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

Fourth Annual Report to the U.S. Forest Service, Tallahassee, FL

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Executive Summary

The Coastal Plains Institute (CPI) and US Forest Service (USFS) entered a 5-year cost-share agreement in October 2010 to address the severe decline of the western striped newt (*Notophthalmus perstriatus*) within the Munson Sandhills of the Apalachicola National Forest (ANF). At that time, CPI and cooperators initiated a multifaceted study to: 1) investigate the cause of the striped newt decline in the ANF, 2) conduct larval repatriations in selected prime wetlands, and 3) investigate and implement precautionary measures to ensure success of repatriations. This report summarizes work conducted in Year 4 (October 2013-September 2014) of the current 5-year study.

After exhaustive and unsuccessful efforts to find remnant striped newts within the ANF both before and after this study's inception, we reported in 2012 (Year 2) what we strongly believed to be extirpation of the western striped newt within our ANF study area. This year (2014), we continued a background monitoring effort within historical striped newt wetlands in the ANF. Despite prime late winter and early spring breeding conditions we continued to observe no western striped newts remaining within historical ANF breeding wetlands. These findings further support our extirpation hypothesis.

Synthetic EPDM pond liners were installed in Year 2 (2012) within three of our four selected repatriation wetlands to lengthen recipient pond hydroperiods so that released newt larvae would have enough time to metamorphose. This year, all liner-enhanced wetlands continued to be ecologically healthy and contained up to five species of larval amphibians at any given time, including the rare ornate chorus frog and gopher frog. They also held water long enough to allow for striped newt metamorphosis. This study's liner technique may prove useful to land managers who wish to manage isolated wetlands within drought-stricken landscapes or to future studies involving rare amphibians.

Last year (2013) we released 58 larval newts into a single wetland, and three efts were observed exiting that wetland. This equated to an eft yield of 5%. Percent eft yield likely would have been higher, but our drift fence flooded, and we suspected that some efts may have trespassed over the top of our fence.

This year (2014), we released 433 larvae during several separate events into all four recipient wetlands. A total of 32 newly-metamorphosed, terrestrial efts were observed exiting our recipient wetlands. This equated to an overall eft yield of 7.4%. Percent eft yields at individual recipient wetlands in 2014 ranged from .08% up to as high as 34.5%, depending on the wetland, season, and degree of drift fence flooding. Actual percent eft yields for 2014 likely were higher than document yields at all sites. Our drift fences, again, became flooded during the prime metamorphosis periods, and we suspect that some metamorphosing efts may have trespassed over the top of our flooded fences.

Despite drift fence flooding, we observed an upward trend toward greater success in 2014 relative to 2013. In 2015, we plan to continue the upward trend by releasing even more individuals per wetland, and applying knowledge gained in the first two years of repatriations. This year's results were very promising and indicate that we have learned much about striped newt husbandry and release techniques, a body of knowledge that was all but completely unknown before our study. We expect to be successful at reaching our long-term study goal with continued repatriation efforts. The field component of our cost-share agreement with USFS officially ends March 2015. We currently are seeking funding to continue our efforts beyond the extent of the current cost-share agreement. Continuing the project beyond March will allow us to measure the success of this year's repatriation efforts as well as release larval newts during the optimal season (late spring/early summer).

ACKNOWLEDGMENTS

We would like to thank many people and institutions for their important intellectual and/or physical contributions to this multifaceted, collaborative conservation project. Numerous 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: Brian Eisele, Sean Wallace, Stefanie Jackson, Emily Mikus, and Steve Gott from Jacksonville Zoo and Gardens; and Steve Bogardy 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.

CONTENTS

FIGURES

TABLES

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 was 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 of 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 southern Georgia, 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.

During the past two 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, JJERC, 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.

CPI has monitored the western striped newt in its ANF stronghold range for over 20 years. The last time we observed larvae in the ANF was in 1998. The last adult was observed in 2007, despite intensive wetland sampling nearly every year since then. By 2010, we believed that the western striped newt in the ANF had likely become extirpated.

One possible cause of the striped newt decline in the ANF is drought. Another is pathogen infection. Other possible causes for decline are 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 in 2011 to create a study that addresses the extirpation of the striped newt population on the ANF. CPI's extirpation hypothesis provided much of the impetus for the current study. Striped newt repatriation coupled with precautionary measures to ensure repatriation success and enhance breeding habitat are being conducted as part of the study. An investigation into the cause of decline also is underway. This study is expected to boost the ANF striped newt population and possibly provide new management strategies for similarly imperiled amphibian species. This report summarizes work conducted in Year 4 (Oct 2013-Sept 2014) 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). Use larvae from the assurance colonies as the source for larval repatriations in the ANF.

2. Continue sampling the ANF for local striped newts (Years 1-5). Although we hypothesized that the ANF striped newt was likely extirpated before this study,

we will continue surveillance sampling to be as certain as possible that the local population is extirpated.

3. Investigate the cause(s) of decline (Years 1-3). 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).

4. Conduct striped newt repatriation efforts in the ANF (Years 3-5). Using captive-bred larvae from striped newt assurance colonies (Objective 1), we will conduct repatriation efforts in four wetlands.

5. Investigate and implement techniques to ensure there are suitable hydrological conditions at selected repatriation wetlands by repatriation time (Years 1-5). Techniques include wetland augmentation and synthetic liner installation.

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 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 (Figure 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 (Figure 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 western 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.

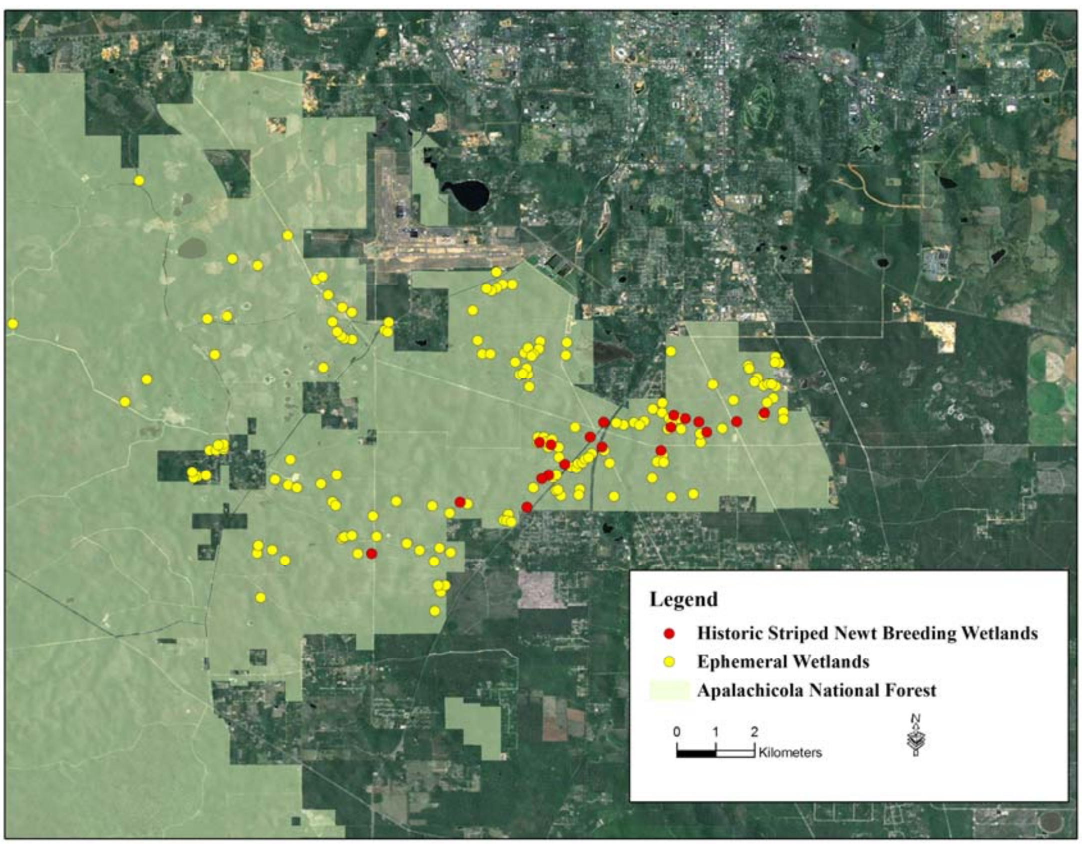

Figure 1. Map of the Munson Sandhills study area. Yellow dots represent the 158 ephemeral wetlands that have been periodically sampled over the last 20 years. Red dots represent the 19 historic striped newt breeding wetlands.

METHODS

Background Monitoring for the Striped Newt

During Year 4, we sampled all 19 historically known striped newt breeding wetlands in the MS region of the ANF with dipnet, as water levels allowed. The first sampling effort took place during February 2014 winter breeding season to search for presence of striped newt aquatic adults. The second sampling effort took place during March and April 2014, what would have historically been spring larval development season.

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. A 6' seine net also was used to supplement our dipnetting efforts. Seine nets are pulled through a large swath of water by two researchers, one on each side of the net. This method has the potential to capture many times more aquatic organisms than a dipnet.

Hydrology and Ecology of Repatriation/Liner Wetlands

During Year 2, we selected four wetlands as repatriation (recipient) wetlands based on their suitability as prime striped newt breeding habitat and suitability for synthetic liner installation: Pond 16, Pond 18, Pond 75, and Pond 182 (Figure 2). Numerical designations were carried over from past CPI surveys of the MS ephemeral pond assemblage. All wetlands were nearby to the expanded gas pipeline corridor within longleaf pine sandhill habitat. Three wetlands 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).

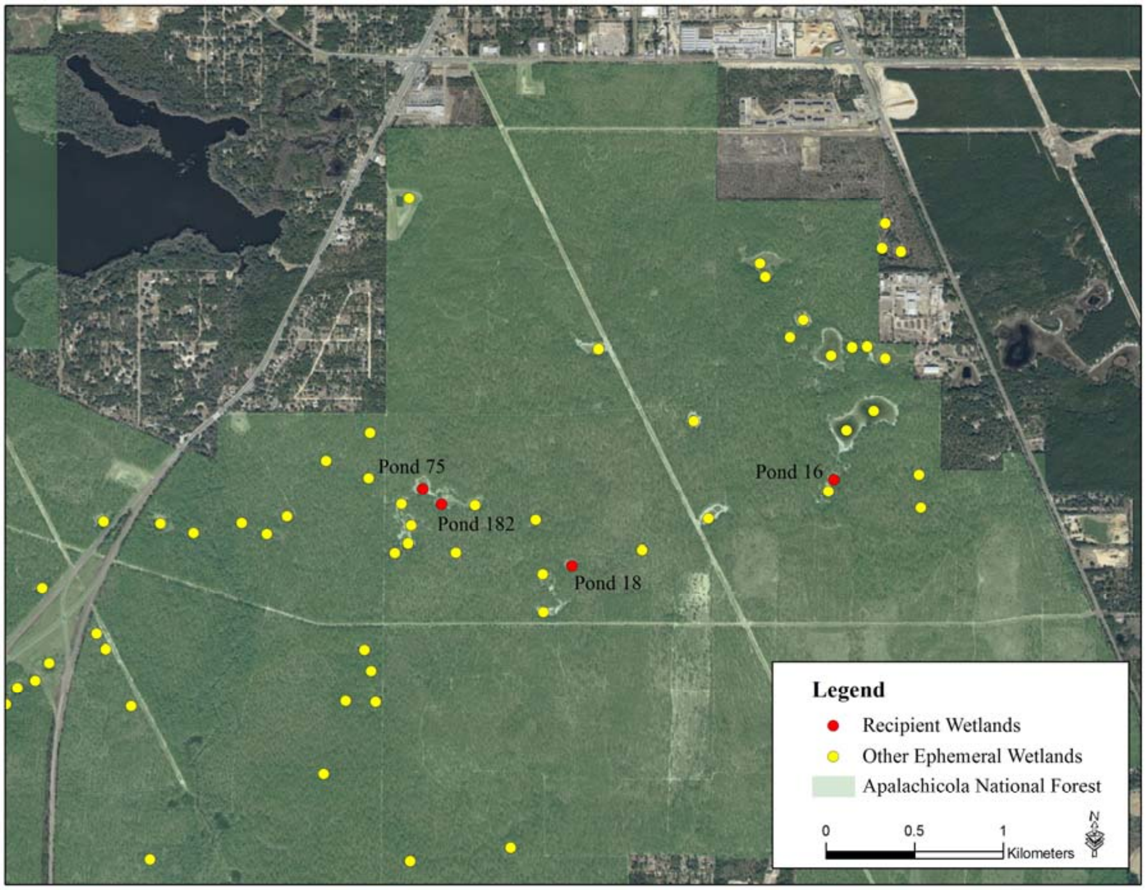

Figure 2. Map depicting location of the four striped newt repatriation/liner wetlands.

Synthetic (EPDM) liners were installed in Year 2 underneath three of our four selected repatriation wetlands (18, 75, 182). Liners were employed as a technique to boost recipient pond hydroperiods and make them more drought resistant, particularly during larval repatriation periods. The fourth, Pond 16, also was scheduled to receive a liner, but conditions there have been too wet to complete installation. A liner will be installed when or if it dries sufficiently enough to enable installation. Four nearby and hydrologically similar wetlands were selectively paired to repatriation wetlands as hydrological reference/control wetlands. Pond 15 is paired with Pond 16, Pond 17 is paired with Pond 18, Pond 75 is paired with 73, and Pond 48 is paired with Pond 182. Observational water presence/absence data have been collected monthly at the four repatriation and four reference wetlands since liner installation.

Repatriation and reference wetlands also were dipnetted periodically, simultaneous and inclusive within background striped newt monitoring, to measure and compare amphibian species richness between liner and reference wetlands.

Striped Newt Husbandry

Jacksonville Zoo and Gardens

Adult striped newts were housed in 21.5 gallon Rubbermaid food storage containers measuring 26" x 18" x 15". We utilized Gladiator 4-shelf storage racks measuring 77" x 73" x 24", with only three of the four shelves in use. All Rubbermaid units had a 1" bulkhead and standpipe for drainage; the standpipe and screen measured approximately 5.5" tall, leading to a total water depth of about 6" or 8 gallons. Standpipes drained into a 2" PVC line leading to a filter sump. All water passed through a 100 micron bag filter; it then passed through the sump which was filled with Bio-Balls, then was pumped through a 20 micron filter and ultraviolet sterilizer before being pumped back into the enclosures via 1" PVC pipes (Figure 3).

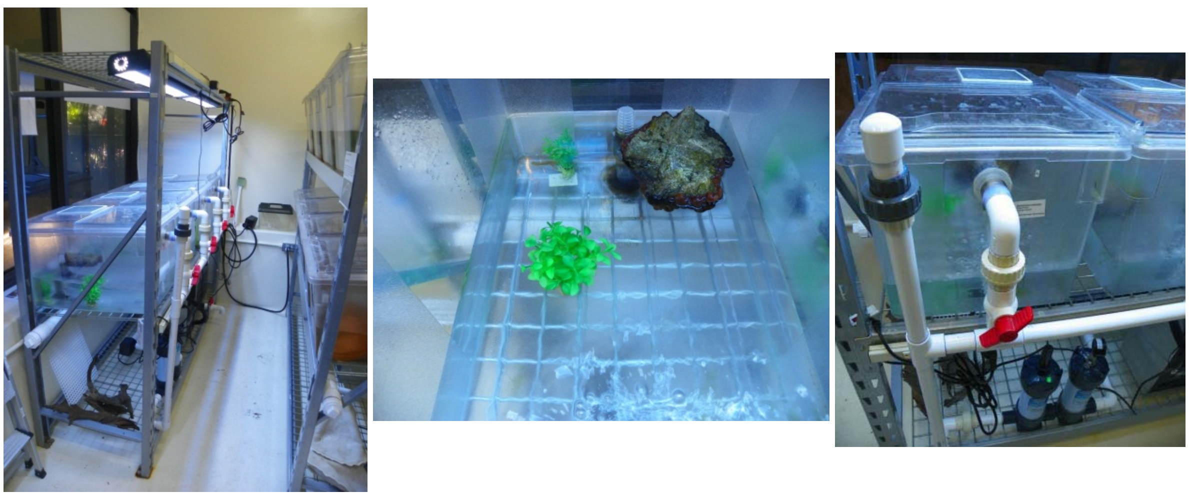

Figure 3. Adult striped newt husbandry operation at Jacksonville Zoo and Gardens.

Temperatures were adjusted seasonally, from 64 degrees in the winter to 75 degrees in the summer months. This was accomplished with the use of a Cyclone Pro drop-in chiller by Trimline; model # TLC-3. Light cycles were also adjusted seasonally from 13 hours of daylight in the summer months to 11 hours of daylight in the winter months. Additionally, the room in which they were held had skylights that allow for further seasonal light cycling. The drop-in chiller unit was adjusted down 2 degrees per day starting in mid-October, and was raised 1 degree per day starting in mid-March.

Currently, newts are housed in pairs (1.1 per unit). All enclosures had submerged plastic plants on which the newts deposit their eggs. The newts historically have laid eggs starting in January, and often continued to lay fertile eggs until May or June. The plants were removed every week and placed in 1 quart deli cups for hatching; these were replaced with fresh plastic plants (Figure 4). Deli cups were removed to a separate room where temperatures were 75 degrees; the warmer temperatures promoted faster hatch rates. By using this procedure, we were better able to count all hatchling newt larvae.


Figure 4. Striped newt egg hatching equipment and set-up used at Jacksonville Zoo and Gardens.

All hatchlings were removed from the deli cups and placed into a 20-gallon aquarium by using a 60 mL syringe. The aquarium was allowed to cycle beforehand and had a sponge filter for biological filtration and aeration. The tank was seeded with blackworms starting in December. Once all eggs hatched from the plants, the plants were disinfected with a 15% Chlorine bleach/water solution, rinsed, and allowed to thoroughly dry before being placed back into the breeding tanks.

Striped newt larvae were kept together in relatively large groups, usually within 2 weeks of hatch date(s). The larvae were small, measuring about 6-8 mm at hatching and often did not eat for several days while they absorbed the remnants of their yolk. Within one week, all larvae had began eating, and many often had visible front limbs. At this time we began feeding live hatchling brine shrimp at least three days per week, as well as chopped blackworms. Blackworms were prepared for the young larvae by placing a small amount (a couple hundred or about 10-20 mL when using a syringe) onto a Petri dish where they were sliced with a straight-edge razor blade into smaller (1mm-4mm) pieces. Many worms started to regenerate, and the smaller ends were what the newt larvae often fed on. Newt larvae were very visual feeders, and if the prey item was not moving they quickly lost interest. Small larvae often were observed shaking and tearing off the ends of blackworms much larger than themselves. Brine shrimp were hatched out using a San Francisco Bay Brand Brine Shrimp Hatchery Kit. Brine shrimp were hatched out approximately three times per week; these were drained from the hatchery via the air hose, separated from the water using a brine shrimp net, and rinsed prior to feeding.

Feeding newt larvae was the most difficult part of the striped newt breeding process. Newt larvae grew quickly, but for the first four to five weeks they required the aforementioned food preparation process(es). After five weeks of age most larvae had partially to well-developed hind limbs and were over 20 mm in length. At this age and size they were capable of hunting adult blackworms. In larger concentrations, it was imperative that the larvae had food available at all times. Cannibalism occurred frequently and tail tips, gills, and limbs went missing often if food was not present in the enclosure(s).

As newt larvae grew, smaller (or larger) animals were removed, depending on their prevalence, into other enclosures. As they grew, animals were often kept together based on size rather than age. Most larval grow-out tanks were set up the same way as the adult breeding tanks, with the addition of free-floating mats of java moss; the moss allowed for great hiding opportunities, as well as some level of biological filtration. Food (primarily whole blackworms) was offered to larger newt larvae three times per week, more often if needed. Many newt larvae began to metamorphose at three to four months of age at the Jacksonville Zoo and Gardens. Ambient room air temperature was maintained at 76 degrees Fahrenheit during the warmer months. Large floating pieces of cork bark were added to the enclosures as the newts began to venture out of the water, and small crickets or fruit flies were offered once per week on these pieces of bark in addition to the blackworms (Figure 5).

Figure 5. Striped newt on floating piece of cork bark. Bark was introduced to enclosures as newts began to venture out of the water.

For all animals, 30-50% water changes were performed once per week, and the 20 micron filters were rinsed weekly as well. All of the water used for these animals was run through a reverse osmosis filtration system with additional water softener and carbon filtration. This water was subsequently reconstituted with SeaChem products: Equilibrium, Acid Buffer and Alkaline Buffer. The pH was maintained at or very near 6.5. The added carbon filter removed any residual chlorine. Newts do not seem to tolerate even relatively low levels of chlorine, therefore, it was imperative to take great measures to ensure that, if present, chlorine be removed from any water that would come in contact with newts. To err on the side of caution, we added an additional SeaChem product, called Prime, to the systems after a water change. Prime dechlorinated as well as detoxified ammonia.

Memphis Zoo

As of September 8, 2014, the breeding group census stood at 23 and included 7.6 (collected and obtained in 2011) and 0.0.10 collected in 2013. They were distributed among seven 10-gallon aquariums in groups of 2.1, 2.1, 1.1, 1.1, and 1.2 (2011 cohort) and 0.0.5 and 0.0.5 (2013 cohort). We currently hold 68 larvae produced in 2014.

The 13 newts in the 2011 cohort were sexually mature and reproducing. The 2013 specimens were only beginning to reach sexual maturity, having displayed breeding behaviors in September 2014 for the first time. These were now able to be sexed, and upon doing so will be divided into pairs or trios as with the 2011 newts to prepare for their first breeding season this fall.

Aquaria were maintained with 4 inches of water, java moss, a bubble filter and a piece of floating cork bark. Breeding activity was stimulated by abruptly dropping the water level by half or less and then "raining" on them for several days in a row, varying the amount of water each time. Once breeding behaviors were observed the artificial rains were reduced, and then terminated upon the onset of oviposition. Eggs were collected and put in a hatching tank to avoid predation by adults, or live zooplankton, which the adults fed on ad lib. As eggs hatched, the larvae were removed and put into 10-gallon tanks set up in the same way as the adults. We limited larvae per aquaria to 25. As larvae grew we move larger animals to prevent predation on smaller individuals. Larvae were reared on a diet of chopped blackworms and *daphni*a.

At present, 68 larvae are being reared for release or dissemination. Twenty were planned for shipment to the Lowry Park Zoo as soon as the temperatures moderate to insure safe transit. This will create a third breeding nucleus, joining Memphis Zoo and Jacksonville Zoo and Gardens. The remaining 48 larvae will be raised through the winter and shipped in January 2015 to be released as adults. Additional larvae may be produced between now and the January release date.

Striped Newt Repatriation and Monitoring

Box enclosures

Similar to last year, we created predator-free box enclosures (i.e. holding boxes) into which we release tiny larvae. The purpose is to reduce predation potential and increase survivorship of young repatriants. We constructed this year's enclosures differently than last year to improve upon two flaws we identified. Last year's screened enclosures were "enclosed" by simply placing masonry bricks around the bottom flared perimeter of enclosures (Means et al. 2013). It was presumed that this would provide a tight enough seal between the screen and irregular, peaty pond bottoms. This probably wasn't the case as evidenced by the unexplained presence of invertebrate predators within enclosures weeks later after removing all predators prior to a newt release. Also, last year's enclosures were staked to the pond bottom and were unable to be moved if necessary. This year, we constructed fully enclosed, net- wrapped, free standing boxes that could be picked up and moved, if necessary (Figure 6). This year's design removed both of last year's concerns.

Holding boxes consisted of cube shaped skeletons constructed out of 1/2 inch diameter PVC pipe. Cube dimensions were approximately 1-meter cubed. Pipe skeletons were then wrapped with 1/16 inch durable delta polyester netting obtained from Memphis Net and Twine Co. Net ends were tucked or folded and numerous 4 and 6 inch plastic cable ties were used to fasten and close any potential openings. One slot at the top of boxes was left as a door opening. This opening also was fastened with cable ties after newt releases to fully enclose boxes while in use (Figure 6a).

One-sixteenth inch sized mesh was the perfect hole size to be restrictive to potentially incoming predatory animals (e.g. mole salamander, predatory macroinvertebrates) but allow adequate sizes and abundances of naturally occurring aquatic zooplankton to enter through the mesh to provide food for small newts (Figure 6b).

a.

b.

Figure 6. Photographs of two striped newt repatriation box enclosures (a.) and a growing larval striped newt observed healthy in a box two weeks after release within enclosure (b.) These predator-free enclosures were improved beyond last year's design.

Drift Fence Construction and Operation

Drift fencing is this study's greatest method of measuring success. Last year, due to low numbers of striped newt larvae available, we released larvae into only one of our four selected repatriation wetlands (Pond 16). This year, we constructed encircling metal drift fences around the other three wetlands (Pond 18, Pond 75, and Pond 182) as well, following the method used for Pond 16 (Means et al. 2013). Each wetland's drift fence was operated from the time of first newt release through the first week of August in order to measure potentially emigrating striped newt efts that leave the wetland after metamorphosis. Drift fence operation methodology is described in Means et al. 2013.

Pond 16 Newt Releases

On February 19, we released 57 larval western striped newts of variable sizes, reared and provided by the Jacksonville Zoo and Gardens. The individuals were transferred from zoo aquaria into plastic bags, placed inside a cooler, and transported by automobile from Jacksonville to the ANF. These individuals then were released at large (i.e. not into holding boxes) and spaced roughly evenly around the pond perimeter.

On February 20, we released 8 more tiny young larvae (SVL 12-14mm) into a single meter cubed, predator free, holding box. These individuals were shipped overnight Fed Ex from the Memphis Zoo carefully packed in a cooler.

On June 13, we released 55 more medium-sized (SVL 18-22mm) larval newts at large into the wetland. These individuals were provided by the Jacksonville Zoo and Gardens using the method described above.

Pond 18 Newt Releases

On April 1, we released 47 larval western striped newts of variable ages/sizes (11-27mm SVL), reared by the Jacksonville Zoo and Gardens. The individuals were transported as described above and the newts were released at large (i.e. not into holding boxes), spaced evenly around the pond perimeter.

On June 13, we released 55 more medium-sized (18-22mm SVL) newts provided by the Jacksonville Zoo and Gardens. The newts were released at large and distributed evenly throughout wetland.

Pond 75 Newt Releases

On February 19, we released 57 larval western striped newts at large into the wetland. Newts were provided by Jacksonville Zoo and Gardens and transported via automobile from Jacksonville to the ANF, as described above for Pond 16.

On February 20, nine more tiny larval newts (11-12mm SVL) provided by the Memphis Zoo were released into a single holding box.

On June 13, we released 55 more larval newts at large into the wetland. These newts were medium-sized (18-22mm SVL) and provided by the Jacksonville Zoo and Gardens.

Pond 182 Newt Releases

On April 2, we released 36 total tiny (12-13mm SVL) western striped newt larvae into the wetland. Newts were reared and provided by Memphis Zoo and shipped overnight to north Florida. The newts were divided evenly and released into two predator-free holding box (18 per box).

On June 13, we released 54 more medium-sized (18-22mm SVL) larval newts into the wetland. These newts were reared and provided by the Jacksonville Zoo and Gardens.

Additional Information

Before release into a given wetland, we gave larvae at least one hour acclimation time in a mixture of pond water and their transport water. Also, any and all remaining blackworms (captive newt food) were carefully removed from transport water to avoid the release of exotic invertebrate species into recipient wetlands. Lastly, after acclimation and blackworm removal, newts were transferred into buckets of pure pond water and observed for several more minutes. This method not only provided additional acclimation time, but also increased assurance that no blackworms were ever released into wetlands.

Marking captured metamorphic newt efts this year remained unnecessary. We know that all individuals we encounter in the fences are from our repatriation efforts--not from the local historical populations that are now believed to be extirpated. Furthermore, we did not want to inflict any injury to these rare individuals so as to decrease their fitness for survival in the surrounding uplands. In this study, we need to measure and compare the numbers of released larvae, emerging terrestrial efts, and returning breeding adults over time in order to measure study success.

Results and Discussion

Weather Conditions

October 2013 through February 2014 saw below average rainfall, and ponds across the study area remained relatively dry. The months of March and April 2014 were extremely rainy in North Florida, and in our study region. Our ponds received 20-25 inches of rainfall within those two months in multiple, successive, multi-inch rains associated with strong frontal passages. This extremely wet weather pattern resulted from a global El Niño climatic event occurring at the time. Drift fences at all four recipient wetlands became entirely flooded and submerged by the end of March 2014. Drift fences were entirely or partially submerged through June and gradually emerged throughout July. High water for area wetlands, including all study ponds, remained in place through June and well into July 2014.

The down side of heavy pond flooding this year was that the resulting inundated drift fences impacted our ability to measure repatriation success. However, on the bright side, flooding provided plenty of water (long hydroperiods) to give our repatriated larvae enough time to metamorphose into efts before pond-drying.

In early May hot, dry weather ensued in our study area. Unusual heat and dryness occurred through August 2014. As a result, area wetlands entered a long drying trend which continued through the end of the project year (September 2014). All wetlands retained pools of water until mid-August, when all three liner ponds briefly went dry (Ponds 18, 75, 182). A month of heavy monsoonal rainfall from mid-August through mid-September, created pools on all liner wetlands. The other MS wetlands all continued a drying trend through this period, thanks to continued heat and evapotranspiration. Our lined wetlands, over the central portions containing the liners underneath pond bottoms, no longer are drawn down by evapotranspiration. Evaporation alone influences these pond levels.

Temperatures between October and December were roughly seasonal, but January through April frequently were well below average in our study region. Pond water temperatures were considered much too cold (upper 40's to low 50's Fahrenheit) for all of January and most of February to release newts. We waited to begin the year's first releases in late February during a warm spell. Unfortunately, temperatures plummeted again after the first releases in February, and possibly negatively impacted our early releases.

Striped Newt Repatriation and Monitoring

Last year, because of a variety of challenges, only 58 larval striped newts were available for repatriations (Means et al. 2013). These were released into only a single wetland (Pond 16).

This year, we produced 433 total western striped newts (early middle and late larval stages) available for release, and we released them in multiple waves at all four repatriation wetlands. A total of 32 terrestrial efts were observed exiting all four wetlands this year, in spite of having flooded, un-operational drift fences for most of the metamorphic periods. Our original Scope of Work stated that fences were to be monitored until the end of June. Because of flooding and our desire to measure success of our repatriation methods, we did not stop monitoring the fences until August.

Below are specific, per pond descriptions of our repatriation results. We report a percentage figure per wetland called "percent eft yield." This quantity is a percentage of the number of observed outgoing efts (in drift fences) and the number of released larvae at a particular wetland.

Pond 16

After releasing 57 variably-aged larval western striped newts on February 19, four newly metamorphosed efts were captured in the drift fence and released into the immediate uplands. Individuals exited very rapidly after release, within 5 days. These individuals clearly represented the oldest members of the release group that was already at or near complete metamorphosis at the time of their release. Three of the four emergent efts had one or more small grayish to pinkish skin spots or lesions (Figure 7). The reason for this is unknown, but we suspect that the most likely cause was cold water stress (Raffel, et al.2006) during metamorphosis. The animal was otherwise robust and healthy.

Figure 7. Emergent striped newt eft with two gray skin spots from Pond 16 observed February 24. One spot is on the center of the back above and just anterior to the hind limb. Another spot is directly over the eye. We do not know what caused the spots, but cold water stress may have been a factor. The individual otherwise appeared healthy and robust.

After releasing eight tiny larvae into a holding box on February 20, we observed that three larvae remained alive in the box after two weeks, and only one out of the original eight remained after another four weeks. On April 2, we released the lone remaining larva into the wetland. It was large, healthy, and robust at release. These box results were not positive and suggest that boxes may not function to protect young larvae from death by predation as was presumed.

By the end of March, the drift fence became flooded and inundated, and remained so until late July. Flooding rendered the drift fence, our primary method to measure success, ineffective for four months. Unfortunately, this flooding corresponded with the time we might expect to see emergent efts exiting the wetland.

On May 8, during the period of fence flooding, we extensively seined this wetland in an effort to detect any remaining newt larvae (i.e. measure short-term repatriation success). We detected no striped newt larvae at that time. This result could be explained by any or a combination of at least three factors: 1) pond flooding caused a dilution effect, reducing our detection ability, 2) metamorphosis and emigration prior to our sampling, and/or 3) death.

After releasing 55 more medium-sized larvae at large into this wetland on June 13, we observed no more metamorphic efts exiting the wetland for the rest of the study period. However, this result likely is influenced by the fact that our drift fence remained flooded until late July. We believe that some efts likely exited the wetland while drift fences were too flooded to accurately measure their exit.

In summary, we released a total of 120 western striped newt larvae at Pond 16 this year, in three separate events (two in February, one in June) and captured four efts in the drift fence exiting the wetland. We therefore documented a 3.3% eft yield from our repatriation efforts. It is likely that our documented yield is lower than the actual yield (i.e. more efts likely exited this wetland) because of our inability to capture emigrating efts in a flooded drift fence.

Pond 18

Shortly after releasing 47 variably-aged western striped newt larvae into this wetland on April 11, the encircling drift fence flooded. It was rendered useless for measuring any potential emigrating efts until mid-June.

On May 8, in an attempt to observe larval newts still in the wetland (i.e. measure short-term repatriation success), we extensively seined Pond 18. We observed no newts while seining. As with our similar effort at Pond 16, we concluded that one or a combination of three factors might have led to our observation of zero newts: 1) dilution due to flooding, 2) newts already having metamorphosed and emigrated, and/or 3) die-off.

As mentioned above in the Methods section, we released 55 more medium-sized larvae from Jacksonville Zoo into Pond 18 on June 13. At the time of second release, our recently flooded drift fence encircling the pond had already emerged above receding water levels, although most pitfall traps were still under water. Larvae were released inside the emergent fence ring such that all individuals would be contained within the wetland should water continue to recede rapidly in June. Fortuitously, water did continue to recede throughout June and July such that wetland drying provided a natural catalyst for newts to metamorphose while having a drift fence in place to measure their exit as newly metamorphosed terrestrial efts.

A total of 19 robust and newly-metamorphosed terrestrial efts were recorded in interior drift fence pitfalls between June 22 and July 25. Individuals were released outside the fence approximately five to 20 feet exterior and upslope from the drift fence in the closest place containing sufficient vegetative or downed log cover.

Water receded rapidly down to the region containing the rubber liner underneath. As hypothesized earlier in this study, the central pool of water remained throughout the summer, resistant to the droughty conditions. This small (i.e. 30' diameter, 2' deep) pool in the pond center acted as a refugium for drought-stressed, metamorphosing larval newts.

In summary, we released a total of 102 newt larvae during two efforts at Pond 18 this year and we captured 19 terrestrial efts exiting the wetland. We therefore documented an 18.6% eft yield from our repatriation efforts. The actual eft yield was likely higher. The first release cohort probably were not present in the pond when the second release occurred, as evidenced by the seining efforts described above. Consequently, the 19 terrestrial efts captured were most likely produced only from the second release of 55 larvae. Therefore, our percent eft yield at Pond 18 may have been as high as 34.5%.

Drift fence results from Pond 18 were very promising, and provided this study's greatest glimpse of success potential to date. We believe that Pond 18's measured success this year resulted from several factors. First, there was a fully operational drift fence intact to measure an emigration event. Second, release of medium aged/sized larvae may have resulted in greater robustness and ability of larvae to avoid predators. Third, the release of larvae late in spring (almost summer), may have provided warmth or food abundance necessary for higher survival rates. We plan to model these efforts next year at all recipient wetlands. Of course, natural events such as flooding or extreme drought are unpredictable and can always influence results.

Pond 75

After releasing 57 variably-aged larval western striped newts on February 19, a single, newly-metamorphosed eft was captured in the drift fence on February 25. This individual exited the wetland rapidly, and clearly was one of the oldest members of the first release cohort. This individual also had a small grayish skin 'lesion' that we suggest may have been caused by cold stress during metamorphosis.

Out of the nine tiny larvae (12-13mm SVL) that were released into a single holding box on February 20, six out of nine remained by April 2. These individuals were robust and had grown relatively large (24-25 mm SVL) and were released at large into Pond 75 at this time. These were the most promising box results this year.

As we stated in the Method section, we released another 55 newts at large into Pond 75 on June 13. Zero emigrating efts were detected by the drift fence because it was flooded during their metamorphosis period. The drift fence became flooded by mid March and remained so until late July, with only 2 weeks remaining of Year 4's active field season. This wetland's drift fence remained flooded for the longest period of time out of the four recipient wetlands, and no doubt eliminated our ability to observe any newt eft emigration out of the wetland.

On May 8, in an attempt to observe larval newts still in the wetland, we extensively seined Pond 75. We observed no newts while seining. As with our similar effort at Pond 16 and Pond 18, we concluded that one or a combination of three factors might have led to our observation of zero newts: 1) dilution due to flooding, 2) newts already having metamorphosed and emigrated, and/or 3) die-off.

In summary, we released 121 western striped newt larvae in three separate events (two in February, one in June) into Pond 75 this year. We captured 1 eft exiting the wetland. We therefore documented a 0.08% eft yield from our repatriation efforts. We believe that more efts likely exited this wetland, but our drift fences were too flooded (March through July) to accurately measure their exit.

<u>Pond 182</u>

A total of 36 tiny larvae from Memphis Zoo were released into two holding boxes on April 2. The 36 individuals were split evenly between the two holding boxes. The boxes were checked 5 weeks later, on May 7. In one box, five out of 18 larval newts remained. In the other box, only 3 out of 18 remained. Combining these results, eight out of the original 36 larvae remained within boxes after 5 weeks. Cannibalism or other unforeseen causes may have played roles in the loss of the majority of this release group. The individuals that remained were robust and healthy in appearance, much larger, and they were released at large that same day into Pond 182.

By mid April, the drift fence became entirely submerged under flood waters and remained so until late June. We likely missed measuring the emigration of any efts produced from the first release because of a flooded drift fence.

On May 8, while we seined the other three recipient wetlands for the presence of newt larvae, we opted not to seine this wetland because we had just released 8 newt larvae at large into the wetland one day prior.

As mentioned above in the Method section, we released 54 more medium-sized larvae from Jacksonville Zoo into Pond 182 on June 13. At the time of second release, our flooded drift fence encircling the pond had partially emerged from pond drying. Although we released the individuals inside the drift fence, it is probable that some larvae swam over the inundated portion of the fence rendering themselves undetectable. Water continued to recede rapidly after the second release. During the rapid pond drying period from July 9 through July 23, eight striped newt efts were captured by drift fence exiting the wetland. This emigration event was concurrent with a similar event at Pond 18.

In summary, we released 90 western striped newt larvae in two efforts at Pond 182 this year. We captured eight terrestrial efts exiting the wetland. We therefore documented an 8.8% eft yield from our repatriation efforts. For reasons similar to events at Pond 18, the actual eft yield likely was much greater than our documented yield. Most of the individuals from the first release likely were not present in the pond at the time of the second release. All eight emigrating efts captured probably were produced by the second release group of 54 larvae. Therefore, our percent eft yield may be as high as 14.8%. Furthermore, it could even be higher due to the likely escape of some individuals of the second release group over the flooded portion of the drift fence.

<u>Repatriation Summary</u>

Year 4 percent eft yield results from Ponds 16 (3.3%) and 75 (0.08%) were not as high as we expected, but results for these two wetlands were severely impacted by drift fence flooding. We believe that the true percent eft yields at these two locations were likely much higher. Cold conditions in late February at these two wetlands also may have negatively impacted the February releases, as evidenced by the presence of skin spots on some of the early, newly-metamorphic efts.

This year's percent eft yield results from Pond 18 and 182 are strongly encouraging and represent measured study success. We believe that our results here are closely representative of a typical r-selected amphibian species that produces hundreds of larvae per breeding pair in a lifetime. We note that, for these two wetlands, we released larvae later in the season, more in line with when larvae would be naturally expected to occur in these wetlands.

This year's results may represent quite successful numbers when viewed within an r-selective ecological framework, considering that the 32 efts observed exiting the wetlands were the offspring of less than 30 captive parents and that many more efts likely exited while fences were flooded. However, this year's terrestrial eft cohorts at Pond 18 and 182 still have many obstacles in the uplands to endure before returning to breed in their natal ponds.

When we first proposed the study nearly five years ago, we proposed that repatriations would occur during Years 3-4, and that Year 5 would conclude the study and see no more repatriations.

Now, at Year 4's end, we see that continued repatriations, and an indefinitely long study extension are necessary in order to reach long term success.

There are a variety of reasons why we need to continue repatriations into Year 5 and beyond. Put simply, we believe the chance for long-term success increases as the number of repatriated larvae increases. Early in the study, we believed that we could produce thousands of larvae from our initial captive parental population and have them available rapidly for repatriations during Year's 3-4. That was not the case. But we have learned much since then, and are heading in a positive, encouraging direction. After two years of repatriations, we have released a total number of 491 larvae (58+433), and have observed a total of 35 efts (3+32) emigrating out of wetlands into surrounding uplands. Even though we have had some success producing larvae and recruiting efts in the uplands, clearly these numbers still need to increase in order to establish self-sustaining populations.

We already are preparing to reach 1,000 releases next year and beyond. Two additional zoological institutions will be involved with this project next year--Central Florida Zoo (Orlando, FL) and Lowry Park Zoo (Tampa, FL). The additional holding space at these zoos is necessary in order to produce and house larger parental and offspring populations. Our next step is to find funding to continue the project beyond March 2015.

Background Monitoring for the Striped Newt

This year, as with last year, our monitoring efforts focused only on the 19 historical striped newt breeding wetlands in our study area. Because of below average winter rainfall, only 5 out of the 19 wetlands held water. No larval amphibians were observed at historical newt wetlands during our wintertime first sampling effort.

In March and April, heavy rains associated with a global El Niño climatic event filled area wetlands to the highest levels observed since the last prominent El Niño event in 1998. All 19 historical striped newt ponds filled to flood levels. We waited six weeks after pond fillings to resample the historical newt wetlands in order to allow plenty of time for any potential newt larvae to become large enough to easily detect. Despite these potentially prime breeding conditions, we observed no striped newts within our study region. This was further evidence in support of our extirpation hypothesis.

Many other pond-breeding amphibians were observed in relative abundance at the 19 historical striped newt ponds this year. A total of 11 species of larval amphibians were observed:

- Two salamander species: mole salamander (*Ambystoma talpoideum*) and central newt (*Notophthalmus viridescens*)

- Nine frog species: southern cricket frog (*Acris gryllus*), oak toad (*Anaxyrus quercicus*), pine woods treefrog (*Hyla femoralis*), barking tree frog (*Hyla gratiosa*), gopher frog

(*Lithobates capito*), bullfrog (*Lithobates catesbeianus*), southern leopard frog (*Lithobates sphenocephalus*), southern chorus frog (*Psuedacris nigrita*), and ornate chorus frog (*Psuedacris ornata*)

Although numbers were lower than last year (Means et al. 2013), ornate chorus frog and gopher frog larvae continued to be well represented in the MS. Out of the 19 wetlands sampled, they were observed at 6 and 4 ponds, respectively. In Florida, both are Species of Special Concern, and both are regarded to be either rare or in decline by most herpetologists. It is worth noting that the MS region of the ANF continues to be a stronghold location for both species.

Hydrology and Ecology of Repatriation/Liner Wetlands

Before liner installment, we hypothesized that liners would boost recipient pond hydroperiods and create a more drought-resistant environment. This effect would act as a tool to avoid wetland dry-up, particularly during larval repatriation periods, and therefore avoid the loss of repatriated larvae due to pond drying. This year's hydrological data continue to suggest that liners have been effective at extending pond hydroperiod (Table 1).

Table 1. Monthly presence (blue shading) or absence (white shading) of water within recipient wetlands and paired, nearby hydrological reference wetlands. Our data clearly indicate that liners are lengthening the hydroperiods of recipient wetlands relative to nearby reference wetlands that once behaved similarly to lined wetlands . The recipient Pond 16 has no liner, and it hydrologically behaves similarly to its reference pond 15. Even though lined wetlands now have extended hydroperiods, it is important to note that liner wetlands still go dry frequently during dry spells, which is exactly what we want in order to preserve the ephemeral nature of these wetlands.

	Pond 16	**Pond 15**		**Pond 18**	**Pond 17**		**Pond 75**	**Pond 73**		**Pond 182**	**Pond 48**
	No Liner	No Liner		Liner	No Liner		Liner	No Liner		Liner	No Liner
Oct 2013											
Nov 2013											
Dec 2013											
Jan 2014											
Feb 2014											
Mar 2014											
Apr 2014											
May 2014											
Jun 2014											
Jul 2014											
Aug 2014											
Sep 2014											

Table 1 shows clearly that liner wetlands hold water approximately 3-5 months longer than nearby similar hydrological reference wetlands. It also shows that liner ponds fill more readily than reference ponds during rainy periods. Water levels also persist 2-4 weeks longer during intense, hot dry-up spells. Note that before liner installation, our reference wetlands behaved hydrologically similarly to our lined wetlands.

The Pond 16/Pond 15 wetland pair both stayed hydrated during the entire Year 4 study period. Pond 16 is the only striped newt recipient wetland with no liner at this time. Pond 16 and 15 water amounts waxed and waned proportional to wet and dry spells. Both these wetlands are considerably deeper than the other wetland pairs and thus hold water much longer throughout the year. Their aquifer-driven hydroperiods are nearly perfectly synchronous since neither has a liner, and because they are both sink depressional wetlands adjacent to one another with nearly the same bottom elevation.

All liner-enhanced wetlands currently appear to be ecologically healthy, containing diverse native vegetation (Figure 8). They also contain the naturally occurring assemblage of pond breeding amphibians that are expected within local ephemeral wetlands. There have been up to five species of larval amphibians observed at any given time within liner wetlands. The amphibian communities of liner wetlands have not changed as a result of the liners, except that there are now more opportunities for local amphibians (including the rare ornate chorus frog and gopher frog) to breed because of lengthened hydroperiods induced by the liners.

Figure 8. Photos of the three lined, recipient wetlands. Pond 18 (a), Pond 75 (b), and Pond 182 (c).

As previously stated, and similar to last year, both the gopher frog and ornate chorus frog were abundantly observed either calling, present as adults, or present as tadpoles within all liner wetlands during February through April 2014 (Figure 9). These species are considered by many, including the authors, to be either rare or declining globally. Their continued relatively abundant presence within our liner wetlands is a good indication of ecological heath of the liner wetlands. Their presence also indicates the positive effect liners are having on reproduction in local pond-breeding amphibians, including rare species. 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 and provide additional breeding habitat for species of concern, particularly in regions that have been impacted by increased drought frequency and longevity.

Figure 9. We detected breeding events of gopher frogs and ornate chorus frogs at all three liner wetlands in winter/spring 2014. Gopher frog tadpoles (a.). Ornate chorus frog male/female amplexed pair with eggs (b).

YEAR 5 EXPECTATIONS

Next year, we plan to continue full-scale repatriations in all four recipient wetlands. We now have experienced an increase in our production abilities and subsequent increase in our repatriation success from Year 3 to Year 4. Continuing that trend upward next year in all aspects should increase our likelihood for long-term success.

We expect at least two additional zoos to join in our striped newt husbandry efforts for this project next year (2015)--Central Florida Zoo, Orlando, FL and Lowry Park Zoo, Tampa, FL.

Drift fences encircling all recipient wetlands will be installed in January and will be operational at least through March 2015, when funding for this project ends. It now is possible for us to observe Years 3 and 4 terrestrial efts returning to recipient wetlands to breed. Drift fencing will help us to determine these events. We are actively pursuing additional funding to continue with all aspects of the study to increase our efforts next year and into future years as mentioned. If we secure additional funding to continue drift fencing, then we will run the fences through June or July.

The 19 historical striped newt wetlands in the ANF will be resampled in winter and in spring 2015. Even though the striped newt is likely extirpated in the ANF, it is still very important to continue a sampling presence now and in the future to continue to add vital data concerning the status of the striped newt in the ANF.

Next year's repatriations will proceed, incorporating what we learned from this year. Larvae will be released during the warmer spring months, March-May, and into June if necessary. Also, we want to experiment with releasing breeding-ready aquatic adults into one or more recipient wetlands sometime in the winter, January-February 2015. This will be the first time adults will have been released in this project. Although much has already been learned, there still is plenty left to learn, and we believe developing new release techniques could only benefit our efforts. We also believe that we should continue with repatriations into subsequent years until sufficient, long-term repatriation success is documented.

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