



# **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*

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. 2018a). 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. Recent results also emphasize that environmental conditions through the migratory corridor are stronger drivers of selection than those at natal sites for steelhead in the Columbia River Basin (Micheletti et al. 2018a).

#### *Objective 2) Genetic Basis for Phenotypic Expression of Traits*

For Objective 2, empirical studies have been implemented to advance our understanding of multiple traits related to recovery of salmonids in the Columbia River. Work has focused on genomic regions associated with resident vs. anadromous *O. mykiss* life histories (Narum et al. 2008; Hecht et al. 2013), run-timing related to maturity in steelhead (Hess et al. 2016; Micheletti et al. 2018c) and Chinook salmon (Narum et al. 2018), age at maturity in Chinook salmon (Micheletti and Narum 2018b), disease resistance in *O. mykiss* (Campbell and Narum 2015), and thermally adapted strains of redband trout under heat stress (Narum et al. 2010; Narum et al. 2013; Garvin et al. 2015; Narum et al. 2015, Chen et al. 2018a; Chen et al. 2018b). Progress has also been made towards investigating the genomic basis for age-at-maturity in Chinook salmon, maturation and run-timing in Chinook salmon and steelhead, and development of studies to investigate thermal adaptation in anadromous stocks of *O. mykiss* and age/size at maturity in steelhead (A vs. B run). As these candidate genes for traits have begun to be identified,

SNP markers from these regions are being incorporated in standard genotyping panels with GTseq in order to validate and monitor genetic variation for these traits in large numbers of individuals.

## 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 SNPs that are distributed across the genome (e.g., Narum et al. 2018), 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, Baird et al. 2008; Pool-seq, Schlotterer et al. 2014) 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/maturation, disease resistance, anadromy, and age-at-maturity. Once these underlying genomic regions are identified, they can be broadly screened in populations 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

See Appendix A for table of critical uncertainties research.

#### 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:** Whole Genome Resequencing

- **Method Link:**
- [Whole Genome Resequencing: Poolseq Individual Barcode v1.0](#) (ID: 6754) Published
- [Whole Genome Resequencing: Poolseq Pooled v1.0](#) (ID: 6775) Published

#### Method Summary:

With reference genome assemblies now publicly available for both Chinook salmon and steelhead, this enables whole genome resequencing for investigating adaptive variation across a large portion of the genome (50-80%) in these species. Whole genome resequencing methods include individually barcoded samples, or pools of samples (Pool-seq; Schlotterer et al. 2014) depending on the study design. For both

methods, sequence data is aligned to the reference genome assembly, and allele frequencies from millions of SNPs are analyzed to detect statistically significant regions of the genome associated with specific traits or adaptation to environmental factors. Putatively neutral regions of the genome are also useful for standard phylogeny and demographic analyses of populations. In most studies, allele frequencies are available for collections but sequencing depth is typically not high enough to provide individual genotypes. However, candidate SNPs may be developed into standard panels with GTseq or other approaches to genotype many individuals to validate trait association, determine inheritance, and estimate linkage disequilibrium.

**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) Environment & Landscape Genetics

##### *Hypotheses:*

Hypothesis 1: Environmental and landscape features act as drivers of selection leading to local adaptation of fish populations. Testing many variables can identify the key environmental drivers of selection.

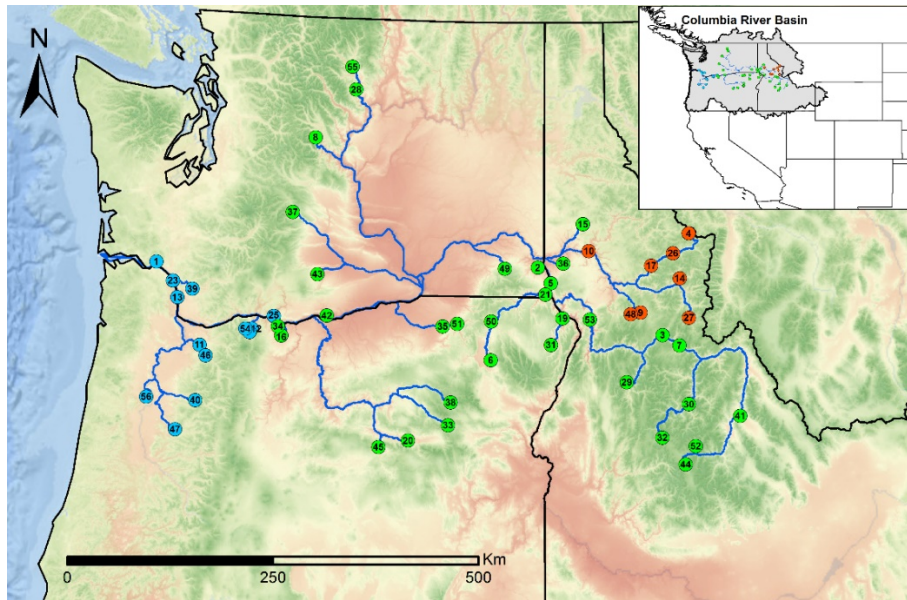
Hypothesis 2: Environmental drivers of selection act on specific genes resulting in different allelic and genotypic frequencies among adapted populations. Genome scans can identify candidate genes involved in local adaptation of fish populations across heterogeneous landscapes.

##### *Activities implemented:*

Multiple studies have been initiated to investigate local adaptation with a landscape genetics/genomics approach to address the two hypotheses above. Our work has focused on steelhead and Chinook salmon throughout the Columbia River Basin. Studies continue to progress as more markers become available throughout the genome of each species.

For steelhead, landscape genetics work has been done at both fine and broad scales. Fine scale landscape genetics approaches were used to identify ecological patterns of residence vs anadromy and found consistent evidence that certain landscape features have led to prevalence of certain life history types in *O. mykiss* (Narum et al. 2008a) but also *O. nerka* (Nichols et al. 2016). Broad scale studies were initially done with panel of 188 SNP markers to investigate patterns of landscape genetics across 145 populations in the Columbia River Basin (Matala et al. 2014). Results indicated that precipitation and temperature were the primary environmental drivers of local adaptation and neutral genetic structure largely reflected isolation by distance. These results were shown to be robust due to replication of multiple populations representing each distinct genetic unit (Hand et al. 2016). A follow-up study (Micheletti et al. 2018a) was done with a much larger number of genetic markers (~20K SNPs) at broad scale and found evidence that the mainstem Columbia River migratory corridor exhibits greater selective pressure on steelhead than natal tributaries (Figure 1). Populations that must migrate long distances through the migratory corridor were under the most intense selection and multiple candidate genes were identified (Micheletti et al. 2018a). Additional studies are underway to further pinpoint candidate genes through whole genome resequencing.

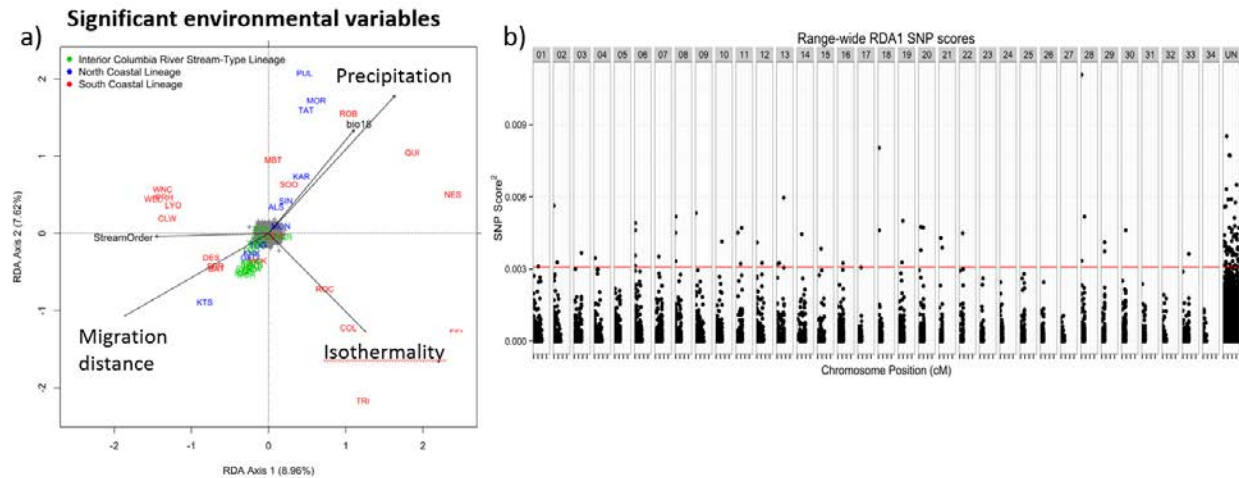
**Figure 1. Steelhead collection localities colored by genetic cluster (from Micheletti et al. 2018a). blue = coastal, green = inland, red = inland-Clearwater. Blue lines indicate each population's migration path to the ocean. Map layer shows mean annual temperature for the warmest quarter with transition from colder (green) to warmer (red).**



For Chinook salmon, landscape genetics work has also been done at both fine and broad scales. Initial studies evaluated the utility of certain marker types for landscape genetics and found consistency in signals among marker types (Narum et al. 2008b; Hess et al. 2011), but that SNPs provided greater potential for identifying candidate genes involved in local adaptation and should be used in subsequent studies (Narum et al. 2013a). Broad scale studies were initially done with panel of 96 SNP markers to investigate patterns of connectivity related to landscape features across 54 populations in the Columbia River Basin (Matala et al. 2011). Results indicated that precipitation, elevation, and temperature were the primary environmental drivers of local adaptation depending on genetic lineage, and neutral genetic structure largely reflected isolation by distance within each lineage (Matala et al 2011). A follow-up study (Hecht et al. 2015) was done with a much larger number of genetic markers (~20K SNPs) at broad scale and found that between 6-22% of genetic variation could be accounted for by environmental features such as precipitation, temperature, and migration distance (Figure 2a). Several candidate markers were associated with local adaptation within and among lineages (Figure 2b; Hecht et al. 2015). A follow-up study to investigate local adaptation through whole genome resequencing has demonstrated that a broad portfolio of diversity persists in Chinook salmon and candidate genes for local adaptation are widespread throughout the genome within and among lineages (Narum et al. 2018). Additional studies are underway to represent genome wide variation for a more thorough set of populations throughout the Columbia River Basin.



**Figure 2.** a) Environmental drivers of selection and b) candidate markers for local adaptation in Chinook salmon (from Hecht et al. 2015). a) Population scores for canonical RDA axis 1 and 2 are represented by the three-letter abbreviation for each population, coloured to represent the lineage assignment of that population. SNP variance is indicated by the position of grey '+' symbols radiating from the plot centroid, each representing a different locus. Environmental factors are depicted as black vectors (arrows), where the length of the vector is a representation of the magnitude of the contribution of that environmental variable in explaining SNP variance. The angle between environmental variable vectors is a representation of the correlation between those variables. Vectors and points are plotted with symmetrical scaling (scale = 3) to preserve the relationship between scores, without focusing on a single score.



### *Summary of accomplishments & lessons learned:*

Steady progress has been made to better understand landscape genomics and neutral vs. adaptive patterns of genomic variation for steelhead and Chinook salmon as reflected in 11 publications (Narum et al. 2008a; Narum et al. 2008b; Matala et al. 2011; Hess et al. 2011; Narum et al. 2013a; Matala et al. 2014; Hecht et al. 2015; Hand et al. 2016; Nichols et al. 2016; Micheletti et al. 2018a; Narum et al. 2018).

Results consistently show specific environmental variables are drivers of selection in both steelhead and Chinook including precipitation, temperature, and migration distance through the mainstem Columbia River. Neutral variation across studies consistently accounts for highly distinct lineages of each species, and genetic structure within each lineage that is related to geographic location by sub-basins. While adaptive genetic variation can be evident with modest numbers of SNP markers, specific candidate genes are best pinpointed by genome resequencing.

### **Objective 2) Genetic Basis for Phenotypic Expression of Traits**

#### *Hypotheses:*

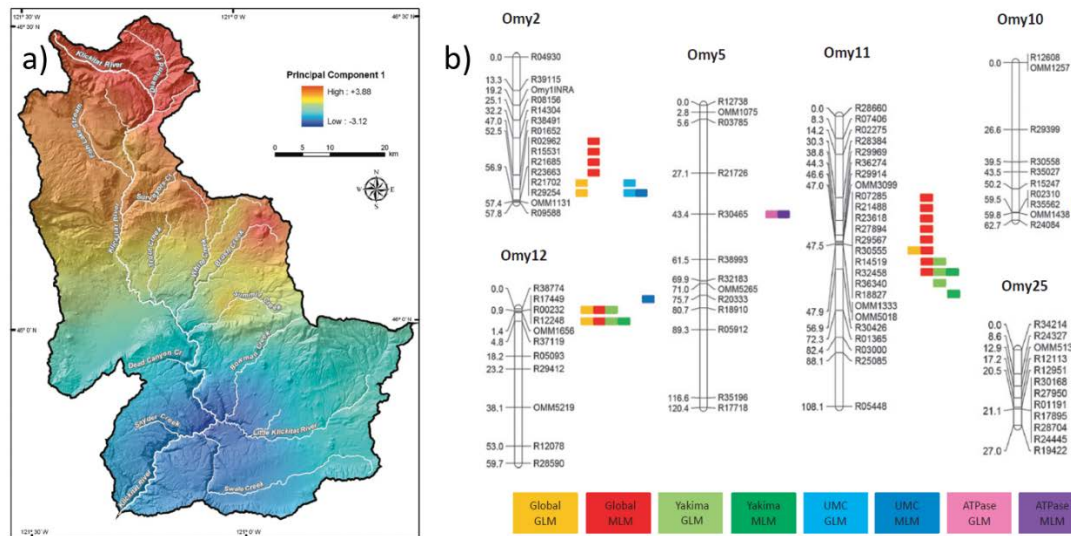
Hypothesis 1: Many phenotypic traits include a heritable component that must have a genetic basis, which can be detected with association mapping and gene expression to identify candidate genes associated with specific traits.

Hypothesis 2: Markers from candidate genes can be developed into cost efficient assays for genotyping large numbers of individuals to monitor genetic variation for phenotypic traits at broad scales, with the potential to predict resiliency of populations to environmental changes.

### Activities implemented:

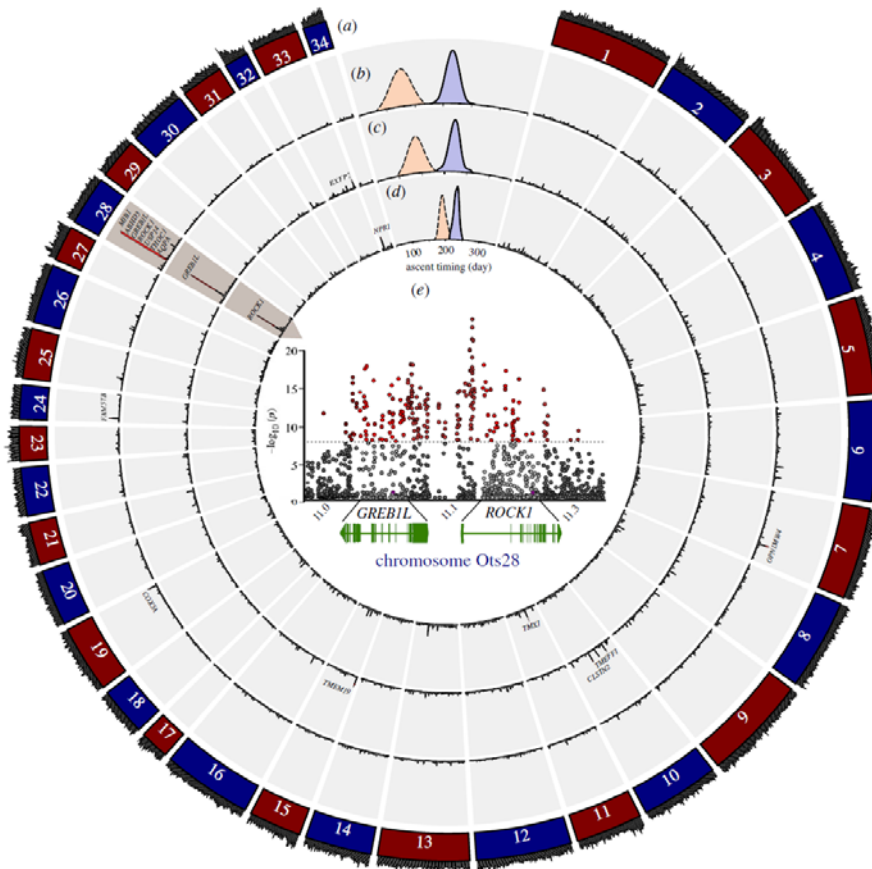
Multiple studies have been initiated to investigate the genetic basis for multiple phenotypic traits using association mapping or gene expression approaches. Work has focused on genomic regions associated with several different traits. Work on resident vs. anadromous *O. mykiss* (Narum et al. 2008a; Hecht et al. 2013) has revealed that a combination of environmental and genetic factors contribute to determination of these life history types (Figure 3).

**Figure 3. a) Synthesis map illustrating interpolation principal component (PC1) that accounts for 59% of variation in resident (red) vs anadromous (red) life history (from Narum et al. 2008a). b) Significant loci detected from eight individual association tests, where each test is represented by a different colored pill and placed to the right of the linkage group. GLM, general linear model; MLM, mixed linear model (from Hecht et al. 2013).**



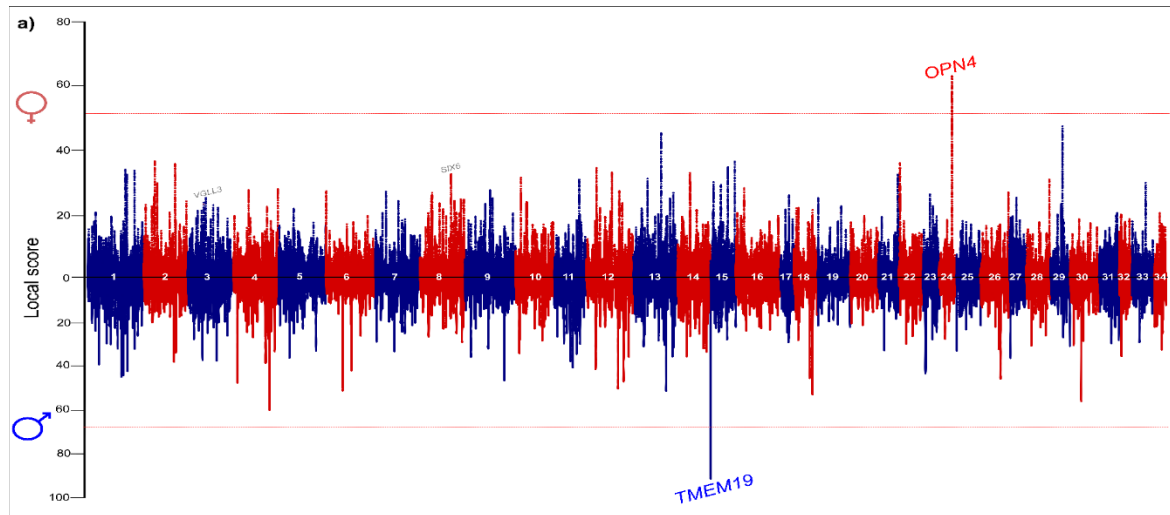
Run-timing related to maturity in steelhead (Hess et al. 2016; Micheletti et al. 2018c) and Chinook salmon (Hess and Narum 2011; Narum et al. 2018) has been shown to be controlled by a genomic region of major effect with the same candidate genes in both species (GREB1L, ROCK1, intergenic region; Figure 4).

**Figure 4.** Manhattan plot for premature and mature collections of Chinook salmon (from Narum et al. 2018). (a) Sequence coverage (black outer ring) for each chromosome. (b) Significant divergence between premature (spring-run) and mature (fall-run) migrating Chinook salmon in the Cowlitz River of the coastal lineage. (c) Significant divergence between premature (Methow River summer-run) and mature (Priest Rapids fall-run) migrating Chinook salmon within the interior ocean-type lineage. (d) Significant divergence for Chinook salmon returning to Johnson Creek (interior stream-type) that enter freshwater premature (spring/summer-run), but the final ascent to spawning grounds is bimodal with early premature and late mature females. (e) Annotation of the 203 Kb region on Ots28 between 11.022 and 11.225 Mb (GREB1 L, ROCK1, and intergenic regions) with significance based on CMH tests. Significant genes are labelled, and corresponding details are in electronic supplementary material, table S5. Timing of ascent to spawning grounds for premature (dashed line, orange) and mature (solid line, blue) collection pairs are shown within the ring for each lineage (b–d). Purple dots show two non-synonymous SNPs.



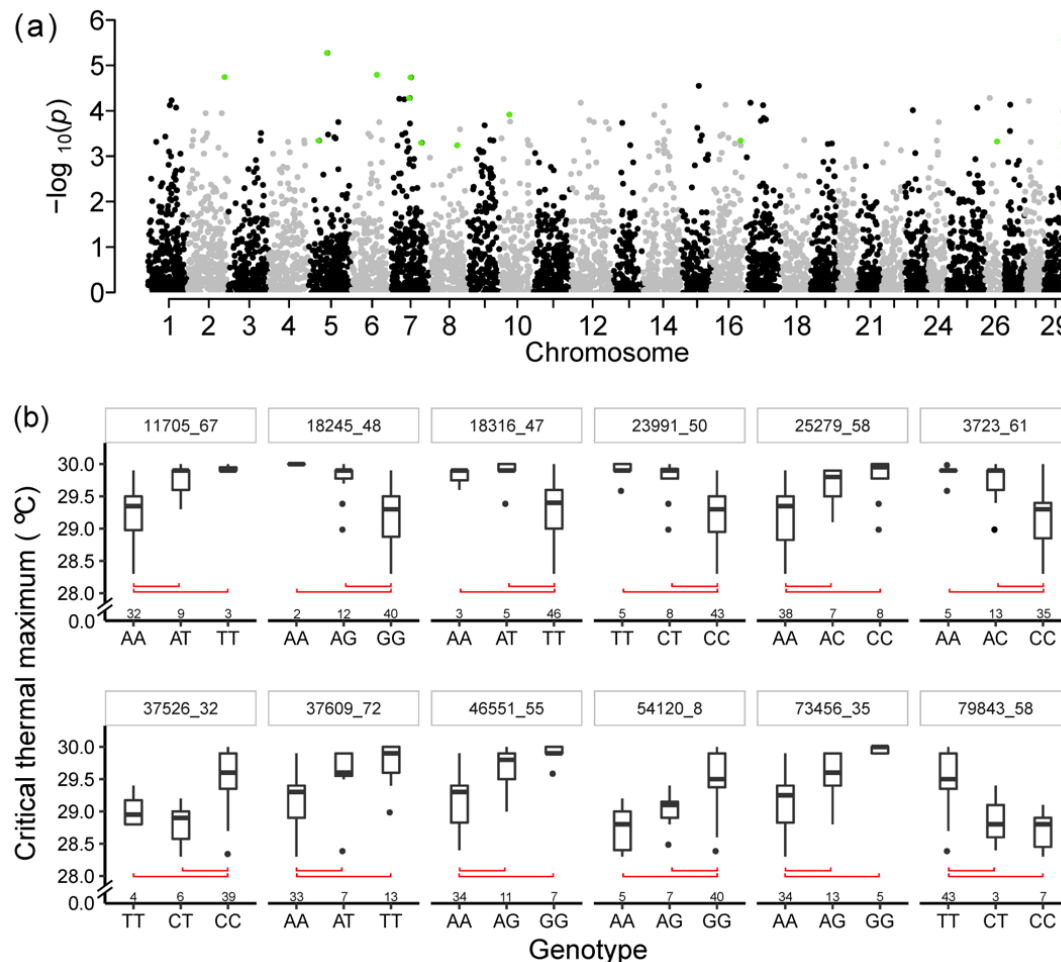
Age at maturity in Chinook salmon appears to be a polygenic trait but genes of greatest effect differ between sexes (females = OPN4, males = TMEM19; Figure 5; Micheletti and Narum 2018b). Disease resistance in *O. mykiss* appears to be highly complex with several genes involved (Campbell and Narum 2015).

**Figure 5.** Manhattan plot (from Micheletti and Narum 2018b) illustrating differentiated regions within age classes of females (top; 4- vs. 5-year-olds) and males (bottom; 3- vs. 4- vs. 5-year-olds). Red dashed lines indicate  $\alpha = 0.01$  significance threshold for the local score test with Bonferroni correction. Only the opsin 4 (OPN4) gene in females and transmembrane protein 19 (TMEM19) in males were significant using Bonferroni corrected thresholds. Relative locations of the VGLL3 (Ch3) and SIX6 (Chr8) genes which correspond to age at maturity in Atlantic salmon, are displayed in grey and were not significant.



Finally, thermal adaptation has evolved in desert strains of *O. mykiss* (Narum et al. 2010) so that fish have higher thermal tolerance and increased capacity to deliver oxygen to tissues by achieving higher maximum heart rate than montane fish (Chen et al. 2018a; Chen et al. 2018b). The genetic basis for thermal adaptation includes response of heat shock proteins (Narum et al. 2013; Narum et al. 2015; Chen et al. 2018a; Chen et al. 2018b) but also other genes involved in efficient uptake oxygen (Garvin et al. 2015; Narum et al. 2015; Chen et al. 2018a; Chen et al. 2018b). As these candidate genes for traits have begun to be identified (Figure 6; Chen et al. 2018a), SNP markers from these regions are being incorporated in standard genotyping panels with GTseq in order to validate and monitor genetic variation for these traits in large numbers of individuals.

**Figure 6. Results from Chen et al. (2018a) that demonstrate association of loci with thermal tolerance in natural populations of redband trout from desert, cool montane, and cold montane environments. Outlier loci and the association with critical thermal maximum (CTMAX). (a) Manhattan plot of calibrated p values, which were derived from the median z-score of results from Lositan, outFLANK and PCAdapt (François et al., 2016). Outliers identified by at least two analyses are in green. (b) significant associations between CTMAX and genotypes of candidate outlier loci (locus name above each panel). Red brackets indicate significant differences at the level of  $\alpha=0.05$  in one-way ANOVA on ranks with subsequent Dunn's post-hoc test. Numbers above genotypes represent the sample size. Results published in Chen et al. (2018a).**



### *Summary of accomplishments & lessons learned:*

Steady progress has been made to identify adaptive genomic variation and genetic basis for phenotypic traits in steelhead and Chinook salmon as reflected in 17 publications: Narum et al. 2008a; Narum et al. 2010; Narum and Campbell 2010; Narum et al. 2011; Hess and Narum 2011; Hecht et al. 2013; Narum et al. 2013; Campbell and Narum 2015; Garvin et al. 2015; Narum et al. 2015; Hess et al. 2016; Chen et al. 2018a; Chen et al. 2018b; Micheletti and Narum 2018; Micheletti et al. 2018b; Micheletti et al. 2018c; Narum et al. 2018).

Candidate genes for several traits have been identified in both steelhead and Chinook salmon. As these candidate genes for traits have begun to be identified, SNP markers from these regions are being incorporated in standard genotyping panels with GTseq to validate and monitor genetic variation for these traits in large numbers of individuals.

## 5. Synthesis of Findings: Discussion/Conclusions

### a. Fish Population RM&E

#### **Management questions/decisions and anticipated outcomes**

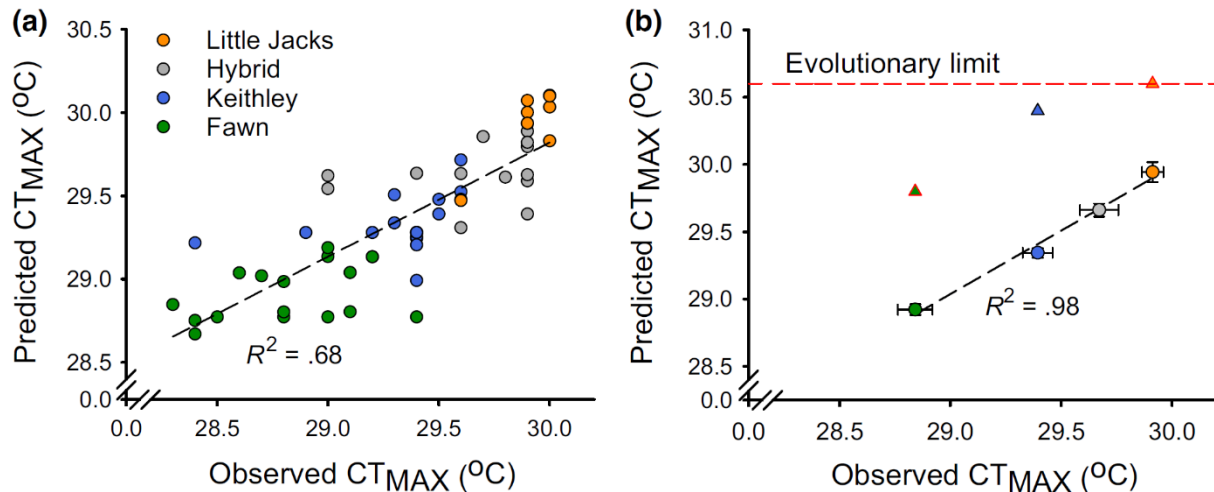
##### *Application of research results:*

Inclusion of candidate markers associated with specific traits allows more detailed genetic monitoring of stocks in the Columbia Basin. Extensive programs are in place that enable genetic identification of the origin of individual fish, but candidate markers from this study also provide the ability to monitor genetic variation for specific traits that are expected to be necessary to maintain life history variation for long term persistence of populations.

Water temperatures are predicted to increase in this century, e.g. approximately 0.27°C per decade for streams where salmonids are distributed. Thus, it is questionable whether species and populations will be able to adapt to future environmental changes, especially for freshwater ectotherms with limited migratory opportunities. Local extirpation events might occur if populations experience extreme temperatures above their maximum adaptive capacity from existing genomic variation. According to our predictions based on standing genetic variation at adaptive loci, natural populations appear to have some capacity to evolve a higher mean  $CT_{MAX}$  to meet challenges of warmer conditions (Figure 7). However, populations that currently live in warm environments may have a narrow safety margin, and therefore are more vulnerable and may need conservation attention. Thus, phenotypic plasticity and behavioral thermoregulation, such as seeking thermal refugia (e.g. deep pools, cool springs and upwelling groundwater), will become critically important for them to temporarily survive or avoid extreme temperatures in the future. To predict the rate and limits of evolutionary adaptation more acutely in the future, more advanced niche models need to incorporate additional factors such as the intensity of selection, effective population size, heritability and phenotypic plasticity. This information can be incorporated into robust adaptive networks that include a broad portfolio of adaptive diversity, connectivity, and meta-population scale management for long-term persistence.

Markers that are associated with specific phenotypic traits will also enable monitoring of genetic variation for traits that are considered important to managers. In particular, markers have been developed for premature vs. mature arrival to spawning grounds (e.g., migration/maturation timing) to monitor genetic variation for this trait in the Columbia Basin.

**Figure 7. Critical thermal maximum (CTMAX) prediction (from Chen et al. 2018a). (a) Correlation between observed and predicted individual CTMAX values. (b) Prediction of population mean CTMAX (SEM) and evolutionary limits for populations (triangle) and species (dash line).**



### Project timeline

This project began in 2009 with studies that utilized genetic data available at the time. As genomic methods have advanced over the last decade, more intensive genomic tools have enabled discovery of adaptive genetic variation and the genetic basis for specific phenotypic traits. Work is ongoing to discover adaptive variation and validate the genetic basis for phenotypic variation that is necessary for long-term persistence of salmonids. Thus, the project does not have a clear end date as more work is necessary into the future.



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## Appendix A: Critical Research Uncertainties

### Uncertainty Database Record:

Research Question	Addressed
What is the magnitude of any demographic benefit to the production of natural-origin juveniles and adults from the natural spawning of hatchery-origin supplementation adults?	Directly
To what extent do tributary habitat restoration actions affect the survival, productivity, distribution, and abundance of native fish populations?	Indirectly
How do artificial production and supplementation impact the maintenance or restoration of an ecologically functional metapopulation structure?	Indirectly
What is the relationship between genetic diversity and ecological and evolutionary performance, and to what extent does the loss of stock diversity reduce the fitness, and hence survival rate and resilience, of remaining populations?	Indirectly
Can integrated ecological monitoring be used to determine how climate change simultaneously affects fish and wildlife and the freshwater, estuarine, ocean, and terrestrial habitats and ecosystems that sustain them?	Directly
What long-term changes are predicted in the Columbia River Basin and the northeast Pacific Ocean, how will they affect the fish and wildlife in the region, and what actions can ameliorate increased water temperatures, decreased summer river flows, and other ecosystem changes	Directly

This list appears incomplete for this project and the fourth item above should be changed from “Indirectly” to “Directly”. Further, the larger list of uncertainties appears to have been updated and additional items and apply to this project:

### F. Population structure and diversity

<b>Question 1.</b> What level of population diversity is necessary to ensure population integrity?	indirect
1.4. How is diversity distributed among populations within a geographic region or biological grouping?	indirect

### L. Harvest

<b>2.1.</b> Do current harvest strategies effectively reduce risk to fish populations, their abundance, productivity population structure and viability?	indirect
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## 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	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		