

**Project Title:** A Conservation Strategy for the Imperiled Striped Newt (*Notophthalmus perstriatus*) in the Apalachicola National Forest, Florida.

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## **Introduction**

The Florida Gas Transmission Company is scheduled to increase the amount of natural gas it transports throughout the U.S. Gulf Coastal region. To accomplish this task, an already existing natural gas pipeline that spans east/west across the Munson Sandhills region south of Tallahassee will be expanded to accommodate additional natural gas transmission. Of particular concern is the expansion of the existing route that runs through the portion of the Munson Sandhills owned by the Apalachicola National Forest (ANF). A significant amount of ANF acreage will be altered in order to accommodate the expansion of the pipeline right-of-way.

The ANF portion of the Munson Sandhills where the pipeline expansion will occur is currently a longleaf pine sandhill ecosystem harboring abundant ephemeral wetlands that serve as breeding sites for the rare striped newt (*Notophthalmus perstriatus*) and many other amphibian species (Means and Means 2008). Longleaf pine sandhill with embedded ephemeral wetlands is the preferred habitat for the striped newt. The native longleaf pine ecosystem of much of the Munson Sandhills outside of the ANF has extensively been altered and reduced by development and incompatible land management over the last several decades, and the striped newt is absent there (Means and Means 2005). The last remaining portion of relatively healthy longleaf pine ecosystem still suitable for striped newts in this region occurs within ANF lands.

Striped newts have a complex life cycle (Johnson 2002). Sexually mature adults migrate from the surrounding uplands to fishless, ephemeral wetlands to breed in mid-winter, November-February. Courtship, copulation, and egg-laying take place from January to April and eggs hatch beginning in April. Larvae grow in the ephemeral pond environment for several months until mid summer when they typically metamorphose. Once larvae reach metamorphosis size, larvae may either undergo metamorphosis and exit the pond or remain in the pond and grow, eventually maturing as a paedomorph (Petranka 1998, Johnson 2005). If the pond goes dry, then larvae must either metamorphose or perish. There is evidence that small larvae can metamorphose by at least 3 months of age, at which time they lose their external gills, develop lungs for air-breathing, and become a relatively dry-skinned animal called an eft (Johnson 2002). The

eft stage is adapted for life in the longleaf pine-wiregrass forest of the adjacent hot and dry sandhills (Means 2007). Minimum water residency time (hydroperiod) in a given wetland suitable for breeding and larval development into the eft stage is approximately 6-7 months, from December-January through June (Means 2007). When efts return to the wetland to breed, they enter the wetland and undergo another metamorphosis into the aquatic adults. Aquatic adults have a long tail fin, larger body size, and slick skin. Males develop swollen vents and robust hind limbs.

The natural global distribution of the striped newt is small and restricted to parts of South Georgia and the northern half of the Florida peninsula, and into the eastern Florida Panhandle (Conant and Collins 1998). New evidence suggests there are 2 genetic variants of the striped newt— “western” and “eastern” groups (May et. al *in review*). The western genetic group is composed of populations from the Gulf Coastal Plain of southwest Georgia and the eastern Florida Panhandle. The eastern group is composed of populations scattered around several public lands in central and north Florida east of the Suwannee River, and a few locations in the Atlantic Coastal Plain of Georgia.

In the past 2 decades, numerous surveys have been conducted to more thoroughly document the occurrence and distribution of striped newts in Florida and Georgia (Dodd and LaClaire 1995, Franz and Smith 1999, Johnson and Owen 2005, Means 2007, K. Enge, FFWCC, pers. comm., L. Smith, JJEC, pers. comm., J. Jensen, GDNR, pers. comm.).

In Georgia, the striped newt is a state-listed threatened species. It has been recorded from approximately 30 wetlands on 15 properties statewide. As of 2007, only 2 properties in the state are known to support viable populations: Joseph W. Jones Ecological Center at Ichauway (JJEC) and Fort Stewart Army Base (Stevenson et al. 2007). The Fort Stewart population lies within the range of the eastern genetic group on the Atlantic Coastal Plain and was represented by approximately 10 known wetlands. Since 2002, striped newts have been found at only 1 wetland (Stevenson et al. 2007). The JJEC population lies within the range of the western genetic group on the Gulf Coastal Plain and is represented by 5 known wetlands. In an annual survey from 2002-2010, researchers confirmed striped newts from only 3 of these 5 known wetlands (L. Smith, JJEC, pers. comm.). Evidence suggests that both the eastern and western striped newt populations in Georgia are rare and declining.

The current distribution and conservation status of the striped newt in Florida is best summarized by 2 ongoing surveys. Since 2005, the Florida Fish and Wildlife Conservation Commission (FFWCC) has collaborated with land managers and other scientists in a state-wide effort to document status and distribution of the striped newt. This campaign concentrates sampling efforts on public lands and represents the widest ranging effort thus far undertaken in Florida to document striped newt occurrence (K. Enge, FFWCC, pers. comm.). The second ongoing survey is being conducted by the Tallahassee based Coastal Plains Institute (CPI). CPI has conducted numerous amphibian surveys in approximately 200 ephemeral wetlands across the Munson Sandhills of the ANF since 1994 (Means et al. 1994a, Means et al. 1994 b, Means 1996,

Means and Printiss 1996a, Means and Printiss 1996b, Means and Means 1997, Means and Means 1998a, Means and Means 1998b, Means 1999, Means 2001, Means and Means 2005, Means 2007, Means 2008, R.C. Means and D.B. Means unpublished data).

There are approximately 124 known historic and active wetlands for the striped newt in Florida, on 19 public lands (K. Enge, FFWCC, pers. comm.). This figure is generated from cumulative data of all past and present striped newt surveys. Of the 19 properties where striped newts have been documented to occur, only 5 are considered “stronghold” sites, where greater than 10 wetlands used for breeding by striped newts have been documented (Johnson and Owen 2005). The 5 Florida stronghold sites and their number of known newt breeding wetlands are: Ocala National Forest (ONF) (39), ANF (21), Camp Blanding Military Reservation (13), Ordway-Swisher Preserve (13), and Jennings State Forest (12). Stronghold sites probably represent metapopulations. The remaining 14 properties have 5 or fewer known striped newt wetlands.

The ONF contains the largest aggregation of striped newt localities in the eastern genetic group’s distribution and also represents the largest grouping of localities globally. The ANF contains the largest aggregation of striped newt localities in the western genetic group’s range and second largest globally. The ANF also is the only stronghold site within the western genetic group.

FFWCC data suggest, at this time, that the striped newt exists as a relatively robust, healthy population on only 1 property in Florida—the ONF. The other 18 properties known to harbor striped newts in Florida appear to have rare, declining, or otherwise imperiled populations that produce newts unreliably or in low abundance (K. Enge, FFWCC, pers. comm.).

CPI sampling data clearly show that, up until 1999, individuals of the western striped newt in the ANF were relatively abundant. However, since that time, the striped newt in the ANF has undergone a mysterious decline. Alarming, during the last 11 years, fewer than 10 adults and 0 larvae have been captured despite repeated sampling efforts by CPI. No individuals have been observed since 2007. CPI’s sampling data strongly indicate a sharp decline in the world’s second largest stronghold, the ANF. There is mounting evidence to suggest that the western striped newt on the ANF is very nearly, or possibly already, extirpated.

CPI’s sampling data from the ANF through 2007, coupled with data from other researchers, was the impetus for the petitioning to federally list the striped newt as “threatened” under guidelines of the Endangered Species Act (Means et al. 2008). In March 2010, the U.S. Fish and Wildlife Service issued a 90-day notice of listing for the striped newt in the Federal Register in response to the petition (Endangered and threatened wildlife and plants, 2010).

One possible cause of the striped newt decline in the ANF is drought. Drought has been linked to some amphibian declines and extirpations of populations (Pounds et al. 1999, Lips et al. 2005). Since 1998, North Florida experienced two prolonged, excessive

droughts during the 10-year period from 1998-2008 (M. Griffin, Florida Climate Center, pers. comm.). Severe droughts lasted from 1998-2001 and 2006-2008. Hydroperiods were much shorter in ephemeral wetlands across the Munson Sandhills during the droughts (R. C. Means and D.B. Means, unpublished data). Rarely were there prime opportunities for striped newts to breed, and when there were opportunities, CPI biologists did not detect larval newts despite considerable sampling effort (Means 2007, Means 2008). When populations become isolated, as is the case for the ANF striped newt and other striped newt localities, the effects of annual reproductive failures may result in extirpation.

Another possible cause of decline in the ANF striped newt is pathogen infection. This past winter breeding season (2010) was the first winter in quite some time that heavy rains and subsequent pond fillings ensued, creating what appeared to be a prime breeding season for striped newts. No newts were observed in any of the 20 historic newt localities nor in 21 other suitable wetlands sampled (R.C. Means and D. B. Means unpublished data). Suspiciously the eastern newt (*Notophthalmus viridescens*) was also virtually missing from this survey. CPI biologists encountered many other pond-breeding amphibian species in relative abundance during the same sampling period, including the ornate chorus frog (*Pseudacris ornata*) and gopher frog (*Lithobates capito*). CPI data suggest that both resident newt species in the area are in decline. Extensive field work by U.S. Geological Survey biologists indicates a similar situation at the nearby St. Marks National Wildlife Refuge (Dodd et al. 2007). That 2 closely related newt species are the only species in decline in the region may suggest that pathogen infection is in play within the ANF.

Other causes for decline could be off-road vehicular disturbances to breeding ponds, incompatible land management techniques, development, and encroachment of woody shrubs and pines into pond basins (Means et. al 2008). It is unknown which single factor or combination of factors is the culprit behind the decline. We suggest that some combination of the above factors is the most likely cause, with emphasis on drought and/or pathogen infection.

Combined sampling data from Florida and Georgia show that the striped newt is in decline globally. This was known by 2004, which prompted the striped newt be listed as NT (“near threatened”) on the IUCN Red List of threatened species (IUCN 2010). Since then, the decline has only worsened in both the eastern and western groups, particularly in the western. The ANF decline, coupled with apparent declines in all other sites containing the western striped newt in Florida and Georgia, indicate that the western striped newt is on the brink of extirpation.

Various techniques are available to as conservation strategies that could benefit the striped newt metapopulation in the ANF. Relocation, repatriation, and translocation (RRT) may be used to help boost populations of imperiled amphibians (Marsh and Trenham 2001, Semlitsch 2002, Denton et al. 2003, Germano and Bishop 2008). As defined in Dodd and Seigel (1991) and Dodd (2005), relocation involves the moving of individuals from a threatened area to where they would be less prone to habitat loss.

Repatriation is the releasing of animals into an area formerly or currently occupied by that species. Translocation involves the release of animals into areas where they historically were not known to occur.

Many factors can affect the success of repatriation including: location of source populations; whether animals are wild-caught or captive-reared; age or life-history stage of the individuals; knowledge of the threats that caused the original decline; and the number, frequency, and timing of the translocation event (Semlitsch 2002, Dodd 2005, Germano and Bishop 2008). These factors need to be considered before a RRT project is initiated. Additionally, long-term monitoring is needed to assure the release has been effective at re-establishing populations (Dodd 2005).

The value of RRT as a conservation tool has been debated widely over the past 20 years. Primary concerns are the lack of proven success and the potential for transmission of disease (Dodd and Seigel 1991, Seigel and Dodd 2002, Dodd 2005). However, in a recent review of 38 amphibian RRT studies, Germano and Bishop (2008) report the success rate of RRT has doubled since the initial 1991 review of RRT by Dodd and Seigel (1991).

The success of a repatriation effort can depend on the conditions at the release site. Emerging infectious diseases can reduce the likelihood of success if an infectious agent is present and the reintroduced species is highly susceptible (Green et al. 2009). There are 2 major pathogens associated with catastrophic amphibian die-offs: *Batrachochytrium dendrobatidis* (*Bd* or the chytrid fungus) and group of viruses belong to the genus *Ranavirus* (Gray et al. 2009a, Kilpatrick et al. 2009). The chytrid fungus is primarily pathogenic to adult anurans, and most die-offs have occurred at high elevations in tropical latitudes (Collins and Crump 2009). Although *Bd* is known to infect eastern newts (Rothermel et al. 2008), chytridiomycosis rarely develops in this species (D. Miller, University of Georgia, unpubl. data) or in other salamanders (Chatfield et al. 2009, Hossack et al. 2010). Ranaviruses have caused mortality in captive and wild amphibian populations on five continents and at all latitudes and elevations that amphibians inhabit (Gray et al. 2009a). Mortality events from ranaviruses have been documented in 8 frog and 3 salamander families, including newts. In North America, ranaviruses are likely a greater threat to amphibians than *Bd* (Gray et al. 2009b). Only few cases exist in the western United States where *Bd* may be playing a role in population declines (Collins and Crump 2009). In contrast, catastrophic die-offs of wild amphibian populations have occurred in >30 U.S. states and 5 Canadian provinces (Green et al. 2002, Gray et al. 2009a), prompting Gray et al. (2009b) to hypothesize that ranaviruses may represent that greatest disease threat to amphibian biodiversity in North America.

Ranaviruses are pathogenic to adult and larval amphibians; however, mortality rates tend to be greater for larvae in North America (Gray et al. 2009a). Hoverman et al. (2010) reported that susceptibility to ranavirus differs among amphibian species and viral strains. The University of Tennessee Center for Wildlife Health recently completed experimental challenges with 19 amphibian species, and determined that several species of Ranidae (*L. capito*, *L. sphenocephalus*), the eastern spadefoot (*Scaphiopus holbrookii*), and 2

ambystomatid salamanders (*Ambystoma tigrinum*, *A. opacum*) were highly susceptible to ranavirus infection (Gray et al., unpubl. data). Most concerning, gopher frog tadpoles experienced 80% mortality, and 100% of gopher frog metamorphs died within 6 days of being exposed to ranavirus (Gray et al., unpubl. data). Gopher frogs are decreasing throughout much of their range, and commonly coexist with striped newts in the ANF (Means 1998). Southern leopard frogs also are highly susceptible (experiencing 50% mortality, Gray et al., unpubl. data) and occur in the ANF. Highly susceptible species can increase the likelihood of an outbreak because high mortality rates amplify virion concentration and increase transmission rates; these species are commonly called “superspreaders” (cf. Lloyd-Smith et al. 2005). Thus, amphibian communities composed of highly susceptible species may be more prone to emergence of ranaviral disease.

The presence of ranavirus in the ANF and susceptibility of striped newts to infection are unknown. Eastern newts have been associated with ranavirus die-offs (Green et al. 2002), but it appears they have relatively low mortality (10%) when exposed to ranavirus in a water bath (Gray et al., unpubl. data). Catastrophic die-offs of alpine newts (*Mesotriton alpestris*) from ranavirus have been documented in Spain (Balseiro et al. 2009), and several species of North American ambystomatid salamanders are highly susceptible (Collins et al. 2004, Cotter et al. 2008). The relative susceptibility of striped newts to ranaviral infection and disease needs to be determined. If this species is susceptible, it is important to identify repatriation sites that are ranavirus-free and composed of amphibian communities with few superspreader species. Alternatively, repatriation could involve postmetamorphic individuals instead of larvae because the former are typically less susceptible, although this remains to be tested for striped newts.

If repatriation is warranted and donor individuals come from an area outside the distribution of the imperiled population, wild-caught individuals can be tested (Gray et al. 2009a) and captive-raised individuals can be obtained from a disease-free source (S. Reichling, Memphis Zoo, pers. comm.). It is crucial that repatriated individuals be disease free (Green et al. 2009).

Wetland augmentation is another technique that can be used to help critically imperiled species, particularly in wetlands impacted by drought or withdrawal for human consumption. In this process, water from a local or distant additional source is pumped into a wetland periodically to increase water residency time (hydroperiod) and water depth. Long-term groundwater augmentation of a wetland suffering from desiccation was conducted for 20 years in southwest Florida to mitigate for damage caused by groundwater withdrawal by municipalities to supply public drinking water (Berryman and Hennigar 1995). However, the wetland water chemistry shifted over time to that of a more calcareous, clear-water wetland system reminiscent of a Florida spring. No one studied the potential responses of amphibians at the augmented wetland.

Altering water chemistry is a concern when considering wetlands augmentation as a conservation strategy for amphibians (Means and Franz 2005). In one study, researchers documented the bioaccumulation of <sup>226</sup>Radium in fishes (bone), unionid mussels (shell and mantle), plants, and lake-bottom sediments from groundwater originating in

phosphate-rich sediments of the Floridan Aquifer System at augmented Round Pond in Hillsborough County (Brenner et al. 2000). Researchers concluded that high levels of radium in soft tissues could represent an important pathway for transfer of radio-nuclides into higher trophic levels in both aquatic and terrestrial food webs.

Clearly, some issues have been raised with respect to long-term wetland augmentation using water of different chemistry. However, research associated with short-term augmentation has provided more encouraging results. In southern Mississippi, researchers used groundwater to augment hydroperiod of a single ephemeral wetland so that larvae of the endangered dusky gopher frog (*L. sevosa*) would have enough water in the pond to complete metamorphosis (Seigel et al. 2006). As a result of the groundwater augmentation, 130 metamorphic frogs were produced, the first successful reproduction for the species since 1998.

Water chemistry effects also were investigated as part of the above study. They tested for effects of well water on tadpole survival in a surrogate species, the southern leopard frog (*L. sphenoccephalus*). They reared tadpoles in well water and in original pond water and found that there was no difference in survival between the 2 groups (Seigel et al. 2006). The researchers rightly were concerned that water chemistry of the wetland may change toward that of added groundwater. However in their study, adding well water to the wetland had only mixed effects on water chemistry. Researchers investigated effects on 2 analytes, pH and dissolved oxygen. Wetland pH was variable both before and after augmentation, but was typically higher after augmentation. Changes in dissolved oxygen were minor.

In an augmentation study in northeast Florida, early indications from one study site mirrored the results from Seigel et al. (2006). Namely, a particular augmentation event at the experimental wetland provided a boost to wetland hydroperiod sufficient enough to allow for the metamorphosis of hundreds of concentrated anuran larvae in a dry-up pool that otherwise would have perished in an impending pond dry-up (Means and Meegan 2004).

The northeast Florida study also provides evidence suggesting that short-term augmentation does not significantly alter water chemistry (CH2M Hill 2004a, Ch2M Hill 2004b). A much longer list of analytes were measured, including the 2 investigated in the Siegel et al. (2006) study. In 2 wetlands augmented with groundwater at different sites, measured water quality parameters were similar both before and after augmentation. Although groundwater chemistry and surface pond water chemistry were initially different, researchers hypothesized that groundwater pumped slowly into the wetland along the wetland edge allowed for the added water to slowly seep through the surface detritus and naturalize to ambient water conditions. This study conducted multiple augmentation boosts, each lasting for 24 hours, and spaced 6 months apart.

Although more studies are needed, we believe that there is enough evidence suggesting that repatriation and short-term wetland augmentation can be used to help critically imperiled amphibian species avoid local extirpation or even extinction. We also believe

that the western striped newt is imperiled enough to warrant the use of repatriation and, if necessary, augmentation (to avoid wetland drying) to boost population size.

Other techniques may be used for striped newt conservation, such as proper habitat management (i.e. summer burning, reduction of encroaching shrubs and trees into suitable breeding marshes, elimination of ATV disturbance of wetlands). CPI has supported proper habitat management for striped newts on the ANF over the years. However, considering the crisis with the western striped newt, habitat management becomes a moot point if the western striped newt becomes extirpated.

A repatriation study in the ANF, coupled with short-term augmentation to avoid wetland dry-up during repatriation, could provide not only a boost to striped newt populations, but also could provide more data on promising conservation methods for imperiled species. Siegel et al. (2006) call for additional replication studies. With a sharp decline underway of one of Florida's rarest amphibian species in its greatest western geographical stronghold (and second greatest globally), it is reasonable to assume that any additional impacts to its local habitat, such as the gas pipeline expansion, may have detrimental, long-term effects on the western genetic group, and on the entire species. If striped newts are already extirpated from the ANF, immediate repatriation is necessary while there are still other western populations in existence to use as repatriation sources and before the loss of the entire western genetic group potentially occurs.

### **Study Proposal**

CPI proposes to conduct a multifaceted 5-year study in the ANF as a conservation strategy that will serve as mitigation for additional damages incurred to the imperiled striped newt by gas pipeline corridor expansion. As part of this study, CPI will continue to monitor ephemeral wetlands in the ANF for the presence of striped newts. Assuming striped newts continue to be absent from ANF wetlands, we propose to conduct larval repatriation in 2 selected wetlands. As a precaution, we propose to conduct a ranavirus surveillance assessment of select ANF wetlands and test the susceptibility of striped newts to ranavirus before repatriation. Short-term groundwater augmentation of repatriated wetlands also is proposed on an as-needed basis to prevent the wetlands from drying during repatriated larval development periods.

### **Study Objectives**

1. Extensively sample the ANF for striped newts during winter breeding and spring larval seasons. Based on 11 years worth of sampling data, we hypothesize that the ANF striped newt is extirpated, or very nearly so. Despite our hypothesis, we believe it is necessary to more thoroughly assess the status of the striped newt in order to either reject or fail to reject our hypothesis, before repatriation occurs. If we detect that newts have rebounded from decline in the ANF (i.e. we reject our hypothesis), we will not proceed with repatriations. If newts are found to exist in low abundance in the ANF (less than 5 wetlands), but high enough abundance to obtain individuals to establish an assurance colony with, then we will repatriate using ANF sourced

individuals. If we fail to reject our hypothesis, then repatriation will proceed as outlined in Objective #4. Approximately 200 wetlands will be sampled twice per year for 2 years.

2. Collect individuals from the most closely related genetic sources to use for the establishment of a captive assurance colony. These source populations may come from southwest Georgia, if no newts can be found in the ANF.
3. Conduct striped newt ranavirus susceptibility tests and sample for the presence of ranavirus in sympatric species at repatriation wetlands and in nearby wetlands.
4. Conduct striped newt repatriation efforts in the ANF. We will conduct repatriation efforts in 2 wetlands using captive-bred striped newt assurance colonies developed in the first 2 years of the study (Objective #2). We believe it is paramount to act as soon as possible to boost the western striped newt before its remaining vestiges in southwest Georgia potentially suffer the same fate as the ANF populations. If too few or no striped newts can be found to establish a western striped newt assurance colony, we will use striped newts from the existing ONF assurance colony for repatriations (S. Reichling, Memphis Zoo, pers. comm.). If newts are susceptible to ranavirus and ranavirus is present in selected repatriation wetlands, we will make a well-informed decision how to proceed with repatriations in such a way as to reduce the potential for repatriation failure. We recognize the potential for failure but believe that repatriation is our only option for the reestablishment of a striped newt population in the ANF. If we do nothing, there will be no striped newts in the ANF, an unacceptable loss.
5. Enhance striped newt habitat and provide suitable breeding conditions at the 2 selected pond wetlands for repatriation. As part of habitat enhancement, wetland augmentation using groundwater from solar powered wells will be utilized at both wetlands to ensure that ponds do not dry up after larvae are repatriated. Any augmentations that occur in this study would be considered short-term augmentations. The use of short-term augmentation reduces the potential for water chemistry changes and subsequent potential ill-effects on wetland fauna. Pond water levels and hydroperiods suitable for larval newt development will be maintained on an as-needed basis only, similar to Seigel (2006). We will monitor water quality before and after augmentations to assess whether water quality changes to wetlands occur. If large changes occur with observable effects to wetland fauna, we will terminate augmentations. Additionally, we will hand remove encroaching woody shrubs and slash pines from the basins of the augmented wetlands to enhance striped newt breeding habitat. Finally, we will recommend a prescribed burn management program favorable for striped newts for both study wetlands and for the Munson Sandhills in general, and provide any consulting assistance to the ANF as needed.

## **Methods and Materials**

### Year 1 (Oct 2010-Sept 2011): Sample for striped newts

Approximately 200 isolated wetlands across the Munson Sandhills region of the ANF will be visited and sampled twice with dipnet and/or seine during the year. The first sampling event will take place during the January-March winter breeding season to

sample for striped newt aquatic adults. The second sampling event will take place during the April-June larval development season in an attempt to detect striped newt larvae.

As part of the larger dipnet sampling process, beginning in Year 1, additional baseline community monitoring of all amphibians and select dragonfly larvae (Order: Odonata) will begin at 19 historical newt ponds, a subset of the above mentioned 200 wetlands. These baseline community data will be used later in the study to compare with post-augmentation community data (see Year 3) to monitor for potential effects of augmentation on faunal communities. Two of the 19 historical newt ponds will eventually be selected as the experimental repatriation and augmentation sites by Year 3. Two of the remaining 17 historical newt ponds will be selected as control wetlands based on which of the 17 has the most similar community compositions to the selected experimental wetlands. Selection of the two community control wetlands will take place by Year 3, after which, post-augmentation community sampling will only take place at the two experimental wetlands and the two selected control wetlands. While dipnet sampling, biosecurity measures similar to those laid out in Green et al. (2009) will be employed.

If newts are detected in abundance in the ANF this year, which is unlikely, we will attempt to collect a total of 16-20 later stage larval individuals to establish a captive raised assurance colony to eventually become the source for later repatriations in this study's third year. The repatriation study would proceed as an internal ANF repatriation. The assurance colony from the ANF would represent the western genetic group. If no newts are detected within the ANF during the first sampling event for adults, CPI biologists will attempt to collect 16-20 later stage larval individuals from JJEC for the establishment of the assurance colony. JJEC is the most reliable southwest Georgia site within the western genetic group. If no newts are found at JJEC, we will sample at Fall Line Sandhills Natural Area and a private site in Irwin County. It is unlikely that we will find all 16-20 individuals at a single Georgia site (J. Jensen, Georgia Department of Natural Resources, pers. comm.); therefore, we will most likely have to procure individuals from multiple sites. All efforts in Georgia to obtain striped newts for repatriations will proceed with collaboration from J. Jensen, Georgia Department of Natural Resources, and L. Smith, JJEC.

S. Reichling, Curator, Memphis Zoo, will rear an assurance colony representing the western genetic group to become the source colony for later repatriations (S. Reichling, Memphis Zoo, pers. comm.). He already has successfully raised an assurance colony sourced from the ONF. The existing ONF assurance colony will become the source for repatriations if we cannot establish a colony representing the western genetic group.

To test the relative susceptibility of striped newts, we will collect up to 5 adult breeding pairs in Year 1 from known sites in the ONF where high densities exist. Newts will be sent overnight to the UT Center for Wildlife Health. Pairs will be housed separately in 20-L containers with native aquatic plants collected from the field and fed *Tubifex* worms *ad libitum*. Eggs will be collected daily and put in separate tanks for development. At one month post-hatch, 40 larvae will be placed in separate 1-L plastic tubs, and  $10^3$  plaque-forming units per mL of an FV3-like ranavirus will be added to half of the tubs

(Hoverman et al. 2010). The other tubs will serve as controls and virus-free media will be added. Larvae will be fed zooplankton *ad libitum*. Water changes will be performed every 3 days, and virus not re-added. A 3-day exposure to ranavirus is sufficient to initiate infection and cause disease (Hoverman et al. 2010). Survival of larvae will be monitored daily for 21 days, which is sufficient duration to observe mortality and consistent with previous studies (Hoverman et al. 2010). All individuals that are alive after 21 days will be humanely euthanized with benzocaine hydrochloride. We also will raise 40 additional larval newts and at one month post-metamorphosis expose them to ranavirus following the same protocol. If larval newts are highly susceptible yet metamorphs have low susceptibility (as commonly observed with ranavirus), it will provide the impetus to consider releasing metamorphs at repatriation sites.

Larval and metamorphosed striped newts from the experimental challenges will be necropsied, and the liver and kidney extracted for ranavirus testing (Miller et al. 2007). These organs are sites of ranavirus infection (Gray et al. 2009a). Genomic DNA (gDNA) will be extracted from a tissue homogenate using the DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA). We will use a Qubit<sup>TM</sup> fluorometer and the Quant-iT<sup>TM</sup> dsDNA BR Assay Kit to quantify the concentration of gDNA in each sample (Invitrogen Corp., Carlsbad, CA, USA). Real-time PCR will be used to test for infection following Picco et al. (2007), and gDNA concentration estimates used to calculate viral load (Hoverman et al. 2010). All necropsies and molecular testing will be performed by the UT Center for Wildlife Health.

First annual report will be submitted September 2011.

#### Year 2 (Oct 2011-Sept 2012): Sampling Continued

We will repeat the ANF dipnet sampling effort conducted in Year 1. At the conclusion of Year 2, the conservation status of the striped newt in this region, and implications for the status of the species as a whole, will be reported. Community monitoring will continue at the 19 historical newt ponds to acquire baseline community data and as efforts continue to select two community control wetlands to be monitored after augmentation begins at experimental wetlands in Year 3.

We will repeat Georgia sampling, if necessary, to make a second attempt to create a western genetic group assurance colony. If we fail in this second effort to create an assurance colony representing the western group, then we will proceed with repatriation beginning Year 3 using individuals from the existing ONF (eastern group) assurance colony.

By end Year 2/beginning Year 3, we will select 2 historically known striped newt breeding wetlands near the expanded gas pipeline to be the recipient wetlands of striped newt repatriations and water augmentations. These sites are anticipated to be Study Pond #1 and Study Pond #18 (Figure 1), 2 ephemeral wetlands formerly studied by CPI. Feasibility studies will be undertaken to determine suitability of the selected wetlands for augmentation. Percolation rates of the wetland soils will be measured and compared to

predicted water input rates. Water input rates must be appreciably greater than percolation rates to ensure that any supplemental water will not be rapidly lost to percolation. If either or both anticipated study wetlands are determined to be unsuitable for augmentation, we will select alternative wetland(s) from the remaining 17 historical striped newt wetlands.

After experimental wetlands have been selected, we will hand-thin encroaching woody shrubs and slash pines from the wetland interiors if necessary. Thinning encroaching vegetation will restore the open marshy character and enhance striped newt breeding habitat. CPI will provide consultation to the ANF, if needed, for a prescribed burn management program favorable for striped newts in the Munson Sandhills of the ANF.

In Years 2 and 3, we will test up to 60 larval amphibians per year at the 2 repatriation sites for ranavirus infection. If superspreader species (e.g., gopher frogs, southern leopard frogs) are present, these will be targeted because of their high likelihood of infection when the virus is present. We will test larvae because of their higher probability of infection compared to adults (Gray et al. 2009a). If individuals are infected with ranavirus, there is a 95% chance of detection with  $n = 60$  samples and a pathogen prevalence of 5% (Green et al. 2009). We also will collect and test 60 individuals at the closest suitable breeding site to each repatriation site, because ranavirus can be transmitted overland by sublethally infected dispersing individuals (Brunner et al. 2004). Thus, 480 individuals will be tested at 4 ANF sites over 2 years (4 sites per year x 60 individuals per site x 2 years). Individuals will be randomly collected with dip nets between March and May, which are the months of intended release. Collected individuals will be put in separate containers, humanely euthanized with benzocaine hydrochloride, packaged in individual Whirl-Paks®, and shipped overnight on ice to the UT Center for Wildlife Health for diagnostic testing following the previously described procedures.

Second annual report will be submitted in September 2012.

### Year 3 (Oct 2012-Sept 2013): Begin Repatriation

Augmentation structures will be constructed beginning in October 2012 at each wetland and will be in place by December 2012. We will oversee and subcontract the installation of a well, pumphouse, and solar powered pump at each of the 2 experimental wetlands. Augmentation equipment will be in place from Year 3 to Year 5 to provide short-term boosts to water levels if wetlands threaten to go dry during critical breeding and larval development periods of the repatriation phase of the study. Groundwater will be the source water for augmentations—either from a surficial aquifer system or from the Floridan Aquifer System—whichever is more feasible, as determined by the subcontractor.

Wetland augmentation will be conducted, only as needed, at both experimental wetlands, beginning during the winter (Dec-Mar) breeding season, concurrent with a frontal passage and heavy rain event. Given the droughty conditions over the past decade, augmentation will be used only as a contingency to hedge against the possibility of

wetland dry-up during repatriation years. We do not want to run the high risk of losing repatriated larvae to wetland dry-up. Also, augmentation will provide a relatively stable hydroperiod in wetlands suitable for returned adults to breed during Years 4-5.

Based on our extensive field experience in the selected wetlands, we will maintain water levels to be suitable for striped newt breeding and larval development. Depth gauges will be installed in the wetland center. Water depth at Study Pond 18's center will be maintained at approximately 1m depth, and 1.5m depth at Study Pond 1.

Water quality of both experimental wetlands and 2 selected water quality control ponds will be monitored during the augmentation period. Control ponds will be the 2 selected community control ponds. Measurements will be taken both before and after augmentation events. Water quality parameters to be monitored will be pH, conductivity, dissolved oxygen, and temperature. Although no ill-effects of short-term augmentations are expected, water quality monitoring will provide control data and allow us to ascertain possible negative effects of augmentations in experimental wetlands over the course of the project. We will terminate augmentations during the contract period in the unlikely event there is evidence that altered water chemistry is having a detrimental effect on wetland ecology.

We will repeat the ranavirus infection testing at the 2 repatriation sites and nearby selected wetlands. Tests will be completed before repatriation occurs.

If we determine after Year 1 and 2 of sampling that striped newt numbers remain critically low in the ANF (<5 wetlands), we will begin repatriation by April 2013. The timing will coincide with the natural larval development season. Larvae from assurance colony sources (either those developed during Year 1 and 2 from western populations or those already in existence from the ONF population) will be introduced into both experimental wetlands concurrently. A minimum of 100 individuals, and up to several hundred, depending on availability, will be repatriated into each wetland. Identical numbers will be introduced into each wetland. Wetland augmentation boosts will take place if the wetlands threaten to go dry until all newts have metamorphosed and exited the wetland. When larvae exit the wetland, augmentation will be terminated and the wetlands will resume natural water fluctuation until the beginning of Year 4 (December).

Encircling drift fences with pitfall traps around both study ponds will be installed before repatriation. Traps will be shaded, and a damp sponge will be maintained in the bucket. Fences will be continuously operational and checked regularly to measure recruitment success of newly metamorphosed terrestrial efts into the surrounding upland ecosystem. Fences will be disabled until the following year after newts have exited the wetland.

We will mark emigrating efts using a combination of visual implant elastomer and minimal toe clips (Hoffman et.al 2008). Marking will allow us to distinguish between the Year 3 and Year 4 repatriation cohorts, as well as potential wild individuals that may appear.

CPI will monitor background newt activity at the 17 other historical striped newt breeding ponds, including the 2 water quality/faunal community control ponds. Sampling will occur in these 17 ponds twice during Year 3. Even though we do not anticipate that the ANF striped newt will rebound on its own, continuing a sampling presence throughout the study in all historical newt ponds will allow us to monitor for possible background striped newt activity and provide further evidence of striped newt status in the ANF.

If at any time during the repatriation phase there is sufficient evidence to suggest that the ANF striped newt emerges out of decline (i.e. we find larvae in >5 wetlands), we will terminate repatriations from outside sources. If it becomes possible to find enough larval newts from within ANF source wetlands, then we will consider moving some into experimental wetlands so that a stable wetland environment can be provided to increase the chance for larval development and recruitment. Drift fence monitoring would continue under this scenario to measure repatriation success. Augmentation also would still continue as needed.

Third annual report will be submitted in September 2013.

#### Year 4 (Oct 2013-Sept-2014): Second Repatriation.

A second and final repatriation effort will be conducted in Year 4 using information we learn in Year 3's efforts. Depending on what level of success we had in Year 3, we will repeat the repatriation as conducted in Year 3. If we determine that using more individuals in Year 4 may increase success, we will increase numbers of individuals released per site. Repatriation will begin by April 2014. The encircling drift fences will be re-activated at the beginning of winter rainy season and operated until newts have left the wetland. Drift fencing in Year 4 will measure the return of the Year 3 cohort to experimental wetlands and measure the emigration of the Year 4 cohort into surrounding uplands.

Wetland augmentation boosts will be used as needed beginning in December 2013 and lasting through June 2014. Water quality and faunal community monitoring will continue as per Year 3.

The 17 other historical striped newt breeding ponds will continue being sampled to measure any possible background striped newt activity (as in Year 3).

Fourth annual report will be submitted in September 2014.

#### Year 5 (Oct 2014-Sept 2015): Study Conclusion.

Wetland augmentation will be conducted, as needed, and experimental and reference wetlands will be monitored for newt activity and water quality as in prior years. Encircling drift fences will be operated during the breeding season (Dec-Mar 2015), and

will be removed at the end of March 2015. Drift fencing in Year 5 will measure the breeding immigration of either or both repatriation cohorts into study wetlands.

We will evaluate the success of repatriation and augmentation over the course of the study. If augmentation has proven to be useful, and there is continued need to provide hydroperiod boosts in a drought-stricken landscape, we will make recommendations to the ANF and discuss options for continued augmentation at study wetlands after the study is completed. If there is no continued need for augmentations on the ANF, we will terminate augmentations upon conclusion of the study.

We will resample for possible background striped newt activity in the 17 other historical newt ponds as in Years 3 and 4.

CPI will continue to monitor the striped newt in the Munson Sandhills after the conclusion of this study, as we have been doing for nearly 2 decades (Means et al. 2008). These data also will help determine the success of repatriation in the current proposed study.

The project final report will be completed and submitted in September 2015. Appropriate statistical analyses of data will be conducted, interpreted, and reported.

### **Expected Benefits**

The proposed study is expected to: 1) provide additional evidence on the conservation status of the ANF striped newt, with implications on the status of the species globally; 2) acquire knowledge on the susceptibility of the striped newt to ranavirus and on ranavirus ecology; 3) help replenish striped newt populations in a region that formerly harbored a robust assemblage of populations; 4) enhance striped newt breeding habitat; 5) and gain more needed data on the feasibility of 2 promising conservation techniques for critically imperiled species. To our knowledge, this study will be the first to utilize repatriation in concert with wetland augmentation to help a critically imperiled species or population.

**Estimated Project Budget:** \$275,412  
Amount requested from USFS: \$215,187  
CPI Match: \$60,225  
Percent Match: 21.9%

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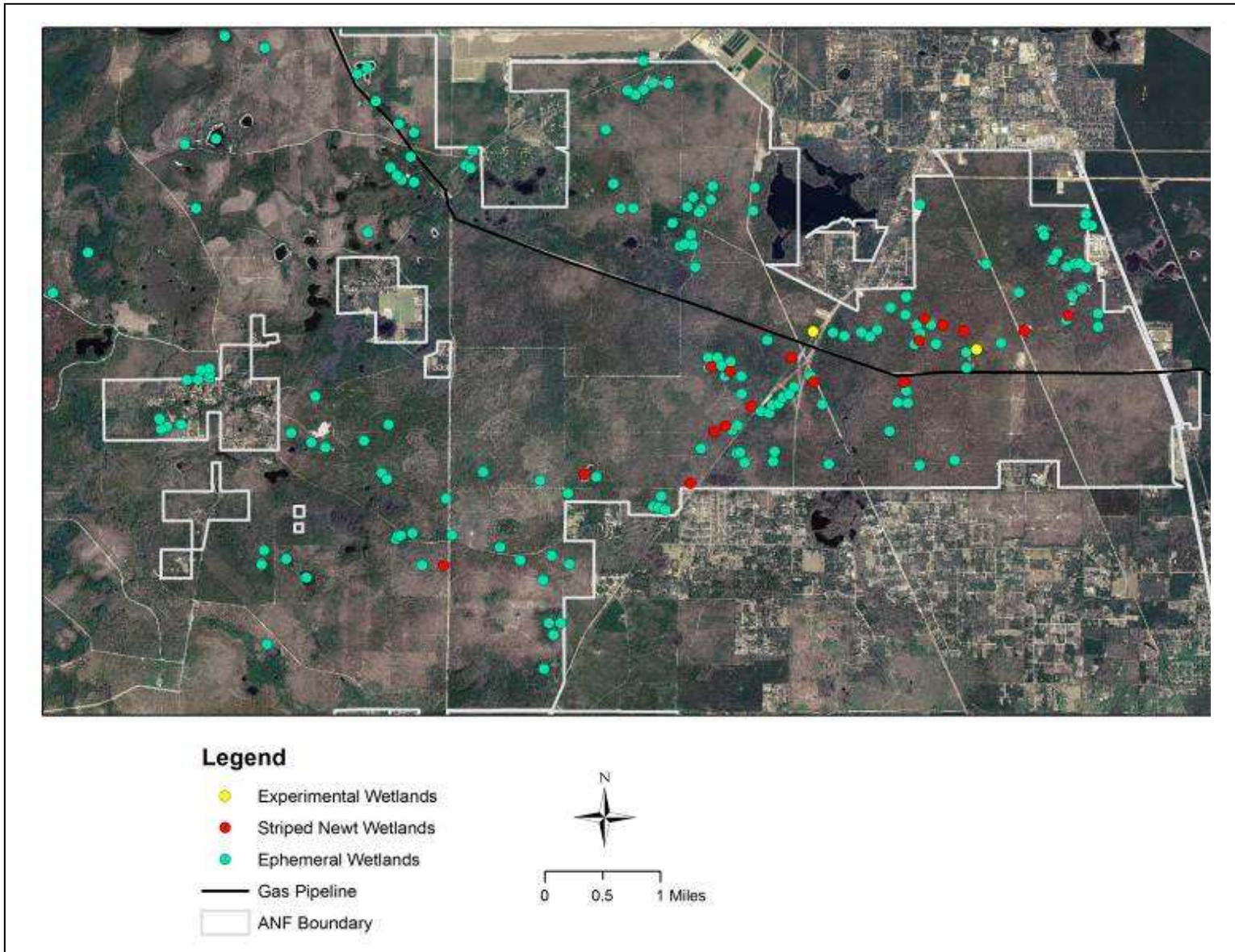


Figure 1. Map of the ANF showing the location of ephemeral wetlands, striped newt breeding wetlands, and proposed experimental wetlands.