## GENETICS MANAGEMENT PLAN FOR THE

ENDANGERED FISHES OF THE SAN JUAN RIVER





Final Report 24 March 2003

Cover Art:

Illustrations of the Colorado pikeminnow and razorback sucker are  $\ensuremath{\mathbb G}\xspace$  Joseph R. Tomelleri.

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Final Report

24 March 2003

Prepared for the: San Juan River Recovery Implementation Program

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#### PREFACE

This Genetics Management Plan provides a conceptual framework for genetics management and recommendations for managing the endangered fish stocks in the San Juan River Basin. This document is not intended as a comprehensive reference document for genetics management for which the reader is referred to the extensive literature that now exists. Rather, this plan is intended to serve as a <u>practical</u> <u>reference</u> <u>document</u> for the San Juan River Recovery Implementation Program (SJRIP), to document important assumptions, considerations and recommendations pertinent to the SJRIP. Boxes are used to highlight important guidelines and recommendations. This management plan is intended to be consistent with U.S. Fish and Wildlife Service (USFWS) policy and genetic management practices employed by the Recovery Implementation Program for the Endangered Fishes of the Upper Colorado River (UCRIP) to the extent that they apply. It borrows heavily from several previously produced genetics management documents including: the Genetics Management Guidelines for Listed and Candidate Fish Species Region 6 (Williamson, Wydoski and Visscher 1996); and the Genetics Management Guidelines (Williamson and Wydoski 1994) and Genetics Management Plan (Czapla 1999), both produced for the Recovery Implementation Program for Endangered Fish in the Upper Colorado River Basin. Large portions of those documents have been reorganized and used verbatim or expanded and modified to fit the San Juan River. A glossary of the terms used herein is provided on pages 43-45 in the back of this document.

Genetic management of the endangered fishes in the San Juan River Basin is detailed at two levels:

<u>Genetics Management Plan</u>: This management plan provides the conceptual framework of genetics management and rationale that will be employed to maintain genetic diversity of endangered fish stocks in the San Juan River Basin. This document also identifies priorities that will be used in the development of specific stocking plans to ensure that genetic diversity of wild endangered fish stocks in the San Juan River will not be compromised and that the best possible practices will be used for augmentation efforts.

This management plan: (1) provides a protocol for conserving the genetic variability in wild endangered fish stocks; (2) states the need for captivereared endangered fish; (3) identifies genetic risks associated with captive propagation; (4) provides breeding strategies for captive propagation; (5) provides guidance in the choice of founders for broodstock development; and (6) discusses ways to maximize the effective population size in captive stocks.

<u>Augmentation Plans</u>: In concert with the Genetics Management Plan, augmentation plans provide the justification and rationale for specific actions regarding the stocking of captive-reared endangered fish in the San Juan River basin including details such as species, size of fish, timing of release, location of release, genetic risks, and a description of how evaluation of the stocking will be made. Stocking plans are prepared for all experimental, augmentation, and restoration stocking and must be approved through the SJRIP before any endangered fish are stocked into the San Juan River. These plans must also be cleared by the U.S. Fish and Wildlife Service for issuance of a Federal Fish and Wildlife Permit or a supplemental permit and Section 7 consultation under the Endangered Species Act to ensure that stocking of captive-reared endangered fish will not jeopardize wild stocks. Augmentation plans for the San Juan River with the exception of short-term, research-related efforts will be planned using a minimum five year planning horizon. Following the adaptive management approach, augmentation plans can be modified as new information becomes available.

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#### I. INTRODUCTION

Water development activities in the Upper Colorado River Basin have led to the construction of multitudinous instream barriers (i.e., dams) and diversions to provide for flood control, power production, irrigation, and municipal water uses. However, these structures fragment once-contiguous sections of fish habitat and prevent fish passage, thus isolating fish populations. Additionally, water development has altered the historic flow regime of the Colorado River system, thereby affecting streamflow magnitude, water temperatures, and channel variability and maintenance crucial to the successful fulfillment of life history requirements of endemic fishes (U.S. Fish and Wildlife Service 1987a, 1987b, 1990a, 1990b, 1991). Numerous nonnative fish species have also been introduced and become established that compete with and prey upon native fishes (Miller et al. 1982, Tyus and Saunders 1996). These biological and environmental changes have resulted in four large endemic Colorado River fishes -- razorback sucker (Xyrauchen texanus), bonytail (Gila elegans), humpback chub (Gila cypha), and Colorado pikeminnow (<u>Ptychocheilus</u> <u>lucius</u>) -- being listed as "endangered" under the Endangered Species Act of 1973, as amended. Only two of the four listed species, the razorback sucker and Colorado pikeminnow are known to occur in the San Juan River. Portions of the San Juan River have been designated as critical habitat for these two species (U.S. Fish and Wildlife Service 1994).

The U.S. Fish and Wildlife Service coordinated an effort to develop a Recovery Implementation Program (Program) for endangered fish in the San Juan River to (1) protect the endangered fishes; and (2) allow water development to continue in the San Juan River Basin to meet the needs of society. The Program includes members from many potentially-effected entities in the San Juan River Basin: the Ute, Jicarilla, and Navajo nations, the States of Colorado and New Mexico; the U.S. Bureau of Reclamation, U.S. Bureau of Indian Affairs, U.S. Fish and Wildlife Service, and U.S. Bureau of Land Management; and water development interests.

#### II. MISSION, GOALS, AND PHILOSOPHY FOR GENETICS MANAGEMENT PLANNING

The mission and goals of this Genetics Management Plan are to maintain the genetic integrity and preserve the evolutionary potential of wild and captivereared endangered fishes in the San Juan River Basin consistent with endangered species management in the Colorado River Basin as a whole (Box 1). If possible, wild San Juan River populations should be managed through preservation of habitats and development of refugia and broodstock programs to prevent loss of locally-adapted genes. Where wild San Juan River populations are depleted to the extent that wild fish are not available for refugia and broodstock development, supplemental introductions from other appropriate sources of genetic material should be utilized to reintroduce fish into suitable wild habitats. The rationale for this philosophy is that natural refugia provide a source of preadapted genetic diversity that increases the probability for successful recovery of the endangered fish (Krueger et al. 1981; Samson 1992; Smith and Rhodes 1992). However, where insufficient wild resources are available for refugia and broodstock development, the Program will endeavor to utilize other appropriate genetic resources, including upper

and lower Colorado River Basin fish in broodstock development and augmentation efforts. The ultimate goal of all augmentation efforts is to achieve recovery of the two endangered fish species in the San Juan River.

Recovery, by definition, implies that endangered fish stocks whether simply being managed or actively augmented, will eventually become self-sustaining. This management plan is intended to provide a sound basis for the establishment of secure self sustaining stocks in the San Juan River. Genetics management goals consistent with the program mission and philosophy are given in (Box 1).

#### Box 1. Mission and goals of the San Juan Recovery Implementation Program for genetics management of endangered fish in the San Juan River

#### MISSION:

To maintain the genetic integrity and preserve the evolutionary potential of wild and captive-reared endangered fishes in the San Juan River Basin.

#### GENETICS MANAGEMENT GOALS

- 1. To prevent immediate extinction of any wild endangered San Juan River fish stocks.
- 2. To conserve genetic diversity of wild endangered fish stocks through recovery efforts that will protect or restore viable wild stocks.
- 3. To maintain genetic diversity of any captive-reared endangered fish broodstocks that are similar to the wild stock used as founders.
- 4. To identify priority stocks for use in augmentation efforts in the San Juan River in the event that native stocks are so depleted that effective genetic management using only the few remaining wild individuals is precluded.

#### III. GENETICS CONSERVATION PRINCIPLES AND ASSUMPTIONS

The following principles and assumptions are inherent in the development of this genetics management plan and the specific augmentation plans based upon it.

- C Stocks of Colorado pikeminnow and razorback sucker in the San Juan River represent a portion of the overall genetic potential of the species in the Colorado River Basin and should be managed, if possible, to preserve potentially unique genes.
- C For the purposes of genetic management and species recovery the San Juan River will be managed to support a single population of both Colorado pikeminnow and razorback sucker. It is assumed that these populations will be geographically and reproductively isolated from other Colorado River Basin populations.
- C Conservation of genetic diversity is fundamental to both short-term ecological adaptation and long-term evolutionary potential of a species since genetic variation is the raw material upon which natural selection acts.
- C Genetic diversity in endangered fish stocks allows for adaptability to various environmental conditions.
- C All management activities in the Colorado River Basin, including the San Juan River, may affect the overall genetic diversity of endangered fish species.
- C Accordingly, all proposed management activities in the Upper Colorado River and San Juan River Basins should be evaluated with consideration to potential genetic risks to endangered fish populations, especially if the risks include genetic losses that may become irreversible.
- C Irreversible loss of genetic diversity in endangered fishes in the Upper Colorado River and San Juan River basins can be avoided or minimized through rational and systematic planning.
- C Adaptive management (Boyce 1993; Ludwig et al. 1993; Walters and Hillborn 1978) should be employed in recovery of the endangered fishes in the San Juan River Basin. All management and recovery actions should be thoroughly evaluated so they are completed systematically and adjustments or refinements are made, as needed, to obtain the desired response from the endangered fishes.

#### IV. GENETICS MANAGEMENT OF WILD RIVERINE STOCKS

Conservation of genetic diversity is fundamental to both short-term ecological adaptation to various environmental conditions and the long-term evolution of a species through natural selection. The extinction of indigenous fish stocks is generally preceded by the loss of genetic diversity within and among

populations. As species decline to the point where they are listed as threatened or endangered, each remaining stock could play an important role in increasing the probability for recovery (Philipp et al. 1986; Rohlf 1991). Natural propagation should <u>always</u> be given the first priority in the recovery of endangered fishes in the Upper Colorado and San Juan River Basins. Lichatowich and Watson (1993) emphasized that stocking of captive-reared endangered fishes may be a useful management tool but should not be used as a substitute for the protection and restoration of habitat to reestablish viable wild populations. They state that management emphasis on existing stable wild stocks of endangered fishes should be directed primarily on protection, preservation, and enhancement of natural habitats needed by all life stages to increase the numbers of fish to that needed for recovery.

However, depressed fish stocks that do not have sufficient recruitment to maintain self-sustaining populations may require captive propagation and augmentation or restoration stocking (Williams et al. 1988). If sufficient numbers of wild fish are available, a broodstock should be developed to produce progeny for augmentation that preserve the genetic makeup of the parent stock to the greatest extent possible.

#### A. Genetic Risks To Wild Stocks Associated With Captive Propagation And

**Stocking.** Any stocking or augmentation has inherent risks associated with it. It is important to carefully assess possible risks of the stocking of captivereared endangered fishes to evaluate the benefits of stocking and to avoid potential adverse affects on genetic diversity of wild stocks (Kapuscinski et al. 1993; Philipp et al. 1993; Riggs 1990). Potential risks from stocking to natural stocks are varied but can include hybridization and introgression, inbreeding depression, and outbreeding depression, all of which can lead to the loss of rare alleles.

A.1. Hybridization and Introgression. Genetic introgression occurs when hybridization and back-crossing lead to an exchange of genetic material between different species or genetically-differentiated populations of the same species. This occurs both as a result of natural events and anthropogenic activities. Two types of human activities can contribute to loss of native populations of fish by encouraging hybridization: habitat alterations and introduction of fishes into locations outside of their natural areas of occurrence (Buth et al. 1987, Echelle 1991). Habitat alteration can increase hybridization by allowing contact between previously separated populations, altering features important to reproductive isolation, or reducing abundance of a species until contact with individuals of another species is more likely than contact with conspecifics (Echelle 1991). Introduction of species into areas where they previously did not occur also has the potential to heighten hybridization through contact of previously isolated populations.

No hybridization has been documented in the Colorado pikeminnow. However, hybridization has been documented for razorback suckers. The frequency of introgressive hybridization of razorback suckers with flannelmouth suckers (<u>Catostomus latipinnis</u>) is low -- 0-5% toward flannelmouth suckers (Buth et al. 1987) -- but could increase if the riverine environment in the upper basin and San Juan River is altered further, forcing the two species to spawn in the same areas or if flow/temperature cues are altered over spawning areas. Razorback sucker, flannelmouth sucker, and bluehead sucker are sympatric in

the San Juan River and the potential for some introgression does exist. However, due to the fact that these species evolved together and efforts are being undertaken to restore historic habitats and flow regimes, large scale introgression of genes seems relatively unlikely to occur in the San Juan River.

A.2. Inbreeding Depression. Inbreeding depression is possibly the most serious and yet most nebulous problem facing endangered fishes managers. Inbreeding is defined as the mating of individuals related by common ancestry. It is a relative concept because the degree of inbreeding is considered relative to a base population and thus there is no absolute measure. In general, populations with small effective population size are more susceptible to the effects of inbreeding. Traits such as fertility, fecundity, clutch size, survivorship, growth, and age at maturity are greatly depressed by inbreeding (Meffe 1986). Inbreeding depression can occur when the effective population size of wild stocks is significantly reduced by removing fish for captive propagation. It can also occur when large numbers of fish derived from a broodstock composed of relatively few parents are produced and stocked back into a wild population.

A.3. Outbreeding Depression. Outbreeding depression is a breakdown of fitness related traits as a consequence of incompatibilities between differentiated parts of the parental genome. An extreme example of outbreeding depression would be the production of sterile offspring following the mating of two different species. Though not as well documented as inbreeding depression, there is evidence to indicate that stocking of fish with different genetic make up can alter or replace the genome of wild stocks being augmented. The "Nearest Neighbor" method of stocking seeks to reduce outbreeding depression through use of fish that are geographically and genetically similar. Often genetic distance is correlated with geographic distance. This is due to the limited opportunity for gene flow and the environmental differences that can occur over large geographic distances.

A.4 Planning To Minimize Genetic Risk. Planning recovery efforts for the endangered fishes in the Upper Colorado River Basin will be accomplished following a logical and systematic approach (Box 2) that will minimize potential genetic risks. A genetic risk is broadly defined as the sum of critical uncertainties associated with a management action or inaction that may change the genetic diversity both within and among wild stocks. Risk assessment consists of (1) estimating risk and (2) managing risk (Lichatowich and Watson 1993). Major genetic risks associated with captive propagation can be identified and reduced through a careful assessment. Risk assessment will be addressed by individual stocking/augmentation plans on a case-by-case basis. The potential to manage potential genetic risks to wild stocks will be essential for a "no jeopardy opinion" under Section 7 consultation of the Endangered Species Act.

Box 2.	Systems	approach f	Eor	genetics	management	of	endangered	fish stocks.	
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<u>Step</u>	<u>Question to ask</u>	Tasks for each step by priority
I. Inventory/scoping	Where are we?	<ol> <li>Review available information.</li> <li>Define mission and goals.</li> <li>Identify and characterize fish stocks.</li> <li>Assess status and trends of stocks.</li> <li>Identify problems or issues.</li> <li>Assign priorities by species or stock.</li> </ol>
II. Strategic planning	Where are we going?	<ol> <li>Determine specific measurable objectives.</li> <li>Identify strategies to overcome problems.</li> <li>Evaluate potential genetic risks.</li> <li>Employ step-down planning (identify actions needed by priority).</li> </ol>
III. Operational planning	How do we get there?	<ol> <li>Prioritize actions from step-down planning.</li> <li>Determine resources needed to accomplish actions.</li> <li>Develop operational plans with budgets.</li> <li>Document need in Statement of Work.</li> <li>Seek approval and funding.</li> </ol>
IV. Evaluation	Did we make it?	<ol> <li>Monitor progress of actions.</li> <li>Revisit status and trend of target species.</li> <li>Evaluate actions to determine if they warrant continuation or if changes are needed.</li> <li>Repeat systems approach if necessary.</li> <li>Document results and disseminate information.</li> </ol>

**B.** Guidelines For Stocking Captive-Reared Endangered Fishes. Suitable habitat must be available in river reaches that are proposed for stocking to increase the likelihood of successful stocking. The reasons for holding, rearing, and stocking endangered fishes are summarized in Box 3. Criteria for when to stock and actions required are provided in Box 4. Guidelines for preparation of stocking plans for release of captive-reared endangered fish into the San Juan River are summarized in Box 5. Precautions that should be taken in conjunction with propagation and stocking of endangered fish are listed in Box 6.

# Box 3. Reasons for holding, rearing, or stocking endangered Colorado River fishes.

- 1. Place endangered fish in refuges to protect genetic diversity in instances where the stock may become extinct in the near future from population declines or due to catastrophic risk
- 2. Production of endangered fishes for experimental stocking to obtain biological, ecological, or environmental information from field experiments that are essential to recovery efforts
- 3. Production of endangered fishes for augmentation stocking to enhance severely depleted stocks
- 4. Production of endangered fishes for restoration stocking of extirpated populations or stocks

#### Box 4. General criteria for holding, rearing, or stocking endangered fish.

Analysis of wild population or stock

Population or stock is increasing and recruitment is sufficient to maintain a self sustaining population or stock

Population or stock is stable and recruitment is sufficient to maintain a self-sustaining population or stock

Population or stock is stable and recruitment is low but still considered sufficient to develop a self-sustaining population or stock, possibly with the aid of proper management actions

Population or stock is declining and recruitment is not considered to be sufficient to develop a self-sustaining population or stock

Population or stock is declining rapidly, already at very low numbers, or at catastrophic risk

Population or stock has been extirpated from part of its historic habitat

Action recommended

Monitor status and trends of population or stocks. Holding of captive reared fish is unnecessary

Monitor status and trends of population or stocks. Holding of captive reared fish is unnecessary

Identify and remove or reduce factor(s) limiting recruitment; Monitor status & trends of population or stock; Consider development of a broodstock in an artificial genetic refuge and an augmentation plan

Identify and remove or reduce factor(s) limiting recruitment; Monitor status & trends of population or stock; Development a broodstock in an artificial genetic refuge and an augmentation plan; Implement the plan if necessary

Place in refugia

If habitat is suitable and genetic risks to the same or other endemic fishes is low develop and implement a restoration stocking plan

# Box 5. Guidelines for preparation of a stocking plan for release of captive-reared endangered fish.

- 1. Describe the need for stocking
- 2. Characterize the endangered fish stock being managed and describe status and trends of target stock.
- 3. Describe limiting factors for target stock if known and corrective steps being taken to remove or reduce them.
- 4. Identify and discuss alternative management options that were considered and rejected.
- 5. Define objectives for stocking of endangered fishes and relationship to recovery effort
- 6. Assess genetic/ecological risk associated with proposed effort.
- 7. Describe origin of fish to be stocked and provide justification for their use in the San Juan River
- 8. Describe specific details of stocking and rationale for: -size of fish -time of year to release -numbers of fish -size of fish -frequency of stocking -location of release
- 9. Identify habitat availability for the fish to be stocked
- 10. Describe monitoring methods and how they will be used to evaluate the stocking effort

#### Box 6. Precautions to be taken during propagation and when stocking endangered fish into the Upper Colorado and San Juan River Basins. Listed in order of priority:

- 1. Precautions must be taken to prevent escapement of endangered fish from refugia or captive propagation facilities
- 2. Stocking of captive-reared fish will be conducted only in locations of the San Juan River were it can be demonstrated that such stocking will not adversely and significantly effect any indigenous fish populations or stocks of the same or different species.
- 3. Supplemental stocking will be restricted to locations with suitable habitat that will support a viable population or stock, or to historic habitats.
- All captive-reared endangered fish should be marked before release into the river, preferably with a permanent identifier such as a PIT Tag.
- 5. As much as possible, fish should be stocked as equal family lots from pedigreed matings to minimize the potential for genetic swamping and so that adequate evaluation of stockings can be made.
- 6. Any proposed stocking of endangered Colorado River fishes in the San Juan River will be described in a stocking plan, approved by the SJRIP and the appropriate regulatory agencies

C. Genetic Management Approaches Applicable To The San Juan River. There are three basic management strategies that can be used to bolster numbers of endangered fishes (Riggs 1990):

- 1. <u>All Natural</u>. Establish a refuge or genetic conservation area to manage a natural or naturalized population or stock without any kind of captive production or supplementation.
- 2. <u>Supplementation</u> (Augmentation). Rebuild natural spawning while minimizing genetic risks through captive propagation and supplemental stocking to augment declining populations or stocks or to restore extirpated populations or stocks.
- 3. <u>All Captive-Reared</u>. Maximize hatchery contribution to maintain populations or stocks with little or no expectation of restoring natural production.

Since the goal of recovery is to develop self-sustaining populations or stocks, strategies 1 and 2 are appropriate strategies to recover the endangered fish stocks in San Juan River Basin. Strategy 1 is based solely on increasing population size through management of the stock without any type of captive production or supplementation of the wild stock. Management could include protection of fish from harvest, reducing densities of nonnative fish that prey on and compete with endangered fish, and restoring the habitat and natural productivity of the river. Natural recruitment is the preferred strategy when adequate spawning adults are present and critical habitat elements can be improved to increase recruitment of young fish to the adult population. This strategy would be most appropriate for stocks that are presently considered to be stable.

Strategy 2 involves an integration of natural and artificial production systems to achieve recovery. Supplemental stocking is used to augment declining populations or to restore extirpated populations or stocks in conjunction with management activities to improve conditions for the endangered fish. Augmentation would be an appropriate strategy where numbers of spawning adults are low and the potential for improving habitat conditions such that self-sustaining populations can be attained is possible. Sources of fish for use in supplemental stocking efforts are varied and there are pros and cons associated with each possible source of fish that could be used for augmentation. Using only fish from the San Juan may result in increased levels of inbreeding due to the small numbers of adult endangered fish remaining in the San Juan River. Using fish from other sources could result in the loss of potentially locally-adapted genes in the population and possible outbreeding depression. The following set of priorities for supplemental stocking in the San Juan was conservatively designed to preserve potentially locally-adapted genes in the San Juan River. Sources of fish used for augmentation in the system should follow the priorities listed below:

- 1- Augment using native stocks from the San Juan River. These could include fish produced from captive adults taken from the San Juan or raised from wild larvae taken from the river. It could also include translocating wild fish (of any life stage) from areas where chances for retention or survival in the river are slim into more suitable upstream habitats.
- 2- Augment with fish from the upper Colorado River Basin (i.e., the Colorado or Green rivers or their immediate tributaries) using a "Nearest Neighbor" approach. This approach would be used in the event that native San Juan River stocks are not available or represent insufficient parents/genetic diversity to prevent inbreeding.
- 3- Augment using fish from the lower Colorado River Basin. Using fish from the lower Colorado River basin would be an extension of the "Nearest Neighbor" approach to augmentation. In the case of razorback sucker this means using fish from Lake Mohave, the area in which the greatest amount of genetic diversity is found among razorback sucker (discussed later). This approach was presented as a viable alternative to the "Nearest Neighbor" approach for razorback sucker by the upper Colorado River basin Genetics Panel due to the large number of fish (and individual haplotypes) found in Lake Mohave and the dearth of razorback sucker (and individual haplotypes) found in upper basin rivers. This option does not apply to Colorado pikeminnow.

The decision on whether to augment and the source of fish used for augmentation should be based on the status of the target population in the San Juan River and the possible impacts of augmentation on that population.

Strategy 3 involves the intensive use of hatchery production to replace natural reproduction in the river system. This approach is not likely to lead to self-sustaining populations since it does not address habitat limitations. However, it may be used as a last resort to maintain adult Colorado pikeminnow and razorback sucker in wild holding areas such as Lake Powell or off-channel impoundments for a number of years. Fish that would be used in Strategy 3 could come from a variety of sources but should follow the priorities outlined under Strategy 2.

#### <u>Guideline</u>:

- On 19 February 2002, the San Juan River Biology Committee agreed that there were too few wild razorback sucker or Colorado pikeminnow remaining in the San Juan River to achieve recovery by using genetic management Strategy 1 (i.e., all natural) at all, or by using genetic management Strategy 2, Priority 1 alone (i.e., augmentation using just San Juan River parent stock). Therefore the Biology Committee agreed to adopt the following genetic management strategies for the two San Juan River endangered fishes:
  - Razorback sucker: Since there are razorback sucker in captivity both from the San Juan River arm of Lake Powell (believed to have originated from wild San Juan River stocks) and from appropriate "Nearest Neighbor" sources, augmentation will proceed using broodstock from both sources (i.e, Strategy 2, Priorities 1-3). Colorado pikeminnow: Since there are no Colorado pikeminnow of San Juan River origin in captivity and appropriate captive "Nearest Neighbor" broodstock are available, augmentation will proceed using "Nearest Neighbor" broodstock (i.e., Strategy 2, Priority 2).

#### V. GENETICS MANAGEMENT OF CAPTIVE STOCKS

When progeny derived from the wild San Juan River parent stock are not available, a "Nearest Neighbor" approach for augmentation or restoration stocking should be used.

Colorado pikeminnow (Holden 2000).

#### A. Determining The Need For Captive Propagation And Stocking Endangered <u>Colorado River Fishes</u>. Depressed, endangered fish stocks that do not have sufficient recruitment to achieve and maintain self-sustaining populations may require captive propagation to produce fish for experiments related to research studies and for augmentation or restoration stocking (Williams et al. 1988). Such is the case in the San Juan River for both razorback sucker and

In the Upper Colorado River Basin needs for captive-reared endangered fish that have been identified include: (1) genetic refugia and broodstock development; (2) research and development; (3) information and education; and (4) augmentation or restoration stocking (Wydoski 1994). Refuges serve a vital function in maintaining endangered fish that may become extinct due to declines in wild populations or from catastrophic risks and to hold wild fish for broodstock development. Captive-reared endangered fish are needed for research to conduct laboratory and field experiments related to ecological requirements, habitat use, interactions with nonnative fishes, response to contaminants in acute or chronic bioassays, homing (i.e., olfaction and chemoreception studies), fish passage, taxonomy, anatomy, and physiology as well as performance, fitness, and hereditary trials. Endangered fish specimens are needed for use in public relations programs and to educate the public about reasons for the fish being listed as endangered and the program to recover declining populations. Augmentation or supplemental stocking may be required to provide a "jump-start" mechanism to supplement fish in habitats where the wild populations are very depressed. Restoration stocking is necessary in river reaches where stocks have been extirpated.

If conducted in a scientifically sound manner, captive propagation of endangered fish will prevent: (1) extinction of the species; (2) loss of genetic diversity within a species, stock, or population; (3) loss of genetic diversity among stocks or populations; and (4) inadvertent artificial selection that may lead to directional succession from inbreeding or genetic swamping of wild stocks (Busak 1990; Kapuscinski et al. 1993). The genetic processes that may be affected by these risks and the hatchery activities that may cause the risks are summarized in Box 7. The extinction of indigenous fish stocks can be caused by stocking captive-reared fish but is generally preceded by the loss of genetic diversity within and between populations.

Reasons For Maintaining Genetic Diversity In Captive Propagation Programs. в. The major objective in captive propagation of threatened and endangered fish species is (1) to develop broodstocks and to produce offspring that have genetic characteristics similar to those of the founding population. Two additional objectives for captive propagation of endangered fishes are (2) to use captive propagation and stocking when necessary as temporary fishery management tools to facilitate recovery (i.e., development of self-sustaining populations) of endangered fishes in their natural habitats while preserving the genetic diversity of wild endangered fish stocks and (3) to maintain artificial genetic refugia for endangered fish species or stocks that may be susceptible to extinction from declining numbers and from catastrophic risk in the wild, until such risks are removed. Breeding, rearing, and maintaining captive stocks such that genetic diversity similar to wild stocks is maintained from generation to generation is important in achieving this objective. Managing captive populations to maintain existing natural genetic diversity preserves future options for effective management and recovery of the taxon by fish and wildlife managers (Ralls and Ballou 1992; Thomas 1990).

The natural genetic diversity within a population is associated with its evolutionary history and is important for adaptation, long-term survival, and maximum production in the wild. Protecting genetic diversity within a hatchery broodstock prevents artificial selection, inbreeding, and genetic drift which frequently occurs in hatchery programs (Allendorf 1993). The concept of "maximally protecting genetic diversity" within a hatchery stock assumes that a sufficient number of randomly sampled individuals from the appropriate donor stock or population were used such that the broodstock truly reflects the genetic characteristics of the donor population. Proper broodstock development during the founding phase of a propagation program is fundamental in the recovery of endangered species because the species must possess sufficient genetic diversity to allow survival and recruitment of the fish in altered and partially restored environments.

The importance of maintaining detailed and accurate propagation records for captive broodstocks cannot be overemphasized. Records should include the origin, genetic profile, and unique identifying number of each adult broodfish as well as its production record and progeny performance. Genetic characteristics of broodstocks and progeny must be known so that augmentation or reintroduction efforts can be monitored and evaluated to determine survival of hatchery-reared fish and their interaction with wild stocks (Gauldie 1991; Hynes et al. 1981).

Box 7. Geneti hatche Modifi	c risks (expressed as changes from natury activities and the related genetic ped from Kapuscinski et al. (1993).	nral patterns) of processes.
<u>Genetic risk</u>	<u>Hatchery activity</u> <u>causing Risk</u>	<u>Genetic process</u>
Extinction	-Excessive numbers of adults removed from wild stock or population for development of broodstock	Increases in genetic drift and inbreeding
Loss of genetic diversity within stocks or populations	<ul> <li>-Low number of parents mated.</li> <li>-Inappropriate breeding design.</li> <li>-Captive breeding changing the natural genetic variation in families.</li> <li>-Founders for broodstocks differ from wild or supplemented stocks or populations in genetic diversity, life history patterns, or adaptability to the environment of the wild stock or population</li> </ul>	Increases in genetic drift and inbreeding
Loss of genetic diversity among stocks or populations	-Broodstock collected from stocks or populations that are genetically unrelated to wild stocks	Immigration: Changes in genetic patterns; Outbreeding depression
Inadvertent artificial selection	<ul> <li>Broodstock not collected throughout the spawning season.</li> <li>mated adults are from a nonrandom sample of their cohort with respect to genetic performance traits because of nonrandom mortality during incubation, rearing, or holding.</li> <li>Genetic swamping of wild stocks through stocking of large numbers of closely related individuals or unequal family lots</li> </ul>	Artificial selection: Disrupted patterns of natural selections; Changes in amounts of natural selection

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It is especially important to obtain a random sample of fish that represents the donor population by sampling adults within and among spawning times throughout the spawning range of that stock. The number of wild fish removed at any one time should be limited so that the remaining wild fish are not adversely affected by their removal unless the species is in immediate danger of extinction. In general, no more fish should be removed than can be spawned at a particular time or maintained in available holding facilities. Following successful contribution to the  $F_1$  generation, wild fish should be returned to the river reach where they were collected. If further stocking is deemed necessary, additional wild fish can be captured and used to supplement the genetic diversity of broodstocks.

c. Choice Of Founders For Broodstock Development. The first priority for obtaining founders for a broodstock is to use founders from the appropriate wild stock or population and to use acceptable breeding strategies that will maintain genetic diversity similar to the wild stock (Kapuscinski et al. 1993). Strategies for broodstock development can be prearranged to reduce genetic risks to wild stocks. Adult fish from each stock evolved life history patterns (other things being equal) that allowed adaptation to their environment in the Upper Colorado River Basin. If the endangered fish stock or population from a specific river reach has been extirpated or is extremely low, then the founders should be obtained from a stock that would be the nearest neighbor from an evolutionary (not necessarily geographic) standpoint. Fish from nearest neighbor stock should exhibit life history patterns similar to the extirpated population and be taken from an environment that has the greatest ecological similarity to the environment where the species is to be restored. If restoration cannot be done by natural reproduction, small numbers of wild founders should be collected from the wild stock to minimize demographic impacts on the genetic resources, life history patterns, and natural recruitment of the wild stock.

D. Estimating The Number Of Fish Required To Produce Broodstocks. The number of endangered fish needed at the end of each growing season to produce broodstocks is dependent upon the attrition (i.e., mortality) rates of the fish at the end of each growing season. Conservative attrition rates were estimated by consensus among members of the upper basin Propagation Subcommittee and Biology Committee. These attrition rates will be refined from empirical data from captive propagation of the fish in different facilities. Estimates of razorback suckers or Colorado pikeminnow needed at the end of each growing season until the fish mature and can be maintained as broodstocks in a primary and a backup refuge are provided. To be certain that about 30 adults (preferably equal sexes) from each family lot are available as broodstocks in primary and backup refuges, the numbers of fish required at the end of each growing season is estimated to be: 1 - 500 fish; 2 - 250 fish; 3 - 200 fish; 4 - 160 fish; 5 - 152 fish; and 6 - 144 fish.

**E.** Important Considerations In Captive Propagation Of Endangered Fishes. Equal sex ratios among breeding adults (Kapuscinski et al. 1993; Lacava and Hughes 1984; Tave 1984), equal numbers of offspring among family lots from pedigreed matings (Allendorf 1993; Kapuscinski et al. 1993), extended intervals between generations, and avoidance of selection are important in maintaining large effective population sizes and genetic diversity of broodstocks in captive breeding programs (Frankham et al. 1992; Kapuscinski et al. 1993). Ignoring these factors has resulted in rapid loss of genetic diversity in broodstocks under captive rearing conditions (Allendorf 1993; Kapuscinski et al. 1993). Equal numbers of fish from pedigreed family lots at the time of release will provide genetic diversity that will increase effective population size and reduce the potential for inbreeding while enhancing the potential for adaptation and survival after stocking (Allendorf 1993; Kapuscinski et al. 1993). Captive rearing of endangered fish results in a gain in total offspring but with a tradeoff in which there may be a simultaneous reduction in the effective population size (Ryman and Laikre 1991). A reduction in effective population size will be accompanied by excessive loss of genetic heterozygosity. This is especially true if the absolute size of the wild population is small. Ryman and Laikre (1991) strongly recommend that the risk of losing genetic variability through captive breeding should be carefully considered even if only a single stocking is to be made. Precautions that will be taken during propagation and stocking are summarized by priority in Box 8.

# Box. 8 Recommended strategies to maximize the effective population size in the captive propagation of endangered Colorado River fishes. (Recommendations in priority order).

- Mate one male with one female as a minimally accepted protocol if wild adults are available until an effective population size of 50 is achieved to maximize the genetic contribution from the wild stock and minimize the rate of inbreeding.
- 2. Breed as many adults as feasible in a given year until an effective population size of 50 is reached by mating one male with one female. If 25 families cannot be obtained during one spawning season, continue in the following years until 25 paired matings have been attained. It is important that all 50 fish used in the matings are different individuals so that all family lots are unique.
- 3. If the numbers of adult endangered fish are low, use the 5X5 breeding matrix or di-allele cross to develop a broodstock (Figure 1). The half-sib family lots provide a safeguard against the potential catastrophic loss of the genetic contribution from any parent.
- 4. When wild fish are extremely rare and represented by unequal sexes, use factorial matings to capture the genetic contribution from all fish of the least numerous sex. For example, if three males and one female were available, three family lots should be produced by mating all three males with the single female. This example will result in the three half-sib family lots.
- 5. If possible, additional wild adults should be obtained to supplement the effective population size of broodstocks developed from the diallele cross or factorial cross. Where facilities are limited, family lots from mating one male with one female should replace halfsib family lots to maximize the genetic contribution from the wild stock.

**F. Effective Population Size In Captive Propagation Of Endangered Fish.** The effective population size (N) is a key parameter in conservation of genetic diversity because the rate of inbreeding and the loss of genetic heterozygosity is proportional to the inverse of the effective population size (Ryman and Laikre 1991). The effective population size can be calculated using the following formula (Lande and Barrowclough 1987):

 $N = \frac{4N \text{ (males) } X 4N \text{ (females)}}{N \text{ (males) } + N \text{ (females)}}$ 

Exact estimates of the effective population size are complicated. For example, the formula given above for the effect of sex ratios assumes random mating, constant population size through time, and variance in fertility that approaches the Poison distribution. Effective population size can be maximized by non-random matings of paired individuals and equalizing the sizes of family lots (Allendorf 1993).

The effective population size should be maximized in every hatchery generation to reduce the risk of loss of within-population genetic variation (Kapuscinski et al. 1993). Strategies for conserving genetic diversity in captive-reared endangered fish are summarized in Box 9.

The rate of inbreeding (F) can be calculated if the N is known using the formula:

$$F = \frac{1}{2 N}$$

By applying this formula, the estimated rate of inbreeding for an effective population size of 50 (equal sexes) is 1 %. For an effective population size of 10 (equal sexes), the estimated rate of inbreeding is 5%. Therefore, it is advantageous to use as many adult fish as possible in developing broodstocks.

It has been suggested that a minimum effective population size of 50 is required to prevent inbreeding depression (Soule 1980) and a minimum effective population size of 500 is required to reduce long-term genetic drift (Franklin 1980). The acceptability of the "50" value is empirical from broad experience of animal breeders. Animal breeders have not encountered problems when the rate of inbreeding was 1% or lower per generation. However, some animal breeders will allow inbreeding to increase between 2% and 3%. A maximum of 1% in the inbreeding rate is recommended for wild populations (Simberloff 1988). The acceptability of the "500" value was proposed for genetic drift based on one trait in a fruit fly. Both values of this "50/500" rule have been disputed and no magic numbers or specific rules appear to be valid for propagation of animals (Simberloff 1988). Inbreeding is of particular importance because it has been demonstrated that a consequence of inbreeding depression in brother-sister matings for some species will result in offspring that are sterile or inviable after several generations (Lande 1988). The 1% maximum inbreeding rate recommended by Simberloff (1988) for wild populations should be used as a target for an effective population size in developing broodstocks of the endangered Colorado River fishes.



Figure 1. Diagram illustrating a 5 x 5 di-allele breeding matrix for maintaining genetic diversity during captive propagation of endangered fish stocks.

## Box 9. The 5 X 5 di-allele breeding matrix (Explanation of Figure 1)

Over generations, genetic diversity can decline in captive populations from the original founders through uncontrolled matings. Controlled breeding using marked broodstock, paired matings, equal sex ratios, and equal numbers from pedigreed family lots can help to reduce any negative effects to the original genetic diversity.

- Ten unrelated parents, five males (columns) and five females (rows) are bred using a 5 X 5 matrix, the "di-allele" cross (Kapuscinski et al. 1993)
- 2. Effective population size is 10.
- 3. The figure illustrates a single locus, multi-allelic (10 unique allele) system. Each unique allele is represented in the figure with a different color.
- 4. Each parent in this illustration is limited to two alleles that are homozygous.
- 5. Twenty five heterozygous  $F_1$  lots or genotypes are produced by the matings, resulting in all possible genotypes.
- 6. The breeding matrix produces half-sib maternal (within rows) and half-sib paternal (within columns)  $F_1$  family lots.
- 7. Breeding within family lots, brother/sister or full-sib matings (i.e., mating an Al male with an Al female)results in inbreeding in the F<sub>1</sub> generation. See lower left illustration in Fig. 1. Inbreeding results in an increased frequency of homozygous genotypes and a decreased frequency of heterozygous genotypes. This results in an increased frequency of familial alleles and a loss of population alleles, with a loss of fitness in offspring because of increasing deleterious recessive alleles.
- If another population is founded from a limited gene pool, the new population (i.e., broodstock) would suffer the effects of "founder event".
- 9. Breeding within a row or column produces half-sib matings(i.e., mating an Al male with and A2 female), that also results in inbreeding, but not to the extent of brother/sister pairings. Homozygosity increases at a reduced rate when compared to full-sib matings. See lower center illustration of figure.
- 10. Mating progeny from outside columns and rows prevents inbreeding. All  $F_1$  family lots are heterozygous and genetic diversity is maintained. See lower right illustration in Figure.

Allendorf (1993) recommended stocking equal numbers of offspring of family lots from pedigreed matings. This practice will reduce the potential for loss of genetic diversity. Philipp et al. (1993) recommended that genetic characterization of captive-reared fish be compared with wild stocks to ensure that the genetic diversity is similar.

**G. Ideal Number Of Founders For Broodstock Development.** The designation of "endangered" to any organism under the Endangered Species Act implies that the numbers of wild organisms are limited. The basic goal in an captive propagation program is to maintain the genetic diversity of captive broodstocks that is similar to the parent wild stock. Simberloff (1988) emphasized that general agreement does not exist about the absolute numbers of animals needed to maintain genetic diversity in captive propagation programs.

To achieve Simberloff's recommendation of keeping the maximum rate of inbreeding at 1% or lower, an effective population size of fifty parents (25 males and 25 females) is needed for development of a broodstock. Mating of one male with one female is desirable to maximize the genetic contribution from all fish used as parents (Allendorf 1993). This strategy of 25 paired matings is recommended as a target for an effective population size in the captive propagation of endangered Colorado River fishes. This strategy was designated as "Priority 1" in Box 8 for the maximization of genetic diversity in broodstock development. The fertilized eggs from mating one male and one female (i.e., 1 family lot) should be divided and reared in separate facilities to avoid the total loss of the genetic contribution from those two parents through an accident or catastrophe.

It is desirable to mate the 25 pairs of endangered fish in one year because broodstocks would mature at the same time so that equal numbers of all family lots could be produced and stocked from parents of the same age. This procedure would eliminate many variables that could influence genetic diversity and stocking success.

If sufficient numbers of endangered fish are in the wild, the goal would be to increase wild recruitment until self-sustaining populations are realized to meet the carrying capacity of available habitat. However, for declining endangered fish stocks with little or no recruitment, captive propagation may be necessary to augment wild stocks since a critical level of abundance of adults or threshold density (Allee Effect) may be needed for successful natural spawning (Lande 1988). In river reaches where the endangered fish have been extirpated, restoration stocking will be necessary, provided that suitable habitat is available for all life stages. If an effective population size of 50 adults cannot be obtained in one year, broodstock development can be accomplished over a number of years by mating one male with one female (Item 2 in Box 8).

H. Breeding Strategy For Captive Propagation When Small Numbers Of Endangered Fish Are Available. If the number of available adult wild fish is low, mating 5 males and 5 females using a 5 X 5 breeding matrix (Figure 1) is reasonable. An Ad Hoc panel of fishery geneticists agreed that the 5 X 5 breeding matrix should be used as the basic minimal breeding strategy model for propagation of the endangered Colorado River fishes when the number of wild adults is limited. This model, the di-allele cross (Figure 1), involves mating 5 males with 5 females of a stock as broodstock founders to produce 25 family lots (Kapuscinski et al. 1993). The entire genetic contribution from mating 5 males and 5 females are represented in the diagonal cells (upper left to lower right). The remaining 20 crosses are various combinations of the 10 parental genotypes.

Pedigreed matings of this nature (a single uniquely-marked male mated with a single uniquely-marked female) ensure that every possible genotype is produced and each parent is genetically represented in the next generation, thus preserving allelic diversity in the offspring of the captive stock (Kapuscinski et al. 1993). The mating of ten unrelated parents in the illustration of a di-allele cross (Figure 1) will maintain the genetic contributions of all parents. Note the genetic diversity in offspring from full-sib (Al X Al) or half-sib (Al X A2) crosses. The resulting offspring of the full-sib cross is illustrated in the lower left diagram and the half-sib cross is illustrated in the lower center diagram. A detailed explanation of the di-allele cross (Box 9) accompanies Figure 1 to explain its use in maintaining genetic diversity.

If space is limited for propagation of all family lots from a 5 X 5 di-allele cross, maintaining separate groups of the 5 unique family lots represented in the diagonal cells as illustrated in Figure 1 would be preferred to maintaining the 20 other matings because these 5 family lots contain the entire genetic contribution from the 10 parents. If adult fish are extremely rare and five fish of each sex cannot be obtained, factorial matings (Kapuscinski et al. 1993) will be used to increase the genetic contribution from the least numerous sex (See Item 4 in Box 8).

After a broodstock has been developed from a 5 X 5 cross or a factorial mating, additional wild endangered fish should be used to supplement the genetic contribution of the parent stock until a minimum effective populations size of 50 is achieved, providing that no adverse affect will occur to the wild stock (See Item 5 in Box 8). Additional lots from mating one male with one female should replace the half-sib lots in the di-allele cross to maximize the genetic contribution from the wild parents.

In some situations, it may be feasible to spawn wild fish at the site of capture and return them to the habitat from where they were collected. However, in most situations, fish will be captured, transported to holding facilities at genetic refuges or hatcheries, and held until they can be spawned. The stress of capture and transporting may prevent potential broodstock from spawning during the year of capture. Even if fish spawn in the year of capture or a subsequent year, the wild adults may be retained at the refuge or hatchery for an additional year to ensure that  $F_1$  progeny survive from the initial spawning.

#### VI. GENETIC MANAGEMENT PLAN FOR SAN JUAN RIVER ENDANGERED FISH

A. Razorback Sucker. The razorback sucker is a large monotypic catostomid species endemic to the Colorado River Basin (Miller 1959). It was once widespread and common in warmwater reaches of many Colorado River Basin streams from Wyoming to Mexico. Distribution and abundance of the species has declined in recent years and it is now extirpated from most of its historic range. Interruptions of natural flow patterns, obstruction of migration routes, and destruction of historic habitat by diversions and impoundments have contributed to the decline of the species. In addition, introductions of

nonnative species that prey on and compete with the razorback sucker (Tyus and Saunders 1996) and adverse effects from various contaminants (e.g., Hamilton 1998, Hamilton and Buhl 1997) have also negatively effected the species. Due to its imperiled status the razorback sucker was listed as an endangered species under the Endangered Species Act of 1973, as amended, effective November 22, 1991.

#### A.1. Status/Distribution And Abundance

#### <u>Historic</u>

**Colorado River Basin**. The razorback sucker was once common throughout 3,500 miles of the Colorado River Basin. Its range included the mainstem river and major tributaries in California, Arizona, Nevada, New Mexico, Colorado, Utah, and Wyoming; and in the State of Baja California Norte and Sonora of Mexico (Ellis 1914, Minckley 1973, McGinnis 1984).

The species was most abundant in the Lower Colorado River Basin downstream of the present day Lake Mead and was very abundant around Yuma, Arizona (Gilbert and Scofield 1898). Known collections and reports of occurrence in the lower basin were summarized by Maddux et al. (1993).

In the Upper Basin, razorback suckers occurred in the Colorado and Green River drainages. In the Colorado River drainage, razorback suckers were present from Lee's Ferry to near Rifle, Colorado on the Colorado River, the Gunnison River and San Juan River (Maddux et al. 1993). In the Green River drainage, historic distribution of the species was from the confluence of the Green and Colorado Rivers upstream to the town of Green River Wyoming, including portions of all the major tributaries to the Green River (Maddux et al. 1993).

San Juan River. The past distribution of the razorback sucker in the San Juan Basin has not been well documented and is not well known. The range of the species in the San Juan River probably included the mainstem river from its confluence with the Colorado River upstream to and including portions of the Animas River. The original range of the species in the San Juan River Basin may have been greater but early surveys and collections are sporadic or lacking. Anecdotal evidence exists that suggests razorback suckers were present at least seasonally in the Animas River (Jordan 1891) and present in the mainstem San Juan River upstream to within a few miles of the Colorado-New Mexico state line (Koster 1960). Lief Ahlm of the New Mexico Department of Game and Fish also investigated reports that suggested the occurrence of razorback sucker in the Cedar Hill area of the Animas River (approximately 7 to 8 miles downstream of the state line, near the present-day location of the Highway 550 bridge) during the 1930s and 1940s. He found evidence of razorback suckers and roundtail chub in the Animas River through the identification of the two species in photographs and descriptions from longtime residents and anglers of the Cedar Hill area (L. Ahlm pers. comm.). The first scientifically verifiable record of razorback sucker in the San Juan River basin was in 1976, when two adults were collected from an irrigation pond near Bluff, Utah (Platania 1990).

#### Current Status

**Colorado River Basin**. Distribution and abundance of the razorback sucker have been reduced to a fraction of their historic standing and appear to be continuing to decline. No significant natural recruitment to any population has been documented in recent years (Maddux et al. 1993).

The formerly large Lower Basin populations have become extirpated from most of the remaining riverine environments, but remnant populations of varying sizes still exist in several lower basin reservoirs (Minckley 1973, Holden et al. 1997). The largest of these reservoir populations, estimated at approximately 60,000 adults, exists in Lake Mohave (Minckley et al. 1991). However, natural recruitment in this population is lacking and the population appears to be in decline. Some managed recruitment, however, is occurring in the Lake Mohave population as a result of intensive efforts to control predation on young fish through the use of predator free grow-out areas adjacent to the lake (W.L. Minckley pers. comm.). Recently, a population of relatively young razorback suckers (when compared to other lower basin reservoirs) has been documented in Lake Mead (Holden et al. 1997). However, the Lake Mead population of razorback suckers is much less numerous than that found in Lake Mohave, totaling less than 500 individuals (Holden et al. 1997, 1999).

The largest concentration of razorback suckers in the Upper Basin occurs in the upper Green River between the Duchesne and Yampa Rivers (Lanigan and Tyus 1989, Bestgen 1990). Lanigan and Tyus (1989) estimated that between 758 and 1,138 razorback suckers were present in the upper Green River. Recent evidence indicates that although there has been some limited recruitment this population is declining. Occasional captures of razorback suckers also still occur in the Colorado River in the Grand Valley but have declined appreciably since 1974 (Maddux 1993). Adults of the species are rarely collected in the lower Green and Colorado Rivers. Larval razorbacks suckers, were collected between 1992 and 1994 from several locations on the Green River downstream from its confluence with the Yampa River, and in the Colorado River Arm of Lake Powell. No verified collections of larval fish have been made in the Colorado River above its confluence with the Green River (Muth pers. comm.). Collections of juvenile fish in the upper basin are rare.

San Juan River. Current distribution of razorback suckers in the San Juan River, including introduced fish, is from approximately the confluence of Ojo Wash (RM 170.8) to the San Juan River Arm of Lake Powell. Numerous, intensive scientific studies since 1987 have failed to collect wild razorback suckers from the San Juan River in Colorado or New Mexico and only rarely have razorback suckers been collected from the riverine portions of the San Juan River in Utah. Most collections of wild fish have occurred in the San Juan River Arm of Lake Powell over suspected spawning locations. Recent reported collections of wild specimens in the San Juan River Basin total 41 different fish and include:

- Two adults collected from an irrigation pond near Bluff, Utah, in 1976 (Platania 1990).
- One adult, 550 mm total length (TL) captured 14 November 1983, at Neskahi Wash by the Utah Division of Wildlife Resources (UDWR) during its annual gill netting to monitor fish populations (UDWR in lit.).
- One adult (545 mm TL) captured 14 November 1984, at Neskahi Wash by the UDWR during their annual gill netting to monitor fish populations (UDWR in lit.).
- One adult captured 25 April 1988, from the mainstem at approximately RM 82, near Bluff, Utah (Platania 1990).
- Twelve different adults were collected from a suspected spawning location in the San Juan River Arm of Lake Powell near Piute Farms (RM -0.5) between 20 March and 5 April 1987. Eight were ripe males and four appeared to be gravid females (Platania 1990).
- Ten adults (six fish were recaptures from 1988) were collected at the same suspected spawning location in the San Juan River Arm of Lake Powell near Piute Farms (RM -0.5) in spring 1988 (Platania 1990).
- One adult (601 mm TL) was captured 14 November 1989, at Neskahi Wash by the UDWR during its annual gill netting to monitor fish populations (UDWR in lit.).
- Fourteen adult razorbacks were collected during April 1990 in a joint effort by the UDWR , U.S. Bureau of Reclamation (USBR), and U.S. Fish and Wildlife Service. Four of the fish collected had been previously tagged. Three had been tagged at Piute Farms during 1987 and 1988 and one had lost the Carlin tag but had retained the attachment thread. It is likely that the fish with the lost tag had also been previously collected at Piute Farms. Fish were collected over suspected spawning locations and ranged in size from 557 mm to 682 mm. Collections were from the San Juan River Arm of Lake Powell at approximately RM -0.5 (1 adult) and RM -5.5 (13 adults). Eleven of the fish were subsequently transported to Ouray National Fish Hatchery for broodstock development and genetic studies (McKay 1990).
- Three adult razorback suckers were collected in early April 1991 near Nokai Canyon on the San Juan River Arm of Lake Powell in a joint effort by the UDWR and USBR (USBR in lit.). All three adults were transported to Ouray National Fish Hatchery (Chart pers. comm.).
- Three adult razorback suckers were collected by UDWR during April 1992 near RM -10 in a cove on the south side of the San Juan River Arm of Lake Powell (Stangl 1993).

Intensive ongoing collections between 1990 and 2002, part of a continuing research program by the SJRIP have not resulted in the capture of any additional wild razorback suckers from riverine habitats in the San Juan River. In addition, an intensive effort to collect adult fish during the spring of 1997 from the San Juan Arm of Lake Powell resulted in no additional fish captures.

However, between 1994 and 2002, 6,975 PIT-tagged razorback sucker were stocked into the San Juan River, first as part of an experimental stocking and monitoring program (n = 940; Ryden and Pfeifer 1994, Ryden 2000) and later as part of a five-year augmentation effort (n = 6,035; Ryden 1997, 2001). The lineage of stocked fish varied widely from progeny of San Juan River arm of Lake Powell adults, to progeny of adults from the Green and Colorado Rivers, to progeny of wild adults from Lake Mohave.

Radio telemetry and capture data from these fish have demonstrated survival of stocked fish in the river over a several year period. Those fish stocked at > 300 mm TL seem to have much higher survival rates, post-stocking (Ryden 2000, 2001). In addition, in spring 1997, 1999, and 2001 spawning aggregations of stocked razorback sucker adults were documented at RM 100.2 downstream of Aneth, Utah (Ryden 2000, 2001). Larval razorback sucker have been collected in five consecutive years, 1998-2002, and larval razorback suckers have been collected both up- and downstream of the suspected spawning area at RM 100.2, indicating that there is at least one other unidentified spawning area upstream of that location. However, despite the continued survival of stocked adult razorback suckers and presence of larval fish in five consecutive years, no recruitment has yet been documented.

A.2. Genetic characterization. Razorback suckers were once abundant and widely distributed throughout the Colorado River Basin. Currently, they are sparsely distributed within portions of their previous range and occur in sizeable numbers in only a few areas (Minckley et al. 1991). Human activities in the last century have severely impacted this species through introduction of exotic species and construction of dams that have modified habitat and disrupted potential corridors for movement throughout the basin. However, some researchers believe that even prior to human modifications razorback suckers may have been distributed discontinuously, tending to be localized in wide, alluvial reaches of the Colorado River system like the Green and Gila Rivers (Minckley et al. 1991). Lack of continuity could have resulted in isolation and divergence of populations, although the longevity, fecundity, and size of the species made it probable that enough individuals dispersed among regions to prevent divergence of subpopulations (Dowling and Minckley 1994). Baseline genetic studies including allozymes and mtDNA have been conducted since 1990 on the remaining populations to quantify the amount of genetic variation within and among existing populations and to help formulate management strategies for the species as a whole. Location of samples and type of analysis conducted are shown in Table 1.

Allozymes and mtDNA represent complementary data sets, measuring different parts of the genome and can act as dual tests of hypotheses involving hybridization, divergence, etc.

In his analysis of allozyme data Buth (1994) found that all groups of samples had heterozygosity levels below the mean for teleost fishes but were average for tetraploid catostomid fishes. Allozyme heterozygosity levels varied geographically but not in a clinal fashion. In addition, though razorback sucker populations are now fragmented and do not comprise a single, randomly breeding metapopulation no fixed or complete allelic differences among geographic samples were found for any loci examined. Buth concluded that

Allozyme Study (Buth 199	4)	mtDNA Study (Dowling & Minckley 1993)		
Location	Sample Size	Location	Sample Size	
Green/Yampa Rivers	51	Green/Yampa Rivers	29	
-Yampa R. @ confl. with Green R	(3)			
-Green R. (RM 311)	(36)			
-Stewart Lake drainage (RM 299)	(5)			
-Old Charley Wash (RM 251)	(4)			
-Duchesne R. (RM 248)	(3)			
Etter Pond (Colorado R.)	55			
Upper Colorado R./Highline	11	Upper Colorado River	29	
-Colorado R. near Grand Junction	(7)			
-Highline Lake	(4)			
Lake Powell	22	Lake Powell	23	
-Lake Powell proper	(19)			
-San Juan Arm	(3)			
		Little Colorado River	12	
		Lake Mead	15	
Lake Mohave	51	Lake Mohave	49	
Senator Wash Res. (Buth et al. 1987)	14			
		Colorado River Indian Tribe Canals	3	
		Dexter National Fish Hatchery	31	

Table 1. Listing of specimens of *Xyrauchen texanus* used in genetic analyses.

there was little allozyme evidence for major structure among populations of razorback sucker and that specific stocks were not recognizable via any marker loci. Buth also analyzed the allozyme database to determine if some locations (populations) are worse than others as sources of broodstock. In his analysis Buth identified a very low level of heterozygosity in the Senator Wash Reservoir sample that was extreme compared to other populations/locations that would limit the suitability of that population as a broodstock. Similarly the Etter Pond sample was highly atypical in its expression of an allele common in *Catostomus latiipinnis* (Buth et al. 1987). Based on the allozyme data he examined Buth (1994) stated that "a conservative recommendation might recognize Green + Yampa and/or Lake Powell and/or Lake Mohave as equally legitimate sources of broodstock." He further stated in his report that criteria beyond allozymes would have to be considered to justify the maintenance of just one, or two, or three separate stocks.

Dowling and Minckley (1994) examined many of the same razorback sucker populations using mtDNA analyses. They found that estimates of haplotype diversity ( $_i$ ) varied dramatically between populations examined with the greatest haplotype diversity occurring in Lake Mohave ( $_i$ =0.97) and the lowest in the upper Colorado River ( $_i$ =0.00) with geographically intermediate samples exhibiting intermediate  $_i$  values ranging from .73 to .82 (Table 2). The number of haplotypes and the number of haplotypes per sample corrected for sample size ( $n_n$ ) showed similar trends.

	Lake Mohave	Lake Mead	Lake Powell	Green River	Upper Colorado River
SAMPLE SIZE <sup>a</sup>	48	13	20	28	27
# OF HAPLOTYPES	32	6	7	4	1
n <sub>h</sub> <sup>b</sup>	0.67	0.46	0.35	0.14	0.04
i	0.98	0.82	0.81	0.73	0.00

Table 2. Characteristics of natural populations of razorback sucker analyzed in mtDNA study (from Dowling and Minckley 1994).

<sup>a</sup>=only samples with complete composite haplotypes and razorback mtDNA were used in calculations.

<sup>b</sup>=number of haplotypes per sample, corrected for sample size

Analyses conducted by Dowling and Minckley indicated that the frequency of specific haplotypes differed among samples; however, the proportion of variation differentiating populations was small, indicating that populations were not divergent. Phylogenetic and phenetic analysis also indicated a lack of population differentiation. Taken together they concluded that analyses of mtDNA restriction site data indicate that existing populations of razorback sucker have been interconnected by considerable gene flow or have become isolated so recently that mtDNA differences have yet to accumulate. While divergence among regions was limited, levels of variation within some populations was considerable. In particular the sample from Lake Mohave was especially variable, exhibiting estimates of  $_{\rm i}$  and  ${\rm n}_{\rm h}$  as high as those for any organism examined to date. Such a high diversity indicates surviving individuals were produced by a large number of females, likely more than exist in the region today. The high diversity and lack of appreciable differentiation of existing populations is consistent with considerable interconnection of regions (Dowling and Minckley 1994).

Dowling and Minckley (1994) postulated that the observed clinal reduction in mtDNA diversity could have resulted from recent serial bottlenecks as razorback suckers colonized further upstream after the last glacial period (Figure 2). If the system has not yet achieved equilibrium, upstream populations would exhibit only a subset of variation found in the downstream source, with the most common haplotype(s) likely to become more common as one moves upstream.

As part of their mtDNA study Dowling and Minckley examined genetic characteristic of the Dexter National Fish Hatchery (DNFH) broodstock relative to its parent source, Lake Mohave. They found that Lake Mohave fish contained more haplotypes and were more genetically diverse than the broodstock at DNFH. They concluded that the best means of preserving the natural genetic variability of the razorback stock in Lake Mohave would be through a program of capturing and rearing of wild caught larvae from the lake. In addition, they recommended that a large diverse captive broodstock be maintained and that the present inbred broodstock at DNFH be replaced with wild caught individuals from Lake Mohave.



Figure 2. Diagram displaying the observed clinal reduction in number of individual haplotypes (i.e., mtDNA diversity) found among populations of razorback sucker in the Colorado River basin (from Dowling and Minckley 1994).

A.3. Recommendations For Razorback Sucker Management. Due to their extremely depressed status, the likelihood of collecting wild razorback suckers in the San Juan River is very low and special efforts to collect additional wild fish for use as broodstock would almost certainly be futile, based on recent efforts. Therefore, augmentation/restoration efforts for razorback suckers in the San Juan River should be implemented using a combination of several stocks already in captivity, including the San Juan River Arm of Lake Powell parental stock and other nearest neighbor stocks. Use of other stocks in addition to fish of San Juan River Arm of Lake Powell parental several reasons, including:

- C Use of only San Juan River Arm of Lake Powell fish represents a significant risk for inbreeding depression due to the very small effective population size of that broodstock.
- C It is unlikely that the effective population size of the San Juan River Arm of Lake Powell broodstock will be significantly increased through the capture of additional wild fish.
- C Genetic studies to date indicate that considerable gene flow has occurred between various razorback sucker stocks and do not provide any evidence for divergence of populations.
- C There is little genetic risk to wild populations in the San Juan River since they have become essentially extirpated upstream of the San Juan River Arm of Lake Powell.

In addition, due to the extremely depressed status of San Juan River stocks and the ready availability of genetically diverse fish from Lake Mohave consideration should be given to using lower basin fish from Lake Mohave as well as upper basin fish in an augmentation effort.

In the unlikely event that a wild adult razorback sucker is collected in the San Juan River, it should be brought into captivity, spawned, and then returned to the river as soon as possible, so that it might participate in spawning events already documented to be occurring among stocked razorback sucker. Since there no longer appears to be a wild razorback sucker population in the San Juan River, the short-term removal of, at most, one or two wild fish should not be detrimental, since spawning events among groups of wild fish are nonexistent. At present, there is no evidence to indicate that there is any razorback sucker haplotype that is specifically unique to the San Juan River. However, if a wild adult razorback sucker is collected, efforts should still be made to include its genetic material in a San Juan River broodstock.

# Box 10. Genetics management recommendations for razorback sucker in the San Juan River.

- 1. Develop broodstock using appropriate sources of fish.
  - a. Retain any existing San Juan River arm of Lake Powell fish in captivity for broodstock development.
    - b. In the unlikely event that a wild adult razorback sucker is collected in the San Juan River, it should be brought into captivity, spawned, and then returned to the river as soon as possible.
    - c. Using San Juan River arm of Lake Powell and appropriate Nearest Neighbor fish, implement a 5X5 di-allele or other appropriate breeding matrix to develop broodstock (see box 8) from any remaining wild fish.
- 2. Develop an augmentation/restoration stocking plan for the San Juan River.
  - a. Evaluate need
  - b. Establish a genetic baseline for introduced stocks
  - c. Evaluate factors affecting recruitment, growth, and survival of introduced fish including; stock used, size/condition of fish at stocking, and time of stocking.
- 3. Monitor genetic status and trends of introduced fish and overall fish community.
  - a. Conduct periodic monitoring among progeny of stocked fish, as they reach adulthood, to ensure that inbreeding depression is not occurring.
  - b. Conduct periodic monitoring for possible introgression between razorback sucker and other native and nonnative suckers.

#### B. Colorado Pikeminnow.

The Colorado pikeminnow is the largest of the four existing species of *Ptychocheilus* and is endemic to the Colorado River Basin. It is also the largest member of the minnow family (family Cyprinidae) native to North America with maximum weights which likely exceeded 80 lbs. This large, predaceous minnow has a complex life history which has allowed it to exploit a variable environment and survive to the present. The species is a generalist adapted to large seasonal flow variations, high silt loads, turbulence, low food bases, and changing riverine subsystems (Smith 1981, Tyus 1986).

Once very common throughout the Colorado River Basin, Colorado pikeminnow have declined from historic levels and it is now found primarily in the Upper Basin of the Colorado River. Various factors have been implicated in the decline of the species including alteration of natural streamflows and temperature regimes, loss of habitat and habitat fragmentation as a result of water development in the Colorado River Basin, and the introduction of nonnative fish species that altered the biological environment in which the Colorado pikeminnow evolved. Additionally, the poisoning of areas below newly created reservoirs in the 1960's to create more favorable conditions for game fish (e.g., Olson 1962) species may have hastened the decline of the species in some locations.

#### B.1. Status/Distribution And Abundance

#### <u>Historic</u>

**Colorado River Basin**. The Colorado pikeminnow originally occurred throughout the Colorado River Basin. In the Lower Basin, the species was recorded in the Colorado River mainstem from the Gulf of California in Baja California del Norte to Lee's Ferry in Arizona. The species has also been recorded from most of the major tributaries to the Colorado River in the Lower Basin and the Salton Sea (Maddux et al. 1993). In the Upper Basin Colorado pikeminnow occurred in the mainstem from Lee's Ferry on the Colorado River to upstream of Rifle, Colorado on the Colorado River and to at least Green River, Wyoming on the Green River. The species was common in the mainstem Green and Colorado Rivers. It also occurred in all the major tributaries to the Green and Colorado Rivers including the San Juan River and probably occurred in numerous smaller streams. A more detailed list of the known distribution in the Upper Basin is presented by Maddux et al. (1993).

San Juan River. In the San Juan River Basin the historic distribution of the Colorado pikeminnow included the entire mainstem San Juan River up to at least Rosa, New Mexico, upstream approximately 25 miles from present day Navajo Dam. In addition the species may have ranged up the Animas River to approximately Durango, Colorado. Wild Colorado pikeminnow have been documented using the lower extremities of the Mancos River prior to spawning (Ryden and Ahlm 1996), and historic seasonal use of other tributaries in the subbasin is also probable.

Historic collections, which for the purposes of this document are defined as prior to the filling of Navajo Reservoir, have been sporadic. Cope and Yarrow (1875) were the first to report on fish from the San Juan River drainage. Roundtail chub were reported by Lt. R. Birnie in 1874, but no reports were made of other species including Colorado pikeminnow. Jordan (1891) reported anecdotal accounts of Colorado pikeminnow, razorback sucker, and flannelmouth sucker ascending the Animas River up to Durango during the spring. Jordan, however, did not collect any specimens. The first substantiated record of Colorado pikeminnow from the San Juan River drainage is three juveniles taken near Alcove Canyon, Utah, located approximately 7 miles upstream of Neskahai Wash, on July 4, 1936 (Platania 1990). Historic collections (summarized by Platania 1990) include:

- Three juveniles (72 to 73 mm SL) taken in the San Juan River at Alcove Canyon, Utah (RM 22) on 4 July 1936.
- One juvenile (278 mm TL) collected prior to 24 September 1941 on the mainstem in New Mexico.
- One adult (12 lbs) that was taken near Four Corners in Colorado (RM 119) in 1955.
- Two Colorado pikeminnow (unknown size) were collected near Rosa, New Mexico (approximately 25 miles upstream of Navajo Dam near RM 250) in 1959. This site was subsequently inundated by Navajo Reservoir.
- Three young fish were collected at Mexican Hat (RM 52) on 21 August 1960.
- At least 8 Colorado Pikeminnow were collected during the preimpoundment survey for Navajo Reservoir in 1962 within the area subsequently flooded by Navajo Reservoir. Four specimens were collected between 175 and 200 mm TL. Four additional specimens weighing a total of 12.0 lbs were also collected and documented by a photograph in a report by Olsen (1962).

#### Current Status

**Colorado River Basin.** Native populations of the Colorado pikeminnow are now restricted to the Upper Basin of the Colorado River. The species occurs in the Green, Yampa, White, Gunnison, Duchesne, and San Juan river basins. For a more complete description of the current distribution of Colorado pikeminnow in the Green and Colorado sub-basins see Maddux et al. (1993).

The greatest concentration of Colorado pikeminnow is found in the Green River Basin. Tyus (1991) suggested that the Green River sub-basin supported about an order of magnitude more Colorado pikeminnow than the Colorado River subbasin. No comparisons have been made with the San Juan River sub-basin but over ten years of catch effort data from intensive fish community studies suggest that populations in the San Juan River are less abundant than populations in either the Green or Colorado River. San Juan River. A small population of Colorado pikeminnow has persisted in the San Juan River since the closure of Navajo Dam in 1962. Preliminary information available from research studies recently completed or currently in progress as part of the SJRIP indicate that the Colorado River pikeminnow is reproducing and recruiting in the river to at least a limited degree. The range occupied by the species in the San Juan River has apparently shrunk since the closure of Navajo Dam and no verified collections of the species have occurred upstream of Shiprock in recent years. Recent collections, however, have not indicated a continuing range contraction. The current range of the Colorado pikeminnow in the San Juan River appears to be from Hogback Diversion (RM 158.6), downstream to Lake Powell. This estimate of current range is based on numerous collections made since 1987, recent radio telemetry studies, credible sightings by qualified biologists and presence of instream barriers.

Due to the low numbers of fish collected it has not been possible to quantify population size or trends for Colorado pikeminnow in the San Juan River. However, the number of adult fish in the system is believed to be small, probably less than 50 wild, adult fish. The largest concentration of wild adults is found in a 23 mile segment of river between Cudei Diversion (RM 142) and Four Corners (RM 119; Ryden 2000). The greatest concentrations of YOY and juvenile Colorado pikeminnow are present in the river downstream of Mexican Hat (RM 52) and in the San Juan River Arm of Lake Powell (Platania 1990, Lashmett 1994). Young fish have been collected relatively consistently but in low numbers since monitoring was initiated in 1991.

Between 1996 and 2000, the Utah Division of Wildlife Resources (UDWR) stocked approximately 827,449 larval and age-0 Colorado pikeminnow into the San Juan River at various stocking locations downstream of Hogback Diversion as part of a study to monitor retention, habitat selection and use, and survival. In addition, the USFWS stocked hatchery-reared adult Colorado pikeminnow at RM 178.8 on 23 September 1997 (n = 49 16-year old fish) and 11 April 2001 (n = 148 10-year old fish) to study retention and habitat use.

Stocked, early-life-stage Colorado pikeminnow demonstrated fairly rapid downstream displacement and low survival rates. However, those young fish that did survive grew quickly and reached total lengths in excess of 300 mm by age-3. Young Colorado pikeminnow also demonstrated a propensity for using shallow (i.e., less than 1 foot deep) shoreline habitats along the river's main and secondary channels in lieu of backwaters. The best observed survival and growth among stocked early-life-stage Colorado pikeminnow was from the 1996 stocking when fish were stocked in November at a mean of 55 mm TL, the largest mean length of all of the UDWR stockings.

Older, hatchery-reared Colorado pikeminnow adults stocked into the San Juan River also demonstrated rapid downstream movements and, based on the dearth of subsequent recaptures, low survival, post-stocking.

**B.2. Genetic Characterization.** Similar to razorback suckers, Colorado pikeminnow were once abundant and widely distributed throughout the Colorado River Basin. Natural populations of the fish have been extirpated from the lower basin but still occur in sizeable contiguous areas of the upper basin. Colorado pikeminnow are known to undergo long distance movements and

migrations of adult fish to spawning locations of over 100 km have been documented. In addition, though fish often appear to exhibit fidelity to a spawning site, and river reach, movement has been documented between the Colorado, Gunnison, and Green river systems (F. Pfeifer, pers comm.), indicating potential for gene flow throughout the upper basin. The small population of adult fish in the San Juan River is likely the most isolated population in the upper basin. Though not physically isolated, Lake Powell is likely a barrier to most fish movement between the San Juan River and the rest of the upper basin. No movement of Colorado pikeminnow has been documented between the San Juan River and other areas of the upper basin.

Baseline allozyme studies have been conducted on the Colorado pikeminnow to quantify the amount of genetic variation within and among various designated stocks. Results indicated that based on allozyme data there appears to be no significant differentiation among the various stocks (Williamson et al. 1997). Lack of allozyme differentiation among stocks would be consistent with a highly migratory species and considerable gene flow among various populations.

B.3. Recommendations For Colorado Pikeminnow Management. Although the Colorado pikeminnow is considered relatively stable in some parts of the upper basin, San Juan River stocks appear to be composed of a limited number of fish and may be declining. Based on the lack of allozyme differentiation observed among various stocks (Williamson et al. 1997), age-0 Colorado pikeminnow (progeny of adults from the Colorado and Green rivers) were experimentally stocked into the San Juan River between 1996 and 2000. In addition, an augmentation plan has been developed for the San Juan River and is currently being implemented using age-0 fish of this same lineage. Because of these two stocking efforts, it is now impossible to discern whether or not age-0 Colorado pikeminnow collected from the San Juan River are progeny of wild or stocked fish. This precludes the practicality of using young Colorado pikeminnow collected from the San Juan River to establish a unique San Juan broodstock or to having them contribute genetic material that is unique to the San Juan River (if indeed such exists) to an already established broodstock. In addition, there is a significant possibility that sufficient numbers of wild adult Colorado pikeminnow cannot be collected from the San Juan River to produce a viable broodstock. In any event the effective population size of a San Juan broodstock so established would likely be too small to prevent inbreeding. It was the majority opinion of members of the SJRIP Biology Committee that removing wild adult Colorado pikeminnow for broodstock production could significantly impact the wild population in the San Juan River, potentially even resulting in the extirpation of this population. Therefore, it was decided that the few wild adult fish remaining in the San Juan River should be left in the river so they might contribute to natural spawning events.

For these reasons, fish used for establishing a broodstock for Colorado pikeminnow augmentation efforts in the San Juan River should follow the Nearest Neighbor approach. Once appropriate adult broodstock are obtained or identified (if already in captivity), they should be mated using a 5X5 diallele breeding matrix or equivalent factorial mating scheme to produce a minimum broodstock. Wild fish should then be returned to their respective river(s) once the di-allele crosses are completed.

	Box 11.	Genetics management recommendations for Colorado pikeminnow in the San Juan River.
1.	Ident augme a. b. c. d.	<pre>ify/obtain appropriate stocks of Colorado pikeminnow for use in ntation efforts. Do not remove wild adult Colorado pikeminnow from the San Juan River. Identify appropriate Nearest Neighbor stocks. If there are none currently in captivity, implement a broodstock development program. Implement a protocol for collection of wild fish for broodstock production, if necessary. Once broodstock are obtained, implement 5x5 di-allele or other</pre>
2.	Devel River a. b. c.	appropriate breeding matrix (see box 8) to develop broodstock. op and implement an augmentation stocking plan for the San Juan Evaluate need. Establish a genetic baseline for wild stock and stocks used for introduction. Evaluate factors affecting recruitment, growth and survival of stocked fish including; stock used, size/condition of fish at stocking and time of stocking.
3.	Monit commu a.	or genetic status and trends of introduced fish and overall fish nity. Conduct periodic monitoring among progeny of stocked fish, as they reach adulthood, to ensure that inbreeding depression is not occurring.

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#### GLOSSARY

- Adaptive Management Making decisions based on available information and allowing flexibility for refinements (i.e., adaptation) to such decisions as new information becomes available.
- Allele One of two or more alternate forms of the same gene; alleles for the same gene occur at the same locus; each individual diploid organism has a maximum of two alleles for a specific gene.
- Allozyme An enzyme produced by an allele at a structural gene locus; different allozymes are produced by different alleles at the same locus.
- Artificial selection The process of choosing parents on the basis of a trait in order to obtain a phenotypic and genetic change in the next generation.
- DNA Abbreviation for deoxyribonucleic acid.
- Dominance The property of an allele that suppresses expression of other alleles at the same locus; a dominant allele is the only allele expressed phenotypically in a heterozygote.
- Effective population size The size of an ideal population that would experience genetic drift and inbreeding at the same rate as the real population under consideration.
- Electrophoresis A laboratory procedure for the separation of proteins (enzymes) that can be used as genetic markers.
- Enzyme A protein produced in living cells that speeds up a specific chemical reaction.
- Full-sibs Individuals having both parents in common.
- Gene A segment of DNA that occupies a specific position (locus) on a chromosome, is heritable, and has one or more specific effects upon the phenotype of an organism.
- Genetic diversity The genetic variation in within and among individuals, populations, or species.
- Genetic drift random or chance changes in the allelic frequencies due to natural or human sampling errors that occur each generation; the rate of genetic drift may increase as the effective population size decreases.
- Genotype The set of alleles at one or more loci in a organism; the entire set of genes carried by an individual.
- Half-sibs Individuals having one parent in common.
- Haploid a cell (i.e., gamete) or organism with a single set of homologous chromosomes.

- Hardy-Weinburg equilibrium the relationship between allelic and genotypic frequencies in a stable population after a single generation of random mating.
- Heterozygote A cell or organism with two different alleles at a particular locus.
- Homozygote A cell or organism with two identical alleles at a particular locus.
- Hybridization Interbreeding of different species and the union of gamete that results in a new organism.
- Inbreeding Mating of closely related individuals (e.g., brothers and sisters).
- Inbreeding Depression The loss in fitness of offspring due to the unmasking of deleterious recessive alleles that results from mating closely related individuals.
- Isozymes Multiple molecular forms of enzymes that promote the same chemical reaction but may be the products of alleles at different loci.
- Locus the position of a particular gene on a chromosome.
- Mitochondrial DNA (mtDNA) Deoxyribonucleic acid (DNA) found in the mitochondria of a cell; mtDNA is inherited from the maternal parent.
- Natural selection The selection of successful genotypes in natural environments on the basis of phenotypic traits related to fitness.
- Naturalized stocks or populations Fish that have become established in river reaches after augmentation or restoration stocking and are completing their entire life cycle in natural environments.
- Outbreeding Depression The loss in fitness of offspring due to the breakdown of coadapted gene complexes from mating individuals that are too distantly related.
- Phenotype The physical characteristics of an individual organism that can be detected visually; phenotypes are influenced by the genotype and environment.
- Polymorphic A gene or qualitative trait that exists in two or more forms in a population.

Population - A group of organisms that freely interbreed.

Presumptive Stock - A group of individuals that are presumed to be a breeding aggregation that has spatial, temporal, or behavior integrity.

Recessive - A trait or allele that is expressed only in homozygotes.

- Supplementation Stocking of captive-reared fish with the goal of augmenting or restoring self-sustaining populations; the genetic diversity of broodstocks is maintained through proper breeding strategies.
- Wild stocks or populations Endangered fish that are naturally reproducing and have the potential for natural recruitment if limiting factors are eliminated.