Proposal 11: Comparative Reproductive Success of Wild and Hatchery Origin Spring/Summer Chinook Salmon that Spawn Naturally in the Pahsimeroi and Upper Salmon Rivers

Sponsor-(IDFG)

This is the Idaho Department of Fish and Game's (Department) and the University of Idaho's responses to comments generated by the Independent Science Review Panel (ISRP) for Proposal #11: "Comparative Reproductive Success of Wild and Hatchery Origin Spring/Summer Chinook Salmon that Spawn Naturally in the Pahsimeroi and Upper Salmon Rivers (IDFG)"

The ISRP reviewers requested responses on six main issues:

1.) **The ISRP pointed out that we did not propose to extend the study beyond the F_2 generation.**
2.) **The ISRP pointed out that we did not propose work to address possible causes for differences in reproductive success between hatchery and wild salmon.**
3.) **The ISRP stated that we were unsure as to whether our results would be applicable to the ESUs specified in the additional criteria of the RFS.**
4.) **The ISRP stated that the proposal would be strengthened by the addition of control streams.**
5.) **The ISRP is concerned about studies that include extant populations with a long history of potential interbreeding with hatchery fish.**
6.) **The ISRP is concerned about quality control and assurance of the genetic data collected in potentially long-term field project.**

The answers to these requests follow:

1.) **In response to the ISRP regarding the idea that the work will not extend beyond the F_2 generation.**

This project can easily and efficiently evaluate the reproductive success of hatchery and natural origin fish beyond the F_2 generation. This project is designed to sample **every adult chinook** returning above the Pahsimeroi and Sawtooth weirs **every year** (this began in 2002) as well as sub-sample juveniles at different life-stages each year (currently in process). This sampling can and will continue indefinitely depending on funding. Currently, the proposal describes sampling of F_2 adults through 2012 (Table 1), originating from 2002 parental crosses. By sampling juveniles in 2009-2014, and adults

in 2011-2017 we will have sampled F_3 progeny (at three separate lifestages, parr/pre-smolt, smolt, and adult (Table 1).

	Age-0, **F_1 parr** sampling	Age-0, **F_1 pre-smolt** 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
Spawn Year						
2002	2003	2003	2004	2005	2006	2007
	Age-0, **F_2 parr** sampling	Age-0, **F_2 pre-smolt** 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**, 1-ocean a**dults** spawn	2007	2007	2008	2009	2010	2011
2007 **F_1**, 1-ocean **adults** spawn	2008	2008	2009	2010	2011	2012
	Age-0, **F_3 parr** sampling	Age-0, **F_3 pre-smolt** sampling	Age-1, **F_3 smolt** sampling	1-ocean, **F_3 adult** sampling	2-ocean, **F_3 adult** sampling	3-ocean, **F_3 adult** sampling
2008 **F_2**, 1-ocean adults spawn	2009	2009	2010	2011	2012	2013
2009 **F_2**, 1-ocean adults spawn	2010	2010	2011	2012	2013	2014
2010 **F_2**, 1-ocean adults spawn	2011	2011	2012	2013	2014	2015
2009 **F_2**, 2-ocean adults spawn	2010	2010	2011	2012	2013	2014
2010 **F_2**, 2-ocean adults spawn	2011	2011	2012	2013	2014	2015
2011 **F_2**, 2-ocean adults spawn	2012	2012	2013	2014	2015	2016
2010 **F_2**, 3-ocean adults spawn	2011	2011	2012	2013	2014	2015
2011 **F_2**, 3-ocean adults spawn	2012	2012	2013	2014	2015	2016
2012 **F_2**, 3-ocean adults spawn	2013	2013	2014	2015	2016	2017

Table 1. Schedule of sampling for filial (F) generations F_1 through F_3 by brood year and life stage**.** Cells in column 1 show brood year adult spawners by filial generation and ocean age at return. Cells in columns 2 –7 show sampling events by fish age and life stage. These sampling event cells are tiered by filial generation (i.e. F_1, F_2, and F_3) and the headings the generational tiers are shaded. For example**,** all adults returning to the Sawtooth and Pahsimeroi River weirs were sampled in 2002 (Spawn Year 2000). Collection of F_1 juvenile offspring of those individuals will take place during 2003 (Age-0, F_1 parr and F_1 pre-smolt sampling) and 2004 (Age-0, F_1 smolt sampling). These individuals will be sampled again as F_1 adults from 2005 to 2007 when they return from the ocean. (1-ocean, F_1 adult sampling, 2-ocean, F_1 adult sampling, 3-ocean**,** F_1 adult sampling). Offspring from those F_1 adults (i.e. F_2 generation) will be sampled as juveniles between 2006 and 2009, and then as adults from 2008 to 2012. Offspring from return F_2 adults (i.e. F_3 generation) will be sampled as juveniles between 2009 and 2014, and then as adults from 2011 to 2017.

One of the comments in the ISRP review was that "Releases of hatchery adults will cease in 2007 and consequently the research will not assess the effects beyond the F_2 generation". The reviewers are correct in stating that 2007 will be the last year in which hatchery origin adults will return to the Pahsimeroi and Sawtooth Weirs and that all fish spawning above the weirs subsequent to 2007 will be of natural origin. However, we are confused as to why the reviewers felt that we would not be capable of assessing the hatchery effects of 2002 spawners beyond the F_2 generation. Importantly, as illustrated in Table 1, not only will we be capable of following spawn year 2002 adults through all life stages of the F_3 generation (assessing the reproductive fitness of the offspring of spawn year 2002) we will also have the ability to evaluate the reproductive success of five additional brood years of hatchery and wild origin crosses (2003-2007).

2.) **The ISRP pointed out that we did not propose work to address possible causes for differences in reproductive success between hatchery and wild salmon.**

We concur with the reviewers about the importance of trying to identify possible causes for differences in reproductive success between hatchery and wild salmon. In fact, IDFG has already identified objectives that relate directly to addressing important possible causes for differences in reproductive success between hatchery and natural origin chinook salmon (run timing, length and age at migration (adult and juvenile), sex-ratio, spawning distribution, and spawning behavior) and we began work on several of these objectives last year as part of a pilot study funded through the Idaho Supplementation Studies project. With a limited time to prepare a proposal, and the understanding that projects would likely be limited to $200,000 to $300,000 we decided against including those additional objectives in our original proposal. Furthermore, we felt that including these studies in our proposal might give the appearance of funding overlap with the existing ISS pilot project goals. However, since this project proposal is designed to enhance and contribute to the wealth of knowledge we already possess on these to chinook populations and compliment studies underway to address other important facets of their life history, we concur that additional objectives should be included. With the understanding that additional funds will be required, we have included objectives needed to expand our existing pilot project to the Sawtooth Basin and extend the work in both

basins through 2017 (the last year of returning F_3 adults). Th additional objectives and methods are listed below:

OBJECTIVES

Objective 5. Determine the run timing of hatchery and natural origin adult chinook salmon returning above the Pahsimeroi and Sawtooth River weirs.

Ho_5: There is no significant difference in the run timing of hatchery and natural origin chinook salmon above the Pahsimeroi and Sawtooth River weirs.

To test the null hypothesis, the date of all returning adults to the Pahsimeroi and Sawtooth River weirs will be recorded.

Objectives 6-8. Determine the sex, age, and length of hatchery and natural origin adult chinook salmon returning above the Pahsimeroi and Sawtooth River weirs.

Ho_6: There is no significant difference in the age at return of hatchery and natural origin chinook salmon returning above the Pahsimeroi and Sawtooth River weirs.

Ho_7: There is no significant difference in the sex ratio of hatchery and natural origin chinook salmon returning above the Pahsimeroi and Sawtooth River weirs.

Ho_8: There is no significant difference in the length of hatchery and natural origin chinook salmon returning above the Pahsimeroi and Sawtooth River weirs.

To test these null hypotheses, scale samples will be collected from all adults returning to the Pahsimeroi and Sawtooth River weirs to determine age. Sex and length of all returning fish will also be recorded.

Objective 9. Determine spawn timing of hatchery and natural origin adult chinook salmon above the Pahsimeroi and Sawtooth River weirs.

Ho_9: There is no significant difference in the spawn timing of hatchery and natural origin chinook salmon above the Pahsimeroi and Sawtooth River weirs.

To test the null hypothesis, multiple spawning ground surveys will be conducted to asses spawn timing of both hatchery and natural adult chinook. Spawn timing will be determined from the observations of predefined spawning activity (Table 2). The relative proportion of each group observed (for each sex) actively spawning over time will result

in a spawn timing distribution. Spawn timing distributions of natural and hatchery adults will be compared to determine differences in spawn timing.

Objective 10. Determine the spawning distribution of hatchery and natural origin adults above the Pahsimeroi River weir.

Ho_{10}: There is no significant difference in the spawning distribution of hatchery and natural chinook salmon above the Pahsimeroi River weir.

To test the null hypothesis, the know historic spawning area will be divided into lower, middle, and upper strata and multiple spawning ground surveys will be conducted throughout the spawning period to include early, peak and post spawning periods. Locations of all known origin adults will be documented during each survey. The Chi squared statistic will be used to determine differences in distribution between the three strata.

Objective 11. Determine the relative spawning success of hatchery and natural origin parents above the Pahsimeroi River weir.

Ho_{11}: There is no significant difference in the relative spawning success of hatchery and natural origin chinook above the Pahsimeroi River weir.

To test the null hypothesis, detailed spawning behavior observations will be conducted and observed spawning activities (Table 2), over fixed time intervals, will be recorded for both groups. Differences in spawning success will be determined by testing for significant differences in the frequency of spawning activity between the two groups using ANOVA.

Table 2. Spawning behaviors used to determine spawn timing and relative spawning success of wild/natural and hatchery origin chinook.

Spawn Time	Behavior	Definition
Pre-Spawn	Holding	Prolonged lack of activity of individuals or groups of chinook. Holding in areas not suitable for redd construction such as deeper pools, and large cobble or boulder habitat.
Spawning	Aggression	Biting, chasing or sudden forward or lateral movements invoking chase or submission
	Quivering	Shuddering or rapid full body shaking usually by males accompanying females during redd construction and fertilization.
	Crossing over	Males swimming back and forth over the backs of females with

		males oriented near the caudal region of the female.
	Cutting	Any of several actions by the female to scour or fill gravel by turning on her side and fanning her tail against the substrate. Includes scouring upstream of redd to cover deposited eggs.
Post-Spawn	Guarding	A female holding over or near a completed redd. Her tail will likely be worn and white in appearance and she will appear thin through the abdominal region indicating she has completed egg deposition. Female will likely not be accompanied by males

METHODS

Adult Marking and Releases

As adult chinook enter the Pahsimeroi and Sawtooth hatchery weir traps, they will be lengthened, sexed, sorted, and ponded or released based on their origin (natural or hatchery) and a predetermined hatchery weir management plan. Hatchery origin adults will be distinguished from natural origin adults based on adipose clips and/or coded wire tags (CWT). Approximately 25% of each sex by group (natural and hatchery) will be marked with a 7/8 inch Peterson Disc tag prior to release. Tags will be attached approximately ½ - ¾" (inch) below the insertion of the dorsal fin rays. A single tag will be attached on both sides of the fish midway between the anterior and posterior insertion of the dorsal fin. Tags will be color coded to differentiate between natural and hatchery origin adults only.

Observation Survey Schedule

Starting at the beginning of the spawning period, spawning ground surveys will be conducted every are you sure we want to do them every day? day. A survey will include at least one sampling transect from each of three strata. Selection of transects to be sampled and the order in which they will be sampled will be randomly assigned. To reduce bias associated with adults moving between strata on successive days, each survey will include one transect from each strata on the same day.

Spawn Timing

Spawn timing will be determined from observations of predefined spawning behavior of all known origin adults during spawning observation surveys. Based on behavioral observations, adults will be assigned to one of two groups (spawning or not spawning). Spawn timing distributions will be determined by tracking the proportion of each group

observed exhibiting spawning behavior over time. Mean spawn timing for each group will be calculated and weighted by observation day. Significant differences in mean timing will be determined by subtracting the means and constructing a 95% confidence interval around the difference. If the interval captures zero, it will be concluded that there is no difference in spawn timing.

Spawning Distribution

During observation surveys, the locations of all known origin adults observed will be recorded on a GPS receiver. Comparisons of spawning distributions over time will be weighted based on the proportions of each group released upstream. The Chi Squared statistic will be used to determine differences in distribution between the two groups for both sexes across three strata by using 2x3 contingency tables representing both populations.

Relative Spawning Success

During observation surveys, spawning behavior of each known origin fish observed will be recorded for ten-minute observation intervals. Frequencies of spawn activity for both groups (natural and hatchery) will be used as a measure of spawning success. Frequencies will be averaged across all sample periods and differences in relative spawn success will de determined using ANOVA. In addition, all female carcasses recovered will be examined for evidence of contribution by measuring all unspawned eggs using a volumetric graduated cylinder. Female pre-spawn mortalities will be compared between the two groups.

Costs

Increased costs for conducting behavior observations and scale/aging analyses are outlined below:

Comments	Salary/hr	Hours/week	Weeks		Total
(2) Techs. for behavior observations	$11.88	40	8	$	7,603
Tech benefits (42.8%)				$	3,254
(4) Bioaides for behavior observations, scale analysis	$7.63	40	16	$	12,486
bio-aide benefits (49.8%)				$	6,218
			Total Personnel Costs	$	29,561

3.) **The ISRP stated that we were unsure as to whether our results would be applicable to the ESUs specified in the additional criteria of the RFS.**

We have to say that the answer to the question: *"Can results from this study be extrapolated to other systems or ESUs?"* is once again *"unknown"*. How could we honestly answer differently? However we would like to reemphasize that the goal of this project is not to simply provide information on one particular system but rather to test whether interactions between hatchery and wild salmon and any resultant differential success can be predicted across different systems. 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 and information gathered from these systems could be extrapolated to other systems and ESUs throughout the Columbia Basin. Again, if the information obtained is not predictive, then each system may have to be evaluated on a case-by-case basis. The advantage of using the Pahsimeroi and Sawtooth chinook salmon stocks to examine these important questions is that these stocks meet the following criteria: 1) adequate sample sizes through the F_2 generation for detection of specific crosses in returning adults, 2) limited enough in scope and geographic area for the study to be 'manageable', 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).

4.) **The ISRP stated that the proposal would be strengthened by the addition of control streams.**

5.) **The ISRP is concerned about studies that include extant populations with a long history of potential interbreeding with hatchery fish**

These two ISRP concerns are related and will be considered to together. In crafting our Comparative Reproductive Success (CRS) proposal we gave careful consideration to the possibility monitoring results of WxW crosses in control streams for comparison with results WxW crosses in the Pahsimeroi and Upper Salmon rivers. Our deliberations drew heavily on the extensive peer review that the ISRP and others have contributed on the topic of control streams for the ISS study. Based upon these deliberations we rejected the idea of including separate control stream in our proposal for the following reasons:

1. Locating true "control" streams among any ESUs in the Columbia Basin is likely problematic. Evaluation of spawner carcass data (redds) from Idaho Supplementation Studies (ISS) study streams indicates that straying occurs in all study streams, is extensive in many streams, and has influenced redd production. As a result, investigators concluded that few "control" streams without hatchery influence exist among streams included in the ISS study. Concern about the impacts of hatchery strays on pair-wise comparisons of ISS treatment and control streams was echoed by ISRP in their review of a recent statistical analysis of production data from that study. Although they were favorably impressed by the prototype analysis that was formulated to statistically treat the effect of hatchery strays on the ISS study design, the ISRP remained concerned that straying was widespread, that no true control streams existed, and they recommended further pilot statistical analyses.
2. Straying is one of many sources of variability that can bias comparisons between treatment and control streams. The ISS was able to cope with the straying variable because it could be quantified but many of the other sources of variability between streams are far less tangible. Consequently the experimental design in our proposal compares productivity between "wild" and "hatchery influenced" fish within the same stream specifically because that design minimizes non-genetic in-basin and out-of basin variables that affect survival. In our study design for example WxW, WxH, and HXH crosses all experience the same freshwater incubation and rearing environment. Treatment and control fish that originate

from a common rearing environment in the same stream also share the same migratory corridor through tributaries and the mainstem Columbia River and they likely share ocean rearing conditions as well. Similarly, our design also minimizes the effects of biological variables such as spawner densities, rearing densities, food availability, inter-species interactions, exposure to disease, etc. that are more or less independent of genetic effects and could bias comparisons between treatment and control groups.

In contrast, regardless of how carefully investigators select separate treatment and control streams for a pair-wise comparison it is virtually impossible to confidently assume that extrinsic environmental and biological do not vary between the two streams in the pair. Even measuring all the possible sources of variability is problematic. Furthermore, as the experience with the straying variable in the ISS study shows, it is a challenge to statistically reduce the effects of differences that do exist even if they can be measured.

Previous studies have tried with varying degrees of success to reduce the bias in productivity comparisons from experiments that includes “treatment” and “control” streams by including more “treatment” and “control” pairs. In theory, bias is reduced if enough streams are selected for each category such that they are representative of the observed variability among streams with respect to environmental and extrinsic biological variables. Increasing the sample size (number of treatment and control streams) also results in improved precision of productivity estimates and the likelihood of detecting differences if they exist. The strength of ISS experimental design, for example, lies in its large-scale evaluations where replication occurs across two subbasins (Salmon and Clearwater Rivers), affording statistical power to detect change in the face of major sources of variation. Other large-scale comparative studies have also adopted this approach. A study conducted by the Alaska Department of Fish and to asses impacts to wild pink salmon populations from the Exxon Valdez Oil Spill, for example, compared pre-emergent fry estimates from multiple pairs of

oiled ("treatment") and un-oiled ("control") streams. While the mortality estimates form the latter study were sufficiently precise to detect differences between pooled "treatment" and "control" streams, the debate still rages on, almost 15 years later, as to whether to observed differences were due to an oil spill effect of some other unidentified sampling related or natural source of bias associated with streams selected for the "treatment" and "control" groups (for example, the possibility that emergence timing was consistently earlier in control streams than in treatment streams).

We concur with the ISRP observation that supplementation of our proposed study streams with progeny of "natural" x "supplementation" crosses that are reared in the hatchery may have reduced the genetic differences between natural and hatchery fish hence the likelihood of demonstrating genetically based productivity differences. We also concur with the ISRP that results of trans-generational comparisons that indicate no detectable differences in productivity between WxW, WxH, and HxH crosses in that situation are equivocal and do not demonstrate conclusively that hatchery introductions into wild population have no effects on productivity. On the other hand, we would be remiss if we did not point out that the converse is also true. That is, if we do demonstrate differences in the F_1 generation or beyond despite prior hatchery influences then the evidence for an effect is very strong indeed.

A strong case could be made that hatchery straying in Columbia Basin ESUs is the rule rather than the exception. It may be that results from the study that we propose that are positive relative to a treatment response in streams having previous or ongoing hatchery influence are more "transferable" to non-study streams or other ESUs than results from a study that incorporates control streams that are truly pristine relative to hatchery influence. Certainly a positive response from our proposed study would suggest that hatchery influence persists even in mixed populations and can likely be reversed or reduced if the hatchery influence ceases. Conversely, if streams that truly have no prior hatchery influence are used

as a control, a positive response indicates an initial effect of a hatchery introduction relative to a pure wild population but is not indicative of whether that difference persist in the situation encountered in most of or natural populations, i.e. some measure of long term hatchery influence.

6.) The ISRP is concerned about quality control and assurance of the genetic data collected in potentially long-term field project.

We concur completely with the database QA/QC concerns voiced by the ISRP. If fact, we have or are in the process of addressing these issues within the context of existing projects. The anadromous research section of IDFG is currently utilizing a database to store and track the disposition of biological sample data. This database is well suited to store and catalogue the suite of genetic and biological samples to be collected in our proposed research. In addition, the IDFG genetics lab utilizes a database designed to track genetic samples from post collection to final analysis. Sample identification numbers that allow samples to be tracked from collection to final analysis links both databases. Finally, IDFG is working in cooperation with agencies coastwide to standardize laboratory analyses and interpretation of chinook genetic samples and data for use in a coastwide stock identification model.

A key component to our proposed research will be the collection of representative genetic samples from out-migrating juveniles to measure life-stage specific productivity. IDFG currently has eleven years of escapement and juvenile production and emigration timing data on both the Upper Salmon and Pahsimeroi Rivers. This a priori production and emigration information will help predict the size and timing of juvenile outmigrations and ensure proper sampling design protocols to obtain representative samples from that outmigration.