



INFLUENCE OF LANDSCAPE AND ENVIRONMENT ON SALMONID GENETICS

BPA Project # 2009-005-00

Report covers work performed under BPA contract #(s) 69549, 76573

Report was completed under BPA contract #(s) 69549, 76573

1/1/2017 - 12/31/2017

Shawn Narum, Zhongqi Chen, Amanda Matala, Andrew Matala, Steven Micheletti

Columbia River Inter-Tribal Fish Commission (CRITFC), Hagerman, ID, 83332

This report was funded by the Bonneville Power Administration (BPA), U.S. Department of Energy, as part of BPA's program to protect, mitigate, and enhance fish and wildlife affected by the development and operation of hydroelectric facilities on the Columbia River and its tributaries. The views in this report are the author's and do not necessarily represent the views of BPA.

This report should be cited as follows: Shawn R. Narum, Zhongqi Chen, Amanda Matala, Andrew Matala, Steven Micheletti. INFLUENCE OF LANDSCAPE AND ENVIRONMENT ON SALMONID GENETICS, 1/1/2017 - 12/31/2017 Annual Report, 2009-005-00.

Table of Contents

Table of Contents.....	2
Acknowledgements.....	3
Executive Summary/Abstract	3
a. Fish Population RM&E	3
2. Introduction.....	4
a. Fish Population RM&E	6
Uncertainty Research.....	6
3. Methods: Protocols, Study Designs, and Study Area	6
4. Results	7
a. Fish Population RM&E	7
5. Synthesis of Findings: Discussion/Conclusions.....	8
a. Fish Population RM&E	8
6. References.....	10
Appendix A: Detailed Results	14
Appendix B: List of Metrics and Indicators	16

Acknowledgements

Funding was provided by the Bonneville Power Administration under agreements reached within the Columbia Basin Fish Accords (2008). We are grateful for extensive contributions in the genetics laboratory from Vanessa Morman, Stephanie Harmon, Lori Maxwell, Amanda Matala, Megan Moore, Travis Jacobson, and Jeff Stephenson. Additional administrative support was provided from CRITFC by Zach Penney, Doug Hatch, Christine Golightly, Melissa Edwards, Chrissy Bynum, and the CRITFC Finance Department. We appreciate the BPA administrative support supplied by Barbara Shields and Dorothy Welch, Contracting Officer Technical Representatives, Kristi Van Leuven and Karen Wolfe, Contracting Office Representative, and Israel Duran, Environmental Compliance Lead.

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. 2017). 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 indicate that environmental conditions through the

migratory corridor are stronger drivers of adaptation than those at natal sites for steelhead in the Columbia River Basin (Micheletti et al. 2017).

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. A recent study on mechanisms of thermal adaptation in redband trout links physiological traits for thermal tolerance with regions of the genome and patterns of gene expression (Chen et al. 2017). Further, progress has 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).

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, 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 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. 2017). Organisms typically show evidence of adaptation to features within their local environment. However, many species undergo long-distance dispersal or migration across larger geographical regions that consist of highly heterogeneous habitats. Therefore, selection may influence adaptive genetic variation associated with landscape features at residing sites and along migration routes in migratory species. We tested for genomic adaptation to landscape features at natal spawning sites and along migration paths to the ocean of anadromous steelhead trout (*Oncorhynchus mykiss*) in the Columbia River Basin. Results from multivariate ordination, gene-environment association, and outlier analyses using 24,526 single-nucleotide polymorphisms (SNPs) provided evidence that adaptive allele frequencies were more commonly associated with landscape features along migration paths than features at natal sites (91.8% versus 8.2% of adaptive loci, respectively). Among the 45 landscape variables tested, migration distance to the ocean and mean annual precipitation along migration paths were significantly associated with adaptive genetic variation in three distinct genetic groups. Additionally, variables such as minimum migration water temperature and mean migration slope were significant only in inland stocks of steelhead that migrate up to 1200 km farther than those near the coast, indicating regional differences in migratory selective pressures. This study

provides novel approaches for investigating migratory corridors and some of the first evidence that environment along migration paths can lead to substantial divergent selection. Consequently, our approach to understand genetic adaptation to migration conditions can be applied to other migratory species when migration or dispersal paths are generally known.

Objective 2) New results:

A recent study on mechanisms of thermal adaptation in redband trout links physiological traits for thermal tolerance with regions of the genome and patterns of gene expression (Chen et al. 2017). Heterogeneous and ever-changing thermal environments drive the evolution of populations and species, especially when extreme conditions increase selection pressure for traits influencing fitness. However, projections of biological diversity under scenarios of climate change rarely consider evolutionary adaptive potential of natural species. We tested for mechanistic evidence of evolutionary thermal adaptation of ecologically divergent redband trout (*Oncorhynchus mykiss gairdneri*) in cardiorespiratory function, cellular response and genomic variation. In a common garden environment, fish from an extreme desert climate had significantly higher critical thermal maximum ($p < 0.05$) and broader optimum thermal window for aerobic scope ($>3^{\circ}\text{C}$) than fish from cooler montane populations. In addition, the desert population had the highest maximum heart rate under thermal stress (20% greater than montane populations), indicating improved capacity to deliver oxygen to internal tissues. In response to acute heat stress, distinct sets of cardiac genes were induced among the ecotypes, which explained differences in cardiorespiratory function. Candidate genomic markers and genes underlying these physiological adaptations were also pinpointed, such as genes involved with stress response and metabolic activity (*hsp40*, *ldh-b* and *camkk2*). These markers were developed into a multi-variate model that not only accurately predicted critical thermal maxima, but also the evolutionary limit of thermal adaptation in these specific redband trout populations relative to the expected limit for the species. This study demonstrates mechanisms and limitations of aquatic species to evolve under changing thermal environments that can be incorporated into advanced models to more effectively predict ecological consequences of climate change for natural organisms.

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. 2017). 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 indicate that environmental conditions through the migratory corridor are stronger drivers of adaptation than those at natal sites for steelhead in the Columbia River Basin (Micheletti et al. 2017).

Objective 2)

Candidate genetic markers that were highly associated with steelhead run-timing and maturation status (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 many 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 and maturation 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 and maturation status of steelhead to facilitate conservation management of this species. Markers identified from Hess et al. (2016) were also validated in Hood River steelhead this year and used to help identify winter or summer-run fish.

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; Chen et al. 2017) 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, progress has 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).

6. References

- Angers, B., Magnan, P., Plantes, M., and Bernatchez, L. 1999. Canonical correspondence analysis for estimating spatial and environmental effects on microsatellite gene diversity in brook charr (*Salvelinus fontinalis*). *Mol. Ecol.* 8: 1043-1053.
- Baird, N. A., Etter, P. D., Atwood, T. S., Currey, M. C., Shiver, A. L., Lewis, Z. A., ... Johnson, E. A. (2008). Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PLoS One*, 3(10), e3376. doi:10.1371/journal.pone.0003376.
- Brannon, E.L., Powell, M.S., Quinn, T.P., and Talbot, A.J. 2004. Population structure of Columbia River Basin Chinook salmon and steelhead trout. *Res. Fish. Sci.* 12: 99-232.
- Campbell, N. R., and S. R. Narum. 2008. Identification of novel SNPs in Chinook salmon and variation among life history types. *Transactions of the American Fisheries Society* 137:96-106.
- Campbell, N.R., and S. Narum. 2009. Identification and characterization of heat shock response related single nucleotide polymorphisms in *O. mykiss* and *O. tshawytscha*. *Molecular Ecology Resources* 9:1460-1559.
- Campbell, N.R., S.E. LaPatra, K. Overturf, R. Towner, S.R. Narum. 2014. Association mapping of disease resistance traits in rainbow trout using RAD sequencing. *G3 Genes, Genomes, Genetics*, 4:2473-2481, doi:10.1534/g3.114.014621.
- Castric, V., Bonney, F., and Bernatchez, L. 2001. Landscape structure and hierarchical genetic diversity in the brook charr, *Salvelinus fontinalis*. *Evolution* 55: 1016-128.

Chen, Z., A.P. Farrell, A. Matala, S.R. Narum. In press. Mechanisms of thermal adaptation and evolutionary potential in conspecific populations to changing environments. *Molecular Ecology*.

Costello, A.B., Down, T.E., Pollard, S.M., Pacas, C.J., and Taylor, E.B. 2003. The influence of history and contemporary stream hydrology on the evolution of genetic diversity within the species: an examination of microsatellite DNA variation in bull trout, *Salvelinus confluentus* (Pisces: Salmonidae). *Evolution* 57: 328-344.

Faubet P. and O.E. Gaggiotti, 2008. A new Bayesian method to identify the environmental factors that influence recent migration. *Genetics* 178: 1491–1504.

Feder, M.E., and G.E. Hofmann. 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. *Annual Review of Physiology* 61:243-282.

Foll, M., and O. E. Gaggiotti, 2005 COLONISE: a computer program to study colonization processes in metapopulations. *Molecular Ecology Notes* 5: 705-707.

Foll, M., and O.E. Gaggiotti, 2006. Identifying the environmental factors that determine the genetic structure of Populations. *Genetics* 174: 875-891.

Funk, W.C., Blouin, M.S., Corn, P.S., Maxell, B.A., Pilliod, D.S., Amish, S., and Allendorf, F.W. 2005. Population structure of Columbia spotted frogs (*Rana luteiventris*) is strongly affected by the landscape. *Mol. Ecol.* 14: 483–496.

Harris, D.J., Arnold, E.N., and Thomas, R.H. 1998. Rapid speciation, morphological evolution, and adaptation to extreme environments in South African sand lizards (*Meroles*) as revealed by mitochondrial gene sequences. *Mol. Phylogenet. Evol.* 10: 37- 48.

Garvin, M.R., G.H. Thorgaard, S.R. Narum. 2015. Differential expression of genes that control respiration contribute to thermal adaptation in redband trout (*Oncorhynchus mykiss gairdneri*). *Genome Biology and Evolution*, 7:1404-1414

Hecht, B.C., N.R. Campbell, D.E. Holecek, and S.R. Narum. 2013. Genome-wide association reveals genetic basis for the propensity to migrate in wild populations of rainbow and steelhead trout. *Molecular Ecology* 22:3061-3076.

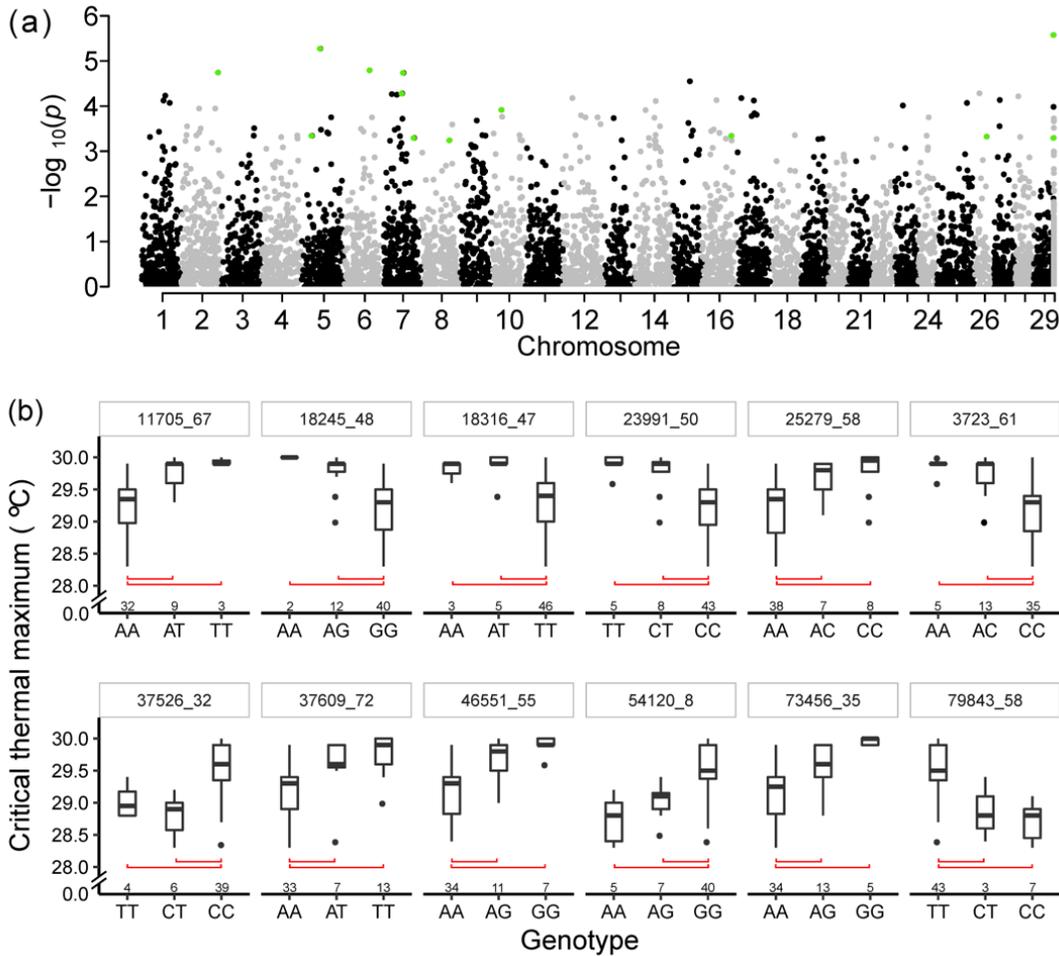
Hecht, B. C., Matala, A. P., Hess, J. E. and Narum, S. R. (2015), Environmental adaptation in Chinook salmon (*Oncorhynchus tshawytscha*) throughout their North American range. *Molecular Ecology*, 24: 5573–5595. doi: 10.1111/mec.13409

Hess, J.E., and S.R. Narum. 2011. SNP loci correlated with run-timing in adult Chinook salmon from the Columbia River Basin. *Transactions of the American Fisheries Society* 140:855-864.

- Kalinowski ST, MH Meeuwig, SR Narum, ML 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*.
- Manel, S., Schwartz, M.K., Luikart, G., and Taberlet, P. 2003. Landscape genetics: combining landscape ecology and population genetics. *Trends Ecol. Evol.* 18: 189-197.
- Matala, A.P., Hess, J.E., and S.R. Narum. 2011. Resolving adaptive and demographic divergence among Chinook salmon populations in the Columbia River Basin. *Transactions of the American Fisheries Society* 140:783-807.
- Matala, A.P., M.W. Ackerman, M.R. Campbell, and S.R. Narum. 2014. Relative contributions of neutral and non-neutral genetic differentiation to inform conservation of steelhead trout across highly variable landscapes. *Evolutionary Applications*, 7:682-701.
- Micheletti, S.J., A.R. Matala, A.P. Matala, S.R. Narum. 2017. Landscape features along migratory routes influence adaptive genomic variation in steelhead (*Oncorhynchus mykiss*). *Molecular Ecology*.
- 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.
- Narum, S.R., and N.R. Campbell. 2010. Sequence divergence of heat shock genes within and among three *Oncorhynchids*. *Journal of Heredity* 101:107-112.
- Narum, S.R., N.R. Campbell, C.C. Kozfkay, and K.A. Meyer. 2010. Adaptation of redband trout in desert and montane environments. *Molecular Ecology* 19:4622-4637.
- Narum, S.R., J. Zendt, C. Frederiksen, N.R. Campbell, A.P. Matala, and B. Sharp. 2011. Candidate genetic markers associated with anadromy in *Oncorhynchus mykiss* of the Klickitat River. *Transactions of the American Fisheries Society* 140:843-854.
- Narum, S.R., N.R. Campbell, K.A. Meyer, M.R. Miller, and R.W. Hardy. 2013. Thermal adaptation and acclimation of ectotherms from differing aquatic climates. *Molecular Ecology* 22:3090-9097.
- Narum, S.R., and Campbell, N.R. 2015. Transcriptomic response to heat stress among ecologically divergent populations of redband trout. *BMC Genomics* 16:103-116. DOI 10.1186/s12864-015-1246-5.
- Nichols, K.M., A.F. Edo, P.A. Wheeler, and G.H. Thorgaard. 2008. The genetic basis of smoltification-related traits in *Oncorhynchus mykiss*. *Genetics* 179:1559-1575.

- Nichols, K.M., C.C. Kozfkay, S.R. Narum. 2016. Genomic signatures among *Oncorhynchus nerka* to inform conservation and management of endangered sockeye salmon. *Evolutionary Applications* 9:1203-1311. DOI: 10.1111/eva.12412
- Overturf, K. S. LaPatra, R. Towner, N. Campbell, and S. Narum. 2010. Relationships between growth and disease resistance in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* 33:321-329.
- Piertney, S.B., MacColl, A.D., Bacon, P.J., and Dallas, J.F. 1998. Local genetic structure in red grouse (*Lagopus lagopus scoticus*): evidence from microsatellite DNA markers. *Mol. Ecol.* 7: 1645-1654.
- Raymond, M., and Rousset, F. 1995. GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *J. Hered.* 86: 248-249.
- Richards, J.G., J.W. Semple, J.S. Bystriansky, and P.M. Schulte. 2003. Na/K-ATPase α -isoform switching in gills of rainbow trout (*Oncorhynchus mykiss*) during salinity transfer. *Journal of Experimental Biology* 206:4475-4486.
- Schlötterer, C., Tobler, R., Kofler, R., & Nolte, V. Sequencing pools of individuals – mining genome-wide polymorphism data without big funding. *Nature Reviews Genetics* 15, 749-763 (2014).
- Storfer, A., Murphey, M.A., Evans, J.S., Goldberg, C.S., Robinson, S., Spear, S.F., Dezzani, R., Delmelle, E. 2006. Putting the 'landscape' in landscape genetics. *Heredity* 2006: 1-15.
- Thrower, F. P., J. J. Hard and J. E. Joyce. 2004. Genetic architecture of growth and early lifehistory transitions in anadromous and derived freshwater populations of steelhead. *J. Fish Biol.* 65: 286–307.

Figure 2: Results from Chen et al. (2017) 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 (CT_{MAX}). (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 CT_{MAX} 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. (2017).



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	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		