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

By

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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 program of humpback chub. Broodstock development is considered within the context of the Endangered Species Act (ESA), 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 (U.S. Office of Federal Register 65:183 [2000]: 56916-56922). Biological risks involved with broodstock development 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 in relation to broodstock development, and we list hatchery attributes required to raise broodstock fish.

Second, we address the feasibility of establishing a program for captive grow-out of wild caught age-0 humpback chub for release into the wild. The primary risks 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 (LCR), and 2) translocation of fish into Bright Angel, Shinumo, and Havasu creeks in Grand Canyon. Translocation of fish above Chute Falls appears to offer potential for a minor gain in the wild census population, but may involve potential genetic risks to the main population of humpback chub in LCR and Grand Canyon. 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 from the Grand Canyon Monitoring and Research Center (GCMRC), the U.S. Fish and Wildlife Service (USFWS) has examined the feasibility of three actions: 1) developing a captive broodstock for humpback chub, 2) establishing a supplemental stocking program for humpback chub in Grand Canyon using wild caught age-0 fish removed from the LCR and grown to a larger size in captivity, and 3) establishing a second spawning (or expanding the current) population of humpback chub in Grand Canyon. 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. Any one of these actions, singly or in concert with the others, is considered by the USFWS to be of mitigative importance to ameliorate impacts to the endangered humpback chub caused by Federal water development in the Colorado River basin.

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 age-0 fish grown out in captivity. Part of the rationale for investigating this approach is that this method is currently being used as a management action to conserve the razorback sucker population in Lake Mohave.

The USFWS only proposes to investigate the feasibility of carrying out these management actions; this document does not constitute a proposal to implement these actions, nor an endorsement by USFWS. Any initiation of management actions will require thorough review both within the USFWS and among the cooperating agencies, as well as additional funding to the agencies carrying out the actions, and would require long-term monitoring. These efforts are coordinated with and reviewed by the Upper Colorado River Endangered Fish Recovery Program in an effort to better unify mitigation, management, and recovery efforts throughout the Colorado River basin.

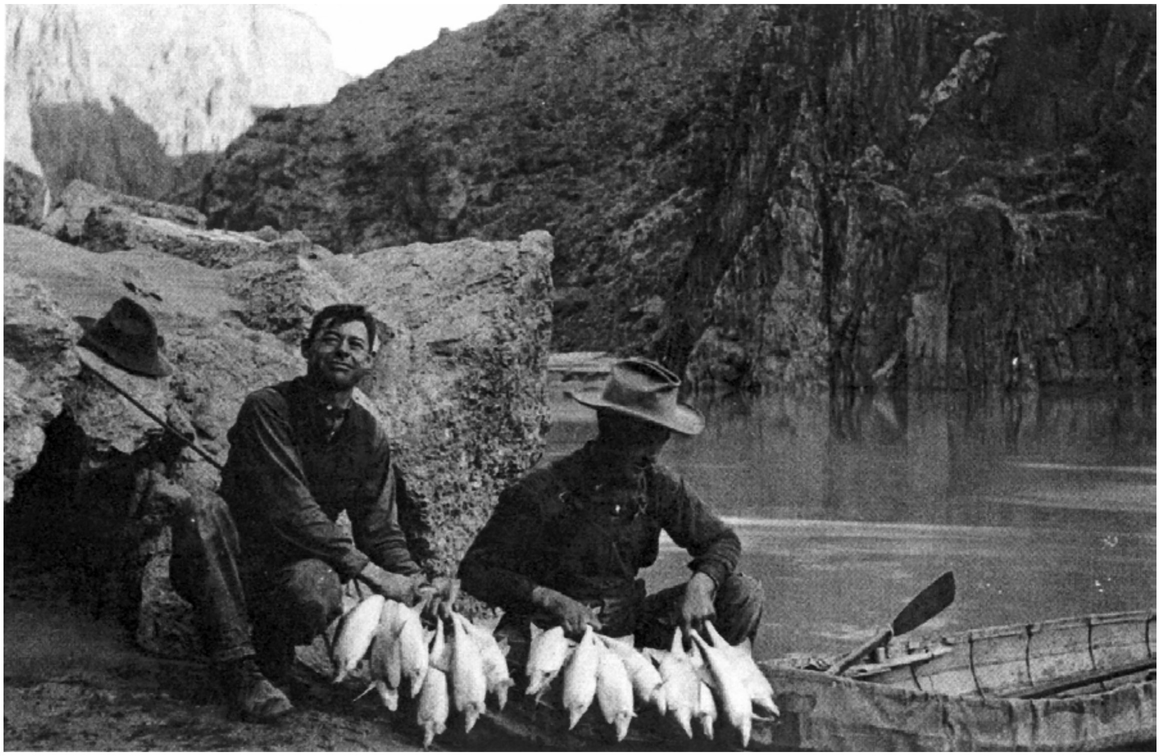
BACKGROUND

Status of Humpback chub

Humpback chub is endemic to the Colorado River basin (Miller 1964, Minckley 1991), with origins extending as far back as Miocene (Miller 1959, Minckley et al. 1986). Three of eight native fish species have become extirpated 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 River. The Grand Canyon population also possesses the life history trait of being migratory spawners, and remains isolated from 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 threats, including habitat loss (Suttkus and Clemmer 1979, Minckley 1991), watershed mismanagement (Abruzzi 1995), cumulative effects of environmental variation (see Gilpin and Soulé 1986), parasite loads (Clarkson et al. 1997), and predation by introduced non-native fishes in the mainstem Colorado River (Valdez and Ryel 1995). Even though multiple causes of population decline have been identified, no known progress has been achieved in elevating population numbers since listing in 1967.

Early accounts of the abundance of humpback chub in Grand Canyon, while sparse, suggest a much higher historical population. The Kolb brothers witnessed humpback chub spawning in the mouth of the LCR in numbers so large that they described the striking of their tails upon the surface waters as sounding like “a slide of shale” (Kolb and Kolb 1914). This simple description suggests very high densities of fish, 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 (RM 87.5; Photograph 1). Since Glen Canyon Dam has been in place (nearly forty years), only a few humpback chub have been captured in this vicinity with high intensity effort (i.e., hundreds of hours of trammel netting and electro-shocking). Minckley et al. (2003) calculated that there were about 200,000 adult humpback chub inhabiting the Colorado River basin during historic pre-dam 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 database of humpback chub in Grand Canyon has confirmed this declining trend (Coggins et al. 2003). This evidence indicates that there has been a 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 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 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 a cold, hypolimnetic, fluctuating flow environment that is not supportive of all life stages of native fish.

As a result of concern over the decline of humpback chub in Grand Canyon, additional management actions (or experiments) are being put forth. One experiment currently initiated by GCMRC is removal of nonnative fishes from the mainstem Colorado River near the LCR (Coggins et al. 2002). However, it is uncertain if this action 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

Augmenting 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 endangered Pacific salmonids (*Oncorrhynchus* sp.; Hedrick et al. 1994, Waples and Drake 2002). Despite these trends, the merits of hatchery production have been 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 economic failures (Hilborn 1998, Naylor et al. 2000).

As a result, managers for threatened or endangered species sometimes face a potential double jeopardy situation. Failure to intervene in a deterministic decline of an endangered species might result in extinction. However, using captive broodstock for supplementation may result in changes (primarily genetic) that reduce sustainability and viability of the wild population (Hynes et al. 1981, Allendorf and Ryman 1987, Waples and Do 1994, Levin et al. 2001). The following section discusses several specific questions pertinent to development of broodstock for humpback chub.

<u>Is a captive adult broodstock currently needed, and what will it contribute</u>?

Captive broodstock is sometimes considered when risk of extinction is high enough that this type of conservation measure is viewed as justifiable (Anders 1998). The goal is that supportive breeding will give a demographic boost to the wild population and, presumably decrease extinction risk. However, this potential for gain is accompanied by genetic and behavioral risks to the wild population. Although there is no clear-cut answer that can be guaranteed correct, the following literature review may be useful in guiding managers to make a decision.

First, we review broodstock development of an endangered species in the context of the ESA and in the context of USFWS policy regarding controlled propagation (U.S. Office of Federal Register 65:183 [2000]: 56916-56922). 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 discuss 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). 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." This document 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." In addition, 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." 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." 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 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 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. In view of the declining humpback chub 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) of humpback chub. At worst, humpback chub in Grand Canyon may currently be in 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 suggests 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 a literature review. 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 depend on 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 important to consider the likelihood for future reintroduction (Seal 1986). If a broodstock is to be developed, several considerations must be faced. First, how soon is reintroduction into the wild to be expected? The longer fish are held in captivity (e.g., especially in terms 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 one generation for humpback chub). Second, once broodstock and supportive stocking activities are initiated, it is critical that these activities are long-term commitments. This is generally because carrying capacity conditions for the species in decline have not been rectified. As a result, 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 (Waples and Do 1994). These reasons are further discussed below. Third, captive broodstock activities can present a suite of risks to the wild population that must be considered in order to prevent costly or irrevocable mistakes.

The literature is replete with 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, Lynch and O'Hely 2001, Ford 2002). Limitations of captive breeding include an array of genetic problems, 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, 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). 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 diminishment of habitat and aquatic ecosystem conservation and restoration (Philippart 1995). A 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 wild populations 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).

One of the main concerns is that supportive breeding via the use of captive broodstocks can pose genetic risks to wild populations (Ryman and Laikre 1991, Busack and Currens 1995, Lynch and O'Hely 2001, Ford 2002). The risks are multiple in nature, and full consideration of these factors should be presented and expanded in a formal broodstock management plan.

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, Flagg et al. 1995), and in order to avoid artificial introgression, broodstock should be obtained 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 implications if broodstock activities are coordinated using other populations of humpback chub from the Upper Basin, as well as for choice of hatchery facilities and hatchery operations (e.g., risks of introgression occurring if other *Gila* spp. are on station or in the watershed).

Second, there is risk of inbreeding occurring within the hatchery population. Traits that frequently exhibit inbreeding depression are quantitative, and are associated with reproductive capacity and physiological efficiency (Kincaid 1983, Lande 1981). In order to maintain variability in hatchery populations, a total of 50 to 500 genetically effective founding breeders has been recommended (Franklin 1980, Frankel and Soulé 1981, Hynes et al. 1981, Kincaid 1983). However, more recent genetics theory suggests these numbers may be at least an order of magnitude too low for preserving quantitative variability (Lande 1995). The danger is that if hatchery fish are deficient in overall genetic variability, this may 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 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). Since captive bred populations are usually created using only a very small proportion of the wild population, 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, 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).

If the underlying problems for population decline have not been initially addressed (e.g., habitat destruction; Meffe 1992), supported populations may exceed carrying capacity and can then be subject to a "supplementation and crash" scenario (Waples and Do 1994). 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 breeding population size, 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.

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 should be a very slow and protracted operation in order to minimize risk.

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 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 offset by decreasing the genetic variability and 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 alleviate the problem; however, the wild population is not protected from a decline in fitness unless gene flow from the captive population approaches zero. This means 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.

Fish that are held in captivity for a substantial portion of their lives are removed from natural learning experience that would ordinarily be gained in the wild. Consequently, 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 (Valdez and Ryel 1995).

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,b, Gosling and Sutherland 2000). Olla et al. (1994, 1998) suggested it is critical for hatcheries to implement methodologies that improve post-release survival. Brown and Day (2002) suggest that environmental enrichment, pre-release training programs, and soft release protocols can assist in making fish more ecologically viable once release occurs. Environmental enrichment means matching captive conditions to natural conditions. This can include 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 fish skills that they will need to survive in the wild (Suboski and Templeton 1989, Brown and Laland 2001). Primarily, these include exposure to predators and natural food types (see reviews in Olla et al. 1998, Brown and Day 2002). Pre-release training does not 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 (e.g., holding fish in pens for a period of a few days at the release site) 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. This acclimatization period can significantly decrease mortality (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 if a captive broodstock is needed, and know what it will contribute. Our best assessment as to the predicted status of humpback chub follows 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, the USFWS and NOAA (U.S. Office of Federal Register 65:183 [2000]: 56916-56922), and the scientific community (e.g., Nehlsen et al. 1991, Philippart 1995, Snyder et al. 1996), make it clear that captive broodstock activities should be considered as a last option for recovery. Although some alternative conservation measures 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 humpback chub population should be made available (e.g., thermal control devices placed in Glen Canyon Dam). Furthermore, recent predator removal efforts 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 (Coggins and Yard *in review*). 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.

Captive broodstock activities are inherently genetically risky. There is risk of creating a worse situation by carrying out captive broodstock activities prematurely or incorrectly. Waples and Drake (2002) caution that even when managers are made aware of all foreseeable risks, uncertainty is high, and that the chances are high that unexpected developments will erase the projected benefits. 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.

The Recovery Goals for humpback chub call for no significant decline occurring in the number of fish within each wild population over a 5-8 year period (as yet, this time frame is unidentified for any population). In addition, each core minimum viable population (MVP) must be self-sustainable, genetically and demographically viable, and contain adult (>200 mm) estimates whose lower 95% confidence interval exceeds 2,100. The sizes for the MVPs were calculated 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 to be an inadequate safeguard against extinction (Shaffer 1981, Simberloff 1988, Boyce 1992, Lande 1995, Minckley et al. 2003). Thus, managers for the humpback chub face a difficult situation. There may be a need to demographically boost populations of humpback chub in order to meet the proposed MVP standards and reach recovery. However, 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; thus further jeopardizing the humpback chub. The literature repeatedly calls for exhausting other means (such as improving habitat) 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 should 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. Prior to implementation, 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.

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

All options for fish collection should be identified in a formal broodstock management plan, and decisions based from there. 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 age-0, or collection of spawning sized adults. Although we offer a rough outline below, 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. 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.

It has been suggested by some to remove the small mainstem aggregation of humpback chub 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 likely 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). However, 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. 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 age-0 from this group of fish (B. Persons, Arizona Game and Fish Department). Post-larval humpback chub have been captured at the spring at 30-mile (Valdez and Masslich 1999), but successful collection may require stabilization of flows from Glen Canyon Dam during the months of June and July.

Another potential source of broodstock is the ~120 humpback chub currently held at Willow Beach NFH. During July 1998, ~450 age-0 humpback chub were removed the LCR, and flown to Willow Beach NFH for use in temperature growth studies (Gorman and VanHoosen 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. 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 age-0 humpback chub from all 14 km of the LCR, over a period of several years. Using age-0 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, 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 March to 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. Selecting fish in a manner that could disrupt timing of natural migration and spawning patterns should be avoided. For instance, if all age-0 fish 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, and shown to be detrimental for salmonid broodstock (Flagg et al. 1995, Fleming et al., 1997).

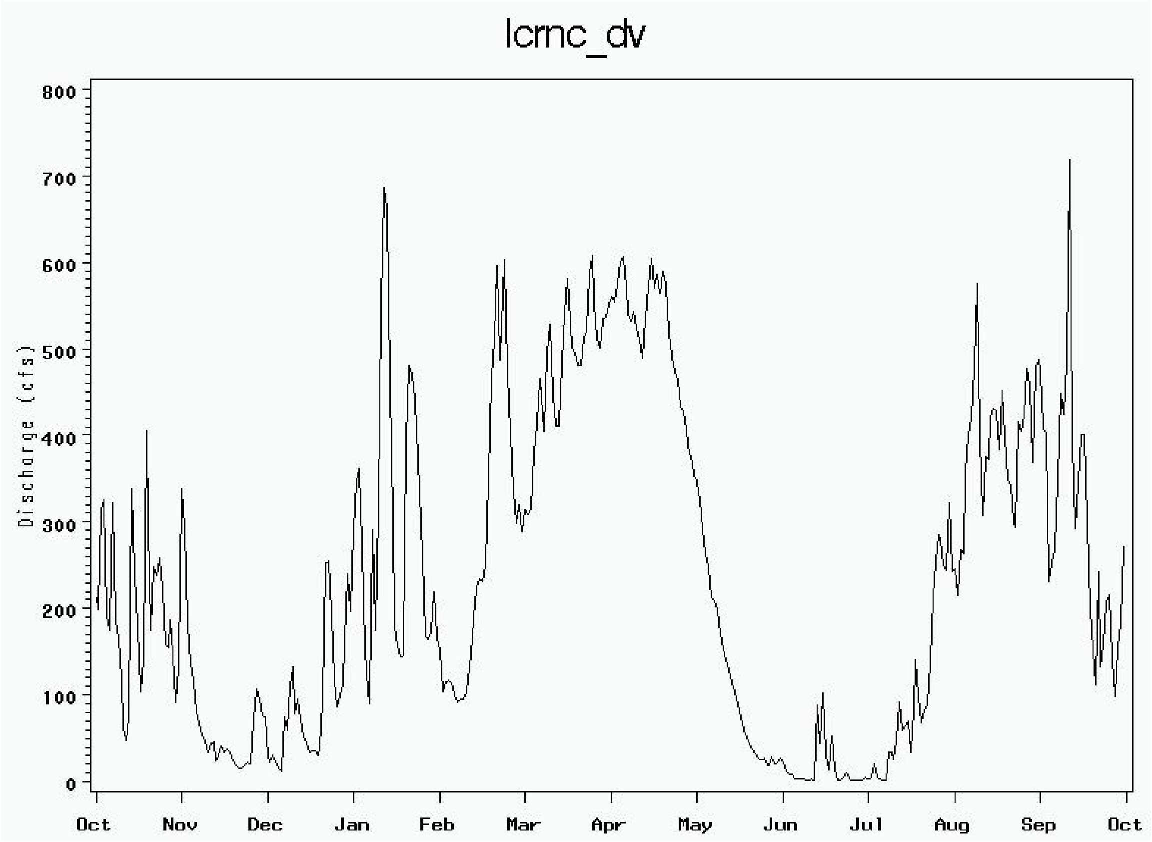
Much as the potential problem that could result from not capturing fish on an appropriate temporal protocol, it should be considered that selective changes could occur from not capturing fish in an appropriate spatial manner. For instance, Douglas and Marsh (1996) hypothesized that the altered regime 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. It could be possible that age-0 fish collected in the lower reaches of the LCR may be more representative of mainstem migrants, while age-0 fish captured in the upper reaches of the LCR may be more representative of humpback chub locally adapted to being residents 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). Capture of age-0 fish may be easiest in the Boulders or Coyote reaches, as catch-per-unit-effort of age-0 humpback chub has been higher in these reaches in the past (Van Haverbeke and Coggins 2003). We suggest keeping the number of fish captured low (500 fish) during the any given year in order to accommodate a temporally spaced collection protocol, and to minimize impact on the wild population. 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 (D. Van Haverbeke, pers. obs.). Although it is possible to capture fish between June and November (USFWS 2000, Van Haverbeke 2001b), 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, USFWS, pers. com., D. Van Haverbeke, pers. obs.). In June, it may be possible to capture large numbers of age-0 fish, but most are likely to be < 50 mm (Gorman 1994, Van Haverbeke 2001a). The modal length of humpback chub reached > 50 mm in 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). 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, age-0 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 age-0 humpback chub were seined in a half-day under turbid water conditions between 10 and 12 km in the LCR, and transported via helicopter to Willow Beach NFH (Gorman and VanHoosen 2000, D. Van Haverbeke, pers. obs.).

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



Thus, flood conditions can actually facilitate capture, since fish appear to be concentrated, and will not “see” the capture gear. However, many age-0 fish are transported out of LCR by late summer and fall flood events (Valdez and Ryel 1995). As flood conditions become more extreme (or more time passes under erratic flood regimes), progressively more age-0 fish may drift into the mainstem, making capture more difficult. The easiest time to capture 50 to 75 mm 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 age-0 cohort has undergone mortality (or been transported to the mainstem).

Streamside spawning could be initiated as another option, and 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 (Hamman 1982). Once ovulation occurs, the extruded eggs can be fertilized with freshly captured ripe males, or with other ripe males also held in captivity. However, the logistics of such an endeavor can be 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 females at the right time, in holding fish in pens without stress if hormones are used to induce ripeness, 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 concerns are 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. Complete genetic studies being performed on humpback chub, and develop a formal broodstock management plan.
2. Consider that captive broodstock should be viewed as a last resort, to be performed when all other available conservation actions have failed.
3. Should captive propagation be 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.
4. Build as large a broodstock as possible (several thousand fish), in order to retain as much genetic variability as possible.
5. Build the main broodstock from age-0 fish collected under a temporal and random spatial design.
6. Consider taking several years (5-10) to collect a sufficient amount of age-0 fish.
7. 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).
8. Once broodstock(s) and supportive activities are begun, realize that this will 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 will need to be quarantined and treated for Asian tapeworm (*Bothriocephalus acheilognathi*), and any other health issues.

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

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 addition, 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 genetic problems 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 *G. elegans*). 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 physical hatchery qualifications for the culture of humpback chub are 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 VanHoosen (2000) found the optimal temperature for growth was at 24 ^{o}C.

It is suggested that methods (reviewed in Brown and Laland 2001, Brown and Day 2002) are followed. 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 to the budget.

FEASIBILITY OF ESTABLISHING A SUPPLEMENTAL STOCKING PROGRAM USING WILD CAUGHT AGE-0 FISH

We investigate the feasibility of capturing wild age-0 humpback chub from the LCR, transporting them to a grow-out facility, marking them with a unique identifier (such as a Passive Integrated Transponder [PIT] tag), and releasing them back into the Colorado River or its tributaries within Grand Canyon.

Since augmentation of a population via the use of broodstock progeny is genetically risky, this document incorporates the concept of capture and grow out of wild age-0 fish, and releasing them in order to attempt to augment the population of humpback chub in Grand Canyon. 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 lack of literature concerning the capture, short-term grow-out, and release of wild progeny. Dowling et al. (1996) 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 design 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, ASU, pers. com.).

The primary concerns with capture and grow out of wild larval (or age-0) fish appear to involve issues related to ethology (behavior) rather than to genetics. 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 after release, and lacking abilities to home to natal areas or knowledge of migratory routes.

In addition to behavioral concerns, some potential exists for a form of domestication 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 of 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). Some level of genetic change relative to the natural population cannot be avoided in a cultured population (Waples 1999). Therefore, we suggest that it is important to reduce (to the maximum extent possible) the time that fish are held in captivity.

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 age-0 humpback chub is to potentially increase the likelihood for survivorship to a larger size class (e.g., > 150 mm). Based on 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 [UBC], 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 (Clarkson et al. 1997). One of the main reasons that age-0 and juvenile humpback chub appear to suffer high mortality in the mainstem is that once displaced into the mainstem, they lack growth and remain vulnerable to the effects of predation for a long period of time (Clarkson and Childs 2000).

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 (<20 mm) should have less impact on the wild population than removal of an equal number of 50 mm fish. Also, the time fish are held in captivity should be kept to a minimum in order to minimize behavioral changes. In addition, growing 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 can be captured with dipnets in the LCR, however, identification to species can be problematic in the field (Childs et al. 1998). Humpback chub 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. Collecting fish > 50 mm may also allow time for imprinting to occur, should this be a factor for humpback chub.

Another alternative could be removing 1-year old fish during spring. These fish would be ~125 mm, and could be grown larger (e.g., 200 mm), for increased survivorship. However, removal of a sufficient number of fish of this size may be more problematic, and should be expected to depress wild recruitment more than removal of smaller fish.

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. As discussed in the captive broodstock section, collection of fish should be performed on appropriate temporal and spatial scales in order to maintain the maximum likelihood for retention of genetic integrity. 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 river water. Aeration of standing water in coolers during the hot ambient air temperatures of July and August is not advised as mortality would occur. Rather, a constant supply of fresh river water is preferred. Fish will need to be treated according to the most recent protocols to relieve stress. Specifics on amounts and types of approved 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 (C. Feigel, USFWS, pers. com.).

We suggest direct transport via helicopter to the appropriate receiving facility. Since several thousand fish will be transported (numbers are discussed below), this will likely require 10 to 12 long distance flights per year just for fish transport. Two large coolers, each containing from 300 to 400 age-0 fish, and supplied with oxygen should be the maximum expectations for transport. More preferable would be one cooler transported per flight, with a technician on board to monitor the oxygen supply.

What is the best size to grow out captive fish before release?

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. In addition, by growing wild age-0 humpback chub to a larger size class before release, they could be expected to have an increased probability of survivorship. Larger humpback chub (>150 mm) 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).

Whereas age-0 humpback chub do not put on growth at 10 ^{o}C (Gorman and VanHoosen 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 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. Therefore, 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, for 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 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.). 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, collecting fewer fish and growing them to a larger size (200 mm) would result in less annual cropping of the wild cohort. In addition, it may be preferable to annually release a smaller number of cultivated fish into the wild. Second, it would be 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 (Coggins et al, 2002). 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.), and one might reason that the less time fish spend in the hatchery, the less chance for mortality to occur.

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 behavioral issues of fish. Even under optimal conditions, these fish will be held under unnatural conditions for a substantial portion of their lives (~ 1 year or more). This will have impacts on anti-predator responses, feeding abilities, and possibly other factors related to migration and spawning behavior (Paszkowski and Olla 1985, Usher et al. 1991, Howell 1994, Brown and Laland 2001, Brown and Day 2002).

<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 an age structured population model for humpback chub. This model (designed by C. Walters,UBC) 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 age-0 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 age-0 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.

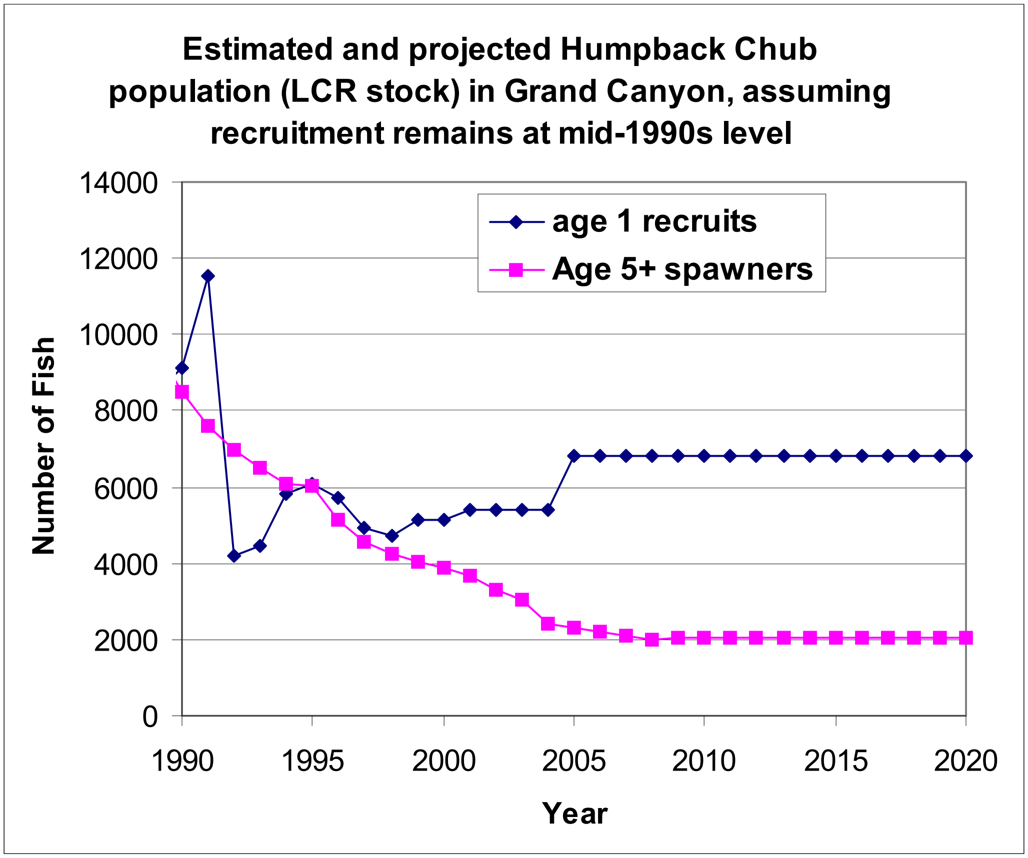
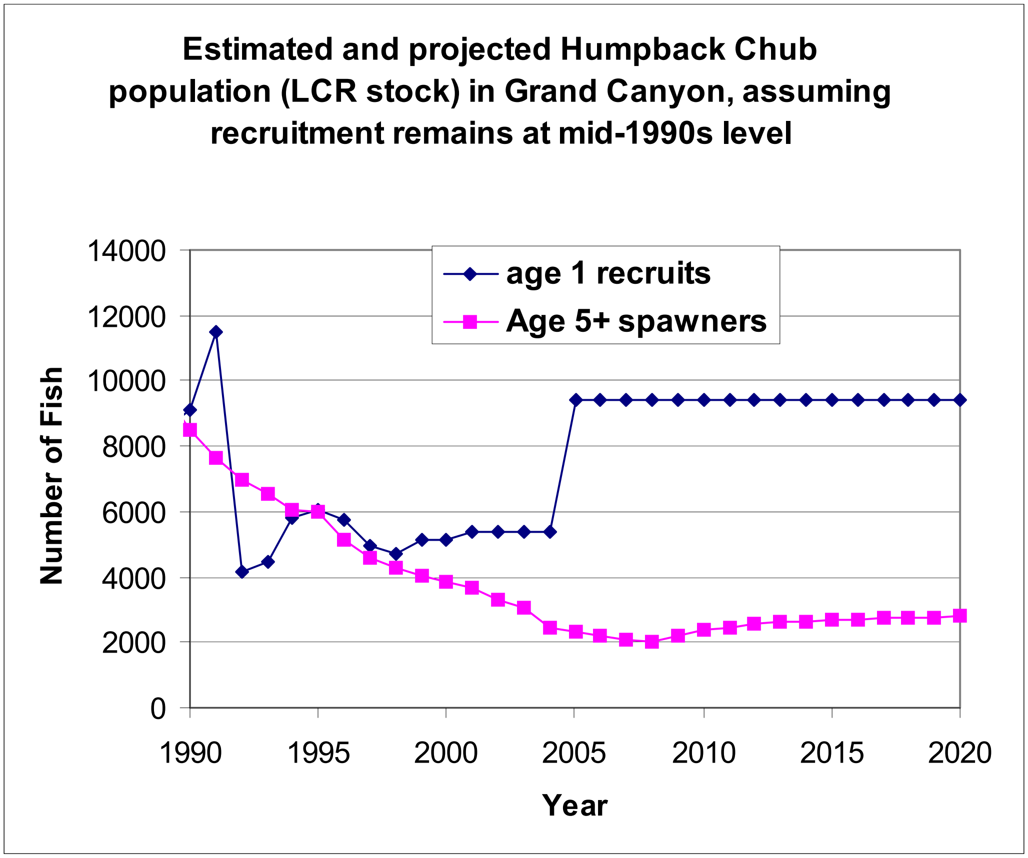


Figure 2 predicts that the decline of age 5+ fish would be arrested and show a minor increase in the year 2009. Figure 3, on the other hand, depicts that 4,000 age-0 fish are captured and grown out to 150 mm per year. In this instance, there is a predicted noticeable increase in 5+ year old spawners.

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 require minor modification, since mortality in captivity could easily average 20%. In addition, about 50 to 60 fish are generally killed for health studies 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. If the very low survival rates of other stocked species are any indication (McNeil 1991, Salvanes 2001), we can only assume that additional post-stocking mortality could be high (i.e., >50%). This suggests that the numbers of age-0 fish collected will need to be doubled (C. Walters, UBC, pers. com.). Primarily, this is because a 50% post mortality rate should be an expected reality, and that it will be easier to perform mark-recapture studies by releasing a larger number of fish. This suggests capturing ~ 3,480 age-0 fish per year for grow out to 150 mm, or ~1,860 age-0 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. Finally, managers may wish to consider that increased recruitment may be realized through predator control efforts rather than fish stocking.

The question has been raised as to what proportion of the wild population will be removed annually for 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, UBC, 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 age-0 fish per year. Thus, 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 age-0 fish (i.e., 3,480/50,000). We caution that annual production of age-0 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 and Ryel 1995), and multiply this by the annual survival rate of 0.1 (Valdez and Ryel 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 age-0 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 age-0 (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 age-0 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 age-0 production. In addition, 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 primary 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 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 year's cohort of age-0 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 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 age-0 cohort via predation and 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.

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				

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 decrease mortality due to predation, and lead to increased survivorship of age-0 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).

Other possible options exist for stocking. 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. Mark-recapture 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 potentially 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 [RM 62.5], in front of Carbon camp [RM 64.8] as well as eddies/return channels above Carbon camp, and in the eddy across from Lava/Chuar camp [65.4]). 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 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.

<u>Where could the supplemental fish be grown</u>?

It is not the purpose of this document to specifically identify a hatchery where supplemental fish could be grown. Attributes that a facility should contain include all those listed under the previous section for captive broodstock (e.g., experienced personnel, committed funding, ability to keep fish unquestionably isolated from other *Gila* spp., appropriate water flow and temperatures, quarantine facility, etc.). Raceways, circulating tanks, or even outdoor ponds may be useful for grow-out of age-0 fish, and the process should be adaptive in nature.

It is strongly recommended that the facility have the capabilities for naturalistic rearing (e.g., 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 Laland 2001 for review).

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 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 that successful translocation included: 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). 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.

Translocation of humpback chub within the Little Colorado River

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 age-0 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 have a greater chance for survival. However, 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). 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 likely 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 catches of 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 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 unknown reasons (e.g., environmental stochasticity) and the species has been unable to successfully re-colonize. 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, 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).

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 closure 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. Because humpback chub 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 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. 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 N_e 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.). As a result, the founding effective population size should be expected to be far less than 600 (i.e., below the minimum viable population standards of $N_e = 500$; Franklin 1980).

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 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 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 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. All populations experience some level of inbreeding (Kincaid 1983). 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 ranges from 0 to 1, with zero being the base level (Kincaid 1983). Assuming that the base level inbreeding coefficient for the main population is zero (as there are no historical data to measure against), it is possible to 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 generations, it should be possible to monitor for these changes, provided that long-term commitments are set and continued. To begin with, arrangements should be made to genetically 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. P. Hedrick, Arizona State University [ASU] and Dr. C. Walters, UBC). 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, ASU, pers. com.). For example, even if only 50 males and 50 females survived to reproduce, this would theoretically result in a rate of inbreeding increase per generation of 0.005 (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). This does appear to assure an appropriate level, 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 8 years (USFWS 2002a), this translates into decades. However, traits such as behavior, morphology, reproductive capacity, and physiological efficiency are likely to involve quantitative genetics (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. 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 without secondarily marking the fish). 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 a precise estimate of survivorship will be unlikely (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.

Equally important, it will be difficult (if not impossible) to accurately measure downstream levels of gene flow from the resulting offspring above Chute Falls. Therefore, 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 age-0 fish, or PIT tag fish once they reach 150 mm, but these efforts are time consuming and may contain a high degree of uncertainty. For example, most offspring should be expected to move downstream (below Chute Falls) at small sizes (before they can be PIT tagged) during flood events, which is when drift nets become problematic (e.g., currents become too strong and nets very quickly fill up with 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 captive-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, ASU, pers. com.). As a result, the contribution of such hypothetical fish is likely to be much less than their numbers would predict (P. Hedrick, ASU, pers. com.).

Nevertheless, offspring produced from higher up in the LCR may 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. Since there is unexploited habitat by humpback chub above Chute Falls, accompanied by increased food resources (Robinson et al. 1996) and decreased predators, this suggests a possibility for high survivorship of offspring fish above Chute Falls.

Generally, managers respond to these 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, 1 to 10 fish 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 initial heterozygosity). 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. This is important since translocation of fish into a situation with unidirectional gene flow suggests that selection could cause other concerns. Fish that survive generations 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 to impact the genotype of the main LCR population, even with minimal movement (see Ford 2002). There are documented cases of subtle decreases occurring in fitness when gene flow occurs between subpopulations experiencing different or conflicting selective forces (review in Storfer 1999). To minimize differential selection, it may be preferable to translocate fish from immediately below Chute Falls rather than from the confluence of the LCR.

Swamping the translocated fish with a high number of fish from the main population each generation may be an alternative to using the One Migrant Per Generation Rule. Managers could repeat the movement of fish above Chute Falls for several years and then continue to move smaller numbers of fish (say 100 age-0) once a fish generation. This approach is suggested by P. Hedrick (ASU), who believes 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 may cause other unknown ecological effects. For example, based on visual observation, the habitat above Chute Falls is conspicuously different from that below Chute Falls (D. Van Haverbeke, pers. obs.). While algal communities exist below Chute Falls, they tend to be meager and substrates are dominated by marle, sand or gravels. In comparison, algal communities above Chute Falls are dense and diverse, 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 total invertebrate densities were 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, and 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, particularly during flood events when many components of this upriver community can be washed downriver (see Grimm and Fisher 1989, Newcombe and McDonald 1991). If food is a limiting factor in LCR, as has been suggested (Kubly and Cole 1979, Haden et al. 1999), the upstream community above Chute Falls could be important for maintaining the carrying capacity for humpback chub below Chute Falls. As Vannote et al. (1980) discussed, downstream communities are fashioned to capitalize on upstream processing inefficiencies.

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 (P. Hedrick, ASU, pers. com.). 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, ASU, 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.

The potential for demographic boost will likely be limited to < 280 humpback chub > 200 mm. Assuming a current population of ~2,000 adults, this translates into a 14% maximum demographic boost. This gain may or may not be viewed as sufficient to offset the preceding genetic concerns.

In addition, the proposed action does offer the potential to expand the current 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 sampled 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 (e.g., toxic spill into the LCR from upstream).

Finally, a successful translocation (if properly done) has potential to further promote a humpback chub population that is self-sustaining. Therefore, it may be advisable to attempt this action prior to enacting other potential options. However, the potential risks and benefits should be thoroughly reviewed before the action is implemented.

A translocation effort above Chute Falls should be accompanied by long-term commitments to manage and monitor these fish. Such commitments may involve mark-recapture efforts once the group of fish becomes established, to maintain an appropriate level of bi-directional gene flow, and to monitor genetic aspects of the fish both above and below Chute Falls (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 floods impossible in many areas.

In summary, a translocation effort above Chute Falls may or may not be successful. If it is successful, uncertainties exist about potential genetic impacts to the main population of humpback chub below the falls (e.g., concerns with inbreeding, effective population sizes, and the ability (or lack of ability) to correct for problems associated with unidirectional gene flow). As Stockwell and Leberg (2002) state, translocations have both short and long term consequences for the evolutionary ecology of the species. The proposed translocation does 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 these fish subject to genetic uncertainties because of unidirectional gene flow and small effective population size. Finally, the translocation effort may have other ecological unpredictable or unexpected consequences. 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. 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 will require long-term commitment and funding.

Translocation of humpback chub to other tributaries within Grand Canyon

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 (RM 1), Bright Angel (RM 87.7), Shinumo (RM 108.7), Tapeats (RM 133.8), Deer (RM 136.2), Kanab (RM 143.5), Havasu (RM 156.8), and Spencer (RM 246) 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 (Valdez et al. 2000).

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 (except Paria) are all well down river from the LCR (Bright Angel being the closest at ~26 miles from the LCR). Consequently, the potential for offspring to genetically swamp the main LCR population should be minimal. A complete analysis of humpback chub movement in Grand Canyon has not been performed to date, however, preliminary investigations suggest that migration of fish from far downriver to the LCR is very minimal (L. Coggins; pers. com.). For instance, only two humpback chub 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 related to an inability to support a viable genetic effective population size of fish (Valdez et al. 2000). 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 attempts to remove predators in Grand Canyon. Continued attempts to remove brown trout via a weir in Bright Angel Creek should be expected to decrease predation and open niche space within the creek. Should a simultaneous effort be made to remove brown trout in the mainstem between Grapevine and Horn Creek rapids (RM 81.7-90.2), this might reduce predators 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. 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 Serpentine and Waltenberg rapids (RM 106 – 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 recruitment in the LCR is being hampered because of age-0 and juvenile mortality, and predator removal efforts in the mainstem near the LCR minimize 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 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 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 (S. Rogers, AGFD, pers. com.). 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 (unpublished data, GCMRC) have been captured in Havasu Creek, and adult humpback chub are still occasionally or captured in Havasu Creek (unpublished data, GCMRC). Small numbers of age-0 humpback chub have been infrequently captured in Shinumo (Valdez and Ryel 1995) and Kanab creeks (D. Van Haverbeke, pers. obs.) in the past 15 years. Third, the presence of spawning 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 removal of predators from the tributaries, 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 initial translocation using 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 risks (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 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.

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 small population with the 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. Captive broodstock in itself does not pose genetic risk to the wild population, but 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, and represents the highest risk of any of the proposed actions regarding genetic matters (Table 3). Supportive stocking from a captive population should not be considered until all other management activities have been attempted and shown to fail. At this point, however, working toward development of a captive broodstock may foster completion of preliminary actions (e.g., genetics work, captive broodstock management plans, etc.). A fully developed captive broodstock will likely entail the construction of substantial isolation facilities, the identification of appropriate locations to hold several thousand fish, and will be a long-term and costly commitment.

This document does not give specific criteria for when broodstock and supplemental stocking activities should commence. 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 should be undertaken. Nevertheless, managers will be compelled to initiate such activities with a continuing population decline, particularly when other recovery measures are failing. 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). Predator removal efforts are proceeding, and appear to be showing promising results (in terms of predator depletion). Given the scale and intensity of the predator removal efforts in Grand Canyon, some benefits might be expected in the near future. For example, catch rates of age-0 and juvenile humpback chub may increase in mainstem sampling, 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, etc. According to earlier literature, a N_e of 500 should be maintained (Franklin 1980). 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 diminish. 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), suggesting a need for some type of action to augment the population of humpback chub in Grand Canyon. Clearly, attempting to establish or initiate captive broodstock and supplemental stocking activities is impossible without knowledge of the N_e of the wild population. Future work should focus on estimating the N_e of the wild population in Grand Canyon (Anders et al. 2001). In addition, much preliminary work is needed (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 age-0 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 primary problem appears to be related to changes in the behavior of fish that are held in captivity for a substantial portion of their lives. Therefore, post-stocking mortality may be high. Actions 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.

Developing of a program for this type of activity will probably require the removal of 2,000 to 4,000 age-0 fish from the LCR annually and 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). The action does, however, immediately address what is thought to be the primary factor for population decline in Grand Canyon (i.e., lack of recruitment).

A supplemental stocking program using wild caught age-0 fish will need to be adaptive in nature. Some level of continued monitoring of the annual age-0 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.). The location 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 the LCR by over-taxing the carrying capacity. 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 (~6 km), however, this expansion may not provide any substantial security from catastrophic loss. Finally, unlike other alternatives, this proposed action does have the potential to further promote a self-sustaining population. However, the action could potentially be accompanied by several genetic risks to the wild population, primarily because this has potential to establish a small reproductively isolated group of fish within 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 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 may be genetically less risky to the main LCR population because these tributaries are 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 may be subject to inbreeding (Valdez et al. 2000). However, they do have potential for acting as refugia (Valdez et al. 2000). If ongoing predator removal efforts by the National Park Service 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 (RM 81.7 - 90.1). 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 Serpentine to Waltenburg rapids; RM 106 - 112.1). Removing predators in Havasu creek (particularly carp and stripped bass), and in the mainstem within the vicinity of Havasu creek may also accomplish a similar result. Each of these small tributaries by themselves appear to have insufficient carrying capacity to support a population of humpback chub not subject to inbreeding. However, 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). Translocation and predator removal efforts by themselves may not be sufficient, and some level of warming mainstem waters may be required to initiate an effect.

As a final consideration, managers should be aware that each potential management actions 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 age-0 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					Potentially 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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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+; $\geq$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 3 km section of the Little Colorado River (LCR) in the Salt Camp Area in July 1998. 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:

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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, 30-60 mm humpback chub, (*Gila cypha*, HBC) to upstream areas of the Little Colorado River to offset the potential impacts on chubs from the proposed project. A reinitiation of the BO in May 2003 expanded this original size range from 30-60 mm to 50-100 mm. 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 (VanHaverbeke and Coggins 2003). 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 whether transplanted fish would remain above Chute Falls. Geomorphology of this section of the LCR includes narrow, canyon bound stretches subject to scouring flows. During high flow events during winter runoff and monsoon storms, small life history stages of HBC may 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 diminish the large-scale loss of HBC in the 50-100 mm 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 mm size range that survival substantially increases. If HBC can remain in the LCR longer to reach these larger sizes, 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 (Valdez and Ryel 1995). 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.

Methods:
A reconnaissance 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.

Dissolved Carbon dioxide measurements will be taken by titration of LCR water mixed with phenolphthalein indicator with 3.636 N Sodium Hydroxide using a HACH digital titrator. A graduated cylinder will be used to collect water and place it in disposable clear plastic cups for each individual titration. This is to alleviate problems with contamination resulting from residue sodium hydroxide and problems associated with $CaCO_3$ precipitation (cleaning the flask with vinegar may also add additional unwanted acid residue). Because CaCO3 may be the primary deterrent of HBC already occupying this region (Mattes 1993, Strength 1997), samples will be taken at numerous upriver locations beginning below Chute Falls and going at least three kilometers upriver and potentially to Blue Springs at river km 21, if time permits.

Dissolved oxygen, conductivity, temperature, and pH will be taken at a single fixed location above Chute Falls using a Hydrolab. Turbidity will be taken daily at this fixed site using a HACH turbidimeter. Robinson et al. (1996) indicated that water temperature remained relatively constant in between LCR reaches in all months but January. Although they did show that dissolved oxygen, pH, and conductivity greatly increased downstream from Blue Spring to Chute Falls, we are not going to attempt to translocate YOY HBC that far upriver. They also indicated that turbidity increased from Blue Spring downstream to 10 km, but their figure indicated that this increase was within but a few nephelometric turbidity units during clear water conditions. Moreover, Strength (1997) showed that from Chute Fall upriver for ~ 3km during June 1996 (clear water conditions) that the conductivity (~4,830 uS) and pH (7.14-7.60) remained relatively stable.

In July 2003, USFWS biologists 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. 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 (Haines et. al 1998, Hale and Grey 1998, Olsen and Vollstad 2001, Close 2002, Close and Jones 2002). 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 (26-40 mm) during cage experiments at river km 15 and 12.5 suggesting some handling induced mortality from transport. Mortality was reduced to 0% when age-1 fish (40-100 mm) 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 sizes, 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 HBC will be measured for length, and implanted with an elastomer tag with a unique color. All other fishes will be returned to point of capture. All captured HBC will be held in 1/8 inch 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. Stress behaviors include rapid opercular movements, gulping at the surface and hyperactivity. 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 retention rates above Chute Falls. 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 km. Idenfication 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 (Thurow 1994). 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 survival through November 2004. 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.

Timeline:
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: Monitoring trip, 5 days
  December 31, 2003: Interim 2003 Report due
Spring 2004: Post winter flow snorkeling surveys, 5 days
June/July 2004: Translocation trip at confluence of LCR and mainstem Colorado, 2-5 days
November 2004: Monitoring, 5 days
  December 31, 2004: Interim 2004 Report Due
Spring 2005: Post winter flow snorkeling surveys, 5 days
  June 2005: Final report due

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