident coho salmon *(Oncorhynchus kisutch)* and sockeye salmon, as well as several case studies supporting both of these conclusions. They also showed evidence that freshwater entry and residence time of the gravid female with respect to egg development may influence otolith core Sr/Ca valUes of their progeny. In combination, all of these studies suggest that where freshwater Sr/Ca values are not unusually high, it is generally possible to distinguish anadromous from resident salmonid progeny on the basis of their otolith core Sr/Ca values.

The purpose of this study was to examine otolith core and freshwater zone Sr/Ca values for four groups of steelhead *(Oncorhynchus mykiss)* representing anadromous and freshwater resident life histories. The four types are 1) anadromous adults, 2) freshwater resident adults spawned by anadromous parents, 3) seaward migrating smolts spawned by anadromous parents and 4) freshwater resident adults spawned by resident fish. Data from otolith core analyses will be evaluated to test the hypothesis that anadromous progeny have elevated Sr/Ca values as a result of maternal parent associations with sea water. Data from the freshwater zones of otoliths will determine whether or not high freshwater strontium levels could be responsible for elevated core Sr/Ca values.

Materials and Methods

Specimens

Juvenile and adult O. *mykiss* were coliected from five locations which represented four different life history types; 1) known anadromous adults (Wallowa and Lsheep), 2) Adults which were spawned by anadromous adults but have residualized in fresh water (residual BC), 3) juvenile smolts captured during their migration to sea (WH) and 4) freshwater resident adults spawned by resident parents (Balm Cr. Rb). Otoliths were removed from all fish and stored in vials prior to analyses.

Otolith Preparation and Analysis

Otoliths (sagittae) were removed from vials, placed sulcus-side down on glass plates, surrounded by rubber molds and cast with a two-part polyester resin forming a solid block encasing several otoliths. The blocks were cut apart so that each otolith was encased within its own resin block. Individual blocks were lapped with 500 grit silicon carbide paper on a rotating wheel until the primordial region of the otolith was exposed in hemi-section. Each sample was polished using 1 µm. aluminum oxide in a water slurry followed by 1/4 µm. diamond paste on a felt pad and ultrasonically cleaned. Only de-ionized water was used in these preparation steps. Prepared specimens were coated with a 250 Å layer of conducting carbon.

Wavelength dispersive elemental analyses were performed with a four spectrometer JEOL model 733 electron microprobe. The bremssstrahlung radiation inherent to electron microprobe analysis limits the study of fish otolith chemistry to a small suite of elements which have the most abundant concentrations, typically Ca, Sr, Na, K and S (Gunn et al., 1992; Kalish1989,1990; Rieman et al., 1994; Volk et al., in press). In this study, we limited our analyses to strontium and calcium.

Data was collected using an accelerating voltage of 15 Kv, a beam amperage of 15 nA and a beam diameter of 10 µm, giving a beam power density of 2.9 $\mu W/\mu m^2$. Counting times for the minor elements were 120 seconds, with calcium, x-rays collected until 40,000 counts (0.5% relative statistical error) was reached, usually 14-16 seconds. Data was collected during several microprobe sessions. Background intensities were collected only on the first analysis of each session and these count rates retained for all subsequent analyses. This is permitted as the background count rate is dominated by Ca-C-O, and is insensitive to fluctuations in minor element concentrations. Raw x-ray intensities were corrected for matrix effects using either the Bence-Albee (Bence and Albee,1968; Albee and Ray, 1970) or CITZAF (Armstrong, 1988) routines.

Gunn et al. (1992) emphasized the importance of beam conditions in WDS analyses of otoliths and demonstrated their role in data quality. They showed that to minimize specimen damage during analysis, a beam power density of less than 3 uW/pm2 was required. Prior to the initiation of this study, we evaluated several analytical conditions by monitoring count rates of Ca and the minor elements in an otolith as a function of beam dwell time. Though pits were always created at analysis sites under our analytical conditions, x-ray intensities determined at five-second intervals for 120 seconds revealed no systematic change for any element except Ca, which increased by 7%. To eliminate any potential error on the concentration of Ca due to beam heating, Ca intensities were always determined during the first 14-16 seconds of beam exposure on the sample, a time period over which our tests showed no Ca intensity modification.

Salmonid otoliths have a multi-nucleated core region typically enclosed by a very dark band. In polished sections, nuclei appear as "pits" creating a very uneven surface, not ideal for microprobe analyses. Analyses from the otolith core region were taken one beam diameter (~10 µm) from an obvious pit at the center of the otolith, which represents embryonic life history. We assume that otolith chemistry is largely dependant upon the yolk chemistry derived from the female parent at this stage (Kalish,1990) since egg membranes are fairly impermeable to large ions after fertilization. Though no attempt was made to standardize the position of these points with respect to spatial axes of the otolith, their position relative to nuclei was consistent and represented a specific time in the life history of the juvenile fish. Any sections which did not include the core region were excluded from analyses. Analyses corresponding to freshwater life history of the juvenile fish were obtained approximately 450 □m. from the otolith core along the dorsal axis of the sectioned otolith. For each location, two replicate analyses were performed.

On two otoliths, we conducted analysis transects every 25 µm beginning at the otolith core and ending nearly 400 µm distant on the dorsal otolith axis. This helped verify that freshwater zone analyses were sufficiently far from the core to sample the effects of freshwater chemistry only. Where replacement of aragonite with vaterite was extensive (see below), analyses were conducted at any site where aragonite could be located. On a sample of specimens from all groups and including both freshwater and core regions, up to six replicate analyses were conducted to examine how mean values changed with the addition of more analyses.

Results and Discussion

Analysis transects were conducted on two specimens to document changes in otolith Sr/Ca values as the maternally-derived yolk sac was absorbed by the juvenile. An example transect analysis is shown in Figure 1. Both specimens showed a pattern of fairly high Sr/Ca values near their otolith cores (0.90-1.20), characteristic of their anadromous maternal parentage, followed by steep declines, reaching values less than 0.50 at 350-375 µm distant from the otolith cores (Figure 2). Final analyses for both specimens were very similar to those taken from the freshwater regions of other specimens in the same group (Table 1). Results from Volk et al. (in press) showed a similar pattern, where mean otolith Sr/Ca values taken at the core region of anadromous coho salmon progeny dropped from 1.57 to 0.50 at an analysis location approximately 400 µm. from the core. Thus, by conducting freshwater otolith analyses at least 400 um beyond the otolith core, we can be confident that they reflect the influence of environmental water chemistry rather than the continued effect of maternally derived yolk material.

A number of specimens in this study exhibited extensive replacement of aragonite with vaterite, a less common form of calcium carbonate. A typical vateritic otolith is shown in Figure 3 where a small region of aragonite was found near the center of the otolith, with the remainder composed mainly of vaterite. While it is very rare for vaterite to occupy the central regions of salmonid otoliths near the core, it is quite common distally in juvenile and adult salmon, particularly with hatchery-produced fish (Volk et al., in prep). Strontium analyses are greatly impacted by the presence of vaterite because its crystal lattice accommodates very little of the Sr^{2+} ion, an amount below the detection limits of the electron microprobe (Volk et al., in press). Thus, where large amounts of vaterite were present, it may not have been possible to sample otolith aragonite 400 µm. from the core. On these specimens, we conducted analyses as far out on the otolith as possible while remaining in aragonite. If we were not able to achieve a distance of at least 350 µm, we did not conduct a freshwater analysis, since it would clearly not just represent the influence of water chemistry on the juvenile otolith. Otolith Sr/Ca data taken from otolith cores and freshwater regions were similarly distributed among groups one, two and three, all of which were spawned by anadromous female parents (Figure 4, Appendix 1). In each group, mean Sr/Ca values for freshwater otolith regions typically ranged between 0.0 and 0.40, with one or two values ranging between 0.55 and 0.90 from each group. Sr/Ca values taken at the otolith core ranged between 0.65 and 1.50 (Figure 4). Mean otolith core and freshwater zone Sr/Ca values for each of the four *O. mykiss* groups is shown in Table 1. Mean core values were significantly greater than mean freshwater zone values for all three groups (ANOVA, $P < 0.05$, Table 2) but mean Sr/Ca values from either the otolith core region or the freshwater zone were not significantly different among the three groups (ANOVA, $P > 0.05$, Table 2).

The mean otolith core Sr/Ca values for the three groups of fish known to have originated from anadromous females were similar to those from three other anadromous summer steelhead populations in Washington State (Wallace River Hatchery, mean Sr/Ca = 1.04, n=16; Skamania Hatchery, mean Sr/Ca = 1.29, n=19; Wells Hatchery, mean Sr/Ca = 1.23, n = 17) and consistent with their anadromous origin (Volk et al., in press). Another possible explanation for high core strontium could be that strontium is abundant in these freshwater systems, which confuse the distinction between anadromous and resident progeny (Rieman et al., 1994; Volk et al., in press). When this occurs, even resident fish would produce progeny with high otolith core Sr/Ca values, in spite of their freshwater life history. While we do not yet have water samples to document this directly, freshwater otolith zone analyses show that these freshwater environments do not typically produce high otolith Sr/Ca, and could not explain the high otolith core Sr/Ca values observed. The similar data from these three groups is consistent with low freshwater strontium abundance and maternal associations with an environment higher in strontium, most likely the sea.

Although the distributions of core and freshwater values in groups one two and three were very distinct (Figure 4), in each group there was one or two specimens where the freshwater zone Sr/Ca value

overlapped with the distribution of higher core values. But, core Sr/Ca values were still at least 30% greater than those recorded in the freshwater zone of these specimens. Also, each of the samples with elevated freshwater zone Sr/Ca values showed extensive aragonite replacement with vaterite, with the latter sometimes completely surrounding the otolith core. Though we tried to conduct analyses at least 400 µm. from the core, we fell short of this goal in these specimens due to the presence of vaterite. It is possible that the analyses were not sufficiently far from the otolith core to be out of the influence of maternally derived yolk chemistry and contributed to elevated freshwater zone Sr/Ca values in these "outliers". If they were eliminated from the data, there would be no overlap in the distributions of core and freshwater zone Sr/Ca values

Otolith core and freshwater zone Sr/Ca values were widely distributed for group 4 and overlapped extensively (Fig. 4). Mean core and mean freshwater Sr/Ca values were not significantly different in these freshwater resident fish (test; $t = 1.36$, $df = 43$; $P > 0.05$). Mean otolith core Sr/Ca values were significantly lower than those from the other three groups while mean freshwater zone Sr/Ca values were significantly higher (ANOVA, $P < 0.01$, Table 2).

The similar mean otolith core and freshwater zone Sr/Ca values for samples from group number four is typical of resident populations because fish are usually not migrating between habitats with radically different strontium concentrations as are anadromous fish. Thus, for both maternally derived otolith core chemistry analyses and those representing juvenile life in freshwater, strontium availability is often roughly the same. The fact that freshwater otolith zone Sr/Ca values are greater than those from the other three groups suggests that water Sr/Ca values from the group four collection location are elevated with respect to the other three. There is a very close relationship between water and otolith Sr/Ca values for resident fish ($Y = 0.37 X - 0.41$, $r^2 = 90$; Volk et al., unpub. data). From this relationship and our measured otolith freshwater zone Sr/Ca values, we predict a water Sr/Ca value of 2.80 from the group number four collection location. However, there will be a long interval between fish and water sample collection and we have no idea how seasonally stable this system is with respect to strontium and calcium.

On some specimens, more than two replicate analyses were conducted to test the effects of increased analyses on mean values. We calculated absolute and percentage change between our two-replicate means and each subsequent mean created by up to four additional analyses. Results are shown in figure 5. The absolute scalar difference between calculated means ranged from 0.0 to 0.15 while the percentage change in calculated means ranged between 0 and 33%. There appeared to be no systematic error in terms of which direction the change occurred Mean Sr/Ca values changed little with the addition of analyses, and means were significantly different only when the original two-replicate mean was compared with those calculated from six replicates (Table 3). Even then, the difference in mean values was small.

Because we expect otoliths to be spatially heterogeneous with respect to calcium and strontium, it is clearly an advantage to have as many replicate analyses from one life history region as possible to adequately sample this variability Performing only two replicate analyses was partially an economic decision, allowing more individuals to be analyzed with available funds. Nevertheless, it would appear from this small sample that two replicates were adequate for the conclusions drawn. Though some percentage differences in means calculated from successive analysis additions were as high as 33%, these were associated with low Sr/Ca values where a small absolute change registers as a large percentage change (Figure 5). Most percentage changes were much smaller and the mean absolute change was .07 or less. Furthermore, a comparison of calculated means from two, three, four, five or six replicate analyses among all groups and otolith analysis locations showed that means only changed significantly in the specimens with six analyses (Table 3). Thus, while we agree that more analyses are always desirable to describe the inherent variability of otolith chemistry, our test indicates that no change in conclusions would have occurred had specimens received more replicate analyses in this study.

Conclusions

1. For sample groups one, two and three, otolith core Sr/Ca data was similar to that found in other summer steelhead populations and is consistent with the hypothesis that the fish were spawned by anadromous female parents. The low Sr/Ca values from freshwater otolith zones in these groups suggests that water Sr/Ca values are too low to explain the high core values if maternal parents were not

anadromous. The few individuals from each group that exhibited high freshwater Sr/Ca values, overlapping with the distribution of core values, could be explained by the extensive replacement of aragonite with vaterite. This may have prevented freshwater analyses from being conducted sufficiently far from the otolith core to escape the influence of maternally derived yolk material on otolith chemistry.

2. For sample group four, where individuals are known to be the progeny of freshwater resident females, results show that otolith core Sr/Ca values were lower than those recorded for anadromous fish in the other groups, but still overlapped with those distributions. The fact that freshwater zone Sr/Ca values were higher than those recorded from other groups and statistically similar to the core values in group four indicates that Sr/Ca values are higher in this water than the other three freshwater systems in this study. Water samples should be collected to verify this. Higher freshwater Sr/Ca values are likely to cause confusion in analyses with summer steelhead because summer run fish typically display lower otolith core Sr/Ca values than other anadromous species and stocks due to their extended freshwater residence prior to spawning (Volk et al., in press).

Literature Cited

Aibee, A.L. and L. Ray. 1970. Correction factors for electron probe microanalysis of silicates, oxides, carbonates, phosphates and sulfates. Analytical Chemistry, vol. 42, 1408-1414.

Armstrong, J.T. 1988. Bence Albee after 20 years: Review of the accuracy of the cc-factor correction procedures for oxide and silicate minerals. In: Microbeam Analysis. D. E. Newbury (ed.).

Bence, A.E. and A.L.Albee. 1968. Empirical correction factors for the electron microprobe analysis of silicates and oxides. J Geol. 76:382-403.

Gunn, J.S, I.R. Harrowfield, C.H. Proctor and R.E. Thresher. 1992. Electron Probe Micro-Analysis of Fish Otoliths-Evaluation of Techniques for Studying Age and Stock Discrimination. J. Exp. Mar. Biol. Ecol. 158: 1-36.

Kalish, J.M. 1989. Otolith Microchemistry; Validation of the Effects of Physiology, Age and Environment on Otolith Composition. Journal of Experimental Marine Biology and Ecology. 132: 151-178.

Kalish, J.M. 1990. Use of Otolith Microchemistry to Distinguish the Progeny of Sympatric Anadromous and Non-Anadromous Salmonids. Fish. Bull. 88: 657-666.

Palmer, M.R. and J.M.Edmond. 1989. The Strontium Isotope Budget of the Modern Ocean. Earth and Planetary Science Letters, 92 (1989) 11-26.

Rieman, B.E., D.L.Myers and R.L.Nielsen.1994. Use of Otolith Microchemistry to Discriminate Oncorhynchus nerka of Resident and Anadromous Origin. Can. J.Fish. Aquat. Sci. 51: 68-77.

Rosenthal, H.L., M.M.Eves and O.A.Cochran. 1970. Common strontium concentrations of mineralized tissues from marine and fresh water animals. Comp Biochem. Physiol. 32: 445-450.

Volk, E.C, A Blakley, S L. Schroder and S.M. KueMner. Otolith Chemistry Reflects Migratory Characteristics of Pacific Salmonids: Using Otolith Core Chemistry to Distinguish Maternai Associations with Sea and FreshWaters. In Press. Fisheries Research.

Acknowledgements

We thank Jeff Grimm, Dana Anderson and Lang Nguyen, Washington Department of Fish and Wildlife Otolith Laboratory, for their skillful preparation of otolith sections for the microprobe. This study was funded under contract no. 48090861 with ODF&W.

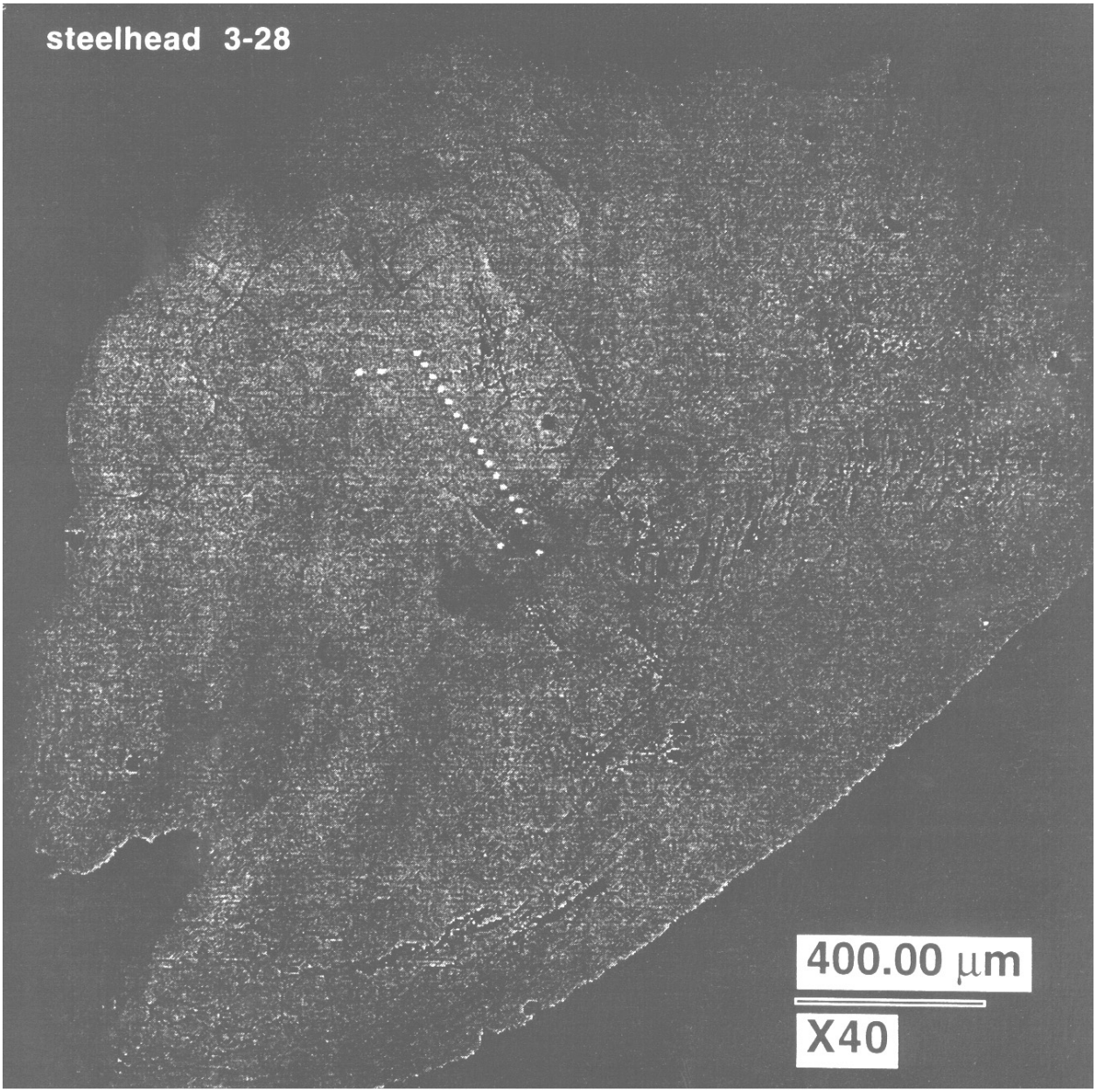

Figure 1. Back-scattered electron image of steelhead smolt otolith. White spots show two core analyses centrally, two freshwater zone analyses distally and a 400 µm transect of points in 25 µm increments. The transect originates at the otolith core and follows a dorsal axis distally.

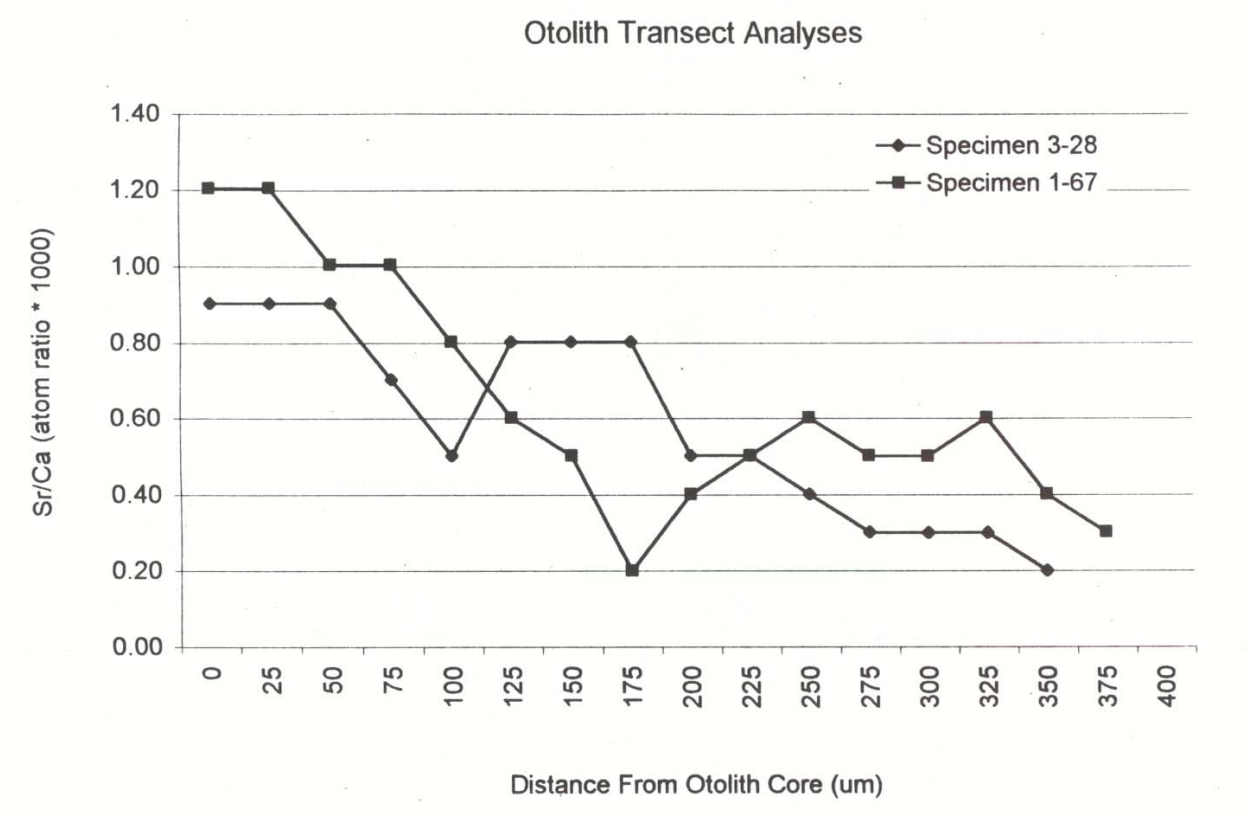

Figure 2. Otolith Sr/Ca values taken at 25 µm. Intervals along a transect running from the otolith core to a point nearly 400 µm distant.

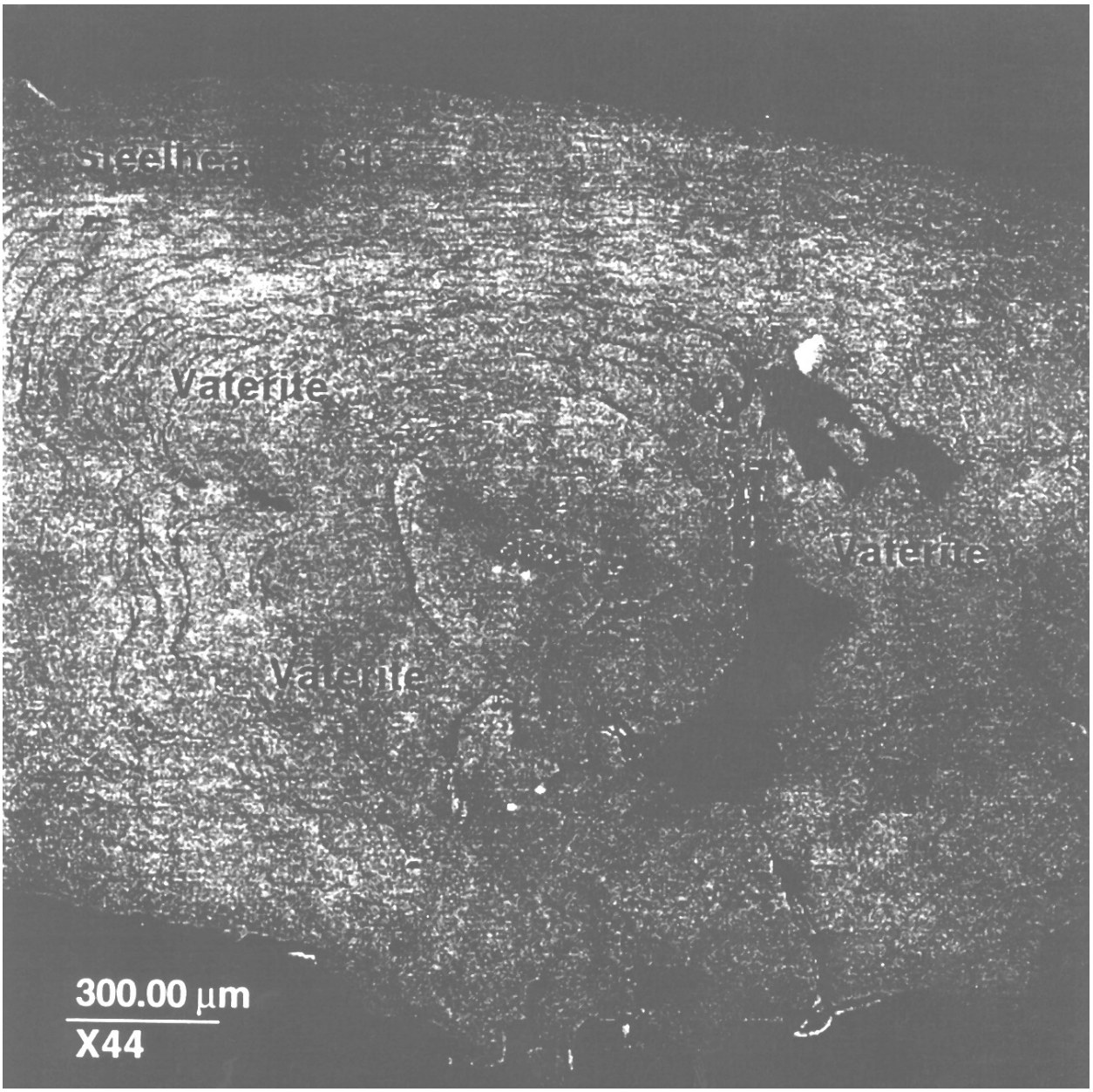

Figure 3 A mostly vateritic steelhead smolt otolith. Two analyses conducted near the otolith core are in aragonite (central pair of white spots). The central aragonite region, about 600 µm in diameter, is surrounded by vaterite where freshwater zone analyses (distal pair of white spots) were located.

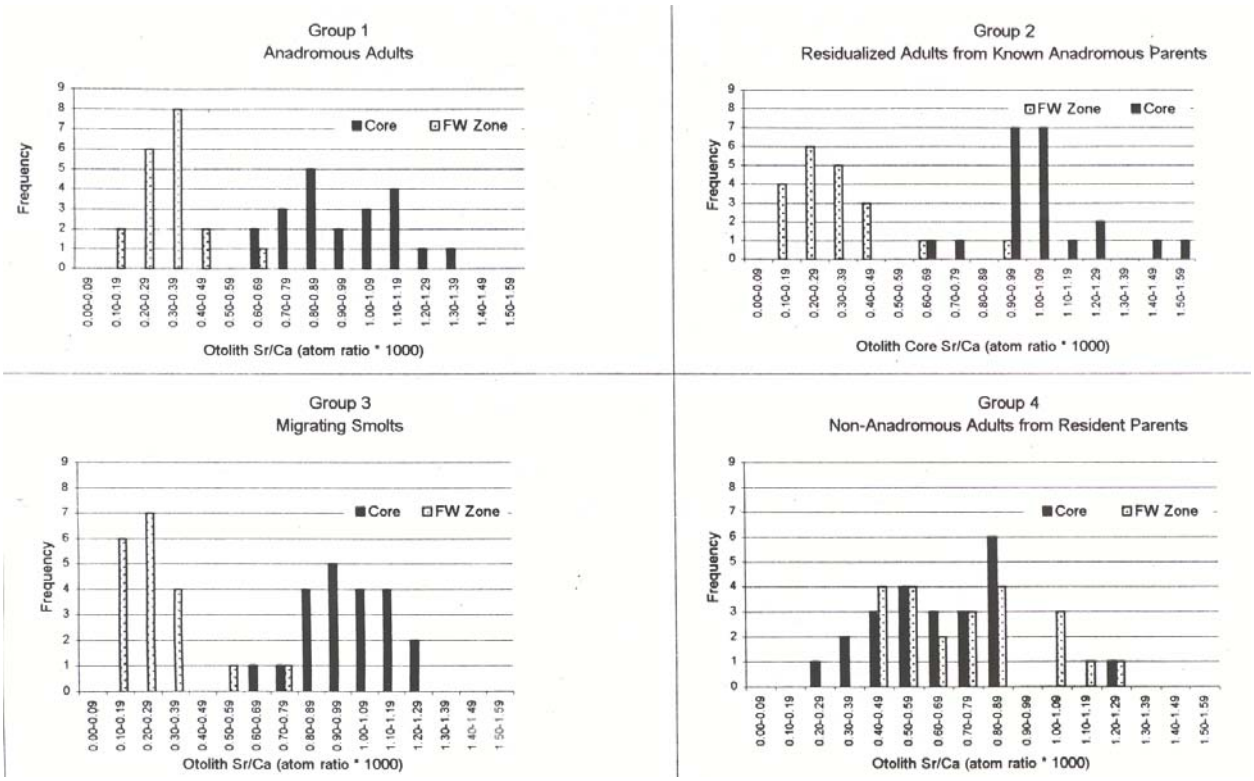

Figure 4. Frequency distributions of otolith core and freshwater zone Sr/Ca values for four populations of anadromous and resident *O. mykiss.*

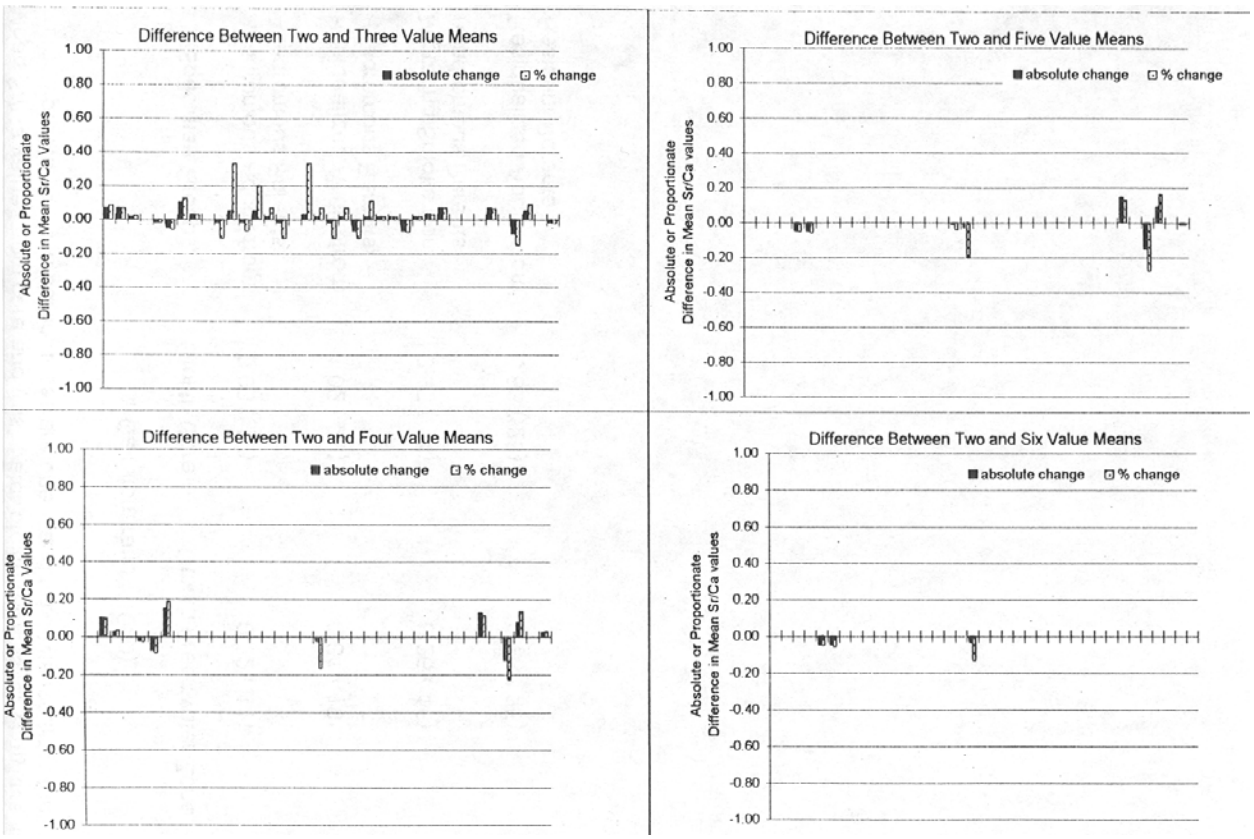

Figure 5. Absolute and percentage difference between means of first two replicate Sr/Ca analyses and those calculated with from one to four additional analyses.

Table 1. Mean Sr/Ca values from core and freshwater otolith regions for *O. mykiss* in each of four groups. Numbers in parentheses are one standard deviation from the mean.

		Sr/Ca (atom ratio · 1000)	
	Specimen Group	Otolith Core	Otolith Freshwater Zone
1	Anadromous adults from anadromous parents.	0.93 (0.19)	0.22 (0.11)
2	Residualized adults from anadromous parents.	1.02 (0.20)	0.31 (0.19)
3	Migrating smolts from anadromous parents.	0.97 (0.14)	0.26 (0.15)
4	Residualized adults from resident parents.	0.63 (0.23)	0.73 (0.25)

Table 2. Results of ANOVA and SNK multiple range comparisons for strontium analyses from otolith cores and freshwater zones in each sample group. The letters C and FW refer to analyses at the otolith core and freshwater zones respectively.

ANOVA	Effects	F	df	P
Strontium/Calcium	Treatment	63.84	7	< 0.01
	Error		158	

Results of SNK tests: C2 C3 C1 > FW4 C4 > FW2 FW3 FW1

Table 3. Comparison of mean otolith Sr/Ca values calculated from our first two analysis replicates and up to four additional analyses. Each comparison of means with n samples was evaluated for significance. Specimens receiving additional analyses were randomly chosen among all groups and otolith analysis regions, thus mean values do not represent specific life history attributes.

Rep. 1-2	Rep. 1-3	Rep. 1-4	Rep. 1-5	Rep. 1-6	n	t	Significance (P= 0.05)
0.58	0.59				37	1.56	NS
0.74		0.76			13	0.88	NS
0.66			0.66		9	0.12	NS
0.69				0.65	4	2.84	*

Appendix 1a. Two replicate analyses and mean Sr/Ca values for all specimens in each group and otolith region. Values are atom ratios*1000. **Otolith Core** Sr/Ca values.

Group 1. Anadromous adults from anadromous parents.					Group 2. Mature residualized fish from anadromous parents.					Group 3. Smolts from anadromous parents.					Group 4. Resident fish from resident parents.			
Specimen	Rep. 1	Rep. 2	Mean		Specimen	Rep. 1	Rep. 2	Mean		Specimen	Rep. 1	Rep. 2	Mean		Specimen	Rep. 1	Rep. 2	Mean
1	0.7	0.6	0.65		1	0.9	1.1	1.00		25	1.1	1.1	1.10		1	0.5	0.4	0.45
6	0.7	1.0	0.85		2	1.0	0.8	0.90		26	0.9	1.0	0.95		2	0.3	0.4	0.35
13	1.0	1.1	1.05		3	1.4	1.4	1.40		27	0.8	1.0	0.90		3	1.2	1.3	1.25
32	1.1	1.2	1.15		4	1.0	0.9	0.95		28	0.6	0.7	0.65		4	0.5	0.5	0.50
34	0.8	0.7	0.75		5	1.0	1.0	1.00		29	1.1	1.1	1.10		5	0.6	0.6	0.60
35	0.8	0.8	0.80		6	1.2	1.2	1.20		30	0.8	0.9	0.85		6	0.6	0.8	0.70
39	1.0	0.9	0.95		8	1.2	1.0	1.10		31	1.1	1.2	1.15		7	0.2	0.3	0.25
45	0.8	0.8	0.80		9	1.0	1.1	1.05		32	1.1	1.0	1.05		8	0.3	0.3	0.30
46	0.9	1.4	1.15		10	1.0	0.9	0.95		33	1.1	1.0	1.05		9	0.6	0.5	0.55
47	1.2	1.2	1.20		11	0.9	1.1	1.00		34	1.0	1.2	1.10		10	0.7	0.5	0.60
49	1.1	0.9	1.00		13	0.8	1.0	0.90		36	0.7	0.7	0.70		11	0.8	0.8	0.80
50	0.8	0.7	0.75		14	1.0	0.9	0.95		37	1.2	1.0	1.10		12	0.6	0.6	0.60
52	1.3	1.3	1.30		15	0.6	0.7	0.65		38	0.9	0.8	0.85		13	0.5	0.6	0.55
54	0.6	0.7	0.65		16	0.7	0.7	0.70		39	1.0	1.0	1.00		14	0.8	0.8	0.80
56	0.7	0.7	0.70		17	0.9	0.9	0.90		40	0.9	1.1	1.00		15	0.8	0.8	0.80
59	0.8	1.1	0.95		18	0.9	1.0	0.95		41	1.0	0.9	0.95		16	0.4	0.5	0.45
62	0.9	0.8	0.85		19	1.0	1.1	1.05		42	0.8	0.9	0.85		17	0.4	0.4	0.40
63	1.1	1.1	1.10		20	1.2	1.2	1.20		43	1.0	0.9	0.95		18	0.8	0.9	0.85
64	1.0	1.0	1.00		21	1.6	1.4	1.50		44	0.8	0.9	0.85		19	0.6	0.5	0.55
66	0.8	0.8	0.80		22	1.0	1.0	1.00		45	1.0	1.2	1.10		20	0.8	0.7	0.75
67	1.1	1.1	1.10		23	1.0	1.0	1.00		47	0.9	1.0	0.95		21	0.7	0.7	0.70
															23	0.8	0.8	0.80
															24	0.9	0.8	0.85
															22	0.6	0.6	0.60

Appendix 1b. Two replicate analyses and mean Sr/Ca values for all specimens in each group and otolith region. Values are atom ratios*1000. **Otolith Freshwater Zone** Sr/Ca values (Does not include vaterite analyses).

Group 1. Anadromous adults from anadromous parents.				Group 2. Mature residualized fish from anadromous parents.				Group 3. Smolts from anadromous parents.				Group 4. Resident fish from resident parents.			
Specimen	Rep. 1	Rep. 2	Mean	Specimen	Rep. 1	Rep. 2	Mean	Specimen	Rep. 1	Rep. 2	Mean	Specimen	Rep. 1	Rep. 2	Mean
1	0.2	0.3	0.25	1	0.2	0.4	0.30	25	0.2	0.3	0.25	1	0.3	0.5	0.40
6	0.1	0.3	0.20	2	0.4	0.4	0.40	26	0.2	0.3	0.25	2	0.4	0.5	0.45
13	0.2	0.1	0.15	3	0.2	0.3	0.25	27	0.4	0.3	0.35	4	0.5	0.6	0.55
32	0.1	0.2	0.15	4	0.1	0.1	0.10	28	0.2	0.2	0.20	5	0.8	0.6	0.70
34	0.2	0.3	0.25	5	0.2	0.2	0.20	29	0.8	0.6	0.70	6	0.5	0.5	0.50
35	0.2	0.3	0.25	6	0.3	0.4	0.35	30	0.2	0.1	0.15	7	0.6	0.5	0.55
39	0.1	0.2	0.15	8	0.8	0.5	0.65	33	0.2	0.3	0.25	8	0.4	0.4	0.40
45	0.2	0.3	0.25	9	0.1	0.4	0.25	34	0.2	0.2	0.20	9	0.9	0.8	0.85
46	0.2	0.1	0.15	10	0.3	0.2	0.25	36	0.2	0.2	0.20	10	0.6	0.6	0.60
47	0.3	0.3	0.30	11	0.1	0.3	0.20	37	0.4	0.3	0.35	11	0.5	0.5	0.50
49	0.3	0.3	0.30	13	0.1	0.2	0.15	38	0.3	0.2	0.25	12	1.0	1.1	1.05
50	0.2	0.2	0.20	14	0.1	0.2	0.15	39	0.1	0.2	0.15	13	1.0	1.0	1.00
54	0.1	0.1	0.10	15	0.3	0.4	0.35	40	0.3	0.3	0.30	14	0.8	0.9	0.85
56	0.1	0.1	0.10	16	0.4	0.2	0.30	41	0.1	0.2	0.15	15	0.6	0.6	0.60
59	0.3	0.2	0.25	17	0.2	0.4	0.30	42	0.1	0.1	0.10	16	0.4	0.4	0.40
62	0.1	0.2	0.15	18	0.3	0.5	0.40	43	0.1	0.1	0.10	17	0.8	0.7	0.75
63	0.2	0.3	0.25	19	0.4	0.4	0.40	44	0.1	0.1	0.10	18	1.0	1.1	1.05
64	0.6	0.6	0.60	20	1.0	0.8	0.90	45	0.7	0.4	0.55	19	0.7	0.8	0.75
66	0.1	0.2	0.15	21	0.2	0.2	0.20	47	0.4	0.2	0.30	20	1.1	1.1	1.10
				23	0.1	0.1	0.10					21	0.9	0.8	0.85
												23	1.3	1.2	1.25
												24	0.8	0.8	0.80
												22	0.9	1.0	0.95