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Lee;

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

## Norton Creek (#2389)

The report dealing with this sample collected August 5, 2002 really did not conclude much but, it does tend to make one feel the population is slightly hybridized. Actually the status of this population should be considered to be uncertain. A PINE fragment usually characteristic of rainbow trout was detected at one of the six diagnostic loci analyzed that usually distinguish rainbow from westslope cutthroat trout. The 'rainbow trout' fragment was detected in only one fish. The other 14 fish in the sample possessed only PINE fragments characteristic of westslope cutthroat trout. Thus, this could be a non-hybridized westslope cutthroat trout population with PINE genetic variation that is electrophoretically indistinguishable from that usually characteristic of rainbow trout or it could be slightly hybridized with rainbow trout. Further analysis will be required to distinguish between these possibilities. At this time, the conservative approach would be to consider this a non-hybridized westslope cutthroat trout population.

## Teepee Creek (#2025)

This sample collected August 9, 1999 was reported as being suspected to be non-hybridized westslope cutthroat trout. The uncertainty was do to the presence of a PINE fragment in the sample usually characteristic of Yellowstone cutthroat trout at one of the four diagnostic loci analyzed that usually distinguish Yellowstone from westslope cutthroat trout. The suspect fragment was detected in five of the 25 fish in the sample. If its presence was due to hybridization, it is highly unlikely (contingency table chi-square, $P<0.01$) we would not have detected evidence of hybridization with Yellowstone cutthroat trout at the other diagnostic loci analyzed. Thus, the presence of this fragment was considered to more likely represent westslope cutthroat trout PINE genetic variation that is electrophoretically identical to that usually characteristic of Yellowstone cutthroat trout rather than evidence of hydridization. There is no reason to alter the conclusion that conservatively this should be considered to be a non-hybridized westslope cutthroat trout population.

## South Fork Warm Springs Creek-upper (#2148)

PINE fragments characteristic of only westslope cutthroat trout were detected in this sample collected September 26, 2001. With the sample size of 27, we have a 96% chance of detecting as little as a one percent rainbow trout and an 89% chance of detecting as little as a one percent Yellowstone cutthroat trout genetic contribution to a hybrid swarm. Thus, this population was appropriately considered to have most likely been non-hybridized westslope cutthroat trout.

## South Fork Warm Springs Creek –lower (#2147)

Initially, this sample collected September 26, 2001 was reported to probably be a mixture of fish of hybrid origin between rainbow and westslope cutthroat trout and possibly some non-hybridized westslope cutthroat trout. Of the five fish in the sample, two of them possessed PINE fragments usually characteristic of rainbow trout at five of the six diagnostic loci analyzed that usually distinguish rainbow from westslope cutthroat trout conclusively indicating these fish to be of hybrid origin. The remaining fish possessed PINE fragments characteristic of only westslope cutthroat trout. The conclusion that these fish are non-hybridized westslope cutthroat trout is somewhat tentative because of the small sample size but, the fact that a non-hybridized westslope cutthroat trout population appears to exist in the upper reaches of the creek adds support to this interpretation. Further support to the conclusion that these fish are westslope cutthroat trout is provided by the fact that the hybrids detected had a very high rainbow trout genetic contribution Thus, very few hybrids would not be distinguishable from westslope cutthroat trout using six diagnostic loci. There is no compelling reason, therefore, to alter the original interpretation that this reach of stream contained a mixture of hybrids and westslope cutthroat trout.

## Gurnett Creek (#158 and#2216)

The most recent sample collected October 30, 2001 (2216) was reported to be a mixture of westslope cutthroat trout and fish of hybrid origin between westslope and Yellowstone cutthroat trout. This interpretation is highly compatible with the data. PINE fragments characteristic of only westslope cutthroat trout were detected in all the fish analyzed except one. The latter individual possessed PINE fragments characteristic of Yellowstone cutthroat trout at two of the four diagnostic loci analyzed that usually distinguish Yellowstone from westslope cutthroat trout. This fish, therefore, was almost undoubtedly of hybrid origin.

These results differ from those obtained from a previous sample collected April 1, 1986 (158). Allozyme analysis of these fish (N=11) provided no evidence of hybridization. Thus, at this time the population was conservatively considered to be non-hybridized westslope cutthroat trout.

There are a few possible reasons for the discrepancy between the two data sets. The population could have been hybridized in 1986, but evidence of it was not detected because of sampling error. The first sample was collected from T8N R3E S2 while the second sample was collected from T8N R3E S1. Thus, another possibility is the creek may contain two genetically different populations with one being hybridized and the other not. Another possible reason for the discrepancy is the population has only recently become hybridized or contains recent migrants from a hybridized population with a substantial Yellowstone cutthroat trout genetic contribution.

Of the above possibilities, the first is the most unlikely. If the population contained hybrid individuals in 1986, then when it was sampled again in 2001 it should largely have appeared to be a hybrid swarm which certainly does not appear to be the case.

Of the remaining two explanations, the last most likely pertains to the most recent sample. It appeared to contain some non-hybridized westslope cutthroat trout and the only definite hybrid individual collected possessed Yellowstone cutthroat trout markers at multiple loci. The hybrid, therefore, possessed characteristics of individuals of recent hybrid origin or individuals from a hybrid population with a substantial Yellowstone cutthroat trout genetic contribution. Because only one definite hybrid individual was collected, in this situation reliably assessing how confidently one could distinguish on an individual basis between hybridized and non-hybridized fish is not possible. Thus, with the available data this reach of stream should simply be considered to contain a hybridized population of westslope and Yellowstone cutthroat trout.

Based on the first sample, whether or not the stream does contain a non-hybridized westslope cutthroat trout population in the area of T8N R3E S2 is an open question. This can obviously be addressed only by further sampling.

## No-named tributary to Hebgen Reservoir (#2291 and #2839)

When these two samples are combined (2839, N=21, collected July 30, 2002?; 2291, N=28, collected September 25, 2002), PINE fragments characteristic of only westslope cutthroat trout were detected at all the loci analyzed except one. A PINE fragment characteristic of rainbow trout was detected in one fish at one of the six diagnostic loci analyzed that usually distinguish rainbow from westslope cutthroat trout. This population, therefore, could be non-hybridized westslope cutthroat trout with PINE genetic variation that is electrophoretically indistinguishable from that usually characteristic of rainbow trout or it could be slightly hybridized with rainbow trout. With this uncertainty, it was suggested that the population conservatively be considered non-hybridized westslope cutthroat trout and this is certainly the most appropriate management approach unless further data indicate otherwise.

## Dutchman Creek-upper (#2345)

PINE fragments characteristic of only westslope cutthroat trout were detected in the sample at all the loci analyzed. With the sample size of 52, there is better than a 99% chance of detecting as little as a one percent rainbow or Yellowstone cutthroat trout genetic contribution to a hybrid swarm. Thus, this population was appropriately reported as appearing to be non-hybridized westslope cutthroat trout.

## Bear Creek (#832 and #2226)

This stream was first sampled August 30, 1993 (832) and allozyme analysis indicated no evidence of hybridization. Since only 10 fish were analyzed, the population was conservatively considered to be non-hybridized westslope cutthroat trout at this time. PINE analysis of the more recent sample collected October 29, 2001 (2226) also provided no evidence of hybridization as fragments characteristic of only westslope cutthroat trout were detected. With the combined sample size of 63, we have better than a 99% chance of detecting as little as a one percent rainbow or Yellowstone cutthroat trout genetic contribution to a hybrid swarm. This population, therefore, as last reported is almost certainly non-hybridized westslope cutthroat trout.

## Ray Creek (Un-numbered and #2344)

PINE analysis of both samples (un-numbered, collected October 18, 2001; 2344, October 7, 2002) detected fragments characteristic of only westslope cutthroat trout. With the combined sample size of 56, we have better than a 99% chance of detecting as little as a one percent rainbow or Yellowstone cutthroat trout genetic contribution to a hybrid swarm. This population, therefore, as last reported is almost certainly non-hybridized westslope cutthroat trout.

## German Gulch (#75 and #2392)

Allozyme analysis of the first sample (75, collected July 27, 1984) provided no evidence of hybridization, and with the sample size of 37 the population was considered to be non-hybridized westslope cutthroat trout. PINE analysis of the more recent sample (2392, N=15, collected July 25, 2002) detected a fragment characteristic of rainbow trout in one fish at one of the six diagnostic loci analyzed that usually distinguish rainbow from westslope cutthroat trout. This could indicate a small amount of hybridization with rainbow trout or it could be westslope cutthroat trout PINE genetic variation that is electrophoretically indistinguishable from that usually characteristic of rainbow trout. In the database, based on this sample the population was reported as being slightly hybridized with rainbow trout, but there is no compelling evidence for this. Because of the uncertainty about the status of the population based on the more recent sample, conservatively the population should be considered to be non-hybridized westslope cutthroat trout.

## Jones Creek (#695 and #2224)

This stream was first sampled August 27, 1992 (695) and allozyme analysis indicated no evidence of hybridization. Because only 10 fish were collected, however, the possibility it may have been slightly hybridized with rainbow or Yellowstone cutthroat trout could not reasonably be excluded. Thus, conservatively it was suggested that the population be considered to be non-hybridized westslope cutthroat trout.

In the more recent sample collected October 30, 2001 (2224, N=25), PINE analysis detected fragments characteristic of Yellowstone cutthroat trout at two of the four diagnostic loci analyzed that usually distinguish Yellowstone from westslope cutthroat trout. The fragments characteristic of Yellowstone cutthroat trout appeared to be randomly distributed (Poisson distribution, $P>0.50$) among the fish in the sample. As initially reported this sample, therefore, appears to have come from a hybrid swarm between westslope and Yellowstone cutthroat trout with about a 2.5% Yellowstone cutthroat trout genetic contribution.

Considering both samples, there are at least two possible explanations for the observed results. When first sampled, the population was hybridized with Yellowstone cutthroat trout, but evidence of this was not detected because of sampling error. This does not seem very likely, however, because with 10 diagnostic allozyme loci between westslope and Yellowstone cutthroat trout and 10 fish there is less than a five percent chance (contingency table chi-square) we would not detect a 2.5% Yellowstone cutthroat trout genetic contribution to a hybrid swarm. Thus, the more likely explanation is that the population was not hybridized with Yellowstone cutthroat trout in 1992, but subsequently it has become hybridized.

PINE fragments characteristic of rainbow trout were detected in the most recent sample at four of the six diagnostic loci analyzed that usually distinguish rainbow from westslope cutthroat trout. The rainbow trout fragments were not randomly distributed ($P<0.001$) among the fish in the sample. In contrast, they were all detected in only one individual. Thus, it appears that hybridization with rainbow trout has only recently begun in the population or the fish definitely of hybrid origin with rainbow trout was a recent migrant. Overall, when this population was last sampled it appears to have contained a mixture of hybrids between westslope and Yellowstone cutthroat trout and a small proportion of hybrids with a high rainbow trout genetic contribution.

## Bean Creek (#696 and #2225)

This stream was first sampled August 27, 1992 (696) and allozyme analysis indicated no evidence of hybridization. Because only 10 fish were collected, however, the possibility it may have been slightly hybridized with rainbow or Yellowstone cutthroat trout could not reasonably be excluded. Thus, conservatively it was suggested that the population be considered to be non-hybridized westslope cutthroat trout.

In the most recent sample collected October 30, 2001 (2225, N=53), a PINE fragment characteristic of rainbow trout was detected in one fish at one of the six diagnostic loci analyzed that usually distinguish rainbow from westslope cutthroat trout. This could indicate a small amount of hybridization with rainbow trout or it could simply be westslope cutthroat trout PINE genetic variation that is electrophoretically indistinguishable from that usually characteristic of rainbow trout. Because of this uncertainty and in 1992 the population appeared to be non-hybridized, it should not have been reported to be hybridized in the August 14, 2002 report. Instead, its status should have been reported as uncertain and, therefore, conservatively it should be considered to be non-hybridized westslope cutthroat trout.

## McClellan Creek (#410, #1063, and #2024)

Allozyme analysis of the first two samples (410, N=10, collected August 1, 1990; 1063, N=10, collected 1995) yielded somewhat ambiguous results. Alleles characteristic of only westslope cutthroat trout were detected at all the loci analyzed except one. At this locus, an allele characteristic of Yellowstone cutthroat trout was also detected in both samples. Thus, we were somewhat uncertain whether this represented a small amount of hybridization with Yellowstone cutthroat trout or it was westslope cutthroat trout genetic variation that was electrophoretically identical to that characteristic of Yellowstone cutthroat trout. The same allele, however, usually characteristic of Yellowstone cutthroat trout was detected in both samples. Furthermore, based on its combined frequency (0.175) it was highly unlikely (contingency table chi-square, $P<0.001$) that if this allele represented evidence of hybridization that no other alleles characteristic of Yellowstone cutthroat trout would be detected. Thus, this allele much more likely appears to represent westslope cutthroat trout genetic variation than hybridization with Yellowstone cutthroat trout. It was recommended, therefore, that the population conservatively be considered to be non-hybridized westslope cutthroat trout.

PINE analysis of the most recent sample collected August 10, 1999 (2024) detected fragments characteristic of only westslope cutthroat trout. These data combined with the previous allozyme data strongly indicate that the allozyme allele normally characteristic of Yellowstone cutthroat trout that was detected actually is simply westslope cutthroat trout genetic variation. After eliminating the variable diagnostic allozyme locus, with the combined sample size of 44, we have better than a 99% chance of detecting as little as a one percent rainbow or Yellowstone cutthroat trout genetic contribution to a hybrid swarm. The McClellan Creek population, therefore, is almost undoubtedly non-hybridized westslope cutthroat trout.

## Middle Fork Cabin Creek (#747, #’s1932-1937, #2019, #2023, and #2744)

Spatially these samples extend from about 0.2 miles above the mouth of the Middle Fork Cabin Creek (1932) to 4.5 miles above the mouth (2019). Allozyme analysis of the first sample collected June 1, 1993 (747) collected about 2.8 miles above the mouth provided no evidence of hybridization. Because only 10 fish were analyzed we could not reasonably exclude the possibility that the population was slightly hybridized with Yellowstone cutthroat trout, rainbow trout, or both taxa, but evidence of this was not detected because of sampling error. Thus, at this time it was suggested that the population conservatively be considered to be non-hybridized westslope cutthroat trout.

Middle Fork Cabin Creek was next sampled July 27, 1999 (1932-1937). These samples represented an altitudinal group of 8 or 10 fish from six locations spanning from about 0.2 to 3.2 miles above the mouth. Among the samples, PINE analysis detected fragments characteristic of rainbow trout at four of the six diagnostic loci analyzed that usually distinguish rainbow from westslope cutthroat trout. The PINE fragments characteristic of rainbow trout, however, were not randomly distributed ($P<0.001$) among the fish in the samples. In contrast, significantly more fish lacked rainbow trout fragments (53) than expected by chance (46.2), significantly fewer fish possessed a rainbow trout fragment at only one locus (1) than expected by chance (10.7), and significantly more fish possessed rainbow trout markers at two or more loci (4) than expected by chance (0.1). The fish possessing rainbow trout markers indicating they were definitely of hybrid origin were dispersed among the sample locations. They were present in samples 1932 about 0.2 miles above the mouth (N=2), 1934 about 2 miles above the mouth (N=1), 1935 about 2.3 miles above the mouth (N=1), and 1936 about 2.6 miles above the mouth (N=1). Based on the non-random distribution of rainbow trout fragments among the fish the population was considered to be a mixture of non-hybridized westslope cutthroat trout and a relatively small proportion of hybrids between westslope cutthroat and rainbow trout. In this situation, conclusively determining which individuals are non-hybridized would be extremely problematic. This would require a large number of markers because the hybrid individuals collected were definitely later than first generation hybrids. Thus, with a relatively small number of markers many hybrids would be indistinguishable from westslope cutthroat trout. This combined with the fact that hybrid individuals were dispersed among sample locations led to the conclusion that from a practical perspective the population should simply be considered to be hybridized between westslope cutthroat and rainbow trout.

Since no evidence of hybridization was detected in the two samples collected more than about 2.6 miles above the mouth (samples 747 at about 2.8 miles and 1937 at about 3.2 miles) the possibility that the upper reaches of the stream contained a non-hybridized westslope cutthroat trout population remained viable. Thus, the upper portions of the stream were sampled in 2001 and 2002. Although no evidence of hybridization based on PINE analysis was detected in samples 2744 (4 miles above the mouth, N=41, collected July 7, 2002) and 2023 (4.3 miles above the mouth, N=26, collected June 15-22, 2001), PINE fragments characteristic of rainbow trout in were detected in sample 2019 (4.5 miles above the mouth, N=55, collected June 15, 2001) at two of the six diagnostic loci analyzed that usually distinguish rainbow from westslope cutthroat trout. Like in the downstream samples in which evidence of hybridization was detected, the PINE fragments characteristic of rainbow trout were not randomly distributed ($P<0.01$) among the fish in sample 2019. Rather, only one fish in the sample possessed rainbow trout markers, but at two loci definitely indicating it to be of hybrid origin. Thus, this reach of stream also appeared to contain a mixture of non-hybridized westslope cutthroat trout and a small proportion of later generation hybrids between westslope cutthroat and rainbow trout. Again, however, conclusively separating the non-hybridized individuals from the hybridized ones would be problematic because it would require a large number of diagnostic loci.

Considering all the data, it definitely appears that Middle Fork Cabin Creek contains a mixture of non-hybridized westslope cutthroat trout and a small proportion of later generation hybrids between westslope cutthroat and rainbow trout. The latter fish appear to be more common in the lower reaches of the stream, but they have also been detected in the upper most reach of the stream sampled. Because non-hybridized individuals cannot reliably be separated from the hybrid fish using our standard PINE analysis, unless significantly more diagnostic loci are analyzed from a practical perspective the fish in the entire reach of stream sampled should be considered to be hybridized. Alternatively, by using a combination of PINE and microsatellite analyses we can screen individuals at 13 diagnostic loci between rainbow and westslope cutthroat trout. With this procedure, we would have a 99% chance of detecting hybrid individuals with as little as a 16% rainbow trout genetic contribution. Based only on the individuals definitely of hybrid origin collected from Middle Fork Cabin Creek these fish appear, on the average, to have about a 25% rainbow trout genetic contribution. Thus, this approach could potentially reliably separate non-hybrid from hybrid individuals in Middle Fork Cabin Creek. If Middle Fork Cabin Creek is to be used as a brood stock or transfer source of fish for westslope cutthroat trout conservation, then screening individuals for evidence of hybridization using a combination of PINE and microsatellite analyses is highly recommended.

Sincerely,

Robb Leary