Recommendations from the Westslope Cutthroat Trout Technical Committee for the Genetic Conservation of the Westslope Cutthroat Trout in the Upper Missouri River Drainage

Prepared by:

The Upper Missouri Westslope Cutthroat Trout Committee

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This report presents the recommendations of the Upper Missouri Westslope Cutthroat Trout (WCT) Technical Committee (Committee). The Committee was formed by Montana Fish, Wildlife and Parks (FWP) in 1995 to make technically sound recommendations for the conservation and restoration of westslope cutthroat trout in the Upper Missouri River basin to FWP and other managers in the basin. The Committee recognizes that the conservation and restoration of any species depends upon preserving and restoring habitats needed by that species. The American Fisheries Society recently adopted a position statement on the conservation of biodiversity (Winter and Hughes 1997). While this position statement recommended that planning occur on an ecosystem, watershed, landscape, or ecoregion basis, rather than on an individual species level, the Committee's intent is to use WCT as an indicator for native cold water stream species assemblages and the habitats and processes which support those assemblages. The assumption inherent with this strategy is that if a WCT population is present and healthy, the aquatic ecosystem which supports that population is healthy and will support the full complement of native species dependent upon cold water ecosystems.

Evolutionary Significant Unit

The following is from Leary et al. (1997) which has been included as Appendix A:

There is some question as to whether or not westslope cutthroat trout, Oncorhynchus clarki lewisi, from the upper Columbia River and upper Missouri River drainages should be treated as distinct units for conservation and restoration purposes. In order to constitute an evolutionary significant unit (ESU), a population or a group of populations must at least satisfy two criteria (Waples 1991):

1) *they must be reproductively isolated from all other conspecific population units; and*
2) *they must represent an important component in the evolutionary legacy of the species or in other words there must be substantial genetic divergence between them and other conspecific populations.*

The United States Fish and Wildlife Service and National Marine Fisheries Service have adopted criteria for the designation of distinct population segments (DPS) for protection under the Endangered Species Act (Federal Register 1996) similar to those proposed by Waples (1991) for an ESU. In order to constitute a DPS, a group of populations must be discrete (markedly separated from other populations of the taxon), significant (ecologically unique for the taxon, extinction would produce a significant gap in the taxon's range, only surviving native population of the taxon, or there is substantial genetic divergence between them and other populations of the taxon), and their status must warrant

protection under the Endangered Species Act.

It is believed that westslope cutthroat trout gained access to the upper Missouri drainage from the upper Columbia drainage shortly after the last glaciation (Roscoe 1974; Trotter 1987). The last draining of glacial Lake Missoula is believed to have isolated the upper Missouri and upper Columbia drainages from each other. Thus, westslope cutthroat trout in the upper Missouri drainage have been reproductively isolated from all other groups of westslope cutthroat trout for thousands of years and clearly satisfy criterion 1 of an ESU and the discrete criterion for a DPS.

It is less clear whether they satisfy criterion 2 of an ESU or the significance criterion of a DPS. Using protein electrophoretic data Leary et al. (1988) reported substantial genetic divergence between westslope cutthroat trout from the upper Missouri and upper Columbia drainages. A potential problem with the previous study is that only one sample of westslope cutthroat trout from the upper Missouri drainage was available for comparison. If this sample was not indicative of the genetic characteristics of the populations throughout the basin, then the results could be misleading. In fact there is a good possibility that this may be the case as substantial genetic differences have been reported among westslope cutthroat trout populations in other river drainages (Allendorf and Leary 1988; Leary et al. 1988).

Of the total amount of genetic variation detected (0.0311) among samples from 16 populations in the upper Missouri drainage and 22 populations in the upper Columbia drainage, 64.95% (0.0202) was attributable to genetic variation within populations, 33.76% (0.0105) to genetic differences among populations within a drainage, and only 1.29% (0.0004) to genetic differences between populations from the upper Columbia River and upper Missouri River drainages. Thus, there is a large amount of genetic divergence among westslope cutthroat trout populations, but this is not due to appreciable differences between populations from the two drainages. Rather it is due to large differences among populations within the drainages indicating that even over short geographic distances westslope cutthroat trout populations can be genetically very different from each other.

The high genetic divergence among the westslope cutthroat trout populations mainly arises from two factors. First, there are a few variant alleles that exist in many populations with widely divergent frequencies among populations. Most of these alleles were detected in both drainages and there is broad overlap in the frequencies between the drainages. The widely divergent frequencies result in substantial genetic differences among populations within a drainage, but the

broad overlap results in little divergence between the drainages. Next, there is a large number of alleles that were detected in only one, two, or three samples. Many of these alleles, however, exist at appreciable frequency (> 0.10) in the populations in which they were detected. The populations with these alleles, therefore, are genetically very different at the particular locus from all other populations of westslope cutthroat trout regardless of where the other populations come from. Such locally distributed but high frequency alleles also result in substantial genetic divergence among populations within a drainage, but little divergence between the two drainages.

We feel that the primary genetic goal of a conservation program should be to ensure that the existing genetic variation of the taxon is maintained. This variation not only represents the evolutionary legacy of the taxon but the loss of genetic variation can have a variety of harmful effects on the characteristics of individuals important for population persistence: growth, survival, fertility, developmental rate, and the ability of individuals to develop properly (reviewed by Mitton and Grant 1984; Allendorf and Leary 1986; Palmer and Strobeck 1986; Zouros and Foltz 1987; Leary and Allendorf 1989). Furthermore, the loss of genetic variation is expected to reduce the ability of populations to adapt to changing environmental conditions and to increase their susceptibility to epizootics (Fisher 1930; Ayala 1965, 1969; Frankham 1980; O'Brien et al. 1985).

Conservation programs should be more concerned about the conservation of alleles than of allele frequencies. Allele frequencies are a temporary characteristic of a population that can be changed by genetic drift, gene flow, or natural selection. In contrast, the loss of an allele represents a permanent loss of genetic variation. Once an allele is lost it can only be recovered by mutation, the probability of which is minuscule.

Allelic variation in westslope cutthroat trout is composed largely of alleles with a very narrow geographic distribution, but these alleles often occur at appreciable frequencies in populations. Maintenance of this allelic diversity will require ensuring the continued existence of many populations throughout the range of westslope cutthroat trout. Thus, the primary goal of a conservation and restoration program for westslope cutthroat trout should be ensuring the continued existence of essentially all remaining populations.

This pattern of allelic diversity also argues against treating fish from the upper Missouri and upper Columbia drainages as separate ESU's or DPS's. First of all there is very little additional genetic divergence at this level so criterion 2 of an ESU or a DPS is not met. More importantly, however, protecting populations in

only a portion of the fish's range could leave a vast amount of allelic diversity less protected and more susceptible to loss.

The allelic diversity of westslope cutthroat trout also suggests that historically there has been very little gene flow among populations, except possibly at a very local level (Wright 1932). In this situation, even fairly weak natural selection can effectively establish local adaptations. Thus, there is a good possibility that some populations of westslope cutthroat trout may have some degree of local adaptation (e.g. Fox 1993; Phillipp and Clausen 1995) which could be broken down, compromising population viability, if the native fish interbreed with westslope cutthroat trout introduced from other populations. It is likely that westslope cutthroat trout conservation and restoration efforts at times will call for the stocking of fish either from a hatchery broodstock or from transplants from native populations. In view of the above possibility, the potential for these efforts to adversely impact native populations needs to be considered before introductions are made.

Conservation and Restoration

The Committee recommends protecting all populations of WCT to maintain as much existing genetic diversity as possible. We suggest that USGS designated sub-basin units (termed fourth-level hydrologic unit codes or HUC's; ie. Madison, Gallatin, Sun river basins) be managed as units. Restoration of WCT populations into currently unoccupied habitats within each of these fourth code HUC's can occur, but priority should be given to securing, protecting, and expanding extant populations. The Committee recommends, **by order of priority**, that:

1. All WCT populations should be conserved to ensure that the existing genetic diversity represented in these populations is preserved (Allendorf and Leary 1988). The documented presence of unique alleles in individual populations constitute potentially important genetic resources. These populations may have a high adaptive significance to the subspecies (Scudder 1989). Conservation of these remaining populations will require:

    a) identifying their location and ensuring that fish, land, and water management activities within tributary drainages (tributary drainages are defined as sub-watershed according to terminology of Maxwell et al. (1995) and typically flow into designated "rivers") are consistent with their preservation or enhancement.

    b) physical isolation of some WCT populations may be required to prevent invasion by potentially hybridizing or competing nonnative salmonids, where that risk is high. Physically isolating any WCT population should only be done after determining that isolation would pose little risk to the continued existence of that

population. Prior to isolating any population using a fish passage barrier, the extent of seasonal movements made by individuals within that population should be determined.

c) where possible, populations of WCT should be expanded, either by expanding the available habitat or enhancing the quality of currently occupied habitats.

2. The number of WCT populations should be increased by restoring WCT to historic habitats which are presently unoccupied. Unoccupied habitats shall be defined as habitats which do not presently contain genetically pure populations of WCT, and which are not connected to habitats occupied by a genetically pure WCT population. We do not now recommend that WCT be introduced into waters containing or connected to waters that contain a pure WCT population unless the existing pure population is the source of the introduced fish. This recommendation will prevent the possibility of breaking down local adaptations due to interbreeding of extant fish with introduced fish. We also recommend that initial conservation efforts concentrate on expanding, where possible, existing WCT populations by correcting factors limiting their range or abundance. This expansion might be accomplished either by watershed (habitat) restoration, enhancing existing recruitment using gametes taken from the WCT population, along with habitat restoration opening access to previously unavailable habitats, or a combination of the above.

Restoring WCT to presently unoccupied habitats will require a donor source or sources of WCT, either fish or gametes. Based on the assessment of genetic variation by Leary et al. (1997; Appendix A) the Committee suggests that any genetically pure source of WCT could be used, as long as it is capable of providing at least 50 fish, ideally at least 25 females and 25 males (Allendorf and Ryman 1987). Since there is presently a relatively high level of uncertainty concerning which donor sources might be best adapted for any particular environment, we suggest that either of the following two alternatives are viable and, if tried, their success needs to be monitored and evaluated:

a) Translocation of fish or gametes from existing populations which are abundant enough to withstand loss of at least 50 fish or 25 pair matings or gametes from these matings (Griffith et al. 1989). Due to disease concerns, it is likely that translocation efforts will use gametes, rather than fish. Translocated gametes should be incubated at the restoration site to maximize the potential for local adaptation. Translocation could be used to replicate a WCT population as a genetic reserve. Translocations would likely occur from either the nearest population or a population inhabiting habitats most similar to the proposed restoration site.

b) A captive WCT brood could be used for restoration, provided that this

captive brood has an appropriate amount of genetic diversity.

Genetic Purity

The Committee recognizes that genetic purity must be a major criteria in classifying populations and recommends that 100% pure WCT populations be given the highest priority for conservation. These populations are the only ones that can serve as potential donor sources for restoration, by either translocation or to be incorporated into a captive brood. Prior to being used as a donor source, genetic purity must be confirmed for the donor population by sampling at least 50 individuals. This genetic sampling can occur over a period of 3 to 4 years (about one generation), if a sample of 50 cannot be taken during a single year due to concerns about impacting the donor population.

For slightly hybridized WCT populations the Committee recommends that habitats supporting these populations be protected. Prior to the replacement of any slightly introgressed population with a 100% pure population, an extensive genetic sampling program must be completed throughout the range of the introgressed population to confirm that no 100% pure populations exist in the area. This recommendation is critical because genetic samples indicating a slight level of introgression are often collected only from a single site, usually in the lower portion of a stream, and it may be possible that genetically pure individuals inhabit the upper portion of the drainage.

Fish Management

WCT are an important fish to Montanans and will continue to play an important role in fish management in the state. The present “catch and release” regulation for this subspecies in the Missouri River basin will likely continue until this subspecies is fully “recovered”. The ultimate goal of “recovery” should be numerous populations which are healthy enough to support some level of angler harvest.

The Committee suggests that WCT genetic reserves, either wild or captive, are an option which could be considered. FWP’s existing captive WCT brood may be used to restore WCT populations throughout the state. The Committee supports FWP’s goal of incorporating as much genetic diversity as possible from WCT populations into this captive brood. The Committee suggests that if FWP wants to use their existing WCT brood as the primary donor source for restoration throughout the state, they should consider incorporating gametes from upper Missouri River drainage populations into it. Cultured WCT can play a role in fish management including: restoration, captive genetic reserves, "put grow and take" (private ponds and state management of lakes) fisheries; and research (rearing techniques, stocking methods, and survival).

Improper stocking of mountain lakes and the permitting of private fish ponds by FWP may place WCT populations at risk. The Committee recommends that FWP review its mountain lake

stocking policy and offers the following suggestions:

1) Within tributary drainages which are not connected to habitats which support WCT populations any suitable species may be used, however, potential risks to WCT population(s) should be assessed on a case-by-case basis;

2) Within tributary drainages that support or are connected to habitats which support WCT populations, we suggest stocking only native fish, either Arctic grayling or westslope cutthroat trout. See #2 on page 6 (first paragraph, third sentence) regarding our recommendation regarding the stocking of WCT over existing genetically pure WCT populations. This recommendation would also apply to mountain lake stocking done by FWP.

The Committee further recommends that within tributary drainages that support or are connected to habitats which support WCT populations, FWP restrict private fish pond licenses to permit releases of only westslope cutthroat trout or Arctic grayling. Other species or subspecies that can potentially inter-breed with WCT including rainbow and Yellowstone cutthroat trout should not be allowed to be stocked into these private ponds. In addition, we recommend that "state of the art" barriers be required at the inlet and outlets of these private ponds to prevent the movement of fish into or out of these private ponds.

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Appendix A

LACK OF GENETIC DIVERGENCE BETWEEN WESTSLOPE CUTTHROAT TROUT FROM THE COLUMBIA AND MISSOURI RIVER DRAINAGES

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Abstract.—There is some question as to whether or not westslope cutthroat trout, *Oncorhynchus clarki lewisi*, from the upper Columbia River and upper Missouri River drainages should be treated as distinct units for conservation and restoration purposes. Electrophoretic analysis of the products of 45 protein coding loci revealed genetic variaiton at 23 of them. There was substantial genetic divergence among populations within these two drainages (34% of total variation), but little additional divergence between drainages (1% of total variation). This pattern of genetic divergence is mainly due to the presence of a few widely dispersed variant alleles with highly variable frequencies among populations and numerous alleles, often at appreciable frequency (>0.10), in a very small proportion of the samples. Principal components analysis of the frequencies of variant alleles also indicated little genetic divergence between the drainages, but substantial divergence within. Along all principal components there was broad overlap in the space occupied by populations from both drainages. All principal components except the first placed a few populations in unique space because they had unusual frequencies of one or a few variant alleles. These results strongly argue against treating the fish from the two drainages as separate units for conservation and restoration purposes.

INTRODUCTION

There is some question as to whether or not westslope cutthroat trout, *Oncorhynchus clarki lewisi*, from the upper Columbia River and upper Missouri River drainages should be treated as distinct units for conservation and restoration purposes. In order to constitute an evolutionary significant unit (ESU), a population or a group of populations must at least satisfy two criteria (Waples 1991):

1) they must be reproductively isolated from all other conspecific population units; and
2) they must represent an important component in the evolutionary legacy of the species or in other words there must be substantial genetic divergence between them and other conspecific populations.

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It is less clear whether they satisfy criterion 2 of an ESU or the significance criterion of a DPS. Using protein electrophoretic data Leary et al. (1988) reported substantial genetic divergence between westslope cutthroat trout from the upper Missouri and upper Columbia drainages. A potential problem with the previous study is that only one sample of westslope cutthroat trout from the upper Missouri drainage was available for comparison. If this sample was not indicative of the genetic characteristics of the populations throughout the basin, then the results could be misleading. In fact there is a good possibility that this may be the case as substantial genetic differences have been reported among westslope cutthroat trout populations

in other river drainages (Allendorf and Leary 1988; Leary et al. 1988). In this report, we re-examine the amount of genetic divergence between westslope cutthroat trout from the upper Columbia and upper Missouri drainages using multiple samples from both drainages.

METHODS

Samples

All samples of fish from the upper Missouri River drainage containing at least fifteen individuals and from genetically pure populations of westslope cutthroat trout were included in the data analysis (Table 1). Only a subset of samples from the upper Columbia River drainage meeting the above two criteria were included in the data analysis to prevent it from becoming unwieldy. We divided the upper Columbia drainage into thirteen regions and randomly chose when possible two samples from each (Table 1). In four cases, only one sample was available from a region (Table 1).

Electrophoresis

Horizontal starch gel electrophoresis was used to determine each fish's genotype (genetic characteristics) at 45 loci (genes) coding for proteins (loci in parentheses) present in muscle, liver, or eye tissue: adenylate kinase (*AK-1**, *AK-2**), alcohol dehydrogenase (*ADH**), aspartate aminotransferase (*sAAT-1**, *sAAT-2**, *sAAT-3,4**), creatine kinase (*CK-A1**, *CK-A2**, *CK-B**, *CK-C1**,*CK-C2**), dipeptidase (*PEPA-1**, *PEPA-2**), glucose-6-phosphate isomerase (*GPI-A**, *GPI-B1**, *GPI-B2**), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH-3**, *GAPDH-4**), glycerol-3-phosphate dehydrogenase (*G3PDH-1**, *G3PDH-2**), iditol dehydrogenase *(IDDH**), isocitrate dehydrogenase (*mIDHP-1**, *mIDHP-2**, *sIDHP-1**, *sIDHP-2**), lactate dehydrogenase (*LDH-A1**, *LDH-A2**, *LDH-B1**, *LDH-B2**, *LDH-C**), malate dehydrogenase (*sMDH-A1,2**, *sMDH-B1,2**), malic enzyme (*mMEP-1**, *mMEP-2**, *sMEP-1**, *sMEP-2**), phosphoglucomutase (*PGM-1**, *PGM-*2*), phosphogluconate dehydrogenase (*PGDH**), superoxide dismutase (*sSOD-1**), tripeptide aminopeptidase (*PEPB**), xanthine dehydrogenase (*XDH**). Electrophoresis followed the procedures described by Leary and Booke (1990). Stains used to reveal the position of particular proteins in the gels after electrophoresis followed the recipes of Harris and Hopkinson (1976) and Allendorf et al. (1977). Nomenclature of loci and alleles (form of a gene) followed the recommendations of Shaklee at al. (1990). Whether the samples came from genetically pure populations of westslope cutthroat trout or ones hybridized with non-native trout was determined using the criteria discussed by Leary et al. (1987).

Data Analysis

Halfway Creek in the Jefferson River drainage was sampled in 1985 and 1991 (Table 1). We used contingency table chi-square analysis to test for heterogeneity of allele frequencies at the polymorphic (genetically variable) loci between the samples. If significant allele frequency differences were present, then this could indicate that more than one population exists in the creek or that the genetic characteristics of the population had changed through time. In either case, it would not be appropriate to combine the samples into a single Halfway Creek sample.

The total amount of genetic variation, in terms of heterozygosity, detected among all 38 samples was divided into that due to genetic differences between samples from the upper Columbia and upper Missouri drainages, genetic differences among samples within the two river drainages, and genetic variation within samples using the procedure of Chakraborty (1980). If there was a large between drainage component, then this would provide evidence for treating populations in the two drainages as distinct units for conservation and restoration.

We used principal components analysis of the covariance matrix of the allele frequencies to examine the proximity of samples to each other in multivariate space. If samples from the upper Columbia and upper Missouri drainages occupied unique regions in multivariate space, this would indicate a substantial amount of genetic divergence between populations from the two drainages. In contrast, if there was wide overlap in the space occupied by samples from the two drainages this would indicate relatively little genetic divergence between populations from the drainages. We excluded the common allele at each locus from the principal components analysis to eliminate redundancy from the data set due to the fact that allele frequencies at a locus must sum to one.

RESULTS

We detected genetic variation at 23 loci among the samples (Table 2; Common alleles at each locus: *sAAT-1*200, sAAT-3, 4*100, ADH*100, CK-A2*84, CK-C1*100, GAPDH-4*100, GPI-A*92, GPI-B1*100, GPI-B2*100, G3PDH-2*100, IDDH*40, sIDHP-1*86, sIDHP-2*100, LDH-A1*100, LDH-A2*100, LDH-B1*100, LDH-B2*100, sMDH-A1, 2*100, sMDH-B1,2*100, mMEP-1*88, PGM-1*100, PGM-2*100, sSOD-1*100.*). A significant allele frequency difference was present between the two Halfway Creek samples at one of six polymorphic loci (Table 3). This could indicate that at least two genetically different populations exist in the creek, the genetic characteristics of the population had not been temporally stable, or this difference could simply be a chance departure from homogeneity due to the number of comparisons that were performed. In order to distinguish between the last and former two possibilities, we compared the chi-square statistic at *sIDHP-1** to that associated with the modified level of significance proposed by Rice (1989). This difference remains significant at the modified level suggesting the existence of genetic differences so the samples were not combined in the following analyses.

Of the total amount of genetic variation detected (0.0311), 64.95% (0.0202) was attributable to genetic variation within populations, 33.76% (0.0105) to genetic differences among populations within a drainage, and only 1.29% (0.0004) to genetic differences between populations from the two drainages. Thus, there is a large amount of genetic divergence among westslope cutthroat trout populations, but this is not due to appreciable differences between populations from the upper Columbia River and upper Missouri River drainages. Rather it is due to large differences among populations within the drainages indicating that even over short geographic distances westslope cutthroat trout populations can be genetically very different from

each other.

The high genetic divergence among the westslope cutthroat trout populations mainly arises from two factors. First, there are a few variant alleles that exist in many populations with widely divergent frequencies among populations (e.g. *CK-C1*38, sIDHP-1*71, sIDHP-2*40*; Table 2). Most of these alleles were detected in both drainages and there is broad overlap in the frequencies between the drainages. The widely divergent frequencies result in substantial genetic differences among populations within a drainage, but the broad overlap results in little divergence between the drainages. Next, there is a large number of alleles that were detected in only one, two, or three samples (Table 2). Many of these alleles, however, exist at appreciable frequency (> 0.10) in the populations in which they were detected (e.g. *sAAT-1*100*, *LDH-A2*140*, *LDH-B2*22;* Table 2). The populations with these alleles, therefore, are genetically very different at the particular locus from all other populations of westslope cutthroat trout regardless of where the other populations come from. Such locally distributed but high frequency alleles also result in substantial genetic divergence among populations within a drainage, but little divergence between the two drainages.

The principal components analysis also indicates there is substantial genetic divergence among populations within a drainage, but relatively little divergence between the drainages. Populations from both drainages occupy a broad (indicating high within drainage divergence) but widely overlapping (indicating little between drainage divergence) region along principal component one (Figs. 1). The only allele highly correlated with this axis which accounts for 41% of the total variation is *sIDHP-2*40* (Table 4). This axis, therefore, essentially depicts genetic divergence among populations for this highly variable allele.

There is less but still a substantial amount of overlap in the space occupied by populations from the two drainages on susbseqent principal components (Fig. 1). Each of these principal components has only a few alleles whose frequency is highly correlated with it (Table 4) and a few populations occupying unique space. These populations represent ones that are genetically very different from most others at a few loci regardless of the drainage the other populations exist in. There is also little geographic relationship among these unique populations. For example, the five very divergent populations along principal component two are the Middle Fork Flathead River, Jock S Canal and Centipede Creek in the lower Flathead drainage, Sleeping Child Creek in the Bitterroot drainage, and the Brushy Fork in the Clearwater drainage. Thus, these axes also depict little genetic divergence between drainages (broad overlap) but substantial genetic divergence among populations within a drainage (the unique populations) mainly due to unusual allele frequencies at one or a few loci in a few populations.

DISCUSSION

We feel the results presented here are pertinent to three aspects of westslope cutthroat trout conservation and restoration: conservation of genetic diversity, the possibility of treating fish

from the upper Missouri and upper Columbia drainages as separate ESU's or DPS's, and the possibility of stocking westslope cutthroat trout.

We feel that the primary genetic goal of a conservation program should be to ensure that the existing genetic variation of the taxon is maintained. This variation not only represents the evolutionary legacy of the taxon but the loss of genetic variation can have a variety of harmful effects on the characteristics of individuals important for population persistence: growth, survival, fertility, developmental rate, and the ability of individuals to develop properly (reviewed by Mitton and Grant 1984; Allendorf and Leary 1986; Palmer and Strobeck 1986; Zouros and Foltz 1987; Leary and Allendorf 1989). Furthermore, the loss of genetic variation is expected to reduce the ability of populations to adapt to changing environmental conditions and to increase their susceptibility to epizootics (Fisher 1930; Ayala 1965, 1969; Frankham 1980; O'Brien et al. 1985).

Conservation programs should be more concerned about the conservation of alleles than of allele frequencies. Allele frequencies are a temporary characteristic of a population that can be changed by genetic drift, gene flow, or natural selection. In contrast, the loss of an allele represents a permanent loss of genetic variation. Once an allele is lost it can only be recovered by mutation, the probability of which is minuscule.

Allelic variation in westslope cutthroat trout is composed largely of alleles with a very narrow geographic distribution, but these alleles often occur at appreciable frequencies in populations. Maintenance of this allelic diversity will require ensuring the continued existence of many populations throughout the range of westslope cutthroat trout. Thus, the primary goal of a conservation and restoration program for westslope cutthroat trout should be ensuring the continued existence of essentially all remaining populations.

This pattern of allelic diversity also argues against treating fish from the upper Missouri and upper Columbia drainages as separate ESU's or DPS's. First of all there is very little additional genetic divergence at this level so criterion 2 of an ESU or a DPS is not met. More importantly, however, protecting populations in only a portion of the fish's range could leave a vast amount of allelic diversity less protected and more susceptible to loss.

The allelic diversity of westslope cutthroat trout also suggests that historically there has been very little gene flow among populations, except possibly at a very local level (Wright 1932). In this situation, even fairly weak natural selection can effectively establish local adaptations. Thus, there is a good possibility that some populations of westslope cutthroat trout may have some degree of local adaptation (e.g. Fox 1993; Phillipp and Clausen 1995) which could be broken down, compromising population viability, if the native fish interbreed with westslope cutthroat trout introduced from other populations. It is likely that westslope cutthroat trout conservation and restoration efforts at times will call for the stocking of fish either from a hatchery broodstock or from transplants from native populations. In view of the above

possibility, the potential for these efforts to adversely impact native populations needs to be considered before introductions are made.

It is important to stress that introductions whose primary purpose is to re-establish westslope cutthroat trout in waters from which they have been extirpated will often need to be preceded by attempts to improve habitat quality. Furthermore, the success or failure of these introductions needs to be documented by subsequent monitoring.

In situations where introduced fish would have the potential to interbreed with an adjacent native population, the most appropriate source of fish would be translocations from the adjacent population or a broodstock founded from it. If either of these options is not possible, then fish from any feasible source will have to be used. In these situations, serious consideration should be given to protecting the genetic integrity of the native population or populations by constructing dispersal barriers. Furthermore, the chances of successful introductions may be enhanced by translocating fish from a number of populations or using fish from a broodstock established from a number of populations. This practice will increase the genetic variation in the fish by converting the substantial among population genetic divergence into within population genetic variability. This increased genetic variation is expected to better allow the introduced fish to adapt to the new environment.

Situations in which successfully introduced fish would have no or little potential to interbreed with native westslope cutthroat trout populations could be used for one of two purposes. They could be viewed as representing a situation analogous to the one above when dispersal barriers were constructed. They could also be used to establish genetic reserves. A genetic reserve is a population established in the wild or maintained in a hatchery to preserve the genetic diversity of a declining native population. If extinction of the native population does occur, and subsequently conditions are again made suitable for the fish, the reserve would represent the most appropriate source for re-introduction attempts. There are a number of factors that need to be considered when establishing a genetic reserve and a number of risks associated with a reserve once established. These are fully discussed by Leary (1991) and the Montana Bull Trout Scientific Group (1996) and will not be reiterated here.

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TABLE 1.— Sample locations, collection date (date), and sample sizes (N) of westslope cutthroat trout populations in the upper Columbia River and upper Missouri River drainages.

Location	Date	N
Upper Missouri River		
Beaverhead drainage		
1. Brays Canyon Creek	10, Aug. 1989	20
2. Buffalo Creek	10, Aug. 1989	20
3. Middle Fork Stone Creek	17, March 1992	16
Big Hole drainage		
4. Lambrecht Creek	15, Aug. 1984	28
Box Elder drainage 5. Collar Gulch Creek June 1981 16		
Jefferson drainage		
6. Halfway Creek (A)	Aug. 1985	36
7. Halfway Creek (B)	7, Nov. 1991	15
Mid Missouri drainage		
8. Elkhorn Creek 8, Aug. 1996	25	
Ruby drainage		
9. Geyser Creek	June 1990	16
Smith drainage		
10. North Fork Deep Creek	1, Aug. 1985	31
Teton drainage		
11. Cow Creek	10, Aug. 1990	15
12. Waldron Creek	1, July 1992	21
13. North Fork Waldron Creek	1, Aug. 1990	23
14. North Fork Willow Creek	9, Aug. 1990	22
Two Medicine drainage		
15. Lee Creek	7, Aug. 1985	21
16. Middle Fork Dupuyer Creek	26, June 1996	34

Location	Date	N
Upper Columbia River		
Bitterroot drainage		
17. Granite Creek	June 1982	27
18. Sleeping Child Creek	5, Sept. 1985	25
Blackfoot drainage		
19. Pierson Creek	1, Aug. 1994	23
Lower Clark Fork drainage		
20. Marten Creek	16, Aug. 1983	27
21. Vermillion River	16, Aug. 1983	27
Middle Clark Fork drainage		
22. O'Keefe Creek	June 1982	51
23. South Fork Little Joe Creek	26, Sept. 1990	30
Upper Clark Fork drainage		
24. Telegraph Creek	4, Nov. 1986	26
25. Warm Springs Creek	16, July 1986	21
Clearwater drainage		
26. Brushy Fork	1993	60
Lower Flathead drainage		
27. Centipede Creek	June 1991	25
28. Jocko S Canal	14, Aug. 1991	26
Kootenai drainage		
29. Dodge Creek	25, Aug. 1983	26
30. Gold Creek, Britsh Columbia	24, July 1986	34
Middle Fork Flathead drainage		
31. Middle Fork Flathead River	2, Aug. 1994	26
North Fork Flathead drainage		
32. Nicola Creek	28, Aug. 1984	25
33. Yackinikak Creek	11, Sept. 1984	26
Salmon drainage		
34. Middle Fork Salmon River	18, July 1989	35

Location	Date	N
South Fork Flathead drainage		
35. Hungry Horse Creek	Oct. 1982	48
36. Tin Creek	30, Aug. 1983	30
Swan Drainage		
37. Groom Creek	June 1983	25
38. Soup Creek	June 1983	25

TABLE 2. — Frequencies of the variant alleles detected among samples from 38 populations of westslope cutthroat trout from the upper Columbia and upper Missouri River drainages. Numbers correspond to sample numbers in Table 1.

	Alleles and allele frequencies					
Sample	*sAAT-1*250*	*sAAT-1*100*	*sAAT-1*n*	*sAAT-3, 4*90*	*sAAT-3, 4*77*	*ADH*n*
Upper Columbia						
33 Yackinikak	0.040	-	-	-	-	-
32 Nicola	-	-	-	-	-	-
35 Hungry Horse	-	-	-	-	-	-
36 Tin	0.050	-	-	-	-	-
31 M.F. Flathead	0.077	-	-	-	-	-
38 Soup	-	-	-	-	-	-
37 Groom	-	-	-	-	-	-
28 Jocko S	0.077	-	-	-	0.058	-
27 Centipede	0.100	-	-	-	-	-
29 Dodge	-	-	-	-	-	-
30 Gold	-	-	-	-	-	-
20 Marten	-	-	-	-	-	-
21 Vermillion	-	-	-	-	-	-
22 O'Keefe	-	-	-	-	0.187	-
23 S.F. Little Joe	0.150	-	-	-	0.042	-
25 Warm Springs	-	-	-	-	-	-
24 Telegraph	-	-	-	-	-	-
17 Granite	-	-	-	-	0.043	-
18 Sleeping Child	-	-	-	-	0.070	-
19 Pierson	-	-	-	-	0.022	-
34 M.F. Salmon	-	-	-	-	0.207	-
26 Brushy	-	-	-	-	0.513	-
Upper Missouri						
1 Brays	-	-	-	-	-	-
2 Buffalo	-	-	-	-	-	-
5 Collar	-	-	-	-	-	-
11 Cow	-	-	-	-	-	-
8 Elkhorn	-	0.540	-	-	-	0.346
9 Geyser	-	-	-	0.125	-	-
6 Halfway (A)	-	-	0.408	-	-	-
7 Halfway (B)	-	-	-	-	-	-
4 Lambrecht	-	-	-	-	-	-
15 Lee	-	-	-	-	-	-
16 M.F. Dupuyer	-	-	-	-	-	0.594
3 M.F. Stone	-	-	0.354	-	-	-
10 N.F. Deep	-	-	-	-	-	-
13 N.F. Waldrow	-	-	-	-	-	-
14 N.F. Willow	-	-	-	-	-	-
12 Waldron	-	-	-	-	-	-

	Alleles and allele frequencies					
Location	*CK-A2*100*	*CKC1*38*	*GAPDH-4*n*	*GPI-A*100*	*GPI-B1*n*	*GPI-B2*145*
Upper Columbia						
33 Yackinikak	-	-	-	-	-	-
32 Nicola	-	0.058	-	-	-	-
35 Hungry Horse	-	-	-	-	0.010	-
36 Tin	-	-	-	-	-	-
31 M.F. Flathead	-	0.135	-	0.019	-	-
38 Soup	-	-	-	-	-	-
37 Groom	-	-	-	-	-	-
28 Jocko S	-	0.019	-	-	-	0.115
27 Centipede	-	0.840	-	-	-	-
29 Dodge	-	-	-	-	0.192	-
30 Gold	-	-	-	-	-	-
20 Marten	-	-	-	-	-	-
21 Vermillion	-	-	-	-	0.019	-
22 O'Keefe	-	-	0.059	0.010	0.069	-
23 S.F. Little Joe	-	-	-	-	-	-
25 Warm Springs	-	0.289	-	-	-	-
24 Telegraph	-	0.115	-	-	0.019	-
17 Granite	-	-	0.138	-	0.052	-
18 Sleeping Child	-	0.200	0.040	0.020	0.080	-
19 Pierson	-	0.022	-	-	-	-
34 M.F. Salmon	-	-	-	-	-	-
26 Brushy	-	0.669	-	-	-	-
Upper Missouri						
1 Brays	-	-	-	-	-	-
2 Buffalo	-	-	-	-	-	-
5 Collar	-	-	-	-	-	-
11 Cow	-	-	0.033	-	-	-
8 Elkhorn	-	-	0.040	-	-	-
9 Geyser	-	-	-	-	0.250	-
6 Halfway (A)	-	-	-	-	-	-
7 Halfway (B)	-	-	-	-	-	-
4 Lambrecht	-	0.429	0.036	0.089	-	-
15 Lee	-	-	-	-	-	-
16 M.F. Dupuyer	-	0.162	-	-	-	-
3 M.F. Stone	-	0.100	-	-	-	-
10 N.F. Deep	0.064	-	0.113	-	-	-
13 N.F. Waldrow	-	0.022	-	-	-	-
14 N.F. Willow	-	-	0.125	-	-	-
12 Waldron	-	-	0.071	-	-	-

	Alleles and allele frequencies					
Location	*G3PDH-2*200*	*IDDH*100*	*sIDHP-1*71*	*sIDHP-1*40*	*sIDHP-1*n*	*sIDHP-2*71*
Upper Columbia						
33 Yackinikak	-	-	-	-	-	-
32 Nicola	-	-	-	-	-	-
35 Hungry Horse	-	-	-	-	0.072	-
36 Tin	-	-	0.016	-	0.018	-
31 M.F. Flathead	-	-	0.423	-	-	-
38 Soup	-	-	-	-	-	-
37 Groom	-	-	-	-	-	-
28 Jocko S	-	-	0.596	-	-	-
27 Centipede	-	-	-	-	-	-
29 Dodge	-	-	-	-	-	-
30 Gold	-	-	-	-	-	-
20 Marten	0.065	-	-	-	-	0.154
21 Vermillion	-	-	-	-	-	-
22 O'Keefe	-	-	-	-	-	-
23 S.F. Little Joe	-	-	0.207	-	-	-
25 Warm Springs	-	-	-	-	-	-
24 Telegraph	-	-	-	-	-	-
17 Granite	-	0.017	-	-	-	-
18 Sleeping Child	-	0.100	0.693	-	-	-
19 Pierson	-	-	0.522	-	-	-
34 M.F. Salmon	-	-	-	-	-	-
26 Brushy	-	-	0.358	-	-	-
Upper Missouri						
1 Brays	-	-	-	-	-	-
2 Buffalo	-	-	-	-	-	-
5 Collar	-	-	-	-	-	-
11 Cow	-	-	-	-	0.033	-
8 Elkhorn	-	-	-	-	0.042	-
9 Geyser	-	0.156	-	-	-	-
6 Halfway (A)	-	-	-	-	-	-
7 Halfway (B)	-	-	0.133	-	-	-
4 Lambrecht	-	-	-	-	-	-
15 Lee	-	-	-	-	-	-
16 M.F. Dupuyer	-	-	-	-	-	-
3 M.F. Stone	-	-	0.219	-	-	-
10 N.F. Deep	-	-	-	-	-	-
13 N.F. Waldrow	-	-	-	0.022	-	-
14 N.F. Willow	-	-	-	-	-	-
12 Waldron	-	-	-	-	-	-

	Alleles and allele frequencies					
Location	*sIDHP-2*40*	*sIDHP-2*20*	*LDH-A1*50*	*LDH-A2*140*	*LDH-B1*88*	*LDH-B1*n*
Upper Columbia						
33 Yackinikak	0.600	-	-	-	-	-
32 Nicola	0.288	-	-	-	-	-
35 Hungry Horse	0.532	-	-	-	-	-
36 Tin	0.484	-	-	-	-	-
31 M.F. Flathead	-	-	-	-	-	-
38 Soup	-	-	-	-	-	-
37 Groom	0.220	-	-	-	-	-
28 Jocko S	0.404	-	-	-	-	-
27 Centipede	0.920	-	-	-	-	-
29 Dodge	-	-	-	-	-	-
30 Gold	-	-	-	-	-	-
20 Marten	0.058	-	-	-	-	-
21 Vermillion	0.148	-	-	-	-	0.037
22 O'Keefe	0.167	-	-	-	-	-
23 S.F. Little Joe	0.328	0.069	-	-	-	-
25 Warm Springs	0.762	-	-	-	-	-
24 Telegraph	0.788	-	-	-	-	-
17 Granite	0.502	-	0.103	-	-	-
18 Sleeping Child	0.560	-	-	-	-	-
19 Pierson	0.783	-	-	-	-	-
34 M.F. Salmon	-	-	-	-	0.014	-
26 Brushy	-	-	-	0.559	-	-
Upper Missouri						
1 Brays	0.175	-	-	-	-	-
2 Buffalo	0.375	-	-	-	-	-
5 Collar	0.531	-	-	-	-	-
11 Cow	0.033	-	-	-	-	-
8 Elkhorn	0.500	-	-	-	-	-
9 Geyser	-	-	-	-	-	-
6 Halfway (A)	0.917	-	-	-	-	-
7 Halfway (B)	0.933	-	-	-	-	-
4 Lambrecht	0.482	-	-	-	-	-
15 Lee	0.983	-	-	-	-	-
16 M.F. Dupuyer	0.353	-	-	-	-	-
3 M.F. Stone	0.406	-	-	-	-	-
10 N.F. Deep	0.290	-	-	-	-	-
13 N.F. Waldrow	0.348	-	-	-	-	-
14 N.F. Willow	0.690	-	-	-	-	-
12 Waldron	0.476	-	-	-	-	-

	Alleles and allele frequencies					
Location	*LDH-B2*112*	*LDH-B2*22*	*sMDH-A1, 2*40*	*sMDH-B1, 2*83*	*sMDH-B1, 2*74*	*mMEP-1*n*
Upper Columbia						
33 Yackinikak	-	-	-	-	-	-
32 Nicola	-	-	0.010	-	-	-
35 Hungry Horse	-	-	0.005	0.010	-	-
36 Tin	-	-	-	-	-	-
31 M.F. Flathead	-	-	-	-	-	-
38 Soup	-	-	-	-	-	-
37 Groom	-	-	-	-	-	-
28 Jocko S	-	-	-	-	-	-
27 Centipede	-	-	-	-	-	-
29 Dodge	-	0.135	-	-	-	-
30 Gold	-	0.353	-	-	-	-
20 Marten	-	-	-	-	-	-
21 Vermillion	0.241	-	-	-	-	-
22 O'Keefe	-	-	-	-	-	-
23 S.F. Little Joe	-	-	-	-	-	-
25 Warm Springs	0.190	-	-	-	-	-
24 Telegraph	0.038	-	-	-	-	-
17 Granite	-	-	-	-	-	-
18 Sleeping Child	-	-	-	0.010	-	-
19 Pierson	-	-	-	-	-	-
34 M.F. Salmon	-	-	0.043	-	-	-
26 Brushy	-	-	-	-	-	-
Upper Missouri						
1 Brays	-	-	-	-	-	-
2 Buffalo	-	-	-	-	-	-
5 Collar	-	-	-	-	-	-
11 Cow	-	-	-	-	-	-
8 Elkhorn	-	-	-	-	-	-
9 Geyser	-	-	-	-	-	-
6 Halfway (A)	-	-	-	-	0.028	0.014
7 Halfway (B)	-	-	-	-	-	-
4 Lambrecht	-	-	-	-	-	-
15 Lee	-	-	-	-	-	0.017
16 M.F. Dupuyer	-	-	-	-	-	-
3 M.F. Stone	-	-	-	-	-	-
10 N.F. Deep	-	-	-	-	-	-
13 N.F. Waldrow	-	-	-	-	-	-
14 N.F. Willow	-	-	-	-	-	-
12 Waldron	-	-	-	-	-	-

Location	Alleles and allele frequencies					
	*PGM-1*110*	*PGM-1*n*	*PGM-2*120*	*PGM-2*85*	*PGM-2*62*	*sSOD-1*152*
Upper Columbia						
33 Yackinikak	-	-	-	-	-	-
32 Nicola	-	-	-	-	-	-
35 Hungry Horse	-	-	-	-	-	-
36 Tin	-	0.033	-	-	-	-
31 M.F. Flathead	-	-	-	-	-	-
38 Soup	-	-	-	-	-	-
37 Groom	-	-	-	-	-	-
28 Jocko S	-	-	-	-	-	-
27 Centipede	-	-	-	-	-	-
29 Dodge	-	-	-	-	-	-
30 Gold	-	-	-	0.029	-	-
20 Marten	-	-	-	-	-	-
21 Vermillion	-	0.148	-	-	-	-
22 O'Keefe	0.118	-	-	0.098	-	-
23 S.F. Little Joe	-	-	-	-	-	0.379
25 Warm Springs	-	-	-	-	0.024	0.024
24 Telegraph	-	-	-	-	-	-
17 Granite	-	-	-	-	-	0.155
18 Sleeping Child	-	-	-	0.060	0.020	-
19 Pierson	-	-	-	-	0.261	-
34 M.F. Salmon	-	-	0.114	-	-	0.543
26 Brushy	-	-	-	-	-	-
Upper Missouri						
1 Brays	-	-	-	-	-	-
2 Buffalo	-	-	-	-	-	-
5 Collar	-	-	-	-	-	-
11 Cow	-	-	-	-	-	-
8 Elkhorn	-	-	-	-	-	-
9 Geyser	-	-	-	-	-	-
6 Halfway (A)	-	-	-	0.222	-	-
7 Halfway (B)	-	-	-	0.300	-	-
4 Lambrecht	-	-	-	0.071	-	-
15 Lee	-	-	-	-	-	-
16 M.F. Dupuyer	-	-	-	-	-	0.074
3 M.F. Stone	-	-	-	0.156	-	-
10 N.F. Deep	-	-	-	-	-	-
13 N.F. Waldrow	-	-	-	-	-	-
14 N.F. Willow	-	-	-	-	-	-
12 Waldron	-	-	-	-	-	-

TABLE 3.— Allele frequencies at the polymorphic loci in samples of westslope cutthroat trout collected from Halfway Creek in 1985 and 1991. X^2 is contingency table chi-square statistic for heterogeneity of allele frequencies between samples with two degrees of freedom. **=P<0.01

		Sample and allele frequencies		
Locus	Alleles	1985	1991	X^2
sAAT-1*	200	0.592	1.000	2.834
	null	0.408	—	
sIDHP-1*	86	1.000	0.867	9.950**
	71	—	0.133	
sIDHP-2*	100	0.083	0.067	0.085
	40	0.917	0.933	
sMDH-B1,2*	100	0.972	1.000	1.706
	74	0.028	—	
mMEP-1*	88	0.986	1.000	0.412
	null	0.014	—	
PGM-2 *	100	0.778	0.700	0.694
	85	0.222	0.300	

TABLE 4. — Alleles with correlations of greater than ± 0.25 with at least one of the first seven principal components. Percent is the percentage of the total variation accounted by the axis.

	Principal component and correlations						
Allele	I	II	III	IV	V	VI	VII
*sAAT-1*100*	-0.02	-0.05	**0.35**	**0.52**	-0.07	0.13	-0.20
*sAAT-1*n*	-0.07	-0.02	-0.06	-0.11	-0.02	**0.69**	**0.49**
*sAAT-3, 4*77*	0.09	**0.29**	0.07	-0.02	**0.31**	**0.33**	**-0.44**
*ADH*n*	0.00	-0.04	0.12	**0.82**	-0.03	0.09	0.18
*CK-C1*38*	-0.13	**0.68**	**0.59**	-0.02	-0.08	-0.16	**0.31**
*sIDHP-1*71*	-0.04	**0.59**	**-0.77**	0.14	-0.07	-0.09	0.09
*sIDHP-2*40*	**-0.97**	-0.07	-0.03	0.00	0.11	-0.02	-0.13
*LDH-A2*140*	0.06	**0.29**	0.13	-0.02	0.06	**0.36**	**-0.48**
*PGM-2*85*	-0.08	-0.01	-0.06	-0.09	-0.01	**0.43**	0.22
*sSOD-1*152*	0.07	-0.02	-0.02	0.07	**0.91**	-0.13	0.24
Percent	0.41	0.19	0.12	0.06	0.05	0.04	0.03
Cumulative Percent	0.41	0.60	0.72	0.78	0.83	0.87	0.90

FIGURE 1. — Histogram of principal component scores for the first seven principal components which account for 90% of the total variation for samples from 22 populations of westslope cutthroat trout in the upper Columbia and 16 populations from the upper Missouri river drainages.