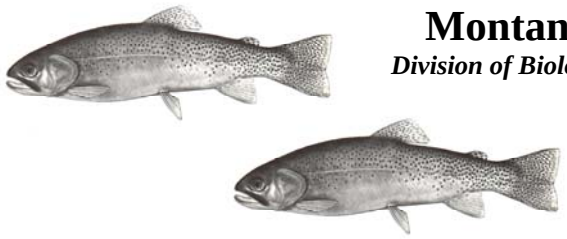

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Mark Deleray
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Mark;

Following is my assessment of the high priority samples you wanted checked for accuracy of data interpretation:

Mount Creek (#2026)

This sample collected June 1, 2000 was originally reported to have come from a hybrid swarm with about a 99% westslope cutthroat and a 1% rainbow trout genetic contribution. I concur with this interpretation. PINE fragments characteristic of rainbow trout were detected at two of the six diagnostic loci analyzed that usually distinguish rainbow from westslope cutthroat trout. Furthermore, the fragments characteristic of rainbow trout appear to be randomly distributed among the fish in the sample as three individuals each possessed a marker characteristic of rainbow trout at a single locus.

Lower Pilgrim Lake (#2337 and #2322)

The sample collected September 5, 2001 (2337) was originally reported as having come from a hybrid swarm with about a 99% westslope cutthroat and a 1% rainbow trout genetic contribution. I somewhat disagree with this interpretation although from a management perspective the disagreement is moot .

There was some trouble obtaining data from the sample and complete data are available for only 11 of the 30 fish collected. These data, however, clearly indicate the presence of hybridization. PINE fragments characteristic of rainbow trout were detected at two of the six loci analyzed that usually distinguish rainbow from westslope cutthroat trout. The fragments characteristic of rainbow trout, however, do not appear to be randomly distributed among the fish in the sample. Rather, they were detected in only two fish with one possessing rainbow trout markers at two loci and another possessed a rainbow trout marker at one locus. This nonrandom distribution of the markers among individuals suggests the sample may have contained some non-hybridized westslope cutthroat trout as well as hybridized individuals. In this situation, conclusively determining which

individuals are non-hybridized will be extremely problematic. This will require a large number of markers because the hybrid individuals collected were definitely late generation hybrids, probably third generation or later, and they appear to contain a relatively small proportion of rainbow trout genetic material. Thus, with a relatively small number of markers many hybrids will be indistinguishable from westslope cutthroat trout. From a management perspective, therefore, at this point in time the population should simply be considered to have been slightly hybridized with rainbow trout.

The second sample collected September 1, 2002 (2322) was initially reported as having come from a hybrid swarm with about a 99% westslope cutthroat and a 1% rainbow trout genetic contribution. I agree with this interpretation. PINE fragments characteristic of rainbow trout were detected at two of the six loci analyzed that usually distinguish rainbow from westslope cutthroat trout. The PINE fragments characteristic of rainbow trout appeared to be randomly distributed among the fish in the sample as only two (initially erroneously reported as three) individuals each possessed a rainbow trout marker at only one locus. Thus, at this point in time the population appears to have mainly, if not solely, been composed of hybridized individuals.

When Lower Pilgrim Lake was first sample August 14, 1986 (sample not in data base) allozyme analysis indicated the population to be a hybrid swarm with about a 95% westslope cutthroat and a 5% rainbow trout genetic contribution. Considering all the samples, the data suggest stocking westslope cutthroat trout into the lake beginning in 1989 and ceasing after 1994 initially resulted in a mixed population of westslope cutthroat trout and hybridized individuals. Subsequently, however, it appears the population has reverted to a hybrid swarm with a lower rainbow trout genetic contribution than originally detected.

Pyramid Lake (#2027 and 1690)

The sample collected July 30, 2001 (2027) was originally reported as appearing to have come from a hybrid swarm with about a 99% westslope cutthroat and a 1% rainbow trout genetic contribution. The sample actually appears to have come from a population containing a mixture of predominantly non-hybridized westslope cutthroat trout and a small proportion of hybridized individuals between westslope cutthroat and rainbow trout. In the sample, one fish possessed PINE markers characteristic of rainbow trout at two of the six diagnostic loci analyzed that distinguish rainbow trout from westslope cutthroat trout. The remaining 26 fish in the sample contained PINE fragments characteristic of only westslope cutthroat trout suggesting that many of them are probably non-hybridized westslope cutthroat trout. For the same reasons as in Lower Pilgrim Lake, conclusively identifying non-hybridized individuals in Pyramid Lake will be extremely problematic. Thus, from a management perspective at this point in time the Pyramid Lake population should simply be considered slightly hybridized with rainbow trout.

When Pyramid Lake was first sampled August 5, 1987 (1690), allozyme analysis indicated it to be a hybrid swarm with a 99% Yellowstone cutthroat and a 1% rainbow trout genetic contribution. Considering both samples, the data indicate stocking westslope cutthroat trout into Pyramid Lake in 1988 and ceasing after 1994 has replaced the Yellowstone cutthroat-rainbow trout hybrid swarm with a population of mainly westslope cutthroat trout and a small proportion of westslope cutthroat-rainbow trout hybrids. This observation suggests at least some partial reproductive isolation between the westslope cutthroat trout and hybrids because with random mating we would expect to observe what appeared to mainly be a hybrid swarm in this situation a few generations after the lake was last stocked.

Haskill Creek (#2166)

This population collected November 1, 2001 was originally reported as being suspected of being non-hybridized westslope cutthroat trout and I agree with this assessment. PINE fragments characteristic of rainbow trout were detected at one of the six loci analyzed that usually distinguish rainbow from westslope cutthroat trout. In the sample, five fish possessed the rainbow trout marker. This could indicate a small amount of hybridization or this could simply be westslope cutthroat trout PINE genetic variation that is electrophoretically indistinguishable from that usually characteristic of rainbow trout. In this case, the data suggest the latter interpretation is much more likely because if the "rainbow trout" fragment detected was due to hybridization it is highly unlikely (contingency table chi-square; $P<0.001$) markers characteristic of rainbow trout would not be detected at the other diagnostic loci analyzed.

Dickey Lake (#2331)

This population sampled July 13, 2001 was originally reported as suspected of being non-hybridized westslope cutthroat trout and I concur with this interpretation. A PINE fragment characteristic of Yellowstone cutthroat trout was detected in only one fish at one of the four loci analyzed that usually distinguish Yellowstone and westslope cutthroat trout. This is certainly not strong enough evidence to conclude the population is hybridized, but the possibility it may be can not reasonably be excluded. In situations of uncertainty like this, the conservative approach is to consider the population to be non-hybridized westslope cutthroat trout unless additional data indicate otherwise.

Gordon Creek (#2126, #2127, #2319, and #308)

When Gordon Creek was first sampled August 2, 1989 (308) allozyme analysis indicated it to be non-hybridized westslope cutthroat trout. Likewise, when lower (2126) and upper Gordon Creek (2127) were sampled August 5, 2000 the population appeared to be non-hybridized westslope cutthroat trout as PINE fragments characteristic of only this fish were detected in the samples. In contrast, the sample collected August 27, 2002 (2319) was initially reported as most likely being hybridized with a 99.5% westslope

cutthroat trout, a 0.3% Yellowstone cutthroat trout, and a 0.2% rainbow trout genetic contribution. I do not completely agree with the latter assessment.

In sample 2319, a PINE fragment characteristic of rainbow trout was detected in one fish at one of the six diagnostic loci analyzed that usually distinguish rainbow and westslope cutthroat trout. A PINE fragment characteristic of Yellowstone cutthroat trout was also detected in only one fish at one of the four loci analyzed that usually distinguish Yellowstone and westslope cutthroat trout. Different individuals possessed the "rainbow" and "Yellowstone cutthroat trout" fragments. Thus, this variation could represent hybridization or it could simply be westslope cutthroat trout PINE genetic variation that is electrophoretically indistinguishable from that usually characteristic of rainbow or Yellowstone cutthroat trout. Thus, the most appropriate interpretation of the population based on this sample would be to consider its status uncertain. In this situation, the conservative approach would be to consider the population to be non-hybridized westslope cutthroat trout unless future data indicate otherwise.

In terms of being used as a potential source of fish for transplants or brood stock purposes for westslope cutthroat trout restoration and conservation, the Gordon Creek population should not be used until its status concerning whether or not it is hybridized is more conclusively determined. Analysis of additional fish using PINEs may not provide a more reliable assessment than is presently available as it is likely we would obtain data very similar to that which we already have. We have available seven microsatellite loci that distinguish westslope cutthroat trout from rainbow trout, but we have no microsatellite loci readily available that distinguish westslope and Yellowstone cutthroat trout. In contrast, we have six allozyme loci available that distinguish westslope cutthroat and rainbow trout and 10 allozyme loci available that distinguish westslope and Yellowstone cutthroat trout. Thus, if it is deemed desirable to obtain a better understanding of the status of the Gordon Creek population allozyme analysis provides the most readily available alternative technique than PINEs.

Lower Seven Acres Lake (#2327 and #2325)

This lake was first sampled July 29, 1986 (not in data base). Results from the allozyme analysis of these fish were somewhat inconclusive. An allele usually characteristic of rainbow trout was detected at one of the six diagnostic loci analyzed that usually distinguish westslope cutthroat and rainbow trout. This could indicate a small amount of hybridization with rainbow trout or it could simply be westslope cutthroat trout genetic variation that is electrophoretically indistinguishable from that usually characteristic of rainbow trout. In this situation, the former interpretation was considered more likely as the lake was probably historically fishless and for a while it appears that Montana Department of Fish, Wildlife, and Parks was maintaining a presumed westslope cutthroat trout broodstock established from Big Salmon Lake that may have been slightly hybridized with rainbow trout (Sage 1993).

The sample collected September 6, 2001 (2327) supports the above conclusion. In the sample, a single individual conclusively contained a PINE fragment characteristic of rainbow trout at one of the six loci analyzed that usually distinguish rainbow trout from westslope cutthroat trout. These data in conjunction with the previous allozyme data strongly suggest that this population is a hybrid swarm with a predominant westslope cutthroat trout genetic contribution and less than a one percent rainbow trout genetic contribution.

In the sample collected September 9, 2002 (2325), only PINE fragments characteristic of westslope cutthroat trout were detected. Thus, this sample was interpreted as appearing to have come from a non-hybridized westslope cutthroat trout population. Considering the previous data and that the lake was last stocked with westslope cutthroat trout in 1993 this interpretation probably is incorrect. The interpretation most consistent with all the available data is that the population is slightly hybridized with rainbow trout (less than a one percent genetic contribution) and evidence of this was not detected in the last sample because of sampling error.

Upper Seven Acres Lake (#2324)

PINE fragments characteristic of only westslope cutthroat trout were detected in this sample. Thus, I agree with the initial interpretation that this sample appears to have come from a non-hybridized westslope cutthroat trout population.

North Biglow Lake (#2334, #2323, and #1634)

PINE fragments characteristic of only westslope cutthroat trout were detected in the samples collected September 5, 2001 (2334) and September 9, 2002 (2323). Thus, these samples were considered to have come from a non-hybridized westslope cutthroat trout population.

Considering the initial allozyme data and the fact that the lake has not knowingly been stocked with westslope cutthroat trout, however, the conclusion this is a non-hybridized westslope cutthroat trout population may be erroneous. The allozyme sample collected September 5, 1984 (1634) conclusively indicated the population to be hybridized with about a 98% westslope cutthroat and a 2% rainbow trout genetic contribution as alleles characteristic of rainbow trout were detected at two of the six diagnostic loci analyzed that usually distinguish westslope cutthroat and rainbow trout.

There are two possible explanations for the apparent discrepancy between the allozyme and PINE data sets. First, the population is slightly hybridized, but evidence for it was not apparent using the PINE markers because it has randomly been lost from the population because of genetic drift (chance loss of genetic variation from populations of finite size). Alternatively, the population actually has successfully been stocked with westslope cutthroat trout, but no official record exists. The available data do not allow us to distinguish between these possibilities. At this time, therefore, the most appropriate

management approach to adopt would be to consider the population largely, if not solely, westslope cutthroat trout.

Whether or not the population actually is non-hybridized westslope cutthroat trout at this time could best be addressed by microsatellite or allozyme analysis. If evidence of hybridization is still apparent, then the obvious conclusion is the population is hybridized and evidence of this was not detected using PINE markers because of the chance loss of genetic variation. In contrast, if no evidence of hybridization is detected and significant genetic changes in the population appear at nondiagnostic allozyme loci, then this would more strongly suggest the population has mainly been replaced by one or more unrecorded westslope cutthroat trout stockings.

Sincerely,

Robb Leary

Literature Cited

Sage, G. K. 1993. Allozymic and parasitic examination of interspecific introgression in *Onchorhynchus* from the South Fork of the Flathead River drainage. Masters Thesis. University of Montana, Missoula, Montana.