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

**First Annual Report to the US Forest Service, Tallahassee, FL**

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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). Striped newt repatriation coupled with precautionary measures to ensure success will be conducted over the 5-year period. This first annual report summarizes work conducted in Year 1 (October 2010-September 2011) of the study.

CPI biologists visited and sampled 158 isolated, ephemeral wetlands scattered across the Munson Sandhills Region of the ANF. Sampling occurred twice this year, once in the winter and once in the spring. These wetlands have been sampled 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 extremely droughty conditions. Out of the 158 wetlands visited in Winter 2011, 50 were hydrated. Out of that 50, only 16 still retained water by the spring sampling effort. Zero striped newts were observed during both efforts. These findings provide further evidence that the striped newt has sharply declined on the ANF, or may be extirpated.

Ranavirus susceptibility testing of larval striped newts took place this year. This was a critical step before repatriations begin later in the study. Our findings suggested that striped newts vary in susceptibility to different ranavirus isolates, which is similar to other amphibian species. They further suggest that susceptibility of striped newt larvae can be classified as low. These preliminary results are promising for the planned repatriations later in this study, and suggest that ranaviruses should have limited impact on repatriation success.

Western clade striped newt larvae genetically similar to ANF striped newts were collected in April from Big Pond in the Fall Line Sandhills Natural Area, Georgia. Larvae were sent overnight to study collaborators at the Memphis Zoo. Twenty-one striped newt larvae were successfully raised into the paedomorphic adult phase and by September 2011, these individuals were being prepared to be stimulated into breeding readiness. The 21 larval striped newts from Big Pond are being utilized for two important roles in the conservation of the striped newt. The first role is that they will serve as the F1 source for the creation of the first western clade striped newt captive assurance colony to be housed at the Memphis Zoo indefinitely. Secondly, hundreds, if not thousands, of larval individuals from this colony will be utilized for repatriations during this study's years 3-4.

Wetlands augmentation using groundwater was slated to be implemented in this study pending the results of a feasibility assessment. Augmentation was to be used as a technique to avoid wetland drying during later repatriations. CPI conducted an augmentation feasibility assessment in one of the proposed repatration wetlands in May 2011. Data from other researchers suggested that a geological confining layer apparently was missing underneath study area wetlands. To test this, we introduced 2500 gallons of water into the dried interior and observed. All 2500 gallons of water rapidly percolated into the wetland bottom over the course of 9 hours at a rate of 237.8 gallons (901 liters) per hour. The lack of a confining layer at the wetland was confirmed. Due to the rapid loss of supplemental water during the test augmentation, we concluded that wetlands augmentation would be unfeasible in this study for a variety of reasons. It would take more water to conduct augmentation than we could logistically produce. Supplemental water would have to be added continuously into repatriation wetlands during dry periods. Ecological impacts of adding such large amounts of groundwater with a different chemistry into an ephemeral wetland had the potential to be severe.

It became necessary to amend the original study design by omitting wetlands augmentation to implementing another technique to avoid wetland drying during repatriations, namely, the use of fish grade, biodegradeable synthetic liners to be installed underneath recipient wetland centers. Liners are expected to act as the missing confining layer that will retain rain water during repatriations and ensure that wetland centers do not go dry during repatriations in the event of drought. CPI and the USFS currently are engaged in the process to amend the original cost-share agreement between USFS and CPI to reflect the omission of augmentation and the installation of pond liners as the replacement technique to avoid pond drying.

# ACKNOWLEDGMENTS

We would like to thank many people for their important intellectual and/or physical contributions to this multifaceted, collaborative conservation project. We thank John Jensen of the Georgia Department of Natural Resources for intellectual assistance in the planning phase of this project and for field assistance. Many thanks to Dr. Lora Smith and the Joseph W. Jones Ecological Research Center for providing project partnership. A huge round of applause goes out to Dr. Steve Riechling of the Memphis Zoo, and numerous additional colleagues for partnering with our project to produce assurance colonies of both Western and Eastern clade striped newts to be used for this project and future conservation efforts. Additional colleagues include: Chris Baker, Steve Bogardy, Andrew Kouba, Brian Summerford, and Shannon White also from the Memphis Zoo; Jen Stabile of the Central Florida Zoo; Jessie Krebs of Omaha's Henry Doorly Zoo; Don Goff and J. T. Warner of the Beardsley Zoo; and Dino Ferri of the Jacksonville Zoo and Gardens. 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. They provided helpful field assistance, important data sharing, and intellectual assistance on hydrogeological aspects of this study.

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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). As of 2010, long-term CPI sampling data suggested that the striped newt had 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.

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 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. The gas pipeline expansion 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 address the severe decline of the striped newt population on the Apalachicola National Forest. Expansion of the gas pipeline corridor was an additional impact to the already imperiled ANF striped newt that provided the impetus for the current study. 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 multiple wetland breeding sites is expected to boost the ANF striped newt population and provide new management strategies for similarly imperiled amphibian species. This is the first annual report that summarizes work conducted in Year 1 (2010-11) of the study.

**Overall Study Objectives**

CPI's multifaceted 5-year repatriation study is a conservation strategy that will attempt to boost the population of the western striped newt within the Munson Sandhills region of the ANF.

1. Extensively sample the ANF for striped newts during 2 winter breeding and 2 spring larval seasons. Based on 11 years worth of sampling data, we hypothesize that the ANF striped newt is extirpated, or very nearly so. However, in order to be as thorough as possible, we believe it is necessary to provide more sampling

evidence on the status of the striped newt 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 do not observe newts after 2 more years of thorough sampling (i.e. 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 will come from southwest Georgia, if no newts can be found in the ANF.

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

4. Conduct striped newt repatriation efforts in the ANF. We will conduct repatriation efforts in multiple 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 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.

5. Investigate and implement techniques to ensure there are suitable larval developmental conditions at the selected repatration wetlands. Short-term wetland augmentation using groundwater from solar powered wells was originally proposed for repatriation wetlands to ensure that wetlands not dry up during larval repatraition periods. However, after conducting an augmentation feasibility test in study Year 1, we concluded that augmentation would not be feasible at repatriation sites (see Results and Discussion section for details).

   Another method to ensure repatriation wetlands do not go dry during the critical repatriation periods later in this study currently is being proposed to replace augmentation. CPI proposes to install biodegradeable, fish grade, synthetic liners under small interior portions of selected repatriation wetlands that will act as catchments for rain water. After liner installation, introduced larvae are expected to have pools in wetland centers as refugia in the event that wetlands threaten to go dry during repatriations.

6. Hand-remove encroaching woody shrubs and slash pines from the basins of repatriation 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.

# 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, 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 western half of the Munson Sandhills is located within the Apalachicola National Forest (ANF). This region of the ANF contains over 150 ephemeral wetlands. These wetlands provide breeding habitat for over 20 amphibian species, including the striped newt. The majority of the uplands are a fire-maintained longleaf pine ecosystem.

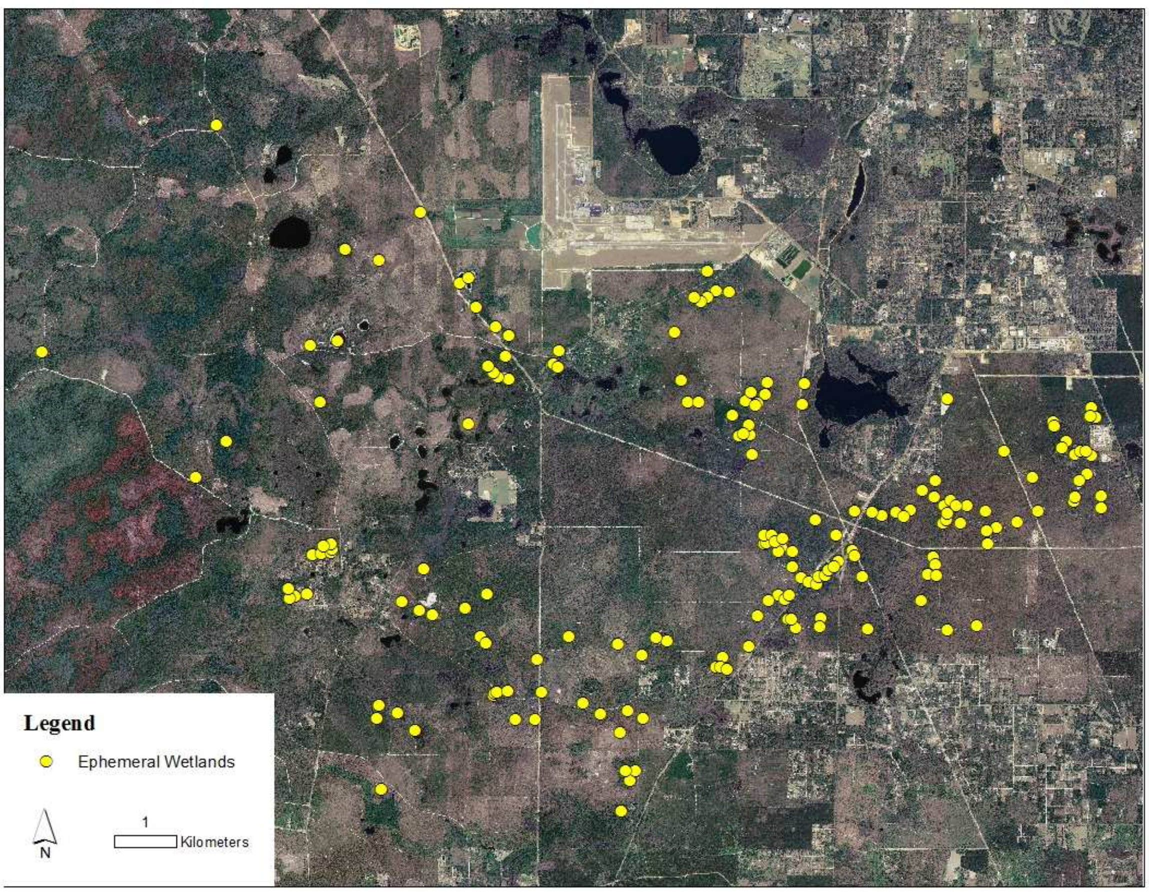


**Figure 1.** Map of the study area depicting the location of ephemeral wetlands.

# METHODS

## Striped Newt Sampling in the Apalachicola National Forest

We visited and sampled 158 isolated wetlands across the Munson Sandhills region of the ANF with dipnet and/or seine. The first sampling event took place during the January-March winter breeding season to sample for striped newt aquatic adults. The second sampling event took place during the April-June larval development season in an attempt to detect striped newt larvae.

Sampling was conducted using a heavy duty dip net (Memphis Net and Twine Co. HDD-2 model) with 3/16" mesh. The number of dip net sweeps per pond varied depending on pond size. The entire pond periphery and the center of smaller ponds was swept and a minimum of 50 sweeps was used for larger ponds. Sweep efforts were concentrated in the herbaceous vegetation where newt larva concentrate.

## Ranaviral Susceptibility Testing

During March 2011, CPI bioligists, along with study collaborator Dr. Steve Johnson (University of Florida), collected 10 eastern striped newt adults/paedomorphs from a single, well known locality known as Round Pond in the Ocala National Forest. The 10 individuals were sent overnight Fed Ex to study collaborators Drs. Gray and Miller at the University of Tennessee. These individuals were slated to become the founders of a captive population of eastern striped newts to be utilized for ranavirus susceptibility testing as part of the current repatriation study. However, within 3 weeks of arrival, all 10 newts died for unknown reasons.

Considering the failed breeding attempt at UT, we solicited additional striped newt larvae from several zoological institutions with the help of Steve Reichling (Memphis Zoo). Between June and September 2011, we received 147 striped newt larvae from four institutions (Connecticut Zoo, Jacksonville Zoo, Central Florida Zoo, and Omaha, NE Zoo). Most larvae arrived in good condition and survived. Eighty larvae that were at least 4 weeks post-hatching were used for the first ranavirus challenge experiment, which was conducted 16 August – 6 September 2011 (see below). Remaining larvae (ca. 20 newts) are being raised through metamorphosis for the second experiment; additional larvae (ca. 60) will need to be acquired.

Ranaviral susceptibility testing is proceeding under the direction of study collaborators Drs. Debra Miller and Matthew Gray of the University of Tennessee.

Larval 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 (no virus) served as the control. Based on our previous experiments with 27 North American amphibian species (Hoverman et al. 2011; R. Brenes, unpubl. data), FV3, SM, and RI can be generally classified as having low, moderate, and high pathogenicity. The experiment consisted of the four treatments with 20 replicate larvae per treatment, totaling 80 experimental units. Larvae were randomly selected and placed individually in 2-L plastic tubs containing 1 L of aged water. After 24 hours acclimation, we added $10^3$ PFU/mL of each virus isolate to 20 tubs corresponding to its treatment. Control larvae were exposed to the same quantity of virus-free media.

Larvae 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.) 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 that exhibit 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 larvae were humanely euthanized by immersion in benzocaine hydrochloride and necropsied. At necropsy, gross changes were noted. Sections of body containing liver were collected for PCR analysis and remaining portions placed into 10% formalin for possible future histopathological evaluation.

**Establishment of the Western Striped Newt Assurance/Repatriation Colony**

In April 2011, CPI biologist Ryan Means, along with Georgia DNR biologist John Jensen, dipnet sampled Big Pond in the Fall Line Sandhills Natural Area near Butler, GA.. Big Pond is the last known perennially reliable source wetland for western striped newts. Twenty-five larval striped newts were collected after approximately 2 hours of dipnet sampling (Figure 2). The newts were shipped overnight Fed Ex to study collaborator Dr. Steve Reichling, Curator, Memphis Zoo.

**Figure 2.** Dipnet sampling for western striped newt larvae at Big Pond, Fall Line Sandhills Natural Area, GA.

# Results and Discussion

## Striped Newt Sampling of the Apalachicola National Forest

During February and early March 2011, CPI biologists extensively revisited and resampled 158 isolated, ephemeral wetlands scattered across the Munson Sandhills Region of the ANF. These wetlands have been sampled in past years by CPI as part of prior efforts to monitor for striped newts in the region. Out of the 158 wetlands visited in Winter 2011, 50 were hydrated. The hydrated wetlands were standardly dipnet sampled for the presence of breeding adult striped newts. Zero striped newts were observed.

Weather conditions during winter 2011 were not prime for striped newt breeding, nor for amphibian breeding in general, but several frontal passages produced light to moderate rains that could have stimulated breeding. All wetlands underwent a drying trend during the period, despite some frontal passages. This was testimony that no siginificantly heavy rains fell during the winter sampling period--at least not significant enough to fill wetlands.

Other pond-breeding amphibians were regularly or sporadically observed regionwide during the winter sampling period, including: mole salamander larvae (*Ambystoma talpoideum*), southern leopard frog larvae and adults (*Lithobates sphenocephala*), gopher frog larvae (*Lithobates capito*), cricket frog adults (*Acris gryllus*). The ornate chorus frog (*Pseudacris ornata*) and southern chorus frog (*Pseudacris nigrita*) were observed calling sporadically, but no larvae or adults were observed during the winter.

Durng late April and Early May, 2011, CPI biologists conducted the second sampling effort for the striped newt in the Munson Sandhills study region of the ANF. Spring is the time of year when larval striped newts can be found; however, larval striped newts have not been observed since the late 1990's in this region--the very observation that is the impetus for the current study. After sampling the region again this spring, our results remained the same--no striped newt larvae were observed.

Area ephemeral wetlands continued the drying trend that began in the winter. Severe drought set in beginning in April. The entire period of April through June was extremely dry, and daytime temperatures soared to 100 degrees regularly by June. Of the 158 wetlands that were visited in the wintertime, only 16 had standing water by May (Figure 3). Breeding conditions for striped newts in 2011 were extremely poor overall.

**Figure 3.** Study pond 54 held water during the winter striped newt sampling effort (left) but went dry by the spring sampling effort (right). Extremely droughty conditions prevailed across the Apalachicola National Forest study area in 2011 and almost all of the area ephemeral wetlands went dry by May.

**Ranaviral Susceptibility Testing Preliminary Results**

Survival curves 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 Mountain isolates, respectively.

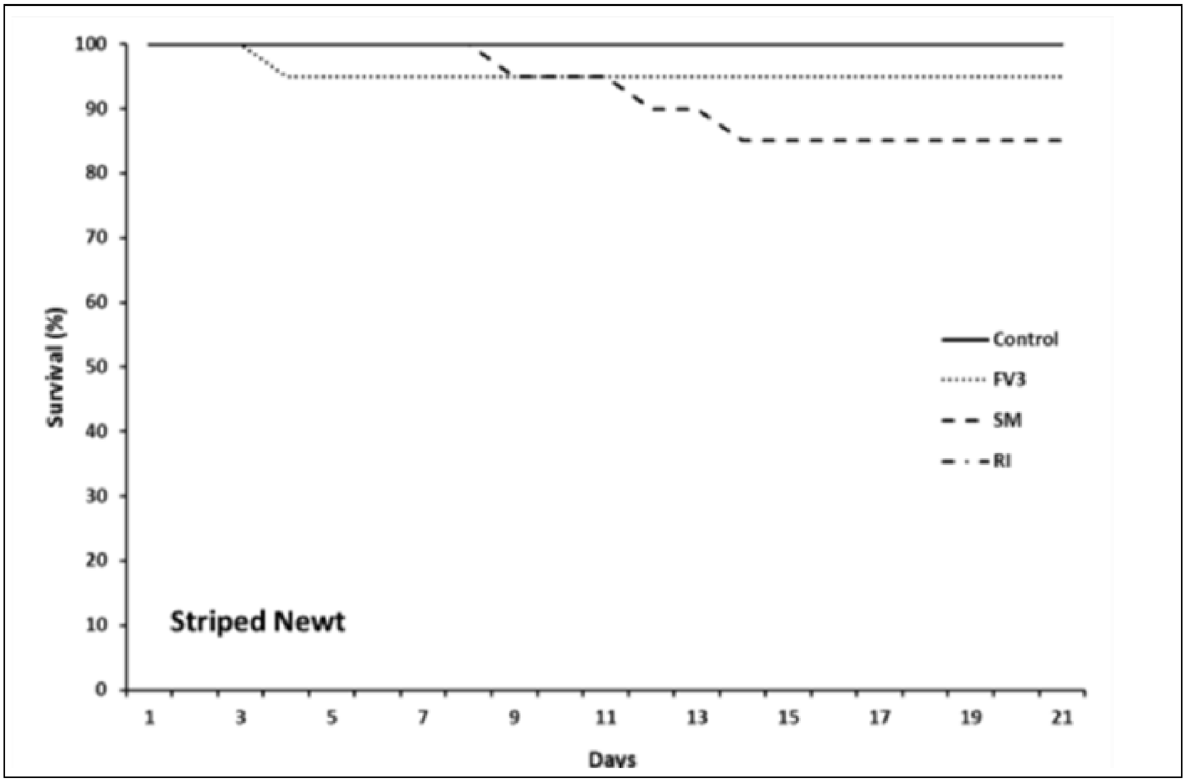


**Figure 4.** Percent survival of individuals (n = 20/treatment) exposed to one of three isolates of ranavirus (FV3 = frog virus 3, SM = Smoky Mountains isolate, RI = Ranaculture Isolate). Survival curves (100%) for controls and RI overlap.

These findings suggest that striped newts vary in susceptibility to different ranavirus isolates, which is similar to other amphibian species (Hoverman et al. 2010, Schock et al. 2010). They further suggest that susceptibility of striped newt larvae may be similar to eastern newt larvae, and in general, can be classified as low (Hoverman et al. 2011). Considering that immune function increases in amphibians following metamorphosis (Robert 2010), we hypothesize that very little mortality from ranaviral disease will be observed during the second experiment with juveniles. These preliminary results are promising for the planned repatriation project, and suggest that ranaviruses should have limited impact on repatriation success. However, we cautiously note that unique ranavirus strains could occur in the ANF that have greater pathogenicity, which emphasizes the importance of the planned surveillance activities. If ranavirus is detected at repatriation sites, we will attempt to isolate and characterize the virus to determine if it is genetically different than the isolates used in the challenge experiments.

**Establishment of the Western Striped Newt Assurance/Repatriation Colony**

Big Pond was completely dry less than one month after our sampling effort.

Twenty-one larvae survived the shipping process and were given excellent care and attention by Memphis Zoo staff. All were successfully raised into the paedomorphic adult phase. At the time of this report writing, 5 months later, these individuals all had developed into adults and were being prepared to be stimulated into breeding readiness.

The 21 larval western striped newts from Big Pond are being utilized for two important roles in the conservation of the western striped newt. The first role is that they will serve as the F1 source for the creation of a substantial western striped newt captive assurance colony that will be housed at the Memphis Zoo indefinitely. This will be the first captive raised assurance colony for the western striped newt in existence. Secondly, hundreds, if not thousands, of larval individuals from this colony will be utilized for repatriations during this study's years 3-4. The creation of the western striped newt assurance/repatriation colony is proceeding under the direction of Dr. Reichling and colleagues at the Memphis Zoo.

It is extremely important to conduct repatriations utilizing indviduals from the same genetic stock, or as closely as possible, to the original population that once occurred in the ANF. This belief was the basis for our collecting individuals from Big Pond within the western clade (same as the ANF) to become the source individuals for the assurance/repatriation colony, instead of utilizing individuals from the eastern clade.

**Wetlands Augmentation Feasibility Assessment at Study Pond 1 in the ANF**

We began communications and data-sharing with researchers from Florida State University and Florida A&M University shortly after the beginning of the current study. Hydrogeologist Dr. Steve Kish from FSU and wetland scientist Dr. Katherine Milla from FAMU generated data that strongly suggested that the hydrology of one of our prospective repatriation/augmentation wetlands (Study Pond 1) is driven by a direct relationship with the underlying Floridan Aquifer System. Data show that Study Pond 1 rises and recedes identically with the known potentiometric surface of the local Floridan Aquifer. It became apparent that the water level of Study Pond 1 is a manifestation of the local groundwater table. This finding suggested that there was no geological confining layer present under Study Pond 1. There would hypothetically be nothing to prevent largescale loss of supplemental water into the aquifer if augmentation were to be attempted.

By late May, 2011, Study Pond 1 had been dry for nearly two months. Study Pond 1 had been considered as being one of two potential recipient sites for later repatriations. On May 21, 2011

CPI conducted a test augmentation within the dried basin of Study Pond 1 as part of the process to assess the feasibility of conducting wetlands augmentations. CPI, with the help of Chuck Hess of the USFS, arranged for a single water truck to introduce 2500 gallons of water into the center of the wetland (Figure 5). The supplemental water puddle occupied an area of 35.6 $m^2$ and a center depth of 24 cm. Water levels were observed hourly and recorded so that the infiltration rate could be calculated.

**Figure 5.** Conducting a test augmentation at a potential augmentation/repatriation wetland.

All 2500 gallons of water rapidly percolated into the wetland bottom over the course of 9 hours at a rate of 237.8 gallons (901 liters) per hour (Figure 6). Water depth decreased by 2.53 cm per hour. Applying these rates to the level that would be needed to maintain our proposed stable wetland conditions for repatriations, 835 gallons (3164 liters) per hour would be continuously needed to maintain stable water levels during dry periods. If wetlands were to remain similarly dry throughout the entire repatriation periods later in this study, then we would need up to 300,527 gallons (1,139,000 liters) of water to maintain suitable water levels within just a single treatment wetland.

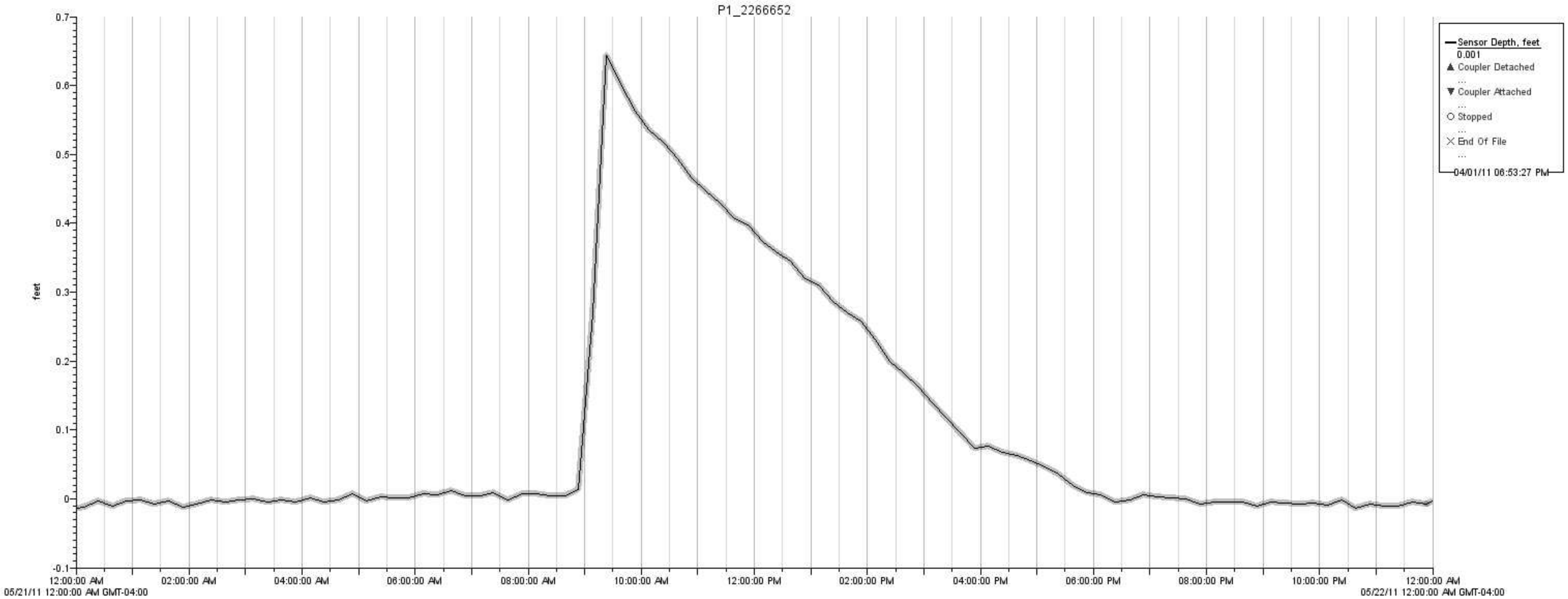


**Figure 6.** Test augmentation results from Study Pond 1, May 2011. Water (2,500 gallons) was introduced into the dry wetland at approximately 0930 hrs. All water had infiltrated into the ground directly into the aquifer by 1800 hrs on the same day. The spike represents the introduction of water into the wetland followed by the rapid infiltration of that water into the ground. The negatively sloping line past the spike represents the infiltration rate of -2.51 cm per hour. The flat lines before and after the spike represent the groundwater table. (Figure courtesy of Dr. Katherine Milla, Florida A&M University).

Because of the rapid percolation of supplemental water during the test augmentation, we concluded that wetlands augmentation would be unfeasible in this study for a variety of reasons. It would take more water to conduct augmentation than we could logistically produce with solar power. Supplemental water would have to be added continuously to recipient wetlands. We believed that the ecological impacts of adding large amounts of cool groundwater with potentially different chemistry into an ephemeral wetland had the potential to be severe. Continuously introducing water into a wetland would create currents within a normally still water environment--the effects of which are unknown. Subtracting large amounts of water from the aquifer nearby to a recipient wetland also would create a relatively large cone of depression in the aquifer surface, which, in turn, would likely increase the water percolation rate in that wetland and further confound augmentation issues.

Our original concept of augmentation only called for the use of short-term, occasional boosts--not for large volumes of continuously running water. CPI concluded that the original study design to utilize wetlands augmentation as a tool to avoid wetland dry up during repatriations needed to be amended.

**Amendment to original cost-share agreement between USFS and CPI**

CPI currently is in the process of amending the original agreement between the USFS and CPI to conduct the 5-year striped newt repatriation study on the ANF. As part of the new study design, CPI will omit wetlands augmentation from the original agreement. This creates the need to replace augmentation with another method to ensure that repatriation wetlands do not dry up during the critical larval repatriation periods later in this study.

As an alternative approach to avoid repatriation wetland dry-up during critical periods, CPI proposes to add biodegradeable, fish-grade, synthetic liners to the central portions of selected ephemeral wetlands. These liners have been successfully used as confining layers in ephemeral wetlands and will be installed as described in Biebighauser (2002).

Using pond liners instead of groundwater augmentation will allow CPI to double the original number of proposed repatriation wetlands from two to four. It is significantly cheaper to install pond liners than to conduct wetlands augmentation; CPI will allocate former augmentation-related funding toward additional repatriation effort. Doubling the number of receipient wetlands also will increase our chance of success by reducing the influence of localized conditions and increasing the total number of newts repatriated.

By May 2012, four locations will be selected within the eastern Munson Sandhills to be the recipient locations for striped newt repatriations. Two of the four repatriation sites will be historical striped newt breeding wetlands. Selected repatriation sites will be nearby to other ephemeral wetlands or wetland clusters that tend to hold water for the longest periods. This selection criterion is needed in order to ensure that repatriated newts will have nearby wetlands acting as recipient wetlands for future population expansion.

Liners will be installed in targeted wetlands for a 10-month observational period before the first repatriations begin. This provides enough time for wetlands to fill either by rainfall alone or by a combination of rainfall and aquifer recharge, and will allow the pond vegetation time to recover. Selected wetlands were dry at the time of this report writing (September 2011) and are anticipated to be dry next May 2012. However, six additional wetlands have been identified as backups in the event that unforseen tropical or heavy winter rains fill area wetlands between now and May 2012. This will ensure we will have additional options from which to choose dry sites for liner installation.

# YEAR 2 EXPECTATIONS

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.

We will repeat Georgia sampling in springtime, if necessary, to add individuals to supplement a colony formed within Year 1.

By May 2012, four locations will be selected within the eastern Munson Sandhills to be the recipient locations for striped newt repatriations. Two of these locations will be sink depressional wetlands that have deep centers with typically longer hydroperiods. Two will be shallower karst depressions in the nearby uplands that have been dry for most of the past decade and can be classified as extremely ephemeral wetlands. Two of the four repatriation sites will be historical striped newt breeding wetlands. An additional six sites will be identified as potential backup sites. Selected repatriation sites will be nearby to other ephemeral wetlands or wetland clusters that tend to hold water for the longest periods. This selection criterion is needed in order to ensure that repatriated newts will have nearby wetlands acting as source wetlands for future population expansion.

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.

During May 2012, CPI will install biodegradeable "fish grade" synthetic liners within each of the four selected repatriation locations. Short term disturbance is anticipated to recover quickly. A 10-month observational period will follow during which vegetation can recover and wetlands can fill with rainwater.

In Years 2 and 3, we will test up to 60 larval amphibians per year at the four 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. 2009). 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). 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. Additionally, the UT Center for Wildlife Health plans to test the susceptibility of juvenile striped newts, assuming sufficient numbers of larval striped newts can be acquired from zoological facilities and raised through metamorphosis.

Second annual report will be submitted in September 2012.

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