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Dear ISRP Members,

We appreciate the comments provided for the Accord project “Influence of Environment and Landscape on Salmonid Genetics” (200900500) which have helped us to identify areas for further development and planning. We have evaluated the comments provided by the ISRP, and have prepared responses to improve or further explain the study design. Our responses are interspersed following each ISRP comment, with new text highlighted in **GREEN**. The responses include more thorough explanation of connectivity among the two objectives, additional details of methods, and potential management implications of this project. We strongly believe that both objectives in this study are critical for understanding functional/biological adaptations that exist among populations of salmonids in the Columbia River Basin, and this information can greatly benefit fisheries management.

Thank you for your time and contribution to this study.

Sincerely,

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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
Subbasin(s)	Basinwide
Contact Name	Shawn Narum
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http://pisces.bpa.gov/release/documents/DocumentViewer.aspx?doc=P108874&session=500441b3-1761-42d2-9431-9802c91e5095

ISRP 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.

ISRP Comments:

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

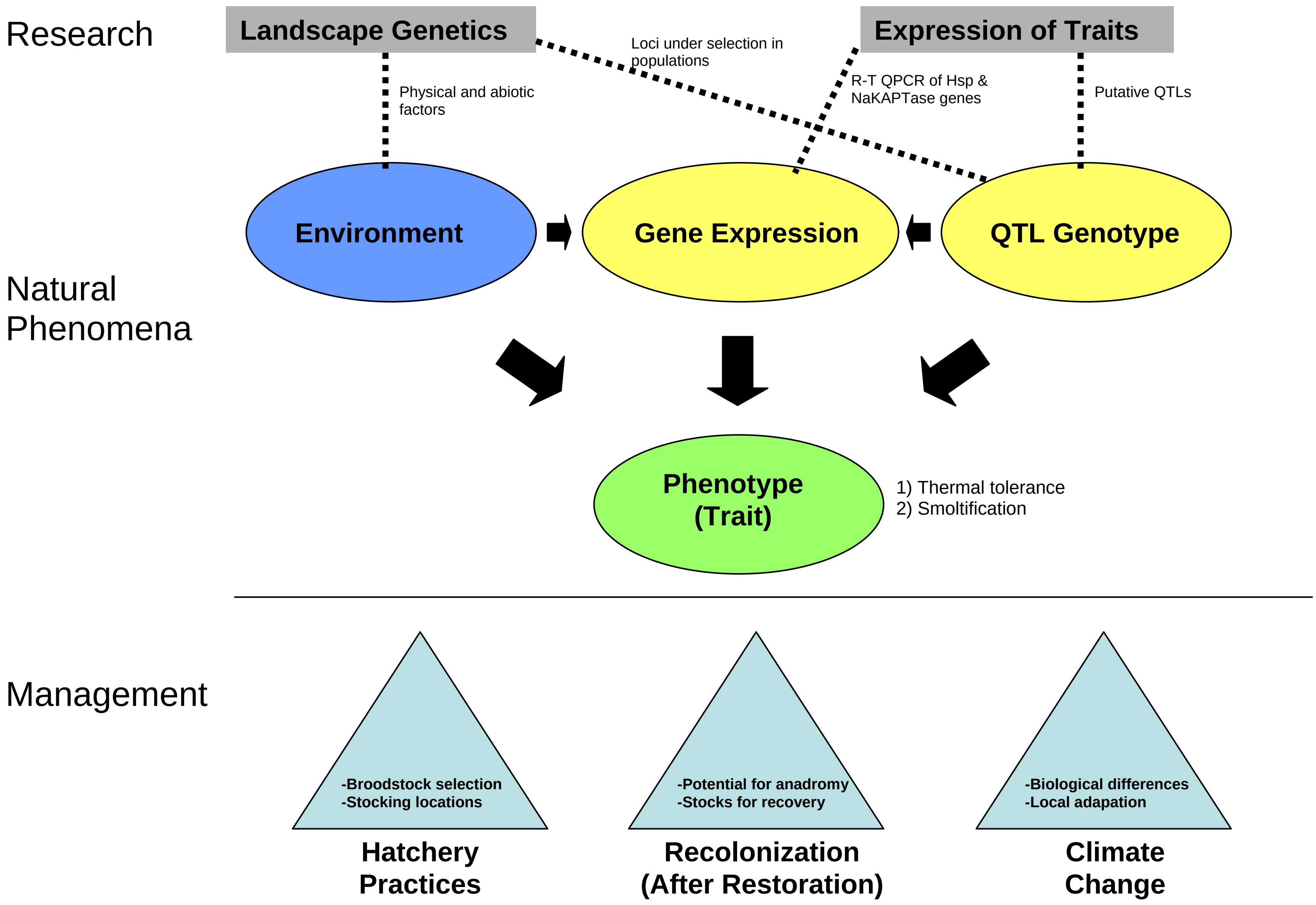
This project initiates two investigations – 1) an overlay of Columbia River Chinook salmon genetic variation with watershed characteristics in the streams inhabited by individual fish and, 2) an experimental description of how environmental conditions influence expression of some smolt physiological traits in steelhead and resident *Oncorhynchus mykiss*.

The narrative includes two objectives that will be referred to as “Landscape Genetics” and “Expression of Traits” throughout the responses.

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.

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.)

Figure 1. Diagram of the connection between projects and general research design.



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.

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.

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.

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.

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.

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.

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?

-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.

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.

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**

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

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).

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.

One of the attributes of Landscape Genetics analysis is the ability to apply this approach on different scales (Figures 2a & 2b) 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.

Figure 2a. Synthesis map from Narum et al. (2008) illustrating geographic interpolation of principal components analysis utilizing physical and genetic data. The first principal component accounts for 59.0% of total variation, and the shading for PC1 demonstrates areas that are most suitable for anadromous (blue) and resident (red) life history types of *O. mykiss* in the Klickitat River sub-basin.

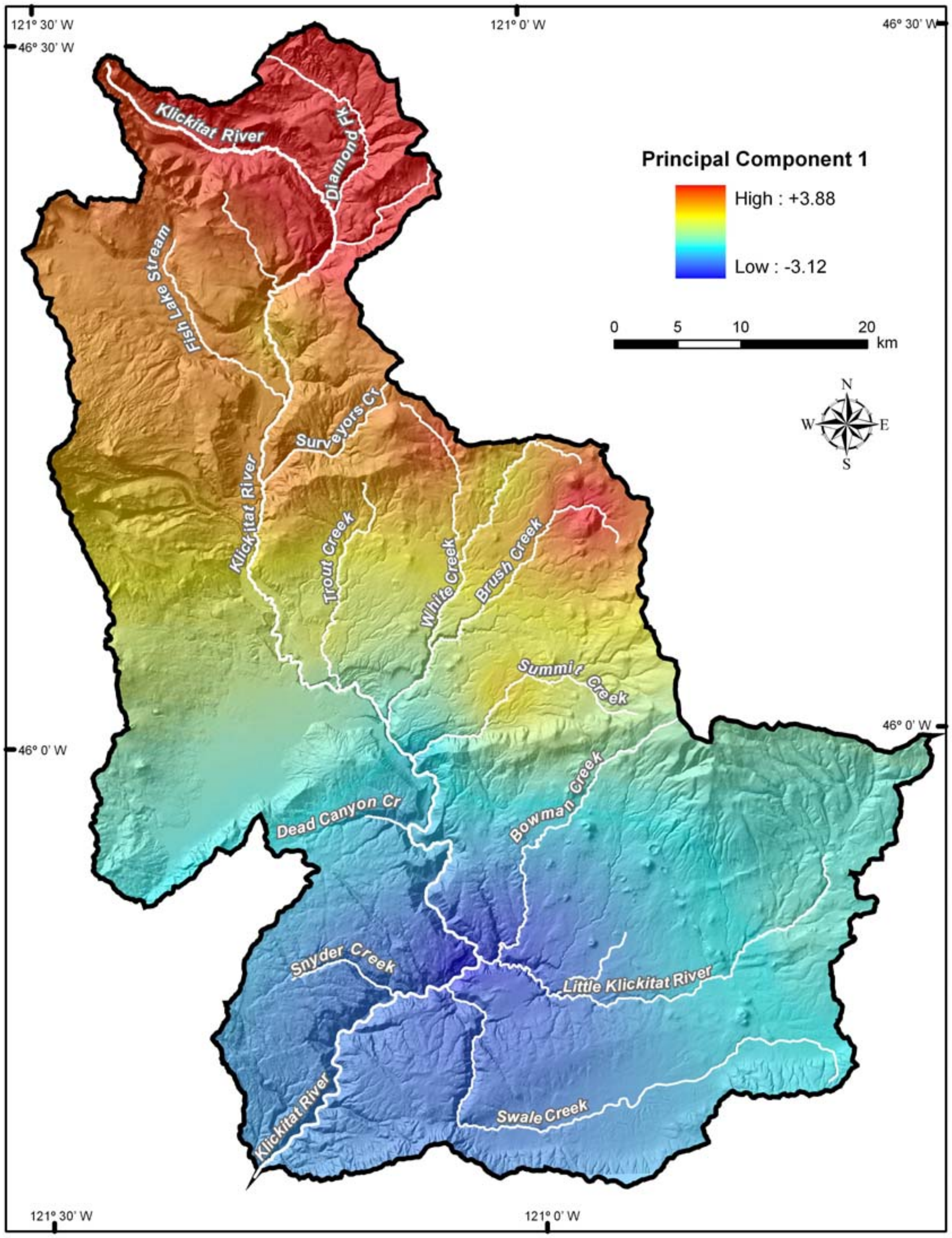
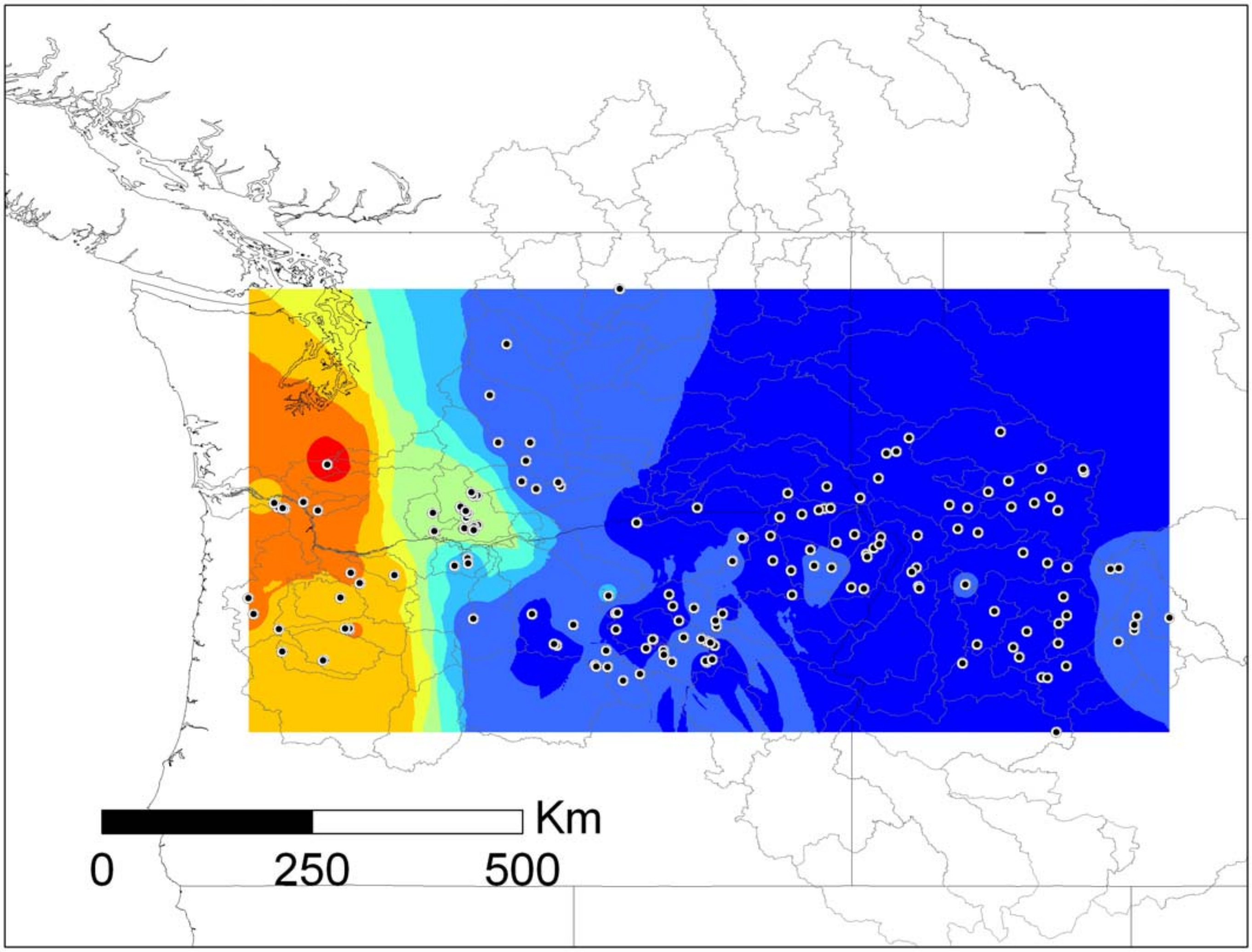


Figure 2b. Example of a broad scale synthesis map (unpublished data, J. Hess and S. Narum), demonstrating how the approach can be utilized basinwide. (This map only includes genetic data by location, but both watershed variables and genetic data will be analyzed in the proposed Accord project.)



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.

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 & Cambpell 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).

3. M&E (sections G and F)

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.

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.

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

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

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).

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.

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.

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