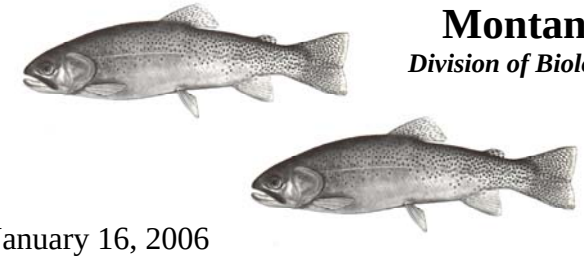

Montana Conservation Genetics Laboratory

Division of Biological Sciences * University of Montana * Missoula, MT 59812
(406)243-5503/6749 Fax (406)243-4184

January 16, 2006

Mike Hensler
Montana Department of Fish, Wildlife, and Parks
475 Fish Hatchery Road
Libby, Montana 59923

Mike;

We have completed the protein electrophoretic analysis of the trout collected from Little Cherry Creek.

Summary of results.

Sample #	Water Name/Location/Collection Date/ Collector	N (a)	# markers (b)	Species ID (c)	Power (d)	(%)
3127	**Little Cherry Creek**	30	R5Y4	CBRT	W95Y99	100
	7/28/2005 Mike Hensler					

a) Number of fish in the sample
b) Number of diagnostic loci. W=westslope cutthroat trout, Y=Yellowstone cutthroat trout, R=rainbow trout
c) CBRT=Columbia Basin redband trout, RBT=coastal rainbow trout, WCT= westslope cutthroat trout, YCT= Yellowstone cutthroat trout. Taxa separated by X indicate hybridization between them was detected.
d) Probability of detecting one percent hybridization with the indicated taxa. Taxa indicated as in b.

Horizontal starch gel electrophoresis was used to determine each fishes genetic characteristics (genotype) at 47 loci (genes) coding for proteins present in muscle, liver, or eye tissue (Table 1). At some of these loci, redband, *Onchorhynchus mykiss gairdneri*, and coastal rainbow trout (collectively termed rainbow trout), *O. m. irideus*, rarely share alleles (form of a gene) in common with westslope cutthroat trout, *O. clarki lewisi* (Table 2). This situation also pertains to a comparison of rainbow and Yellowstone cutthroat trout, *O. c. bouvieri* (Table 2). Loci at which such fixed genetic differences exist between taxa are commonly termed diagnostic loci because the alleles detected at them can be used to help determine whether a sample came from a non-hybridized population of one of these fishes or a population in which hybridization between two or all three of them has or is occurring.

Populations of Columbia Basin redband (redband) and coastal rainbow trout are usually genetically distinguishable from each other. Redband populations usually possess *LDH-B2*76* at a frequency greater than 0.25 and *sSOD-1*152* at a frequency less than 0.10 (Knudsen et al. 2002). In contrast, coastal rainbow trout populations usually possess *LDH-B2*76* at a frequency less than 0.10 and *sSOD-1*152* at a frequency greater than 0.15 (Knudsen et al. 2002). The frequency of these two alleles in a sample, therefore, can be used to help determine whether it came from a redband trout population, coastal rainbow trout population, or a population in which hybridization between these fishes has or is occurring.

Results and Discussion

Little Cherry Creek 3127

Fish were collected from two reaches of Little Cherry Creek. The upstream sample (N=20) was collected in the Little Cherry Creek loop near the crossing of road 6212. The downstream sample (N=10) was collected about one-half to three quarters of a mile upstream from the confluence of Little Cherry Creek and Libby Creek. Contingency table chi-square analysis for heterogeneity of allele frequencies indicated that they were statistically homogeneous between these two samples at all the loci that showed evidence of genetic variation (data not shown). Thus, there was no evidence of genetic differences between the fish collected from the different areas. The two samples, therefore, were combined into a single sample for further analysis.

With the exception of *sAAT-1**, alleles characteristic of only rainbow trout were detected in the sample (Table 3). The *sAAT-1*200* allele detected in the sample is usually characteristic of westslope cutthroat trout. Thus, its presence could indicate hybridization with westslope cutthroat trout or it could simply be rainbow trout genetic variation that is electrophoretically indistinguishable from that usually characteristic of westslope cutthroat trout. In this case, we strongly favor the latter interpretation because if its presence was due to hybridization then it is highly unlikely (contingency table chi-square, $P<0.001$) we would not detect alleles characteristic of westslope cutthroat trout at the other diagnostic loci for this fish that were analyzed. With the sample size of 30, we have about a 95% chance of detecting as little as a one percent westslope and better than a 99% chance of detecting as little as a one percent Yellowstone cutthroat trout genetic contribution to a hybrid swarm. Thus, Little Cherry Creek almost certainly contains a non-hybridized rainbow trout population.

The allele frequencies at *LDH-B2** and *sSOD-1** in the sample were highly characteristic of redband trout (Table 3). Thus, the rainbow trout population in Little Cherry Creek strongly appears to be redband trout.

Little Cherry Creek was previously sampled in 1991 (sample # 572, N=5) and 1992 (sample # 644, N=25). There was no evidence of genetic differences (contingency table chi-square, data not shown) between these two samples so they were combined into one for further analysis.

Evidence of genetic variation was detected at five loci between the two Little Cherry Creek samples (Table 3). Contingency table chi-square analysis indicates that the allele frequencies were statistically heterogeneous between the samples at three of these loci (Table 3). This heterogeneity could indicate that genetic differences exist between the samples or it could simply represent chance departures from homogeneity due to the number of comparisons that were performed. In order to distinguish between these two possibilities, we compared the chi-square statistic at the loci showing heterogeneity to the modified level of significance proposed by Rice (1989). The differences remain significant at the modified level so we conclude that genetic differences exist between the two samples. The genetic characteristics of the Little Cherry Creek population, therefore, do not appear to have been temporally stable.

In order to place the amount of genetic divergence between the two Little Cherry Creek samples into perspective, we partitioned the total amount of genetic variation detected into that due to genetic variation within the samples and genetic differences between them using the procedure of Chakraborty (1980). Genetic differences between the samples account for nine percent of the genetic variation detected. Using allozyme data from six redband populations from throughout the Kootenai River drainage, Knudsen et al. (2002) estimated that about 16% of the total genetic variation detected was due to genetic differences among populations. Thus, the amount of genetic change that has occurred in the Little Cherry Creek population between 1992 and 2005 is about half as much as that observed among redband populations throughout the drainage. The genetic changes observed in the Little Cherry Creek population, therefore, are far from trivial.

The most likely explanation for the marked genetic changes observed in the Little Cherry Creek population is that it has a very low effective population size. With low effective population size there is substantial sampling error involved in what particular alleles get passed on to subsequent generations (genetic drift). Thus, with low effective population size over time the allele frequencies at genetically variable loci will change by chance. The amount of change expected by genetic drift is inversely related to effective population size. The substantial changes observed in the Little Cherry Creek population over a relatively short period of time, therefore, suggest significant genetic drift and thus low effective population size.

The *PGM-1*null* allele was not detected in the 1991 and 1992 samples. The presence of this allele in the 2005 sample and evidence of significant genetic changes in the population suggest that this allele probably was present at a lower frequency in the population when it was sampled in 1991 and 1992. It was probably not detected because its presence can only conclusively be determined when homozygous individuals, that is those with two copies of the allele, are included in the sample. For example, with a sample size of 30 and a frequency of 0.10 there is about a 50% chance of not collecting

homozygous individuals and, therefore, not detecting this allele. Thus, the apparent absence of this allele in the earlier samples is more likely due to its low frequency rather than its actual absence.

You mentioned last week that there was some belief that the previous data indicated that the Little Cherry Creek population was hybridized with westslope cutthroat trout and coastal rainbow trout. The report addressing the 1991 sample (Sage 1992) indicated the status of the population was uncertain because the *sAAT-1*200* allele was detected in one fish and this could indicate a small amount of hybridization with westslope cutthroat trout or simply be rainbow trout genetic variation that was electrophoretically indistinguishable from that usually characteristic of westslope cutthroat trout. Because of the small sample size, it was not possible to distinguish between these possibilities and conservatively it was suggested the population be considered hybridized with westslope cutthroat trout unless future data indicated otherwise. The 1992 (Sage 1993) and 2005 samples clearly indicate the population is not hybridized with westslope cutthroat trout.

The report addressing the 1992 sample (Sage 1993) indicated the population was probably hybridized with coastal rainbow trout. This conclusion was based on the observation that the *LDH-B2*76* frequency (0.800) was significantly lower in the 1993 Little Cherry Creek sample than the mean frequency (0.957) observed among seven redband trout samples from the Yaak River drainage above the falls. This comparison was made because at this time about the only apparent redband trout populations sampled from the Kootenai River drainage were from the Yaak River drainage above the falls. Extensive subsequent sampling throughout the drainage, however, has conclusively indicated this comparison was misleading. It is now clear that redband trout populations in the Yaak River drainage above the falls generally have a significantly higher mean *LDH-B2*76* frequency than other redband populations in the Kootenai River drainage (Table 4). Thus, considering all the data it now appears clear that Little Cherry Creek contains a non-hybridized redband trout population.

Robb Leary

Literature Cited

Chakraborty, R. 1980. Gene-diversity analysis in nested subdivided populations. Genetics 96:721-726.

Knudsen, K. L., C. C. Muhlfeld, G. K. Sage, and R. F. Leary. 2002. Genetic structure of Columbia River redband trout populations in the Kootenai River drainage, Montana, revealed by microsatellite and allozyme loci. Transactions of the American Fisheries Society 131:1093-1105.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223-225.

Sage, G. K. 1992. Letter to Doug Perkinson, United States Forest Service, July 2 1992.

Sage, G. K. 1993. Letter to Doug Perkinson, United States Forest Service, February 23, 1993.

Table 1

Enzymes and loci examined. Tissues: E=eye, L=liver, M=muscle.

Enzyme	Loci	Tissue
Adenylate Kinase	*AK-1*, AK-2**	M
Alcohol Dehydrogenase	*ADH**	L
Aspartate Aminotransferase	*sAAT-1*, sAAT-2**	L
	*sAAT-3,4**	M
Creatine Kinase	*CK-A1*, CK-A2**	M
	CK-B, CK-C1*, CK-C2**	E
Dipeptidase	*PEPA-1*, PEPA-2**	E
N-acetyl-beta-Glucosaminidase	*bGLUA**	L
Glucose-6-phosphate Isomerase	*GPI-A**	E
	GPI-B1, GPI-B2**	M
Glyceraldehyde-3-phosphate Dehydrogenase	*GAPDH-3*, GAPDH-4**	E
Glycerol-3-phosphate Dehydrogenase	*G3PDH-1*, G3PDH-2**	L
Iditol Dehydrogenase	*IDDH**	L
Isocitrate Dehydrogenase	*mIDHP-1*, mIDHP-2**	M
	*sIDHP-1,2**	L
Lactate Dehydrogenase	*LDH-A1*, LDH-A2**	M
	LDH-B1, LDH-B2*, LDH-C**	E
Malate Dehydrogenase	*sMDH-A1,2**	L
	*sMDH-B1,2**	M
Malic Enzyme	*mMEP-1*, mMEP-2*, sMEP-1**	M
	*sMEP-2**	L
Phosphoglucomutase	*PGM-1*, PGM-2**	M
	*PGM-1r**	L

Table 1-continued

Enzyme	Loci	Tissue
Phosphogluconate Dehydrogenase	*PGDH**	M
Superoxide Dismutase	*sSOD-1**	L
Tripeptide Aminopeptidase	*PEPB**	E
Xanthine Dehydrogenase-*like*	*XDHl**	L

Table 2

Alleles at the diagnostic loci that differentiate westslope cutthroat trout and rainbow trout, westslope and Yellowstone cutthroat trout, and rainbow and Yellowstone cutthroat trout. When more than one allele exists at a locus within a taxon, the most common allele is listed first.

Locus	Taxa and characteristic alleles	
	Westslope	Rainbow
*sAAT-1**	*200,250*	*100*
*CK-A2**	*84*	*100*
*GPI-A**	*92,100*	*100*
*IDDH**	*40,100*	*100,200,40*
*sIDHP-1**	*86,71*	*100,114,71,40*
*mMEP-1**	*100*	*null*
	Westslope	Yellowstone
*sAAT-1**	*200,250*	*165*
*CK-C1**	*100,38*	*38*
*GPI-A**	*92,100*	*100*
*IDDH**	*40,100*	*100*
*mIDHP-1**	*100*	*-75*
*sIDHP-1**	*86,71*	*71*
*mMEP-1**	*100*	*null*
*sMEP-1**	*100*	*90*
*sMEP-2**	*100*	*110*
*PEPA-1**	*100*	*101*
*PEPB**	*100*	*135*
*PGM-1**	*100,null*	*null*

Table 2- continued

Locus	Taxa and characteristic alleles	
	Rainbow	Yellowstone
*sAAT-1**	*100*	*165*
*CK-A2**	*100*	*84*
*CK-C1**	*100,38,150*	*38*
*mIDHP-1**	*100*	*-75*
*sIDHP-1**	*100,114,71,40*	*71*
*sMEP-1**	*100*	*90*
*sMEP-2**	*100,75*	*110*
*PEPA-1**	*100,115*	*101*
*PEPB**	*100,120*	*135*
*PGM-1**	*100,null*	*null*

Table 3

Allele frequencies at the loci showing evidence of genetic variation in the combined 1991 and 1992 and the 2005 samples of redband trout from Little Cherry Creek. X^2= contingency table chi-square statistic for heterogeneity of allele frequencies between samples. *=P<0.05. **=P<0.01. ***=P<0.001. D.F.= degrees of freedom.

		Sample and allele frequencies			
Locus	Alleles	1991+1992	2005	X^2	D.F.
*sAAT-1**	*100*	0.950	0.683	14.248***	1
	200	0.050	0.317		
*IDDH**	*100*	0.983	0.967	0.342	1
	200	0.017	0.033		
*sIDHP-1,2**	*100*	0.500	0.500	10.909**	2
	71	--	0.083		
	40	0.500	0.417		
*LDH-B2**	*100*	0.217	0.100	3.064	1
	76	0.783	0.900		
*PGM-1**	*100*	1.000	0.592	5.455*	1
	null	--	0.408		

Table 4

Frequency of *LDH-B2*76* in 37 samples of what appear to be redband trout populations in the Kootenai River drainage.

Drainage and Sample		*LDH-B2*76* Allele Frequency
Wolf Creek		
Weigal Creek		0.211
Wolf Creek		0.315
Lower Brush Creek		0.361
Wolf Creek		0.870
Syrup Creek		0.639
Upper Little Wolf Creek		0.250
Lower Little Wolf Creek		0.636
Calx Creek		0.600
	Mean Frequency	0.485
Pleasant Valley Fisher River		
Pleasant Valley Fisher River above Loon Lake		0.367
McGinnis Creek		0.420
	Mean Frequency	0.394
Libby Creek		
Libby Creek above Ramsay Creek		0.533
Poorman Creek		0.720
Little Cherry Creek 1991+1992		0.783
Little Cherry Creek 2005		0.900
Upper Bear Creek		0.150
	Mean Frequency	0.617
Big Cherry Creek		
Granite Creek below falls		0.740
Big Cherry Creek below Deep Creek		0.460
Big Cherry Creek		0.600
	Mean Frequency	0.600
Parmenter Creek		
Parmenter Creek		0.717

Table 4- continued

Drainage and Sample	*LDH-B2*76* Allele Frequency
Callahan Creek	
South Callahan Creek	0.280
North Callahan Creek	0.635
Callahan Creek	0.300
Mean Frequency	0.405
Yaak River below falls	
Yaak River	0.240
Yaak River above falls	
Yaak River between Vinal Creek and West Fork Yaak River	0.920
Basin Creek	0.964
West Fork Basin Creek	0.911
East Fork Basin Creek	0.944
Porcupine Creek	0.896
Lower North Fork Yaak River	0.981
Upper North Fork Yaak River	1.000
Boyd Creek	1.000
Solo Joe Creek 2004	0.800
Caribou Creek 2004	0.875
East Fork Yaak River 2004 above and below bridge	0.908
Mean Frequency	0.927
Saddle Creek	
Saddle Creek	1.000
Grass Creek	
Grass Creek	0.480