Rio Grande Silvery Minnow

Genetics Management and Propagation Plan

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Introduction

The Rio Grande silvery minnow (RGSM), *Hybognathus amarus* is restricted to a 280 km portion of the Rio Grande (the "middle Rio Grande") in New Mexico, which represents approximately 7% of the species historic range. Within the middle Rio Grande, multiple factors including habitat loss, water management, and recurring drought continue to threaten this species with extinction. A critical management strategy for alleviating jeopardy and helping to recover RGSM relies on captive rearing and propagation to augment the population in the middle Rio Grande. Additionally, reintroduction sites are under evaluation for restoration activities that will use propagated fish, either from captive spawns or egg salvage, to reintroduce the species to historic habitat such as the Pecos River. The first restoration effort, an experimental nonessential stocking (10J) of RGSM in the Big Bend reach of the Rio Grande was initiated in December 2008. This effort will continue for another 4 years to establish a second self-sustaining population in the Rio Grande.

The propagation strategy for the RGSM is based on two key elements: 1) the collection of eggs from the middle Rio Grande to meet the majority of targeted stocking numbers and 2) maintaining fish from the annual wild egg collection as broodstock for captive propagation and as refuge populations' in the event catastrophic changes occur in the river. These actions minimize the risk to the extant wild population by preventing broodstock mining and maximize the potential to replicate as closely as possible a natural recruitment cycle. Broodstock mining is the practice of collecting wild reproductive adults which can impact the reproductive and genetic reserves of the population. The propagation program will be contingent on an orchestrated balance between the use of wild caught eggs and captive propagation that will require ongoing monitoring of river populations and genetic monitoring of wild and captive stocks.

The propagation program will use a combination of wild egg collections and hatchery spawning of fish from those eggs (F_1) to produce fish for stocking. To the extent possible, eggs will be collected in the river every spring from natural spawning events and delivered to propagation facilities. The amount of eggs removed during spawning events represents a small percentage of the available pool of eggs and occurs

during the lifestage that is inherently represented by low survival. The eggs will be hatched, and larval fish reared to adulthood in captivity. A small portion from each year class will be retained as captive broodstock. If recruitment fails in any given year, the captive stock can be used to produce fish to maintain the species through the next year.

Additionally, paired or communal captive spawning will be conducted annually. Ongoing genetic monitoring will be used to ensure a minimum number of breeding animals contribute to the next generation. We expect that in low egg collection years, when natural spawning does not yield adequate numbers of eggs for the program, captive propagation will be required to maintain the genetic effective population size, and to meet targeted stocking numbers.

The RGSM *Genetics Management and Propagation Plan* (Plan) is designed to provide a strategy for maintenance of genetic diversity in RGSM. In concert with strategies to address the underlying cause of the species' decline, fish from collected eggs and F_1 propagated fish will enhance the long-term survival and support recovery for the RGSM. The actions presented in this document are necessary to provide offspring appropriate for reintroduction as identified in the draft revised RGSM recovery plan (USFWS 1999; USFWS 2007) and in the Services' conservation strategy for the species (USFWS 2003).

An extensive review of the species' life history, including the distribution, habitat requirements and spawning behavior is available in the draft revised Recovery Plan (2007). Section 1.0 of the RGSM Recovery Plan provides background information, current distribution and population status and presents a recovery strategy that addresses three major goals: prevention of extinction, downlisting, and delisting of the species.

Propagation and Management Facilities

Currently, RGSM are held at the locations described below. Maintaining stocks at several facilities minimizes the extinction risk due to stochastic events and serves to mitigate the impact of any one facility on the genome of the RGSM. Several other facilities, including the U.S. Fish and Wildlife Service's Bozeman Fish Technology Center, the state of New Mexico's Rock Lake State Fish Hatchery, and the University of

New Mexico's Museum of Southwestern Biology have provided technical assistance to the propagation program.

The **Dexter National Fish Hatchery and Technology Center** in Dexter, New Mexico, (Dexter) serves as the lead federal facility for propagation of the species. Activities include the establishment of an ad hoc propagation work group, development of a captive propagation plan, refinement of captive propagation and rearing techniques, maintenance and expansion of refugial populations, and research on life history, feed formulation, feeding trials, fish marking, and tag retention. Dexter uses modified warm-water fish culture techniques to rear the fish. The RGSM propagation program uses 11 ponds, two 2000-gallon recirculation systems with temperature control and independent bio-filtration, ten 3-foot-diameter circular tanks, two 10-foot-rectangular flow-through tanks, and twelve 40-gallon aquaria. The facility annually produces more than 250,000 (>35 mm) RGSM for the middle Rio Grande. The propagation program at Dexter began in 2001, and has made significant advances in developing appropriate and consistent propagation and culture methods. Dexter maintains a captive refugium/broodstock of approximately 15,000 adults from wild collected eggs. The facility relies on egg collections in the middle Rio Grande and captive broodstock to meet targeted augmentation numbers.

The **City of Albuquerque's Rio Grande Silvery Minnow Rearing and Breeding Facility** (BioPark) began experimental propagation of RGSM in 2000 in cooperation with the U.S. Fish and Wildlife Service. In 2003, the BioPark added new facilities, including an indoor breeding and hatching system, twelve 20,000-gallon outdoor rearing and holding tanks, and an outdoor naturalized stream which houses a captive population for spawning, rearing, and research. The facility produces about 75,000 fish annually and holds approximately 30,000 fish as a captive population. The BioPark is the first stop for wild-caught eggs, which are then distributed to other propagation facilities. In June 2003, the BioPark's naturalized refugium was brought online, and is intended to produce fish that are better able to adapt to river conditions upon release. This outdoor system is an oval-shaped channel with a volume of 50,000 gallons. The refugium features include sand substrate, plunge pools, backwaters, runs,

and debris piles. The refugium is used primarily for grow-out of fish for augmentation and for holding broodstock.

The **New Mexico Fish and Wildlife Conservation Office** (NMFWCO) in Albuquerque oversees Rio Grande silvery minnow management activities, including determining the propagation needs for augmentation and restoration stockings. NMFWCO develops propagation and augmentation plans, conducts augmentation monitoring activities, and assists with wild egg collections for propagation facilities. A 1,000-gallon recirculation system at the facility is used to hold juvenile and adult RGSM for research and broodstock maintenance.

The New Mexico Cooperative Fish and Wildlife Research Units' **Rio Grande Silvery Minnow Propagation and Research Facility** began captive breeding, rearing, and research activities at the Geothermal Fish Culture and Research facility (A-Mountain) in Las Cruces, New Mexico in 2001. The facility has the ability to vary water temperature with a geothermal water source. The culture component of the system is enclosed in a double-wall arched greenhouse (2,880 ft^2). Two independent culture units support multiple system comparisons, operational flexibility, and offset production schedules. A-Mountain maintains a backup broodstock, and is used to conduct applied and basic research. A-Mountain also spawns fish in support of the captive propagation program. The eggs/larvae produced are transferred to Dexter for hatching, rearing in ponds and subsequent distribution to stocking locations.

An additional research facility is located on the NMSU campus, with an 870-gallon recirculation system. This laboratory includes an indoor 2500 ft^2 building with high quality well water for single pass or closed reuse systems. The research focuses on developing and refining fish culture methods for RGSM. Feed studies to improve survival and performance of captive specimens were initiated at A-Mountain and Dexter through a cooperative effort with the U.S. Geological Survey (USGS) and Bozeman Fish Technology Center. Test diets were formulated by the Bozeman Center, while A-Mountain and Dexter evaluated the feed.

Personnel from the USGS Yankton Ecotoxicology Research Center, Yankton, South Dakota, also conducted research with the species. Studies began in 1998, comparing relative sensitivity and acute toxicity of RGSM to a variety of waterborne

inorganic contaminants. Yankton maintains an aging population of RGSM collected in 1998. Their longevity is attributed to cold water temperatures at the facility.

In addition, two new facilities have been added to the RGSM program. The **Rio Grande Silvery Minnow Sanctuary** is located in Albuquerque, New Mexico, within the levee system of the Rio Grande near River Mile 181. The sanctuary is about 1,300 ft long, with water supplied from the Albuquerque Riverside Drain. The sanctuary is to be completed in 2009. The NMFWCO is responsible for long-term operation and management of the sanctuary, with assistance from the Middle Rio Grande Endangered Species Collaborative Program and U.S. Bureau of Reclamation, Albuquerque. The basis for the sanctuary design is to provide suitable habitat for maintaining RGSM. Specific environmental components that may be supported by the sanctuary are 1) self sustaining food web, 2) spawning habitat, 3) early life stage rearing, and 4) adult habitat. Geomorphic variability of constructed sanctuary habitats, (i.e., sloughs, swales, ephemeral side channels) will allow for habitat diversity and acclimation of captive reared RGSM prior to release.

The **Los Lunas Silvery Minnow Refugium**, in Los Lunas, New Mexico began construction in 2007, and will be operational in 2009. The facility will consist of a 0.28 acre outdoor refugium and an indoor facility. The design includes a 458 ft X 10 ft channel that will feed 5 ponds. In addition, there will be a 0.05 acre overbank area that can be flooded. The refugium was designed to provide a habitat similar to a backwater area of the main river channel. The refugium will maintain a captive stock that can be used for restoration purposes if catastrophic losses occur in the river or other facilities.

Recovery Goals and Objectives

The goals of the recovery plan (USFWS 1999; 2007) that are partially addressed in this plan include:

1) **Stabilize and enhance populations of RGSM (and associated habitat) in the middle Rio Grande Valley**.

The RGSM population in the middle Rio Grande is imperiled primarily due to loss of eggs into river reaches that cannot provide safe harbor for larval fishes. The

middle Rio Grande population of RGSM should be the highest priority for conservation planning and to meet recovery goals. This population survives and spawns, but limited recruitment and habitat availability has thus far prevented the population from becoming self sustaining. The captive propagation and genetics management strategy defined in this Plan relies heavily on this wild resource.

This Recovery Goal has been addressed by ongoing propagation activities, with notable success. The propagation program produces 250,000-300,000 RGSM annually for augmentation/reintroduction stocking. The program requires collection of approximately 500,000 eggs to meet annual stocking commitments. When possible, the eggs are collected from the wild and hatched at cooperating facilities, and those offspring are used to meet priority stocking commitments (Alò and Turner 2005; Osborne et al. 2005; Turner et al. 2006). A small proportion of these wild-caught eggs are held over for broodstock. A new augmentation plan for the middle Rio Grande was finalized in 2008, which includes a new, dynamic algorithm to determine augmentation numbers. Stocking densities are based on *catch per unit effort* (CPUE) data, which is also listed as a criteria for Recovery Goals in the DRAFT Recovery Plan (USFWS 1999; 2007). The previous five-year plan targeted an increase in census numbers such that the middle Rio Grande population could be considered self-sustaining. The current plan is intended to monitor the status of the population in the middle Rio Grande, and adjust stocking commitments according to census estimates and conditions in the river on an annual basis. For planning purposes, an initial estimate for propagation needs from the Program will be calculated each January 15th. This initial estimate will use flow estimates of the river in conjunction with the stocking numbers of the previous year to determine the augmentation number. The algorithm to maintain a target density of 1 fish / 100 m^2 will be 0.5 X the previous year's stocking for a wet year, 1 X the previous year's stocking for an average year, and 1.5 X the previous year's number for a dry year. The final propagation request will be calculated annually on November 1st. This final estimate will take into account September CPUE data relative to the target density (1 fish / 100 m^2). Using these calculations, the range of the propagation request could be between 0 and 310,000 fish annually for the middle Rio Grande (USFWS 2008).

Although the specifics of RGSM spawning are not known, conservation geneticists have suggested that paired matings would be optimal to conserve the effective population size. However, recent empirical research on the genetic effects of paired mating versus communal spawning in RGSM indicates little genetic difference between the two strategies (M. Osborne and T. Turner, University of New Mexico, pers. comm.). The ad Hoc propagation and genetics management committee has agreed to continue with communal spawning with the caveat that no more than 20 pairs be spawned in a given tank. The fish to be used for captive propagation will be the result of the previous year's egg collection, combined with the adults held over from prior years. Spawning of multiple year classes will provide a mechanism to offset temporal variation resulting from differential reproductive success in any one year class (Whitlock 1992). Captive fish live longer than wild fish and are still reproductively capable at 4+ years of age. A mixed year class of captive fish, combined with paired or communal spawning techniques, may help mitigate the sweepstakes-mismatch recruitment (Osborne et al. 2005) that results in low N_e.

Temporal genetic variation, extinctions and re-colonization, as well as widely fluctuating census numbers are common in nature and can impact the standing genetic variation at any given point in time (Whitlock 1992; Fiumera et al. 2004). Population stability provided in the hatchery environment can meet the assumptions of population genetics models more aptly in some cases than natural populations, resulting in the preservation of genetic diversity (Fiumera et al. 2004; Alò and Turner 2005; Osborne et al. 2005; Turner et al. 2006). In addition to egg collections, each year captive spawning should be conducted using no less than 600 fish.

2) Reestablish the RGSM in at least two other areas within the historic range.

The ad Hoc propagation and genetics committee is in support of NMFWCO's proposal to use propagated fish to reestablish a population of RGSM near Big Bend National Park. This reach delineates the border of Texas and Mexico, approximately 600 miles downstream from the middle Rio Grande population. A population established in the Big Bend area would provide a second natural reservoir for genetic diversity and an

opportunity to replicate the current population in suitable habitat. Other sites are also under consideration for restoration of additional populations, such as the Cochiti reach of the Rio Grande and the Pecos River in New Mexico.

In December 2008 a reintroduction of Rio Grande silvery minnow (RGSM) occurred into the Big Bend, TX reach of the Rio Grande. This stocking was to establish a 10(j) experimental, nonessential population, using 395,000 fish from Dexter and an additional 35,296 fish from Albuquerque's BioPark. The stocking strategy involved pooling all age 0 fish and age 1 fish from Dexter and dividing each age class into five stocking trucks. Fish were stocked at 4 locations: 67,400 age 0 and 28,000 age 1 fish stocked at Santa Elena Canyon; 50,300 age 0 and 42,300 age 1 fish stocked at Adam's Ranch; 72,300 age 0 and 32,600 age 1 stocked at Grassy Banks and 68,000 age 0 and 35,000 age 1 fish were stocked at Rio Grande Village. The BioPark distributed age 0 fish, with 11,764 released at Adam's Ranch and 23,532 at the Rio Grande Village. All broodstock spawned were fish produced from the Rio Grande egg salvage operations, with Dexter's age-1 fish produced from 400 individuals from the 02 year class and age-0 fish derived from 344 individuals - (264 from the 02 & 03 year classes and 80 from the 04 & 05 year classes). Dexter used communal spawning, with no more than 20 fish per tank, and recorded the number of females that spawned. This allowed a more accurate estimate of the lots progenitors.

Genetic Risks for Captive Broodstocks, Propagation, and Stock Augmentation

The topics below are factors that influence the genetic structure of a population. This is measured statistically by comparing the changes that occur under different evolutionary pressures to an idealized population. This 'perfect' population is infinitely large, not under selection of any kind and individuals mate randomly. It also suffers no mutation and fish never move out of the population and no new individuals move into the population. This perfect population is said to be in Hardy-Weinburg equilibrium (Hardy 1908; Weinburg 1908). The topics listed below cause a natural population to deviate from this perfect model and by focusing on the differences between the model and the

'real' population, statistical measures provide a means to identify and predict trends in the population genetics of the target species. Many of these phenomena exhibit the greatest impact on small populations, such as endemic populations or captive stocks of endangered fish.

Founder effects

"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 the 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 implies that a few individuals from a population are the source for a new population and the new population diverges as a result from the donor population.

For example, if you have a jar filled with 50% yellow marbles and 50% blue marbles and you grab a handful of three, your new population might have two of a yellow variety and one of a blue variety. The 'founders' and subsequent offspring of this population would visibly differ from the original population in the first generation and more so in later generations.

It is easy to envision two individuals with a seine and an objective of capturing 10 fish. In any given stream, they might find all ten fish on the first seine haul, or they might have to sample five miles of stream to get ten fish. The chance of all individuals in one pool being related and thus sharing the same few alleles, is much greater than if the entire stream is sampled (Hansen et al. 1997). The potential consequence of this type of sampling error is loss of alleles and genetic diversity and a gradual erosion of the similarity between the captive stock and the donor stock. Indeed, this type of genetic change, combined with natural selection and sufficient time, can result in speciation (Ferguson and Mason 1981, Ferguson and Taggart 1991).

Potential risks associated with a **founder effect** in RGSM captive stocks include:

1) Loss of alleles, or reduction in genetic diversity
2) Loss of genetic identity with the donor stock

Genetic drift

Random genetic drift reflects genetic changes in a population associated with chance events. Loss of within population diversity due to the effects of genetic drift is typically associated with small population size (Lande 1995). Random genetic drift is common in nature in marginal, fragmented habitat, where populations are small and conditions suboptimal (Rieseberg et al. 2003). In this instance, a few individuals will contribute to the next generation and pass on their genes, not as a result of Darwinian selection, but by chance. In the example above, the new captive population may be started with the ten fish captured, but only three fish are still reproductively active, the others senescent. The genetic changes associated with this small population over time will result in the loss of rare alleles, fixation of some alleles and a shift in genetic makeup of the new population such that the new population 'drifts' away from the source population.

Mitigating the loss of genetic diversity requires adequate numbers of founding individuals (Minckley 1995). To overcome the effects of drift and founder effects, it is important to maintain large census numbers, both in the wild and the captive population. Managers are frequently told to maintain a minimum of 50 and an optimum of 500 individuals (Franklin 1980). However, recent research suggests that an order of magnitude greater (i.e. 5000) may be required to offset genetic risks associated with small populations (Lande 1995, Lynch and O'Hely 2001)

Ibrahim et al. (1996) theorized that genetic diversity is resilient to population contractions, but long range dispersal of a few founders results in a loss of genetic diversity. However, Lenormand (2002) suggests even small numbers of migrants per generation (gene flow) into isolated populations will minimize adaptive processes and genetic changes. When numerous metapopulations occur, genetic diversity is maintained in the species, if not the specific subpopulation (Nichols et al. 2001) supporting the importance of developing additional self recruiting populations (Minckley 1995).

An additional concern within a small population is that drift may fix mildly deleterious alleles (mutational meltdown) and increase the risk of extinction (Lynch and Gabriel 1990). Given the numerous discussions regarding mutational loads, a number closer to 5000 appears to be more realistic for long term population viability for RGSM.

Potential risks associated with **genetic drift** in RGSM captive stocks include:

3) Loss of alleles, or reduction in genetic diversity
4) Increase in genetic distance from donor stock
5) Fixation of deleterious mutations

Inbreeding and inbreeding depression

Mating of closely 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. Matings between close relatives is common in nature 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 long-term inbreeding depression is a purging of deleterious alleles and a subsequent increase in population fitness (Lynch et al. 1999, Kirkpatrick and Jarne 2000). A rapid, drastic population reduction can result in a decrease in inbreeding depression, but a bottleneck in a population causes an increase in the genetic load (accumulation of deleterious mutations) (Kirkpatrick and Jarne 2000). However, Garcia-Dorado (2003) suggests captive breeding 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).

Programs such as the propagation of RGSM require intensive genetic management to avoid inbreeding and loss of diversity (Fiumera et al. 2004, Alò and Turner 2005, Osborne et al. 2005, Turner et al. 2006 and Waite et al. 2005). Waite et al. (2005) and Lynch and O'Hely (2001) suggest that the greater the number of individuals contributing to the gene pool, the less inbreeding will impact the population. Given the

small physical stature of RGSM, the ability to maintain large numbers of individuals compensates for many of the genetic risks associated with captive rearing.

Potential risks associated with **inbreeding and inbreeding depression** in the RGSM captive stocks include:

6) Exposure of deleterious alleles in the homozygote
7) Fitness of population decreases initially, (population may or may not recover)

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 a continuum and includes both interspecific and intraspecific crosses.

Outbreeding depression can occur in the first generation by affecting the adaptation to fine scale environmental conditions. Loss of local adaptation will result in a decrease in the overall fitness of the population (Lynch et al. 1998, Lynch et al. 1999). Outbreeding depression as a result of hybrid breakdown occurs in the second generation, when recombination produces a montage of maladaptive progeny (Burton 1990).

Loss of among population genetic diversity (species homogenization) occurs in a captive propagation program when different metapopulations are mixed and interbreeding occurs (Tufto 2001). This can also occur when hatchery stocks are used to augment wild populations without consideration for the genetic subdivision that may be present. Outbreeding is fundamentally as significant a problem as inbreeding (Burton 1990; Kephart 2004).

Potential risks associated with **outbreeding and outbreeding depression** in the RGSM captive stocks include:

8) F_1-loss of fine-scale environmental adaptations, reduction in fitness
9) F_2-Recombination produces genomic shuffle, reduction in fitness
10) Skewed sex ratios, sterility

11) Homogenization of population structure

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). Rarely do managers have specific information regarding genes related to fitness of a given species. Instead we rely on 'neutral' genetic markers to provide insight into subtle distinctions within and between populations. These markers are, at best, a surrogate for fitness related characters (Milligan et al. 1994).

Longterm genetic evaluations of RGSM indicate the middle Rio Grande population is one panmictic population (Alò and Turner 2005, Osborne et al. 2005, Turner et al. 2006). These studies suggest that current management strategies are unlikely to result in outbreeding depression. Census numbers of the RGSM are sufficiently high that inbreeding, given the current management strategy, is not likely to impact the current population either (Alò and Turner 2005, Osborne et al. 2005, Turner et al. 2006).

Demographics of captive populations

Typically, a population of either captive or wild organisms change over time due to the unique characteristics of the population: fecundity, fertility, reproductive life cycle, death rate, sex ratio and age dynamics. Rates and types of harvest can also change the structure of a population (Laikre and Ryman 1996). Captive populations often have differing demographics than wild populations, for example, RGSM appear to live longer in captivity, successfully spawn past age 4 (M. Ulibarri pers. comm.) and have survived to 9 years of age (K. Buhl, USGS, Yankton, S.D. pers. comm.). Combined with an increase in survival from eggs to adult, it is easy to envision how the character of a population differs between wild stocks and captive populations (Whitlock 1992; Laikre and Ryman 1996).

Potential risks associated with the **changing demography** of captive and wild RGSM stocks include:

12) Survival of progeny that would readily succumb to selective pressures in the wild

Domestication/artificial selection in captive populations

The genetic constituency 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). Domestication is thought to result in genetic changes that affect the fitness of a population that may include a high proportion of captively reared fish (Lynch and O'Hely 2001; Ford 2002). Domestication can occur because of culture practices that favor certain genetic backgrounds, or domestication can occur because of the relaxation of selection, thereby allowing maladaptive genotypes to persist (Hard 1995; Lynch and O'Hely 2001; Tufto 2001).

Domestication selection is a problem if propagated fish are less adapted to the natural environment than wild fish and negatively impact overall population fitness of wild stocks by inundating wild genomes with genetic backgrounds adapted to the hatchery environment (Ford 2002; Lynch and O'Hely 2001; Tufto 2001). However, McPhee and Silverman (2004) suggest this loss of fitness may be compensated for by calculating an optimum release ratio. For example, they calculated from 130 to 150 captively reared mice were the genetic equivalent of 100 'wild' mice (McPhee and Silverman 2004). While empirical data for fish are scant, fisheries managers have long realized that release numbers do not equal the number of individuals surviving. Typically, managers assume a survival of released fish between 1% and 10%. Some of the initial losses are the result of stochastic events and unlikely to reflect a strong selection factor. After the initial loss, selection may help to compensate for the relaxation of selection, at least for older life history traits of adaptive advantage.

Potential risks associated with **domestication selection** of the captive stocks include:

13) Relaxation of selection-prevents selective pressure that would normally 'cull' less fit individuals from the gene pool
14) Inadvertent selection-adaptation to the hatchery environment, less fit when returned to natural environment

Augmentation of wild stocks

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. Many species of fish have high fecundity, as most eggs and young will perish before becoming reproductively capable. For example, striped bass can provide an average of 75,000 eggs a year and spawn annually for 10 years, producing 750,000 offspring in one reproductive lifetime. In a stable population, only one of those eggs will result in a replacement reproductive female. Captive propagation to augment wild stocks is typically undertaken to assist the species by relaxing the selective pressure placed on eggs and larval fish. This serves to increase the survival of progeny and subsequently increase the census numbers of a wild population. As an example, if recovery of a population of striped bass were the goal and the target stocking number was 100,000 fish, two paired matings would accomplish that objective. The population would have an increased number of individuals (census number), but if in later years when the breeding population was dominated by the result of those two paired matings, the effective population size (N_e) would be greatly reduced.

Short term propagation programs for RGSM include a large captive propagation and rearing component and provide for water flow regimes that will provide the environment necessary for survival of hatchery propagates. Long term objectives should include a targeted augmentation number that substantially reduces the reliance on captive propagation (McPhee and Silverman 2004; Tufto 2001) and focuses instead on providing a reliable environment conducive to long term population stability (USFWS 2007).

The advent of conservation genetics and subsequent application to fisheries management resulted in the identification and mitigation of many negative effects of stock augmentation. For example, the Ryman-Laikre effect (Ryman and Laikre 1991; Laikre and Ryman 1996) causes a reduction in the genetic effective population size when a few individuals are used for production and stocking of an existing population as illustrated above. Natural populations frequently have a similar 'lottery' reproduction strategy, exemplified by high fecundity and wide variances in family sizes (Fiumera et al. 2004). Current management practices no longer rely on targeting numbers of offspring, instead the goal is to equalize the numbers of parental contributions and maximize the number of progenitors (Allendorf 1993). The RGSM propagation program demonstrates the benefits of the strategic application of genetic information to the recovery process

(Alò and Turner 2005; Osborne et al. 2005; Turner et al. 2006 but see Osborne et al. 2006).

A second consideration is the potential for increasing the mutational load of the wild population (Lynch et al. 1998). Obviously, if 750,000 eggs yield one replacement individual, natural selection is extremely harsh and effective in shaping a population (Riesberg et al. 2003). Captive rearing is intended to relax the impact of selection on critical life stages to ensure greater numbers of adults. The risk associated with that practice is the proliferation of deleterious or maladaptive mutations that would be selected against in the wild population, but survive in the benign captive environment (Lynch and O'Hely 2001; Tufto 2001).

Tufto (2001), Lacy (1987) and Kirkpatrick and Jarne (2000) suggest that mutation is a risk that should be considered in conservation programs, but inbreeding, loss of genetic diversity and adaptation to the hatchery 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 also 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 via inbreeding 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 will result 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 have a greater negative consequence to managed populations more destructively. 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."

The third risk of stock augmentation is the potential transfer of disease or nuisance species when moving fish from one site to another. The RGSM propagation program is actively involved in research to identify and mitigate sources of potential health risks in transport of wild and stocked fish. In 2007, the work group incorporated fish health inspections in the operation of propagation facilities which will be conducted annually by the USFWS Fish Health Unit at Dexter.

Genetic Status of RGSM

Early studies focused on allozymes to describe the systematic and taxonomic relationships among RGSM and closely related congeners (Cook and Yates 1988; and Cook et al.1992). Genetic monitoring of the RGSM using microsatellite and mitochondrial DNA commenced in 1999 and has continued annually (Turner et al. 2003; Alò and Turner 2005; Osborne et al. 2005; Osborne et al. 2006; Turner et al. 2006). Critical information gained from ongoing genetic monitoring has been used to adaptively manage the propagation program. These studies have provided insight into the mechanisms affecting levels and patterns of diversity in the wild population and captive stocks of RGSM. Major findings of the monitoring program include:

- The effective size of the wild population is several orders of magnitude less than the census size (Alò and Turner 2005). Additional data collected annually between 2002 and 2006 indicate the genetic effective size is around 100.
- There is little genetic structure among RGSM collected in three river reaches. This indicates that the middle Rio Grande population can be considered panmictic.

- Eggs collected from the downstream end of the current range of RGSM throughout the spawning period effectively capture the genetic diversity extant in the species.
- Wild caught eggs reared in captivity are more genetically diverse than captively spawned fish.
- Supportive breeding and augmentation have captured RGSM genetic diversity and restored that diversity in the wild fish (Turner and Osborne 2006 but see Osborne et al. 2006).
- Due to the *mismatched sweepstake* reproduction strategy of RGSM, in the event of a catastrophic loss of the wild population, approximately 1 million RGSM would be needed to reestablish a comparable population to the current middle Rio Grande population (Osborne et al. 2006.)

Genetic Management of RGSM

A comprehensive strategy to fulfill the goals outlined in the Plan and minimize the impact of captive propagation on RGSM wild populations involve three major areas of oversight: captive propagation, broodstock management and stock augmentation. The RGSM captive stock is multipurpose and will be used to increase the census size of breeding adults, to increase N_e and to serve as a genetic backup in the event of a catastrophic loss in the middle Rio Grande. The Plan provides guidance by defining goals, risks and tasks to manage RGSM captive propagation. However river conditions and the RGSM populations are neither stable nor static and will require ongoing monitoring and coordination of the captive propagation programs for all facilities to ensure continued successful mitigation of ongoing management activities.

Captive Propagation Goals:

The RGSM captive propagation and genetics workgroup recommend the following goals and objectives be considered to successfully stabilize and enhance

populations of RGSM throughout the middle Rio Grande basin and in restored populations.

Provide genetically appropriate RGSM for recovery efforts.

1) Coordinate and facilitate genetic management and production of RGSM for all facilities involved in RGSM propagation. This is the most parsimonious approach to identifying donor source locations, augmentation needs and annual production requirements.
   Task: Fund and hire a RGSM propagation coordinator
2) Maintain a captive stock of at least 5,000 and up to 15,000 adults to represent the middle Rio Grande.
   Task: Target no less than 10,000 adults at Dexter
   Task: Maintain at least 5,000 adults between other locations: BioPark, A-Mountain, Los Lunas and the Sanctuary
3) Maintain and maximize the effective population size and genetic diversity in captive stocks and in the river.
   Task: Conduct annual genetic monitoring of river population
   Task: Conduct annual genetic monitoring of broodstocks
   Task: Provide annual report with estimates of census numbers and population genetic parameters of all stocks
   Task: Annual stocking should target a minimum of 1,000 total progenitors (combined egg collection and captive spawn)
4) Ensure production fish contain acceptable genetic variation such that the overall diversity of the augmented population is not reduced.
   Task: Split eggs from each captive spawn whether paired mating or communal spawn into multiple groups. Those may be designated for:
      i. Middle Rio Grande stocking
      ii. Big Bend stocking
      iii. Other purposes (such as broodstock)
   Task: Document historic levels of allelic diversity and track changes over the course of the program.

Task: Ensure a combination of wild caught eggs and high numbers of captive parents such that no one section of river is overwhelmed with progeny from just a few parents.

5) Establish a monitoring program that will identify and minimize the sources of genetic change (domestication selection) in production fish for all facilities.

Task: Ongoing genetic monitoring will provide information on genetic changes (allele loss or change in frequencies) at broodstock facilities.

Task: Genetic data will be used to establish culture procedures that minimize the genetic loss or changes associated with intensive hatchery production.

Task: Ensure funding for genetic monitoring.

Production Goals:

1) Provide up to 300,000 adult RGSM for release into the middle Rio Grande annually. Primary source is eggs collected in the wild, held in captivity for 18 months and supplemented with captive propagation to reach stocking goals. Captively propagated fish would be held for 6 months prior to release as captive propagation starts prior to the natural spawning season and these individuals could be more easily raised to the minimum targeted size.

Task: Annual collection of a minimum of 500,000 eggs for captive rearing and subsequent stocking of RGSM in the middle Rio Grande. Egg collections will continue with eggs going to the BioPark and then distributed to Dexter. Dexter will supply juvenile fish to other facilities. $3/5^{ths}$ of eggs collected will go to Dexter; the remainder to other facilities. The production of adults from these eggs/larvae will provide the basis for releases in the following year. (For example, adults raised from eggs collected in 2008 will be released in fall 2009.)

2) Hatchery spawning and propagation with captive broodstock will be conducted annually to supplement production from Production Goal #1 above. (For example, if the total request for 2009 is 160,000 fish for the Middle Rio

Grande and the resulting adults from eggs collected in 2008 is 50,000, then captive spawning will be conducted to reach the total of 150,000

Task: Dexter will conduct paired or communal matings until 200 pairs successfully reproduce.

Task: The BioPark will conduct paired or communal matings until 50 pairs have successfully reproduced.

Task: The A-Mountain facility will conduct paired or communal matings until 50 pairs have successfully reproduced.

Task: All fish spawned or attempted to spawn will be removed from the broodstock. These adults will be stocked in the middle Rio Grande or the Big Bend area.

Task: Excess fish resulting from paired matings will be used for Recovery Goal 2.

3) Retain from 3,000 to 10,000 individuals from annual production from wild caught eggs as a backup broodstock at Dexter. The combination of A-Mountain, the BioPark and other facilities will retain no less than 5000 adults jointly.
4) Conduct all propagation and genetic management under the guidance of the RGSM workgroup and propagation coordinator.
5) Assess additional facilities such as Uvalde NFH, Los Lunas Refugium and the Albuquerque Sanctuary to determine the capabilities and roles to facilitate the RGSM propagation program.

Reestablish the RGSM in at least two other areas within the historic range.

Action: Maximize the genetic diversity of the potential population. Fish reared for this recovery goal are in addition to the production required for the middle Rio Grande program and numbers of eggs collected and paired matings will have to be adjusted accordingly.

1) Replicate the middle Rio Grande population in the Big Bend reach of the Rio Grande.

Year 1-2008) **Target Age 1 fish: 150,000**

Target Age 0 fish: 50,000

Dexter: 200,000

BioPark: 50,000

A-Mountain: Maintain backup broodstock

Age 1 fish were stocked at 4 locations in 2008: 28,000 age 1 fish stocked at Santa Elena Canyon; 42,300 age 1 fish stocked at Adam's Ranch; 32,600 age 1 stocked at Grassy Banks and 35,000 age 1 fish were stocked at Rio Grande Village. All broodstock spawned were fish produced from the Rio Grande egg salvage operations, with Dexter's age-1 fish produced from 400 individuals from the 02 year class.

Age 0 fish were stocked at 4 locations in 2008: 67,400 fish stocked at Santa Elena Canyon; 50,300 at Adam's Ranch; 72,300 age 0 at Grassy Banks and 68,000 age 0 fish were stocked at Rio Grande Village. The BioPark distributed age 0 fish, with 11,764 released at Adam's Ranch and 23,532 at the Rio Grande Village. All broodstock spawned were fish produced from the Rio Grande egg salvage operations, with Dexter's age-0 fish derived from 344 individuals - (264 from the 02 & 03 year classes and 80 from the 04 & 05 year classes).

Year 2-5) **Target Age 0 fish: 200,000 from wild caught eggs and captive spawning of no less than 300 fish combined.**

Task: The stocking effort should be conducted annually for no fewer than five years to capture and replicate the extant genetic diversity in the middle Rio Grande reach (Big Bend Reintroduction Plan – FWS)

Task: Pair matings or communal spawning should provide the progeny from no fewer than 150 pairs annually, with no more than 20 pairs per communal tank.

Task: Annual capture of 250,000 eggs for captive rearing and subsequent

stocking of RGSM in Big Bend and complement egg capture with communal spawning to reach targeted augmentation numbers.

2) No or minimal genetic changes resulting from ongoing culture activities.

1) Maintain bidirectional gene flow to ensure captive and wild fish remain genetically similar.

   Task: Egg collections should be made every year and the broodstock augmented even if no production or stocking is planned for the year.

   Task: Stocking commitment should rely on adults from wild caught eggs, but paired or communal matings can be used to reach a minimum target of 300 breeders annually.

2) Genetic monitoring should be conducted to ensure genetic changes are detected quickly and adjustments made to the program to prevent the divergence of captive and wild stocks.

   Task: Loss of genetic diversity as measured by the number of allelic variants greater than 10% in any one year is not acceptable. If this occurs, the propagation committee should meet and design a strategy to mitigate and restore diversity during the next spawning season.

3) Complete the comparison of communal spawning vs. paired mating spawning.

   Task: Optimize recommendation for paired matings, or designate numbers for communal spawning to maximize number of parental contributors.

4) Document genetic variation in wild stocks and hatchery stocks and annually compare with historic levels to maintain current levels of genetic variation.

   Task: Conduct annual genetic monitoring of river population.

   Task: Conduct annual genetic monitoring of production stocks.

   Task: Provide annual report with estimates of census numbers and population genetic parameters of all stocks.

5) Annually determine the targeted numbers of pairs of RGSM to be spawned at all facilities.

Task: No fewer than 300 pairs in a given year.

Task: Target should be at least 100 pairs for every 100,000 stocking commitment.

Task: All fish used in captive spawning efforts will be stocked out, or provided to other facilities as research animals. No fish will be spawned in captivity twice.

Summary

The genetic risks outlined in the Plan can be managed and, to some extent, mitigated if we have sufficient information. The ability to monitor genetic changes associated with multiple neutral genetic markers has allowed the development of an organized captive breeding program that considers all facilities involved in propagation and release of RGSM. Failure to follow a unified comprehensive strategy that focuses on recovery goals and the objectives outlined in the Plan could result in any one facility negatively impacting the overall program. In this document, we have outlined issues associated with augmentation strategies to enhance census numbers of wild populations. The facilities currently involved with RGSM propagation mutually agree on a centralized approach and together strive to mitigate the impacts of captive propagation. Current conservation genetics theory suggests that failure to follow the synergistic approach provided in the plan may negatively impact the RGSM recovery efforts.

The information provided in the Plan delineates the potential impact of non-adherence to the policies outlined within. The dynamic process of the RGSM propagation program provides a benchmark that illustrates the positive impact of a captive rearing program on the genetics of the wild population. The captive propagation workgroup will continue to use the best science available to adaptively manage the recovery process by integrating genetic information into the recovery and restoration of RGSM. Demographic data generated from ongoing research in the river and facility records will drive the decision process to determine *how many* fish to spawn in a given

year and effective genetic monitoring will provide the information to decide *who* to spawn. Joint decisions will determine which facilities will contribute progeny, which in conjunction with an annual estimate of N_e in the river, will provide a genetic snapshot of the RGSM population in the middle Rio Grande (Recovery Goal 1) and any newly established populations (Recovery Goal 2).

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