POPULATION STUDIES OF REDBAND TROUT: GENETIC INVESTIGATION OF POPULATION STRUCTURE

FY2004 Progress Report

Prepared by:

Christine Cegelski, Conservation Geneticist
Matthew Campbell, Senior Fisheries Research Biologist
Kevin Meyer, Principal Fisheries Research Biologist
and
Daniel J. Schill, Fisheries Research Manager

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By
Christine Cegelski
Matthew Campbell
Kevin Meyer
Daniel J. Schill

Idaho Department of Fish and Game
600 South Walnut Street
P.O. Box 25
Boise, ID 83707

To
U.S. Department of the Interior
Bureau of Land Management
Lower Snake River Ecosystem
Boise District Office
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INTRODUCTION

Idaho Department of Fish and Game (IDFG) and the Bureau of Land Management (BLM) have both identified redband trout *Oncorhynchus mykiss*, residing in arid southwest Idaho basins, as a sensitive subspecies. These populations of redband trout in Idaho were proposed for listing under the Endangered Species Act (ESA) during the mid-1990s, but the petition was not found to be warranted at that time (USFWS 1995). The original petition that involved Idaho fish did not distinguish between desert redband trout and other interior forms, including steelhead. Strong interest in the preservation and status of these populations in Idaho remains among environmental groups, and the potential for a future petition submission remains high. As a group, populations of redband trout residing in Idaho desert basins are one of the least studied Idaho salmonids (Schill et al. 2002). Although population inventories have been conducted in select drainages (e.g., Allen et al. 1996; Zoellick 1999; Zoellick et al. 2005), basic life history and population dynamics information is lacking for most populations. Knowledge of suspected spawning areas and associated habitat currently only exists for a few streams (B. Zoellick, BLM, personal communication).

Genetic analyses are a complimentary method to demographic and ecological studies, which can provide relevant information regarding population status and persistence. Genetic diversity levels can be good indicators of population health and evolutionary adaptability (Primmer et al. 2003; Reed & Frankham 2003; Borrell et al. 2004). Low levels of variability are seen as limiting a species’ ability to respond to short-term and long-term demographic and environmental changes and are often a consequence of inbreeding or genetic drift in small populations. Diversity may be gained through mutation or gene flow from a neighboring population, and the balance between gene flow and genetic drift is important to the maintenance of genetic diversity in small populations. In addition, the level of genetic exchange between populations can provide information regarding the potential for recolonization of extirpated populations (Fraser et al. 2004).

An understanding of the relationship between genetic structure and environment can help develop predictions for how genetic variation is partitioned and be an important step in defining practical units for management. Whiteley et al. (2004) outlines three ways in which genetic structure may be predicted: 1) physical template of stream network, 2) distance, and 3) patch size. Population structure may mirror the physical template of the system where branching patterns dictate levels of genetic differentiation (Meffe and Vrijenhoek 1988). This pattern allows for delineation of population units based upon stream networks. Secondly, population structure may mirror geographic distance where dispersal distances are constrained in salmonids leading to increased genetic differentiation with increasing geographic distance. In this instance, dispersal distance can dictate the delineation of management units. Thirdly, population structure may correspond to patch size, whereas the presence and absence of suitable habitat dictate levels of genetic differentiation. In this scenario, unsuitable habitat would serve as a barrier to migration and lead to increased genetic differentiation. The scale for differentiation would then be dependent upon the amount of suitable habitat. This scenario is likely the most difficult to generalize across landscapes since it is highly dependent upon migration barriers.

In this study, we analyze 13 microsatellite loci to understand the patterns of genetic differentiation for 26 populations sampled in the Owyhee, Bruneau, and Salmon Falls drainages in southern Idaho. This report is a progress report of our initial findings. A final report will be
OBJECTIVES

1. To evaluate levels and patterns of genetic diversity and genetic differentiation among desert redband populations.

2. To compare these results to other *O. mykiss* population genetic studies.

METHODS

**Sampling and DNA Extraction**

During 2001-2005, over 3,000 redband trout tissue samples were collected from over 150 sample sites in the Upper Snake River basin by IDFG personnel. For this report, 721 tissue samples were analyzed from 26 sample sites. Sample sizes and locations of each sample site are presented in Table 1 and Figure 1. Samples were stored in 100%, non-denatured ethanol until DNA extraction. DNA was extracted using a salt-chloroform method described by Paragamian et al. (1999).
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Table 1. Sample locations, major drainage, site number in Figure 1, and sample size (N) for redband trout populations along with genetic diversity estimates. $H_e = \text{average expected heterozygosity across 13 loci}; A = \text{average number of alleles across 13 loci}; R_t = \text{average allelic richness across 13 loci}.$
Microsatellite Amplification

Thirteen polymorphic microsatellite loci were amplified: Oki23 (Genbank Accession #AF272822), Ssa289 (McConnell et al. 1995), Omy1011 (P. Bentzen, unpublished), Oke4 (P. Bentzen, unpublished), Ssa408 (Cairney et al. 2000), Ssa407 (Cairney et al. 2000), Ots4 (Banks et al. 1999), Oneu8 (Scribner et al. 1996), Ogo1a (Olsen et al. 1998), Omy27 (Heath et al. 2001), Ogo4 (Olsen et al. 1998), Omy325 (O’Connell et al. 1997), and Oneu14 (Scribner et al. 1996), using fluorescently labeled primers. PCR reaction conditions and cycling profiles are available from the authors upon request. PCR products were separated electrophoretically using an ABI 3100 automated sequencer (Applied Biosystems) platform. PCR products from multiplex 1 (Oki23, Ssa289, Omy1011, Oke4, Ssa408, Ssa407) were electrophoresed together. PCR products from multiplex 2 (Ots4, Oneu8, Ogo1a, Omy27) were electrophoresed together, and PCR products from multiplex 3 (Ogo4, Omy325, Oneu14) were electrophoresed together. Fragments were sized against GS500 ROX size standard (Applied Biosystems) using GeneMapper® 3.5 software (Applied Biosystems).
Statistical Analyses

Each population was tested for Hardy-Weinberg equilibrium and linkage disequilibrium using Genepop on the web (Raymond and Rousset 1995). A sequential Bonferroni correction was used to adjust significance for multiple comparisons (see Rice 1989). An alpha value of 0.05 was chosen for statistical significance for all analyses.

Genetic diversity was measured by the number of alleles per locus (A) and expected heterozygosity (He) using FSTAT version 2.9.3 (Goudet 2001). As the detected number of alleles per locus is highly dependent upon sample size, a rarefaction option was performed by FSTAT 2.9.3. Corrected estimates of allelic diversity (Rt) were obtained based upon the smallest sample size at a given locus in this study (n = 5).

Patterns and levels of population differentiation were evaluated using both traditional distance estimates and new Bayesian clustering methods. Pairwise FST estimates (Weir and Cockerham 1984) were generated using ARLEQUIN 2.0 with significance based upon a permutation process. A sequential Bonferroni correction was used to adjust significance for multiple, simultaneous comparisons (see Rice 1989). An unrooted neighbor-joining (NJ) tree using Cavalli-Sforza and Edward’s (1967) chord distance (Dce) was used to display the clustering relationship among populations using the software POPULATIONS 1.2.14 (Langella 2001) and TreeView (Page 1996).

The Bayesian method of STRUCTURE 2.0 (Pritchard et al. 2000) was also used to determine levels of fine-scale structuring without any prior knowledge of population origin and assign individuals to inferred population clusters (K). Ten independent runs of K = 1 to 20 were run at 100,000 Markov Chain Monte Carlo (MCMC) repetitions and 100,000 burn-in. The most probable number of K was then chosen as having the highest log likelihood value (loge probability of the data). Individuals were assigned to a population cluster or multiple population clusters based upon their multi-locus genotype.

RESULTS

Hardy-Weinberg and Linkage Disequilibrium

Tests for Hardy-Weinberg equilibrium revealed that genotypes were in expected proportions, except for 30 of the 325 tests. While these results are higher than expected by chance (16.3 tests expected from Type I error of 0.05), none of the tests were associated with a particular locus and no more than two tests were rejected per population except for one population (Wickahoney Cr.; 5 loci rejected). A total of 2,028 tests for linkage disequilibrium were performed, and 156 of the tests were rejected at $\alpha = 0.05$, which also was slightly higher than expected by chance (101 expected from Type I error of 0.05). None of the tests clustered around a particular locus or population. Therefore, there appear to be no problems with physical linkage of loci or deviations from Hardy-Weinberg expectations.

Genetic Diversity

The number of alleles per locus ranged from six alleles (Omy27) to 22 alleles (Omy325). Genetic diversity varied widely within populations (Table 1). Expected heterozygosity ranged
from 0.48 in Crab Creek to 0.80 in Jarbidge River, and allelic richness ranged from 2.86 alleles in Crab Creek to 5.31 alleles in Jarbidge River. Due to unequal sample sizes in these sampled sites, the allelic richness values were substantially different from the number of alleles per locus in most cases.

**Genetic Differentiation and Gene Flow**

An overall Fst value of 0.137 (95% C.I. 0.112 to 0.163), indicated significant population differentiation. All of the pairwise comparisons revealed significant differentiation except for the following comparisons: Willow Cr., Bruneau R., MF Willow Cr. (Fst >0.05). Two of these populations have small sample sizes (Willow Cr. N = 7; MF Willow Cr. N = 7), which may reflect nonsignificance for these comparisons. Population pairwise comparisons ranged from 0.01 for Deer Cr. and Deer Cr. comparison to 0.30 for Salmon Falls Cr. and Little Jacks Cr. comparison.

An unrooted dendrogram was constructed to illustrate population relationships (Figure 2). There was not a strong association between genetic structure and the physical template of the watershed. If the dendrogram mirrored stream network structure, we would expect that all of the populations would cluster with other populations in the sampled drainage, and the populations that are farther apart within a drainage would have larger genetic distances (longer branches on the dendrogram). Instead, we see that only some of the populations clustered with neighboring populations in geographic proximity (outlined in circles on the tree).

The Bayesian clustering program, STRUCTURE, identified 15 clusters as the most likely number of clusters in this dataset (Figure 3). The majority of sample sites were assigned to unique population clusters, indicating significant genetic differentiation. Exceptions to this were Williams Cr. a, b, and c; Deer Cr. a, b; Duncan Cr. 2002 and 2003; Duncan and Big Jacks Cr; Willow, Willow, MF Willow and Bruneau R.; Shack and upper Cedar Cr; and Cottonwood, NF Salmon Falls, and MF Shoshone Cr. The clustering results suggest that these populations are not genetically differentiated.
Figure 2. Neighbor-joining dendrogram (unrooted) of the genetic relationships among 26 redband trout populations based on Cavalli-Sforza and Edward’s (1967) chord distance. Populations are color-coded based upon drainage location.
Figure 3. Structure results for 26 redband trout sample locations. Population memberships into each cluster (displayed as a different cluster) are shown for each sample location.
DISCUSSION

Redband trout are highly structured within these drainages. Significant differentiation was observed at small spatial scales unlike other *O. mykiss* (e.g., steelhead) populations. Patterns of population differentiation do not follow stream-network structure. Genetic distance estimates indicate that the populations are highly differentiated from one another and that genetic drift has likely altered population relationships. Patterns of population differentiation also do not correlate with geographic distance, as some of the populations separated by smaller geographic distances have high Fst estimates. The sample sites, MF Willow Cr., Willow Cr., Willow Cr., and Bruneau R. appear to be less differentiated at a scale of 10-45 km. Other populations that were less differentiated were Deer Cr. a, b.; Williams Cr. a, b, c; Duncan Cr. 2002 and 2003; Cedar Cr.; and Sinker Cr.

Heterozygosity estimates were similar to those previously reported in other *O. mykiss* studies of population genetic structure (Moran 2003; Heath et al. 2001). However, anadromous steelhead populations experience higher levels of gene flow across a larger scale. In the Clearwater River basin, low to moderate levels of genetic differentiation were reported (Fst >0.05) for steelhead populations sampled within an entire drainage (Moran et al., unpublished data). Similar levels of genetic differentiation have been reported for steelhead, where populations within drainages are not differentiated from one another (Heath et al. 2001; Nielsen et al. 2004). In contrast, we have observed significantly higher Fst estimates within one sampled drainage. Our estimates of genetic differentiation were more similar to those observed for fragmented populations of cutthroat trout (Neville-Arsenault 2003; Wofford et al. 2005; Cegelski et al. in press).

Genetic differentiation could be due to the following contributing factors: life history, barriers to movement, habitat suitability, and/or hybridization with hatchery-origin fish. Currently, it is not known whether these populations are comprised of resident or migratory fish, but most likely, there is a mixture for at least some of the populations. The degree of hybridization is also not known, but hybridization could result in genetic differentiation due to the presence of nonnative alleles, which are not shared with the nonhybridized populations. Intermittent stream flows characterize many streams in these areas and likely contribute to the patterns of population differentiation and observed fragmentation. For example, Little Jacks Cr. and Wickahoney Cr. appear to be isolated due to intermittent flows in the lower parts of the streams. Absence or low abundance of redband trout in large streams such as the NF Owyhee Cr. that connects smaller tributary streams (Zoellick et al. 2005) may also lead to genetic differentiation at small scales.

A number of the sites were sampled at large geographic distances, and while they appear to be highly differentiated and isolated, we did not sample intermediate sites that would offer insights into the scale for population differentiation. For example, Williams Cr. appears to be isolated from the rest of the populations in the Owyhee drainage, but it is 200 km from the next closest sampled site (in river distance). If we had analyzed intermediate populations, we would be able to conclude with certainty if Williams Cr. was isolated or the scale for genetic differentiation. Other populations may be connected to Williams Cr. in the Jordan Cr. system. This information may be necessary to quantify population abundance and identify discreet management units.

Populations do not appear to be structured by geographic distance or stream network structure in these basins, and this is most likely due to differences in habitat suitability, which
serve as barriers to movement and increase genetic drift. Under this scenario, we are unable to predict how genetic variation is partitioned for unsampled sites in these drainages, unless we can determine the factors (life history, barriers to fish movement, habitat, thermal tolerance, hybridization) influencing genetic differentiation. Assuming that redband trout are structured at the individual stream level may be assuming more differentiation than actually exists in some places and splitting a population too finely for management purposes. Alternatively, failure to recognize and manage distinct populations as separate may result in local extinctions or inadequate representation of species diversity. Ongoing work that will be completed for the final report will shed more light on these questions.

Future work should aim at correlating habitat features and barriers to movement to our understanding of the processes influencing genetic structure in these drainages so that generalizations can be made regarding the genetic structure of unsampled populations. One possibility is to correlate the likelihood of encountering redband trout (K. Meyer, unpublished data) on the landscape to levels of genetic differentiation. In this model, the prediction would be that areas in which there are no redband trout are unsuitable habitat patches that likely limit movement from adjacent areas. Genetic differentiation should show a discontinuity at the same scale as the predicted discontinuities in habitat and lead to higher gene flow within the patches than between the patches. Future work will also attempt to evaluate hybridization in these drainages. We will try to find microsatellite or SNP alleles that are diagnostic between coastal-hatchery strains and interior redband trout to assess whether a population is hybridized.


Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3).


USFWS. 1995. Endangered and threatened wildlife and plants: 90 day finding for a petition to list Desert Redband trout in the Snake River drainage above Brownlee Dam and below Shoshone Falls as threatened or endangered. Federal Register 60:49819-49821.


