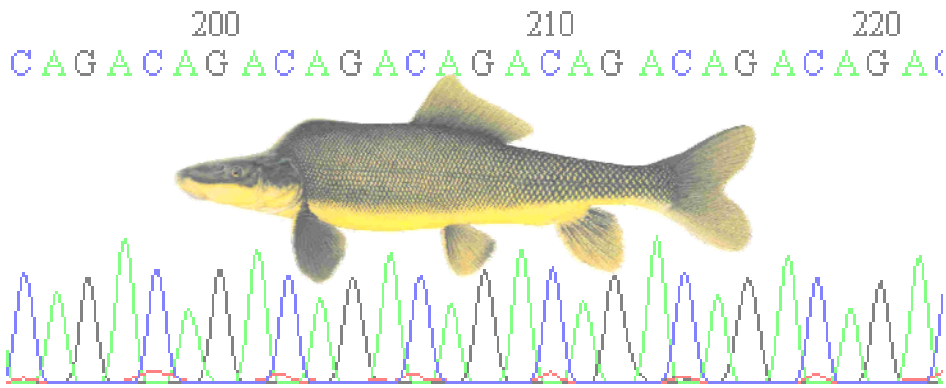
Razorback sucker (*Xyrauchen texanus*)

Genetics Management and Captive Propagation Plan

Dexter National Fish Hatchery and Technology Center

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## Executive Summary

The razorback sucker, *Xyrauchen texanus,* was listed as an endangered species in 1991 under the Endangered Species Act of 1973, as amended. During the early 20th century the species was abundant and distributed throughout the upper and lower Colorado River basin and its tributaries. The decline of the species to the verge of extinction is attributed to the construction of massive dams and water diversion projects that drastically changed the natural hydrology and aquatic environments of the Colorado River. Competition with, and predation by, introduced nonnative fish also add to the lack of recruitment by wild populations.

The Razorback Sucker Recovery Goals (U. S. Fish and Wildlife Service 2002) geographically delineate the management efforts in the Colorado River basin as two recovery units: the upper basin, including the Green River, upper Colorado River, and San Juan River subbasins; and the lower basin, including the mainstem and its tributaries from Glen Canyon Dam downstream to the international boundary with Mexico. The largest and most genetically diverse aggregation of razorbacks resides in the lower Colorado River basin in Lake Mohave, Arizona. This aggregation is considered to be the primary source for genetic material needed to recover the species in the lower basin and perhaps throughout the entire Colorado River basin. The immediate conservation goals for razorback sucker in the lower basin are to avert extirpation of the Lake Mohave population and to conserve it as a genetic resource. Controlled propagation of the species at designated facilities and reintroduction of fish into historic habitats are viable strategies and approaches to prevent imminent loss and reestablish populations. Dexter National Fish Hatchery & Technology Center (Dexter NFHTC) has contributed to the overall conservation effort since 1981.

This Genetic Management and Captive Propagation Plan (Plan) describes the guidelines used at the Dexter NFHTC for developing and maintaining razorback broodstocks, experimental populations and refugia maintenance. The Plan focuses primarily on genetic broodstock reserves and captive

propagation of stocks from the lower Colorado River basin in Lake Mohave, Arizona, and relates broodstock development at Dexter NFHTC to upper and lower basin needs. The purpose of this document is twofold: to provide the framework for maintaining genetic reserve populations of razorback that serve as broodfish, and to provide a captive propagation strategy that ensures production fish adequately represent the available razorback genome.

Dexter's genetic management goals and captive propagation objectives include maintaining a Lake Mohave razorback sucker genetic refuge represented by 3,000 individuals derived from the 1981 captive stock, ninety unique paired matings from wild-caught adults and six year classes of wild-caught larvae. A major component to the program is genetic monitoring, and production of known genetic quality fish in sufficient numbers to assist in the recovery, research and other approved management needs as defined in the Razorback Sucker Recovery Plan (U. S. Fish and Wildlife Service 1998) and Razorback Sucker Recovery Goals (U. S. Fish and Wildlife Service 2002). This Plan incorporates genetic guidelines provided by the Recovery Implementation Program for Endangered Fish Species in the Upper Colorado River Basin (U. S. Fish and Wildlife Service 1994), and the Genetics Management Plan for the Endangered Fishes of the San Juan River (Crist and Ryden 2003).

Authority:

Work activities identified in this document are conducted under authority of the Endangered Species Act of 1973, as amended, and the Federal Fish and Wildlife Permit # TE676811-8 held for such purposes.

# Introduction

Razorback sucker, *Xyrauchen texanus* (RBS), is a unique catostomid exhibiting extreme morphological adaptation to the Colorado River. RBS are at risk of extinction throughout their range, primarily due to the transition of the Colorado River from a rigorous lotic environment to a series of large manmade impoundments (Minckley 1995; Starnes 1995). This habitat transformation, coupled with the introduction of exotic fish, has severely impacted the most unique assemblage of fish species in North America. Small populations of RBS are currently found in the Green River, upper Colorado River, and San Juan River subbasins; lower Colorado River; Lakes Havasu, Mead and Mohave; and in small tributaries of the Gila River subbasin (Verde River, Salt River, and Fossil Creek). Extant populations of RBS are small with little or no recruitment. Threats to RBS include loss of spawning and rearing habitat resulting from water development projects, stream flow regulation, competition with, and predation by, nonnative fish species, and pesticides and pollutants (U.S. Fish & Wildlife Service 2002).

The RBS was listed as endangered in 1991 under the Endangered Species Act of 1973 (U. S. Fish and Wildlife Service 1998). A recovery plan for RBS was completed in 1998 and recovery goals finalized in 2002. The short-term recovery goal, as identified by both documents, is to prevent extinction of the species. The long-term goals are to recover the species and facilitate down-listing and eventual delisting. Captive propagation has been identified as a vital component for accomplishing short and long term goals. The development of hatchery broodstocks to produce genetically appropriate fish for recovery efforts is essential to establish new and augment existing populations.

This Genetic Management and Captive Propagation Plan (plan) provides clear guidelines for developing and maintaining RBS broodstocks at Dexter National Fish Hatchery and Technology Center (Dexter NFHTC). The purpose of this document is twofold: to provide the framework for maintaining genetic reserve populations of RBS that serve as broodfish, and to provide a captive propagation strategy that ensures production fish adequately represent the available RBS genome.

The plan focuses primarily on genetic broodstock reserves and captive propagation of stocks from the lower Colorado River basin in Lake Mohave, Arizona. However, a brief description of stocks currently and historically held at other facilities is included.

The plan is guided by the RBS Recovery Plan (U. S. Fish and Wildlife Service 1998) and RBS Recovery Goals (U. S. Fish and Wildlife Service 2002). The plan incorporates genetic guidelines provided by the Recovery Implementation Program for Endangered Fish Species in the Upper Colorado River Basin (U. S. Fish and Wildlife Service 1994), Genetics Management Plan For The Endangered Fishes of the San Juan River (Crist and Ryden 2003), and the Conservation Plan for the Colorado River Multi-Species Conservation Program (MSCP) (SAIC/Jones & Stokes 2001). Goals and objectives of the MSCP were developed based on conservation recommendations presented in Biological Opinions for the lower Colorado River, primarily Lake Mohave (1994; 1997) and Lake Havasu (1993).

The RBS Recovery Goals geographically delineate the management efforts in the Colorado River basin as two recovery units: the upper basin, including the Green River, upper Colorado River, and San Juan River subbasins; and the lower basin, including the mainstem and its tributaries from Glen Canyon Dam downstream to the international boundary with Mexico (Figure 1). The following is a discussion of the current status of wild and captive stocks based on those geographic distinctions, and relates broodstock development at Dexter NFHTC to upper and lower basin needs.

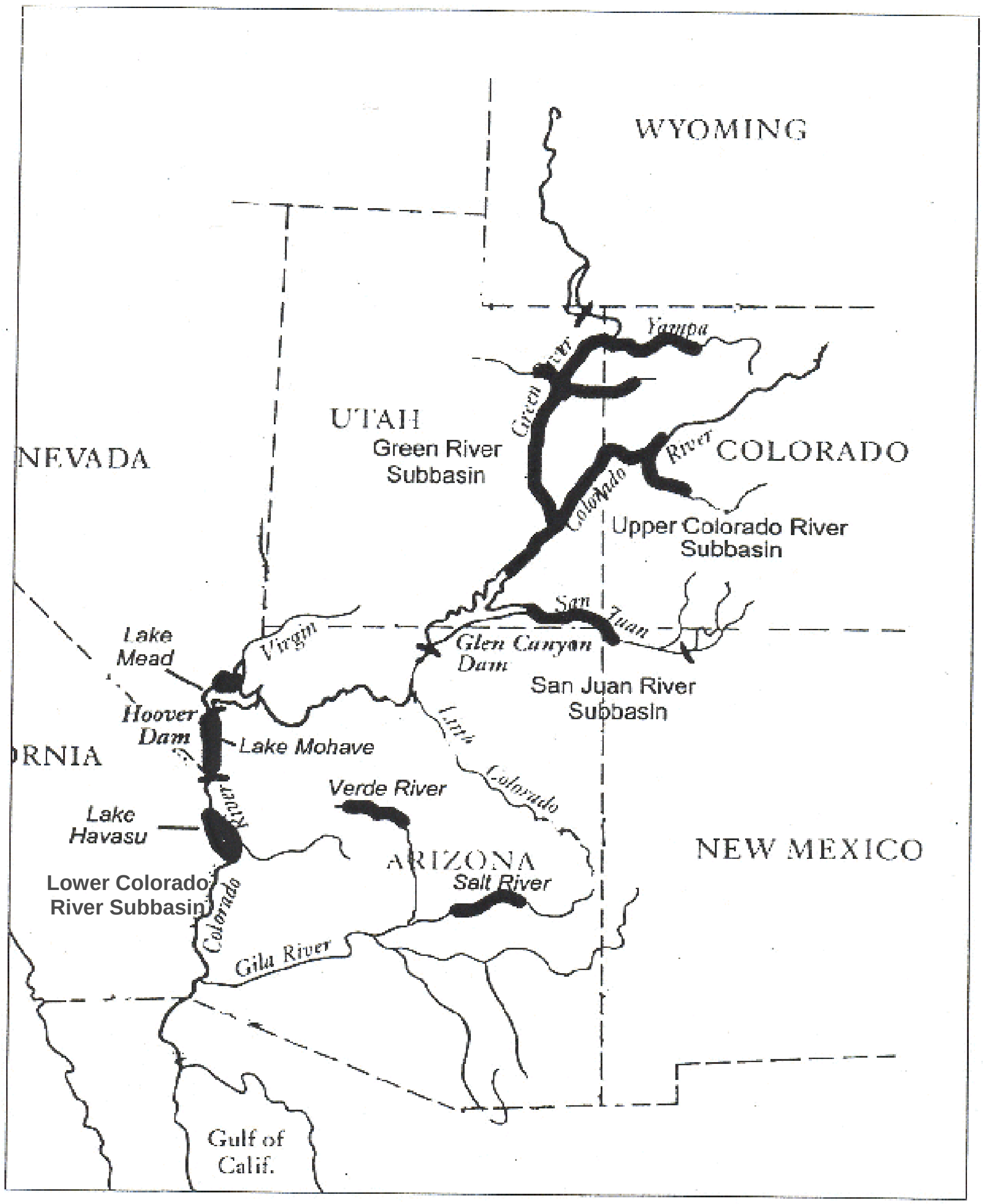


Figure 1. Distribution of wild or stocked razorback sucker in the Colorado River Basin.
(U. S. Fish & Wildlife Service 2002)

# Geography of Razorback Sucker Distribution

## Upper Colorado River Basin Recovery Unit

Green River/Yampa River System

Today, the RBS in this system exist as a small, non-recruiting, riverine population supporting 500 to 1000 individuals (Lanigan and Tyus 1989; Modde et al. 1995), and a diminishing riverine population of less than 100 adults in the middle Green River, between the confluences of the Yampa River and the Duchesne River (Modde et al. 1996; Bestgen et al. 2002). Historically, the lower Yampa River and Green River provided numerous spawning and rearing sites for RBS (Holden 1973; Minckley et al. 1991). It is unknown if this reach supported RBS populations that were genetically isolated from the mainstem Colorado River population. Evidence is provided that as recently as the late 1980s several putative spawning aggregations were present in the middle Green River and lower Yampa River (Tyus 1987; Tyus et al. 1982). Recently, a few larval and juvenile razorbacks were collected in the Green River, and it appears that these fish originated from the middle section (Chart et al. 1999, Muth et al. 2000). In 1989, wild adults were collected from the middle Green River at Jensen, Utah, downstream of the Ouray National Wildlife Refuge, and 20 adults from the San Juan arm of Lake Powell were also collected to begin development of a captive broodstock at the Ouray National Fish Hatchery (Ouary NFH) on the Green River near Vernal, Utah. The San Juan adults were later transferred to the Grand Valley Endangered Fish Facility. Captive spawning strategies used at Ouray are those identified in the Genetic Guidelines, Recovery Implementation Program for Endangered Fish Species in the Upper Colorado River Basin (U.S. Fish & Wildlife Service 1994). From 1989 to 1991 three lots of future broodstock (one annually) were created using a mixed stock of 15 females and 13 males of Green River origin. From 1993 to 2001, 25 unique family lots were created using predominantly the Green River stock. Two of the lots created in 2001 are from F1 parents of the San Juan and Colorado River arms of Lake Powell. Fish were obtained for these matings from the Grand Valley Endangered Fish Facility. Each family lot is represented by a minimum of 10 adult females and 10 adult males. In the future, the 25 family lots will be used in a 25 X 25 breeding matrix. Parental fish are stocked out after use; that is, an individual fish is only used for production once.

The Wahweap State Fish Hatchery (Wahweap SFH) maintains a portion of the 1996 through 2001 year classes for backup of Ouray's Green River broodstock, but at this time they have no production commitments for these fish.

Upper Colorado Mainstem/Gunnison River System

Minckley et al. (1991) reported that RBS abundance in Lake Powell was low. After inundation of the riverine habitat, spawning aggregation locations in the reservoir were assumed to be indicative of fish migration into the reservoir from the respective upstream tributaries**.** These aggregations are referred to as the Colorado River arm, the San Juan River arm, and Dirty Devil River arm populations. A few individual RBS were captured from the San Juan and Colorado River arms of Lake Powell and used as initial founders for respective broodstocks at Ouray NFH, Wahweap SFH, and Grand Valley facilities in the early 1990s; however, those wild populations are now considered extinct.

With the exception of a few wild RBS in isolated off-stream ponds, and fish in the Colorado River arm of Lake Powell, the wild population has been extirpated in the mainstem. The last captured wild individuals from the mainstem river were taken into captivity in the late 1980s and early 1990s. Seven of those wild adults were taken to Dexter in 1990, and spawned in 1991. Efforts to develop an Upper Colorado River mainstem captive broodstock at Dexter were unsuccessful. In 1993 a small population of razorback suckers were found in Etter Pond near Debeque, Colorado, in an off-stream backwater in the Grand Valley area. Genetic analysis suggested those fish were derived from very few individuals (perhaps only one female) and could be full or half siblings. Buth et al. (1987) found RBS from Etter Pond had atypically high levels of an allozyme variant common in flannelmouth sucker. Buth et al. (1987) also suggest that allozymes, while informative, may not provide all the information necessary to differentiate stocks of RBS. Ten of those individuals were kept at the Horsethief Ponds Fish Culture Facility for broodstock. The same year, two wild females and one wild male were captured from the mainstem Colorado and added to the broodstock pool (M. Baker, FWS, Pers. comm. 2002). In 1995 one wild female was collected from the mainstem Colorado River near Grand Junction, Colorado, and added to the captive broodstock held at the Grand Valley Endangered Fish Facility.

Captive reared RBS have been stocked into the Colorado/Gunnison subunit for several years (Burdick 2002). Production facilities that have contributed fish to the augmentation effort in this subunit include the Grand Valley Endangered Fish Facility, Grand Junction, Colorado; Ouary NFH, Vernal, Utah; Wahweap SFH, Utah; and Colorado's Mumma Native Aquatic Species Restoration Facility. Fish from the Colorado River mainstem, Green River, the Colorado River and San Juan River arms of Lake Powell, and Etter Pond have been stocked by these facilities into the Upper Colorado River.

**Representative broodstocks**

Upper Colorado River basin captive broodstocks are maintained at the Grand Valley Endangered Fish Facility, Ouray NFH, and Wahweap SFH (Table 1). The captive broodstock held at the Grand Valley Endangered Fish Facility is comprised of Colorado River/Lake Powell stocks derived from nine females and eight males (C. McCada, FWS, Pers. comm. 2002). These fish were wild fish captured from the Colorado mainstem, Etter Pond, and the Colorado River arm of Lake Powell as described above. In 1994, two wild females and one wild male collected near Grand Junction and an Etter Pond male were mated in a 2 X 2 cross, resulting in four lots that form the basis of the current captive broodstock. Concurrently, six mature wild RBS captured in the San Juan arm of Lake Powell being held at Wahweap SFH, and four wild RBS from the Colorado River arm of Lake Powell held at Ouray NFH were transferred to Horsethief Ponds for future propagation efforts. In 1995, an additional wild female collected from the mainstem near Grand Junction was added to the founding broodstock pool. In 1999, 20,000 wild caught fry and 1,050 wild caught fingerlings (85mm) of Lake Mohave origin were transferred from Willow Beach NFH to Bounds Pond, a U. S. Fish and Wildlife Service owned pond near Clifton, Colorado, for growout. These fish serve a dual purpose as future broodstock and for augmentation of the San Juan River System. In February, 2003, utilizing the "nearest neighbor" approach, the Upper Colorado River Endangered Fish Recovery Program (UCREFRP) Biology Committee approved a strategy to incorporate the Lake Mohave fish into the captive broodstock to increase the number of paired matings, and genetic diversity of the existing stock (UCREFRP, Biology Committee Meeting notes, 2003). Lake Mohave fish however, will only be used in paired matings with hatchery broodfish of upper basin origin. Essentially, no Lake Mohave X Lake Mohave crosses will be created.

<u>San Juan River System</u>

Wild RBS are considered extirpated from the San Juan River mainstem. The last recorded collection of a wild RBS in the San Juan River was in 1988 near Bluff, Utah (Ryden 2000). The U.S. Fish and Wildlife Service initiated development of a captive broodstock for the San Juan River from fish collected in the San Juan arm of Lake Powell in the early 1990s at the Ouray NFH, Utah. The fish were spawned at Ouray in 1992, the eggs were hatched at the Belview Research Laboratory, Belview, Colorado, and reared at Wahweap SFH. In the early 1990s, all adults and offspring were transferred from Ouray NFH to ponds maintained at Wahweap SFH near Lake Powell. In 1995, ninety RBS from the1992 spawning effort conducted at Ouray NFH and reared at Wahweap SFH were transferred to the Grand Valley Endangered Fish Facility and used in a selenium toxicity study (M. Baker, FWS Pers. comm. 2002). Currently, Wahweap SFH maintains backup broodfish lots of Green River origin; however, no captive propagation is planned at this time. In 1994 captive reared RBS from Lake Mohave and Grand Junction stocks were the first RBS stocked into the mainstem San Juan River by the U.S. Fish and Wildlife Service (Ryden 2000). Production facilities that have contributed fish to the augmentation effort in this subunit are Dexter

NFHTC and Willow Beach NFH (from the lower basin) and Grand Valley Endangered Fish Facility (from the upper basin).

## Table 1. Upper Basin Razorback Sucker Captive Broodstocks

**Grand Valley Endangered Fish Facility**

| Year Class | Origin | Numbers on hand | Founders Represented | Lot Designation |
|---|---|---|---|---|
| 1995 | CR,EP,GV,SJ | *285 | 19 pairs | 95 |
| 1995 | GR | *20 | 2 & 1% | 95 GR $F_1$ |
| 1996-98 | Willow Beach/Mohave | *285 | 19 pairs | 98 LB |
| 1999 | CR,EP,GR, GV,SJ | *375 | 25 pairs | 99 |

**Ouray National Fish Hatchery**

| | | | | |
|---|---|---|---|---|
| 1989-1991 | GR | 67 | 15♀ 13♂ (3 lots) | 89-91 GR RBS |
| 1993-2000 | GR | 849 | 19 pairs | 93-00 GR RBS |
| 2001 | GR,CR,SJ | 360 | 3 pairs | 01 GR RBS |

**Wahweap State Fish Hatchery**

| | | | | |
|---|---|---|---|---|
| 1997 | Ouray | 71 | 1 pair | |
| 1998 | Ouray | 301 | 5 pairs | |
| 1999 | Ouray | 342 | 9 pairs | |
| 2000 | Ouray | 68 | 2 pairs | |
| 1991-1995 | Ouray | 143 | 6 pairs | |

*CR- Colorado River, EP- Etter Pond, GV- Grand Valley, GR-Green River, SJ- San Juan River, LB- Lower Basin.*
* estimated numbers.

### Lower Colorado River Basin Recovery Unit

In the lower basin, the Colorado River system was drastically altered during the 20th century in the course of major water development and construction. The mainstem river and the Gila and Salt/Verde tributaries were impounded by impassable dams, creating a chain of reservoirs linked by cold, clear, high velocity waters. The lower basin recovery unit exists as a series of discrete reservoirs that present management opportunities markedly different than waters in the upper basin. Razorback sucker management in the lower basin is dependant on augmentation stocking in an attempt to enhance remaining RBS populations, and to conserve extant genetic material of the largest remaining stock of RBS in the Colorado River system.

Recruitment failure is the most critical challenge in the lower basin reservoirs. Inundation of nursery areas and predation by non-native fish on eggs, larvae, and early juvenile stages are major causes of recruitment failure (Minckley et al. 1991). Without natural habitat, captive rearing of early life history stages and advanced juveniles is required to maintain current populations status. Wild caught and hatchery spawned RBS fry are reared in hatcheries and ponds to a minimum length of 300 mm, PIT (passive integrated transponder) tagged, and stocked to restore extirpated or augment remnant populations of RBS.

All lower basin reservoirs (Mead, Mohave, Havasu, and Imperial) have documented historical populations of RBS (Minckley 1991). The resident RBS populations in Lake Havasu and Imperial Reservoir are considered extirpated, and management efforts to reestablish a population through augmentation in Lake Havasu began in the early 1990s. Since 1993, Lake Havasu Fisheries Improvement Program, Bureau of Land Management, U. S. Fish and Wildlife Service, and the Arizona Department of Game & Fish have cooperated to release over 30,000 subadult RBS into the reservoir. Visual observations by U. S. Fish and Wildlife Service sampling crews indicate the fish are surviving and adult fish are utilizing numerous spawning sites throughout the lake (C.O. Minckley, FWS, Pers comm. 2002).

The Lake Mead population, abundant in the 1960s, is estimated at less than 150 adults inhabiting two distinct areas (Las Vegas Bay and Echo Bay) Holden et al. 1999; Abate et al. 2002). The Nevada Division of Wildlife released 26 subadult RBS fish into Las Vegas Bay in 1994, and 14 into Echo Bay in 1995. All the fish were PIT tagged and had originated from the

Dexter NFHTC 1984 year class reared at Floyd Lamb State Park in Nevada (Abate et al. 2002). Larval RBS have been collected at several spawning locations in Lake Mead since 1995. In 2002, using fin ray sections, several fish were aged at 6-10 years, indicating recent recruitment to the Lake Mead population (Abate et. al 2002).

Currently, the larger Lake Mohave population provides the benchmark for the success of RBS recovery throughout the basin. The recovery effort, as reflected by the intense management program to restore the RBS population in Lake Mohave, relies on augmentation of the existing population with hatchery reared fish.

Lake Mohave

The largest aggregation of RBS in the Colorado River resides in this mainstem reservoir. The population represents the majority of the genetic diversity remaining in the species, and is essential to the recovery of RBS. The Lake Mohave population consists of a declining number of old adults, estimated to be at least 50 years of age. Adult population estimates have ranged from 60,000 individuals in the 1980s to fewer than 2,500 today (T. Burke. Bureau of Reclamation, Pers. comm. 2002). The Lake Mohave Native Fishes Work Group, in a multi- agency conservation effort with the U. S. Fish and Wildlife Service, has implemented an augmentation program to maintain the Lake Mohave population since 1992. Rearing strategies include the use of federal, state, county and city captive rearing facilities, in addition to off-channel coves to effectively hold and spawn adults, hatch and rear wild caught larvae, juveniles, and sub-adults. Using these many strategies, the Biological Opinion goal (USFWS 1994) to stock 50,000 RBS in Lake Mohave was met in 2001. The current management approach for captive rearing is based on the collection of wild spawned larvae from Lake Mohave adults during the spring spawning season. The larvae are transported to Willow Beach NFH for rearing. Fingerlings are then distributed to Dexter NFHTC and Arizona's Bubbling Ponds SFH for grow out to 300 mm. Fish are then PIT tagged and repatriated to Lake Mohave.

At the onset of the restoration program for RBS, management biologists recommended the development and maintenance of unique genetic reserve populations endemic to targeted drainages. The strategy was to preserve the genetic uniqueness of the stocks, each of which may have evolved novel adaptations to local environments. Since the turn of the century and extirpation of most of the wild populations in the Upper Colorado River mainstem, San Juan mainstem, and Lake Powell, and the decline of the middle Green River population, the primary source for genetic material needed to recover the species throughout the Colorado River basin has become the Lake Mohave stock. Some geneticists believe that Lake Mohave RBS contain all the available genetic diversity from inundated populations above the dam. Dowling and Minckley (1993) reported a continuum of mtDNA haplotypes from the upper Colorado down to Lake Mohave with an increase in genetic diversity from 0 in the upper Colorado River to 0.98 in Lake Mohave.

Undesignated Tributary Systems

Although the Recovery Goals (U.S. Fish & Wildlife Service 2002) do not identify goals specific to tributaries of the Lower Basin recovery unit, stocking RBS will establish new populations and increase chances for recovery. These tributaries are the Gila, Salt/Verde, Little Colorado, and the Bill Williams river systems.

Natural populations of RBS were extirpated from the Gila River basin and lower Colorado River by 1960 (Hendrickson 1994). Minckley (1973) indicated the last known collection of RBS from the Verde River occurred in 1954 at Pecks Lake and from the lowermost reservoir of the Salt River (Saguaro Lake) in 1949. In 1980, the Service and the Arizona Department of Game and Fish signed an agreement to cooperatively reintroduce the species into the Gila River Basin.

The fry and fingerling stockings began in 1981 from fish produced at Dexter NFHTC using broodstock collected from Lake Mohave (Brooks 1986). Over the next ten years, more than 11 million hatchery produced fish were released into the Salt and Verde Rivers (Hendrickson 1994). The stockings were considered unsuccessful as large populations of RBS were not established in either system. Field surveys conducted by the Arizona Department of Game and Fish have found no evidence of natural reproduction. The lack of recruitment and minimal survival is attributed to habitat fragmentation, predation by exotic piscivores, downstream displacement due to high velocity flows, lack of isolated backwaters, and heavy parasite infestations (Hendrickson 1994). An assessment of stocking success conducted by the Arizona Department of Game and Fish for the ten year augmentation effort indicated that very few RBS survived in either system for more than one year.

Due to these threats, the augmentation program was adjusted to incorporate the stocking of larger fish. Biologists believed larger fish would be less susceptible to predation and better able to acclimate to the riverine habitat. Since 1993, stocking records indicate that approximately 20,000 RBS averaging 300 mm in length have been stocked into the Verde River near Childs and Beasley Flats, Arizona. The fish stocked were of Lake Mohave origin and reared at the Bubbling Ponds SFH and Dexter NFHTC. The majority of the fish stocked were marked with coded wire tags for future monitoring activities. The Arizona Department of Game and Fish collected nine razorback suckers averaging 400 mm in length in 2002 during the annual field surveys indicating that a small number of stocked fish are surviving. We are unable to provide population estimates due to insufficient data. Current management plans address stocking 2000 fish 300 mm in length annually (Lake Mohave stock) in the Verde River.

Sampling efforts to date have found no RBS in the Salt River. Due to limited sampling contact with any RBS in the Salt River and tribal concerns, all stockings were discontinued in

1996 following a release of 2,000- 285 mm fish at Horseshoe Bend on the upper Salt River. The fish released were of upper basin origin from Ouray NFH and reared at Bubbling Ponds SFH.

Over the past 50 years, fewer than twenty RBS have been collected from the Grand Canyon (Maddux et al. 1987, Hendrickson and Kubly 1990). The last known RBS to be captured from the mouth of the Little Colorado River was in 1989 (Hendrickson and Kubly 1990). There are no records indicating historical RBS stockings in the Grand Canyon and no current plans exist to augment the Little Colorado River RBS population.

**Dexter NFHTC Lake Mohave Broodfish**

Captive broodstocks representing the Lake Mohave population exist at Dexter NFHTC. According to station records, the initial broodstock was founded with progeny from 136 wild adult fish collected from Lake Mohave in 1981. An additional 147 wild individuals were collected from the lake in 1982, spawned that year, and contributed fry to the stocking efforts in the Gila, Salt and Verde rivers and Lake Mohave, but were not incorporated into the captive broodstock. In 1984, Dexter's RBS captive broodstock consisted of 360 three-year old fish derived from the wild adults spawned at Dexter NFHTC in 1981. Station records do not show any additional augmentation of the captive stock with wild caught or captively produced individuals, and the captive broodfish inventory does not reflect an increase in fish numbers. Wild caught adults collected in 1981 and 1982 had expired by the end of 1985.

The first captive broodstock of RBS at Dexter NFHTC will be referred to as the '81 broodstock. The '81 broodstock currently contains 133 adult fish (Table 2). Initial spawning of this broodstock occurred in 1984 (Hamman 1985). It should be noted that no progeny of the '81 broodstock are currently held as broodstock at any facility. Since the broodstock's inception, all offspring have been stocked to meet production commitments. Over the past 19 years, offspring from this stock have been stocked into Lake Mohave and Lake Havasu; Gila, Salt, Verde and San

Juan rivers; Niland-Imperial Valley Hatchery, California; Page Springs SFH, Arizona; Buenos Aires, Cibola, Imperial, Havasu National Wildlife Refuges; and the Colorado River Fisheries Project (CRFP) at Vernal, Utah.

The second broodstock will be referred to as the Paired Matings (PM) broodstock. This stock, comprised of approximately 90 unique family groups is the product of paired matings of wild caught adults spawned at Willow Beach NFH from 1994 to 2003. Those efforts resulted in 1,200 fish currently held as PM future broodstock at Dexter NFHTC (Table 2).

A third broodstock has been developed at Dexter NFHTC, and consists of six year classes of juvenile wild-caught fish from Lake Mohave. These fish were captured as fry from eight locations throughout Lake Mohave and given the designation of Wild Caught (WC) future broodstock
(Table 2).

The breeding program at Willow Beach NFH incorporates the rearing of wild caught larvae from Lake Mohave, and paired mating of adults collected during the annual RBS roundup. Offspring from paired matings are shipped to Dexter NFHTC for growout and incorporation into the PM future broodstock. Wild caught larvae collected by the Lake Mohave Native Fishes Cooperative Work Group are reared at Willow Beach NFH, Dexter NFHTC, and Bubbling Ponds SFH to 300 mm, PIT tagged, and released back into Lake Mohave. Larval fish collections are scheduled to continue for several years while repatriated fish survival and adult population estimates are calculated. On an annual basis, Willow Beach NFH maintains five year classes of wild caught fish (Table 2). These fish also serve as a potential broodstock source of Lake Mohave origin.

## Table 2. Lower Basin Razorback Sucker Captive Broodstock

| Year Class | Origin | Numbers on hand | Founders Represented | Lot Designation |
| --- | --- | --- | --- | --- |
| **Dexter National Fish Hatchery & Technology Center** | | | | |
| 1981 | $F_1$ Mohave | 133 | adults / Mohave | '81 |
| 1994-2003 | Mohave | 1200 | 90 / Mohave | PM |
| 1999-2004 | Mohave | 1000 | fry /Mohave | WC |
| 2003 | $F_2$ Mohave | 1000 | 25/ '81 captive stock | $F_2$ |
| **Willow Beach National Fish Hatchery** | | | | |
| 1999 | Mohave | 20,000 | | WC |
| 2000 | Mohave | 48,000 | | WC |
| 2001 | Mohave | 25,000 | | WC |
| 2002 | Mohave | 16,000 | | WC |
| 2003 | Mohave | 26,000 | | WC |
| **Bubbling Ponds State Fish Hatchery** | | | | |
| 1999 | WB | 2,770 | | $9WBF_1P$ |
| 2000 | WB | 1,000 | | $0WBF_1P$ |
| 2001 | WB | 6,650 | | $1WBF_1P$ |
| 2001 | Mohave | 15,000 | | $1WBF_2P$ |
| 2000 | WC | 1,750 | | $0WBF_2P$ |

*'81-1981 year class, Mohave-Lake Mohave, AZ, PM-Pair Matings, WC-Wild Caught, WB-Willow Beach, P- Production.*

**Relationship to Recovery Plan and Goals**

Fish are more readily reared in-situ than many other endangered species (Minckley 1995). The propagation effort for RBS must carry the responsibility of judiciously managing the entire species, from an individual's genetic potential to the gene pool of the species (Philippart 1995). Minckley (1995) states that the goal of conservation propagation is, "the re-establishment of a population size, dispersion, and structure that will define itself by allowing an organism to proceed along independent evolutionary pathways comparable with those it followed prior to disruption by human interference". This goal would be impossible to achieve without long-term research of the genetics of wild populations and hatchery stocks. While it is beyond the scope of Dexter NFHTC to modify habitat, or alter the Colorado River environment to support naturally recruiting populations of RBS, one of its objectives is to produce genetically diverse fish that contain the maximum potential for successfully integrating into the current Colorado River environment.

**Immediate Management Actions Needed (RBS Recovery Goals 2002)**

1. Reestablish populations with hatchery-produced fish.
2 Identify and maintain genetic variability of RBS in Lake Mohave.

These actions are specific to objectives contained within the plan. It is incumbent on Dexter NFHTC and cooperators to identify the current genetic status of existing captive and wild stocks of RBS, and develop an appropriate strategy to ensure the remaining genetic material in RBS persists into perpetuity. This will fulfill the management need to identify and maintain the genetic variability of RBS in Lake Mohave. The goal in management of the captive RBS stocks is to provide a hatchery product that is genetically viable, and appropriate for introduction at targeted stocking sites.

To achieve recovery of the species, self-sustaining populations should be established in the upper and lower Colorado River basins by captive propagation and augmentation. Recovery goals will be considered achieved when the decline of the three extant stocks of RBS in Lake Mohave (AZ, NV), the lower Yampa River, (CO), and the middle Green River (UT), has been reversed as indicated by increasing population sizes through natural recruitment (U.S. Fish & Wildlife Service 2002).

Two of the five Recovery Priorities listed in the RBS Recovery Plan (U. S. Fish & Wildlife Service 1998) contain captive propagation elements. These essential components provide guidance in achieving the goals for recovery of the species.

*Priority 1). Prevent extinction of major extant RBS populations and permanent loss of genetic diversity of existing populations. Specific tasks to be addressed through captive propagation include:*

1.1 Protect fish in refugia and maintain genetic diversity.

1.4 Augment wild populations.

1.5 Monitor populations and habitat status.

1.1 Protect fish in refugia and maintain genetic diversity. This element calls for the maintenance of adequate refugia, collection of RBS for refugia, and management of the genetic composition of the fish held in refugia. Razorback sucker captive refugia currently exist at the Dexter NFHTC, New Mexico; Willow Beach NFH, Arizona; Ouray NFH, Utah; Grand Valley Endangered Fish Facility, Colorado; Mumma Native Aquatic Species Restoration Facility, Colorado; and Wahweap SFH, Utah.

1.4 Augment wild populations. This element calls for the collection and rearing of wild larvae and reintroduction of juvenile or adult fish large enough to avoid predation. Strategies to address this element have been incorporated into production programs and augmentation plans. As discussed previously, Dexter NFHTC has been an active participant in this endeavor since 1994.

1.5 Monitor populations and habitat status. This element calls for the monitoring of populations and habitat status to determine success and refinement of the captive propagation and recovery program. Although this element is beyond the scope of the Dexter NFHTC, the Center will continue to work closely with both Service and non-Service cooperators charged with this task.

*Priority 2) Establish and protect additional wild populations.*

2.6 Augment or reintroduce RBS in recovery areas.

2.6 Augment or reintroduce RBS in recovery areas. This element calls for the refinement of propagation techniques to improve culture methods, address life history requirements, maintain genetic diversity of captive stocks and increase fish survival. Captive management objectives to facilitate meeting the above priorities are listed below.

**Broodstock Management Objectives**

1) Develop a RBS broodstock program that conserves the genetic diversity of Lake Mohave RBS, and a captive propagation program to produce offspring reflective of that diversity for recovery efforts.

2) Establish a genetic broodstock management plan that actively monitors and maintains genetic variability and long-term fidelity of each broodstock.

3) Establish a marking system for all broodstocks to allow an unambiguous identification of each broodfish.

4) Establish an active genetic monitoring plan that identifies and minimizes sources of domestication in production fish for the hatchery environment.

5) Establish culture procedures that minimize risks associated with intensive fish production.

6) Establish a breeding program, using a genetic database, to maintain and ensure that the effective population size of broodstock is adequate to offset the results of genetic drift in small populations.

7) Establish a genetics monitoring program to assess the long-term genetic integrity of production fish.

8) Address biological opinion requirements for captive propagation and augmentation.

9) Refine spawning, maintenance, and marking techniques of RBS to ensure genetic longevity of the stocks.

10) Identify locations, augmentation needs, and current broodstock production capabilities.

## Genetic Considerations in RBS Broodstock Management and Captive Propagation Program

### Maintenance of current levels of genetic variation

The overall goal of genetics management of native fishes is to protect natural variation and patterns of genetic diversity, and to prevent extinction as a result of deleterious changes to the genome by anthropogenic actions. This plan calls for specific genetics management objectives to maintain existing levels of genetic diversity of RBS throughout the process of broodstock maintenance and captive propagation.

Hatcheries, through captive breeding, have prevented the extinction of many species of imperiled fish. Captive breeding and rearing have enhanced wild populations and prevented population extirpation (Maitland 1995). Hatchery programs are particularly important when habitat alterations, such as those in the Colorado River, prevent wild populations from reproducing and recruiting naturally (Maitland 1995). The goal in maintaining a refugium broodstock and captive propagation program for RBS is to maintain, to the extent possible, the genetic characteristics of wild fish in the captive populations. This requires a captive breeding program that monitors and identifies all critical life history stages at which changes in genetic composition as a result of culture practices may occur (Doyle et al. 1995).

This plan lists the genetic risk factors associated with maintenance of captive broodstocks and captive propagation, and outlines strategies to identify, monitor and minimize the risk. Breeding strategies and fish rearing practices are designed and conducted to reduce the effects of drift, selection, inbreeding, outbreeding, and mutational load (Doyle 2001; Doyle et al. 1995).

**Maintenance of Genetic Refugia as a Captive Broodstock**

Over 130 wild adult fish were captured in the early 1980s, and each fish was PIT tagged and transported to Willow Beach NFH and Dexter NFHTC (Hamman 1985). Progeny from those fish represent the ‘81 broodstock, and the genetic reserves of those wild individuals. Current recovery efforts preclude the process of broodstock mining of adult fish from remaining Lake Mohave RBS populations. However, the ongoing collection of naturally produced larvae from throughout Lake Mohave provides a means of increasing the survival of naturally recruited fish, and a protocol to ensure the genetic diversity of the RBS in Lake Mohave is adequately sampled. For example , adult RBS congregate on spawning bars throughout the lake; larval fish taken from those areas, if sampled throughout the spawning period, should reflect the genome of the current Lake Mohave RBS reproducing population.

Fry taken from spawning sites that are destined for future broodstock are grown in aquaria until large enough to PIT tag. Current studies at Dexter NFHTC are examining the use of VIE (visible implant elastomer) tags on smaller life stages of RBS which will allow early mixing of family lots, while still enabling identification at a later date. If the implants are retained, this technique would allow more efficient use of rearing space and mixing of family lots would allow for the use of culture methodologies that more closely resemble a natural habitat.

"Nature's" rearing is a culture methodology developed in the Pacific Northwest on salmon hatcheries. This practice advocates that by mimicking the natural environment hatchery fish have 1) reduced hatchery selection for potential fitness related parameters, such as light tank backgrounds when the stocking environment is dark, and 2) enhanced survival of hatchery reared fish by imprinting hatchery fish to an appropriate environment. Dexter NFHTC's large earthen ponds serve as 'Nature's rearing habitat for RBS. Before RBS are stocked into ponds, vegetation is allowed to flourish, and the ponds are fertilized to enrich the plankton food base. Aquatic insects inhabit the ponds and serve as both food and predators. Bird predation is also present, as are other predators such as raccoons and turtles. This practice should provide a method of pre-acclimation for cove or backwater environment where the fish are to be stocked. The sooner fry can be moved into these rearing environments, the greater the potential for imprinting young fish to a naturalized environment. VIE tagging would allow the earliest transition possible to the 'Nature's environment.

Non-lethally sampled tissues will be taken from each potential broodfish at Dexter, and those brought into the facility in the foreseeable future. Tissues will be used for PCR-based DNA technologies in genetic identification, characterization and monitoring (Amos and Hoelzel 1992; Ferguson et al. 1995; O'Reilly and Wright 1995). Genetic data will facilitate the management of broodstock and provide guidance for propagation activities.

The goals for maintaining a refugia and broodfish population of RBS at Dexter NFHTC are based on conservation genetic principles as outlined by current conservation genetics theory (Blouin et al. 1996; Lynch 1996; Ford 2002; Lande 1995; Doyle 2001). Although the statistical sampling of random molecular markers does not conclusively have a direct correlation with fitness of populations or individuals (Waples 1991a), high levels of molecular variation provide “strong evidence” that the chance for adaptive potential exists (Lynch 1996; Lynch et al. 1998, but see Pearman 2001 and Milligan at al. 1994). Lynch suggests the goal of maintaining 90% of genetic variation is a short-term goal and that long-term maintenance of evolutionary potential should focus on a population size that allows the average genetic variance to become independent of population size. A genetically effective population size of 1000 (Ne), which corresponds to a total population size of between 5,000 and 10,000 breeding adults is a realistic goal to attempt to defray the effects of mutational load, drift, and selection (Lande 1995; Lynch 1996). However, Lynch (2001) theoretically outlines substantial problems with mutational load and domestication selection even in large captive populations. Other empirical research suggests that additive genetic variance, which is directly correlated with many fitness components, has been shown to increase when populations expand after a bottleneck event (Goodnight 1988). Given those caveats, while any one facility does not have room or funding to maintain 10,000 adult RBS, collectively the five facilities (Ouray NFH, Dexter NFHTC, Willow Beach NFH, Grand Valley Endangered Fish Facility and Wahweap SFH) involved in RBS recovery could approach that optimum total population size.

Our goal is to maintain a minimum pool of 3,000 adults at Dexter NFHTC that will be used in a rotational pedigree breeding program to ensure production fish will represent the genome of the RBS, and most importantly, not adversely affect the Ne or fitness of wild RBS populations (Ford 2002; Lande 1995; Lynch et al. 1998; Ryman and Laikre 1990; Laikre and Ryman 1996; Doyle 2001). To that end, collections of wild caught larvae and paired matings of the ’81 broodstock will continue until sufficient individuals are held to meet the target of 3,000 adult broodstock. Dexter NFHTC broodstock development has encompassed more than two decades, and will continue as outlined above. This process will achieve adequate representation of wild RBS genetic material. Once genetic baselines are established, Dexter NFHTC will have a better estimate of genetic diversity of the refugium broodstock which will guide future broodstock development.

**Methods used to monitor gene pool maintenance**

The molecular ecology program at Dexter NFHTC will initiate a long-term program of genetic assessment of RBS broodfish and production fish in order to achieve our objectives (Allendorf and Phelps 1981; O'Reilly and Wright 1995). The genetic character of the wild and captive populations will be monitored based on the results of a genetic survey of broodstocks currently held at captive propagation facilities. A suite of microsatellite markers will be used to produce a genotype of each individual broodfish. This information will be used to assess and monitor the refugium stocks, and identify any changes in genetic character (Allendorf and Phelps 1981).

To accomplish the objectives outlined here, Dexter NFHTC will use microsatellite markers, as the most sensitive method for detecting genetic variation discovered to date. Genetic markers, when used to define the status and character of a population, should be reliable, reproducible, preferably codominant and highly variable. Vertebrate microsatellite loci average 8.7 alleles per locus (Blouin et al. 1996; Estoup et al. 1998) and are selectively neutral. Microsatellites are codominant markers, so population structure, levels of heterozygosity, and relatedness are easily measured (Gertsch et al. 1995; Paxton et al. 1996). Microsatellites are short motifs of 2-6 bases that are repeated within a given segment of DNA. The number of repeats appear to be under evolutionary constraint and rarely more than 30 (Goldstein and Pollock 1997).

Much controversy has been generated in recent years concerning the use of neutral genetic markers, such as microsatellites, as a statistical surrogate for overall genetic variability, and the assumption that heterozygosity at neutral loci may imply overall fitness (Waples 1991b; Hansson and Westerberg 2002; Allendorf and Leary 1988). This theory has caused concern in the fisheries community because of the apparent loss of genetic variation in some hatchery stocks and the implications of this loss to the augmented population (Waples 1991b). Allendorf and Phelps (1980) suggest this loss may result in a decrease in fitness, such as a loss of alleles that are important in disease resistance. Genetically, the effects of population supplementation with hatchery stocks range from no effect (no survival of stocked fish) to a swamping of the wild genome with hatchery stocks as indicated by marker data (Skaala et al. 1990; Hindar et al.1991). Many authors have modeled potential losses of genetic diversity associated with wild stock augmentation (Laikre and Ryman 1990; Waples 1991a; Ryman and Laikre 1996); however, the direct relationship between genetic marker diversity and fitness components are rarely addressed and still controversial (Milligan et al. 1994; Waples 1991). Seldom have hatchery stocks been subjected to extensive genetic monitoring as a management, rather than a research tool. We propose to genetically characterize and monitor broodstocks and production fish to prevent the loss of or a change in genetic diversity.

## Genetic Risks for Broodstock Management and Captive Propagation

### Founder effects

"The fundamental genetic hazard associated with broodstock management within a gene pool maintenance program is loss or undesired changes in the genetic variation or identity of the hatchery population with respect to its donor source" (Williamson 2001). One of the earliest recognized genetic risks of captive rearing on native fish was the potential of a founder effect in the hatchery population. This effect occurs when an inadequate sample of the wild genome is used to develop the captive stock. Typically, this is the result of using too few individuals. Thus, the genetics of future generations do not adequately reflect the donor population. This risk can be minimized by genetic analysis of wild and hatchery stocks to ensure that hatchery stocks contain a similar genetic character, based on allele frequencies at neutral markers, to reflect an adequate statistical sample of the genome (Allendorf and Phelps 1981; Pearman 2001; and Milligan et al. 1994).

**Loss of adaptive variation by random genetic drift**

Loss of within population diversity due to the effects of genetic drift is a common event associated with small populations (Lande 1995). Random genetic drift typically occurs in nature in marginal habitat, where populations are small, and conditions suboptimal. This change in genome is again associated with sampling error. In this instance, a few individuals will contribute to the next generation by chance and not as a result of Darwinian selection. The genetic changes are similar to a founding effect, although it is more appropriate to say that genetic drift is typically subsequent to a founding event. Drift is of particular concern when populations are small, such as some hatchery broodstocks, and too few individuals are used to contribute to the next generation. It is incumbent on managers of captive stocks to ensure that population size is large enough, and progeny produced contain a large enough sample of the available genome to overcome the effects of random genetic drift.

**Inbreeding and inbreeding depression**

Inbreeding is correlated with population size. Mating of related individuals can lead to altered genetic structure in small populations. Larger populations contain more individuals, and the chance of mating by individuals that are not related is statistically greater (Blouin et al. 1996). Inbreeding does not lead to a reduction in allelic diversity, but to the partitioning of alleles into homozygotes at the expense of heterozygotes (Lynch et al. 1999). Matings between relatives is not necessarily bad; inbreeding is a problem only when it results in inbreeding depression.

Inbreeding depression is a reduction in fitness associated with the partitioning of *deleterious* alleles in the homozygous condition (Lande 1995). However, the result of ongoing inbreeding depression is a purging of deleterious alleles, and over time, an increase in population fitness (Lynch et al. 1999). Genetic data generated in RBS broodstock development will allow managers to preclude the mating of related individuals (Blouin et al. 1996; Norris et al. 2000), and prevent both inbreeding and inbreeding depression.

**Outbreeding and outbreeding depression**

Outbreeding is the sexual combination of divergent genomes. Extreme outbreeding results in developmental instability, and is often associated with sterility, particularly in the heterogametic sex. Mules are an example of outbreeding. Outbreeding depression occurs when the offspring have a reduced fitness because of the combination of diverse genomes.

Outbreeding depression can occur in the first generation by affecting the adaptation to fine scale environmental conditions. Loss of local adaptation will result in a decrease in the overall fitness of the population. Outbreeding depression as a result of hybrid breakdown occurs in the second generation, when recombination produces a montage of maladaptive progeny (Burton 1990). Loss of population diversity or loss of population identity occurs in a captive propagation program when different populations are mixed and interbreeding occurs. Outbreeding is fundamentally as significant a problem as inbreeding (Burton 1990). The RBS population in Lake Mohave is predicted to be a sump for inundated populations throughout the basin. If this is true, then outbreeding is an ongoing process occurring in every spawning aggregation and not a major concern in controlled propagation programs.

**Domestication/artificial selection in captive populations**

Genetic variation of captive populations can be altered on a hatchery when the populations adapt to the hatchery environment, a process called inadvertent or domestication selection (Doyle et al. 1995). Domestication is thought to result in genetic changes that affect the fitness of a population (Ford 2002). Domestication can occur because of culture practices that favor certain genetic backgrounds or occur because of the relaxation of selection, thereby allowing maladaptive genotypes to persist (Hard 1995; Lynch and O'Hely 2001). Domestication selection is viewed as a problem when hatchery fish are less adapted to the natural environment than wild fish. The negative impact occurs in wild populations when the overall population fitness of wild stocks genomes are swamped with genetic backgrounds adapted to the hatchery environment (Ford 2002; Lynch and O'Hely 2001). "Natures" rearing practices initiated at Dexter NFHTC in conjunction with an intensive genetic monitoring program will provide the information and culture methodology necessary to mitigate domestication selection.

**Strategies to minimize risks**

Dexter NFHTC basic strategy to propagate and manage captive RBS broodstock populations will be based on the best scientific information available: the use of molecular genetic data (Ferguson et al. 1995; O'Reilly and Wright 1995). Dexter's plan acknowledges that standard hatchery practices are sources of genetic hazards as outlined above (Lynch and O'Hely 2001; Ryman and Laikre 1990). Captive propagation and broodstock management plans must address genetic hazards, and provide management strategies to reduce the risk of undesirable or hazardous genetic effects on broodstock and production fish. These genetic hazards are defined as lost genetic variation or altered genetic composition as measured by allelic frequencies, and reduction in fitness of hatchery stocks and offspring as a result of the altered genetic composition (Allendorf and Phelps 1981).

Proper application of the plan's guidelines will assist in identifying necessary captive propagation activities, assessing their potential for genetic impacts, and developing and using proximate genetic management safeguards that reduce genetic risks associated with routine breeding and rearing activities (Williamson and Wydoski 1994).

The risk of genetic hazards or losses resulting from these processes will be reduced by the application of an ongoing comprehensive genetic management and monitoring program. This plan outlines the strategy to produce baseline information on captive and wild stocks, using a set of management objectives, actions and tasks that will allow informed management decisions to be made based on the results of ongoing genetic analysis.

The basic management activities of broodstock development and captive propagation are (Williamson and Wydoski 1994):

1) selection of the proper donor source;
2) mining wild fish or gametes for broodstock, reproducing existing stocks;
3) rearing broodfish to maturity;
4) choosing and applying the proper breeding or mating system;
5) rearing young for various management activities; and
6) augmentation stocking or reintroduction stocking.

The U. S. Fish and Wildlife Service considers the Lake Mohave RBS population to be the highest priority genetics conservation unit in the lower basin, in terms of its ultimate value to species identity and integrity, its recoverability, and its long-term viability and sustainability. This rationale has provided the impetus for Dexter NFHTC to select the Lake Mohave population as the proper donor source upon which to found a captive production broodstock. Over the past two decades, Dexter NFHTC has developed a diverse, multi-year class sample that will contain an adequate representation of the genetic diversity of the Lake Mohave population of RBS.

Biologists will continue to bring in wild caught larvae from spawning aggregations until 2004. The target of 200 individuals per year will be pooled from all spawning aggregations over the duration of the spawning period. These individuals will be reared until large enough to PIT tag and placed in ponds that contain an aggregation of young WC RBS broodstock.

Management activities in the rearing environment include incubation and rearing to maturity offspring of hatchery produced fish and future broodstock. Dexter NFHTC will minimize the effects of selection on offspring by maintaining low densities and family lot integrity until individuals can be marked by family and put into 'Nature's grow out ponds for stocking as fingerlings. Equal numbers of individuals from each family lot will be maintained. Historically, up to 100,000 fry/acre were stocked in Dexter NFHTC ponds for growout, and survival was excellent. In an attempt to maximize growth and minimize the effects of density as a selective factor and to initiate 'Nature's rearing strategy, Dexter NFHTC will stock RBS fry at 50,000/acre, and after the first year reduce that number to 25,000/acre. Fish are placed in rearing ponds where hatchery conditions mimic a natural backwater area or nursery habitat (Lynch and O'Hely 2001). Dexter NFHTC pond environments include predation, natural pond populations of aquatic insects as food and predators, diurnal fluctuations of temperature and oxygen, and aquatic vegetation. These captive stock selection factors mimic many natural impoundments, and are arguably cognate with many historical larval RBS rearing habitats (Lynch and O'Hely 2001).

This plan outlines a breeding strategy for RBS that maximizes effective population size of the captive parental population and minimizes the genetic risks associated with hatchery practices. Our breeding strategy will use data from a genetic screening to assess the relatedness of all broodfish, produce a matrix that categorizes the relatedness of female and male broodfish (Quellar and Goodnight 1989), and prohibit the use of broodfish fish that have a high genetic similarity (Blouin et al. 1996; Quellar and Goodnight 1989; Norris et al. 2000). The matrix will be used to provide the statistical basis for selecting or discarding potential broodfish. This will minimize inbreeding within the hatchery population of RBS (Blouin et al. 1996; Norris et al. 2000). The cutoff for the discrimination of potential pairs will be defined after the initial genetic survey is completed, but is expected to be 0.2.

Outbreeding depression, or the loss of coadapted gene complexes, is also of concern within the conservation community. The RBS population in Lake Mohave is predicted to be a sump for inundated populations throughout the basin. If this is true, then outbreeding is an ongoing process occurring in every spawning aggregation. If only distantly similar fish are considered as potential mates, the possibility of outbreeding depression will be exacerbated.

Dexter will produce fish for reintroduction that reflect as accurately as possible the genetic identity of the donor populations. The aforementioned activities outline a variety of proximate genetic management safeguards that will promote management success while reducing the risk of genetic hazards inherent in all captive breeding programs (Williamson and Wydoski 1994). We anticipate a dynamic process of identification of selective factors, and adjustments to hatchery protocols to defray the genetic consequence on refugia and production fish.

**Genetics research needs**

Inbreeding depression and outbreeding depression are difficult to quantify. However, the importance of both genetic hazards necessitates an effort to quantify the results of our breeding strategy. Staff will initiate a research project to quantify the effects of inbreeding and outbreeding after the initial genetic survey is completed. Staff will produce paired matings of the most and least genetically similar fish with replicates of twenty pairs in each group. Offspring will be reared in identical environments as production fish. Survival and growth will be assessed at 2 months, 6 months, 1 year, and 2 years of age. The hypothesis will assume that related and divergent matings are not statistically different in growth and survival than production fish maintained in the same manner. If the hypothesis is accurate, inbreeding and outbreeding of RBS in the hatchery environment at Dexter NFHTC will not be an issue. Disposition of research fish will follow recommendations noted in the "Guidelines for Use of Fishes in Field Research" (ASIH/AFS/AIFRB, 1988).

**Genetic Broodstock Management Objectives**

**(many tasks and management actions may be identical to address different objectives)**

1) Develop a RBS broodstock program that conserves the genetic diversity of Lake Mohave RBS, and produces offspring reflective of that diversity for recovery efforts.

Management Action: Establish a captive broodstock of at least 3,000 adults to represent the Lake Mohave population, and characterize the genetic diversity present.

Management Action: Maintain genetic diversity extant in Lake Mohave RBS stocks.

Management Action: Ensure production fish contain acceptable genetic variation such that the overall diversity of the augmented population is not reduced.

**A. Task**: Broodstocks from all facilities currently holding RBS will be inventoried and compared using microsatellite loci, and broodstocks will be compared to archived tissues and past genetic analysis of RBS stocks if available. This will include Dexter NFHTC, Ouray NFH, Wahweap SFH, and Grand Valley Fish Culture Facilities broodstocks. **Target date: July 2003-July 2005**

**B. Task**: Replicate technique using microsatellite primers used by Dr. Tom Dowling in RBS evaluation, and develop a suite of additional microsatellite primers. **Target date: July 2004**

**C. Task**: Replicate technique used in mtDNA RFLP analysis used by Dr. Tom Dowling in screening RBS. **Target date: October 2004**

**D. Task**: Produce baseline genotype of all RBS broodstock at Dexter and other facilities. **Target date: July 2005**

**E. Task**: Provide statistical analysis of results to determine the extent of differentiation of hatchery stocks. **Target date: December 2005**

*Alternative*: If broodstocks appear to be distinct, such as Lake Mohave and the Green River stocks, separate stock identities will be designated in collaboration with the holding facilities, and mating strategies developed to ensure genetic integrity is maintained.

*Alternative*: If stocks are not significantly different, movement of gametes and fry between hatcheries will be part of ongoing broodstock management strategy to maximize diversity of captive populations.

2) Establish a genetic broodstock plan that actively monitors and maintains genetic variability and long-term fidelity of each broodstock at Dexter NFHTC.

Management Action: prevent loss of genetic diversity as measured by microsatellite loci, and mtDNA.

**A. Task**: active pairing of genotyped fish based on interactive database to maintain heterozygosity and all allelic variants present in broodstock.

**Target date: April 2006**

**B. Task**: monitor production fish and stocked fish annually to ensure no loss of allelic diversity, or no significant change in allelic frequency.

**Target date: July 2004-July 2008**

3) Establish a marking system for all broodstocks to allow identification of each broodfish.

Management Action: All broodfish will be pit-tagged. A pedigree database will be established for all broodfish, with a microsatellite genotype of each fish. This database will represent the baseline for broodfish from all facilities.

**A. Task**: Genetic analysis will be performed on all broodfish, beginning with the oldest stocks. **Target date: July 2005**

**B. Task**: Paired matings will provide a set of allele frequency expectations of the progeny of each pair based on recorded genotypes. As fish are pooled into populations (ponds for growout), each pond will have a known expected allelic frequency. **Target date July 2004-July 2008**

**C. Task**: Fish in grow out ponds will be sampled annually and genetically analyzed to determine if lineage sorting or allelic loss is occurring. If allelic loss is occurring, management plans may be adjusted accordingly.

**Target date: July 2004-July 2008**

## Captive Propagation Objectives

4) Establish an active management/monitoring plan that will identify and minimize the sources of domestication selection in production fish for the hatchery environment.

Management Action: Monitoring of all hatchery broodstocks and progeny to determine changes in allele frequency as indicators of loss of 'wild' characteristics.

Task: Hatchery broodstocks will be inventoried annually to determine mortality, and statistical analysis performed to determine if lineage sorting is occurring.

**Target date: July 2004-July 2008**

**A. Task**: A sample of 1% of hatchery production fish will be sampled annually, and allelic frequencies will be compared to expected frequencies to determine if, and at what stage selection (as assessed by allelic loss) is occurring.

**Target date: July 2004-July 2008**

**B. Task**: Annual report will be prepared to assess status of broodstocks and progeny, and strategies identified to minimize culture impacts.

**Target date: December 2005-December 2008**

5) Establish culture procedures to minimize the risks associated with intensive hatchery production:

Management Action: A minimum of 5,000 fish from any one parent will be produced for stocking in one location, whether the parent is used in a one on one paired mating, or two by two mating.

Management Action: When more than 10,000 fish are needed for a production commitment, a two-by-two mating scheme will be employed, with a minimum of ten pairs.

**A. Task**: spawn from two females will be split, and two males will be used to fertilize the eggs, each male fertilizing ½ of each female's spawn. This will result in four ½ sibling families, as opposed to two full sibling families.

**Target date: February 2004**

**B. Task**: when parents are used in a one on one paired mating production strategy, or two by two strategy, contributions of each cross will be equalized. Multiple stocking commitments can be met from the same matings, but no more than 5,000 fish will be stocked in any one area from one fish. Excess eggs from each paired mating will be maintained for research purposes, sent to other facilities or disposed of. **Target date: February 2004-July 2008**

**C. Task**: Broodfish will be rotated so that no fish will be spawned twice in five years. **Target date: February 2004-July 2008**

**D. Task**: Expected allelic frequencies will be developed for each paired mating, and for each pool of production fish. **Target date: July 2005-July 2008**

6) Establish breeding program, using the database to maintain and ensure that the effective population size of broodstock is adequate to offset the results of small populations:

Management Action: Maintain at least 3,000 adult broodstock at Dexter NFHTC.

**A. Task**: Follow allelic frequencies of individual broodfish to prevent loss of alleles. **Target date: July 2005-July2008**

**B. Task**: As part of annual report, present population genetic parameters of broodstocks and production fish, and document changes over time.

**Target date: July 2005-July 2008**

**C. Task**: To ensure no loss of allelic diversity, as needed sperm, eggs, or individuals may be transferred between facilities or wild populations. (Based on alternatives on page 35) **Target date: July 2005-July 2008**

**D. Task**: Estimate relatedness of individual broodfish. Do not spawn fish with a relatedness estimate greater than 0.2. (if genetic survey indicates sufficient diversity to use that cutoff) **Target date: July 2005-July 2008**

7) Establish a monitoring program to assess the long-term genetic integrity of captive broodstocks and wild populations.

Management Action: Annual genetic sampling of production fish, 'wild' populations, and inventory of all broodfish.

**A. Task**: Request tissues from fish collected during wild population monitoring.

**Target date: July 2004**

**B. Task**: Access archived tissues from all available sources to determine historical and current baselines of populations. **Target date: July 2004**

8) Address biological opinion (R2/ES-SE 2-21-95-F-216, 1997) requirements for captive propagation and augmentation.

Management Action: Reasonable and Prudent Alternatives provisions require 50,000 RBS over a five-year period to augment populations below Parker Dam. pg 159.

**A. Task**: Continue to produce genetically appropriate progeny for growout at Dexter and other rearing facilities for stocking below Parker Dam.

Management Action: "put 50,000 razorback suckers into Lake Mohave by 2000" pg 135.

**B. Task**: Continue to produce genetically appropriate progeny for growout at Dexter and other rearing facilities for stocking in Lake Mohave.

Management Action: "proposed action will put 25,000 young fish in Lake Havasu and 50,000 into Lake Mohave." pg 154.

**C. Task**: Continue to produce genetically appropriate progeny for growout at Dexter and other rearing facilities for stocking in Lake Mohave and Lake Havasu.

Management Action: Objective for Lake Havasu Biological Opinion (1993) is to "release 30,000 razorback sucker by 2003" in up to ten native fish rearing coves. pg 3.

**D. Task**: Continue to produce genetically appropriate progeny for growout at Dexter and other rearing facilities for stocking in Lake Havasu.

9) Refine spawning, maintenance and marking techniques of the captive stocks to ensure genetic integrity of production fish is maintained.

Management Action: Continue to develop, refine and initiate spawning, and marking protocols, and proceed with proceeding research initiatives outlined in previous actions.

**A. Task**: Initiate research project to assess outbreeding and inbreeding depression.

**Target date: February 2005**

**B. Task**: Develop a comprehensive database to use in monitoring any changes associated with hatchery practices. Annual statistical analysis of ongoing monitoring activities will direct the research and management strategies needed to minimize changes associated with captive propagation.

**Target date: July 2005-July 2008**

**C. Task**: When spawning RBS to meet stocking commitments, a two female by two male spawning will be prepared in four separate bowls, and only 10,000 fry from each spawning bowl will be kept. Spawns will be placed in separate hatching trays until swimup. **Target date: February 2004**

10) Identify locations, augmentation needs, and current broodstock production capabilities.

Management Action: Strategy for stocking commitments when more than 100,000 larval fish are required.

**A. Task**: A minimum of 30 pairs of adult fish will be used when 100,000 fry are needed. Stocking will take place in March. **Target date: February 2004**

Management Action: Strategy for stocking commitments when more than 10,000 fingerlings fish are required.

**B. Task**: minimum of ten pairs will be used in a two-by-two matrix. When small numbers are needed, fewer eggs will be kept from each mating. Fish will be reared from March until October, and stocked in October.

**Target date: February 2004**

Management Action: Strategy for stocking commitments when more than 1,000 8”-12” fish are required.

**A. Task**: If stocking commitment is for 8-12 inch fish, grow out will be from March of the current year until October/November of the following year. While numbers of fish are fewer, no less than ten mated pairs in a two-by-two matrix will be used for stocking any one area at any one time.

**Target date: February 2004**

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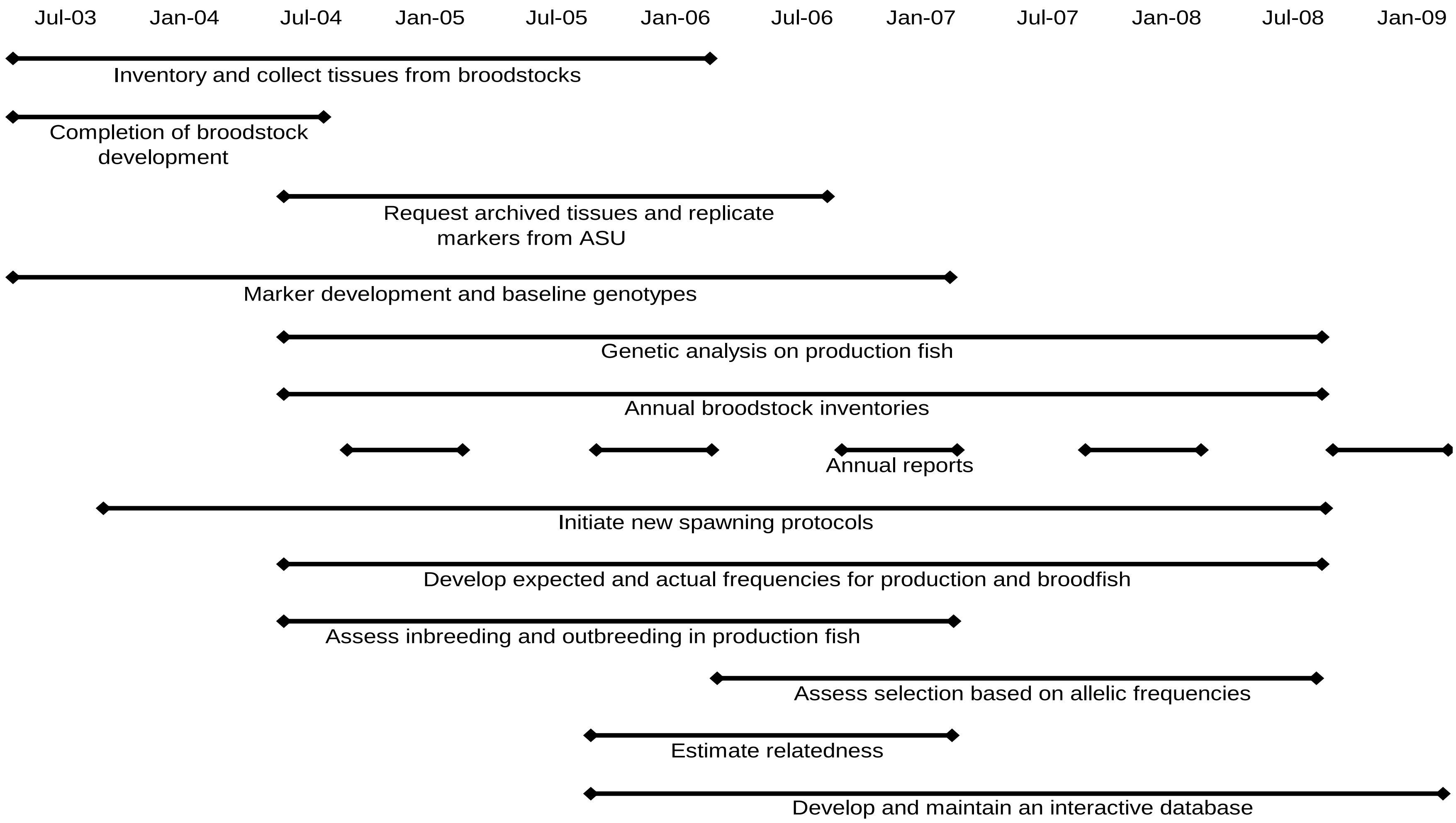
## **Appendix Table 1.**

Proposed augmentation stocking plans for upper and lower basins.*

| Location | Numbers to stock | Age/Size | Time Frame |
|---|---|---|---|
| Colorado R. Rifle-Debeque Canyon. | 3,310 | 2YO/300mm | 2003-2009 |
| Colorado R. Palisade-Stateline | 3,310 | 2YO/300mm | 2003-2009 |
| Gunnison R. Hartland-Redland dams | 3,310 | 2YO/300mm | 2003-2009 |
| Mdl. Green R. (RM 249-302) | 9,930 | 2YO/300mm | 2003-2009 |
| Low. Green R. (RM 120-249) | 9,930 | 2YO/300mm | 2003-2009 |
| San Juan R. | 11,400 | 2YO/300mm | 2004-2011 |
| Gila R. basin/ Verde R. | 2-10,000 | 2YO/300mm | indefinite |
| Lake Mohave | 10-15,000 | 2YO/300mm | target: 50,000 adults From wild larvae |
| Lake Havasu | as needed | 2YO/300mm | target: 30,000 adults |
| Below Parker Dam | 10,000 | 2YO/300mm | indefinite |
| Grand Canyon | n/a | n/a | n/a |
| Lake Mead | n/a | n/a | n/a |
| Low Colorado R. La Paz & Yuma Co. AZ, San Bernadino, Riverside, Imperial Co, CA) | n/a | n/a | n/a |

* information courtesy of Nesler et al. 2003, RIPSWG 2003 & Ryden 2003

**Appendix Table 2**
**Timeline for Completion of Major Tasks:**



## Appendix Table 3.

## Dexter National Fish Hatchery and Technology Center
## ' 81 Razorback Sucker Spawning Protocols: 2003

Roger Hamman

### March 3, 2003
▪screen, board and start filling pond 1A

**March 15, 2003**
▪start draining pond 6A

**March 16, 2003**
▪continue draining pond 6A

**March 17, 2003**
▪finish draining pond 6A and bring all broodstock to Fish Culture Building
▪sort males/females and place in separate tanks
▪record pit tag numbers, lengths, weights and take genetic samples of each fish
▪inject 25 females with 0.1cc HCG/lb in preparation for spawning
▪inject 25 males if necessary with 0.3 cc HCG/lb in preparation for spawning
▪take remaining broodstock not used in spawning trials to pond 1A

**March 18, 2003**
▪inject 25 females with 0.1cc HCG/lb

**March 19, 2003**
▪inject 25 females with 0.1cc HCG/lb
▪prepare incubation system to receive eggs
▪gather other equipment and supplies needed for spawning trials

**March 20, 2003**
▪spawn razorbacks using 1 female X 1 male spawning procedure
▪inventory each individual spawn
▪place eggs in incubators
▪take fish used in spawning trails to pond 1A
▪subset of each spawn for broodstock development to aquaria for hatching

**March 21, 2003**
▪check eggs in incubators
▪individual egg lots can be moved at this time
▪prepare a minimum of two 12' tanks to receive fry

**March 22, 2003**
▪check eggs in incubators

**March 23, 2003**
▪check eggs in incubators
▪commence filling 12' tanks with heated water

**March 24, 2003**
▪check incubators (morning and afternoon) and transfer fry to 12' tanks

**March 25, 2003**
▪check incubators (morning and afternoon) and transfer fry to 12' tanks

**March 26, 2003**
▪transfer remaining fry to 12' tanks
▪clean incubators

**March 27, 2003**
▪observe fry in 12' tanks

**March 28, 2003**
▪observe fry in 12' tanks

**March 29, 2003**
▪observe fry in 12' tanks

**March 30, 2003**
▪observe fry in 12' tanks
▪clean 12' tanks in preparation for shipment

**March 31, 2003**
▪fry can be shipped at this time to meet production commitments
▪all fry shipped no later than April 4, 2003

United States Department of the Interior

FISH AND WILDLIFE SERVICE

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April 14, 2004

**Appendix A.**

Finalization of the document **Razorback Sucker (*Xyrauchen texanus*) Genetics Management and Captive Propagation Plan, Dexter National Fish Hatchery and Technology Center,** and response to comments received on the draft final version of the Plan.

Hard copies of the draft final plan were mailed to the individuals or agencies listed below on March 31, 2003, with a 60-day peer review comment period. Five individuals provided verbal or written comments. The authors have summarized the comments received into three categories and responded accordingly. We would like to thank all the individuals that responded. Your assistance greatly improved the quality of the final document.

Comment responses:

The majority of the comments received on the document were editorial and format in nature. Those applicable were incorporated into the document. A map of the geography of razorback sucker distribution was added to the document and any missing references from the text and literature cited section were added.

Several reviewers expressed concern over omission of management strategies that address enhancing survival of production fish through conditioning. The authors agree that conditioning fish to flows, and acclimating fish to stocking sites prior to release are essential components of a stock management program. We, however, do not agree that those strategies should be included in this genetics management and captive propagation plan. The major focus of this document is to establish goals, objectives and strategies to effectively maintain razorback sucker captive stocks and their genetic integrity at Dexter. It is our recommendation that fish conditioning strategies be addressed in augmentation plans for the species.

The Lake Mohave razorback population is estimated to be fewer than 2,500 individuals with recruitment of approximately 5% of all stocked fish into the reservoir. In this plan, Dexter has proposed to establish a captive broodstock of 3,000 adult fish that will be used in a rotational pedigree breeding program to ensure production fish represent the Lake Mohave razorback genome. A large captive stock also helps defray the effects of mutational load, drift and selection over time. Normal attrition of captive reared razorbacks at Dexter is estimated to be 30% over a 23-year period (approximately 1.3% loss annually), based on survival rates of the 1981 year class. With this in mind a broodstock comprised of 3000 individuals and eight year classes could potentially contribute to the recovery program for over 75 years.

The broodstock developed will also serve as a captive refugia population and be maintained at Dexter indefinitely. All broodfish will be rotated into the spawning program and no individual fish will be spawned twice in 5 years.

Peer Reviewers:

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