

Narrative

Influence of Environment and Landscape on Salmonid Genetics

Table 1. Proposal Metadata

Project Number	2009-005-00
Proposer	Columbia River Inter-Tribal Fish Commission
Short Description	Influence of Environment and Landscape on Salmonid Genetics
Province(s)	Basinwide
Subbasin(s)	Basinwide
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Information transfer:

A. Abstract

Environmental and landscape features can greatly contribute to population structure, life history diversification, and adaptation of salmonids. This proposal combines two projects from the Fish & Wildlife Program Accords with the following objectives: 1) Landscape Genetics - determine correlation of watershed characteristics such as elevation, barriers, migration distance, and temperature to genetic structure of Chinook salmon and steelhead populations; and 2) Expression of Traits - evaluate how environmental conditions influence the genetic expression of physiological traits (i.e., smoltification and thermal tolerance) that are related to recovery of steelhead populations. This information will facilitate understanding of adaptation of natural populations of salmonids to their environment. We expect this to benefit future management/recovery of natural and supplemented populations, along with reintroduction programs. The work related to objective 1 (Landscape Genetics) will be completed by CRITFC and University of Idaho (UI) staff at the Hagerman Fish Culture Experiment Station. Tissue samples for objective 2 (Expression of Traits) will be collected and analyzed by CRITFC and UI staff in Hagerman. Geneticists with CRITFC have adequate expertise to complete these objectives, and have published related peer-reviewed papers (Narum et al. 2008, Campbell et al. 2008).

B. Technical and/or scientific background

Landscape features can greatly contribute to the population structure and life history diversification of organisms in both aquatic and terrestrial habitats (reviewed in Storfer et al. 2006). Geographic barriers to dispersal are associated with reproductive isolation among intraspecific populations, and have both contemporary and paleogeological sources (e.g., Castric et al. 2001). This includes recent events that may have been human induced (i.e., roads and dams) as well as ancient events such as glaciations and formation of mountain chains. However,

other landscape characteristics such as elevation, temperature, forest cover, and precipitation may influence distribution, adaptation, and gene flow of species (Funk et al. 2005). For example, the geographic distributions of species ranges' are often determined by thermal tolerance (Brannon et al. 2004) and may necessitate adaptations in extreme environments (Harris et al. 1998).

Evaluation of environmental features has progressed beyond traditional Mantel tests of isolation-by-distance (genetic distance correlated to geographic distance) to determine critical landscape characteristics that influence genetic diversity and structure. Recent advances in both spatial and genetic analysis methods provide the opportunity to better determine the correlation between landscape features and genetic structure at fine scales. Further, a variety of statistical models have been developed to address specific questions related to landscape genetics (Manel et al. 2003; Storfer et al. 2006). For example, ordination models with canonical correspondence analysis have been used as an alternative to Mantel tests to simultaneously evaluate drainage, altitude, and human impacts to genetic diversity of salmonid fishes (Angers et al. 1999; Costello et al. 2003). Recent applications of interpolation models that utilize multivariate analyses such as principal components analysis (PCA) have also demonstrated that habitat and landscape features can identify and predict spatial patterns associated with restricted gene flow (Piertney et al. 1998). When PCA results are interpolated and overlaid with GIS data, synthesis maps can identify genetic patterns related to landscape (e.g., Narum et al. 2008). In this study, we plan to apply these approaches at a basinwide scale for Chinook salmon and steelhead in the Columbia River.

Genetics and environment can also interact to influence the expression of genes controlling physiological traits such as smoltification (Thrower et al. 2004; Nichols et al. 2008) and response to high temperatures (Feder and Hofmann 1999). These traits play a role in recovery of ESA listed populations, and this research project is directed at determining the underlying genetic X environmental mechanisms associated with these traits. Experiments in this study will take place under both controlled and natural environments. This approach will allow us to isolate variables in captivity, and then apply those results to samples collected from natural populations.

Smoltification is an important trait to study since resident rainbow trout and anadromous steelhead life history types (*Oncorhynchus mykiss*) interbreed and thus there is potential for rainbow trout to contribute to the recovery of ESA listed steelhead stocks in the Columbia River Basin. Both environmental and genetic factors determine if individual *O. mykiss* remain as resident rainbow trout, or undergo the necessary physiological changes (smoltification) to prepare for anadromy. While some of the associated environmental factors (i.e. photoperiod and temperature) have been evaluated, the genetic mechanisms that contribute to migratory selection are not well known. Recent studies have confirmed that genetic factors do play a role in smoltification (Thrower et al. 2004; Nichols et al. 2008), and quantitative trait loci (QTL) have been identified that are associated with phenotypic traits of smolts (Nichols et al. 2008). However, further research is needed to extend these discoveries with studies that quantify genetic expression and frequency of QTL alleles associated with smoltification in *O. mykiss*.

Another important trait is the response of fish to stressors such as high water temperature. In recent history, water temperatures in parts of the Columbia River basin have increased due to a wide range of factors including habitat destruction, dams, and possibly climate change. The physiological response and adaptation of steelhead to these circumstances may be critical to persistence of stocks in the Columbia River Basin. Expression of a class of genes involved in stress response to high temperatures (heat shock proteins) will be evaluated in controlled and natural conditions. Putative QTL loci will also be tested for association with adaptation to high

temperatures. This information will assist our understanding of the ability of populations to adapt to increasing temperatures.

C. Rationale and significance to regional programs

This research proposal affects all stocks in the Columbia River Basin and therefore is considered a basinwide application. The projects in this proposal address needs for management of natural and supplemented populations, along with reintroduction programs. These needs have been identified in multiple “Reasonable and Prudent Alternatives” (RPA) in the BiOp:

- page 57, RPA No. 41, Preserve genetic resources
- page 69, RPA No. 50, Fish population status monitoring
- page 89, RPA No. 63, Monitor hatchery effectiveness
- page 89, RPA no. 64, Investigate hatchery critical uncertainties

D. Relationships to other projects

This research project is directly related to Genetic Assessment of Columbia River Stocks (2008-511-00) since baseline genetic data from that study will be utilized for landscape genetic analyses. Additionally, our project is related to Management Scenarios for Climate Change (2008-514-00) since our study addresses the ability of populations to adapt to increasing temperatures.

Table 2. Relationship to existing projects

Funding Source	Project #	Project Title	Relationship (brief)
BPA	2008-511-00	Genetic Assessment	Baseline genetic data will be utilized in landscape genetics analyses.
BPA	2008-514-00	Management Scenarios for Climate Change	Understanding of adaptation to increasing temperatures.

E. Project history (for ongoing projects)

This is a newly funded project with no history.

F. Proposal biological/physical objectives, work elements, methods, and metrics

Related to all Objectives (1 & 2)

Work Elements:

119. Manage and Administer Projects: Project Management

This will include project administration and contract development.

132. Produce (Annual) Progress Report: Submit Progress Report for 1/1/2009 to 12/31/2009

The progress report will summarize the project objectives, hypotheses, completed and uncompleted deliverables, problems encountered, lessons learned, and long-term planning. Date range 1/1/2009 to 12/31/2009.

183. Produce Journal Articles: Submit for Publication

As deemed appropriate by project leaders, results from studies will be submitted for publication in peer-reviewed journals.

185. Produce Pisces Status Reports: Periodic Status Reports for BPA

CRITFC shall report on the status of milestones and deliverables in Pisces. Reports shall be completed either monthly or quarterly as determined by the BPA COTR. Additionally, when indicating a deliverable milestone as COMPLETE, CRITFC shall provide metrics and the final location (latitude and longitude) prior to submitting the report to the BPA COTR.

Objective 1) Landscape Genetics - Test for correlation of landscape/watershed characteristics with genetic structure of Chinook salmon and steelhead populations.

Work Elements:

156. Develop RM&E Methods and Designs: Determine Landscape and Genetic Variables

Landscape genetics analyses methods will be utilized to evaluate hypotheses that landscape features influence genetic structure and life history variation of Chinook salmon and steelhead. Physical variables such as elevation, stream gradient, water temperature, and geographic distance of each sample site will be included as potential landscape features that influence genetic diversity. Physical and genetic data will be analyzed following Narum et al. (2008) and other pertinent studies.

157. Collect/Generate/Validate Field and Lab Data: Gather Landscape and Genetic Data
Genetic Data:

This objective does not include genotyping new samples, but rather accessing existing genetic data. Thus, microsatellite and SNP genotypes for Chinook salmon and steelhead will be utilized from existing and ongoing genetic baseline projects for each species. Populations will be analyzed from throughout the Columbia River Basin, depending on availability from genetic databases.

Landscape Data:

Landscape features for genotyped populations will be determined using Geographic Information Systems (GIS) analysis of each sample site. This stream layer will provide elevation, migration distance, geographical stream distance among sites, and stream gradient. Precipitation and temperature for each site will be estimated with simulations from PRISM (Parameter-elevation Regressions on Independent Slopes Model; <http://www.ocs.orst.edu/prism/>) of the Oregon Climate Service.

162. Analyze/Interpret Data: Landscape Genetics Analyses/Correlation

Tests for correlation among landscape and genetic variables will be completed with pairwise and multivariate analyses. Results from principle components analysis will be interpolated across the Columbia River drainage.

161. Disseminate Raw/Summary Data and Results: Distribute Results

Results from landscape genetics analyses will be made available in annual reports and publications. Studies will also be presented at professional meetings and seminars as appropriate.

Objective 2) Expression of Traits - Evaluate how environmental conditions influence the genetic expression of physiological traits that are related to recovery of steelhead populations

156. Develop RM&E Methods and Designs: Sampling Plan and Gene Expression/QTL Analysis

The first year of this study will begin to address two key traits related to recovery of steelhead, smoltification and thermal tolerance. Expression of genes related to these functions will be quantified in fish under controlled environments, and putative quantitative trait loci (QTL) for smoltification and heat shock proteins (HSP) will be genotyped. Subsequent years of this project may include additional traits such as immune response and growth.

157. Collect/Generate/Validate Field and Lab Data: Tissue Sampling

Tissues will be sampled at multiple developmental stages from fish reared under controlled environments (temperature and photoperiod). Samples will be collected at 3-4 time periods of development, with up to 30 fish per time (3 replicate tanks of 10 fish). Fish will be sacrificed with an overdose of MS-222, and then immediately dissected to remove tissues such as brain, liver, gill, and fin tissues for analysis. Archived tissues collected from fish in natural populations with varying temperature (cool headwater tributaries vs. warmer mainstem sites) and smoltification (resident vs. anadromous) profiles will be identified for potential analysis.

157. Collect/Generate/Validate Field & Lab Data: Quantify Gene Expression&QTL Frequencies

The expression of three HSP and two Na/K-ATPase genes will be quantified with real-time PCR methods in tissues such as brain, liver, and gill that are sampled from fish in controlled environments. Putative QTL markers will also be genotyped in fin tissues collected from each fish. A total of approximately 1,500 samples will be analyzed by gene expression or QTL methods.

162. Analyze/Interpret Data: Gene Expression Related to Traits

The expression of Na/K-ATPase genes is expected to change relative to photoperiod during smoltification, and we will test for significant differential expression over the course of development. Allele frequencies of QTL markers will also be tested for association with gene expression and smoltification traits. The expression of genes known to play a major role in smoltification (i.e., Na/K-ATPase) will be evaluated to determine smolt quality and the potential of resident fish to contribute to anadromy in natural populations.

Gene expression of HSPs is expected to vary in response to thermal stress and high temperatures. We will test for differential expression of each HSP gene under variable temperature conditions, and the association with survival. Allele frequencies of putative QTL markers will also be tested for association with gene expression and survival. This information will provide insight regarding genetic variability and the potential of fish to adapt to increasing temperatures.

161. Disseminate Raw/Summary Data and Results: Distribute Results

Results from gene expression and QTL analyses will be made available in annual reports and publications. Studies will also be presented at professional meetings and seminars as appropriate.

G. Research – Methods

Objective 1) Landscape Genetics

Hypothesis:

- Landscape features are correlated with genetic diversity/structure of salmonid populations.

Experimental Design

Landscape genetics analyses methods will be utilized to evaluate hypotheses that landscape features influence genetic structure and life history variation of Chinook salmon and steelhead. Physical variables such as elevation, stream gradient, water temperature, and geographic distance of each sample site will be included as potential landscape features that influence genetic diversity. Genetic data will be obtained from existing baselines for Chinook and steelhead in the Columbia River Basin. Landscape features for genotyped populations will be determined using ArcGIS and simulations from PRISM. As appropriate, physical and genetic data will be analyzed following Narum et al. (2008) and new methods available in recent literature (e.g., Faubet and Gaggiotti 2008; Kalinowski et al. 2008).

Quality Control

Known information from previous studies will be used to validate results determined from this new analysis.

Statistical Analyses

Mantel tests will be utilized to test for genetic isolation by fluvial distance among sites. The regression of the pairwise $F_{ST}/(1 - F_{ST})$ on geographic distance (GENEPOP; Raymond and Rousset 1995) will be used to determine significance of Mantel tests. Correlations between landscape features and heterozygosity will be tested with Spearman's r . A principle components analysis (PCA) will be completed with the R! statistical package (<http://www.r-project.org/>) to determine which landscape features account for the majority of genetic diversity. Variables in the PCA include heterozygosity, elevation, stream gradient (both general and below), temperature, precipitation, and upstream distance. Values from PC1 and PC2 will be interpolated across the Columbia River Basin with the inverse distance squared method implemented by ArcGIS. The program STREAMTREE (Kalinowski et al. 2008) will be used to map genetic distances among populations. As possible, other analysis algorithms will also be utilized such as BIMr (Faubet and Gaggiotti 2008), GESTE (Foll and Gaggiotti 2006), and COLONISE (Foll and Gaggiotti 2005) to infer environmental factors that influence recent migration and colonization in metapopulations.

Communication of Results

Results from this project will be included in annual reports and submitted for publication as warranted. Studies will also be presented at professional meetings and seminars as appropriate.

Objective 2) Expression of Traits

Hypothesis:

- Gene expression and allele frequencies of QTL markers are associated with traits such as smoltification and thermal tolerance.

Experimental Design

The first year of this study will begin to address two key traits related to recovery of steelhead, smoltification and thermal tolerance. Expression of genes related to these functions will be quantified in fish under controlled environments, and putative quantitative trait loci (QTL) for smoltification and heat shock proteins (HSP) will be genotyped. Subsequent years of this project may include additional traits such as immune response and growth.

Tissues will be sampled at multiple developmental stages from fish reared under controlled environments (temperature and photoperiod). Samples will be collected at 3-4 time periods of development, with up to 30 fish per time (3 replicate tanks of 10 fish). Fish will be sacrificed with an overdose of MS-222, and then immediately dissected to remove tissues such as brain, liver, gill, and fin for analysis. Archived tissues collected from fish in natural populations with varying temperature (cool headwater tributaries vs. warmer mainstem sites) and smoltification (resident vs. anadromous) profiles will be identified for potential analysis.

The expression of three HSP (Wacyk unpublished data) and two Na/K-ATPase (Richards et al. 2003) genes will be quantified with real-time PCR methods in tissues that are sampled from fish in controlled environments. Putative QTL markers (Campbell et al. 2008; Nichols et al. 2008) will also be genotyped in fin tissues collected from each fish. A total of about 1,500 samples will be analyzed by gene expression or QTL methods.

Quality Control

Positive and negative controls, standard quantification curves, and dissociation analysis will be utilized to assure quality of gene expression data. Genotype data for QTLs will be checked for quality by utilizing positive and negative controls in each run. Repetitive genotyping of randomly selected individuals will be completed to ensure repeatability of genotyping results.

Statistical Analyses

Gene expression in tissues will be quantified relative to a standard curve of known amounts of cDNA, and normalized to a ubiquitous gene (i.e., "arp"). Gene expression among time periods and treatments will be tested for significant differences with t-tests and ANOVA. Allele frequencies will be estimated from QTL genotypes and tested for correlations with expression data and physical traits (i.e., condition factor, growth, survival).

Communication of Results

Results from this project will be included in annual reports and submitted for publication as warranted. Studies will also be presented at professional meetings and seminars as appropriate.

H. Facilities and equipment

Genetic analysis will be completed at the Hagerman Fish Culture Experiment Station (HFCES) in Hagerman, ID, operated by Columbia River Intertribal Fish Commission and University of Idaho staff. The Hagerman site houses multiple laboratories (including fish genetics, nutrition, and culture) and sufficient office space for the staff in this project. In addition to salaries, funding from this project will provide money for genetic laboratory supplies, wet lab supplies for fish culture, and field sampling gear. Existing equipment at HFCES will be sufficient to accommodate gene expression analysis and QTL genotyping for this study.

I. References

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J. Key personnel

The Lead Geneticist (Dr. Shawn Narum) will oversee this project and both of the objectives. Department Manager (Phil Roger) and Habitat Specialist/Senior Scientist (Dale McCullough) will facilitate coordination and administration of tasks related to the project. Key staff for completing objectives includes a Genomics Researcher (Nate Campbell), Conservation Geneticist (Andrew Matala), and a post-doctoral fellowship (TBD). Additional field and lab technicians will be also be important to completing objectives, but are not listed individually (see budget spreadsheet). Time allocation to this project for each key staff member is below:

- Shawn Narum, Lead Geneticist, 0.1 months (FTE)
- Dale McCullough, 0.5 months (FTE)
- David Graves, 0.5 months (FTE)
- Nate Campbell, 5.5 months (FTE)
- Andrew Matala, 2 months (FTE)
- Post-doc, 6 months (TBD)

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Education

Ph.D., Natural Resources, University of Idaho, 2006
M.S., Marine Science, University of San Diego, 2000
B.S., Fishery Biology, Colorado State University, 1996

Appointment

2002-present Lead Geneticist, Columbia River Inter-Tribal Fish Commission

Selected Publications

Narum, S. R., J. Zendt, D. Graves, and B. Sharp. 2008. Influence of landscape on resident and anadromous life history types of *Oncorhynchus mykiss*. *Canadian Journal of Fisheries and Aquatic Sciences* 65:1013-1023.

Campbell, N. R., K. Overturf, and S.R. **Narum**. 2008. Characterization of 22 novel single nucleotide polymorphism markers in steelhead and rainbow trout. *Molecular Ecology Resources* (*In Press*).

Narum, S. R., M. Banks, T.D. Beacham, M.R. Bellinger, M.R. Campbell, J. DeKoning, A. Elz, C.M. Guthrie III, C. Kozfkay, K.M. Miller, P. Moran, R. Phillips, L.W. Seeb, C.T. Smith, K. Warheit, S.F. Young, J.C. Garza. 2008. Differentiating salmon populations at broad and fine geographic scales with microsatellites and SNPs. *Molecular Ecology* 17:3464-3477.

Narum S. R., D. Hatch, A. J. Talbot, P. Moran, and M. S. Powell. 2008. Conservation of iteroparous salmonids in complex mating systems. *Journal of Fish Biology* 72:45-60.

Kalinowski, S. T., M. H. Meeuwig, S. R. **Narum**, M. L. Taper. 2008. Stream trees: a statistical method for mapping genetic differences between populations of freshwater organisms to the sections of streams that connect them. *Canadian Journal of Fisheries and Aquatic Sciences* (*In Press*).

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Education

B.S., Biology w/ Chemistry Minor, Eastern Michigan University

Appointments

2008-present Genomics Researcher, Columbia River Inter-Tribal Fish Commission
2006-2008 Genetics Laboratory Technician, University of Idaho

Selected Publications

Fang M, Li J, Blauwkamp T, Bhambhani C, **Campbell N**, Cadigan K (2006) C-terminal-binding protein directly activates and represses Wnt transcriptional targets in *Drosophila*. *EMBO Journal* **25**: 2735-2745

Campbell N, and Narum S (2008) Identification of Novel Single-Nucleotide Polymorphisms in Chinook Salmon and Variation among Life History Types. *Transactions of the American Fisheries Society* **137**: 96-106

Campbell, N. R., K. Overturf, and S.R. Narum. (2008) Characterization of 22 novel single nucleotide polymorphism markers in steelhead and rainbow trout. (*In Press at Molecular Ecology Resources 06-2008*)

Campbell N, and Narum S (2008) Quantitative PCR assessment of microsatellite and SNP genotyping with variable quality DNA extracts. *Conservation Genetics*, DOI 10.1007/s10592-008-9661-7.

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Education

B.S., Biology, Pacific Lutheran University, 1990
B.S., Microbiology, Washington State University, 1995
M.S., Fisheries Genetics University of Alaska Fairbanks, 2002

Appointments

2008-present	Conservation Geneticist, CRITFC
2004-2008	Fishery Biologist/Geneticist, USFWS, Abernathy

Selected Publications

Microsatellite Variation Indicates Population Genetic Structure of Bocaccio. *North American Journal of Fisheries Management* 24:1189-1202, 2004.

Two Genetically Distinct Forms of Rougheye Rockfish (*Sebastes aleutianus*) are Different Species. *Transactions of the American Fisheries Society* 134:242-260, 2005.

A genetically distinct wild redband trout (*Oncorhynchus mykiss gairdneri*) population in Crane Prairie Reservoir, Oregon, persists despite extensive stocking of hatchery rainbow trout (*O. m. irideus*). Andrew P. Matala Æ Steven Marx Æ Ted G. Wise. Conservation Genetics, DOI 10.1007/s10592-008-9527-z, 2008.

Genetic Distinction of Winter-Run and Summer-Run Steelhead in the Hood River, Oregon: Feasibility of Using Genetic Assignment Tests to Identify Ecotype. Bonneville Power Admin. Report, Contract No. 00013429 and 00018702. *Submitted for Publication to: Transactions of the American Fisheries Society.*