A Genetic Management Plan for Captive and Translocated Endangered Humpback Chub in the Lower Colorado River Basin

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Contents

Executive Summary

Recovery planning for endangered humpback chub (*Gila cypha*) includes the potential for captive holding and assurance population development (U.S. Fish and Wildlife Service (USFWS) 1990, 2002). This plan, which follows requirements of the U.S. Fish and Wildlife Service Policy Regarding Controlled Propagation of Species Listed Under the Endangered Species Act (Propagation Policy, USFWS 2000), defines management actions and tasks necessary for the development of captive assurance populations, as well as for their use in a captive breeding and stocking program should the need arise. Since translocations of humpback chub to tributaries other than the Little Colorado River are being undertaken to establish *in situ* refuge populations within Grand Canyon National Park, recommendations to help guide these actions are also covered in this plan. Risk factors associated with these actions are addressed based on the most current scientific information regarding humpback chub in the lower Colorado River basin, specifically in the Little Colorado River and the Colorado River in the Grand Canyon area (the Lower Colorado River Basin Recovery Unit, USFWS 2002). The purpose of establishing assurance populations is to develop and maintain representative captive stocks to ensure the retention of the genetic diversity present in the lower Colorado River Basin in the event of a catastrophic decline in the wild population. Translocations are intended to expand the demographic range of humpback chub in Grand Canyon and to provide warmer, lower predator rearing areas in tributaries. To accomplish that goal, we provide a strategy to maintain gene flow from the wild population to the captive and translocated stocks to counter the effects of evolutionary processes and stochastic genetic changes known to occur in fish populations. As discussed in the Recovery Plan (USFWS 1990), there may be a need to use an assurance population as broodstock to develop a propagation program. This would provide animals for restoration purposes in river reaches that no longer contain humpback chub, and/or to augment stocks that have become so critically imperiled that no other option is available to prevent the extirpation of the population. We provide guidance in this plan under the assumption that no stocking or augmentation will rely on

the captive stock until the Recovery Team and Regional Directorates have determined a need for a propagation and stocking program. With that caveat, we provide management strategies to mitigate genetic risks associated with the development and maintenance of captive stocks to ensure the retention of extant genetic diversity in the lower Colorado River Basin, and the use of assurance population stocks for propagation of humpback chub for restoration and recovery efforts in a captive breeding and stocking program. Genetic risks that are addressed, as per the Propagation Policy (USFWS 2000), include: broodstock mining**,** inbreeding and inbreeding depression, population homogenization, domestication selection, hybridization, and outbreeding.

In addition, strategies to mitigate known risk factors associated with captive assurance populations and propagation are addressed. These risks include genetic drift, founder effect, and the Ryman-Laikre effect. Other issues associated with the maintenance of captive stocks, such as disease transmission, disposition of surplus animals, potential for escapement, record keeping, and stock redundancy are also discussed. Guidance provided to minimize risks include the development of a genetic management and captive propagation committee to identify and mitigate new issues should they arise in the program, evaluate research proposals, determine fate of incidental spawns and older broodstock, and provide support and recommendations to the Regional Directorate regarding humpback chub assurance population maintenance and captive propagation.

We provide a strategy that considers 1) the maintenance of genetic diversity of fish in the wild, 2) the establishment of two assurance populations to maintain a total species census (captive and wild) on the order of 5,000 individuals to offset the accumulation of deleterious mutations, and 3) ensure population cohesion via gene flow. A population of 5,000 fish is not a “recovery” objective, but rather a census target based on two genetic considerations: mutational meltdown theory and the genetic effective population size. We identify risks in common with captive, translocated and wild populations of humpback chub, and then provide guidance to mitigate common risks and address hazards specific to each population type. We recommend the use of software developed by the zoological community to maintain records and facilitate future genetic management. We also identify a course of action for the development of

assurance populations, translocations and monitoring for facilities engaged in humpback chub cultivation.

Introduction

The discipline of population genetics compares a theoretically ideal population to a real population (Diniz-Filho et al. 2008). This comparison allows managers to focus on the specific evolutionary pressures causing the population to deviate from the 'norm.' When the organism in question is an imperiled species additional constraints must be considered, and managers enter the realm of conservation genetics (Diniz-Filho et al. 2008). Currens and Busack (1995) define genetic risk assessment as "ecological risk assessment that emphasizes the systematic identification and characterization of vulnerability to losses of genetic diversity." They further define types of genetic loss as: (1) extinction, (2) loss of within-population genetic variability, (3) loss of between-population genetic variability, and (4) domestication or the loss of fitness in the wild fish propagated in an artificial environment or their offspring (Currens and Busack 1995). Populations often face the same consequences associated with being small whether the population occurs naturally (endemism) or is a captive stock such as a hatchery or zoo population. Restored or translocated populations are also faced with the consequences of a limited population size.

This plan contains a series of management actions and tasks to mitigate hazards inherent in the process of genetic management of an imperiled species. It is important to realize that we provide a template for the genetic management of humpback chub (*Gila cypha*) and that because population management is a dynamic process, our recommendations will not cover all situations, or new risks and hazards. Adaptive management strategies should be based on these guidelines, but current conditions in the Colorado River and Little Colorado River, as well as ongoing monitoring of humpback chub populations, will dictate the specific actions required to meet the targets contained within this plan.

Recovery planning for humpback chub included the potential for a captive component as early as 1990 when the second revision of the Recovery Plan

was approved (USFWS 1990). The Recovery Plan provided guidance to establish and maintain assurance populations, and to "assess potential reintroduction or augmentation sites and implement stocking when deemed necessary and feasible (USFWS 1990)." The Glen Canyon Dam Adaptive Management Program (GCDAMP) in 2004 identified the need to provide genetic guidance for any captive component for humpback chub and identified a genetic management plan as a necessary precursor to guide the establishment of captive assurance populations, translocation efforts, and stocking from captive breeding stocks. The opportune time to initiate a captive assurance population program is prior to a catastrophic event or precipitous decline in an imperiled population. This allows managers to conserve the genetic resources available in a healthy, genetically viable population such as currently exists for humpback chub in the lower Colorado River Basin. This plan provides needed guidance in this regard, and is consistent with the USFWS guidelines established in the Propagation Policy (USFWS 2000).

As discussed in the Recovery Plan (USFWS 1990) and in the GCDAMP's Draft Comprehensive Plan for the Management and Conservation of Humpback Chub (*Gila cypha*) in the Lower Colorado River Basin (2009), there may be a future need to use an assurance population as a broodstock to provide animals for restoration purposes in river reaches that no longer contain humpback chub and/or to augment stocks that have become so critically imperiled that no other option is available to prevent the extirpation of the population. The Propagation Policy (Federal Register 2000) provides the following guidance to determine when that action is necessary:

> "Our policy is that the controlled propagation of threatened and endangered species 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. All reasonable effort should be made to accomplish conservation measures that enable a listed species to recover in the wild, with or without intervention,

prior to implementing controlled propagation for reintroduction or supplementation."

The guidance provided here assumes that no stocking or augmentation will rely on the captive stock until the Regional Directorate have indicated the need for propagation and stocking must proceed to prevent extinction of resident wild populations. With that caveat, we address two distinct foci: the creation and maintenance of assurance population stocks to ensure the retention of extant genetic diversity as represented by the recent genetic baseline of humpback chub in the lower Colorado River basin; and the use of such assurance populations for propagation of humpback chub for restoration into historical waters. An additional caveat to this discussion is our use of neutral markers (Milligan et al. 1994). Rarely do managers have information regarding genes related to fitness of a given species for use in conservation genetic management. Instead, neutral genetic markers are relied upon to provide insight into subtle distinctions within and between populations. These markers are commonly used as a surrogate for fitness related characters (Milligan et al. 1994) and provide a random statistical sample of the genome. This plan employs neutral genetic markers to assess genetic risks. Genetic risks that must be addressed, as per the Propagation Policy (USFWS 2000), include:

Broodstock Mining. Removal of natural parental (adult) stock that may result in an increased risk of extinction by reducing the abundance of wild individuals and reducing genetic variability within naturally occurring populations (USFWS 2000).

Inbreeding and inbreeding depression. Adverse genetic effects within populations that may decrease the utility and long term viability of the population (USFWS 2000).

Population Homogenization. Potential erosion of genetic differences between populations as a result of mixed stock transfers or supplementation (USFWS 2000).

Domestication Selection. Exposure to novel selection regimes in controlled environments that may diminish a listed species' natural capacity to survive and reproduce in the wild (USFWS 2000).

Hybridization and Outbreeding. Genetic introgression, which may diminish local adaptations of the naturally occurring population (USFWS 2000) in the first filial (F1) generation and lead to hybrid breakdown in the genetic shuffle of the second (F2) generation.

Other risk factors include those associated with captive assurance populations and propagation. These include **founder effect**, which occurs when a new population is started from a few individuals and by chance the genetic constituency does not reflect the normal distribution of alleles in the donor population. Founder effects in many populations have been implicated for high prevalence of certain diseases in small or isolated populations, including multiple sclerosis (Marrosu et al. 2002) and Tay-Sachs (Chase and McKusick 1972) in human populations. A related phenomenon, **genetic drift**, is a process that affects small populations by changes in genetic diversity as some alleles become lost to the population and others become more common, not as a result of evolutionary selective pressures, but solely by chance (Wright 1931; Wright 1932).

The **Ryman-Laikre effect** (Ryman and Laikre 1991; Laikre and Ryman 1996) is an anthropogenic impact on the genetics of a wild population as a result of augmentation stocking. Census numbers do not necessarily correlate with the **genetic effective population size**, which can be described in terms of the number of individuals that successfully pass on their genetic material. Swamping of a wild genome with large numbers of genes from just a few individuals can have a severe impact on the genetic diversity of the wild stock. The process became known as the Ryman–Laikre effect, defined as the reduction in the effective population size caused by augmentation stocking. Eldridge and Killebrew (2008) suggest "the possibility of inflicting genetic damage to a population through supplementation is greatest for organisms that are capable of producing large numbers of offspring under captive conditions" as with many

cultivated fish species. The Ryman-Laikre effect is the primary reason this plan discourages the supplementation of existing humpback chub populations with fish produced through captive propagation.

Additional issues to be discussed include the potential for disease transmission, translocated populations, NATURES rearing, disposition of surplus animals, record keeping, and stock redundancy. Other documents provide extensive information on demographics, food habits, and life history of humpback chub and this plan defers to those sources. Those documents include:

> U. S. Fish and Wildlife Service. 1990. Humpback chub recovery plan. U.S. Fish and Wildlife Service, Denver, Colorado. 43 pp.
>
> 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.

Translocation has become an important tool in the conservation of imperiled fish species, but one with genetic risks which must be addressed (Minckley 1995). Historically, Minckley and Brooks (1985) documented no translocations of humpback chub with the exception of a few fish taken to Willow Beach National Fish Hatchery in the early 1980s for research. More recently, translocations of humpback chub have included moving individuals from the downstream portion of the Little Colorado River upstream to reaches above a natural barrier, Chute Falls, thought to be unoccupied by humpback chub (Stone 2009), and translocation of humpback chub from near the mouth of the Little Colorado River to Shinumo Creek in Grand Canyon in 2009 and 2010. This plan provides guidance and suggests protocols to enhance translocation efforts, and to minimize any potential negative genetic consequences of translocation activities.

Finally, it should be noted that there is a plethora of theoretical and empirical conservation genetic literature addressing domestication or artificial selection and the perils and pitfalls of captive propagation and subsequent release of fish. The vast majority of the work accomplished to date relative to domestication of captive stocks has occurred in coldwater fish culture in the Pacific Northwest. Those facilities rear

salmonids at high densities in concrete raceways. This plan addresses those concerns and to the extent possible, outlines methods to mitigate some of the negative impacts associated with the type of culture activities necessary with facilities using raceway propagation. We recommend to the extent practical, managers of captive stocks implement NATURES rearing practices (Maynard et al. 1995). Maynard et al. (1995) provides a complete discussion of this subject at http://pisces.bpa.gov/release/documents/documentviewer.aspx?doc=20651-1. Essentially, this is an approach to rearing fish in captivity that strives to mimic the natural environment to raise fish that retain wild characteristics by providing raceways with cover, structure, and natural substrates, and feeding methods to simulate natural foraging behavior. The technique also suggests predator conditioning and providing live foods to improve foraging ability. Maynard et al. (1995) assumptions "are that NATURES will: (1) promote the development of natural cryptic coloration and antipredator behavior, (2) increase post-release foraging efficiency, (3) improve fish health and condition by alleviating chronic, artificial rearing habitat-induced stress and, (4) reduce potential genetic selection pressures induced by the conventional salmon culture environment."

In addition, there is much research and literature from the zoo and aquarium community related to the captive propagation and maintenance of endangered species, including gene pool preservation, inbreeding, and avoidance of mutational load. This plan introduces some of the concepts and methods used worldwide by the Association of Zoos and Aquariums (AZA) for the conservation of imperiled animals in captivity. The Service advocates managers follow the criteria and protocols for captive propagation currently in use in the zoological community as stated in the Propagation Policy:

> "Controlled propagation protocols will follow accepted standards such as those employed by the American Zoo and Aquarium Association, the Center for Plant Conservation, and Federal agency protocols such as fish management guidelines to the extent practical (USFWS 2000)."

Genetic Status of Humpback Chub in the Little Colorado River and Grand Canyon

The lower Colorado River Basin contains the largest remnant population of humpback chub, and the most stable. Genetic assessments of humpback chub in the Little Colorado River and Grand Canyon have been performed twice in the last decade. Douglas and Douglas (2007) examined 77 fish from the Little Colorado River as part of a comprehensive range-wide analysis of humpback chub and roundtail chub (*G. robusta*). Dexter National Fish Hatchery and Technology Center has also completed a genetic analysis of over 300 humpback chub from the Little Colorado River, including fish at Willow Beach National Fish Hatchery that were collected from the Little Colorado River in 1999. Both analyses suggest the populations in the Little Colorado River and the Grand Canyon mainstem area can be treated as a single management entity, referred to in this document as the Little Colorado River aggregate (this is also the Lower Colorado River Basin Recovery Unit). Results of both efforts are considered in the management scenarios provided in this plan.

Summary of Genetic Risks and Implications for Captive Stocks

Founder effect

"The fundamental genetic hazard associated with broodstock management within a gene pool maintenance program is loss or undesired changes in the genetic variation or identity of the hatchery population with respect to its donor source" (Williamson 2001). One of the earliest recognized genetic risks of captive rearing of native fish was the potential founder effect in the hatchery population. A founder effect, as defined previously, occurs when too few individuals from a donor population are the source for a new population, which results in a population that differs from the donor population.

Genetic drift

Genetic drift produces genetic changes in a population associated with random events. Loss of within population diversity due to the effects of genetic drift is typically associated with small population size (Lande 1995). Genetic drift is common in marginal fragmented habitat where populations are small and conditions suboptimal (Rieseberg et. al 2003). Under these circumstances a few individuals may contribute to the next generation, not as a result of Darwinian selection, but by chance. The genetic changes associated with small populations over time result in the loss of alleles, fixation of other alleles, and a pervasive shift in the genetic makeup of the population such that the new population randomly 'drifts' away from the source population. The subsequent loss of diversity poses a risk for the population because genetic diversity, (i.e., heterozygosity and polymorphisms), theoretically allow an organism and a population to respond to environmental changes by having the ability to express alternate forms of the same gene.

Mitigating this loss of genetic diversity requires sufficient numbers of founding individuals. To overcome the combined impacts of drift and founder effects, it is important to maintain as many individuals both in the wild and the captive population as practical. Numbers that have been used in past decades suggest a minimum of 50 and an optimum of 500 individuals (Franklin 1980). However recent research suggests that an order of magnitude greater (5,000) may be required to offset genetic risks associated with the accumulation of deleterious mutations in small populations (Lande 1995; Lynch and O'Hely 2001; Reed et al. 2003).

Inbreeding and inbreeding depression

Mating of related individuals can lead to altered genetic structure in small populations. Inbreeding does not necessarily lead to a reduction in allelic diversity, but to the partitioning of alleles into homozygotes at the expense of heterozygotes. Mating between relatives is common in nature (Hudson et al. 1990), but becomes a problem when it results in inbreeding depression (Waite et al. 2005). Inbreeding depression is a reduction in fitness associated with the exposure of deleterious alleles in the

homozygous condition (Lande 1995; Lynch et al. 1995). However, the result of ongoing inbreeding depression is a purging of deleterious alleles and, over time, an increase in population fitness (Lynch et al. 1999; Kirkpatrick and Jarne 2000). A drastic population reduction can result in a decrease in inbreeding depression (homozygotes for deleterious alleles drop from the population), but a bottleneck in a population does cause an increase in the genetic load (accumulation of slightly deleterious mutations) (Kirkpatrick and Jarne 2000). Nevertheless, Garcia-Dorado (2003) suggests captive programs should recognize the immediate genetic threats of inbreeding, loss of diversity, and inadvertent selection as the most critical factors, with mutational load a long term management issue. Given those caveats, it is important to note that in endangered species management, the time and population stability required to expunge deleterious mutations is usually not available (Kephart 2004), and strategies to minimize inbreeding in the captive population should be deployed.

Outbreeding and outbreeding depression

Outbreeding is the sexual combination of divergent genomes. Extreme outbreeding results in developmental instability and is often associated with sterility, particularly in the heterogametic sex (Haldane 1922). Outbreeding depression occurs when offspring have reduced fitness as a result of the combination of diverse genomes. Outbreeding is synonymous with introgression and hybridization as discussed in the Propagation Policy (USFWS 2000). Outbreeding depression can occur in the first generation by affecting the adaptation to fine scale environmental conditions. The subsequent loss of local adaptation results in a decrease in the overall fitness of the population (Edmands 2007; Lynch et al. 1999; Lynch 1991). Outbreeding depression as a result of hybrid breakdown occurs in the second or later generations when recombination produces a montage of maladaptive progeny (Edmands 2007; Burton 1990).

Humpback from the lower Colorado River Basin appear to be genetically similar, and analysis indicates a high numbers of migrants between reaches within the Little Colorado River and the mainstem Colorado River in the Grand Canyon (Douglas and

Douglas 2007; Keeler-Foster et al. 2009). While these studies indicate little difference between stocks of humpback chub in the lower Colorado River Basin, differences between fish from the upper basin (the Green River) and the lower basin suggest anthropogenic movement between those populations should be avoided (Douglas and Douglas 2007; Keeler-Foster et al. 2009).

Domestication and artificial selection in captive populations

The genetic makeup of captive populations can be altered in a captive stock when the population adapts to the hatchery environment, a process called inadvertent or domestication selection (Doyle et al. 1995; Tufto 2001). This can result from differential survival of wild fish brought into the hatchery or from differential survival of progeny in the hatchery. Domestication is thought to result in genetic changes that impact the fitness of a wild population that include a high proportion of captively reared fish (Lynch and O'Hely 2001; Ford 2002). Domestication in captive stocks can occur because of culture practices that favor some genetic backgrounds, allowing genotypes to persist that thrive in captivity, but may perform poorly in the wild (Hard 1995; Lynch and O'Hely 2001; Tufto 2001). Domestication selection is viewed as a problem when propagated fish are less adapted to the natural environment than wild fish and, subsequent to release, inundate wild genomes with genetic backgrounds adapted to the hatchery environment (Lynch and O'Hely 2001; Tufto 2001; Ford 2002).

Augmentation

Perhaps the most important consideration when the decision has been made to augment a wild population with captively-reared animals is to avoid 'swamping' the resident gene pool with the progeny of a few captive fish (Ryman and Laikre 1991; Laikre and Ryman 1996). Captive propagation to augment wild stocks is typically undertaken to assist in species recovery by relaxing the selective pressure placed on early life history stages. This serves to increase the survival of progeny and subsequently increase the census numbers of a wild population.

Discussion of Genetic Risks

Lynch (1991) suggests that the difference between outbreeding depression and inbreeding depression can be viewed as interactions within loci (inbreeding) and interactions between loci (outbreeding). Independent genetic evaluations of humpback chub indicate the Little Colorado River and the Grand Canyon contain one population (Keeler-Foster et al. 2009; Douglas and Douglas 2007). Tufto (2001), Lacy (1987), and Kirkpatrick and Jarne (2000) suggest that while mutation is a risk that should be considered in conservation programs, inbreeding, loss of genetic diversity and adaptation to the captive environment are of greater concern. Kirkpatrick and Jarne (2000) address the impact of the small numbers of individuals used in the conservation of many critically endangered species. Their calculations indicate that deleterious mutations are a factor in conservation programs, but, while a bottleneck of 10 individuals produces an immediate increase in the mutation load, over time the result is a greater purging of deleterious mutations than would occur in a larger stable population (Kirkpatrick and Jarne 2000). Lynch and O'Hely (2001) surmise that the long-term impact of the relaxation of selection experienced in captive populations results in an increase in mutational load that accumulates over time, exacerbating the potential for extinction. However, Lacy (1987) and Garcia-Dorado (2003) suggest that other factors impact managed populations more imminently. Lacy (1987) used simulation modeling to predict the impact of small population size on captive programs. He found:

> "Genetic drift was the overriding factor controlling the loss of genetic variation. Mutation had no noticeable effect on populations of the size typically managed in zoos and nature preserves. Immigration from a large source population can strikingly slow, halt, or even reverse the loss of genetic variation, even with only one or a few migrants per generation. Unless selection is stronger than commonly observed in natural populations, it is inefficient in countering drift when population sizes are on the order of 100 or fewer. Subdivided populations rapidly lose variability from within each population but retain variation across subpopulations better than does a panmictic population."

Another factor that may impact the debate of mutational load and captive propagation is the role of sensitive versus tolerant genomes (Garcia-Dorado 2003). The concept of mutational load and subsequent mutational meltdown is based on (a) theory or (b) empirical findings based on studies of *Drosophila* or similar laboratory animals. Species such as Florida panthers or cheetahs are often invoked as examples, but to this author's knowledge, no empirical evidence exists for fish. Garcia-Dorado's (2003) review is based on both a and b, but with slightly different models, very different outcomes emerge. Summarizing his results:

> "Under the tolerant model, fitness decline due to deleterious fixation is generally low, indicating conservation programs should give priority to the avoidance of inbreeding, loss of genetic variability and adaptation to captive conditions, even if this reduces the strength of selection against new mutations."

Also, his analyses suggest:

> "Thus, weak stabilizing selection on the morphological trait(s) would cause tiny deleterious effects for these mutations. However, these tiny deleterious mutations can become advantageous if an environmental change moves the trait's optimum away from the population average."

Heterozygosity constitutes a genetic load, as populations of organisms that are adapted to an environment would be homozygous at many loci related to fitness and adaptation. However, the same population would not be adapted to change, the essence of the requisite of conserving genetic diversity. Willis (2001) suggests that closely estimating the relatedness of captive animals is the best management strategy for the maintenance of genetic diversity in captive stocks and outlines the risks associated with over or underestimating familial relationships. This plan relies on genetic analysis to reconstruct relatedness estimates for individual fish and recommends development of a studbook for recordkeeping. Pedigree information will be maintained from that point onward to minimize the mating of related individuals.

Garcia-Dorado (2003) recommends several steps to enhance fitness in conservation programs:

- “avoiding quick inbreeding, as well as preserving genetic variability, requires the maintenance of a large effective population size."
- “in captive endangered population management, a recommended genetic management for breeding is to minimize kinship,”
- “fragmenting populations has also been recommended both to avoid loss of genetic variability and to prevent adaptation to captive conditions...”

These recommendations are consistent with the program outlined in this plan, but the concept of mutational load should still be considered as a long-term risk for the program.

Current genetic theory suggests that a population of over 5,000 adults might be necessary to minimize the impact of deleterious mutation accumulation on the 100-year survivorship of the population (Lynch and O’Hely 2001). Targeting a larger census number for maintenance of genetic reservoirs for future adaptability is supported when we include the consideration of the genetic effective population size (N_e). Theoretical and empirical research suggests the actual number of genetic contributors to a wild population range from 0.5 (Nunney 1991) to 0.11 (Frankham 1995) of the census population. Our conservative value taken from the compilation of empirical estimates was 0.14 (Palstra and Ruzzante 2008). The recovery goals target 2,100 adults in the river, which equates to an N_e of between 105 and 1,050. This document targets 5,000 adults, including the proposed captive assurance populations and wild stocks, as an attainable criteria to meet the minimum number of animals suggested for a long-term genetically viable population (Lynch and O’Hely 2001). N_e approximations suggest that target equates to an N_e of 700 (Palstra and Ruzzante 2008). However, it should be noted that the numbers suggested are a theoretical abstract and actual numbers may be lower or higher.

The establishment of captive assurance populations are undertaken to serve as reservoirs for genetic resources, and for providing a means to continue gene flow from

wild to hatchery stocks to protect that reserve. This gene flow is unidirectional, but provides a mechanism to ensure that captive stocks continue to reflect the donor population. Current estimates of humpback chub in the Little Colorado River are near 6,000 adults, however, previous numbers remained steady at around 4,000 for several years. Maintaining 2,000 adults in captive assurance populations with active genetic monitoring and maintenance should provide a buffer from the accumulation of deleterious mutations, as well as maintaining genetic diversity currently available in the Little Colorado River. This plan should be reviewed by the genetic management and captive propagation committee and updated on a biennial basis to incorporate new technologies and theoretical approaches for retaining and optimizing genetic diversity.

Recommendations

- A genetic management and captive propagation committee should be developed to identify and mitigate new issues should they arise in the program, evaluate research proposals, determine fate of incidental spawns, and provide support and recommendations to the Regional Directorate regarding humpback chub assurance population maintenance and captive propagation. Incidental or unplanned spawns should be targeted for research or disposed of rather than committing space to rearing fish with no future as broodstock.
- A second assurance population should be developed.
- Young-of-year should be collected and 200+ individuals provided annually to each assurance population on alternate years for 10 years.
- Incidental spawns from captive stocks should be collected annually and used for purposes to be determined by the propagation committee.
- Any fish stocked, translocated, or used in a captive program should be PIT-tagged and genotyped.
- An active cryopreservation program should be developed to harvest the genetic reserves of the Little Colorado River. Use of cryopreserved sperm would only be initiated if numbers of humpback chub decline below 100 fish in the Little Colorado River and Grand Canyon.

- At most one facility using raceway culture should maintain a captive stock; the second assurance population should use netted outdoor ponds for rearing. Preferably both stocks would be reared in pond culture.
- Comparisons of genetic changes and allele loss should be ongoing for the duration of the captive program.
- All facilities involved with captive stocks of humpback chub should develop site-specific NATURES rearing practices, especially facilities involved in raceway culture.
- Identify a closed basin system that can be used to maintain older broodstock once they have been intentionally spawned twice. This experimental population can serve many purposes as determined by the genetic management and captive propagation committee.
- A “studbook keeper” should be designated to maintain pedigree record information for all assurance populations and propagation events for humpback chub. This necessitates facilities becoming members of the International Species Information System (ISIS), and training the individual in the Zoological Information Management System (ZIMS) record keeping system.
- The studbook keeper will provide onsite spawning assistance and designate preferred spawning pairs if propagation is initiated.

Assurance Populations and Captive Propagation

The need exists to have a process to evaluate and develop refuge locations for humpback chub. Willow Beach NFH successfully held humpback chub for several years. Ouray NFH is currently maintaining humpback chub collected in 2007. Dexter NFH is also currently maintaining humpback chub collected in 1997, 2008, 2009 and 2010 from the lower Little Colorado River. Other facilities have indicated an interest in holding humpback chub, but little is known regarding optimum conditions for maintaining larval, juvenile, and adult humpback. Gene pool maintenance relies on minimal mortality, although as previously discussed this carries the risk of accumulation of

deleterious mutations. When fish die that don't adapt well to captive conditions, inadvertent selection results in survivors that carry genes that favor the conditions in the captive facility, not the wild habitat. However, humpback chub in captivity have demonstrated retention of allelic diversity greater than the newly translocated population in Chute Falls. As per Garcia-Dorado (2003) and Lacy (1987), greater risks prevail and the relaxation of selection in the captive environment is of secondary concern. Preventing domestication selection begins at the most basic level: provide conditions that result in minimal mortality. This is achievable by providing a variety of diet, including live foods, and maintaining densities that prevent stress on the fish. Wild fish appear to be maintaining a stable population in the Little Colorado River, however, broodstock mining and taking young-of-year fish may potentially impact the local population (USFWS/NPS 2009). The need for new captive assurance populations and preventing the loss of valuable wild fish are in direct conflict. Resolution of this conflict can be avoided by a series of planning and facility development steps.

Assurance Population Preplanning and Site Assessment

Appendix 2 provides a flow chart to indicate key components to assurance population development. In addition, the following recommendations are provided to enhance the potential for successful assurance population development.

- Develop a captive assurance population and propagation team for humpback chub.
- Identify potential assurance population locations.
- New facilities should be assessed by the team to provide a facility inspection and report prior to the release of wild humpback chub to a facility. This should include a list of required modifications, concerns and positive attributes of each potential facility.
- Dexter maintains adult humpback chub. If possible, fish should be intentionally spawned to provide fish to new facilities to test their systems and train personnel prior to providing them with wild fish. This would allow a “test” phase to

determine the facilities best suited for maintenance of a captive assurance population.

- Subsequent approval for captivity of wild fish should be in a formal recommendation by the review team based on the combination of their site assessment and demonstrated success with the species.
- Test phase fish that survive for the first year should be PIT-tagged and used for further research as determined by the genetic management and captive propagation team.

Assurance Population Development Objectives, Management Actions and Tasks

- Goal: Develop a humpback chub assurance population program that conserves the genetic diversity of the Little Colorado River and Grand Canyon humpback chub and is capable of producing offspring reflective of that diversity for recovery efforts.

Management Action: Establish captive stock(s) of at least 2,000 adults to represent the Little Colorado River and Grand Canyon population and characterize the genetic diversity present.

Task: Develop two assurance populations of 1000 adults each.

Task: Dexter currently has ~30 adults (and ~400 young-of-year), add 200 wild caught young-of-year annually on alternating years for ten years.

Task: All fish should be PIT-tagged and fin clipped.

Task: All fish should be genotyped and entered into studbook.

Task: Designate second assurance population location, add 200 wild caught young-of-year on alternating years for ten years.

Task: Identify closed waters for experimental population that serve as a backup refuge population of older fish when they are expunged from the program. This population should be available as a research stock to enhance recovery efforts after proposal approval by genetic management

and captive propagation committee and as directed by the Regional Directorate.

- Goal: Establish and implement a genetic management plan that actively monitors and maintains genetic variability and long term fidelity of each captive stock.

  Management Action: NATURES rearing practices will be followed in facilities using raceway propagation.

  Task: Addition of structure to raceways, painting of raceways to mimic Little Colorado River substrate, and overhangs or shades placed over portions of each raceway.

  Task: Live food will be offered to all life stages at least weekly.

  Task: Minimize the density of fish in holding facilities to no greater than 20% of comparable trout hatchery densities.

  Management Action: NATURES rearing practices will be followed in facilities using pond propagation.

  Task: Addition of structure to ponds, and provide overhangs or shades in ponds.

  Task: Unlined ponds are preferred.

  Task: Live food will be offered to all life stages at least weekly.

  Task: Minimize the density of fish in ponds.

  Management Action: Prevent loss of genetic diversity as measured by microsatellite loci and one mtDNA marker.

  Task: Monitor wild fish and captive fish to ensure no loss of allelic diversity or no significant change in allelic frequency.

  Task: Tissues will be collected annually from 48 individuals of each new year class and location. Genetic analysis will be completed every three years.

Task: Raise wild young-of-year separately until old enough to PIT-tag, fin clip, and enter into studbook.

Task: Studbook keeper should provide an annual report to the genetic management and captive propagation committee on allele frequencies, heterozygosity, private alleles and changes in wild and captive stocks.

- Goal: Establish a chain of custody for captive stocks to allow identification and to track individual fish from integration into the captive stock until final disposition.

Management Action: Assurance population fish will be PIT-tagged. A ZIMS database will be established for broodfish with a microsatellite genotype of each fish. This database will represent the baseline for broodfish from different facilities.

Task: Genetic analysis will be performed on assurance population fish.

Task: Genetic data will be used to determine the relatedness of fish within and between captive stocks.

Task: Spawns, transfers, deaths and growth will be recorded for each fish, and data provided to the studbook keeper monthly for entry in ZIMS.

Captive Propagation Objectives, Management Actions and Tasks

- Goal: Initiate a captive propagation program only as directed by the Regional Director of the Southwest Region.

Management Action: Develop a humpback chub production program that reflects the genetic diversity of Little Colorado River and Grand Canyon humpback chub and produces offspring that reproduces that diversity for recovery efforts. The exception to this would be intentional spawning for research and development of cultures techniques. Any spawning for research

should be overseen by the genetic management and captive propagation committee.

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

Task: Assurance population s from facilities currently holding humpback chub will be inventoried every two years, and reports generated by ZIMS will determine the success of the gene pool maintenance program.

Task: Replicate technique used by Douglas and Douglas (2007) for mtDNA analysis used in screening humpback chub.

Task: Produce baseline multilocus genotype of humpback chub at propagation facilities.

Task: Provide statistical analysis of results to determine the extent of differentiation between captive stocks and compare to wild stocks.

Task: Active pairing of genotyped fish based on ZIMS database to maintain heterozygosity and allelic variants present in broodstock.

Task: Captive stocks from the lower Colorado River Basin should retain their separate stock identity.

- Transfers of fish or gametes between lower basin facilities holding the Little Colorado River and Grand Canyon stock may occur as recommended by the studbook keeper.
- Mating strategies should be developed to ensure genetic integrity is maintained.
- No transfer of fish or gametes should occur between Upper Basin and Lower Basin stocks.

- Goal: Develop a cryopreservation program to harvest the genetic reserves currently available in the Little Colorado River and Grand Canyon.

Management Action: Identify and fund a project to take samples of wild adult males annually and cryogenically store them for perpetuity.

Task: Collect sperm from 20-100 wild males annually, PIT-tag those males, and fin clip them for genetic analysis.

Task: Use of cryopreserved sperm would only be initiated if numbers of humpback chub decline below 100 fish in the Little Colorado River and Grand Canyon population.

Task: Genotype and other information will be included in the studbook.

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

Management Action: Monitoring of hatchery production to determine changes in allele frequency as indicators of loss of 'wild' characteristics.

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

Task: A sample of 5% of production fish will be sampled annually, and genetic analysis will be conducted every three years to determine allelic frequencies. Comparisons to expected frequencies will determine if, and at what stage, selection (as assessed by allelic loss) is occurring.

Task: Annual report will be prepared by the studbook keeper to report the status of broodstocks and progeny, and strategies identified to minimize culture impacts.

- Goal: Establish culture procedures to minimize the risks associated with intensive hatchery production.

Management Action: NATURES rearing practices will be used in facilities using raceway propagation.

Task: Production fish will be reared in raceways with a naturally appearing substrate and structure in the raceway and shade/covers

provided. Any additional options to mimic the natural environment should be identified and tested.

Task: Densities should be minimal, roughly 20% for the equivalent size and age density of a trout facility.

Management Action: A maximum of 2,000 fish from any one pair will be produced for stocking in one location, whether the pair is used in a one-on-one paired mating, or two-by-two mating.

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

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

Task: When parents are used in a one-on-one paired mating production strategy, or two-by-two strategy, contributions of each cross will be equalized. Multiple stocking commitments (locations) can be met from the same mating, but no more than 5,000 fingerlings will be stocked in any one area from one pair. Excess eggs from each paired mating will be maintained for research purposes, sent to other facilities or disposed of according to the genetic management and captive propagation committee recommendations.

Task: Broodfish will be rotated so that no fish will be spawned more than twice in five years, and after second spawning broodfish will not be used again. Disposition of older broodfish will be determined by the propagation committee.

Task: Expected allelic frequencies will be developed for each paired mating and for each pooled lot of production fish by the studbook keeper.

- Goal: Establish breeding program using the ZIMS database to maintain and ensure that the effective population size of broodstock is adequate to offset the consequences of small populations.

  Management Action: Maintain at least 2,000 adult broodstock.

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

  Task: Recommendations by the studbook keeper will guide transfer of sperm, eggs, or individuals to manage net loss of allelic diversity.

  Task: Estimate relatedness of individual broodfish. Do not spawn any two fish with a relatedness estimate greater than 0.2.

  Task: Spawning pairs will be determined by studbook keeper in keeping with protocols established by the AZA.

- Establish a monitoring program to provide comparisons of captive broodstocks with wild populations.

  Management Action: Annual genetic samples of production fish, 'wild' populations, and inventory of broodfish with genetic analysis completed every three years.

  Task: Request tissues from individuals involved in monitoring wild populations.

  Task: Access archived tissues from available sources to determine historical and current baselines of populations.

  Task: Maintain records of analyses, and synthesize data sets from any new research.

  Task: Use a standard set of primers for the duration of the assurance population program. New markers and techniques may be used, but at a minimum the same suite of microsatellite markers used by Douglas and Douglas (2007) and Keeler-Foster et al. (2004) should be used.

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

  Management Action: Continue to develop, refine, and initiate spawning and marking protocols, and proceed with new research initiatives.

  Task: Incidental spawns will occur, and annual inventory should remove those fish from the assurance population stock. Those fish should be made available for research purposes as determined by the genetic management and captive propagation committee.
  Task: Fish from incidental spawns should be retained until large enough to PIT-tag and entered in the Chain of Custody to determine use and fate of individuals.
  Task: Intentional spawns of F2 for research purposes may occur with approval of the genetic management and captive propagation committee. Chain of Custody requirements still apply.
  Task: ANY research using captive stock is under the guidance of the genetic management and captive propagation committee and the Regional Directorate and will contain a caveat that all raw data will be provided to the studbook keeper annually.
  Task: The genetic management and captive propagation committee should provide a specific request to the propagation facility that identifies stocking locations, size and augmentation numbers a full year in advance.

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

  Task: When 100,000 fry are requested, at a minimum use 10 pairs of adult fish.

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

Task: Minimum of ten pairs will be used in a two-by-two matrix. When small numbers are needed, fewer eggs will be kept from each mating.

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

Task: If stocking commitment is for 8-12" fish, ultimate numbers of fish are fewer, however, no less than ten mated pairs in a two-by-two matrix will be used for stocking any one area at any time.

Translocation

Translocation is a tool in conservation biology where animals from one population are captured and moved to a new location, typically within the species range but where the species is no longer found. Translocations have fewer genetic risks than augmentation programs, but a few are noteworthy. One particularly important consideration is the source and number of the fish that form the basis of the new population. Typically the "nearest neighbor" approach is applied, that is the donor source for the new population is the closest geographical neighbor. In fish, this can be complicated by other landscape features such as drainages, natural dams or barriers that impede the flow of genetic material from one location to another. Douglas and Douglas (2007) and Keeler-Foster et al. (2009) found the Little Colorado River and Grand Canyon population is a homogenous admixture, and aggregates remain linked by gene flow. Restoration of native sites within the area of the Little Colorado River and Grand Canyon can be conducted without risk of introducing fish that are genetically disjunct, which could result in outbreeding depression. This is important for restocking purposes, as it allows managers' access to natural reproduction of humpback chub for restoration to areas that remain geographically connected.

The previous draft genetic management plan for humpback chub (Czapla 2005) suggests a minimum of 100 fish, whereas we are recommending 200 fish, but both documents concur that long-term commitment to the effort is essential. Our

recommendation of 200 fish per year is based on multiple considerations, including the high initial mortality or emigration noted in the first translocation of humpback chub to the Little Colorado River above Chute Falls. Of over 1,500 fish translocated since 2003, approximately 162 remain. Another factor important to consider is the genetic effective population size (N_e). Moving 100 fish for five years would result in a census number of 500 individuals, assuming no mortality or emigration. Without empirical estimates of N_e for humpback chub, we again use the average from Palstra and Ruzzante (2008) suggesting perhaps 14 fish from every 100 adult fish are recruiting offspring to the next generation. Using that average, a census number of 500 equates to an N_e of 70, and if even minimal mortality has occurred, that number approaches 50, a threshold commonly invoked as the minimum necessary for retention of long-term genetic diversity and population viability (Franklin 1980; but see Lynch and O'Hely 2001). Reed et al. (2003) report "approximately 5,800 adult animals are needed for a 95% chance of persistence over 40 generations, 4700 for 90% persistence and 550 for a 50% chance of persistence." Our goal of 200 fish every year for five years (or every other year for 10 years) would produce a census value of 1,000 (assuming 100% survival and no emigration) and an estimated N_e of 140 if the calculation is based on values provided by Palstra and Ruzzante (2008). The target of 200 individuals per year for five years should be viewed as a minimum number for the long-term maintenance of the genepool, but this value should in no way negate other ecological considerations such as carrying capacity of the system.

The founders of a new population should reflect the extant genetic diversity of the donor stock, span the temporal spawning range of the donor stock (i.e. multiple collections of fish throughout the spawning period), and represent an age structure typical of a healthy viable population. To achieve this, multiple years of collections are required with fewer individuals collected over a longer time frame rather than a larger single effort. This also minimizes the impact of the collection on the donor population, and prevents the collection of larger fish (broodstock mining). Young-of-year are typically targeted for translocation efforts as it is assumed most will not survive to maturity in a stable population. Genetic analysis, in conjunction with long-term population sampling, indicate the confluence of the Little Colorado River with the

mainstem Colorado River may be ideal for sampling young-of-year as fish move downstream from spawning sites above and congregate in the area. The confluence reach known as the lower 1200 m, along with any additional known spawning sites in the mainstem Colorado River in the Grand Canyon should be targeted for capture of young-of-year fish for future broodstock or translocation efforts. Young-of-year may be transported to a hatchery to rear until a size suitable for restoration, but this is not to be confused with captive propagation, rather the facility would serve as a holding facility until the restoration activity takes place.

Translocation Objectives, Management Actions and Tasks

The development of additional stable populations is essential to recovery and ultimate downlisting of humpback chub. To date, two successful translocations have occurred. The first translocation involved moving fish from the lower portion of the Little Colorado River to above Chute Falls, still within the Little Colorado River. Not only have adult fish remained above the barrier, but fish have been documented navigating the falls (Stone 2009). The second success marked the first translocation of humpback chub outside the Little Colorado River. A total of 302 fish were moved into Shinumo Creek in July 2009 and preliminary data analyses suggest that over half of these have remained in Shinumo nearly 10 months later. The success of these translocations results in an expansion of the current distribution of humpback chub and should be continued. The translocation effort should provide for a continual influx of fish to span a generation and establish a reasonable approximation of a natural population; that is a normal size and age distribution, as well as gene flow from the donor source. This requires a continued commitment to the effort. Collections for translocations should span the spawning window and occur across the entire Little Colorado River. In addition, fish from the mainstem may reflect additional genetic diversity, and young-of-year or year 1 fish should be taken as close to the confluence of the Little Colorado River/Grand Canyon as possible. If any of these younger size classes are found within the mainstem proper, those fish should be prioritized.

- Goal: Develop yearly stocking rates based on surveys of total available habitat and management practices of other successful humpback chub translocations.

  Management Action: Determine the initial targeted census number with a minimum of 200 individuals.

  Task: Young-of-year should not be stocked until of sufficient size that individuals can be PIT-tagged and fin clipped.

  Task: Restoration and translocation should occur over a minimum of 5 years, with young-of-year stocked at age 0, or later, but recruited from the wild annually if possible.

  Task: If captive spawned fish are used, the studbook keeper will provide recommendations on preferred matings and maintain records of founders for new populations.

  Task: Establish an age structure including a minimum of 5 year classes, with the oldest fish reproductively capable.

  Management Action: Determine the potential for immigration and emigration into the restored population.

  Task: Estimate the level of emigration from the management unit.

  Task: Estimate the level of immigration into the management unit.

  Task: Refine annual stocking rates based on management units' population dynamics.

  Management Action: Refine and adapt criteria for successful translocation and restoration.

  Task: Determine generation interval.

  Task: Estimate annual survival rate for each stocked year class.

  Task: Determine optimum size of released fish for increased survival.

  Task: Develop protocol for assessing year class strength of wild-spawned and translocated humpback chub.

Task: Assess relative year class strength of wild-spawned humpback chub to determine impact of artificial recruitment of young-of-year on the donor population.

Management Action: As multiple locations are restored, ongoing monitoring should include fin clips to follow genetic reorganization of newly established population.

Task: During ongoing population monitoring activities, fin clip up to 30 fish per year for the duration of the management activity.

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Appendix 1. Quarantine and Disease Treatment of Humpback Chub

Excerpt from Childs, M., and B. Persons 2006. DRAFT. Protocol for Collection, Transport, Quarantine, Disease Treatment and Maintenance of the Grand Canyon Population of Humpback Chub: Assurance population Establishment and Long-Term Genetics Management. Arizona Game and Fish Department.

- The condition of fish upon arrival at the assurance population facility will determine if therapeutic treatments will start immediately or the following day or days. Weak fish (e.g. swimming erratically, exhibiting loss of equilibrium) will not be treated immediately.
- Fish that die during transport will be inspected for fish pathogens. This will be done by visual observation, skin scrapes, and dissection. A fin clip will be collected from each dead fish and preserved in 95% ethanol for subsequent genetic analysis. The bodies of dead fish will be preserved in 10% formalin and accessioned into an appropriate museum.
- Any fish that die in quarantine tanks will be inspected for fish pathogens. For recent mortalities (within 1-h postmortem), bacterial samples will be collected from the kidney and shipped to the fish health lab in Pinetop, AZ, or Dexter, NM, for later identification.
- Arizona Game and Fish Department and National Wildlife Health Center personnel have previously identified fish pathogens for humpback chub (Table A1).
- Cestodes can be treated with Praziquantel, which is FDA approved according to Sigma-Aldrich Chemical Co. Cestodes will be treated with Praziquantel at 6 parts per million for 24 hours. Only one treatment for cestodes will be needed at the quarantine area. Cestodes killed in this manner will be preserved in 95% ethanol and accessioned into an appropriate museum.
- Chemical treatments for copepods will consist of 1-h salt baths (uniodized), starting at a concentration of 1% and slowly increasing to 3.5% depending on tolerance of the fish. Adult parasites will be physically removed from the fish. This procedure will continue for several weeks depending on the life cycle of the

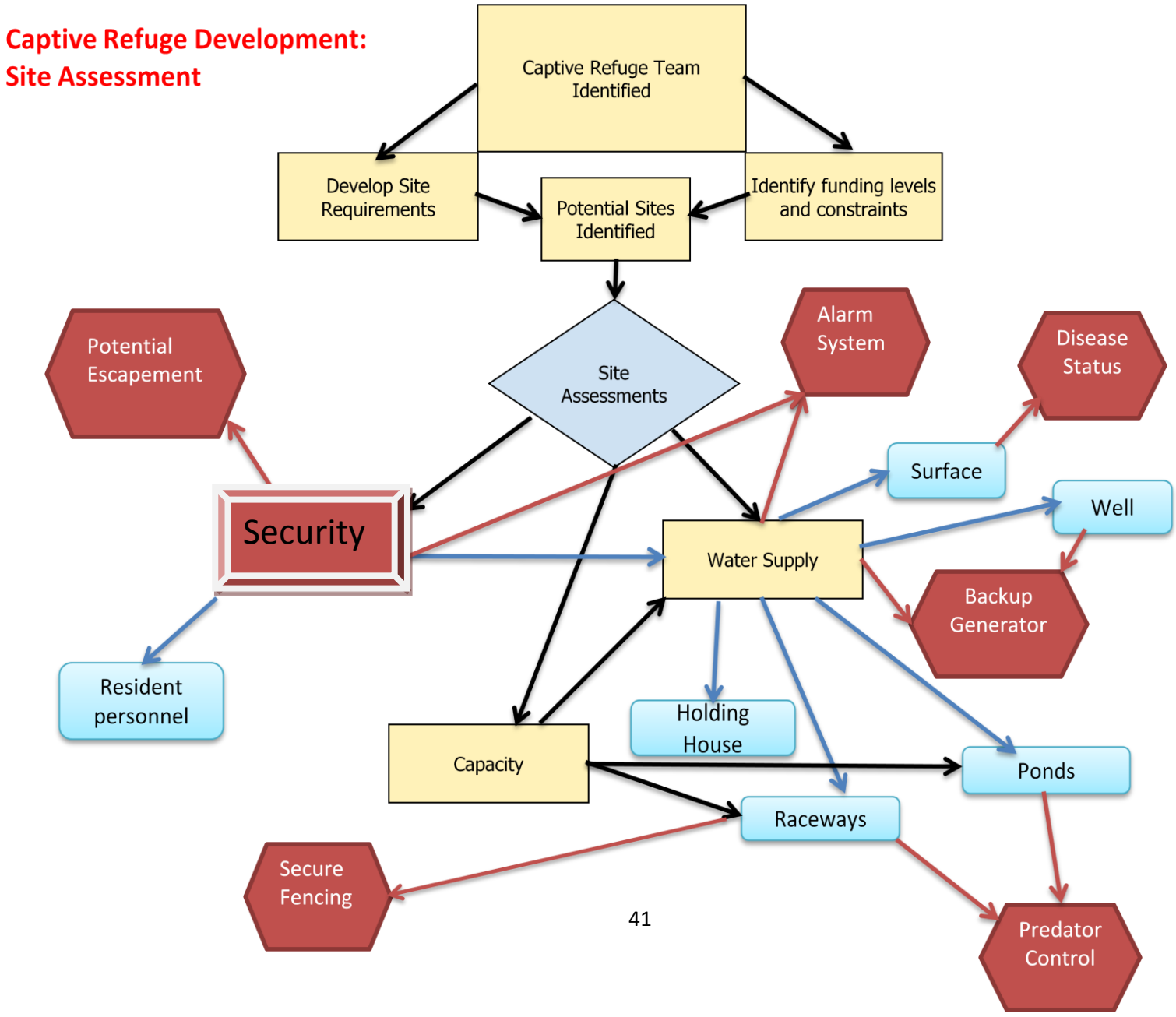
parasite being treated. Copepods killed in this manner will be preserved in 95% ethanol and accessioned into an appropriate museum.

- Chemical treatments for trematodes will consist of formalin, starting at a concentration of 100 parts per million for one hour. Tolerance to formalin will be monitored, and concentrations will be slowly increased to as high as 167 parts per million for one hour. Treatments with formalin will be administered for 3 to 5 consecutive days. Following treatments, fish will be examined for external parasites using visual observations and skin scrapes. Trematodes killed in this manner will be preserved in 95% ethanol and accessioned into an appropriate museum.
- All internal bacterial infections will be treated with oxytetracycline (TM) in a prepared diet or chemical bath. Treatment with TM will be administered for not less than 10 consecutive days. Chloramine-T is also available for treatment of external bacterial infections at 10 parts per million for 1 h.
- Fish will be held in quarantine until all known pathogens are removed, and until the fish have been certified as pathogen-free by a qualified fish pathologist.

Table A1. Parasites found in humpback chub in the Little Colorado River (Hoffnagle et al. 2000).

Cestoda	Bothriocephalus acheilognathi
	Unidentified plerocercoid
Copepoda	Lernaea cyprinacea
Trematoda	Ornithodiplostomum sp.
	Rhabdochona sp.

Appendix 2. Flow Charts for Captive Assurance population Development Site Assessment, and Captive Stock Development



Captive Stock Development