Final Report

The Feasibility of Developing a Program to Augment the Population of Humpback Chub (*Gila cypha*) in Grand Canyon

By

David R. Van Haverbeke

and

Robert L. Simmonds Jr.

U.S. Fish and Wildlife Service
Arizona Fishery Resources Office – Flagstaff
323 N. Leroux
Flagstaff, AZ 86001
928-226-1289

July 2004

Document No. - USFWS-AZFRO-FL-03-007

Table of Contents

List of Tables

List of Figures

Executive Summary

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

First, we address the feasibility of establishing a captive broodstock program for humpback chub. Broodstock development is considered within the context of captive propagation policy as defined by the U.S. Fish and Wildlife Service and the National Oceanic and Atmospheric Association, and within the context of the scientific literature. Part of this policy requires that captive broodstock activities are based on the specific recommendations of recovery strategies identified in recovery plans whenever practical; a feature for which the current Recovery Goals for the species make no provision.

Establishment of a captive broodstock may serve as a genetic refugium, and the activity by itself poses no risk to the wild population. However release of captive propagated individuals into the wild poses serious biological risks, and as such should not be considered as a management strategy unless all other conservation measures fail. Potential biological risks involved with release of captive propagated fish into the wild include introgression, inbreeding depression, domestication, and potential to decrease the genetic effective population size of the wild population.

A captive broodstock program will require being preceded by completion of genetics studies being performed on humpback chub, development of a formal captive broodstock management plan, identification of a an adequate site equipped with a quarantine facility and capable of holding several thousand fish, development of a formal reintroduction plan, and commitment of significant long term funding. This document discusses basic questions in relation to broodstock development, and lists hatchery attributes required to raise broodstock fish. The humpback chub currently held at Willow Beach National Fish Hatchery may serve as a starting point for such an activity.

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. Modeling suggests that if ~960 to 1,400 age-0 humpback chub were captured per year from the Little Colorado River (LCR), and grown out to ~170 mm before release, this should arrest further population decline and maintain the abundance of age-4+ humpback chub at levels estimated in 2000. Alternatively, the model predicts that if ~4,300 to 6,350 age-0 fish were captured per year from the LCR, and grown out to ~170 mm before release, this may achieve a positive increase in the abundance of age-4+ fish to the level estimated in 1990. These predicted numbers may need to be inflated, depending upon the rate of post-release mortality (i.e., they may need to be doubled). These predicted effects should be fully realized by the year 2020. The primary biological risks associated with this activity 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 effect density-dependant dynamics in the wild population. It is recommended that if this action is performed, and is to succeed, a naturalistic rearing facility and methodologies are used. Although the action, if properly performed, appears to pose no serious biological risks to the wild population, the action would not promote a self sustaining population, a feature required in the recovery criteria for the species. In addition, this management action would likely require significant long term effort and funding (years to decades) to achieve a sustained positive population response.

Third, we address the feasibility of augmenting the Grand Canyon population of humpback chub via translocation. Considered are 1) translocation of fish within the LCR, and 2) translocation of fish into other tributaries (Bright Angel, Shinumo, and Havasu creeks) in Grand Canyon.

Translocation of humpback chub within the LCR appears to offer potential for minor gain in the wild census population, and may significantly expand the current spawning range of the species, but may pose some potential for genetic risk to the main population in Grand Canyon. These risks are related to inbreeding and depression of genetic effective population size. Corrective measures to avoid these problems (should they occur) appear to be available, provided monitoring and funding is emplaced to detect them.

Translocation of humpback chub to other tributaries in Grand Canyon may offer potential for augmenting mainstem aggregations, and some potential exists for creation or significant expansion of downstream aggregations. Translocation efforts to these tributaries may need to be accompanied by sizeable predator removal efforts to effect a change. Genetic risks to the main population of humpback chub in Grand Canyon appear to be minor.

It is recommended that if any of these actions are pursued, they be carried out with the priorities in mind of: 1) posing least potential for genetic harm to the wild population, and 2) having the best potential for promoting a self-sustaining wild population. This suggests that translocations might be a first priority, followed by supplemental stocking of wild caught age-0 fish, and as a last resort the release of captive propagated fish. Maintaining fish in captivity for refugium purposes poses no genetic risks to the wild population, however, the release into the wild of captive reared individuals does pose numerous genetic risks that need to be seriously evaluated.

Objectives and Rationale

At the request 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 Little Colorado River (LCR) and grown to a larger size in captivity, and 3) using translocation to establish a second spawning population (or expand 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 formerly been proposed as a potential conservation action (USFWS 1990), as has establishing a second population of humpback chub (USFWS 1990, USFWS 1994, USBR 1995). However, the current amended Recovery Goals for the species make no provision for captive broodstock activities (USFWS 2002a).

This report only considers 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 cooperating agencies, as well as additional funding to the agencies carrying out the actions, and would require long-term monitoring of results. 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). 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 is comprised of individuals that live some portion of their life history in the mainstem Colorado River and migrate to the LCR for spawning purposes.

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 large humpback chub captured during a day of angling in the mainstem Colorado River just above Bright Angel Creek (RM 87.5; Figure 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. Minckley et al. (2003) calculated that there were about 200,000 adult humpback chub inhabiting the Colorado River basin during historic pre-dam times.

Recent population estimates indicate that during the past twenty years, humpback chub in Grand Canyon have declined. Population estimates have dropped from around 7,500 fish >200 mm total during the late 1970s (Kaeding and Zimmerman 1982), to ~4,500 fish >150 mm during spring in the LCR in the early 1990s (Douglas and Marsh 1996). Recent closed estimates from the LCR for humpback chub ≥150 mm have been 2,082 fish (SE = 242) in spring 2001 (Van Haverbeke and Coggins 2003), 2,666 fish (SE = 463) in spring 2002 (Van Haverbeke 2003), and 3,419 fish (SE = 480) in spring 2003 (Van Haverbeke 2004). Modeling based on the database of humpback chub in Grand Canyon has confirmed this overall 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.

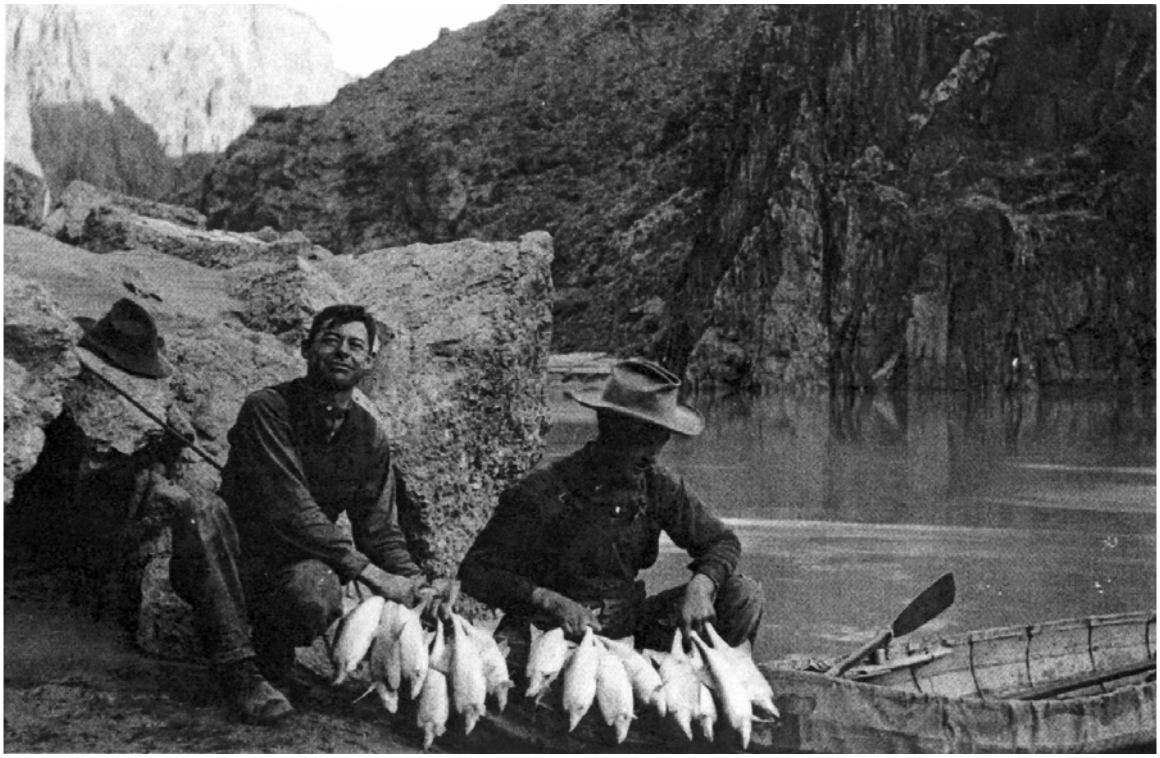

Figure 1. Early photograph of humpback chub taken on the mainstem Colorado River, a short distance upriver from Bright Angel Creek. Photograph from Grand Canyon Archive, Rust Collection.

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, hypolimnetically-derived, fluctuating flow environment that is not supportive of all life stages of native fish.

Three actions that have been proposed for humpback chub are: 1) development of a captive broodstock (USFWS 1990), 2) supplemental stocking of wild fish from the LCR, and 3) translocation of fish to upstream reaches of the LCR or to other tributaries. We here evaluate the feasibility of each of these potential actions.

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 the management alternatives for the conservation and recovery of endangered Pacific salmonids (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). Waples and Drake (2002) caution that even when managers are aware of all foreseeable risks, the chances are high that unexpected developments will erase projected benefits. Hilborn (1998) states that based upon historical experience, politicians, managers and advocates of new stocking programs should realize that there are limited empirical data that support the long-held belief that supportive stocking has ever been biologically successful.

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).

Policy and priority considerations

Within the context of the ESA, the USFWS and the National Oceanic and Atmosphere Administration (NOAA) 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 report. 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.

The scientific literature clearly coincides with the perspective of USFWS's and NOAA's policy on captive propagation. 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 stocking should not be a factor that leads to the diminishment of habitat conservation and restoration (Philippart 1995). There is concern 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), largely 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). Philippart (1995) emphasizes that movement of animals from the wild to a captive breeding station is considered the most extreme form of relocation, and Snyder et al. (1996) emphasize that captive breeding should be viewed as a last resort to species recovery.

Primary objectives

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 long term conservation of genetic variability applies to a species whose wild habitat may be lost and whose whole future may depend on captive maintenance. For humpback chub, although large portions of habitat have been altered or lost, untested options still remain for improving degraded habitat. Nevertheless, some may view that a refugium is necessary in the event of catastrophic loss. This view should include the conservation and retention of maximum genetic variability.

In the case of the second objective (captive breeding for release into the wild), 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 near future. 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.

Biological concerns

The literature is replete with warnings concerning the pitfalls of captive breeding programs (e.g., Waples and Do 1994, Busack and Currens 1995, Philippart 1995, Utter 1998, 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 (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).

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. One of the main concerns is that supportive breeding via the use of captive broodstocks can pose genetic risks (or hazards) to wild populations (Ryman and Laikre 1991, Busack and Currens 1995, Lynch and O'Hely 2001, Ford 2002). 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, 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 studies imply 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, 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. Furthermore, this risk appears to be contributing to the demise of fisheries on a worldwide basis (Tringali and Bert 1998).

If the underlying problems for population decline are not 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 (Waples 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). 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). However, this potential for gain is accompanied by genetic and behavioral risks to the wild population. 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).

Is a captive broodstock of humpback chub needed?

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. 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). Such options as simultaneously running steady flow experiments with removal of 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.

The Recovery Goals for humpback chub call for no significant decline occurring in the number of adult fish within each wild 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 does not appear to be a need to demographically boost populations of humpback chub in order to meet the proposed MVP standards and reach recovery. And undertaking a management action (such as release of captive propagated fish) has the potential to further reduce $N_{e,}$ or lead to introgression of deleterious alleles; thus further jeopardizing the humpback chub. Yet, waiting to take action until humpback chub fall below an arguably insufficient MVP standard does pose some risk for a loss of opportunity for maintaining full genetic variability.

In view of this, 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, the ongoing genetics work on humpback chub should be completed. A formal and comprehensive captive broodstock development plan as well as a reintroduction plan should be completed. The genetic variability of any humpback chub in captivity should be compared to the genetic variability of the wild population at large in the LCR. It would be advisable to complete, and to perform similar genetics work on the 30-mile aggregation of humpback chub in the Colorado River. 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 that document. 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 minimum 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.

A potential source of broodstock is the ~80 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 80 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).

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-recaptures, 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.

The strategy involved in using either the Willow Beach NFH or the 30-mile aggregation 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.

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 not all fish may spawn each year. 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, peaked in April and waned in 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 imposed 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, 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 (e.g., 300 fish) during any given capture event 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 broodstock 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 in the age-0 cohort to minimize impacting the wild population. In June, it may be possible to capture large numbers of age-0 fish, but most will likely be < 50 mm (Gorman 1994, Van Haverbeke 2001a). The modal length of age-0 humpback chub reached > 50 mm in late July and early August during 1993 and 1994 (Gorman 1994). 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.). It is possible to capture this size class of fish between June and November (USFWS 2000, Van Haverbeke 2001b), but the optimal months are probably from mid July through the end of August (D. Stone, USFWS, pers. com., D. Van Haverbeke, pers. obs.).

In addition, the logistics of capturing a sufficient number of age-0 fish can be complicated by the hydrograph of the LCR (Figure 2). 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 fish >50 mm 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.). Thus, flood conditions can actually facilitate capture, since fish appear to be concentrated, and will not “see” the capture gear.

By late summer and fall, many age-0 fish are transported out of LCR by 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. Flows in the LCR generally return to base flow conditions during November; however, by then much of the age-0 cohort has undergone mortality (e.g., transported to the mainstem), or grown > 75 mm. Again, flexibility will be required to accommodate an appropriate temporal scale for collection.

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 much less frequent (Gorman and Stone 1999, Van Haverbeke 2004). As a result, large females must usually 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 ripe males, much easier to capture. 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, in egg survivorship, 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.

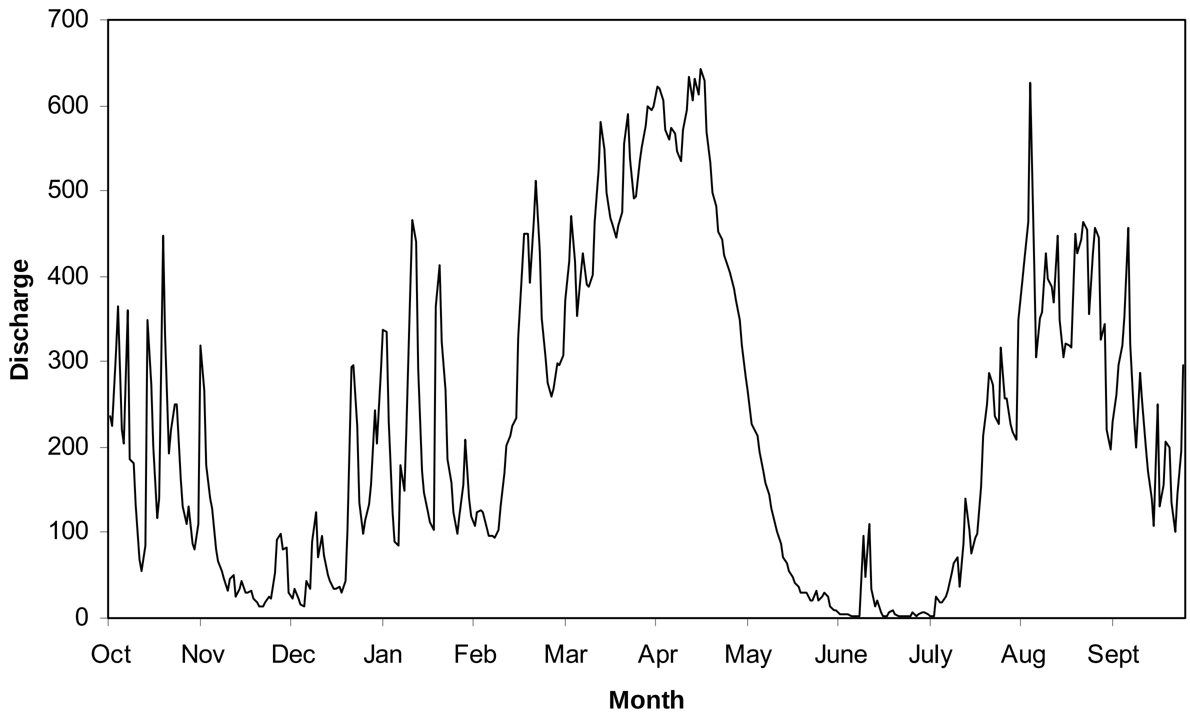

Figure 2. Daily mean discharge (cubic feet per second) of Little Colorado River for water years 1947-2003. Data from USGS gage station 0940200 near Cameron, Arizona.

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 some 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 should be large enough to hold from 2,500 to 3,000 fish (M. Ulibarri and C. Keeler-Foster, USFWS, DNFH & TC, pers. com.). This should allow for the incorporation and maintenance of sufficient genetic variability. This implies large space requirements that need to be 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 1982). 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 to help reduce domestication. This implies that flowing water will be needed to raise the fish or at least to condition them prior to release. 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. This factor by itself could add enormously to the budget.

Summary

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 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.
9. Realize that release of captive grown fish into the wild will likely need to be a slow, protracted process (many years), in order to avoid potential problems with negatively effecting the wild N_e.

Feasibility of Establishing a Supplemental Stocking Program Using Wild Caught Age-0 Fish

Objective and rationale

Below, 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. 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.

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 at larger sizes 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 progress toward the goal of establishing a stock of no fewer than 50,000 repatriated fish (P. Marsh, ASU, pers. com.).

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, 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).

Biological considerations

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 factor after release, and lack of 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.

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, 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 ability 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).

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

A basic premise in fisheries ecology 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. In addition, growing fish from 50 to 150 mm rather than from 30 to 150 mm will take less time in the hatchery (perhaps a month). Two important considerations are to 1) 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), and 2) minimize the time that fish are held in captivity in order to minimize behavioral changes.

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 captured and handled with minimum mortality using seines, minnow-traps, or hoop nets.

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

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

Another alternative could be removing 1-year old fish during spring. These fish would be ~93 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. Protocols will need to be established to ensure mortality is kept to levels specified in permits. For instance, holding pens will need to be established at each camp, and methods will need to be established for moving fish safely from capture sites to the holding pens. An alternative to setting up holding pens 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. Fiegel, USFWS, pers. com.).

We suggest direct transport via helicopter to the appropriate receiving facility. Since several hundred to several thousand fish will be transported (numbers are discussed below), this will likely require several 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 oxygen levels.

In summary, we recommend capturing fish at age-0 (50-70 mm), from multiple localities within the LCR during the months of July and August.

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

We recommend 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.

Whereas age-0 humpback chub show minimal 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). This discrepancy may not be real, or it could be that once fish reach 150+ mm, there is more food available in the mainstem, allowing faster growth even at lower temperatures.

Whether to grow fish beyond 150 mm becomes a question subject to debate. Larger humpback chub (>150 mm) should be even 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). Wild fish that are 200 mm are estimated to have a greater annual survivorship than wild fish at 150 mm (Table 1). Note that to obtain even a 52% annual wild survival rate, fish would need to be 134 mm. Therefore, fish grown to 150 mm could optimally have a post-release annual survival rate of ~55%, while fish grown to 200 mm could have an optimal post-release annual survival rate of 67% (Table 1).

Unfortunately, the above survival rates are likely highly optimistic since they are estimated wild survival rates. 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 roughly estimated as high as ~25% (F. Pfieffer, USFWS, pers. com.); however, these fish were grown to 400 mm.

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. 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 logistically easier to collect fewer fish. It is unlikely that this would reduce hatchery space requirements or costs, since growing fish to 200 mm will result in holding multiple cohorts on station.

In summary, we recommend that fish are grown out to a minimum of 150 mm for initial efforts. If problems with survivorship rates upon release at this size appear to be insurmountable in achieving augmentation, efforts may need to be taken to grow fish to 200 mm before release. Our rationale for recommending 150 mm is based on 1) a need to PIT tag and monitor these fish once released, and 2) minimizing the chance for hatchery mortality and domestication issues.

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

We have investigated an Age Structured Mark-Recapture (ASMR) population model (designed by C. Walters, UBC) for humpback chub. This model provides an estimate of the number of fish that may be needed to arrest a continuing decline or to augment the population of humpback chub in Grand Canyon via the use of supplemental stocking using wild caught fish. The model can be set up using alternative assumptions about wild survivorship rates. For example, the model can use the age at length and estimated survival rates from ages 1 to 30 (partially shown in Table 1), and with the use of historical data, run from 1989 to 2020. The model can operate by inputting the number of aged fish that are stocked (e.g., ages-1, 2, 3, etc.) on top of the estimated average wild recruitment between 1994 and 1999. Since the model uses annual age at length data, it will not predict exactly how many humpback chub at 150 mm would need to be stocked (i.e., it can only input the age specific lengths - for example age-3 fish at 171 mm). In this report, the model is not intended to provide a final definitive number; it is dynamic and is subject to assumptions and future refinements that may or may not be considered necessary. The model accounts for removal of age-0 fish from the wild cohort (i.e., cropping), and assumes a 20% mortality rate in the captive phase (as based on communications with M. Ulibarri, Dexter NFH&TC, on grow out of bonytail chub). The model also assumes that wild mortality rates remain in place once the fish are stocked (i.e., there is no additional post release mortality). The model is useful in that it provides a magnitude for the number of stocked fish that might be needed to arrest a continued expected decline or to achieve a positive population response. We have used the model to predict the number of fish that may need to be stocked to 1) arrest decline and maintain the abundance of age-4+ fish at levels estimated in 2000, and 2) achieve a positive population response and return to the abundance of age-4+ fish estimated in 1990. As stated above, since the model can be set up using varying assumptions about wild survivorship rates, we present a range of numbers (Table 2) that include a best and worst case scenario.

With no additional stocking, the three variations of the ASMR model (i.e., using three different assumptions about wild survivorship rates inclusive of a best and worst case scenario) predict that if wild annual recruitment continues to be stable, the spawning population will soon stop declining, and will stabilize at an average spawning abundance ~1,900 to 2,300 fish. This is in agreement with the initial predictive assessment made by Coggins et al. (2003).

Table 2. Predicted numbers of humpback chub using Age Structured Mark-Recapture modeling that would need to be removed at age-0, grown in captivity to 171 mm and released into the wild in order to stabilize the abundance of age-4+ fish at the level estimated in 2000 (arrest further decline) and to increase the abundance of age-4+ fish back to levels estimated in 1990 (effect a positive population response).

	Stabilize at 2000 level		**Recover to 1990 level**	
	Remove age-0	Stock age-3	Remove age-0	Stock age-3
ASMR base model	1,350	1,080	4,294	3,435
ASMR+ high mortality	960	768	6,358	5,086
ASMR+low mortality	1,414	1,131	5,963	4,770

The model suggests that if ~960 to 1,400 age-0 humpback chub were captured per year from the LCR, and grown out to ~170 mm before release, this should arrest a decline and maintain the abundance of age-4+ humpback chub at levels estimated in 2000 (Figure 3). Alternatively, the model predicts that if ~4,300 to 6,350 age-0 fish were captured per year from the LCR, and grown out to ~170 mm before release, this should effect a positive increase in the abundance of age-4+ fish estimated in 1990. It should be kept in mind that these numbers may need to be inflated somewhat if the fish are only grown to 150 mm, or deflated somewhat in fish are grown to 200 mm. It should also be recognized that the terminal abundance estimates that the model is predicting apply to the year 2020 with stocking beginning in the year 2005, suggesting these effects will take over a decade to fully achieve. Prior to any final decisions being made, the designer of the model should be further consulted. This is in part because one of the key assumptions in using the model for these predictive purposes is that future recruitment rates continue to remain at the mid-1990s level; a factor that may or may not be realized in the years to come.

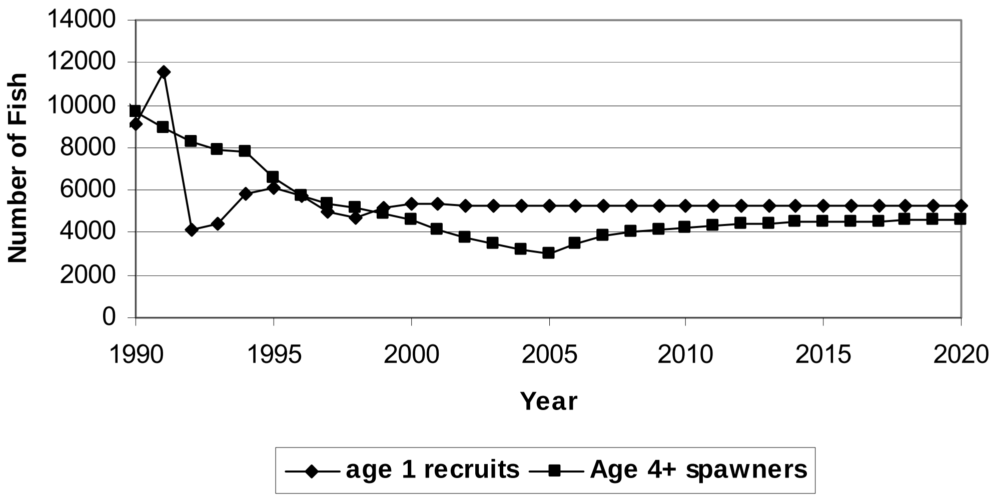

Figure 3. Provisional estimated and projected humpback chub population (Little Colorado River [LCR] stock) in Grand Canyon, assuming recruitment remains at mid 1990s level. This example figure of the base Age Structured Mark-Recapture model depicts the predicted effect of capturing 1,350 age-0 humpback chub per year from the LCR, growing them in captivity to 171 mm, and beginning release in 2005. The model predicts that by the year 2020, abundance estimates of age-4+ fish would equal the abundance estimated to be present in the year 2000.

As mentioned above, based on culturing bonytails, mortality in the hatchery system could easily average 20%, and one might reason that the less time fish spend in the hatchery, the less chance for mortality to occur. Also, if the low survival rates of other stocked species are any indication (McNeil 1991, Salvanes 2001), we can only assume that post-stocking mortality of humpback chub could be high (i.e., >50%, or much higher than wild survival rates for an equivalent sized fish). This suggests that the numbers of age-0 fish collected may need to be doubled (C. Walters, UBC, pers. com.). 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 wild recruitment failure have not been solved. In addition, about 50 to 60 fish are generally killed for health studies each time fish are brought from the wild into a hatchery station (J. Thoesen, USFWS, Fish Health, pers. com.).

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 an annual wild production of ~50,000 age-0 fish per year. Thus, removing about 960 to 1,400 age-0 fish on an annual basis (to arrest decline) might be the equivalent of removing 2 to 3% of the annual age-0 production. Removing 4,300 to 6,350 age-0 fish per year (to achieve a positive increase to 1990 levels) might be the equivalent of cropping 9 to 13% of the annual wild production. As explained above, the model does account for this cropping in estimating the projected future stock. Also as explained above, this cropping may need to be doubled to account for post-release mortality. Finally, we caution that annual production of age-0 fish in the LCR is likely highly variable (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 (based on Valdez and Ryel 1995). Thus in some years, the cropping may be insignificant, while in other years it could be significant (i.e., > 10%), depending on the goals to be achieved.

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 3 shows fall population abundance estimates that have been obtained in the LCR for fish > 150 mm. These estimates are thought to estimate the number of humpback chub that “overwinter” in the LCR, and may be somewhat representative of the carrying capacity in this system. Consider if only 768 to 1,131 (Table 2) humpback chub > 150 mm were released into the LCR each fall in an attempt to arrest further decline. Also consider that the average point population estimate for humpback chub > 150 residing in the LCR during the fall since 1992 is 1,729 fish (Table 3). This translates into a 44 to 65% increase in numbers of fish that would suddenly inhabit the system. Should 3,435 to 5,086 fish be released in an attempt to reach 1990 age-4+ levels, this translates into a 199 to 294% increase. If food is limiting in the LCR, there is concern that the sudden increase in abundance of humpback chub > 150 mm could cause secondary effects related to carrying capacity and density dependence, cropping the next year’s age-0 cohort via predation and potentially eliminating any gain from the augmentation effort. Another obvious concern would be the accidental introduction of diseases or parasites into the wild, again potentially negating positive effects. One positive aspect of releasing supplemental fish back into the LCR could be gaining immediate familiarity with habitat, breeding grounds, and migration routes.

Table 3. 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), 2002 estimate is from Van Haverbeke (2003), and 2003 estimate is from Van Haverbeke (2004).

			95% Confidence Interval				
Date	Abundance Estimate	SE	Lower	Upper	Reach (rkm)	Size (mm)	# per km
October 1991	2,038	518	1,276	3,368	0 - 14.9	≥ 150 mm	137
November 1991	1,989	489	1,264	3,235	0 - 14.9	≥ 150 mm	133
October 1992	1,099	60	990	1,224	0 - 14.9	≥ 150 mm	74
November 1992	1,417	408	839	2,500	0 - 14.9	≥ 150 mm	95
October/November 2000	1,590	297	992	2,552	0 - 14.2	≥ 135 mm	107
October/November 2001	1,064	33	999	1,129	0 - 14.2	> 150 mm	71
October/November 2002	2,774	209	2,364	3,184	0 - 14.2	≥ 150 mm	186
September/October 2003	1,862	206	1,459	2,265	0 - 14.2	≥ 150 mm	125
Average	1,729						

Another option for release of supplemental stocked 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. First, carrying capacity issues should be much less of concern. This may be especially true so since ongoing trout removal efforts should be opening up niche space. Since January 2003, ~13,400 trout have been removed from Kwagunt Rapid (RM 56) to Lava Canyon (RM 65.4). It is thought that this is maintaining trout at ~80 to 90% of the level formerly residing in this reach of the river (L.G. Coggins, GCMRC, pers. com.). 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). Spread of other unexpected diseases to the LCR might be of less concern than releasing fish directly into the LCR. Many diseases, including Asian tapeworm, should be expected to subside in the cooler mainstem waters before fish re-enter the LCR.

Other options could be stocking a proportion of fish into the LCR, and another proportion into the mainstem. 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 eddies and backchannels of the mainstem, with equal numbers of fish released above and below the LCR. Mark-recapture efforts might 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. From here, the fish would have limited access to the LCR, or full access to the mainstem Colorado River. An important factor determining release sites should be the ability (or lack thereof) to set up soft-release protocols (i.e., short-term holding pens).

Efforts 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 of low flows with decreased velocities. To do otherwise may invite undue mortality. An adaptive approach should be taken (i.e., release times should be flexible and dictated by riverine conditions and flows, rather than by inflexible schedules). Paying attention to current and expected hydrographs, both in the LCR and in the mainstem will be important.

In summary, because of concerns about potential unanticipated secondary effects related to carrying capacity, density dependence, and disease, and in an effort to increase recruitment to the main LCR population, it is suggested that the first attempts to release supplemental stocked fish are done in the mainstem Colorado River between river mile 58.8 and 64.5 (i.e., in the backchannels and eddies between Kwagunt and Lava/Chuar Rapids). Should these attempts prove unproductive, less conservative measures may need to be taken (i.e., release near the confluence region, or higher up in the LCR watershed).

Where could the supplemental fish be grown?

It is not the purpose of this document to specifically identify a hatchery where supplemental fish could be grown. Attributes that a facility should possess include all those listed under the previous section for captive broodstock (e.g., experienced personnel, committed long term 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, marl, sands, gravels and substrate rocks could be obtained from the LCR. This could be accomplished by transporting them via boat. In addition, natural food types and some exposure to predators would be desirable (see Brown and Laland 2001 for review).

Summary

In summary, we suggest the following for establishing a program for supplemental stocking of wild caught fish:

1. Use criteria based on the most current trend and abundance analyses to determine whether or not supplemental stocking is needed as a management action in order to 1) arrest a future predicted decline in numbers of humpback chub, or 2) to achieve a positive increase in predicted long term abundance for the species.
2. If action is determined necessary to arrest a decline or to achieve a positive population response, identify a rearing facility that is adequately funded, equipped, and staffed for such an activity to take place.
3. Depending on the goals to be achieved, consider that at least 960 to 6,350 age-0 fish will need to be cropped annually from the LCR, that these numbers may need to be doubled to account for post release mortality, and that this effort will need to be carried out for many years (probably a decade or two) to effect a change.
4. Capture age-0 fish from the LCR on appropriate spatial and temporal scales.
5. Grow these fish to at least 150 mm TL before release into the wild, and PIT tag all supplemental fish before release.
6. Grow the fish out using naturalistic rearing techniques.
7. Use soft release protocols.
8. Continue long term monitoring of the population in order to detect results of the management action.

Feasibility of Expanding the Humpback Chub Population Via Translocation in the Little Colorado River 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 14.9 km) in the LCR, and establishing (or augmenting) fish in Bright Angel, Shinumo, or Havasu creeks.

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 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). Griffith et al. (1989) also point out that the chance for a successful translocation increases if there is more than one potential translocation area, and that substantial gain is achieved by splitting the animals between areas.

Translocation of humpback chub within the Little Colorado River

Rationale

In a December 6, 2002 Biological Opinion (USFWS 2002b), and through subsequent Section 7 re-initiation in March 2003, a conservation action was proposed by U.S. Bureau of Reclamation, Grand Canyon National Park, Glen Canyon National Recreation Area, and GCMRC to translocate three hundred 50 to 100 mm total length humpback chub from near the mouth of the LCR to a reach within the LCR above a natural travertine dam structure referred to Chute Falls (14.9 km). Since results of the translocation effort in July 2003 were deemed successful (i.e., translocated fish were found to survive; Stone and Sponholtz 2003), a second translocation of 300 humpback chub will be conducted in summer 2004. The action is intended 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.

Background

The habitat above Chute Falls is most 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 well established in the LCR above Chute Falls. Until the translocation of humpback chub above Chute Falls in 2003, the only native species known to recently exist between Chute Falls and Blue Springs (at 21 km) was the speckled dace (*Rhinichthys osculus*). Non-native fish that been captured or observed include carp, fathead minnow (Kaeding and Zimmerman 1983), plains killifish, red shiner, black bullhead, channel catfish, rainbow trout, and green sunfish (Stone and Sponholtz, 2003). The reason humpback chub were not formerly found in recent studies above Chute Falls is unknown. Chute Falls may have been a physical barrier (Robinson et al. 1996), which implies that if humpback chub were historically above Chute Falls, local extinction occurred (e.g., environmental stochasticity) and the species was unable to successfully re-colonize.

The recent translocation results (Stone and Sponholtz 2003) thus far suggest that there is 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.

Potential risks of the Chute Falls translocation

The translocation effort has potential for establishing a reproductively isolated population of humpback chub above Chute Falls. This holds some potential genetic implications. First, since humpback chub apparently did not formerly reside above Chute Falls, this suggests 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). Even when numerous individuals are translocated, bottlenecks may occur early in population establishment, leading to reduced genetic diversity (Stockwell et al. 1996).

Second, if the effort is not continued, the founder population will consist of < 600 fish (i.e., some will not survive the translocations). Since the translocated fish are small (50 to 100 mm), there is 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 result in 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).

Third, should the translocation continue to be successful, and a large number of offspring occur from an insufficient number of founders, the action may have potential to decrease the 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). This potential problem is considered by some as 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. 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 above Chute Falls 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. In addition, offspring from the translocated fish may demonstrate increased survivorship because of the unexploited habitat and the apparently much higher food resources above Chute Falls.

Fourth, there are potentials for selection to act upon the translocated fish (i.e., move away from the main genotype), and thus 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). If fish are successful at reproducing and remaining above Chute Falls, this might be expected to impose selection for a resident genotype. Douglas and Marsh (1996) hypothesized that there may be a resident genotype developing in the LCR since closure of Glen Canyon Dam. 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 will move toward the mainstem. However, Gorman and Stone (1999) found that smaller adults (< 300 mm) tend to remain as residents in the LCR. In addition, the unusually high growth rates already observed in the fish translocated in 2003 (Stone and Sponholtz 2003) suggest that within year or two some fish may commence spawning activities. If Chute Falls is a barrier against upstream movement of fish (and no data has convincingly shown otherwise), 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. Water discharged from Blue Springs comes from an aquifer dominated by limestone, 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 travertine dams where release of CO_2 to the atmosphere occurs, and large amounts of calcium carbonate precipitate on the river's 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).

Finally, the translocation of fish above Chute Falls may precipitate 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 marl, 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 one or two orders of magnitude higher than those below Chute Falls (D. Van Haverbeke, pers. obs., D. Stone, pers. com.). Many of these differences may be because humpback chub presumably did not formerly inhabit this area, and primary production and prey were 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 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 main question that needs to be asked is whether or not establishment of a small breeding 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). 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 (P. Hedrick, ASU, and Dr. C. Walters, UBC, pers. com.). 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).

In addition, 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.).

Potential benefits of the Chute Falls translocation

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 demographic gain can be roughly estimated by considering that since 2001 there has been an average of 740 humpback chub ≥ 200 mm residing in the lower 14.2 km of the LCR during the fall (Van Haverbeke 2004). Assuming that these fall abundance estimates are representative of year round residence, this translates into an average of 52 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 312 fish > 200 mm (i.e., 4+ year old fish of presumed breeding age). This represents a potential increase to the presumed resident adult portion of the LCR population of ~30%. Alternatively, using the average estimate since 2001 for the abundance of humpback chub ≥ 200 mm (1,631 fish; Van Haverbeke 2004), this translates into a 16% demographic boost, more representative of the LCR population as a whole. Thus, the potential demographic increase resulting from the translocation could be viewed as a positive conservation measure from a demographics standpoint. On the other hand, it also suggests that the founder population may have some power to influence the genetics of the LCR population. Regardless, the above illustrates that should a group of reproductively isolated humpback chub establish above Chute Falls, it is probably destined to remain small (i.e., $N_e << 500$).

In addition to a demographic gain, the proposed action offers the potential to expand the current range of the species in the LCR by ~ 5 km. Speckled dace are sampled up to about one km below Blue Springs (Mattes 1993), suggesting that km 20 may be the uppermost reach that humpback chub would be expected to survive. Nevertheless, a 5 km expansion would represent a 25% increase in available LCR habitat for humpback chub. 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 has potential to further promote a humpback chub population that is self-sustaining, as required in the current Recovery Goals for the species (USFWS 2002a). Therefore, it may be advisable to continue with this action prior to enacting other potential options.

Recommendations for the translocated fish above Chute Falls

The translocation effort above Chute Falls should be accompanied by long-term commitments to manage and monitor these fish. Such commitments may include mark-recapture efforts, maintaining an appropriate level of bi-directional gene flow, monitoring genetic aspects of the fish both above and below Chute Falls (particularly changes in heterozygosity), and monitoring the algal and invertebrate communities above and below Chute Falls.

An effort should be made to track the abundance of the founding population. Thus far, translocated fish have only been batch marked with fluorescent elastomer tags, and there appears to be some tag loss (21%; Stone and Sponholtz 2003). This precludes mark-recapture efforts to track the population. Once fish reach 150 mm, they can be PIT tagged; allowing mark-recapture studies to precede, however, a funded program to do so must be established, and we recommend that Chute Falls monitoring be a part of the routine monitoring in the LCR.

Generally, managers respond to a potential threat of decreasing a wild N_e by artificially imposing gene flow into the smaller population (i.e., the One Migrant Per Generation rule [OMPG]; 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). Additionally, the OMPG assumes no natural selection is occurring in either population (i.e., only drift and gene flow are in operation).

Swamping the translocated fish with a high number of fish from the main LCR population each generation may be an alternative to using the OMPG. 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-1 fish) once a fish generation. This approach is suggested by P. Hedrick (ASU, pers. com.), who believes that the OMPG is inappropriate in this instance and that the numbers should be higher.

Some effort should be made to monitor for potential inbreeding (loss of heterozygosity in either the translocated population or the main LCR population). Since the processes of inbreeding takes generations, it should be possible to monitor for these changes, should they occur. However, tissues must be taken to genetically analyze the fish and a funding mechanism identified to analyze samples.

It will be difficult (if not impossible) to accurately measure downstream levels of movement from the resulting offspring above Chute Falls. 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 will likely 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 batch marked or tagged) during flood events, which is also when drift nets become problematic (e.g., currents become too strong and nets very quickly fill up with debris; D. Van Haverbeke, pers. obs.). However, if genetic samples are obtained, it may be possible to detect changes in the populations.

Summary

In summary, 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. Future efforts should be accompanied by long-term commitments to manage and monitor for genetic uncertainties. Finally, the translocation effort may have other unexpected ecological consequences, such as a potential founder population explosion, or changes in the upstream food community. The Biological Opinion and accompanied re-initiation called for the translocation of 600 fish above Chute Falls. Managers are proposing translocating more fish in the future, and will do so in consultation with USFWS. This effort will require long-term commitment and funding, and the potential risks and benefits should be thoroughly considered.

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 less risk to the main population of humpback chub than the translocation effort above Chute Falls. This is because 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 of several hundred 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 (recall LCR above Chute Falls is estimated to carry 312 fish ≥ 200 mm). 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. Given that Havasu Creek, above its barrier, may be able to sustain more adults than the Chute Falls translocation, this may be an alternative that managers might wish to consider.

Three places that were largely discounted by Valdez et al. (2000) as being viable options were Bright Angel, Havasu, and Shinumo creeks (the later two below their waterfall barriers). Bright Angel Creek was discounted because of large numbers of predators (i.e., brown and rainbow trout), and Shinumo and Havasu creeks (below their barriers) were discounted because of access to only 100 to 200 m of stream.

However, it may be worthwhile to revisit these options in view of recent attempts to remove predators in Grand Canyon. 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 (e.g., between Grapevine and Horn Creek rapids [RM 81.7- 90.2]), this might reduce predators in the mainstem sufficiently 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 removal 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 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 to remove exotics, 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. Large humpback chub were occasionally captured between Havasu Creek and Last Chance Camp in the mainstem during the early 1990s (Valdez and Ryel 1995), and two large ripe males were captured in 2004 a short distance above the mouth of Havasu (L. Johnstone, SWCA, pers. com.).

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 creeks 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 the spectacular photograph taken on the Rust expedition (Figure 1). 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 in these mainstem reaches suggests some affinity to these tributaries. The decline in catch rates of these fish in the past decade also suggests that lack of recruitment from these tributaries may be 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 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 4).

Establishing a captive broodstock of humpback chub followed by supportive stocking should be viewed as a last recovery option. This is based on legal and biological considerations. Legal considerations stem from USFWS and NOAA policy that captive broodstock and supportive stocking activities need to be included as recovery strategies in approved recovery plans. 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 potential for: 1) inbreeding occurring within the captive population, 2) reducing the N_e of the wild population, and 3) impacting the wild population by input from fish that have become genetically domesticated in a hatchery. Additional problems associated with behavior of captive bred fish are largely linked to 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 actions regarding genetic matters (Table 4). Supportive stocking from a captive population should not be considered until all other management activities have been attempted and shown to fail.

However, at this point, working toward development of a captive broodstock may foster completion of preliminary actions (e.g., genetics work, captive broodstock management plans, reintroduction 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 if other recovery measures are failing. Meanwhile, 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 a minimum (i.e., reduce artificial selection). The primary problems appear 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 likely require the removal of ~960 to 1,400 age-0 fish from the LCR annually to arrest decline and maintain levels of age-4+ humpback chub at the abundance level estimated in 2000. It may require the removal of ~4,300 to 6,400 age-0 humpback chub from the LCR to achieve a positive population response to return the abundance of age-4+ fish to the level estimated in 1990. These numbers may need to be roughly doubled if post-release mortalities are high. Both scenarios will require a long term commitment (i.e., it may take a decade or more to realize a change in the wild population). The action does, however, address what is thought to be the primary factor for population decline in Grand Canyon; that is lack of recruitment.

Furthermore, a supplemental stocking program using wild caught age-0 fish will need to be adaptive in nature. 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.). Release locations for the fish will require an adaptive management approach. For example, releasing too many fish into the LCR may 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. Some level of continued monitoring of the annual age-0 and age-1 cohorts will need to be maintained in the LCR to ensure that this activity does not result in significantly cropping wild recruitment.

Continuing the 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 demographic boost, however, this long term gain might be expected to be < 300 individuals ≥ 200 mm. There is also potential to expand the range of the species (~5 km), however, this expansion should not be expected to provide any substantial security from catastrophic loss. However, unlike other alternatives, this action does have the potential to further promote a self-sustaining population. But, the action could potentially be accompanied by some genetic risks to the wild population, primarily because it has potential to establish a small reproductively isolated group of fish within proximity to the main LCR population. Specifically, 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 being undertaken, this action could result in long-term uncertainties about the genetic impact to the wild population. Finally, the action may have other unexpected and unpredictable ecological consequences related to the food base and carrying capacity of the LCR.

Carrying out a program for the translocation of humpback chub into other tributaries in Grand Canyon should not pose genetic risks 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 wild 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 in the mainstem. 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). 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 appears 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 population 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 keep in mind that habitat destruction is generally the primary cause for the decline of most endangered species. Reasonable and prudent elements to help ameliorate this in Grand Canyon primarily included modifications in flow and temperature regimes. Each potential management action discussed in this document involves unique potentials for demographic boost or for enhancing recruitment for the humpback chub. However, 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 habitat and natural recruitment will do much to benefit the humpback chub, and will do much to achieve eventual downlisting and delisting of the species.

Table 4. 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 stocking	X	X	X	X	X	Potentially large	Potential to increase densities in mainstem
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.

Acknowledgements

We are appreciative of discussions and communications held with Lew Coggins, Marlis Douglas, Chester Fiegel, Connie Keeler-Foster, Paul Marsh, Bill Persons, Nils Ryman, Pam Sponholtz, Dennis Stone, Manuel Ulibarri, and Carl Walters. We are especially appreciative for the initial discussion and ideas from C.O. Minckley concerning grow out and release of age-0 humpback chub. We are grateful of review comments provided by Tom Czapla, Jennifer Fowler-Propst, Steve Gloss, Phil Hedrick, Craig Paukert and Bill Persons. This report was funded by U.S. Geologic Survey, Grand Canyon Monitoring and Research Center, under Interagency Acquisition No. 01-3022-R1009.

Literature Cited

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

Allendorf, F.W. and N. Ryman. 1987. Genetic management of hatchery stocks. Pp. 141-159 *In*: N. Ryman and F. Utter (eds.). Population genetics and fishery management . University of Washington Press, Seattle, Washington.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Flagg, T.A., F.W. Waknitz, D. J. Maynard, G.B. Milner, and C.V.W. Mahnken. 1995. The effect of hatcheries on native Coho salmon populations in the Lower Columbia River. American Fisheries Society Symposium 15: 366-375.

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

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

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

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

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

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

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

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

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

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

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

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

Grimm, N.B. and S.G. Fisher. 1989. Stability of periphyton and macroinvertebrates to disturbance by flash floods in desert streams. Journal of the North American Benthological Society 8: 293-307.

Haden, A., D.W. Blinn and J.P. Shannon. 1999. Food base studies and stable isotope analysis of the diet of humpback chub (*Gila cypha*) in the Little Colorado River, Coconino County, AZ: Final Report submitted to U.S. Fish and Wildlife Service, Flagstaff.

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

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

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

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

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

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

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

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

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

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

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

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

Kaeding, L. R. and M. A. Zimmerman. 1983. Life history and ecology of the humpback chub in the Little Colorado and Colorado Rivers in Grand Canyon. Transactions of the American Fisheries Society 112:577-594.

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

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

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

Kolb, E. and E. Kolb. 1914. Experiences in the Grand Canyon. National Geographic Magazine XXVI (2): 99-184.

Kubly, D.M. and G.A. Cole. 1979. The chemistry of the Colorado River and its tributaries in Marble and Grand Canyons. In: Proceedings of the first annual Conference on Scientific research in the National Parks. U.S. National Park Service Transactions and Proceedings. Series 5: 565-572.

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

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

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

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

Lande, R. and G.F. Barrowclough. 1987. Effective population size: genetic variation, and their use in population management. pp. 87--123. *In*: M.E. Soulé (ed.) Viable Populations for Conservation, Cambridge University Press, Cambridge, UK.

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

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

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

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

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

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

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

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

McNeil, W. 1991. Expansion of cultured Pacific salmon into marine ecosystems. Aquaculture 98: 173-183.

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

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

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

Miller, R.R. 1959. Origin and affinities of the freshwater fish fauna of western North America. pp. 187-222. *In*: C.L. Hubbs (ed.) Zoogeography. American Assoc. for the Advancement of Sci., Publ. 51. Washington DC.

Mills, L.S. and F.W. Allendorf. 1996. The One-Migrant-per-Generation Rule in conservation and management. Conservation Biology 10: 1509-1518.

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

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

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

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

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

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

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

Newcombe, C.P. and D.D. McDonald. 1991. Effects of suspended sediment on aquatic systems. North American Journal of Fisheries Management 11: 72-82.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Soulé, M.E. 1980. Thresholds for survival: maintaining fitness and evolutionary potential. pp. 151-169. *In*: M.E. Soulé and B.A. Wilcox (eds.) Conservation Biology:

An evolutionary-ecological approach, Sinauer Associates, Sunderland, Massachusetts.

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

Stockwell, C.A., M. Mulvey, and G.L. Vinyaard. 1996. Translocations and preservation of allelic diversity. Conservation Biology 10:1133-1141.

Stone, D. and P. Sponholtz. 2003. Translocation of young-of-year humpback chub above Chute Falls in the Little Colorado River, AZ: 2003 Interim Report. Prepared for Grand Canyon Monitoring and Research Center, Flagstaff, AZ. U.S. Fish and Wildlife Service, Arizona Fishery Resources Office-Flagstaff. AZFRO Document # USFWS-AZFRO-FL-04-006. 20 pp.

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

Strand, J.G. 1998. An analysis of the Homol'ovi fauna with emphasis on ritual behavior. Dissertation submitted to the Department of Anthropology, University of Arizona, Tucson. 540 pp.

Subonski, M.D. and J.J. Templeton. 1989. Life skills training for hatchery fish: social learning and survival. Fisheries Research (Amsterdam) 7:343-352.

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

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

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

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

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

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

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

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

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

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

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

Valdez, R.A., S.W. Carothers, M.E. Douglas, M. Douglas, R.J. Ryel, K.Bestgen, and D.L. Wagner. 2000. Final research and implementation plan for establishing a second population of humpback chub in Grand Canyon. Grand Canyon Monitoring and Research Center, U.S. Department of the Interior, Flagstaff. 56 pp.

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

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

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

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

Van Haverbeke, D.R. 2003. Stock assessment and fisheries monitoring in the Little Colorado River within Grand Canyon during 2002. Final Report submitted to the Grand Canyon Monitoring and Research Center, Flagstaff, AZ from USFWS, Arizona Fishery Resources Office-Flagstaff, AZ. AZFRO Document No. USFWS-AZFRO-FL-03-002. 87 pp.

Van Haverbeke, D.R. 2004. Stock assessment and fisheries monitoring activities in the Little Colorado River within Grand Canyon during 2003. Annual Report submitted to the Grand Canyon Monitoring and Research Center, Flagstaff, AZ from USFWS, Arizona Fishery Resources Office-Flagstaff, AZ. AZFRO Document No. USFWS-AZFRO-FL-04-007. 84 pp.

Van Haverbeke, D.R. and L.G. Coggins, Jr. 2003. Stock assessment and fisheries monitoring activities in the Little Colorado River within Grand Canyon during 2001. Final Report submitted to the Grand Canyon Monitoring and Research Center, Flagstaff, AZ from USFWS, Arizona Fishery Resources Office-Flagstaff, AZ. AZFRO Document No. USFWS-AZFRO-FL-02-002. 87 pp.

Vannote, R.L., G.W. Minshall, K. Cummins, J.R. Sedell, and C.E. Cushing. 1980. The River Continuum Concept.Canadian Journal of Aquatic Science 37: 130-137.

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

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

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

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

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

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

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

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

Appendix 1 – Assess suitability of humpback chub currently at Willow Beach NFH as broodstock

Draft Document

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 humpback chub 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.

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 80 humpback chub currently held at Willow Beach National Fish Hatchery (NFH). These fish were collected from a 2 km section of the Little Colorado River (LCR) in the Salt Camp Area in July 1998. A total of approximately 450 age-0 fish were removed and transported to Willow Beach NFH. These fish have been the subject of various experiments (primarily temperature related), and approximately 80 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.

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.

Study area: Willow Beach NFH.

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.

Task Description and Schedule:

1. Collect genetics samples from humpback chub at Willow Beach NFH, 2004.

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).
3. Process all samples, June/July 2005.
4. Analyze data and write report, Aug/Sep 2005.

References:

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

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

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