FISHERY RESEARCH

An Updated Study Design and Statistical Analysis of Idaho Supplementation Studies

IDFG Report Number 05-35
August 2005

An Updated Study Design and Statistical Analysis of Idaho Supplementation Studies

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Prepared for:

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Project Numbers 1989-098-00, 1989-098-01, 1989-098-02, 1989-098-03
Contract Numbers 00020863

IDFG Report Number 05-35
August 2005

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August 26, 2005

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Dear Mr. Fritsch:

Enclosed you will find the Updated Study Design and Statistical Analysis of the Idaho Supplementation Studies as requested by the Council. This document is intended to satisfy the concerns of the Independent Scientific Review Panel (ISRP) about the Idaho Supplementation Studies (ISS) following their review of the Evaluation and Statistical Review of the Idaho Supplementation Studies (Lutch et al. 2003).

This updated study design represents the cooperative input of all four agencies that make up the ISS: the Idaho Department of Fish and Game, the Nez Perce Tribe, the Shoshone-Bannock Tribes, and the U.S. Fish and Wildlife Service. Representatives from each agency have signed the attached document thus indicating their support and commitment to the findings and recommendations contained within.

The development of this updated study design and additional statistical treatments of ISS data address specific ISRP comments and Council recommendations contained in a memo from you to the other Fish and Wildlife Committee Members dated July 9, 2003. Specific recommendations included,

1. the development of a final design for Phase III,
2. expand carcass collection to all ISS study streams to better estimate the effects on production by hatchery strays,
3. evaluate DNA-based assessment in ISS treatment and control streams to further identify parental contribution from the three classes of adults in Phase III analysis.

The updated study design contains five distinct sections. A brief summary and the salient points from each follow.

In the first section, the original objectives and research questions posed in the original study design (Bowles and Leitzinger 1991) and the data collected to date to answer them are reviewed. Based on this review, we conclude that there will be sufficient data in the form of redd counts from nearly all ISS study streams and juvenile production estimates for almost half of the streams to evaluate the effects of supplementation on natural production. Based on these estimates of production and productivity, is should also be possible for us to provide recommendations on the usefulness of supplementation as a recovery tool. However, due to low adult escapement early in the program and the resulting inability to provide all the prescribed treatments, it will be difficult to assess the relative effectiveness of the various supplementation strategies (e.g., treating streams with parr, pre-smolts, or smolts).

Section two contains the updated study design and statistical methodologies proposed for implementation through the end of Phase III. Two statistical approaches to the analysis of ISS data are presented. The first is a mixed model ANOVA as described by Bowles and Leitzinger (1991), which will remain the primary analysis technique to evaluate ISS data. Lutch et al. (2003) demonstrated that this method is statistically robust and sensitive to treatment responses. Continuing the expanded carcass collection protocol initiated in 2003 will enhance this analysis. A regression analysis is the second technique developed, and was completed at the request of the ISRP. The regression approach will provide an excellent companion analysis to the mixed model ANOVA. Finally, a graphical technique is presented for the evaluation of juvenile migration and survival data. This may be the most appropriate approach for use on these data sets ***at the time of this writing***. However, as more Phase III data become available, regression (and possibly ANOVA) should become appropriate analysis tools for these data as well.

The third section contains information on how juvenile migration and survival data were compiled for use in developing the regression analysis. Information from the additional carcass collection effort in 2003 is also presented here. This information will be a key component in future analyses of the effect of hatchery straying on production in ISS study streams.

Section four contains supporting information on how the regression analysis was developed to predict redd density, juvenile migration estimates, and juvenile survival as recommended by the ISRP. Variables included in the models are treatment proportion, stray rate, baseline redd production, and stream groupings from Lutch et al. (2003). The result of this effort suggests that a regression approach is feasible for the analysis of ISS data, and this analysis can partially adjust for the effects of hatchery straying.

Finally, in section five, the ISRP recommendation to include DNA level parentage tests to evaluate the reproductive success and contribution of natural, ISS hatchery, and general production hatchery adults is addressed. Brief summaries of ISS tissue collection to date and the level of effort necessary to undertake this type of analysis are presented. Streams where sufficient escapement monitoring would be possible to make this type of analysis feasible are then identified, and a generalized study design and

timeframe to address this issue are presented. While we agree parentage tests are powerful tools for investigating the effects of supplementation, additional or an alternate funding source is needed for the ISS program to address this recommendation from the ISRP.

I believe this updated study design satisfies both ISRP and Council concerns about the ISS program and completes the Programmatic Issue 10 Review process. If you have any questions about the updated study design please feel free to contact me at 208-334-3791 or David Venditti at 208-465-8404.

Sincerely,

Virgil Moore
Chief of Fisheries

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ABSTRACT

This paper summarizes a cooperative effort by the Idaho Supplementation Studies (ISS) project to address the second technical review of ISS by the Independent Science Review Panel (ISRP 2003-8) for the programmatic Issue 10 in the Mountain Snake Province. In their review, ISRP concluded that the ISS should extend considerable effort in adjusting their study design by reviewing current data sets and considering DNA assignment tests for evaluating the effects of general production (GP) hatchery straying into ISS study streams. Furthermore, the ISRP recommended that ISS place more emphasis on modeling and point estimation to assess the effects of supplementing Chinook salmon *Oncorhynchus tshawytscha*. In response to the ISRP concerns, the ISS completed the following: 1) we reviewed the remaining data types to determine whether the ISS can meet the original study objectives, 2) we compiled data sets for additional statistical treatment of the ISS, 3) we developed a regression analysis and qualitative graphical approach to modeling the effects of treatment, 4) we pursued DNA parentage analysis as an alternative method for evaluating the effects of GP hatchery straying, and 5) we provided an updated study design through the final study phase. Reviewing project data sets and the research questions defined in the original study design suggest that ample data are available to meet the two main objectives for ISS: evaluating the effects of supplementation on Chinook salmon natural production and evaluating changes in natural productivity due to hatchery treatments. Using these newly compiled data sets, we determined that a multiphased regression analysis is appropriate for modeling the effects of GP hatchery straying and compliments the mixed model analysis. A DNA parentage analysis that more precisely evaluates the effects of stray hatchery Chinook salmon is proposed for the ISS, assuming that the identified study streams are used and the prescribed number of tissue samples for juveniles and adults are collected. However, additional funding sources would be required for such an extensive analysis. Finally, using the findings from reviewing data sets and the results from the statistical analyses, an updated study design through the final study phase is presented.

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INTRODUCTION

The collaborative research project known as the Idaho Supplementation Studies (ISS) was designed to evaluate the benefits and risks of using artificial propagation to supplement populations of Chinook salmon to increase or establish natural production. In the Salmon River subbasin, we are evaluating the effects of supplementation for the purpose of augmenting existing populations (supplementation-augmentation), while in the Clearwater River subbasin supplementation is being evaluated as a restoration tool to reestablish natural production in streams where Chinook salmon have been extirpated (supplementation-restoration). Experimentally, the research examines the response in natural production and productivity of Chinook salmon between treated and control streams over three study phases. Phase I addressed baseline data collection needs concurrent with the construction of locally adapted broodstocks. During Phase II, the supplementation phase, returning ISS adults augmented production in treatment streams by 1) being passed at a maximum prescribed proportion along with natural origin adults to traditional spawning areas to supplement natural production, and 2) being crossed with natural origin adults in hatcheries to produce juveniles that were later out-planted (at various life stages) to natural rearing areas. In Phase III, juvenile treatments are terminated, the remaining adults returning from juvenile ISS releases are allowed to supplement natural production, and production and productivity measures are monitored for one generation after the final adult treatment.

As the project transitions from the supplementation phase (Phase II) to the evaluation phase (Phase III), ISS managers have recognized several challenges to the study design, including straying of general production (GP) hatchery fish, conflicts with other research and management projects, subsequent loss of study streams from the original study design, and the inability to construct broodstocks and maintain prescribed treatments during Phases I and II. These issues were highlighted by the Independent Scientific Review Panel (ISRP) (ISRP 2001–12A) during the 2001 Provincial Review for the Mountain Snake Province as being problematic for the evaluation of ISS. The Northwest Power and Conservation Council (Council), acting on technical review from ISRP, advised the ISS to complete a technical review of the study as part of the Fiscal Year 2002 Programmatic Issues document (Issue 10) to resolve the concerns stated above.

On March 23, 2003, ISS cooperators (Idaho Department of Fish and Game, the Nez Perce Tribe, the Shoshone Bannock Tribe, and the U.S. Fish and Wildlife Service) submitted a response to the Council to address ISRP concerns and recommendations. Through statistical consultation with the University of Idaho, a technical review of the ISS study was completed that included a preliminary statistical treatment of ISS data (Lutch et al. 2003). In this document we demonstrated a coordinated effort in compiling project data across multiple agencies for the purpose of technical project review and determined statistically how GP hatchery straying affects the ISS study. Furthermore, we completed a power and sensitivity analysis to predict outcomes due to loss of study streams and developed a prototype statistical analysis to treat challenges to the ISS study, such as differences in the timing and levels of supplementation treatments.

On May 22, 2003, the ISRP completed their review of the ISS statistical paper (ISRP 2003-8) and accepted the following responses: 1) the statistical prototype certified by Dr. Kirk Steinhorst, University of Idaho, satisfied the recommendation of a written protocol for statistical analysis using an independent statistical team, 2) ISRP agreed with ISS in the need to address general production hatchery and ISS hatchery production straying using carcass data to

estimate the density of hatchery origin strays, and 3) the ISRP agreed that the timetable presented is an appropriate plan for implementation of Phase III of the ISS. However, ISRP also indicated that technical questions remained regarding the ability of ISS to meet some of the original study objectives. They concluded that data collected on other important performance measures such as juvenile abundance and survival are somewhat incomplete. They also noted that the statistical analyses in Lutch et al. (2003) were completed using only redd density in streams where carcass information was sufficient to estimate the GP hatchery stray rate. The ISRP then expressed concern that ISS may not be able to use even redd density as an analysis variable in the remaining streams because of insufficient carcass data. Furthermore, the ISRP was uncertain of the status of tissue samples collected from Chinook salmon for the purpose of using genetic analysis in the evaluation of ISS. They recommended the ISS must adjust the study design for Phase III, specifically assessing the status of tissue collection and the use of DNA assignment tests for evaluating the effects of GP hatchery straying into ISS study.

Using the results from the ISRP (2003-8), the Council provided a draft memorandum to ISS sponsors on June 27, 2003 that described the following recommendations:

1. Council staff recommends that during 2003 the sponsors conduct an analysis and develop an updated final design for the start of Phase III in 2004,

2. Council staff recommends that collection of carcass data be required in 2003 on as many ISS study streams as feasible for estimation of abundance of strays and abundance of ISS supplementation fish,

3. Council recommends that DNA-based assessment of ISS treatment and control populations be evaluated and addressed as part of the final design for Phase III,

4. Council staff recommends funding of the Idaho Supplementation Studies for one year subject to the above stated requirements for carcass data collection in 2003 and development of a final design for Phase III. Further, the analysis following the 2003 field season and the final design of the Phase III segment be reviewed and approved before the 2004 field season.

ISS collaborators prepared this document in order to resolve the technical recommendations provided by Council. We used the statistical review (Lutch et al. 2003) as a foundation to review other aspects of the ISS study, such as the status of other data types and the ability to use genetic analyses for project evaluation purposes. Independent statistical consultation was provided through the University of Idaho Statistical Consulting Center.

In this report, we address the following objectives:

1. Review additional data types available to determine whether the ISS can meet its original research objectives,

2. Compile additional project data for the statistical treatment of the ISS,

3. Develop and apply additional statistical analyses to ISS data,

4. Evaluate the usefulness of using DNA based assessments in the evaluation of the ISS,

5. Present an updated study design that includes methods for the statistical analysis of ISS data through Phase III.

In the following sections, we prepare data sets and describe methods feasible for both a quantitative and qualitative evaluation of the ISS study. In section one, we present an overview of the datasets maintained by the ISS program. In section two, we present an updated study design using these datasets that includes a regression analysis as recommended by the ISRP. Section three provides additional supporting information on how data was compiled to test the additional statistical treatments presented in the updated study design. Section four describes the development of the prototype regression analysis. Finally, in section five, we address additional ISRP recommendations that ISS implement DNA microsatellite parentage analyses to assess the reproductive contribution of hatchery strays into ISS streams. We identify those ISS study streams where this type of parentage analysis would be feasible and provide a generalized study design outlining how this could be done. However, it must be stated that without additional funding, the ISS program cannot undertake this type of analysis without compromising other critical aspects of the program.

SECTION ONE

Study Objectives and Research Questions

As the ISS project nears the completion of Phase II, we felt that it would be useful to first review the questions posed in the original ISS study design and the data types that have been collected to date for the purpose of determining whether they are appropriate to adequately address the original study objectives. As described in Bowles and Leitzinger (1991), the four study objectives are:

1. Monitor and evaluate the effects of supplementation on presmolt and smolt numbers and spawning escapements of naturally produced Chinook salmon,

2. Monitor and evaluate changes in natural productivity and genetic composition of target and adjacent populations following supplementation,

3. Determine which supplementation strategies provide the quickest and highest response in natural production without adverse effects on productivity,

4. Develop supplementation recommendations.

Below we address each of the research questions posed in the original study design (Appendix G pages 158 to 165 of Bowles and Leitzinger 1991) to address the project objectives and describe which streams and data sets will be used in comparisons. Discussions will reference Appendix A, which summarizes available data for all ISS study streams.

Question One: Does supplementation of existing Chinook salmon populations enhance natural production?

This question evaluates responses in adult escapement and juvenile production from Phases I through III of the ISS project. Presently, the ISS study measures production response using redd counts, juvenile abundance, and adult escapement where possible (Table 1.1).

Question Two: Does restoration using existing hatchery stocks establish natural production?

This question evaluates responses from Phase I and II of the ISS project in supplementation-restoration streams. Evaluation of this question will be limited to streams in the Clearwater River subbasin where hatchery stocks have been used to re-establish self-sustaining populations. For this question, response may be evaluated using redd counts, juvenile abundance, escapement, and/or survival wherever possible (Table 1.2). Survival measures include estimates of juvenile survival for PIT tag groups released at rotary screw traps, and in some cases, juvenile to adult survival (typically from the Lower Granite Dam [LGR] juvenile bypass facility back to the LGR adult fish ladders).

Question Three: Does supplementation of existing populations reduce natural productivity below acceptable levels?

This question evaluates responses in Phase I through III of the ISS project in supplementation-augmentation streams. Response variables that are available to address this question include adult or redd to juvenile ratio and juvenile survival in streams where data are

available (Table 1.3). Juvenile survival refers to expanded survival estimates to LGR obtained by PIT tagging juvenile Chinook salmon captured at rotary screw traps.

This question is somewhat ambiguous given that an "acceptable" decrease in productivity is undefined. One solution would be simply to note where a detectable change in productivity is noted following supplementation-augmentation activities. Where possible, a mean estimate of the change in a specific parameter will be reported, with enumeration of 90% confidence intervals.

Table 1.1. Streams and data types available for analysis of ISS question one: Does supplementation of existing Chinook salmon populations enhance natural production? Block refers to the life-stage of juveniles released into treatment streams. Control streams received no juvenile treatments (supplementation).

Stream	**Block**	**Redd Counts**	**Juvenile Abundance**	**Adult Escapement**
West Fork Yankee Fork	Presmolt	X	X	
Pahsimeroi River	Smolt	X	X	X
Lolo Creek	Smolt	X	X	X
East Fork Salmon River	Smolt	X	X	X
Clear Creek	Smolt	X	X	X
American River	Smolt	X	X	
Johnson Creek[a]	Smolt	X	X	X
South Fork Salmon River	Multiple	X	X	X
Upper Salmon River	Multiple	X	X	X
Red River	Multiple	X	X	X
Brushy Fork Creek	Control	X		
Crooked Fork Creek	Control	X	X	X
White Cap Creek	Control	X		
Herd Creek	Control	X		
Lemhi River	Control	X	X	
Marsh Creek	Control	X	X	
North Fork Salmon River	Control	X		
Lake Creek	Control	X	X	X
Secesh River	Control	X	X	
Slate Creek	Control	X		
Bear Valley Creek	Control	X		
Valley Creek	Control	X		
Total Number of Streams Available	**10 Treatment 12 Control**	**10 Treatment 12 Control**	**10 Treatment 5 Control**	**8 Treatment 2 Control**

[a] Johnson Creek was originally a control stream, but is currently part of the NPT Johnson Creek Artificial Production Evaluation program. Continuing annual supplementation activity will likely preclude this stream from most Phase III analyses.

Table 1.2. Streams and data types from the Clearwater River subbasin available for analysis of ISS question two: Does restoration using existing hatchery stocks establish natural production? Block refers to the life-stage of juveniles released into treatment streams. Control streams received no juvenile treatments (supplementation).

Stream	**Block**	**Redd Counts**	**Juvenile Abundance**	**Adult Escapement**
Big Flat Creek	Parr	X		
Colt Killed Creek	Parr	X	X	
Fishing Creek	Parr	X		
Pete King Creek	Parr	X		
Legendary Bear Creek	Smolt	X		
Clear Creek	Smolt	X	X	X
American River	Smolt	X	X	
Crooked River	Multiple	X	X	X
Lolo Creek	Multiple	X	X	X
Newsome Creek	Multiple	X	X	X
Brushy Fork Creek	Control	X		
Crooked Fork Creek	Control	X	X	X
White Cap Creek	Control	X		
Total Number of Streams Available	**10 Treatment 3 Control**	**10 Treatment 3 Control**	**6 Treatment 1 Control**	**4 Treatment 1 Control**

Question Four: Can existing hatcheries and broodstocks be used effectively to supplement existing populations within local or adjacent subbasins?

This question evaluates responses during ISS Phases I and II in supplementation-augmentation streams. Streams supplemented with hatchery derived broodstocks in existence at the inception of the ISS project will be compared to control streams to test for responses in redd counts, adult escapement, juvenile abundance, juvenile survival to LGR, and adult or redd to juvenile productivity wherever possible (Table 1.4).

Table 1.3. Streams and data types available for analysis of ISS question three: Does supplementation of existing populations reduce natural productivity below acceptable levels? Block refers to the life-stage of juveniles released into treatment streams. Control streams received no juvenile treatments (supplementation).

Stream	**Block**	**Redd Counts**	**Juvenile Abundance**	**Adult Escapement**
West Fork Yankee Fork	Presmolt	X	X	
Pahsimeroi River	Smolt	X	X	X
Lolo Creek	Smolt	X	X	X
East Fork Salmon River	Smolt	X	X	X
Clear Creek	Smolt	X	X	X
Johnson Creek[a]	Smolt	X	X	X
American River	Smolt	X	X	
South Fork Salmon River	Multiple	X	X	X
Upper Salmon River	Multiple	X	X	
Red River	Multiple	X	X	X
Brushy Fork Creek	Control	X		
Crooked Fork Creek	Control	X	X	X
White Cap Creek	Control	X		
Herd Creek	Control	X		
Lemhi River	Control	X	X	X
Marsh Creek	Control	X	X	X
North Fork Salmon River	Control	X		
Lake Creek	Control	X	X	X
Secesh River	Control	X	X	
Slate Creek	Control	X		
Bear Valley Creek	Control	X		
Valley Creek	Control	X		
Total Number of Streams Available	**9 Treatment 12 Control**	**9 Treatment 13 Control**	**9 Treatment 5 Control**	**6 Treatment 4 Control**

[a] Johnson Creek was originally a control stream but is currently part of the NPT Johnson Creek Artificial Production Evaluation program. Continuing annual supplementation activity will likely preclude this stream from most Phase III analyses.

Table 1.4. Streams and data types available for analysis of ISS question four: Can existing hatcheries and broodstocks be used effectively to supplement existing populations within local or adjacent subbasins? In these cases, streams with functional populations were initially treated with progeny of hatchery broodstock crosses.

Stream	Block	Redd Counts	Juvenile Abundance	Adult Escapement
West Fork Yankee Fork	Treatment	X	X	
Pahsimeroi River	Treatment	X	X	X
Lolo Creek	Treatment	X	X	X
East Fork Salmon River	Treatment	X	X	X
Clear Creek	Treatment	X	X	X
Red River	Treatment	X	X	X
South Fork Salmon River	Treatment	X	X	X
Upper Salmon River	Treatment	X	X	
American River	Treatment	X	X	
Brushy Fork Creek	Control	X		
Crooked Fork Creek	Control	X	X	X
White Cap Creek	Control	X		
Herd Creek	Control	X		
Lemhi River	Control	X	X	X
Marsh Creek	Control	X	X	X
Johnson Creek	Control	X	X	X
North Fork Salmon River	Control	X		
Lake Creek	Control	X	X	X
Secesh River	Control	X	X	
Slate Creek	Control	X		
Bear Valley Creek	Control	X		
Valley Creek	Control	X		
Total Number of Streams Available	**9 Treatment 13 Control**	**9 Treatment 13 Control**	**9 Treatment 6 Control**	**6 Treatment 5 Control**

Question Five: Is there an advantage to developing new, localized broodstock with a known natural component for supplementation of existing natural populations?

This question will evaluate whether supplementation-augmentation activities benefit from the collection of broodstock from within the populations to be supplemented. The complexity of this analysis is increased by the fact that localized broodstocks were established for only two streams during Phase II of the ISS study. Therefore, we will decompose this question into two analyses to assess whether this question is still feasible for the ISS. First, we will determine whether Phase I treatment streams that were initially supplemented using local broodstock exhibited a larger response to treatment than Phase I streams that were initially supplemented with non-local broodstock (Table 1.5a). Second, we will compare responses among treatment streams that have always been supplemented using a local broodstock versus those streams for which local broodstocks were derived (localized) in Phase II (Table 1.5b). Each of these tests will also include comparisons of treatment streams versus control streams. We will test for responses in redd counts, adult escapement, juvenile abundance, juvenile survival, and/or adult or redd to juvenile productivity wherever possible.

Question Six: What life stage released (smolt, presmolt, parr) provides the quickest and highest response in natural production?

This question was posed to evaluate whether parr, presmolts, or smolts stimulate the greatest response in natural production. Smolt releases have resulted in the largest adult return (Brent Snider, IDFG Hatchery Manager, Personal Communication), but questions about potential reproductive differences in the F_1 generation adults released at the various life stages remain. However, the number of replicates per release strategy is low in most cases because of the inability of ISS to meet prescribed treatments. Therefore, we recognize that we will not likely be able to definitively address this question, but we are not advocating foregoing the analysis at this time. Response may be tested using redd counts, adult escapement, and juvenile abundance (Table 1.6).

Table 1.5a. Streams and data types available for analysis of ISS question five part one: Is there an advantage to developing new, localized broodstock with a known natural component for supplementation of existing natural populations? These streams had initial hatchery broodstocks sourced from an endemic population (Local) or from an out of drainage source (Non-Local).

Stream	**Phase I Brood Source**	**Redd Counts**	**Juvenile Abundance**	**Adult Escapement**
Pahsimeroi River	Local	X	X	X
E. Fork Salmon River	Local	X	X	X
S. Fork Salmon River	Local	X	X	X
Upper Salmon River	Local	X	X	
Crooked River	Local	X	X	X
Red River	Local	X	X	X
Colt Killed Creek	Non-Local	X	X	
W. Fork Yankee Fork	Non-Local	X	X	
Clear Creek	Non-Local	X	X	X
Legendary Bear Creek	Non-Local	X		
Lolo Creek	Non-Local	X	X	X
Newsome Creek	Non-Local	X	X	X
American River	Non-Local	X	X	
Fishing Creek	Non-Local	X		
Pete King Creek	Non-Local	X		
Big Flat Creek	Non-Local	X		
Brushy Fork Creek	Control	X		
Crooked Fork Creek	Control	X	X	X
White Cap Creek	Control	X		
Herd Creek	Control	X		
Lemhi River	Control	X	X	X
Marsh Creek	Control	X	X	X
Johnson Creek	Control	X	X	X
N. Fork Salmon River	Control	X		
Lake Creek	Control	X	X	X
Secesh River	Control	X	X	
Slate Creek	Control	X		
Bear Valley Creek	Control	X		
Valley Creek	Control	X		
Total Number of Streams Available	6 Local 10 Non-Local 13 Control	6 Local 10 Non-Local 13 Control	6 Local 6 Non-Local 6 Control	5 Local 3 Non-Local 5 Control

Table 1.5b. Streams and data types available for analysis of ISS question five part two: Is there an advantage to developing new, localized broodstock with a known natural component for supplementation of existing natural populations? These streams had Phase II hatchery broodstocks sourced from an endemic population (Local) or from descendants of out of drainage brood sources used during Phase I (Localized).

Stream	Phase II Brood Source	Redd Counts	Juvenile Abundance	Adult Escapement
Pahsimeroi River	Local	X	X	X
Crooked River	Local	X	X	X
Red River	Local	X	X	X
South Fork Salmon River	Local	X	X	X
Upper Salmon River	Local	X	X	
Johnson Creek[a]	Local	X	X	X
Lolo Creek	Localized[b]	X	X	X
Newsome Creek	Localized[b]	X	X	X
Brushy Fork Creek	Control	X		
Crooked Fork Creek	Control	X	X	X
White Cap Creek	Control	X		
Herd Creek	Control	X		
Lemhi River	Control	X	X	X
Marsh Creek	Control	X	X	X
North Fork Salmon River	Control	X		
Lake Creek	Control	X	X	X
Secesh River	Control	X	X	
Slate Creek	Control	X		
Bear Valley Creek	Control	X		
Valley Creek	Control	X		
Total Number of Streams Available	6 Local 2 Localized 12 Control	6 Local 2 Localized 12 Control	6 Local 2 Localized 5 Control	5 Local 2 Localized 4 Control

[a] Johnson Creek was originally a control stream, but is currently part of the NPT Johnson Creek Artificial Production Evaluation program. Continuing annual supplementation activity will likely preclude this stream from most Phase III analyses.

[b] Due to low escapement to these streams supplementation broodstocks included out of drainage adults until 2003.

Table 1.6. Streams and data types available for analysis of ISS question six: What life stage released (smolt [S], presmolt [PS], parr [P]) provides the quickest and highest response in natural production? Streams receiving some combination of life stage treatments are designated as Multiple (M).

Stream	Life Stage Planted	Redd Counts	Juvenile Abundance	Adult Escapement
Big Flat Creek	Parr	X		
Colt Killed Creek	Parr	X	X	
Pete King Creek	Parr	X		
Fishing Creek	Parr	X		
Legendary Bear Creek	Smolt	X		
American River	Smolt	X	X	
Newsome Creek	Multiple	X	X	X
West Fork Yankee Fork	Presmolt	X	X	
Pahsimeroi River	Smolt	X	X	X
Lolo Creek	Smolt	X	X	X
East Fork Salmon River	Smolt	X	X	X
Clear Creek	Smolt	X	X	X
Red River	Multiple	X	X	X
South Fork Salmon River	Multiple	X	X	X
Upper Salmon River	Multiple	X	X	
Brushy Fork Creek	Control	X		
Crooked Fork Creek	Control	X	X	X
White Cap Creek	Control	X		
Herd Creek	Control	X		
Lemhi River	Control	X	X	X
Marsh Creek	Control	X	X	X
North Fork Salmon River	Control	X		
Lake Creek	Control	X	X	X
Secesh River	Control	X	X	
Slate Creek	Control	X		
Bear Valley Creek	Control	X		
Valley Creek	Control	X		
Total Number of Streams Available	4 Extinct P	4 Extinct P	1 Extinct P	0 Extinct P
	0 Extinct PS	0 Extinct PS	0 Extinct PS	0 Extinct PS
	2 Extinct S	2 Extinct S	1 Extinct S	0 Extinct S
	1 Extinct M	1 Extinct M	1 Extinct M	1 Extinct M
	0 Extant P	0 Extant P	0 Extant P	0 Extant P
	1 Extant PS	1 Extant PS	1 Extant PS	0 Extant PS
	4 Extant S	4 Extant S	4 Extant S	4 Extant S
	3 Extant M	3 Extant M	3 Extant M	2 Extant M
	12 Control	12 Control	5 Control	4 Control

Question Seven: How often is supplementation required to maintain populations at satisfactory levels?

This question proposes to evaluate how often supplementation must occur in order to maintain adult abundance. Since “satisfactory” represents an indefinable value judgment, we will not attempt to provide one here. However, the Technical Recovery Team is currently developing delisting criteria based on abundance, productivity, spatial structure, and diversity (ICBTRT 2004). Once finalized, these criteria may provide a useful working definition of

satisfactory levels. This question will require that monitoring of ISS treatment and control streams continue for a minimum of two salmon generations subsequent to the final out-plant of juvenile treatment fish in 2004.

Question Eight: What life stage released (parr, presmolt, smolt) results in least deleterious effects on existing natural productivity and genetic integrity?

This question proposes to evaluate whether supplementation has reduced survival, productivity, or genetic diversity, and determine which life stage results in the least deleterious impact (if observed). Genetic impacts would likely have to be analyzed using a separate study design. Comparing juvenile survival and juvenile abundance per redd or adult among streams treated with parr, presmolts, or smolts versus control streams would be a likely scenario for this evaluation (Table 1.7).

Table 1.7. Streams and data types available for analysis of ISS question eight: How often is supplementation required to maintain populations at satisfactory levels?

Stream	Life History Out-planted	Redd Counts	Juvenile Abundance	Adult Escapement
Big Flat Creek	Parr	X		
Colt Killed Creek	Parr	X	X	
Fishing Creek	Parr	X		
Pete King Creek	Parr	X		
W. Fork Yankee Fork	Presmolt	X	X	
Pahsimeroi River	Smolt	X	X	X
Legendary Bear Creek	Smolt	X		
Lolo Creek	Smolt	X	X	X
E. Fork Salmon River	Smolt	X	X	X
Clear Creek	Smolt	X	X	X
American River	Smolt	X	X	
Red River	Multiple	X	X	X
S. Fork Salmon River	Multiple	X	X	X
Upper Salmon River	Multiple	X	X	
Newsome Creek	Multiple	X	X	X
Crooked River	Multiple	X	X	X
Brushy Fork Creek	Control	X		
Crooked Fork Creek	Control	X	X	X
White Cap Creek	Control	X		
Herd Creek	Control	X		
Lemhi River	Control	X	X	X
Marsh Creek	Control	X	X	X
N. Fork Salmon River	Control	X		
Lake Creek	Control	X	X	X
Secesh River	Control	X	X	
Slate Creek	Control	X		
Bear Valley Creek	Control	X		
Valley Creek	Control	X		
Total Number of Streams Available	4 Parr 1 Presmolt 6 Smolt 5 Multiple 12 Control	4 Parr 1 Presmolt 6 Smolt 5 Multiple 12 Control	1 Parr 1 Presmolt 5 Smolt 5 Multiple 5 Control	0 Parr 0 Presmolt 4 Smolt 4 Multiple 4 Control

Results and Discussion

Given the research questions and data sets reviewed in this section, we believe that a robust evaluation of the ISS is still feasible because most of the original study objectives can be met (for a detailed description of objectives, see Bowles and Leitzinger 1991). For objective one, we can clearly evaluate the effects of supplementation on natural production, because redd count data were collected in nearly all treated and control streams and juvenile migration estimates can be generated for nearly half of the total streams. Each of these data types represent the baseline and treatment phases for ISS and will be monitored through the evaluation phase following the stream specific protocols for research components established in Lutch et al. (2003). Using adult escapement estimates that are measured directly at hatchery weirs may be problematic because of the limited number of weirs on control streams. However, these data may be useful for more stream specific evaluations that are described in section three of this report. For objective two, productivity evaluation points can be generated using the same data sets applied to objective one. Hence, temporal and spatial coverage is adequate for determining how supplementation influences the natural productivity of Chinook salmon.

For objectives three and four, providing supplementation recommendations will be an important component in the final analysis of the ISS. Clearly, the primary goal is to contribute evidence as to how Chinook salmon respond to hatchery supplementation and the mechanisms responsible for any observed effects. However, it may be difficult for this study to assess different supplementation strategies (e.g., releasing different life stages) because the number of prescribed treatments using all life stages (parr, presmolt and smolt) was not met due to low adult escapement and our resulting inability to construct broodstocks annually during Phase II. Combining the parr and presmolt treatments into a single category for analysis will likely improve our ability to make this comparison.

SECTION TWO

Research Objectives

One of the main objectives of this report is to describe an updated design and analysis that is feasible for the ISS given the types and status of data sets that will be available through Phase III. For this purpose, we define the primary objectives that the ISS will likely meet using the data types that were reviewed and statistically analyzed in the previous sections. The following original objectives for ISS will be maintained:

1. Monitor and evaluate the effects of supplementation on Chinook salmon natural production,

2. Monitor changes in productivity due to supplementation activities,

3. Develop supplementation recommendations.

Using the current study design, much attention will focus on objectives one and two in order for the ISS to best determine what role supplementation can play in the recovery of spring and summer Chinook salmon in Idaho. Below, we describe the evaluation points and statistical analyses that will be used to monitor our progress toward achieving the ISS program objectives.

Data will be collected for each of these evaluation points through Phase III as prescribed in Lutch et al. (2003).

It is important to note that this is a programmatic recommendation resulting from a statistical review of the ISS project during Phase II. Clearly, the ISS needs to continue to be adaptive to the future status of Snake River spring and summer Chinook salmon stocks and potential conflicts with other research and management projects occurring in the Snake River basin.

Statistical Analysis of the Idaho Supplementation Studies

We propose using two approaches for the statistical analysis of the ISS: 1) the experimental evaluation of treatment and control streams to determine cause and effect relationships, and 2) a modeling approach to estimate treatment effects. The first approach maintains the original experimental analysis prescribed in Bowles and Leitzinger (1991). The second approach considers the ISS as an observational study per recommendation by ISRP and places more emphasis on modeling and estimating effects from supplementation treatments.

Mixed Model ANOVA—The mixed model ANOVA will be the principal statistical method for the experimental evaluation of ISS through Phase III (Table 2.1). Considering the statistical treatment of ISS data completed through more than half of Phase II (Lutch et al. 2003), this model best compares responses among treated and control streams and is capable of compensating for changes in study stream classification, varying levels of treatment, and biological, geographic, and habitat based effects on production. The utility of this method is further supported by the significance of a treatment effect in partially treated streams using redd density as the response variable (Lutch et al. 2003). Since all ISS treated and control streams contained adequate data and were included in this partial Phase II analysis, we believe that this method will be critical in the final analysis of ISS data. While we caution against using these results to make inferences regarding the final outcome of the study, the results do suggest that the technique is statistically robust and sensitive to responses resulting from supplementation treatments. Continued, rigorous collection of escapement (e.g., redds) and GP hatchery straying by ISS cooperators through the remainder of Phase II and all of Phase III will only enhance this analysis.

The ISS cooperators recognize that a thorough evaluation of the effects of straying will be critical for the final analysis, particularly when the mixed model is applied. Lutch et al. (2003) investigated how GP hatchery straying compromised treatment affects in several years of Phase II prior to developing the “prototype” mixed model by selecting a subset of streams for analysis of covariance. For the purpose of this initial analysis, streams were selected that contained ample stream-years of data (e.g., those that were most productive) and experienced a wide range of stray rates. Results from this analysis indicated an insignificant effect on treatment measures over time. Based on this, the mixed model was developed using all treated and control streams. Most study streams have sufficient carcass data available during Phase II in years with documented adult escapement (redds) to apply the mixed model analysis. Only the East Fork Salmon River and White Cap, Herd, and Valley creeks contain redd data but lack sufficient carcass data. In 2003, the ISS programmatically prescribed expanded carcass data collection for all study streams through the remainder of the study. As such, adequate data should be available for most of Phase II and all of Phase III to uniformly adjust for effects of GP

hatchery strays in all streams as necessary when using redd densities or other measures of treatment affects (e.g., juvenile migration).

Regression—In streams where a response variable (e.g., redds) was measured consistently and both treated and control streams are adequately represented over time, a regression analysis is feasible for treating the ISS as an observational study. This method allows us to model the effect of GP hatchery strays and provides a good companion analysis to the statistical prototype when using redd density as the primary response variable. Both methods detect a treatment effect and agree that GP hatchery straying is important, and each can easily be extended as Phase III data become available. Furthermore, redd count and salmon carcass data critical for these analyses can be maintained consistently across all study streams through Phase III in a cost efficient manner, as demonstrated in 2003. At this time, we do not feel the regression approach is appropriate for comparing juvenile survival or the number of migrating juveniles. However, as more data become available during Phase III, regression (and possibly the mixed model) will likely become an appropriate analysis method (Table 2.1).

Graphical Analysis—Considering the current state of the smolt migration and survival data, a graphical representation would be appropriate for the analysis of these ISS datasets. Smolt migration data were collected over time for eight of the 15 streams in the Clearwater subbasin (one control, seven treated), but three treated streams (American River, Clear Creek and Colt Killed Creek) have only three years of data to date. Of the 15 Salmon River streams, four controls and four treated streams have migration data. Two of the controls (Secesh River and Lake Creek) have data only since 1996. Although the ISS project was originally conceived as an experiment, these data are more observational than experimental because of the constrained randomization of the streams with migration data and the varying lengths of the data series. Model building may be more appropriate than hypothesis testing (Burnham and Anderson 1998). Adding redd counts, stream flows, and other variables (e.g., temperature, size at tagging, tag date) to these figures may improve the model representation for these data (Table 2.1).

Evaluation Levels

Treatment effects will be evaluated globally using treated and control streams distributed across both the Salmon and Clearwater subbasins with relatively complete ISS data sets (e.g., redd densities). This will provide inferences on the effects of supplementation statewide. Treatment effects will also be tested and compared between restoration (Clearwater River subbasin) and augmentation (Salmon River subbasin) strategies (Bowles and Leitzinger 1991) as more data become available. As described in Lutch et al. (2003), statistical blocking where streams are grouped based on stream habitat, productivity, and geographic similarities will be considered in the evaluations.

We prescribe a second evaluation approach for the ISS using a much finer scale that will focus on individual study streams. This includes the newly proposed DNA parentage study and the ongoing reproductive success study in the Pahsimeroi River (and similar data being collected in the upper Salmon River pending additional funding) that is examining the parental genetic contribution of ISS supplementation and natural origin Chinook salmon to F_1 generation offspring and their survival to adulthood (as measured by smolt-to-adult return rates).

Table 2.1. Proposed ISS evaluation, data set, and statistical method for meeting ISS project objectives one and two.

Objective	Evaluation Measure	Evaluation Approach	Study Phase	Statistical Analysis
Supplementation effects on Natural Production	Redd Counts	Global	I, II, III	Mixed Model Regression Regression
	Juvenile Migration	Global	I, II, III	Mixed Model Graphical
	DNA Parentage	Small Scale	II, III	Parentage Analysis
	ISS Parental Exclusion	Small Scale	II, III	Parental Exclusion
Supplementation effects on Productivity	Min. Juvenile Survival	Global	I, II, III	Regression Graphical
	Smolt to adult	Global	I, II, III	Regression Graphical
	Recruits per spawner	Global	I, II, III	Regression Graphical
	DNA Parentage	Small Scale	II, III	Parentage Analysis
	ISS Parental Exclusion	Small Scale	II, III	Parental Exclusion

Study Phases

Natural production and productivity evaluation at the global and subbasin levels will be evaluated across each of three study phases for the purpose of determining the short-term benefits and long-term effects of hatchery supplementation and will differ only in the number of study streams evaluated. This maintains the original experimental design using treatment and control streams by comparing response measures between baseline, treatment, and post treatment periods.

The third approach, using the small-scale genetic study, will focus on treatment effects through one or two Chinook salmon generations. We consider these as comprehensive evaluations that will directly measure the performance of ISS supplementation origin salmon relative to naturally produced populations, and more importantly, will demonstrate how hatchery supplementation influences naturalized populations of Chinook salmon.

Natural Production Evaluation Points

The ISS study will continue to estimate both adult escapement and juvenile production through the final study phase for the purpose of evaluating supplementation effects on Chinook salmon natural production (Figure 2.1). Redd counts standardized as the number of redds/km will be used as the primary index for adult escapement because this presently represents our most complete data set. Adult return to hatchery weirs is our most direct measure of escapement and will be used where available. However, since few control streams contain weirs, evaluations will be limited to a graphical approach for select grouped comparisons. Juvenile migration estimates that were generated by using a combination of rotary screw and scoop traps will provide a measure of juvenile production. For analysis purposes, juvenile

migration will be estimated for both the number of smolts and the total number of juveniles from a specific brood year. Intensified spawning ground surveys will continue through Phase III to provide much needed Chinook salmon carcass data for the purpose of evaluating the effects of GP hatchery straying.

Productivity Evaluation Points

Changes in the natural productivity of Chinook salmon will be evaluated using several estimates of survival among select streams to address objective two for the ISS (Figure 2.2). Juvenile survival to the Lower Snake River hydroelectric complex will be estimated using the SURPH2 model (Lady et al. 2001) in streams where PIT tag data are available. Smolt-to-adult survival between the period when smolts are detected at the four lower Snake River dams and returning adults are detected at the same locations will also be evaluated. Other datasets we consider as meaningful for the evaluation of ISS that will be compiled during Phase III include an estimate of recruitment (e.g., number of smolts produced per female), fecundity, and age structure of adults returning to spawn in ISS treatment and control streams.

The ongoing parental exclusion analysis in the Pahsimeroi River and pending in the Upper Salmon River will describe the reproductive performance of ISS supplementation and natural origin Chinook salmon allowed to spawn volitionally. We will examine parental contribution to the F1 progeny at both the juvenile and adult life stages. This will enable ISS researchers to identify differences in survival among different parental combinations at several life stages (parr, presmolt, smolt, and adult). While these studies on the Pahsimeroi and upper Salmon rivers are similar to the DNA assessments described in Section Four, the latter were developed primarily to quantify the contribution of GP hatchery strays to natural production to more precisely evaluate ISS treatment affects.

Figure 2.1. Streams and data sets used for evaluating natural production for the ISS.

Natural Production

Adult Escapement – Redds

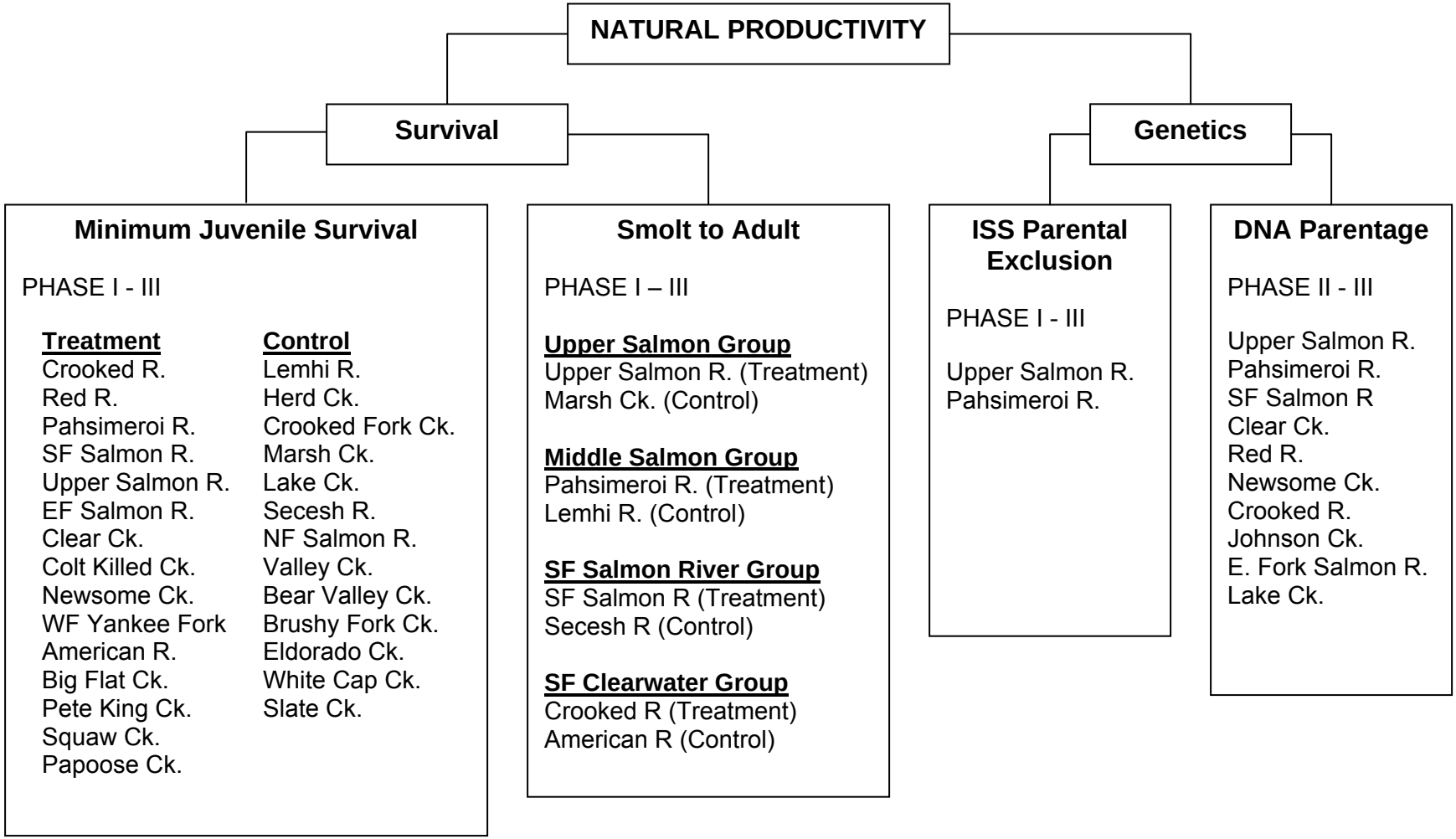
PHASE I – III

Treatment	**Control**
Crooked R.	Lemhi R.
Red R.	Herd Ck.
Pahsimeroi R.	Crooked Fork Ck.
SF Salmon R.	Marsh Ck.
Upper Salmon R.	Lake Ck.
EF Salmon R.	Secesh R.
Clear Ck.	NF Salmon R.
Colt Killed Ck.	Valley Ck.
Newsome Ck.	Bear Valley Ck.
WF Yankee Fork	Brushy Fork Ck.
American R.	Eldorado Ck.
Big Flat Ck.	White Cap Ck.
Pete King Ck.	Slate Ck.
Squaw Ck.	
Papoose Ck.	

Juvenile Production - # of migrants

PHASE I – III	PHASE II – III
Treatment	**Treatment**
Crooked R.	Crooked R.
Red R.	Red R.
Pahsimeroi R.	Pahsimeroi R.
SF Salmon R.	SF Salmon R.
Upper Salmon R.	Upper Salmon R.
Lolo Ck.	Lolo Ck.
EF Salmon R.	EF Salmon R.
Clear Ck.	Clear Ck.
	Colt Killed Ck.
	Newsome Ck.
	WF Yankee Fk.
	American R.
Control	**Control**
Lemhi R.	Lemhi R.
Crooked Fork Ck.	Crooked Fork Ck.
Marsh Ck.	Marsh Ck.
	Lake Ck.
	Secesh R.

Figure 2.2. Streams and datasets used to evaluate natural productivity in ISS study streams.



SECTION THREE

Data Compilation

ISS cooperators compiled several data sets for validating additional statistical treatment of both natural production and productivity response variables. In the first ISS statistical review (Lutch et al. 2003), Chinook salmon redds standardized as the number counted per km of stream surveyed were used in constructing a prototype (mixed model) analysis. For this report, we prepared juvenile migration and survival estimates to develop a regression analysis as recommended by the ISRP. These data have also proved useful in developing the updated study design described in the following sections of this report. Furthermore, we reviewed carcass data collected in 2003 to determine if the study recommendation to collect complete carcass data (Lutch et al. 2003) occurred in all streams.

Juvenile Migration

For the purpose of performing additional statistical treatment of ISS data, juvenile migration data were reviewed and abundance estimates were generated in streams containing screw traps. We estimated the number of smolt migrants because screw traps were operated consistently in the spring when smolts were migrating downstream.

Trapping data were arrayed by stream and year so that the numbers of naturally produced smolt migrants could be estimated for any given brood year (years when adults returned and spawned naturally). The daily number of juveniles that were captured, marked, released upstream of traps, and subsequently recaptured were grouped into strata. Each stratum contained a minimum of seven recaptured juveniles. In most cases, daily screw trap data were first stratified by week. We then compared weekly trapping efficiencies and grouped adjacent weekly strata into larger periods when their trapping efficiency estimates were similar. In some cases, brood year data were not stratified because few marked fish were recaptured throughout the smolt trapping period. When no marked fish were recaptured, we applied an average trapping efficiency to those data to generate a migration abundance estimate.

We used Gauss software (Aptech 2002) to estimate the number of migrating smolts from brood years 1991–1999 (Appendix B). The Gauss program uses the Lincoln-Petersen estimator and modifications (e.g., Bailey's estimator) for calculating abundance and the profile and bootstrap methods for calculating confidence intervals (Steinhorst et al. 2004; Hong 2002; Steinhorst 2000). The daily numbers of captured, marked, and recaptured fish are used to estimate efficiency, with the assumption that marked fish are released far enough upstream to permit random mixing with unmarked juveniles. Gauss uses an iterative solution to calculate the abundance estimate (Steinhorst 2000). When enough iterations are run (typically 1,000), the estimates stabilize and a maximum likelihood estimate is obtained.

Juvenile Survival

In the original study design, Bowles and Leitzinger (1991) prescribed PIT tagging a minimum of 500 parr annually in ISS control streams. In addition, in streams with juvenile traps, a minimum of 300 fall (presmolt) and 100 spring (smolt) migrants were to be tagged annually. Minimum tagging goals were formulated using assumed life history specific survival relationships to ensure a minimum of 35 PIT tag detections per juvenile life history group at

LGR. Given the uncertainty associated with original survival assumptions and to provide more accurate survival and migration estimates, annual ISS PIT tag goals have been increased substantially. Preseason migration estimates are made for each stream, and available PIT tags are distributed to each stream in proportion to their migration estimates. Every X^{th} fish from each life stage is then PIT tagged to distribute tags over the entire trapping season. In-season migration estimates and tag usage are monitored regularly at each trap, and tagging frequency is adjusted as necessary.

During migration, some juvenile fish pass through PIT tag interrogation facilities located at Snake and Columbia River dams. PIT tag interrogation efficiencies differ by dam because of differences in design or by changes in operation (e.g., spill). The PIT tag detections recorded at these facilities are stored and disseminated from the PIT Tag Information System (PTAGIS) database (PSMFC 1998; www.ptagis.org). We queried the PTAGIS database for information on the cumulative number of fish from each PIT tag group that are detected at all interrogation sites. Queries include PIT tag numbers, dates of detections, and travel times. Fish from all three juvenile life history stages (parr, presmolt, and smolt) are captured at ISS tributary trapping locations. Subsequent tag detections in the hydrosystem are pooled by life history stage at tagging for analysis.

The life history stage data are evaluated to determine survival probabilities to LGR. The Survival Under Proportional Hazards (SURPH2) model (Lady et al. 2001 based on Smith et al. 1994) is used to calculate these survival and detection probabilities. The SURPH2 model uses survival and detection probabilities for release recapture group survival estimates. A baseline probability is defined by the model and main effects, and group and individual covariate coefficients are evaluated against the baseline for relational differences (Lady et al. 2001). The survival probability is reported for Lower Granite Dam and to Bonneville Dam.

Carcass Data Collection

In their first statistical review (Lutch et al. 2003), ISS researchers identified the need to collect Chinook salmon carcass data to develop a covariate adjustment for hatchery straying in the statistical analysis of ISS data. As a result, a program decision was made to increase monitoring effort for salmon carcasses beyond spawning surveys in all streams for the remaining years of study. For this report, we review and present carcass data collected in 2003 for each stream to demonstrate that the ISS can apply programmatic recommendation across multiple cooperative agencies and collect consistent data (Table 3.1).

Data were collected from Chinook salmon carcasses in 2003 to determine their origin (GP hatchery, ISS supplementation, or naturally produced) and ocean age. Sex, fork length, mid-eye to hypural length, presence of external marks or PIT tags, date, and stream name were recorded. Carcasses were visually examined for external marks (e.g., fin clips) and tested for coded-wire tags by either collecting snouts for laboratory analysis or by scanning fish with detectors in the field. The visceral cavities of carcasses were also visually inspected to determine the degree of egg retention. Several structures were collected for age and DNA analysis using methods outlined in Kiefer et al. (2002). Carcass origin data summarized in this report will be a key component to constructing a covariate to account for production attributable to hatchery straying.

Table 3.1. Chinook salmon carcass recoveries by origin type collected during 2003. IDFG = Idaho Dept. of Fish and Game, NPT = Nez Perce Tribe, SBT = Shoshone Bannock Tribe, and USFWS = U.S. Fish and Wildlife Service.

Study Stream	Agency	Category	Natural	ISS Hatchery	Production Hatchery	Unknown
Colt Killed Cr	IDFG	Treatment	3	0	1	0
Big Flat Cr	IDFG	Treatment	0	0	0	0
Crooked R	IDFG	Treatment	47	2	4	12
Pahsimeroi R	IDFG	Treatment	82	88	0	4
Red R	IDFG	Treatment	3	0	3	8
SF Salmon R	IDFG	Treatment	878	455	75	34
Upper Salmon R	IDFG	Treatment	140	46	0	0
Legendary Bear Cr	NPT	Treatment	3	0	3	0
Fishing Cr	NPT	Treatment	2	0	0	1
Lolo Cr[a]	NPT	Treatment	55	10	1	18
Newsome Cr	NPT	Treatment	37	28	4	11
WF Yankee Fork	SBT	Treatment	20	0	0	0
EF Salmon R	SBT	Treatment	22	0	0	0
Clear Cr	USFWS	Treatment	1	3	10	0
Pete King Cr	USFWS	Treatment	0	0	0	0
American R	IDFG	Treatment	21	0	14	7
Johnson Cr above weir[b]	NPT	Treatment[c]	396	95	5	8
Johnson Cr below weir	NPT	Treatment[c]	28	1	1	0
Eldorado Cr	NPT	Control	0	0	0	0
Lemhi R	IDFG	Control	9	0	0	0
Brushy Fork Cr	IDFG	Control	3	0	0	0
Crooked Fork Cr	IDFG	Control	8	0	1	0
Marsh Cr	IDFG	Control	233	0	0	0
Knapp Cr	IDFG	Control	16	0	0	0
NF Salmon R	IDFG	Control	9	0	0	0
White Cap Cr	IDFG	Control	3	0	3	8
Lake Cr	NPT	Control	257	0	0	14
Secesh R	NPT	Control	256	0	2	11
Slate Cr	NPT	Control	1	0	1	0
Bear Valley Cr	SBT	Control	318	0	0	0
Herd Cr	SBT	Control	27	0	0	0
Valley Cr	SBT	Control	121	0	0	0

[a] Includes carcasses recovered in Yoosa Creek
[b] Includes carcasses recovered in Burnt Log Creek
[c] The entire Johnson Creek drainage was originally designated a control stream. However, the Nez Perce Tribe implemented an independent supplementation program in 1998. Cooperators will continue to monitor and report data for Johnson Creek following ISS data collection protocols.

SECTION FOUR

Analysis of the Idaho Supplementation Studies

We developed a statistical prototype (categorical mixed model) in Lutch et al. (2003) to test our ability to compensate for changes in ISS study stream designation, varying levels of treatment, and geographic and habitat based effects on production. Redd densities were used as the response variable for evaluating treatment effects on natural production because they represent the most complete ISS data set. In this report, we use redd density, juvenile migration estimates, and juvenile survival data to develop a multiphased regression analysis as recommended by ISRP (Appendix B). This was completed with the following goals in mind:

1. Demonstrate that the statistical analysis of ISS data is feasible given the current data types that will be collected through Phase III,

2. Use the results to develop a more appropriate study design and statistical analysis that would include modifying monitoring procedures utilized by the ISS program as necessary prior to the start of Phase III.

Regression Analysis

We developed a prototype, multiphase regression analysis that incorporates treatment proportion, straying, redd production, and grouping of streams by proximity/habitat. To complete this study the following points were considered:

1. Any regression model that incorporates GP hatchery straying cannot include Phase I data as straying numbers were not collected before 1995,

2. Phase I redd production can be used, however, to indicate baseline stream productivity and as such can appear as an independent variable in the analysis. Since no uniform measures of primary productivity are available, average redd production in Phase I provides a surrogate variable to represent this dimension of the system,

3. Adult treatment percentages were lagged by four years based on the assumption that the majority of fish return the fourth year,

4. Straying was analyzed using the proportion of strays reported in Lutch et al. (2003) expressed as strays per kilometer (straying in treatment streams included GP adults, and in control streams included GP and ISS supplementation adults),

5. In using treatment percentage as a variable, we are assuming that the prescription for each stream is accurate so that a treatment proportion in stream A of 45% is comparable to a treatment proportion in stream B of 20%, for example.

Using baseline redd production (the average of 1992, 1993, and 1994), percent adult treatment (lagged by 4 years), and percent GP hatchery straying and groupings from Lutch et al. (2003) we considered the following models:

1. $\log(rpk + 1) = \text{constant} + T + S$
2. $\log(rpk + 1) = \text{constant} + T + S + B$

3. log(rpk +1) = constant + T + S + grouping (G2-G9)
defined as a contrast of each group compared to G1).

where rpk is redds per kilometer, T is percent adult treatment, S is general production hatchery straying, B is baseline redd production, and G2-G9 are grouping variables developed in Lutch et al. (2003) based on habitat/proximity similarities between streams.

All models were compared using Akaike's Information Criterion (AIC; Franklin et al. 2001). The AIC was selected because it allows the simultaneous evaluation of multiple candidate models and identifies the most appropriate one given the data.

Regression Analysis Results and Discussion

The regression analysis was run using SAS (SAS 2003). The results for redd densities, juvenile migration estimates, and juvenile survival are reported in Appendix C, Appendix D, and Appendix E, respectively.

The results of these analyses suggest that a regression approach as suggested by ISRP is feasible and would provide a complementary analysis to the prototype ANOVA analysis developed in Lutch et al. (2003). For redd densities and juvenile migration size, the analyses suggest a treatment effect and the importance of hatchery straying. When one looks at the Least Square Means of redd densities (Appendix C), for example, we see that as treatment proportion, hatchery straying proportion, and the group 8 indicator variable increase, production (log [redds per kilometer +1]) values increase. Assuming that straying complicates the evaluation of ISS treatment effects on natural production, this analysis can partially adjust for the effects of hatchery strays.

We caution that these results are preliminary since only Phase II data were included. When Phase III data become available, the technique can be extended easily.

A regression approach will be an important tool for the statistical analysis of ISS through Phase III, assuming that the appropriate data continue to be collected. It will be important to maintain redd counts on all ISS streams. Chinook salmon carcasses are critical for determining GP hatchery stray rates into treated and control streams. Thorough carcass sampling should be conducted in as many treatment and control streams as possible. Juvenile migration and survival should, at minimum, be estimated on the representative treated and control streams. However, the ISS study will consider expanding juvenile monitoring activities to achieve a more balanced evaluation of treated and control streams if additional funding becomes available.

SECTION FIVE

DNA Assessments for the Evaluation of ISS

Program cooperators are collaborating in response to the Council's recommendation for using DNA based assessments for the evaluation of the ISS project. The materials included in this section address concerns voiced by the ISRP. In their review of the Evaluation and Statistical Review of Idaho Supplementation Studies (ISRP 2003-8), the ISRP suggested:

"There is a need to use DNA level microsatellite analysis to identify parentage relationships between spawning adults, out-migrating smolts, and adults that return to spawn in the next generation (including the use of assignment tests) in order to separate non-ISS strays from natural production within distinct tributary systems. A second step, and subset of this analysis, would be to then separate ISS supplementation fish from natural-origin fish in the same system using the same methods."

As a first step in this process, the ISRP recommended:

"The principal investigators should evaluate the current status of Chinook tissue collections throughout the ISS populations and stream types (treatment, control) to determine if collections are sufficient to allow this type of DNA analysis. If not, project sponsors should assess the overall project and identify locations, opportunities, and schedules that will provide for this critically needed analysis."

This section was prepared in response to the ISRP recommendation to include assignment tests as a task under the larger program umbrella. The first part of this section reviews the spatial and temporal distribution of DNA samples collected to date for the ISS study. We then provide a general literature review for determining the level of sampling effort necessary to pursue a DNA based parentage analysis. The third part of this section identifies which of the 29 ISS study streams have the potential to provide the level of escapement monitoring necessary for an expanded DNA based parentage analysis to assess the contribution of GP hatchery strays, as recommended by the ISRP. We then present a generalized study design and timeframe for completion of parentage analyses. A study proposal and estimated budget for the completion of parentage analyses in two locations (the Pahsimeroi and upper Salmon rivers) is presented in Appendix F. This proposal could be easily expanded to include additional streams. However, without additional funding the ISS program cannot undertake these investigations without compromising other aspects of the program the ISRP has also deemed critical.

Spatial and Temporal Distribution of DNA Samples

As described in Bowles and Leitzinger (1991), the collection of tissue samples was aimed at answering two questions:

1. What is the relatedness/level of differentiation between spawning aggregates occupying ISS treatment and control streams, as well as the hatcheries currently used and proposed for use as a source of treatment juveniles and adults?

2. Following supplementation, what has been the rate and direction of introgression?

In order to address these questions, Bowles and Leitzinger (1991) proposed the annual collection of 50 presmolts from a subset of treatment and control streams (Table 5.1) and 100 smolts per year from hatchery facilities used as a source of treatment fish. Samples were to be assayed at a number of loci and visualized using starch gel electrophoresis. To evaluate supplementation effects, samples were then to be collected from adult carcasses recovered during redd count surveys on each study stream.

Table 5.1. Original ISS genetic sample design.

Study Stream	Race	T/C	n	Priority	Agency
Upper Salmon River	Spring	T	50	1	NMFS
Alturas Lake Creek	Spring	T	50	1	NMFS
West Fork YFSR	Spring	T	50	1	IDFG
Upper East Fork SR	Spring	T	50	1	IDFG
Lemhi River	Spring	T	50	1	IDFG
Pahsimeroi River	Summer	T	50	1	IDFG
South Fork Salmon	Summer	T	50	1	IDFG
Crooked Fork Creek	Spring	T	50	1	IDFG
Red River	Spring	T	50	1	IDFG
Lolo Creek	Spring	T	50	1	NPT/IDFG
North Fork SR	Spring	C	50	2	IDFG
Upper Valley Creek	Spring	C	50	2	IDGF/NMFS
Herd Creek	Spring	C	50	2	IDFG/SBT
Camas Creek	Spring	C	50	2	IDFG
Marsh Creek	Spring	C	50	2	NMFS
Bear Valley Creek	Spring	C	50	2	IDFG
Secesh/Lake Creek	Summer	C	50	2	NMFS
Lower Johnson Creek	Summer	C	50	2	NMFS
Brushy Fork Creek	Spring	C	50	2	IDFG
Sawtooth Hatchery	Spring	-	100	1	NMFS
EFSR Hatchery Weir	Spring	-	100	1	IDFG
McCall Hatchery	Summer	-	100	1	NMFS
Rapid River Hatchery	Spring	-	100	1	NMFS
Dworshak Hatchery	Spring	-	100	1	IDFG

A preliminary analysis of baseline ISS genetic samples from 15 ISS study streams (Table 5.2) was completed by the Washington Department of Fish and Wildlife (WDFW) in 1994 (Marshall 1994). Twenty-six enzymes were screened via starch-gel electrophoresis yielding data for 57 putative loci. Of the 57 putative loci assayed, 53 were resolved, of which 31 were polymorphic. Spatial and temporal (where possible) genetic homogeneity was tested using G-tests, and genetic distances were visualized with dendrograms.

In general, dendrograms did not reveal consistent separation between sample groups from the Clearwater River subbasin and Salmon River subbasin. Temporal comparisons among repeated samples from the same spawning aggregate were statistically significant in all cases ($P < 0.05$), as were all pairwise (spatial) comparisons among sample groups. Of the ISS baseline sample groups, four (Table 5.3) have been maintained in cold storage for possible re-analysis (Anne Marshall, Washington Department of Fish and Wildlife, Personal Communication).

Table 5.2. Temporal and spatial distribution of samples analyzed by Marshall (1994).

	Annual Samples				
Study Stream	**1991**	**1992**	**1993**	**1994**	**Sum of Samples**
Bear Valley Creek	50	75	50		175
NF Salmon River	30	56	50		136
Red River		61[a]	50		111
Lemhi River	50	74	54		178
Dworshak Hatchery		102	100	100	302
Sawtooth Hatchery		90	100	100	290
Pahsimeroi River	50	39	29		118
Brushy Fork Creek	13	19	13		45
Lolo Creek	36	23	40		99
W.F. Yankee Fork	50	55			105
Herd Creek	50	53			103
Camas Creek	50	56			106
Crooked Fork Creek	50	52			102
EF Salmon River	20	54			74
SF Salmon River	51				51

[a] Samples from 1991 and 1992 combined into a single sample group.

Table 5.3. Baseline ISS sample groups maintained in cold storage by WDFW.

Study Stream	Sample Size
WF Yankee Fork	53
EF Salmon River	50
Herd Creek	51
Crooked Fork Creek	49

As prescribed in Bowles and Leitzinger (1991), tissue samples have been collected from adult Chinook salmon in most study streams since 1996 (Table 5.4). However, many years are not represented, particularly in treated streams, and sample sizes vary greatly across years. This is not surprising given the annual fluctuations in adult escapement and the precariously low adult returns during the mid-1990s. In short, the ISS study was not designed to include parentage analyses. Therefore, with the exception of the Upper Salmon and Pahsimeroi Rivers, parentage analyses cannot be pursued retrospectively.

Juvenile Chinook salmon have been sampled for genetic analysis in only a few streams. Only recently, beginning in 2002, have similar evaluations been applied on the Pahsimeroi River as part of a small-scale study to evaluate reproductive success of hatchery origin and naturally produced Chinook salmon. Tissue samples from the Upper Salmon River have been collected since 2002 with the hope of implementing a parentage analysis in that location. However, this analysis is not possible under the current level of funding without compromising other aspects of the program that the ISRP also identified as critical (e.g., carcass collections, juvenile emigrations estimates).

Table 5.4. Adult genetic samples collected from 1996–2001 for genetic monitoring purposes in the ISS study.

Study Stream	Category	Adult Samples
Bear Valley Creek	Control	1996-2001
Brushy Fork Creek	Control	1997-1998, 2000-2001
Crooked Fork Creek	Control	1996-2001
Herd Creek	Control	1997
Lake Creek	Control	1996-2001
Lemhi River	Control	1997-1998, 2000-2001
Marsh Creek	Control	1996-2001
NF Salmon River	Control	1998, 2000
Secesh River	Control	1996-2001
Slate Creek	Control	2001
Valley Creek	Control	1996-2001
American River	Treatment	2000-2001
Clear Creek	Treatment	1998, 2000-2001
Colt Killed Creek	Treatment	1997-1998
Crooked River	Treatment	2000-2001
EF Salmon River	Treatment	1997-1998, 2000-2001
Newsome Creek	Treatment	1996
Pahsimeroi River	Treatment	1996-2000
Papoose Creek	Treatment	1996-1997, 1999-2000
Pete King Creek	Treatment	2001
Red River	Treatment	2000-2001
SF Salmon River	Treatment	1996-2001
Upper Salmon River	Treatment	1997, 1999, 2000-2001
Walton Creek[a]	-	1992-2001
WF Yankee Fork	Treatment	1996, 1999-2001

[a] Walton Creek is not an ISS study stream but enters near the confluence of Colt Killed Creek and Crooked Fork Creek.

Sample Effort Required for Parentage Analysis

The sampling requirements for parentage analysis are somewhat dependent on the statistical model used. In general, parentage analyses operate by either assigning an individual (a juvenile or adult) to a parent or parents in the previous generation (e.g., PARENTE; Cercueil et al. 2002), or by excluding potential parents sampled in the previous generation (e.g., CERVUS; Marshall et al. 1998). With both assignment and exclusion tests, an investigator can attempt to identify both parents giving rise to a sampled individual (a "triplet") or default to finding only a single parent (maternal or paternal). Regardless of the type(s) of parentage analyses employed, the probability of correctly identifying the parent(s) or correctly excluding potential parent(s) for a given sampled individual increases as the proportion of candidate adults sampled in the previous generation increases and, to a lesser extent, as the number of polymorphic loci assayed increases[1]. In other words, if all of the adults contributing gametes to

[1] In general, it is advisable to use the minimum number of loci necessary to achieve parental matches/exclusions, since genotyping error is expected to increase as the number of assayed loci increases (Mike Ford, NOAA Fisheries, personal communication).

a given brood year are sampled, the probability of identifying the adults that gave rise to progeny from that brood year increases, and likewise, the risk of erroneously identifying/excluding a parental pair decreases.

In most cases, sampling every adult returning to a given stream is not possible. High spring runoff events can preclude the installation of weirs prior to the arrival of the first adult and/or weirs may not be 100% effective at directing adults to a trap structure. In other cases, the operation of a weir might be undesirable for practical or aesthetic reasons. In locations where the capture rate of adults is less than 100%, estimating the number of erroneous assignments/exclusions is difficult because it depends on the distribution of alleles among all adults, including those that were not sampled. Models suggest that statistical power decreases drastically when fewer than 50% of the candidate parents are sampled (Mike Ford, NOAA Fisheries, personal communication).

However, assignment and or exclusion tests can still be used if it is acceptable to determine single parents as opposed to triplets (since both parents may not have been sampled). In addition, if one can reasonably assume that hatchery and natural origin individuals are sampled with the same efficiency, one can also assume that assignments or exclusions are an unbiased sample from the larger population (i.e., the proportion of individuals assigned to hatchery or natural origin parents in the sampled group is an unbiased estimate of those proportions in the population as a whole). This assumption might be violated in cases where hatchery origin adults exhibit earlier run timing and spawn timing than natural origin adults. For example, if hatchery origin adults exhibit advanced run timing relative to natural origin adults, a potentially large proportion of hatchery origin adults might pass a weir site before the weir can be installed. In addition, if spawning by hatchery origin adults is advanced relative to natural origin adults, carcass surveys might under-represent hatchery origin fish due to loss from the system or advanced decay (which might decrease the chances of extracting high quality DNA). However, even in cases where less than 100% of the candidate parents were sampled in the previous generation, the relative reproductive success of hatchery versus natural origin parents can still be assessed, although doing so requires greater juvenile sampling effort relative to cases where all candidate parents can be sampled, as discussed later. The increase in juvenile sampling effort is required to maintain a reasonable probability of sampling juveniles arising from the subset of adults sampled in the previous generation.

Potential Locations for Implementation of Parentage Analysis

As discussed previously, the ISS study was not designed to implement parentage analysis. As such, tissue sampling effort has been insufficient to retroactively pursue parentage analyses in most of the study streams. The following will identify ISS treatment and reference streams for which existing infrastructure will allow sampling a minimum of 50% of the total adult escapement and a representative sample of juvenile migrants. Following this discussion, we will identify locations that could potentially meet sampling needs with additional infrastructure.

Selection of candidate streams for parentage analysis was based on the following considerations:

1. Existing infrastructure (e.g., screw traps and/or weirs),

2. Adult capture or sampling efficiencies of 50% or greater,

3. Straying rates of non-ISS Chinook salmon,

4. Sample sizes for migrating adults and juveniles.

Streams containing existing screw traps were first considered for juvenile sampling purposes (Table 5.5). Streams containing weirs were also considered a high priority since they enable researchers to manage adult escapement in ISS study reaches, and more importantly, effectively enumerate and sample candidate parents. Our preliminary analysis validates our earlier assumption that higher sampling efficiencies are obtained in streams with weirs. In streams without weirs, sampling 50% of the total adult escapement generally was not (and will not be) possible. Therefore, all streams considered for this analysis currently have weirs that function during some portion of the Chinook salmon spawning run.

For capture efficiency, we estimated the proportion of adults that were historically sampled for the ISS study. In streams with weirs that are less than 100% effective, adult returns and carcass counts by origin type (ISS supplementation, GP hatchery, and naturally produced adults) were modeled to predict the number of adults that escaped prior to weir installation. Using carcass data, the equation that explains the proportion (P) of ISS observed fish to the total is:

$$P= C+L / C+L+(R*L)$$

where R is the ratio of non-ISS adults to ISS adults observed at the trap and C is the number of adults intentionally released above the weir.

We then solve for L, which is the number of ISS fish that escaped past the weir uncounted.

$$L = (C - PC) / (P + PR - 1)$$

Again, P is calculated from observed ISS and non-ISS Chinook salmon in carcass collections. For this exercise, we assume that R represents the ratio that escaped upstream, but there may be differences in run timing that might affect these data. We then calculate the number of non-ISS fish that escaped uncounted by multiplying by R.

The selection of Johnson Creek for DNA parentage analysis warrants further discussion because in their statistical review, ISS sponsors recommended removing Johnson Creek from phase III analysis (Lutch et al. 2003). Previously a control stream, Johnson Creek now receives supplementation releases as part of the Johnson Creek Artificial Production Enhancement Project (JCAPE, Vogel and Hesse 2000), which are expected to continue through the ISS evaluation phase when treatments in other ISS stream will be discontinued. In their latest review of ISS, the ISRP proposed modeling stray rates as an alternate scenario for statistical analysis, stating that:

> *"there are no controls in the ISS, i.e., there are simply streams with various levels of two types of supplementation, at levels that range from 0% to somewhat less than 67% for non ISS strays and 0% to some unreported level X% for ISS fish*."

Table 5.5. Study streams selected for DNA parentage analysis. Average expected adult returns are calculated for no more than five years beginning with 2004. The level of treatment received is designated T–treatment, C–control, or P–partial treatment.

Study Stream	T/C/P	Weir	Screw Trap	Stray Rate	Weir Efficiency (%)	ISS Grouping	Average Return Nat.	Average Return Hat.
Pahsimeroi R	T	X	X	0	100	Lower Salmon	279	370
Upper Salmon R	T	X	X	0	100	Upper Salmon	563	350
S. Fork Salmon R		X	X	0.67	74	S. Fork Salmon	1,293	722
Johnson Cr	T	X	X	0.02	79	S. Fork Salmon	405	750
Red R	T	X	X	0.43	64	S. Fork Clearwater	662	614
Newsome Cr.	T	X	X	0.44	97	S. Fork Clearwater	237	828
Crooked R	T	X	X	0.3	81	S. Fork Clearwater	368	651
Clear Cr	T	X	X	0.31	78	Lower Lochsa	33	486
E. Fork Salmon R	P	X	X	0	100	Middle Salmon	76	0
Lake Cr	C	X[a]	X	0.03	100	S. Fork Salmon	12	400

[a] Adult escapement into Lake Creek is monitored with a video weir.

They suggested modeling redds or other dependent variables to provide inferences concerning the effects of changing levels of straying (supplementation of a control stream in this case). They suggested that:

> *"Continued supplementation in Johnson Creek and other streams might contribute important information concerning the complex interactions between naturally produced fish,* [GP hatchery] *strays, and ISS fish."*

The ISS sponsors recognize that Johnson Creek presents an opportunity for evaluating the relative reproductive success of natural x natural origin adults spawning in the stream versus the relative success of F_1 x F_1 adult hatchery returns (resulting from natural x natural origin adults spawned and reared to smoltification in the hatchery) allowed to spawn in the stream. Infrastructure presently supported by JCAPE could facilitate adequate juvenile and adult sampling.

Other streams could provide the opportunity to compare the relative reproductive success of various origin type adults (i.e., natural, ISS supplementation, and GP hatchery stray). Crosses of interest include 1) natural x natural in both treatment and control streams, 2) ISS x ISS and ISS x natural crosses in treatment streams or those that result from ISS adults straying into control streams, and 3) GP x GP, GP x ISS, and GP x natural crosses resulting from the general production adults straying into either treatment or control streams. Typically weired treatment streams in the ISS study are not good candidates for this kind of analysis because GP hatchery strays are intentionally excluded. Nevertheless, not all weirs are completely fish-tight. At some locations (e.g., the South Fork Salmon River), enough GP hatchery strays make it past the weir in some years that the site may be a candidate. Other streams considered for this kind of DNA analysis include the American River, Lemhi River, and Lake Creek. These streams contain screw traps that would allow sampling for juvenile Chinook salmon but lack trapping weirs (Lake Creek has a video weir). Adult sampling efficiency through carcass recoveries is low in American River and extremely low in the Lemhi River, but opportunities exist for additional infrastructure at these locations.

Study Design and Timeframe

In the previous section, we identified locations with adult and juvenile sampling facilities that could provide the level of tissue sampling effort necessary to pursue parentage analyses. In this section, we will identify the primary questions that we can address with a parentage analysis and the types of data necessary to address those questions. Following that discussion, we will identify the number and distribution of tissue samples required for parentage analyses in each location.

We foresee two primary questions that can be addressed by parentage analyses within the framework of the ISS study:

1. What is the relative reproductive success (measured as proportional juvenile production and returning adults) of natural origin adults, ISS supplementation origin adults, and GP hatchery stray adults?

2. Do adults of different origins spawn with one another in the proportions expected under the assumption of random mating?

Adult Sampling Requirements

The distribution and number of adult tissue samples necessary to answer question one is relatively straightforward. Researchers should attempt to sample every adult entering a targeted stream. Ideally, adults will be sampled at weirs and marked in a manner that indicates that a tissue sample has been collected (e.g., an operculum punch could provide the necessary mark and could be preserved for DNA extraction). In cases where weirs are less than 100% effective, carcasses lacking an operculum punch (those adults not sampled at the weir) could be sampled during carcass surveys. The combination of adult tissue samples collected at weirs and from carcasses would provide a number of “candidate” parents that could be matched to or excluded from progeny (either juveniles or adults) arising from that brood year. Continuous sampling of adults over time will allow adults to be matched to candidate parents from previous years and/or will allow candidate parents to be excluded as true parents of sampled adults.

Juvenile Sampling Requirements

The distribution and number of juvenile samples necessary to answer question one is dependent on 1) the proportion of adults sampled in the previous generation (as discussed previously); 2) the number of origin categories of those adults (natural, ISS supplementation, and GP hatchery stray); and 3) the relative efficiency of juvenile and adult sampling based on the origin category of candidate adults. In general, juvenile sampling should occur at a rate that will reasonably ensure that progeny of each origin category of candidate parents are sampled. To calculate a minimum juvenile sampling size, we make two simplifying assumptions: 1) progeny of natural origin, ISS supplementation origin, GP hatchery stray, and all possible cross types are sampled with the same efficiency (i.e., there are no differences in behavior among origin and cross types that result in differential vulnerability to sampling methods such as rotary screw traps); and 2) reproductive success among all origin and cross types is equivalent (this assumption will be tested in the next step). For the purposes of determining reproductive success, we will concentrate on paternal or maternal identification rather than identification of “triplets.” Using single-parent matches will allow us to draw conclusions regarding relative

reproductive success even in streams where adult sampling efficiency is low (i.e., those locations where identification of triplets would be difficult due to a large number of unsampled adults).

In a stream where all adults can be sampled, employing the previous assumptions allows us to use the binomial distribution to calculate a minimum sample size. For example, if 75% of the adults passed above the weir are natural origin, 20% are ISS supplementation origin, and 5% are GP hatchery strays, we would expect 75% of sampled juveniles to be assigned to a natural origin parent, 20% to an ISS parent, and 5% to a GP hatchery stray parent. This is by necessity a hypothetical example, because in the ISS study all GP hatchery strays trapped are denied access to natural spawning areas. If the weir did permit *all* adults to be sampled, no GP hatchery stray adults would be allowed to pass. However, it is instructive to consider a case of allowable straying past a perfect weir to illustrate the best case example from a sampling perspective. Obviously, in this example, where the hypothetical stray rate is low, random events dictate that a fairly large sample of juveniles may be required to ensure that at least one juvenile from smallest group (GP hatchery strays in this case) is sampled. For reasons discussed later, we wish to ensure with a reasonable probability that at least five individuals of the least common category are sampled. This sample can be calculated as follows:

n = the number of juvenile samples,
p = the probability of sampling a juvenile of GP hatchery stray origin,
q = the probability of sampling a juvenile of either natural or ISS origin,
np = the long-run average number of GP hatchery stray origin juveniles expected to occur in a sample of size n,
$\sqrt{n*p*q}$ = the standard error of the mean np, *and*
2.95*standard error of np = 99% confidence interval.

Using this methodology, we find that 99 out of 100 juvenile samples of 336 individuals will contain at least five GP hatchery stray origin juveniles, assuming that they are present in the population at the expected rate of 5%.

The previous example assumed that all adults passing into a targeted tributary could be sampled. In the more realistic case where 5% of the spawners are GP hatchery strays because adult sampling efficiency is less than 100%, the number of juvenile samples necessary to estimate the relative reproductive success of GP hatchery strays increases. This is because some of the sampled juveniles will likely be the result of spawning by unsampled adults. In locations where adult sampling devices (e.g., weirs) are less than 100% efficient, a mark-recapture (or alternative method) must be used to determine the proportion of the adult population sampled. For example, if 200 adults are sampled and mark-recapture estimates indicate that 50% of the total adult return was not sampled, we will be unable to determine the parentage of approximately 50% of the sampled juveniles. So, to continue with the example shown above, if we require a minimum of 336 juveniles to be assigned, our sample size must at least double to ensure that we maintain the ability to assign 336 juveniles to an adult of known origin. In fact, the number of juvenile samples required more than doubles due to chance. In this case, to ensure that at least 50% of the juveniles sampled can be assigned to a sampled parent, we again employ the binomial distribution and 99% prediction interval. In this case, 99 out of 100 juvenile samples of 710 individuals will include at least 336 juveniles that can be assigned to a parent sampled in the previous generation.

Question One: Testing the Assumption of Equal Reproductive Success

X2 Contingency Table—If adult and juvenile sampling effort follows the guidelines previously established, we can assume with very high confidence that the juvenile sample includes tissues of all origin and cross-types within a targeted tributary (i.e., there is a very low probability that "rare" individuals—general production origin juveniles in this example—would be excluded by chance from juvenile samples). In addition, if the *a priori* assumption that adults experience equivalent reproductive success regardless of origin holds true, we have maintained a high probability of sampling at least five individuals from the least common class (GP hatchery stray adults in this case). Therefore, we can employ a simple χ^2-test to determine if the proportion of sampled juveniles matches expectations based on the proportion of natural, ISS, and GP hatchery stray origin adults passed above the weir in the previous generation. Following the example above, if reproductive success were equivalent among adults of all origin types, 75% of the sampled progeny would be assigned to a natural origin parent, 20% to an ISS parent, 5% to GP hatchery strays, and 50% to unknown (unsampled) parents. To test the null hypothesis that reproductive success is equivalent, we will employ a simple χ^2-test contingency table as follows (Table 5.6):

$\mathbf{H_o}$: P1=P2=P3=P4; the proportion of juveniles assigned to each parental category is equivalent to the proportion of adults enumerated in that category.

Table 5.6. Chi-square contingency table populated by hypothetical data representing expected values under the assumption of equivalent reproductive success between natural, Idaho Supplementation Studies (ISS) supplementation, hatchery general production (GP), and unknown origin adult Chinook salmon.

	Natural	ISS	GP	Unknown	Total
Adult	75	20	5	100	200
Juvenile	266	71	18	355	710
Total	341	91	23	455	910

Using the hypothetical data from Table 4.6, we achieve a P-value of 0.999; hence, we fail to reject the null hypothesis. In this case, the observed proportion of juveniles in each category precisely matches expectations under the assumption of equivalent reproductive success. Alternatively, Table 5.7 contains hypothetical data illustrating nonequivalent reproductive success. In the case of Table 5.7, the P-value is 0.0002; hence, we reject the null hypothesis of equivalent reproductive success among adult classes.

Table 5.7. Chi-square contingency table populated by hypothetical data representing unequal reproductive success between naturally produced, Idaho Supplementation Studies (ISS) supplementation, hatchery general production (GP) hatchery strays, and unknown origin adult Chinook salmon.

	Natural	ISS	GP	Unknown	Total
Adult	75	20	5	100	200
Juvenile	380	100	3	300	783
Total	455	120	8	400	983

Dunnett's Test—If the null hypothesis is rejected, a two-tailed Dunnett test will be employed to determine if the reproductive success of ISS, GP hatchery strays, and unknown adults differ from the reproductive success of natural origin adults.

It is possible that one or all of the alternative hypotheses will be accepted. For example, we could find that all other origin types exhibit lesser reproductive success compared to natural origin adults. The two-tailed Dunnett test is also sensitive to departures in which alternative origin types exhibit higher reproductive success compared to natural origin adults. In the case of the hypothetical data in Table 5.7, we find that the reproductive success of GP hatchery strays and unknown adults is less than natural origin adults; however, we fail to reject the null hypothesis that natural origin adults have greater reproductive success than do ISS origin adults. In this situation, it is informative to view the change in proportions from the adult to juvenile generation (Table 5.8).

As we can see from the change in proportions from the adult to juvenile generation, both natural and ISS origin adults produced a greater proportion of the total offspring than expected under the assumption of equivalent reproductive success. Although natural origin adults produced proportionately more sampled offspring than did ISS origin adults, the difference is not statistically significant. Alternatively, both GP hatchery strays and unknown origin adults produced fewer offspring than expected under the assumption of equivalent reproductive success.

Table 5.8. Change in proportions from the adult to juvenile generation in the hypothetical reproductive success dataset comparing natural origin adults to ISS supplementation, general production (GP) hatchery stray, and unknown origin adults.

	Natural	**ISS**	**GP**	**Unknown**
Proportion Adult	0.38	0.10	0.03	0.50
Proportion Juvenile	0.49	0.13	0.004	0.38

Power—Failure to reject the null hypothesis of equivalent reproductive success among origin types does not indicate that the null hypothesis is in fact true. We intend to use a simple power analysis (Zar 1984) to calculate power, the probability rejecting the null hypothesis when it is false. Following Equation 22.52:

$$P_w = P\left[Z < \frac{-Z_{\alpha(2)}\sqrt{\overline{pq}/n_1 + \overline{pq}/n_2 - (p_1 - p_2)}}{\sqrt{p_1q_1/n_1 + p_2q_2/n_2}}\right] + P\left[Z > \frac{Z_{\alpha(2)}\sqrt{\overline{pq}/n_1 + \overline{pq}/n_2 - (p_1 - p_2)}}{\sqrt{p_1q_1/n_1 + p_2q_2/n_2}}\right]$$

where:

$$\overline{p} = \frac{n_1 p_1 + n_2 p_2}{n_1 + n_2},$$

$$q_1 = 1 - p_1,$$

$$q_2 = 1 - p_2,$$

and

$$\overline{q} = 1 - \overline{p}$$

(Zar 1984), the power to detect a change in proportion from the adult to juvenile generation in our previous example is (Table 5.9):

Table 5.9. Power to detect a change in hypothetical proportional reproductive success dataset comparing natural, ISS supplementation, general production (GP) hatchery stray and unknown origin adult Chinook salmon.

	Natural	ISS	GP	Unknown
Power	0.80	0.17	0.74	0.85

Of primary interest in this case is the relatively low power associated with the change in proportion of the ISS class. While we failed to reject the null hypothesis that natural and ISS origin adults experienced equivalent reproductive success, the relatively low power associated with the ISS class would indicate that it is inappropriate to assume that reproductive success is indeed equal. In this case, the small difference between adult and juvenile proportions within the ISS class (0.10 versus 0.13) is the primary contributor to the lack of statistical power. Typically, increasing sample sizes can increase power; however, due to the small difference in initial and final proportions, increasing the juvenile sample size ten-fold increases power to only 0.18. So, despite failing to reject the null hypothesis of equivalent reproductive success between natural and ISS origin adults, we have not proven that reproductive success is in fact equal. The only means to address this question is to accumulate multiple years of data to see if the relationship holds true.

Binomial Likelihood—In addition to computing the power analysis, it may be useful to visualize the distribution of probabilities associated with alternative results. To do so, we will employ the binomial likelihood function:

$$\mathrm{P}\left(p \middle| n, y\right) = \binom{n}{y} p^{y} (1-p)^{n-y}$$

For example, if 20% of the adults sampled in generation one were ISS hatchery origin fish, and reproductive success was equivalent among all origin and cross types, we would expect 20% of the progeny sampled in generation two to be assigned to an ISS adult. Using the binomial likelihood function, a distribution of probabilities can be generated. The observed result can be compared to this distribution to evaluate how closely the observed data match expectations. For example, if only 5% of the sampled progeny are assigned to ISS origin adults, it would suggest that the hypothesis of equivalent reproductive success is unlikely to be true (Figure 5.1). In fact, the probability of such a result is only 0.000015, compared to 0.099 for the expected value of 20%.

Question Two: Assortative Mating

Aside from the goal of determining relative reproductive success of adult classes, it will be important to determine whether adults spawn with one another randomly or, alternatively, maintain some degree of reproductive isolation based on origin type (assortative mating). If ISS and natural origin adults do not spawn together, rather than increasing the size of a targeted

spawning aggregate, supplementation could create two coexisting but isolated spawning aggregates.

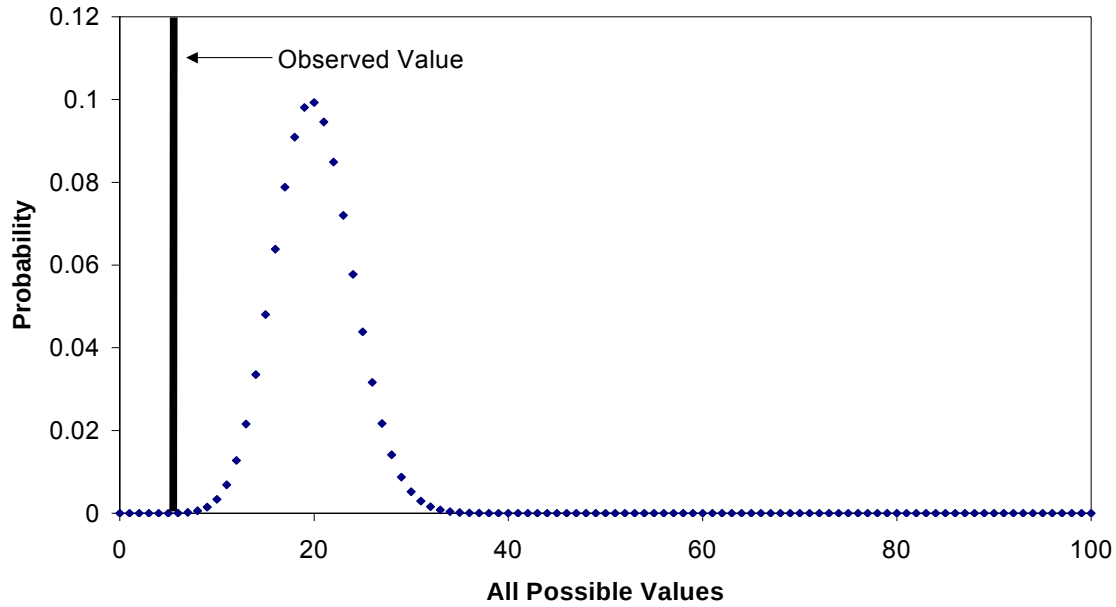


Figure 5.1. Comparison of an observed value to a binomial probability distribution constructed assuming equivalent reproductive success of all origin and cross types.

Directly testing the assumption of random mating is more difficult than testing for equivalency in reproductive success, because it requires the identification of triplets (mother and father matches for a given juvenile sample). Given this requirement, direct tests of random mating likely will be successful only in locations where nearly all adults can be sampled (e.g., the Upper Salmon and Pahsimeroi rivers). For illustrative purposes, we present two hypothetical examples of sample sizes required and minimum cost estimates to run these samples. The first example is a situation where there is a weir in place that is less than 100% effective (we assume 50%). This would be a reasonable representation for implementing an assortative mating analysis in the South Fork Salmon River. The second is an example of what we would expect if implementing an assortative mating program on the Pahsimeroi or Upper Salmon rivers; a "best case scenario."

Statistical Analysis of Parentage Data: Testing the Assumption of Random Mating

Determining Expected Cross Proportions—The first step in directly testing for deviations from random mating is determining the expected proportions of cross types within progeny (either juvenile or adult). For the purposes of the first example, we expect four classes of adults: natural, ISS, GP hatchery strays, and unknown origin (adults not sampled). Within each class of adults, it is possible to have males and females, yielding eight possible classes. From these eight possible classes, we can expect 16 possible cross-types among the progeny (Table 5.10). For the purposes of maintaining a reasonable sample size within each category, these cross types can be collapsed into eight categories (Table 5.11). For the second example,

we expect three classes of adults: natural, ISS, and unknown origin. Because the weir in this example is fish-proof, the unknown category in this example represents precocial males (i.e., there are no unknown females). Precocial males will be assumed present at 5% of the natural population. Again, males and females are present in each class (except unknown), yielding six possible cross-types (Table 5.12), which can be collapsed into five categories (Table 5.13).

Table 5.10. Potential juvenile cross-types possible in Idaho Supplementation Study (ISS) study streams given the four possible origins of the potential parents in streams with weirs that are 50% effective. General production hatchery stray adults are designated (GP). Adults of "unknown" origin are those that escaped past the weir without being sampled and are of either natural, ISS, or GP origin.

	Natural Male	**ISS Male**	**GP Male**	**Unknown Male**
Natural Female	NxN	NxISS	NxGP	NxU
ISS Female	ISSxN	ISSxISS	ISSxGP	ISSxU
GP Female	GPxN	GPxISS	GPxGP	GPxU
Unknown Female	UxN	UxISS	UxGP	UxU

Table 5.11. Collapsed potential juvenile cross-types possible in Idaho Supplementation Study (ISS) study streams given the four possible origins of the potential parents in streams with weirs that are 50% effective. General production hatchery stray adults are designated (GP). Adults of "unknown" origin are those that escaped past the weir without being sampled and are of either natural, ISS, or GP origin.

Collapsed Cross-Types
Natural x Natural
Natural x ISS
Natural x GP
ISS x ISS
ISS x GP
GP x GP
Natural x Unknown
ISS x Unknown
GP x Unknown
Unknown x Unknown

Table 5.12. Potential juvenile cross-types in the Pahsimeroi River and Upper Salmon River given the possible origins of the potential parents. Unknown males represent precocials that would not be sampled under the current design.

	Natural Male	**ISS Male**	**Unknown Male**
Natural Female	N x N	N x ISS	N x U
ISS Female	I x N	I x I	I x U

Table 5.13. Collapsed cross-type juvenile categories possible in the Pahsimeroi River and Upper Salmon River given the possible origins of the potential parents. Unknown males represent precocials that would not be sampled under the current design.

Collapsed Cross-Types
Natural x Natural
Natural x ISS
ISS x ISS
Natural x Unknown
ISS x Unknown

The expected proportion of sampled progeny falling into each cross-type can be calculated by multiplying the proportion of male adults of a given class to the proportion of female adults of a given class. We will assume equal sex ratios in all classes for simplicity and illustration. For the first example, given a sample of 200 natural origin adults, 100 ISS origin adults, 50 GP origin adults, and 200 unknown adults, we expect 13% of the sampled progeny to result from matings between natural origin adults (Table 5.14). In the second example, we again have a sample of 200 natural origin adults, 100 ISS origin adults, and 15 unknown (precocial) males. In this case, we expect approximately 40% of the sampled juveniles to be from natural x natural crosses (Table 5.15).

Table 5.14. Expected cross proportions in Idaho Supplementation Study (ISS) study streams given the four possible origins of the potential parents in streams with weirs that are 50% effective. General production hatchery stray adults are designated (GP). Adults of "unknown" origin are those that escaped past the weir without being sampled and are of either natural, ISS, or GP origin.

	Natural Male	**ISS Male**	**GP Male**	**Unknown Male**
Natural Female	0.132	0.066	0.033	0.132
ISS Female	0.066	0.033	0.017	0.066
GP Female	0.033	0.016	0.008	0.033
Unknown Female	0.132	0.066	0.033	0.132

Table 5.15. Expected cross proportions in the Pahsimeroi and Upper Salmon rivers given the possible origins of the potential parents. Unknown males represent precocials that would not be sampled under the current design.

	Natural Male	**ISS Male**	**Unknown Male**
Natural Female	0.404	0.202	0.061
ISS Female	0.202	0.101	0.030

Sample Size Requirements—As the number of categories of expected cross-types increases, the expected proportions of progeny within each cross-type decreases and sample size requirements become prohibitive. In the first example above, only eight out of 1,000 sampled progeny would be expected to result from a mating between two GP hatchery stray adults (Table 5.14). Probabilistically, ensuring that even one GPxGP progeny would be recorded would require a minimum of 1,318 juvenile samples (assuming equal reproductive

success among adult classes). Therefore, directly testing for assortative mating will be restricted to only the most common cross-types (natural and ISS origin adults in this example2). To expect to get at least five ISS x ISS juveniles in 99 out of 100 samples would require a collection of 1,222 juveniles (adjusted somewhat based on 50% weir efficiency and 50% carcass collection for an effective 75% adult sampling efficiency). The estimated cost to process this number of samples (given a per sample cost of $60) would be $73,320. However, this is a conservative estimate since it assumes we only collect the minimum number of juveniles, all samples amplify, and we have no genotyping error. Even after restricting classes, several years of data may be required to obtain reasonable statistical power.

Sampling under our best-case scenario would be much more cost effective and likely would result in reasonable statistical power sooner. (To expect to get at least five ISS x ISS juveniles in 99 out of 100 samples would require a collection of 351 juveniles.) The estimated cost to process this number of samples (given a per sample cost of $60) would be $21,060. Again, this is a conservative estimate since it assumes we only collect the minimum number of juveniles, all samples amplify, and we have no genotyping error.

Testing for Deviation from Expected Cross Proportions—Once the expected proportions of progeny cross-types are calculated and the number of cross-types to be tested is restricted, testing for deviation among cross-types will follow the same procedures outlined for testing relative reproductive success. In this example, excluding rare cross-types containing GP hatchery stray adults and those crosses containing parents of unknown origin, we would use a two-sided $\chi 2$–test to evaluate the following null hypothesis:

H_o: The proportion of natural x natural, natural x ISS, and ISS x ISS progeny is equivalent to the proportion of natural and ISS adults sampled in the previous generation.

Rejection of the null hypothesis would suggest that mating was not random or that one of the parental classes suffered lower relative reproductive success. We can exclude (or compensate for) the latter explanation using the relative reproductive success values calculated by the analyses outlined in the previous section. For example, if we know from previous tests of relative reproductive success that ISS adults demonstrate only 50% of the reproductive success of natural origin adults, we would adjust expected values to represent actualized expectations.

Power—Failure to reject the null hypothesis of random mating between origin types does not indicate that the null hypothesis is in fact true. As with tests of equal reproductive success, we will use a simple power analysis (equation 22.21, Zar 1984) to determine the probability of rejecting the null hypothesis when it is, in fact, false.

Timeframe for Parentage Analyses

The ISS study currently operates rotary screw traps in each of the locations recommended for implementation of parentage analyses. A subset of juveniles captured in these screw traps is PIT tagged for estimation of survival to LGR (Bowles and Leitzinger 1991).

We recommend that a tissue sample be nonlethally collected from PIT-tagged juveniles in these locations to determine the relative juvenile production of adult classes sampled at the

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2 We exclude “unknown” origin adults from these tests, because they do not yield useful information.

weir the previous generation. Since tissue samples will correspond with PIT tags, it may be possible to track the survival of juveniles by adult class in real-time. Doing so would enable researchers to follow life stage specific survival from in-stream juvenile capture to LGR and to adult return.

Where sample collections for parentage analyses are implemented in adult return year 2004, a complete sample of brood year 2004 juveniles could be collected by spring 2006 (excluding yearlings). Adult returns from brood year 2004 would commence with jacks in 2007 and would end with age-5 returns in 2009 in most streams. So, an analysis of relative reproductive success of adult classes (measured as relative juvenile production) could be completed as early as 2006. Relative survival from juvenile to adult could be estimated as early as 2009. This timeline will apply to the Pahsimeroi and upper Salmon rivers where DNA collection is currently underway. If funding for reproductive success analyses is made available for other streams, simple arithmetic can be used to compute when the collection of each component would be complete.

We propose to collect the samples necessary to conduct the parentage assignment study for one full generation. Therefore, by 2010 we would have relative adult-to-juvenile reproductive success for five complete juvenile cohorts. By 2013, we would have information on relative adult-to-adult return success for one complete generation. The samples necessary to conduct this investigation can be collected under the current ISS program scope. However, a new funding mechanism will need to be identified to finance this major change of program scope, and a laboratory identified with the time and throughput capacity to handle the large number of samples that this investigation will require.

ACKNOWLEDGEMENTS

This effort would not have been possible without the assistance and cooperation of far too many people to list here. Kim Apperson, Justin Bretz, Arnie Brimmer, Nathan Brindza, Jay Hesse, Andy Kohler, Brian Leth, Jill Olson, and Doug Taki all provided and summarized the data necessary for these analyses. Hatchery staff at the Clearwater, Kooskia, McCall, Pahsimeroi, and Sawtooth fish hatcheries made much of this work possible by creating the broodstocks and managing the returns. We must also acknowledge the efforts of the field crews from each of the cooperating agencies that have worked so hard to collect these data. We also appreciate the efforts of the Nez Perce Tribal Hatchery (BPA Project 1983-350-03), the Johnson Creek Artificial Production Monitoring and Evaluation project (BPA Project 1996-043-00), and the Natural Production Monitoring and Evaluation project (BPA Project 1991-073-00) for collecting ISS data. Cheryl Leben formatted the document. We would like to extend special thanks to Peter Lofy for all his assistance with contracting and making the administrative aspects of the program run smoothly. Finally, we appreciate the Bonneville Power Administration for funding the program.

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APPENDICES

Appendix A. Summary of the current status and data availability for all ISS study streams.

Study Stream	Current Status	Available Data (years data were collected)
Big Flat Creek	Treatment/Restoration Parr Non-Local Broodstock	Redd Counts (1992-ongoing) Carcass Recoveries (1992-ongoing) Parr Abundance (1991-1996)
Colt Killed Creek	Treatment/Restoration Parr Non-Local Broodstock	Redd Counts (1992-ongoing) Carcass Recoveries (1992-ongoing) Juvenile Emigration/Abundance (1998-ongoing) Parr Abundance (1991-1996)
Fishing Creek	Treatment/Restoration Parr Non-Local Broodstock	Redd Counts (1992-ongoing) Carcass Recoveries (1992-ongoing) Parr Abundance (1992-1993)
Pete King Creek	Treatment/Restoration Parr Non-Local Broodstock	Redd Counts (1991-ongoing) Carcass Recoveries (1992-ongoing) Parr Abundance (1991-1996)
West Fork Yankee Fork Salmon River	Treatment/Augmentation Presmolt Non-Local Broodstock	Redd Counts (1992-ongoing) Carcass Recoveries (1992-ongoing) Juvenile Emigration/Abundance (1998-ongoing) Parr Abundance (1991-1996)
Pahsimeroi River	Treatment/Augmentation Smolt Local Broodstock	Redd Counts (1992-ongoing) Carcass Recoveries (1992-ongoing) Juvenile Emigration/Abundance (1992-ongoing) Rack Returns Parr Abundance (1991-1993)
Legendary Bear Creek	Treatment/Restoration Smolt Non-Local Broodstock	Redd Counts (1992-ongoing) Carcass Recoveries (1992-ongoing) Parr Abundance (1992-1996)
Lolo Creek	Treatment/Augmentation Smolt Non-Local Broodstock	Redd Counts (1992-ongoing) Carcass Recoveries (1992-ongoing) Juvenile Emigration/Abundance (1992-ongoing) Rack Returns Parr Abundance (1992-1996)
East Fork Salmon River	Treatment/Augmentation Smolt Local Broodstock	Redd Counts (1992-ongoing) Carcass Recoveries (1993-1999) Juvenile Emigration/Abundance (1993-ongoing) Adult Returns (2004-ongoing)
Clear Creek	Treatment/Augmentation Smolt Local Broodstock	Redd Counts (1991-ongoing) Carcass Recoveries (1993-2000) Juvenile Emigration/Abundance (1998-ongoing) Parr Abundance (1991-1996) Rack Returns
Crooked River	Treatment/Restoration Parr and Presmolt Local Broodstock	Redd Counts (1991-ongoing) Carcass Recoveries (1992-1999) Juvenile Emigration/Abundance (1992-ongoing) Rack Returns Parr Abundance (1991-1997)

Appendix A. Continued.

Study Stream	Current Status	Available Data (years data were collected)
Red River	Treatment/Augmentation Presmolt and Smolt Local Broodstock	Redd Counts (1991-ongoing) Carcass Recoveries (1992-ongoing) Juvenile Emigration/Abundance (1992-ongoing) Rack Returns Parr Abundance (1991-1999)
South Fork Salmon River	Treatment/Augmentation Parr, Presmolt, and Smolt Local Broodstock	Redd Counts (1992-ongoing) Carcass Recoveries (1992-ongoing) Juvenile Emigration/Abundance (1992-ongoing) Rack Returns Parr Abundance (1991-1996)
Upper Salmon River	Treatment/Augmentation Presmolt and Smolt Local Broodstock	Redd Counts (1992-ongoing) Carcass Recoveries (1993-ongoing) Juvenile Emigration/Abundance (1993-ongoing) Parr Abundance (1992-1996)
Newsome Creek	Treatment/Restoration Parr and Smolt Non-Local Broodstock	Redd Counts (1992-ongoing) Carcass Recoveries (1992-ongoing) Juvenile Emigration/Abundance (1998-ongoing) Rack Returns Parr Abundance (1991-1996)
American River	Treatment/Restoration Smolt Non-Local Broodstock	Redd Counts (1992-ongoing) Carcass Recoveries (1992-ongoing) Juvenile Emigration/Abundance (1998-ongoing) Parr Abundance (1991-1999)
Brushy Fork Creek	Control	Redd Counts (1992-ongoing) Carcass Recoveries (1992-ongoing) Parr Abundance (1991-1997)
Crooked Fork Creek	Control	Redd Counts (1992-ongoing) Carcass Recoveries (1992-ongoing) Juvenile Emigration/Abundance (1992-ongoing) Rack Returns Parr Abundance (1991-1997)
White Cap Creek	Control	Redd Counts (1992-ongoing) Parr Abundance (1991-1997)
Herd Creek	Control	Redd Counts (1992-ongoing) Carcass Recoveries (1992-ongoing) Parr Abundance (1992-1996)
Lemhi River	Control	Redd Counts (1992-ongoing) Carcass Recoveries (1992-ongoing) Juvenile Emigration/Abundance (1992-ongoing) Rack Returns Parr Abundance (1991-1997)
Marsh Creek	Control	Redd Counts (1992-ongoing) Carcass Recoveries (1992-ongoing) Juvenile Emigration/Abundance (1993-ongoing) Rack Returns Parr Abundance (1992-1997)

Appendix A. Continued.

Study Stream	Current Status	Available Data (years data were collected)
Johnson Creek	Control	Redd Counts (1991-ongoing) Carcass Recoveries (1992-ongoing) Juvenile Emigration/Abundance (1998-ongoing) Rack Returns Parr Abundance (1992-1995)
North Fork Salmon River	Control	Redd Counts (1991-ongoing) Carcass Recoveries (1992-ongoing) Parr Abundance (1991-1995)
Lake Creek	Control	Redd Counts (1991-ongoing) Carcass Recoveries (1992-ongoing) Juvenile Emigration/Abundance (1997-ongoing) Video Weir (1997-ongoing) Parr Abundance (1992-1996)
Secesh River	Control	Redd Counts (1991-ongoing) Carcass Recoveries (1992-ongoing) Juvenile Emigration/Abundance (1997-ongoing) Parr Abundance (1991-1996)
Slate Creek	Control	Redd Counts (1991-ongoing) Carcass Recoveries (1992-ongoing) Parr Abundance (1992-1995)
Bear Valley Creek	Control	Redd Counts (1992-ongoing) Carcass Recoveries (1992-ongoing) Parr Abundance (1991-1996)
Valley Creek	Control	Redd Counts (1992-ongoing) Carcass Recoveries (1992-ongoing) Parr Abundance (1992-1996)
Eldorado Creek	Control	Redd Counts (91-ongoing) Carcass Recoveries (91-ongoing) Parr Abundance (91-ongoing)

Note: American River was reclassified in the ISS statistical review as treatment for statistical analysis of ISS data but has not been treated beyond brood year 1993. Parr abundance estimates were obtained via snorkeling and exhibit substantial variation, hence parr abundance estimates will generally not be considered in evaluations. Non-local broodstock indicates that fish outplanted for treatments were from an existing hatchery program and are not likely to represent the targeted spawning aggregate. Localized broodstock refers to management activities that initially outplanted non-local stocks and subsequently captured adults returning to a targeted stream for use as broodstock. Local broodstock refers to the practice of collecting broodstock from a targeted spawning aggregate and outplanting progeny back to the stream of parental origin.

Appendix B. Data used to develop the multiphased regression analysis recommended by the ISRP. Year code provides an alternative input to calendar year in which the zero value corresponds to the calendar year prior to an expected response resulting from ISS treatments. Years before an anticipated ISS response are sequentially negatively numbered from zero; years following an expected ISS response are sequentially positively numbered. Time values of one correspond to calendar years for which a change in the response variable could not have been the result of ISS treatments (i.e., there were no treatments in the previous generation). Time values of two correspond to calendar years in which a change in the response variable could be the result of ISS treatments. Year code and time values denoted by an X indicate likely gaps in changes to the response variable due to missed ISS treatments in the previous generation. Productivity estimates are based on smolt carrying capacities for each stream and the number of stream miles included in ISS evaluation. Treatment proportion = actual treatment/prescribed treatment. Group denotes the nine groupings into which streams were placed based on habitat similarity and geographic proximity. This was calculated for redd and smolt response variables.

Stream	Treatment Level	Year	Year Code	Time	Productivity smolt/mile[a]	Treatment Proportion	Straying	Group (redd)	Redds/ Km	Group (juvenile)	Smolts	Survival Wpar	Survival Wprs	Survival Wsmlt	Survival Wtotal
American R.	Partial	1991	-5	1	1471			SF Clearwater		SF Clearwater					
American R.	Partial	1992	-4	1	1471			SF Clearwater	0.15	SF Clearwater					
American R.	Partial	1993	-3	1	1471			SF Clearwater	6.04	SF Clearwater		0.100			
American R.	Partial	1994	-2	1	1471			SF Clearwater	0.26	SF Clearwater					
American R.	Partial	1995	-1	1	1471	0	0	SF Clearwater	0.00	SF Clearwater					
American R.	Partial	1996	0	1	1471	0	0	SF Clearwater	0.26	SF Clearwater					
American R.	Partial	1997	1	2	1471	1.73	0.857	SF Clearwater	8.99	SF Clearwater	9411	0.000	0.119	0.463	0.336
American R.	Partial	1998	2	2	1471	0	0.468	SF Clearwater	3.24	SF Clearwater	5579	0.000	0.139	0.451	0.251
American R.	Partial	1999	X	X	1471	0	0	SF Clearwater	0.03	SF Clearwater	413	0.000	0.142	0.434	0.320
American R.	Partial	2000	X	X	1471	0	0.755	SF Clearwater	3.76	SF Clearwater		0.000	0.173	0.644	0.402
American R.	Partial	2001	5	2	1471	0	0.659	SF Clearwater	11.27	SF Clearwater		0.000	0.016	0.502	0.423
Bear Valley Ck.	Control	1991	-3	1	4284			Upper Salmon							
Bear Valley Ck.	Control	1992	-2	1	4284			Upper Salmon	0.73						
Bear Valley Ck.	Control	1993	-1	1	4284			Upper Salmon	3.87						
Bear Valley Ck.	Control	1994	0	1	4284			Upper Salmon	0.11						
Bear Valley Ck.	Control	1995	1	2	4284	0	0	Upper Salmon	0.08						
Bear Valley Ck.	Control	1996	2	2	4284	0	0	Upper Salmon	0.34						
Bear Valley Ck.	Control	1997	3	2	4284	0	0	Upper Salmon	0.84						
Bear Valley Ck.	Control	1998	4	2	4284	0	0	Upper Salmon	1.79						
Bear Valley Ck.	Control	1999	5	2	4284	0	0	Upper Salmon	0.73						
Bear Valley Ck.	Control	2000	6	2	4284	0	0	Upper Salmon	1.65						
Bear Valley Ck.	Control	2001	7	2	4284	0	0	Upper Salmon	4.29						
Big Flat Ck.	Partial	1991	-4	1	3170			Upper Lochsa							
Big Flat Ck.	Partial	1992	-3	1	3170			Upper Lochsa	1.00						
Big Flat Ck.	Partial	1993	-2	1	3170			Upper Lochsa	0.50						
Big Flat Ck.	Partial	1994	-1	1	3170			Upper Lochsa							
Big Flat Ck.	Partial	1995	0	1	3170	0	0	Upper Lochsa	0.00						
Big Flat Ck.	Partial	1996	1	2	3170	0.766	0	Upper Lochsa	0.00						
Big Flat Ck.	Partial	1997	2	2	3170	0.937	0.5	Upper Lochsa	1.46						
Big Flat Ck.	Partial	1998	3	2	3170	0	0	Upper Lochsa							

Appendix B. Continued.

Stream	Treatment Level	Year	Year Code	Time	Productivity smolt/mile[a]	Treatment Proportion	Straying	Group (redd)	Redds/ Km	Group (juvenile)	Smolts	Survival Wpar	Survival Wprs	Survival Wsmlt	Survival Wtotal
Big Flat Ck.	Partial	1999	X	X	3170	0	0	Upper Lochsa							
Big Flat Ck.	Partial	2000	5	2	3170	0	0	Upper Lochsa	0.00						
Big Flat Ck.	Partial	2001	6	2	3170	0	0.75	Upper Lochsa	2.92						
Brushy Fork Ck.	Control	1991	-3	1	2861			Upper Lochsa							
Brushy Fork Ck.	Control	1992	-2	1	2861			Upper Lochsa	0.58			0.000			
Brushy Fork Ck.	Control	1993	-1	1	2861			Upper Lochsa	2.07			0.048			
Brushy Fork Ck.	Control	1994	0	1	2861			Upper Lochsa	0.00						
Brushy Fork Ck.	Control	1995	1	2	2861	0	0	Upper Lochsa	0.59						
Brushy Fork Ck.	Control	1996	2	2	2861	0	0	Upper Lochsa	0.41						
Brushy Fork Ck.	Control	1997	3	2	2861	0	0.622	Upper Lochsa	6.12						
Brushy Fork Ck.	Control	1998	4	2	2861	0	0.125	Upper Lochsa	1.57						
Brushy Fork Ck.	Control	1999	5	2	2861	0	0.666	Upper Lochsa	0.25			0.000			
Brushy Fork Ck.	Control	2000	6	2	2861	0	0.666	Upper Lochsa	1.32			0.000			
Brushy Fork Ck.	Control	2001	7	2	2861	0	0.108	Upper Lochsa	10.50						
Clear Ck.	Treatment	1991	-3	1	1122			Lower Lochsa		Clearwater					
Clear Ck.	Treatment	1992	-2	1	1122			Lower Lochsa	0.06	Clearwater			0.334	0.000	0.335
Clear Ck.	Treatment	1993	-1	1	1122			Lower Lochsa	0.39	Clearwater		0.000	0.235	0.588	0.268
Clear Ck.	Treatment	1994	0	1	1122			Lower Lochsa	0.06	Clearwater			0.000	0.000	0.000
Clear Ck.	Treatment	1995	1	2	1122	0	0	Lower Lochsa	0.00	Clearwater					
Clear Ck.	Treatment	1996	2	2	1122	0	0	Lower Lochsa	0.17	Clearwater	2692		0.436	0.511	0.453
Clear Ck.	Treatment	1997	3	2	1122	1.007	1	Lower Lochsa	0.66	Clearwater	1759	0.000	0.265	0.750	0.506
Clear Ck.	Treatment	1998	4	2	1122	1.014	0	Lower Lochsa	0.06	Clearwater	412	0.194	0.260	0.750	0.329
Clear Ck.	Treatment	1999	5	2	1122	0	1	Lower Lochsa	0.00	Clearwater					
Clear Ck.	Treatment	2000	6	2	1122	0.687	0	Lower Lochsa	1.04	Clearwater		0.161	0.347	0.511	0.316
Clear Ck.	Treatment	2001	7	2	1122	1.021	0.837	Lower Lochsa	6.98	Clearwater		0.066	0.115	0.421	0.198
Colt Killed Ck.	Treatment	1991	-3	1	7321			Upper Lochsa		Clearwater					
Colt Killed Ck.	Treatment	1992	-2	1	7321			Upper Lochsa	0.26	Clearwater					
Colt Killed Ck.	Treatment	1993	-1	1	7321			Upper Lochsa	0.17	Clearwater					
Colt Killed Ck.	Treatment	1994	0	1	7321			Upper Lochsa	0.04	Clearwater					
Colt Killed Ck.	Treatment	1995	1	2	7321	0.845	0	Upper Lochsa	0.04	Clearwater					
Colt Killed Ck.	Treatment	1996	2	2	7321	0.75	0	Upper Lochsa	0.04	Clearwater					
Colt Killed Ck.	Treatment	1997	3	2	7321	0.936	0.769	Upper Lochsa	0.71	Clearwater	2814	0.000	0.232	0.611	0.449
Colt Killed Ck.	Treatment	1998	4	2	7321	0	0	Upper Lochsa	0.00	Clearwater	1528		0.580	0.396	0.446
Colt Killed Ck.	Treatment	1999	5	2	7321	0	0	Upper Lochsa	0.00	Clearwater	470	0.000	0.444	0.723	0.645
Colt Killed Ck.	Treatment	2000	6	2	7321	0	1	Upper Lochsa	0.08	Clearwater		0.154	0.197	0.431	0.207
Colt Killed Ck.	Treatment	2001	7	2	7321	2.804	0.515	Upper Lochsa	2.91	Clearwater		0.000	0.094	0.347	0.153
Crooked Fork Ck.	Control	1991	-3	1	6524			Upper Lochsa		Clearwater	5802				
Crooked Fork Ck.	Control	1992	-2	1	6524			Upper Lochsa	0.67	Clearwater	6149	0.251	0.311	0.608	0.331
Crooked Fork Ck.	Control	1993	-1	1	6524			Upper Lochsa	0.61	Clearwater	1083	0.099	0.214	0.491	0.218
Crooked Fork Ck.	Control	1994	0	1	6524			Upper Lochsa	0.00	Clearwater	84		0.301	0.455	0.305
Crooked Fork Ck.	Control	1995	1	2	6524	0	0	Upper Lochsa	0.24	Clearwater	801	0.000	0.245	0.571	0.264
Crooked Fork Ck.	Control	1996	2	2	6524	0	0.732	Upper Lochsa	4.55	Clearwater		0.346	0.543	0.587	0.547
Crooked Fork Ck.	Control	1997	3	2	6524	0	0.943	Upper Lochsa	6.91	Clearwater	6411	0.189	0.320	0.582	0.357
Crooked Fork Ck.	Control	1998	4	2	6524	0	0.315	Upper Lochsa	1.03	Clearwater	5655	0.000	0.322	0.585	0.365
Crooked Fork Ck.	Control	1999	5	2	6524	0	0.562	Upper Lochsa	0.49	Clearwater	4160	0.346	0.380	0.521	0.419
Crooked Fork Ck.	Control	2000	6	2	6524	0	0.544	Upper Lochsa	6.06	Clearwater		0.114	0.244	0.396	0.223
Crooked Fork Ck.	Control	2001	7	2	6524	0	0.387	Upper Lochsa	13.88	Clearwater		0.124	0.104	0.407	0.131

Appendix B. Continued.

Stream	Treatment Level	Year	Year Code	Time	Productivity smolt/mile[a]	Treatment Proportion	Straying	Group (redd)	Redds/ Km	Group (juvenile)	Smolts	Survival Wpar	Survival Wprs	Survival Wsmlt	Survival Wtotal
Crooked R.	Treatment	1991	-5	1	2647			SF Clearwater		SF Clearwater	240				
Crooked R.	Treatment	1992	-4	1	2647			SF Clearwater	2.47	SF Clearwater	3999	0.140	0.263	0.485	0.279
Crooked R.	Treatment	1993	-3	1	2647			SF Clearwater	2.47	SF Clearwater	10271	0.203	0.122	0.449	0.285
Crooked R.	Treatment	1994	-2	1	2647			SF Clearwater	0.18	SF Clearwater	779	0.138	0.091	0.408	0.199
Crooked R.	Treatment	1995	-1	1	2647	0	0	SF Clearwater	0.00	SF Clearwater				1.000	
Crooked R.	Treatment	1996	0	1	2647	0	0	SF Clearwater	0.27	SF Clearwater	1067		0.272	0.623	0.552
Crooked R.	Treatment	1997	1	2	2647	0.498	0.097	SF Clearwater	2.97	SF Clearwater	6337	0.000	0.236	0.459	0.370
Crooked R.	Treatment	1998	2	2	2647	0	0	SF Clearwater	1.44	SF Clearwater	903		0.162	0.530	0.299
Crooked R.	Treatment	1999	X	X	2647	0	0.5	SF Clearwater	0.05	SF Clearwater	511	0.000	0.000	0.537	0.485
Crooked R.	Treatment	2000	X	X	2647	0	0.807	SF Clearwater	4.45	SF Clearwater		0.000	0.250	0.430	0.390
Crooked R.	Treatment	2001	5	2	2647	0.405	0.504	SF Clearwater	6.51	SF Clearwater		0.130		0.330	0.320
EF Salmon R.	Partial	1991	-3	1	4446			Middle Salmon		Upper Salmon	1691				
EF Salmon R.	Partial	1992	-2	1	4446			Middle Salmon	0.04	Upper Salmon	77	0.000	0.138	0.429	0.160
EF Salmon R.	Partial	1993	-1	1	4446			Middle Salmon	0.70	Upper Salmon	2597	0.130	0.185	0.510	0.245
EF Salmon R.	Partial	1994	0	1	4446			Middle Salmon	0.19	Upper Salmon	1098	0.000	0.300	0.476	0.403
EF Salmon R.	Partial	1995	1	2	4446	0.201	0	Middle Salmon	0.00	Upper Salmon					
EF Salmon R.	Partial	1996	2	2	4446	0.071	0	Middle Salmon	0.07	Upper Salmon					
EF Salmon R.	Partial	1997	3	2	4446	0.282	0	Middle Salmon	0.00	Upper Salmon					
EF Salmon R.	Partial	1998	4	2	4446	0	0	Middle Salmon	0.78	Upper Salmon	5493			0.558	
EF Salmon R.	Partial	1999	5	2	4446	0	.	Middle Salmon	0.30	Upper Salmon	446		0.299	0.564	0.348
EF Salmon R.	Partial	2000	6	2	4446	0	.	Middle Salmon	0.07	Upper Salmon		0.084			
EF Salmon R.	Partial	2001	7	2	4446	0	.	Middle Salmon	0.93	Upper Salmon		0.000		0.494	0.469
Eldorado Ck.	Control	1991	-9	1	.			Lower Lochsa							
Eldorado Ck.	Control	1992	-8	1	.			Lower Lochsa	0.00						
Eldorado Ck.	Control	1993	-7	1	.			Lower Lochsa	0.57						
Eldorado Ck.	Control	1994	-6	1	.			Lower Lochsa	0.00						
Eldorado Ck.	Control	1995	-5	1	.	0	0	Lower Lochsa	0.00						
Eldorado Ck.	Control	1996	-4	1	.	0	0	Lower Lochsa	0.00						
Eldorado Ck.	Control	1997	-3	1	.	0	0	Lower Lochsa	0.00						
Eldorado Ck.	Control	1998	-2	1	.	0	0	Lower Lochsa	0.00						
Eldorado Ck.	Control	1999	-1	1	.	0	0	Lower Lochsa	0.00						
Eldorado Ck.	Control	2000	0	1	.	0	0	Lower Lochsa	0.00						
Eldorado Ck.	Control	2001	1	2	.	0	0.214	Lower Lochsa	1.14						
Fishing Ck.	Treatment	1991	-3	1	447			Upper Lochsa							
Fishing Ck.	Treatment	1992	-2	1	447			Upper Lochsa	0.17						
Fishing Ck.	Treatment	1993	-1	1	447			Upper Lochsa	0.00						
Fishing Ck.	Treatment	1994	0	1	447			Upper Lochsa	0.00						
Fishing Ck.	Treatment	1995	1	2	447	0.633	0	Upper Lochsa	0.00						
Fishing Ck.	Treatment	1996	2	2	447	0.75	0	Upper Lochsa	0.17						
Fishing Ck.	Treatment	1997	3	2	447	0.936	1	Upper Lochsa	2.83			0.182			
Fishing Ck.	Treatment	1998	4	2	447	0	0	Upper Lochsa	1.83						
Fishing Ck.	Treatment	1999	5	2	447	0	0	Upper Lochsa	0.67						
Fishing Ck.	Treatment	2000	6	2	447	0	0	Upper Lochsa	0.67						
Fishing Ck.	Treatment	2001	7	2	447	0.802	0	Upper Lochsa	10.67			0.144			
Herd Ck.	Control	1991	-3	1	2156			Middle Salmon							
Herd Ck.	Control	1992	-2	1	2156			Middle Salmon	0.21						
Herd Ck.	Control	1993	-1	1	2156			Middle Salmon	2.52						

Appendix B. Continued.

Stream	Treatment Level	Year	Year Code	Time	Productivity smolt/mile[a]	Treatment Proportion	Straying	Group (redd)	Redds/ Km	Group (juvenile)	Smolts	Survival Wpar	Survival Wprs	Survival Wsmlt	Survival Wtotal
Herd Ck.	Control	1994	0	1	2156			Middle Salmon	0.23						
Herd Ck.	Control	1995	1	2	2156	0	0	Middle Salmon	0.00						
Herd Ck.	Control	1996	2	2	2156	0	0	Middle Salmon	0.00						
Herd Ck.	Control	1997	3	2	2156	0	0.08	Middle Salmon	0.82						
Herd Ck.	Control	1998	4	2	2156	0	0	Middle Salmon	0.59						
Herd Ck.	Control	1999	5	2	2156	0	0.08	Middle Salmon	0.18						
Herd Ck.	Control	2000	6	2	2156	0	0.08	Middle Salmon	0.18						
Herd Ck.	Control	2001	7	2	2156	0	0.08	Middle Salmon	1.29						
Johnson Ck.	Control	1991	-3	1	3412			SF Salmon							
Johnson Ck.	Control	1992	-2	1	3412			SF Salmon	2.78						
Johnson Ck.	Control	1993	-1	1	3412			SF Salmon	7.87						
Johnson Ck.	Control	1994	0	1	3412			SF Salmon	1.20						
Johnson Ck.	Control	1995	1	2	3412	0	0	SF Salmon	0.23						
Johnson Ck.	Control	1996	2	2	3412	0	0.05	SF Salmon	1.02						
Johnson Ck.	Control	1997	3	2	3412	0	0.019	SF Salmon	4.54			0.257	0.305	0.621	0.413
Johnson Ck.	Control	1998	4	2	3412	0	0	SF Salmon	3.80			0.268	0.297	0.493	0.319
Johnson Ck.	Control	1999	5	2	3412	.	0	SF Salmon	0.95			0.313	0.341	0.619	0.422
Johnson Ck.	Control	2000	6	2	3412	.	0.157	SF Salmon	1.30			0.328	0.269	0.491	0.295
Johnson Ck.	Control	2001	7	2	3412	.	0.131	SF Salmon	15.28			0.120	0.140	0.380	0.230
Lake Ck.	Control	1991	-3	1	2820			SF Salmon		SF Salmon					
Lake Ck.	Control	1992	-2	1	2820			SF Salmon	2.42	SF Salmon					
Lake Ck.	Control	1993	-1	1	2820			SF Salmon	2.47	SF Salmon					
Lake Ck.	Control	1994	0	1	2820			SF Salmon	0.67	SF Salmon					
Lake Ck.	Control	1995	1	2	2820	0	0	SF Salmon	0.67	SF Salmon					
Lake Ck.	Control	1996	2	2	2820	0	0	SF Salmon	1.74	SF Salmon	917		0.386	0.577	0.421
Lake Ck.	Control	1997	3	2	2820	0	0.111	SF Salmon	2.65	SF Salmon	478	0.203	0.255	0.404	0.253
Lake Ck.	Control	1998	4	2	2820	0	0.086	SF Salmon	2.41	SF Salmon	876	0.288	0.292	0.415	0.303
Lake Ck.	Control	1999	5	2	2820	0	0.058	SF Salmon	1.16	SF Salmon	536	0.268	0.390	0.494	0.360
Lake Ck.	Control	2000	6	2	2820	0	0.011	SF Salmon	8.62	SF Salmon	486	0.113	0.149	0.427	0.148
Lake Ck.	Control	2001	7	2	2820	0	0.054	SF Salmon	16.23	SF Salmon		0.082	0.097	0.286	0.116
Legendary Bear Ck.	Treatment	1991	-4	1	451			Upper Lochsa							
Legendary Bear Ck.	Treatment	1992	-3	1	451			Upper Lochsa	3.33						
Legendary Bear Ck.	Treatment	1993	-2	1	451			Upper Lochsa	5.00			0.117			
Legendary Bear Ck.	Treatment	1994	-1	1	451			Upper Lochsa	0.00						
Legendary Bear Ck.	Treatment	1995	0	1	451	0	1	Upper Lochsa	0.33						
Legendary Bear Ck.	Treatment	1996	1	2	451	0.322	0	Upper Lochsa	2.33						
Legendary Bear Ck.	Treatment	1997	2	2	451	1.106	0.309	Upper Lochsa	9.12			0.167			
Legendary Bear Ck.	Treatment	1998	3	2	451	0	0	Upper Lochsa	1.91			0.131			
Legendary Bear Ck.	Treatment	1999	X	X	451	0	0	Upper Lochsa	0.67						
Legendary Bear Ck.	Treatment	2000	5	2	451	0	0.526	Upper Lochsa	6.83			0.142			
Legendary Bear Ck.	Treatment	2001	6	2	451	0.959	0.042	Upper Lochsa	32.33			0.101			
Lemhi R.	Control	1991	-3	1	11794			Lower Salmon		Lower Salmon					
Lemhi R.	Control	1992	-2	1	11794			Lower Salmon	0.29	Lower Salmon	824		0.266	0.322	0.275
Lemhi R.	Control	1993	-1	1	11794			Lower Salmon	0.72	Lower Salmon	1215	0.146	0.369	0.710	0.381
Lemhi R.	Control	1994	0	1	11794			Lower Salmon	0.39	Lower Salmon	398		0.394	0.792	0.469
Lemhi R.	Control	1995	1	2	11794	0	0	Lower Salmon	0.17	Lower Salmon	95			0.472	
Lemhi R.	Control	1996	2	2	11794	0	0	Lower Salmon	0.56	Lower Salmon	1726		0.547	0.754	0.587

Appendix B. Continued.

Stream	Treatment Level	Year	Year Code	Time	Productivity smolt/mile[a]	Treatment Proportion	Straying	Group (redd)	Redds/ Km	Group (juvenile)	Smolts	Survival Wpar	Survival Wprs	Survival Wsmlt	Survival Wtotal
Lemhi R.	Control	1997	3	2	11794	0	0	Lower Salmon	0.97	Lower Salmon	5987	0.286	0.411	0.730	0.464
Lemhi R.	Control	1998	4	2	11794	0	0	Lower Salmon	0.79	Lower Salmon	2113	0.246	0.431	0.466	0.440
Lemhi R.	Control	1999	5	2	11794	0	0	Lower Salmon	0.93	Lower Salmon	1188	0.083	0.344	0.338	0.336
Lemhi R.	Control	2000	6	2	11794	0	0	Lower Salmon	1.80	Lower Salmon		0.000	0.416	0.502	0.512
Lemhi R.	Control	2001	7	2	11794	0	0	Lower Salmon	6.56	Lower Salmon		0.054	0.231	0.500	0.267
Lolo Ck.	Treatment	1991	-9	1	2393			Lower Lochsa		Clearwater	617		0.313	0.774	0.346
Lolo Ck.	Treatment	1992	-8	1	2393			Lower Lochsa	0.90	Clearwater	1742		0.276	0.690	0.380
Lolo Ck.	Treatment	1993	-7	1	2393			Lower Lochsa	1.14	Clearwater	9249		0.215	0.706	0.354
Lolo Ck.	Treatment	1994	-6	1	2393			Lower Lochsa	0.33	Clearwater	872		0.143	0.713	0.245
Lolo Ck.	Treatment	1995	-5	1	2393	0	0	Lower Lochsa	0.28	Clearwater					
Lolo Ck.	Treatment	1996	-4	1	2393	0	0	Lower Lochsa	1.00	Clearwater			0.444	0.794	0.549
Lolo Ck.	Treatment	1997	-3	1	2393	0	0.761	Lower Lochsa	5.21	Clearwater	69443		0.180	0.661	0.281
Lolo Ck.	Treatment	1998	-2	1	2393	0	0.15	Lower Lochsa	1.47	Clearwater	12183		0.189	0.856	0.497
Lolo Ck.	Treatment	1999	-1	1	2393	0	0.875	Lower Lochsa	0.43	Clearwater	1720		0.213	0.656	0.298
Lolo Ck.	Treatment	2000	0	1	2393	0	0.22	Lower Lochsa	4.74	Clearwater			0.120	0.880	0.490
Lolo Ck.	Treatment	2001	1	2	2393	0.841	0.26	Lower Lochsa	20.28	Clearwater			0.190	0.660	0.300
Marsh Ck.	Control	1991	-3	1	3460			Upper Salmon		Upper Salmon	655				
Marsh Ck.	Control	1992	-2	1	3460			Upper Salmon	6.74	Upper Salmon	962	0.299	0.315	0.352	0.311
Marsh Ck.	Control	1993	-1	1	3460			Upper Salmon	4.09	Upper Salmon	912	0.168	0.261	0.421	0.266
Marsh Ck.	Control	1994	0	1	3460			Upper Salmon	0.82	Upper Salmon	187	0.000	0.397	0.000	0.401
Marsh Ck.	Control	1995	1	2	3460	0	0	Upper Salmon	0.00	Upper Salmon					
Marsh Ck.	Control	1996	2	2	3460	0	0	Upper Salmon	0.55	Upper Salmon	72	0.593	0.580	0.551	0.577
Marsh Ck.	Control	1997	3	2	3460	0	0	Upper Salmon	3.46	Upper Salmon	1705				
Marsh Ck.	Control	1998	4	2	3460	0	0	Upper Salmon	3.73	Upper Salmon	1626	0.258	0.411	0.690	0.325
Marsh Ck.	Control	1999	5	2	3460	0	0	Upper Salmon	0.00	Upper Salmon				0.311	
Marsh Ck.	Control	2000	6	2	3460	0	0.038	Upper Salmon	2.73	Upper Salmon		0.300	0.391	0.340	0.281
Marsh Ck.	Control	2001	7	2	3460	0	0.016	Upper Salmon	10.00	Upper Salmon		0.148	0.188	0.317	0.181
Newsome Ck.	Treatment	1991	-5	1	1259			SF Clearwater		SF Clearwater					
Newsome Ck.	Treatment	1992	-4	1	1259			SF Clearwater	0.13	SF Clearwater			0.183		
Newsome Ck.	Treatment	1993	-3	1	1259			SF Clearwater	3.64	SF Clearwater					
Newsome Ck.	Treatment	1994	-2	1	1259			SF Clearwater	0.00	SF Clearwater					
Newsome Ck.	Treatment	1995	-1	1	1259	0	0	SF Clearwater	0.00	SF Clearwater					
Newsome Ck.	Treatment	1996	0	1	1259	0	0.666	SF Clearwater	0.27	SF Clearwater				0.429	
Newsome Ck.	Treatment	1997	1	2	1259	1.896	0.551	SF Clearwater	4.44	SF Clearwater	3682		0.174	0.500	0.176
Newsome Ck.	Treatment	1998	2	2	1259	0	0.09	SF Clearwater	2.12	SF Clearwater	2648		0.153	0.559	0.161
Newsome Ck.	Treatment	1999	X	X	1259	0	0	SF Clearwater	0.00	SF Clearwater			0.000	0.000	0.000
Newsome Ck.	Treatment	2000	X	X	1259	0	0.167	SF Clearwater	0.33	SF Clearwater			0.118	0.479	0.156
Newsome Ck.	Treatment	2001	5	2	1259	0.741	0.69	SF Clearwater	14.64	SF Clearwater			0.070	0.318	0.111
NF Salmon R.	Control	1991	-3	1	2650			Lower Salmon							
NF Salmon R.	Control	1992	-2	1	2650			Lower Salmon	0.33						
NF Salmon R.	Control	1993	-1	1	2650			Lower Salmon	0.46						
NF Salmon R.	Control	1994	0	1	2650			Lower Salmon	0.08						
NF Salmon R.	Control	1995	1	2	2650	0	0	Lower Salmon	0.03						
NF Salmon R.	Control	1996	2	2	2650	0	0	Lower Salmon	0.14						
NF Salmon R.	Control	1997	3	2	2650	0	0	Lower Salmon	0.27						
NF Salmon R.	Control	1998	4	2	2650	0	0	Lower Salmon	0.08						
NF Salmon R.	Control	1999	5	2	2650	0	0	Lower Salmon	0.05						

Appendix B. Continued.

Stream	Treatment Level	Year	Year Code	Time	Productivity smolt/mile[a]	Treatment Proportion	Straying	Group (redd)	Redds/ Km	Group (juvenile)	Smolts	Survival Wpar	Survival Wprs	Survival Wsmlt	Survival Wtotal
NF Salmon R.	Control	2000	6	2	2650	0	0	Lower Salmon	0.72						
NF Salmon R.	Control	2001	7	2	2650	0	0	Lower Salmon	2.77						
Pahsimeroi R.	Treatment	1991	-3	1	12268			Lower Salmon		Lower Salmon	3781				
Pahsimeroi R.	Treatment	1992	-2	1	12268			Lower Salmon	1.21	Lower Salmon	10564	0.140	0.274	0.278	0.259
Pahsimeroi R.	Treatment	1993	-1	1	12268			Lower Salmon	3.82	Lower Salmon	19814	0.170	0.301	0.463	0.313
Pahsimeroi R.	Treatment	1994	0	1	12268			Lower Salmon	1.07	Lower Salmon	61	0.000	0.308	0.706	0.559
Pahsimeroi R.	Treatment	1995	1	2	12268	0.627	0	Lower Salmon	0.67	Lower Salmon	60	0.175	0.508	0.503	0.415
Pahsimeroi R.	Treatment	1996	2	2	12268	0.347	0	Lower Salmon	0.79	Lower Salmon	602	0.308	0.242	0.727	0.711
Pahsimeroi R.	Treatment	1997	3	2	12268	1.1	0	Lower Salmon	1.44	Lower Salmon	3948	0.364	0.386	0.677	0.547
Pahsimeroi R.	Treatment	1998	4	2	12268	0	0	Lower Salmon	1.57	Lower Salmon	2576	0.351	0.385	0.575	0.501
Pahsimeroi R.	Treatment	1999	5	2	12268	0.911	0	Lower Salmon	3.437	Lower Salmon	1937	0.176	0.266	0.201	0.241
Pahsimeroi R.	Treatment	2000	6	2	12268	0	0	Lower Salmon	2.58	Lower Salmon	4876	0.115	0.230	0.626	0.572
Pahsimeroi R.	Treatment	2001	7	2	12268	1.013	0	Lower Salmon	5.96	Lower Salmon		0.126	0.208	0.531	0.381
Pete King Ck.	Treatment	1991	-4	1	643			Lower Lochsa							
Pete King Ck.	Treatment	1992	-3	1	643			Lower Lochsa	0.00						
Pete King Ck.	Treatment	1993	-2	1	643			Lower Lochsa	0.00						
Pete King Ck.	Treatment	1994	-1	1	643			Lower Lochsa	0.00						
Pete King Ck.	Treatment	1995	0	1	643	0	0	Lower Lochsa	0.00						
Pete King Ck.	Treatment	1996	1	2	643	0.692	0	Lower Lochsa	0.00						
Pete King Ck.	Treatment	1997	2	2	643	0.87	0	Lower Lochsa	0.13						
Pete King Ck.	Treatment	1998	3	2	643	0	0	Lower Lochsa	0.00						
Pete King Ck.	Treatment	1999	X	X	643	0	0	Lower Lochsa	0.00						
Pete King Ck.	Treatment	2000	4	2	643	0	0	Lower Lochsa	0.25						
Pete King Ck.	Treatment	2001	5	2	643	0.744	0	Lower Lochsa	2.13						
Red R.	Treatment	1991	-3	1	2236			SF Clearwater		SF Clearwater	3596				
Red R.	Treatment	1992	-2	1	2236			SF Clearwater	1.02	SF Clearwater	2976	0.261	0.293	0.521	0.343
Red R.	Treatment	1993	-1	1	2236			SF Clearwater	1.79	SF Clearwater	7445	0.111	0.164	0.529	0.290
Red R.	Treatment	1994	0	1	2236			SF Clearwater	0.54	SF Clearwater			0.265	0.653	0.358
Red R.	Treatment	1995	1	2	2236	0.075	0.333	SF Clearwater	0.40	SF Clearwater	322		0.226	0.667	0.521
Red R.	Treatment	1996	2	2	2236	0.278	0.437	SF Clearwater	1.20	SF Clearwater	1252		0.321	0.667	0.387
Red R.	Treatment	1997	3	2	2236	0.997	0.397	SF Clearwater	7.78	SF Clearwater	18849	0.000	0.168	0.429	0.236
Red R.	Treatment	1998	4	2	2236	0	0.307	SF Clearwater	2.10	SF Clearwater	14086	0.127	0.263	0.478	0.353
Red R.	Treatment	1999	5	2	2236	0	0	SF Clearwater	0.35	SF Clearwater	815	0.167	0.229	0.502	0.318
Red R.	Treatment	2000	6	2	2236	0.64	0.455	SF Clearwater	5.93	SF Clearwater		0.000	0.111	0.699	0.435
Red R.	Treatment	2001	7	2	2236	0.826	0.726	SF Clearwater	7.87	SF Clearwater		0.000	0.036	0.332	0.186
Secesh R.	Control	1991	-3	1	2820			SF Salmon		SF Salmon					
Secesh R.	Control	1992	-2	1	2820			SF Salmon	5.55	SF Salmon					
Secesh R.	Control	1993	-1	1	2820			SF Salmon	7.65	SF Salmon					
Secesh R.	Control	1994	0	1	2820			SF Salmon	1.77	SF Salmon					
Secesh R.	Control	1995	1	2	2820	0	0	SF Salmon	1.51	SF Salmon					
Secesh R.	Control	1996	2	2	2820	0	0.022	SF Salmon	3.45	SF Salmon	3700		0.334	0.364	0.336
Secesh R.	Control	1997	3	2	2820	0	0.16	SF Salmon	6.22	SF Salmon	3152	0.154	0.293	0.300	0.277
Secesh R.	Control	1998	4	2	2820	0	0.142	SF Salmon	4.20	SF Salmon	1402	0.379	0.334	0.402	0.327
Secesh R.	Control	1999	5	2	2820	0	0	SF Salmon	2.86	SF Salmon	2058	0.310	0.373	0.398	0.354
Secesh R.	Control	2000	6	2	2820	0	0	SF Salmon	8.74	SF Salmon	1636	0.163	0.211	0.497	0.218
Secesh R.	Control	2001	7	2	2820	0	0.053	SF Salmon	20.08	SF Salmon		0.096	0.108	0.206	0.116
SF Salmon R.	Treatment	1991	-3	1	2919			SF Salmon		SF Salmon	1577				

Appendix B. Continued.

Stream	Treatment Level	Year	Year Code	Time	Productivity smolt/mile[a]	Treatment Proportion	Straying	Group (redd)	Redds/ Km	Group (juvenile)	Smolts	Survival Wpar	Survival Wprs	Survival Wsmlt	Survival Wtotal
SF Salmon R.	Treatment	1992	-2	1	2919			SF Salmon	22.48	SF Salmon	9086	0.195	0.194	0.347	0.230
SF Salmon R.	Treatment	1993	-1	1	2919			SF Salmon	34.36	SF Salmon	1083	0.000	0.124	0.443	0.190
SF Salmon R.	Treatment	1994	0	1	2919			SF Salmon	3.76	SF Salmon	3225	0.000	0.212	0.410	0.239
SF Salmon R.	Treatment	1995	1	2	2919	0.558	0.701	SF Salmon	3.02	SF Salmon	2016	0.227	0.168	0.444	0.233
SF Salmon R.	Treatment	1996	2	2	2919	0.991	0.127	SF Salmon	3.86	SF Salmon	1688	0.316	0.284	0.486	0.330
SF Salmon R.	Treatment	1997	3	2	2919	0.161	0.151	SF Salmon	13.07	SF Salmon	6976	0.144	0.165	0.522	0.248
SF Salmon R.	Treatment	1998	4	2	2919	0.985	0.287	SF Salmon	7.38	SF Salmon	6349	0.174	0.213	0.397	0.255
SF Salmon R.	Treatment	1999	5	2	2919	0.266	0.607	SF Salmon	12.82	SF Salmon	6876	0.184	0.193	0.477	0.266
SF Salmon R.	Treatment	2000	6	2	2919	0.175	0.416	SF Salmon	14.36	SF Salmon	3927	0.098	0.112	0.533	0.239
SF Salmon R.	Treatment	2001	7	2	2919	0.69	0.335	SF Salmon	21.29	SF Salmon		0.038	0.092	0.461	0.152
Slate Ck.	Control	1991	-3	1	2902			Slate							
Slate Ck.	Control	1992	-2	1	2902			Slate	0.72						
Slate Ck.	Control	1993	-1	1	2902			Slate	0.18						
Slate Ck.	Control	1994	0	1	2902			Slate	0.36						
Slate Ck.	Control	1995	1	2	2902	0	0	Slate	0.54						
Slate Ck.	Control	1996	2	2	2902	0	0	Slate	0.00						
Slate Ck.	Control	1997	3	2	2902	0	0.5	Slate	0.90						
Slate Ck.	Control	1998	4	2	2902	0	0	Slate	1.09						
Slate Ck.	Control	1999	5	2	2902	0	0	Slate	0.36						
Slate Ck.	Control	2000	6	2	2902	0	0	Slate	0.72						
Slate Ck.	Control	2001	7	2	2902	0	0.142	Slate	3.26						
Upper Salmon R.	Treatment	1991	-3	1	4268			Upper Salmon		Upper Salmon	2739				
Upper Salmon R.	Treatment	1992	-2	1	4268			Upper Salmon	0.46	Upper Salmon	3992	0.091	0.136	0.321	0.260
Upper Salmon R.	Treatment	1993	-1	1	4268			Upper Salmon	2.15	Upper Salmon	10887	0.112	0.207	0.563	0.313
Upper Salmon R.	Treatment	1994	0	1	4268			Upper Salmon	0.37	Upper Salmon	3615	0.000	0.333	0.719	0.457
Upper Salmon R.	Treatment	1995	1	2	4268	0.494	0	Upper Salmon	0.00	Upper Salmon					
Upper Salmon R.	Treatment	1996	2	2	4268	0.145	0	Upper Salmon	0.24	Upper Salmon	798	0.323	0.407	0.745	0.613
Upper Salmon R.	Treatment	1997	3	2	4268	0.411	0	Upper Salmon	0.14	Upper Salmon	315	0.303	0.302	0.655	0.450
Upper Salmon R.	Treatment	1998	4	2	4268	0.049	0	Upper Salmon	0.42	Upper Salmon	6364	0.252	0.271	0.553	0.367
Upper Salmon R.	Treatment	1999	5	2	4268	0.009	0	Upper Salmon	0.24	Upper Salmon	2830	0.188	0.267	0.403	0.313
Upper Salmon R.	Treatment	2000	6	2	4268	0.086	0	Upper Salmon	2.48	Upper Salmon		0.141	0.241	0.423	0.271
Upper Salmon R.	Treatment	2001	7	2	4268	0.212	0	Upper Salmon	4.36	Upper Salmon		0.074	0.153	0.455	0.290
Valley Ck.	Control	1991	-3	1	2806			Upper Salmon							
Valley Ck.	Control	1992	-2	1	2806			Upper Salmon	0.21						
Valley Ck.	Control	1993	-1	1	2806			Upper Salmon	1.40						
Valley Ck.	Control	1994	0	1	2806			Upper Salmon	0.09						
Valley Ck.	Control	1995	1	2	2806	0	0	Upper Salmon	0.00						
Valley Ck.	Control	1996	2	2	2806	0	0	Upper Salmon	0.02						
Valley Ck.	Control	1997	3	2	2806	0	0	Upper Salmon	0.15						
Valley Ck.	Control	1998	4	2	2806	0	0	Upper Salmon	0.99						
Valley Ck.	Control	1999	5	2	2806	0	0	Upper Salmon	0.54						
Valley Ck.	Control	2000	6	2	2806	0	0	Upper Salmon	0.69						
Valley Ck.	Control	2001	7	2	2806	0	0	Upper Salmon	1.83						
WF Yankee Fork	Partial	1991	-5	1	6743			Middle Salmon		Upper Salmon					
WF Yankee Fork	Partial	1992	-4	1	6743			Middle Salmon	0.52	Upper Salmon					
WF Yankee Fork	Partial	1993	-3	1	6743			Middle Salmon	1.21	Upper Salmon		0.129			
WF Yankee Fork	Partial	1994	-2	1	6743			Middle Salmon	0.78	Upper Salmon					

Appendix B. Continued.

Stream	Treatment Level	Year	Year Code	Time	Productivity smolt/mile[a]	Treatment Proportion	Straying	Group (redd)	Redds/ Km	Group (juvenile)	Smolts	Survival Wpar	Survival Wprs	Survival Wsmlt	Survival Wtotal
WF Yankee Fork	Partial	1995	-1	1	6743	0	0	Middle Salmon	0.00	Upper Salmon					
WF Yankee Fork	Partial	1996	0	1	6743	0	0	Middle Salmon	0.60	Upper Salmon	1201			0.436	
WF Yankee Fork	Partial	1997	1	2	6743	0	0	Middle Salmon	0.52	Upper Salmon	1570	0.000	0.283	0.592	0.388
WF Yankee Fork	Partial	1998	2	2	6743	0	0	Middle Salmon	1.03	Upper Salmon	3222	0.192	0.303	0.479	0.370
WF Yankee Fork	Partial	1999	X	X	6743	0	0	Middle Salmon	0.00	Upper Salmon		0.000		0.000	
WF Yankee Fork	Partial	2000	X	X	6743	0	0	Middle Salmon	0.35	Upper Salmon				0.228	
WF Yankee Fork	Partial	2001	5	2	6743	0	0	Middle Salmon	3.10	Upper Salmon				0.548	
White Cap Ck.	Control	1991	-3	1	.			Selway							
White Cap Ck.	Control	1992	-2	1	.			Selway	0.10						
White Cap Ck.	Control	1993	-1	1	.			Selway	0.30						
White Cap Ck.	Control	1994	0	1	.			Selway	0.10						
White Cap Ck.	Control	1995	1	2	.	0	.	Selway	0.00						
White Cap Ck.	Control	1996	2	2	.	0	.	Selway	0.15						
White Cap Ck.	Control	1997	3	2	.	0	.	Selway	0.00						
White Cap Ck.	Control	1998	4	2	.	0	.	Selway	0.20						
White Cap Ck.	Control	1999	5	2	.	0	.	Selway	0.00						
White Cap Ck.	Control	2000	6	2	.	0	.	Selway	0.40						
White Cap Ck.	Control	2001	7	2	.	0	.	Selway	0.96						

[a] Productivity estimates were taken from Bowles and Leitzinger (1991) and were not converted to metric units.

Appendix C. SAS output of Regression using redd densities.

We considered the following models:

1. log(redds per kilometer +1) = constant + treatment percent(T) + straying percent(S)
2. log(rpk +1) = constant + T + S + baseline (B)
3. log(rpk +1) = constant + T + S + grouping (as a categorical variable; G2-G9 defined as a contrast of each group compared to group 1).

The results are as follows:

Model	No. of parameters	AIC
T, S	3	-92.4
T, S, B	4	-123.2
T, S, G2 G4-G9	10	-128.7
T, S, G2, G8	5	-135.3

Note that grouping 3 (White Cap Creek) is not represented here because there were no estimates of straying. Among these models, we would choose the last one based on the Akaike Information Criterion. This model includes treatment and straying percentages and the effects of being in group 2 (lower redd production) and group 8 (higher redd production). All of the rest of the groups are considered the same (average production relative to groups 2 and 8).

If one looks at the least squares analysis of this model, we obtain,

Dependent Variable: **logrpk**

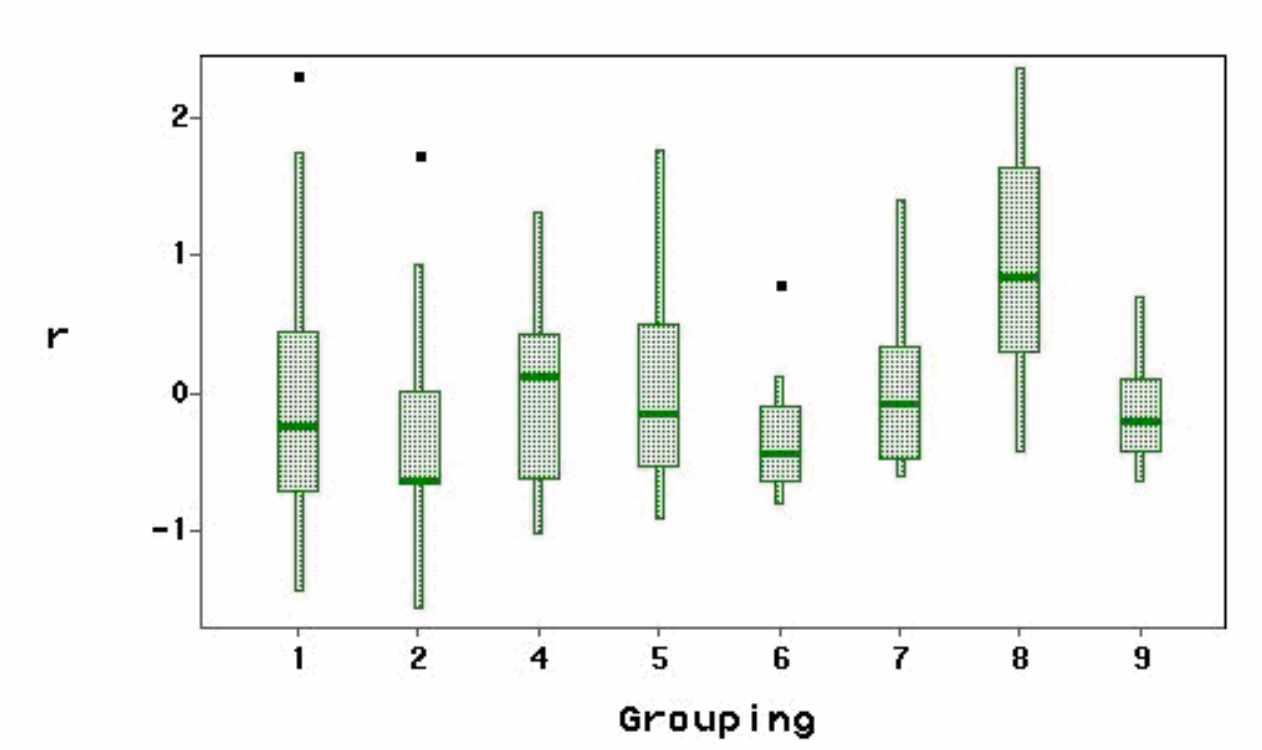
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	47.83441	11.95860	24.55	<.0001
Error	190	92.56457	0.48718		
Corrected Total	194	140.39898			

Root MSE	0.69798	R-Square	0.3407
Dependent Mean	0.87238	Adj R-Sq	0.3268
Coeff Var	80.00917		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	**Intercept**	**1**	**0.54860**	**0.06759**	**8.12**	**<.0001**
TrtPercent	**TrtPercent**	**1**	**0.61024**	**0.15702**	**3.89**	**0.0001**
Straying	**Straying**	**1**	**0.91322**	**0.18545**	**4.92**	**<.0001**
g2		**1**	**-0.35029**	**0.14489**	**-2.42**	**0.0166**
g8		**1**	**0.95534**	**0.15145**	**6.31**	**<.0001**



Box plots of residuals for log (redds + 1) from regression of treatment percent and straying percent using grouping as group variable.

Appendix D. Results of the regression analysis of smolt migration data. Straying is expressed as a percent.

```
                    The SAS System
                                        The GLM Procedure
                                     Class Level Information
          Class         Levels    Values
          JuvGroup           5    Clearwater Lower Salmon SF Clearwater South Fork Salm Upper Salmon
                                 Number of observations    330
NOTE: Due to missing values, only 53 observations can be used in this analysis.
                                        The SAS System                          09:14 Friday, July 30, 2004 532
                                        The GLM Procedure
Dependent Variable: migrants
                                                  Sum of
                Source                  DF       Squares     Mean Square    F Value    Pr > F
                Model                    6     703124780       117187463       1.27    0.2911
                Error                   46    4254766687        92494928
                Corrected Total         52    4957891467

                          R-Square     Coeff Var      Root MSE    migrants Mean
                          0.141819      200.5081      9617.428         4796.528

                Source                  DF     Type III SS     Mean Square    F Value    Pr > F
                JuvGroup                 4     127947135.4      31986783.9       0.35    0.8456
                TrtPercent               1      53274453.8      53274453.8       0.58    0.4518
                Straying                 1     213973618.2     213973618.2       2.31    0.1351
```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Appendix D. Continued.

```
                                   The SAS System
                  Plot of residual*yhat.  Legend: A = 1 obs, B = 2 obs, etc.

 residual ,
    60000 ^                                                                                                  A
          ,
          ,
          ,
          ,
          ,
          ,
    40000 ^
          ,
          ,
          ,
          ,
          ,
          ,
    20000 ^
          ,                                   A
          ,
          ,                                                 A
          ,                         A      A
          ,              A  A          A   AAAB                                 AA
        0 ^                            D      DBA   A     A        A
          ,                               A AAACC  A        A        A   A            A
          ,                                                 A AA       A
          ,                                                                                  A     A                    A
          ,                                                                                                A
          ,                                                                                                      A
          ,
   -20000 ^
          ,
          Sff^fffffffffff^fffffffffff^fffffffffff^fffffffffff^fffffffffff^fffffffffff^fffffffffff^fffffffffff^fffffffffff^ff
           -2000         0         2000        4000        6000        8000        10000       12000       14000       16000

                                                                yhat

NOTE: 277 obs had missing values.
```


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Appendix D. Continued.

Note: The following analysis is used only to get SAS to compute AIC values. We are not interested in “all variables regression” via R values. We would in practice formulate a set of *a priori* models and compare their AIC values.

```
                                  The REG Procedure
                                    Model: MODEL1
                           Dependent Variable: migrants

                             R-Square Selection Method

Number in
  Model      R-Square           AIC    Variables in Model

      1        0.1021      971.0527    Straying
      1        0.0874      971.9139    g4
      1        0.0413      974.5244    Reddspkm
      1        0.0205      975.6633    g5
     ---------------------------------------------------------------------------
      2        0.1598      969.5290    Reddspkm g4
      2        0.1301      971.3740    Reddspkm g5
      2        0.1272      971.5505    Straying g4
      2        0.1194      972.0181    Straying Reddspkm
      2        0.1160      972.2240    TrtPercent Straying
      2        0.1136      972.3702    Straying g5
      2        0.1033      972.9831    Straying g2
      2        0.1024      973.0345    Straying g3
      2        0.1022      973.0439    Straying g6
      2        0.0972      973.3396    g2 g4
      2        0.0901      973.7564    g4 g5
      2        0.0883      973.8608    g4 g6
      2        0.0880      973.8775    g3 g4
      2        0.0878      973.8917    TrtPercent g4
      ---------------------------------------------------------------------------
      3        0.2075      968.4311    Reddspkm g4 g5
      3        0.1791      970.3021    Reddspkm g2 g4
      3        0.1705      970.8501    Straying Reddspkm g5
      3        0.1701      970.8757    Straying Reddspkm g4
      3        0.1668      971.0876    TrtPercent Reddspkm g4
      3        0.1617      971.4089    Reddspkm g3 g4
      3        0.1610      971.4535    Reddspkm g4 g6
      ---------------------------------------------------------------------------
      4        0.2194      969.6313    TrtPercent Reddspkm g4 g5
      4        0.2113      970.1774    Straying Reddspkm g4 g5
      4        0.2085      970.3635    Reddspkm g2 g4 g5
      4        0.2081      970.3944    Reddspkm g4 g5 g6
      4        0.2077      970.4206    Reddspkm g3 g4 g5
      4        0.1927      971.4121    TrtPercent Reddspkm g2 g4
      4        0.1920      971.4612    TrtPercent Straying Reddspkm g5
      4        0.1884      971.6982    Reddspkm g2 g3 g4
      4        0.1865      971.8188    Reddspkm g2 g4 g6
      4        0.1853      971.8967    TrtPercent Straying Reddspkm g4
      ---------------------------------------------------------------------------
```


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We present an analysis of one such *a priori* model with juvenile group terms deleted via the AIC analysis above. This preliminary analysis suggests that smolt emigration is tied closely to redd production regardless of treatment or straying.

The REG Procedure
Model: MODEL1
Dependent Variable: migrants

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1137895447	227579089	2.80	0.0271
Error	47	3819996020	81276511		
Corrected Total	52	4957891467			

Root MSE	9015.34864	R-Square	0.2295
Dependent Mean	4796.52830	Adj R-Sq	0.1475
Coeff Var	187.95571		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	1665.42401	1996.58313	0.83	0.4084
TrtPercent	1	-3880.99954	3684.57013	-1.05	0.2976
Straying	1	4372.89420	5570.40473	0.79	0.4364
Reddspkm	1	1081.06707	467.25002	2.31	0.0251
g4	1	5997.28706	3963.98282	1.51	0.1370
g5	1	-5522.48927	3363.53353	-1.64	0.1073

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Appendix E. Results from the Regression analysis of juvenile survival data.

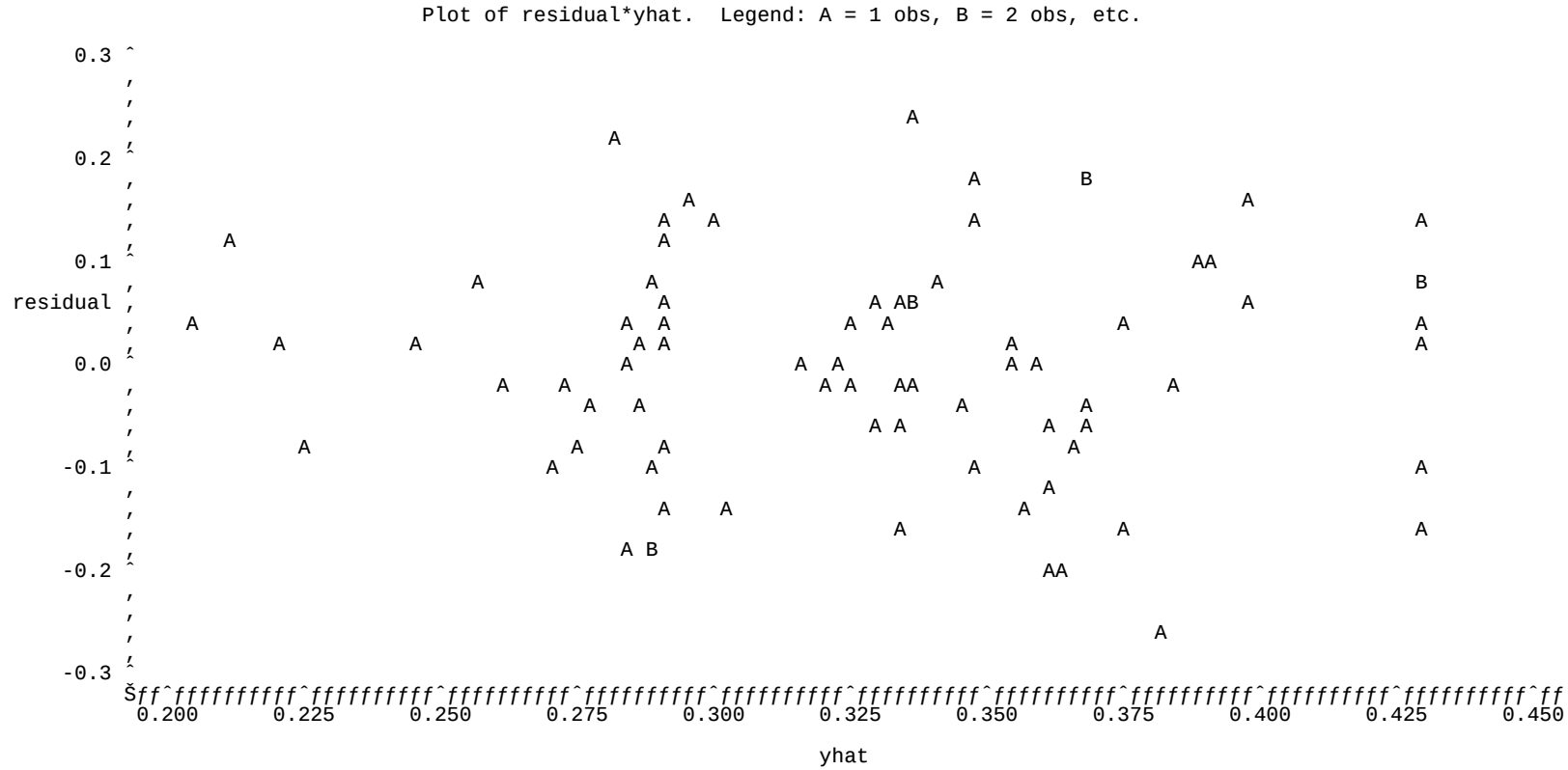
```
                                The SAS System                          09:14 Monday, August 2, 2004  27
                                              The GLM Procedure
                                           Class Level Information
                Class         Levels    Values
                JuvGroup           5    Clearwater Lower Salmon SF Clearwater South Fork Salm Upper Salmon

                                       Number of observations    330
NOTE: Due to missing values, only 80 observations can be used in this analysis.
                                              The SAS System                          09:14 Monday, August 2, 2004  28
                                              The GLM Procedure
Dependent Variable: Wtotal
                                                   Sum of
                    Source                    DF       Squares     Mean Square    F Value    Pr > F
                    Model                      6    0.22455553      0.03742592       2.98    0.0116
                    Error                     73    0.91531486      0.01253856
                    Corrected Total           79    1.13987039

                              R-Square     Coeff Var      Root MSE    Wtotal Mean
                              0.197001      34.02612      0.111976       0.329088

                    Source                    DF     Type III SS     Mean Square    F Value    Pr > F
                    JuvGroup                   4     0.16890053      0.04222513       3.37    0.0138
                    TrtPercent                 1     0.05660041      0.05660041       4.51    0.0370
                    Straying                   1     0.00751801      0.00751801       0.60    0.4412
```


64



NOTE: 250 obs had missing values.

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The REG Procedure
Model: MODEL1
Dependent Variable: Wtotal

R-Square Selection Method

Number in Model	R-Square	AIC	Variables in Model
1	0.2869	-363.1451	Reddspkm
1	0.0853	-343.2181	g5
1	0.0621	-341.2219	g6
1	0.0478	-340.0080	TrtPercent
1	0.0138	-337.2030	g4
1	0.0097	-336.8672	Straying
1	0.0001	-336.0975	g3
1	0.0001	-336.0973	g2
2	0.3171	-364.5977	Reddspkm g6
2	0.2992	-362.5297	TrtPercent Reddspkm
2	0.2987	-362.4724	Reddspkm g5
3	0.3295	-364.0721	TrtPercent Reddspkm g6
3	0.3287	-363.9736	Reddspkm g4 g6
3	0.3234	-363.3385	Reddspkm g5 g6
3	0.3187	-362.7899	Reddspkm g3 g6
3	0.3181	-362.7210	Straying Reddspkm g6
3	0.3171	-362.5980	Reddspkm g2 g6
3	0.3160	-362.4775	TrtPercent Reddspkm g5
4	0.3458	-364.0325	TrtPercent Reddspkm g4 g6
4	0.3397	-363.2938	TrtPercent Reddspkm g5 g6
4	0.3344	-362.6598	TrtPercent Straying Reddspkm g6
4	0.3331	-362.4964	TrtPercent Reddspkm g3 g6
4	0.3311	-362.2548	Reddspkm g2 g4 g6
4	0.3304	-362.1709	Reddspkm g4 g5 g6
4	0.3297	-362.0920	TrtPercent Reddspkm g2 g6
4	0.3293	-362.0407	TrtPercent Reddspkm g3 g5
5	0.3520	-362.8041	TrtPercent Reddspkm g2 g4 g6
5	0.3491	-362.4415	TrtPercent Reddspkm g4 g5 g6
5	0.3472	-362.2085	TrtPercent Reddspkm g3 g5 g6
5	0.3466	-362.1364	TrtPercent Reddspkm g3 g4 g6
5	0.3465	-362.1189	TrtPercent Straying Reddspkm g4 g6

The REG Procedure
Model: MODEL1
Dependent Variable: Wtotal

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.38888	0.07778	7.66	<.0001
Error	74	0.75099	0.01015		
Corrected Total	79	1.13987			

Root MSE	0.10074	R-Square	0.3412
Dependent Mean	0.32909	Adj R-Sq	0.2966
Coeff Var	30.61194		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	0.38620	0.02166	17.83	<.0001
TrtPercent	1	-0.04537	0.03210	-1.41	0.1617
Straying	1	0.01726	0.04263	0.40	0.6868
Reddspkm	**1**	**-0.01085**	**0.00254**	**-4.27**	**<.0001**
g5	1	-0.02609	0.03004	-0.87	0.3880
g6	1	0.06573	0.03955	1.66	0.1008

As in the case for smolt migration, the most significant term in this preliminary regression model is redds per kilometer.

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Appendix F. A proposal to determine the reproductive contribution of Chinook salmon of natural and ISS supplementation hatchery origin in the absence of general production hatchery (GP) straying. This study could be expanded (with additional funding) to include additional streams where GP straying is more common in order to address concerns raised by the Independent Scientific Review Panel.

STUDIES TO DETERMINE THE REPRODUCTIVE SUCCESS OF HATCHERY SPAWNERS
(FCRPS BiOp Action #182)

SUBMITTED TO:

Bonneville Power Administration

SUBMITTED BY:

IDAHO DEPARTMENT OF FISH AND GAME
EAGLE FISH GENETICS LABORATORY
1800 TROUT RD.
EAGLE, ID 83616

NAMPA RESEARCH OFFICE
1414 EAST LOCUST LANE
NAMPA, ID 83686

UNIVERSITY OF IDAHO
CENTER FOR SALMONID AND FRESHWATER SPECIES AT RISK
HAGERMAN FISH CULTURE EXPERIMENT STATION
3059 F NATIONAL FISH HATCHERY ROAD
HAGERMAN, ID 83332

PROJECT TITLE:

COMPARATIVE REPRODUCTIVE SUCCESS OF WILD AND HATCHERY ORIGIN SPRING/SUMMER CHINOOK SALMON COMBINATIONS THAT SPAWN NATURALLY IN THE PAHSIMEROI AND UPPER SALMON RIVERS

Principal Investigators:
Matt Campbell, IDFG, Fisheries Research Biologist
Sam Sharr, IDFG, Principal Fisheries Research Biologist
Jeff Lutch, IDFG, Fisheries Research Biologist
Madison Powell, University of Idaho, Assistant Professor

PROJECT SUMMARY

The Idaho Department of Fish and game (IDFG) and the University if Idaho (UI) are collaborating in response to the Bonneville Power Administration's request for proposals addressing the NMFS Biological Opinion RPA 182. We propose a multiphase, comprehensive evaluation project to measure reproductive success and influence of hatchery origin salmonids upon wild fractions of the same population. Compelling evaluation of hatchery influence upon wild salmonids is a complex problem requiring substantial preparation since generation times are long and variable within a cohort, and individuals are both semelparous and r-selected. Additionally, long-term evaluation of hatchery influence upon a salmonid population through the F_2 generation requires much forethought for predicted returns to make statistically valid comparisons throughout the lifespan of the project (i.e. there will be enough projected returns in coming years to evaluate statistically). Accordingly, an investigation of this scope would be most cost effective provided the infrastructure for such an investigation were already in place. Likewise, it would be advantageous to integrate this largely genetic investigation with an ongoing, long-term project that can provide both logistical support for the collection of samples and collateral information regarding ecological and population dynamics within the same system.

In 2002, the IDFG and the UI, funded in part under the Idaho Supplementation Studies (ISS) project, began behavioral and genetic investigations on the Pahsimeroi River to evaluate the reproductive success of natural-origin and wild-spawning hatchery-origin Chinook salmon through the F_1 generation. The hatchery origin recruits returning to Pahsimeroi River originated from supplementation broodstock that is constructed from adult hatchery origin and naturally produced Chinook salmon. All adults are intercepted at the weir, with hatchery (supplementation) adults passed upstream to spawn naturally at a level that numerically does not exceed the wild/ natural component, as part of the ISS experimental design. This project utilizes the heretofore unrealized potential of genetically identifying every adult passed over the Pahsimeroi weir of both wild and hatchery origin and subsequently identifying their offspring as they pass through the system on their outward migration.

The ISS project also funds adult and juvenile monitoring activities for numerous other spring and summer Chinook populations sites including the population that spawns naturally above the weir at Sawtooth Hatchery on the upper Salmon River. At the Sawtooth site, the ISS project enumerates and collects biological samples from all natural and supplementation adults that ascend the river and are allowed to spawn above the weir. Project personnel conduct periodic foot surveys of spawning grounds above the weir to record numbers and distribution of spawners. They also subsample juveniles that migrate downstream past the weir site. Consequently, the infrastructure already exists to implement behavioral and genetic investigations on the Upper Salmon River identical to those already being conducted on the Pahsimeroi River.

Based upon the existing infrastructure at the ISS sites on the Pahsimeroi and Upper Salmon rivers and the demonstrated feasibility of the ongoing pilot project on the Pahsimeroi River, this proposal requests funding to expand Pahsimeroi investigations to the F_2 generation and to test models developed from this Pahsimeroi data to predict reproductive success of various crosses between fish that are allowed above the Sawtooth weir (Hatchery ♀X Hatchery ♂; Hatchery ♂X Wild ♀; Wild ♀ X Wild ♂; and Wild ♂ X Hatchery ♀). Thus, the much larger data set collected from interactions at Sawtooth would serve as a replication and validation of the work on the Pahsimeroi. This project described in this proposal, hereafter referred to as the Comparative Reproductive Success (CRS) project, provides a unique opportunity since returns from future

cohorts elsewhere are likely problematic with respect to sufficient numbers of adult fish to make statistical comparisons. Moreover, since a pilot project already exists, methods detailing all behavioral and genetic analyses have already been worked out and the parental generations at both locations (Pahsimeroi and Sawtooth) have already been sampled. Thus, this study is capable of most cost-effectively addressing the questions specifically asked within the RFP:

- Are there statistically significant differences in reproductive success between natural-origin and hatchery-origin fish when measured at the second generation (F_2)?

- Do F_1 progeny with HxW parents differ from F_1 progeny with HxH parents in the production of F_2 progeny?

- What are possible hypotheses to explain this difference? For example, can the difference be attributed to reduction in genetic fitness of hatchery-origin fish compared to natural-origin fish?

- Are differences more significant during any specific life history stages?

- What is the likely effect of any difference, in terms of population growth, population recovery, and genetic diversity/fitness in subsequent generations according to the Viable Salmonid Population (VSP) criteria?

PROJECT DESCRIPTION

Existing Infrastructure

Field activities supporting the CRS project will be integrated with the ISS project. As stated above, the ISS study presently maintains monitoring and evaluation activities in the Pahsimeroi River and the Upper Salmon River as part of their study design, but genetic evaluations proposed in the CRS project significantly expand on the scope of work of the ISS project. Equipment and personnel needed for performing a variety of ISS tasks (e.g., estimating juvenile Chinook salmon out-migration and adult returns, collecting tissue samples) are located on site from mid-March through November. As the lead coordinating agency for ISS, the IDFG would function as the representative cooperator to CRS and provide logistical support for field sample and data collections.

ISS is an ongoing cooperative research project that was initiated in 1991 to evaluate supplementation as a recovery tool for Snake River Chinook salmon stocks returning to Idaho. ISS research activities are distributed among four cooperative agencies that are financially supported by the Bonneville Power Administration (contract numbers: 1989-089-00, 1989-089-01, 1989-089-03, 1989-089-04). Presently, the research is entering the evaluation phase. Following completion of a programmatic review and statistical treatment of ISS data for review by the Independent Science Review Panel, new study timelines were developed (Lutch et al. 2003). Further, recommendations were made for evaluating an additional generation of Chinook salmon and extending the project through 2012.

The significance of the ISS study to CRS relates directly to objectives in the ISS study design that focus on evaluating the effects of supplementation/augmentation on existing wild/natural Chinook salmon populations. Pursuant to these objectives are specific tasks that are currently identified in the ISS Statement of Work for CY 2003. For the purpose of evaluating changes in

natural production and productivity of Chinook salmon, the IDFG representative for ISS operates rotary screw traps at both locations to estimate juvenile production, applies weir management and escapement criteria for adults returning to study reaches above the satellite hatcheries, and enumerates escapement (redd counts, adult returns to weirs).

In 2002, the ISS study extended their research activities to more directly evaluate the affect of hatchery reared supplementation broodstock on Chinook salmon productivity. The Pahsimeroi River and the Upper Salmon River were selected as case studies since escapement weirs are nearly 100% effective at these locations. The existing ISS infrastructure was used to collect tissue samples from adult Chinook salmon released upstream to spawn naturally. Predictive power using forecasted numbers for adult returns and juvenile out-migration was examined prior to sample collections. Data were also collected to examine temporal and spatial aspects of spawning activity between hatchery and wild natural Chinook salmon. Presently, this additional ISS research has provided adult tissue samples from all potential parentage combinations of naturally spawning Chinook salmon for 2002. These samples are stored at the IDFG Genetics Laboratory, Eagle Hatchery, Idaho, and await funding sources for processing and analysis. Logistical support for CRS project will be provided directly through ISS research activities.

Laboratory activities supporting the CRS project will be integrated with ongoing genetic studies at the University of Idaho's Center for Salmonid and Freshwater Species at Risk which utilizes several, high-throughput, multiplex genotype sets of microsatellite loci specifically developed for Chinook salmon. The center currently employs these molecular markers to address numerous genetic questions on Chinook populations as project sponsor or subcontractor to several BPA funded projects including the Johnson Creek supplementation project, the Salmon River Chinook salmon captive rearing research project, and others. The laboratory has already used all the molecular and statistical procedures outlined in the methods section to successfully conduct parentage analysis on spring/summer Chinook salmon from the Pahsimeroi River. Research proposed under the CRS project is not within the scope of work outlined in any of these other activities.

Study Design

Weirs in position on both the Pahsimeroi and Upper Salmon Rivers allow for sampling and enumeration of all returning adults with essentially 100% efficiency. This includes the parental generation, first filial (F_1) and second filial (F_2) returning adults to be examined in this project. Screw traps operated in these systems will allow for the timely capture of juveniles of different life stages to be sampled for genetic analyses. Parentage analysis (parental exclusion analysis) will be used to assign offspring back to parental crosses. Assignment need not be 100%, only robust enough to assign proportions of different possible crosses to juveniles and subsequent generations. If hatchery adults exhibit the same spawning success as their wild counterparts, and they randomly interbreed, then the observed proportions of offspring from each possible cross should not be significantly different from the proportions of wild and hatchery fish among male and female adults placed over the weir(s). If, however, mating is not random or there is differential spawning success between hatchery and wild fish, then this will manifest itself in two ways. First, nonrandom mating would be evidenced by observed genotypic proportions being out of Hardy-Weinberg equilibrium with expected heterozygous and homozygous genotypic proportions. Secondly, differential spawning success would also be observed in significant departures from the probabilities of expected, random crosses (i.e. if 70% of males and females placed over the weir are hatchery origin we would expect a similar proportion of juveniles from those parents in the F_1 population).

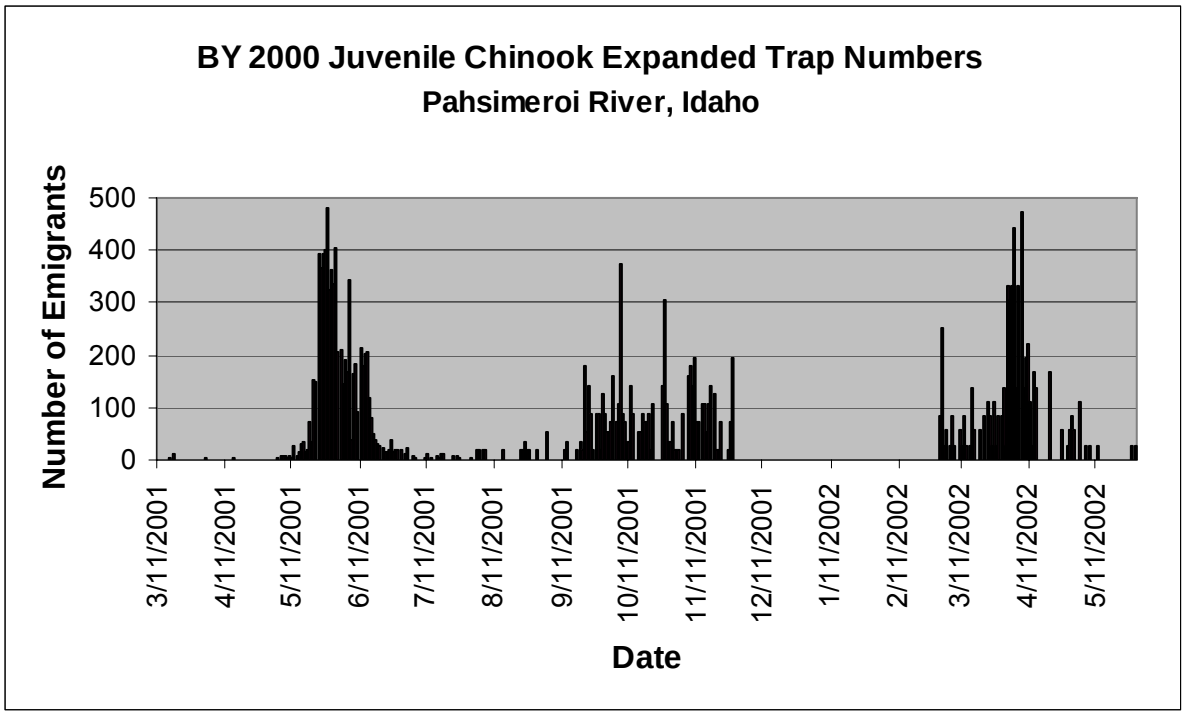

Figure 1. Expanded trap numbers and timing for juvenile Chinook in the Pahsimeroi River, Idaho.

These types of analyses will be tested via reject-support type hypothesis testing as outlined in the methods section. Sample sizes required for statistical significance and power have been calculated. Parentage assignment also allows for an even greater detailed analysis of hatchery vs. wild spawning success since the success of males and females from each origin can be assessed. Thus, it may be, for instance, that wild males contribute more to the F_1 generation than hatchery males regardless of the female's origin. Alternatively, hatchery males may be preferentially selected by hatchery females, thus providing evidence that hatchery fish selectively breed amongst themselves rather than with wild counterparts. All possible crosses and their departure(s) from random mating can be assessed.

Juvenile life stages will be sampled at more than one time since parr, presmolts and smolts are distinguishable on the basis of size and timing in these systems as in Figure 1. This design allows for the examination of changes through time of allelic and genotypic proportions in the juvenile population. For example, spawning success may not differ significantly between hatchery and wild origin parents and their crosses, but juvenile mortality or their timing may differ. Sampling the juvenile population at more than one life stage (parr = T_0, presmolt = T_1, smolt = T_2) allows for the detection of this potential differential success.

Critical Uncertainties and Study Rationale

Why use Pahsimeroi and Sawtooth stocks as opposed to ESU's listed in the RFP?
The best science would provide not only information about a biological system but also infer a set of predictive outcomes given similar circumstances. Thus, information gathered from one system could be extrapolated to another. Alternatively, if the information obtained is not predictive, then each system must be evaluated on a case-by-case basis. Whether interactions

between hatchery and wild salmon and any resultant differential success can be predicted across different systems remains unknown. Studies examining hatchery influence on a wild population have been used to predict the interaction on other systems but none has been empirically tested. Ongoing, BPA funded projects addressing specific Snake River spring/summer Chinook salmon ESU's under RPA 182 have not been replicated. Pahsimeroi and Sawtooth Chinook salmon stocks were selected for this proposal because of the following criteria: 1) adequate sample sizes through the F_2 generation for detection of specific crosses in returning adults, 2) small enough sample sizes and geographic area for the study to be 'manageable' in size, 3) populations with sufficient numbers in parental returns such that density dependent effects and/or Allee effects are minimized, 4) allogenic factors or outside influence from different alleles (i.e. straying) are minimal, 5) existing infrastructure of weirs and collection equipment, 6) a collaborative, ongoing, long-term evaluation of population dynamics and ecology in those systems (ISS), and 7) an ongoing pilot project (Pahsimeroi) from which information can be used to predict the outcome of a much larger, replicated data set (Sawtooth).

Table 1. Forecasted returns of adult Chinook salmon and estimated juvenile production in the Pahsimeroi River and the Upper Salmon River return years 2003-2012. Adult forecasts based on brood year production estimates and smolt-to-adult return rates of 0.6% for Pahsimeroi River and 0.5% for Upper Salmon River. Brood Year Juvenile production estimates are calculated from expected wild/natural and hatchery females released above escapement weirs, then applying parr/presmolt per female and smolt per female estimates specific to each stream. nr = no supplementation returns expected since ISS releases ceased with brood year 2002.

	Adults		Juvenile Production	
	Wild/Natural	Hatchery	Parr, Presmolts	Smolts
Pahsimeroi R.				
2003	154	361	49,126	19,866
2004	108	378	34,452	13,932
2005	374	609	119,306	48,246
2006	362	404	115,478	46,698
2007	323	100	67,469	27,283
2008	226	nr	36,047	14,577
2009	785	nr	125,208	50,633
2010	760	nr	121,220	49,020
2011	444	nr	70,018	28,638
2012	237	nr	37,802	15,286
Upper Salmon R.				
2003	128	171	64,512	20,736
2004	318	375	160,272	51,516
2005	914	434	339,696	109,188
2006	1,248	473	461,160	148,959
2007	205	118	81,396	26,163
2008	509	nr	128,268	41,229
2009	1,078	nr	271,656	87,318
2010	1,377	nr	347,004	111,537
2011	258	nr	65,016	20,898
2012	407	nr	102,564	32,967

Will there be sufficient returns in coming years to insure adequate numbers of fish to examine statistically? Principal evidence for differential reproductive success between hatchery origin and wild natural Chinook salmon lies in the ability to detect relative differences in fitness variables (e.g. survival) measured between the two groups (Roff, 1997) (also see [Endler 1986] for a comprehensive review of methods for detecting differential fitness in the wild]. The power to detect such differences depends largely on adequate sample sizes for detection of all combinations of parental crosses (e.g., Hatchery ♀X Hatchery ♂; Hatchery ♂X Wild ♀; Wild ♀ X Wild ♂; and Wild ♂ X Hatchery ♀). As a first step to predicting statistical power for this project, adult escapement was forecasted for wild natural and hatchery origin Chinook salmon through 2012 at both Pahsimeroi and Sawtooth weirs. As demonstrated in Table 1, sufficient Chinook salmon adults representing both groups are expected to return through the adult sample collection phase. Using these estimates and applying a recruit per spawner estimate, the representative brood year out-migration of juveniles is also predicted to be adequate for second-generation (F_2) genetic analyses.

Adjustments to forecasts of adult escapement will be coordinated with the ISS study as more data become available (e.g., PIT tag data). Refined estimates will be also applied to subsampling methods for collecting juvenile Chinook salmon in the second generation.

Has parentage analysis been used in similar studies? Yes. Parentage analysis has been used successfully in several other fish studies (Bernatchez and Duchesne 2000; Eldridge et al. 2002; Estoup et al. 1998; Letcher and King 2001; Norris et al. 1999; O'Reilly et al 1998) including Chinook salmon from the Snake River (Stephenson submitted). All laboratory and data analysis methods required for this project have been successfully utilized by the Center for Salmonid and Freshwater Species at Risk.

Is there sufficient genetic variation between hatchery and wild components in the proposed stocks for parental exclusion to be useful? Yes. Current evidence from other ongoing projects in the region (Stephenson submitted) suggests more than sufficient genetic variation to conduct parentage assignment tests even from these closely related groups. In this instance, the population components of the supplementation program would likely be too close for population assignment but not for parental assignment. Similar work on estimates of relative survival between two groups of fish (hatchery vs. natural origin) has been successful using microsatellites to separate those two closely related groups (Eldridge et al. 2002).

If differential reproductive success is observed, will this study tell us why? No. This study is designed to detect statistically significant differential reproductive success among four potential genetic crosses with a high degree of power. It is not designed to examine possible causes for that differential reproductive success. All the intrinsic and environmental parameters (both stochastic and deterministic) that may affect reproductive success are beyond the scope of this project.

Can results from this study be extrapolated to other systems or ESU's? Unknown. Presumably, if results obtained from the Pahsimeroi system can successfully be used to predict hatchery vs. wild interactions in the Sawtooth system, this would provide evidence that such interactions are indeed predictable. Whether this extends across Chinook stocks out of the Snake River or to different species such as steelhead would require additional studies.

Objectives and Testable Hypotheses

Objective 1.0 Determine the relative reproductive success of hatchery and natural origin parents to the production of F_1 smolts. *Since tissue samples will be collected from smolts through 2009 (Table 5), analysis of reproductive success to F_1 smolt production will be replicated using brood year 2002 through 2005.*

Testable hypothesis: There are no significant differences in the reproductive success of hatchery and natural origin parents to the production of F_1 smolts.

Task 1.1 Collection of adults (parental types). *Importantly, all adults allowed above the weir in 2002 (hatchery and natural origin) have already been sampled and resulting F_1 progeny will be representatively sampled as smolts in 2004.*
All returning, prespawn adults collected and passed over the Pahsimeroi (n = 299) and Sawtooth weirs (n = 1340) in the fall of 2002 (differentially marked and unmarked males and females) were genetically sampled. All tissue samples have been stored in Lysis buffer at the Eagle Fish Genetics Laboratory pending genetic analysis.

Task 1.2 Collection of smolts.
A subsample of smolts originating from parents spawning above the Sawtooth weir in 2002 will be collected as they emigrate past the Sawtooth juvenile trap site in 2004 (n ≥460). Smolts will also be collected at the Pahsimeroi juvenile trap site in 2004 (n ≥460) from parents that spawned above the Pahsimeroi weir in 2002. All tissue samples will be stored in Lysis buffer at the Eagle Fish Genetics Laboratory pending genetic analysis.

Task 1.3 Generation of genetic data and analysis.
Genomic DNA will be extracted from tissues samples taken from adults and juveniles. Multilocus genotypes of all adults and juveniles will be generated using highly polymorphic microsatellite loci. Juveniles will be assigned back to individual parents using maximum likelihood and Bayesian procedures to exclude adult genotypes. The expected proportions versus observed proportions of parents contributing to the smolt population will be compared statistically. Funds are not being requested for the genetic analysis of Pahsimeroi adults sampled in 2002 or Pahsimeroi juveniles sampled in 2003 and 2004.

Objective 2.0 Determine parental proportions among resulting F_1 progeny at the parr, presmolt and smolt life stages. *Parental proportions will be analyzed using brood year 2002-2005 production data.*

Testable hypothesis: Parental proportions are not significantly different among F_1 progeny life stages (parr, presmolt and smolt).

Task 2.1 Collection of various juvenile life stages.
Genetic samples from parr (n >90), and presmolt (n >90) life stages from parental spawning in 2002 are currently being collected. As stated above, smolts from the same 2002 parental spawning will be collected in 2004.

Task 2.2 Generation of genetic data and analysis of various juvenile life stages.
Using the same procedures in Task 1.3 above, juveniles will be assigned to individual parents. The expected proportions vs. observed proportions of F_1 progeny at different life stages will be statistically compared.

Objective 3.0 Determine the relative reproductive success of hatchery and natural origin parents to the production of F_1 adults. *Since tissue samples will be collected from adults through 2012 (Table 5), analysis of reproductive success to F_1 adult production will be replicated using brood year 2002 through 2005.*

Testable hypotheses: There are no significant differences in relative reproductive success of hatchery and natural origin parents to resultant F_1 adults.

There are no significant differences in relative reproductive success of hatchery and natural origin F_1 adults from juvenile life stages of the same year class.

Task 3.1 Collection of returning F_1 adults.
Genetic samples of all F_1 adults originating from the 2002-2005 parental crosses will be collected from 2005 to 2010 as they return to the Pahsimeroi and Sawtooth weirs.

Task 3.2 Generation of genetic data and analysis of various F_1 year classes.
Using the same procedures in Task 1.3 above, F_1 adults will be assigned to individual parental crosses. The expected proportions vs. observed proportions of F_1 adults will be statistically compared to the parental crosses and to the proportion of genotypes present in different juvenile life stages.

Objective 4.0 Determine the relative reproductive success of hatchery and natural origin parents to the production of juveniles and adults when measured at the second generation (F_2 juveniles and adults).

Testable hypothesis: There is no significant difference in relative contribution of hatchery and natural origin parents to resultant F_2 smolts.

Task 4.1 Collection of various juvenile life stages.
Genetic samples of emigrating parr (n >90), presmolt (n >90), and smolt (n ≥460) life stages (F_2s) will be collected from the Pahsimeroi and Sawtooth systems from 2006 to 2009, in the same manner as they were collected in 2003 and 2004.

Task 4.2 Collection of F_2 adults.
Genetic samples of F_2 adults returning to the Pahsimeroi and Sawtooth weirs will be collected from 2008 to 2010.

Task 4.3 Generation of genetic data and analysis of various F_2 juveniles and adults.
F_2 juveniles and adults will be assigned to individual parents (F_1s) sampled as part of Objective 3.0. The expected proportions vs. observed proportions of F_2 progeny at different life stages will be statistically compared.

Sampling Methods

Adults-

Fin clips were sampled from all returning adults allowed above the Pahsimeroi and Sawtooth weirs during the summer and fall of 2002 (Tables 3 and 4). Subsequently, nonlethal fin tissue will be sampled from all adult Chinook salmon that return to the Pahsimeroi and Sawtooth weirs during the summer and fall of 2003-2012, (samples in 2003 and 2004 are collected exclusively

in relation to the ISS study, whereas samples from 2005-2012 will be collected since they will contain a proportion of fish that originate from 2002 parental spawners and also relevant to the CRS project).

Table 3. Sex and origin of Chinook released above Pahsimeroi Weir.

Origin	Males	Females
Natural	91	66
Supplementation	46	96
TOTAL	**137**	**162**

Table 4. Sex and origin of Chinook released above Sawtooth Weir.

Origin	Males	Females
Natural	480	314
Supplementation	236	310
TOTAL	**716**	**624**

Table 5. Summary of F1 and F2 life history stage present in each sampling year for cohorts originating from brood year 2002, 2003, 2004, and 2005.

Life history stages of brood year cohorts by sampling year 1/								
Sample Year 4/	**2002**		**2003**		**2004**		**2005**	
	J 2/	**A 3/**	**J 2/**	**A 3/**	**J 2/**	**A 3/**	**J 2/**	**A 3/**
2002		P02						
2003	F1-0+			P03				
2004	F1-1+		*F1-0+*			P04		
2005		F1-3	F1-1+		F1-0+			P05
2006		F1-4		F1-3	F1-1+		F1-0+	
2007	F2-0+	F1-5		F1-4		F1-3	F1-1+	
2008	F2-0+,1+		F2-0+	*F1-5*		*F1-4*		*F1-3*
2009	F2-1+	F2-3	F2-0+,1+		F2-0+	F1-5		F1-4
2010		F2-3,4	F2-1+	F2-3	F2-0+,1+		F2-0+	F1-5
2011		F2-4,5		F2-3,4	F2-1+	F2-3	F2-0+,1+	
2012		F2-5		F2-4,5		F2-3,4	F2-1+	F2-3
2013				F2-5		F2-4,5		F2-3,4
2014						F2-5		F2-4,5
2015								F2-5

1/ Cell labeling conventions are: F = filial, * = generation (i.e. F_1 or F_2), and, ** denotes age of fish (0+ and 1+ are parr/presmolt and smolt respectively, 3, 4, & 5 are adults)

2/ Stippled juvenile cells occur in occur among F_2's when the juveniles present include production that results from F_1 jack that cross with adults from a preceding brood year that is not included in this study. For example the stippled juvenile cell for sample year 2005 indicates that age-0 juveniles are present that result from brood year F_1 male jacks crossing with adults from brood years 2001 or 2000. These are not pure F_2 fish from brood year 2002 but should be sampled.

3/ Stippled adult cells indicate sample years when adult returns are present that are unrelated to the brood year at the top of the column but must be sampled to track parentage of brood year 02 fish through the F_2 generation or to track F_1 adult returns from brood years 03, 04, and 05.

4/ Note that analysis through F_2 for brood year 2002 requires sampling and analysis of juvenile for 03-09 and adults from 02-12. These same collected and analyzed samples can be used to complete F_1 juvenile and adult analyses for brood years 03, 04, and 05 as well at no additional cost. Gray shaded sample years are not included in this proposal but if sampling were completed in these years, analyses of F_2's from brood years 03, 04, and 05 could be also completed.

Juveniles (parr, presmolts, and smolts)-

Rotary screw traps were installed near the Sawtooth Hatchery and Pahsimeroi Hatchery weirs in March 2003 to sample emigrating juvenile Chinook. Fin samples from brood year 2002 production will be collected during 2003 and 2004 from three discernable life stages as they migrate downstream past these traps: parr will be sampled from .May through July, presmolts will be sampled from September through November, and smolts will be sampled the following spring in March through mid April. Because there may be inherent differences in parental contribution to the three groups, they will be treated separately. In order to obtain a representative sample from all production above the weir, sampling will be conducted proportionally across the entire out-migration (spring 2003 thru spring 2004). The exception will be the three-month period (December-February) when the trap will not be operational. During this period, very little movement of juveniles occurs. In order to accurately sample juveniles proportionately across the migration period, historical records of emigration timing (10 years) collected from ongoing production research by Idaho Fish and Game will be employed. Similar collections of parr, presmolts, and smolts will take place from 2005 to 2009.

Sample Sizes

To compare the relative distribution of crosses contained in the sample set, the distribution of alleles, and the contribution of individual parents for the specific number of adults passed over the Sawtooth and Pahsimeroi weirs requires extensive sampling of smolts. A sample size of n = 460 was chosen for smolt collection using the Power analysis program in STATISTICA (Statsoft, Inc.) and information on the proportions of each group released above the Pahsimeroi and Sawtooth weirs in 2002, as well as anecdotal behavioral evidence. A putative hatchery ♂X wild ♀ cross is expected to have the smallest probability of occurrence and therefore detection. Thus, sample sizes were based upon the low probability of this cross occurring. Power analysis indicated that a sample size of n ≥460 would allow the observation of all possible alternate outcomes of crosses occurring with a frequency as low as 1% with 99% accuracy. It would also allow the detection of changes from expected frequencies at a true difference of >1% with 95% accuracy while maintaining 88% power.

In testing whether parental proportions are significantly different among F_1 progeny life stages (parr, presmolt and smolt), sampling effort will not need to be as intensive since we wish only to compare the relative proportions of crosses as they change or remain unchanged through time. In this instance, a sample set of at least 90 individuals will ensure we can detect changes in the proportions of crosses with greater than 95% probability.

Genetic Analyses

DNA will be extracted following a Qiagen tissue protocol (Qiagen Laboratories). Ten to twelve microsatellite loci will be amplified for each individual following procedures outlined by (Narum et al. *submitted*; Williamson et al. 2002). These loci have demonstrated high levels of allelic variation and heterozygosity in Chinook salmon populations (Table 2, adapted from Williamson et al. 2002).

Summary of Adult and Juvenile Collections

Spawn Year	**Age-0, F_1 parr sampling**	**Age-0, F_1 presmolt sampling**	**Age-1, F_1 smolt sampling**	**1-ocean, F_1 adult sampling**	**2-ocean, F_1 adult sampling**	**3-ocean, F_1 adult sampling**
2002	2003	2003	2004	2005	2006	2007
	Age-0, F_2 parr sampling	**Age-0, F_2 presmolt sampling**	**Age-1, F_2 smolt sampling**	**1-ocean, F_2 adult sampling**	**2-ocean, F_2 adult sampling**	**3-ocean, F_2 adult sampling**
2005 F_1, 1-ocean adults spawn	2006	2006	2007	2008	2009	2010
2006 F_1, 2-ocean adults spawn	2007	2007	2008	2009	2010	2011
2007 F_1, 3-ocean adults spawn	2008	2008	2009	2010	2011	2012

Table 2. Chinook salmon microsatellite alleles currently available for parentage assignments.

Locus	**Repeat motif of original clone**	**Allele size range (bp)**	**No. of alleles**	**Heterozygosity**		
				H_O	H_E	**HWE**
OtsG3	$(GAAT)_8$-GATAGATTAATA-$GATA)_{11}$-GATTAATAGAGA-$(GATA)_{26}$	146-246	5	0.33	0.37	ns
OtsG68	$(GATA)_{30}(TAGA)_1$	184-296	12 (17)	0.88	0.97	ns
OtsG78b	$TAGA(TATA)_2$-N_{12}-$(TAGA)_{31}$	216-356	13	0.88	0.95	ns
OtsG83b	$(TGTC)_7$-N_{51}-$(TATC)_{34}$	155-303	15	1.0	0.98	ns
OtsG243	$(TAGA)_{63}(CAGA)_{12}(GACA)_7(GA)_{22}$	190-466	12	1.0	0.96	ns
OtsG249	$(TAGA)_{19}$	192-310	13 (14)	1.0	0.95	ns
OtsG253b	$(GACA)_{10}$ $(GATA)_{14}$	141-301	12	1.0	0.96	ns
Ots311	$(GATA)_{30}$-GACA-$(GATA)_2$-$(GAGTGATA)_7$-GATA	278-374	12	0.88	0.95	ns
OtsG409	$(GA)_9(TAGA)_6$-GGTA-$(GATA)_{16}$	116-282	10	0.77	0.91	ns
OtsG422	$(GATA)_{24}$	264-414	15	1.0	0.97	ns
OtsG432	$(GATA)_3$-GGAT-$(GATA)_8$	122-202	12	0.88	0.95	ns
OtsG474	$(GATA)_6$	155-191	6	0.66	0.75	ns

Products from PCR amplification will be run out on an ABI 3100 Genetic Analyzer (Applied Biosystems). Allele sizes and genotypes will be determined using the software programs Genescan 3.0 and Genotyper 2.1 (Applied Biosystems).

Statistical Methods

Juveniles will be assigned to parental crosses via comparison of multilocus microsatellite genotypes among candidate parents. Maximum likelihood (Marshall et al. 1998) and Bayesian (Neff et al. 2001; Lange 1997) procedures will be used to exclude possible crosses and parents (parental exclusion analysis). Observed versus expected parental contributions will be analyzed with Goodness-of-fit tests ($\chi 2$, Fisher's Exact Test, G-Test) (Motulsky 1995; Zar 1996). Differences among life stages will be analyzed with paired t-tests between groups (parr, presmolt, smolt) (Motulsky 1995; Zar 1996). Changes in allele and/or genotypic frequencies will be examined using statistical software for population genetics (Genepop [Raymond and Rousset 1995]; GDA [Lewis and Zaykin 1999]) and a Bayes estimation of allele frequencies (Dirichlet-multinomial distributions) to assess linkage and provide predictive distributions (Lange 1997).

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Timeline

September 2002	Fin-clips samples collected from all adults passed above Pahsimeroi (N = 299) and Sawtooth (N = 1340) weirs **(COMPLETED)**
Mar 2003-Dec 2004	Sample F_1 parr (n >90 at each site), F_1 presmolts (n >90 at each site) (ONGOING) and F_1 smolts (n ≥460) at Pahsimeroi and Sawtooth for genetic fin-clips
January 2004	Completion of data collection of 10-12 microsatellite loci on 2002 adults from Pahsimeroi (N = 299) and adults from Sawtooth (N = 1340), **preliminary report to BPA on project status**
August 2004	Completion of data collection of 10-12 microsatellite loci for F_1 parr, F_1 presmolts (n ≥90) and F_1 smolts (n ≥460) from Pahsimeroi and Sawtooth, **preliminary report to BPA on reproductive success of hatchery and wild spawners on the production of F_1 parr, presmolts, and smolts**
Jun 2005-Oct 2007	Fin-clips samples collected from all F_1 adults passed above Pahsimeroi and Sawtooth weirs
Mar 2006-Dec 2009	Sample F_2 parr (n >90 at each site), F_2 presmolts (n >90 at each site) and F_2 smolts (n ≥460) at Pahsimeroi and Sawtooth for genetic fin-clips
January 2008	Completion of data collection of 10-12 microsatellite loci on F_1 adults from Pahsimeroi (N = 299) and adults from Sawtooth (N = 1340), **preliminary report to BPA on project status**
August 2008	Completion of data collection of 10-12 microsatellite loci for F_2 parr (n ≥90) and F_2 presmolts (n ≥90) from Pahsimeroi and Sawtooth, **preliminary report to BPA on reproductive success of hatchery and wild spawners on the production of F_2 parr and F_2 presmolts**
January 2010	Completion of data collection of 10-12 microsatellite loci for F_2 smolts from Pahsimeroi and Sawtooth, **preliminary report to BPA on reproductive success of hatchery and wild spawners on the production of F_2 smolts**
Jun 2008-Oct 2012	Fin-clips samples collected off of all F_2 adults allowed above Pahsimeroi and Sawtooth weirs from original 2002 parental crosses
December 2012	Completion of data collection of 10-12 microsatellite loci for F_2 adults from Pahsimeroi and Sawtooth, **completion report to BPA on reproductive success of hatchery and wild spawners on the production of F_2 adults**

Facilities and Equipment

Only limited field equipment costs and tissue collection costs are necessary during the entire term of this project. Adult tissue samples were already collected in 2002 and current juvenile collections are supported by the existing ISS budget.

The genetic work described in this proposal will be conducted out of the Idaho Department of Fish and Game's fish genetics laboratory at Eagle, Idaho and the Salmonid and Freshwater Fish Genetics Research Laboratory at the University of Idaho's Hagerman Fish Culture Experiment Station. Between the two facilities, all of the necessary molecular genetic analysis equipment and expertise for this work is already in place.

The only capital equipment requested for this project is a centrifuge to run 96 well PCR plates and a PCR thermal cycler. We are also requesting as part of operating expenses the lease of a ABI 3100 fragment analyzer to expedite the generation of multilocus, microsatellite genotypic data for approximately 3,000 genetic samples. The ABI 3100 fragment analyzer currently owned and in operation at the University of Idaho Hagerman's laboratory can complete all proposed analyses. However, the timeliness of the project would be greatly facilitated by the lease of an additional instrument for a fixed period of time. Analyses of costs associated with personnel and equipment indicates the lease of an additional instrument would be more cost effective than the retention of extra personnel throughout the year to operate a single instrument.

QUALIFICATION OF PARTICIPANTS

Dr. Madison Powell received his Ph.D. in the Systematics & Evolutionary Biology program at Texas Tech University in 1995 and is currently an Assistant Professor in the Department of Fish and Wildlife Resources and Department of Animal and Veterinary Sciences at the University of Idaho. Dr Powell is also the director of the Center for Salmonid & Freshwater Species at Risk at the University of Idaho. He supervises UI molecular genetic laboratories at the Aquaculture Research Institute in Moscow, Idaho and at the Hagerman Fish Culture Experiment Station in Hagerman, Idaho. The laboratories' primary goals are to provide timely genetic information to applied conservation genetic questions and provide genetic advice and consultation to state, federal, and tribal agencies regarding endangered fishes and fisheries management. Dr. Powell is currently the Principal investigator of several genetic projects examining reproductive success of hatchery and wild fish using microsatellite DNA analyses including sockeye (project BPA #199107200) and Chinook captive broodstock (project BPA #199009300). Dr. Powell will assist in the development of the research study design, supervise genetic lab work, analyze data, and report results.

Education
Ph.D. Zoology, Texas Tech University (1995)
M.S. Zoology, University of Idaho (1990)
B.S. Zoology/Biology, University of Idaho (1985)
Expertise:
Fishery/Genetics Research:
UI Assistant Professor researching conservation genetics of salmonids (2 years)
Expertise Specific to this Project:
UI Research Scientist studying endangered sockeye populations in Snake River Idaho (7 years)
Dissertation using genetic fragment analysis to discriminate populations

Matthew Campbell (IDFG) is currently employed by IDFG as a fisheries biologist/geneticist and oversees genetic projects at IDFG's Eagle Fish Genetics Lab. Current projects include using microsatellite analyses to assess the reproductive success of hatchery and wild Chinook salmon at the Pahsimeroi River and to assess the reproductive success of hatchery and wild spawning sockeye salmon at Redfish Lake, Idaho. Matt received a M.S. degree in Fisheries (emphasis in genetics) from the University of Idaho, examining hybridization and introgression issues in cutthroat trout populations using molecular markers. He previously worked at the University of Idaho's genetics lab for over six years examining hybridization, genetic diversity, and genetic population structure of fish species throughout the Pacific Northwest using mtDNA and microsatellite DNA analyses. Matthew Campbell will perform genetic work with assistance from one scientific aide and will assist Matt Powell with data analysis and reporting of results.

Education:
BSc (Fisheries Research) from University of Idaho (1995)
MSc (Fisheries Research-emphasis in fish genetics) from University of Idaho (2001)
Expertise:
Population Genetics Research:
IDFG geneticist-current
University of Idaho–Biological Aide (Genetics Lab), Center for Salmonid and Freshwater Species at Risk (5 years)-Moscow, Idaho
Expertise Specific to this Project:
Supervises State's Chinook salmon genetic projects
Proficient in generating and analyzing microsatellite data on an ABI 310 and ABI 3100 fragment analyzer

Jeffrey Lutch is a Senior Fishery Research Biologist with the IDFG at the Nampa Research Facility. As the lead biologist for the Idaho Supplementation Studies project, he is evaluating benefits and risks of different Chinook salmon supplementation strategies on natural production and productivity. Jeff was previously employed as a fishery biologist with the National Park Service in Yellowstone National Park, where he performed status assessments for cutthroat trout populations while documenting the extent of genetic hybridization with nonnative salmonids. Previously, he worked as a fishery biologist with Bureau of Land Management in Alaska and studied the effects of recreational use on fisheries. Jeff received his B.S. from the University of Pittsburgh and an M. S. from Clarion University, where he investigated aggressive interactions between native and introduced trout and the effects on reproductive success. Jeffrey Lutch will coordinate sample and data collections supported by the Idaho Supplementation Studies project and will assist in data analysis and report writing.

Education
BS (Biology) from the University of Pittsburgh (1990)
MS in Biology (emphasis in fish ecology) from Clarion University of Pennsylvania (1994).
Expertise
Fishery Research
Species Interactions
Population Dynamics
Hatchery Supplementation
Management
Exotic species control
Recreational Fisheries

Population monitoring and evaluation

Expertise to this project

Thesis examining reproductive success of sympatric native and introduced salmonids

Coordinates the Idaho Supplementation Studies project

Proposed and supervises the small-scale reproductive success study between hatchery and wild Chinook salmon at the Pahsimeroi River

Budget

Reproductive Success of wild and hatchery Chinook salmon-BPA FY2003 (IDFG 2004)

		Personnel Costs			
	Comments	Salary/hr	Hours/week	Weeks	Total
Temporary	Genetic lab assistant	$18.00	40	32	$23,040
	Assistant benefits (35.0%)				$8,064
Temporary	(2) Techs for trap op., sample collection	$11.88	40	32	$30,413
	Tech benefits (42.8%)				$13,017
Temporary	(2) Bio-aides for trap op., sample collection	$7.63	40	32	$19,533
	Tech benefits (49.8%)				$9,727
				Total Personnel Costs	$103,794
		Operating Costs			
Supplies (not Cap Outlay)	Chemicals, pipette tips, gloves, primers, etc.		Cost/sample	# of samples	
HFCES (U of I)	DNA extractions, quantifications, normalization		$5.00	1900	$9,500
Eagle Genetics Lab (IDFG)	PCR amplifications, usat electrophoresis		$55.00	1900	$104,500
Equipment Lease	ABI 3100 fragment analyzer (2003-2007)				$41,058
Misc.	Equipment repair, misc.				$5,000
				Total Operating Costs	$160,058
		Capital Outlay Costs			
1 PCR machine (2003)					$5,000
1 Centrifuge (2003)					$8,000
				Total Cap Outlay Costs	$13,000
				Subtotal	$276,852
				Overhead (20.9% of operating and personnel)	55,145
				Total Costs	**$331,**997

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