Independent Scientific Review Panel
for the Northwest Power & Conservation Council
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Memorandum (ISRP 2014-2) **February 10, 2014**

To: Bill Bradbury, Council Chair

From: Greg Ruggerone, ISRP Chair

Subject: Review of Progress Report for the Yakama Nation's Accord Proposal, Upper Columbia Nutrient Supplementation (#2008-471-00)

Background

In response to the Council's December 13, 2013 request, the ISRP reviewed the Interim Project Progress Report (November 2013) for the Yakama Nations' Upper Columbia Nutrient Supplementation Project (#2008-471-00). The progress report is intended to address a condition placed on this project as part of the Council decision made on May 12, 2010 that concurred with the ISRP recommendation on the project. Specifically, the ISRP found that the project had the potential to be a useful nutrient experiment (ISRP 2010-8). However, the ISRP recommended that a complete study plan was needed that included the following critical project components:

- identification of the form in which nutrients will be added
- power analyses of the detection of a response in fish production
- stable isotope work details
- securing of permits for sampling fish

The Council agreed with the ISRP and stated that these issues could be addressed over two to three years as the Yakama Nation gathered pre-treatment data. The Council noted that the information could then be included in an updated study plan by 2013 and submitted to the ISRP for review. Based on this understanding, the Council recommended to Bonneville to implement the pre-treatment activities and that implementation of the nutrient enrichment study depends on favorable scientific review of an updated study plan.

The ISRP notes that the study has changed considerably from the previously reviewed proposal. The revised proposal includes a set of pilot experiments on Hancock Springs to be followed by a nutrient treatment on a section of the Twisp River. The initial proposal was to add nutrients to a much longer reach of the Twisp River. As a result of the fundamental restructuring of the study, the ISRP feels it is appropriate to review this submission as a new project. Although we do provide comments relative to the four issues identified during the previous review, many of our comments concern the revised experimental design.

Recommendation

Does Not Meet Scientific Review Criteria

The ISRP recommends that any future proposal carefully consider the comments below, especially those related to experimental design and statistical analysis. Should a future proposal be developed, it should fully address the issues raised in this ISRP review and be presented as a stand-alone study proposal rather than combined with a progress report.

Summary

Nutrient enhancement has become an increasingly popular technique in the Columbia Basin over the last decade. However, the effectiveness of this technique is poorly understood. The authors propose to implement two, large-scale, unreplicated field experiments in an attempt to evaluate food web response to nutrient augmentation with carcass analogs. In addition, one of the study sites (Hancock Springs) also will evaluate food web response to habitat restoration and removal of brook trout. Unfortunately, in the ISRP's judgment, the design of both the Hancock Springs and Twisp River components of this study are seriously flawed. The design issues appear to be fundamental problems that cannot be corrected by using different sampling or data analysis methods. Consequently, the ISRP does not believe the proposed studies, as they are currently structured, will provide a rigorous test of the potential benefits from habitat restoration, nutrient addition, and invasive species removal.

The ISRP did find the emphasis placed on food web response to the application of various restoration treatments a very attractive element of the study approach. We appreciate how difficult it is to compile the data required to generate the detailed energy flow analyses summarized in figures 21 and 22. In general, this aspect of system response to restoration treatments has not received the degree of attention that it should. Trophic response to all types of restoration designs, not just nutrient addition, may make an important contribution to the treatment effectiveness. A better understanding of this aspect of stream restoration will be very important in refining restoration strategies in the future. The project sponsors are to be commended for recognizing the significance of this issue.

The ISRP visited Hancock Springs during a field tour in 2013. The ISRP did feel this study site offered opportunities for a detailed study of system response to various experimental manipulations due to the manageable size of the study site and the wealth of data that were being collected on trophic system dynamics. However, the ISRP was not fully aware of the experimental design issues, detailed below, at the time of the tour. Review of the proposal made these problems evident.

Experimental Design

A key challenge with the design and statistical analysis is that both the Hancock Springs and Twisp River components of the study are unreplicated experiments. Large-scale, unreplicated experiments are sometimes a reasonable option, especially if important community or ecosystem-scale processes can be measured only at large scales (see papers by Steve Carpenter on whole lake studies; citations below). If this approach is used, however, experimental designs and statistical analyses must be adapted to this constraint. The ANOVA designs being used at Hancock Springs are likely not valid. With only one treatment and one control section, the repeated measures in different seasons are not independent and cannot be used in this design as replicates. An even greater problem is using pseudo replicates (sites within reaches) and assuming these are true replicates; this is not valid. True replicates would be either additional treatment and control sites on Hancock Springs that are far enough away to be considered independent (not possible given the short length of this stream), or additional sites on other streams that are similar (i.e., spring streams).

There are some methods appropriate for the analysis of unreplicated experiments with one treatment and one control section. Randomized Intervention Analysis, which was used in the whole lake studies by Carpenter et al. (1989), is one possibility (*Carpenter, S. R., T. M. Frost, D. Heisey and T. K. Kratz. 1989. Randomized intervention analysis and the interpretation of whole-ecosystem experiments. Ecology, 70(4): 1142-1152.*). Another useful paper may be (*Carpenter et al. 1998. Evaluating alternative explanations in ecosystem experiments. Ecosystems 1: 335-344*.). However, given the fact that one of the planned treatments at Hancock Springs has already been applied, it may not be possible to meet the requirements of these analytical options.

Hancock Springs, in particular, lacks adequate experimental controls. The habitat reconstruction treatments were done without any pre-treatment monitoring. It is unclear whether or not the two study reaches were comparable before treatment. Without some understanding of pre-treatment conditions, it is impossible to attribute the observed differences in trophic organization and fish productivity between reaches 1 and 2 to the treatment. Another confounding factor is that the channel reconstruction treatment at Hancock Springs was done on the upstream study reach. As a result, the sediment released during construction of the new channel in reach 1 potentially moved downstream to affect the control reach. The comparison of the two reaches post-treatment will not provide an accurate estimate of the response to treatment of reach 1 because the habitat of the control reach may have been degraded during application of the treatment.

The planned nutrient and brook trout removal treatments on Hancock Springs will occur without a spatial control; both treatments will be applied to both study reaches, one of which already has had channel reconstruction. Pre- and post-treatment monitoring are proposed, but there would be no way to rule out possible confounding time trends in biological responses that might be unrelated to the treatments.

In retrospect, a better design for Hancock Springs would have been to implement channel reconstruction on the downstream reach, to avoid the confounding effects of sediment, and then to add nutrients and remove brook trout only from the downstream reach. This approach would provide a true control upstream for the full suite of treatments. Unfortunately, this option is now not possible. The only other option would appear to be replicating the restoration of physical habitat in reach 2 immediately, foregoing or deferring nutrient addition and brook trout removal. If other spring streams in the Methow River watershed could be found that could serve as control sites, it would be possible to assess response to nutrient and brook treatments at Hancock Springs, once the response to physical habitat manipulation is fully understood. Replicating the physical habitat treatment in reach 2 could also serve to distinguish a change in abundance due to redistribution from one reach to another versus an increase in overall abundance through improved survival, and in successive years, through reproduction. Clearly, the differences in fish population metrics between reaches 1 and 2 observed to date cannot be attributed to increased survival or reproduction since 2011, but such increases might be observed when surviving progeny mature after 2015.

The second component of this proposal is for a nutrient enhancement study on two sections of the Twisp River. Given that channels like the Twisp River study sites may be more available for treatment in the Columbia Basin than small spring creeks, like Hancock Springs, this aspect of the study would provide information on the effectiveness of nutrient addition more broadly applicable to other systems. However, the Twisp River experimental design suffers from the same basic issue described above for Hancock Springs; it represents an unreplicated experiment. The control reach on the Twisp is above the treatment reach, avoiding the problem with the placement of the study reaches on Hancock Springs. However, another concern with the Twisp River design is the high annual variability in water chemistry and macroinvertebrates revealed by the pre-treatment monitoring. This variability raises doubts about the adequacy of the pre-treatment monitoring and will limit the ability to detect a response to the treatment. In addition, the introductory letter submitted for review along with the report and proposal states that estimates of fish production and abundance in the Twisp River study sites could not be made because of low capture efficiencies and depletion rates during electrofishing (although levels of depletion that would be considered adequate are not mentioned) . The study proposal provides no suggestions that might help resolve this challenge. Given that fish response is the parameter of most interest, the inability to measure the response of salmon, steelhead and bull trout represents a serious limitation.

The Hancock Springs site is ecologically interesting and its small size is conducive to the application of treatments. However, spring streams may not be widespread in the region, so opportunities for restoration of these systems might be very limited. An extensive analysis of the Upper Columbia Province to identify the number of spring creek sites and their potential for restoration would be most useful. It may be that Hancock Springs is somewhat unusual or the habitat type occurs only on private land (being in the valley bottom), making it very difficult to implement enough restoration at these sites to significantly contribute to restoration of salmonid populations. Nonetheless, a more thorough understanding of the distribution of spring streams in the province would provide a better understanding of the potential for restoration of sites like Hancock Springs to contribute to recovery of listed fishes.

Due to the distinctive characteristics of Hancock Springs, the expectation that knowledge gained from this system will help inform the study on the Twisp River may be overly optimistic. These systems have very different characteristics. In particular, the larger size and higher transport capacity of the Twisp River suggests that the retention of the analogs and the nutrients they generate may be much less efficient than at Hancock Springs. Therefore, responses may be muted or shorter-term relative to those at Hancock Springs. Coupled with the high inter-annual variability in water chemistry and macroinvertebrates seen in the pre-treatment data from the Twisp River, detection of a response may be very difficult. The study design could partially account for this possibility by examining downstream transport of the analogs and nutrients and more intensive sampling during the period of time immediately after the analogs are placed (see comment about possible pathways of analog incorporation below).

Numerous ongoing projects in the Upper Columbia Province are assessing system response to the application of various restoration methods. This study would benefit from better integration with these projects. Such integration is the responsibility of both the project sponsor and the entities involved with recovery efforts in the region: the Upper Columbia Salmon Recovery Board, NOAA Fisheries, and the Council. The proposal also does not mention some of the large stream fertilization studies outside the Upper Columbia area. There is a wealth of information being generated by these efforts that would help inform the design of a similar experiment on the Twisp River. It would be worthwhile to incorporate stream fertilization literature both from outside the Columbia Basin (e.g., Arctic streams, Keogh River study in British Columbia [*McCubbing, D.J.F., and B.R. Ward. 1997. The Keogh and Waukwaas rivers paired watershed study for B.C.'s Watershed Restoration Program: juvenile salmonid enumeration and growth 1997. Province of British Columbia, Ministry of Environment, Lands and Parks, and Ministry of Forests. Watershed Restoration Project Report No. 6: 33p.*]) as well as within the basin (e.g., Kootenai River).

Response to Issues Identified in the Previous ISRP Review

1. The form of nutrient additions

The explanation of nutrient treatments and subsequent analysis of response is insufficient for ISRP assessment. The explanation of nutrient enrichment on page 34 does not identify the level of enrichment the experiment wishes to achieve. Are the sponsors planning to simply add a pre-determined number of carcass analogues or are they seeking to raise nutrient levels to a pre-determined concentration throughout the treatment reach? Without a more complete understanding of nutrient cycling and transport, at the study sites, it is not clear how a desired nutrient concentration could be specified and the appropriate level of analog addition to achieve this level determined. In order to identify proper application rates, a detailed nutrient budget for the study sites should be developed. There are numerous sources of essential nutrients, and these have often been disrupted by human activities (e.g., riparian clearing, browsing). The proposal would be stronger if the sponsors had a reliable estimate of the average natural flux of N or P from various sources, including marine-derived nutrients. There was no indication in the proposal that a nutrient budget was being assembled.

In addition, the proposal only addresses the addition of N and P. Salmon carcass analogs contain not only N and P but also C (an energy source) and other important micronutrients (K, Mg, Ca, and others). Total amount of all added nutrients and elemental ratios can affect biotic responses. The analogs also may contain small amounts of toxins. The proposal does not indicate whether or not the carcass analogs will be analyzed for total nutrients, nutrient ratios, and toxins before being placed in the stream, but these are critical measurements.

An important consideration for this study is that the analogs are not simply nutrients; they are pellets of processed fish tissue. Carcass analogs have been used previously in the Columbia Basin and elsewhere and these studies have established that fish consume the analogs directly. In fact, direct consumption of carcass analogs is a key factor in the effect this material has on fish growth (*Guyette, M. Q., C. S. Loftin, and J. Zydlewski. 2013. Carcass analog addition enhances juvenile Atlantic salmon (Salmo salar) growth and condition. Can. J. Fish. Aquat. Sci. 70: 860–870; Kohler, A., T. Pearsons, J. Zendt, M. Mesa, C. Johnson and P. Connolly. 2012. Nutrient enrichment with Salmon Carcass Analogs in the Columbia River Basin, USA: A Stream Food Web Analysis. Trans. Amer. Fish. Soc. 141:802–824*). There has been relatively little research evaluating the effects of analogs on inorganic nutrient levels at sites where they have been added. A more complete understanding of the relative contribution to fish growth and productivity, of direct consumption of analogs versus bottom-up stimulation of the food web, as a result of nutrients derived from decomposition of the analogs, would help in the development of effective nutrient enhancement protocols. In order to capture the relative significance of these two incorporation pathways, studies should employ sampling protocols that ensure responses from direct consumption and bottom-up stimulation can be differentiated. The measurement of nutrient concentrations and primary and secondary productivity, as proposed for this study, will be critical to assessing any bottom-up system response. However, the sampling procedures outlined in this proposal may not be adequate to capture responses due to direct consumption of analogs by the fish. Sampling fish stomach contents and growth rates should be concentrated in the period immediately following analog placement to ensure this uptake pathway is captured.

2. Power analyses of the detection of a fish production response

The proposal indicates that power analyses will be conducted for most of the metrics being measured at Hancock Springs and the Twisp River. Presumably, a power analysis was already completed for Hancock Springs, given how far that project has already advanced. However, results of this analysis were not provided. In fact, no data on variability at Hancock Springs are provided for response variables reported in Table 9 or Figures 5 through 11. The pre-treatment data from the Twisp River study site are still being collected. However, there are sufficient data from the Twisp River site to conduct a preliminary assessment of the level of response that will be detectable for most of the study parameters. No indication that this evaluation had been conducted was included in the proposal. No statistical analyses of any of the Twisp River data were presented in the report. For a number of the Twisp River parameters, statements were made about longitudinal gradients or treatment-control reach differences but no statistical verification of these statements was provided. In fact, no indication of variation or uncertainty was provided for many of the Twisp River response variables (Figures 29, 31-36).

3. Stable isotope work

More detail on the manner in which stable isotope data will be used needs to be incorporated into the proposal. There is a brief description of how samples for stable isotopes will be collected and analyzed. The stable isotope data will be used primarily to quantify the amount of N and/or C, from the analogs, that is incorporated in different components of the food web at the treated study sites. The isotope data will augment the information on primary, secondary, and fish production. The $\delta^{15}N$ and $\delta^{13}C$ values of the analogs must be distinct from that of N and C delivered from other sources in order to determine the rate of analog incorporation.

However, there is no information in the proposal indicating that natural isotope levels in the analogs, and from other sources, are different enough to be detectable. If there is insufficient distinction in isotopic ratios, a possible option is to enrich the analogs with $\delta^{15}N$ and/or $\delta^{13}C$. The project sponsors also might consider examining S stable isotope ratios. Stable isotope data can be difficult to interpret, but frequently a clearer understanding of trophic system organization can be obtained by examining the isotopic ratios of multiple elements.

4. Fish sampling permits

The required permits have been obtained.

5. Other comments on the study plan

Additional clarifications and corrections that should be incorporated into this document:

1. In several places, comparisons are described as additive, such as in Table 5 – effect of brook trout with and without nutrient addition. Since there is no control without nutrients, this comparison cannot be made (also, see p. 34, third paragraph for a similar incorrect statement about individual and combined effects, and p. 43 under carrying capacity). The design is not suitable for separating individual (main) effects and interactions (combined effects). A statistician should be consulted to help the authors design a suitable study.

2. Fish will be sampled by electrofishing 6 times annually. This level of sampling raises concerns about the possible effect of the sampling itself on the fish population response (and the invertebrates and microbes too – the base of the food supply) (See *Kruzic, L.M., Scarnecchia, D.L., and Roper, B.B. 2001. Comparison of midsummer survival and growth of age-0 hatchery coho salmon held in pools and riffles. Trans. Am. Fish. Soc. 130:147-154.).* The sponsors should take a careful look at the sampling frequency to be absolutely sure that the effects are minimal.

3. The data collected so far appear to show that: 1) the habitat reconstruction apparently had a strong effect on spawning anadromous salmonids, but 2) despite potential improvements in habitat for anadromous species, most invertebrate biomass is being consumed by brook trout. Given the dominance of brook trout, it would seem that they are the species most likely to respond to the application of nutrients. It is not clear that developing a detailed understanding of the response of a non-native species to the application of nutrients will provide information that will improve understanding of the effectiveness of nutrient addition for the target species: Chinook, steelhead, and bull trout.

4. Invertebrate drift density is being measured at mid-day. Drift rates typically peak at dusk. As a result, the drift sampling may not adequately represent the availability of invertebrates at the study sites. Consider modifying the sampling protocol for drift to address this issue.

5. It is not clear what would happen should the project leader not be involved with the project for its duration. This is a long-term project that, in effect, is largely led by a single

person. Should the project leader leave, would the program stay the same or be as productive? What are the contingency plans?

6. Some additional consideration of the challenges presented by working in larger systems should be incorporated into the proposal.

7. Some misunderstanding of N/P ratios was apparent. On page 93 is the statement "TN:TP ratio values were intermediate to TN and TP values and ranged from 7.8 to 35.1µ ug/l…." N/P ratios are unitless values expressing the relative availability of these two elements; they are not expressed in µg/l and cannot be intermediate to the TN and TP values. N/P ratios do provide a preliminary indication of the nutrient limiting primary production. But bioassays to establish limiting nutrients are a much more reliable method of identifying the limiting nutrient. It might be valuable to identify an advisor with expertise in this area to help guide this part of the project.

8. A project like this generates massive amounts of data. How are the data managed? How do the sponsors address QA/QC issues? The proposal needs a section that explicitly addresses data management in addition to the proposed statistical analyses.

9. Some references listed in the text were missing, including key ones like Sanderson et al. (2008), Hershey et al. (2007) and Lang et al. (2006).

10. More detail should have been provided on the channel reconstruction project on Hancock Springs. There was no discussion in the document of what caused the channel to be degraded, so it is unclear what the goal was in channel reconstruction. A key question with channel engineering is whether processes that were lost have been restored, or whether only structure was restored. Without restoring key processes, such as hyporheic flow or large woody debris recruitment for example, improving the appearance of the channel may have little long-term effect.

11. There is no discussion of other habitat restoration or supplementation projects occurring on the Twisp River that might either share data or impact the execution and analysis of the work proposed. Although the proposal states the Twisp River is not jeopardized by altered physical habitat, the ISRP believes that both wood structures and some flood plain habitat manipulations have been done on this system. The ISRP has also reviewed a Washington Department of Fish and Wildlife study on steelhead relative reproductive success in the Twisp River. Some level of supplementation and fish sampling is taking place within this watershed. The study plan would be improved by inclusion of information on other work occurring at or near the study sites.

12. In Table 1, for fry, suffocation is listed as a problem under physical habitat, which seems unlikely, and reduced food availability is reported as not limiting, which is also not likely.

13. Page 16. The NOAA 2012 link does not lead to a document.

14. Page 35: What are the hypotheses to be tested? These should be listed in a Table along with quantifiable ecological objectives (including timelines for meeting those ecological

objectives). For example, how many more Chinook and steelhead are expected to be produced from the experimental manipulations, and when?

15. Page 37. The methods for estimating fish abundance appear to be outdated, based on regression techniques (Seber and Zippin), rather than maximum likelihood methods incorporated in the MARK software (see the following):

    a. *White, G. C. 2008. Closed population estimation models and their extensions in Program MARK. Environmental and Ecological Statistics 15:89-99.*

    b. *White, G. C., D. R. Anderson, K. P. Burnham, and D. L. Otis. 1982. Capture-recapture and removal methods for sampling closed populations. Report LA-8787-NERP. Los Almos National Laboratory, Los Alamos, New Mexico, USA.*

    c. *White, G. C., and K. P. Burnham. 1999. Program MARK: survival estimation from populations of marked animals. Bird Study 46:S120-S139.*

16. Page 37. Growth is described as an instantaneous rate, which is the value required for production estimates. But the equation given is for relative growth rate. In addition, the equation for instantaneous growth rate (*G*) on the next page is not correct.

17. Pages 38 – 43. The description of calculations and the symbol choices for estimating production and energy flow are confusing. P, rather than PPi, is defined on page 42. How are estimates of periphyton (measured per unit area) combined with estimates of macroinvertebrates (measured per unit volume) and estimates of fish to arrive at an estimate of total production (expressed per unit area)? The supporting reference for Hayes et al. (2007) is missing.

18. Page 48. It is unclear how nearly opposite pool:riffle ratios in the two reaches can nevertheless have similar percentages of pools. There is no indication here about whether the data are calculated using areas or volumes.

19. Page 56. Fish abundance in Hancock Springs is presented as fish/m^2. It would be useful to provide actual numbers of fish partitioned by size or age class.

20. Page 62. It is not clear how annual growth rate for steelhead could be significantly higher in reach 2 than reach 1 when, earlier in the paragraph, it was established that there were no significant differences in daily growth rates between reach 1 and 2 for any species.

21. Page 62. How does mean annual growth rate for steelhead of 53 and 73.3 g/year compare with other waters in the Methow River and Upper Columbia region? Is this growth for young-of-the-year?

22. Page 63. Figure 19 – Fish growth, expressed as increase in length, has a different scale on the figure and legend; one is cm/week, the other mm/week. This is a huge difference in growth. Also, the text gives weight gain expressed as cm/year, an obvious error.

23. The life-histories of the steelhead and Chinook are not explained. When do they spawn and how long do they rear in Hancock Springs. If the Chinook are subyearling type (summer/fall), the data presentation should concentrate on growth/survival/condition of the fish while they are resident. If the Chinook are yearling migrants (spring Chinook), summer and winter growth and survival might be considered. For steelhead, the age and residency before smolting, and information on resident rainbow trout, should be considered. How results for brook trout, which are presumably year round residents, would be different from species that are seasonal residents needs to be discussed.

24. Page 69. Sentence 2 and 3 in the invertebrate production, consumption, and fish production paragraph needs revision. Sentence 2 states that 12.9g $DM/m^2/y$ of insects were consumed in reach 2. Sentence 3 states that aquatic and terrestrial insect consumption of 2.5g $DM/m^2/y$ in reach 2. Both cannot be correct.

25. Summary information on nitrogen and phosphorus concentration, and their ratio, is presented for Hancock Springs. This descriptive information is not interpreted or used to establish that nutrients are limiting production in Hancock Springs.

26. The thermal regime in Hancock Springs is very different from the Twisp River, and presumably different from the Methow River; warmer in winter and cooler in summer – but generally cool overall. How this would influence seasonal growth, and compatibility of spawning in Hancock Springs, with final rearing and smolt production in the Methow River system, should be considered. Egg incubation and alevin growth would be faster in Hancock Springs, with subsequent YOY growth slower.

27. Page 91, paragraph 2, sentence 2: "These differences in food consumption and energy flow between reaches likely contributed to the observed increases in fish abundance, biomass, and production in Reach 1 compared to Reach 2." The logic of this assertion is questionable. From the site visit and this report, it seems more supportable that physical changes in the habitat after restoration in 2011 resulted in more suitable physical habitat conditions for fish with modest increases in primary and secondary production. The larger energy transfer from primary and secondary production to fish is primarily a product of improved physical habitat suitability for fish.