Response to the ISRP comments, Project 198909600
Aug. 9th 2002

1. Sample sizes are based on a combination of simulation results (Kalinowski 2002) and practical experience working with genetic data from these Snake River populations. Early in the project sample sizes for allozymes were 100 or more individuals per population, per year. It quickly became clear that a great deal of power was still possible with much smaller numbers. Highly polymorphic microsatellite gave us new concerns about sampling. However, Kalinowski's work, both published and unpublished, showed us that for allele frequency monitoring (tier 2 sites), 48 individuals per year examined at 12 to 16 loci with 8 to 12 alleles each gave a reasonable distribution of effort (i.e., uniform control of various sources of error), and substantially better estimates of F_{ST}, genetic distance, and other parameters than were possible with a full battery of allozyme loci. (Please also see 4. below)

2. All returning adults passed over the weirs at all three tier 3 sampling sites would be sampled, including progeny of captive brood stock. We would not, however, DNA-type all adults reared and juveniles released from the captive program. Managers know how families perform while in captivity. Our study will provide that information for the stream environment above the weir and put it in the context of wild fish performance.

3. If one had an infinite number of markers one would know parentage with virtual certainty, even if only half the true parents were sampled. Obtaining an accurate and precise estimate of selection coefficients would then only be a matter of collecting more juveniles than thought to be required for that estimate (twice as many on average). In other words, you simply keep DNA-typing juveniles until you have enough parent-pair-offspring triplets to obtain the desired power. Obviously we don't have an infinite number of loci, but we can run enough that we're confident the triplets are real. This is a bit tricky because it depends on allele frequencies we don't know (those of the unsampled parents). However, in the Little Sheep steelhead data we see almost no ambiguous triplets (one measure of power).

4. Regarding the size and scope of the reproductive success studies, they are daunting. Adult chinook returns were projected for Lostine River and Catherine Creek by Pat Kinery, ODFW. We expect to genotype between 500 and 3000 fish per year from each of those rivers and nearly as many steelhead from Little Sheep Creek. With current and projected projects, this number will exceed the current genotyping capability of our laboratory. Although a single sequencing instrument is more than enough to handle this load, the purchase of one sequencer over the duration of this study is by far the most efficient use of resources. The consequence of not funding this purchase would be a compromise in sample numbers and the power to obtain unbiased and precise estimates of selection coefficients. Mike Ford has done extensive simulations in estimating selection

gradients and actually favors rather higher sample sizes than we proposed in the current study. My sense from the work on Little Sheep Creek is that effect size will be sufficient to overcome the loss of power related to sub-optimal sample sizes (at least with respect to hatchery/wild relationships, though perhaps not finer relationships between success and phenotype). I feared that financial limitations would pre-empt an ideal sampling design and elected to make the sacrifice early (we may actually collect more samples than we can afford to run in the hope that costs will continue to decline). The screw trap was recommended by the comanagers at a meeting we held recently in LaGrande. Rich Carmichael, ODFW, favored running the trap year-round because so little is known about migration timing in this system. More importantly DNA-typing migrants might shed light on the problem of missing parents and genetic relationships between anadromous and resident *O. mykiss* in Little Sheep Creek. As indicated above, we have a great deal of confidence in our ability to assign parentage if the true parent is sampled. The "limited success of assigning parentage" cited in the RME review is more likely a reflection of the biology of the system (i.e., resident adults contributing to our parr samples), rather than a lack of power in our analysis. We're not surprised that resident *O. mykiss* apparently contribute to parentage, and we've taken steps to address it (collecting resident adults, larger juvenile samples, etc.). We expect less difficulty with chinook salmon; however, we are prepared to deal with precocial parr that we know are present in many Snake River populations.

Kalinowski, S.T. 2002. How many alleles should be used to estimate genetic distance. Heredity, in press.