



INFLUENCE OF LANDSCAPE AND ENVIRONMENT ON SALMONID GENETICS

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Executive Summary/Abstract

a. Fish Population RM&E

This project addresses two objectives related to environmental and landscape features that contribute to population structure, life history diversification, and local adaptation of salmonids in the Columbia River.

Objective 1) Environment & Landscape Genetics – Evaluate genetic structure of natural populations of salmonids relative to their environment and identify candidate markers associated with traits that are related to local adaptation of steelhead and Chinook salmon populations.

For Objective 1, work has progressed on sequencing Chinook salmon and steelhead throughout the Columbia River Basin to evaluate neutral and adaptive genetic variation related to environmental features. Our recent results indicate that environmental features are strong drivers of adaptive genomic divergence in salmonid species, and provide a foundation to investigate how populations might respond to global environmental change (Matala et al. 2014; Hand et al. 2015; Hecht et al. 2015; Micheletti et al. *in prep*). Broad geographic patterns of neutral and non-neutral variation demonstrated in these studies can be used to accommodate priorities for regional management and inform long-term conservation of Chinook salmon and steelhead in the Columbia River. Results from these studies indicate that temperature and precipitation are consistently the main environmental factors influencing genetic variation in salmonids, but other variables may also contribute for specific populations.

Objective 2) Controlled & Natural Experiments – experiments with controlled environmental variables to validate phenotypic response of fish with given genotypes.

For Objective 2, empirical work was done to further advance our understanding multiple traits related to recovery of salmonids in the Columbia River. Recent work focused on genomic regions associated with run-timing in steelhead and Chinook salmon, and thermally adapted strains of redband trout under heat stress. Results from association mapping of steelhead migration run-timing identified a major effect gene that can be used for broad examination of this trait in the Columbia River Basin (Hess et al. 2016). For thermal adaptation, results from genome scans and gene-expression profiles (Narum et al. 2010; Narum et al. 2013; Narum et al. 2015; Garvin et al. 2015) suggest an adaptive response of *Oncorhynchus mykiss gairdneri* to survive under increasing temperatures, and this is valuable information for fisheries and conservation efforts. Further, progress was also made towards developing projects to investigate the genomic basis for age-at-maturity in Chinook salmon and expansion of work to investigate thermal adaptation in anadromous stocks of *O. mykiss* in addition to resident stocks.

2. Introduction

Environmental and landscape features can greatly contribute to the population structure, life history diversification, and local adaptation of organisms in aquatic habitats (reviewed in Storfer et al. 2006). Geographic barriers to dispersal include recent events that may have been human induced (e.g., dams) as well as ancient events such as glaciations and formation of mountain chains (e.g., Castric et al. 2001). However, other environmental characteristics such as elevation, temperature, forest cover, and precipitation may influence distribution, adaptation, and gene flow of species (Dionne et al. 2008; Narum et al. 2008). For example, the geographic distributions of species ranges' are often determined by thermal tolerance (Brannon et al. 2004) and may necessitate adaptations for survival in extreme environments (Rodnick et al. 2004).

Screening with many genetic markers provides the opportunity to investigate local adaptation in natural populations and identify candidate genes under selection (Beaumont and Nichols 1996; Beaumont and Balding 2004; Excoffier et al. 2009). This has become a commonly employed approach in ecological and population genetics studies to detect outlier loci that are putatively under selection (e.g., Vasemagi and Primmer 2005; Nosil et al. 2008). Additionally, correlation methods can be highly informative to identify markers in coding and cis-regulatory regions of known functional genes that are associated with specific selective pressures or

phenotypes (Lyman and Mackay 1998; Chase et al. 2009; Torgerson et al. 2009). With increasing genomic information available for non-model organisms, single nucleotide polymorphisms (SNPs) have begun to see increased use as genetic markers for population genetic studies (e.g., Morin et al. 2004). These sequence polymorphisms are densely scattered throughout the genome of most organisms, and are commonly observed in both coding and non-coding regions of functional genes making them ideal markers to study adaptive molecular variation (e.g., Akey et al. 2002). In a large suite of unlinked SNPs that are distributed across the genome (e.g., Campbell et al. 2009), it is possible to utilize both functionally neutral and adaptive markers within a single study. This combination of information provides a powerful approach to study questions in ecological genetics since both demographic processes (i.e., gene flow and genetic drift) and local adaptation (i.e., selection) may be inferred.

Molecular techniques such as RNA-seq (Wolf 2013) also provide the opportunity to investigate transcriptional response to thermal stress and further identify mechanisms for thermal adaptation. Patterns of gene expression under heat stress are important to determining evolutionary adaptation among conspecific populations that occupy various environments. Multiple genes have been shown to be involved in heat tolerance across many species, including highly conserved heat shock proteins (hsps) that are upregulated under stressful conditions such as exposure to heat (Morimoto et al. 1992; Sorensen et al. 2003). An adaptive heat shock response has additionally been shown to occur among conspecific populations that occupy variable environments (e.g., Dahlhoff and Rank 2000; Sorensen et al. 2001). However, many genes are known to have a role in regulating the effects of temperature and are likely to be involved in thermal adaptation (Sorensen et al. 2005; Kassahn et al. 2007). Thus, RNA-seq provides the opportunity to investigate differential expression across the transcriptome and identify biological pathways involved in evolutionary response to thermal stress.

Thus, genome scans with large numbers of SNP markers (e.g., RAD sequencing) and gene expression (e.g., RNA-seq) approaches may be effective tools for identifying the genetic architecture underlying specific traits such as thermal tolerance, run-timing, disease resistance, and age-at-maturity. Once these underlying genomic regions are identified, they can be broadly screened throughout the Columbia River Basin to facilitate management for long term conservation and recovery of salmonids.

a. Fish Population RM&E

F&W Program Strategy: Assess the status and trend of diversity of natural and hatchery origin fish populations.

F&W Program Management Question: What are the status and trend of diversity of natural and hatchery origin fish populations?

Uncertainty Research

Identify and compare adaptive genetic variation relative to neutral variation in salmonid stocks in the Columbia River.

Project Map:

<http://www.cbfish.org/Project.mvc/Map/2009-005-00>

Contract Map(s):

<http://www.cbfish.org/Contract.mvc/Map/61839>

<http://www.cbfish.org/Contract.mvc/Map/65575>

3. Methods: Protocols, Study Designs, and Study Area

Method Title: RAD sequencing v1.0

Method Link: <http://www.monitoringmethods.org/Method/Details/4144>

Method Summary:

RAD sequencing is a technique for tagging DNA at restriction enzyme cut sites with adapters used in massively parallel sequencing. This method allows thousands of SNPs to be discovered and genotyped in several individuals. Through the use of sample specific DNA barcodes included in the adapters, information for specific samples can be separated in silico following sequencing. This method effectively reduces sequence complexity by targeting only sequence surrounding restriction enzyme cut sites making alignments among sequencing reads far less computationally intense. The sequence alignments among samples can then be analyzed for both identification and genotyping of SNPs (Single Nucleotide Polymorphisms). This method was first described by Baird et al. (2008).

Method Title: Obtain gene expression data via RNAseq v1.0

Method Link: <http://www.monitoringmethods.org/Method/Details/607>

Method Summary:

Compare gene expression between fish of different genetic backgrounds but raised in the same environment. Molecular techniques such as RNAseq provide the opportunity to investigate transcriptional response and further identify mechanisms for thermal adaptation. Patterns of gene expression are important to determining evolutionary adaptation among conspecific populations that occupy various environments.

4. Results

a. Fish Population RM&E

Objective 1) New results:

For steelhead in the Columbia River, new environmental variables for migratory paths in mainstem rivers were incorporated into landscape genomics analyses. Results indicate that the most influential drivers of adaptive divergence were represented by variables along migration paths rather than at-site conditions (Micheletti et al *in prep*). Anadromous fish experience environmental conditions along their migration path to the ocean that may not coincide with conditions in freshwater spawning and maturing sites. Consequently, migration-path habitat may pose stronger selective pressures than habitat within tributaries. We used landscape genomic analyses to determine if conditions encountered along migration-paths drive adaptive genomic divergence more so than at-site conditions in 2548 anadromous steelhead trout (*Oncorhynchus mykiss*) collected from 56 sites across the Columbia Basin. We first compiled 21 non-correlated environmental variables that are hypothesized to be associated with adaptive genomic variation and extracted measurements of these environmental variables at freshwater collection sites, as well as along migration paths to the ocean for each population. We then used a multivariate method, redundancy analysis (RDA), to determine which landscape variables contribute the most to adaptive variation in 24,526 single nucleotide polymorphisms (SNPs). Finally, in conjunction with RDA, we implemented a series of outlier analyses and gene-environment association tests to identify SNPs that show the strongest evidence for selection to environments across the landscape. Our results show that the most influential drivers of adaptive divergence were overwhelmingly represented by variables along migration paths rather than at-site conditions. These variables include the migration distance to the ocean, the maximum temperature encountered along migration paths, and the mean precipitation encountered along migration paths. Furthermore, we identified 81 evident outlier SNPs that which are primarily associated with temperature and precipitation along migration paths. Together, our results indicate riverscape features that are strong drivers of adaptive genomic divergence in *O. mykiss*, and provide a basis to investigate how *O. mykiss* might respond to predicted scenarios of global climate change.

Objective 2) New results:

Results from association mapping of steelhead migration run-timing identified a major effect gene that can be used for broad examination of this trait in the Columbia River Basin. Migration traits are presumed to be complex and to involve interaction among multiple genes, thus we employed both univariate analyses and multivariate Random Forest (RF) machine learning algorithm to conduct association mapping of 15,239 single nucleotide polymorphisms (SNPs) for adult migration-timing phenotype in steelhead (*Oncorhynchus mykiss*). Our study focused on a model natural population of steelhead that exhibits two distinct migration-timing life histories with high levels of admixture in nature. Neutral divergence was limited between fish exhibiting summer- and winter-run migration owing to high levels of interbreeding, but a univariate mixed linear model found three SNPs from a major effect gene to be significantly associated with migration-timing ($P < 0.000005$) that explained 46% of trait variation. Alignment to the annotated *S. salar* genome provided evidence that all three SNPs localize within a 46 kb region within GREB1-like (an estrogen target gene) on chromosome Ssa03. Additionally, multivariate analyses with RF identified that these 3 SNPs plus 15 additional SNPs explained up to 60% of trait variation. These candidate SNPs may provide the ability to predict adult migration-timing of steelhead to facilitate conservation management of this species and this study demonstrates the benefit of multivariate analyses for association studies. These results are published in Hess et al. (2016).

5. Synthesis of Findings: Discussion/Conclusions

a. Fish Population RM&E

Objective 1)

Our recent results indicate that environmental features are strong drivers of adaptive genomic divergence in salmonid species, and provide a foundation to investigate how populations might respond to global environmental change (Matala et al. 2014; Hand et al. 2015; Hecht et al. 2015; Micheletti et al. *in prep*). Broad geographic patterns of neutral and non-neutral variation demonstrated in these studies can be used to accommodate priorities for regional management and inform long-term conservation of Chinook salmon and steelhead in the Columbia River. Results from these studies indicate that temperature and precipitation are consistently the main environmental factors influencing genetic variation in salmonids, but other variables may also contribute for specific populations.

Objective 2)

Candidate genetic markers that were highly associated with steelhead run-timing (Hess et al. 2016) may help predict the adult migration timing of individual steelhead

throughout the entire course of their life cycle, which would benefit long-term conservation management of this protected species. Ability to identify the genetic propensity for migratory traits in steelhead would be useful for a multitude of applications including characterizing differences associated with these adult alternative migration tactics that pertain to pre-adult life stages (e.g. juvenile migration and size-at-age), and categorizing adults on spawning grounds into migration categories. Currently, steelhead are categorized into summer- or winter run based on the timing when they enter streams near the mouth of their natal tributary. However, steelhead may overwinter in freshwater areas outside of their natal tributary, which complicates their classification as summer- or winter-run, and therefore underscores the need for a method of genetic classification. Thus, these candidate SNPs may provide the ability to predict adult migration timing of steelhead to facilitate conservation management of this species.

Results from genome scans and gene-expression profiles (Campbell and Narum 2009; Narum and Campbell 2010; Narum et al. 2010; Narum et al. 2013; Narum et al. 2015; Garvin et al. 2015) suggest an adaptive response of *Oncorhynchus mykiss gairdneri* to survive under increasing temperatures, and this is valuable information for fisheries and conservation efforts. These patterns could be used to monitor the health of populations of fish that are exposed to higher temperature regimes as the climate continues to warm. Genetic markers that have been determined to be associated with thermal tolerance and local adaptation are being screened more broadly in populations throughout the Columbia River to evaluate the adaptive potential of specific populations under scenarios of climate change. This information is expected to identify conservation priorities and assist in determining management plans for long term persistence of salmonids.

Additional traits that have been studied include residence vs. anadromy in *O. mykiss* and *O. nerka* (Narum et al. 2011; Hecht et al. 2013; Nichols et al. 2016), and disease resistance in *O. mykiss* (Overturf et al. 2012; Campbell et al. 2014). Results from those studies have identified genetic markers that are associated with specific traits that are important for long-term conservation of life history variation in the Columbia River. Further, projects continue to develop to investigate the genomic basis for age-at-maturity in Chinook salmon and steelhead, run-timing in Chinook salmon (Hess et al. 2011) and steelhead, and expansion of work to investigate thermal adaptation in Chinook salmon and anadromous stocks of *O. mykiss* in addition to resident stocks.

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Appendix A: Detailed Results

Figure 1. Results from Hess et al. (2016) in that demonstrate candidate SNPs from GREB1 gene associated with adult migration timing in winter- and summer-run steelhead. STRUCTURE plot of individual ancestry to two clusters for (a) 18 candidate SNPs and (c) 180 non-candidate SNPs. Individuals were ordered by their migration timing into the Klickitat River and grouped by the summer-run (May-Aug), transitional (Sep-Nov), and winter-run (Dec-Apr) periods. Plots of the Mantel test correlation between individual ancestry and day of migration (units are ordinal day as reordered summer-winter) are shown for (b) 18 candidate SNPs, and (d) 180 non-candidate SNPs.

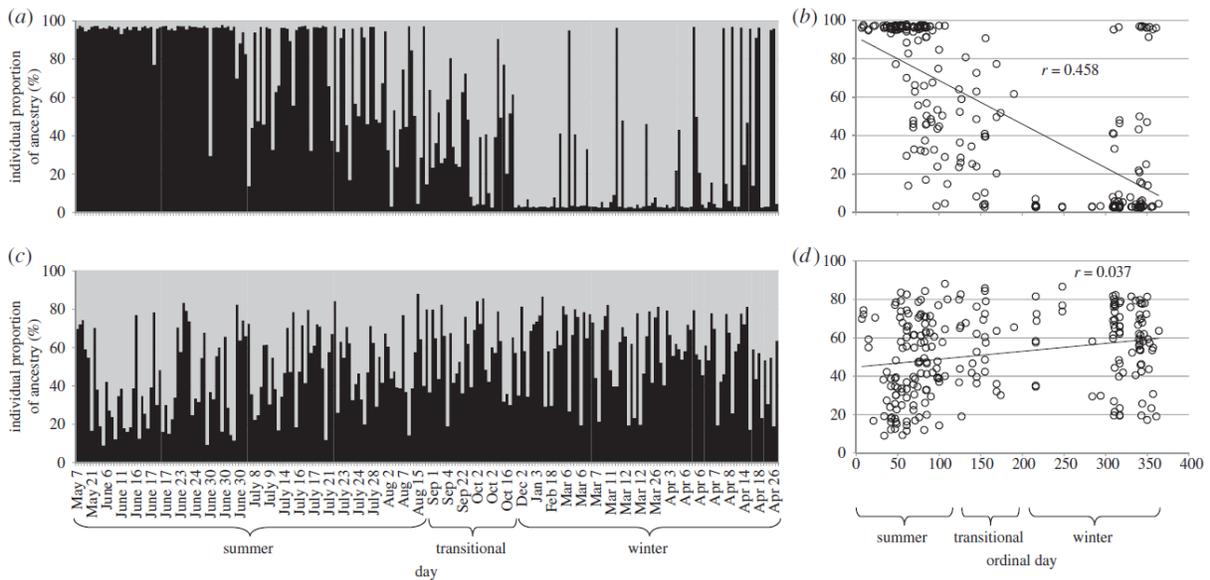
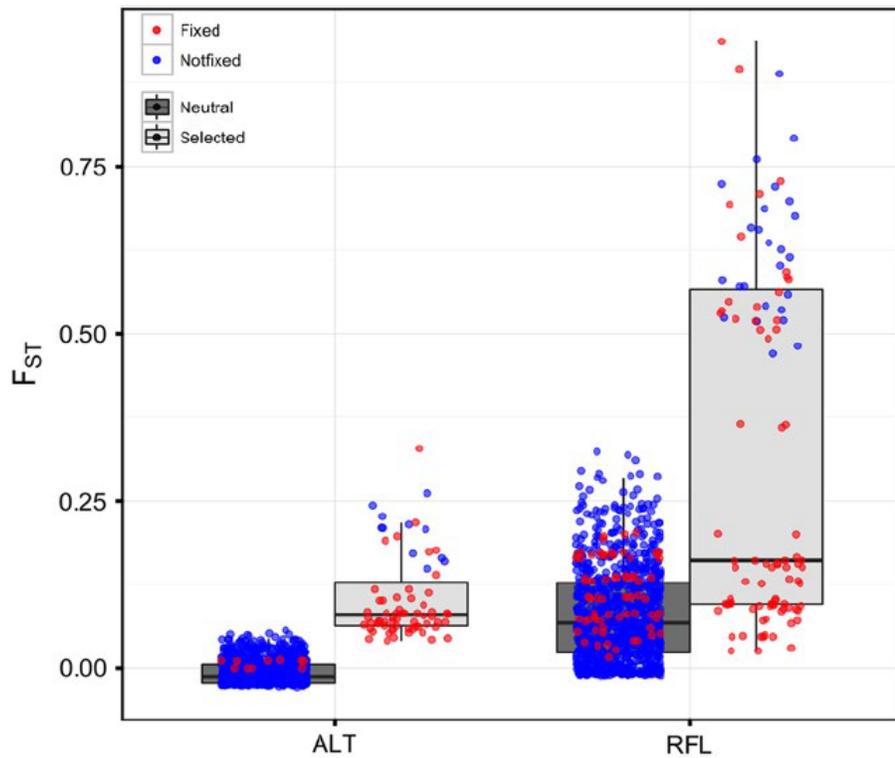


Figure 2: Results from Nichols et al. (2016) in that demonstrate divergence of neutral and outlier SNPs for resident and anadromous *O. nerka* in Alturas Lake (ALT) and Redfish Lake (RFL). Boxplots for pairwise F_{ST} calculated from the neutral ($n = 1036$) and divergent selected loci in each population pair. F_{ST} values were calculated in LOSITAN. Red and blue colors denote whether or not the locus was fixed in one of the two populations considered in each pairwise comparison.



Appendix B: List of Metrics and Indicators

Category	Subcategory	Subcategory Focus 1	Subcategory Focus 2	Specific Metric Title
Fish	Composition: Fish Species Assemblage	Fish Life Stage: Juvenile - Alevin	Fish Origin: Natural	Fish stock analysis based on genetics
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		Understand genetic relationship of steelhead
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Natural		Steelhead diversity and variation based on genetics
Fish	Presence/Absence: Fish	Fish Life Stage: Juvenile - Stream Type		Hatchery/out of basin wild steelhead presence based on genetics
Fish	Stray Rate	Fish Origin: Both		out of basin stray spawning or introgression rate
Fish	Tissue Sample: Fish			Fish tissue samples for genetics
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		
Fish	Composition: Fish Species Assemblage	Fish Life Stage: Juvenile - Alevin	Fish Origin: Natural	Fish stock analysis based on genetics
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		Understand genetic relationship of steelhead

Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Natural		Steelhead diversity and variation based on genetics
Fish	Presence/Absence: Fish	Fish Life Stage: Juvenile - Stream Type		Hatchery/out of basin wild steelhead presence based on genetics
Fish	Stray Rate	Fish Origin: Both		out of basin stray spawning or introgression rate
Fish	Tissue Sample: Fish			Fish tissue samples for genetics
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		
Fish	Genetics: Fish Diversity, Fitness or Variation	Fish Origin: Both		