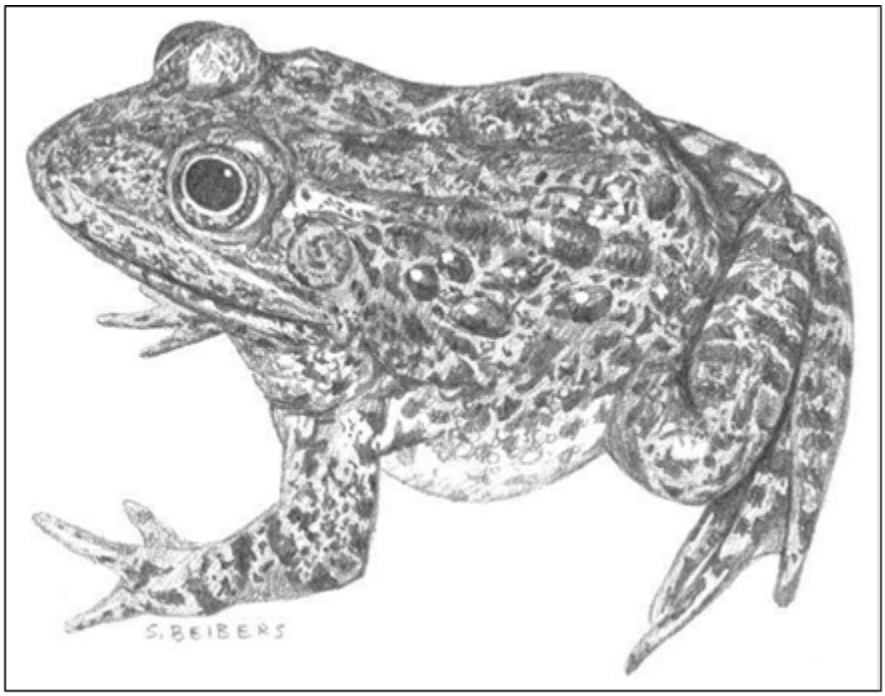
DUSKY GOPHER FROG

CONTROLLED PROPAGATION

AND

REINTRODUCTION PLAN



Prepared by:

Linda LaClaire
U.S. Fish and Wildlife Service
Jackson, Mississippi

June 23, 2017

The following reviewed this plan and provided helpful comments:

Kelly Bibb, U.S. Fish and Wildlife Service
Jim Lee, The Nature Conservancy
Joe Pechmann, Western Carolina University
Steve Reichling and Kim Terrell, Memphis Zoo

Cover Illustration:
Sam Beibers, Mississippi Museum of Natural Science

TABLE OF CONTENTS

PURPOSE 1
BACKGROUND 1
Listing and Critical Habitat… 1
Range… 1
Biology and Habitat… 1
Threats… 2
Population Trends… 3
Dusky Gopher Frogs in Captivity 2001-2016… 3
Dusky Gopher Frog Captive Colonies 3
History of Dusky Gopher Frog Captive Propagation 4
PURPOSE OF CAPTIVE PROPAGATION 4
GOALS AND OBJECTIVES OF THE CONTROLLED PROPAGATION PROGRAM… 5
PARTNERS… 5
CONSIDERATIONS FOR CONTROLLED PROPAGATION AND POPULATION REINTRODUCTION 5
Population Restoration Measures… 5
Justification of Population Restoration Measures… 6
GUIDELINES FOR DUSKY GOPHER FROG POPULATION RESTORATION EFFORTS 6
MANAGEMENT OF CAPTIVE DUSKY GOPHER FROGS 7
Species Survival Plan®… 7
Genetic Considerations 7
Genetics Management Guidelines… 8
Disposition of Excess Progeny… 8
Considerations for Releasing Captive-bred Dusky Gopher Frogs… 8
Need 8
Habitat Quality and Site Selection 9
Biosecurity and Housing… 10
Disease Prevention 10
Risk of Disease Transmission 10
Population Restoration Efforts Approval and Reporting… 10
Permitting 10

Reintroduction Release Plan 11
Reintroduction Monitoring and Habitat Management Plan 11
Reporting 12
CONTROLLED PROPAGATION AND REINTRODUCTION PLAN REVIEW 12
LITERATURE CITED 12

DUSKY GOPHER FROG
CONTROLLED PROPAGATION AND REINTRODUCTION PLAN

PURPOSE

The purpose of this document is to provide guidelines for dusky gopher frog (*Rana sevosa*) captive propagation and population restoration efforts in accordance with the *Policy Regarding Controlled Propagation of Species Listed Under the Endangered Species Act* (65 FR 56916) jointly published by the U.S. Fish and Wildlife Service (Service) and the National Marine Fisheries Service. This policy addresses the role of controlled propagation in the conservation and recovery of Federally-listed species (U.S. Fish and Wildlife Service and National Marine Fisheries Service 2000). This plan is also intended to facilitate coordination among all parties involved in captive propagation and population restoration efforts supporting recovery of the dusky gopher frog and to ensure those efforts are based on sound science. This document may be updated periodically as new scientific information becomes available.

BACKGROUND

Listing and Critical Habitat

The dusky gopher frog was listed as an endangered species under the Endangered Species Act of 1973, as amended, on December 4, 2001 (U.S. Fish and Wildlife Service 2001). Approximately 6,477 acres (2,621 hectares) in Louisiana and Mississippi have been designated as critical habitat for the dusky gopher frog (U.S. Fish and Wildlife Service 2012). The critical habitat is located in St. Tammany Parish, Louisiana, and Forrest, Harrison, Jackson, and Perry Counties, Mississippi.

Range

Historically, populations of dusky gopher frogs occurred in at least nine counties or parishes across the lower coastal plain ranging from the Mississippi River in Louisiana, through Mississippi, to the Mobile River delta of Alabama. At the time of listing in 2001, only one breeding site was known, located on the DeSoto National Forest (Glen's Pond). The last documented observation of a gopher frog in Louisiana was in the mid-1960s (Landry 2011), and in Alabama, it was last seen in 1922 (Bailey 1994). Since 2005, only two naturally-occurring dusky gopher frog populations have been verified through documented breeding. These populations occur in Harrison and Jackson Counties, Mississippi (U.S. Fish and Wildlife Service 2015).

Biology and Habitat

Dusky gopher frogs are amphibians with a complex life cycle that consists of aquatic eggs/larvae and terrestrial adults. They are endemic species of the longleaf pine ecosystem. Adult dusky gopher frogs spend most of their lives underground in forested habitat consisting of fire-maintained, open-canopied woodlands historically dominated by longleaf pine and having a grassy understory. Stump holes or small mammal burrows are the underground habitats where we currently observe them. Active and abandoned gopher tortoise burrows were

historically used as below-ground retreats (Allen 1932), and head-started metamorphic dusky gopher frogs released at a current translocation site have been observed using active gopher tortoise burrows (J. Lee, The Nature Conservancy, pers. comm. 2017). During the breeding season, dusky gopher frogs leave the uplands and migrate to their open-canopied isolated wetland (ponds not connected to any other water body) breeding sites that dry completely on a cyclic basis. The dominant source of water to dusky gopher frog breeding sites is rainfall within each pond's small, localized watershed. Adequate, frequent rain is needed to ensure that the ponds remain sufficiently full to allow hatching, development, and metamorphosis of larvae.

Breeding typically occurs from December through March after rains associated with passing cold fronts (Young 1997). According to published records, the number of eggs per egg mass in Mississippi range from 500 to 2,800 (Richter and Seigel 1997, 1998; Young 1997, Richter 1998). Dusky gopher frog egg masses take 9 to 21 days to complete hatching; the hatching rate is driven by water temperature (Richter and Seigel, unpublished data, Baxley and Qualls 2007). Metamorphosis occurs from mid-May to early August (Richter *et al.* 2003, Sisson *et al.* 2008) and may occur as early as 94 days after hatching (J. Pechmann pers. comm. 2014).

Threats

The dusky gopher frog was listed as an endangered species primarily due to threats of habitat loss and degradation, and small population size. These factors continue to affect the species' long-term viability. The quality of the available upland habitat within the historical range of the frog continues to decline, primarily through fire suppression and habitat fragmentation. The numbers of small wetlands that are potentially appropriate breeding sites have been reduced, especially by fire suppression and hydrological alterations.

Connectivity of dusky gopher frog breeding and nonbreeding habitat within the geographic area occupied by the species, and gene flow among local populations, are important for the species' survival. Extant dusky gopher frog populations are widely separated from each other by unsuitable habitat. Studies have shown that the loss of small, fragmented populations is common, and recolonization is critical for their regional survival (Fahrig and Merriam 1994, Burkey 1995, Marsh and Trenham 2001). Unfortunately, existing dusky gopher frog populations are too widely separated by distance and unsuitable habitat to be able to colonize most unoccupied sites.

A lethal disease killed most gopher frog tadpoles (mortality of adults from this disease has not been recorded) at their primary breeding site in 2003 (Overstreet and Lotz 2004). Recent monitoring indicates that this disease, an unnamed protist (*Dermomycoides* sp., also known as "*Perkinsus*-like" disease (Green *et al.* 2003, Jones *et al.* 2012)) is still present at the pond and is widely distributed in ponds across Mississippi and many other states. Annual mortality has been sporadic, however low recruitment in 2016 was likely attributable to this disease (J. Pechmann pers. comm. 2016). Ranavirus (*Ranavirus* spp.) is another species that has the potential to be a serious disease problem for the dusky gopher frog. Results from laboratory studies indicate that the dusky gopher frog is highly susceptible to infection from Ranavirus and, once infected, mortality approaches 100 percent (Sutton *et al.* 2014). As yet, no wild dusky gopher frogs have been found infected with Ranavirus. Chytrid fungus (*Batrachochytrium dendrobatidis*) causes

Chytridomycosis, a fungal disease that is responsible for decimating amphibian populations worldwide (Daszak *et al*. 2000). This disease has been detected in cricket frogs (*Acris gryllus*) and chorus frogs (*Pseudacris nigrita*) at Glen's Pond (D. Green, pers. comm. 2004), but thus far, no dusky gopher frogs have tested positive for the disease.

The genetic and population ecology data available for the dusky gopher frog illustrate the consequences of geographic range collapse and geographic isolation of populations: reduced overall population sizes, increased negative effects of variation in reproductive success, inbreeding-related mortality, low genetic diversity, and elevated probability of extinction (Richter *et al.* 2009, Richter and Nunziata 2014). Small populations are at increased threat from natural processes and stochastic events (genetic isolation, inbreeding, and drought) as well as the threats listed above and below.

Annual variability in rainfall influences how frequently a dusky gopher frog breeding pond fills, and, if it holds water long enough for metamorphosis of tadpoles to occur. Breeding events can be unpredictable and the likelihood that recruitment will occur in a given year cannot be predicted. Although rainfall variability is a result of natural processes, extreme weather events such as drought may have serious consequences for such isolated and small populations. During the drought that occurred in the early to mid-2000's, the dusky gopher frog was saved from extinction by using cattle tanks to head-start tadpoles after a breeding event occurred and releasing the resulting metamorphic frogs at Glen's Pond (Pechmann *et al.* 2017).

Population Trends

The Glen's Pond metapopulation on the DeSoto National Forest (DNF) remains the largest dusky gopher frog population. The two other naturally-occurring populations discovered in 2004 were both very small and appear to have died out during the drought of the 2000's. Nevertheless, the Service and our partners have been continuing with translocations of Glen's Pond head-started frogs to appropriate habitat. Initial releases were on a preserve owned by The Nature Conservancy (TNC); additional releases have occurred on multiple DNF sites, an additional TNC property, and on the Mississippi Sandhill Crane National Wildlife Refuge. Calling frogs have been heard at five of these seven sites and egg masses have been found at three sites. The dusky gopher frog's current distribution remains restricted to the state of Mississippi. Presently, we estimate that a minimum of 135 individual adult frogs survive in the wild, the majority of which occur in the original population known at the time of listing (U.S. Fish and Wildlife Service 2015).

Dusky Gopher Frogs in Captivity 2001 - 2016

Dusky Gopher Frog Captive Colonies

In 2001, due to the extreme rarity of the dusky gopher frog, tadpoles were sent to the Audubon Zoo and the Detroit Zoo in the first attempt to establish the species in captivity. In 2003, the Memphis Zoo was sent tadpoles as part of an effort to rescue them during the first disease outbreak of *Dermomycoides sp*. Memphis Zoo became the permit holder for management of the captive dusky gopher frog populations and personnel at the Memphis Zoo provide leadership for the Association of Zoos and Aquariums (AZA) Species Survival Plan® (SSP®; see further

discussion below under **MANAGEMENT OF CAPTIVE DUSKY GOPHER FROGS**). Subsequently, dusky gopher frogs, primarily tadpoles hatched from eggs collected at Glen's Pond, have been removed from the wild and placed in a number of zoos in an effort to capture the natural genetic variation in the species. In addition, zoo populations have increased due to captive propagation (see below). Memphis Zoo, as a part of their Federal permit responsibilities, must coordinate with each AZA facility which houses dusky gopher frogs and ensure AZA standards are met. They must also provide annual reports to the Service including for each facility: facility name; numbers of dusky gopher frogs in the captive population; mortalities; propagation attempts and successes; and any other conditions/observations important to successful propagation of the species. As of December 2016, captive populations are housed at 14 AZA institutions in the United States and one in Canada (Reichling 2016).

History of Dusky Gopher Frog Captive Propagation

Steve Reichling, Memphis Zoo, tried for many years to create artificial habitats with conditions that would encourage natural reproduction in dusky gopher frogs; however, he was unsuccessful in these efforts. In 2006, Dr. Andy Kouba, at that time also located at the Memphis Zoo, led the successful effort in developing in vitro fertilization (IVF) techniques for propagating the species. These techniques were successfully implemented and led to viable dusky gopher frog tadpoles both at the Memphis Zoo and at Omaha's Henry Doorly Zoo (Aaltonen *et al.* 2012, Kouba *et al.* 2012). Using protocols developed from these techniques, captive propagation is being used to maintain the genetic diversity in the captive population (Reichling and Sullivan 2016). The current dusky gopher frog AZA SSP® guidance is for retention of 5 individual frogs from each breeding pair at each breeding event and for euthanasia of the balance of the eggs not needed for other approved purposes (Reichling and Sullivan 2016).

PURPOSE OF CAPTIVE PROPAGATION

The primary purposes of dusky gopher frog captive propagation are to conserve the genetic diversity in the species and to contribute to its recovery by increasing the number of frogs available for reintroductions. Current policy (U.S. Fish and Wildlife Service and National Marine Fisheries Service 2000) states that our first priority is to recover threatened and endangered species in their native habitat, wherever possible, without resorting to the use of controlled propagation. We have made extensive improvements in unoccupied habitat to improve it for dusky gopher frogs; however, we believe that the rarity of the dusky gopher frog and its restricted distribution limit our ability to protect existing genetic diversity and increase the number of wild populations. Beyond our primary goals, there are other benefits to maintaining captive breeding populations of dusky gopher frogs. Frankham *et al*. (2004) provides the following as additional purposes of captive breeding populations: (1) to establish populations in secure *ex situ* locations; (2) to educate and engage the public on conservation issues; and (3) to provide opportunities for research of the species, yielding knowledge that can be applied to conservation in the wild.

GOALS AND OBJECTIVES OF THE CONTROLLED PROPAGATION PROGRAM

1. Enable additional restoration of wild dusky gopher frog populations by using captive-bred dusky gopher frogs in order to offset population declines, prevent extinction, and achieve recovery of the species.

2. Monitor and maintain dusky gopher frog genetic diversity within the captive population to reflect source dusky gopher frog populations in the wild and protect existing genetic variation in the species. Bring additional frogs into captivity, as necessary, for this purpose.

3. Maximize the genetic representation of wild dusky gopher frog populations in captivity for use in establishing new wild populations.

PARTNERS

A group of partners is working with the Fish and Wildlife Service on population maintenance and breeding of dusky gopher frogs in captivity. The Memphis Zoo has the lead responsibility for coordinating dusky gopher frog issues within the zoo community and communicating these to the Service recovery lead. Currently, zoo members of the dusky gopher frog SSP® include the Bergen County Zoo (Paramus), Birmingham Zoo, Como Zoo (St. Paul), Dallas Zoo, Detroit Zoo, Disney Animal Kingdom, Hutchinson Zoo, Lowry Park Zoo, Memphis Zoo, Omaha's Henry Doorly Zoo, Rio Grande Zoo, Toronto Zoo, Zoo Atlanta, and Zoo Knoxville. In 2017, facilities having biosecure housing and involved in breeding and raising frogs for release into the wild include: Dallas Zoo, Detroit Zoo, Memphis Zoo, and Omaha's Henry Doorly Zoo.

CONSIDERATIONS FOR CONTROLLED PROPAGATION AND POPULATION REINTRODUCTION

<u>Population Restoration Measures</u>

- Augmentation – describes the action of increasing numbers of a species within an occupied area of habitat through the transplantation of adults from other locations, or through the release of captive-bred individuals. Augmentation is appropriate when the population size of a listed species is minimal within an occupied area, and/or the population is experiencing recruitment failure or localized catastrophic events. At this time, augmentation of the original dusky gopher frog population with captive-bred progeny is not being considered as a recovery tool because the population is being sustained by natural reproduction and the use of head-starting (see below).

- Head-starting – a type of supplementation designed to decrease juvenile mortality. It involves collecting individuals of a particular life stage, usually a young and more vulnerable life stage (such as eggs, tadpoles, and juveniles), in the wild and hatching and/or rearing those individuals in a captive setting to release them back into their native habitat after they reach a certain age or size. We are using this technique to raise a portion of the dusky gopher frog eggs laid in the wild. Tadpoles are raised to metamorphosis in outdoor tanks near the breeding pond and the resulting metamorphic frogs are released back at their point of origin. When the number of metamorphic frogs is in excess of what is needed to maintain the primary breeding population, the excess frogs are released at previously unoccupied sites in an effort to establish new populations.

- Reintroduction – the movement of a species into historically occupied habitat where that species no longer occurs, and which is not accessible to natural immigration from extant populations. Reintroductions are intended to reestablish populations, provide genetic refugia, and reduce the potential of extinction due to random catastrophic events. Successful reintroductions may move a species closer to a recovery threshold and potential delisting. Reintroductions may be accomplished by translocating adults from extant populations, or through the release of captive-bred individuals.

- Translocations – deliberate and mediated movement of wild individuals or populations from one part of their range to another, a specific type of reintroduction (IUCN/SSC 1998).

Justification of Population Restoration Measures

The dusky gopher frog is in danger of extinction due to its restricted distribution and low population numbers. Due to the isolation of existing populations from one another, there was no avenue for natural immigration and emigration that would lead to the establishment of new populations or re-establishment of extirpated prior to initiating translocations. Together with our partners, we have worked extensively to improve unoccupied habitat within the historical distribution of the dusky gopher frog in Mississippi. Through a program of reintroductions, we have used head-started metamorphic frogs to attempt to establish new recovery populations. The Dusky Gopher Frog Recovery Plan also includes the action of revising and implementing a controlled propagation and reintroduction plan to facilitate use of captive dusky gopher frogs in our recovery program (U.S. Fish and Wildlife Service 2015). As we continue head-starting wild tadpoles to generate metamorphic frogs for reintroductions, we believe using captive-bred progeny from controlled propagation as part of our population restoration efforts provides another tool with the potential to expedite dusky gopher frog recovery.

GUIDELINES FOR DUSKY GOPHER FROG POPULATION RESTORATION EFFORTS

Population restoration efforts will follow established guidelines, such as those set forth by the AZA and IUCN/SSC (International Union for the Conservation of Nature/Species Survival Commission), and will be based upon the principles set forth in the Endangered Species Act. These guidelines include, but are not limited to, the following:

- Population restoration efforts will only take place within the dusky gopher frog's known historic range and at sites where the species no longer occurs.

- The habitat where the dusky gopher frog relocation is to occur must be suitable and under long-term protection. Human activities that may lead to the species' extirpation at the relocation site have been identified and discontinued.

- Captive-bred dusky gopher frogs will only be released into the wild as long as these population restoration efforts will not reduce the survivability of existing wild dusky gopher frog populations.

- There should be minimal risk of disease from population restoration efforts. Dusky gopher frogs will undergo rigorous health screening by qualified veterinarians and other personnel prior to their release (see *Risk of Disease Transmission* section below).

- There should be minimal disturbance at the relocation site from activities of the reintroduction managers and observers (AZA 1992). Any individuals involved in the release of dusky gopher frogs will be trained to minimize disturbances to the species and its habitat during release and monitoring activities.

- We will attempt to release large numbers of individuals (1,000+) during each population restoration effort in order to establish a population size necessary for normal social interactions and mating behavior. The number of individuals released (either at one time at the same site or during repeated releases over a period of time at the same site) has been shown to significantly affect success rates for amphibian and reptile population restoration programs (Griffith *et al*. 1989, Germano and Bishop 2008). We will also attempt to release successive cohorts at release sites to maximize the establishment and survival of the population (AZA 1992).

- Release protocols for each reintroduction event will be developed and included in associated reintroduction plans (see sections on *Reintroduction Release, Monitoring, and Habitat Management Plans* below). Partners in reintroduction actions should be made aware that captive breeding and reintroduction programs require long-term commitments from all parties to ensure success (Griffiths and Pavajeau 2008).

MANAGEMENT OF CAPTIVE DUSKY GOPHER FROGS

Species Survival Plan®

A Population Analysis and Breeding and Transfer SSP® has been developed for the dusky gopher frog by the AZA. The mission of an AZA SSP® Program is to cooperatively manage specific, and typically threatened or endangered, species populations within AZA-accredited zoos and aquariums, certified related facilities, and other AZA-approved participants (AZA 2009). Each SSP® Program is responsible for developing a comprehensive population studbook and a Breeding and Transfer Plan, which summarizes the current (short-term) demographic and genetic status of the captive population and identifies breeding or non-breeding recommendations with considerations given to each animal's social and biological needs as well as transfer feasibility. Steve Reichling of Memphis Zoo has taken the lead for generating the dusky gopher frog SSP® and Deanna Lance of Memphis Zoo is maintaining the dusky gopher frog studbook (Reichling and Schad 2012, Reichling and Sullivan 2016).

Genetic Considerations

All efforts should ensure that the genetic makeup of propagated individuals is representative of that in the free-ranging population and that propagated individuals are behaviorally and physiologically suitable for release back into the wild (U.S. Fish and Wildlife Service and National Marine Fisheries Service 2000). Dr. Stephen Richter of Eastern Kentucky University, and the Memphis Zoo, collaborated on a genetic analysis of the captive dusky gopher frog population. Results indicated that the genetics of the captive population reflected the wild population, and that many captive individuals were unrelated based on relatedness measures (Richter 2012, Hinkson *et al.* 2016). Since 2012, some of the original founders have died and other individuals have been added to the captive population. A second study of the genetic diversity of the captive population is being initiated in 2017 by Kristin Hinkson of the Memphis Zoo.

Genetics Management Guidelines

Captive propagation efforts should be based on sound scientific principles to conserve genetic variation and species integrity (U.S. Fish and Wildlife Service and National Marine Fisheries Service 2000). Population restoration using captive-bred frogs will follow established guidelines (such as those set forth by the AZA and IUCN/SSC) for balancing the maintenance of genetic diversity while retaining genetic lineages that are representative of wild populations.

- Steps should be taken to reproduce captive dusky gopher frogs before individuals within each population/ lineage approach natural senescence or post-reproductive stages (ages 5 to 6 years) to ensure each genetic lineage is maintained in captivity.
- Information from genetic studies and the dusky gopher frog studbook will be used to develop potential crossings for captive breeding purposes. These data will be important to avoid potential inbreeding affects.
- Priority for captive propagation should be given to lineages that will be used for population restoration efforts and for maintenance of genetic diversity in captive populations.
- The genetic identity of source parents used in all captive propagation efforts will be

recorded. Likewise, efforts will be made to verify the parentage of captive-bred frogs by genotype prior to their release into the wild.

Disposition of Excess Progeny

Propagation efforts will likely result in dusky gopher frogs in excess of needs for maintaining the captive population or conducting reintroduction activities. This may occur due to the following instances: (1) there are individuals found to be unfit for release into the wild, (2) there are individuals found to be unfit to serve as brood stock, (3) there are surplus individuals such as when the number of frogs generated during captive propagation is more than required for program needs; and/or (4) there are individuals considered surplus to the recovery needs of the species (for example, to prevent genetic swamping).

In the above instances, participating zoological institutions will confer with the Memphis Zoo (Steve Reichling) and the Service dusky gopher frog recovery lead (Linda LaClaire, Mississippi Ecological Services Field Office) to determine the best course of action for the disposition of excess dusky gopher frogs. Some options may include: (1) using unfit individuals in zoo exhibits for display and public education; (2) transferring individuals to other Service-approved facilities for display, research, or other approved purposes; (3) using individuals in research (including releasing individuals into exclosures in the wild for research purposes); and (4) euthanizing individuals. Frogs used in laboratory research or experimentation may not be introduced into the wild unless the studies are being conducted specifically to improve fitness of release animals.

Considerations for Releasing Captive-bred Dusky Gopher Frogs

Need

Dusky gopher frogs from the primary breeding population have been released at a number of sites designated as critical habitat (translocations). However, a drought during the 2000's limited the amount of natural reproduction and most of the head-started frogs raised during that time were used to support the primary population. Drought and other natural factors, such as on-going disease issues, may limit future attempts to establish additional recovery populations. Decisions to use captive-bred dusky gopher frogs in reintroduction efforts will be the made at the discretion of the Service and the Mississippi Department of Wildlife, Fisheries, and Parks after evaluation of the following factors: (1) need, based on an insufficient amount of wild dusky gopher frog progeny to meet recovery goals (translocations); (2) the quality of the habitat into which individuals are being placed; (3) risk of disease transmission; and (4) genetic concerns.

Habitat Quality and Site Selection

Because the threats of habitat loss, alteration, and fragmentation are thought to be the most consequential factors in the dusky gopher frog's decline, it is essential that any releases of captive animals for reintroduction purposes occur only where habitat quality is sufficient to sustain those populations and a long-term commitment has been made by the landowners to provide ongoing management activities to maintain it and participate in dusky gopher frog conservation. Sites for reintroduction activities will be selected based on criteria including

historical and current distribution of the species; habitat conditions; and past, present or future threats. Primary habitat considerations include presence of longleaf pine forested habitat with an open canopy and herbaceous groundcover, sandy soils, and appropriate breeding site availability (i.e., ephemeral wetlands with adequate hydroperiod and emergent vegetation). Priority will be given for those release sites with quality habitats where the threats facing the species have been eliminated or ameliorated, and where there is minimal probability of considerable pollution, manmade disaster, or exposure to exotic or introduced predators, competitors, or diseases (AZA 1992).

Biosecurity and Housing

Captive dusky gopher frogs shall be housed at facilities that are maintained under careful biosecurity (security from the introduction of non-native or novel disease agents) from other exotic amphibian and reptile species. Dusky gopher frogs must be held under biosecure conditions from the time they are brought into captivity if they are to be used for captive reproduction, will be housed with individuals that will be used for captive reproduction, or will be housed with individuals that will be reintroduced to the wild. Biosecurity measures must be included as an important component of any captive propagation program. Training programs for keepers should be reviewed annually and updated as required to ensure that biosecurity measures are being implemented to control the risk of disease occurring in dusky gopher frog captive colonies and to prevent the spread of disease through reintroductions. Program managers and staff must understand the importance of biosecurity and how to implement relevant protocols and keep abreast of new information and protocols as they become available.

Pessier and Mendelson (2010) have written a manual describing best management practices for controlling the spread of infectious disease within amphibian captive colonies and reintroduction programs. For example, Omaha's Henry Doorly Zoo, Dallas Zoo, Detroit Zoo and Memphis Zoo have biosecure buildings on their grounds dedicated solely to dusky gopher frog conservation. Biosecure areas must have rigorous health management and climate control as recommended by Griffiths and Pavajeau (2008) and outlined by Pessier and Mendelson (2010); these types of biosecure areas should be considered standard for dusky gopher frog captive facilities.

Disease Prevention

Risk assessment and addressing the potential of disease transmission are principal concerns when maintaining captive dusky gopher frogs for use in population restoration efforts. All facilities involved in the dusky gopher frog SSP® will conduct stringent veterinary examinations of dusky gopher frogs as they are brought into captive colonies in order to prevent the introduction of non-native or novel pathogens into captive populations (Pessier and Mendelson 2010), and prior to reintroduction efforts, as recommended by the AZA (1992). This would include implementation of a disease surveillance program that includes routine fecal screening, histopathology, and molecular testing designed to prevent the spread of disease and pathogens. Screening for known infectious agents, such as "chytrid fungus" and Ranaviruses, will be conducted prior to releases of dusky gopher frogs into the wild. Individuals will not be

released until they have been diagnostically screened and confirmed, or confidently presumed, to be healthy enough for released (Pessier 2008) by an experienced veterinarian. The primary goal of pre-release screening is to ensure that released animals will not introduce any novel pathogens into wild populations of amphibians. All captive dusky gopher frogs should also be inspected regularly for signs and symptoms of disease. We currently do not have a screening technique for *Dermomycoides sp.* in adult frogs; however, as tadpoles develop in captivity, they must be monitored for the disease.

The only potential sources for a novel pathogen to enter captive colonies would be from material or people that have moved into dusky gopher frog facilities from cosmopolitan collections housed in other areas of facilities, a zoo keeper with contaminated clothing from a collection of exotic amphibians at home, or contaminated materials (husbandry supplies, such as moss or soil, feeder invertebrates, or other supplies) from a source outside the host institution. All facilities involved in housing dusky gopher frogs will take every precaution to prevent disease and the transfer of novel pathogens from these or any other sources, in accordance with protocols presented by Pessier and Mendelson (2010).

Population Restoration Efforts Approval and Reporting

Permitting

Memphis Zoo holds a threatened and endangered species recovery permit issued by the Service under section 10(a)(1)(A) of the Endangered Species Act of 1973, as amended. Memphis Zoo will coordinate transfers to and from zoos in the dusky gopher frog SSP® program. As dusky gopher frogs leave Mississippi (frogs from the wild) or enter Mississippi (captive progeny to be reintroduced), additional Federal coordination with the species' recovery lead (Linda LaClaire, Mississippi Ecological Services Field Office) and State permits (State permit coordinator, Scott Payton) will be needed. If it is determined that a reintroduction will occur in Alabama or Louisiana, the appropriate Service Field Office and State agency will be involved in the decision and permitting process.

Reintroduction Release Plan

Reintroduction release plans will be developed through coordination between Federal and State agencies, the appropriate land management agency or non-governmental organization. Plans must be approved by the Mississippi Ecological Services Field Office (MSFO) prior to any population reintroduction effort that involves the release of captive-bred dusky gopher frogs into the wild. In some situations, releases of head-started dusky gopher frogs into an area different from their collection site may necessitate development of a reintroduction release plan; these situations will be handled on a case by case basis through coordination between MSFO and partners. Reintroduction plans should be designed to ensure adequate survival rates and to avoid genetic bottlenecks or swamping. Each reintroduction plan should include the following information:

- Location of release site – including landowner name and property location information (such as tract size, GPS coordinates, and map)
- Copy of written landowner permission for dusky gopher frog releases and access to property for post-release monitoring, if necessary

- Number of cohorts to be released
- Approximate number of individuals to be released from each cohort
- Life stage/age of each cohort to be released (tadpoles, juveniles, or adults)
- Source of captive stock of each cohort to be released – including facility name and genetic identification (if available) or geographic locality of source parents for each cohort to be released
- Results of health and disease screening of cohorts to be released
- Release protocols – including any pertinent information such as any pre-release conditioning, how the frogs will be transported, and/or whether soft or hard releases will be used

- Certification that all captive-bred progeny are pathogen-free at the time of release into the wild

Reintroduction Monitoring and Habitat Management Plan

Prior to moving forward with the release of dusky gopher frog captive-bred progeny in Mississippi, the Service and the Mississippi Department of Wildlife, Fisheries, and Parks will work together to develop a site-specific Reintroduction Monitoring and Habitat Management Plan. If reintroductions are proposed for Alabama or Louisiana, the corresponding state wildlife agency will partner with the Service in developing a plan for their state. If property habitat managers are part of another agency or non-governmental organization, they will also be included in the planning process. All recovery partners, and any other affected private or public entities identified by the partners, will be notified of planned reintroduction activities, and will be provided with the site-specific plan prior to relocating or releasing animals into the wild. At a minimum the plans will include:

- Brief explanation of how population restoration efforts at the site are expected to enhance the survivability and recovery of the species
- The exact location where animals are to be introduced
- Relationship of the reintroduction site to other populations of the target species
- Current habitat conditions (including threat assessment) at the reintroduction site
- Monitoring plan. Monitoring is critical to determine success and usefulness of reintroduction programs. At a minimum, reintroduction sites will be monitored annually for 10 years, or until there is evidence of success or failure
- Habitat management plan
- Predator management plan, if necessary
- Cooperating and responsible partners
- Any other pertinent information

Reporting

All reintroduction actions should be carefully documented. Partners conducting propagation studies, reintroduction releases, monitoring studies, or habitat management will provide an annual report of activities to the Service, the Mississippi Department of Wildlife, Fisheries, and Parks or other appropriate state agency, and any other involved partners. Depending on the action or study, examples of the information that should be included in an annual report include

the following:

- A description of the propagation program, including objectives and status
- Any deviations from the reintroduction plans, as approved, and explanations for why these deviations occurred
- List of cooperators, if any
- Information on disposition of individuals not used in the reintroduction, if applicable
- Results of post-release monitoring, if conducted
- Activities conducted, prospects for, or obstacles to, achieving the research, propagation, or reintroduction efforts
- A brief description of the status of the targeted reintroduced populations, if appropriate

CONTROLLED PROPAGATION AND REINTRODUCTION PLAN REVIEW

This plan is a working document that is subject to modification based on results of current and future research, and recovery activities involving dusky gopher frog propagation or reintroduction. Recovery partners are encouraged to provide comments and suggestions to the Service species' recovery lead. The Service will conduct an annual review of the Plan and will incorporate new information, protocols, and other pertinent information as it becomes available.

LITERATURE CITED

Aaltonen, J.T., T. Bohlender, W. Snyder, J. Krebs, L. Linoff, M. Snoza, S. Plesub, A. Kouba, J. Germano, S. Reichling, and N.M. Loskutoff. 2012. The developmental competence of tadpoles produced in vitro from the endangered dusky gopher frog (*Rana sevosa*) using exogenous hormone treatment. Reproduction, Fertility and Development 24:172.

Allen, M. J. 1932. A survey of the amphibians and reptiles of Harrison County, Mississippi. American Museum Novitiates 542:1-20.

AZA (Association of Zoos and Aquariums). 1992. AZA guidelines for reintroduction of animals. Available at: http://www.aza.org/reintroduction/. Date Accessed: April 19, 2017.

AZA (Association of Zoos and Aquariums). 2009. Species Survival Plan® Programs. Available at http://www.aza.org/species-survival-plan-programs/. Date Accessed: April 25, 2017.

Bailey, M. A. 1994. An investigation of the dusky gopher frog in Alabama. Unpublished report submitted to U.S. Fish and Wildlife Service, Jackson, Mississippi. 9 pp. + figure

Baxley, D. and C. Qualls. 2007. Monitoring, reproduction, and translocation of the Mississippi gopher frog (*Rana sevosa*). Unpublished report submitted to Mississippi Department of Wildlife, Fisheries, and Parks, Jackson, Mississippi. 21 pp. + tables and figures.

Burkey, T. V. 1995. Extinction rates in archipelagoes: implications for populations in fragmented habitats. Conservation Biology 9:527-541.

Daszak, P., A.A. Cunningham, A.D. Hyatt. 2000. Emerging infectious diseases of wildlife – threats to biodiversity and human health. Science 287: 443-449.

Fahrig, L. and G. Merriam. 1994. Conservation of fragmented populations. Conservation Biology 8:50-59.

Frankham, R., J.D. Ballou, and D.A. Briscoe. 2004. A primer of conservation genetics. Cambridge University Press, Cambridge, United Kingdom.

Germano, J.M. and P.J. Bishop. 2008. Suitability of amphibians and reptiles for translocation. Conservation Biology 23: 7-15.

Green, D.E., S. H. Feldman, and J. Wimsatt. 2003. Emergence of a Perkinsus-like agent in anuran liver during die-offs of local populations: PCR detection and phylogenetic characterization. Proceedings of the American Association of Zoo Veterinarians 2003:120-121.

Griffith, B., J.M. Scott, J.W. Carpenter, and C. Reed. 1989. Translocation as a species conservation tool: status and strategy. Science 245: 477-480.

Griffiths, R.A. and L. Pavajeau. 2008. Captive breeding, reintroduction, and the conservation of amphibians. Conservation Biology 22:852-861.

Hinkson, K. M., N. L. Henry, N. M. Hensley, and S. C. Richter. 2016. Initial founders of captive populations are genetically representative of natural populations in critically endangered dusky gopher frogs. Zoo Biology 35:378-384.

IUCN/SSC (International Union for the Conservation of Nature/Species Survival Commission). 1998. Re-introduction practitioners directory. 11 pages.

Jones, M.E.B., J.G. Armien, B.B. Rothermel, and A.P. Pessier. 2012. Granulomatous myositis associated with a novel alveolate pathogen in an adult southern leopard frog (*Lithobates sphenocephalus*). Diseases of aquatic organisms 102:163-167.

Kouba, A., E. Willis, C. Vance, S. Hasenstab, S. Reichling, J. Krebs, L. Linhoff, M. Snoza, C. Langhorne, J. Germano. 2012. Development of assisted reproduction technologies for the endangered Mississippi gopher frog (*Rana sevosa*) and sperm transfer for in-vitro fertilization. Reproduction, Fertility and Development 24:170.

Landry, K. 2011. Email correspondence between Linda LaClaire and Keri Landry, Louisiana

Natural Heritage Program, Louisiana Department of Wildlife and Fisheries, Baton Rouge, Louisiana. October 4, 2011.

Marsh, D.M. and P.C. Trenham. 2001. Metapopulation dynamics and amphibian conservation. Conservation Biology 15:40-49.

Overstreet, R. M., and J. M. Lotz. 2004. Initial results of a study involving a Perkinsus-like organism infecting the Mississippi gopher frog. Unpublished report to the U. S. Fish and Wildlife Service.

Pechmann, J.H.K., J.A. Tupy, M.S. Atkinson, and J.E. Smith. 2017. Research on conservation and recovery of the endangered dusky (Mississippi) gopher frog, 1 January 2012 – 31 December 2016. Unpublished final report for Grant # F11AP00652 submitted to U.S. Fish and Wildlife Service, Jackson, Mississippi. 74 pp.

Pessier, A.P. 2008. Management of disease as a threat to amphibian conservation. International Zoo Yearbook 42:30-39.

Pessier, A.P. and J.R. Mendelson (editors). 2010. A Manual for Control of Infectious Diseases in Amphibian Survival Assurance Colonies and Reintroduction Programs. IUCN/SSC Conservation Breeding Specialist Group: Apple Valley, Minnesota.

Reichling, S. 2016. Annual report for activities under Permit #TE 171493. Unpublished report to Mississippi Ecological Services Field Office, Jackson, Mississippi. 3 pp.

Reichling, S. and K. Schad. 2012. Population analysis and breeding and transfer plan. Dusky gopher frog (*Rana sevosa*) AZA Species Survival Plan®, Yellow Program. Association of Zoos and Aquariums. Population and Management Center, Chicago, Illinois. 117 pp.

Reichling, S. and S. Sullivan. 2016. Population analysis and breeding and transfer plan. Dusky gopher frog (*Rana sevosa*) AZA Species Survival Plan®, Yellow Program. Association of Zoos and Aquariums, Silver Spring, Maryland. 117 pp.

Richter, S.C. 1998. The demography and reproductive biology of gopher frogs, *Rana capito*, in Mississippi. Master's Thesis, Southeastern Louisiana University, Hammond, Louisiana. 94 pp.

Richter, S.C. 2012. Genetics of captive and natural populations of dusky gopher frogs. PowerPoint presentation given March 10, 2012, at the Mississippi Gopher Frog Conservation through Reintroduction and Translocations Workshop, Memphis Zoo, Memphis, Tennessee. 23 pp.

Richter, S.C., B.I. Crother, and R.E. Broughton. 2009. Genetic consequences of population reduction and geographic isolation in the critically endangered frog, Rana sevosa. Copeia

2009:801-808.

Richter, S.C. and S.O. Nunziata. 2014. Survival to metamorphosis is positively related to genetic variability in a critically endangered amphibian species. Animal Conservation 17:265-274.

Richter, S.C. and R.A. Seigel. 1997. Demography and reproduction of gopher frogs in Mississippi. Project No., E-1, Segment 11, Museum Technical Report No. 50. Unpublished report submitted to the U.S. Fish and Wildlife Service, Jackson, Mississippi. 21 pp.

Richter, S.C. and R.A. Seigel. 1998. Demography and reproduction of gopher frogs in Mississippi. Project No., E-1, Segment 12, Museum Technical Report No. 51. Unpublished report submitted to the U.S. Fish and Wildlife Service, Jackson, Mississippi. 24 pp.

Richter, S. C., J. E. Young, G. N. Johnson, and R.A. Seigel. 2003. Stochastic variation in reproductive success of a rare frog, *Rana sevosa*: implications for conservation and for monitoring amphibian populations. Biological Conservation 111: 171-177.

Sisson, M.A., M. Murphy, and J.A. Tupy. 2008. Unpublished final report prepared for the U. S. Fish & Wildlife Service, Jackson, Mississippi, in partial fulfillment of Mississippi Endangered Species Project E-1, Segment 24 [Museum Technical Report No. 142]. 17 pp.

Sutton, W. B., M. J. Gray, R. H. Hardman, R. P. Wilkes, A. J. Kouba, and D. L. Miller. 2014. High susceptibility of the endangered dusky gopher frog to ranavirus. Diseases of Aquatic Organisms 112:9-16.

U.S. Fish and Wildlife Service. 2001. Endangered and threatened wildlife and plants; final rule to list the Mississippi gopher frog distinct population segment of dusky gopher frog as endangered. Federal Register 66:62993-63002.

U.S. Fish and Wildlife Service. 2012. Endangered and threatened wildlife and plants; designation of critical habitat for dusky gopher frog (previously Mississippi gopher frog). Federal Register 77:35118-35161.

U.S. Fish and Wildlife Service. 2015. Dusky Gopher Frog (*Rana sevosa*) Recovery Plan. Atlanta, Georgia. 86 pp.

U.S. Fish and Wildlife Service and National Marine Fisheries Service. 2000. Policy regarding controlled propagation of species listed under the Endangered Species Act. 65 Federal Register 56916-56922.

Young, J.E. 1997. Taxonomic status of *Rana capito* and *Rana sevosa*: Reproduction and movements of *Rana sevosa* in Mississippi. Unpublished M.S. thesis, Southeastern Louisiana University, Hammond, Louisiana. 80 pp.