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To: **KEW-4**

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WDFW Response to ISRP/HHS Review of

Proposal #4

"Reproductive Success of Natural-Origin, Endemic Hatchery Origin, and Reconditioned Kelt summer Steelhead in the Tucannon River (WDFW)"

ISRP General Recommendations Regarding Kelt Reconditioning and Reproductive Success

We disagree with the ISRP's recommendation that all questions (even the "if possible") be addressed in each study. That seems unfair to expect all sponsors to address these questions when some may not be possible (facility or study constraints), and many of the questions are "speculation" that might not be addressed by the tools in hand.

The RFS specifically stated it wanted proposals to examine the "reproductive success" of the variant groups. Examining long-term fitness of the study population, as indicated by the ISRP, will not be accomplished in 3-5 years, and WDFW would agree with that assessment. Long-term fitness changes might not be realized for 20-50 years. We doubt BPA or the ISRP would recommend a study of that duration. As far as this study (Proposal #4) is concerned, we wanted to determine the **reproductive success** for the variant groups and making sure the viable gametes were produced from reconditioned kelts, not the reproductive fitness of the entire population. We do recognize that genetic data obtained from the study could potentially be used in the future to examine long-term fitness of the natural population. However, this study is not a first time introduction of hatchery origin steelhead in the Tucannon River. Previous hatchery releases (Lyons Ferry Stock), and new endemic broodstock hatchery releases, have and will continue to play a part in the long-term reproductive fitness of the Tucannon River natural steelhead population, and will have to be considered. Further, the actual contribution of reconditioned

kelts in the system during the course of the study will likely be minor compared to other natural and hatchery origin fish spawning in the system, and we feel that kelts will not likely have negative genetic effects on the natural population.

When asking questions about reproductive fitness, or trying to just answer them in regards to kelt reconditioning using only the Tucannon River without an appropriate control would not be scientifically sound and we fully agree with the comments provided by the ISRP. Thus our emphasis on determining gamete quality of the reconditioned kelts for the first year of the study, followed by determining reproductive success from the various spawning groups in the upper Tucannon River in the following years.

Lastly, we applaud the ISRP for recognizing that sponsors had very little time to properly develop proposals. Likewise, the ISRP was limited on their time to review and consolidate comments back to the sponsors. Hence, it was likely a struggle for all the project sponsors and the ISRP to fully dedicate their time and thoughts to all aspects of the proposed work. Therefore, comments such as "It is unfortunate that we do not see a more thoughtful consideration of these points in any of the proposals" are not constructive.

Specific Comments for Proposal #4
Section 1: ISRP Recommendation:

"Priority for the project would be higher if a smolt trap was available for this project, and evidence existed that the process of reconditioning kelts had been successfully undertaken"

Smolt Trap Option: This was not fully developed in the proposal because of the cost of operation for smolt trapping, and other concerns regarding trap efficiencies and sample sizes required for DNA analysis to determine reproductive success. Based on the estimated budget for smolt trapping included in the Basinwide Proposal for Reproductive Success of Spring Chinook (Pearsons and Ford), it would cost ~$120,000 to get started in the first year, and ~$40,000-50,000/year after that just to operate the smolt trap. This ***does not*** include the DNA analysis and interpretation for DNA samples collected (~120,000/year). The project sponsors also have some concerns about trap efficiency for smolts in the upper Tucannon River study area. Trapping efficiencies of maybe 10-20% are expected based on lower Tucannon River smolt trapping with a rotary screw trap. Site location (water turbulence and noise) are believed to greatly affect smolt trap efficiency, and it's unclear at this point whether or where a smolt trap could be operated in the upper Tucannon River with these conditions. Also, water clarity (which is generally excellent in the upper Tucannon River in the study area) will also affect trap efficiencies; with clearer water lowering trap efficiencies.

Based on the potential number of adults spawning in any given year, we determined that ~2,700 Age 1+ presmolts would have to be sampled by electrofishing techniques to provide adequate statistical testing to determine reproductive success. Since we normally only capture 2,000-3,000 natural origin steelhead in the lower Tucannon River smolt trap, it seemed unlikely that enough samples could be obtained from smolt trapping in the study area. This would likely occur since the majority of natural steelhead production occurs below the study area. Because of these potential problems, we felt that smolt trapping was not the best option for the proposal to obtain the genetic samples required to determine reproductive success.

Successfully Reconditioned Kelts: WDFW was fully upfront in the proposal that previous attempts to recondition kelts at Lyons Ferry Hatchery in 2000 failed miserably. Hence, we backed away from the strategy as others in the basin with more time and resources seemed better prepared to evaluate and succeed in the process. Since that time, CRITFC has demonstrated the ability to recondition kelts, although the overall success rate to this point still remains very low (20-30% survival). At this point, WDFW would not consider 20-30% survival to the next year as "successfully undertaken", but we remain optimistic that improvements can be made in their survival. While CRITFC has demonstrated released kelts have made redds, it remains to be determined whether their reconditioned kelts that do survive are producing viable gametes. The ISRP seem to have missed this point in regard to this proposal, as the focus for the kelt reconditioning during the first year of our study will be to examine gamete quality after reconditioning (also includes spawn timing evaluation), and to start looking at reproductive success of the natural and hatchery origin fish above the Tucannon Fish Hatchery adult trap. Reconditioned kelts would added to the area above the Tucannon adult trap after it was demonstrated that viable gametes are obtained.

Section 2: ISRP Review Comments

"The Tucannon situation is directly applicable to a listed ESU and presents an important opportunity for satisfying the requirements of the RFP even given the difficult sampling problem of finding offspring of wild fish with which to estimate reproductive success."

Agree with the ISRP comment that it may be difficult to obtain samples from wild fish to determine reproductive success. However, we are confident that genetic samples can be collected either through electrofishing (if ESA approved by NOAA/NMFS for such extensive sampling), or through smolt trapping if that is the recommended option (however sample sizes may be limited for the reasons stated above).

"The project geneticist is well qualified. However, the questions about genetic consequences in the RFP aren't addressed in the proposal. The proposal does not describe a plan to answer the questions or address the potential long-term genetic risk of kelt reconditioning"

We believe that investigation of the use of kelt reconditioning should occur in a logical sequence. Failure to successfully recondition kelts that produce viable gametes negates the need for a full examination of the potential genetic impacts of such actions on small populations. Moreover, repeat spawners are a natural life history pattern of steelhead. Since we have not documented repeat spawners in the Tucannon River natural population, that does not mean they never existed. It is likely that decreased mainstem survival from dams and other factors have removed them from the natural spawning population in the Tucannon River. By adding a few (if we can succeed in the hatchery at reconditioning) repeat spawners back into the population will likely have little, if any, genetic effect considering that many other wild and hatchery origin fish will be present on the spawning grounds. WDFW anticipates that the endemic hatchery program in the Tucannon River will potentially have more of a negative impact to the natural spawning population than the reconditioned kelts, based on total number of spawners expected alone.

"A smolt trap would be a better approach, although they are already likely getting some of their sampling with ongoing monitoring via electrofishing"

Smolt trapping might be a better approach for a study of this sort, but the limitations in the study area do not make the best option – See above. The project sponsors initially looked at the smolt trap option, but then decided we would likely have a better chance to succeed with juvenile sampling from electrofishing.

"The proposal is not thoroughly written, is a bit disorganized, and seems to place little emphasis on examining possible risks of kelt reconditioning."

See above statement of what potential WDFW thought there would be to the natural population and risks from kelt reconditioning.

"After spawning, fish will be PIT tagged for unique identification and transported to a 20' circular tank at LFH, and the reconditioning process will begin." Shouldn't the fish be marked and identified earlier so that fitness of their offspring rearing the hatchery can be compared to the offspring produced from reconditioned adults? Maybe they are doing this, but it is not clear. Will each individual female be incubated separately at LFH the first year so that results can be compared to results after reconditioning."

The ISRP is correct, this was not very clear in the proposal. Every natural origin female steelhead spawned at Lyons Ferry Hatchery is incubated separately to the eye-up stage for disease control of IHNV. However, after eye-up, eggs are combined into larger groups. Hatchery design does not allow for further rearing by individual female.

The study plan to track fish will be as follows: Immediately following gamete collection from live spawned fish from the endemic program (females will be air-spawned), each fish (male and female) will receive a PIT tag (either in the dorsal sinus area, or in the cheek behind the maxillary bone) to track the individual and its gametes success through the study. The PIT tag number will be written on the bucket of eggs or bags of semen collected. This will allow for tracking how each adult's gametes are used in the matrix crosses. Fertilization success (i.e. egg mortality/survival to eye-up) will be documented for each egg lot. Reconditioned kelts (male and female) that survive to the next spawning year (or if not mature, until year 2) will then be spawned again. Given the reconditioned success rate anticipated and to remove some uncertainty, reconditioned male/female kelts will be spawned with newly trapped wild fish from the Tucannon River. Again, fertilization success will be tracked for gametes from individual fish to the eye-up stage, which can then be compared to the first year spawning results. Any newly trapped fish will be PIT tagged upon spawning as previously described.

"Are tissue samples need from both the juveniles (Task 2b) and smolts (Task 2c- costs are not included because the trap is proposed in another study and may or may not be funded) if the smolt trap is funded. Which best?"

Tissue samples from the juveniles would only be required at one stage (juveniles or smolts). See previous answers provided above for the problems associated with sampling either juveniles or smolts. From a budget standpoint, it will be less expensive to collect samples from the juveniles by electrofishing. However, this does not mean it is the best.

"Task 2d. Collect DNA tissues from all natural origin returning adults at the TFH adult trap from study year fish to conduct pedigree analysis from adult to adult." Shouldn't they also collect DNA tissues from hatchery produced adults that are allowed to spawn naturally."

This is just a misunderstanding of the Task. The intent of this task was to collect tissues from natural origin fish that would have been produced from fish that spawned in the river from the three variant groups (natural origin, hatchery origin, reconditioned kelts) and returned as adults to the TFH adult trap. We would then use the samples collected to look at the genetic profiles of these fish to determine which of the three variant groups they were produced from originally to determine reproductive success. Sampling hatchery origin adults at this time will likely be done, but would not be included as part of the study as they were not part of the study populations.

Just to be clear, hatchery origin adults passed above the TFH adult trap during the study years of 2004-2009 (see Task 2a) will have a DNA sample collected, as they will be one of three variant groups.

"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."

From our interpretation of the RFS, we were supposed to design a study to look at reproductive success to the F1 stage. Following fitness to F2 and beyond was not considered in the proposal. That would require additional undetermined funding for several more years.

As far as a control stream to follow fitness of the population, WDFW would suggest using Asotin Creek. Asotin Creek has been designated by WDFW as a wild steelhead refuge. No hatchery releases occur and reconditioned kelts would not be planted into Asotin Creek in the future. A proposal to further expand our understanding of salmonids in the Asotin Creek basin is currently being negotiated with BPA and will likely be funded very soon. The Asotin Creek project will consist of an adult trap, smolt trap, and extensive electrofishing surveys to determine the status and health of salmonids within the basin. DNA samples will be collected from that project to determine structure and fitness of the Asotin Creek population.

Section 3: RFS Review Criteria:

- *Will the study determine the relative reproductive success of reconditioned steelhead kelts spawning in the wild compared to natural-origin adults, hatchery-origin adults, and cross matings of these three variants, in one or more populations.*

ISRP Response: [Yes, in the Tucannon River population of steelhead, if successful in each of several components – 1) reconditioning kelts, 2) sampling offspring successfully (will a representative sample of natural-origin parr be obtained from eight 'Index Sites' in the Tucannon River? 3) How will additional sites be chosen? The proposal is bit vague. 4) For example on whether or not funds have been included for DNA tissue analysis from the parents of the hatchery origin adult – or, is this a good idea?**]**

1) Previously discussed on Page 3 of this response.

2) Yes, we believe offspring can be sampled successfully from electrofishing surveys. While only a small portion will/can be obtained from the eight index sites, additional sites will supplement the index sites to fulfill sample needs (See next paragraph).

3) Taken directly from Page 8 of the proposal. "Multiple pass electrofishing surveys are conducted annually by WDFW as part of the LSCRP monitoring and evaluations in the Tucannon River. Surveys are conducted to estimate densities and derive population

estimates for Age 0 and Age 1+ summer steelhead in the Tucannon River. Surveys are conducted at 16 index sites from rkm 22 (HWY 12 Bridge) to rkm 79 (Winchester Creek). Currently, eight of the index sites are in the natural production areas above the TFH adult trap (rkm 59). The eight index sites are located approximately 2-3 miles apart, so additional sample sites will be added to collected individuals from many locations/habitats." … "Collecting Age 1+ fish approximately every 400 meters between the TFH adult trap and Winchester Creek, with approximately 30 Age 1+ fish collected at each location, will bring the maximum samples size to ~2,700 samples".

While not stated precisely in the proposal, the total number sites where samples would be collected is 80 (8 index and 72 additional sites). Such random sampling will ensure collection of fish from multiple habitat types throughout the upper Tucannon River, and should likely have representatives from the three variant spawning groups.

4) Previously discussed on Page 3 of this response. Again, it comes back to a misunderstanding of Task 2d. We have included funds for DNA tissue analysis from the hatchery origin adults passed above the TFH adult trap for years 2004-2009 (Task 2a). Beyond that time removes them from the scope of the study. Wild origin returns beyond that time would have DNA samples collected to determine reproductive success to the adult stage from the study groups. Collection of DNA samples from wild origin adults beyond the scope of the study years could also be included if it was desired to examine success to the F2 and F3 generations.

- *Does the proposal employ the use of microsatellite DNA analysis in order to ascertain the pedigree of resulting progeny and subsequent returning adult steelhead. Other methods may be acceptable if they can provide quantification of reproductive success of equal or better power than microsatellite DNA analysis.*

ISRP Response: [Yes**]**

- *The study should include analysis of the potential genetic consequences of repeat-spawning steelhead on small populations.*

ISRP Response: [Not included in this proposal**]**

Correct. WDFW did specifically include a detailed plan to study the genetic consequences of repeat spawning steelhead in small populations. See previous discussions provided in this response as to why this was done.

As a side note, we would like to provide the following information to relate to the discussion regarding risks to small populations. In the Tucannon River study area, we anticipate that maybe from 50-200 wild adults, and 50-200 hatchery adults will be returning to the project area each year. The anticipated number of kelts at full endemic broodstock program (80 adults and using the current success rate of say 25%) would equate to only 20 reconditioned kelts returned to the river. If 100 total fish (wild and endemic brood hatchery origin fish) are in the study are, then 20% would be repeat spawners. If 400 total fish returned, then 5% of the spawning population would be repeat spawners. Given that the re-conditioned kelts would not likely be directly related to other spawning adults, how much harm is likely if they contribute at the 5% level.

In the Touchet River system (Mid-Columbia ESU – Walla Walla Basin) WDFW has documented a 5% repeat spawner rate in the natural population. This population is considered depressed due to the habitat loss within the basin. The four main-stem Columbia River dams also play a factor in overall survival, and repeat spawner rates were likely higher in the past before the dams. The Touchet River has an average run size from 200-400 fish annually. Would this be considered a small population? If so, there seems probable evidence that natural repeat spawners (which equate to reconditioned kelts) have contributed at proposed or greater levels in the past. We agree that the topic of genetic effects could and maybe should be evaluated, but there was insufficient time and need to include it in this proposal.

- *Other research topics, which should be addressed in the proposed study **if possible**, include: how increasing iteroparity might increase inbreeding in the target population, particularly if it is small; how reconditioning kelts might increase domestication selection in the target population; and how the reconditioning program might alter age structure and life history structure in the target population.*

ISRP Response: [Not in the proposal or Not addressed well in the proposal**]**

In the RFS, it stated these other research topics should be addressed, ***if possible***. By keeping in the phrase "if possible", left it open to the project sponsors to address these other research topics if they wanted to. We **did not** interpret this statement as a mandated part of the study design.

Taken directly from the project proposal #4 in the Project Summary on Page 1 "As part of the study, project leaders will provide data analysis (where possible) to discuss other questions posed in the RFCS about 1) increasing inbreeding by use of reconditioned kelts, 2) how this might increase domestication selection, and 3) how the reconditioning program might alter age and life history structure of the target population." At the time of the proposal writing, it seemed that all of these questions would be valuable to look at in the future when there was credible genetic data that could potentially answer some of them. Other than giving short speculations on what "might" happen regarding these was not possible given the short time to develop the proposals. Further, the project sponsors did not feel short, incomplete speculations of this nature would be of value to the proposal, and would likely open the door for greater criticism.

- *Research site(s) must offer the ability to capture and sample sufficient outmigrating offspring and all, or nearly all, returning adult steelhead.*

ISRP Response: [Yes for adults, but not for outmigrating smolts unless the smolt trap is funded in a chinook study. Electrofishing for juveniles might not give adequate sample size and might not be completed (ESA listed, NMFS Approval). Smolt monitoring might be less than ideal unless a new trap is installed**]**

Smolt trapping and juvenile sample previously discussed in this response.

- *Proposed studies should be directly applicable to one or more of the following ESUs: Upper Columbia, Mid-Columbia, and Snake River steelhead.*

ISRP Response: [Yes**]**

- *Cost-effectiveness (e.g. the ability to take advantage of existing fish production, research, monitoring or evaluation activities will be an important consideration in the proposal selection process.*

ISRP Response: [Potentially highly efficient by adding marginal effort to other projects. Budget seems sketchy but suggest large effort devoted here. ~$140K (only adult sampling and without juvenile sampling) and ~$260K (with

juvenile sampling) seems reasonable. Would the $140K include funds for sampling smolts if the smolt trap is approved?**]**

Budget seems sketchy? We are unsure how to respond to this comment. The $140,000 with adult sampling only is a maximum cost and will vary depending on the DNA analysis required from samples collected off returning adults. The $140,000 **does not** include funds for sampling/analyzing smolts if the spring chinook project was approved. Smolt trapping would require some personnel time to assist with sampling and tracking of samples, DNA analysis of samples ($86,000 for DNA analysis, 2,700 samples @ $32/sample), and genetic interpretation of results. As indicated earlier, we anticipate this could cost as much as an additional $170,000/year if the decision was to use smolt samples instead of juvenile sampling for DNA analysis.