

Currently, there are 10 smolt traps operating in the Upper Columbia region, including a trap at the lower end of each population (Table 1). Having a trap at the lower end of each population allows for the opportunity to evaluate population level productivity status and trend, if appropriate expansions can be made. Having smolt traps in sub-watersheds allows for comparisons between Major Spawning Areas, comparing Major Spawning Areas to the population as a whole, and potentially comparing Major Spawning Area or population estimates to reference watersheds.

## Results and Discussion

The smolt trap survey in the Chiwawa River Basin provides the longest term data set in the Upper Columbia, with 19 years of operation (Table 1). Annual status as well as longer term trends can be evaluated from data sets of this length. For example, the density dependent response in juvenile productivity is suggested by the much higher smolts/redd observed during the low adult escapement years of the mid-late 1990s (Figure 1). Data sets for most other smolt traps are not long enough to evaluate long-term trends or have confidence that the mean across years shows the year-to-year environmental variability that is natural and expected (Table 1). The Chiwawa River has received very little habitat restoration and is considered highly functional habitat, so it may be a good reference watershed, if the contribution of hatchery origin fish spawning in the wild can be accounted for.

When picking a reference stream, it is important that inherent differences in productivity due to watershed specific climate, geology, geomorphology, and hydrology are understood. Analyses still need to be conducted to determine exactly how to use and compare the juvenile productivity data between subbasins. For example, it is reasonable to assume, based on an examination of the graph to conclude that the Wenatchee Subbasin as a whole is not as productive as the Chiwawa River, since every year the population level estimate from the Monitor smolt trap is less than the Chiwawa River estimate (Figure 1). A statistical analysis of a comparison between traps within and among subbasins is needed (Figure 2). Duration (years), variance, and autocorrelation (not shown on any of the graphs) will be important considerations in these comparisons. Likewise, additional information is needed to determine the definition of trend with respect to this and other juvenile fish data in order to definitively answer the key management question. However, given the length of time and quantity of habitat restoration actions that are planned and the long-term commitments in place to continue smolt monitoring, it is likely that this will prove to be a useful measure for increases in habitat productivity in the future. ➔

## Genetic Variation in *Oncorhynchus mykiss* in Tributaries to the Lower Methow Basin

Dana Weigel<sup>2and3</sup>, M. Powell<sup>1</sup>, P. Connolly<sup>1</sup>, and K. Martens<sup>2</sup>

<sup>1</sup>Columbia River Research Lab, U.S. Geological Survey, Cook, WA

<sup>2</sup>Aquaculture Research Institute, University of Idaho, Moscow, ID

<sup>3</sup>Grangeville Field Office, U.S. Bureau of Reclamation, Grangeville, ID

## Background

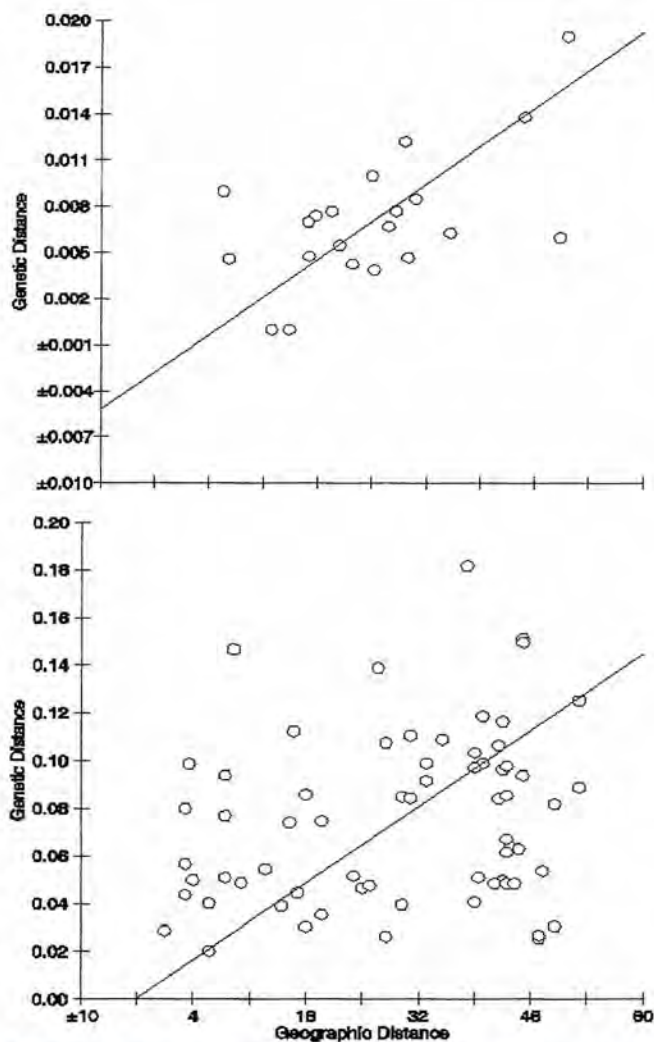
Genetic processes such as selection, mutation, and drift interact with various behavioral and environmental factors to promote reproductive opportunities for individuals in a landscape. Genetic differentiation is determined by population isolation. Salmonids are known to exhibit several isolating mechanisms such as: life history variation separated spatially and temporally, homing to natal streams, and assortative mating.

*Oncorhynchus mykiss* exhibit sympatric life history strategies: resident/fluvial and anadromous. Studies have shown some inter-breeding between these life history types, and plasticity in their derivation (such as resident fish parenting anadromous offspring). Hatchery fish are present in the Methow Basin, and introgression between the wild and hatchery populations is thought to occur. However, hatchery fish have much lower relative fitness in natural environments. For example, wild (W) female steelhead were found to produce about 20 times the smolts than hatchery (H) females in a study in Forks Creek, Washington (McLean et al. 2004). In addition, the cross and direction can determine the relative survival to age 1 with WxH having 58%, HxW 30%, and HxH 14% of the relative survival of WxW offspring (Miller et al. 2004).

The purpose of this study was to: describe genetic diversity and genetic differentiation in *O. mykiss* from 3 natal tributaries (Beaver, Libby and Gold creeks) in the lower Methow Basin, and explore the relative contribution of environmental and biological attributes. The null hypothesis tested was that *O. mykiss* populations in the study area were panmictic (or no detectable population differentiation). The alternative hypotheses was that population differentiation was detectable and was related to life history and/or habitat attributes.

## Methods

Juvenile and adult trout were collected from 19 sites in the study area during 2004 and 2005 using electrofishing. Genetic material were collected and PIT tag interrogation data were used to determine the dominant life history strategy at each site. Trout were considered to exhibit anadromous migration patterns when PIT tags were detected at John Day/McNary dams or downstream on the lower Columbia River. Anadromous sites had >3%, and resident sites <1%, of the proportion of total tags deployed detected on the Columbia River or estuary. Stream distances between sites were calculated using the distance tool in ArcView GIS software.



Figures 1 and 2. Mantel test for isolation by distance for anadromous sites (top) and for resident sites (lower) in the lower Methow tributaries. The test is significant ( $p < 0.05$ ) with an  $R^2 = 0.35$  for anadromous sites, not significant ( $p > 0.05$ ) and  $R^2 = 0.05$  for resident sites.

DNA was extracted and amplified using standardized protocols and loci for the Columbia Basin *O. mykiss* (Stephenson et al. 2009). Sixteen microsatellite loci were used. The later 5 loci provided some differentiation between *O. mykiss* and *O. clarki*. Putative full siblings were identified and removed using ML Relate set to 10,000 permutations (Kalinowski et al. 2006). A Bonferroni correction was used for all multiple comparisons. Exact tests for Hardy Weinberg and linkage disequilibrium were performed using Genepop set to default values (Raymond and Rousset 1997). Genetic diversity indices (heterozygosity and allelic richness) were calculated using HP Rare (Kalinowski et al. 2005). Genetic differentiation was measured using pairwise  $F_{st}$  values and exact tests were calculated using Genepop set to default values. Isolation by distance, examining whether there is increased genetic differentiation with geographic distance, was tested with a Mantel test using IBD Web Service (Bohanak et al. 2002). Arlequin was used to partition variation using AMOVAS (Excoffier et al. 2005).

### UCRTT Deliberations

Weigel et al. have demonstrated that populations of *Oncorhynchus mykiss* are more diverse in the Methow Sub-basin, and presumably in the Upper Columbia, than had been previously understood. The limited geographic scope of this study does not give us a complete estimate of status that is of most interest to recovery assessment and would not provide much of a baseline against which to elucidate trends. These findings recall the question of what are the normative processes and conditions against which the spatial structure and diversity (SS/D) metrics should be compared? A spatially-balanced sampling program for steelhead throughout the Upper Columbia should be initiated to give us much better insight into the status and trends related to SS/D. Existing genetic monitoring programs that focus on the comparison between hatchery vs. natural populations are powerful and need to be included in any future genetic monitoring program. However, additional investigation would be required to 1) develop a reference condition or an idea about what is the desired condition for SS/D, 2) describe the status and trends in SS/D for steelhead for the entire ESU, and 3) elucidate the contribution of rainbow trout production and diversity to steelhead, something that recent studies suggest may be significant.

	Basin (B, L, G)	Life History (A, R)
Among group	0.40%	0.10%
Among pop. within group	3.90%	4.10%
Within Pop.	95.70%	95.80%

Table 1. The amount of variation at the basin scale (Beaver, Libby, Gold) to life history (anadromous vs. resident) (AMOVA).

### Results

A total of 693 trout were collected from 19 sites. Suspected full siblings detected at each site ranged from 0 to 62% (average 23%), with more full siblings detected at resident sites. After removing all but one suspected full sibling from each site, 590 trout from 19 sites were used for the subsequent analyses. Sample sizes ranged from 7 to 55 (average 28). Of the 19 sites, 6 were anadromous, 6 were resident, 4 were resident *O. m.* with *O. m.* x *O. c.* hybridization present, and 2 were anadromous *O. m.* with *O. m.* and *O. c.* hybridization present. Three additional sites in Beaver Creek have not been analyzed yet.

Four sites had one locus each out of Hardy Weinberg Equilibrium and 7 of 2,280 comparisons showed significant linkage disequilibrium. There was no pattern to these loci or sites. Sample-wide pairwise  $F_{st}$  values ranged from 0 to 0.18 across all the sites. Pairwise  $F_{st}$  values ranged from 0 to 0.019 across the anadromous sites with about half the sites significantly different ( $p < 0.003$ ). Generally, adjacent sites tended not to be significantly different. The Mantel test showed significant isolation by

distance for all the pairwise site comparisons in the study area with an  $R^2 = 0.18$ . However, when we separated the data set by life history type, anadromous sites showed a stronger ( $R^2 = 0.35$ ) and significant isolation by distance relationship, whereas resident sites did not (Figures 1 and 2). The AMOVA indicated that > 95% of the genetic variation is at the individual level, and a small proportion is at the basin or life history level (< 0.5%). Basin explained four times the variation in the genetic data than life history, additional indication that genetic diversity is related to distance or other landscape variables more so than life history (Table 1).

	# sites	# anad sites	# loci	He (Ho)	AR	Fst	Sig IBD
<b>Methow B. L. G</b>	19	8	16	0.67-0.83	4.53-6.88	0-0.18	Y anad N resid
<b>Klickitat (Narum et al 2008)</b>	20	7	13	0.46-0.82	2.8-9.0	0-0.377	Y anad N resid
<b>Grande Ronde (Narum et al 2006)</b>	4	4	20	(0.76-0.81)	11.2-12.4	0.005-0.016	
<b>Walla Walla (Narum et al 2004)</b>	14	12	6	0.8, 0.78	14.5, 13.7	0.001-0.018	
<b>Snake (Neilsen et al 2009)</b>	79	75	11	0.55-0.73	4.1-6.2	0.003-0.05*	Y
<b>Skeena, Nass, Dean - Canada BC (Heath et al. 2001)</b>	10	10	6	0.75-0.85		Avg 0.04*	Y
<b>Kamchatka - Russia (McPhee et al. 2007)</b>	7	5	10	0.24-0.54	1.9-9.8	0-0.19	Y

Table 2. Comparison of *O. mykiss* data from Beaver, Libby and Gold creeks genetic diversity and differentiation measures and isolation by distance to other studies in the Columbia Basin, British Columbia, Canada, and Kamchatka, Russia. Asterisk indicates data that were pooled by subbasin and are not a direct site level comparison to the values reported in this study.

## Discussion

The genetic diversity measures (heterozygosity, allelic richness), genetic differentiation measure (Fst), AMOVA and isolation by distance results from the lower Methow tributaries are similar to other studies documented in various Columbia Basin tributaries, British Columbia, Canada, and Kamchatka, Russia (Table 2). Our study is most similar in sample intensity, genotypic data and spatial extent to the Klickitat Basin, Washington study (Narum et al. 2008), aside from some low heterozygosity, allelic richness and higher Fst values that were likely the influence of more isolated resident populations sampled in the Klickitat study. The Klickitat populations were shown to have no detectable hatchery introgression thought to be due to little reproductive overlap between the native steelhead and stocked Skamania steelhead in the basin (Narum et al. 2006).

The *O. mykiss* sampled from the Grande Ronde basin in Oregon had higher genetic diversity (heterozygosity and allelic richness), but similar genetic differentiation (Fst) values (Narum et

## Adaptive Management Recommendations

A spatially balanced genetic sampling program for Chinook salmon and steelhead should be established throughout the Upper Columbia that can be repeated at intervals to understand the status and trends in genetic diversity. This program would be particularly useful if it was designed 1) to monitor the influences of hatchery impacts to population genetic structure, 2) to help understand what the desired condition for SS/D might be, and 3) elucidated the contribution of rainbow trout production and diversity to steelhead, something that recent studies suggest may be significant.

Status data from the Canadian portions of the Okanogan steelhead population should be incorporated into the overall status assessment that has until now focused on the portion of the Okanogan subbasin within the U.S. Included within this assessment should be the identification, delineation, and monitoring of major and minor spawning areas within Canada. Likewise, the Canadian portions of the Okanogan should be included within a spatially balanced genetic sampling program (see above point).

al. 2006). This study only compared data from two wild and two hatchery populations. Hatchery populations could be increasing these genetic diversity values due to brood management practices such as random mating. The Walla Walla basin, Snake River basin and British Columbia basins (Narum et al. 2004, Neilsen et al. 2009, Heath et al. 2001) have similar genetic results to our study. The Kamchatka populations show lower levels of heterozygosity and allelic richness, but similar levels of genetic differentiation to our study (McPhee et al. 2007).

Several of the populations tested in the Snake River basin (Neilsen et al. 2009) showed introgression between *O. m.* and *O. c.* from other studies (Weigel et al. 2002), indicating that this is not a phenomenon limited to the Methow Basin study. In conclusion, spatial genetic diversity in *O. mykiss* in the lower Methow tributaries is present, and most sites were significantly different. Spatial genetic diversity and differentiation is generally similar to other *O. mykiss* studies. Basin explained more genetic variation than life history, and isolation by distance was significant for anadromous sites, but not resident sites. ←

# **Upper Columbia Regional Technical Team 2010 Analysis Workshop Synthesis Report**

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Edited by:

**Michael B. Ward, Terraqua, Inc.**

**Julie Morgan, Upper Columbia Salmon Recovery Board**

**Casey Baldwin, Upper Columbia Regional Technical Team  
(and WDFW)**

Prepared for:

**Upper Columbia Salmon Recovery Board**

Prepared by:

**Upper Columbia Regional Technical Team**

and

**Terraqua, Inc.**

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