Responses to ISRP and HHS Questions Regarding Proposal No. 9

An Evaluation of the Efficacy of Steelhead Kelt Reconditioning to Address Biological Opinion Action 184b: The reproductive success of hatchery-origin and wild-origin repeat spawners.

Sponsors

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Individual Responses to the Independent Science Review Panel

Response to direct questions:

(1) *Following fitness of the various crosses to F2 and F3 is not mentioned. Are control streams needed to follow fitness over time without hatchery and reconditioned steelhead?*

We agree with the assertion that following fitness to the F2 and beyond is desirable, especially if a major deficit in reproductive performance of the reconditioned kelts or hatchery fish is apparent. Negative consequences of a decrease in reproductive competence would be buffered somewhat by a rapid recovery to wild type reproductive success in subsequent generations. Conceptually, extension of the assessment of fitness of crosses to F2 and F3 progeny is relatively straightforward. It would involve extending the sampling regimen proposed for the Phase II work (see response to ISRP question 6; Phase II methods) by one or two steelhead generations (DNA-type every adult in each year for 5-10 years, beginning in 2011). The pedigree analyses from one generation to the next is used to link parents to progeny to grand-progeny, and so on. However, it also seems likely, given the pace at which molecular techniques are being developed and becoming available to field researchers, that fundamentally different (and more powerful and/or efficient) technologies might be applied a decade from now. For example, it is currently not feasible on a large scale to establish the grand-parentage of specimens without establishing the multi-locus genotypes of the potential grandparents and parents. Too many hypervariable loci for too many specimens would need to be screened at a very high cost per fish to make a direct pedigree assignment from, for example, F2 progeny to grandparents. On the other hand, micro-array DNA typing is an emerging technology that may hold promise for complex studies to track pedigrees spanning generations with less comprehensive sampling regimens. It is very plausible, if not likely, that the micro-array technology or a completely different tool will permit this important work to be completed more effectively than is possible with tools in common use today. We are very interested in such future potentials and would vigorously pursue their application to trans-generational pedigree analyses as appropriate.

We also agree in theory that the use of control streams is a useful experimental design approach, to the extent that it would permit comparisons of productivity over time as a measure of the population response to the treatment (presence of hatchery and reconditioned steelhead). However, in practice, we do not believe that the control stream approach can achieve its intended purpose, because it is very unlikely to be a true control and the degree to which it fails to do so will not be known. That is, it is highly probable that there will be factors other than the treatment effect itself that differ among control and treatment stream(s) and have an effect on the response measure (reproductive success). For example, the quality and quantity of habitats used by the fish from spawning to smolt emigration are likely to vary even among streams that are subjectively judged to be very similar. Likewise, there could be “population effects” between treatment and control streams due to inherent differences in the productivity of the particular fish in the streams, particularly for relatively small streams with small

populations. As a result, the degree to which stream/population effects influence observed differences (or lack thereof) between productivity in control and treatment streams is likely to be unknown. The only way to assess those influences would be to include enough control and treatment streams to assess variability both within and between treatment groups, which would be prohibitively expensive and logistically intractable. We therefore assert that the use of control streams could only provide the very coarsest comparison of control vs. treatment effects, and is not amenable to these types of studies.

(2) *Why pool milt – is intent to examine variability of potency? H s W?*

It has been shown that > 90% of steelhead kelts are female (Evans et al. 2001; Anders et al. 2002); therefore, the probability of a 2nd-time female spawner mating with a 2nd-time male spawner is small. The following example is based on a system with natural wild- and hatchery-origin repeat spawners—assuming: (1) a population of 1000 spawning pairs, (2) 10% of which are natural repeat spawners (3) 90% of natural repeat spawners are females, then the probability of a 2nd-time male mating with a 2nd-time female is only 0.36%. For this reason we are focusing on females in our assessment of gamete viability. Assuming that the numbers of naturally spawning hatchery-origin and wild-origin fish in a stream are not seriously skewed toward one group or the other, a 2nd-time female (hatchery or wild) could reasonably mate with a 1st-time spawning male of either hatchery or wild origin (or both). Kalama River female steelhead spawn once sometime in winter or early spring (but may have eggs fertilized by more than one male), while male fish are able to spawn repeatedly over the same time period.

In order to reduce the variability in fertilization success based on differences in sperm motility or viability of individual males, we will (1) use the same groups (hatchery and wild) of males throughout the spawning period and (2) pool milt from all of the males in a group at each spawning. We will determine sperm motility and viability for each pool at each spawning.

Response to technical comments:

(3) *Reviewers assessed a lower rank to this investigation as it may not constitute an adequate test of gamete viability when tested only within the controlled environment. … Reproductive success may be as much a function of spawning behavior, synchrony of gamete development, and local stresses, which cannot be naturally controlled in the laboratory environment.*

We agree that reproductive success, for fish in the wild, may be influenced by many factors. However, in Phase I of our study, we are not testing gamete viability and reproductive success of fish in the wild, but are assessing the process of reconditioning and its influence on gamete viability. Obviously, such reconditioning programs take place in hatchery or laboratory environments and our assessment is focused within this context. In fact, we believe that gamete viability can be reliably measured only in a

laboratory setting where selected environmental variables can be controlled as much as is possible.

Gamete viability is a measure of how fish cope with environmental variation and partition available energy resources into physiological functioning, somatic growth and gamete production. To accurately measure the capacity of reconditioned fish to produce quality gametes, we must exert some control over energy resources (i.e., food availability) and physiological functioning (e.g., controlling temperature). Such procedures would be standard practice in a kelt reconditioning program. In our proposed research, we are concerned with the quality of eggs after reconditioning (see answer to ISRP question ***2*** above). To minimize variability in sperm, we will use pools of sperm from hatchery and wild fish and will attempt to control for the effects of sperm concentration by doing a sperm dilution series and fertilizing the eggs from each female with each dilution. Such a test is more ecologically realistic and should minimize obscuring the true quality of the eggs by swamping them with sperm. All eggs will be exposed to identical environmental conditions so we can directly compare the gamete quality between an individual fish after its first spawning, that same fish after it has been reconditioned, and virgin spawners from that same year. Data will also be collected from a limited number of naturally returning repeat spawners (*ca*. 8 to18 females), providing another important maturational category for comparisons. After these comparisons are made, we will be in a position to examine other variables regulating reproductive success—see answer to ISRP question ***7*** below.

(4) *The studies associated with BiOp RPA Action #184 do not incorporate an analysis of the potential long-term genetic consequences of increasing repeat spawners (e.g., inbreeding, domestication selections, altering age structure or life history structure)…* paraphrased from ISRP review criteria.

We agree with the reviewers that the potential for long-term genetic impacts of a kelt reconditioning program was not adequately addressed in any of the proposals. Furthermore, we agree with the ISRP that a true evaluation of the long-term genetic consequences (e.g., fitness effects) of a reconditioning program will require longer commitments, additional funding, and more adequate genetic tools to answer (ISRP Proposal Review, p.6). Our proposal only implicitly dealt with this issue: we placed a contingency on a transition from Phase I research to Phase II research based on an exploration of the potential for the kelt reconditioning study to negatively impact the well-being of the extant steelhead population in the Kalama River (see response to HHS question). We expect that negative genetic impacts of the study are less of a concern in the Kalama River than in rivers further up the Columbia Basin because of the strength of the steelhead run in the Kalama. In addition, genetic and life history data (e.g., run timing, maturation timing, sex ratios, ocean residence timing, outmigration and spawning age) collected from natural returning repeat spawners will provide important clues into any inherent difference present in a population prior to releasing reconditioned kelts (Phase I) and reconditioned kelts are released for natural spawning (Phase II).

It would seem perhaps more prudent to approach this particular issue from a modeling perspective first. Based on the literature and our internal discussions, it seems to us that the modeling effort will have to be quite sophisticated. The modeling will need to simultaneously address the potential for artificial selection (e.g., via non-random selection of kelts for reconditioning), domestication selection (via mortality or failure to recondition some of the kelts) and perhaps an increased rate of inbreeding (via increase in variance in family size due to increased rate of iteroparity). Importantly, the model will have to account for varying probabilities of negative outcomes given varying run sizes in systems where kelt reconditioning might be implemented. Again, an evaluation of these factors will likely depend upon the demographics of the population(s) receiving additional repeat spawners (above and beyond the population's current number) and will likely require consideration on a case-by-case basis. We suggest that the ISRP and HHS consider the idea of supporting a separate, independent modeling effort directly and rigorously addressing the issue of long-term genetic impacts, using data collected from funded studies.

In summary, the Kalama Research Program is focused on examining genetic and ecological consequences of hatchery practices and will continue to do so. However, at this time a long-term genetic evaluation of kelt reconditioning—especial given the limitations of detecting fitness effects—is premature and perhaps not feasible at this time, as noted by the ISRP. We welcome the opportunity to collaborate on such a project in the future and, for our part, are confident that we will be collecting and reporting on information that will be essential to addressing these issues.

Response to clarify design and/or uncertain information:

(5) *This proposal is of the lowest priority of the three reconditioning proposals because it does not have as direct application to the ESU's*

We disagree that our proposal—especially Phase I—does not have broad-based, direct application to the ESUs listed in the RFS and are concerned that our proposal would receive a low priority because of this assertion. We discuss here some reasons why we think our work in Phase I is applicable to the ESUs of concern, and explain why it may be important to do such work in a "surrogate" ESU. We are also curious about the specific reasons for the low priority given our proposal by the ISRP regarding the ESU applicability issue.

A. The purpose of our research during Phase I is to assess the influence of reconditioning on several egg and progeny variables within different mating groups of steelhead kelts. We believe that the results obtained from this research—including such things as the influence of reconditioning on fecundity, egg size, fertilization success, hatching success, and early progeny survival—will be applicable to summer run steelhead throughout the Columbia River basin. We are attempting to address key, universal uncertainties regarding the efficacy of the reconditioning *process*. We argue that such basic information on the reconditioning process itself should be known *before* a complete

evaluation of kelt rehabilitation programs and reproductive success can be achieved. Further, our laboratory-based assessment—using established reconditioning protocols—should be independent of the ESU. Empirical evidence suggests that factors such as diet, water temperature, and photoperiod can affect the maturation process and, ultimately, the survival of progeny from reconditioned fish. However, such differences have emerged regardless of the fish's origin, age, sex, or even species. Although many of the factors that may influence iteroparity cannot be controlled within our experimental design, we believe our work in Phase I represents a good first step towards a more complete understanding of reconditioning steelhead kelts. Information gathered during Phase I of our study should be known *before* reconditioned steelhead are released to the wild.

B. We are proposing to use a summer-run race of steelhead, both hatchery and wild origin, for the work described in Phase I. This is of course a logical surrogate race of fish to use for the target ESUs (i.e., as opposed to winter-run fish). The wild broodstock hatchery program on the Kalama River uses protocols that should be universally accepted throughout the Columbia River basin, thereby increasing the applicability of our results to other ESUs.

C. Adequate facilities to capture, monitor, and conduct research on the entire life cycle of steelhead are rare, but do exist at the Kalama facility. That is, this facility has the capability to handle and collect data and samples from entire runs of adult steelhead, and also monitor juvenile outmigrations (5-10% of outmigrants captured annually in smolt traps), which would of course greatly facilitate future aspects of our study. Also, any permitting needed to accomplish our study should be facilitated within the existing Kalama project with only minor modification to the existing Hatchery Genetic Management Plan.

D. One major reason we decided to use a population of fish for our research that was outside the target ESUs was because of anticipated difficulties in obtaining permits and, more importantly, the risks associated with sampling individuals from the target ESUs. In fact, during some early discussions with colleagues about this matter, we determined that the odds were very low, or non-existent, that we would be able to sample an adequate number (e.g., for statistical comparisons) of wild individuals from the target ESUs. Runs of Kalama River steelhead, although listed as threatened within the Lower Columbia ESU, are still relatively strong runs (~90% of escapement goal in the 2002 return year). This fact, coupled with our design of incorporating our sampling within the existing Kalama River research program, should help to minimize any risk of detrimental effects of this type of work on extant populations.

E. Another advantage to working with Kalama R. steelhead is the rare opportunity to investigate fecundity, gamete viability, and early ontogeny in naturally occurring repeat spawners. Scale analysis of steelhead from the Kalama suggests that upwards of 15% of the summer-run steelhead can be repeat spawners. Conversely, very few - if any - naturally returning repeat spawners persist in up river ESUs. In our opinion, a rigorous evaluation of reconditioning should incorporate natural repeat spawners, thereby providing a true base line in which to evaluate the efficacy of a kelt reconditioning

program. For example, there may be inherent differences between virgin spawners and naturally occurring repeat spawners that influence or regulate population growth and plasticity (e.g., differences in fish age, size, maturational rates, fecundity, egg quality, progeny survival and emergence timing). Such differences would go undetected and/or unaccounted for if natural repeats were excluded from a study population. Given that it is reproductive contribution of repeat spawners that reconditioning efforts are intended to mimic, it is important that natural repeats be included in these evaluations.

F. Based on our perusal of other proposals and reviewer comments, it is not clear to us why simply proposing to work with a run of steelhead that lies outside the ESUs of interest is sufficient reason for the relatively low priority given to our proposal. Numerous proposals received under this RFS have also chosen to work with runs of fish that were outside the target ESUs, yet, to our knowledge, none was given a lower priority specifically because of this. We assert that working outside the target ESUs, specifically for Phase I of our study, should not tacitly result in our proposal receiving a lower priority rating. Our assertion would be more consistent with the apparent application of the weight of that criterion to other studies proposing to work outside of target ESUs. Moreover, we maintain that the strengths of conducting the proposed work in the Kalama basin, coupled with the applicability of results to target ESUs, collectively result in a design that will have high value in meeting the objectives of the current RFS to address RPA Action #184. Nevertheless, if the reviewers have suggestions as to where we might better conduct our work within the target ESUs, we would welcome the opportunity to discuss them.

(6) Methods *for the design of or analysis of Phase II are not given.*

Phase II involves the insertion of reconditioned hatchery- and natural-origin kelts into the spawning aggregate in the Kalama watershed. A DNA sample (fin clip), scale sample, and other biological information (fork length, sex, general condition, run timing, and presence of identifying marks) will be obtained from all adult steelhead passed upstream.

The aggregate will be comprised of natural-origin virgin and repeat spawners, hatchery origin virgin and repeat spawners, hatchery origin reconditioned spawners, natural origin reconditioned spawners, resident rainbow trout, and residuals (from earlier hatchery releases). Abundance of individuals in the latter two elements relative to the anadromous adult components of the spawning aggregate is only coarsely known (based on snorkel surveys, electrofishing and angling surveys). For the purposes of this proposal, the effects of successful spawning by resident and residual trout that results in the production of adults expressing an anadromous life history is assumed to be negligible or, at the least, not a significant source of bias in understanding patterns of production by the other elements of the aggregate. The authors are aware of the importance of this assumption and suggest that sampling of resident and residual trout and DNA screening could be used to assess their potential contribution to returning anadromous adult offspring.

Anadromous adult offspring will return two to six years after conception, depending on length of freshwater and saltwater residency. The majority will return as four or five year

old fish. Essentially all naturally produced returning adults will themselves be sampled as described above.

The parentage of each returning adult will be unambiguously established using the best available DNA-based technology available at the time. Currently, we anticipate applying the msDNA techniques used successfully by the WDFW DNA laboratory for other work in the Kalama and elsewhere.

Each brood will be reconstructed using a combination of the DNA-based pedigrees and scale analyses. The actual abundance of adult offspring from each of the spawner types and combinations thereof will be compared to the expected abundances derived from numbers of each type of spawner in the parental spawning aggregate. As a simplified example, using recent escapement estimates and projected releases of reconditioned spawners, we might expect that of approximately 1000 total adults, 475 will be natural-origin virgin spawners, 475 will be hatchery-origin virgin spawners, and 50 will be female reconditioned kelts. Assuming equal sex ratios of the virgin spawners, under the null hypothesis (equal reproductive success), ancestry of the returning adult offspring should be binomially distributed according to the original abundance of the parental spawner types. A chi-square or G-test can be used to test for significant deviations from expected frequencies.

Statistical power of the anticipated tests is an important issue for any assessment of natural reproductive success. We expect to obtain a near census of the returning adults. Preliminary power analyses have shown that we can likely subsample for DNA-typing and still achieve a sufficiently robust design to, for example, get 80% statistical power to detect a 25% deficit in reproductive success between virgin and reconditioned spawners (Table 1). However, as we partition the spawners in various ways to explore patterns of variance in reproductive success among, for example, reconditioned kelts vs. natural repeats (Table 2) or virgin wild vs. reconditioned wild (Table 3), statistical power may under some scenarios be less than desired. It is the authors' experience that these modeling exercises tend to overestimate actual statistical power because of unknown or unforeseen variance components. Nevertheless, it is our intent here to illustrate that success with the design as laid out is reasonably within reach. The authors are aware that a number of other researchers assessing reproductive success are struggling with the issue of how high variability in reproductive success among individuals- can confound attempts to make statistically robust comparisons of reproductive success among different groups within a spawning aggregate. We anticipate that we will both contribute to and benefit from those discussions in the coming years before we have a need to apply the methods in our system. We further contend that the statistical power in the study proposed here will likely be greater than that in study sites in severely depressed populations that are more the norm within many of the target ESUs (though assessment of the latter would clearly be required to demonstrate or refute our contention).

Table 1. The statistical power of comparing the fitness of virgin spawners to that of reconditioned spawners. All recapture scenarios assume unambiguous pedigree determination, an adult-to-adult replacement ratio of 1:1 for virgin spawners, and between a 10% and 50% reduction in fitness from adults produced from reconditioned spawners. Statistical significance is set at 0.05 (a).

	Treatment Groups	Run/Release size	Power (ß) at Fitness Deficits for Reconditioned Adult Progeny		
			10%	25%	50%
	Possible Scenarios				
1)	Virgin spawners Reconditioned spawners	1000 50	95.4%	> 99.9%	>99.9%
2)	Virgin spawners Reconditioned spawners	500 50	92.7%	>99.9%	>99.9%
3)	Virgin spawners Reconditioned spawners	1000 25	88.4%	99.0%	>99.9%
4)	Virgin spawners Reconditioned spawners	500 13	77.5%	93.1%	99.8%

Scenarios:

(1) Normal run size; high survival during reconditioning
(2) Low run size; high survival during reconditioning
(3) Normal run size; low survival during reconditioning
(4) Low run size; low survival during reconditioning

Table 2. The statistical power of comparing the fitness of reconditioned spawners (i.e., cultured repeats) to that of naturally occurring repeat spawners. All recapture scenarios assume unambiguous pedigree determination, an adult-to-adult replacement ratio of 1:1 for natural repeats, and between a 10% and 50% reduction in fitness from progeny from reconditioned spawners. Statistical significance is set at 0.05 (a).

	Treatment Groups	Release/Run size	Power (ß) at Fitness Deficits for Reconditioned Adult Progeny		
			10%	25%	50%
	Possible Scenarios				
1)	Reconditioned spawners Natural repeats	50 100	70.4%	98.9%	>99.9%
2)	Reconditioned spawners Natural repeats	50 50	42.6%	94.7%	>99.9%
3)	Reconditioned spawners Natural repeats	25 100	57.6%	93.1%	>99.9%
4)	Reconditioned spawners Natural repeats	13 50	30.8%	69.6%	97.0%

Scenarios:

(1) Normal run size; high survival during reconditioning
(2) Low run size; high survival during reconditioning
(3) Normal run size; low survival during reconditioning
(4) Low run size; low survival during reconditioning

Table 3. The statistical power of comparing the fitness of virgin spawners to that of naturally returning repeat spawners. All recapture scenarios assume unambiguous pedigree determination, an adult-to-adult replacement ratio of 1:1 for virgin spawners, and between a 10% and 50% reduction in fitness from progeny produced by naturally returning repeat spawners. Statistical significance is set at 0.05 (a).

Treatment Groups	Run size	Power (ß) at Fitness Deficits for Natural Repeat Adult Progeny		
		10%	25%	50%
Possible Scenarios				
1) Virgin spawners Natural repeats	1000 100	99.4%	>99.9%	>99.9%
2) Virgin spawners Natural repeats	500 50	92.7%	>99.9%	>99.9%
3) Virgin spawners Natural repeats	250 25	75.4%	97.3%	>99.9%

Scenarios:

(1) Normal run size
(2) Moderate run size
(3) Low run size

(7) *The very brief discussion of work to potentially follow Phase I and II may be the most unique part of the study, namely comparison of the reproductive viability of gametes and progeny from reconditioned spawners relative to naturally returning repeat spawners.*

Preliminary information regarding the reproductive success of natural repeat spawners will be obtained in Phase I and Phase II of this proposal (see response to ISRP question ***3***, ***5*** and ***6***, above). Based on the on-going research and the elements of this proposal, we believe that the Kalama River offers unique opportunities to conduct controlled experiments addressing reconditioning methodology, and the effects of hatchery and natural inputs on reproductive success. We included this list of potential future work to illustrate the value of our proposal beyond our Phase I and II objectives.

Individual Responses to the Hatchery/Harvest Research, Monitoring and Evaluation Subgroup

Response to direct questions:

All respondents are requested to explicitly identify the Principle Investigator(s) and provide non-BPA references if the PI does not have a history of leading complex projects under BPA funding.

Drs. Alec G. Maule and Matthew G. Mesa are the lead PIs on the project and both have long histories of managing complex research programs for BPA. Maule has been the leader of the Physiological Ecology Section at the Columbia River Research Lab since 1991. This section currently has about 15 projects funded by BPA, US Army Corps of Engineers, Bureau of Reclamation, National Science Foundation, NOAA-Fisheries and others. Among these projects are *Assessment of Smolt Condition* (BPA No. 1987-401), which has been in placed since 1987 (BPA COTR: John Piccininni) and *Gas Bubble Disease and Monitoring of Juvenile Salmonids* (BPA No. 1996-021), which has been in place since 1996 (BPA COTR: Bill Maslen).

Dr. Mesa has been the principal investigator for numerous BPA-funded projects during the 1990's, the majority of which fell under large-scale programs such as predator-prey interactions and gas bubble trauma in juvenile salmonids. In recent years, he has served as PI for research studies funded by the USACE, the U. S. Fish and Wildlife Service, and the U. S. Forest Service, among others. Among the topics being studied are energy expenditure in adult salmon, swimming performance and physiology of bull trout, and reproductive development and performance in Pacific lampreys.

Response to technical comments:

The uncertainty regarding the future implementation of Phase II (uncertainty over manageability of risk to the existing research program and extant wild Kalama River steelhead population) is a major weakness of this proposal. A funding investment in Phase I could not be made without complete assurance that Phase II would be feasible from a policy/management perspective.

The HHS review of this proposal expressed concern over a short list of threshold criteria we provided to describe what must occur before the research transitions from Phase I (an evaluation of the effect of kelt reconditioning on reproductive potential) to Phase II (a direct estimation of natural reproductive success of reconditioned kelts). Those threshold criteria were (1) during Phase I there must be adequate survival and recovery of kelts, (2) reconditioned kelts must produce gametes qualitatively equal to or nearly equal to those of virgin spawners, and (3) work proposed here and elsewhere must indicate manageable risk to the existing research program and the extant wild steelhead in the system. We argue that these criteria are absolute limiting factors for all of the kelt reconditioning

proposals under consideration by the ISRP and the HHS and that the fact that our proposal articulated the concerns should not be considered a weakness of the proposed design.

None of the proposed projects can succeed if, in the initial phases of work, the numbers of successfully reconditioned kelts produced (live, healthy spawners with viable gametes) are inadequate. None of the projects should be initiated if the proposed work clearly and substantially conflicts with the well being of extant populations or the plethora of other ongoing recovery efforts in the watersheds. The latter concerns are the most difficult to deal with since the magnitude of genetic risks posed by a kelt reconditioning program is unknown and, indeed, an evaluation of the genetic risks is an integral element of the RFS.

We are completely confident that given time and support we can rigorously and objectively address the concerns. For now, we are reasonably confident that because our Phase II research involves release of a proportionately small number of kelts into a relatively healthy endemic population (as opposed to release of proportionately large numbers into distinctly depressed populations), the risks to the endemic populations and to concurrent research will be well within acceptable limits and the work can and will go forward.

In support, we can (at your request) provide a document from an appropriate WDFW manager(s) expressing assurance from a policy/management perspective that the work as proposed is expected to be useful and that WDFW is committed to the work as constrained from a responsible research/prudent conservation perspective.