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

Second Annual Report to the US Forest Service, Tallahassee, FL

Submitted by: Ryan C. Means[1], Rebecca P.M. Means[1], Debra L. Miller[3], Matthew J. Gray[3], Steve Reichling[4], Steve A. Johnson[2], D. Bruce Means[1], Roberto Brenes[3]

[1]Coastal Plains Institute and Land Conservancy, Tallahassee, FL
[2] University of Florida, IFAS/Dept. of Wildlife Ecology & Conservation, Gainesville, FL
[3] University of Tennessee, Institute of Agriculture, Center for Wildlife Health, Knoxville, TN
[4] Memphis Zoo, Memphis, TN

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Executive Summary

The Coastal Plains Institute (CPI) and US Forest Service entered a 5-year cost-share agreement in October 2010 to address the severe decline of the striped newt (*Notophthalmus perstriatus*) population on the Apalachicola National Forest (ANF). CPI has initiated a multifaceted study to: 1) investigate the cause of striped newt decline in the ANF; 2) conduct larval repatriations in selected prime wetlands; 3) investigate and implement precautionary measures to ensure success of repatriations. This report summarizes work conducted in Year 2 (October 2011-September 2012) of the current 5-year study.

In Year 2, CPI continued exhaustive efforts to find remnant striped newts within the ANF. Identical to last year's efforts, CPI biologists visited and, if hydrated, sampled 158 ephemeral wetlands scattered across the Munson Sandhills of the northeastern ANF once in winter and once in spring. These wetlands have been sampled many times in past years by CPI as part of prior efforts to monitor for striped newts in the region. Hydrated wetlands were standardly dipnet sampled for the presence of breeding adult (winter) or larval (spring) striped newts. Both sampling periods were characterized by ongoing drought. Out of the 158 wetlands visited in Winter 2012, only 9 were hydrated and dipnetted. During Spring 2012, 14 out of the 158 visited wetlands had water and were sampled. Identical to last year, zero striped newts were observed during both efforts. These findings provide further evidence that the striped newt likely is extirpated within the ANF--its former western geographic stronghold.

The cause(s) for extirpation of the western striped newt on the ANF is unknown. Before proceeding with a repatriation effort, it is imperitive that cause(s) for extirpation be ascertained. Likely causes include pathogen infection from ranavirus and/or long-term drought. We are investigating both.

The presence of ranavirus in the Apalachicola National Forest (ANF) and the susceptibility of striped newts (*Notophthalmus perstriatus*) to ranavirus infection are unknown. Thus, the first part of this study (laboratory portion) was to determine the relative susceptibility of striped newt larvae and metamorphs to ranaviral infection and disease using controlled experimental challenges, which was performed in Year 1 and Year 2. Our results indicate that post-metamorphic striped newts (<1 year in age) are highly susceptible to ranaviral disease, experiencing 60 – 100% mortality when exposed to ranavirus (three unique isolates) in water. Larval striped newts experienced 5 – 10% mortality among isolates, which is similar to eastern newt larvae. Interestingly, 10 – 40% of larval striped newts that survived were infected with ranavirus, suggesting that this age class may serve as a reservoir. Our results indicate that ranaviruses could significantly impact adult recruitment of striped newts by affecting survival during the juvenile stage, and if ranavirus is present in an aquatic system, striped newt larvae could contribute to the disease's persistence. Our next step, early in Year 3, will be to test for the presence of ranavirus in our study area to ascertain whether ranavirus could have been the culprit for decline.

Twenty -one western clade striped newt larvae genetically similar to ANF striped newts were collected in Year 1 from Big Pond in the Fall Line Sandhills Natural Area, Georgia. These animals would become the parental generation that would start a captive assurance colony. Larvae were sent overnight to study collaborators at the Memphis Zoo. These larvae were successfully raised into yearling juveniles and housed at the Memphis Zoo during all of study Year 1. In June, late in study Year 2, the parentals became paedopmorphic adults and began to reproduce rapidly. Three months later, by the beginning of September 2012, 545 F1 larvae were in existence. However, the population suffered an enigmatic crash just before preparation of this report, and the number of F1's shrunk to approximately 45 individuals. The reason for the crash was unknown. The parentals all remained healthy. This captive western striped newt colony serves two important roles. It is the first assurance colony ever created for the highly imperiled western genetic variant of the striped newt and serves obvious conservation needs. Second, larvae produced from this colony will be utilized for this study's repatriation efforts later in Years 3-4.

Habitat enhancement techniques to ensure recipient wetlands do not go dry during the critical larval repatriation periods are being investigated. In Year 1, we determined that wetlands augmentation using local groundwater would not be a feasible tool to avoid pond-drying for a variety of reasons. This year, another promising technique was investigated and implemented. In May 2012, while ponds were dry, 40' X 40' synthetic rubber pond liners were installed underneath the central, deepest portions of three pre-selected future repatriation wetlands. Liners were hypothesized to extend wetland hydroperiods by catching rainwater more effectively and providing an impermeable confining layer underneath wetlands that were found to be lacking natural geological confining layers.

In late June, one month after liner installation, heavy rain from Tropical Storm Debbie formed pools within only the lined central portions of all three wetlands, as was hypothesized. Open water remained within the lined portion of wetlands for nearly 2 months, perched atop the underlying water table, until early August. Without liners, ponds would have been dry those two months. These data preliminarily suggest that liners were effective at extending pond hydroperiods. All liner-enhanced wetlands currently appear to be ecologically healthy, containing rapidly regenerated natural vegetation and up to four species of larval amphibians. Liner wetlands will be observed closely for another six months until repatriations begin in April 2013 (study Year 3). This study's liner technique potentially could become a useful tool for land managers that wish to enhance natural wetlands to become more drought-resistant, particularly in regions that have been impacted by increased drought frequency and longevity.

ACKNOWLEDGMENTS

We would like to thank many people and institutions for their important intellectual and/or physical contributions to this multifaceted, collaborative conservation project. Dino Ferri of the Jacksonville Zoo and Gardens, as well as numerous additional zoo colleagues are greatly thanked for their colossal efforts and expertise in producing assurance colonies of both Western and Eastern striped newts to be used for this project and for future conservation efforts. Additional colleagues include: Chris Baker, Steve Bogardy, Andrew Kouba, Brian Summerford, and Shannon White from the Memphis Zoo. The work of these individuals and zoological institutions is vital to this project and to the conservation of the striped newt. We greatly thank Dr. Katherine Milla of Florida A&M University and Dr. Steve Kish of Florida State University for their important contributions to this study. Dr.'s Kish and Milla provided helpful field assistance, important data sharing, and intellectual assistance on hydrogeological aspects of this study. We thank Shaun Dorothy of Coastal Plain Land and Lakes, LLC for expertise and labor in the installation of synthetic liners in our recipient wetlands. W. James Barichivich is greatly thanked for donating five captive adult western striped newts to increase the genetic robustness of our small, but growing assurance colony.

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INTRODUCTION

The Florida Gas Transmission Company has increased 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 spanned east/west across the Munson Sandhills region south of Tallahassee recently was expanded to accommodate additional natural gas transmission. Of particular concern is the expansion of the existing route that ran through the portion of the Munson Sandhills owned by the Apalachicola National Forest (ANF). A significant amount of ANF acreage was altered in order to accommodate the expansion of the pipeline right-of-way.

The ANF portion of the Munson Sandhills where the pipeline expansion occurred is a longleaf pine sandhill ecosystem harboring abundant ephemeral wetlands that serve as breeding sites for the globally rare striped newt (*Notophthalmus perstriatus*) and many other amphibian species (Means and Means 2005). Longleaf pine sandhill with embedded ephemeral wetlands is the preferred habitat for the striped newt. The native longleaf pine ecosystem of almost all of the Munson Sandhills outside of the ANF has extensively been altered 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.

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 may be 2 genetic variants of the striped newt— “western” and “eastern” groups or clades (May et. al 2011). The western genetic group is composed of populations from the Gulf Coastal Plain of southwest Georgia and the eastern Florida Panhandle, including the ANF. 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 the striped newt 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.). These surveys indicated that the striped newt is rare globally and reliably found only in a few wetlands, primarily within the eastern group. Striped newts were once common in its greatest western stronghold--the ANF; however, it has sharply declined there since the late 1990's for unknown reasons (Means et. al 2008).

In 2004, the striped newt was listed as NT (“near threatened”) on the IUCN Red List of threatened species (IUCN 2010). CPI petitioned the US Fish and Wildlife Service 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). That is where it remains.

As of 2010, long-term CPI sampling data suggested that the striped newt had likely become extirpated within the ANF. The ANF decline, coupled with apparent declines in all other sites containing the western striped newt in Florida and Georgia, indicated that the western striped newt was on the brink of extirpation--or even extinction--depending on the level of genetic uniquenness of western striped newts.

One possible cause of the striped newt decline in the ANF is drought. Another possible cause of decline in the ANF striped newt is pathogen infection. 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 whether a 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. The gas pipeline expansion and subsequent upland habitat alteration is the latest in a lengthy list of probable impacts to the ANF striped newt population.

The Coastal Plains Institute and the US Forest Service entered a 5-year cost-share agreement to create a study that addresses the severe decline or extirpation of the striped newt population on the Apalachicola National Forest. Striped newt repatriation coupled with precautionary measures to ensure repatriation success and enhance breeding habitat will be conducted as part of the study. Repatriation at four prime ephemeral wetland habitats is expected to boost the ANF striped newt population and possibly provide new management strategies for similarly imperiled amphibian species. This is the second annual report that summarizes work conducted in Year 2 (Oct 2011-Sept 2012) of the current 5-year study.

Overall Study Objectives

1. Collect individuals from the most closely related genetic source(s) to use for the establishment of a captive assurance colony (Year 1 and/or 2). House, maintain, and grow the assurance colony within collaborating zoological institutions (Years 1-5 and beyond). Larvae from the assurance colony will be the source for larval repatriations in the ANF.

2. Continue sampling the ANF for local striped newts (Years 1-5). Even though we hypothesized that the ANF striped newt was likely extirpated before this study, it is still necessary to continue surveillance sampling the ANF for newts before and after repatriations to be as certain as possible that the local metapopulation is gone before bringing individuals in from other populations. If at anytime newts rebound in the ANF, we will modify the study design. If newts are found to exist in low abundance in the ANF (less than 5 wetlands), but high enough abundance to obtain individuals for establishment of an assurance colony, then we will establish the colony and repatriate using ANF sourced individuals. If we cannot find any more ANF newts, then repatriation will proceed as outlined in Objective #4. Up to 200 wetlands will be sampled twice per year during the first 2 study years, then the 19 historical newt ponds will be sampled yearly in study Years 3-5.

3. Conduct striped newt ranavirus susceptibility tests and conduct surveillance testing for ranavirus in sympatric species at repatriation wetlands and in nearby wetlands (Years 1-3). This will be done as a precaution to ensure we do not introduce striped newts into a potentially hazardous environment. If newts are determined to be 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.

4. Conduct striped newt repatriation efforts in the ANF (Years 3-4). We will conduct repatriation efforts in four wetlands using captive-bred larvae from striped newt assurance colonies developed in the first 2 years of the study (Objective #1). 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 metapopulation.

5. Investigate and implement techniques to ensure there are suitable hydrological conditions at selected repatriation wetlands by repatriation time (Years 1-3). Wetlands augmentation originally was considered as being our primary method to ensure stable wetland environments for larval repatriations, but the method was tested and rejected as an option last year. This year, we implemented another method to ensure repatriation wetlands do not go dry during the critical repatriation periods later on. CPI installed synthetic liners under small interior portions of selected repatriation wetlands that create longer pooling of rain water and provide a boost to wetland hydroperiods in an otherwise drought-stricken landscape. Later in this study, these pools are expected to act as hydrated refugia as a contingency if wetlands threaten to go dry during repatriations.

6. Enhance striped newt breeding habitat, including hand-removal of encroaching woody shrubs and trees from the basins of repatriation wetlands (Year 3,4 or 5). CPI will be available throughout the study as needed to provide the USFS with any management recommendations favorable for the long term ecological management of the striped newt.

7. Measure repatriation success with the use of encircling drift fences and continued dipnet sampling (Years 3-5).

STUDY AREA

The study area is a west-to-east trending belt of sandy hills in the southern portion of Leon County, Florida, and just south of the capital city of Tallahassee (Fig. 1). The hills form a small physiographic region called the Munson Sand Hills (MS), a subdivision of the larger Gulf Coastal Lowlands. They represent deep sands (up to 30 ft) capping Pliocene Jackson Bluff Formation limestones that overlie late Miocene limestones of the St. Marks Formation.

The MS run through the northeastern portion of the Apalachicola National Forest (ANF) immediately south of Tallahassee. The uplands within the ANF -owned MS are a native longleaf pine-wiregrass ecosystem on rolling sandy hills. The area contains approximately 200 ephemeral wetlands depicted below as yellow dots (Fig. 1). CPI has extensively studied and regularly sampled most of these wetlands for ephemeral pond-breeding amphibians over the past 20 years. The wetlands provide breeding habitat for over 20 amphibian species, historically including the striped newt--our current study focus. The prominent, light-colored, L-shaped figure in the upper (northern) center of the study area is the Tallahassee Regional Airport.

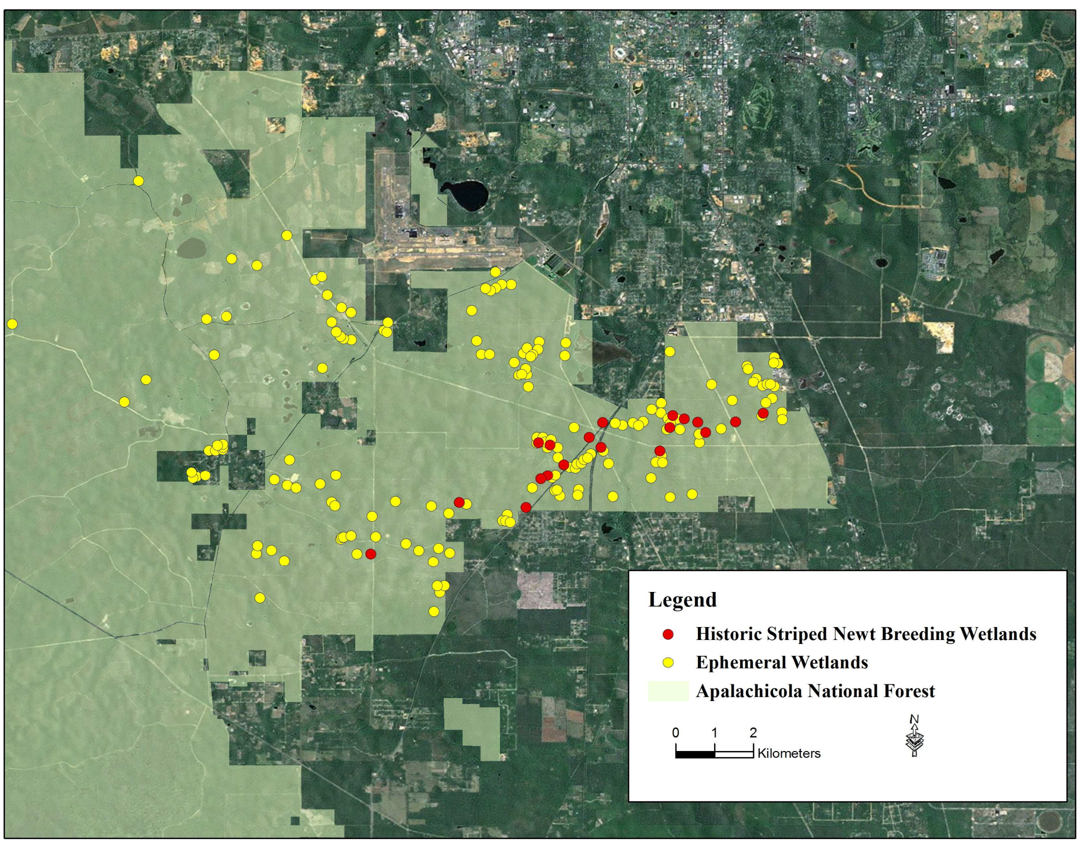

Figure 1. Map of the Munson Sandhills study area. Yellow dots represent the 158 ephemeral wetlands that have been periodically sampled over the last 20 years. Red dots represent the 19 historic striped newt breeding wetlands.

METHODS

Striped Newt Sampling in the Apalachicola National Forest

During Year 2, we twice visited and sampled 158 isolated wetlands across the MS region of the ANF with dipnet and/or seine. The first sampling effort took place during the January-March 2012 winter breeding season to search for presence of striped newt aquatic adults. The second sampling effort took place during the April-June 2012 spring larval development season to detect striped newt larvae.

Sampling was conducted using a heavy duty dipnet (Memphis Net and Twine Co. HDD-2 model) with 3/16" mesh. The number of dipnet sweeps per pond varied depending on pond size. For small ponds, the entire pond periphery and the center thoroughly was swept. Large ponds were given 50 dipnet sweeps around the perimeter. Sweep efforts were concentrated in submerged or emergent herbaceous vegetation where newt larvae tend to concentrate.

Ranavirus Susceptibility Testing

Between January and June 2012, we received 69 striped newt larvae from the Jacksonville Zoo. Similar to 2011, most larvae arrived in good condition and survived (Table 1). Thirty five larvae obtained from the zoo were combined with 20 larvae remaining from the 2011 experiments, and raised through metamorphosis. The ranavirus challenge experiment was conducted between 4 – 25 July 2012. All metamorphs were <1 year in age and hence belonged to the juvenile age class.

Table 1. Number of striped newt larvae received from the Jacksonville Zoo for the metamorph experiment and their survival.

Date	Institution	Number Received	Alive	Dead
1/30/12	Jacksonville Zoo	28	28	0
4/27/12	Jacksonville Zoo	25	25	0
6/20/12	Jacksonville Zoo	16	9	7
Total		**69**	**62**	**7**

Experimental challenges in 2011 and 2012 followed the same procedures. Larval and metamorphosed (juvenile) newts were exposed to one of three ranavirus isolates known to cause disease in North American amphibians (frog virus 3 [FV3], Smoky Mountains isolate [SM], Ranaculture Isolate [RI]). Frog virus 3 is the type species for *Ranavirus* and was isolated in 1963 from clinically normal adult northern leopard frogs (*Lithobates pipiens*, Granoff et al. 1965) collected from Wisconsin, SM was isolated in 2009 from dead larval marbled salamanders (*Ambystoma opacum*, Todd-Thompson 2010) during a die-off in the Great Smoky Mountains National Park, Tennessee, and RI was isolated from morbid American bullfrog (*L. catesbeianus*) juveniles at a ranaculture facility in Georgia (Miller et al. 2007). A fourth treatment (virus growth media) served as the control. Based on our previous experiments with 35 North American amphibian species (Hoverman et al. 2011a; R. Brenes, unpubl. data), FV3, SM, and RI can be classified generally as having low, moderate, and high pathogenicity. The experiment conducted in 2011 consisted of the four treatments with 20 replicate larvae per treatment, totaling 80 experimental units. The experiment in 2012 consisted of the four treatments, but with 10 replicate metamorphs per virus treatment and five controls, totaling 35 experimental units.

For all experiments, larvae and metamorphs were randomly selected and placed individually in 2-L plastic tubs containing 1 L and 0.25-L of aged water, respectively (Figure 2). We used a lower volume of water in the metamorph tubs so individuals could stand with their nares above the surface. After 24 hours acclimation, we added 10^3 PFU/mL of each virus isolate to the tubs corresponding to its treatment. Controls were exposed to the same quantity of virus-free media. Exposure duration was 3 days, which is sufficient duration to cause infection (Hoverman et al. 2011a), then individuals were placed in new containers with water containing no virus or media. We added a plastic floating platform to the metamorph tubs to allow individuals to crawl from the water.

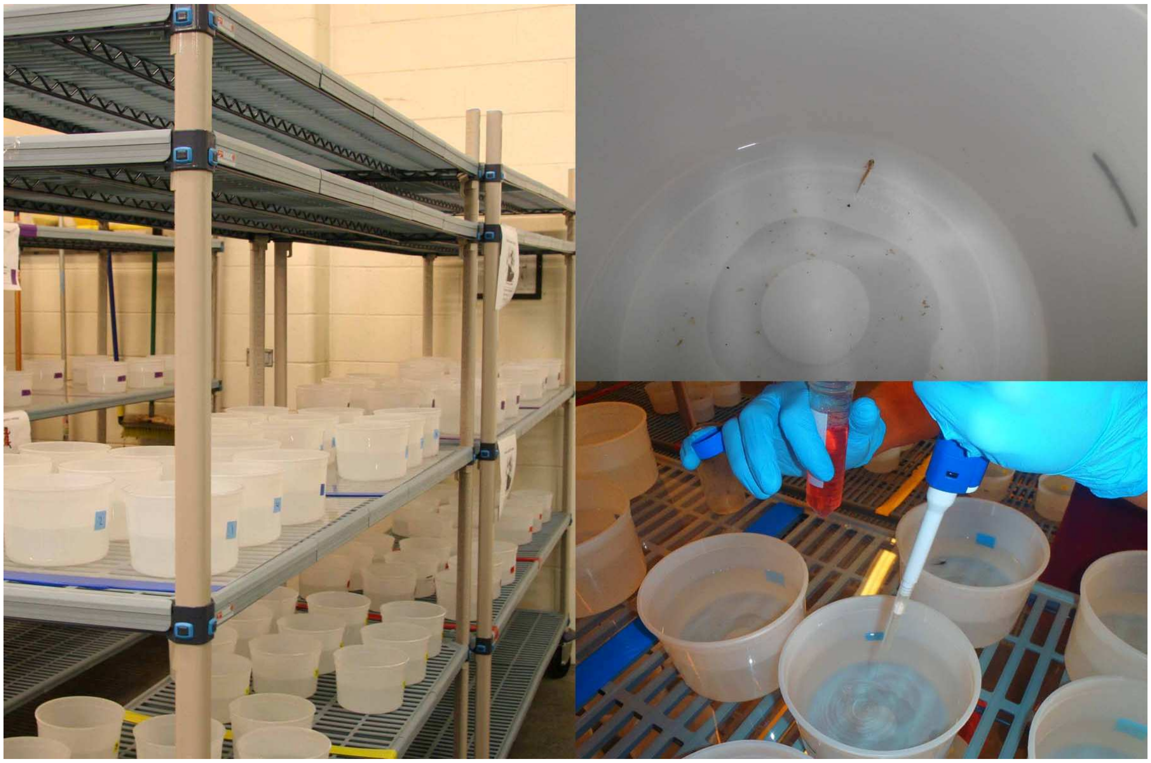

Figure 2. Amphibian Disease Laboratory at the University of Tennessee Center for Wildlife Health where experimental challenges occurred. Plastic 2-L tubs containing 1-L of aged water were randomly arranged (left) and one larva or metamorph was placed in each tub (upper right). Each individual was exposed to one of three isolates of ranavirus (frog virus 3, Smoky Mountains isolate, ranaculture Isolate) by adding a quantity of the respective virus to the water (lower right) that resulted in a final concentration of 10^3 PFU/mL.

Individuals were monitored 2X daily for morbidity and mortality. Water was changed (100% of volume) every three days to maintain water quality (Hoverman et al. 2010). Larvae were fed 3 mL of concentrated zooplankton (predominately *Daphnia* spp. and *Artemia* sp.) and juveniles were fed 3 black worms (*Lumbriculus variegatus*) every 3 days. The duration for all trials was three weeks (21 days), which is sufficient duration for morbidity to be observed from ranavirus infection (Brunner et al. 2004, Hoverman et al. 2010). Larvae or metamorphs that exhibited morbidity consistent with terminal ranaviral disease (i.e., petechial hemorrhages, edema, and loss of equilibrium) before the end of the trial were humanely euthanized and necropsied. At the end of the experiment, surviving individuals were humanely euthanized by immersion in benzocaine hydrochloride and necropsied. At necropsy, gross changes were noted. Liver and kidney were collected for PCR analysis and remaining portions of the specimen placed in 10% formalin for possible future histopathological evaluation. To test for ranavirus infection, genomic DNA was extracted from a homogenate of liver and kidney tissue and real-time quantitative PCR was performed following Hoverman et al. (2011a) to estimate prevalence.

Western Striped Newt Assurance/Repatriation Colony

Twenty-one western striped newt larvae genetically similar to ANF striped newts were collected in Year 1 from Big Pond in the Fall Line Sandhills Natural Area, Georgia. These individuals would become the original parental generation of the planned assurance colony. The larvae were successfully raised through metamorphosis and housed at the Memphis Zoo during all of study Year 1.

In Year 2, the 21 parentals continued to be housed, and bred for a repatriation colony, by study collaborators at the Memphis Zoo.

Repatriation Site Selection

In the Fall of 2011, CPI identified several natural ephemeral wetlands in the Munson Sand Hills that would be suitable for repatriations and liner installation. By May 2012, four of these 10 wetlands were selected to become this study's repatriation (recipient) wetlands based on their suitability as prime striped newt breeding habitat and siutability for synthetic liner installation. The wetlands were given numerical designations as: Pond 16, Pond 18, Pond 75, and Pond 182 (Fig. 3). Numerical designations were taken from past surveys conducted by CPI on the MS ephemeral pond assemblage over the past 20 years. All wetlands were nearby to the expanded gas pipeline corridor within prime upland longleaf pine habitat. Three were historical striped newt breeding wetlands (16, 18, and 75), while the fourth (182) was adjacent to two historical newt ponds, including one of the other selected wetlands (75).

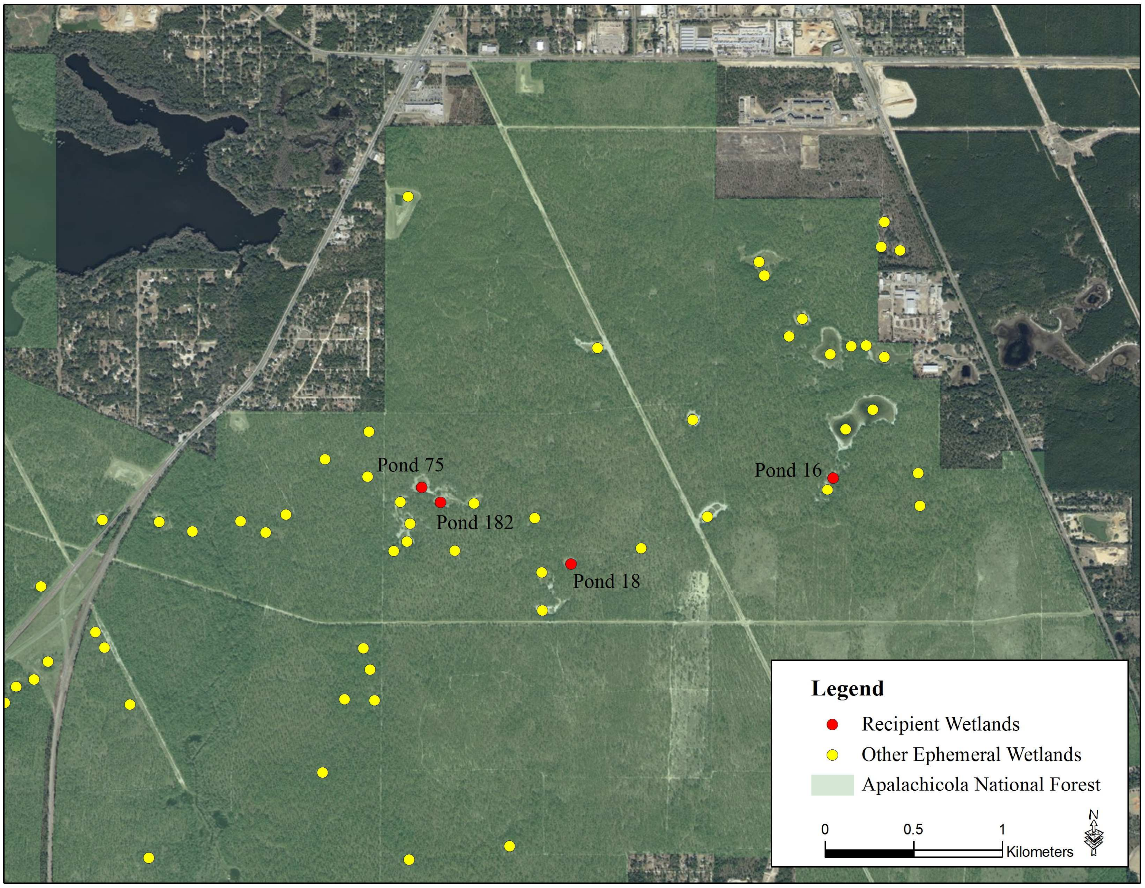

Figure 3. Map depicting location of the 4 striped newt recipient wetlands.

Synthetic Pond Liner Installation

By May 2012, four pond liners were purchased from www.pondliner.com. Liners were delivered to the ANF's Wakulla Work Center. They were identically shaped 40' X 40' squares, constructed of 45 mil EPDM material. In May 2012, we installed liners within three selected repatriation wetlands (18, 75, and 182). The fourth pre-selected wetland (16) had become too moist in its central portion to install a liner without potentially incurring soil damage. We decided to wait for it to dry out more before attempting to install a liner, and we continued to wait through the remainder of Year 2. We will attempt to install the fourth liner in Pond 16 as soon as conditions permit.

We developed a procedure for installing a synthetic liner to make an ephemeral wetland from Biebighouser (2002) and with intellectual assistance from the following: hydrogeologist Dr. Steve Kish from Florida State University, wetland scientist Dr. Katherine Milla of Florida A&M University, and Mr. Shaun Dorethy of Coastal Plain Land and Lakes, LLC (CPLL). Our liner installation procedure differs from

Biebighouser (2002) in that we are utilizing liners in already existing wetlands to extend their hydroperiods, unstead of creating new wetlands outright.

CPLL was contracted to assist with liner installations. CPLL utilized a tractor with front end bucket to perform the majority of soil removal and soil replacement on top of deployed liners. Liner installation procedure was identical for all wetlands. We created shallow, liner-underlain depressions in dried wetland centers that were slightly more than a foot deeper than the original wetland bottom. Liner installation was conducted when recipient wetlands were extremely dry to avoid any unnecessary rutting or soil damage.

Liner installation procedure is described below. Please see Appendix A. for chronological photographs depicting the process. In a selected wetland, a 40' x 40' square in the center, deepest portion was staked out and well-marked so that the tractor operator could easily determine the area of ground to be excavated. A 4' long reference stake was marked in foot increments, then driven into the exact center of the square such that the tractor operator could use it as a guide for the exact depth of soil to be removed.

All native herbaceous vegetation, including four to six inches of underlying soil containing intact root systems, was carefully scooped out of the staked area by the operator using the tractor bucket and placed delicately nearby. Sod patches were stored in shade if possible.

Dirt was removed throughout the entire staked area with the tractor, creating a 2' deep in center, shallowly-sloped depression. Excavated dirt was temporarily stored in mounds along the wetland-upland ecotone. After dirt was removed, the operator then sculpted the depression into a perfect shallow bowl, 2' deep in center, gently sloping up to the edges.

A rolled pond liner was placed on the depression edge using a fork lift extension off the tractor. The liner then was unrolled and spread into the new depression to precisely fill the excavated area. Although a single liner wieghed 500 lbs., two men easily accomplished deployment of the liner once the liner was placed on the depression edge by tractor. Liner edges were folded over and staked with 6" zinc-coated nails fitted into large washers. EPDM material for liners was chosen based on its superior quality, durability and its capacity to stretch--precisely fitting into all the contours of the excavated depression bottom. EPDM is among the highest quality materials available for pond liners, and we recommend its use in similar conservation applications.

After deployment of the liner, the tractor/operator covered the liner with approximately 8"of the previously removed soil, preserving the shallow bowl shape of the new depression. The operator was very careful not to drive the tractor on top of bare liner. Dirt bridges into the center were created, gaps were filled in, then dirt was raked smooth and even across the depression.

The operator/tractor placed the sod patches carefully back on top of the soil covering the liners. After sod replacement, there were a few areas of bare soil for two reasons. The first is due to unavoidable disintegration of some sod during removal and replacement. Second, the ground surface area in the depression increased after making it a foot deeper. The operator utilized a 4-pronged fork lift to transport the original sod patches back into the depression. We were able to salvage approximately 90 % of the original native herbaceous groundcover and get it transported safely back into all wetlands.

An operator used an ORV work vehicle with an attached 300 pound roller to smooth out the contours of the newly created depression. The approximately 1200 cubic feet of leftover dirt per wetland was then spread evenly and thinly over patches of adjacent uplands. The center of a newly created and lined interior depression was 12-16"deeper than the original pond bottom.

Results and Discussion

Striped Newt Sampling on the ANF

Out of the 158 wetlands visited in winter 2012, only 15 were hydrated substantially enough to dipnet. Zero striped newts were observed in winter 2012.

In comparison to Year 1, Year 2 wintertime was much drier overall. In Year 1 winter, 50 wetlands were hydrated compared to just 15 in Year 2. In the calendar year of 2011, the nearby Tallahassee Regional Airport received just 36"of annual rainfall, which is only 60 % of the annual average of 60 inches. Weather conditions during winter 2012 were not prime for striped newt breeding, nor for amphibian breeding in general.

However, in February, at least three frontal passages produced substantial enough rains to increase the number of hydrated wetlands in our study area from 9 to 14. Even though there was a small spike in water levels for the deepest wetlands whose bottoms are closest to the local water table, conditions in the wintertime still were best characterized as extremely dry relative to "normal" conditions.

The ornate chorus frog (*Pseudacris ornata*), southern chorus frog (*Pseudacris nigrita*), and southern leopard frog (*Lithobates sphenocephalus*) were observed calling frequently at a double pond system (Ponds 178 and 12) within our study region throughout February. Ponds 178 and 12 are among the most drought resistant wetlands in the study area, and they continue to support all the local rare and common pond breeding amphibians, except the striped newt.

From winter to spring 2012, the number of hydrated wetlands in the region remained the same at 14. However, as spring progressed up until the third week of June (beginning of summer), there was an overall seasonal drying trend, and the number of hydrated wetlands in the region decreased back to 10.

During the spring 2012 larval sampling effort in April and May, zero striped newt larvae were observed. However, we encountered larvae of other obligate ephemeral pond-breeding amphibian species in relative abundance in subsets of the 15 hydrated wetlands. Species encountered were: gopher frog (*Lithobates capito*) in six wetlands, ornate chorus frog in seven wetlands, mole salamander in six wetlands (*Ambystoma talpoideum*), barking treefrog (*Hyla gratiosa*) in six wetlands. The non-ephemeral pond-breeding obligates southern cricket frog (*Acris gryllus*) (14 wetlands) and southern leopard frog (six wetlands) continue to be extremely common in the study area as well. Other species were encountered either as adults or larvae in the springtime. Bullfrog larvae were abundant (*Lithobates catesbeianus*) in two wetlands. One dwarf salamander (*Eurycea quadridigitata*) larva was found in one wetland. One eastern newt (*Notophthalmus viridescens*) larva was encountered in one wetland. Southern toad (*Bufo terrestris*) larvae were common in three wetlands.

Over the past 13 years, local annual rainfall totals have, on the average, reduced. February's substantial rains still were not enough to reverse the overall trend of dryness, and there was not enough winter or spring rain to induce widespread amphibian breeding across the MS. But ponds that did develop shallow pools as a result of February's rains did support breeding amphibians, including other rare amphibians such as ornate chorus frog and gopher frog.

On June 24, 2012, well after striped newt breeding and sampling season had concluded, Tropical Storm Debbie produced extremely heavy rainfall across our study area. The Tallahassee Regional Airport, located within the study area, recorded 8.94"of rainfall from Debbie. Following this tropical storm event, our normal summertime rainy season was active and produced at least 10 more inches of rainfall for the area through September. This may set up much needed wetter conditions for next year's planned striped newt larval repatriations--provided that the upcomming Fall 2012-Winter 2013 seasons are not dry enough to erase the current rainfall surplus.

Western Striped Newt Status in the ANF

Since 1998, despite repeated regionwide sampling efforts, exactly zero striped newt larvae and less than 10 adults have been observed in the Munson Sand Hills region of the northeastern ANF region (Means and Means 1998a, 1998b, 2005, Means 2007, Means et al. 2008, 2011). The last adults were observed in 2007 from two of the 19 wetlands. All too often, since 1998, winter breeding conditions have not been prime for striped newts because of frequent drought. However, during one seemingly prime 2009-2010 El Niño winter that featured abundant rain and widespread pond fillings in the MS, no striped newts were detected even though the other pond-breeding obligate species, such as the mole salamander, gopher frog, and ornate chorus frog, were observed in relative abundance (R. Means, pers. obs.).

Intensive sampling in the first two years of our current study also has failed to turn up any striped newts in the Munson Sandhills. Although we recognize that the absence of proof does not necessarily signify a proof of absence, we believe it is reasonable to conclude, based on 20 years worth of CPI sampling data, that the western striped newt now is extirpated within its former ANF stronghold. Such extirpation of the world's largest known western striped newt metapopulation also signifies a crisis globally for the western genetic variant of the striped newt as well as crisis for the entire species (both eastern and western groups).

Our study was designed to provide even more evidence on the decline of the ANF western striped newt before proceeding ahead with repatriations. Determining the conservation status of the western striped newt in the ANF was key in order for the current repatriation study to proceed ahead into the repatriation phase. Based on all available data, we believe that repatriation in the ANF is now a necessary and warranted approach in the effort to conserve the western striped newt from global extirpation/extinction.

Ranaviral Susceptibility Testing

Survival curves of striped newt larvae for each isolate are presented below (Figure 4). No mortality occurred in the control group or the group exposed to the ranaculture isolate. Final mortality was 5% and 15% for larvae exposed to FV3 and the Smoky Mountains isolates, respectively. Although mortality was low, 10% and 40% of the larvae exposed to the Smoky Mountains and ranaculture isolates, respectively, that survived were infected (Figure 5a).

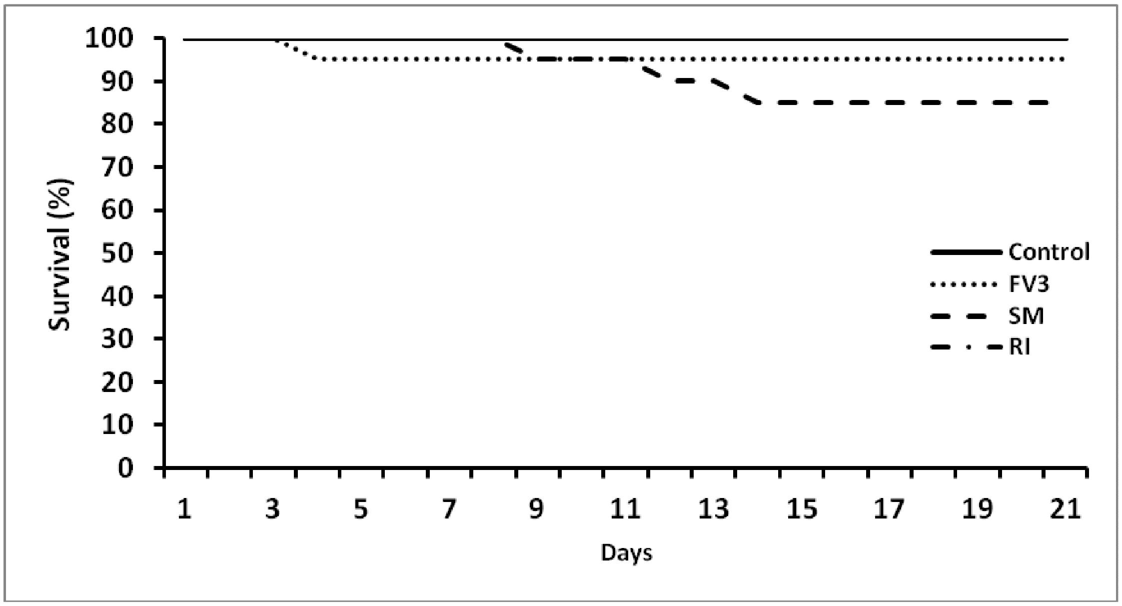

Figure 4. Percent survival of striped newt larvae (n = 20/treatment) exposed to one of three isolates of ranavirus (FV3 = frog virus 3, SM = Smoky Mountains isolate, RI = Ranaculture Isolate). Survival curves (100%) for controls and RI overlap.

Survival curves of striped newt metamorphs for each isolate are provided in Figure 6. No mortality occurred in the control group. Final mortality was 60%, 80% and 100% for metamorphs exposed to FV3, Smoky Mountains and ranaculture isolates, respectively. For metamorphs, only 10% of the survivors exposed to FV3 were infected with ranavirus (Figure 5b); thus, the majority of individuals that became infected experienced mortality unlike larvae.

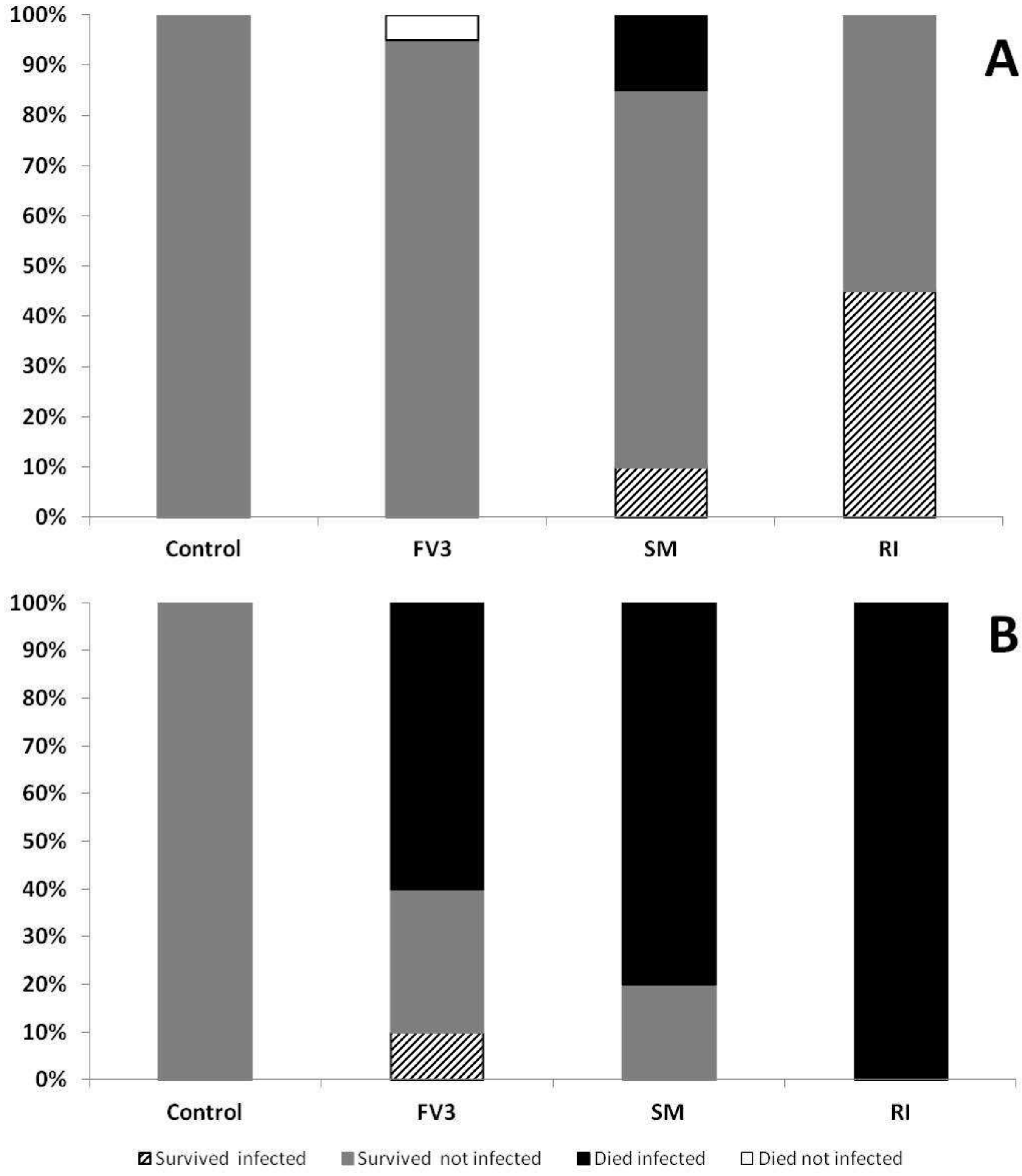

Figure 5. Percent survival and infection of striped newt larvae (A) and metamorphs (B) exposed to one of three isolates of ranavirus (FV3 = frog virus 3, SM = Smoky Mountains isolate, RI = Ranaculture Isolate).

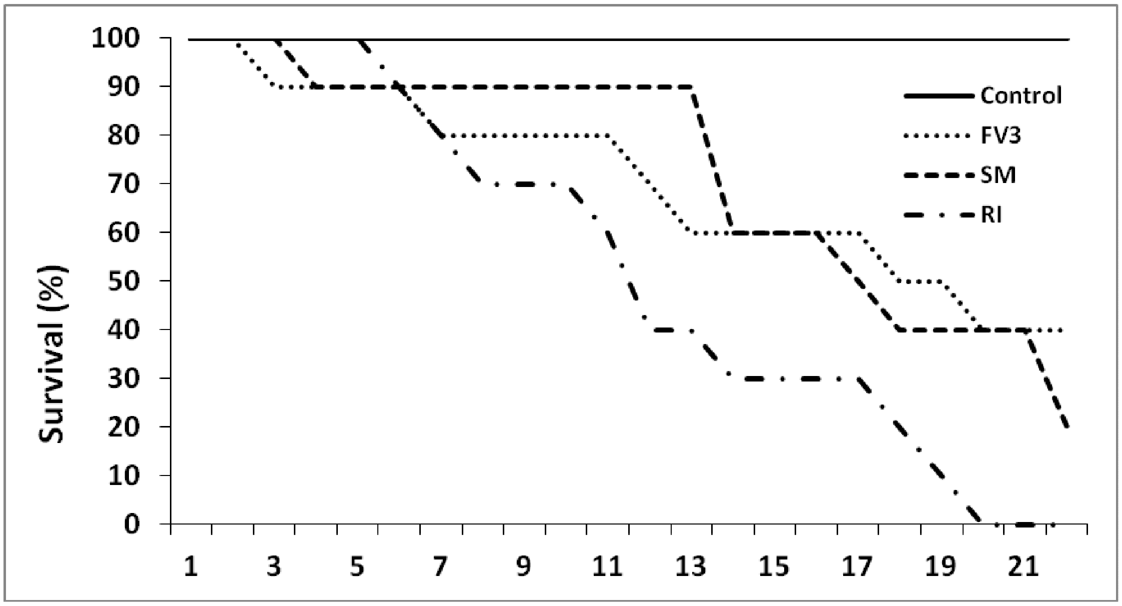

Figure 6. Percent survival of metamorphs (n = 10/virus treatment, n=5 controls) exposed to one of three isolates of ranavirus (FV3 = frog virus 3, SM = Smoky Mountains isolate, RI = Ranaculture Isolate).

Our results suggest that recruitment of adult striped newts into a population could be limited by high susceptibility to ranavirus during the juvenile stage if they are exposed to the pathogen. High susceptibility of juvenile and adult alpine newts (*Mesotriton alpestris*) has been reported in Spain (Balseiro et al. 2010). The susceptibility of adult eastern newts is unknown; however, there are field cases of ranavirus-associated mortality (Green et al. 2002) as well as reports of apparent sublethal infection (Uyehara et al. 2010, Hoverman et al. 2011b). Eastern newt larvae appear to have low susceptibility (Hoverman et al. 2011a). Although patterns of susceptibility to ranavirus can follow phylogenetic lineages, species with limited distributions can be highly susceptible to ranaviral disease, perhaps due to a loss of genetic diversity (Pearman and Garner 2005, Hoverman et al. 2011a). Striped newts have experienced significant population declines and possibly a genetic bottleneck. Moreover, the individuals used in our study were from captive populations, which are commonly used in repatriation studies, but often have lower genetic diversity than wild populations (Frankham et al. 2002). Thus, the success of striped newt repatriation at ANF may depend on the existence of ranavirus-free sites. Considering that striped newt larvae had low susceptibility and could be sublethally infected with ranavirus, this age class could serve as a reservoir for the pathogen similar to the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*; Rachowicz and Vredenburg 2004, Smith et al. 2007).

Based on these findings, our recommendation is to select striped newt repatriation sites that are ranavirus free. We also recommend that sites with hydroperiods <9 months are selected. The likelihood of ranavirus presence may be lowest at sites with seasonal hydroperiods, because virions in the environment likely become inactivated after 30 days of dry conditions (Langdon

1989, Nazir et al. 2012). Permanent water bodies also provide habitat for various species of ectothermic vertebrates (e.g., chelonians, squamates, osteichthyan fish) that can be suitable hosts for ranavirus (Miller et al. 2011), and contribute to virion shedding in the environment. Efforts in 2013 will focus on estimating ranavirus prevalence in amphibian communities at ANF repatriation sites.

Western Striped Newt Assurance/Repatriation Colony Status

In June 2012, nearly 14 months after their capture as young larvae from the wild, the parentals began to breed as paedomorphic adults. Their progeny were the F1 generation. Breeding fared very well throughout the summer of 2012. The number of larval F1's increased rapidly to 545 individuals by the beginning of September. Then, an enigmatic setback occurred. There was a rapid die-off of the larval F1's back down to about 45 individuals. Cause for the die-off was unknown. The parental generation remained in good condition.

By the end of Year 2, Dino Ferri and colleagues at Jacksonville Zoo and Gardens were working with the Memphis Zoo to acquire part of the assurance colony in an effort to help expand the colony. Also at this time, W. James Barichivich of the USGS in Gainesville, FL was collaborating to donate 5 captive striped newt adults to the Jacksonville Zoo to add to the genetic robustness of the assurance colony. These individuals had been in captivity for several years, were healthy, and breeding ready. They also were from the same locality as the parentals--Big Pond in Georgia.

Our captive western striped newt colony serves two important roles. It is the first assurance colony ever created for the highly imperiled western genetic variant of the striped newt and serves obvious conservation needs. Second, larvae produced from this colony will be utilized for this study's repatriation efforts later in Years 3-4.

Synthetic Pond Liner Installation

After liner installation, each ephemeral wetland contained shallow, bowl-shaped, 40' diameter depressions in their interiors that were 12 to 16"deeper than the original pond bottoms (Fig. 7).

Pond 16 before liner installation

Pond 16 immediately after liner installation

Pond 75 before liner installation

Pond 75 immediately after liner installation

Pond 182 before liner installation

Pond 182 immediately after liner installation

Figure 7. Images depicting before and immediately after liner installation at the three recipient wetlands.

Liners were hypothesized to extend wetland hydroperiods by providing an impermeable confining layer underneath wetlands that, in study Year 1, were found to be lacking natural geological confining layers (Means et. al 2011). Wetland soils in our study area are primarily sand and extremely porous. Liners were expected to catch and pool rain water that normally would be lost to infiltration in the porous, sandy local soils.

Liner wetlands remained dry for nearly a month after installation, because low rainfall and dryness persisted into early summer. However, in late June, one month after liner installation, Tropical Storm Debbie produced extremely heavy rain in our study area. This single rainfall event created pools, 14-18' deep, only within the lined portion of recipient wetlands, perched atop the local water table. These pools remained for nearly 2 months until early August (Fig. 8). Most other nearby and similar wetland depressions remained dry because the local water table had not risen high enough to expose water at the surface. Pond 16, the fourth recipient wetland, is one exception because of its relatively long, natural hydroperiod. These data preliminarily suggest that liners already are effective at extending pond hydroperiods.

Figure 8. Photos of recipient wetlands one month after Tropical Storm Debbie. Most nearby and similar wetland depressions remained dry because the local water table had not risen high enough to expose water at the surface. These data preliminarily suggest that liners already are effective at extending pond hydroperiods.

MS ephemeral wetlands are primarily water-table driven. When the local water table (i.e. surficial aquifer system) rises, wetlands begin to fill. If the water table recedes, wetlands go dry. MS wetlands normally respond moreso to longer-term climatic trends than to single rainfall events. However, evidence shows our recipient liner wetlands now may respond to single rain events.

Summer (May-September in north Florida) is the hottest portion of the year, and also has the highest evapotranspiration rates. It had been a concern, before installing liners, that evapotranspiration may be so high during April-June planned repatriation periods that our new shallow, lined pools may still dry up rapidly if drought continued. Year 2 liner results are promising and indicate that open water can persist on the liners during a hot summer period.

From mid August until mid September, increased summer thunderstorm activity increased wetland water levels to beyond the liners in ponds 18 and 75, which indicated a rise in the local water table. The highest (perched) wetland (182) continued to hold water only on the liner, still perched above the underlying water table.

By the end of Year 2 (September 2012), all three recipient wetlands contained rapidly regenerated natural and original vegetation (Fig. 9). By this time, it was impossible to tell visually that any mechanical disturbance of the wetland occurred. The wetlands also contained up to four species of larval amphibians--barking treefrog, southern toad, southern cricket frog, and southern leopard frog. Young juveniles of a fifth amphibian species, the eastern spadefoot toad (*Scaphiopus holbrookii*), were observed abundantly around the edges of all recipient wetlands. They apparently were produced as a result of Tropical Storm Debbie. A sixth amphibian species also was recorded only at Pond 18. A single adult male mole salamander was observed crawling in live oak leaf litter on the south side, approximately 40' from the liner pool in July. All three liner-enhanced wetlands appear to be healthy functioning ephemeral wetlands. Liner wetlands will be observed closely for another six months until repatriations begin in April 2013.

Pond 18

Pond 75

Pond 182

Figure 9. Photos of the recipent wetlands, just three months after liner installation, depicting the rapid regrowth of native vegetation.

As far as we know, our study is the first amphibian larval repatriation study to utilize synthetic liners within natural recipient wetlands to act as insurance against pond-drying during repatriations. Preliminary data from this year already show that liners can extend hydroperiods within natural wetlands. This study's liner technique could become a useful land management tool to create more drought-resistant wetlands within drought-stricken landscapes as a response to ongoing global climate change.

YEAR 3 EXPECTATIONS

We will begin ranavirus surveillance testing in the study area in the Fall of 2012 (beginning of Year 3). Thanks to Tropical Storm Debbie and heavy summer thunderstorm activity, currently there is sufficient water in wetlands and amphibian larvae present to conduct our surveillance monitoring.

If ranavirus is found to be prevalent 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 will continue to produce as many western striped newts as possible in the assurance colony such that there will be abundant larvae available by next April's (Year 3) planned repatriations.

Repatriation of striped newt larval in the ANF will begin in April of 2013. Drift fences, encircling all 4 recepient wetlands, will be installed. We will conduct repatriation efforts in four wetlands using captive-bred larvae from striped newt assurance colonies developed in the first two years of the study. Wetlands will be continuously monitored through metamorphosis to measure recruitment of striped newts into the uplands.

The 19 historical striped newt wetlands in the ANF will be resampled in winter and in spring 2013. 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.

CPI will enhance striped newt breeding habitat, including hand-removal of encroaching woody shrubs and trees from the basins of repatriation wetlands.

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APPENDIX A. Photographs, in chronological order, depicting the installation of a pond liner. Order is depicted left to right, top to bottom.

Appendix A (con't)