



Independent Scientific Review Panel
for the Northwest Power & Conservation Council
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Memorandum (ISRP 2009-3)

February 19, 2009

To: W. Bill Booth, Council Chair
From: Eric Loudenslager, ISRP Chair
Subject: Review of CRITFC Fish Accord Proposal: Influence of Environment and Landscape on Salmonid Genetics, 2009-005-00

Background

This memo is the ISRP's review of the Columbia River Inter-Tribal Fish Commission's proposal, Influence of Environment and Landscape on Salmonid Genetics, 2009-005-00. This project is called for in the Columbia River Fish Accords. On December 15, 2008, the ISRP completed its initial review of the original proposal and sent a memo to the project sponsor requesting additional information that would allow us to complete our scientific review. The ISRP emphasizes that asking for a response to clarify issues occurs with a majority of proposals and is not indicative of the failure of a proposal. It is a constructive process that adds significant value to the review process, improving the proposal's justification and the ISRP's understanding. Our recommendation and review summary are provided below, followed by the complete project review discussion.

200900500 - Influence of Environment and Landscape on Salmonid Genetics

Proposer	Columbia River Inter-Tribal Fish Commission
Short Description	Influence of Environment and Landscape on Salmonid Genetics
Province(s)	Basinwide

<http://pisc.es.bpa.gov/release/documents/DocumentViewer.aspx?doc=P108874&session=500441b3-1761-42d2-9431-9802c91e5095>

ISRP Recommendation

Does Not Meet Scientific Review Criteria

The proposal and ensuing response do not provide a sufficient level detail for the ISRP to conclude that the project will meet the five ISRP review standards: 1. based on sound science principles; 2. benefit fish and wildlife; 3. have clearly defined objective and outcome; 4. provisions for monitoring and evaluation; and 5. consistent with the Council's fish and wildlife program.

If this project proceeds, a detailed study design should be prepared and reviewed by the ISRP before any tasks or work elements associated with the landscape genetic or the expression of traits objective are initiated. This will establish the scientific basis of both objectives; provide clearly defined objectives and outcomes; and consistency with the Council's fish and wildlife program. The ISRP understands that development of the study design will take time and, likely, resources.

For the landscape genetic objective the study design needs to include:

- the specific hypotheses for the focal populations the analysis is intended to address
- the field locations where genotypic data will be taken from
- the sources and type of genotypic data for each site
- the type and location of environmental data
- the specific correlative analyses that will be performed on the data with an explanation of how the analysis of genetic data with environmental data from those sites resolves the questions posed in the hypothesis

For the expression of traits objective the study design needs to explain the specific methods that will be employed:

- the breeding design that is going to be used to identify QTL
- how alleles at QTL will be identified
- how the frequencies of these alleles will be estimated in natural populations
- how populations that are going to be screened for QTL allele frequencies and expression of HSP and Na/K ATPase will be selected
- how many populations will be screened

ISRP Review Summary

The ISRP appreciates the Sponsor's effort to respond to its review comments; however, for the most part the response misses the important critique elements in our December 15, 2008 Review.

To be sure, the emerging field of "landscape genetic" analysis has the potential to contribute important information to salmon management and recovery actions for Chinook, steelhead, or other salmon. Moreover, a broader understanding of the genetic underpinnings and expression of traits critical to viability and recovery has the potential to shape our understanding of resident/anadromous life-history variation and thermal tolerance in steelhead/rainbow trout, as well as to define the boundaries of "local" adaptation.

Unfortunately, the sponsor's responses do not provide a sufficient level of additional detail or support. As a result, the ISRP cannot conclude that the proposed research efforts and analyses (either independently or together) will transform management in a way advertised by the sponsor. The benefits for fish and wildlife (specifically, Chinook salmon and steelhead/rainbow trout) are vague and provisional. The sponsor would make a more compelling case if the proposal presented a short list of real management situations where expected results would be used in a specific rather than a very general way.

For example, the proposal implies the kinds of management activities or decisions that would benefit from the project's (both objectives) results. These include more closely matching source-populations for hatchery broodstock and subsequent release sites; an improvement in the fine-scale application of artificial production for recovery of listed species; and restoration of extirpated species that are not currently listed (e.g., coho salmon above Bonneville and spring Chinook in various locations). Managers already have considerable fine-scale phylogeographic information on salmon species throughout the Columbia River Basin, and yet there is not a universal acceptance that this is an important concern for management. For example, hatchery stocks of Snake River spring Chinook are currently segregated on a stream-specific basis. Is even finer-scale production anticipated? Would the Basin have the water to increase production facilities? If the current level of artificial production for restoration and conservation (in contrast to mitigation) is not successful, will the region wish to expand that strategy? Thus, even if delivered, it remains to be seen whether a finer level of discrimination would be valued and injected into management decisions.

The landscape genetics objective needs to be linked to management actions and decisions, and the genetic and environmental and spatial databases demonstrated to be sufficient to provide robust analysis to support the management. The genetic and environmental sampling needs to be compatible with the analytical methods. As an example, the sponsor states that the proposal will contribute to fulfilling the 2008 Federal Columbia River Power System (FCRPS) Biological Opinion Reasonable and Prudent Alternative 41: Preserve and rebuild genetic resources through safety-net and conservation actions to reduce short-term extinction risk and promote recover (hatchery strategy 2); implement safety net programs to preserve genetic resources and reduce short-term extinction risk. The RPA calls for specific elements to be completed in 2009, 2013, and 2016. It would be helpful if the proposal explained how and why these data and analyses are going to be used to undertake these three elements.

For the expression of traits objective, a more complete description of the investigation is needed. Particularly, the justification of the hatchery stocks used in laboratory testing of expression of heat shock and Na/Ka ATPase enzymes, the method of identifying alleles at QTL, the methods of estimating frequencies of QTL, and the choice of natural populations for evaluation of QTL frequencies, and finally the application of interpretation of the experiments.

Finally, the relationship between the studies is still not completely clear. The expression of traits study will focus on steelhead and the landscape genetics work will examine variation in Chinook. The difference in species would suggest that the two parts of the project are very different studies. If the authors believe that the results from one part of this study will enhance the interpretation of results from the other, this connection should be described more explicitly (Figure 1 is at best broadly conceptual and is not specific to the two elements of this study).

Complete Review Discussion

The complete review discussion follows below and is organized by ISRP December 15, 2008 comments, CRITFC response, and final ISRP February 19, 2009 comments with each iterative comment labeled and sequentially indented.

ISRP December 15 Recommendation: Response Requested - Does Not Meet Scientific Review Criteria. The proposal is insufficient for technical review. The landscape genetics and QTL investigations have the potential to be useful contributions to understanding how and why the diverse life-history variation in Chinook salmon and steelhead/resident rainbow trout is distributed in the Columbia River Basin. This information could lead to improved planning in the face of climate change and human population growth. This, however, is an omnibus proposal, with two distinct parts. It is not clear how they fit together. The proposal should be rewritten to address the criticisms identified below in the ISRP Comments.

Specific Comments and Responses:

1. Technical Justification, Program Significance and Consistency, and Project Relationships (sections B-D)

ISRP December 15, 2008 Comments: This project initiates two investigations – 1) an overlay of Columbia River Chinook salmon genetic variation with watershed characteristics in the streams inhabited by individual fish and, 2) an experimental description of how environmental conditions influence expression of some smolt physiological traits in steelhead and resident *Oncorhynchus mykiss*. Both investigations are “interesting” to biologists and decision-makers in the basin. It is unclear, however, what the sponsors are really after. It is also unclear how the two studies relate to each other.

CRITFC Response: The narrative includes two objectives that will be referred to as “Landscape Genetics” and “Expression of Traits” throughout the responses. These two projects were combined in one narrative partly for ease of contracting, and that these projects will share some data and staff. However, there are some important connections among portions of each study (i.e., Landscape Genetics - water temperature, and Expression of Traits – thermal tolerance). Most phenotypes are influenced by genetic X environmental interactions, and each objective in the project will contribute to the overall understanding of the traits being studied (see Figure 1. [not included in this memo])

ISRP February 19, 2009 Comments: The relationship between the studies is still not completely clear. The laboratory work will assess steelhead and the landscape genetics work will examine Chinook. The difference in species would suggest that the two parts of the project are very different studies. If the authors feel that the results from one part of this study will enhance the interpretation of results from the other, this connection should be described. The ISRP appreciates the Figure 1 diagram as providing a minor conceptual level of interconnectivity; however, the full relationship of the two objectives remains unclear and not compelling. The issues of contracting and staff sharing, while

important to administrators, is not a really an issue for scientific review and does not diminish the need for demonstrating scientific relations between proposed objectives.

a) Landscape genetics – the nature and structure of the genetic and ecological data sets are not presented in a format that the ISRP can reasonably review. For example, genetic data may be presented as allele frequencies, as heterozygosities, as percent polymorphisms, etc. Using water temperature as an example of ecological data, at any location we might expect that water temperature can vary daily, seasonally, or annually. Even if we accept extant environmental conditions as important in shaping the genetic background of salmon populations relative to historical conditions prior to alteration, there is no way to tell what is being related (and why).

For example, correlating the frequency of a specific allele to a temperature at a point in time (or perhaps daily or seasonal fluctuation, and so on) may or may not give a significant result. Ultimately, the sponsor does not present what the data fields will look like or what will be correlated (or what analytical approach is proposed) to appropriately evaluate statistical rigor. Moreover, it is difficult to determine the importance of a correlation between an allele and an environmental variable.

b) QTL screening – two traits are proposed as being searched for a QTL relationship: temperature tolerance and smoltification. There is no real design here to evaluate. For example, there may be two or more isolated populations (or brood stocks or other) of a species that express different tolerances (perhaps overlapping, but significantly different temperature ranges). This implies that perhaps there is a QTL basis worthy of screening. However, the sponsor provides no real information about the “set up” for this approach (including how many populations or lineages or brood stocks, etc. and how many candidate loci will be screened). Finally, the sponsor’s feedback includes a comment on the GxE interaction of traits, but provides no quantitative genetics design for determining the genetics and environment as main effects or GxE as an interaction effect.

Also, on its surface, smoltification seems to be a binary character (an individual smolts or it does not). The Sponsor may be thinking about rates of smoltification or some other characteristic, but because no information about the data field is provided, it is difficult to evaluate the design.

ISRP December 15, 2008 Comments: The approach of the first study seems to be a “lets correlate everything and see what pops out” type of approach that throws every conceivable statistical test at the data to see if there is something worthwhile. In this way, it appears to be a data mining exercise. While this approach may ultimately lead to some interesting and valuable results that help to narrow the field of important watershed variables, it can also suggest relationships simply on random chance.

CRITFC Response: The Landscape Genetics objective will focus only on variables that have been demonstrated to be critical for salmonids, not simply random parameters. These critical variables include elevation, migration barriers, migration distance, and temperature. Scientific literature is abundant regarding the importance of these variables and we have

focused our statistical analysis methods to those demonstrated in published studies, including those from our laboratory (Narum et al. 2008; Kalinowski et al. 2008) and others involved in this field of landscape genetics (e.g., Faubet and Gaggiotti 2008).

ISRP February 19, 2009 Comments: There needs to be a more detailed explanation and description of the data fields to be mined. The sponsor indicates, for example, that “elevation” is a critical variable. Is this elevation of extant spawning grounds (vs. historical) or elevation of hatchery or elevation where sampled? As far as the main channel goes, elevation likely correlates strongly with migration distance, thus the rationale for the critical data choices is not provided in a way that argues its importance.

Also, the Sponsor needs to provide some treatment of the specific analytical or statistical approaches employed for finding “causative” associations.

ISRP December 15, 2008 Comments: The approach for the second study is a Quantitative Trait Loci (QTL) screening exercise for genes associated with smolt physiological traits such as temperature tolerance. The sponsors present no data/results that indicate that such a trait has variation, regardless of whether it has a genetic component. Here too, the approach appears to screen lots and hope for something significant.

CRITFC Response: The second project, Expression of Traits, is intended to initially focus on two important traits of smoltification and thermal tolerance. Both traits have been shown to have extensive variation in natural populations as anadromy in *O. mykiss* is often plastic (Zimmerman and Reeves 2000; Narum et al. 2004; Thrower et al. 2004; Olsen et al. 2006) and thermal tolerance varies not only among species of *Oncorhynchus*, but also among populations of the same species (Brett 1956; Mosser and Bols 1988; Bear et al. 2007). Additionally, each trait has a clear genetic component (Nichols et al. 2008; Basu et al. 2002) that can be utilized as putative QTLs.

ISRP February 19, 2009 Comments: Some basic summary of the results from the published works would be appropriate. For example, inclusion of some summary information reflecting the nature and magnitude (or overall “shape”) of among-population variation in the traits of interest would have greatly strengthened this aspect of the original proposal. Ultimately, there is no real analytical design here to evaluate. For example, there may be two or more isolated populations (or brood stocks or other) of a species that express different tolerances (perhaps overlapping, but significantly different temperature ranges). This implies that perhaps there is a QTL basis worthy of screening. However, the sponsor provides no real information about the “set up” for this approach (including how many populations or lineages or brood stocks, etc. and how many candidate loci will be screened). Finally, the Sponsor’s feedback includes a comment on the GxE interaction of traits, but provides no quantitative genetics design for determining the genetic and environment as main effects or GxE as an interaction effect.

Also, on its surface, smoltification seems to be a binary character (an individual smolts or it does not). The sponsor may be thinking about rates of smoltification or some other

characteristic, but because no information about the data field is provided, it is difficult to evaluate the design.

ISRP December 15, 2008 Comments: Technical Justification: The technical and scientific background (section B pages 1, 2, and 3) introduce generally the topic of landscape genetics, and expression analysis of QTLs (quantitative trait loci), but do not convincingly identify how this information will be used to inform management decisions in the near and medium term. Landscape genetics is an emerging field of analysis – synthesizing and analyzing genotypic (and other genetic variation metrics) data together with habitat and environmental data, and spatial position to try to understand how historic landscapes and contemporary conditions produce the genetic associations observed today. The methods have potential to help with planning for ESA recovery, human population growth, and climate change. These applications, however, have not been realized yet for any species, and the proposal does not address this important challenge.

CRITFC Response: Previous studies with neutral molecular markers provide an understanding of genetic diversity as it relates to gene flow and genetic drift within and among populations (i.e., Narum et al. 2007). This study will expand on this previous knowledge by evaluating the biological differences and adaptation of populations that have been determined to have genetic structure. For example, two adjacent tributaries may each contain steelhead populations that have significantly different allele frequencies at neutral markers, but no functional differences exist and thus may be good candidates for “composite” hatchery broodstock. Likewise, two populations with similar allele frequencies at neutral markers may in fact have adaptive differences at functional genes. These adaptive differences may provide fitness advantages that should be taken into consideration.

This information is critical for management decisions such as determining appropriate hatchery broodstock and stocking/outplanting locations, appropriate stocks for recolonization following habitat restoration, and potential for resident fish to contribute to anadromous recovery (Figure 1). Without this research, managers are left with limited scientific information needed to help make decisions.

ISRP February 19, 2009 Comments: The response does not adequately address the ISRP’s concern regarding the management applicability of the expected results. The response appears to focus more on the feasibility of the method than on how the data will be used directly. Here, the ISRP recognizes the utility of spatial distributions of genetic diversity for management decisions (such as designations of GMUs and ESUs and so on). What remains unclear is whether the sponsor’s analyses will be used by the managers beyond what is currently available in the various species and coastwide databases presently being developed.

ISRP December 15, 2008 Comments: Program Significance: The sponsors believe data from this project would contribute to meeting the objectives of four RPAs (41, 50, 63, 64) in the most recent Biological Opinion. The proposal should provide a more detailed explanation of what is contained in the RPAs, and how the data from the project will be used to meet the RPAs’ objectives. How will information from the project be applied to the RPA, by whom?

CRITFC Response

-page 57, RPA No. 41, Preserve genetic resources:

Previous studies with neutral markers are helpful to determine genetic diversity and differentiation as a measure of gene flow and genetic drift, but these studies do not identify biological differences among populations. These new projects will help to identify biological differences and adaptation among populations, in addition to existing information on neutral genetic diversity. This will help identify important genetic resources throughout the Columbia Basin.

-page 69, RPA No. 50, Fish population status monitoring:

Determination of population specific thermal tolerance and smoltification traits will provide an evaluation of responses to a variety of disturbances. For example, if stream temperature has increased due to deforestation/loss of riparian habitat, evaluation of Hsp expression and QTLs will determine if populations are adapting to different thermal regimes relative to undisturbed areas.

-page 89, RPA No. 63, Monitor hatchery effectiveness:

Evaluation of both hatchery and natural stocks will determine if hatcheries are maintaining biological and adaptive characteristics that are important to natural populations.

page 89, RPA no. 64, Investigate hatchery critical uncertainties:

These studies will help to address if biological and adaptive differences exist in supplementation areas and identify genetically appropriate broodstock.

ISRP February 19, 2009 Comments: The connections outlined in the response are presented as statements of fact that should, perhaps, be obvious and acceptable at face value, but the sponsor provides no real support as to how project results will be applied to meet RPA objectives.

For example, the landscape genetics objective needs to be linked to management actions and decisions, and the genetic and environmental and spatial databases demonstrated to be sufficient to provide robust analysis to support the management. The genetic and environmental sampling needs to be compatible with the analytical methods. As an example, the Sponsor identifies that the proposal will contribute to fulfilling the 2008 Federal Columbia River Power System (FCRPS) Biological Opinion Reasonable and Prudent Alternative 41: Preserve and rebuild genetic resources through safety-net and conservation actions to reduce short-term extinction risk and promote recovery (hatchery strategy 2); implement safety net programs to preserve genetic resources and reduce short-term extinction risk. The RPA calls for specific elements to be completed in 2009, 2013, and 2016. This MOA proposal should explain how or why these data and analyses are needed for these three elements.

ISRP December 15, 2008 Comments: Project Relationships: The sponsor identifies that two accord projects are related to this one – 2008-511-00 (Genetic Assessment) and 2008-514-00

(Management Scenarios for Climate Change). The first project will provide baseline genetic data to be used by the landscape genetic analysis, and the second has the potential to use results from the landscape genetic analysis in scenario testing.

This section needs to provide additional support. First, this proposal states that they will be using past databases of genetic data for Chinook salmon and steelhead in the landscape analysis. The ongoing projects funded by the Fish and Wildlife Program and others (e.g., Pacific Salmon Treaty Southern and Northern Fund) that will supply data should be cited, and what kind of contribution they will make. That is, are these projects essential to the execution of the work in the proposal? Second, the projects and data sources that provide environmental and geospatial data should be mentioned.

CRITFC Response: Past genetic data for Chinook salmon and steelhead are available through shared databases. Our laboratory was a key contributor to these multi-agency efforts in both species. Funding for these previous efforts was provided by the Pacific Salmon Commission (Southern Boundary Fund and Chinook Technical Committee LOA). The collaborative efforts to create these shared databases have been published for Chinook salmon (Seeb et al. 2007; Narum et al. 2008) and steelhead (Stephenson et al. 2008), with CRITFC scientists as either lead or secondary authors for these publications. The genetic and location data from these databases are critical for the first stages of the landscape genetics project that will rely heavily on this shared information. As the baseline expands through the Genetic Assessment project (2008-907-00), this information will be used in further analyses. Environmental and geospatial data will initially be downloaded from the PRISM database (Parameter-elevation Regressions on Independent Slopes Model; <http://www.ocs.orst.edu/prism/>) of the Oregon Climate Service. As the project develops, the “Monitoring Habitat Recovery Trends” study (2008-517-00) may provide additional information.

In addition, this project will contribute to, and benefit from, two other Accord projects: “Monitoring Habitat Recovery Trends” (2008-517-00), and “Evaluating Climate Change Impacts in Tribal Ceded Areas” (2008-514-00) as follows:

1. This project will help managers understand how populations respond genetically to environmental conditions.
2. The Monitoring Habitat Recovery Trends project will provide information about the factors controlling temperature, sedimentation, and streamflow and how those metrics affect fish distribution, abundance, and productivity (three critical metrics needed for delisting decisions). The genetic information obtained from this project will complement the biological information gained from the Habitat Monitoring Project to give managers a deeper understanding of the inter-relationships between fish populations and habitat conditions.
3. The Climate Change Impacts project will evaluate how key habitat metrics and conditions are likely to change under the pressures of climate change and population growth.
4. These anticipated habitat changes, plus the knowledge of habitat-fish interactions gained from the first two projects, can be used to help managers:
 - a. Identify potential future salmon strongholds and areas at high risk;

- b. Effectively incorporate the impacts of climate change, population growth and hatchery programs in natural resource plans and objectives. This was not adequately done in the 2004 subbasin plans;
- c. Identify protection and restoration strategies to effectively address management objectives, for example;
 - i. determining appropriate hatchery broodstock and stocking/outplanting locations,
 - ii. determining appropriate stocks for recolonization following habitat restoration
 - iii. identifying areas and land use practices most likely to preserve and restore temperature and sediment levels necessary to maintain and rebuild populations

ISRP February 19, 2009 Comments: The description here implies the sponsor’s project will monitor changes to population genetic variation associated with habitat conditions. The sponsor provides no information about what constitutes the temporal baseline and how often such changes will be probed to observe an effect. There is also no support given for the premise that the genetic monitoring is sufficiently sensitive to detect effects associated with which environmental condition (reference vs. treatment data sets).

2. Objectives, Work Elements, and Methods (section F)

ISRP December 15, 2008 Comments: There are two objectives for this proposal – 1. Landscape genetics: test for correlation of landscape/watershed characteristics with genetic structure of Chinook salmon and steelhead populations; and 2. Expression of Traits – Evaluate how environmental conditions influence the genetic expression of physiological traits that are related to recovery of steelhead populations.

Work elements and methods for objective 1. The work elements 156, 157, and 162 are not sufficiently detailed to make a determination of whether the tasks are technically justified. The sponsor states that they are going to analyze physical and genetic data following Narum (2008) and other pertinent studies. The choice of which individuals and locations to use in any landscape analysis will determine to some extent the outcome of the analysis, but also what questions are appropriate for the scale – both temporally and spatially. The specific goals of the analysis – how is it hoped that this information will inform management and provide prediction about the future state of the species – are not stated. Under this circumstance, the investigation is likely to pursue measuring and analyzing everything obtainable from everywhere. The result is often contradictory evidence that does not lead to improved management.

CRITFC Response: The Landscape Genetics objective will focus only on variables that have been demonstrated to be critical for salmonids, not simply random parameters. These critical variables include elevation, migration barriers, migration distance, and temperature. Scientific literature is abundant regarding the importance of these variables and we have focused our statistical analysis methods to those demonstrated in published studies, including those from our laboratory (Narum et al. 2008; Kalinowski et al. 2008) and others involved in this field of landscape genetics (e.g., Faubet and Gaggiotti 2008). These approaches will help

identify priority areas for conservation, restoration, and recovery of species and life history types of concern. For example, the study of *O. mykiss* in the Klickitat River (Narum et al. 2008) identified areas most likely to support anadromous versus resident ecotypes (Figure 2a).

ISRP February 19, 2009 Comments: There needs to be a more detailed explanation and description of the data fields to be mined. The sponsor indicates, for example, that “elevation” is a critical variable. Is this elevation of extant spawning grounds (vs. historical) or elevation of hatchery or elevation where sampled? As far as the main channel goes, elevation likely correlates strongly with migration distance, thus the rationale for the critical data choices is not provided in a way that argues its importance.

ISRP December 15, 2008 Comments: The implication of the narrative is that all Chinook within the Columbia River Basin are going to be potential candidates for inclusion in the analysis. This seems inappropriate given our understanding that we have several ESUs that rarely interbreed. Why would you include Willamette River Chinook in an analysis with Snake River Chinook in an effort to identify the landscape features and environmental variables that contribute to maintaining the distinctiveness of these fish? It would seem you would want to focus on gene exchange and important source – sink populations and environmental correlates across independent populations and major population groups within ESUs. For example, gaining a greater understanding of the dynamics of spring/summer Snake River Chinook spanning the Tucannon, Grande Ronde/Imnaha, and Salmon River systems would be more important. Finally, the specific set of analysis should be identified ahead of time, and some sort of approach directed at specific questions of population structure, limiting factors, and predicting anthropogenic influences.

CRITFC Response: One of the attributes of Landscape Genetics analysis is the ability to apply this approach on different scales (Figures 2a & 2b [not included in this memo]) and integrate both biological and physical data. The analyses can be completed for any scale that data points are collected, even entire continents where appropriate (i.e., Hannote et al. 2002). In the case of salmon and steelhead, we plan to utilize baseline genetic data collected throughout the Columbia Basin. With the known location of those collections, we can include data on elevation, migration barriers, migration distance, and temperature. This analysis provides results that distinguish populations by regions (Willamette and Snake R. are highly distinct), and identifies connectivity not only by geographic proximity but also genetic similarity and watershed/physical characteristics. Figures 2a & 2b demonstrate how these approaches can be utilized on different geographic scales.

ISRP February 19, 2009 Comments: The ISRP appreciates the explanation that landscape genetic analysis can be conducted across a broad geographic area, and still address hypotheses specific to subsets of the region. However, two important concerns remain for the ISRP. One is ensuring that the sampling of genotypic and environmental data and the form of the analysis are actually sufficient to address the specific restoration constraints for individual populations. The second has to do with confounding variables and non-causal co-variation. The greater the number of variables, the more likely a

statistically significant interaction will be observed simply out of chance. This does not appear to be addressed in the current study design.

ISRP December 15, 2008 Comments: Work elements and methods for objective 2. Work elements 156, 157 (1), 157 (2), and 162 are insufficient for a technical analysis. The study proposes to analyze samples from fish reared in a controlled environment to determine the influence of environmental conditions on the genetic expression of physiological traits. The basic outline of the experimental is to assay individuals for the enzymatic activity as a measure of gene expression under controlled conditions; to genotype these individuals for QTLs for smoltification and heat tolerance; and finally to statistically assess correlation between specific QTL alleles and the gene expression as measured as enzymatic activity (or heat shock protein).

Archived samples from natural populations across a range of environmental conditions will then be analyzed to determine if the patterns observed in the laboratory trials are evident in situ. This approach assumes that the archived samples have been collected across a sufficient range of environmental conditions to enable this comparison. Some evaluation of the archived samples to determine if they represent a sufficient range of environmental conditions should have been included in the proposal. It is also important to consider the source of the fish, any breeding scheme, and the testing regime when determining the sufficiency of the experimental design for the laboratory investigation. The “hypothesis” for objective 2 in section G is too vague.

CRITFC Response: The general intent is to test if a significant correlation can be identified between genetic factors (QTL genotypes and gene expression) and specific traits under controlled environmental conditions (i.e., changing photoperiod or differing water temperatures). The putative QTLs were chosen as markers since they are highly associated with the traits being studied (i.e., smoltification, Nichols et al. 2008; thermal tolerance, Narum & Campbell in review). The source of fish for the thermal tolerance & Hsp gene expression study will include a strain native to the Columbia River. The source of fish for the smoltification & Na/K-ATPase gene expression (Richards et al. 2003) study will be from the upper Salmon River, reared at the Hagerman National Fish Hatchery.

Archived samples of natural populations at fine geographic scale will also be genotyped with putative QTLs to test for differential allele frequencies relative to neutral markers. This includes *O. mykiss* samples from the Klickitat River that have been characterized as primarily resident or anadromous and have corresponding temperature data (see samples sites on Figure 2a). Other potential sites with appropriate archived tissues include tributaries of the Grande Ronde River (Narum et al. 2006), and desert vs. mountain locations from the Snake River with corresponding temperature data (desert = Owyhee & Bruneau rivers; mountain = Boise & Payette rivers).

ISRP February 19, 2009 Comments: The additional information is important, but the proposal still does not adequately present an analytical/experimental design.

3. M&E (sections G and F)

ISRP December 15, 2008 Comments: This proposal is for a descriptive set of studies to learn about the “patterns of genetic variation” at a finer scale than presently available. Thus, no management treatment/action is undertaken to monitor or evaluate, so a separate M&E section is not essential. However, even though greater detail on the experimental designs and methods are provided in section G (Research – Methods) than in earlier sections, the problems identified in work elements and methods still persist. The presentation is not sufficient to establish that the proposal has a high likelihood of success and will contribute to improving the status of fishery resources in the Columbia River basin. Another key element that is not fully developed in the proposal is the process by which the information developed during the study will be used to inform management decisions. Some discussion of the adaptive management process that will be employed to this end would have provided a more complete justification of the potential value of this research.

CRITFC Response: Previous studies with neutral molecular markers provide an understanding of genetic diversity as it relates to gene flow and genetic drift within and among populations (i.e., Narum et al. 2007). This study will expand on this previous knowledge by evaluating the biological differences and adaptation of populations that have been determined to have genetic structure. For example, two adjacent tributaries may each contain steelhead populations that have significantly different allele frequencies at neutral markers, but no functional differences exist and thus may be good candidates for “composite” hatchery broodstock. Likewise, two populations with similar allele frequencies at neutral markers may in fact have adaptive differences at functional genes. These adaptive differences may provide fitness advantages that should be taken into consideration.

This information is critical for management decisions such as determining appropriate hatchery broodstock and stocking/outplanting locations, appropriate stocks for recolonization following habitat restoration, and potential for resident fish to contribute to anadromous recovery (Figure 1). Without this research, managers are left with limited scientific information needed to help make decisions.

ISRP February 19, 2009 Comments: The ISRP recognizes the utility of information on spatial distributions of genetic diversity for management decisions (such as designations of GMUs and ESUs and so on). What remains unclear is whether the Sponsor’s analyses will be used by the managers beyond what is currently available in the various species and coastwide databases presently being developed. Furthermore, we reiterate the need for a more complete description of the design to permit review of the proposal’s technical merits.

4. Overall Comments - Benefit to F&W (all proposal)

ISRP December 15, 2008 Comments: Objective 1. Landscape analysis incorporating genotypic and other genetic data with spatial and environmental data should be considered as one approach to gaining a better understanding about the relationships and processes that have produced the extant distribution of breeding populations of Chinook salmon. The framework for

this analysis should be developed further in hopes of achieving this goal, before initiating collections of environmental data or genetic data. Complicated analysis will not necessarily provide better information than simple analysis.

The landscape genetics analysis has the potential to provide benefits to fish and wildlife.

CRITFC Response: This analysis provides results that distinguish populations by regions and identifies connectivity not only by geographic proximity, but also genetic similarity and watershed/physical characteristics. This can be highly informative for determining appropriate stocks for recovering specific populations. It is important to consider donor stocks that not only have similar levels of genetic diversity, but also occupy similar habitat (Objective 1: Landscape Genetics) and may have similar adaptations (Objective 2: Expression of Traits).

ISRP February 19, 2009 Comments: The ISRP agrees in principle that fine-scale information could be useful for intensive and localized management. However, the project's benefits are described at only the most general of levels and connections to current information needs by co-managers are somewhat diffuse and insufficiently supported in spite of their potential.

ISRP December 15, 2008 Comments: Objective 2. It is not clear what the real objective of this work is, why it is combined in a proposal to conduct landscape analysis, and whether the approach is adequate. It is difficult to assess the potential for benefits to fish from this work.

CRITFC Response: This study will expand on this previous knowledge by evaluating the biological differences and adaptation of populations that have been determined to have genetic structure. This information is critical for management decisions such as determining appropriate hatchery broodstock and stocking/outplanting locations, appropriate stocks for recolonization following habitat restoration, and potential for resident fish to contribute to anadromous recovery (Figure 1). Without this research, managers are left with limited scientific information needed to help make decisions.

ISRP February 19, 2009 Comments: The ISRP agrees in principle that fine-scale information could be useful for intensive and localized management. However, the project's benefits are described at only the most general of levels and connections to current information needs by co-managers is somewhat diffuse and unsupported.