Kings River Genetics Study

Program Year 2011 - 2015

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KRFMP GENETICS STUDY 2011 – 2015

INTRODUCTION

The distribution of both steelhead and resident rainbow trout (*Oncorhynchus mykiss*) populations in the Central Sierra Mountains have been forever changed through the workings of human intervention. Prior to extensive trout planting programs since the late 19th and early 20th centuries, most streams and lakes in elevations above 1,800m (6,000ft.) were without fish. The only major exceptions to this were the upper reaches of the Kern River, where golden trout (*Oncorhynchus aguabonita*) evolved, and those tributaries to the Pit and McCloud Rivers that contained redband trout (*Oncorhynchus mykiss gairderii*) (Moyle, 2002). Artificial propagation of *O. mykiss* began in the Central Valley more than 125 years ago with the establishment of the Baird Station on the McCloud River, and many billions of fish have been released in Central Valley rivers, streams, lakes and reservoirs since then. This massive propagation and planting effort, much of it sparsely documented, significantly clouds efforts to disentangle residual historic structure from effects of these hatchery rainbow trout and steelhead (Garza and Pearse 2008).

The Kings River below Pine Flat Dam has been stocked with hatchery-produced rainbow trout since construction was completed in the mid-1950s; while stocking in the upper watershed had been occurring long before that time. The trout strains and hatcheries utilized by the California Department of Fish and Game (DFG) have varied over the years. Despite very low numbers of "wild" rainbow trout collected in the 1990s some trout have managed to establish themselves through natural reproduction. This is evidenced by the wild rainbow trout that are collected each fall during the Kings River Fisheries Management Program (KRFMP) annual population survey. The "wild" designation refers to a rainbow trout that has inhabited the river from birth regardless of its lineage. This is opposed to what some may call a "native" trout which is assumed to have direct genetic ties to the original ancestor of the region.

The wild trout population inhabiting the Kings River below the dam is believed to be small but fluctuates annually. Edge habitat surveys also indicate that natural reproduction is occurring (unpublished data) in the winter and spring. It is believed that the wild population is derived from hatchery trout that have reverted to a feral condition and have established themselves as residents. In order to better understand the rainbow trout residing in the tail water fishery and the differences between the seemingly more fit rainbow trout above the reservoir, the KRFMP launched a comparative genetics study in the fall of 2011.

METHODS

One hundred genetic samples were taken from wild rainbow trout residing above Pine Flat Dam whereas Seventy-three genetic samples were taken from wild rainbow trout residing below Pine Flat Dam. Data collection rested heavily on the efforts of local volunteer fishermen. These volunteers along with KRFMP staff were asked to take a small clipping (about the size of a shelled sunflower seed) from the trout's tail and place it in a pre-labeled vial of 95% ethanol alcohol. The vial number, date and location of capture were recorded on a data sheet and added to an Access database. When possible, trout were also measured, sexed and/or photographed.

Fin clips were sent to the laboratory of Dr. Andres Aguilar at CSU Los Angeles where samples were stored in individual collection vials until DNA was extracted (Agulair, 2014). DNA was extracted using a commercially available kit following the manufacturer's protocol (DNeasy – Qiagen Inc.).

The KRFMP requested that twelve microsatellite loci (Appendix A) be examined in the lab. Microsatellites, also known as simple sequence repeats (SSRs), are repeating sequences of 2-5 tandem pairs of DNA which create an identifiable pattern (Figure 1). The position of the sequences on a chromosome is referred to as the locus. Generally, species can be accurately differentiated using as few as eight loci, but some researchers have used up to eighteen. After filtering out monomorphic loci and those that did not amplify consistently across individuals a total of nine microsatellite loci were used in subsequent analyses (Aguilar, 2014).

Initial descriptive statistics including numbers of alleles, *allelic richness, observed and expected *heterozygosity (HO and HE), conformance to *Hardy-Weinberg Equilibrium (HWE), and *linkage disequilibrium (LD) were all generated with the program GENETIX (Belkhir et al. 2004). Allelic richness was adjusted for sample size using HP-RARE (Kalinowski 2005). GENETIX was also used to assess population level differences between the two sampling locations using pairwise $*F_{ST}$ estimates.

STRUCTURE v2.3 (Pritchard el al. 2000) was used to determine if any genetic clusters existed among the above and below Pine Flats Dam samples. Parameters in STRUCTURE were set to produce posterior probabilities with 50,000 steps discarded as burn in and 500,000 replicates recorded after the burn in stage. Default settings were used with the 'admixture' option. We also simulated structure runs with K values that ranged from 2-4 and identified optimal K using the online software Structure Harvester v6.92.

(Aguilar, 2014)

^{*}Allelic richness: the number of alleles per locus. *Heterozygosity: (Ho = observed, H_E = expected) having dissimilar pairs of genes for any hereditary characteristic (as would be expected from any organism with two unrelated parents) *Hardy Weinberg Equilibrium: the mathematical assumption that alleles within a population remain constant given that mating is random, no mutation, selection or migration occurs and a large population without genetic drift remains intact. *Linkage disequilibrium (LD): non-random similarity + amount of genetic drift. *F_{ST} estimates: a calculation of drift where the expected level of reduced heterozygosity is statistically compared to Hardy Weinberg Equilibrium.



Figure 1: Example of 5 Simple Sequence Repeats in tandem, using nucleotides G, A, T, C (the basic building blocks of DNA).

RESULTS

Reliable genotypes were obtained for one hundred individuals sampled above and seventy-three individuals below Pine Flat Dam (Aguilar, 2014). Of the one hundred samples taken above the reservoir the uppermost included twenty-three from the area around Cedar Grove, eight caught just upstream of Boyden and four caught just above Roaring River at an elevation of 4,850ft. All upper river samples were taken from the South-fork and main drainage of the Kings. Zero trout were captured from the Middle-fork or the North-fork of the river.

Of the samples taken on the lower river, fifteen were collected by Kings River Conservation District (KRCD) staff during routine surveys. All other samples were collected by local volunteer fishermen. The furthest downstream samples were taken from two trout captured at the Greenbelt fishing access at an elevation of about 430ft. The distance between our uppermost and lowermost sample sites was approximately 54miles.

Samples taken below Pine Flat Dam showed an elevated level of genetic variation indicating that there is little to no inbreeding occurring in the fishery. The levels of genetic variation were slightly higher in the samples taken below the reservoir than from those taken above. Pairwise population genetic differentiation, as estimated by F_{ST} was 0.037 (95% confidence interval: 0.022-0.053) (Aguilar, 2014), indicating that genetic differences between the above dam population and below dam population are minimal.

DISCUSSION

In 2008 John Carlos Garza and Devon E. Pearse published a paper entitled "Population genetic structure of *Oncorhynchus mykiss* in the California Central Valley". Their study genotyped more than sixteen hundred steelhead and rainbow trout across eighteen microsatellite loci:

Analyses of these data examined population structure within the region, relationships between populations above and below barriers to anadromy, relationships of Central Valley populations with coastal steelhead populations, and population genetic diversity. Analysis focused on 17 initial "population" samples, comprised of fish sampled from the Kings, Tuolumne, Stanislaus, Calaveras, American, Yuba, Feather, Butte, Deer, Battle and McCloud River sub-basins. Additional analyses were conducted with data from the same microsatellite markers in rainbow trout hatchery stocks.

The Kings River samples came from two distinct sites, Deer Cove Creek, a very small tributary of the main stem near the entrance to the National Park, and Mill Flat Creek, a larger tributary that branches near Pine Flat Reservoir. The population from the Kings River sampled at Deer Cove Creek, clusters with hatchery strains in most analyses, indicating likely hatchery trout ancestry (Further investigation of the Mill Flat origins proved unsuccessful).

In general, although structure was found, all naturally-spawned populations within the Central Valley basin were closely related, regardless of whether they were sampled above or below a known barrier to anadromy. This is due to some combination of preimpoundment historic shared ancestry, downstream migration and, possibly, limited, anthropogenic, upstream migration.

(Garza and Pearse 2008)

The magnitude of F_{ST} values are slightly higher in previous studies than in the Kings River study; however direct comparison of F_{ST} values across studies is not statistically appropriate given that the same microsatellite loci were not used here. However, the general trend from these studies is that within watershed/basin collection of rainbow trout in California, regardless of if they were sampled above/below dams, are genetically more similar than samples from between watersheds (Aguilar 2014).

In 1990 the California Department of Fish and Game (CDFG) published a report which detailed the trout strains distributed by each of California's hatcheries. According to the publication the Mt. Whitney, Hot Creek and Coleman strains were propagated and stocked seasonally by the San Joaquin Hatchery in Fresno County. The report coincided with data acquired by the Kings River Fisheries Management Program in 2005 which listed Mt. Whitney, Hot Creek, Coleman and Eagle Lake strains as the fishes obtained for use by the KRFMP telemetry study (Kings River Fisheries Management Program, 2007). This suggests that viable populations of these strains had been stocked in the Kings River for at least 15 years. Although Garza and Peasrse (2008) only collected samples above Pine Flat Reservoir, the phylogeographic trees created by their study (Appendix B) may suggest that the wild population above the reservoir share similar origins with those stocked by CDFG.

This research, in conjunction with the results from the KRFMP genetics study suggests that there is little difference between the "wild" rainbow trout populations residing above or below Pine

Flat Dam. Aguilar offers that this may be due to the dam not being in place long enough for a strong genetic divergence to occur between the two populations. This may also indicate that the same trout from the San Joaquin Hatchery were planted above and below the reservoir post construction of the Dam. On the flip side, Aguilar concluded that "It is highly unlikely that the samples typed above or below the Pine Flat Dam contain any fish of hatchery origin. Additionally these studies find that introgression from hatchery strains is minimal". This statement seems to contrast with the premise just established, however further inquiry suggests that post stocking, the trout that do go on to reproduce themselves create a residualized population which shows little interest in mating with newly introduced hatchery stock over time. Despite sharing a genetic lineage with hatchery produced rainbow trout, the resident population has reverted to a more wild state and thus, doesn't behave in a manner similar to recently stocked fish.

In January 2013, S.1149, (2012), Sec. 12(e) became effective. The new law mandated that only non-reproductive trout be planted in California streams with few exceptions. Throughout 2013 the San Joaquin Hatchery phased out the remaining diploid trout (with the exception of brood stock) and was stocking triploid trout by 2014. It is likely that any wild fish captured after 2014 are product of residual diploid hatchery stock.

References

Aguilar, Andres. (2014). Report to the Kings River Conservation District. Unpublished, CSU Los Angles.

- Barngrover, Bruce G. (1990). Rainbow Trout Strains in California State Hatcheries. California Department of Fish and Game 76 (2).
- Garza, J.C., Pearse, D.E. (2008). Population genetic structure of Oncorhynchus mykiss in the California Central Valley. California Department of Fish and Game, Final Report for Contract PO485303, Santa Cruz.
- Kings River Fisheries Management Program, Technical Steering Committee. (2007). Habitat Selection, Behavioral Movement, and Fate of Adult Rainbow Trout within the Kings River Downstream of Pine Flat Dam: Results of the 2006-2006 Radio Tagging Investigation. Unpublished, Internal Document.
- Moyle, Peter B. (2002) Inland Fishes of California, Revised and Expanded. University of California Press.

Appendix A

Microsatellites Tested

- 1. OMM1127
- 2. OMM1130
- 3. OMM1054
- 4. OMM1081
- 5. 0MM1082
- 6. OMM1083
- 7. OMM1101
- 8. OMM1104
- 9. OMM3027
- 10. OMM5041
- 11. OMM5092
- 12. OMM5010

Appendix B



Figure A: Neighbor joining phylogeographic tree created from all samples taken by Garza, Pearse 2008.



Figure B: Neighbor joining phylogeographic tree created from all samples, all sites taken by Garza, Pearse 2008.





Figure D: No CV below barrier pops (Garza; Pearse. 2008)