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SIGNATURE PAGE

We, the undersigned, enter into this Conservation Strategy to share the benefits derived by acting cooperatively to protect and enhance populations of Colorado River Cutthroat trout in southwest Colorado. This is not a financially or legally binding agreement.

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ID: 88073783

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COLORADO RIVER CUTTHROAT TROUT CONSERVATION STRATEGY FOR SOUTHWEST COLORADO

TO REESTABLISH COLORADO RIVER CUTTHROAT

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November, 1994

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Citation of this report should be:

Langlois, D., J. Cameron, C. S. Hutchinson, M. Japhet, D. Smith, and J. Castellano. 1994. Colorado River cutthroat trout conservation strategy for southwestern Colorado. Colo. Div. of Wildl., Montrose, CO and U.S. For. Serv., Delta, CO. 42 pp.

TABLE OF CONTENTS

ADAPTIVE MANAGEMENT iv
EXECUTIVE SUMMARY v
INTRODUCTION
NATURAL HISTORY
Description
Historic Range
Current Distribution
Habitat and Water Quality Requirements
Factors Causing the Decline
THE CONSERVATION STRATEGY
Inventory of Habitat and Populations
Reestablish Populations
Habitat Improvement and Restoration
Protection of Habitat and Populations
INFORMATION AND EDUCATION
16
LITERATURE CITED
APPENDICES
Appendix A. Potential Cutthroat Waters on USFS Lands
Appendix B: Selection Criteria for Streams
Appendix C: Selection Criteria for Lakes
Appendix D: Protocol For Preparing Fish Specimens in the Field

ADAPTIVE MANAGEMENT

The Colorado Division of Wildlife (DOW), U.S. Forest Service (USFS) and Bureau of Land Management (BLM) developed this Conservation Strategy for Colorado River cutthroat trout. Experts from universities, organizations and other individuals also reviewed the plan. Agency personnel used the best written information available to them and collective knowledge, experience and professional judgment of others in producing this Conservation Strategy. It is hoped the ideas can be used by all concerned with the Colorado River cutthroat trout to restore populations of ColoradoRiver cutthroat trout, protect sensitive habitats, and coordinate conservation activities. Goals and objectives will be attained and funds spent depending on appropriations, priorities, and other operational constraints.

This Conservation Strategy and its objectives and actions must be responsive to change to be effective. The document is subject to modification as dictated by new findings in conservation biology, changes in species status and completion of tasks in the plan. Revisions are the responsibility of the authors. The Strategy does not necessarily represent the views of all personnel of the agencies nor official positions or approvals of cooperating agencies, organizations and individuals. Potentially affected interests in the southwestern Colorado should view this draft plan as an interactive information document that will aid the decision making process as various alternatives for recovering Colorado River cutthroat trout are considered.

EXECUTIVE SUMMARY

This Conservation Strategy suggests cooperative efforts by the Southwest Region of the Colorado Division of Wildlife, the San Juan and Grand Mesa-Uncompahgre-Gunnison National Forests of the U. S. Forest Service, and Montrose District of the Bureau of Land Management to preserve Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*). These local agencies have collaborated in preparation of this Conservation Strategy with the intent to achieve the following in southwestern Colorado:

- 1. Reestablish populations of Colorado River cutthroat trout to ensure their long term survival.
- 2. Protect key streams and watersheds through management practices that provide optimum trout habitat.
- 3. Coordinate management of this trout and its habitat through interagency conservation efforts.

GOAL: To ensure the long term survival of Colorado River cutthroat trout in southwestern Colorado. The conceptual idea is to protect the genetic purity and preserve the genetic variability of remaining stocks of this trout.

OBJECTIVE: Create at least three metapopulations¹ of Colorado River cutthroat trout: one for each of the major river basins that include the Gunnison (Uncompahgre-North Fork), the San Juan (Piedra-Pine-Navajo-Animas-LaPlata-Mancos), and Dolores (West Fork-San Miguel) drainages. Ancillary to this work it would be desireable to also manage a number of refugia that include populations in isolated streams and populations in high lakes that must be maintained by periodic stocking from genetically acceptable stocks.

ACTIONS:

- Inventory of habitat and populations a database of potential waters and populations, population sampling and monitoring.
- Reestablish populations identify potential waters, make transplants, manage wild broodstocks.
- Habitat improvement and restoration manage streams and lakes in selected watersheds to create optimum trout habitat.
- Protect of habitat and populations exclude nonnative fishes, set fishing regulations, improve land management practices.
- Public information build consent for Conservation Strategy elements.

¹A metapopulation is a collection of localized smaller populations with some amount of dispersal possible for individuals to move among populations.

INTRODUCTION

"We are charged with the perpetuation of native species insofar as possible. Historically, most of the reducton of cuthroat trout habitat area in the higher elevations has been traceable to our own stocking activities and tose other conservation agencies such as the Forest Service. I refer mainly to introductions of brook and rainbow into cuthroat waters. <u>I feel it is high time we make a</u> <u>listing of remaining pure cuthroat waters and set them aside as inviolate native trout</u> <u>waters regarding stocking</u>." This quotation from Wayne R. Seaman, state fishery manager for the DOW, was in a memo from the southwest regional manager, C.E. Till, to the hatchery superintendents and the WCOs. <u>It was dated March 9, 1964</u>!

The cutthroat trout (Oncorhynchus clarki) is the only trout native to Colorado. Four subspecies have been identified: Colorado River cutthroat trout (O. c. pleuriticus); greenback cutthroat trout (O. c. stomias); Rio Grande cutthroat trout (O. c. virginalis) and the now extinct yellowfin cutthroat (O. c. strained). During the past 100 years, Colorado River cutthroat trout have been adversely affected by stocking of non-native salmonids and development of land and water resources. Like many of the native trouts in the American west, the present distribution of the Colorado River cutthroat trout is a small fraction of what is was historically. As of this writing, pure Colorado River cutthroat trout in southwestern Colorado exist in only six isolated headwater tributaries in the San Juan and Gunnison drainages. This subspecies is currently a U. S. Fish and Wildlife Service Category 2 species (may be appropriate for listing as federally threatened or endangered), is considered a Species of Special Concern by the DOW, and is on the USFS regional sensitive species list.

State and Federal agencies are becoming increasingly cognizant of the critical nature of the issues facing us today in the management of threatened or endangered fish wildlife and sensitive species populations and their habitat. On January 25, 1994, the Chief of the USFS, Jack Ward Thomas, signed a Memorandum of Understanding made and entered into by the USFS, BLM, National Park Service, U. S. Fish & Wildlife Service and National Marine Fisheries Service to establish a general framework for cooperation and participation in the conservation of species that are tending toward federal listing under the Endangered Species Act . It is a significant agreement wherein the agencies agree to work together to achieve a common goal of species conservation through protection and management of the habitats and ecosystems. Also in 1994, the International Association of Fish and Game Directors signed an agreement with the federal agencies to cooperate towards the same end. Implementation of Conservation Strategies such as this one for Colorado River cutthroat trout will ensure the requirements of federal planning systems and the National Environmental Policy Act are fulfilled. This Strategy completes coverage of conservation efforts for the Rocky Mountain region in Colorado. It is an interim Strategy on a regional level that complements other interagency planning efforts for Colorado River cutthroat trout. This Strategy is consistent with the USFS plan amendment (pp. III 33-34, Gen. Dir. 01 and 01c).

The USFS and BLM are responsible for managing public land habitats for this subspecies in southwest Colorado. The Grand Mesa, Uncompahyre and Gunnison National Forests encompass nearly 3 million acres of land which contain approximately 3,657 miles of perennial streams. The San Juan National Forest covers nearly 2.7 million acres of land which contain approximately 1,000 miles of perennial stream. The BLM administers 2.1 million acres of land containing approximately 1,250 miles of streams. In addition to these stream miles, the San Juan National Forest has 9600 acres of lake, the BLM has 92 acres, and the Grand Mesa-Uncompany Gunnison National Forest contains approximately 12,000 acres of lake habitat.

The primary mission of the Colorado DOW is protection of fish and wildlife including Colorado River cutthroat trout populations. This responsibility is mandated by Colorado statutes and regulations under the authority of the Colorado Wildlife Commission.

NATURAL HISTORY

DESCRIPTION

Colorado River cutthroat trout are yellow with a pale to brilliant red band which runs horizontally along both sides of the body. Males become much more crimson along their ventral region during spawning. The spotting pattern is variable, depending on geographic locality, but consists of large, black spots located mostly above the lateral line and posteriorly. It is very infrequent that spots are present on the head. Behnke and Zam (1976) provided a taxonomic description of Colorado River cutthroat trout. This subspecies differs from other trouts of the southern Rocky Mountain basins in its higher scale counts (170-205+ in lateral series, 38-48+ above lateral line). Pyloric caeca typically number 25 to 45 with averages in the 30 to 40 range. The number of gill rakers ranges from 17 to 21 and averages 19. Vertebrae number 60 to 63 with mean value usually 61 or 62.

In 1977, Binns published a rating system to determine the degree of purity of Colorado River cutthroat trout in Wyoming. The rating system categorized populations into grades of purity based on degree of suspected hybridization (Table 1). The rating assigned grades from A (most pure) to F (obvious hybrids) using the range of variability of meristic characters and spotting pattern.

Meristic character	Grade						
and spotting pattern	А	В	С	D	F		
Scales ¹	180+	168-179	155-167	142-154	120-141		
Pyloric caeca	≤40.9	41.0-44.5	44.6-48.5	48.6-53.0	53.1+		
Basibranchial teeth2	0-10%	10-20%	20-40%	40-75%	75-100%		
Spotting pattern ³	Uniform, no variability	Slight variability	Some variability yet still <i>pleuriticus</i>	Quite variable yet still <i>pleuriticus</i>	Obvious hybrid spotting		

Table 1. Purity rating criteria used to determine degree of purity of Colorado River cutthroat trout. Range given for scales and pyloric caeca represent mean values. An A represents the most pure populations; an F the least pure.

¹Number of scales two rows above lateral line.

² Percent of specimens lacking basibranchial teeth.

3 Variability in size, number, shape and position of spots among specimens from the same population.

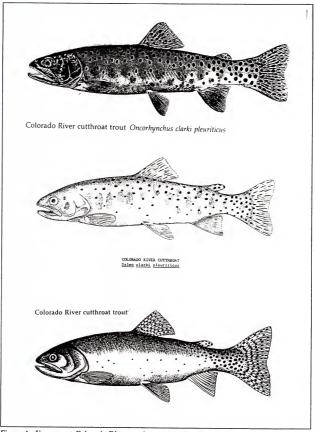


Figure 1. Uppermost Colorado River cutthroat trout drawing from Behnke (1992). Middle and lower drawings from Behnke and Zarn (1976).

Proebstel (1994) provided a description of 82 populations in western Colorado. This work used classical taxonomic methods. It also set the stage for continued development of techniques using molecular genetic analysis.

HISTORIC RANGE

The historic range of Colorado River cutthroat trout extended from the headwaters of the Colorado River basin downstream to the Dirty Devil River, Utah and the San Juan River in Colorado and New Mexico (Fig. 2). The distribution of Colorado River cutthroat trout began above a point where the distribution of warmer water species such as Colorado River squawfish (Pychocheilus lucius) left off (Behnke, 1980).

CURRENT DISTRIBUTION

Eleven populations of Colorado River cutthroat trout have been identified in southwestern Colorado since 1987 (Table 2). This includes remnant populations in the headwaters of the Navajo, Animas, Piedra and Gunnison hydrologic units (Fig. 3). In 1994, a brood fish lake was established in the Animas River drainage using 300 fish transplanted from the headwaters of the East Fork of the Piedra River. This brood lake is the result of a cooperative effort among the DOW, USFS and a private landowner. In the future, eggs collected from this spawntake operation should make a San Juan basin strain of Colorado River cutthroat trout available for additional transplants in the basin.

Water	Basin	Land Status	Management ¹	Purity rating	Biomass Ib/acre	Survey Date	Water Code
Augustora Cr.	SJ	PRIV/USFS	P, NoSt, R	A	14.7	07/09/91	44486
Big Bend Cr.	SJ	USFS	P, NoSt	B+	87.0	07/13/87	47325
Deer Cr.	SJ	USFS	P, NoSt	B?	LOW	07/12/90	47591
Hermosa Cr., E. Fk.	SJ	USFS	P, St	A	LOW	09/20/90	47628
Hermosa Cr., S. Fk.	SJ	USFS	P, No St	B?	19.3	07/16/87	40674
Navajo R. #2	SJ	PRIV/USFS	P, NoSt, R	А	9.9	07/09/91	49064
Piedra R., E. Fk.	SJ	USFS	P, NoSt, R	А	96	08/04/92	44486
Cottonwood Lake	SJ	PRIV	P, St, R	А	300 fish	08/13/94	
Beaver Cr., W. Fk.	GU	USFS/BLM	P, St	А			44355
Second Cr.	GU	USFS/PRIV	P, NoSt, R	A-			48771
Gunnison R., L. Fk #4	GU	BLM	P, St	A?			48080

Table 2. Current distribution of Colorado River cutthroat trout in southwestern Colorado.

¹ Management recommendation based on Proebsel (1994) system. P = protect; NoSt = no stocking from any source; St = stocking from source of pure Colorado Kiver cutthroat trout is acceptable; R = population has potential use in recovery efforts pending further examination and molecular genetic examinations.

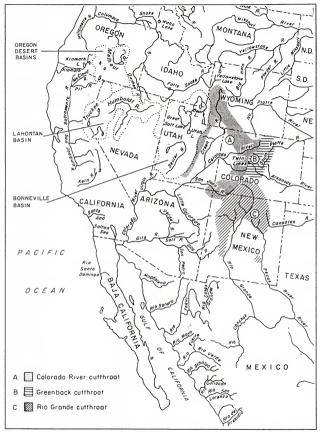


Figure 2. Historical range of Colorado River cuttroat trout, greenback cutthroat trout and Rio Grande cutthroat trout (Behnke 1992).

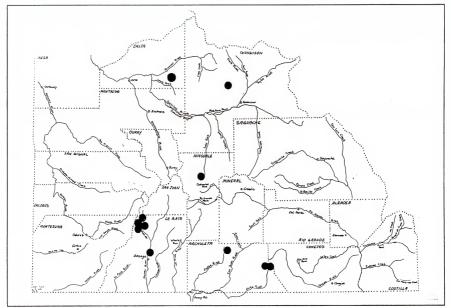


Figure 3. Current distribution of Colorado River cutthroat trout in southwestern Colorado.

7

In northwestern Colorado there are currently 42 populations of Colorado River cutthroat trout judged to be either in the "A category" (Proebstel 1994). That work showed a slight decline in the number of essentially pure populations. In Trappers Lake, Colorado, the largest formerly "pure" population of Colorado River cutthroat trout has become hybridized with rainbow trout (Martinez, 1988). Approximately 30,000 eggs per year have been collected from Lake Nanita in Rocky Mountain National Park for restoration programs outside the Park of since 1990 (Bruce Rosenlund, pers comm).

HABITAT AND WATER QUALITY REQUIREMENTS

Optimal cutthroat trout riverine habitat is characterized by clear, cold water, a silt free rocky substrate in rifle-run areas, an approximately 1:1 pool-rifle ratio with areas of slow, deep water; well vegetated stream banks; abundant instream cover, and relatively stable water flow and temperature regimes (Raleigh and Duff 1981). Not all excellent cutthroat trout streams will have all of these characteristics. While there is much variability from headwaters to foothills regions along the river continuum, this short list captures essential features of cutthroat trout stream habitat.

Optimal lacustrine habitat is characterized by clear, cold, deep lakes that are typically oligotrophic, but may vary in size and chemical quality, particularly in reservoir habitats. Cutthroat trout are stream spawners and require tributary streams with gravel substrate in riffle areas for reproduction to occur (Hickman and Raleigh, 1982).

Cover is recognized as an essential component of trout streams. Cover is provided by overhanging and submerged vegetation, undercut banks, substrate, woody debris, depth and turbulence. Cover utilization for resting and feeding may vary seasonally as requirements during low flow (fall & winter) are different from those during spring run-off, summer or spawning conditions. Spawning substrate located near cover is important and the gravels need to be free of fines for successful egg incubation and emergence of fry. Streams with a wide diversity and complexity of habitat will be able to provide for variable requirements. In some streams, the major factor limiting salmonid densities may be the amount of overwintering habitat rather than summer rearing habitat (Bustard and Narver, 1975).

Cutthroat trout feed primarily on aquatic insects. Quality insect production requires rubble dominated substrate in riffles, oxygenated water and less than 10 percent fines in those riffle areas.

There is a definite relationship between the annual flow regime and the quality of trout habitat. The most critical period is typically the base flow (lowest flows of late summer to winter). A base flow \geq 50 percent of the average annual daily flow is considered excellent, a base flow of 25-50 percent is considered fair and a base flow of \leq 25 percent is considered poor for maintaining quality trout habitat (adapted from Binns and Eiserman 1979; Wesche 1980).

Water temperatures need to be cool, optimally in the range of 12-15 C° (54-60° F). Dissolved oxygen requirements vary somewhat but optimal levels appear to be > 7 mg/l at temperatures $\leq 15^{\circ}$ C and ≥ 9 mg/l at temperatures > 15° C. Most cutthroat populations can tolerate a pH range of 5 to 9.5 with an optimal range of 6.5 to 8.0 (Hickman and Raleigh, 1982)

Cutthroat trout are intolerant of chemical and metal contaminants and perish at lower concentrations than other trout species.

FACTORS CAUSING THE DECLINE

The great reduction of Colorado River cutthroat trout populations in southwest Colorado is due primarily to introduction of nonnative species and habitat losses. In addition to these problems, over-harvest by anglers has further reduced the populations.

Since the late 1800's, widespread stocking of nonnative fish species has occurred. These nonnative species typically out-compete the cutthroat for space and food, prey on fry (especially in lakes and reservoirs) or interbreed. Introduced cutthroat and rainbow trout spawn at the same time and hybridize with Colorado River cutthroat trout, causing a loss of genetic integrity.

Habitat alterations have occurred on private and public lands. Cattle grazing, timber harvest, road building, stream de-watering and other development have created losses of and destruction to habitats, as well as changed flow regimes or blocked migration routes. Mining has been responsible for acute toxicity in many streams and lakes.

Fishing pressure has taken a toll on vulnerable Colorado River cutthroat trout populations. Cutthroat are more susceptible to being caught than other trout. In some cases, catch rates exceed 10 fish per hour and some studies have reported that it would be possible to remove nearly all of the adult fish population in a kilometer of stream in less than 24 angler hours.

Due to the factors mentioned above, the remaining genetically pure populations of the various subspecies of cutthroat trout are left in low order stream at high elevations. This fragmentation may lead to greater susceptibility to natural population fluctuations due to environmental factors.

THE CONSERVATION STRATEGY

GOAL: To ensure the long term survival of Colorado River cutthroat trout in southwestern Colorado. The conceptual idea is to protect the genetic purity and preserve the genetic variability of remaining stocks of this trout. The long term view is to ensure stability of the native aquatic wildlife community.

OBJECTIVE: Create at least three metapopulations of Colorado River cutthroat trout: one for each of the major river basins that include the Gunnison (Uncompahgre-North Fork), the San Juan (Piedra-Pine-Navajo-Animas-LaPlata-Mancos), and Dolores (West Fork-San Miguel) drainages. Ancillary to this work it would be desireable to also manage a number of refugia that include populations in isolated streams and populations in high lakes that must be maintained by periodic stocking from genetically acceptable stocks. A key feature of this objective will be the development and analysis of population viability criteria.

INVENTORY OF HABITAT AND POPULATIONS

1. Create a database of streams and lakes that have Colorado River cutthroat trout or reestablishment potential. The resources management agencies currently use several databases that include attribute systems as well as spatial mapping systems. These include paper file archives, dBase translations of these files, GIS data storage, ARC-INFO and ADAMAS within the DOW (Mark Jones, pers comm), Informix and IRI/CWU and the six state Habitat Conservation Assessment Strategy for cutthroat trout used by the USFS (Schmal, pers comm). The data and records will be available for use by other agencies, organizations and individuals involved in resource management decisions.

A. Inventory of individual waters and watersheds should start with a background check of the physical and biological characters that comprise the preferred habitat selection criteria. The background check consists of examination of U.S. Geological Survey topographic maps, past fish stocking records, and any previous field surveys. Details of how to do background checks are presented in Appendices B and C.

B. Prepare list of candidate waters for field surveys. Priority will be given to waters that appear to meet the habitat needs of Colorado River cutthroat trout and that have no stocking records.

C. A minimum target for field surveys each year would be to check 10 potential Colorado River cutthroat trout waters. Field surveys should minimally include a check for species richness, data describing biomass and community structure, and physical and chemical characters. Acceptable formats for these data include: standardized stream and lake survey forms used by DOW, the Basinwide Inventory used by the USFS and BLM D. Inventory results should be mapped on USGS topographic maps and future electronic database systems. Computerized records and maps will be available on a DOW network. Paper records will be housed at the offices in Durango, Montrose and Delta.

2. Identify potential Colorado River cutthroat trout populations. An efficient way to gain additional pure populations of CRN is simply to identify previously undiscovered remnant populations. This requires use of techniques ranging from standard classical taxonomy to experimental procedures that look at cell contents and products. The purpose of the identification work is to describe the genetic purity and variability of potential populations. These data can be used to make decisions about the level of protection needed or the utility of various stocks in the conservation program.

A. Collect and prepare specimens for analysis. A standard museum label and collection catalog should accompany the collections. This includes fixing whole specimens in formalin, with permanent storage in denatured alcohol. Tissue prepared for mtDNA analysis can be preserved in pure ethyl alcohol or frozen on dry ice. Specimens prepared for protein electrophoresis must be kept on dry ice and stored in a very cold deep freeze. Detailed protocol for specimen preparation is in Appendix D

B. Develop techniques to describe the various stocks or families of Colorado River cutthroat trout throughout it's range. Techniques used may be standard taxonomic techniques, analysis of mitochondrial DNA, and protein electrophoresis or some combination of these.

3. Monitor existing or reestablished Colorado River cutthroat trout populations and their habitat. One purpose is to collect population data such as biomass, age structure, and density. This biological information can be used to assess the viability of various stocks. Combined with physical-chemical data it should be possible to assess land management problems.

A. Conduct periodic electroshocking or trammel net surveys to identify trends in Colorado River cutthroat trout. Protocol for these surveys is presented in 1C and DOW manuals and handbooks.

B. Conduct habitat surveys as mentioned in 1C above.

C. Conduct creel and other surveys to monitor angler use, fish harvest and angler attitudes. Interviews and other information collected from anglers and other interested publics can be used to assess the effectiveness of the conservation program.

REESTABLISH POPULATIONS

1. Establish, maintain and manage at least three wild broodstocks in Gunnison, San Juan and Dolores river drainages.

A. Develop criteria to select and manage brood fish waters. See Appendix C.

B. Develop techniques and procedures to spawn and rear genetically acceptable fish that are disease free. It is important to study spawning and rearing problems that lead to genetic alteration (Mike Young, pers comm). Loss of genetic variation decreases the ability of a population to respond to environmental changes. Current topics for concern include deciding how many individuals to use to found a population, genetic drift in a hatchery, using unequal numbers of offspring from different parents, and artificial selection in the hatchery.

Another area for research is how to reincorporate the wild genome into the brood stock. If only milt from wild males is used, then variation of the genome will decrease because mitochondrial DNA is not passed paternally. Also, it is difficult to incorporate the genetic variation found in populations of Colorado River cutthroat trout into a broodstock. There is variation among watershed and even within a single watershed.

Identify or create stream refugia using stream selection criteria. Streams should have an average gradient of less than 10%, some sort of barrier to prevent migration of other trout species, good habitat and water quality and need to meet the selection criteria in Appendix B.

3. Identify or create lake refugia.

A. Use lake selection criteria. See Appendix C.

B. Consider changing stocking regime of non-native fish in high mountain lakes. See below item 4D.

4. Transplant fish to appropriate habitats.

A. Plan fish reclamation projects and prepare the necessary environmental assessments and chemical treatment plans. In some cases, it can be possible to restore the biological balance that will allow transplanted cutthroat trout to prosper.

B. Remove non-native fish with piscicides following Colorado DOW Chemical Treatment Operation Procedure (1987). Registered fish reclamation chemicals include rotenone, antimycin and potassium permanganate.

C. Use wild salvaged fish from donor waters that have been determined to have adequate populations. There are advantages to transplanting fish from wild stocks primarily because hatchery propagation problems are minimized. Disease certification, commitments of hatchery space and culturists time, hatchery accidents, and human-influenced selection are avoided. To use wild fish as transplant stock it is essential to first ensure that the fish are genetically pure. Costs of using salvaged fish to create a new cutthroat trout population are higher. Also, fewer fish are available for transplant because most wild populations of Colorado River cutthroat trout are comprised of few

individuals. At the same time, these fish may be better adapted to surviving in the new habitat. Additional transfers could be made later to increase the genetic variation and size of the reestablished population until it is self-sustaining.

D. Use hatchery propagated fish from the established wild brood stocks. The strategy for this tool is to stock large numbers (100,000 per year) of fry produced from eggs collected from wild brood stocks. The eggs and fry would be held in the hatchery environment for as short a time as possible to minimize adverse genetic selection, e.g., a September plant from eggs collected in June. In this way, possible loss of wild characteristics that seem to be concurrent with mass propagation would be minimized which would also help ensure survival in natural environments. Such fish would be especially useful for establishing Colorado River cutthroat trout populations in backcountry lakes. Although nonnative trouts are already established in most backcountry lakes in southwestern Colorado, natural reproduction does not occur because spawning habitat is missing. Thus, over a period of approximately 15 years as the nonnative trouts fail to recruit, populations of Colorado River cutthroat trout approximating the indigenous genome would be established. These refugia would be widespread through the Gunnison, San Juan and Dolores basins and perhaps provide an element of short term persistence and insurance against extirpation. Cultured fish would not be stocked into wild populations or habitat that have no history of stocking.

HABITAT IMPROVEMENT AND RESTORATION

1. Improve quality of habitat in Colorado River cutthroat trout streams where needed. Colorado River cutthroat trout habitat improvements should be included in watershed management decisions made by the USFS and BLM. Watersheds with populations of Colorado River cutthroat trout will be identified in Forest Plans, grazing allotments, RMPs and AMPs, activity plans and integrated resource plans. They will be surveyed and site plans developed to mitigate adverse impacts to water quality, instream habitats, channel morphology, riparian areas, and the stability of the population. This can be accomplished by: road improvements including outsloping and construction of waterbars, developing and implementing measures to mitigate impacts from old timber sales, grazing, mining, and recreation areas.

A. Create or improve migration barriers at bottom of existing or potential Colorado River cutthroat trout streams. Barriers will be designed to insure that no upstream migration of other fish will be possible. These habitats should be considered short term refugia that have value towards creating the critical biomass needed to develop populations of Colorado River cutthroat trout.

B. Create Colorado River cutthroat trout habitat in watersheds comprised of several tributaries that are linked by migration routes. These metapopulations would be resistant to extirpation because not all streams in the watershed are likely to suffer an

environmental catastrophe simultaneously, such as a large, hot forest fire, thus some populations will remain despite habitat loss nearby. Even if some subpopulations are extirpated because of poor habitat, they could be recolonized by migrants from larger subpopulations. An essential feature of this activity is to identify and prioritize candidate streams for reconnection using the stream selection criteria in Appendix B. This means the eventual removal or modification of migration barriers and should be viewed as a positive sign of increasingly secure Colorado River cutthroat trout populations.

C. Improve instream cover, pools or spawning gravel with structural improvements. Prerequisite to habitat improvements in individual waters is correction of problems caused by past land management activities. A specific item is to upgrade or replace existing culverts which prevent fish passage during spawning or low flow periods.

D. Improve stream bank stability and riparian vegetation conditions. The cause of the problem needs to be identified and remedied. Bank protection structures and vegetation planting may speed up the recovery process. It may be necessary to revise Allotment Management Plans to include protection of stream or riparian habitat.

E. Improve water quality where needed. Where high summer temperatures are a problem, improving flow conditions through acquisition of water rights or protection of reasonable instream flows will be required. Restoration of streamside vegetation to shade the stream bottom will help. If pollution from agriculture, mining or domestic uses is a problem, reduction of these contaminants would be necessary.

2. Improve habitat conditions in lakes that contain Colorado River cutthroat trout or have reintroduction potential. Lakes that are naturally barren or frequently winterkill should not be considered part of the conservation program. Barren lakes may contain indigenous aquatic biota that evolved without fish. Despite apparent lack of humanistic values do have value simply because they exist (Ehrenfeld 1981). Lakes that regularly winterkill are difficult to maintain. These lakes should be managed by allowing natural recolonization from inlet or outlet spawning streams.

A. Create or improve migration barriers below outlets of lakes to isolate Colorado River cutthroat trout from hybridization or competition with nonnative trout. Where the objective can be expanded to include metapopulation management, there should be a migration route into and out of the lake, rather than a barrier.

B. Improve spawning habitat in inlets or outlets. Excavation of an inlet stream to the lake would provide an area for spawning. The channel should be designed to handle the expected high spring flow without eroding the banks. Placement of one to three inch diameter gravel in the channel to a depth of 6 to 12 in will provide suitable spawning and egg incubation substrate. The spawning channel must also be designed to flow water from June through August in order incubate eggs and hatch fry.

C. Maintain lake water levels. Protection of in-lake water rights can be accomplished by making appropriate filings through the Colorado Water Conservation Board.

PROTECTION OF HABITAT AND POPULATIONS

1. Prevent reintroduction of nonnative fishes in waters managed for Colorado River cutthroat trout. To maintain the stability of the Colorado River cutthroat trout population and perhaps the whole native fish community it is important to prevent the reintroduction of nonnative fishes. Brook char, brown trout and white sucker are thought to outcompete Colorado River cutthroat trout for food and cover as well as prey on them. Nonnative cutthroat trouts and rainbow trout can hybridize with Colorado River cutthroat trout. This results in introgression of the gene pool and thus loss of pure stocks.

The DOW (as determined by the Colorado Wildlife Commission) has regulatory authority over introductions of trouts into flowing waters of Colorado. Nonnative fishes have become established in former Colorado River cuthtroat trout waters mainly through fish stocking programs conducted by DOW, USFS and others over the years. And, sometimes nonnative trouts have become established through illegal introductions by individuals. In fact, there is precedent for this occurring in waters that have already been reclaimed for indigenous cutthroat trouts. Such appears to have been the case in the headwaters of the Lake Fork of the Conejos River where large numbers of small brook char and brown trout suddenly appeared in a thriving population of newly established Rio Grande cutthroat trout. Thus the decision whether to reclaim a water a second time might include additional social and legal aspects.

2. Promote and enforce fishing regulations to match the needs of the Colorado River cutthroat trout populations. In general cutthroat trouts are vulnerable to overharvest by angling. Recommendations concerning fishing regulations such as bag limits, fishing season, size restrictions and terminal tackle restrictions will be recommended to protect the stability of the Colorado River cutthroat trout populations. This will be done on a water by water basis since the fishability, access and angling pressure for most waters are slightly different. Appropriate regulations range from a standard eight fish bag limit with no terminal tackle restrictions in remote areas, a catch and release with flies and lures only regulation in an easily accessible area, or a closure to all fishing to protect a newly created Colorado River cutthroat trout populations. Decisions on fishing regulations are the responsibility of the Colorado Wildlife Commission but input will be solicited from all affected interests.

3. Implement land management practices conducive to habitat preservation and improvements.

A. Revise and set forth standards and guidelines for Colorado River cutthroat trout habitat management in USFS and BLM land management plans. Guidelines will address the entire watershed in areas with populations of Colorado River cutthroat trout. One biological element of managing watersheds is to provide connections for fish passage among populations in individual streams. In this way, two or more individual populations comprise a larger metapopulation that is resistant to extinction.

B. Develop cooperative management agreements with public agencies, organizations and individuals that have an interest or impact on Colorado River cutthroat trout. Cooperative agreements for spawntaking and brood lake management are examples.

C. Work towards securing instream flow rights in all Colorado River cutthroat trout waters on Federal, State and private lands by filing for water rights through the Colorado Water Conservation Board.

D. Maintain or improve water quality to standards needed by Colorado River cutthroat trout. Stream and lake water quality standards and classifications are under the jurisdiction of the Colorado Health Department, Water Quality Control Division.

4. Monitoring of the effectiveness of actions implemented to protect Colorado River cutthroat trout. The purpose of periodic monitoring is to provide information about the viability of various populations and the stability of the habitat. These data can be used to make decisions about fishing regulations, whether transplants of cutthroats from the various stocks can be made, and the success of land management plans and practices

INFORMATION AND EDUCATION

Colorado River cutthroat trout present a moderate challenge as a public relations client. They are be perceived by many people near their habitat areas as "sport fish" that have great worth. They lack some appealing qualities of threatened or endangered species such as the bald eagle or grizzly bear. The trout is viewed by some as an obstacle to economic development. Local public sentiment may characterize this species as an impediment to water management and development in portions of southwestern Colorado. Finally, many people are unaware of the species' existence and residents are not adept at identifying the species. Given this view, the information and education program goals would take the form outlined by the USFWS (1990) in the Five-Year Plan for Information and Education relating to endangered fishes in the Colorado drainage.

1. Educate the public on the uniqueness and value of rare fishes.

2. Increase public understanding and support regarding the recovery of Colorado River cutthroat trout, including support at the local, state and national level for continued funding for the conservation effort.

3. To promote communication and cooperation among the various potentially affected interests of the conservation effort.

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APPENDICES

- A. POTENTIAL CUTTHROAT WATERS ON USFS LANDS
- **B. SELECTION CRITERIA FOR STREAMS**
- C. SELECTION CRITERIA FOR LAKES
- D. PROTOCOL FOR PREPARING FISH SPECIMENS

APPENDIX A. POTENTIAL CUTTHROAT WATERS ON USFS LANDS

Colorado River Cutthroat Trout Waters on

GRAND MESA. UNCOMPAHGRE and GUNNISON NFs

Table 1. Streams containing natural (N) or manmade (M) barriers and are high priority for field surveys.

Stream	CDOW	Major	Barrier	Land 1/	Stocked
Name	Code	Watershed	N/M, Ft.	Status	(Y/N)
	 				
Jones Creek	40840	Cl.Fk.Muddy	N, 5'	F	?
Second Creek	48771	Smith Fk.Gun.	M, 6'	F/P	N
WFk.Beaver Cr.	48061	Upper Gunn.	N, 70'	F/W	Y
Waterfall Cr.	38200	San Miguel	N, ?	F/P	?
Up.LkFk.Gunn.#4	48080	San Cristobal	N, 12'	B/P	Y
Bilk Creek	38403	San Miguel	N, 50'	F	?
NFk.Tabeguache	43492		N, 60'	F/B	N
Coal Creek (W.Elk)	39152	NFK.Gunnison	N, 40'	F	?

 $\underline{l/}$ F = Forest Service, B = BLM, D = Division of Wildlife, P = Private W = Wilderness

Table 2. Streams reportedly containing cutthroat trout. Stocking unknown. (Source: CDOW and USFS personnel)

Stream	CDOW	Major	Stream	CDOW	Major
Name	Code	Watershed	Name	Code	Watershed
Waterfall Creek	38200	C		2050/	
		San Miguel	Deadman Creek	39594	Upper Taylor
Bilk Creek	38403		Illinois Creek	40763	
Fall Creek #1	40131		Spring Creek	43288	Upper Ceb.
Wilson Creek	47274	" "	Cebolla Cr. #2	38895	
W.Beaver Creek	38338		Road Beaver Cr.	38182	Cebolla
NFk.Tabeguache Cr.	43492		NFk.Road Beaver	38183	99
E.Elk Creek	39962	Upper Gunn.	WFk.Terror Cr.	43606	NFk.Gunnison
W.Antelope Creek	48016		Cliff Creek	39114	
Little Cimarron	39051	Lower Gunn.	Cow Creek	39380	Uncompahgre
Van Boxel Creek	46599		Spring Creek	43303	

 Table 3.
 Highest priority streams for inventory work. Surveys show that

 Cutthroat species are present but there are no records of the streams

 being stocked (#1-#13), or no surveys have been completed and there

 are no records of streams ever being stocked (#14-#41). (Source: CDOW)

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Colorado River Cutthroat Trout Waters

SAN JUAN NATIONAL FOREST

 $\frac{\text{Table 1.}}{\text{priority for field surveys.}} \qquad \text{Streams containing natural (N) or manmade (M) barriers and are high priority for field surveys.}$

Stream	CDOW	Major	Barrier	Land 1/	Stocked
Name	Code	Watershed	N/M, Ft.	Status	(Y/N)
	1				
Bear Creek	38136	Dolores		F	
Bear Creek	42646	San Juan		F	
Engine Creek	40030	Animas		F	
Falls Creek	43131	San Juan		F	
Grasshopper Cr.	40383	Animas		F	
Hope Creek	47642	Animas		F	
FlgFk.Junction Cr.	43410	Animas		F	
Kilpacker Creek	43422	WFk.Dolores		w	
Lone Spruce Dr.	43460	Animas		F	
Morrison Creek	38243	WFk.Dolores		F	
Salt Creek	43941	Animas		F	
Sierra Vandera Cr.	44032	Los Pinos		w	
Snowslide Creek	44070	Los Pinos		w	

 $\underline{1/}$ F = Forest Service, B = BLM, D = Division of Wildlife, P = Private W = Wilderness

Table 2. Highest priority streams for inventory work. (Source: CDOW/FS)

Stream	CDOW	Major	Stream	CDOW	Major	
Name	Code Watershed		Name	Code	Watershed	
Truby Creek	49963	Dolores	Corral Draw	47806	Animas	
Morrison Creek	38243	Dolores	Cascade Creek #2	48985	Animas	
Missouri Gulch	41715	Florida	Dark Canyon	42987	E.Fk.Piedra	
Virginia Gulch	43911	Florida	Deadman Creek	47351	E.Fk.Piedra	
W.Virginia Gulch	43923	Florida	Puerto Blanco Cr.	43826	E.Fk.Piedra	
Crazy Woman Gulch		Animas	WFk.Himes Cr.		San Juan	
Lightner Creek	45078	Animas				

APPENDIX B: SELECTION CRITERIA FOR STREAMS

SELECTION CRITERIA FOR COLORADO RIVER CUTTHROAT STREAM INVENTORY/REINTRODUCTION

BACKGROUND CHECK OF EXISTING RECORDS. SURVEYS AND MAPS

Α.

	1. Stream gradient greater than 10\$ Stream gradient less than or equal to 10\$		
	 Stream section less than 7,000' or greater than 11,500' Stream section between 7,000' and 11,500' 		
	3. 1st or 2nd order stream w/lakes or reservoirs above 1st or 2nd order stream w/\underline{no} lakes or reservoirs above		
	4. (Stocked w/rainbow and/or cutthroat trout (Stocked w/brown and/or brook trout Not stocked w/any fish species	Part	В
	 History of population surveys for this stream exist No history of population surveys for this stream exist 		в
	6. Headwaters contain stocked trout Headwaters not stocked or stocking records are unclear		
в.	FIELD SURVEY		
	 No natural fish barrier at lower terminus of stream section Natural fish barrier is present at lower terminus of section 		??

- Electrofishing yields trout below, but not above, barrier Stop Electrofishing yields trout are present above barrier B.3
- Reclamation and re-introduction w/CRN is not feasible Stop Reclamation for CRN reintroduction is feasible Part C

KEY to

C. RECOVERY/REINTRODUCTION

1.	Streamflows less than 1 cfs year-round Streamflows greater than or equal to 1 cfs year-round	
2.	Existing trout standing crop less than 20 lbs/ac Standing crop greater than 20 lbs/ac	
3.	Stream section located in wilderness area Stream section located outside wilderness area	
4.	Stream accessible, high fishing pressure expected Remote site, low fishing pressure expected	
5.	Special regulations are <u>not</u> feasible Feasible to implement special regulations	
6.	Models indicate habitat is currently suitable for CRN Habitat would be suitable w/improvements Habitat not suitable, improvements not feasible	C.7
7.	Reclamation and improvements are feasible Only reclamation is feasible Reclamation and/or improvements are not feasible	C.8
8.	Landowners/agencies <u>totally</u> support reintroduction plan a. No	op ndidate ream

INSTRUCTIONS FOR KEY TO STREAM SELECTION CRITERIA

The following instructions are intended to aid biologists and administrators in using the "Selection Criteria" keys to prioritize streams that may have the potential for Colorado River cuthroat trout (CRN) inventory or reintroduction efforts. Application of the selection criteria to specific streams should allow us to select those streams most suitable for CRN reintroduction. These instructions are meant to clarify specific items in the key and should be referred to whenever necessary to determine which path to take.

Part A: BACKGROUND CHECK OF EXISTING RECORDS, SURVEYS AND MAPS

1. Self-explanatory

2. Self-explanatory

3. If lakes are present above the stream section of interest, the investigators should check the stocking history of any waters above the stream section. If they find that the lakes have been stocked with rainbow and/or cutthroat trout, they should STOP if they are using this key for inventory purposes only. If they find that brown and/or brook have been stocked above the section of stream, they should proceed to PART B, if using this key to consider reintroduction potential. If the lakes have not been stocked with any species, they should proceed to PART B.

4. Refer above, Item 3.

5. Investigators need to check the records in Regional CDOW offices to determine if there is a history of fish stocking, creel census and/or electrofishing on this stream section. Long time residents with personal knowledge of the area may also be contacted for information. This must be done prior to considering Item #6.

6. If the headwaters of this stream contain non-native stocked trout and the investigators are using this key for inventory purposes only then they should STOP at this point. If the key is being used to determine reintroduction potential and the headwaters contain non-native stocked trout, or there are no trout stocked at all, then proceed to PART B. If, after checking all available records, the stocking status in unknown, the investigators should proceed to PART B.

Part B: FIELD SURVEY

1. If there are no natural fish barriers at the lower terminus of the stream section, the investigators should STOP and complete a Level 1 CDOW stream survey and mention in the comments that no natural fish barriers esist. Unless an artificial barrier is constructed, the stream section of interest has no further potential, at this point, to serve as a CRN reintroduction site. (Note: it could be considered at a later date, if the criteria in Part C are met and barrier construction is feasible.)

Examples of natural fish barriers are waterfalls greater than or equal to 6 feet high, toxic mine tailings zones, underground flow, etc.

If there is a natural fish barrier present, the investigators will indicate its location on a 7.5 minute quad map, take a photograph of the barrier, and preceed to 1tem #2.

2. Conduct electrofishing population estimates (two-pass removal method) and biomass estimates above and below the fish barrier. If trout are present above the barrier, proceed to Item #3. If trout are not present above the barrier (or below it) STOP and complete a CDOW stream survey report. In comments, speculate as to the reasons why trout are not present, either below the barrier or above it, or both. Are there critical limiting factors that prevent trout from surviving, or is it because of the barrier. Factors to consider are: steep gradient, water quality, water temperatures, seasonal variations in stream flow, spawning habitat availability, aquatic insect food base, etc. Address the question of whether this stream would be a candidate for reintroduction of pure CRN.

3. If no cutthroat trout are present, i.e. only non-native trout species were found, complete a CDOW stream survey an electrofishing summary and address the feasibility of reclaiming this stream section (re: Item B.4 in Key) with rotenone to remove non-native trout and restocking it with pure CRN.

If cuthroat trout <u>are</u> present, collect 10-15 adult fish (at least 6 inches long) for taxonomic evaluations. Follow instructions below for general guidance in the preservation of fish species. Where necessary, the investigators will check with CDOW personnel to insure they are following the proper protocol for preserving specimens and will make adjustments on a case-by case basis where needed.

a. Take close-up color photographs of live adult cutthroat trout for a permanent record of color and spotting pattern.

b. Using dry ice, immediately freeze a tissue specimen (i.e. adipose fin) from each of the 10-15 trout for DNA analysis.

c. Initially, preserve the adults in 10% formalin. Place a label in the container with the trout and formalin indicating date and origin

of the fish, and collector's name. If possible, inject 10% formalin into the body cavity of each fish.

d. After 3 days, place the formalin treated trout speciment into a jar filled with 100\$ ethanol.

e. Label and transfer the preserved trout specimens to CDOW fish biologists for taxonomic evaluations.

Drainage: Nearest landmark: Sample No.:Date: Collector(s)	Water: County: Elevation: ft. T. R. S.
COLLECTING GEAR:	HABITAT:
COLLECTOFISHING	Water temp
Cill net	Velocity
Dip net	Substrate
Course mesh seine	Water depth
Fine mesh seine	Acres sampled
Other (specify)	EFFICIENCY: Good Fair Poor

Example of LABEL

f. Complete a CDOW stream survey and electrofishing summary. Indicate exact electrofishing location(s) and fish barrier location on a 7.5 minute series topographic map. Include color photographs of any cutthroat trout collected and fish barriers present. Assuring taxonomic evaluations confirm a good phenotype cutthroat, no reintroduction work is necessary. ("A" phenotype should be used for transplant, "B" phenotype should be protected, and "C" phenotype would probably not receive any special attention within the scope of this conservation plan). If taxonomic evaluations reveal non-native authroat trout are present, adress Item B.4 in the key and proceed to PART C if considering reclamation and CRN reintroduction.

 If this is a candidate for reclamation and/or reintroduction, proced to PART C.

PART C: RECOVERY (REINTRODUCTION)

1. Self-explanatory

2. Self-explanatory

3. Stream sections located in wilderness areas are not candidates for reintroduction at this time. They may be further investigated in the future, provided they meet the necessary criteria.

4. Refer to CDOW fishing records and creel censuses to determine fishing pressure. High fishing pressure is generally viewed as anything greater than 300 hours/acre/year.

5. Self-explanatory

 Refer to CDOW and U.S. Forest Service records. If no stream habitat models are available, i.e. PHABSIM, IFIM or Basin-wide, conduct evaluations to answer this question.

7. Determination to be made after evaluations are complete and habitat has been assessed as being suitable for reintroduction of CRN.

8. All landowners and agencies must support the reintroduction efforts. If there is not total support, meet with the agencies and/or individuals to collaborate on how to gain the support required.

APPENDIX C: SELECTION CRITERIA FOR LAKES

SELECTION CRITERIA FOR COLORADO RIVER CUTTHROAT MANAGEMENT IN LAKES

•	PRES	SENCE OR ABSENCE OF TROUT IN WATER BODY		
	1.	Trout absent Trout present		В
	2.	Record of trout stocking No record of trout stocking		
	3.	Level 1 and 2 CDOW lake surveys completed No lake surveys exist (Surveys must be completed prior to continuing to A.5)	A.5 Stop	
	4.	a. Natural lake, history of stocking, no fish, WINTER-KILL . b. Natural lake, no stocking history, BARREN c. Natural lake, no stocking history, CUTTHROAT PRESENT (Collect samples. See instructions.) d. Newly constructed or manmade lake	Sto A.5	p
	5.	Tests confirm CRN present Tests confirm trout present are <u>not</u> CRN	Part Part	C C
	POTI	ENTIAL FOR TROUT SURVIVAL		

E

A

1. Suitability criteria for newly constructed or manmade lake: a. Year-round inflow is adequate - Y___, N__ b. Surface water temp. is w/in normal range of 33-65'F - Y_ , N c. DO is greater than or equal to 5 ppm year-round - Y ____, N d. Heavy metal concentrations are not limiting - Y ____, N ___ e. pH ranges from 6.5-9.0 - Y , N f. At least 25% of lake area is 20' or greater in depth - Y ____, N ____ g. Surface area is at least 2 acres - Y ____, N h. Lake is b/n 6,500' and 12,500' elevation - Y____, N____ (Proceed to B.2)

2.	a.	Any	one o	or al	l ans	vers	for	B.1.a-h	are	"No"	 	• • • • • • • •	Stop	
	b.	A11	answe	ers f	or B.	1.a-h	are	e "Yes"			 		Part	С

C. POTENTIAL FOR CRN TO BE ISOLATED FROM OTHER TROUT

 Natural lake located on 1st or 2nd order stream w/downstream barrier, OR located near the headwaters of a drainage with a natural barrier present downstream from the lake outlet:

> NO to both, not a CRN candidate Stop YES to either C.3

 Manmade lake located off-channel, not a natural flowing drainage, AND it is feasible to install screens in the lake inlet(s) and outlet(s) to prevent other trout from entering the lake:

> NO to either, not a CRN candidate Stop YES to both C-3

3. Compatibility a. All fish present are <u>compatible</u> with CRN Part D b. Some or all fish present are incompatible w/CRN C.4

4. Feasibility of treatment

a. Lake reclamation is feasible Part D b. Lake reclamation is not feasible C.4.b.1

1. Lake contains brown, brook, mackinaw or splake Stop 2. Lake contains only rainbow or cutthroat Part D

D. SPAWNING HABITAT

 Trout spawning habitat does exist and/or it is feasible to construct spawning habitat Part E

E. COMPATIBILITY OF OTHER USES W/CRN REINTRODUCTION

- Located within a wilderness area, candidate for refugium only E.2 Not located within wilderness, candidate for refugium or brood E.2
- Vehicular access during June, suitable as CRN brood lake Y_, N_ Limited access during June, suitable as CRN refugium only Y_, N_
- Lake is open to public fishing and fishing mortality is high a. Yes ___, possible refugium b. No __, broad lake

- 4. Other uses of this lake are compatible w/reintroduction plans a. Yes ___, proceed to E.5 b. No __, Stop
- Surrounding land uses are compatible w/reintroduction plans a. Yes ___, proceed to E.6
 - b. No __, Stop
- 6. Landowners/agencies support reintroduction of CRN
 - a. Yes ____, proceed with plans as refugium or brood lake
 - b. No ____, Stop. Not a candidate for reintroduction

Note: Answers to the questions in Part E will determine whether the lake will be used as a brood lake, refugium or the plans to reintroduce CRN are halted.

Sec.

INSTRUCTIONS FOR KEY TO LAKE SELECTION CRITERIA

The following instructions are to be used in conjunction with the "Selection Criteria" key for prioritizing lakes that may have the potential for Colorado River cuthroat trout reintroduction efforts. Application of these selection criteria will allow us to determine suitability of the lake for refugia or brood stock pruposes. These instructions should be referred to to supplement the information contained in the key.

Standing bodies of water serve two primary roles in recovery of Colorado River cutthroat trout (CRN): 1) as brood lakes with spawntaking potential, and 2) as genetic refugia. Recreational fishing is not considered a compatible use in most trout brood lakes. However, lakes with low angling mortalith and light fishing pressure (less than 10 hours/acre/year) may be wuitable as genetic refugia. As reintroduction of this species preceeds, it is anticipated that CRN will be stocked in selected high lakes and streams with suitable habitat to provide special use fishing opportunities.

Part A: PRESENCE OR ABSENCE OF TROUT IN WATER BODY

Level 1 (basic) lake surveys have been completed by CDOW on most lakes and reservoirs, with the potential to support fish, in southwest Colorado. These surveys comprises approximately 700 lakes. Of these 700 lakes, 430 contained fish, and 270 of these 430 lakes are back country lakes. While several strains of cuthrout trout have been widely stocked and are present in many lakes, no standing waters in southwest Colorado are identified in the lake survey database as containing exclusively CRN.

1. Self-explanatory

2. Make a note of whether or not trout stocking records exist and proceed to Item #3.

3. Check both CDOW regional office records for existing fish surveys and USFS/BLM records for existing physical habitat surveys. If no surveys exist, complete CDOW Level 1 and Level 2 lake surveys. Check for existing trout spawning areas and naural fish barriers, and assess the potential for adding manmade spawning areas and fish barriers before proceeding to Item #4.

4.a. WINTER KILL suspected: If there are records of regular trout stocking, but subsequent lake surveys show no trout were present, or periodic fish kills occur, STOP, this lake is not a candidate for CRN reintroduction.

- b. BARREN LAKE: If there is no documented trout stocking history and this is a natural lake that is barren of trout, STOP, this lake is not a candidate for CRN recovery.
- c. CUTTHROAT PRESENT: If there is no documented trout stocking history and this is a natural lake with <u>cuthroat trout present</u>, these trout could be CRN. Collect 10-15 adult cuthroat trout (at least 6 inches long) for taxonomic evaluation, and proceed to Item 45. SEE INSTRUCTIONS FOR COLLECTING TROUT SPECIENSIN "INSTRUCTIONS FOR KEY TO STREAM SELECTION CRITERIA". If tests confirm that CRN are already present, proceed to parts C, D, and/or E, to assess ways to protect or improve conditions for existing CRN in this lake, and/or to determine suitability for brood stock or refugia lake. If tests confirm that cuthroat trout in this lake are not CRN, then proceed to Part C.
- d. If a, b or c do not apply, or this is a newly constructed manmade lake, proceed to Part B.

Part B: POTENTIAL FOR TROUT SURVIVAL

If rainbow or cutthroat trout are already present in this lake, it is not necessary to complete this section. Proceed to Part C. If the water is a newly constructed impoundment or is a manmade lake that contains no trout, then complete this section.

- 1. Self-explanatory
- 2. Self-explanatory

Part C: POTENTIAL FOR CRN TO BE ISOLATED FROM OTHER TROUT

For many lakes, additional field surveys will be needed to determine if natural barrier(s) are present, and to assess the feasibility of retenone treatment before this section can be completed. Examples of barriers include waterfalls greater than 6-10 feet high, toxic mine tailings zones that are barren of trout, or underground flow that prevents trout movement. If no natural fish barriers are present, assess the feasibility of constructing a fish barrier. Do not overlook the possibility of reclaiming an entire drainage, is feasible.

- 1. Self-explanatory
- 2. Self-explanatory
- 3. Self-explanatory
- 4.a. & b. In order to be feasible for chemical reclamation, the lake should be motorboat accessible, not over 30 feet deep with a volume less than 200 acre feet, and have low inflow and outflow to aid in rapid detoxification. If it is feasible to consider rotenone treatment, draining, or some other method to remove existing fish from this lake and re-stock with CRN, then proceed to Part D.

4.b.1 & 2 If the lake contains brown, brook, mackinaw or splake, STOP, this lake is not suitable for CRN, since these trout are long-lived and may reproduce naturally in lakes with no apparent stream spawning habitat. If the lake contains rainbow or cutthroat trout. proceed to Part D.

Part D: SPAWNING HABITAT

Additional field surveys may be needed to determine if spawning habitat is present. If no natural spawning habitat exists, consider the feasibility of constructing a spawning channel or adding spawning gravel to the lake inlet or outlet.

1. If rainbow trout or non-native cutthroat trout are present, it may take a number of years for these non-natives to die out before a pure CRN refugium can be established. In any event, periodic stocking will be necessary to maintain a CRN refugium at this lake. If spawntaking is planned, it will be necessary to devise a way to capture spawners at this lake. Make note of these factors, and proceed to Part E.

 If natural spawning habitat for trout exists, OR a spawning channel can be added, this lake is potentially a self-wustaining refugium or brood lake for CRN. Proceed to Part E.

Part E: COMPATIBILITY OF OTHER USES W/CRN REINTRODUCTION

1. This lake is a candidate for refugium, but probably not suitable as a CRN brood lake because of motorized access limitations. Note these factors and proceed to ℓ^2 .

2. If there is deive-to access to this lake during the month of June, then this lake is suitable as a CRN brood lake. If the lake is not accessible by vehicle, then is is peobablynot suitable as a brood lake but may be a CRN refugium. Note these factors and proceed to 43.

3. If the lake is open to publec fishing, it is not wuitable as a brood lake, but may be suitable as a refugium if the fishing pressure is light (i.e. less than 10 hours/acre/year). Make note of these factors and proceed to #4.

4. If other uses, such as domestic water supply and livestock watering are probably compatible w/CRN survival, while irrigation, snowmaking and other uses resulting in water drawdown are generally not compatible. If other uses are compatible, proceed to 45, if they are not, STOP.

5. If other land uses, such as livestock grazing, logging, mining or human settlement within the immidiate proximity of the lake are judged to be incompatible w/CRN survival, STOP. If these uses are considered compatible, proceed to #6. 6. If all landowners and management agencies support the concept of a Colorado River cutthroat reintroduction project on this water, then preceed with reintroduction plans. If not, STOP.

APPENDIX D: PROTOCOL FOR PREPARING FISH SPECIMENS IN THE FIELD

Preparation of cutthroat trout for laboratory analysis using standard taxonomy, mitochondrial DNA or protein electrophoresis

The clever biologist will collect 10 specimens of each species from each location of interest. Trout specimens should be adult fish that are at least 6 inches long. Screen the specimens in the field to see that they conform to the characters and variability you would expect to see from pure populations. No need to preserve and submit specimens showing obvious signs of introgression, e.g., "cutthroats" showing many small spots on the head typical of rainbow x cutthroat hybrids. Careful screening of the fish in the field will ensure that efficient laboratory analyses conducted only on the best looking phenotypes rather than obvious hybrids.

PHOTOGRAPHS

Take close up color prints of the live adult fish. Plexiglass fish viewing tanks work well for getting quality photographs of live fish. Tanks for trout have outside dimensions of 12" wide X 6" high X 2" deep OD. Photos taken with the specimen on the measuring board are acceptable also. It is important to take two color photos of any fish that are non-lethal sampled for tissue so spotting pattern is obvious: one from the side and one looking down at the head. The prints should accompany the stream or lake write up in your permanent survey file. These are useful to make judgments about coloration and spotting of specimens that may fade over time in the preservative. See non-lethal sampling section also.

RECORDS OF THE COLLECTION

Use the standard Division of Wildlife lake and stream survey forms for reporting physical, chemical and biological data. In addition, assign a code number to each collection. For example, the code MJ-1 might be assigned to the collection done on Himes Creek in 1994. A second collection at another water might be coded MJ-2, and so on. The collection code should be written on the top of the tally sheet (see example) used to record length-frequencyweight data in the field. Transfer the code to the standard stream and lake report forms. Having the collection code on the survey write up will be useful in the museum when sorting and curating the collections in permanent storage.

Example	Example 1. Length-frequency field tally sheet.					
WATER:	CODE:	STATION:	DATE:			
LOCATION:						
PERSONNEL:						
LENGTH OF STATION:		AVG. WIDTH:	ACREAGE:			
POP EST MADE? Yes	No	COLLECTION CODE NO.				

Specimen Code	Species	Length	weight	Specimen Code	Species	Length	Weight
	1						
					1		
					1	1 1	

LENGTH-FREQUENCY RECORD (INCHES)

LABELING SPECIMENS

Prepare a museum style label for each specimen. A collection of 10 fish would require 10 labels. The label should be waterproof paper. The data can be handwritten using a water proof pen or pencil. However, a supply of preprinted waterproof labels will make the field preparation of the collection go faster. See example 2. *Each fish should be assigned a specimen code number* that is also recorded on the length-frequency tally sheet. The label should be folded and tucked in the mouth of each trout specimen.

Example 2. Museum sty	le label.
Water:	Date:
Scientific Name:	Collection No:
Common Name:	Specimen No:
County: State: Drainage	:
Topo Name: T	R S
Site Description:	
Collectors:	

WHOLE SPECIMENS FOR STANDARD TAXONOMY

The specimens should be euthanized using an overdose of MS-222 [NEED TREATMENT RATE]. *Do not drop live fish into a formalin solution* because this is out of compliance with AFS protocol for humane treatment of animals. It also renders them unacceptable for protein electrophoresis. Place the euthanized specimens with labels in a one gallon plastic jug. Wear goggles and gloves. Fill the jug with 10% formalin solution prepared by mixing nine parts water with one part 38% formaldehyde solution. Include 1/2 tespoon borax to buffer the formalin. Fix the specimens for five days in the formalin solution. After five days, drain the formalin solution and replace it with denatured alcohol for permanent storage.

The specimen collections can be shipped UPS in the plastic one gallon jugs if they are placed in a box. However, it is better to ship the collection wrapped in moistened cheesecloth to save weight and the cost of possibly replacing the plastic jugs. Soak the cheesecloth in enough alcohol to keep the specimens moist. Triple bag the wrapped specimens in zip loc plastic bags to prevent desiccation or leaks. Box up the bags in cardboard boxes for shipping.

TISSUE REMOVAL AND PRESERVATION FOR mtDNA ANALYSIS

This field technique requires the removal of fresh body tissue before fixing the specimens in formalin. It also requires proper labeling of the tissue samples. Tissues should be sampled while the trout is alive or as soon as possible after its death.

Slit the belly to open the body cavity and get to the heart and muscle tissue. Be careful not to cut away any pyloric caeca on the trout as they will be useful later for identifying hybrids. The slit will also aid penetration of the preservative. Remove the heart. Also remove a "V" of muscle tissue about the size of a pea from the inside the body cavity above the lateral line. Careful dissection makes for a clean looking specimen that can be used for other purposes in a museum collection. Place the heart and muscle tissue together in a small vial containing 100% ethanol (not denatured alcohol) or a solution of DMSO and salt [HOW TO PREPARE SOLUTION?]. Put a small waterproof tag (example 3) inside the vial that shows the collection code number and specimen code number linked to the field tally sheet. Put a label on the outside of the vial showing the collection locations. It's good to refrigerate the vials while waiting to relay them for analysis, but not essential.

Example 3. Waterproof tag used for labeling tissue samples in vials.

Collection No:
Specimen No:

NON-LETHAL SAMPLING TECHNIQUE

It is possible to collect fish tissue for mtDNA analysis without scrificing the fish. This may be useful for work on very small population that may be damaged if even ten specimens were removed. It also would be handy for studies of analyzing brood fish without sacrificing any individuals. The tissue sample should consist of a small muscle plug, a piece of adipose fin or eggs if the trout is female. A 2 mm biopsy punch should be used to remove a muscle plug from the dorsal side of the fish next to the dorsal fin. Do not reuse the biopsy punch as this would contaminate other samples. The tissue should be preserved n a vial with 100 percent ethanol and properly labelled.

Oocytes contain 100 times the number of mitochondria per cell as somatic cells and are the best sources of mtDNA. If mtDNA is going to be the only genetic analysis carried out and the trout are in spawning condition, collection of cegs may be an easy source of mtDNA.

WHOLE SPECIMENS FOR PROTEIN ELECTROPHORESIS

Note that the freezing technique used to prepare specimens for protein electrophoresis is also suitable for mtDNA analysis. Frozen specimens can be used to conduct both electrophoretic and DNA analyses.

Place a euthanized specimen on a small sheet of aluminum foil. Add a waterproof museum style label. Completely wrap the specimen and label in the foil. Group the wrapped fish in plastic bags according to collection site. Immediately put the bagged specimens in a cooler with dry ice. It takes about 40 lbs. of dry ice in an 80-quart cooler to keep the specimens frozen solid for three days in summer. It is helpful to wrap the entire cooler with fiberglass insulation. Specimens must be shipped quickly to avoid thawing. Permanent storage awaiting analysis must be in a super cold deep freze.

