Montana Conservation Genetics Laboratory

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

January 12, 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 caught by anglers from Lake Koocanusa during the 2005 fishing derby.

Summary of results.

Sample #	Water Name/Location/Collection Date/ Collector	a N	b # markers	c Species ID	d Power (%)	%
3126	**Lake Koocanusa**	33	W6Y10	RBT	W98Y99	
	5/15/2005 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) RBT= 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 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, coastal rainbow trout, or a population in which hybridization between these fishes has or is occurring.

Results and Discussion

Lake Koocanusa 3126

Alleles characteristic of only rainbow trout were detected in the sample. With a sample size of 33, we have about a 98% chance of detecting as little as a one percent westslope cutthroat and better than a 99% chance of detecting as little as a one percent Yellowstone cutthroat trout genetic contribution to a hybrid swarm. The fish caught in the derby, therefore, were almost undoubtedly non-hybridized rainbow trout.

Evidence of genetic variation was detected in the fish at five loci (Table 3). Although the allele frequencies at *LDH-B2** and *sSOD-1** (Table 3) are characteristic of redband trout, we suspect that these fish also possess a coastal rainbow trout genetic component. We can not be certain of this because we do not know what the genetic characteristics of the fish were prior to any possible hybridization with coastal rainbow trout, but previous analyses (Phelps and Allendorf 1980; Leary et al. 1988) have conclusively indicated that fish collected from the reservoir and those now stocked in the reservoir (Leary 2005) do contain a coastal rainbow trout genetic component. Despite the uncertainty about whether or not the fish caught by anglers in 2005 contain a coastal rainbow trout genetic contribution, the data clearly indicate they have a substantial redband trout genetic component.

The next issue to address is whether or not it appears that the fish caught were stocked from the Murray Springs State Trout Hatchery or originated from natural reproduction. In order to do this, we compared the genetic characteristics of the fish caught in 2005 to those of the 1997 Murray Springs yearclass, the 1987 Duncan redband trout yearclass from the Ennis National Fish Hatchery, the combined 1994, 1996 and 1998 yearclasses of Gerrard redband trout from the Bull River Hatchery, Wardner, British Columbia, and what appeared to be non-hybridized rainbow trout collected from the north end of Lake Koocanusa in 1983 (Table 3). Duncan and Gerrard redband trout were used in the comparisons because they are native to Kootenay Lake in British Columbia and were commonly reared at the Bull River Hatchery which is suspected to have been the original, albeit inadvertent, source of redband trout that invaded Lake Koocanusa. The invasion probably occurred in the mid 1970's (Leary et al. 1988). Although we have data available from the 2004 Murray Springs yearclass, the fish caught in 2005 were too big to have originated from this yearclass so it was not included in the comparisons. We also did not use the data from a sample of trout collected from the Rexford area of Lake

Koocanusa in 1986 because these fish were mainly hybrids between westslope cutthroat, coastal rainbow, and redband trout. Thus, they obviously would not be a source of the rainbow trout caught in 2005.

We used two approaches to compare the genetic characteristics of the samples. First we used contingency table chi-square analysis to test for heterogeneity of allele frequencies at the loci showing evidence of genetic variation between The 2005 Lake Koocanusa sample and the other four. In order to account for the possibility of encountering chance departures from homogeneity due to the number of comparisons performed for each pair of samples, significant differences were compared to the modified level of significance proposed by Rice (1989). Differences that remained significant at the modified level were considered to indicate that genetic differences exist between the samples. Next, we simultaneously compared the allele frequencies among all the loci showing evidence of genetic variation among all the samples using principle components analysis. In this analysis, we eliminated the common allele at each of the genetically variable loci from the data. This was done to eliminate redundancy from the data set because allele frequencies at a locus must sum to one.

At the modified level of significance, no significant allele frequency differences were detected between the 2005 Lake Koocanusa rainbow trout and those collected in 1983 (Table 4). In contrast, there were significant allele frequency differences between the 2005 Lake Koocanusa rainbow trout and the 1997 Murray Springs yearclass at two loci and the Gerrard and Duncan redband trout at one locus (Table 4). These results suggest that compared to the 2005 Lake Koocanusa sample the genetically most similar sample was rainbow trout collected from the northern end of the reservoir in 1983.

The principle components analysis indicates that the 2005 Lake Koocanusa sample is genetically about equidistant from the 1983 Lake Koocanusa sample and the 1997 Murray Springs sample (Figure 1). These results suggest that the most likely source of the fish caught in 2005 was from Lake Koocanusa itself, Murray Springs State Trout Hatchery, or both. Thus, overall we can not definitively state whether or not stocking rainbow trout from Murray Springs is significantly contributing to the Lake Koocanusa trout fishery.

The ambiguity in the data arises from the fact that the 2005 Lake Koocanusa, 1983 Lake Koocanusa, and 1997 Murray Springs samples are all genetically quite similar to each other. Data from the 2004 Murray Springs yearclass indicates that significant genetic changes have occurred in the broodstock between 1997 and 2004 (Leary 2005). For example, the frequency of *LDH-B2*100* increased from 0.508 to 0.782, the frequency of *sSOD-1*152* increased from 0.017 to 0.113, the frequency of *G3PDH-1*140* increased from 0.028 to 0.172, and the frequency of *mIDHP-1*140* increased from 0.036 to 0.202. Because of these changes, in the future we should be able to better determine whether or not fish from Murray Springs are significantly contributing to the Lake Koocanusa trout fishery. If they are, then the observed differences between the 1997 and 2004 yearclasses should be reflected in fish caught from the reservoir. If they are not, then fish caught

from the reservoir should appear substantially different from the Murray Springs broodstock.

Robb Leary

Literature Cited

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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 rainbow trout collected from Lake Koocanusa in 2005 and 1983, the 1997 yearclass of rainbow trout from the Murray Springs State Trout Hatchery, the 1987 yearclass of Duncan redband trout from the Ennis National Fish Hatchery, and the combined 1994, 1996, and 1998 yearclasses of Gerrard redband trout from the Bull River Hatchery, Wardner, British Columbia.

		Sample and Allele Frequencies				
		Lake Koocanusa				
Locus	Alleles	2005	1983	Murray	Duncan	Gerrard
*CK-A1**	*100*	1.000	1.000	0.991	1.000	1.000
	76	--	--	0.009	--	--
*G3PDH-1**	*100*	1.000	1.000	0.972	1.000	1.000
	140	--	--	0.028	--	--
*bGLUA**	*100*	0.985	1.000	1.000	1.000	1.000
	70	0.015	--	--	--	--
*!DDH**	*100*	1.000	1.000	0.989	1.000	1.000
	200	--	--	0.011	--	--
*mIDHP-1**	*100*	1.000	0.967	1.000	1.000	1.000
	140	--	0.033	--	--	--
*sIDHP-1,2**	*100*	0.577	0.667	0.608	0.500	0.540
	114	0.008	0.033	0.017	--	--
	71	0.317	0.183	0.258	0.450	0.322
	40	0.098	0.117	0.117	0.050	0.138
*LDH-B2**	*100*	0.424	0.667	0.508	0.250	0.218
	76	0.576	0.333	0.492	0.750	0.782
*LDH-C**	*100*	1.000	0.967	1.000	1.000	1.000
	95	--	0.033	--	--	--
*sMDH-A1,2**	*100*	1.000	0.983	1.000	1.000	1.000
	40	--	0.017	--	--	--
*sMDH-B1,2**	*100*	0.886	0.883	0.987	0.955	0.835
	125	0.106	0.067	0.009	0.040	0.165
	83	0.008	0.050	0.004	0.003	--

Table 3-continued

		Sample and Allele Frequencies				
		Lake Koocanusa				
Locus	Alleles	2005	1983	Murray	Duncan	Gerrard
*PEPA-1**	*100*	1.000	1.000	0.930	1.000	0.988
	115	--	--	0.070	--	0.012
*PGM-2**	*100*	0.985	0.967	0.991	1.000	1.000
	90	0.015	0.033	0.009	--	--
*sSOD-1**	*100*	1.000	1.000	0.983	1.000	1.000
	152	--	--	0.017	--	--

Table 4

Results of contingency table chi-square test for heterogeneity of allele frequencies between rainbow trout caught by anglers from Lake Koocanusa in 2005 and rainbow trout collected from Lake Koocanusa in 1983, the 1997 yearclass of rainbow trout from the Murray Springs State Trout Hatchery, the combined 1994, 1996, and 1998 yearclasses of Gerrard redband trout from the Bull River Hatchery, Wardner, British Columbia, and the 1987 yearclass of Duncan redband trout from the Ennis National Fish Hatchery. NS=comparison non-significant. *=P<0.05. **=P<0.01. ***=P<0.001. -- =locus was not genetically variable in either sample.

Locus	1983 Koocanusa	1997 Murray Springs	Gerrard	1987 Duncan
*CK-A1**	--	NS	--	--
*G3PDH-1**	--	NS	--	--
*bGLUA**	--	NS	NS	NS
*IDDH**	--	NS	--	--
*mIDHP-1**	--	NS	--	--
*sIDHP-1,2**	NS	NS	NS	NS
*LDH-B2**	NS	NS	**	*
*LDH-C**	NS	--	--	--
*sMDH-A1,2**	NS	--	--	--
*sMDH-B1,2**	NS	***	NS	NS
*PEPA-1**	--	*	NS	--
*PGM-2**	NS	NS	NS	NS
*sSOD-1**	--	NS	--	--

Figure 1

Plot of first two principle component axes derived from the allele frequencies at the genetically variable loci in rainbow trout caught by anglers from Lake Koocanusa in 2005 and rainbow trout collected from Lake Koocanusa in 1983, the 1997 yearclass of rainbow trout from the Murray Springs State Trout Hatchery, the combined 1994, 1996, and 1998 yearclasses of Gerrard redband trout from the Bull River Hatchery, Wardner, British Columbia, and the 1987 yearclass of Duncan redband trout from the Ennis National Fish Hatchery. Number in parentheses indicates the percentage of the total genetic variation accounted for by each axis.