INFLUENCE OF LANDSCAPE AND ENVIRONMENT ON SALMONID GENETICS

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Executive Summary

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 adaptation of salmonids.

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 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 adaptive genetic variation related to environmental features.

Objective 2) Controlled 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. Work focused on patterns of gene expression of various strains of redband trout under heat stress, run-timing in Chinook salmon and steelhead. Further, progress was also made towards developing projects to investigate the genomic basis for age-at-maturity in Chinook salmon and thermal tolerance in *O. mykiss*.

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) 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.

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. 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 in 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 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) We evaluated genetic variation among steelhead trout of the Columbia River Basin, which supports diverse populations distributed among dynamic landscapes. We categorized 188 SNP loci as either putatively neutral or candidates for divergent selection (non-neutral) using a multi-test association approach. Neutral variation distinguished lineages and defined broad-scale population structure consistent with previous studies, but fine-scale resolution was also detected at levels not previously observed. Within distinct coastal and inland lineages, we identified 9

and 22 candidate loci (respectively) commonly associated with precipitation or temperature variables, and putatively under divergent selection.

Objective 2)
In this study, we tested for differential transcriptional response of ecologically divergent populations of redband trout (*Oncorhynchus mykiss gairdneri*) that have evolved in desert and montane climates. Each pure strain and their F1 cross were reared in a common garden environment and exposed over four weeks to diel water temperatures that were similar to those experienced in desert climates within the species' range. Gill tissues were collected from the three strains of fish (desert, montane, F1 crosses) at the peak of heat stress and tested for mRNA expression differences across the transcriptome with RNA-seq. Strong differences in transcriptomic response to heat stress were observed across strains confirming that fish from desert environments have evolved diverse mechanisms to cope with stressful environments. As expected, a large number of total transcripts (12,814) were differentially expressed in the study (FDR $\leq$ 0.05) with 2310 transcripts in common for all three strains, but the desert strain had a larger number of unique differentially expressed transcripts (2875) than the montane (1982) or the F1 (2355) strain. Strongly differentiated genes (> 4 fold change and FDR $\leq$ 0.05) were particularly abundant in the desert strain (824 unique contigs) relative to the other two strains (montane = 58; F1 = 192).

5. Synthesis of Findings: Discussion/Conclusions

a. Fish Population RM&E

Objective 1)
Observed patterns of non-neutral variation suggest overall climate is likely to shape local adaptation (e.g., potential rapid evolution) of steelhead trout in the Columbia River region. Broad geographic patterns of neutral and non-neutral variation demonstrated here can be used to accommodate priorities for regional management and inform long-term conservation of this species.

Objective 2)
This study of *Oncorhynchus mykiss gairdneri* demonstrated patterns of acclimation (i.e., phenotypic plasticity) within strains and evolutionary adaptation among strains in numerous genes throughout the transcriptome. Key stress response genes such as molecular chaperones (i.e., heat shock proteins) had adaptive patterns of gene expression among strains, but also a much higher number of metabolic and cellular

process genes were differentially expressed in the desert strain demonstrating these biological pathways are critical for thermal adaptation to warm aquatic climates. The results of this study further elucidate the molecular basis for thermal adaptation in aquatic ecosystems and extend the potential for identifying genes that may be critical for adaptation to changing climates.

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Appendix A: Detailed Results

Figure 1. Neighbor joining trees depicting Nei's genetic distances among inland lineage populations of steelhead. Numbers at branch ends correspond to population reference numbers (Supplemental 1) Trees are based on a) neutral variation – 146 SNPs, and b) non-neutral variation – 22 SNPs. Bootstrap support exceeding 50% appears at nodes. Population symbols correspond with DPS: triangles – Snake River, circles – upper Columbia River, squares – middle Columbia River. Major drainage sub-basins are labeled on the left. The symbol (*) represents climate related distinction among Lower Clearwater River populations and (£) represents differences among Yakima River populations based on climate. The symbol (®) identifies the branch adjoining Clearwater River and M. F. Salmon River populations. The color scale indicates population ranking by climate (1=coldest, 115=warmest) for 115 inland populations identified by reference number (Supplemental 1). (Matala et al. 2014)

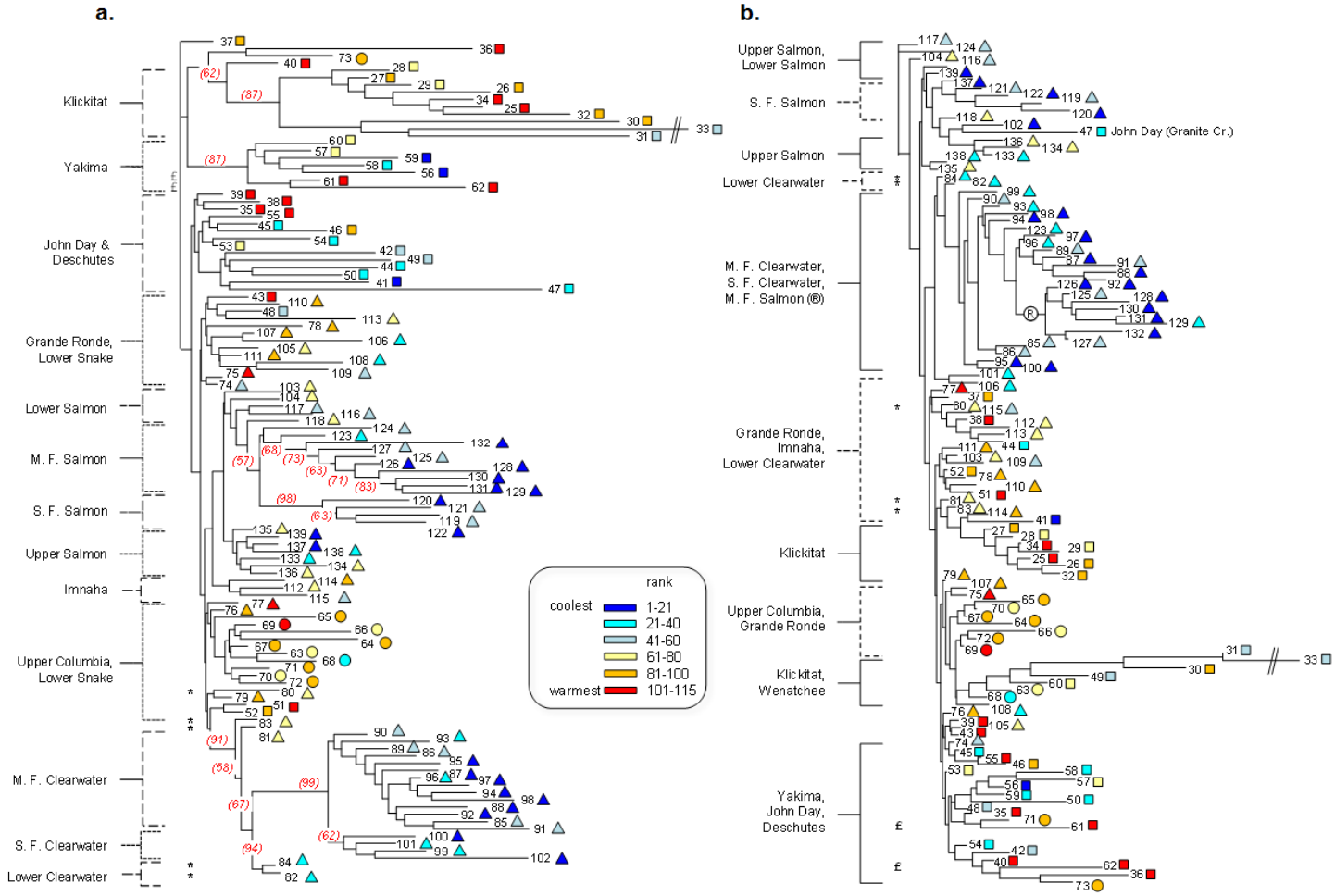
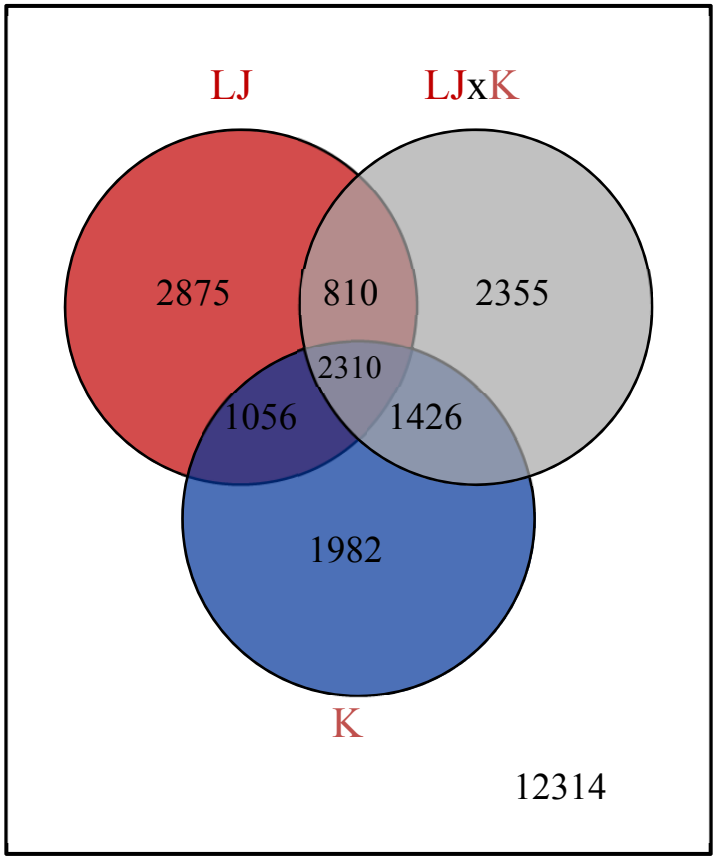
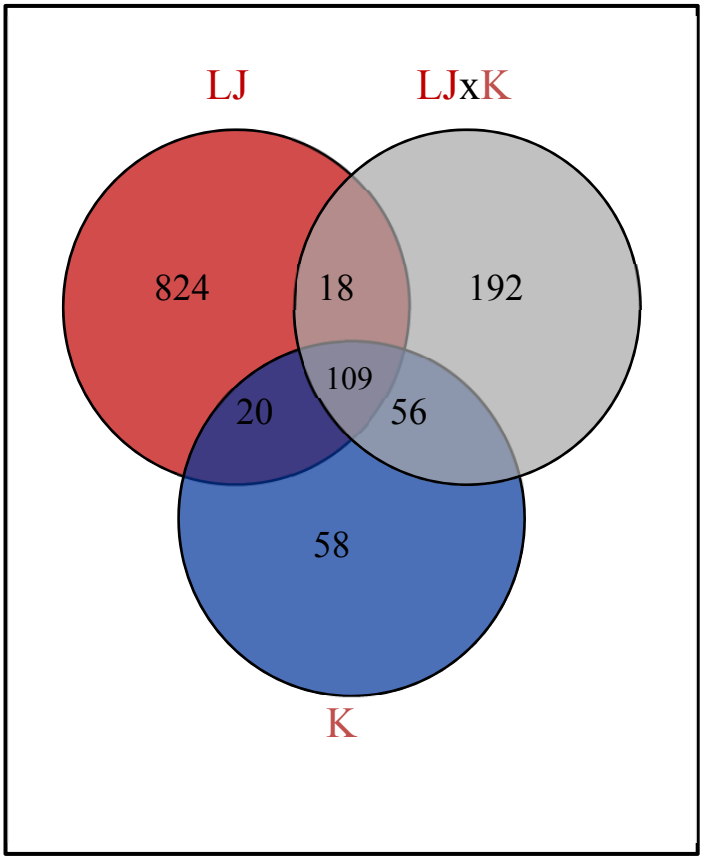


Figure 2: Venn diagrams of differentially expressed genes. Results of each strain of redband trout for a) all significant transcripts (FDR ≤ 0.05); and b) strongly differentiated transcripts (> 4 fold change and FDR ≤ 0.05). Circles are color coded to represent fish by their environment: desert strain (red; LJ=Little Jacks Cr.), F1 crosses (gray; LJxK), montane strain (blue; K=Keithley Cr.). For a) there were 12,314 genes that were not statistically significant at either level and are listed outside of the circles on the Venn diagram. (Narum et al. 2014)

a) All significant genes



b) Strongly differentiated genes (>4 fold)



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 diveristy 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	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 diveristy 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		