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4 June 2003

Dr. Steve Gloss
Program Manager, Biological Resources Program
GCMRC
2255 N. Gemini Dr., MS-5000
Flagstaff, Arizona 86001

Dear Steve:

Attached please find a draft report entitled: The Feasibility of Developing a Program to Augment the Population of Humpback Chub (*Gila cypha*) in Grand Canyon. This report falls under the auspices of Interagency Acquisition No. 98-AA-40-0040. To finalize this draft as quickly as possible, we will promptly address any comments identified following your review process. Should this document receive external review, we are requesting that Dr. Craig Stockwell (Craig.Stockwell@ndsu.nodak.edu), Dr. Carl Walters (c.walters@fisheries.ubc.ca) and Dr. Robin Waples (robin.waples@noaa.gov) be considered as potential reviewers. Please contact me if you have any questions and I look forward to continuing working with GCMRC.

Sincerely,

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cc: Rob Simmonds, Assistant Project Leader, AZFRO

attachment

DRAFT VERSION
FOR REVIEW ONLY

THE FEASIBILITY OF DEVELOPING A PROGRAM TO AUGMENT THE POPULATION OF HUMPBACK CHUB (*Gila cypha*) IN GRAND CANYON

By

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and

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EXECUTIVE SUMMARY

This report summarizes findings by the U.S. Fish and Wildlife Service on the feasibility of performing three management actions in order to promote the conservation of the humpback chub (*Gila cypha*) in Grand Canyon.

First, we address the feasibility of establishing a captive broodstock of humpback chub. Broodstock development is considered within the context of the Endangered Species Act, and within the context of captive propagation policy as defined by the U.S. Fish and Wildlife Service and the National Oceanic and Atmospheric Association. A literature search has been performed that discusses the potential biological risks involved with broodstock development. Such risks include (but are not limited to) introgression (loss of among population genetic variability), inbreeding depression, domestication, and potential to decrease the genetic effective population size in the wild population. Basic questions are discussed and answered concerning an approach to broodstock development (i.e., how many fish, where to collect, etc.). Finally, we list attributes that a hatchery would require in order to raise broodstock fish.

Second, we address the feasibility of establishing a program for captive grow out of wild caught young-of-the-year humpback chub for release into the wild. The primary risks associated with this potential management action appear to be related to ethological issues, such as lack of anti-predator responses or lack of ability to feed efficiently. In addition, depending on where the fish are released, a potential exists to impact density-dependant dynamics in the wild population.

Third, we address the feasibility of augmenting the Grand Canyon population of humpback chub via translocation. Considered are 1) translocation of fish above Chute Falls (14.2 km) in the Little Colorado River, and 2) translocation of fish into Bright Angel, Shinumo, or Havasu creeks in Grand Canyon. Translocation of fish above Chute Falls appears to offer some potential for a minor gain in the wild census population, but may involve potential genetic risks to the main population of humpback chub in Little Colorado River. Translocation of fish to other tributaries in Grand Canyon may offer potential for augmenting the mainstem aggregations of humpback chub, and genetic risks appear to be minor.

GENERAL OBJECTIVE STATEMENT

At the request of Dr. Steven Gloss from the Grand Canyon Monitoring and Research Center (GCMRC), the U.S. Fish and Wildlife Service (USFWS) has developed this report that examines the feasibility of three actions: 1) developing a captive broodstock to be used for captive breeding program for humpback chub, 2) establishing a supplemental stocking program for humpback chub in Grand Canyon using wild caught young-of-the-year (YOY) fish removed from the Little Colorado River (LCR) and grown out to a larger size in captivity, and 3) establishing a second spawning (or expand the current) population of humpback chub in Grand Canyon. Any one of these actions, singly or in concert with the others, is considered by the Service to be of primary mitigative importance to ameliorate impacts to the endangered humpback chub caused by Federal water development in the Colorado River basin.

The request from GCMRC stemmed from a request by the Adaptive Management Work Group for the operation of Glen Canyon Dam to perform a feasibility study for establishing a captive broodstock program. The establishment of a captive broodstock for humpback chub has been proposed as a potential conservation action (USFWS 1990), as has establishing a second population of humpback chub (USFWS 1990, USFWS 1994, USBR 1995). In addition, this report investigates the potential for supplemental stocking using wild caught fish grown out in captivity. Part of the rationale for investigating this approach is because this method is currently being used as a successful management action to conserve the razorback sucker population in Lake Mohave.

We stress that we only propose to investigate the feasibility of carrying out the above management actions; this document does not constitute a proposal to implement these actions, nor an endorsement by USFWS of these actions. The ultimate performance of any of the actions identified herein, and by whom, is not an element of this proposal. Any initiation of management actions will require thorough review both within the USFWS and among the appropriate cooperating agencies, as well as requiring additional funding to the agencies carrying out the actions, and would require long-term monitoring of the action. However, as a cooperating agency with GCMRC, we have agreed to perform this feasibility study. These efforts are coordinated with the Upper Colorado River Endangered Fish Recovery Program in an effort to better unify mitigation, management, and recovery efforts throughout the Colorado River Basin

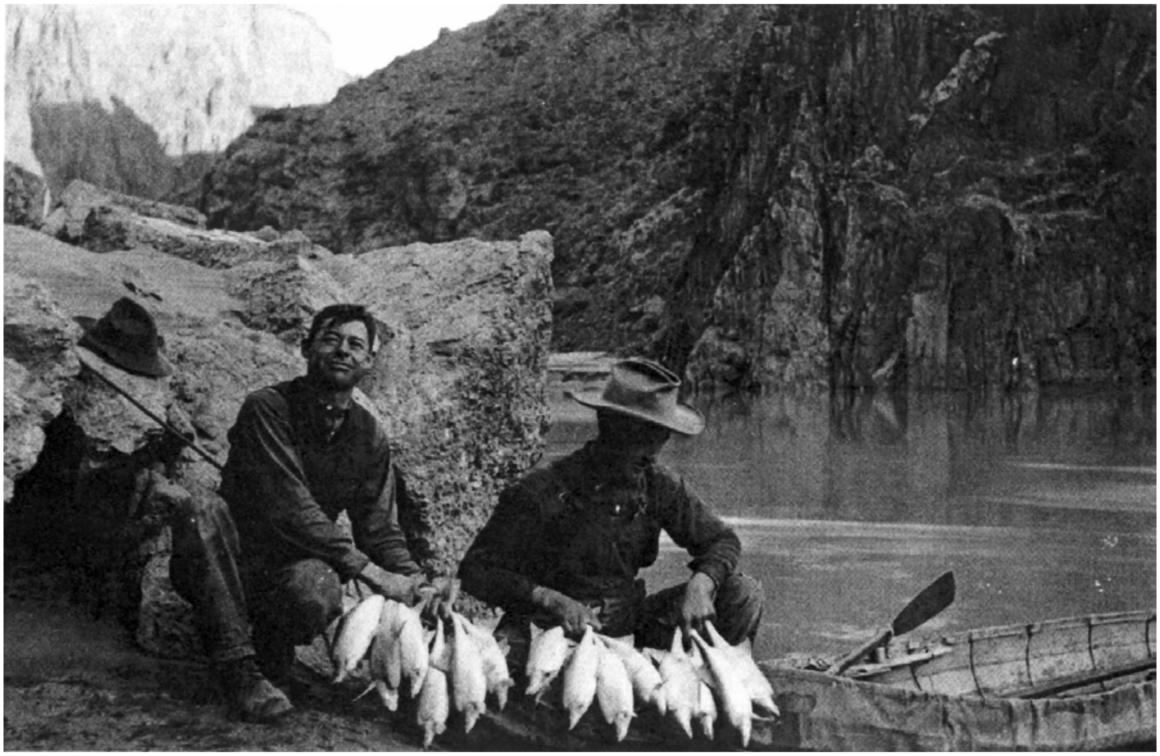
BACKGROUND

Status of Humpback chub

Humpback chub is a morphologically unique fish endemic to the Colorado River basin (Miller 1964, Minckley 1991), with origins extending as far back as Miocene (Miller 1959, Minckley et al. 1986). The species is a member of a relict native fish community, many members of which are locally extinct or declining. For example, three of eight native fish species have become extinct in Grand Canyon since the closure of Glen Canyon Dam in 1963, including the Colorado pikeminnow (*Ptychocheilus lucius*), bonytail (*Gila elegans*), and roundtail chub *(G. robusta*). A fourth, razorback sucker (*Xyrauchen texanus*), may also be extirpated in Grand Canyon (Minckley 1991). In Grand Canyon, humpback chub occupy unusual habitat relative to other populations in the watershed, largely inhabiting the LCR, a saline tributary to the mainstem Colorado. The Grand Canyon population also possesses the life history trait of being migratory spawners, and remains isolated from the dangers of hybridization with other species (although this is now the result of habitat fragmentation and local extinction of other congeneric species rather than natural biological processes). As such, these fish hold important ecological and evolutionary legacies.

Humpback chub was listed as endangered in 1967 (U.S. Office of the Federal Register 32:48 [1967]: 4001). In Grand Canyon, the species faces multiple threats, including widespread habitat loss (Suttkus and Clemmer 1979, Minckley 1991), watershed mismanagement (Abruzzi 1995), cumulative effects of environmental variation (see Gilpin and Soulé 1986), parasite loads in the LCR (Clarkson et al. 1997), and predation by introduced non-natives in the mainstem Colorado River (Valdez and Ryel 1995). Even though multiple deterministic causes of population decline have been identified, no known progress has been achieved in elevating population numbers since listing in 1967. In fact, evidence suggests the contrary.

Early accounts of the abundance of humpback chub in Grand Canyon, while sparse, suggest a much higher population than is present today. For example, the Kolb brothers witnessed humpback chub spawning in the mouth of the LCR in numbers so large that they described the thrashing of their fins upon the surface waters as sounding like “a landslide of shale” (Kolb and Kolb 1914). This simple description suggests very high densities of fish, reminiscent of fish feeding in a hatchery raceway; something not currently observed in the LCR. The two brothers referred to the fish as “bonytails,” but photographs show them to be humpback chub. Another photograph taken by the Rust expedition shows numerous very large chub captured during a day of angling in the mainstem Colorado River just above Bright Angel Creek (Photograph 1). As a dramatic comparison, since Glen Canyon Dam has been in place (nearly forty years), only a few humpback chub have been captured in this vicinity with very high intensity effort (i.e., hundreds of hours of trammel netting and electro-shocking). Minckley et al. (2003) calculated that there were some 200,000 humpback chub inhabiting the Colorado River during historic times.



Photograph 1. Early photograph of humpback chub taken on the mainstem Colorado River, a short distance upstream from Bright Angel Creek. Photograph from Grand Canyon Archive, Rust Collection.

Population estimates indicate that during the past twenty years, humpback chub in Grand Canyon have declined. Point population estimates have dropped from around 7,500 fish (>200 mm total length) during the late 1970s (Kaeding and Zimmerman 1982), to ~4,500 fish (>150 mm) in the early 1990s (Douglas and Marsh 1996), to ~2,090 fish (>150 mm) in spring 2001 (Van Haverbeke and Coggins 2003). Modeling based on the historic database of humpback chub in Grand Canyon has independently confirmed this declining trend (Coggins et al. 2003). The available evidence indicates that there has been an ongoing decline in the abundance of humpback chub in Grand Canyon since the emplacement of

Glen Canyon Dam, and that proactive management actions may need to be undertaken to reverse this decline.

Reasonable and prudent management actions expected to benefit the humpback chub in Grand Canyon were included in the Final Biological Opinion on the Operation of Glen Canyon Dam (USFWS 1994, USBR 1995). Primary among these was moving toward the attainment of riverine conditions that support all life stages of endangered and native fish species (i.e., primarily achievement of optimal flow and temperature regimes). Other reasonable and prudent elements included the development of a management plan for the LCR, and establishing a second population of spawning humpback chub downstream of Glen Canyon Dam (USFWS 1994, USBR 1995). To date, the Colorado River in Grand Canyon remains under a regime dominated by cold, hypolimnetic, fluctuating flows that are not supportive of all life stages of native fish.

As a result of increasing concern over the continued decline of humpback chub in Grand Canyon, additional management actions (or experiments) are being put forth. One such experiment that is being initiated by GCMRC is mechanical removal of nonnative fishes from the mainstem Colorado River near the LCR (Coggins et al. 2002). However, it is uncertain that this action alone will be sufficient to result in increased recruitment of humpback chub (Coggins et al. 2002). Three other actions that have been proposed for humpback chub are: 1) development of a captive broodstock (USFWS 1990), 2) supplemental stocking of wild fish, and 3) translocation of fish to currently uninhabited upstream reaches of the LCR or to other tributaries.

FEASIBILITY OF ESTABLISHING A SUPPORTIVE STOCKING PROGRAM USING HATCHERY PRODUCED FISH FROM A CAPTIVE BROODSTOCK

Below, we investigate the feasibility of establishing a captive broodstock of adult humpback chub and using fish produced from this broodstock to supplement the Grand Canyon population of humpback chub.

Augmenting (or supporting) wild populations through the release of captive bred individuals is increasingly being used in conservation (World Conservation Union 1987). In 1990, 27% of Federal recovery programs in the USA for endangered freshwater fishes included captive breeding as an element of recovery (Andrews and Kaufman 1994), and supportive breeding is a component of many of the management alternatives for the conservation and recovery of Pacific salmonids (*Oncorrhynchus* sp.; Hedrick et al. 1994, Waples and Drake 2002). Despite these trends, the merits of hatchery production have been increasingly challenged on grounds that supportive breeding often contributes to the problem of threatened or endangered species rather than being a solution (Hilborn 1992, Meffe 1992, Lichatowich et al. 1999, Levin et al. 2001, Levin and Williams 2002), and that the majority of such activities have been dismal economic failures (Hilborn 1998, Naylor et al. 2000).

As a result, managers for threatened or endangered species sometimes face a potential double jeopardy situation. On one hand, failure to intervene in the face of a deterministic decline for an endangered animal might result in extinction. On the other hand, using captive broodstock for supplementation purposes has a potential to result in changes (primarily genetic) that may reduce sustainability or viability of the wild population. The following section discusses several specific questions pertinent to development of broodstock for humpback chub.

<u>Is a captive adult broodstock needed at this point in time, and what will it contribute</u>?

A central question currently regarding humpback chub in Grand Canyon is whether or not it is appropriate at this point in time to develop a broodstock, and what will it contribute? Captive broodstock is sometimes considered when risk of extinction is high enough that this type of conservation measure is viewed as justifiable. The hope is that supportive breeding will give a demographic boost to the wild population and, hence, presumably decrease risk of extinction. Unfortunately, this potential for gain is accompanied by numerous and substantial genetic and behavioral risks to the wild population. Although there is probably no clear-cut answer that can be guaranteed correct, the following discussion has taken its base from the literature and may be useful in guiding managers to make a correct decision.

First, we review broodstock development of an endangered species in the context of the Endangered Species Act (ESA) and in the context of USFWS policy regarding controlled propagation. The purpose of the ESA is “to provide a means whereby the ecosystems upon which endangered species depend may be conserved.” The ESA does not specifically treat controlled propagation in detail, but does provide some exception guidelines for propagation and experimental populations. However, the USFWS and the National Oceanic and Atmosphere Administration (NOAA) do provide policy guidelines regarding controlled propagation of listed species (U.S. Office of Federal Register 65:183 [2000]: 56916-56922). This document unambiguously explains that “controlled propagation is not a substitute for addressing factors responsible for an endangered or threatened species’ decline”, and that the “first priority is to recover wild populations in their natural habitat wherever possible, without resorting to the use of controlled propagation.” Policy within this document also states that controlled propagation “will be used as a recovery strategy only when other measures employed to maintain or improve a listed species’ status in the wild have failed, are determined to be likely to fail, are shown to be ineffective in overcoming extant factors limiting recovery, or would be insufficient to achieve full recovery.” Furthermore, “all reasonable effort should be made to accomplish conservation measures that enable a listed species to recover in the wild, with or without intervention (e.g., artificial cavity provisioning), prior to implementing controlled propagation for reintroduction or supplementation.” Importantly, the policy also states that controlled propagation will be “based on the specific recommendations of recovery strategies identified in approved recovery plans or supplements to approved recovery plans whenever practical.” Furthermore, the “recovery plan, in addressing controlled propagation, should clearly identify the necessity and role of this activity as a recovery strategy.” As defined in the document, controlled propagation includes the production of individuals for “reintroduction to the wild to establish new populations”, and to the “holding of offspring for a substantial portion of their development or through a life-stage that experiences poor survival in the wild.” Additionally, controlled propagation must not be carried out as a recovery option without addressing potential benefits and risks (both genetic and ecological); and that prior to release of propagated individuals, controlled propagation must be tied to the development of a reintroduction plan. In short, the policy statement repeatedly emphasizes that controlled propagation (including the holding of offspring and reintroduction) should not be undertaken until all other less intrusive recovery options to recover the species in the wild have been tried and shown to fail. Furthermore, the document is quite clear that controlled propagation should be identified as a recovery option in an approved recovery plan document.

From a legal perspective, this could be problematic concerning many of the options discussed in this proposal. The 1990 Recovery Plan for humpback chub identifies broodstock development, reintroduction and augmentation as specific recovery needs and strategies (USFWS 1990). However, the most recent Recovery Goals for humpback chub (USFWS 2002a) make no such provisions. In addition, the most recent recovery goals call for self-sustaining populations in order to meet downlisting and delisting criteria, as opposed to population augmentation via hatchery production.

Second, we review broodstock development within the context of the priorities of a conceptual plan for managing fishes of the lower Colorado River (Minckley et al. 2003): 1) prevent extinction; 2) perpetuate existing genetic variability; 3) stabilize population(s); 4) expand population(s); 5) achieve self-sustaining population(s); and 6) work toward recovery. At this point in time, Grand Canyon has a declining population of humpback chub. In view of this population trend, it could be proffered that actions for expanding or stabilizing the population should be undertaken (i.e., actions should be taken to address #3 or #4 in the list of priorities stated above). These include developing or creating habitats of sufficient physical, chemical, and biological quality (or improving already existing habitat), followed by the obtainment of sufficient numbers, population structure, and genetic viability (Minckley et al. 2003). At worst, humpback chub in Grand Canyon may currently be in a situation whereby there is a need to perpetuate the existing genetic variability. This is assuming that there may already be some genetic risk posed to the humpback chub in Grand Canyon because the current population level of adults may be lower than the minimum viable population standards given in the recovery goals for humpback chub (Van Haverbeke 2003, *in review*). This would suggest there could be a need to plan and implement genetic management, and to develop broodstock. Although few would argue that the humpback chub is not at risk of extinction, which would definitely indicate a need to secure broodstock, immediate extinction of the humpback chub is probably not imminent.

Third, we review broodstock development through the context of some of the current literature. A thoroughly exhaustive review of the literature is beyond the scope of this document; however, an effort has been made to be as thorough as practical within the bounds of this document.

The first step in designing a captive breeding program is to clearly define its objectives (Frankham et al. 1986). For humpback chub, the following two objectives seem most relevant: 1) long term conservation of genetic variability, and 2) captive breeding for release back into the wild. Generally, the first objective applies to a species whose wild habitat may be lost and whose whole future may lie in captive maintenance. For humpback chub, untested options are available for improving degraded habitat in order to reverse population decline. Nevertheless, a primary goal in development of a captive broodstock for humpback chub should be the conservation and retention of maximum genetic variability. In the case of the second objective, it is of prime importance to consider the likelihood for future reintroduction (Seal 1986). Hence, if a broodstock is to be developed, several important considerations must be faced. First, how soon is reintroduction into the wild to be expected? The longer fish are held in captivity (especially in terms of numbers of generations), the more likely that divergence from the wild population will occur within the captive population via processes of inbreeding, drift, domestication, etc. This implies reintroduction in the very near future (i.e., probably within 4 to 8 years, or within one generation for humpback chub). Second, once broodstock and supportive stocking activities are initiated, it is often critical that these activities are long-term commitments (i.e., essentially permanent commitments that are very costly). This is generally because carrying capacity conditions for the species in decline have not been rectified. Hence, the demographic boost achieved by supportive breeding can be short term, and followed by collapse to pre-stocking levels, creating a worse situation than if supportive breeding had never been performed. These reasons are further discussed below. Third, captive broodstock activities can present a suite of risks to the wild population that must be seriously considered in order to prevent (if possible) costly or irrevocable mistakes. The above concerns by necessity lead into a discussion and review of genetic and ecological considerations.

It should be understood that the literature is replete with redundant unambiguous warnings concerning the pitfalls of captive breeding programs (e.g., Ryman and Laikre 1991, Waples and Do 1994, Busack and Currens 1995, Philippart 1995, Snyder et al. 1996, Utter 1998, Ford 2001, Lynch and O'Hely 2001). Limitations of captive breeding include an array of problems associated with genetics, difficulties in achieving self-sustaining captive populations, failure to breed well in confinement, inability to achieve successful reintroduction back into the wild, problems with domestication of animals (i.e., loss of wild traits), disease transmission, high financial costs, and concern for administrative continuity associated with developing and maintaining a proper broodstock facility (Snyder et al. 1996). Movement of animals from the wild to a captive breeding station is considered the most extreme form of relocation (Philippart 1995), and captive breeding should be viewed as a last resort to species recovery (Snyder et al. 1996). Furthermore, the use of captive broodstocks should not be considered as an effective means for the long-term safeguard of most species and strains (Nehlsen et al. 1991), and should only be used when all other possibilities aimed at conserving a species in its natural environment have been exhausted (Philippart 1995). In addition, captive broodstock stocking activities should not be a factor that leads to the lessening of habitat and aquatic ecosystem conservation and restoration (Philippart 1995). A very real concern is that funding and attention expended for ex situ recovery efforts (i.e., captive broodstock) often preempts funding and attention for in situ recovery efforts (e.g., improvement of habitat; Snyder et al. 1996). This is because long term solutions to conserve populations in the wild are often politically more difficult than captive breeding solutions, tempting managers to de-emphasize efforts for wild populations once captive broodstocks are in place (Snyder et al. 1996). Clearly, much of the literature coincides with the perspective of USFWS's and NOAA's policy on captive propagation (U.S. Office of Federal Register 65:183 [2000]: 56916-56922); namely, that captive propagation should only be attempted when all other conservation actions for restoring the population in the wild have failed.

One of the main concerns raised in the literature is that supportive breeding via the use of captive broodstocks can pose genetic risks (or hazards) to wild populations (Ryman and Laikre 1991, Busack and Currens 1995, Ford 2001, Lynch and O'Hely 2001). Below, we briefly identify some of the major genetic risks and offer solutions for these risks (as recommended in the literature). We caution that the risks are multiple in nature, and that full consideration of these factors should be presented and expanded in a formal broodstock management plan, should such an action be initiated.

First, there could be risk of artificial introgression (for instance, introducing genes from other humpback chub populations outside Grand Canyon or from congenerics). Potential loss of among population variability should be a major concern (Busack and Currens 1995), and in order to avoid artificial introgression, broodstock should be obtained directly from the population into which their offspring will be released (Krueger et al. 1981, Hindar et al. 1991, Ryman et al. 1995). This factor holds major implications, if broodstock activities are coordinated using other populations of humpback chub from the Upper Basin. It also can hold major implications for choice of hatchery and for hatchery operations (for instance, the risks of introgression occurring if other species of *Gila* are on station).

Second, there is risk of inbreeding occurring within the hatchery population. Traits that frequently exhibit inbreeding depression are quantitative or multilocus, and are associated with reproductive capacity and physiological efficiency (Kincaid 1983, Lande 1981). In order to maintain sufficient variability in hatchery populations, a total of 50 to 500 genetically effective founding breeders have been recommended in the past (Franklin 1980, Frankel and Soulé 1981, Hynes et al. 1981, Kincaid 1983). However, current genetics theory suggests that these numbers may be at least an order of magnitude too low for preserving quantitative variability (Lande 1995). The primary danger is that if hatchery fish are deficient in overall genetic variability, this may in turn decrease genetic variability in the population into which they are released. Lande and Barrowclough (1987) point out that once quantitative variability is lost, a population must regain and sustain high abundance for many hundreds to thousands of generations until that variability is replaced by new mutations. The above implies that 1) in order to fully retain genetic variability in a captive broodstock, several thousand individuals may be needed, and 2) if quantitative variability is reduced in the wild because of inappropriate hatchery actions, the loss is very long term (i.e., an irrevocable mistake can be made).

Third, there is a concept often not considered by managers concerned with captive propagation, but that appears to be contributing to the demise of fisheries on a worldwide basis (Tringali and Bert 1998). Namely, genetic hazard can be imposed upon wild populations via the release of broodstock individuals, resulting in a reduction in effective population size (Ryman and Laikre 1991, Waples and Do 1994, Ryman et al. 1995, Wang and Ryman 2001). Captive bred populations are usually created using only a very small proportion of the wild population. Hence, the captive portion of the population has a low genetic effective population size (N_e). The danger comes from a large portion of the captive bred offspring breeding upon release with the wild population (Ryman and Laikre 1991, Lynch and O'Hely 2001). Hence, the overall N_e (and genetic fitness) of the wild population can be reduced to levels dramatically lower than it would have been with no captive propagation and supplemental stocking (Ryman and Laikre 1991, Waples and Do 1994, Ryman et al. 1995, Wang and Ryman 2001).

A low N_e in the wild becomes an accurate predictor of extinction, because of linked mechanisms of reduced gene flow, genetic drift, reduced within population variability, and inbreeding depression (Lacey 1987, Lynch et al. 1995). Because of this effect (termed the Ryman-Laikre effect), genetic variation in supported populations may be at risk, even when presumably adequate numbers of breeders are used. This risk is especially high for fishes, that have high and variable reproductive rates (Tringali and Bert 1998).

Importantly, if the underlying problems for population decline in the first place have not been addressed, such as habitat destruction (Meffe 1992), supported populations of fish can be subject to a "supplementation and crash" scenario (Waples and Do 1994). This is basically because the newly augmented population exceeds carrying capacity. The supplemented population can then become susceptible to the combined effects of a reduction in N_e, swamping of wild-population alleles by those from hatchery fish, and future drift-associated changes caused by the population crash (Tringali and Bert 1998). If supportive breeding does not result in substantial and continuous increase of the census size of the breeding population, it might be genetically harmful because of an overall drop in N_e, and elevated rates of inbreeding and genetic drift (Waple and Do 1994, Wang and Ryman 2001). The end result is that supported populations can end up being more at risk to extinction then they would have been with no captive propagation and supplementation activities in the first place.

Some guidelines for avoiding reductions of N_e are given in Tringali and Bert (1998). For example, in wild populations with an initial N_e greater than 500, a relative hatchery contribution of less than 17% should not drive the total N_e to or below 500, provided a sufficient number of hatchery breeders are used (≥ 50). However, even using 100 effective hatchery breeders, and regardless of the original wild N_e, hatchery contributions larger than ~45% will result in values of N_e below 500. This implies that hatchery supplementation, if it is used, should be a very slow and protracted operation in order to minimize risk.

Perhaps even more serious (because of their small numbers, and relaxation of wild selective forces), captive bred individuals can undergo domestication, a process of rapid and significant evolutionary change in morphological, behavioral, and physiological traits that compromise fitness in a natural setting

(Kohane and Parsons 1988, Arnold 1995, Frankham and Loebel 1992, Ruzzante and Doyle 1993). Captive populations can rapidly accumulate deleterious alleles (i.e., they can rapidly accumulate behavioral or morphological traits that are conducive to living in a hatchery situation, but are deleterious in the wild; Lynch and O'Hely 2001). With sufficient gene flow of deleterious alleles from the captive population, the wild population can ultimately become transformed into a genetic state such that complete collapse can occur in the absence of continued supplementation (Lynch and O'Hely 2001). Thus, the benefit of achieving a demographic boost to the wild population can be easily offset by the potential to simultaneously decrease the genetic variability (heterozygosity) of the wild population (hence reducing fitness of the wild population). This problem increases over time, because serious depletion of heterozygosity is more likely when a population is supported for multiple generations by hatchery-raised fish (Ryman and Laikre 1991).

Sometimes, these problems are addressed by continually introducing wild individuals into the captive stock (Utter 1998). However, Ford (2002) found that substantial phenotypic changes and fitness reductions can occur even if a large fraction of the captive broodstock is brought in from the wild every generation. He suggests that regularly bringing in wild-origin broodstock into captive populations cannot be relied upon to eliminate the effects of inadvertent domestication, although the rate will be reduced compared to a completely closed captive population. Ford (2002) also pointed out that attempting to minimize selection for domesticated traits in captivity can help to alleviate the problem; however, the wild population is not protected from a decline in fitness unless gene flow from the captive population approaches zero. What these types of results mean is that the very populations in need of supplementation (such as endangered species with low population abundances) can easily become the most susceptible to the deleterious effects of gene flow from captive propagation (i.e., the fraction of surviving captive offspring entering the wild population becomes larger, together with the increasing associated risks).

Given the above cautions, there is a term called “conservation aquaculture” or “conservation reintroduction” (Anders 1998, Brown and Day 2002). Conservation aquaculture is the use of aquaculture for conservation and recovery of endangered fish populations. Its goal is to conserve wild fish populations and their locally adapted gene pools, including the characteristic phenotypes and behaviors (Anders 1998). In theory, it differs from standard hatchery production practices that traditionally focus on production of large numbers of fish. Conservation aquaculture is considered justified by some when fish populations in the wild become too small (i.e., when N_e in the wild becomes too small; Anders 1998). Ideally, conservation aquaculture should be performed before populations in the wild reach critically low levels (i.e., low N_e). The practice should be complimentary (rather than in lieu of) other conservation measures designed to improve seriously degraded habitat (Anders 1998). Furthermore, if hatchery programs ignore the risks associated with aquaculture (inbreeding depression, domestication selection, disease, etc.), failure is certain (Brannon 1993, Anders 1998).

Conservation aquaculture should (in theory and in practice) reduce common risks associated with standard hatchery procedures, such as competitive feeding behaviors, reduced growth rates, domestication selection, and increased incidence to disease (Anders 1998). Brown and Day (2002) discuss some specific techniques that can be used to overcome some of these problems, including environmental enrichment, life skills training, and soft release protocols. Basically, these techniques are used to overcome ethological (behavioral) problems rather than genetic problems. These behavioral problems are discussed below.

Fish that are held in captivity for a significant portion of their lives are removed from natural learning experience that would ordinarily be gained in the wild. Hence, their behavior can be altered in ways that severely impact survivorship, and ability to reproduce upon release into the wild (Brown and Day 2002). The most important effects appear to be lack of development of anti-predator responses (Vincent 1960, Olla et al. 1998, Brown and Day 2002), lack of ability to feed efficiently (Ersbak and Haase 1983, Brown and Day 2002), and reduced reproductive performance (Jonsson et al. 1990, Fleming et al. 1997). For instance, early life experience for migrating salmon has been shown to be important for ascending their natal river to spawn (Hasler and Scholz 1983, Hansen and Jonsson 1994, Jonsson et al. 1994), and for locating breeding sites (Jonsson et al. 1990). These types of effects might be particularly relevant to humpback chub in Grand Canyon, since a large portion of the population migrates.

To offset some of these concerns, conservationists are calling for an interface between ecology and behavior, particularly in reintroduction biology (Olney et al. 1994, Clemmens and Buchholz 1997, Caro 1999a and b, Gosling and Sutherland 2000). Olla et al. (1994, 1998) suggested it is critical for hatcheries to implement methodologies that improve post-release behavioral survival. Brown and Day (2002) suggest a number of hatchery procedures that can assist in making fish more ecologically viable once release occurs. Among these are environmental enrichment, pre-release training programs, and soft release protocols. Environmental enrichment simply means matching captive conditions to natural conditions. It can include any number of variables, including matching natural photoperiods, water flow rates, substrates, submerged and overhead cover types, turbidity levels, temperature, water chemistry, etc. (Wiley et al. 1993, Maynard et al. 1995). Pre-release training programs are designed to teach skills to fish that they will need to survive in the wild (Suboski and Templeton 1989, Brown and Leland 2001). Primarily, these include exposure to predators and natural foraging behaviors (i.e., using natural food types; see Olla et al. 1998, Brown and Day 2002 for reviews). Pre-release training does not necessarily need to be cost intensive, and can be initiated only a few days prior to release in order to obtain positive results (Brown and Day 2002). Soft release protocols enable fish to recover from stress of transport, become accustomed to the natural environment (temperature, water chemistry, current, etc.), and allows them to develop social bonds where appropriate. Essentially, this involves holding fish in pens for a period of a few days at the release site in order for them to adjust to their new environment. This acclimatization period can significantly decrease mortality, and can be accomplished in just a few days (see Brown and Day 2002 for review). Other major concerns relating to release of captive bred individuals into the wild relate to transmission of parasites and changes in habitat utilization (Utter 1998, Waples and Drake 2002).

Given the above information, managers will still need to decide whether or not a captive broodstock is needed, and know what it will contribute. Our best assessment as to the predicted status of humpback chub follows verbatim from Coggins et al. (2003): "Straight-line extrapolation of the recent trend estimates would imply a significant risk of extinction for the LCR spawning population within the next 10-15 years. However, this prediction is not supported by estimates of recruitment rates of 2-year old fish. Those rates appear to have been relatively stable since the early 1990s, though at considerably lower levels than would be needed to maintain the spawning population at 1989 levels. If recruitments continue to be stable, we predict that the spawning population will soon stop declining, and will stabilize at an average spawning abundance of roughly 50% of its current level, and that average will most likely be between 1,000 and 2,500 fish. That is, the assessment data do not in fact support demands for emergency policy actions."

Captive propagation is not included in the most recent Recovery Goals for the humpback chub, and therefore should not be undertaken as a recovery option. Since Recovery Plans must be reviewed every five years (ESA 1973), and the species is not in immediate risk of extinction, development of a captive broodstock could be listed as an option for recovery in the near future (i.e., within a generation time for humpback chub). It should primarily be considered at this point as a significant commitment to mitigate the past 50 years of Federal water development in the basin. From a policy standpoint, USFWS, NOAA, and the scientific community make it clear that captive broodstock activities should be considered as a last option for recovery, and that all other options should be exhausted. Although some actions have been undertaken (i.e., fluctuating flows have been modified, and some short-term flow experiments have been performed), other options that may improve the situation for humpback chub should be made available (e.g., thermal control devices placed in Glen Canyon Dam). Furthermore, within the past year options for predator removal in Grand Canyon have been increasingly considered as viable management options. Although in their infancy, large-scale predator removal efforts appear to be showing promising results (in terms of predator depletions), and should be given time. Such options as simultaneously warming mainstem waters and removing predators have not yet been attempted. In short, the efforts to date to improve natural recruitment of humpback chub in Grand Canyon have been minimal, or have only begun, and many of the major options have not yet been attempted.

From a genetics standpoint, captive broodstock activities are inherently very risky. Simply put, there is considerable risk of creating a worse situation by carrying out captive broodstock activities prematurely, or incorrectly. Waples and Drake (2002) strongly caution that even when managers are made aware of all foreseeable risks, uncertainty is high, and that the chances are good that unexpected developments will erase the projected benefits. Concerning the economic aspect alone, Hilborn (1998) states that based upon historical experience, politicians, managers and advocates of new stocking programs should realize that there is very little empirical data that supports the long held belief that supportive stocking has ever been biologically successful, and that these people should begin with about 80% expectation that the program will not be economically viable.

The target number for downlisting/delisting of humpback chub is 2,100 fish per population, based upon a N_e of 500 (USFWS 2002a). This number is considered a minimum in terms of viable population standards (Soulé 1980, Franklin 1980), and is considered by many authors to be an inadequate safeguard against extinction (Shaffer 1981, Simberloff 1988, Boyce 1992, Lande 1995, Minckley et al. 2003). Hence, managers for the humpback chub face a difficult situation. On one hand, there may be a need to demographically boost populations of humpback chub in order to meet the proposed minimum viable population standards and reach recovery. On the other hand, undertaking a management action (such as release of captively propagated fish) has the potential to further reduce $N_{e,}$ or lead to introgression of deleterious alleles (by the processes mentioned above); thus further jeopardizing the humpback chub. It is stressed again that the literature repeatedly calls for exhausting other means (such as improving habitat), in conjunction with, or before broodstock activities are undertaken.

The above should not be construed to preclude preliminary efforts toward a captive broodstock. The main benefit of a captive broodstock at this point in time should probably not be to significantly contribute toward a demographic boost in the population of humpback chub in the LCR. Rather a primary contribution of a captive broodstock should be to capture and maintain maximum genetic variability. Here, there is much work to be finished. For example, the ongoing genetics work on humpback chub should be completed. A formal and comprehensive captive broodstock development plan should be completed. The genetic variability of any humpback chub in captivity should be compared to the genetic variability of the population at large in the LCR. It would be advisable to complete, and to perform similar genetics work on the aggregation of humpback chub at 30-mile. In other words, to capture and maintain maximum genetic variability in the event of initiating a future captive broodstock program, it is advisable to accelerate and complete as much preliminary genetics work as is needed.

<u>How many fish will be needed, what size fish should be collected, when, where, and how?</u>

For the purposes of securing broodstock fish, all options should be identified in a formal broodstock management plan, and decisions based from there. Briefly, there are a number of approaches that could be taken to start a broodstock including streamside spawning (i.e., collection of fertilized eggs), collection of younger fish such as YOYs, or collection of spawning sized adults. As stated above, the primary objective of a broodstock program should be to retain maximum genetic diversity. We offer a very rough outline below, but caution that until genetic concerns are addressed, very little can be considered as a concrete plan of action. In other words, development of a captive broodstock will by necessity need to be an adaptive management process.

An appropriate broodstock for humpback chub might entail holding up to several thousand fish. As mentioned above, although a total of 50 to 500 genetically effective founding breeders has been recommended in the past for broodstock development, genetics theory indicates that these numbers are too low for maintaining quantitative variability (Lande and Barrowclough 1987, Lynch et al. 1995, Lande 1995). For example, Lynch et al. (1995) suggests maintaining long term population sizes > 1,000 in order to avoid problems with mutation loads, and Lande (1995) suggested that the Franklin-Soulé number (N_e = 500) should be increased by a factor of ten, to N_e = 5,000. As a result of a desire to hold as many fish as possible to maintain genetic integrity, and as a result that few hatchery managers would entertain the idea of holding more than a few thousand fish on site for a broodstock, ~3,000 fish may be a rough goal (C. Keeler-Foster, Dexter National Fish Hatchery and Technology Center [DNFH & TC], pers. comm.).

As a starting point for broodstock collection for humpback chub in Grand Canyon, it has been suggested by some to remove the small mainstem aggregation from the Fence Fault area (near river mile [RM] 30). This aggregation is suspected of being a last remnant of the mainstem spawners in Grand Canyon, but recruitment is thought to be absent (Valdez and Masslich 1999). Based on multiple mark-recapture, the small aggregate is thought to be comprised of about 50 adult fish (Valdez and Masslich 1999). This may be a potential starting point in order to begin development of a broodstock (C. Keeler-Foster, DNFH, pers. comm.). It should be mentioned here that until genetic analyses indicate that these fish are not distinct from LCR fish, these fish may have to be maintained as a separate broodstock in order to avoid potentially swamping this presumed mainstem genotype with the LCR genotype. It should also be mentioned that there has been documented movement of fish between 30-mile and LCR, indicating that these fish are not totally isolated. Hence, without substantive genetic information, removal of fish from 30-mile may only serve to needlessly extinguish this group of fish from the wild. An alternative to capturing the last remaining adults at 30-mile might be collecting eggs or YOY from this group of fish (B. Persons, Arizona Game and Fish Department).

Another potential source of broodstock is the ~120 humpback chub currently held at Willow Beach NFH. During July 1998, ~450 YOY humpback chub were removed the LCR, and flown to Willow Beach NFH for use in temperature growth studies (Gorman and Van Hoosen 2000). About 120 of these fish remain. Although these fish came from the LCR, they were never intended to form the nucleus of a breeding program. They were all collected during a single day within a short reach of the LCR (10 to 12 km), and may not fully reflect the genetic variability in the population as a whole. Developing the genetic "fingerprint" of these fish and comparing it with reference samples from throughout Grand Canyon would be absolutely necessary (see Appendix 1).

The strategy involved in using either the 30 mile aggregation or the Willow Beach NFH fish as a starting point for developing a captive broodstock entails 1) determine the genetic constitution of the original group(s) of captive fish 2) compare these respective small captive populations with the respective genetic constitution of the wild population(s) in the LCR or Grand Canyon, and 3) develop methodologies to ensure that the genetic constitutions of the original captive fish come to equal those of the wild population(s). This implies supplementing the small original captive populations with wild fish. This also implies using genetic techniques with high resolution, such as micro-satellite technology, or a combination of mtDNA and micro-satellite technology (Cross 2000).

Provided that either the Willow Beach NFH or the 30-mile fish are used to begin an initial broodstock(s), the next step might entail augmenting these broodstocks at a facility yet to be identified to increase genetic variability. Obviously, in order to build a broodstock of several thousand individuals that equal the genetic constitution of the wild population, more fish would be needed, regardless of what existing or new captive propagation facility is utilized.

Augmenting the 30-mile broodstock (if it is identified as a unique genotype from the wild LCR population), might be problematic since there may be no other fish in the Colorado River that are not part of the LCR complex, or other mainstem aggregations could be unique unto themselves. If the Willow Beach NFH fish are selected as an initial broodstock, we suggest capturing up to several thousand YOY humpback chub from the full reach of the LCR, over a period of several years. Using YOY fish will avoid depletion of wild adult fish, which are crucial for recovery by means of natural recruitment. The main purpose of temporal spacing would be to maximize the probability of capturing genetic variability. Since humpback chub are long lived, and all fish may not spawn each year (i.e., there may be some skip-spawning), collecting over a period of years might increase capturing variability. In addition, it may be advisable to collect fish over a series of months within each year. Gorman and Stone (1999) reported that spawning activity of humpback chub in the LCR commenced in late March, peaked in mid-April, and waned in mid-May. We know that the LCR hydrograph is variable from year to year, and assume that peak abundance of spawning fish is variable from year to year. One problem that may have to be considered in more depth is to avoid selection of fish in a manner that could disrupt timing of natural migration and spawning patterns. For instance, if all YOY collected came from a March spawn, this might select for fish that will only spawn in March. Such changes in natural migration and spawning patterns have been documented for salmonid broodstock (Fleming et al., 1997).

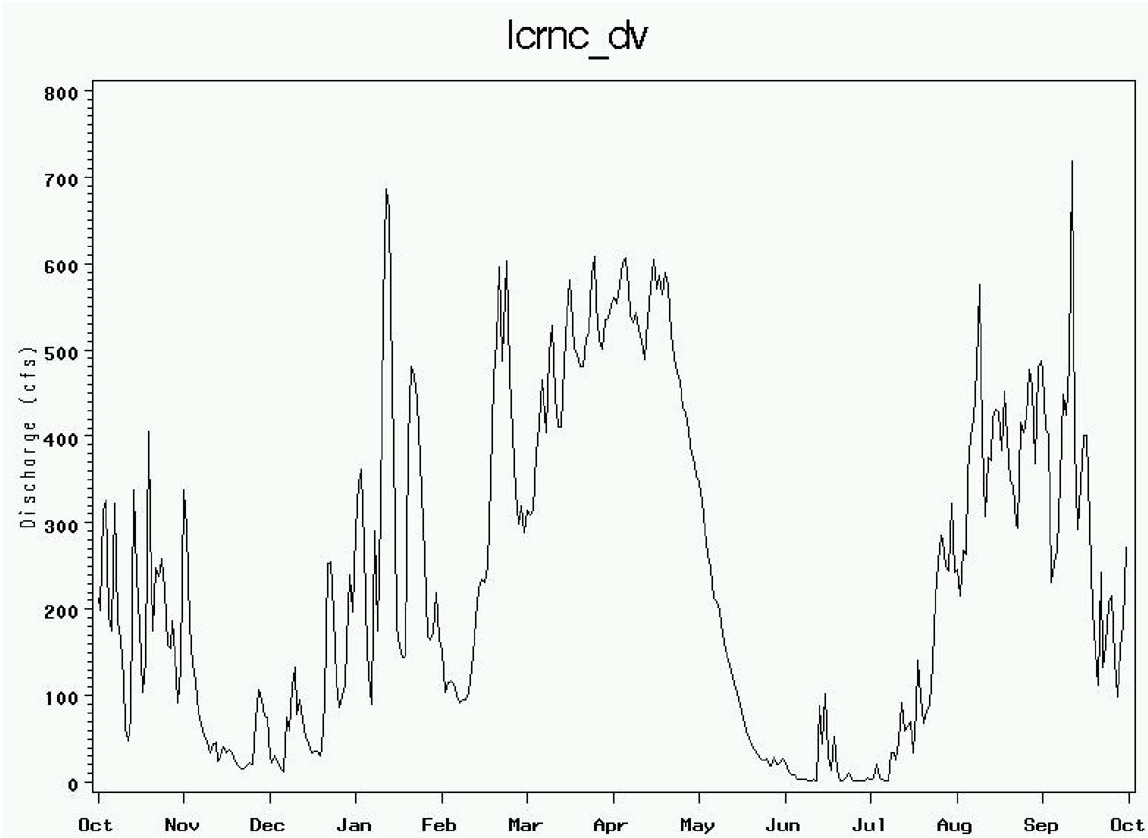
Additionally, we suggest that YOY fish are captured randomly from within the full reach of the LCR (i.e., from the confluence to 14.2 km). Again, this is to maximize the probability for increasing the genetic variation of the stock. Much as the potential problem that could result from not capturing fish on an appropriate temporal protocol, it should be considered that selective changes could occur from not capturing fish in an appropriate spatial manner. For instance, Douglas and Marsh (1996) hypothesized that the altered regime of the mainstem may be forcing humpback chub to adjust its life history, and that fish are being selected to be residents in the LCR. If this is the case, it could be possible that YOY fish collected in the lower reaches of the LCR may be more representative of mainstem migrants, while YOY fish captured in the upper reaches of the LCR may be more representative of humpback chub locally adapted to being residents in the LCR. Hence, there is a possibility of disrupting ongoing selective regimes via capturing an inappropriate proportion of fish from any given stretch of the LCR.

In order to maximize diversity by collecting fish spawned from maximum parental stock, we suggest random capture of fish between 0 to 14.2 km in the LCR. We suggest a first year attempt to capture an equal number of fish from Boulders, Coyote and Salt reaches (0 - 5 km, 5 - 10 km and 10 - 14.2 km respectively). It is likely that capture of YOY fish may be easiest in the Boulders or Coyote reaches, as catch-per-unit-effort of YOY humpback chub has been higher in these reaches in the past (Van Haverbeke and Coggins 2003). It may be advisable to keep the number of fish captured low during the first year (300 fish) in order to minimize impact on the wild population. Also, before the first fish is captured, all logistics, protocols, methods, etc. must be in place. The facility must also go through and pass a testing phase with surrogate species before the first humpback chub arrives.

Should a facility be selected for captive propagation and fully equipped to ensure compliance with health and genetic protocols, we suggest capturing fish within the 50 to 75 mm size class in order to reduce mortalities, although recognize that other options are possible such as collecting larval fish < 50 mm. Humpback chub within the 50 to 75 mm size class can be captured relatively easily with seines, and transported with minimum mortality (pers. obs.). Potential times for capturing YOY in this size class appear to be from June through November. This is when "spikes" of 50 to 100 mm YOY show up in the LCR (USFWS 2000, Van Haverbeke 2001b). Again, we suggest that in any given year, it may be advisable to capture the quota of fish from the main spring spawn over a series of months (or at least several weeks). Although it is possible to capture fish between June and November, the optimal months for capturing sufficient numbers of humpback chub in the 50 to 75 mm size class are probably from late July through the end of August (D. Stone, pers. com., D. Van Haverbeke, pers. obs.). For instance, in June, it may be possible to capture large numbers of YOY, however, most are likely to be < 50 mm (Van Haverbeke 2001a). The modal total length of YOY humpback chub in June 1993 was only 30 to 40 mm (Gorman 1994). The modal length of humpback chub did not reach > 50 mm until late July and early August during 1993 and 1994 (Gorman 1994).

In addition, the logistics of capturing a sufficient number of fish within this size class can be complicated by the hydrograph of the LCR (Figure 1). For example, looking at the hydrograph, it may seem that June would be the optimal month for capturing fish, when the LCR is most likely to be running at base flow. However, during spates, YOY humpback chub appear to concentrate in zero velocity, near shore habitat, and can easily be seined (D. Van Haverbeke, pers. obs.). For example, during late July 1998, about 450 YOY humpback chub were seined in a half-day under turbid water conditions between 10 and 12 km in the LCR (D. Van

Figure 1. Annual discharge and mean daily flow for water years 1948-1999 of the Little Colorado River at Cameron, AZ.



Haverbeke, pers. obs.). Hence, flood conditions can actually facilitate capture, since fish appear to be concentrated, and will not "see" the capture gear coming. On the other hand, many YOY are transported out of LCR by these late summer and fall flood events (Valdez and Ryel 1995). Hence, as flood conditions become more extreme (or more time passes under erratic flood regimes), progressively more YOY become lost to the mainstem, making capture of YOY fish increasingly more difficult. The easiest time to capture YOY chub seems to be during the initial stages of minor flood events (usually in late July or August). Flows in the LCR generally return to base flow conditions during November; however, by then, much of the YOY cohort has undergone mortality (or been transported to the mainstem) because of the often erratic and sometimes severe flood events in September and October (hence, capturing a large number of fish becomes problematic).

As a final option for building a broodstock, streamside spawning could be initiated. Streamside spawning generally entails capturing adult fish during the spawning season. Ripe males are easy to capture, but capture of ripe females is seldom (D. Van Haverbeke, pers. obs.). As a result, large females can be held in a holding pen and injected with carp pituitary hormone to induce ovulation. Once ovulation occurs, the extruded eggs can be fertilized with freshly captured ripe males, or with other ripe males also held in captivity. This sounds simple enough, however, the logistical difficulties of such an endeavor are enormous (R. Hammon, USFWS, DNFH & TC, pers com; B. Persons, AGFD, pers. com.; C.O. Minckley, USFWS, pers. com.; D. Van Haverbeke, pers. obs.). Difficulties inevitably occur in capturing a sufficient number of ripe fish at the right time (particularly females), difficulties in holding fish in pens without unduly stressing them if artificial hormones are used to induce ripeness, difficulties in egg survivorship and transport out of the Grand Canyon, etc. All of these difficulties, and more have been present in past efforts to collect eggs from Grand Canyon. Finally, many critical concerns are very difficult to address in an attempt to perform streamside spawning, such as controlling for family size, etc. These concerns are critical in affecting the N_e of the broodstock (Doyle et al. 2001), and should not be ignored.

In summary, we suggest the following for establishing captive broodstock(s) of humpback chub in Grand Canyon:

1. Development of a broodstock should be viewed as a last resort, to be performed when all other available conservation actions have been attempted and shown to fail.
2. Should captive propagation be further considered and an adequately sized and funded facility be dedicated to that propagation, consider beginning with the already existing small captive group of LCR fish currently being held at Willow Beach NFH, and consider the aggregation of adult humpback chub at 30-mile as another potential source.
3. Build as large a broodstock as possible (at least several thousand fish), in order to retain as much genetic variability as possible.
4. Build the main broodstock from YOY fish collected under a temporal and random spatial design.
5. Consider taking several years to collect a sufficient amount of YOY fish.
6. Once broodstock fish are captured, a complete genetic analysis must be performed under the directions of a formal broodstock management plan in order to avoid problems with introgression, inbreeding, and reduction of N_e (both within the broodstock and into the wild population upon release of these fish).
7. Once broodstock(s) and supportive activities are begun, realize that this may be a long-term commitment.

Finally, any receiving facility would have to be sufficiently large to ensure each individual lot of fish brought in would remain in isolation until health and genetic concerns are fully addressed. For example, all fish brought into a hatchery situation will need to be quarantined and treated for Asian tapeworm, and any other health issues.

<u>Identification of components necessary to develop a broodstock management plan and of a suitable hatchery to hold fish.</u>

Given the time and funding constraints associated with this project, it is beyond the scope of this document to develop a broodstock management plan. We have, however, addressed many of the concerns that need to be considered in developing a broodstock management plan (e.g., genetic considerations, how many fish might be needed, etc.). In additional we can list some of the basic components that a hatchery should possess in order to develop a broodstock.

First, the objectives of a broodstock management plan must be clearly identified. As discussed above, a primary objective should be maintaining maximum genetic variability. Once this is achieved in a broodstock, a secondary objective would be to release fish in order to gain a demographic boost in the wild population. This secondary objective must be attended with strict measures to prevent the genetic problems discussed above from occurring. All potential genetic risks should be listed and thoroughly discussed in the broodstock management plan, and clearly defined methodologies and protocols should be included in the document to prevent or to minimize these risk factors.

A suitable hatchery must possess adequate staff, and personnel expertise in genetics and methodologies for the culture of humpback chub. In addition, a suitable hatchery must have committed and long-term funding. To even attempt to do so without adequate funding would be placing the humpback chub in danger.

Ideally, a suitable hatchery should be in a closed basin where accidental release of fish and risk of introgression with other *Gila* spp. will not be a problem. In addition, the hatchery should possess the necessary safe guards to prevent accidental introgression with other *Gila* spp. being held on station (such as bonytail). This implies completely isolated space requirements with separate raceways or holding tanks and completely separated plumbing components from other *Gila* spp. holding facilities on station, as well as no chance for accidental placement of fish.

A suitable hatchery for a broodstock of humpback chub should be large enough to hold from 2,500 to 3,000 fish (M. Ulibarri and C. Keeler-Foster, DNFH & TC). This should allow for the incorporation and maintenance of sufficient genetic variability. This implies large space requirements that need to fully explained in a broodstock management plan.

Primary among the physical hatchery qualifications for the culture of humpback chub is water availability and quantity, and the ability to regulate water temperature. Requirements for adequate water supply and water quality are identified in Piper et al. (1989). To induce spawning, water temperatures should be 18 to 19 ^{o}C, and optimal temperature for hatching and survival of swim up fry are between 19 to 22 ^{o}C (Hamman 1981). Optimal temperatures for growth of humpback chub are between 16 to 22 ^{o}C (SWCA 1997), although in temperature growth studies at 12, 18 and 24 ^{o}C Gorman and Van Hoosen (2000) found the optimal temperature for growth was at 24 ^{o}C.

It is strongly suggested that methods are followed to prevent domestication. These methods are reviewed in Brown and Day (2002) and Brown and Leland (2001), and were discussed above. Briefly, this might entail growing fish in raceways with current, or in outdoor ponds, and providing natural substrates, cover, and food types. It may also entail providing periodic exposures to high turbidity (as occurs the wild), and exposure to predators. Since many different options and techniques exist, the process will need to be adaptive in nature.

Finally, a broodstock facility for humpback chub will also need to have a quarantine facility to prevent spread within the hatchery of Asian tapeworm, or other parasites. This factor by itself could add enormously (> $1.7 million) to the budget.

Where to hold broodstock, where to raise fish, what size to raise fish, how many, where/when to release?

It is not a purpose of this document to identify a unique hatchery in which to develop a broodstock, or to evaluate available hatcheries for their appropriateness to raise humpback chub. However, the characteristics of a suitable hatchery are presented above, and the remaining concerns (what size to raise fish, how many and where to release) are addressed in the section below regarding supplemental stocking using wild caught YOY humpback chub.

FEASIBILITY OF ESTABLISHING A SUPPLEMENTAL STOCKING PROGRAM USING WILD CAUGHT YOUNG OF THE YEAR FISH

Below, we investigate the feasibility of capturing wild YOY humpback chub from the LCR, transporting them to a grow out facility until they reach a larger size, marking them with a unique identifier (such as a PIT tag), and releasing them back into the Colorado River or its tributaries within Grand Canyon and Tribal lands.

By growing wild YOY humpback chub to a larger size class before release, they could reasonably be expected to have an increased probability of survivorship. Larger humpback chub should be less prone to the effects of predation by nonnative fishes (Valdez and Ryel 1995), and to the detrimental effects associated with cold, fluctuating river flows (Clarkson and Childs 2000). If fish were PIT tagged prior to release, ongoing monitoring activities should be able to detect the success or failure of this type of effort within a relatively short period of time (i.e., within two or three years). Basically, this would be accomplished by estimating the proportions of wild fish and hatchery supplemented fish > 150 mm during ongoing mark-recapture population estimate studies.

From a biological standpoint, augmentation of a population via the use of broodstock progeny is inherently risky, because of numerous genetic concerns discussed above. For this reason, this document has incorporated the concept of capture and grow out of wild YOY fish, and releasing them in order to attempt to augment the population of humpback chub in Grand Canyon. Put simply, many of the potential problems associated with inbreeding and reduction of N_e in the wild population should be avoided since there is no captive breeding of offspring. In addition, this method is expected to be more cost effective.

Unfortunately, there appears to be a dearth of literature concerning the capture, short-term grow-out, and release of wild progeny. Dowling et al. (1996) briefly discussed direct capture and grow out of larvae in order to augment the population of razorback sucker (*Xyrauchen texanus*) in Lake Mohave. This approach, rather than supportive stocking via standard hatchery broodstock procedures, was suggested by Dowling et al. (1996) in order to maximize genetic variation. The assumption is that collection of wild larvae in a temporally and geographically spaced manner will result in maximization of parental representation, and maximum genetic variability should be retained. Such an approach has been ongoing on Lake Mohave since 1993, and in 1999 repatriates from this program constituted approximately 12% of the adult population (Minckley et al. 2003). Hence, the decline that has been occurring in the Lake Mohave razorback population may be showing some promising signs of reversal (T. Burke, USBR, pers. com; P. Marsh, pers. com.).

The primary concerns with capture and grow out of wild larval (or YOY) fish appear to involve issues related to ethology (behavior) rather than to genetics.

These issues were discussed above in the captive broodstock section; however, we stress again that juvenile experience (or lack thereof) can have profound influence on their success in the wild (Curio 1996, Maynard et al. 1995, Fleming et al. 1997). There are a multitude of mechanisms that can impair survivorship, including lack of anti-predator responses, lack of knowledge about feeding and food types, tendencies to be excessively active and aggressive, characteristic drops in condition factor after release, and lacking abilities to home to natal areas or knowledge of migratory routes.

In addition to behavioral concerns, it should be mentioned that some potential does exist for a form of domestication selection to occur. It is sometimes mistakenly viewed that domestication selection can be avoided if there is no mortality in culture (Waples 1999). However, fish held in captivity will have natural selection regimes relaxed, which can lead to problems with domestication (Busack and Currens 1995, Waples 1999, Brown and Day 2002). For instance, mortality for humpback chub in the wild occurs, meaning that wild selection is occurring (surviving floods, predation, disease, etc.). By transferring fish into a hatchery environment during this period of their life history, this wild selection is removed. Temporary relaxation of wild selection may not lead to genetic change within the captive population, provided hatchery mortality is kept to zero, but it does lead to genetic change compared with the high mortality in the early life history stages in wild populations (Waples 1999). In short, some level of genetic change relative to the natural population cannot be avoided in a cultured population (Waples 1999). Hence, we suggest that it is important to reduce (to the maximum extent possible) the time that fish are held in captivity. For example, in this respect, capturing fish at 60 mm and holding until 150 mm would be preferred to capturing fish at 30 mm and holding until 200 mm.

What size fish should be collected, how, from where, and when?

The main purpose for establishing a program for the capture and grow out of YOY humpback chub is to potentially increase the likelihood for survivorship to a larger size class (such as to 150 to 200 mm). Based on extensive modeling, it is believed that recruitment failure is the main factor causing decline in the humpback chub population of Grand Canyon (C. Walters, Univ. British Columbia, pers. com.). Recruitment failure is thought to be caused by a myriad of factors, including mainstem Colorado River habitat degradation and predation (Minckley 1991, Valdez and Ryel 1995, Clarkson and Childs 2000), or parasites such as the Asian tapeworm (*Bothriocephalus acheilognathi)*. One of the main reasons that YOY and juvenile humpback chub appear to suffer high mortality in the mainstem is that once displaced into the mainstem, they lack growth (hence, it is thought that they remain vulnerable to the effects of predation for a long period of time). It is hoped that by growing humpback chub out to a larger size, some early life mortality factors might be negated; hence resulting in increased recruitment to the spawning population.

A basic premise is that survival rates increase with age (e.g., Table 1), and that removal of individuals from a population should be expected to have the least impact upon the population at increasingly younger life stages. For example, removal of larval humpback chub should have less impact on the wild population than removal of an equal number of 50 mm fish. Another basic premise is that the time fish are held in captivity should be kept to a minimum in order to minimize behavioral changes such as those discussed above. For example, 50 to 70 mm humpback chub might be expected to have had more time to acquire wild behavior traits than 30 mm fish. Another factor might include time for which imprinting occurs. In addition, growing a fish from 50 to 150 mm will take less time in the hatchery than from 30 to 150 (perhaps a month). An important consideration is to attempt to keep fish held on station for only a year (or less) in order to logistically accommodate the arrival of the next year's stock (i.e., prevent stacking of year classes at a facility).

Age	1	2	3	4	5	6	7	8	9	10
Mean length	93	134	171	204	232	258	280	300	318	334
Estimated survival rate	0.35	0.52	0.61	0.67	0.71	0.73	0.75	0.77	0.78	0.79

Table 1. Age (in years), mean total length (mm), and estimated survival rates (based from wild survival rates in the 1990s) for humpback chub. Data provided by C. Walters.

Larval humpback chub (< 30 mm) can be captured with dipnets in the LCR, however, identification to species can be problematic in the field (Childs et al. 1998). On the other hand, humpback chub that are 50 to 70 mm can be easily identified by most field personnel, and can be readily captured and handled with minimum mortality using seines, minnow-traps, or hoopnets.

There are other options that could be considered. For example, removing 1 year old fish during spring. These fish would be ~125 mm, and could be grown larger, say 200 mm, for better survivorship. However, removal of a sufficient number of fish of this size class may be more problematic, and should be expected to crop wild recruitment even more.

An important consideration will be maintaining the maximum likelihood for retention of genetic integrity. This implies capturing fish over temporal and spatial scales. As with potential collection of fish for broodstock, we suggest that fish be collected over a period of months (e.g., late July through September), and collected over as wide a geographic reach as possible. For example, an equal number of fish could be randomly collected from each of three reaches in the LCR (0 to 5 km, 5 to 10 km, and 10 to 14.2 km).

Other considerations will include the logistics of obtaining fish from a variety of locations, the gear types for collecting a desired length of fish, and the logistics of keeping fish alive from the time of collection to arrival at their captive destination. All necessary protocols will need to be in place in order to ensure mortality during the operation will be kept to levels specified in permits. For instance, live carrs (holding pens) will need to be established at each camp, and protocols will need to be established for moving fish from capture sites to the holding pens. An alternative to holding pens set up in the river may be large plastic coolers (e.g., 178 quart capacity Gott coolers) supplied with a power source (small Honda generators) and pumps for supplying fresh water or aeration. Fish will need to be treated according to the most recent protocols to relieve stress. Specifics on amounts and types of chemicals used to treat fish and relieve stress need to be included in camp protocols, but this generally includes adding salt (19 g/gallon), Stress Coat (1 mL/gallon), and other chemicals such as Furacin.

We suggest direct transport via helicopter to the appropriate receiving facility. Since several thousand fish will be transported, this will require multiple flights. For example, two large coolers, each containing from 300 to 400 YOY fish, and supplied with oxygen should be the maximum expectations for transport. More preferable (and probably mandatory for safety reasons) would be one cooler transported per flight, with a technician on board to monitor the oxygen supply (i.e., it is critical not to super-saturate the water with oxygen, as this can result in mass mortality). Hence, this will likely require 10 to 12 long distance flights per year just for fish transport.

The above is only a rough idea of some of the logistics and methods involved, and much more detailed protocols will need to set in place before such an operation is carried out. The logistics and costs involved just to collect and transport the fish will be considerable. It should be expected that collecting an adequate number of (~2,000 to 4,000; see discussion below) YOY fish per year from the LCR will be no small undertaking, and that considerable difficulties could be presented in collecting this many fish, and with keeping mortalities to a minimum. It should also be realized that even with detailed protocols in place, the operation will need to be adaptive in nature.

<u>What is the best size to grow out captive fish before release</u>?

We suggest that fish are grown out to a minimum of 150 mm for initial efforts. There is consensus that it is imperative to have the ability to monitor the released fish. Fish ≥ 150 mm can be PIT tagged, and individually tracked with ongoing monitoring efforts once released into the wild. Hence, researchers will have the ability to distinguish wild produced fish from captive fish, and track the proportions comprising the population as a whole over time. Furthermore, mark-recapture studies are easier to perform on individually marked fish using PIT tags, whereas batch marked fish present much more difficulties in tracking numbers.

Although YOY humpback chub do not put on growth at 10 ^{o}C (Gorman and Van Hoosen 2000), once humpback chub reach 150+ mm, they will grow in mainstem waters (Valdez and Ryel 1995). For example, monthly growth rates of 2.25 mm, and 2.79 mm were calculated for mainstem fish between 150 to 200 mm, and between 200 to 250 mm, respectively (Valdez and Ryel 1995). In contrast, monthly growth rates of 1.42 mm and 1.33 mm were calculated for 150 to 200 mm, and for 200 to 250 mm fish in the LCR (Minckley 1992).

Whether to grow fish beyond 150 mm (for example to 200 mm) becomes a question subject to debate. Wild fish that are 200 mm are estimated to have a greater survivorship than wild fish at 150 mm (Table 1). Note that to obtain even a 52% wild survival rate, fish would need to be 134 mm. Hence, fish grown to 150 mm could optimally have a post-release survival rate of ~55%, while fish grown to 200 mm could have an optimal post-release survival rate of ~60% (Table 1).

Unfortunately, the above survival rates are likely highly optimistic since they are based on survival rates of fish that have grown up in the wild. Actual survivorship rates of released fish grown in captivity will likely be much lower. For example, in the case of salmonids, typically less than 5% of all hatchery-reared fish make it to adulthood (McNeil 1991). For other species released from hatcheries, the number is commonly far lower (e.g., chum salmon 1-3%, and cod <1%; Salvanes 2001). Low returns for hatchery-reared trout have been reported for more than 100 years (Wiley et al. 1993). Considering the size or age class at which most hatchery fish are released, the magnitude of mortality is especially great compared to wild mortality rates (Maynard et al. 1995). Post-release survival rates for razorback sucker released in the San Juan River have been seen as high as ~25% (F. Pfieffer, USFWS, pers. com.); however, these fish were grown to 400 mm. It may be that warm water species have higher post-release survival rates than cold-water species (C. Keeler-Foster; USFWS, DNFH & TC, pers. com.).

Additionally, growing fish to 200 mm may take additional space, entail an additional cost, and add an additional six months of growing time minimum (M. Ulibarri, DNFH & TC, pers. com.). This time factor could be crucial, since it is estimated that in order to grow humpback chub much beyond 150 mm, more than a year will be needed. If fish are cultured on a yearly basis, it would be optimal to free space in time for the arrival of new fish. An important factor that could negate some of these concerns is that about half as many fish would need to be grown to 200 mm as to 150 mm in order to accomplish the same objective; that is to increase recruitment to sufficient levels. The reason for this is discussed below. However, this holds at least three important implications. First, this would result in less annual cropping of the wild cohort, and it may be preferable to annually release a smaller number of cultivated fish into the wild. Second, it would be far easier logistically to collect fewer fish. Third, this may have the potential to reduce hatchery space and financial costs, if about half as many fish are cultivated (but they will need to be cultivated for a longer time).

One factor that should be considered is that trout removal efforts are ongoing in Grand Canyon at present. With removal of these predators, there may be less need to grow fish to 200 mm to avoid predation risk.

A primary goal is to minimize mortalities within the hatchery regime. This minimizes the chance for genetic problems (i.e., artificial selection and domestication issues) to occur prior to release in the wild. Based on culturing bonytails, mortality in the hatchery system could easily average 20% (M. Ulibarri, DNFH & TC, pers. com.). Hence, one might reason that the less time fish spend in the hatchery, the less chance for mortality (and hence artificial selection) to occur. Optimally, the goal is to absolutely minimize mortalities, both within the hatchery regime, and during post-stocking activities. No mortalities equals no selection, whether natural or anthropogenically induced (C. Keeler-Foster, DNFH & TC, pers. com.). However, as mentioned above, the absence of wild selection in itself could have implications.

Another important goal is to minimize post-release mortality. Poor survival of hatchery-reared fish is a major concern, and greatly reduces the ability of using hatchery stocks to supplement wild production, whether for commercial or conservation purposes (Maynard et al. 1995, Olla et al. 1988, Brown and Day 2002). Generally, larger fish have a higher survival rate (Brown and Day 2002). All factors being equal, one might assume that survival of 200 mm fish would be greater than survival of 150 mm fish. However, increased time spent under hatchery regimes leads to issues with behavior of fish. Even under optimal conditions, these fish will be held under a regime of unnatural conditions for a significant portion of their lives (up to a year or more). This will have unavoidable impacts on important life history functions, such as anti-predator responses, feeding abilities, and possibly other factors related to migration and spawning behavior (Brown and Day 2002). For instance, following release, many captive reared salmonids may not eat for days, or weeks (Paszkowski and Olla 1985, Usher et al. 1991), and predation is thought to be the principle cause of mortality of hatchery-reared fish (Howell 1994). Most of this mortality appears to occur within the first few days after release, and is caused by lack of anti-predator responses, and inabilities to feed in the wild environment (see Brown and Laland 2001, Brown and Day 2002 for reviews).

In summary, we suggest that fish will need to be grown to 150 mm at a minimum for tagging purposes. To avoid problems associated with artificial selection, and behavior, we suggest that fish are kept in captivity for as short a time as possible.

<u>How many fish will need to be released into the wild in order sufficiently supplement the population of humpback chub in Grand Canyon</u>?

Based on communications with Dr. Carl Walters, we have investigated the most recent model for humpback chub. This model (designed by C. Walters) provides a rough estimate of the number of fish needed to augment the population of humpback chub in Grand Canyon. The model operates primarily by inputting the number of age 1 fish that are stocked. The model also accounts for estimated survival rates from ages 1 to 30, and with the use of historical data, runs from 1989 to 2020. For example, by collecting 1,400 YOY fish and growing them out

150 mm, and assuming natural mortality rates remain in place once the fish are stocked (i.e., there is no additional mortality of fish during growth in captivity and no additional mortality upon release), the abundance of age 5+ spawners first shows a minor increase (~100 individuals) in the year 2009 (Figure 2). The same effect could theoretically be achieved by collecting 725 YOY fish per year, and growing them out to 204 mm (age 3; Table 1), again assuming no mortalities in captivity or post-release.

Figure 2. Estimated and projected humpback chub population (LCR stock) in Grand Canyon, assuming recruitment remains at mid-1990s level. This example figure of the model assumes a successful stocking of 1,400 grown out to 150 mm fish per year, beginning in year 2004, with mortality not exceeding those found in the wild (i.e., no additional mortalities in captivity or post-release). Model was designed and provided by Dr. C. Walters, University of British Columbia.

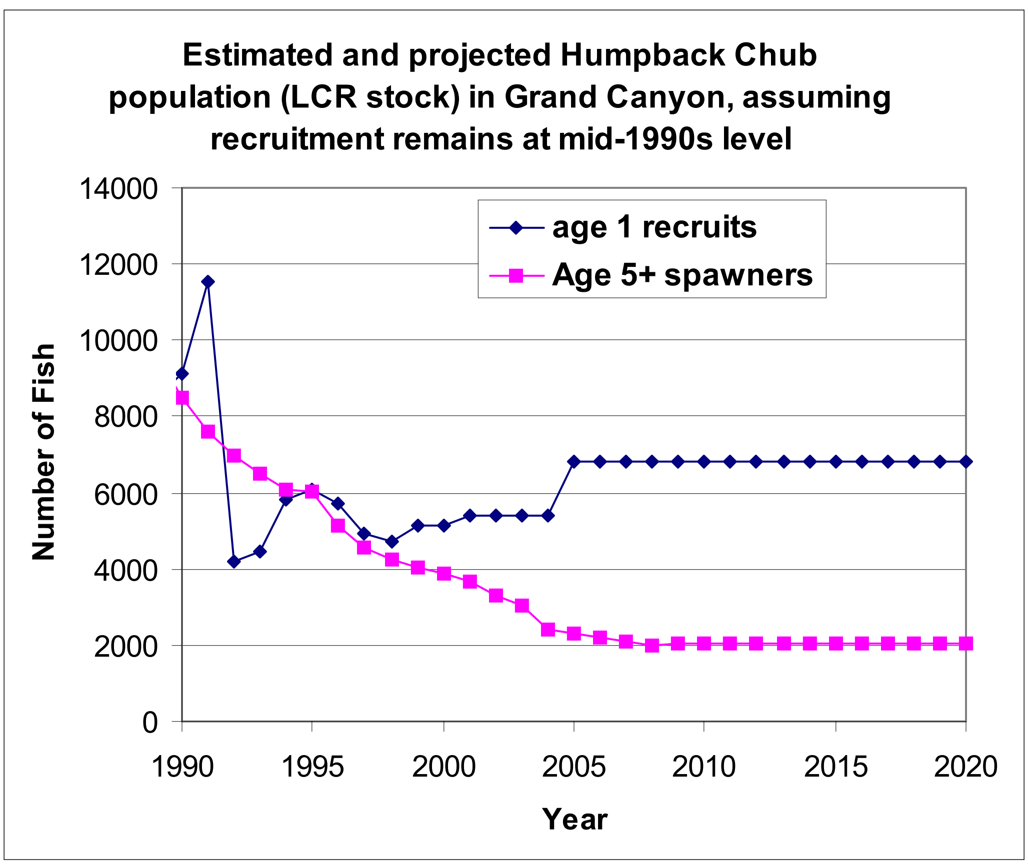
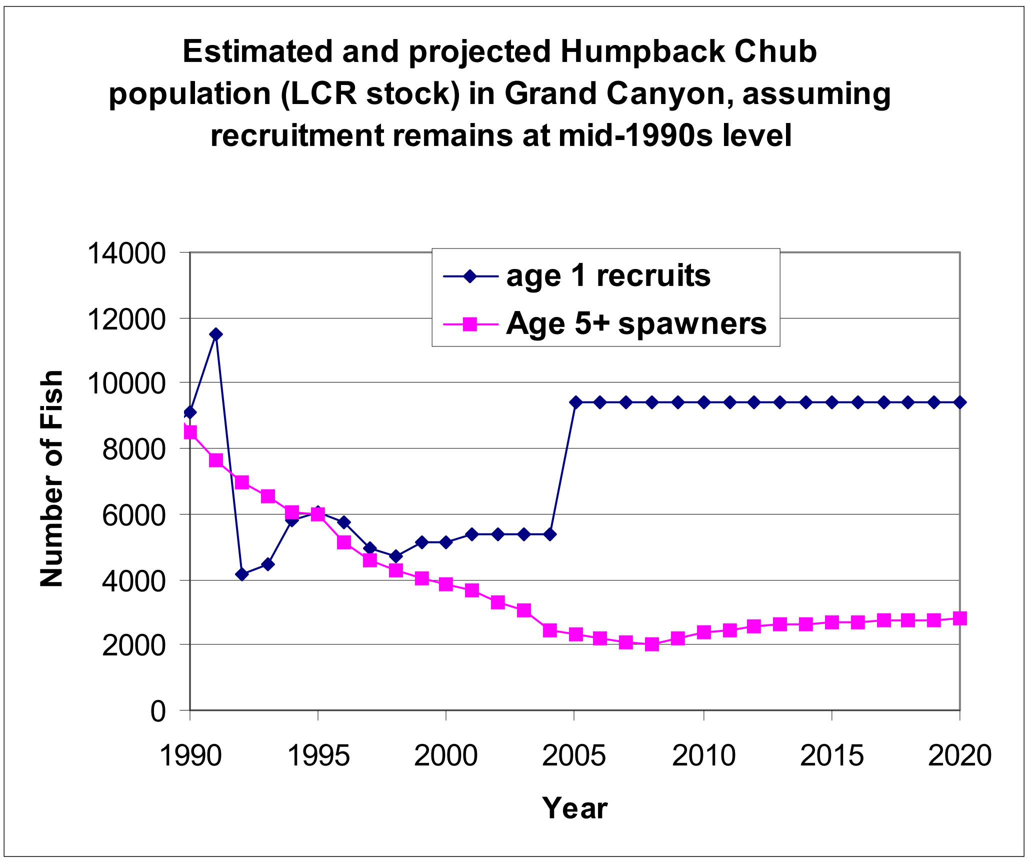


Figure 2 predicts that the decline of age 5+ fish would be arrested and show a minor increase in the year 2009. Figure 3, on the other hand, depicts that 4,000 YOY fish are captured and grown out to 150 mm per year. In this instance, there is a predicted noticeable increase in 5+ year old spawners.

Figure 3. Estimated and projected humpback chub population (LCR stock) in Grand Canyon, assuming recruitment remains at mid-1990s level. This example figure of the model assumes a successful stocking of 4,000 150 mm) grown out to 150 mm fish per year beginning in year 2004, with mortality not exceeding those found in the wild (i.e., no additional mortalities in captivity or post-release). Model was designed and provided by Dr. C. Walters, University of British Columbia.



With no additional stocking, the trend as predicted by Coggins et al. (2003) occurs. Namely, that if recruitments continue to be stable, it is predicted that the spawning population will soon stop declining, and will stabilize at an average spawning abundance of roughly 50% of its current level, and that average will most likely be between 1,000 and 2,500 fish.

Such scenarios as are presented in Figures 2 and 3 are probably unrealistic, since mortality in captivity could easily average 20%, as mentioned above. In addition, about 50 to 60 fish are generally inspected for health studies (killed) any time fish are brought from the wild into a hatchery station (J. Thoesen, USFWS, Fish Health, pers. com.). Adding a rough estimate of 20% captive mortality, and 60 mortalities for health inspection (a minimum, since collection of fishes should be temporally spaced over several collections per year, requiring multiple health inspections) 1,740 fish per year would need to be collected to provide 1,400 fish for grow out to 150 mm, or 930 fish per year for grow out to 200 mm.

Finally, it is unknown how high post-stocking mortality rates will be at this time. If the very low survival rates of other stocked species are any indication, we can only assume that additional post-stocking mortality could be high (i.e., >50%). Hence, it is very likely that the numbers of YOY collected will need to be doubled. In fact, this approach of doubling the numbers is suggested by C. Walters. Primarily, this is for reasons that a 50% post mortality rate should be an expected reality, and that by releasing a larger number of fish it will be easier to perform mark-recapture studies on this portion of the population. Hence, this suggests capturing ~ 3,480 YOY fish per year for grow out to 150 mm, or ~1,860 YOY fish per year for grow out to 200 mm (these numbers account for 50% post release mortality, 20% captivity mortality, and an additional 120 mortalities for two health inspections). Again, this effort would need to be maintained for a period of many years to achieve any results. It should also be understood that if positive results are achieved, they may not be self-sustaining if the original causes for the decline in recruitment failure have not been solved.

The question has been raised as to what proportion of the wild population will be removed annually to conduct this endeavor. Estimates of 1 year old recruits (~93 mm; Table 1) in the past few years have been ~4,000 to 5,000 fish (C. Walters, pers. com.). Assuming an average annual survival rate of about 0.1 in age 0 to 3 fish (Valdez and Ryel 1995), this translates into ~50,000 YOY fish per year. Hence, removing about 3,480 fish on an annual basis (for grow out to 150 mm) might be the equivalent of removing 7% of the annual production of YOY (i.e., 3,480/50,000). We caution that annual production of YOY fish is probably highly variable from year to year (in part because of the highly stochastic nature of the LCR), and that this approach assumes a 0.1 survival rate during the first year of life. An alternative approach is to use an average of the population estimates for humpback chub ≥ 200 mm fish (4+ year old adults), assume a 1:1 sex ratio, multiply this by an average of 3,333 eggs/female (see Valdez et al. 1995), and multiply this by the annual survival rate of 0.1 (Valdez et al. 1995). Spring LCR population estimates for humpback chub ≥ 200 mm for the past two years have been 1,470 (Van Haverbeke and Coggins 2003) and 2,002 fish (Van Haverbeke, *in review*), or an average of 1,736 fish. Assuming a 1:1 sex ratio, this equals 868 females. Hence:

(868 females)(3,333 eggs)(0.1) = 289,304 YOY fish

Of course, this assumes that each female ≥ 200 mm successfully contributes 3,333 eggs that survive (an unlikely scenario). Nevertheless, using this approach suggests that removing about 3,480 fish on an annual basis might be the equivalent of cropping 1.2% of the annual production of YOY (3,480/289,304), rather than 7%. Again, both of these approaches make gross assumptions; however, both suggest that such an effort may not be harvesting a large percentage of the wild production of YOY fish. We caution that if survivorship from egg to 50 mm were < 0.1 (for instance 0.05), then this could amount to cropping ~14 % of the annual YOY production. It should also be kept in mind that the fecundity estimate of 3,333 eggs/female (Hamman 1982) was based on fish with a mean length of 395 mm, and that assuming this estimate for fish > 200 mm is probably highly optimistic. Realistically, survivorship is likely to be highly variable from year to year because of the stochastic nature of the LCR, meaning that during some years, cropping 3,480 fish would be insignificant, while during other years, it could significant (i.e., > 10 %).

Where and when will fish be released back into the wild?

There appear to be two general approaches that can be taken for stocking fish back into the wild once the desired growth has been obtained at whatever facility is ultimately selected and funded for the propagation effort. Namely, release into the LCR or release into the mainstem Colorado River. Release of fish into other small tributaries in Grand Canyon could be a third option, however, this approach should not be expected to solve the main problem of lack of recruitment in the LCR population. In addition, until problems with predators are dealt with in these other tributaries, this approach may largely be a waste of resources.

Problems with release of fish back into the LCR may be primarily associated with carrying capacity of the LCR, and potential for impacting the resident wild population. Table 2 shows spring and fall population abundance estimates that have been obtained in the LCR for fish > 150 mm. Consider if 2,800 captive humpback chub > 150 mm were released into the LCR each fall. Also consider that the average point population estimate for humpback chub > 150 residing in the LCR during the fall since the year 2000 is 1,823 fish (Table 2). This translates into a 153% increase in numbers of fish that would be suddenly introduced into the system. Such numbers raise serious concerns about potential impacts to the resident wild population. An immediate concern would be whether or not carrying capacity is suddenly exceeded in the LCR, and if so, what would be the resulting density dependant effects on the survival of next

Table 2. Spring and fall point population estimates of humpback chub > 150 mm in Little Colorado River. 1991 & 1992 estimates are from Douglas and Marsh (1996); 2000 estimate is from Coggins and Van Haverbeke (2001); 2001 estimate is from Van Haverbeke and Coggins (2003) and 2002 estimate is from Van Haverbeke, *in review*.

Spring population estimates in Little Colorado River

			95 % Confidence Interval		
Date	Abundance Estimate	SE	Lower	Upper	Reach (rkm)
Apr-92	5,555	671	4,416	7,067	0 - 14.9
May-92	4,363	1,216	2,594	7,523	0 - 14.9
Average April and May 92	4,959				
April/May 2001	2,090	244	1,611	2,569	0 - 14.2
April/May 2002	2,666	98	2,474	2,858	0 - 14.2
Average April and May 01-02	2,378				

Fall population estimates in Little Colorado River

			95% Confidence Interval		
Date	Abundance Estimate	SE	Lower	Upper	Reach (rkm)
October 1991	2,038	518	1,276	3,368	0 - 14.9
November 1991	1,989	489	1,264	3,235	0 - 14.9
October 1992	1,099	60	990	1,224	0 - 14.9
November 1992	1,417	408	839	2,500	0 - 14.9
Average Oct. & Nov. 91-92	1,636				
October/November 2000	1,590	297	992	2,552	0 - 14.2
October/November 2001	1,106	172	934	1,179	0 - 14.2
October/November 2002	2,774	209	2,364	3,184	0 - 14.2
Average Oct. & Nov. 00-02	1,823				

year’s cohort of YOY humpback chub? One could argue that the LCR is capable of holding many more fish > 150 mm (as evidenced by the April 1992 population estimate of 5,555 fish; Table 2). However, this would still represent a 50% increase, a significant amount. In addition, the captive fish are likely to be added in the fall, rather than in the spring (a time when point population estimates for humpback chub > 150 mm have averaged < 2,000 fish since 1991). If food is limiting in the LCR, there is a concern that the increased abundance of humpback chub > 150 mm could crop the next years YOY cohort via predation, and hence, potentially eliminate any gain from the augmentation effort.

On the other hand, positive aspects of releasing supplemental fish back into the LCR could be gaining immediate familiarity with habitat, breeding grounds, and migration routes.

Another option for release of supplemental captive fish is the mainstem Colorado River. Extensive monitoring of the mainstem Colorado River in Grand Canyon between 1990 to 1993 showed that 99% of sub-adult humpback chub (<200 mm) were captured between river mile (RM) 58.8 to 92.1 (Valdez and Ryel 1995). Of these, only 2% were captured above the LCR, 68% were between the LCR (RM 61.3) and Lava Canyon (RM 65.4) and 30% were between Lava Canyon and Salt Creek (RM 92.1). It may be preferable to stock supplemental fish in the mainstem Colorado rather than in the LCR (where the main abundance of sub-adult humpback chub reside). First, carrying capacity should be much less of an issue. This should be particularly so since ongoing trout removal efforts are opening up niche space. Since January 2003, ~6,700 trout have been removed from Kwagunt Rapid (RM 56) to Lava Canyon (RM 65.4). It is thought that this may constitute ~80 to 90% of the trout formerly residing in this reach of the river (L.G. Coggins, GCMRC, pers. com.). Such a large-scale removal effort is hoped to significantly decrease mortality due to predation, and lead to increased survivorship of YOY and juvenile humpback chub. Second, disease transmission should be less of a concern. Infestation rates by the Asian tapeworm (a major parasite to humpback chub) are lower in cooler mainstem waters (Brouder and Hoffnagle 1997). Also, spread of other unexpected diseases to the LCR might be less of a concern than releasing fish directly into the LCR (i.e., many diseases, including Asian tapeworm, should be expected to subside in the cooler mainstem waters before fish re-enter the LCR).

It should be mentioned that an effort such as this should be adaptive in nature. In other words, many options are available. For example, although it may not be advisable to over stock the LCR with supplemental fish, a small proportion of the fish could be stocked into the LCR, while another proportion could be stocked into the mainstem. Monitoring efforts might then reveal which is the optimal strategy. Another option may be to stock fish in the left hand channel of the LCR at the confluence region. This is a fairly large pool (probably > 2 acres), generally with very slow currents and some shoreline ledges and vegetation for cover. Here, it may be possible to re-acquaint the fish to LCR waters without overburdening the LCR itself. From here, the fish would have limited access to the LCR, or full access to the mainstem Colorado River. Finally, it may be advisable to release fish in multiple localities. For example, a portion of fish could be released into the LCR within each of the three reaches (i.e., Boulders, Coyote and Salt camps). Another portion of fish could be released into the mainstem, with equal numbers of fish released above the LCR (such as in the small return channels/backwaters above the LCR to 60 mile rapid), and below the LCR (in Crash Canyon eddy, in front of Carbon camp [as well as eddies/return channels above Carbon], and in the eddy across from Lava/Chuar camp). In order to facilitate logistics (avoid multiple landings of a helicopter along the mainstem), fish could be landed at the LCR confluence landing pad, and boated up or down the river to release sites. A critical factor determining release sites may be the ability (or lack thereof) to set up soft-release protocols (i.e., short-term holding pens).

An effort should be made to avoid releasing fish under harsh environmental extremes. For example, fish should probably not be released under flooding conditions in the LCR (although mild-flowing turbid conditions may be acceptable). Likewise, fish should probably be released in the mainstem under periods of minimal fluctuating flows, or periods with low flows and associated decreased velocities. To do so otherwise may invite undue mortality. An adaptive approach should be taken (i.e., helicopter flight times should be flexible and dictated by riverine conditions and flows, rather than flights being scheduled in an inflexible manner). Paying attention to current and expected hydrographs, both in the LCR and in the mainstem will be important.

Where could the supplemental fish be grown?

It is not the purpose of this document to specifically identify a hatchery where supplemental fish could be grown. However, we have provided the type of information that an informed decision can be made regarding this matter. The facility will need to be to be staffed with personnel experienced with culture and rearing techniques associated with humpback chub, or surrogates such as bonytail or roundtail chub. The facility will need to be capable for grow out of ~2,000 to 4,000 fish per year. This could be accomplished in raceways, circulating tanks, or in outdoor ponds. The facility should have a demonstrated ability to keep the fish completely and unquestionably isolated from other *Gila* spp., and to prevent accidental escape of humpback into a watershed with other *Gila* spp. In order to obtain sufficient growth within a year, it will probably need to have the ability to maintain water temperatures up to 22 to 24 ^{o}C, and will certainly need to maintain water temperatures > 16 ^{o}C.

It is strongly recommended that the facility have the capabilities for naturalistic rearing. To recount, this might include exposing fish to moving water currents, natural substrates, cover types, periodic elevated levels of turbidity, etc. For example, marle, sands, gravels and substrate rocks could be collected from the

LCR. This could be accomplished by flying out these materials, or by transporting them via boat and truck. In addition, natural food types and some exposure to predators would be desirable (see Brown and Leland 2001 for review).

Finally, the facility will need to have a quarantine facility for Asian tapeworm, since YOY humpback chub collected in the field will be infested with the parasite.

FEASIBILITY OF EXPANDING THE HUMPBACK CHUB POPULATION VIA TRANSLOCATION IN THE LCR OR OTHER GRAND CANYON TRIBUTARIES.

The biological factors necessary to establish a second population in Grand Canyon have previously been addressed (Valdez et al. 2000). Although tributaries were not deemed optimal for establishment of a second population of humpback chub (Valdez et al. 2000), further investigation may be of value. Specifically, we explore the feasibility of transplanting fish above Chute Falls (i.e., above 14.2 km) in the LCR, and establishing (or augmenting) fish in Bright Angel, Shinumo or Havasu creeks. We are exploring concerns that are associated with performing such management actions. Primarily this entails meeting with the appropriate Tribal and Park personnel to discuss their concerns and issues. In addition, a brief literature review is provided below on the subject of translocation.

Translocation is the intentional release of animals into the wild in an attempt to establish, reestablish, or augment a population (World Conservation Union 1987); and in the face of increasing extinction rates, translocations of rare species may become an important conservation tool (Minckley 1995, Griffith et al. 1989). A translocation is a success if the founder population becomes self-sustaining; alternatively the founder population goes extinct.

A number of variables are known to influence the probabilities of success for translocation efforts (Griffith et al. 1989). Theoretical considerations predict that population persistence will be higher if the number of founders is large, the rate of population increase is high, and the effect of competition is low (Wilson 1988). Other factors that may enhance persistence are: 1) low variance in rate of increase, 2) reduced environmental variation (Leigh 1981), 3) presence of refugia (Goodman 1987), and 4) and high genetic diversity among founders (Stockwell and Leberg 2002).

In a survey of bird and mammal translocations, Griffith et al. (1989) found the following patterns associated with successful translocation: 1) native game species were more likely to be successful as transplants than threatened, endangered, or sensitive species, 2) increased habitat quality was associated with higher success, 3) translocations into the core of the species historic range were more successful than translocations into peripheral range or outside the species historic range, 4) herbivores were more successful at translocation than either carnivores or omnivores, 5) translocations into areas without competitors or with congeneric competitors were more successful than translocations into areas with morphologically similar competitors, 6) early breeders with high fecundity were more successful than late breeders with low fecundity, and 7) translocations of exclusively wild caught animals were more likely to succeed than were those of captive-reared animals.

In addition, Griffith et al. (1989) found that the increase in success associated with releasing larger numbers of animals quickly becomes asymptotic (i.e., a threshold is reached beyond which the release of more organisms does little to increase the likelihood for success). This asymptote appears to be reached at 20 to 40 individuals for large native game animals, 80 to 120 for birds, and is strongly dependant upon other variables (such as habitat quality). The authors clearly discourage against extrapolation of these numbers to conditions other than those represented by their data, as well as against species-specific extrapolation. The point is that with a limited resource for translocations, it should be realized that such an asymptote may exist, and that going beyond that asymptote will do little to increase chance for success. A weakness in Griffith et al. (1989) appears to be that they do not address numbers of animals to be released in terms of the probability for inbreeding to occur in the translocated population.

Finally, Griffith et al. (1989) point out that the chance for a successful translocation increases if there is more than one potential translocation area, and if the animals released are split. For example (using their data), the probability that 300 released birds will fail in excellent habitat is 0.257; whereas two releases of 150 birds each in excellent habitat have failure probabilities of 0.312 each. The probability that both will fail is 0.312 X 0.312 = 0.097; showing that substantial gain is achieved by splitting the birds between areas.

<u>Translocation of humpback chub within the Little Colorado River</u>

In a December 6, 2002 Biological Opinion, a conservation action has been proposed by U.S. Bureau of Reclamation, Grand Canyon National Park, Glen Canyon National Recreation Area, and GCMRC to translocate three hundred 30 to 60 mm total length young-of-the-year humpback chub from near the mouth of the LCR to a reach within the LCR above a natural travertine dam structure referred to Atomizer Falls (USFWS 2002b). The action is to serve as mitigation for the possible effects resulting from experimental flows from Glen Canyon Dam, and from mechanical removal of rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), and other non-native fishes from the Colorado River from above and below the confluence of the LCR and the Colorado River. The purpose of the translocation is to increase survivorship of the translocated fish. It is hypothesized that by moving fish higher up in the watershed, they will be retained longer in the LCR, have more time for growth, and hence have a greater chance for survival. It is stated here for the record that this goal might be just as well accomplished by moving fish to just below Chute Falls. Reinitiation of Section 7 consultation for this proposed action began in March 2003. It will change the size of the translocated fish from 30 to 60 mm to 50 to 100 mm, and calls for moving the fish to above the Atomizer/Chute Falls complex in the LCR (14.9 km). Also, depending on the results of a translocation effort scheduled for July 2003, a second translocation of 300 humpback chub will be conducted in summer 2004. A proposal has been submitted to implement this action in July 2003 (Appendix 3), and a memo has been sent to the Navajo Nation to address the proposed action.

A factor that managers (including the Navajo Nation) should be aware of is that critical habitat for humpback chub is listed at 8 miles (12.87 km) above the LCR confluence (Federal Register 59:54 [1994]: 13374-13400). Hence, a translocation effort to move fish above Chute Falls would involve moving fish into habitat outside its current range and into habitat outside of designated critical habitat.

The habitat above Chute Falls is probably within the historic range of humpback chub. Skeletal remains of Colorado pikeminnow, razorback sucker, bonytail, and humpback chub have been recovered from the Homol'ovi archaeological ruins near Winslow, Arizona (Strand 1998). Miller (1963) reported that S. Sykes caught Colorado pikeminnow and bonytails (*G. elegans*) at the base of Grand

Falls in the early 1900s (~120 km above the LCR confluence). These reports suggest that a historic native fish community was established in the LCR above Chute Falls. Currently, the only native species known to exist between Chute Falls and Blue Springs (at 21 km) is the small cyprinid, speckled dace (*Rhinichthys osculus*). Non-native carp (*Cyprinus carpio*) and fathead minnow (*Pimephales promelas)* also reside in this stretch of river (Kaeding and Zimmerman 1983).

The reason humpback chub do not currently reside above Chute Falls is unknown. Chute Falls may be a physical barrier (Robinson et al. 1996), which implies that if humpback chub were historically above Chute Falls, local extinction occurred for some reason (e.g., environmental stochasticity) and the species has been unable to successfully re-colonize the habitat. Although Chute Falls might be viewed within a geological context as being temporary in nature, in the context of a species' generation time (and time for which dynamics of colonization, isolation and selection to act), the fall should be viewed as more permanent in nature. An alternative hypothesis for why humpback chub are not found above Chute Falls is that elevated levels of CO_2 may preclude the existence of humpback chub above the falls. Water discharged from Blue Springs comes from an aquifer dominated by limestones, and contains high levels of dissolved CO_2 (> 348 mg/l; Robinson et al. 1996). As this water flows toward the confluence, it passes over a series of small and large travertine dam structures where release of CO_2 to the atmosphere occurs (the water is aerated by the mechanical action of the these falls), and large amounts of calcium carbonate precipitate on the rivers substrates (Johnson and Sanderson 1968). The levels of free CO_2 progressively diminish downriver from Blue Springs, apparently being above or near the lethal limit for fish within the first kilometer, and decreasing thereafter (i.e., 196 mg/l at 17.5 km, 192 mg/l at 15 km, etc.; Robinson et al. 1996). As a result of successful acclimatization studies, Robinson et al. (1996) hypothesized that humpback chub could inhabit and utilize the lower portions of the river between Chute Falls and Blue Springs.

In addition, there may be suitable habitat available for humpback chub to persist above Chute Falls. The region is characterized by pool, riffle and run habitat; densely abundant algal communities (particularly during extended periods of base flow); and an abundant prey source (i.e., aquatic invertebrates and speckled dace). Robinson et al. (1996) concluded that neither food nor water chemistry were factors that should preclude humpback chub from above Chute Falls.

The proposed translocation effort does have potential for establishing a reproductively isolated population of humpback chub above Chute Falls. Since humpback chub do not currently reside above Chute Falls, this means that gene flow (by natural means) from the main LCR population to the founder population will be zero. Offspring from the translocated fish will have only one direction to go (downstream). In addition, even when numerous individuals are translocated, bottlenecks may occur early in population establishment, leading to reduced genetic diversity (Stockwell et al. 1996). Largely because of this, we have presented a somewhat lengthy discussion below about the potential genetic and ecologic concerns related to translocating fish above Chute Falls. It is not the purpose of the discussion to over-emphasize these concerns. Rather, it is viewed as a responsibility to present them.

Establishing a reproductively isolated population of humpback chub above Chute Falls holds some potential genetic implications. First, Douglas and Marsh (1996) hypothesized that there may be a resident genotype developing in the LCR since the advent of Glen Canyon Dam. If fish are successful at reproducing and remaining above Chute Falls, this might be expected to further impose selection for a resident genotype. For instance, it is known that a portion of the LCR humpback chub do migrate between the mainstem Colorado and the LCR during their life-history (Valdez and Ryel 1995, Gorman and Stone 1999). This has led some to speculate that before reproduction occurs, the translocated fish would move toward the mainstem. However, Gorman and Stone (1999) found that many smaller adults (< 300 mm) tend to remain as residents in the LCR. This suggests that humpback chub could remain above Chute Falls long enough to reproduce.

Second, the founder population will consist of < 300 fish (i.e., some will not survive the translocation), or up to 600 fish if the effort is carried out for two years. Since the translocated fish will be small (50 to 100 mm), there will be no ability to determine sex of the individuals during collection. This means a possibility to transfer unequal sex ratio. In the best-case scenario, if all 600 fish survive the translocation, and there is a 1:1 sex ratio, and all fish have an equal probability of contributing offspring to the next generation, this would be a founder population with a maximum effective population size of 600. A more realistic scenario is that a large proportion of fish will not survive the translocation effort (because of stress, out-migration, floods, etc.), the sex ratio will not be 1:1, and there will be differential reproduction (because of multiple year classes, unequal family sizes, etc.). Hence the founding effective population size should be expected to be far less than 600 (i.e., below the minimum viable population standards of N_e = 500).

How likely is it that the translocated population will remain less than a N_e of 500? The potential demographic gain can be roughly estimated by considering that since 2001 there has been an average of 661 humpback chub > 200 mm residing in the in the lower 14.2 km of the LCR (range equals 483 to 839 humpback chub > 200 mm for fall point population estimates in 2001 and 2002, respectively). Assuming that these fall population abundance estimates are representative of year round residence, this translates into an average of 47 humpback chub > 200 mm per km of river.   Considering that there may be an additional 6 km of potential habitat above Chute Falls, this translates into a potential demographic gain of 282 fish > 200 mm (i.e., 4+ year old fish of breeding age). However, this might be an overly optimistic estimate of the potential for demographic gain, since increasing levels of CO_2 may preclude humpback chub in some areas above Chute Falls (Mattes 1993, Robinson et al. 1996). Regardless, this rough calculation serves to illustrate that should a group of breeding fish establish above Chute Falls, it is probably destined to remain small ($N_e << 500$). However, this does represent a potential increase to the resident portion of the LCR population of ~43% (i.e., 282/661). This increase could be viewed as a positive conservation measure from a demographics standpoint. On the other hand, it also suggests that the founder population may have some considerable power to influence the genetics of the resident LCR population.

The main question that needs to be asked is whether or not establishment of a small population (likely well below minimum viable population standards) has any potential to detrimentally affect the main population of humpback chub below the falls. In particular, we ask if there is potential to: 1) increase the proportion of inbred fish into the main LCR population (i.e., increase the inbreeding coefficient), and 2) decrease the N_e of the main LCR population (see Ryman and Laikre 1991, Wang and Ryman 2001).

Inbreeding in an infinitely large population is defined as the mating of individuals that are more closely related to each other than individuals mating at random within a population (Kincaid 1983). All finite populations experience some level of inbreeding. In order to measure the increased level of inbreeding that could potentially occur in a translocation procedure, it is first necessary to know the base level inbreeding coefficient. An inbreeding coefficient can only range from 0 to 1, with zero being the base level (Kincaid 1983). Hence, we will assume that the base level inbreeding coefficient for the main population is zero (as there are no historical data to measure against). Against this assumption, it is possible to then determine increases in the inbreeding coefficient that could occur above Chute Falls (because of a small founder size) or below Chute Falls (because of movement of offspring from above the falls and subsequent interbreeding with the main population). Since the processes of inbreeding take time (generations), it should be possible to monitor for these changes, provided that long-term administrative commitments are set in place and continued. To begin with, arrangements should be made to analyze fin clips that will be taken from the translocated fish. These could be compared to the genetic constitution of the main LCR population once this work is completed.

The question of concern now becomes whether or not inbreeding is likely to become a problem. There should be recognition of the power of selection to eliminate detrimental variation (Dr. Phil Hedrick, Arizona State University and Dr. C. Walters, University of British Columbia). If inbreeding due to finite effective population size occurs, and the population size is in the hundreds, the negative effect of fitness would probably be small for generations, and this detrimental effect may be eliminated by selection (P. Hedrick, pers. com.). For example, even if only 50 males and 50 females survived to randomly reproduce, this would theoretically result in a rate of inbreeding increase per generation of 0.0050 (Kincaid 1983). For wild stocks, Soulé (1980) states that the maximum inbreeding rate should probably not exceed 0.01. Unless the translocated population fell to < 25 pairs, this number (0.01) should not theoretically be exceeded (Kincaid 1983). Hence, this does appear to assure a level of comfort, provided that the number of breeders in the translocated population remains sufficiently large from year to year (i.e., > 25 pairs, or $N_e > 50$). Nevertheless, a population held in check at $N_e = 50$ for 20 to 30 generations will lose about 25% of its genetic variation (Soulé 1980). What the preceding discussion means is that severe effects of inbreeding (loss of heterozygosity) should probably not be a concern for many generations. Since humpback chub have a generation time of > 4 years (C. Walters, pers. com.), this translates into decades. However, traits such as behavior, morphology, reproductive capacity, and physiological efficiency are likely to involve quantitative genetics (gene complexes governed by allelic frequencies; Kincaid 1983). From this respect, maintaining a translocated population at 250 pairs ($N_e = 500$), or higher, would be desirable (Franklin 1980, Lande 1995, Lynch et al. 1995).

Another factor that may negate these concerns is that the LCR is highly stochastic in nature. A small group of founders subject to high environmental stochasticity might not be expected to persist (Leigh 1981). From this perspective, the genetic concerns about inbreeding may be minimal (i.e., the founding population may have a high probability of going extinct before genetic problems have time to develop).

Nevertheless, concerns remain about the ability to track and monitor any potential effects that could occur from the proposed translocation effort. This is because fish will be translocated above Chute Falls at small sizes (50 to 100 mm); and will be batched marked (with visible implant flourescent elastomer), rather than individually marked with PIT tags. This means that it will be impossible to determine the survivorship of the founders (mark-recapture experiments will not be immediately possible). In addition, uncertainties exist about the retention time of flourescent elastomer at this point in time (B. Persons, AGFD). Even if an effort is made to PIT tag the translocated fish once they reach 150 mm in their new habitat, the low initial numbers (i.e., < 300) suggest that an accurate estimate of survivorship will not be possible (i.e., confidence intervals on a population estimate will likely be very large). Without this knowledge, a high degree of uncertainty will always exist as to the number of founders that survive. If, for example, only a few pairs survive to produce a large number of offspring, it will not be known.

Equally important, it will be difficult (if not impossible) to accurately measure downstream levels of gene flow from the resulting offspring above Chute Falls. Hence, the potential impacts on the main LCR population will remain unknown until a change is detected (if an effort is made to detect a change). Attempts could be made to monitor downstream drift of larvae with drift nets, batch mark YOY fish, or PIT tag fish once they reach 150 mm, but these efforts should be expected to be time consuming and contain a high degree of uncertainty. For example, most offspring should be expected to move downstream (below Chute Falls) during flood events. This is precisely when monitoring using larval drift nets will become exceedingly problematic (flood currents can quickly become too strong to set drift nets and nets fill up within a few seconds with large amounts of debris; D. Van Haverbeke, pers. obs.).

Should the translocation be successful, and a large number of offspring occur from an insufficient number of founders, the action may have some potential to decrease the genetic N_e of the main population of humpback chub below Chute Falls. This could happen if a large enough number of offspring from the founder population (with low N_e) survive, and interbreed with fish below Chute Falls (with a higher N_e). Much as depletion of heterozygosity is more likely when a population is supported for multiple generations by captively-raised fish (Ryman and Laikre 1991), this problem could increase over time. However, this potential problem is considered highly unlikely because, if the fish above had reduced fitness from inbreeding, they would be less likely to survive, mate, and reproduce than the fish in the main LCR population (P. Hedrick, pers. com.). As a result, the contribution of such hypothetical fish is likely to be much less than their numbers would predict (P. Hedrick, pers. com.).

It is generally thought that offspring produced from higher up in the LCR have an increased chance for survivorship, since they are less proximate to the mainstem Colorado River and less likely to be transported by flood events into the mainstem Colorado River. In fact, this is the main reason for translocating the fish in the first place (i.e., increase survivorship probabilities for some YOY fish as a mitigation measure). There is unexploited habitat by humpback chub above Chute Falls, accompanied by increased food resources and decreased predators. These factors suggest a possibility for high survivorship of offspring fish above Chute Falls.

Generally, managers respond to these sorts of genetic threats by artificially imposing gene flow into the smaller population (i.e., the One Migrant Per Generation rule; Mills and Allendorf 1996). For example, a number of fish (1 to 10) might be moved each generation above Chute Falls. Although such remedial tactics appear to prevent fixation or further loss of heterozygosity within the small population (Mills and Allendorf 1996), it does not appear to address the initial problem (i.e., a very small founder population will likely have decreased heterozygosity from the start).

In addition, the One Migrant Per Generation Rule assumes no natural selection is occurring in either population (i.e., only drift and gene flow are in operation). However, there is selection occurring in the LCR already and will continue to be. This is important since translocation of fish into a situation with unidirectional gene flow suggests that selection could cause other subtle concerns. For instance, fish that survive generation after generation to reproduce above Chute Falls will undergo selection for being non-migratory. In addition, the translocated fish may experience selection forces because of the elevated levels of CO_2 above Chute Falls, or other environmental factors. Because of the potentials for selection to act upon the translocated portion of fish (i.e., move away from the main genotype), there is potential for migrants leaving this isolated group of fish to impact the genotype of the main LCR population, even if the amount of movement is small (see Ford 2000). There are many documented cases of subtle decreases occurring in fitness when gene flow occurs between subpopulations experiencing different or conflicting selective forces (Storfer 1999).

As an alternative to using the One Migrant Per Generation Rule, it may be advisable to essentially continue swamping the translocated fish with a high number of fish from the main population each generation. For example, managers could repeat the movement of fish above Chute Falls for several years and then continue to move smaller numbers of fish (say 100 YOY) once a fish generation. Such an approach is suggested by P. Hedrick, who thinks that the One Migrant Per Generation Rule is inappropriate in this instance and that the numbers should be higher.

Finally, a translocation of fish above Chute Falls should be expected to cause other unknown (and unexpected) ecological effects. For example, based on visual observation, the habitat above Chute Falls is conspicuously different from that below Chute Falls. While algal communities exist below Chute Falls, they tend to be meager and the substrates are dominated by marle, sand or gravels. In comparison, the algal communities above Chute Falls are dense, diverse, and luxuriant, often covering the substrates. Robinson et al. (1996) found that chlorophyll *a* biomass was significantly greater above Chute Falls. They also found that eight taxa of aquatic invertebrates were found above Chute Falls that were not found below, and that densities of invertebrates were significantly higher above Chute Falls. In addition, densities of speckled dace above Chute Falls may be an order of magnitude or two higher than those below Chute Falls (D. Van Haverbeke, pers. obs.). Many of these differences may be because humpback chub do not currently inhabit this area (hence primary production and prey are not cropped to the degree they are below the falls). The lush community above Chute Falls may be an important food source for humpback chub below the falls during floods or during post flood events (i.e., many components of this upriver community are washed downriver during flood events). If the portion of the LCR currently inhabited by humpback chub is limited by carrying capacity (as some hypothesize), the upstream community above Chute Falls could be important for maintaining the carrying capacity for humpback chub in the LCR during flood and post flood times. In short, translocating fish and establishing a population above Chute Falls could have important and unpredictable ecological effects below the falls, should this community begin to show changes because of the translocated fish.

The above risks should be tempered with the realization that overall rapid decline in the humpback chub population could potentially have significant genetic impacts and that action to slow this is important. A reduction in fitness because of contemporary population decline appears to be a particular problem in species with large ancestral populations (as the humpback chub), and consequent high historical variation in fitness (P. Hedrick, pers. com.). The speculated potential gains in establishing fish above Chute Falls would be to: 1) achieve a demographic boost in the main LCR population, 2) expand the range of the species, and 3) contribute to a self-sustaining wild population. Concerning the first, there is some potential for a demographic gain. It should be realized that the potential for demographic boost will likely be limited to < 280 humpback chub > 200 mm. Assuming a current population of ~2,000 breeders, this translates into a 14% maximum demographic boost. This gain may or may not be viewed as sufficient to offset the preceding genetic concerns.

Concerning the potential to expand the range of the species, the proposed action does offer the potential to expand the range of the species in the LCR by another 6.8 km. Much more likely, the range expansion would be restricted to ~1-3 km above Chute Falls, as CO_2 levels continue to increase further upriver, and fish communities begin to dwindle. Speckled dace are seen up to about a kilometer below Blue Springs (Mattes 1993), suggesting that km 20 may be the uppermost reach that humpback chub would be expected to survive. Nevertheless, even a 1 km expansion would represent a 5% increase in occupied habitat in the LCR, and a 6 km expansion would represent a 30% increase. Unfortunately, expansion into this range should not be expected to function as a refuge from catastrophic loss in the LCR.

Third, unlike captive propagation and supportive stocking or supplemental stocking using wild caught YOY fish, a successful translocation (if properly done) has potential to further promote a humpback chub population that is self-sustaining. Largely because of this factor, it may be advisable to attempt this action prior to enacting other potential options. However, the potential risks and benefits should be thoroughly peer reviewed before the action is implemented.

A translocation effort above Chute Falls should be accompanied by several long-term commitments to manage and monitor the fish above Chute Falls. For example, a long-term commitment should be made to monitor this group of fish via mark-recapture efforts once the group of fish becomes established. A long-term commitment should be made to maintain an appropriate level of bi-directional gene flow, which should be accompanied by a long-term commitment to monitor genetic aspects of the fish both above and below Chute Falls (i.e., particularly changes in heterozygosity). A long-term commitment should also probably be made to monitor the algal and invertebrate communities above and below Chute Falls. It should be realized that helicopter support logistics may be difficult or impossible during many times (i.e., it is often difficult or impossible to land a helicopter anywhere near Chute Falls). The fact of the matter is that working in the LCR above Chute Falls becomes difficult at best, and can be lethal because the Canyon narrows, making escape from flood waters impossible in many areas.

In summary, a translocation effort above Chute Falls may or may not be successful. If it is successful, some uncertainties exist about potential genetic impacts to the main population of humpback chub below the falls, since translocations have both short and long term consequences for the evolutionary ecology of the targeted species (Stockwell and Leberg 2002). These uncertainties revolve around concerns with inbreeding, effective population sizes, and the ability (or lack of ability) to correct for problems associated with unidirectional gene flow. These uncertainties should be expected to remain in place, since *a priori* base genetic information on the main population is not known, and the genetic factors involved are complex. The proposed translocation does, however, have potential to create a small demographic boost, to expand the current range of the species, and to further promote a self-sustaining population. This effort should be accompanied by long-term commitments to manage and monitor a small group of fish subject to genetic uncertainties because of unidirectional gene flow and small effective population size. Finally, it should be expected that the translocation effort will have other ecological consequences that may be unpredictable and unexpected. If the underlying goal is to establish a group of fish above Chute Falls, the objectives should be clearly stated in terms of a potential to establish a reproductively isolated population, and the additional risks carefully considered and weighed. It should be further understood that the Biological Opinion calls for the translocation of only 300 fish above Chute Falls. Although managers are proposing translocating more fish in the future, these proposals are premature unless consultation with USFWS is first clarified. This effort, if initiated, will require long-term commitment and funding.

<u>Translocation of humpback chub to other tributaries within Grand Canyon</u>

In addition to translocation of fish within the LCR, some proponents have advocated translocation of fish to other tributaries in Grand Canyon. The main tributaries of interest have been Paria, Bright Angel, Shinumo, Deer, Tapeats, Kanab, Havasu and Spencer creeks (Valdez et al. 2000). Of these, Havasu, and Shinumo creeks (above the waterfall barriers in both creeks) were identified as the most likely candidates.

From a genetics standpoint, any of these tributaries should be expected to be of much less risk to the main population of humpback chub than a translocation effort above Chute Falls. These tributaries are all well down river from the LCR (Bright Angel being the closest at ~26 miles from the LCR). Hence, the potential for offspring to genetically swamp the main LCR population should be minimal (probably non-existent). A complete analysis of humpback chub movement in Grand Canyon has not been performed to date, however, preliminary investigations do suggest that migration of fish from far downriver to the LCR is very minimal (L. Coggins; pers. com.). For instance, only two fish have been identified as moving from Bright Angel creek or below to the LCR (i.e., one from Shinumo creek vicinity and one from Havasu creek).

The main concern of establishing a small group of humpback chub in other tributaries is again related to an inability to support a viable genetic effective population size of fish (Valdez et al. 2000). For example, the authors estimated that Havasu Creek might be able to sustain 462 adults, while Shinumo might sustain 110 adults. Both numbers fell well below their genetic viability guidelines, indicating that inbreeding would be a problem. Nevertheless, the authors did recommend an experimental test of establishing humpback chub in at least one, and preferably more than one, tributary. This was primarily because a small tributary “population” would have value as a backup against catastrophic loss and function as a refuge.

Three places that were largely discounted by Valdez et al. (2000) as being viable options were Bright Angel Creek, and Havasu and Shinumo creeks (the later two below their respective waterfall barriers). Bright Angel Creek was discounted because of large numbers of predators (i.e., brown and rainbow trouts), and

Shinumo and Havasu creeks (below their barriers) were discounted because of access to only 100 to 200 m of stream. However, it might be worthwhile to revisit these options in view of recent serious attempts to remove predators in Grand Canyon. For example, continued attempts to remove brown trout via a weir in Bright Angel Creek should be expected to open niche space within the creek. Should a simultaneous effort be made to remove brown trout in the mainstem between Zoroaster and Horn Creek rapids (RM 84.7- 90.2), this might open up enough niche space in the mainstem to support a viable number of humpback chub. If the population of brown trout in the mainstem near Bright Angel is primarily supported by Bright Angel Creek spawning activity, mainstem efforts may not need to be carried out for extended periods (i.e., a few removal passes may accomplish much). The same tactics could be employed for establishing viable numbers of fish in Shinumo and Havasu creeks. For example, a weir could be placed in Shinumo Creek, while a few simultaneous efforts are made to remove mainstem predators (brown and rainbow trouts) between Bass and Waltenberg rapids (RM 107.9 – 112.1). A weir in Havasu Creek would probably do little, since most fish that spawn in the mouth of Havasu are flannelmouth and bluehead sucker. However, mainstem efforts to remove predators between Last Chance Camp to a few miles below Havasu Creek (e.g., RM 156 – 159) may open niche space for humpback chub. For example, large humpback chub were occasionally captured between Havasu Creek and Last Chance Camp in the mainstem during the early 1990s (Valdez and Ryel 1995), but efforts in the late 1990s showed no such catches. If the assumption is correct that recruitment in the LCR is being hampered because of YOY and juvenile mortality, and that predator removal efforts in the mainstem near the LCR may alleviate this problem, then these tactics might want to be considered elsewhere. Although the carrying capacities of Bright Angel, and Shinumo and Havasu creeks below the barriers may be too small to support viable numbers of humpback chub within the creeks themselves, opening niche space in the mainstem near these tributaries may allow the support of viable population numbers. The problem of visitor impact in Shinumo (and possibly Havasu) would need to be addressed. In addition, rather than posing any genetic risks to the LCR population, establishment of humpback chub in these areas would more likely be accompanied by continued immigration downriver from the LCR population, keeping downstream aggregations of fish swamped with genes from the LCR fish (and slowing or preventing inbreeding depression in the local downstream aggregations).

There are additional reasons why the above scenarios could in fact be viable options. First, aggregations of humpback chub are known to have existed near the mouths of all of these tributaries. Some historical evidence for Bright Angel Creek comes from a spectacular photograph taken on the Rust expedition (Photograph 1). The picture clearly shows that a large number of humpback chub were captured at Roy’s Beach (a short distance above Bright Angel Creek), during a day of fishing. It is not known if these fish were mainstem spawners or tributary spawners, but the picture does indicate that enough niche space formerly existed in this reach of river to support a large number of adult humpback chub at some part of their life history. Aggregations of humpback chub were more recently reported as existing near Bright Angel, Shinumo and Havasu creeks (Valdez and Ryel 1995). The presence of adults residing year round in these mainstem reaches suggests some affinity to these tributaries. The decline in catch rates of these large fish in the past decade also suggests that lack of recruitment from these respective tributaries may the cause. Second, all three tributaries are known to support spawning populations of native fish. Bright Angel Creek sees annual spawning runs of bluehead sucker and flannelmouth sucker. Adult bluehead sucker, flannelmouth sucker, and occasionally humpback chub are still captured in Shinumo Creek during the summer months (unpublished data, GCMRC). Large spawning aggregations of flannelmouth sucker (Douglas and Douglas 2000), and bluehead sucker have been captured in Havasu Creek, and adult humpback chub are still occasionally seen (or captured) in Havasu Creek (unpublished data, GCMRC). Very infrequently in the past 15 years, small numbers of YOY humpback chub have been captured in Shinumo (Valdez and Ryel 1995) and Kanab creeks (D. Van Haverbeke, pers. obs.). Third, the presence of mass spawning of salmonids (particularly in Bright Angel and Shinumo creeks), along with large numbers of these fish found in the mainstem Colorado River near these tributaries suggests that even though spawning habitat may be limited within the tributaries themselves, it may be sufficient to support large populations of adult fish in the surrounding mainstem. Taken as a whole, the above observations suggest that:

1) some small tributaries in Grand Canyon still support aggregations of native fish, and may have historically supported viable aggregations of humpback chub 2) predation and other environmental concerns (such as cold and fluctuating mainstem flows) have resulted in recruitment failure 3) these tributaries could be revisited in the context of efforts to re-establish (or augment) humpback chub aggregations 4) such efforts may require, at a minimum, removal of predators from the tributaries themselves, and from the surrounding mainstem, and 5) if the current abundances of salmonids in the mainstem near these tributaries represents potential niche space to support adult fish, significant population gains in humpback chub abundances might be attainable (provided this niche space is first opened). In addition to predator removal, efforts to re-establish these aggregations may require initially “jump starting” these aggregations with the LCR fish, and may require flow modifications or thermal modifications in the mainstem Colorado.

CONCLUSIONS

This document has reviewed several potential options for augmenting the population of humpback chub in Grand Canyon. Each option appears to have some potential for success, and appears to involve some levels of risk (Table 3).

Establishing a captive broodstock of humpback chub followed by supportive stocking should be viewed as a last recovery option. This is based on both legal and biological considerations. Legal considerations stem from USFWS and NOAA's policy on captive broodstock, and the fact that captive broodstock and supportive stocking activities are not incorporated in the latest Recovery Goals for humpback chub. Biological considerations stem from a wide range of genetic and behavioral problems that can result from using captive bred individuals for supportive stocking. Major genetic problems include: 1) potential for inbreeding to occur within the captive population, 2) potential to reduce the N_e of the wild population, and 3) potential to impact the wild population by input from fish that have become genetically domesticated in a hatchery. Problems associated with behavior of captive bred fish largely are associated with poor post-stocking survivorship, although this problem represents less risk to the wild population, and there are options available that can potentially overcome this problem.

The humpback chub at Willow Beach NFH may be considered as potential future broodstock. Before such consideration can proceed, several steps, currently unfunded, would be required: 1) determine the genetic constitution of the Willow Beach fish, 2) compare this respective small population with the respective genetic constitution of the wild population in Grand Canyon, and 3) develop protocols and methodologies to ensure that the original captive fish come to equal those of the wild population. A similar tactic could be taken to develop a broodstock from fish taken from 30-mile, however, this may entail keeping a separate broodstock from LCR fish (should they show genetic differences).

Development of a captive broodstock by itself may be a relatively benign (although expensive) activity. For instance, a captive broodstock in itself does not pose genetic risk to the wild population. Furthermore, a captive broodstock would help ensure against extinction by catastrophic loss, and serve as a genetic refugium. However, development of a captive broodstock followed by supportive stocking activities holds potential for multiple genetic risks to the wild population. Supportive stocking activities from a captive population within the near future should not be considered an option until all other management activities have been attempted and shown to be insufficient. At this point in time, however, working toward development of a captive broodstock may foster completion of preliminary actions (e.g., genetics work, captive broodstock management plans, etc.). It should be realized that a fully developed captive broodstock will likely entail the construction of significant isolation facilities, the identification of appropriate locations to hold several thousand fish, and will be a long-term and very costly commitment.

This document does not presume to give specific criteria for when broodstock and supplemental stocking activities should commence. Obviously at some point, risk of extinction in the very near future dictates that more extreme conservation measures are taken. For example, complete lack of natural recruitment and an inability to rectify this situation would dictate that captive broodstock and supplemental stocking activities are undertaken. Nevertheless, managers will be compelled to initiate such activities in the face of a continuing population decline, particularly when other measures to rectify the situation are failing, or are viewed as likely to fail. Fortunately, humpback chub is a long-lived species, and there appears to be time to make appropriate decisions. In addition, major alternative conservation options that have been put forth have not yet been attempted (e.g., thermal control device). However, these options must be tried. Predator removal efforts have only just begun, but appear to be showing promising signs (in terms of predator depletion). Given the scale and intensity of the predator removal efforts in Grand Canyon, some effects might be expected in the near future. For example, catch rates of YOY and juvenile humpback chub may increase in mainstem hoop-net sets, larger spawning aggregations of native fish may be detected in the LCR, catch rates of adult humpback chub may increase in the mainstem near the vicinity of LCR as habitat formerly occupied by salmonids becomes available, etc. All of these would be positive indicators, however, managers cannot afford to let demographic numbers of adults dwindle to a dangerously low population level.

But what is this level? According to earlier literature, a N_e of 500 should be maintained (Franklin 1981). This might suggest to some that the population status of humpback chub in Grand Canyon is fine, as long as numbers do not continue to drop. According to more recent literature, minimum viable population levels should be maintained at N_e = 1,000 (Lynch et al. 1995) or even N_e = 5,000 (Lande 1995). This suggests a need for some type of action to augment the population of humpback chub in Grand Canyon. Clearly, attempting to establish a "magic number" to initiate captive broodstock and supplemental stocking activities is impossible without knowledge of the N_e of the wild population. It might be prudent to work toward estimating the N_e of the wild population in Grand Canyon, as has already been suggested (Anders et al. 2001). It may also be prudent to prepare for the development of a captive broodstock (i.e., complete ongoing genetics work or initiate more genetics work as needed, develop a captive broodstock management plan, identify potential hatchery site, procure significant construction and operating funds, etc.).

Establishing a program for capture of YOY fish, followed by grow out and release into the wild appears to hold minimal genetic concerns, provided that mortality is kept to an absolute minimum (i.e., no artificial selection). The main problem with such a program appears to be related to changes in the behavior of fish that are held in captivity for a significant portion of their lives. Because of this, post-stocking mortality should probably be expected to be high. Actions can and should be taken to minimize hatchery and post-stocking mortality including: 1) matching captive conditions to wild conditions to the extent possible (e.g., providing conditioning to appropriate water currents, temperatures, substrates, turbidity levels, food types, predators, etc.), and 2) following soft release rather than hard release protocols.

It should be realized that developing of a program for this type of activity will probably require the removal of some 2,000 to 4,000 YOY fish from the LCR on an annual basis, and that such an effort will require a long term commitment (i.e., it may take one or two decades to see a reversing upward trend in the wild population). Hence, it will be a costly commitment that may or may not prove beneficial. The action does, however, immediately address what is thought to be the primary factor for population decline in Grand Canyon, that is lack of recruitment.

It should also be realized that a supplemental stocking program using wild caught YOY fish will need to be adaptive in nature. For example, some level of continued monitoring of the annual YOY cohort will need to be maintained in the LCR to ensure that this activity does not result in significantly cropping wild recruitment. In addition, various methods for grow out and release of fish will need to be tried (e.g., growing fish in stream tanks vs. ponds, experimenting with different natural food types, exposing the fish to various levels or types of pre-conditioning training for predators, attempting different soft release protocols, etc.). It should also be realized that the place of release for the fish will require an adaptive management approach. For example, releasing too many fish into the LCR could impose risks to the wild population in LCR by suddenly over-taxing the carrying capacity in LCR. Accidental release of hatchery parasites into the wild population will always remain a risk, as with any supplemental stocking activities.

Carrying out a program for the translocation of humpback chub above Chute Falls in the LCR should be met with cautious optimism. There is potential to gain a small demographic boost, however, this gain should be expected to be < 300 individuals > 200 mm. There is also some potential to expand the range of the species (maximum of ~6 km), however, this expansion should not be viewed as providing any significant security in terms of providing a refugium from catastrophic loss. Finally, unlike other alternatives, this proposed action does have the potential to further promote a self-sustaining population. On the other hand, the action could potentially be accompanied by several genetic risks to the wild population. This is primarily because the action has potential to establish a small reproductively isolated group of fish within immediate proximity to the main LCR population. The action may have potential to: 1) increase the inbreeding coefficient of the wild population, and 2) decrease the N_e of the wild population. These potential negative effects are expected to be minimal or unlikely, and should be viewed in the context that they would probably take decades to occur. Nevertheless, because of a lack of knowledge about the genetic constitution of the wild population, and because of methodologies (as currently proposed), this action could result in long-term uncertainties about the genetic impact to the wild population. Finally, the action may have other unexpected and unpredictable ecological consequences related to the food base and carrying capacity of the LCR.

Carrying out a program for the translocation of humpback chub into other tributaries in Grand Canyon should be expected to be of less risk to the main LCR population (in terms of genetics). This is because these tributaries are much less proximal to the main spawning population in LCR. Some potential exists for establishing small populations of humpback chub (< 500 individuals per creek) in Havasu or Shinumo creeks above their barriers, although these populations will be subject to inbreeding (Valdez et al. 2000). They do have potential, however, for acting as refugia (Valdez et al. 2000). If ongoing predator removal efforts prove to be successful in Bright Angel Creek, there may be potential for establishing humpback chub in this tributary. The potential for gaining a demographic boost may be enhanced by simultaneously removing mainstem predators between Grapevine and Horn Creek rapids. The same tactic could be taken in Shinumo Creek below the barrier falls (i.e., install a weir, remove spawning predators within the creek, and remove mainstem predators from Bass to Waltenburg rapids). Since larger numbers of native fish currently spawn in Havasu Creek (below the barrier), removing predators in the mainstem within a few miles of the vicinity of Havasu creek may also accomplish a similar result. It should be realized that each of these small tributaries by themselves appear to have insufficient carrying capacity to support a population of humpback chub not subject to inbreeding. Yet, each tributary may have enough spawning habitat to support a viable group of humpback chub (provided niche space is opened in the mainstem (e.g., via predator removal). It should also be realized that translocation and predator removal efforts by themselves may not be sufficient, and that some level of warming mainstem waters may be required to initiate an effect.

As a final consideration, managers should be aware that each of the potential management actions discussed above involves unique potentials for demographic boost or for enhancing recruitment for the humpback chub. Achieving small demographic boosts attended with high genetic risks should not be a goal. Rather, achieving continuous and self-sustaining gains in demographics via improvement of natural recruitment will do much to benefit the humpback chub, and will do much to achieve eventual downlisting and delisting of the species.

Table 3. Summarized risks and benefits associated with various potential management actions.

Action	Risks					Benefits	
	Risk of inbreeding depression	Risk of inbreeding depression to wild population	Risk of decreasing Ne in the wild population	Genetic domestication issues	Behavioral concerns	Potential for demographic boost	Potential to expand range of the species or act as a genetic refuge
Captive broodstock	X			X	X		Genetic refugia
Captive broodstock followed by supportive stocking	X	X	X	X	X	Potentially large	Potential to increase densities in mainstem
Supportive stocking using wild YOY fish				Should be minimal unless high hatchery mortality occurs**	X	Potentially large enough to reverse declining trend over time	Genetic refugia
Translocation of fish above Chute Falls	X	Minor risk over long term	Minor risk over long term			Small (< 300 adults)	1-6 km potential range expansion
Translocation of fish to Bright Angel Creek	X					Could be large if proximal mainstem area becomes colonized	Potential to increase density in nearby mainstem
Transocation of fish to Shinumo or Havasu creeks above barriers	X					Small (< 500 individuals per creek)	Very small range expansion (< a few km), and genetic refugia
Transocation of fish to Shinumo or Havasu creeks below barriers	Probably not a concern since there should be migrants from LCR population					Could be large if proximal mainstem area becomes colonized	Potential to increase density in nearby mainstem

**However, relaxation of wild selection will occur during the culture phase.

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LITERATURE CITED

Abruzzi, W.S. 1995. The social and ecological consequences of early cattle ranching in the Little Colorado River basin. Human Ecol. 23: 75-98.

Anders, P.J. 1998. Conservation aquaculture and endangered species: Can objective science prevail over risk anxiety? Fisheries 28 (No. 11):28-31.

Anders, P., M. Bradford, P. Higgins, K.H. Nislow, C. Rabeni, and C. Tate. 2001. Grand Canyon Monitoring and Research Center: Protocols Evaluation Program. Final Report of the Aquatic Protocol Evaluation Program Panel. 43 pp.

Andrews, C. and Kaufman, L. 1994. Captive breeding programmes and their role in fish conservation. Pp. 338-351 *In*: P.J.S. Olney, G.M. Mace and A.T.C. Feistner (eds.) Creative Conservation: Interactive Management of Wild and Captive Animals. Chapman and Hall, London.

Arnold, S.J. 1995. Monitoring quantitative genetic variation and evolution in captive populations. pp. 295-317 *In*: J.D. Ballou, M. Gilpin and T.J. Foose (eds.), Population Management for Survival and Recovery. Columbia Press, New York.

Boyce, M.S. 1992. Population viability analysis. Annu. Rev. Ecol. Syst. 23: 481-506.

Brannon, E.L. 1993. The perpetual oversight of hatchery programs. Fishery Research 18: 19-27.

Brown, C. and K.L. Laland. 2001. Social learning and life skills training for hatchery reared fish. Journal of Fish Biology 59: 471-493.

Brouder, M.J. & T.L. Hoffnagle. 1997. Distribution and prevalence of the Asian fish tapeworm, *Bothriocephalus acheilognathi*, in the Colorado River and tributaries, Grand Canyon, Arizona, including two new host records. J. Helminthological Soc. Wash. 64: 219-226.

Busack, C.A. and K.P. Currans. 1995. Genetic risks and hazards in hatchery operations: Fundamental concepts and issues. American Fisheries Society Symposium 15: 71-80.

Brown, C. and R.L. Day. 2002. The future of stock enhancement: lessons for hatchery practice from conservation biology. Fish and Fisheries 3: 79-94.

Caro, T. 1999a. Behavioral Ecology and Conservation Biology. Oxford University Press, Oxford, 582 pp.

Caro, T. 1999b. The behavior-conservation interface. Trends in ecology and evolution 14: 366-369.

Childs, M.R., R.W. Clarkson, and A.T. Robinson. 1998. Resource use by larval and early juvenile native fishes in the Little Colorado River, Grand Canyon, Arizona. Trans. Am. Fish. Soc. 127: 620-629.

Clarkson, R.W. and M.R. Childs. 2000. Temperature effects of hypolimnial release dams on early life stages of Colorado River Basin big river fishes. Copeia 2000: 402-412.

Clarkson, R.W., A.T. Robinson, and T.L. Hoffnagle. 1997. Asian tapeworm (*Bothriocephalus acheilognathi*) in native fishes from the Little Colorado River, Grand Canyon, Arizona. Great Basin Nat. 57: 66-69.

Clemmons, J.R. and R. Buchholz. 1997. Behavioral Approaches to Conservation in the Wild. Cambridge University Press, Cambridge, 398 pp.

Coggins, L., C. Walters, C. Paukert and S. Gloss. 2003. An overview of status and trend information for the Grand Canyon population of the humpback chub (*Gila cypha*). Prepared by the Grand Canyon Monitoring and Research Center, USGS, Flagstaff AZ for the Glen Canyon Dam Adaptive Management Work Group Ad Hoc Committee on Humpback Chub March 12 2003. 23 pp.

Coggins, L., M. Yard and C. Paukert. 2002. Piscivory by non-native salmonids in the Colorado River and an evaluation of the efficacy of mechanical removal of non-native salmonids. Grand Canyon Monitoring and Research Center, U.S. Geologic Survey, Flagstaff, AZ. 40 pp.

Cross, T.F. 2000. Genetic implications of translocation and stocking of fish species, with particular reference to Western Australia. Aquaculture Research 31: 83-94.

Curio, E. 1996. Conservation needs ethology. Trends in Ecology and Evolution 11: 260-263.

Douglas, M.R. and M.E. Douglas. 2000. Late season reproduction by big-river Catostomidae in Grand Canyon (Arizona). Copeia 2000: pp. 238-244.

Douglas, M.E. and P.C. Marsh. 1996. Population estimates/population movements of *Gila cypha*, an endangered Cyprinid fish in the Grand Canyon region of Arizona. Copeia 1996: 15-28.

Dowling, T.E., W.L. Minckley, P.C. Marsh, and E.S. Goldstein. 1996. Mitochondrial DNA variability in the endangered razorback sucker (*Xyrauchen texanus*): Analysis of hatchery stocks and implications for captive propagation. Conservation Biology 10: 120-127.

Doyle, R.W., R. Perez-Enriquez, M. Takagi, and N. Taniguchi. 2001. Selective recovery of founder genetic diversity in aquacultural broodstocks and captive, endangered fish populations. Genetica 111: 291-304.

Ersbak, K. and B.L. Haase. 1983. Nutritional deprivation after stocking as a possible mechanism leading to mortality in stream-stocked brook trout. North American Journal of Fisheries Management 3: 142-151.

Fleming, I.A., A. Lamberg, and B. Jonsson. 1997. Effects of early experience on the reproductive performance of Atlantic salmon. Behavioral Ecology 8: 470-480.

Ford, M.J. 2002. Selection in captivity during supportive breeding may reduce fitness in the wild. Conservation Biology 16: 815-825.

Frankel, O.H. and M.E. Soulé. 1981. Conservation and evolution. Cambridge. Cambridge University Press.

Frankham, R. and D.A. Loebel. 1992. Modeling problems in conservation genetics using captive *Drosophila* populations: rapid genetic adaptation to captivity. Zoo Biology 11: 333-342.

Frankham, R., H. Hemmer, O.A. Ryder, E.G. Cothran, M.E. Soulé, N.D. Murray, and M. Snyder. 1986. Selection in captive populations. Zoo Biology 5: 127-138.

Franklin, I.R. 1980. Evolutionary change in small populations. pp. 135--149. *In*: Soulé, M.E. and B.A. Wilcox (eds.) Conservation Biology: An evolutionary-ecological perspective, Sinauer Associates Inc., Sunderland, Massachusetts.

Gilpin, M.E. & M.E. Soulé. 1986. Minimum viable populations: Processes of species extinction. pp. 19--34. *In*: M.E. Soulé (ed.) Conservation Biology: The science of scarcity and diversity, Sinauer Associates Inc., Massachusetts.

Goodman, D. 1987. How do any species persist? Lessons for Conservation biology. Conservation Biology1: 59-62.

Gorman, O.T. 1994. Habitat use by the humpack chub, *Gila cypha*, in the Little Colorado River and other tributaries of the Colorado River. Final Reprt for Glen Canyon Environmental Studies Phase II. U.S. Fish and Wildlife Service, Flagstaff, AZ.

Gorman, O.T. and D.M. Stone. 1999. Ecology of spawning humpback chub, *Gila cypha*, in the Little Colorado River near Grand Canyon, Arizona. Environmental Biology of Fishes 55: 115-133.

Gorman, O.T. and R. R. VanHoosen. 2000. Experimental growth of four native Colorado River fishes at temperatures of 12, 18, and 24 ^{o}C. Draft Final Report submitted to Grand Canyon Monitoring and Research Center, Flagstaff Arizona. 26 pp.

Gosling, L.M. and W.J. Sutherland. 2000. Behavior and Conservation. Cambridge University Press, Cambridge, 438 pp.

Griffith, B., J. M. Scott, J.W. Carpenter, and C. Reed. 1989 Translocation as a species conservation tool: Status and strategy. Science 243: 477-480.

Hamman, R.L. 1982. Spawning and culture of humpback chub. Progressive Fish Culturist 44(4): 213-216.

Hansen, L.P., B. Jonsson. 1994. Homing of Atlantic salmon: effects of juvenile learning on transplanted post-spawners. Anim. Behav. 47: 220-222.

Hasler, A.D. and Scholz. 1983. Olfactory imprinting and homing in salmon. Berlin: Springer-Verlag.

Hedrick, P.W., D. Hedgecock, and S. Hamelberg. 1994. Effective population size in winter-run Chinook salmon. Conservation Biology 9: 615-624.

Hilborn, R. 1992. Hatcheries and the future of salmon in the Northwest. Fisheries 17: 5-8.

Hilborn, R. 1998. The economic performance of marine stock enhancement projects. Bulletin of Marine Science 62(2): 661-674.

Hindar, K., N. Ryman, and F. Utter. 1991. Genetic effects of cultured fishes on natural populations. Can. J. Fish. Aquat. Sci. 48: 945-957.

Howell, B.R. 1994. Fitness of hatchery-reared fish for suvival in the sea. Aquaculture and Fisheries Management 25 (suppl 1): 3-17.

Hynes, J.D., E.H. Brown, Jr., J.H. Helle, N. Ryman, and D.A. Webster. 1981. Guidelines for the culture of fish stocks for resource management. Can. J. Fish. Aquat. Sci. 38: 1867-1876.

Johnson, P.W. and R.B. Sanderson. 1968. Spring flow into the Colorado River Lees Ferry to Lake Mead, Arizona. Arizona State Land Department, Water-Resources Report No. 34.

Jonsson, B., N. Jonsson, and L.P. Hansen. 1990. Does juvenile experience affect migration and spawning of adult Atlantic salmon? Behav. Ecol. Sociobiol. 26: 225-230.

Jonsson, N., L.P. Hansen, and B. Jonsson. 1994. Juvenile experience influences timing of adult river ascent in Atlantic salmon. Anim. Behav. 48: 740-742.

Kaeding, L.R. and M.A. Zimmerman. 1982. Life history and ecology of the humpback chub in the Little Colorado and Colorado River of the Grand Canyon, Arizona. pp. 281-320 *In*: W.H. Miller, J.J. Valentine, D.L. Archer, H.M. Tyus, R.A. Valdez, and L.R. Kaeding (eds.), Colorado River Fishery Project, Part 2. Field Investigations. Final Report, US Bureau of Reclamation contract 9-07-40-L-1016, and US Bureau of Land Management Memorandum of Understanding CO-910-MU-933. US Fish and Wildlife Service, Salt Lake City. 324 pp. Copy on file at the GCMRC library.

Kincaid, H.L. 1983. Inbreeding in fish populations used for aquaculture. Aquaculture 33: 215-227.

Kohane, M.J. and P.A. Parsons. 1988. Domestication: evolutionary change under stress. Evolutionary Biology 23: 31-48.

Kolb, E. and E. Kolb. 1914. Experiences in the Grand Canyon. Natl. Geogr. Mag. XXVI (2): 99-184.

Krueger, C.C., A.J. Gharrett, T.R. Dehring, and F.W. Allendorf. 1981. Genetic aspects of fisheries rehabilitation programs. Can. J. Fish. Aquat. Sci. 38: 1887-1881.

Lacey, R.C. 1987. Loss of genetic diversity from managed populations: Interacting effects of drift, mutation, immigration, selection, and population subdivision. Conservation Biology 1: 143-158.

Lande, R. 1995. Mutation and conservation. Cons. Biol. 9: 782-791.

Lande, R. 1981. The minimum number of genes contributing to quantitative variation between and within populations. Genetics 99: 541-553.

Leigh, E.G. 1981. The average lifetime of a population in a varying environment. Journal of Theoretical Biology. 90: 213-239.

Levin, P.S. and J.G. Williams. 2002. Interspecific effects of artificially propagated fish: an additional conservation risk for salmon. Conservation Biology 16: 1581-1587.

Levin, P.S., R.W. Zabel, and G. Williams. 2001. The road to extinction is paved with good intentions: negative associations of fish hatcheries with threatened salmon. Proceedings of the Royal Society of London B, Series B 268: 1-6.

Lichatowich, J., L. Mobrand, and L. Lestelle. 1999. Depletion and extinction of Pacific salmon (Oncorhynchus spp.): a different perspective. International Council for the Exploration of the Sea (ICES). Journal of Marine Science 56: 467-472.

Lyles A.M. 1987. Problems in leaving the ark. Nature 326:245-246.

Lynch, M. and M. O'Hely. 2001. Captive breeding and the genetic fitness of natural populations. Conservation Genetics 2: 363-378.

Lynch, M., J. Conery and R. Bürger. 1995. Mutation accumulation and the extinction of small populations. Amer. Nat. 146: 489-518.

MacArthur, R.H. and E.O. Wilson. 1967. The Theory of Island Biogeography. Princeton University Press, Princeton New Jersey.

Mattes, W.P. 1993. An evaluation of habitat conditions and species composition above, in, and below the atomizer falls complex of the Little Colorado River. M.S. Thesis submitted to School of Renewable Natural Resources, University of Arizona, Tuscon, AZ. 105 pp.

Maynard, D., T. Flagg, and C. Mahnken. 1995. A review of semi-culture strategies for enhancing the post-release survival of anadromous salmonids. American Fisheries Society Symposium 15: 307-314.

McNeil, W. 1991. Expansion of cultured Pacific salmon into marine ecosystems. Aquaculture 98: 123-130.

Meffe, G.K. 1992. Techno-arrogance and halfway technologies: salmon hatcheries on the Pacific coast of North America. Conservation Biology 6: 350-354.

Miller, R.R. 1964. Fishes of dinosaur. Naturalist 15: 24-29.

Miller, R.R. 1963. Distribution, variation, and ecology of *Lepidomeda vittata*, a rare cyprinid fish endemic to Eastern Arizona. Copeia 1963: 1-5.

Minckley, W.L. 1995. Translocation as a tool for conserving imperiled fishes: experiences in Western United States. Biological Conservation 72: 297-309.

Minckley, C.O. 1992. Observed growth and movement in individuals of the Little Colorado population of the humpback chub (*Gila cypha*). Proceedings of the Desert Fishes Council 22: 35-36.

Minckley, W.L. 1991. Native fishes of the Grand Canyon region: an obituary? pp 124-177 In Colorado River ecology and dam management. National Academy Press, Washington D.C.

Minckley, W.L., D.A. Hendrickson, and C.E. Bond. 1986. Geography of western North America freshwater fishes: Description and relationships to intracontinental tectonism. pp. 519--613 *In*: C.H. Hocutt & E.O. Wiley (eds.) The Zoogeography of North American Freshwater Fishes, Wiley Interscience, New York.

Minckley, W.L., P.C. Marsh, J.E. Deacon, T.E. Dowling, P.W. Hedrick, W.J. Mathews, and G. Mueller. 2003. A conservation plan for native fishes of the lower Colorado River. Bioscience 53: 219-234.

Naylor, R.L., R.J. Goldburg, J.H. Primavera, N. Kautsky, M.C.M. Beveridge, J. Clay, C. Folke, J. Lubchenco, H. Mooney, and M. Troell. 2000. Effect of aquaculture on world fish supplies. Nature 405: 1017-1024.

Nehlsen, W., J.E. Williams, and J.A. Lichatovitch. 1991. Pacific salmon at the crossroad: stocks at risk from California, Oregon, Idaho and Washington. Fisheries 16: 21.

Olla, B.L., M.W. Davis, and C.H. Ryer. 1998. Understanding how the hatchery environment represses or promotes the development of behavioral survival skills. Bulletin of Marine Science 62: 531-550.

Olla BL, M.W. Davis, and C.H. Ryer. 1994. Behavioral deficits in hatchery-reared fish: potential effects on survival following release. Aquacult. Fish. Manag. 25 (suppl. 1): 19-34.

Olney, P.J.S., G.M. Mace, and A.T.C. Feistner. 1994. Creative Conservation:Interactive Management of Wild and Captive Animals. Chapman and Hall, London, 517 pp.

Paszkowski, C.A. and B.L. Olla. 1985. Foraging behavior of hatchery produced coho salmon (*Onchorhynchus kisutch*) smolts on live prey. Canadian Journal of Fisheries and Aquatic Sciences 42: 1915-1921.

Philippart, J.C. 1995. Is captive breeding an effective solution for the preservation of endemic species? Biological Conservation 72: 281-295.

Piper, R.G., I.B. McElwain, L.E. Orme, J.P.McCraren, L.G. Fowler, and J.R. Leonard. 1989. USFWS Fish Hatchery Management.

Reirez, L, A.G. Nicieza and F. Brana. 1998. Prey selection by experienced and naïve juvenile Atlantic salmon. Journal of Fish Biology 53:100-114.

Robinson, A.T., D.M. Kubly, R.W. Clarkson, and E.D. Creef. 1996. Factors limiting the distributions of native fishes in the Little Colorado River, Grand Canyon, Arizona. Southwestern Naturalist 41:378-387.

Ruzzante, D.E. and R.W. Doyle. 1993. Evolution of social behavior in a resource-rich structured environment: selection experiments with medaka (*Oryzias latipes*). Evolution 47: 456-470.

Ryman, N. 1994. Supportive breeding and effective population size: differences between inbreeding and variance effective numbers. Conservation Biology 8: 888-890.

Ryman, N. and L. Laikre. 1991. Effects of supportive breeding on the genetically effective population size. Conservation Biology 5:325-329.

Ryman, N., P.E. Jorde, and L. Laikre. 1995. Supportive breeding on the genetically effective population size. Conservation Biology 9:1619-1628.

Salvanes, A.G.V. 2001. Ocean Ranching. In J. Steele, K.K. Turkian and S.A. Thorpe (eds.). Encyclopedia of Ocean Sciences. Academic Press 4:1973-1982.

Seal, U.S. 1986. Goals of captive propagation programmes for the conservation of endangered species, Int. Zoo Yb. 24/25:174-179.

Shaffer, M.L. 1981. Minimum viable populations for species conservation. Bioscience 31:131-134.

Shaffer, M. 1987. Minimum viable populations: Coping with uncertainty. pp. 69-86. *In*: M.E. Soulé (ed.) Viable Populations for Conservation, Cambridge University Press, Cambridge, UK.

Simberloff, D. 1988. The contribution of population and community biology to conservation science. Annu. Rev. Ecol. Syst. 19:473-511.

Snyder, N.F.R., S.R. Derrickson, S.R. Beissinger, J.W. Wiley, T.B. Smith, W.D. Toone, and B. Miller. 1996. Limitations of captive breeding in endangered species recovery. Conservation Biology 10:338-348.

Soulé, M.E. 1980. Thresholds for survival: maintaining fitness and evolutionary potential. pp. 151--169. *In*: M.E. Soulé & B.A. Wilcox (eds.) Conservation Biology: An evolutionary-ecological approach, Sinauer Associates, Sunderland, Massachusetts.

Stockwell, C.A. and P.L. Leberg. 2002. Ecological genetics and the translocation of native fishes: emerging experimental approaches. Western North American Naturalist 62(1): 32-38.

Storfer, A. 1999. Gene flow and endangered species translocations: a topic revisited. Biological Conservation 87:173-180.

Subonski, M.D. and J.J. Templeton. 1986. Life skills training for hatchery fish: social learning and survival. Fisheries Research (Amsterdam) 7:343-352.

Sutkuss, R.D. and G.H. Clemmer. 1979. Fishes of the Colorado River in Grand Canyon National Park. pp. 599--604. *In*: Proceedings of the First Annual Conference on Scientific Research in the National Parks. U.S. National Park Service Transactions and Proceedings Series 5.

SWCA. 1997. Grand Canyon data integration project: Preliminary draft. Submitted to Bureau of Reclamation, Salt Lake City, UT from SWCA, Inc., Environmental Consultants, Flagstaff, AZ . 199 pp.

Tringali, M.D. and T.M. Bert. 1998. Risk to genetic effective population size should be an important consideration in fish stock-enhancement programs. Bulletin of Marine Science 62(2):641-659.

USBR [U.S. Bureau of Reclamation]. 1995. Operation of Glen Canyon Dam: Final Environmental Impact Statement. U.S. Bureau of Reclamation. 337 pp. plus attachments.

Usher, M.L., C. Talbot, and F.B. Eddy. 1991. Effects of transfer to seawater on growth and feeding Atlantic salmon smolts (*Salmo salar* L.). Aquaculture 94:309-326.

USFWS [U.S. Fish and Wildlife Service]. 2002a. Humpback Chub (*Gila cypha*) Recovery Goals: Amendment and Supplementation to the Humpback Chub Recovery Plan. Denver, CO: U.S. Fish and Wildlife Service Mountain-Prairie Region 6.

USFWS. 2002b. Section 7 Consultation on Proposed Experimental Releases from Glen Canyon Dam and Removal of Non-native Fish. Memorandum to Regional Director, Bureau of Reclamation, Salt Lake City, UT; Superintendent, Grand Canyon National Park, Grand Canyon, AZ; Superintendent, Glen Canyon National Recreation Area, Page, AZ and Chief, Grand Canyon Monitoring and Research Center, USGS, Flagstaff, AZ from Field Supervisor. 19 pp.

USFWS. 2000. Monitoring of native fishes of the Colorado River ecosystem in Grand Canyon: Trip report Little Colorado River 31 May – 9 June. Prepared for Grand Canyon Monitoring and Research Center, Flagstaff, AZ by USFWS, Arizona Fishery Resources Office-Flagstaff, AZ. 12 pp.

USFWS. 1994. Final Biological Opinion: Operation of Glen Canyon Dam as the modified low fluctuating flow alternative f the final environmental impact statement. Ecological Services Arizona State Office, Phoenix. 56 pp.

USFWS. 1990. Humpback chub Recovery Plan. U.S. Fish and Wildlife Service, Denver, 43 pp.

Utter, F. 1998. Genetic problems of hatchery-reared progeny released into the wild, and how to deal with them. Bulletin of Marine Science 62(2):623-640.

Valdez, R.A., S.W. Carothers, M.E. Douglas, M. Douglas, R.J. Ryel, K.Bestgen, and D.L. Wagner. 2000. 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, Flagstaff. 56 pp.

Valdez, R.A. and W.J. Masslich. 1999. Evidence of reproduction by humpback chub in a warm spring of the Colorado River in Grand Canyon, Arizona. Southwest. Nat. 44:384-387.

Valdez, R.A. and R.J. Ryel. 1995. Life history and ecology of the humpback chub (*Gila cypha*) in the Colorado River, Grand Canyon, Arizona. Final Report to Bureau of Reclamation, Salt Lake City, Utah. Contract No. 0-CS-40-09110. BIO/WEST Repot No. TR-250-08. 286 pp.

Van Haverbeke, D.R. 2001a. Monitoring of native fishes of the Colorado River ecosystem in Grand Canyon: Trip Report Little Colorado River 30 April to 11 May and 4 to 15 June 2001. Prepared for Grand Canyon Monitoring and Research Center, Flagstaff, AZ. U.S. Fish and Wildlife Service, Arizona Fishery Resources Office-Flagstaff. AZFRO Document # USFWS-AZFRO-FL-01-004. 21 pp.

Van Haverbeke, D.R. 2001b. Monitoring of native fishes of the Colorado River ecosystem in Grand Canyon: Trip Report Little Colorado River 1-12 October and 5-16 November 2001. Prepared for Grand Canyon Monitoring and Research Center, Flagstaff, AZ. U.S. Fish and Wildlife Service, Arizona Fishery Resources Office-Flagstaff. AZFRO Document # USFWS-AZFRO-FL-01-006. 14 pp.

Van Haverbeke, D.R. and L.G. Coggins, Jr. 2003. Stock assessment and fisheries monitoring activities in the Little Colorado River within Grand Canyon during 2001. Final Report submitted to the Grand Canyon Monitoring and Research Center, Flagstaff, AZ from USFWS, Arizona Fishery Resources Office-Flagstaff, AZ. AZFRO Document No. USFWS-AZFRO-FL-02-002.

Vincent, R.E. 1960. Some influences of domestication upon three stocks of brook trout (*Salveninus fontinalis* Mitchell). Trans. Am. Fish. Soc. 89:35-52.

Wang, J. and N. Ryman. 2001. Genetic effects of multiple generations of supportive breeding. Conservation Biology 15: 1619-1631.

Waples, R. 1999. Dispelling some myths about hatcheries. Fisheries 24(2): 12-21.

Waples, R.S. and J. Drake. 2002. Risk/benefit considerations for marine stock enhancement: A Pacific salmon perspective. Proceedings of the Second International Symposium on marine stock enhancement, Kobe, Japan, January 2002. National Marine Fisheries Service, Seattle, WA. 100 pp.

Waples, R.S. and C. Do. 1994. Genetic risk associated with supplementation of Pacific salmonids: captive broodstock programs. Canadian Journal of Fisheries and Aquatic Sciences 51: 310-329.

Wiley, R.W., R.A. Whaley, J.B. Satake, and M. Fowden. 1993. An evaluation of the potential for training trout in hatcheries to increase post-stocking survival in streams. North American Journal of Fisheries Management 13:171-177.

Wilson, E.O. 1988. Biodiversity. National Academy Press, Washington, DC.

World Conservation Union. 1987. The IUCN position statement on translocation of living organisms: introduction, re-introductions and re-stocking. Gland, Switzerland.

APPENDIX 1

DRAFT DOCUMENT

GLEN CANYON DAM ADAPTIVE MANAGEMENT PROGRAM

I. Title: Assess Suitability of Humpback Chub Currently at Willow Beach NFH as Broodstock.

II. Relationship to Programs: This section provides insight on the relationship between the proposed action and the Adaptive Management Program goals and objectives, Recovery Goals for humpback chub, and the Biological Opinion RPAs on Glen Canyon Dam operations.

Adaptive Management Program: The goals and management objectives of the Adaptive Management Program that apply are:

Goal 2. Maintain or attain viable populations of existing native fish, remove jeopardy for humpback chub and razorback sucker, and prevent adverse modification to their critical habitats.

Management Objective 2.1: Maintain or attain humpback chub abundance and year-class strength in the LCR and other aggregations at appropriate target levels for viable populations and to remove jeopardy.

Management Objective 2.2: Sustain or establish viable HBC spawning aggregations outside of the LCR in the Colorado River ecosystem below Glen Canyon Dam to remove jeopardy.

Recovery Goals: 5.3.1.1.2.1a. The Grand Canyon population is maintained as a core over a 5-year period, starting with the first point estimate acceptable to the Service, such that: the trend in adult (age 4+; ≥200 mm TL) point estimate does not decline significantly.

Biological Opinion: Elements of the Reasonable and Prudent Alternative that apply are as follows. Successful completion of the RPA is necessary to remove jeopardy to the humpback chub from the proposed action (operation of Glen Canyon Dam under a Modified Low Fluctuating Flow alternative described in the Final EIS and ROD).

Element 2: Establish a second spawning aggregation of humpback chub downstream of Glen Canyon Dam.

III. Study Background/Rationale and Hypotheses: Humpback chub populations in Grand Canyon have undergone substantial decline over the past decade. If this decline continues, and if other management actions are unable to stem the decline in an acceptable time frame, then it will likely be necessary to augment the population with some form of captive raised fish. One option would be to develop a hatchery based broodstock from which offspring would be produced, raised to a sufficient size, and stocked in Grand Canyon. This broodstock must be made up of fish that reflect the genetic characteristics of the wild population. One potential source of broodstock are approximately 120 humpback chub currently held at Willow Beach National Fish Hatchery (NFH). These fish were collected from a 3km section of the Little Colorado River (LCR) in the Salt Camp Area in July 1998. The LCR was flooding at the time these fish were collected, so only a small number of fish were obtained in each seine haul (generally 0-5). A total of approximately 400 young-of-year fish were removed and transported to Willow Beach NFH. These fish have been the subject of various experiments (primarily temperature related), and approximately 120 fish remain. Developing the genetic “fingerprint” of these fish and comparing it with reference samples from throughout Grand Canyon would determine whether these fish were suitable to make up a portion of the captive broodstock.

IV. Study Goals, Objectives, End Product:

Study Goal: Determine the genetic suitability of humpback chub currently at Willow Beach NFH for use as portion of a captive broodstock.

End Product: Report comparing the levels of heterozygosity, polymorphism, Nei’s genetic distances, relatedness, and F statistic between humpback chub at Willow Beach NFH and reference samples collected from other humpback chub in Grand Canyon. Report would contain recommendations regarding the suitability of the captive fish for use as part of a captive broodstock. Project, including report, could be completed within 6-8 months.

V. Study area: Willow Beach NFH.

VI. Study Methods/Approach: We will take a fin clip from each of the potential broodfish, and produce a genetic fingerprint for each fish with 8-12 polymorphic microsatellite markers already screened for applicability to humpback chub research goals. This genotype will be used to determine polymorphism, heterozygosity, Nei’s genetic distances between populations, and levels of relatedness at selectively neutral markers. Microsatellites are codominant markers, so population structure, levels of heterozygosity, and paternity are easily assessed, and comparable to other ongoing research. Based on other research the use of microsatellites should be highly successful in meeting the objectives of this research and in elucidating questions of populations structure. Statistical analysis programs are rapidly being developed to optimize the use of microsatellites in population genetic studies and the use of microsatellites in paternity studies is well established. Baseline data will prove invaluable in future recovery efforts.

VII. Task Description and Schedule:

1. Collect genetics samples from humpback chub at Willow Beach NFH, 2002.
2. Collect genetics samples from reference humpback chub (collected from existing museum samples and/or incidental to other collections in the Colorado and Little Colorado rivers), May/June 2003.
3. Process all samples, June/July 2003.
4. Analyze data and write report, Aug/Sep 2003.

VIII. FY_2003 Work:

_ Process genetics samples, $6,800 (supplies and labor).
_ Analyze data and write report, $10,000 (labor, travel, misc)

IX. Budget Summary:

- FY_2003 - $16,800

- Total: $16,800 (does not include overhead)

X. Reviewers:

XI. References:

Adaptive Management Work Group, Glen Canyon Adaptive Management Program. Final Draft Information Needs, November 7, 2002.

U.S. Fish and Wildlife Service. 1993. Biological Opinion on Operation of Glen Canyon Dam.

U.S. Fish and Wildlife Service. 2002. Humpback chub (Gila cypha) Recovery Goals: amendment and supplement to the Humpback Chub Recovery Plan. U.S. Fish and Wildlife Service, Mountain-Prairie Region (6), Denver, Colorado.

APPENDIX 2

DRAFT DOCUMENT

Proposal: Humpback chub translocation to above Chute Falls

Background:
In the December 6, 2002 Biological Opinion (BO) on the proposed experimental releases from Glen Canyon Dam and removal of nonnative fish, a conservation action was identified by the U.S Bureau of Reclamation, GCMRC and the National Park Service to relocate approximately 300 30-60mm humpback chub, (*Gila cypha*, HBC) to upstream areas of the Little Colorado River to offset the potential impacts on chubs from the proposed project. The conservation action called to relocate HBC to perennial areas upstream in the Little Colorado River, to an area referred to as Chute Falls. Historically, HBC and other native fishes were dispersed throughout the Little Colorado River below Grand Falls, however, due to vegetation changes and flow modifications, the Little Colorado River is no longer perennial below Grand Falls. Flows in the LCR become perennial at Blue Springs, at river kilometer 21. Reduced water volume prevents dilution of highly saline springs like Blue Springs and causes free CO_2 levels to exceed fish tolerance levels. In the past, HBC have been found just below Chute Falls at river kilometer 14.5 (Mattes 1993). More recently, HBC have only been found further downstream, below the complex of travertine dams known as the Atomizer Falls complex (USFWS, unpublished data). Experimental transplants of native fishes at river kilometer 15, 17.5 and 20 found that stress behaviors were apparent at river kilometer 20 but that other, more downstream locations appeared to provide suitable conditions (Robinson et. al. 1996). CO_2 concentrations below river kilometer 17.5-river (196mg/L in Robinson's study and Mattes 1993) are likely below the critical tolerances for HBC and may provide additional rearing habitat during some seasons.

Objectives:
The short-term objective of this project would address the question of whether or not transplanted fish would remain above Chute Falls. Geomorphology of this section of the LCR includes narrow, canyon bound stretches subject to scouring flows. Small life history stages of HBC may not be able to maintain position in high flows and be washed downstream. Yet despite these conditions, native speckled dace have maintained a population above Chute Falls for many years. However, if lower volume flows and baseflow conditions occur over the 2003 and 2004 seasons, HBC may be able to exploit available habitat and remain in this upstream section until they reach larger sizes. The second objective of this project is a direct management action to try and diminish the large-scale loss of HBC in the 30-60mm size class. Data suggest that once smaller life history stages enter the Colorado River either through high flows or downstream drift, that a combination of cold temperatures and predation significantly reduce recruitment. It appears that once HBC exceed the 150-200 size range that

survival significantly increases. If HBC can remain in the LCR longer to reach these larger size classes, they may have an increased chance of survival once they enter the mainstem Colorado. Since food resources do not appear to be limiting (Robinson 1996) and warmer temperatures exist as compared to the mainstem Colorado, the longer they remain in the LCR, the higher the likelihood of surviving until adulthood. The longer-term objective of this project is the establishment of a spawning population above Chute Falls. This situation would require the relocated fish to remain in this section for approximately 3-4 years before they reached sexual maturity. Although this situation is unlikely due to the high flows in the LCR and the canyon bound areas above Chute Falls, genetic considerations would need to be explored should survival rates of translocated fish create a spawning population. Since the LCR is the first place to try this approach, we expect that results of this project could eventually be applied to other tributaries to build a larger HBC population in the mainstem Colorado.

<u>Methods:</u>
A reconnaissance-level trip will be performed in June 2003 to assess water quality (CO_2, pH, temperature, turbidity), densities of nonnative fishes and to determine potential helicopter landing/sling loading areas for subsequent fish transfer above Chute Falls. Capture methods used will include seining, minnow traps and snorkeling surveys. Although water quality above the Atomizer Falls Complex has been adequately documented (Mattes 1993, Robinson et. al 1996, Strength 1997), we propose to obtain limited samples to ensure water quality conditions for subsequent fish release.

In July 2003, USFWS biologists (3) will be taken to the lower end of the Little Colorado River at Boulder's Camp to obtain approximately (300) 50-100mm HBC. Near the confluence of the Colorado River, HBC are most vulnerable to being washed into the mainstem and long-term survival is reduced. While this size range is outside the range identified by the BO, it is imperative that all fish are individually marked so that monitoring efforts can detect movement of translocated fish into areas downstream of Chute Falls. The minimum size that HBC can be elastomer marked is approximately 50mm total length. Due to the limited number of fish being moved, every opportunity to detect fish movement downstream and be able to identify translocated individuals needs to be pursed. In addition, Robinson 1996 found between 20-30% mortality of age-0 fish (26-40mm) during cage experiments at river kilometer 15 and 12.5 suggesting some handling induced mortality from transport. Mortality was reduced to 0% when age-1 fish (40-100mm) were used. Larger size classes may increase survival in transplanted sections.

Capture methods used will include seining, minnow traps and hoop nets. Since it is unknown how long it will take to capture this many HBC within the specific size class, logistics of subsequent helicopter contact and transport will have to be further developed. Due to the warm ambient air temperatures in the LCR during summer, all capture efforts will be conducted during early morning and late afternoon to reduce stress and mortality of captured fishes. Captured fish will be measured for length, and implanted with an elastomer tag with a unique color. Pending approval by the Navajo Nation, all captured nonnative fishes will be sacrificed. All other fishes will be returned to point of capture. All captured HBC will be held in 1/8 mesh live cars until transport upstream. Fish will be transported to the release site in an aerated tank or cooler stored within the helicopter. At the release site, fish will be tempered both for temperature and CO_2 levels until differences between parameters are within 1 mg/l and 1°C. Following tempering, translocated fish will be held in live cars at several locations in the LCR between river kilometer 15 and 17.5. At each location fish will monitored for stress and mortality for a minimum of 24 hours. Following 24 hours of monitoring, fish will be released into the LCR.

Monitoring of released fish will occur in November 2003 for 5 days to determine whether or not any retention above Chute Falls has occurred. Capture methods used will include seining, minnow traps baited hoop nets and snorkeling. Captured HBC will be measured for length and if they exceed 150 mm total length, be implanted with a pit tag. In addition, USFWS population estimate trips will occur in September and October 2003 as well as in spring 2004 and could potentially capture transplanted fish during sampling along the lower 14 kilometers. Unique identification via elastomer tags will provide insight as to how many fish were transported downstream during the 2-3 month time frame. An interim report will be submitted by December 31, 2003 that summarizes the June 2003 reconnaissance trip, July 2003 translocation trip and November 2003 monitoring efforts. This report can then be used to determine subsequent levels of effort and size classes based on initial effort in 2003.

To evaluate how transplanted fish persist following winter flows, monitoring of transplanted fish will occur in late spring 2004. To reduce handling effects on fish, spring monitoring will consist of snorkeling surveys as the primary method to assess presence/absence of transplanted fish. Other methods such as baited minnow traps and seines may be used should turbid water conditions exist during spring monitoring efforts. In June/July 2004, an additional translocation trip will occur using similar methods as described above. Monitoring will occur to assess post monsoon survival in November 2004. The specific date will depend on when the spring 2004 spawn occurred for HBC. An interim report will be submitted by December 31st 2004 that summarizes the spring 2004 monitoring, June/July 2004 translocation trip and the 2004 November monitoring.

Final monitoring will occur in spring 2005, followed by a final report that will be submitted in June 2005. The final report will include a synthesis of all translocations, monitoring efforts and recommendations for future action.

<u>Timeline:</u>
June 2003: Reconnaissance survey to collect water quality, nonnative fish densities and helicopter staging areas, 5 days

July 2003: Translocation trip at confluence of LCR and mainstem Colorado, 3-5 days
November 2003: Post monsoon monitoring trip, 5 days

December 31, 2003: Interim 2003 Report due

Spring 2004: Post winter flow monitoring (snorkeling surveys), 5 days
June/July 2004: Translocation trip at confluence of LCR and mainstem Colorado, 2-5 days
November 2004: Post monsoon monitoring, 5 days

December 31, 2004: Interim 2004 Report Due

Spring 2005: Post winter flow monitoring (snorkeling surveys), 5 days

June 2005: Final report due

<u>Literature Cited:</u>

Mattes, W.P. 1993. An evaluation of habitat conditions and species composition above, in and below the Atomizer Falls complex of the Little Colorado River. The University of Arizona. 105pp.

Robinson, A.T., D.M. Kubly, R.W. Clarkson, and E.D. Creef. 1996. Factors limiting the distributions of native fishes in the Little Colorado River, Grand Canyon, Arizona. The Southwestern Naturalist. 41: 378-387.

Strength, D.A. 1997. Travertine deposition in the Little Colorado River, Arizona and habitat for the endangered humpback chub. Northern Arizona University. 99pp.