FINAL

RESEARCH AND IMPLEMENTATION PLAN FOR ESTABLISHING A SECOND POPULATION OF HUMPBACK CHUB IN GRAND CANYON

Grand Canyon Monitoring and Research Center

U.S. Department of the Interior

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DISCLAIMER STATEMENT

The conclusions of this document indicate that the most likely successful establishment of a second self-sustaining population of humpback chub in the Grand Canyon is in the mainstem Colorado River if the water is sufficiently warmed by implementation of the proposed temperature control device on Glen Canyon Dam. This finding should not be construed as a recommendation for that implementation.

CONTENTS

EXECUTIVE SUMMARY v

1.0 INTRODUCTION 1
- 1.1 Background 1
- 1.2 Purpose 1
- 1.3 Concept of a Second Population 1
- 1.4 Common Factors 2
  - 1.4.1 Temperature Control Device on Glen Canyon Dam 2
  - 1.4.2 Experimental Steady Releases from Glen Canyon Dam 3
  - 1.4.3 Non-Native Fish Control 4
  - 1.4.4 Long-Term Monitoring Plan 4
- 1.5 Document Organization 5

2.0 LIFE HISTORY OF THE HUMPBACK CHUB 6
- 2.1 Status 6
- 2.2. Distribution 6
- 2.3 Population Size 7
- 2.4 Spawning 9
- 2.5 Habitat and Movement 9
- 2.6 Food Habits and Parasites 12

3.0 CRITERIA FOR ESTABLISHING A SECOND POPULATION 12
- 3.1 Criteria for Success 13
  - 3.1.1 Genetic Viability 13
  - 3.1.2 Inbreeding Rate 13
  - 3.1.3 Genetic Effective Population Size 14
  - 3.1.4 Population Structure 16
- 3.2 Evaluation Criteria 16
  - 3.2.1 Habitat Suitability 16

4.0 ALTERNATIVES FOR ESTABLISHING A SECOND POPULATION OF HUMPBACK CHUB 19
- 4.1 Description of Alternatives 19
- 4.2 Evaluation of Alternatives 19
  - 4.2.1 Existing Mainstem Aggregation 19
  - 4.2.2 Metapopulation Approach 24
  - 4.2.3 Tributaries 25
  - 4.2.4 Tributary and Mainstem 29
- 4.3 Approaches Eliminated from Further Consideration 29
  - 4.3.1 Cryopreservation 29
  - 4.3.2 Habitat Augmentation 29

CONTENTS (Continued)

5.0 RESEARCH AND IMPLEMENTATION PLAN 30
5.1 Steps Outlining Plan 30
5.2 Principal Phases and Description of Steps 33
5.2.1 Phase I - Primary Efforts 33
5.2.2 Phase II - Contingency Measures 36
5.2.3 Phase III - Re-Evaluation 37
5.3 Monitoring 37
5.3.1 Monitoring Program for the Mainstem 37
5.3.2 Monitoring Program for Tributaries 38

6.0 ADMINISTRATION AND COSTS 41
6.1 Administrative Requirements 41
6.2 Estimated Cost for Second Population Plan 42

LITERATURE CITED 44

APPENDIX A: Grand Canyon Monitoring and Research Center Fy99 Request for Proposals
APPENDIX B: Role of Hatcheries and Genetic Considerations

TABLES

Table 1. Locations of nine mainstem aggregations of humpback chub with numbers of fish captured and estimated numbers of adults, 1990-93 (Valdez and Ryel 1995) 7
Table 2. Rates of inbreeding for effective population sizes. Rate of inbreeding (Simberloff 1988) calculated as: $\Delta F = 1/2N_e$, where N_e is effective population size 14
Table 3. Numbers of humpback chub by age group necessary to maintain a population size of 1,667 adults under two assumed survival scenarios for ages 1 to 2, 2 to 3, and 3 to 4; survival of ages 0 to 1 and 4+ from Valdez and Ryel (1997) 16
Table 4. Criteria matrix for mainstem aggregations 22
Table 5. Potential rates of inbreeding for mainstem aggregations in Grand Canyon 23
Table 6. Estimated numbers of adult and sub-adult humpback chub that can be supported in the Stephen Aisle/Middle Granite Gorge (SA/MGG) region, based on current estimated densities of fish in the Colorado River near the LCR inflow 24
Table 7. Suitability criteria for tributaries in Grand Canyon as sites for a second population of humpback chub, compared to the Little Colorado River (LCR) 27
Table 8. Criteria matrix for tributaries 26
Table 9. Estimated numbers of adult and sub-adult humpback chub that can be supported in Havasu Creek based on current estimated densities of fish in the Little Colorado River (LCR) 28

TABLES (Continued)

Table 10. Transfer schedule of humpback chub from the LCR to Havasu and Shinumo Creeks 35
Table 11. Estimated cost for second population plan 42

FIGURES

Figure 1. The Colorado River through Grand Canyon, Arizona, with locations of nine mainstem aggregations of humpback chub 8
Figure 2. Percentage of adult humpback chub in spawning condition from monthly samples in (A) the LCR inflow and (B) eight disjunct mainstem aggregations 10
Figure 3. Suitable and optimal temperature range for spawning by humpback chub compared (A) to predam temperatures of the Colorado River at Phantom Ranch; (B) temperature of the LCR and postdam Colorado River at Glen Canyon Dam, LCR, and Diamond Creek without temperature control; and (C) with temperature control . . 11
Figure 4. Longitudinal warming patterns of the Colorado River from Glen Canyon Dam to Diamond Creek with and without dam modifications to control downstream temperatures. 21

BOXES

Box 1. Steps Required for Establishing a Second Population of Humpback Chub in Grand Canyon 31
Box 2. A Monitoring Program for Establishing a Second Population of Humpback Chub in the Mainstem Colorado River in Grand Canyon 39
Box 3. A Monitoring Program for Establishing a Second Population of Humpback Chub in One or More Tributaries of the Colorado River in Grand Canyon 40

EXECUTIVE SUMMARY

INTRODUCTION

The humpback chub (*Gila cypha*) is an endangered cyprinid fish species endemic to the Colorado River Basin in Colorado, Utah, and Arizona. Six populations remain, including five in the upper basin and one in the lower basin in Grand Canyon. The Grand Canyon population consists of nine mainstem Colorado River aggregations and one spawning aggregation in the Little Colorado River (LCR), a major tributary located about 123 km downstream of Glen Canyon Dam. Humpback chub that spawn in the LCR, which includes fish from the mainstem aggregation around the LCR inflow, are considered a single successfully reproducing population. Fish in the remaining eight mainstem aggregations are unable to spawn successfully, presumably because of cold water temperatures from Glen Canyon Dam releases. Establishing a second self-sustaining population in Grand Canyon will reduce the risks associated with a catastrophic loss of the LCR population, thereby aiding in conservation of the species, and helping to address the "jeopardy" determination of the Biological Opinion on the Operation of Glen Canyon Dam.

This plan for establishing a second population of humpback chub in Grand Canyon was developed jointly by a team of biologists, population ecologists, and geneticists. Only biological factors were taken into consideration; potential cultural and political constraints were disregarded. The plan identifies specific time-line action items with contingencies in case a particular action fails or is ineffective. A description of hatchery needs and availability is provided, along with estimated costs for each element of the plan.

CRITERIA FOR A SECOND POPULATION

The primary criteria for establishing a second population of humpback chub in Grand Canyon are (1) establishing successfully reproducing adults of sufficient number to maintain the maximum genetic viability of the species and (2) achieving long-term demographic stability in suitable habitat reasonably protected from threats and catastrophes. A genetic effective population size (N_e) was determined to be 1,667 adults and is presented in this document to serve as a guideline to help evaluate alternative approaches to establishing a second population. Demographic structure is presented as the estimated numbers of adults (age 4+) and juveniles (ages 3, 2, 1, and 0) that are thought to constitute long-term demographic stability. Suitable habitat is ascertained from evaluation of various parameters, including water quantity, water quality, barriers to movement, presence of non-native fishes, and proximity to other populations of humpback chub.

ALTERNATIVES

The plan evaluates four alternatives: (1) Existing Mainstem Aggregation, (2) Metapopulation Approach, (3) Tributaries, and (4) Tributary and Mainstem. Preliminary habitat analyses show that genetic criteria (i.e., target population size and structure) are unlikely to be met in a tributary, but may be met in two contiguous aggregations (Stephen Aisle/Middle Granite Gorge) or in the mainstem taken as a whole (the metapopulation concept, which assumes sub-populations periodically exchange individuals and, hence, are linked genetically). The metapopulation concept is thought to present the greatest likelihood for success in establishing a new, genetically viable population of humpback chub in Grand Canyon.

Existing Mainstem Aggregation. The principal factor now limiting successful reproduction and recruitment of humpback chub in the Colorado River in Grand Canyon is thought to be cold water temperatures released from Glen Canyon Dam. Thus, implementation of the U.S. Bureau of Reclamation's proposal to modify the dam penstocks to warm downstream water temperatures is the key feature of this element. Providing warmer water temperatures of 16-22°C at aggregation sites from May through September is expected to promote successful reproduction and enhance survival and growth of young. Although the proposal to warm dam releases is likely to affect all eight non-spawning mainstem aggregations, we believe the greatest chance of success is with the two largest existing mainstem aggregations: Stephen Aisle and Middle Granite Gorge (210-235 km downstream from Glen Canyon Dam). Assuming that habitat for adults is determined by availability of eddy complexes, we estimate that the 25-km reach occupied by these two aggregations could support enough fish to meet the criteria of genetic viability and long-term demographic stability. The Middle Granite Gorge aggregation by itself appears to have a sufficient number of adults to minimize the effect of inbreeding and this aggregation receives downstream migrants from the LCR. Transfer or stocking of fish is not a feature of this alternative. The aggregations would be monitored for 5-10 years to evaluate success, based on the criteria for success.

Metapopulation Approach. This alternative acknowledges possible responses to warming by fish from most of the mainstem aggregations. The criteria of a second population would be met as the sum of proximate aggregations which exhibit some exchange of individuals and, hence, genetic linkage. This alternative was selected as the most feasible for meeting the goals of a second population.

Tributaries. Establishing a resident population of humpback chub in one or more tributaries other than the LCR is the third alternative of the plan. Two seasonally warmed tributaries, Havasu Creek and Shinumo Creek, were selected as most likely to meet the life history needs of the species. Introductions would be made in both streams in reaches above falls that prevent passage of non-native fishes upstream from the Colorado River. Of the two tributaries, Havasu Creek is preferred because it is more similar in hydrology and habitat to the currently occupied reach of the LCR. Shinumo Creek is recommended as an additional site because it appears to have appropriate water quality, low densities of non-native fish predators, and little human disturbance. These recommendations are preliminary; detailed assessments of habitat suitability and current fish communities in these two streams would have to be conducted before a final determination is made.

Since these tributaries do not currently support humpback chub, fish would be transferred from the LCR to initiate and possibly augment a population. We do not advocate use of a hatchery as a primary tool for establishing a new population of humpback chub in Grand Canyon because hatchery programs may result in reduced genetic viability in the target species. Instead, fish would be transferred from the LCR in totals of 500 young-of-year (50-100 mm total length) and 100 juveniles

(100-250 mm) to each tributary annually for 3 consecutive years. If the numbers of young fish from the LCR are inadequate and if genetic variability can be assured, hatchery-reared fish would be used. These initial transfers would be experimental to determine the best release methods for survival and residence of fish. Positive results of these field experiments could lead to additional transfers of fish to these tributaries over a 3-year period. As a contingency, if transfer of LCR fish fails, hatchery-reared humpback chub would be released in large numbers over a 2-year period. Releasing young hatchery-reared fish into small earthen ponds in the lower Paria River or lower Bright Angel Creek may also be considered a contingency.

Tributary and Mainstem. This alternative considers establishing a population of fish in a tributary with free access to the mainstem. The new population would be expected to move to and from the mainstem in the same manner as the existing LCR population. No tributary in Grand Canyon meets the criteria for this alterative since those with suitable habitat have natural fish barriers near their outflows (Havasu Creek, Shinumo Creek); cold water temperatures (Tapeats Creek); support large populations of non-native fish predators, such as brown trout or rainbow trout (Bright Angel Creek); or lack sufficient flow volume and suitable habitat (Paria River, Kanab Creek).

HATCHERIES AND GENETIC CONSIDERATIONS

We recommend initiating a hatchery program at the outset of this plan (1) as a refuge for unique genetic stocks if they are found to exist, (2) to develop a brood stock of humpback chub, and (3) to produce fish for supplementation, if necessary. Fish and/or gametes from at least the 30-Mile aggregation should be taken into a hatchery refuge in the first year of the plan for genetic assessment and to protect unique genetic material if it is found. These fish are behaviorally distinct from the LCR population and may be relicts of mainstem stocks. Genetic profiles should be developed to determine if significant genetic differences exist among any of the nine aggregations in Grand Canyon, and if transfer of fish among aggregations or use of hatchery products is feasible. If various genetic markers show no significant differences, a brood stock will be developed from progeny of LCR fish. Approximately 200 young-of-year fish would be captured and transferred from the LCR for rearing at a hatchery facility. Assuming losses from handling and mortality over time, 100-150 would be expected to reach adulthood at 4+ years of age (assume 50:50 gender ratio). Fish for supplementation (F1 progeny of wild fish) would be produced from paired matings of this brood stock to maximize genetic diversity. The numbers of males and females needed would be determined by assessing the genetic diversity of the population. Once established, this brood stock would be capable of producing approximately 200,000 young annually for stocking.

CONTINGENCIES AND COSTS

This plan contains several alternative actions and contingencies to provide the maximum likelihood of success. If efforts to establish a mainstem population fail, the concept of a second population and the efficacy of the temperature control device on Glen Canyon Dam will have to be re-evaluated. If all efforts to establish a new population fail, the feasibility of establishing a second population of humpback chub in Grand Canyon will have to be re-evaluated. Total estimated cost of establishing a second population of humpback chub in Grand Canyon is $4.7-$6.2 million. This cost assumes that existing hatchery facilities will be used and that a new hatchery will not need to be constructed. Estimated costs are divided into four elements: mainstem monitoring, tributary stocking and monitoring, hatchery program, and genetic profiles. At the full development scenario for the program, the most expensive elements would be the mainstem monitoring ($2.0-$2.5 million) and the hatchery program ($2.0-$2.4 million). Monitoring for a second population of humpback chub would be merged with monitoring programs for the temperature control device and experimental steady flows, both of which require mainstem monitoring of fish assemblages. To the extent possible, requirements of all three efforts should be satisfied with one monitoring program.

1.0 INTRODUCTION

1.1 BACKGROUND

A new population of humpback chub (*Gila cypha*) in Grand Canyon is identified as a common element of the Environmental Impact Statement (EIS) on the Operation of Glen Canyon Dam and its Record of Decision (U.S. Bureau of Reclamation [Reclamation] 1995, 1996), as well as an element of the reasonable and prudent alternative of the associated "jeopardy" Biological Opinion (U.S. Fish and Wildlife Service [FWS] 1994). Currently, the only successfully reproducing and recruiting population of humpback chub in Grand Canyon spawns in the Little Colorado River (LCR), a tributary of the Colorado River. A second population would provide a backup of similar genetic material in case catastrophic events in the LCR were to reduce the viability of this spawning population. Establishing a new population could also aid in the basinwide conservation and recovery of this endangered fish species (FWS 1990a). The population of humpback chub in Grand Canyon is one of only six in existence (Valdez and Clemmer 1982).

1.2 PURPOSE

The purpose of this document is to present a plan to establish a second self-sustaining population of humpback chub downstream of Glen Canyon Dam. The plan includes an experimental component, step-by-step management actions, estimates of associated costs, and contingencies in case recommended actions fail or are ineffective. Consideration was given to several alternative strategies, the importance of conservation genetics and long-term demographic stability, and the use of hatcheries. This plan was developed jointly by a team of biologists, population ecologists, and geneticists in fulfillment of Agreement No. 99-FC-40-2270 between Reclamation and SWCA, Inc., Environmental Consultants (see Appendix A for the Request for Proposals that initiated this project). Only biological factors were taken into consideration; potential cultural and political constraints were disregarded.

1.3 CONCEPT OF A SECOND POPULATION

The concept of establishing a second spawning population of humpback chub in the Grand Canyon was first proposed as one of seven conservation measures in response to a draft "jeopardy" Biological Opinion on Glen Canyon Dam operations issued by the FWS in 1987 (FWS 1990b, as cited in FWS 1994). These conservation measures were developed jointly by Reclamation, FWS, Arizona Game and Fish Department, Grand Canyon National Park, and the Navajo Nation Natural Heritage Program. During their deliberations, the authors of the measure discussed a second population in terms of a refuge population, most likely located in Havasu Creek, to serve as an emergency source of fish in case a catastrophic incident, such as a spill of hazardous material, decimated fish and habitat in the LCR (Pers. Comm., Frank Baucom, FWS 1999). They did not address specific parameters such as target population size and age structure, nor did they address the issue of genetic viability.

In the Final Biological Opinion of 1994, the concept was presented as element 4 of the Reasonable and Prudent Alternative (RPA): "Establish a second spawning aggregation of humpback chub downstream of Glen Canyon Dam" (FWS 1994). Neither the word "population" in the 1987 draft opinion nor the word "aggregation" in the final 1994 opinion were defined in terms of numbers of fish, sustainability, or genetic viability. However, we assume that the intent of these opinions was to establish a self-sustaining, reproducing, and genetically viable population of humpback chub geographically separated from the LCR population.

1.4 COMMON FACTORS

Several proposed, recommended, or ongoing river management programs may affect implementation of the second population plan presented in this document. Each of these programs is important as either a vital component for establishing a second population of humpback chub or as an underlying assumption of dam operations that will affect efforts to establish the second population. Each is, therefore, a common element to all alternatives evaluated in this document. Two of the programs–installation and operation of a temperature control device on Glen Canyon Dam, and experimental steady releases from Glen Canyon Dam–are elements of the RPA. Two additional program–development and implementation of a non-native fish control program, and a formal long-term fish monitoring plan–are recommendations resulting from this plan. These four river management programs and their relevance to establishing a second population of humpback chub are discussed at greater length below.

In addition, seven elements were identified in the Glen Canyon Dam EIS as common to the alternatives considered in that document, including the alternative selected in the Record of Decision (Reclamation 1995, 1996). As such, these elements affect dam operations and could potentially influence attempts to establish a second population of humpback chub. One of the elements, a new population of humpback chub, is the subject of this plan. A second, further study of a temperature control device, is discussed below. The remaining five elements are Adaptive Management, Monitoring and Protecting Cultural Resources, Flood Frequency Reduction Measures, Beach/Habitat-Building Flows, and Emergency Exception Criteria. For more information about these elements, consult the GCDEIS (Reclamation 1995).

1.4.1 Temperature Control Device on Glen Canyon Dam

Reclamation (1999) is considering modifying Glen Canyon Dam to allow control of downstream temperatures of the Colorado River. Studies show that cold summer water temperatures (8-11°C) created by hypolimnetic dam releases currently constrain endangered and other native fishes by preventing reproduction in the mainstem, possibly killing small YOY entering the mainstem from warmer tributaries, and slowing growth (Valdez and Carothers 1998). These cold water releases are also thought to constrain non-native warm water fishes for the same reasons. A selective withdrawal program was identified as Element 1B of the Biological Opinion on the Operation of Glen Canyon Dam. One possible means for achieving warmer releases is to modify the existing trashrack structures that cover the eight power penstocks by installing fixed openings to allow for withdrawal of warmer water near the surface of Lake Powell. Releases of warm water would be possible only when the reservoir surface elevation is between 3,700 feet (full pool) and 3,670 feet, a condition projected to occur in 85 of 100 years. Withdrawal elevation for the fixed-level intakes would be about 3,630 feet, depending on lake elevation. The intake structures must be covered by at least 40 feet of water before they can become operational.

Based on preliminary modeling with existing data since filling of the lake (Pers. Comm., Susan Hueftle, GCMRC, June 1999), water at the 3,630-foot elevation (1106 m) warms to an average of 15°C on June 1 ± 20 days. At lower lake elevations, this temperature is usually reached during the third week of May; at higher lake elevations, June to early July is more typical. These times neglect the periods when the operation would not be possible because the structure is not covered by the recommended 40 feet of water. Water at the 3,630-foot elevation cools to below 15°C about November 16 ± 8 days. Surface lake water in August may reach 18°C. Reclamation (1999) is proposing to release a mix of this warm surface water with deeper, colder water to achieve a temperature of about 15°C during the months of June through August, then decrease temperature during September. We recommend releasing warm waters as early as available in late May and early June to provide sufficient temperatures for spawning by mainstem aggregations of humpback chub, particularly the fish in Middle Granite Gorge, and to provide warm water for recently hatched young emerging from the LCR. Warm water releases of 15°C should be maintained until about mid-September. A temperature control device should be used for a positive biological response by native and endangered fishes (i.e., successful reproduction, increased survival and growth), but should be inactivated periodically to flush the system with cold water and disadvantage non-native fishes.

A temperature control device was installed on Flaming Gorge Dam in 1976 (Holden and Selby 1979, Holden and Crist 1981), allowing for warmed releases down the Green River beginning in 1978. Cold dam releases had caused reproductive failure and slowed growth of the warmwater native fishes in the Green River. Recent studies show a reinvasion by native fishes into former habitat and reproduction as a result of these warmer releases (Bestgen and Crist 2000).

1.4.2 Experimental Steady Releases from Glen Canyon Dam

Element 1A of the reasonable and prudent alternative of the Biological Opinion on the Operation of Glen Canyon Dam (FWS 1994) identified that "A program of experimental flows will be carried out to include high steady flows in the spring and low steady flows in summer and fall during low water years (releases of approximately 8.23 maf [million acre feet]) . . . ." The primary purpose of experimental flows is to determine if steady flows would improve conditions for young endangered and other native fish in Grand Canyon by creating warmer, more stable shorelines along the Colorado River. While we believe that experimental flows are likely to promote survival and growth of young native fishes along shorelines and in backwaters, mainstem longitudinal warming would produce adequate temperatures for spawning only in the most downstream reaches of Grand Canyon. A temperature control device is essential for providing sufficient warming to allow spawning on the scale needed to result in a second population of humpback chub.

1.4.3 Non-Native Fish Control

A non-native fish control program is an important consideration before attempting to establish a second population of humpback chub in Grand Canyon. Non-native predator and competitor fishes are recognized by some as the most important factor leading to the decline and continued endangerment of the native fishes of the Colorado River Basin (Minckley 1991, Tyus and Saunders 1996). Suppressing the abundance of non-native fish that compete with or prey on young native fish and reduce survival and recruitment may make the difference between success and failure of the second population effort. Non-native fish control is particularly important if a temperature control device is placed on Glen Canyon Dam. Most non-native fish species in the Grand Canyon are warm-water species that are expected to benefit from increased river temperatures. Pre- and post-dam fish surveys clearly show that non-native warm-water fishes declined in distribution and abundance with conversion to cold, hypolimnetic releases following dam construction (Valdez and Ryel 1995, Valdez and Carothers 1998). A reversal of this decline is likely if river temperatures increase.

A non-native fish control program should evaluate (a) response risk associated with each existing non-native fish species, and (b) efficacy of control programs to reduce non-native fishes in case of a response to dam operations that threatens endangered and other native species. A non-native fish control program can be expected to provide only short-term benefits for native fishes by reducing non-native predators and competitors. A non-native fish control program should not last more than 3-5 years, with the criteria for discontinuing the program based on achieving pre-experiment levels of distribution and abundance of non-native fishes. Ongoing non-native fish control is not practical because these populations may recover quickly from reductions. However, short-term reductions of non-natives can provide an advantage to young native fishes, allowing them to survive to a size too large for predators and providing recruitment to the adult portion of the population. Although a non-native fish control element is not identified in this plan, we recommend developing procedures to suppress non-native fish populations in advance of plan implementation. We recommend that this program target both the non-native predators (e.g, brown trout and channel catfish) known to feed on humpback chub, and the small-bodied non-native competitors (e.g., red shiner, fathead minnow, etc.) known to occupy nearshore habitats similar to those used by larval and juvenile native fish. Methods of control may include direct reduction of channel catfish in the LCR and its inflow, where large numbers are known to congregate, and brown trout in spawning areas. Control measures may also include efforts to displace non-native fish in the mainstem through Glen Canyon Dam flow modification. Procedures should be in place to implement when and where necessary to provide an advantage to the newly starting population of humpback chub.

1.4.4 Long-Term Monitoring Plan

A formal, standardized fish monitoring program is a vital part of evaluating the success of establishing a second population of humpback chub in Grand Canyon. Such a program should be in place by the year 2001 in order to gather at least one year of baseline information prior to implementation of actions recommended in this plan. The plan should specify sampling protocols, data recording and archiving, data analyses, and integration of past information. The plan should annually evaluate fish abundance estimates and approaches for reducing the variance of those estimates.

Stock-recruitment models have recently been recommended as a vehicle for assimilation and synthesis of fishery data from Grand Canyon (Pers. Comm., Carl Walters, University of British Columbia 2000). Mark-recapture data can be used in these models to assess cohort strength and recruitment that results from a variety of demographic and environmental conditions, such as reproductive success, and growth, survival, and predation rates. During the years 2000 and 2001, stock recruitment models will be developed for each of the native fish species as well as the more significant non-native fish species. Data collected from past investigations will be used in these models, and future data collection needs will be identified.

1.5 DOCUMENT ORGANIZATION

This document is organized as follows:

- Section 1: Provides background and describes document purpose and organization.
- Section 2: Summarizes the life history of the humpback chub to provide context and background for subsequent discussions.
- Section 3: Discusses the criteria for a second population, including genetics and habitat considerations.
- Section 4: Evaluates alternative approaches to establishing a second population of humpback chub in Grand Canyon.
- Section 5: Presents a research and implementation plan for establishing a second population of humpback chub in Grand Canyon that includes guidelines for existing and future monitoring.
- Section 6: Summarizes administrative requirements and estimated implementation costs of this plan.
- Appendices: Appendix A is the Request for Proposals that initiated this project, and Appendix B discusses the role of hatcheries and genetic considerations for implementation of the plan.

2.0 LIFE HISTORY OF THE HUMPBACK CHUB

2.1 STATUS

The humpback chub is a member of a distinctive ichthyofauna of the Colorado River with species-level endemism of 74% (Miller 1946, 1959). It is one of four mainstem Colorado River fish species listed as endangered by the FWS. The others are razorback sucker (*Xyrauchen texanus*), Colorado pikeminnow (*Ptychocheilus lucius*), and bonytail (*Gila elegans*). The humpback chub was included in the first List of Endangered Species issued by the Office of Endangered Species on 11 March 1967 (32 FR 4001) and is afforded protection under the Endangered Species Act of 1973, as amended. A Recovery Plan was approved 19 September 1990 (FWS 1990a), and critical habitat was designated for this and the three other endangered Colorado River mainstem species on 21 March 1994 (59 FR 13374). Six populations of humpback chub are recognized, including Black Rocks and Yampa Canyon in Colorado; Westwater Canyon, Desolation-Gray Canyon, and Cataract Canyon in Utah; and Grand Canyon in Arizona (Valdez and Clemmer 1982). Decline of this species is attributed to flow regulation, habitat alteration, cold-water releases by dams, and competition and predation by non-native fishes (FWS 1990a, Minckley 1991).

2.2. DISTRIBUTION

Based on identification of osteological specimens found in Stanton's Cave, 52.5 km downstream of Lees Ferry, humpback chub are known to have lived in the Colorado River in Grand Canyon for at least 4,000 years (Miller and Smith 1984). They were likely distributed throughout the length of the mainstem in Grand Canyon before Glen Canyon Dam was built; soon after dam closure, specimens were caught from near the dam to Separation Canyon about 425 km downstream. Whether these fish successfully spawned and recruited in the mainstem is unknown, but it is considered probable since other populations in the upper basin are strictly mainstem residents. Since construction of the dam, most juvenile humpback chub found in the Colorado River have been in the LCR inflow or downstream, suggesting they originated from the LCR; however, juveniles have been captured upstream of the LCR as well (Suttkus et al. 1976, Carothers and Minckley 1981, Valdez and Ryel 1997).

Evidence of pre-dam use of tributaries in Grand Canyon by humpback chub is limited. Fish that may have been humpback chub were observed upstream in the Paria River by residents of Lees Ferry in the early years of this century (Pers. Comm., L. Stevens, GCMRC 1999). In May 1911, Ellsworth and Emery Kolb (1914) observed and photographed numerous fish, which they called "bony tail" but which were almost certainly humpback chub, at the mouth of the LCR (Kolb Brothers Collection, Special Collections Library, Northern Arizona University). In 1932, a National Park Service ranger caught a fish, later identified by Robert Rush Miller as a humpback chub, at the mouth of Bright Angel Creek (Miller 1946, 1972). The most suggestive evidence of spawning in a tributary other than the LCR is a collection of nine YOY humpback chub (48-57 mm) captured about 1,000 m upstream in Spencer Creek by O.L. Wallis in October 1955 (Pers. Comm., Gerald Smith, University of Michigan, Museum of Zoology 2000). Since construction of Glen Canyon Dam, humpback chub have been caught in small numbers in the confluence reaches of Bright Angel Creek, Shinumo Creek, Kanab Creek, and Havasu Creek (Kubly 1990, Gorman and Bramblett 1998, Valdez and Carothers 1998).

2.3 POPULATION SIZE

Humpback chub in Grand Canyon comprise nine mainstem Colorado River aggregations and one spawning aggregation in the LCR (122 km downstream of Glen Canyon Dam) (Table 1, Figure 1; Valdez and Ryel 1997). Except for the mainstem aggregation near the LCR inflow, all mainstem aggregations are composed primarily of adults with little or no successful reproduction, presumably because of cold mainstem temperatures. The estimated number of adults (≥200 mm total length [TL]) in the mainstem during 1990-93 was 3,750; the estimated number in the LCR inflow area was 3,482 (Valdez and Ryel 1997); and the estimated number of adults (>150 mm TL) in the LCR in 1992 was 4,508, based on closed population models (Douglas and Marsh 1996). It is believed that most of estimated 3,482 adults in the LCR inflow area were included in the estimated 3,750 adults in the LCR.

The relationship between the mainstem aggregation of humpback chub and the LCR population is unclear. Fish from the mainstem aggregation immediately adjacent to the inflow ascend the LCR seasonally to spawn (Kaeding and Zimmerman 1983, Douglas and Marsh 1996). Most young remain in the LCR, but many descend into the mainstem during late summer monsoonal freshets; although survival of these young in the mainstem is low because of thermal shock and predation (Valdez and Ryel 1997). It is thought that most mainstem recruitment occurs among fish that descend from the LCR as large juveniles or young adults (Douglas and Marsh 1996, Valdez and Ryel 1997).

Table 1. Locations of nine mainstem aggregations of humpback chub with numbers of fish captured and estimated numbers of adults, 1990-93 (Valdez and Ryel 1995).

Aggregation	**Kilometers From Glen Canyon Dam**	**Number Captured, 1990-93**				**Estimated Number of Adults[2] (95% confidence interval)**
		YOY[1]	**Juveniles**	**Adults**	**Totals**	
1. 30-Mile	73.7-75.8	14	0	26	26	52 (28-136)
2. LCR Inflow	117.1-130.6	1,830	1,293	1,524	4,647	3,482 (2,682-4,281)
3. Lava to Hance	131.1-148.2	778	226	15	1,019	no estimate[3]
4. Bright Angel Inflow	160.2-173.7	13	2	9	24	no estimate
5. Shinumo Inflow	199.3-200.1	4	13	27	44	57 (31-149)
6. Stephen Aisle	210.3-218.6	0	7	17	24	no estimate
7. Middle Granite Gorge	228.3-233.0	1	4	124	129	98 (74-153)
8. Havasu Inflow	276.1-277.5	0	0	7	7	13 (5-70)
9. Pumpkin Spring	367.3-368.4	0	0	6	6	5 (4-16)

[1] YOY = Young-of-year

[2] Based on Chao (1987, 1989) closed population estimator.

[3] No estimate possible because of small numbers of captures and recaptures.

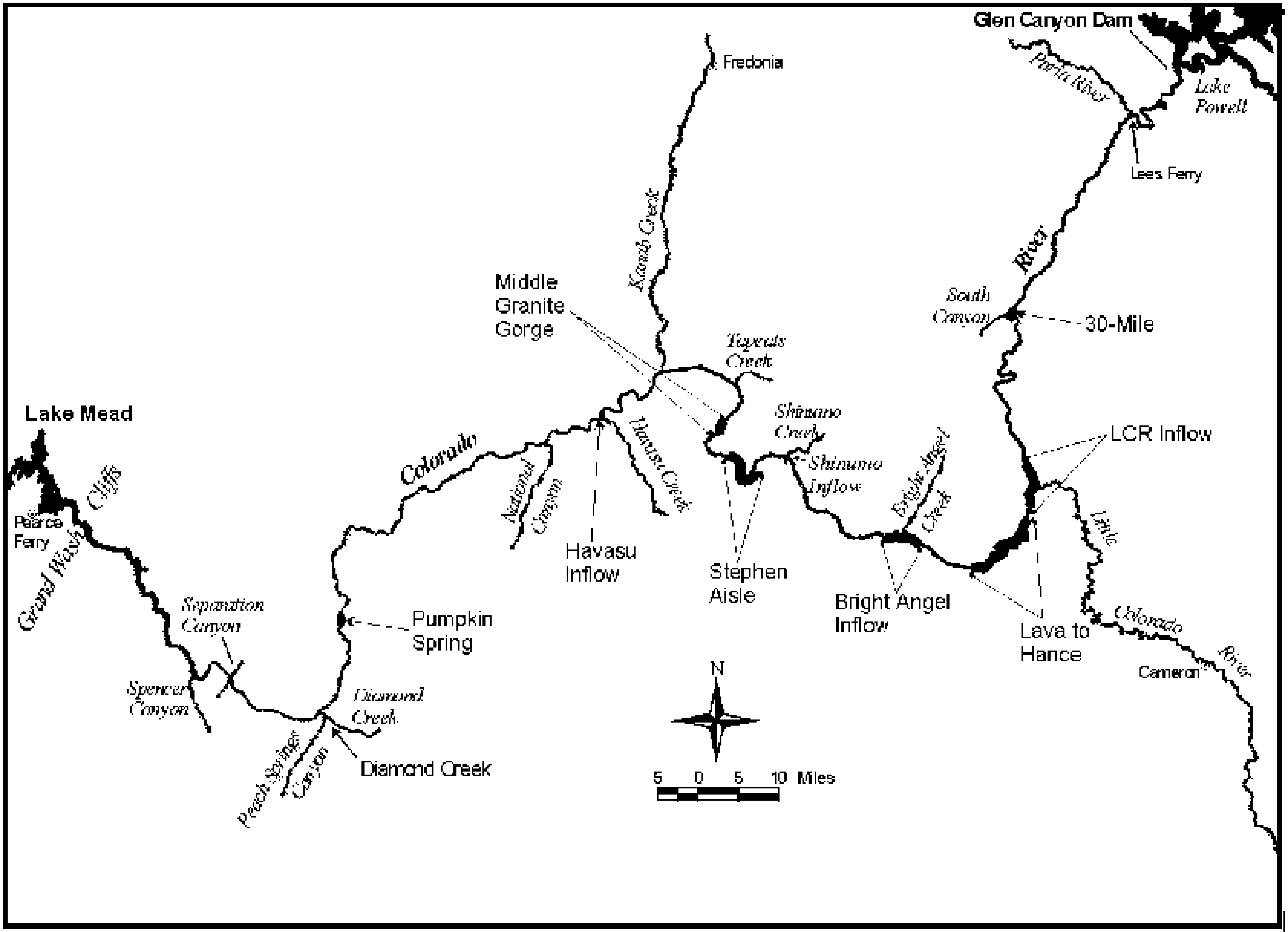

Figure 1. Locations of nine aggregations of humpback chub in the Colorado River though Glen and Grand Canyons. (From Valdez and Ryel 1995)

2.4 SPAWNING

Humpback chub are obligate warm-water species with preferred spawning, hatching, and growth temperatures of 16-22°C (Hamman 1982). Average fecundity is about 2,523 eggs/female or 5,262 eggs/kg of body weight. The eggs are adhesive and are broadcast over cobble and clean gravel bars in complex channel configurations strewn with large angular boulders and characterized by plunge pools, chutes, runs, and eddies (Gorman and Stone 1999), and possibly over rocky substrate along talus shorelines or debris flows (Valdez and Williams 1993). Embryos incubate and hatch in 5-6 days (Hamman 1982).

Spawning in the LCR occurs primarily in March or April, but usually spans March-May (Kaeding and Zimmerman 1983, Robinson et al. 1995, Douglas and Marsh 1996). Relatively early spawning in this tributary may be a function of warm water (a constant 21°C) from Blue Springs, which feeds the lower LCR (Minckley 1996). In contrast, fish in the mainstem outside the LCR inflow were found in spawning condition primarily in May (Figure 2; Valdez and Ryel 1995), which is consistent with historic warming patterns of the mainstem prior to river regulation and with observations of upper basin populations (Valdez and Clemmer 1982, Kaeding et al. 1990). Reproduction by mainstem aggregations in Grand Canyon is largely unsuccessful because cold hypolimnetic dam releases preclude spawning by either failing to provide a needed thermal cue, causing resorption of eggs, or preventing deposited eggs from hatching (Kaeding and Zimmerman 1983; Figure 3). Lack of suitable substrate for spawning is not thought to be a limiting factor in the mainstem..

Some spawning does take place in the mainstem. Collection of post-larval humpback chub about 74 km downstream of Glen Canyon Dam (i.e., 30-Mile aggregation) indicates successful mainstem spawning by fish in association with warm, riverside springs (Valdez and Masslich 1999). Some spawning may also take place in seasonally warmed tributary inflows, although there is no evidence of successful reproduction and recruitment from such sites. Survivorship of larval and post-larval fish is likely to be low because of cold water temperatures, possibly flows, and large numbers of predaceous rainbow trout and brown trout in the mainstem.

2.5 HABITAT AND MOVEMENT

Humpback chub are whitewater canyon inhabitants (Valdez and Clemmer 1982, Kaeding et al. 1990, Karp and Tyus 1990, Valdez and Ryel 1997). Unlike the highly migratory Colorado pikeminnow, which may migrate hundreds of miles to and from spawning sites (Tyus 1990, 1991), the humpback chub is relatively sedentary. Tagging studies in the upper basin show little apparent exchange between populations and little movement within populations (Valdez and Clemmer 1982, Kaeding et al. 1990), although lack of apparent genetic differences and little morphometric variation suggest long-term genetic linkage (McElroy and Douglas 1995, Douglas et al. 1998). Radiotelemetry and tagging studies of adult humpback chub in the mainstem near the LCR confluence showed high fidelity for specific locales of less than 2 km river distance and consistent use of eddy complexes formed by debris fans (Valdez and Ryel 1995, 1997). Although minimum flow preferences are not known, the smallest stream in which this species has been captured regularly is the LCR, which has

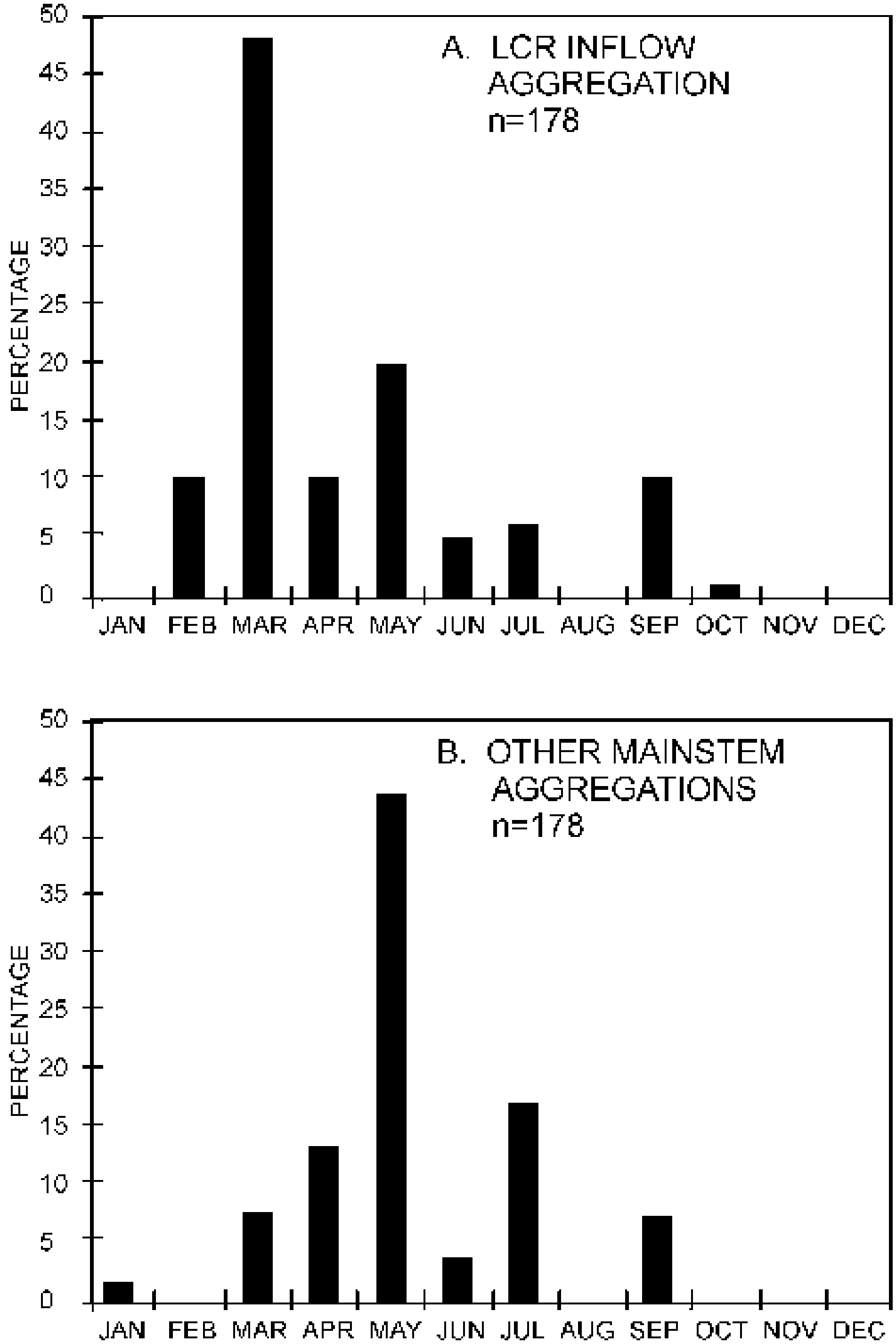

Figure 2. Percentage of adult humpback chub in spawning condition from monthly samples in (A) the LCR inflow aggregation and (B) eight mainstem aggregations.

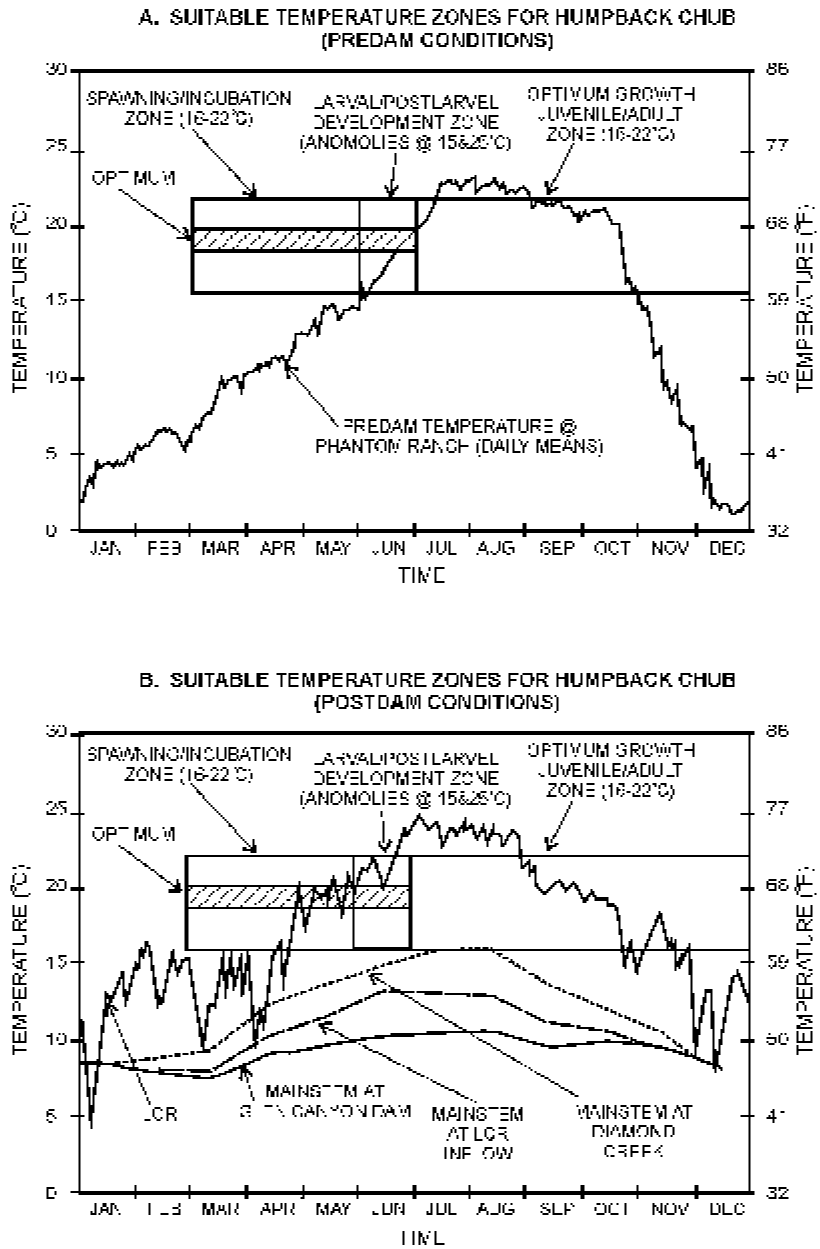

Figure 3. Suitable and optimal temperature range for spawning by humpback chub compared to (A) predam temperature of the Colorado River at Phantom Ranch, and (B) the temperature of the LCR and postdam Colorado River at Glen Canyon Dam, LCR, and Diamond Creek. Spawning, egg, incubation, and larval development periods are shown for present LCR inflow aggregation.

a base flow of about 250 cubic feet per second (cfs). In the LCR, adults prefer deeper water with high cover and structural complexity in daylight hours and shallower, more open areas at night (Gorman 1994). In the Yampa River (Karp and Tyus 1990) and in the Colorado and Green Rivers of the upper basin, adults also occupy deep eddies and pools (Kaeding et al. 1990, Valdez et al. 1990).

Young and juvenile humpback chub use shallow, sheltered shorelines, which can be provided by interstitial spaces in debris fans, talus slopes, or vegetated banks (Gorman 1994, Valdez and Ryel 1997, Converse et al. 1998). They also use shallow backwaters formed by eddy return-current channels (Arizona Game and Fish Department 1996).

2.6 FOOD HABITS AND PARASITES

Humpback chub are omnivorous, but consume primarily insects and crustaceans (Valdez and Carothers 1998). Aquatic foods are most common in the diet, but terrestrial insects can compose a significant proportion of the diet following rainstorm events or insect emergences (Kaeding and Zimmerman 1983, Tyus and Minckley 1988, Valdez and Ryel 1997, Valdez and Hoffnagle 1999). Diet information comes from studies in both regulated and unregulated reaches of river in both the upper and lower basins; however, no information on humpback chub diet was collected in Grand Canyon before dam construction. The principal macroparasites of humpback chub are the external parasitic copepod *Lernaea cyprinacea* and the internal Asian tapeworm *Bothriocephalus acheilognathi*. *L. cyprinacea* requires 25°C for maturation, and *B. acheilognathi* requires 20°C to complete its life cycle (Brouder and Hoffnagle 1997, Clarkson et al. 1997).

3.0 CRITERIA FOR ESTABLISHING A SECOND POPULATION

The analysis in this report involves two sets of overlapping criteria. The first are criteria for success. A successfully established second population of humpback chub in Grand Canyon is defined as a group of humpback chub that (1) is located in an area other than the LCR and its inflow, (2) exhibits successful reproduction, (3) recruits to all age groups, (3) exhibits genetic viability, and (4) has the potential for long-term demographic stability. Genetic viability determines the minimum number of adults necessary for a population to maintain low inbreeding rates and sufficient genetic variability and plasticity for individuals to cope with extreme conditions in environmental stochasticity. Because this concept is important in current scientific thinking about species recovery and because its theoretical underpinnings are both complex and rapidly evolving, genetic viability is discussed at some length in this section.

The second set of criteria are the factors used in Section 4.0 to evaluate alternative strategies for achieving the goal of establishing a second population of humpback chub. These criteria primarily concern habitat suitability, which describes the amount of appropriate living space and environmental conditions necessary for the species to successfully complete its life cycle. Other evaluation criteria include the potential threat of competition and predation from non-native fish species and possibility of human disturbance. The criteria for success and the evaluation criteria overlap in the sense that each alternative strategy is evaluated according to its ability to achieve the target population size (N_m, i.e., our estimate of the minimum number of adult humpback chub necessary to maintain genetic variability).

3.1 CRITERIA FOR SUCCESS

3.1.1 Genetic Viability

The importance of conservation genetics has become evident in efforts to maximize adaptive flexibility, particularly in management of diminished or imperiled populations. Genetic aspects have traditionally been considered in conservation of existing populations (Franklin 1980, Soulé 1980, Soulé 1986, Gilpin and Soulé 1986, Lande 1995), and in predicting long-term sustainability, such as population viability analyses (Gilpin 1993).

Establishing a new population of individuals requires similar genetic considerations. A second wild population of humpback chub in Grand Canyon will require a pool of genetic diversity adequate to allow the population to survive environmental pressures that exceed the limits of developmental plasticity (Frankel and Soulé 1981); hence, genetic viability is important for developing a target population size and structure. A goal of genetics studies has traditionally been the maintenance of 90% of the genetic variability present in the ancestral (pre-disturbance) population for 200 years (Franklin 1980; Soulé 1980, 1987; Soulé 1986; Lande 1995).

Genetic variation consists of within-population genetic diversity and genetic variability among populations or stocks. Genetic variation is important for individuals to maintain physical, physiological, and behavioral traits that enable them to survive the rigors of their environment and to complete their life histories. Forces that erode genetic variation include small population size, population bottlenecks, genetic drift, inbreeding depression, artificial selection in captivity, and mixing of distinct genetic stocks (Meffe 1986). Founder effects, gene flow, nonrandom mating, and mutation are also mechanisms of genetic change (Currans and Busack 1995).

The threshold at which a stock or population of individuals is able to maintain genetic viability when subjected to random events will vary depending on the characteristics of the population and the species. Many random events establish feedback loops, or "extinction vortices," that increase the likelihood of population failure from other stochastic events (Gilpin and Soulé 1986). The consequences of environmental stochasticity are most pronounced with short-lived species. Long-lived fishes, such as humpback chub, may be more resilient in response to random stochastic events.

3.1.2 Inbreeding Rate

For the new population of humpback chub to be considered genetically viable, it must have sufficient numbers of successfully reproducing adults to minimize the negative effects of inbreeding. Rate of inbreeding is an index of the amount of genetic exchange between related siblings. Inbreeding is of particular importance because it has been demonstrated that sib-sister matings for some species will result in offspring that are sterile or inviable after one to several generations. Although a maximum of 1% inbreeding (N=50) is recommended for wild populations (Table 2; Simberloff 1988), larger numbers are recommended to maintain genetic viability. Rate of inbreeding (ΔF; Simberloff 1988) is calculated as:

$$\Delta F = 1/2N_e$$

where: N_e is genetic effective population size (see Section 3.2.3. below).

Table 2. Rates of inbreeding for effective population sizes. Rate of inbreeding (Simberloff 1988) calculated as: $\Delta F = 1/2N_e$, where N_e is effective population size.

Effective Population Size (N_e)	Rate of Inbreeding (ΔF)
10	5.00%
20	2.50%
30	1.67%
40	1.25%
50	1.00%
100	0.50%
200	0.25%
500	0.10%
1,000	0.05%
2,000	0.025%

3.1.3 Genetic Effective Population Size

To maintain genetic viability, we propose to use the concept of "genetic effective population size" (N_e), which is the number of adults contributing genes to the next generation (Gilpin and Soulé 1986, Soulé 1987, Allendorf et al. 1997). This concept presumes that not all adults in a population reproduce successfully each year. We find that N_e is a good and helpful indicator of the magnitude of adults needed to maintain genetic viability; however, we caution against too much reliance on a specific population size target using this approach because of the inexact science and assumptions in developing and computing N_e. Much remains to be known about this genetic concept, particularly for long-lived fish species, such as the humpback chub. For example, it is possible that through time the effective population size of humpback chub has changed drastically (notably in response to dam-caused environmental changes that have occurred relatively recently), calling into question the validity of a firm estimate based on point-in-time observations (see Lavery et al. 1997). Therefore, we apply the concept of N_e in this plan to arrive at a value, the minimum number of adults necessary for a population to maintain genetic variability, that should serve as a guideline and not necessarily as a strict criterion for success or failure. The more a group of fish equals or exceeds the genetic effective population size, the more surety managers will have that the group will be genetically viable over the long term.

N_e likely differs by species (Meffe 1986), but lack of genetic structural characterization with functional relationships for humpback chub precludes a specific determination at this time. In the absence of this information, we derive N_e from contemporary thinking in the field of genetics, which considers the potential rate of inbreeding, as described above. Geneticists have surmised that a population with N_e of $\leq$50 adults will experience short-term inbreeding depression (Soulé 1980, Nelson and Soulé 1987), whereas a population with $N_e \geq 500$ will maintain long-term genetic diversity (Franklin 1980), restricting loss of genetic variation by random drift to 0.1% per generation (Currans and Busack 1995). Although it has been suggested that 1,000 provides a more conservative estimate of N_e (Lynch 1996), the more commonly used value is 500 for fish species (Waples 1990, Bartley et al. 1992, Allendorf et al. 1997), as well as other vertebrate species (Mace and Lande 1991, Ralls et al. 1996). We used the most commonly used N_e value of 500 as the minimum genetic effective population size needed to maintain genetic variability in a second population of humpback chub. We assume a 1:1 sex ratio for this species (Valdez and Ryel 1997) and that reproducing males and females contribute equally to the next generation. Hence, it is estimated that the N_e of 500 adults includes 250 males and 250 females. An uneven sex ratio would increase the value of N_e.

If all adults in a population were to breed every year and contribute genes to the following generation, N_e would equal the total number of adults (N_m). However, N_e derives from the premise that not all individuals in a population contribute gametes with equal probability to the next generation. As with most populations, it is believed that N_e for humpback chub is not equal to N_m. To determine the target N_m for a second population of humpback chub, it is important first to determine a reasonable ratio (C) of genetic effective population size to total adult population size for this species:

$$C = N_e/N_m$$

where: N_e = genetic effective population size as numbers of adults, and
N_m = total population size of adults.

For various fish species, the ratio C varies from 0.013 to 0.90 (Bartley et al. 1992, Avise 1994, Hedrick et al. 1995, Allendorf et al. 1997). However, these estimates are based largely on theoretical values and may not be realistic for an evaluation of *G. cypha*. Lentsch et al. (1997) used a theoretical value of 0.50 as C for developing "interim management objectives"(IMOs) for humpback chub in the upper basin. We used a ratio of 0.30 as an average of values derived for various fish species, including Pacific salmon (Waples et al. 1990a, 1990b; McElhany et al. 2000) Based on this ratio of C, we estimated that N_m, or the number of adults needed to maintain an N_e of 500, is 1,667 (N_e/C = 500/0.30).

We reiterate that this is a guideline for genetic viability of humpback chub in Grand Canyon and should not necessarily be used as a rigid criterion for success or failure of attempts to establish a new population. There are currently wild populations of humpback chub in the upper basin with fewer numbers of adults that appear to be healthy, stable, and self-sustaining. The estimated number of adults in Black Rocks, Colorado, is 1,528 (McAda et al. 1999); in Westwater Canyon, Utah, is 5,186 (Chart and Lentsch 1999); in Yampa Canyon, Colorado, is 600 (Karp and Tyus 1990); in Desolation/Gray Canyons is 1,500 (Chart and Lentsch 2000); and in Cataract Canyon is 500 (Valdez 1990).

3.1.4 Population Structure

Presented in Table 3 are numbers by age group necessary to maintain a population with 1,667 adults. Two scenarios are provided with different annual survival rates to demonstrate the variability of population structure as a function of survival of given age fish, and the need to monitor such a population over a long term. Survival rates for age 0 to 1 and age 4+ are from Valdez and Ryel (1997), while the others are intermediate estimates assuming approximately linear changes in high (scenario 1) and low (scenario 2) survival rates with age. Age 3 fish are assumed to produce enough recruits (1,667 x (1-0.755) = 408) to offset losses from natural mortality in the 4+ age group. Consequently, the estimated total number of sub-adults needed to maintain 1,667 adults would range between approximately 68,000 (scenario 1) and 272,000 (scenario 2).

Table 3. Numbers of humpback chub by age group necessary to maintain a population size of 1,667 adults under two assumed survival scenarios for ages 1 to 2, 2 to 3, and 3 to 4; survival of ages 0 to 1 and 4+ from Valdez and Ryel (1997).

Age	Scenario 1		Scenario 2	
	Number	Survival	Number	Survival
0	68,000	0.1	272,000	0.1
1	6,800	0.3	27,200	0.1
2	2,040	0.4	2,720	0.3
3	816	0.5	816	0.5
4+	1,667	0.755	1,667	0.755

3.2 EVALUATION CRITERIA

3.2.1 Habitat Suitability

Little information is available on fish habitat available for the Colorado River and its tributaries in Grand Canyon. The only assessment of mainstem habitat showed adult humpback chub occupying primarily eddy complexes formed by debris flows. Radiotagged adults disproportionately used eddy habitat; 88% of adults captured and 74% of radio contacts were from eddy complexes over a range of 5,000 to 30,000 cfs flows (Valdez and Ryel 1997). At flood flows of 45,000 cfs, radiotagged adults remained in eddy complexes, using low-velocity vortices presumably for resting and feeding (Valdez and Hoffnagle 1999). Juvenile humpback chub commonly occupy shorelines of talus, debris fans, and vegetation at a depth of <1 m and velocity of <0.10 m/s (Converse et al. 1998). There are no published data on fish habitat of tributaries in Grand Canyon. Habitat data were collected in the LCR and other tributaries (Gorman 1994, Bramblett and Gorman 1998), but no analyses have yet been performed on these data.

Mainstem. To assess habitat availability and carrying capacity in the mainstem Colorado River in Grand Canyon, we assumed a relationship between fish density and eddy complexes associated with debris fans by using the area near the LCR inflow as an index. A total of 27 persistent debris fans and associated eddy complexes were identified for the 13.5-km reach near the LCR (Melis and Webb 1993). This reach was estimated to support 3,482 (95% C.I.=2,682-4,281) adult humpback chub (>200 mm TL; Valdez and Ryel 1997), or about 129 adults/eddy complex, or 258 adults/km.

A similar analysis was performed to determine the potential numbers of sub-adults that could be supported by the mainstem. Carrying capacity for sub-adult humpback chub appears to depend on availability of seasonally warmed, productive shorelines of debris fans, talus, and shoreline vegetation (Converse et al. 1998). Shoreline seining in the 6.7-km reach of the mainstem immediately downstream from the LCR confluence yielded peak estimates of 65,980; 230,930; and 857,750 sub-adults in 1991, 1992, and 1993, respectively (Valdez and Ryel 1995). Exponential decreases in bimonthly densities showed that survival of these fish was low, casting doubt on use of the higher number as an index of carrying capacity. We therefore used the average of the three values to yield an estimate of 384,887 sub-adults as the average number of young fish that could be supported in the 13.5-km reach of the LCR Inflow, or about 28,510 sub-adults/km.

These estimates of habitat carrying capacity in the LCR Inflow reach were used in Section 4.2 as an index to evaluate the possibilities of establishing a second population of humpback chub in other mainstem reaches in Grand Canyon. Other evaluation criteria include:

- Potential Inbreeding Rate of Existing Fish. Inbreeding rates of the existing aggregations reflect their size: larger aggregations have lower inbreeding rates; smaller ones, larger rates. This is a Fatal Flaw criterion: inbreeding rates of approximately 1% or below are acceptable; aggregations with greater than approximately 1% are eliminated from consideration.

- Distance Downstream from the Dam. Benefit from longitudinal warming should increase with distance downstream from the dam.

- Proximity to the LCR. Greater distance from the LCR confluence is considered an advantage because it provides greater protection from any spill in the LCR that might adversely affect the existing population.

- Presence of Non-Native Predators/Competitors. Areas with lower densities of non-native predators/competitors receive higher scores.

- Proximity to Other Existing Aggregations. Aggregations near other aggregations receive higher scores because they are likely to benefit from the exchange of new individuals and genetic material. Closely neighboring aggregations, like those in Middle Granite Gorge and Stephen Aisle, may merge into one population if numbers of fish and their distribution increase significantly with warmer water temperatures.

Tributaries. Carrying capacity of tributaries in Grand Canyon was determined on the basis of existing densities of fish in the LCR and a comparison of stream flow and linear distance. The LCR population of humpback chub for 1992 was an estimated 4,508 adults (>150 mm TL; Douglas and Marsh 1996). These fish occupied a reach of 14.9-km upstream of the confluence with the Colorado River for an estimate of 303 adults/km.

These estimates of carrying capacity were used in Section 4.2 as an index to evaluate the possibilities of establishing a second population of humpback chub in tributaries other than the LCR. Other evaluation criteria include:

- Water Quantity. Inadequate base flow may be a problem for a resident population. Resident populations of humpback chub have not been documented from any stream smaller than the LCR or Yampa River, which have a base flow of about 250 cfs. Water quantity was a Fatal Flaw criterion since some streams in Grand Canyon have minimum measured flow of 0.

- Water Quality. As evidenced by their occupation of the LCR, humpback chub can tolerate high levels of salts and carbonates. The major water quality issue of concern in Grand Canyon is the possibility of human-caused contamination.

- Temperature. Temperature requirements for humpback chub are 16-22°C for hatching, larval survival, and growth.

- Fish Barrier. The presence of a fish barrier (a waterfall) that prevents passage from the Colorado River into the tributary provides protection from mainstem non-native competitors and predators but also precludes natural augmentation by mainstem humpback chub.

- Non-Native Fish. The presence of non-native competitors and predators, especially in large abundances, can limit or preclude reproductive success and/or recruitment.

- Human Disturbance. Heavy use by recreationists can damage habitat, contaminate water, or disturb fish.

4.0 ALTERNATIVES FOR ESTABLISHING A SECOND POPULATION OF HUMPBACK CHUB

4.1 DESCRIPTION OF ALTERNATIVES

We have focused this plan on the Colorado River and its tributaries between Glen Canyon Dam and the Lake Mead inflow, and have discounted regions downstream of Grand Canyon as unsuitable habitat for humpback chub and lacking significant evidence as historic range of the species.[1] Four alternatives were evaluated to determine if the criteria for establishing a second population of humpback chub in Grand Canyon could be met:

1. Existing Mainstem Aggregation: at least one existing mainstem aggregation would be allowed to expand into a self-sustaining population by warming river water with installation of a temperature control device on Glen Canyon Dam.

2. Metapopulation Approach: same as the preceding alternative except the criteria for a second population would be met by the sum of aggregations.

3. Tributaries: humpback chub would be transferred from the LCR into one or more seasonally warmed tributaries to establish a resident population of humpback chub. The population would reside solely within the tributary rather than move to and from the mainstem. This scenario was evaluated as an alternative because some tributaries in Grand Canyon have natural barriers to upstream movement near their mouths, and we did not want to eliminate them from consideration.

4. Tributary and Mainstem: same as the preceding alternative except the new population would be expected to move between a tributary and the mainstem in the same manner as the existing LCR population.

4.2 EVALUATION OF ALTERNATIVES

4.2.1 Existing Mainstem Aggregation

Relatively large numbers of humpback chub currently inhabit the mainstem Colorado River in Grand Canyon as eight non-spawning aggregations (excluding the LCR Inflow aggregation). Mainstem reproduction in Grand Canyon is currently impeded by cold water temperatures, and the only known successful reproduction is reported from a warm, riverside spring about 74 km downstream of the dam (Valdez and Masslich 1999). Humpback chub may have routinely reproduced in the mainstem prior to Glen Canyon Dam as suggested by studies from unregulated reaches of the Colorado River in the

[1] The only evidence of distribution below Grand Canyon consists of two pharyngeal arches identified as humpback chub found in a prehistoric archaeological site (Catclaw Cave) now inundated by Lake Mohave, about 25 km downstream of Hoover Dam (Miller 1955).

upper basin—analogous in many ways to the pre-dam river in Grand Canyon—which show that humpback chub spawn and recruit successfully in the mainstem (Valdez and Clemmer 1982, Kaeding et al. 1990, Valdez and Williams 1993, Karp and Tyus 1990). Other than post-larval fish caught in the 30-Mile aggregation and a few young fish collected far downstream of the LCR, evidence of successful mainstem spawning in Grand Canyon is lacking.

This alternative hinges upon implementation of a temperature control device on Glen Canyon Dam (proposed by Reclamation [1999]), which would warm river temperatures and allow at least one of the eight existing mainstem aggregations to reproduce and expand. The ninth mainstem aggregation, the one in the LCR inflow area, is not considered a candidate for a second population because it is a component of the existing LCR population and, of all mainstem aggregations, is most subject to catastrophic events originating in the LCR.

Once the temperature control device is operational, warm releases (15°C) during late spring and summer months (May or June-September), combined with longitudinal warming, should provide appropriate spawning and incubation temperatures (16-22°C) for downstream aggregations of humpback chub. Aggregations are located 74 to 368 km downstream of Glen Canyon Dam (Table 1, Figure 1). As shown in Figure 4, releases of 15°C would be expected to warm at a rate of between about 1°C/46 km and 1°C/51 km downstream of the dam (Valdez and Ryel 1995, Arizona Game and Fish Department 1996, Korn and Vernieu no date) and produce desirable temperatures for all but the 30-Mile aggregation of fish (74 km downstream from the dam). Although mainstem water temperature at the 30-Mile aggregation is expected to be marginal for spawning, the increased temperature could enhance survival and growth of young hatched in the limited area of the warm springs. Low steady summer flows (element 1A of the 1994 Biological Opinion, FWS 1994) may improve existing conditions for humpback chub in the mainstem by making shoreline conditions more favorable for survival and growth of young fish, but mainstem reproduction depends on warmer mainstem temperatures that can only be provided by dam modifications. Such warming may also benefit non-native fish species that prey on or compete with humpback chub. The effect of an increase in the populations of such fish on the establishment of a second population of humpback chub is unknown, but the potential for detrimental impacts should be mitigated through ongoing monitoring and a non-native fish control program.

Developing a second population of humpback chub from an existing mainstem aggregation relies entirely on responses by the existing resident stocks of wild fish. Transferring fish from other wild stocks or augmentation with hatchery stocks is considered a contingency only if it is determined that numbers of fish in a given aggregation are insufficient to initiate a new spawning population of fish. Transferring fish from other wild stocks or use of hatchery stocks would be considered only after genetic characterizations of aggregations of *Gila cypha* in Grand Canyon are conducted to reduce the risk of losing genetic variability and/or structure.

The authors evaluated the potential of each aggregation to develop into a self-sustaining population. Each physical and biological criterion described in Section 3.0 was scored from 0 (unfavorable) to 5 (favorable). The resulting matrix is presented in Table 4. The criteria are weighted equally except for potential inbreeding rate; an excessively high rate is considered a fatal flaw.

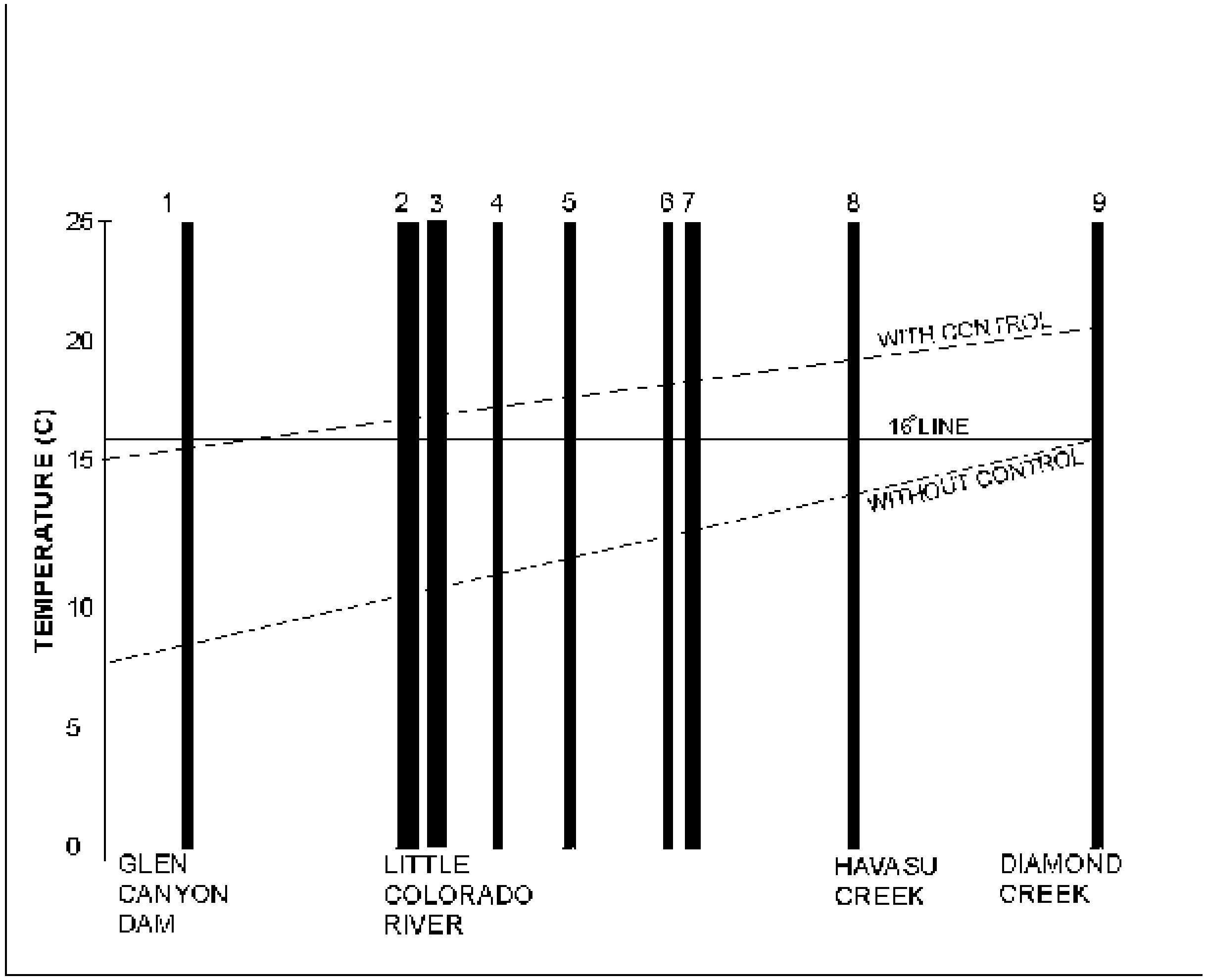

Figure 4. Longitudinal warming patters of the Colorado River from Glen Canyon Dam to Diamond Creek with and without dam modifications to control downstream temperatures.

Table 4. Criteria matrix for mainstem aggregations.

AGGREGATION	GENETIC CONSIDERATIONS (Fatal Flaw)	HABITAT CONSIDERATIONS					TOTAL SCORE[2]	RANK
	Potential Inbreeding Rate of Existing Fish[1]	Habitat Carrying Capacity	Distance from Dam (km)	Proximity to LCR	Non-Native Predators/ Competitors	Proximity to Existing Aggregations		
30-Mile	excessive (2.17%)	2	1 (74)	5	2	0	-	-
Lava to Hance	excessive (no est.)	3	2 (131)	1	3	4	-	-
Bright Angel Inflow	excessive (no est.)	2	2 (160)	4	2	3	-	-
Shinumo Inflow	excessive (2.17%)	2	3 (199)	5	2	3	-	-
Stephen Aisle	excessive (no est.)	4	3 (210)	5	4	4	-	-
Middle Granite Gorge	5 (1.16%)	4	3 (228)	5	4	4	25	**1**
Havasu Inflow	excessive (8.33%)	2	4 (276)	5	4	2	-	-
Pumpkin Spring	excessive (25.0%)	2	5 (367)	5	4	0	-	-

1 Based on numbers from Table 2.

2 Aggregations with a fatal flaw (in this case an excessive inbreeding rate) are eliminated from further consideration and are not given a total score or ranking.

Inbreeding Rate. Only one aggregation, Middle Granite Gorge, was identified as having sufficient numbers of adults to minimize the effect of inbreeding (Table 5). This is the largest mainstem aggregation other than the one in the LCR inflow area. In 1990-93, one YOY, four juveniles, and 124 adults were captured in Middle Granite Gorge (Valdez and Ryel 1995). The total number of adults in this aggregation is an estimated 98 (95% C.I. = 74-153), resulting in an estimated inbreeding rate of 1.02%, which is near the maximum acceptable rate of 1%. Although the genetic characteristics for this group of fish are unknown, mark-recapture studies indicate that some of these fish originate from the LCR (Valdez and Ryel 1995), suggesting periodic genetic infusion. Recent studies of these aggregations by FWS have not been completed and preliminary information were not available for comparison with past data for this report.

Table 5. Potential rates of inbreeding for mainstem aggregations in Grand Canyon.

Mainstem Aggregation – Estimated Number of Adults	Effective Population Size[1]	Rate of Inbreeding[2]
30-Mile – 52	16	3.13%
LCR Inflow[3] – 3,482	1,045	0.05%
Lava to Hance - no estimate	-	-
Bright Angel Inflow - no estimate	-	-
Shinumo Inflow – 57	17	2.94%
Stephen Aisle - no estimate	-	-
Middle Granite Gorge – 98	29	1.72%
Havasu Inflow – 13	4	12.50%
Pumpkin Spring – 5	2	25%

[1] Assume that only 30% of adults are contributing to genes of next generation (i.e., a N_e/N_m ratio of 0.30) to compute effective population size as described in Section 3.2.3.

[2] Rate of inbreeding is calculated as described in Section 3.2.2 ($\Delta F = 1/2N_e$)

[3] The LCR Inflow aggregation is included here for purposes of comparison.

Habitat Carrying Capacity. The carrying capacity of habitat in the vicinity of each aggregation was evaluated using the relationship between fish density and eddy complexes determined for the reach near the LCR inflow. As explained in Section 3.3.1, this reach was estimated to support 3,482 adult humpback chub and 384,887 sub-adults. The only other section of river in Grand Canyon with comparable densities of debris fans and eddy complexes is a 25-km reach through Stephen Aisle and Middle Granite Gorge (210-235 km downstream from Glen Canyon Dam), which includes 28 debris fan/eddy complexes (Melis and Webb 1993). Assuming that habitat for adults is determined by availability of eddy complexes, we estimate that the 25-km reach could support about 3,611 adults, i.e., (3,482/27 x 28) (Table 6). This is more than the calculated genetic effective population size of 1,667 adults needed to achieve long-term genetic viability.

Table 6. Estimated numbers of adult and sub-adult humpback chub that can be supported in the Stephen Aisle/Middle Granite Gorge (SA/MGG) region, based on current estimated densities of fish in the Colorado River near the LCR inflow.

Region	Number of Debris Fans	Number of Adults	Number of Sub-adults
LCR Inflow[1]	27/13.5 km	3,482	384,887[3]
SA/MGG	28/25 km	3,611[2]	712,750[4]

[1] Data source: Valdez and Ryel 1995
[2] Assumes a density of 129 adults per debris fan/eddy complex
[3] Computed as average of three estimates of sub-adults (Valdez and Ryel 1995)
[4] Computed as proportion of available habitat from number of sub-adults per kilometer in LCR Inflow

Shoreline geomorphology for the Stephen Aisle/Middle Granite Gorge reach and the LCR reach are similar, and we assume the same density of 28,510 sub-adults/km as carrying capacity for both reaches. This equates to a carrying capacity of about 712,750 sub-adults (28,510/km x 25 km) for Stephen Aisle/Middle Granite Gorge. This number is well above the estimated range of 68,000 and 272,000 sub-adults needed to support a population of 1,667 adult humpback chub.

Other Criteria. In addition to a relatively low inbreeding rate and sufficient suitable habitat to support a second population of humpback chub, the combined Stephen Aisle/Middle Granite Gorge aggregations offer the following advantages: (1) they are near a third aggregation (Shinumo Inflow) and could, with population growth, merge into one aggregation; (2) non-native fish are in relatively low abundance; (3) this reach is relatively far downstream (228-233 km) from the dam, particularly compared to the 30-Mile aggregation, which will experience very little longitudinal warming with a temperature control device; and (4) they are far enough from the LCR to reduce the risk of a catastrophic event from that source.

Conclusion. We believe that, with operation of the proposed thermal control device on Glen Canyon Dam, it is possible that a genetically viable second population of humpback could develop from the combined Stephen Aisle/Middle Granite Gorge aggregations.

4.2.2 Metapopulation Approach

The metapopulation approach is based on the premise that a temperature control device would promote mainstem spawning for most, if not all, existing aggregations, but resultant numbers of fish for a single aggregation may not reach sufficient numbers to satisfy the criteria of a reproducing, self-sustaining population. Instead, the criteria for a second population would be met by the sum of aggregations. The metapopulation approach predicts responses by individuals of all aggregations and recognizes some exchange of individuals among two or more of these aggregations that would promote genetic variability. Such an exchange of individuals was documented by Valdez and Ryel (1995) who observed 2% of humpback chub from the LCR aggregation moving to other downstream aggregations over a 3-year period. This rate of movement suggests an ongoing genetic link between the LCR and downstream sub-populations.

The greatest chance of success for a second population of humpback chub will likely be couched in a metapopulation framework. The minimum population size guideline of 1,667 adults and 68,000 to 272,000 sub-adults could be satisfied within this framework, assuming habitat carrying capacity of the entire mainstem is adequate to support these numbers of fish. The metapopulation approach may also involve small numbers of fish spawning in tributary inflows or lower reaches of accessible tributaries. However, the numbers of fish in tributaries outside of the LCR is not expected to be large because of low seasonal water volume, limited habitat, blocked passage, and non-native predators.

4.2.3 Tributaries

This alternative examines the use of a seasonally warmed tributary in Grand Canyon as a target site for establishing a second population of humpback chub. The population would reside solely within the tributary rather than move to and from the mainstem (that alternative is evaluated separately). While establishing a second population in a tributary may be possible and has been recommended in the past (Gorman 1994), this alternative appears, because of habitat and space limitations, to be less promising than developing a population in the mainstem. Resident populations of humpback chub have not been documented from any stream smaller than the LCR, although, historically, humpback chub have had access to every tributary in Grand Canyon. The fact that this species has not established populations in tributaries smaller than the LCR suggests habitat and perhaps space limitations. Arizona Game and Fish Department collected water quality data from tributaries considered the best potential sites and concluded that all the streams possessed characteristics that could make establishment of a second population of humpback chub difficult or unlikely (Arizona Game and Fish Department 1996).

It may not be possible to establish any number of humpback chub in a tributary other than the LCR, and if it is possible, such a population, in and of itself, would likely be too small to constitute genetic viability. Still, a small tributary "population" would have value as a backup and refuge. It would provide an isolated group of fish not subject to perturbations of the mainstem, enhance genetic diversity for the Grand Canyon population (or super population if a second population becomes established in the mainstem), and provide redundancy and added insurance against extinction of the species in the lower basin. Although the probability of success in a tributary may be low, the value of a new, smaller group of fish should not be discounted. We therefore recommend an experimental test of the proposition in at least one, and preferably more than one, tributary.

Because humpback chub are not currently found in tributaries other than the LCR, this alternative depends on stocking fish to initiate and possibly supplement the new population. We advocate transferring young fish from the LCR rather than using hatchery-reared fish because hatchery programs may result in reduced genetic viability of stocked fish (see Appendix B). A hatchery program is included in this plan, however, and hatchery-reared stocks would be used if insufficient numbers of fish are available from the LCR (e.g., low reproduction, declining population numbers, catastrophic event, etc.).

Transfers of fish would take place only after an analysis of the genetic structure of all aggregations in Grand Canyon is completed. This analysis would ensure that genetic structural characterization is not dramatically different between the LCR and aggregations proximate to the target tributary. New technology with microsatellite analyses has not been fully employed with the Colorado River *Gila*, and is necessary to ensure that all fish in Grand Canyon are genetically similar. This genetic analysis is particularly necessary for the 30-Mile aggregation of humpback chub, which may have originated from fish spawned prior to dam closure, and may have been isolated from the LCR for nearly five generations (one generation time for humpback chub is 8 years). Hatchery fish could be used as a contingency in case natural reproduction of fish transferred from the LCR or of existing mainstem aggregations fails to produce a new population.

Eight tributaries in Grand Canyon were evaluated for their suitability as sites for a second population: Paria River, Bright Angel Creek, Shinumo Creek, Deer Creek, Tapeats Creek, Kanab Creek, Havasu Creek, and Spencer Creek. Table 7 (see following page) summarizes the suitability of each tributary according to the criteria listed in Section 3.3.1, and Table 8 provides numerical ratings, with each criterion scored from 0 (unfavorable) to 5 (favorable). Since humpback chub do not currently occupy these tributaries, inbreeding rates were not considered in the evaluation matrix. All criteria are weighted equally except water quantity. Insufficient flow is considered a fatal flaw.

Table 8. Criteria matrix for tributaries.

Tributary	Habitat Carrying Capacity	Water Quality	Water Quantity (Fatal Flaw)*	Temp.	Fish Barrier**	Non-Native Predators/ Competitors	Human Disturbance	Total Score	Rank
Paria	2	3	-	5	0	5	3	18	-
Bright Angel	2	4	2	5	0	2	2	17	5
Shinumo	2	3	1	5	5	5	5	26	2
Deer	2	3	1	5	0	5	4	20	3
Tapeats	2	3	2	4	0	4	4	19	4
Kanab	2	2	-	4	0	3	4	15	-
Havasu	4	4	4	5	5	4	2	28	1
Spencer	2	3	2	5	0	3	4	19	4

* Paria River and Kanab Creek are eliminated because flow has dropped below level measurable at the U.S. Geological Survey gauges.
** Presence of a fish barrier is considered positive because it precludes incursion of non-native fish from the mainstem.

Two potential tributary sites were identified as a result of this evaluation: Havasu Creek (the lower 6 km downstream of Beaver Falls) and Shinumo Creek (approximately 10 km above the lower falls). Havasu Creek was identified as the most favorable site because it is most similar to the LCR in hydrology, geomorphology, and some water quality parameters, notably ionic composition (Table 7) (Gorman 1994, Melis et al. 1996, Kubly and Cole 1979). Of all rated tributaries, Havasu Creek is the deepest and widest, although water flow averages only 25-33% of the LCR (Gorman 1994).

Table 7. Suitability criteria for tributaries in Grand Canyon as sites for a second population of humpback chub, compared to the Little Colorado River (LCR).

Tributary	Habitat Suitability	Water Quantity[1]	Water Quality[1]	Water Temperature[1]	Fish Barrier	Non-Native Fishes	Human Disturbance
Paria River	Pools, runs, riffles, medium size stream	0 - 16,100 cfs; Avg. 30.1 cfs	High sulfate, gypsum contaminated with magnesium; upstream urban/agric. use	Warm - Suitable	No fish barriers in lower reaches	None in tributary; large number of rainbow trout at outflow	Moderate use by hikers
Bright Angel Cr.	Deep pools, runs, riffles, medium size stream	10 - 4,400 cfs; Avg. 35.4 cfs	Calcium-magnesium carbonate	Cool - Suitable 1-24°C	No fish barriers in lower reaches	Large number of brown and rainbow trout in tributary and near outflow	Heavy use by hikers, river runners, fishermen
Shinumo Cr.	Deep pools, runs, small stream	5 - 15.5 cfs; Avg. 9.1 cfs	Calcium-magnesium carbonate	Cool - Suitable 1-23°C	Fish barrier ~200 m above outflow	Brown and rainbow trout near outflow; rainbow trout above barrier	Very light use by river runners above falls
Deer Cr.	Deep runs, small stream	5.4 - 8.2 cfs; Avg. 7.2 cfs	Calcium-magnesium carbonate	Cool - Suitable	Fish barrier ~50 m above outflow	Brown and rainbow trout near outflow	Light use by river runners and hikers above falls
Tapeats Cr.	Pools, runs, riffles, medium size stream	51.4 - 283 cfs; avg. 100.1 cfs	Calcium-magnesium carbonate	Cold - Limited	No fish barriers in lower reaches	Brown and rainbow trout in tributary and near outflow	Light use by river runners, fishermen
Kanab Cr.	Pools, runs, riffles, medium to small size stream	0 - 4,360 cfs; Avg. 5.7 cfs	High nutrient load, low dissolved oxygen, upstream urban/agric. use	Warm - Suitable 0-35°C	No fish barriers in lower reaches	Fathead minnow, green sunfish, carp, Plains killifish	Light use by river runners and hikers
Havasu Cr.	Deep pools, runs, riffles–similar to LCR	59.3 - 74.5 cfs; Avg. 63.8 cfs	Magnesium-calcium carbonate with large amounts of sulfates, chloride, and sodium; upstream urban/agric. use	Warm - Suitable 9-23°C	Fish barrier ~200 m above outflow	Brown and rainbow trout in tributary and near outflow	Heavy use by river runners and hikers 1-2 km above falls; moderate use above that point
Spencer Cr.	Deep pools, runs, riffles, stream small and habitat limited	1.1 - 4.4 cfs; Avg. 2.7 cfs	No information available	Warm - Suitable 2-24°C	No fish barriers in lower reaches	Plains killifish, red shiner, carp, fathead minnow	Light use by river runners
LCR[2]	Deep pools, runs, riffles, stream size medium to large	240-24,900 cfs; 205 Avg. (about 250 cfs base flow)	Sodium chloride plus significant calcium carbonate; upstream urban/agric. use	Warm - Suitable 2-25°C	Fish barrier 14.5 km upstream	Carp, channel catfish, plains killifish, red shiner, fathead minnow, black bullhead	Heavy use by river runners 1-2 km above confluence; moderate use above that point

[1] Source: Kubly and Cole 1979

[2] The LCR is included for comparison purposes

After Havasu Creek, Shinumo Creek above the lower falls is thought to have the highest potential as a site for humpback chub. This reach of stream is relatively free of predators and competitors (rainbow trout are the only non-native fish), and non-native fish in the mainstem and lower 100 m of the stream are prevented from moving upstream by the lower falls. Water quality and temperature in Shinumo Creek are suitable. Arizona Game and Fish Department (1996) found that this stream was more similar to the LCR than Havasu Creek for five of seven water quality parameters. Gorman (1994) found some suitable habitat characteristics in Shinumo Creek, notably vertical structure and cover; however, the stream is relatively shallow, narrow, and small. Measured flow ranged between 5 and 15.5 cfs, averaging 9.1 cfs (Kubly and Cole 1979). This is <4% of LCR base flow.

Carrying capacity of Havasu Creek and Shinumo Creek was estimated on the basis of existing densities of fish in the LCR and a comparison of stream flow and linear distance (Table 9). The density of humpback chub in the LCR is estimated to be 303 adults/km. We cannot assume the same densities of fish for either Havasu Creek or Shinumo Creek because of smaller streamflow and less available habitat area. Assuming that average streamflow is directly proportional to habitat availability, numbers of fish in Havasu Creek (approximately 25.5% of LCR base flow) are expected to be about 77 adults/km. Based on available stream habitat in lower Havasu Creek (6 km), total estimated adults in this reach would be 462. Using the same reasoning, the numbers of fish in upper Shinumo Creek (approximately 3.6% of LCR base flow) are expected to be about 11 adults/km, or 110 adults in the full 10-km reach above the falls. These numbers fall well below the genetic viability guideline of 1,667 adults.

Table 9. Estimated numbers of adult and sub-adult humpback chub that can be supported in Havasu Creek based on current estimated densities of fish in the Little Colorado River (LCR).

Tributary	**Reach of Available Habitat (km)**	**Number of Adults/km**	**Total Number of Adults in Tributary**	**Number of Sub-adults/km**	**Total Number of Sub-adults in Tributary**
LCR	14.9	303	4,508	33,570	500,000
Havasu Creek	6	77	462	8,560	51,360
Shinumo Creek	10	11	110	1,209	12,090

Carrying capacity of Havasu Creek and Shinumo Creek for sub-adult humpback chub is also determined by comparing habitat and existing LCR densities (Table 9). The numbers of sub-adults in the LCR is estimated at 500,000 fish for the 14.9-km reach, or about 33,570 sub-adults/km. Assuming the same relationship of flow and habitat, numbers of sub-adults in Havasu Creek and Shinumo Creek are expected to be about 8,560 sub-adults/km and 1,209 sub-adults/km, respectively. Based on available stream habitat reaches for Havasu Creek and Shinumo Creek, total estimated sub-adults in these streams would be 51,360 and 12,090, respectively. Transferring LCR fish to either Havasu Creek or Shinumo Creek is not expected to significantly affect existing native fish communities in those tributaries; however, the status of those communities should be monitored throughout the program to identify any impact.

The following tributaries were eliminated from further consideration for the stated reasons: Paria River (occasional inadequate water quantity; flow has dropped below level measurable at the U.S. Geological Survey gauge; Weiss 1993); Bright Angel Creek (large numbers of predators, frequent human disturbance; Otis 1994); Deer Creek (limited water volume); Kanab Creek (flow has dropped below measurable levels, degraded water quality, predators; Otis 1994); Tapeats Creek (cool water temperatures, large numbers of predators); and Spencer Creek (limited water volume).

4.2.4 Tributary and Mainstem

This alternative considers establishing a population of fish in a tributary with free access to the mainstem. The new population would be expected to move to and from the mainstem in the same manner as the existing LCR population. Although humpback chub may not become established in a tributary, the species may be able to complete part of its life cycle (such as spawning and rearing of young) in a small stream. No tributary was considered suitable for this alternative because fish barriers prevent use of large portions of the larger streams in Grand Canyon. Waterfalls near the mouths of Shinumo Creek and Havasu Creek act as barriers for fish from the mainstem and preclude use by mainstem fish of all but the lowest 100-200 m of stream. The Paria River and Bright Angel, Deer, Tapeats, Kanab, and Spencer Creeks are also not likely to support very large numbers of fish and were eliminated from consideration for the reasons stated in the preceding section. Tributary populations of humpback chub would be expected to currently exist if streams in Grand Canyon were suitable under current conditions; a source of fish is present throughout much of the canyon and mainstem river operations do not affect tributary conditions.

4.3 APPROACHES ELIMINATED FROM FURTHER CONSIDERATION

4.3.1 Cryopreservation

Cryopreservation is not considered to have a role in establishment of a second population of humpback chub in Grand Canyon. Although sperm from humpback chub in the LCR have been collected and cryopreserved (Pers. Comm., Owen Gorman, FWS 1999), the only purpose at this time is to determine the feasibility and logistics of cryopreservation for this endangered species, and to preserve genetic material (sperm of males) in a repository. Cryopreservation does not allow for storage of female ova because of damage to the embryos from freezing.

4.3.2 Habitat Augmentation

Habitat augmentation beyond temperature control is not recommended as part of this plan, with one possible exception. Grow-out ponds built along the lower Paria River or lower Bright Angel Creek might be considered as a continency if preferred approaches fail. This would allow hatchery fish to be raised in protected environments for release into the wild. The Colorado River through Grand

Canyon flows in a channel that is either canyon-bound or confined in meander by canyons, and there is little opportunity for instream or riverside modification. Even if such modifications were possible, humpback chub do not often use riverside features (flooded bottomlands, wetlands, alluvial flood plains) that lend themselves to construction. Instead, they prefer debris fan/eddy complexes–features that cannot easily be constructed.

Removal or modification of existing natural fish barriers may be a consideration for expansion of available tributary habitat. The larger, more suitable tributaries in Grand Canyon, such as Bright Angel Creek, Havasu Creek, Shinumo Creek, all have natural falls and barriers that prevent upstream movement of fish from the mainstem. Removing or modifying these barriers may allow for mainstem populations to use these tributaries for spawning, rearing, or some other aspect of their life history. Removing or modifying these features would require compliance with at least the National Park Service.

5.0 RESEARCH AND IMPLEMENTATION PLAN

The principal approach of this plan is to establish a new population of humpback chub in Grand Canyon by allowing reproduction and recruitment to take place in the mainstem from warming river water with a temperature control device on Glen Canyon Dam. A secondary, but parallel effort is to establish a new small population in at least one tributary by transferring young fish from the LCR. Before any fish are moved, a genetic assessment must be conducted to prevent mixing of distinct genetic stocks that could erode genetic diversity and lead to increased homozygosity and reduced fitness. Mark-recapture studies show movement from the LCR to downstream aggregations indicating genetic stocks in Grand Canyon are mixed, except perhaps for the 30-Mile aggregation. If using LCR fish is not feasible, or successful, or prudent for genetic reasons, hatchery-reared fish may be used as a contingency. This requires developing a hatchery program relatively early in the process, as soon as the genetic assessment is complete.

5.1 STEPS OUTLINING PLAN

Steps outlining a research and implementation plan for establishing a second population of humpback chub in Grand Canyon are listed in Box 1, illustrated in Figure 5, and then described in greater detail in the following sections.

BOX 1

Steps Required for Establishing a Second Population of Humpback Chub in Grand Canyon

This plan assumes that a fish monitoring plan will be in place and ongoing in Grand Canyon.

PHASE I: Primary Efforts

1. Identify and address all administrative and legal requirements.

2. Initiate genetic assessment of all aggregations of humpback chub in Grand Canyon.

3. Assess suitability of habitat in Havasu Creek and Shinumo Creek.

4. Initiate a hatchery program for unique genetic stocks if they exist and to start a brood stock.

5. Establish a Mainstem Population. Following installation of a temperature control device on Glen Canyon Dam, monitor mainstem aggregations to determine if natural reproduction and recruitment are taking place in one or more mainstem aggregations. If 5 years of monitoring show no response, implement Phase III; if response is inadequate, evaluate criteria for a metapopulation and consider supplementing with transfer of fish from the LCR or from hatcheries.

6. Establish a tributary population. Assuming genetic profiles show no significant differences in genetic markers, transfer fish from the LCR to Havasu Creek and Shinumo Creek to conduct experiments on best release methods. Repeat for 3 years, closely monitoring the introduced fish. If release methods are successful in tributaries, evaluate the degree of success to determine if additional transfers are necessary. If release methods are unsuccessful, implement Phase III.

PHASE II: Contingency Measures

1. Transfer hatchery-reared fish to one or more tributaries on an experimental basis first. If transfer and survival of fish are successful, continue stocking for supplementation of the new population. If unsuccessful, re-evaluate program.

2. Consider supplementing one or more mainstem aggregations with hatchery-reared fish.

PHASE III: Re-Evaluation

1. If Phase I or Phase II efforts are unsuccessful, the concept of a second population should be re-evaluated.

While approximate time frames are provided for each phase of this plan, it should be kept in mind that this program will be executed within the context of adaptive management. Results will be evaluated annually, and the program adjusted in response to biological responses and data analyses. Time frames, therefore, will likely vary somewhat from those provided here. This plan assumes that a long-term fish monitoring program will be in place and ongoing in Grand Canyon.

The plan is divided into three time phases:

Phase I - Primary Efforts

> Estimated at 5-10 years, Phase I begins with genetic assessments and initiation of a hatchery program. Primary efforts, however, are directed toward meeting the age/size structure and abundance criteria for a new, genetically viable population in the mainstem and to establish a satellite population in Havasu Creek and/or Shinumo Creek. Monitoring to identify progress toward success will take place throughout this phase.

Phase II - Contingency Measures

> If Phase I efforts fail, Phase II will comprise 2-4 years of contingency measures. If these measures also fail, the second population program and the temperature control device should be re-evaluated. Monitoring for evidence of success will continue through this phase.

Phase III - Re-Evaluation

> If the goals for a establishing a second population are met in either Phase I or Phase II, long-term monitoring will continue over an indefinite period in five-year increments to determine if the population endures. If these efforts are unsuccessful, the concept of a second population of humpback chub in Grand Canyon should be re-evaluated.

Phase I could start in the year 2001 with initiation of genetic assessments. Timing of a hatchery program and tributary stocking would depend on the results of the genetic work, which could take up to 2 years to complete. The time frame for establishing a population in the mainstem depends on the temperature control device, which, if constructed, is estimated to become operational in the year 2002 or 2003 (Reclamation 1999).

5.2 PRINCIPAL PHASES AND DESCRIPTION OF STEPS

5.2.1 Phase I - Primary Efforts

Step 1. Identify and address all administrative and legal requirements (See Section 6.0 of this report.)

Step 2. Develop Genetic Profiles

Because mixing distinct genetic stocks can erode genetic variation and lead to increased homozygosity and reduced fitness (Meffe 1986, Walters 1986), genetic profiles need to be developed for the larger aggregations of humpback chub in Grand Canyon (i.e., 30-Mile, LCR, Shinumo, Middle Granite Gorge, and Havasu) to determine if significant genetic differences exist among these aggregations. Profiling should require about 2 years to collect and analyze materials. Development of genetic profiles is necessary before transfers of fish can be made or before hatcheries can produce fish for supplementation. If significant differences are found in allele frequencies of fast-evolving genetic markers, such as microsatellite DNAs, then aggregations will need to be treated as "management units." If differences are significant, a program should be initiated to protect the integrity of the unique aggregations. Previous genetic analyses have not detected differences in genetic makeup of populations throughout the Colorado River Basin. However, these analyses included fish primarily from the LCR aggregation and did not include and segregate fish from various Grand Canyon aggregations. Furthermore, these analyses did not employ the more sensitive microsatellite techniques currently available. Possibly, fish from the 30-Mile aggregation may be progeny of pre-dam mainstem spawners and have unique genetic characters.

Step 3. Assess suitability of habitat in Havasu Creek and Shinumo Creek

We have identified the lower 6 km of Havasu Creek and upper 10 km of Shinumo Creek as potentially the most suitable localities for attempts to establish a second population of humpback chub in a tributary. Preliminary studies determined that Havasu Creek contains suitable habitat (Gorman 1994), but a detailed habitat assessment, including an estimate of habitat carrying capacity and identification of specific release sites, should be completed before any fish are transferred. A previous study in Shinumo Creek (Allan 1993) evaluated the lower reach, below the barrier falls; we feel that (for reasons explained in Section 4.2.3) the best opportunity for a new population of fish in this stream is above the lower falls. A complete habitat assessment and identification of specific release sites are also required for Shinumo Creek.

Step 4. Initiate a Hatchery Program

Hatcheries can play a role in establishing a second population of humpback chub in Grand Canyon, but they should not be used as a primary tool. A hatchery program should be implemented for the following purposes:

- As a refuge for unique genetic stocks if they are identified.

- To develop a brood stock of humpback chub to be used as a contingency if primary efforts to establish a second population fail.

- To produce fish for supplementation. Supplementation is defined as "the use of artificial propagation in an attempt to maintain or increase natural production while maintaining the long-term fitness of the target population, and keeping the ecological and genetic impacts on nontarget populations within specified biological limits" (Regional Assessment of Supplemental Project 1992).

Maintaining hatchery stocks was listed as the second item in a set of conservation measures developed after the FWS issued their draft Biological Opinion in 1987. Providing a refuge for unique genetic stocks (if they are found) and maintaining a brood stock would implement that conservation measure while simultaneously supplying fish for second population contingency efforts.

A brood stock can be developed by transferring randomly captured young-of-year wild fish from the LCR. The brood stock must be sufficiently large to annually produce about 200,000 young; approximately 100 females and 100 males are required. Proper paired matings should be made to ensure maximum genetic diversity in the progeny. The number of adults required to start the program will be determined from analysis of genetic characteristics of each aggregation. The progeny are able to reproduce as age 4+. Hatchery-reared fish of that age should be available in case attempts to move LCR fish to tributaries fail.

Step 5. Establish a Mainstem Population

Step 5A. Single Aggregation

The criteria for establishing a second population of humpback chub in Grand Canyon may be met in the mainstem by expanding either a single existing aggregation or the sum of aggregations (metapopulation). Achieving the criteria in a single aggregation would be preferred, but we believe that establishing a self-sustaining, recruiting group of fish that range, for example, from the Lava-Chuar area to the Havasu Creek inflow would also satisfy the intent of the 1994 Biological Opinion and RPA. We recommend monitoring the mainstem aggregations as described in Section 5.3 with a focus on the Middle Granite Gorge aggregation to determine if the criteria for a second population have been met. At best, it would take about 6-10 years for the population (growing at 25-50% per year) to meet the population criteria and age/size structure. If a non-native control program is being implemented simultaneously with temperature augmentation, we recommend waiting until the affected non-native populations stabilize without control (limited control period) before judging the final viability of the second population.

Step 5B. Metapopulation Approach

If, at the end of Phase I, the criteria for a second population cannot be met by the Middle Granite Gorge aggregation, abundance of all the aggregations in Grand Canyon should be estimated to determine if the criteria are met by a metapopulation. Since all aggregations will be monitored throughout Phase I, the necessary data will be available. If the metapopulation approach does not meet the criteria, we recommend re-evaluation of second population program and the temperature control device.

Step 6. Establish a Tributary Population

If no significant differences in genetic markers are found among aggregations and sufficient suitable habitat is found in Havasu Creek and Shinumo Creek, then fish will be taken from the LCR and placed into both streams. Throughout Phase I, the tributaries should be monitored as described in Section 5.3.

Step 6A. Experimental Stage. The initial transfer of fish from the LCR (Table 10) into one of these tributaries should be conducted as an experiment to (1) determine if the fish will remain in the area released, (2) determine if a significant proportion of the fish survive, and (3) identify habitat use. Transfers should be made for 3 years with an annual evaluation of the efficacy of the program before additional fish are transferred from the LCR.

Table 10. Transfer schedule of humpback chub from the LCR to Havasu and Shinumo Creeks.

Size Fish (mm TL)	**Marking Method**	**Havasu Creek**	**Shinumo Creek**
50-100	Latex injection[1]	500	500
100-250	PIT tag[2]	100	100
250+	Radio[3]/Sonic tag[4] PIT tag	5	0

[1] Haines and Modde (1996)
[2] Burdick and Hamman (1993)
[3] Valdez and Ryel (1997)
[4] McIvor and Thieme (1999)

Experimental design will evaluate release of different sizes of fish by determining (a) distance moved from release site, (b) survival, (c) habitat use, (d) predation/competition from non-native fishes, (e) effects on existing native fish community. Totals of 500 (50-100 mm TL) and 100 (100-250 mm TL) fish will be transferred from the LCR or from a hatchery for these tests. Additionally, five radiotagged adults (250+ mm TL) will be released and tracked to evaluate release of adults. Adults and large sub-adults (150+ mm TL) will be marked with PIT tags, sub-adults and YOY (30+ mm TL) will be marked with latex dye injections. All fish will be weighed and measured.

We believe that the numbers of fish identified for removal under this program are too small to adversely affect the LCR population. Certain measures, however, should be taken to reduce the possibility of negative impact. For example, YOY fish should be removed from the LCR near the inflow rather than farther upstream so that individuals captured will be those most likely to leave the LCR in their first year; studies show that these fish have low survival in the mainstem (Valdez and Ryel 1997). The fish farther upstream are more likely to remain in the LCR longer and have a high probability of survival and recruitment. Throughout the program, the status of humpback chub in the LCR should be monitored. If fish transfers appear to negatively affect the LCR population, transfers should cease and contingency stocking with hatchery-reared fish implemented. To minimize stress on the subject fish, transfers should be made quickly via helicopter. Transfers should be made in three lots spaced about 2 weeks apart during May-June, when YOY fish are abundant and large enough to transfer. If, after 3 years, fish do not stay in the tributary or survival is unacceptably low, transfers should cease and Phase II contingency stocking with hatchery-reared fish should begin.

Step 6B. Post-Experimental Stage. If the fish remain in the area of release and survival is acceptable, annual transfer of 500 young fish should continue for another 3 years for a total of up to 6 years. Six years allows enough time for the progeny of the first YOY transferred to reproduce. Long-term monitoring should continue to determine if the population persists.

5.2.2 Phase II - Contingency Measures

Step 1. Mainstem

If, at the end of the first 6 years after a temperature control device is installed, the criteria for a second population of humpback chub are not met in the mainstem, either by a single aggregation or by the metapopulation approach, but monitoring results show substantial progress toward that goal, one or more aggregations may be augmented by hatchery-reared fish. Such fish could also be used experimentally to help identify factors that limit recruitment in the mainstem. The decision to augment or not (and where, when, and for how long) should be made at that time.

Step 2. Tributaries

Contingency measures in tributaries should not be implemented if Phase I efforts in the mainstem have succeeded. However, if the criteria have not been met in the mainstem, the following action is recommended.

If fish transferred from the LCR do not remain in either tributary during the 3-year experimental stage or if fish fail to reproduce and recruit and become established by the end of the post-experimental stage (but the habitat appears suitable), large numbers of hatchery-reared fish should be released in one or both tributaries. The numbers and age structure of stocked fish would be determined based on information gathered during the experimental stage. If it is found that genetic markers differ significantly among aggregations, then progeny from the Havasu inflow should be used to stock Havasu Creek and progeny from the Shinumo Creek inflow should be used in Shinumo Creek.

Another possible contingency is stocking hatchery-reared humpback chub in grow-out ponds built along the Paria River near its confluence with the Colorado River (Pers. Comm., L. Stevens, GCMRC 1999). This action would have to be explored in greater detail before implementing. Fish for stocking Paria River ponds could come from the LCR-based brood stock unless the 30-Mile aggregation (the nearest mainstem aggregation) showed distinct genetic markers. If insufficient or no progress is made toward establishing a second population after 3 years, all stocking should cease.

5.2.3 Phase III - Re-Evaluation

If the measures identified in Phases I and II described above fail, the concept of a second population of humpback chub in Grand Canyon should be re-evaluated. If reasonable efforts prove unsuccessful, it may become necessary for Reclamation and the FWS to reconsider element 4 of the RPA of the 1994 Biological Opinion.

5.3 MONITORING

A long-term fish monitoring program should be in place by the time this plan is implemented. That program should include provisions for evaluating establishment of a second population of humpback chub in Grand Canyon. The only aspect of this plan not likely to be covered under a long-term monitoring program is the need to monitor fish transferred into Havasu and Shinumo creeks. Those efforts would have to either be incorporated into an existing monitoring plan or programmed as a component of that plan.

A monitoring program should (1) determine if/when criteria are met for a second population in the mainstem, (2) determine the best methods for releasing fish into tributaries, and (3) determine if/when recruitment takes place in tributaries. A monitoring plan should contain sufficient sampling frequency and sound methodology to determine with confidence the numbers of fish present in a given reach of river or tributary. The program should not rely entirely on relative density, but should employ reliable mark-recapture population estimators, when possible. Monitoring programs for the mainstem and for tributaries are outlined on the following pages. The monitoring program should continue without a time limit until the population criteria are met in the mainstem, a recruiting population is established in a tributary, or establishing a second population is judged infeasible.

5.3.1 Monitoring Program for the Mainstem

Parameters to be monitored include expected population responses: (1) evidence of successful reproduction, (2) increased numbers of young, and (3) evidence of recruitment. Lack of significant recruitment (measured as an increase in adult population) in 10 years would be the criteria for ending the program. The criteria for an independent, self-sustaining, recruiting population may be re-evaluated before that time.

Monitoring for a second population and for the effects of a temperature control device and experimental flows on fish would largely overlap and should be merged to the extent possible. Requirements of all three efforts should be satisfied within one flexible monitoring program.

Components of a monitoring plan in the mainstem are listed in Box 2 and summarized here. A total of three sampling trips should be conducted per year through Grand Canyon to conduct a population estimator program for humpback chub at all aggregations. Sampling trips should be conducted in May, July, and October for a period of 20 days each. During each sampling trip, crews should sample each aggregation with trammel nets, electrofishing, hoop nets, minnow traps, and seines as part of a mark-recapture program to estimate total numbers of adults, sub-adults, and YOY in each aggregation. Adults and large sub-adults (120+ mm TL) should be marked with PIT tags; sub-adults and YOY (30+ mm TL) should be marked with latex dye injections. All fish should be weighed and measured. Sympatric species should be enumerated, weighed, and measured to track abundance and distribution of other native and non-native species. Appropriate population estimators or catch indices should be used to derive total numbers of adults, sub-adults, and YOY in each aggregation.

5.3.2 Monitoring Program for Tributaries

The monitoring program for tributaries presented here is based on an experimental component to first determine if transferring fish from the LCR or from a hatchery would be effective. An experimental design would evaluate release of different sizes of fish by determining (a) distance moved from release site, (b) survival, (c) habitat use, (d) predation/competition from non-native fishes, and (e) effects on existing native fish community. The design must consider season of release, methods of release, fish size at release, marking techniques, and release locations.

Components of a monitoring plan in the mainstem are listed in Box 3 and summarized here. Field teams should be prepared to monitor the fish consistently for at least the first 2 weeks following each release to ensure that fish movements are documented. Innovative release methods to minimize “fright response” (Pers. Comm., Gordon Mueller, U.S. Geological Survey 1999) should be employed to maximize survival and residence of transferred fish. These methods may include using cages or block nets to hold fish at release sites for up to 2 days after introduction. If fish remain in the tributary, monitoring would continue for 3 years. Efforts would focus on establishing a population by either introducing more fish or by allowing for natural reproduction. During this period, three sampling trips would be conducted annually to each tributary to conduct a population estimator program for humpback chub. Sampling trips should be conducted in May, July, and October for a period of 10 days each to each tributary. During each sampling trip, crews would sample each tributary with trammel nets, electrofishing, hoop nets, minnow traps, and seines as part of a mark-recapture program to estimate total numbers of adults, sub-adults, and YOY. All fish would be weighed and measured. Sympatric species would be enumerated, weighed, and measured to track abundance and distribution of other native and non-native species. Appropriate population estimators would be used to derive total numbers of adults, sub-adults, and YOY in each tributary. Population responses include (1) evidence of successful reproduction, (2) increased numbers of young, and (3) evidence of recruitment.

BOX 2

A Monitoring Program for Establishing a Second Population of Humpback Chub in the Mainstem Colorado River in Grand Canyon

1. Under this program, three 20-day sampling trips through Grand Canyon would be conducted per year, one each in May, July, and October.

2. Sampling would be conducted throughout Grand Canyon to determine if fish are responding in other aggregations or areas. Emphasis would be placed on a population estimator program for humpback chub at the 30-Mile, Shinumo Inflow, and Middle Granite Gorge/Stephen Aisle aggregations.

3. In each aggregation, appropriate abundance estimators (population estimates or catch rates) would be used to derive total numbers of adults, sub-adults, and YOY. During each trip, crews would sample each aggregation with trammel nets, electrofishing, hoop nets, minnow traps, and seines as part of a mark-recapture program to estimate total numbers of adults, sub-adults, and YOY in each aggregation.

4. Adults and large sub-adults (120+ mm TL) would be marked with PIT tags, sub-adults and YOY (30+ mm TL) would be marked with latex dye injections. All fish would be weighed and measured.

5. Sympatric species would be enumerated, weighed, and measured to track abundance and distribution of other native and non-native species.

6. Population responses include (a) evidence of successful reproduction, (b) increased growth rates, (c) increased survival, (d) increased numbers of young, (e) evidence of recruitment. The second population plan would be evaluated every year. If four or more of these responses are not detected annually, the second population program would be discontinued.

7. This sampling protocol would continue for a 5-year period, at which time, the criteria for an independent, self-sustaining, recruiting population would be evaluated.

8. If the criteria for an independent mainstem population cannot be met, criteria would be applied to the metapopulation approach to determine if two or more aggregations, in sum, meet the second population criteria.

BOX 3

A Monitoring Program for Establishing a Second Population of Humpback Chub in One or More Tributaries of the Colorado River in Grand Canyon

This monitoring program for tributaries is based on an experimental component to first determine if transferring fish from the LCR or from a hatchery would be effective.

1. Evaluate survival and movement of humpback chub transferred from the LCR for 2 weeks following releases of fish.

2. Conduct three 10-day sampling trips per year (May, July, and October) to each tributary to evaluate survival, movement, and evidence of reproduction.

3. During each trip, crews would sample each tributary with trammel nets, electrofishing, hoop nets, minnow traps, and seines as part of a mark-recapture program to estimate total numbers of adults, sub-adults, and YOY.

4. Sympatric species would be enumerated, weighed, and measured to track abundance and distribution of other native and non-native species.

5. Appropriate population estimators would be used to derive total numbers of adults, sub-adults, and YOY in each aggregation; newly produced fish would be marked appropriately.

6. If it is determine that introducing fish from other tributaries or from a hatchery is successful, efforts would focus on establishing a population by either introducing more fish or by allowing for natural reproduction.

7. Hatchery products would be used only if there are insufficient fish in the LCR for transfer.

8. Population responses would include (a) evidence of successful reproduction, (b) increased growth rates, (c) increased survival, (d) increased numbers of young, (e) evidence of recruitment. The second population plan would be evaluated every year. If four or more of these responses are not detected annually, the second population program would be discontinued.

9. This sampling protocol would continue for a 5-year period, at which time, the criteria for an independent, self-sustaining, recruiting population would be evaluated.

10. If the criteria for an independent tributary population cannot be met, criteria would be applied to the metapopulation approach to determine if two or more aggregations, in sum, meet the second population criteria.

6.0 ADMINISTRATION AND COSTS

6.1 ADMINISTRATIVE REQUIREMENTS

The following is an outline of administrative requirements for implementing a second population plan. It is assumed that Reclamation would be the lead agency responsible for coordinating these efforts.

1. National Environmental Policy Act
    a. Determine level of significant effects
    b. Develop Environmental Assessment (EA) on the specific federal action
    c. Integrate into Programmatic EA
    d. Coordinate with State and Federal entities

2. Coordinate with Fish & Wildlife Service
    a. Ensure that program is consistent with Biological Opinion
    b. Continue informal consultation through program evaluation
    c. Procure scientific collecting permits for transferring fish
    d. Coordinate with hatchery program

3. Coordinate with Reclamation
    a. Ensure that dam modifications provide desired downstream temperatures
    b. Coordinate releases temperatures to achieve downstream goals

4. Coordinate with Arizona Game and Fish Department
    a. Procure scientific collecting permits for transferring fish
    b. Conduct ongoing liaison and coordination
    c. Coordinate with hatchery program

5. Approval from Grand Canyon National Park
    a. EA approval
    b. Research permits for each study and trip permits for field work
    c. Procure scientific collecting permits for transferring fish

6. Advise Glen Canyon National Recreation Area of Plan
    a. EA approval
    b. Research permits for appropriate studies and trip permits for field work

7. Cultural Resource considerations
    a. Grand Canyon National Park
    b. Glen Canyon National Recreation Area
    c. Tribal groups
    d. Coordinate with State Historic Preservation Officer

8. Funding
    a. Develop contracts and agreements for funding specific studies
    b. Coordinate with Glen Canyon Technical Work Group and GCMRC programs
        i. Coordinate with ongoing programs
        ii. Coordinate with long-term fish monitoring program

6.2 ESTIMATED COST FOR SECOND POPULATION PLAN

The total estimated cost of establishing a second population of humpback chub in Grand Canyon is \$4.7-\$6.2 million (Table 11). This cost assumes that existing hatchery facilities will be used and that a new hatchery will not need to be constructed. Estimated costs are divided into four elements: mainstem monitoring, tributary monitoring, hatchery program, and genetic profiles. At the full development scenario for the program, the most expensive elements would be the mainstem monitoring (\$2-\$2.5 million) and the hatchery program (\$2-\$2.4 million). Costs of mainstem monitoring would be included in costs of the existing long-term fish monitoring program for Grand Canyon, when one is implemented. Costs of monitoring include field trips, data analyses, reports, and logistics.

Hatchery and rearing facilities are available at the Willow Beach National Fish Hatchery, Arizona (Pers. Comm., Manuel Ulibarri, Hatchery Manager 1999), Dexter National Fish Hatchery, New Mexico (Pers. Comm., Roger Hamman, Hatchery Manager 1999), Bubbling Pond State Fish Hatchery, Arizona (Pers. Comm., Roger Sorenson, Hatchery Manager 1999), and rearing facilities are available at the Hualapai Fish Holding Facility near Peach Springs, Arizona (Pers. Comm., Ben Zimmerman, Facilities Coordinator 1999). Other facilities may be available.

Table 11. Estimated cost for second population plan.

Element	Cost Per Year	Term of Element	Estimated Costs
Mainstem Monitoring	\$400,000-\$500,000	5 years	\$2.0-\$2.5 million
Tributary Monitoring	\$100,000-\$200,000	5 years	\$500,000-\$1.0 million
Hatchery Program	\$250,000-\$300,000	8 years	\$2.0-\$2.4 million
Genetic Profiles	\$100,000-\$150,000	2 years	\$200,000-\$300.000
TOTAL ESTIMATED COSTS	\$850,000-\$1.15 million		\$4.7-\$6.2 million

Total cost of this plan may vary, depending on several factors, including (a) construction and implementation of the temperature control device on Glen Canyon Dam, (b) the outcome of the genetic profiles, and (c) the need to implement a full hatchery program. If the temperature control device is constructed and implemented, a mainstem monitoring program and efforts to establish a mainstem population can proceed. If that effort succeeds, the cost of the program would be reduced by about \$1.5 million, which is the cost of a full-scale hatchery program. This is also true if the genetic profiles show no significant differences in genetic markers and fish are transferred from the

LCR to establish a new population in a tributary. If a hatchery program is necessary, the program should be used only to initiate a second population and not to supplement the population long-term.

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APPENDIX A
GRAND CANYON MONITORING AND RESEARCH CENTER FY99 REQUEST FOR PROPOSALS

APPENDIX B
ROLE OF HATCHERIES AND GENETIC CONSIDERATIONS

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Artificial hatchery propagation has long been a tool of fishery managers to meet increasing demands for recreational and commercial fishing (Incerpi 1996, Schramm 1996). More recently, hatcheries have been used as mitigation tools to offset habitat losses and to augment natural populations of threatened and endangered fishes. Use of hatcheries to augment or replace dwindling populations has come under increasing scrutiny with concerns over maintenance of genetic diversity (Hindar et al. 1991, Hilborn 1992, Meffe 1992, Nehlsen et al. 1991, Waples 1999).

B1.0 HATCHERIES IN RECOVERY OF ENDANGERED COLORADO RIVER FISHES

Hatcheries have been variously incorporated into recovery efforts for the Colorado River fishes, including development of culture techniques (Toney 1974; Inslee 1982; Hamman 1981a, 1981b, 1982a, 1982b, 1985a, 1985b, 1986, 1987, 1989; Jensen 1986) and for producing animals to use in laboratory experiments (Pimentel and Bulkley 1983a, 1983b; Marsh 1985; Berry 1984, 1988; Berry and Pimentel 1985; Black 1982; Thompson 1989; Black and Bulkley 1985a; 1985b; Muth and Nesler 1989). A Coordinated Hatchery Facility Plan (Wydoski 1994) and Genetics Management Guidelines (Williamson and Wydoski 1994) have led to the development of a Genetics Management Plan (Czapla 1998) for the Upper Colorado River Basin. A hatchery augmentation plan is being developed for the San Juan River by the San Juan River Recovery Implementation Program.

Four species of mainstem Colorado River fishes have been cultured in hatcheries: Colorado pikeminnow (*Ptychocheilus lucius*), razorback sucker (*Xyrauchen texanus*), bonytail (*Gila elegans*), and humpback chub (*Gila cypha*). Fewer humpback chub have been cultured and propagated in hatcheries than any of the other species.

B1.1 History of Humpback Chub in Hatcheries

Humpback chub were first taken to hatchery facilities as adult brood stock in 1978 (Hamman 1982a). Fourteen wild adults were collected from the Little Colorado River (LCR), Arizona, on 17 May 1978, 17 October 1979, and 13 June 1980; and 16 wild adults were collected on 5 November 1979 from the Colorado River at Black Rocks, Colorado, near Grand Junction. These fish were transferred to the Willow Beach National Fish Hatchery, Arizona, where they were held in concrete raceways lined with boulders and cobble as spawning substrate. Spawning was induced with intraperitoneal injections of 4 mg acetone-dried carp pituitary per kilogram of body weight. Some adults (9 females, 5 males) were allowed to spawn naturally on the cobble substrate and were removed from the raceways as soon as the eggs began to hatch. Other adults (9 females, 7 males) were injected with the same dose of carp pituitary at 24-hour intervals until they could be manually stripped. The eggs were held in hatching trays, and the young were reared in fertilized raceways that produced a plankton bloom. Average fecundity was 2,523 eggs/female or 5,262 eggs/kilogram of body weight. Hatching success was highest at 19-20 C, and percent swim-up was highest at 21-22 C. Totals of 600 eggs and

2,700 fry were transferred to Utah State University (Bulkley et al. 1982, Berry and Pimentel 1985) and University of Idaho, respectively, as well as Arizona State University (Marsh 1985) for laboratory experiments. A total of 7,600 juveniles (75-150 mm TL; marked with coded-wire nose tags) were released in December 1981 by the U.S. Fish and Wildlife Service (FWS) in Cataract Canyon (Pers. Observation, Richard A. Valdez, SWCA).

Adults from Black Rocks were also used in crosses with bonytails from Lake Mohave, Nevada, and roundtail chub from Black Rocks (Hamman 1981b). These crosses demonstrated the viability of hybrid crosses, and the possibility that hybridization may occur among the three Colorado River forms of *Gila*.

In June 1980, three female humpback chub at Black Rocks, Colorado, were injected intraperitoneally with acetone-dried carp pituitary daily for 3 days and stripped of 4,000; 4,000; and 10,000 eggs (Valdes-Gonzales 1982). The eggs were fertilized with local wild adult males and transferred to Willow Beach National Fish Hatchery, Arizona, where they were incubated at 20-21 C with a hatching success of about 50%. The progeny and adults of these culture efforts were transferred from Willow Beach National Fish Hatchery to Dexter National Fish Hatchery in about 1980, when the latter assumed the role as the principal endangered fish hatchery for the Colorado River Basin.

Efforts to take humpback chub to a hatchery facility have been primarily for use in experimentation rather than for development of brood stock and subsequent propagation of large numbers of fish. On 26-27 April 1993, gametes were collected from pituitary-injected, manually stripped fish caught in the lower LCR (Clarkson 1993). Fertilized eggs were incubated 1-3 days in screens in the field and transported to Bubbling Ponds State Fish Hatchery, Arizona. The young were used locally for temperature experiments and some were transferred to Colorado State University for electrofishing experiments (Ruppert 1997).

None of the fish collected or propagated in hatcheries, as described above, remain today. The only humpback chub in hatcheries are about 175 of 350 juveniles transferred from the lower LCR to Willow Beach National Fish Hatchery in 1998 for experimental use by the FWS. These fish have been exposed to a variety of temperature experiments, and may not be suitable for release back to the wild as stock or initiating a new population. Additionally, seven adults captured in Black Rocks in 1997, were held temporarily at the FWS's Horsethief facility near Grand Junction, Colorado, and are now live on display at Oceans Journey in Denver, Colorado. Native fish hatcheries are under construction in the San Luis Valley of southern Colorado and under consideration in Utah.

B1.2 Release of Hatchery-Reared Colorado River Fishes

Release of hatchery-reared Colorado River endangered fish into the wild have had little success. Of 1,500 Carlin-tagged sub-adult Colorado pikeminnow (age 6) released in the Colorado River near Moab in April 1980, 13 were recaptured up to 14 months later and 74 km away, but none were found afterward. Of 7,600 coded nose-wire tagged humpback chub released in the Colorado River in Cataract Canyon in 1981, none were captured (Valdez 1990). Between 1981 and 1990, more than

11 million hatchery-produced razorback suckers and over 750,000 Colorado pikeminnow were released in historic ranges in the Verde and Salt Rivers in Arizona, where natural populations had been extirpated (Hendrickson 1993). Only 519 razorback suckers and 444 Colorado pikeminnow were recaptured in several years of intensive sampling. Most fish did not live more than a few months in the wild primarily because of poor adaptability by domestic fish to a wild environment and predation by non-native flathead catfish (*Pylodictis Olivaris*) and smallmouth bass (*Micropterus dolomieui*). Approximately 75,000 hatchery-produced razorback suckers and 100,000 Colorado pikeminnow were released into the San Juan River from 1994 to 1997 (Ryden 1997); sampling continues to evaluate the success of these releases.

B2.0 GENETIC CONSIDERATIONS

The emerging field of native and endangered fish management is beginning to recognize the importance of conservation genetics in recovery of populations (Meffe 1986, Soulé and Wilcox 1980). Genetic aspects must be considered and understood at the outset of management and recovery programs in order to maximize adaptive flexibility. Forces that erode genetic variability, including reduced population size, genetic drift, inbreeding depression, artificial selection, and mixing of distinct genetic stocks, can lead to increased homozygosity, loss of quantitative genetic variation, emergence of deleterious recessive alleles, and reduced fitness (Meffe 1986). Strategies that conserve genetic diversity are outlined below:

1. Conserve humpback chub populations or aggregations in Grand Canyon for their genetic potential for recovery, applicable legal mandates, and social or cultural values.

2. Facilitate natural reproduction and recruitment, where possible, to develop and expand self-sustaining populations.

3. Use, when possible, fish from related genetic aggregations for transfer to initiate or supplement new aggregations.

4. Maintain natural genetic diversity of humpback chub aggregations in situations where supplementation with hatchery-reared fish is necessary.

5. Employ breeding strategies that result in genetic diversity similar to that of wild aggregations where stocking of hatchery-reared endangered fish is intended to complement natural recruitment.

6. Conduct and evaluate experimental transfers or releases of fish prior to large-scale augmentation or supplementation.

B2.1 The Colorado River *Gila*

The cyprinid fishes of the genus *Gila* are a morphologically diverse group restricted to Western North America (Smith 1966, Minckley 1973, Minckley et al. 1986, Douglas et al. 1989, McElroy and Douglas 1995). Humpback chub (*Gila cypha*) inhabit canyon regions of the Colorado River Basin and are one of three morphologically distinct sympatric forms. Bonytail (*G. elegans*) are rare, but were once reported from most of the basin's largest rivers, and roundtail chub (*G. robusta*) inhabit large and small rivers of the basin. All three forms of *Gila* exhibit at least some extreme morphologies (e.g., nuchal humps, depressed occipitals, falcate fins, embedded scales, leathery skin), presumably reflecting adaptations to high variable flows, variable water temperature, and high turbidity (Miller 1946, Minckley 1973).

Morphological analyses (Douglas et al. 1989, McElroy and Douglas 1995) indicate that the three forms of Colorado River *Gila* maintain their distinctiveness in sympatry, with phenotypically intermediate individuals commonly noted (Valdez and Clemmer 1982, McElroy and Douglas 1995). Genetic inter-relationships among and within these species require clarity in order to develop appropriate conservation strategies that can eventually lead to recovery.

Genetics of *Gila cypha* are poorly understood. Allozymic and mitochondrial DNA (mtDNA) characteristics are unique among presently allopatric populations of *G. cypha*, *elegans*, and *robusta*, with evidence of local introgression through hybridization (Dowling and DeMaris 1993). It is hypothesized that introgressive hybridization has played a significant role in generating genetic diversity by providing additional genetic variation for selection and drift to mold into locally distinctive phenotypes. However, distinctive genetic markers within the phenotype recognized as *G. cypha* have not been identified, either for the five upper basin and one lower basin populations or for the nine recognized aggregations within Grand Canyon.

Morphological variation within and among populations of the *Gila* complex is extensive, and some have suggested that introgressive hybridization has contributed to the evolutionary history of this group of fishes (DeMaris et al. 1992, Dowling and DeMaris 1993). Nevertheless, preservation of genetic diversity is paramount in consideration of establishing new populations or expanding existing ones. *Gila cypha* and *G. robusta* show distinct morphological phenotypes, both in sympatry and allopatry (McElroy and Douglas 1995, Douglas et al. 1998). Intraspecific analyses indicate population divergence in both forms and considerable variation in morphology, suggesting as with genetic analyses, introgressive hybridization.

Clearly, studies of the genotype of humpback chub aggregations in Grand Canyon are imperative before fish from one aggregation can be used to augment another. Mixing of distinct genetic stocks can erode genetic variation and lead to increased homozygosity and reduced fitness (Meffe 1986, Walters 1986).

B2.2 Lessons from the Pacific Salmon Experience

Much of the recent debate about appropriate use of hatcheries has focused on potential genetic risks to Pacific salmon populations (Allendorf and Ryman 1987, Clune and Dauble 1991, Currans and Busack 1995). In the last 50 years, artificial hatchery propagation was viewed as appropriate mitigation for habitat loss, blocked fish passage, and increased commercial harvests. Some feel that large-scale hatchery programs for salmonids in the Pacific Northwest have largely failed to provide the anticipated benefits; rather these programs may pose the greatest single threat to the long-term maintenance of salmonids (Hilborn 1992, Meffe 1992). In the last decade, scientists have recognized that salmon stocks and stock diversity in the Pacific Northwest have declined at an alarming rate (Nehlsen et al. 1991, Baker et al. 1996).

Three major concerns are identified with hatchery augmentation of Pacific salmon: (1) levels of genetic variability in hatchery and wild populations may differ, (2) hatchery fish may become increasingly homozygous as compared to their wild counterparts, and (3) negative consequences may be associated with stocking hatchery fish on wild fish of a different stock, thereby altering the genetic makeup of locally adapted gene pools (Waples et al. 1990a, 1990b, 1991). Evidence also suggests competitive interactions between hatchery fish and dwindling wild fish for food, spawning and rearing areas, and other finite resources (Williams and Williams 1995). There is also concern for genetic consequences of artificial selection and swamping of remaining wild genes by domesticated hatchery fish genes. Perhaps the greatest threat is the perception that hatchery programs provide part of the permanent solution to declining fish populations. This may be true for certain species, but the likely causes for population decline of environmental degradation and overexploitation must be corrected first (Meffe 1992).

Campton (1995) and Busack and Currans (1995) recognized three factors that lead to genetic change in cultured populations: (1) intentional or artificial selection for a desired trait (e.g., rapid growth rate or large body size), (2) selection resulting from nonrandom sampling of broodstock, and (3) unintentional or natural selection that occurs in the hatchery environment. Waples (1999) suggested a fourth factor: (4) temporary relaxation during the culture phase of selection that otherwise would occur in the wild (i.e., altered mortality profile).

Lichatowich et al. (1995) recognized the need to approach restoration and management of renewable resources from an ecosystem perspective (Nehlsen et al. 1991, Doppelt et al. 1993, Snake River Salmon Recovery Team 1993) instead of a single species management perspective. Recently, restoration planners have proposed a new rebuilding objective for hatcheries. Instead of circumventing degraded habitat, hatcheries should be used to restore natural production. The use of hatcheries, called supplementation, account for 50% of the planned increases in salmon production in the Columbia River Basin (Regional Assessment of Supplemental Project 1992). Supplementation is defined as "the use of artificial propagation in an attempt to maintain or increase natural production while maintaining the long-term fitness of the target population, and keeping the ecological and genetic impacts on nontarget populations within specified biological limits" (Regional Assessment of Supplemental Project 1992). To reflect this new direction, The Northwest Power Planning Council (NWPPC)

(1982, 1994) revised its interim goals of doubling the size of salmon and steelhead in the Columbia River Basin to include the provision that this would be accomplished “without loss of biological diversity,” as a reflection of the belief that long-term sustainability of fisheries resources depends on conservation of natural populations. Nevertheless, minimum effective population size is a factor when considering Pacific salmon, and must be determined for stocks. The U.S. National Marine Fisheries Services (1987) determined that 200 adult Sacramento River winter chinook were needed to avoid irretrievable genetic loss.

Hatcheries are being increasingly used to supplement at-risk salmonid stocks in the Columbia River Basin (Bugert 1998). Under the supplementation concept, hatcheries programs are required to collect locally adapted populations for broodstock and to release their progeny in the same waters. Supplementation is the use of artificial propagation in an attempt to maintain or increase natural production while maintaining long-term fitness of the target population and keeping the ecological and genetic impacts on non-target populations within specified biological limits (Regional Assessment of Supplemental Project 1992). Supplementation is not considered the solution to declining salmon stocks, but is used in concert with actions that address limiting factors including habitat loss, passage mortalities, and over harvest of weak stocks. To make hatchery programs more effective, broodstock collections are diversified and smolt release strategies correspond to changing environmental conditions and population demographics. These efforts are being coordinated with local watershed restoration initiatives.

Where hatcheries are used as part of restoration and supplementation, specific goals are specified such as post-release survival of propagated fish relative to wild fish, reproductive success of released adults, or long-term fitness or genetic structure of hatchery and wild populations (Lichatowich et al. 1995). Sustainable restoration cannot be achieved through programs that focus entirely on numbers of fish. The specifications of resource quality (e.g., life histories, age structure, distribution) must be included in restoration objectives. To protect genetic diversity of existing fish populations, policies are being adopted that require hatchery programs to stock only progeny obtained from fish from the water system being augmented (Stickney 1994).

Nehlsen et al. (1991) caution against interbasin transfers of stocks because of the potential for introducing ill-adapted individuals into a foreign environment. They also recommend that artificial propagation in hatcheries should be greatly curtailed and reorganized in order to protect existing genetic diversity of salmon stocks. Walters (1986) identified the risks of mixing stocks on sustainability of enhancement production of chinook and coho salmon.

Currans and Busack (1995) recommend a genetic risk assessment based on vulnerability that emphasizes resilience and reliability in management of natural populations. They recommend incorporating known parameters of life history of hatchery-reared fish (e.g., survival of stocked fish, growth, genetic contribution, effect on wild fish) into probabilities of extinction. They recognize “vulnerability to domestication” may be greater than vulnerability to extinction and hence pose a greater threat to the population.

Some have incorporated the concept of watershed restoration into salmon recovery. Rahr et al. (1998) recommend “salmon refuges” as a watershed that contains (1) sufficiently complex and connected habitats, and biophysical processes to create and maintain those attributes through time (Stanford et al. 1996), (2) native populations of Pacific salmon capable of expressing a major part of their historical phenotypic (or life history) diversity, and (3) adequate protection to ensure persistence through time. An Independent Scientific Review Group (1999) recently concluded that restoration of salmonid fishes in the Columbia River depends on increase in normative conditions and management of existing natural aggregations to enhance their life history characteristics.

For several decades, hatchery production for population enhancement has been limited to relatively few species, mostly freshwater or anadromous fishes (Stickney 1994). The notable exception is the red drum (*Sciaenops ocellatus*), a marine form that has been successfully cultured and released to augment spawning populations in the Gulf of Mexico. In general, hatchery stocks of salmon were being released in large numbers as the wild populations were continuing to decline. Maintaining endangered fish stocks in hatcheries for one or a few generations may produce sufficient fish to introduce into the wild and either initiate new populations or augment existing ones. Such hatchery programs will not be effective if life history constraints persist, such as poor habitat, unnatural predation or competition, blockage to movement, limited food supplies, etc.

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