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

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

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September 2013

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 within the Munson Sandhills (MS) 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; 3) investigate and implement precautionary measures to ensure success of repatriations. This report summarizes work conducted in Year 3 (October 2012-September 2013) 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 last year what we strongly believed to be extirpation of the western striped newt within our ANF study area--its former geographical stronghold. This year, we continued a background monitoring effort within historical striped newt wetlands in the ANF. Conditions during winter sampling for breeding adults were extremely dry and not prime for sampling. However, a 15-inch rain event in mid-February created prime conditions for spring larval sampling. Still, as expected, we found zero western striped newts within their historical ANF breeding wetlands. These findings further support our extirpation hypothesis.

Efforts to investigate cause of decline continued this year. Striped newt susceptibility to ranavirus was tested and reported in last year's (Year 2) study efforts. Testing for the prevalence of ranavirus in our study area was undertaken this year. Three of the four repatriation sites contained ranavirus within sampled amphibian (anuran) larvae. Prevalence of ranavirus at each site varied between 0% (Pond 75) and 15% (Pond 16). Although ranavirus was present in our study area, prevalence levels at all four recipient wetlands were well below the infection prevalence of 40% attributed to ranavirus outbreak hotspots. Next year's repatriations will proceed cautiously and will be emphasized at Pond 75 (0% ranavirus prevalence) followed by Ponds 182 and 18. Pond 16 also may receive newts depending on whether there are large numbers available.

In early May 2013, we captured 12 more wild striped newt larvae from our original source wetland within the Fall Line Sandhills Natural Area, Georgia, to boost genetic robustness of our original zoo captive population of founder individuals captured in 2011. Our captive western striped newt colony continues to serve two important roles. First, it is the first assurance colony ever created for the highly imperiled western genetic variant of the striped newt and serves obvious conservation needs. Second, larvae produced from this colony are being utilized for this study's repatriation efforts.

Synthetic (EPDM) liners were installed last year (2012) underneath three of our four selected repatriation wetlands. Observational water presence/absence data collected since

their installation preliminarily suggest that liners have been effective at extending pond hydroperiods. Furthermore, all liner-enhanced wetlands currently appear to be ecologically healthy and vegetatively diverse, and contain up to five species of larval amphibians at any given time. Both the gopher frog (*Lithobates capito*) and ornate chorus frog were abundantly observed as tadpoles in March and April 2013 within all liner wetlands during a time when most of the other area wetlands were dry and unsuitable as breeding habitat. These species are considered by many, including the authors, to be either rare or declining. Their relatively abundant presence within our liner wetlands is a good indication of ecological heath of the liner wetlands and 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/or suitable for rare or imperiled species.

The first repatriation attempt of larval western striped newts into our study area occurred this year. On 1 May 2013, 58 larvae were introduced into a single wetland (Pond 16) into predator-free screen enclosures. Larvae remained in enclosures for 2-4 weeks depending upon rapidly lowering pond water levels. Enclosures were dipnetted periodically to monitor larval development. Few were observed. Those that were exhibited rapid growth. All enclosures were opened after 4 weeks due to pond drying.

An encircling drift fence was constructed around the repatriation wetland to measure any outgoing terrestrial efts. Three terrestrial efts were detected exiting the pond in late June. Heavy and frequent rain fell in July and August. The drift fence flooded and was terminated in late July, nearly three months after repatriation. We periodically dipnetted and seined the wetland afterward to continue to measure for newt occupation of the wetland. No more newts were observed. This finding may either reflect a die-off of the rest of the repatriated newt population or may reflect a dilution effect of wetland flooding. This year's monitoring after repatriation concluded on 20 August 2013.

ACKNOWLEDGMENTS

We would like to thank many people and institutions for their important intellectual and/or physical contributions to this multifaceted, collaborative conservation project. Dino Ferri and Mark Beshel of the Jacksonville Zoo and Gardens, as well as numerous additional zoo colleagues are greatly thanked for their colossal efforts and expertise in producing assurance colonies of both western and eastern striped newts to be used for this project and for future conservation efforts. Additional colleagues include: Chris Baker, Steve Bogardy, Andrew Kouba, Brian Summerford, and Shannon White from the Memphis Zoo. The work of these individuals and zoological institutions is vital to this project and to the conservation of the striped newt. W. James Barichivich and Susan Walls of the U.S. Geological Survey are greatly thanked for valuable field assistance and insights during the first striped newt repatriation period. Many thanks to John Jensen (Georgia DNR) and colleagues for field assistance at Fall Line Sandhills Natural Area.

CONTENTS

TABLE OF 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 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 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 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, 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.

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

One possible cause of the striped newt decline in the ANF is drought. Another is pathogen infection. Other possible causes for decline could be off-road vehicular disturbances to breeding ponds, incompatible land management techniques, development, and encroachment of woody shrubs and pines into pond basins (Means et. al 2008). It is unknown whether a single factor or combination of factors is the culprit behind the decline. We suggest that some combination of the above factors is the most likely cause, with emphasis on drought and/or pathogen infection. The gas pipeline expansion and subsequent upland habitat alteration is the latest in a lengthy list of probable impacts to the ANF striped newt population.

The Coastal Plains Institute and the US Forest Service entered a 5-year cost-share agreement to create a study that addresses the severe decline or extirpation of the striped newt population on the Apalachicola National Forest. Striped newt repatriation coupled with precautionary measures to ensure repatriation success and enhance breeding habitat will be conducted as part of the study. This study is expected to boost the ANF striped newt population and possibly provide new management strategies for similarly imperiled amphibian species. This is the third annual report that summarizes work conducted in Year 3 (Oct 2012-Sept 2013) of the current 5-year study.

Overall Study Objectives

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

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

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

3. Conduct striped newt ranavirus susceptibility tests and conduct surveillance testing for ranavirus in sympatric species at repatriation wetlands and in nearby wetlands (Years 1-3). This will be done as a precaution to ensure we do not introduce striped newts into a potentially hazardous environment. If newts are determined to be susceptible to ranavirus, and ranavirus is present in selected repatriation wetlands, we will make a well-informed decision how to proceed with repatriations in such a way as to reduce the potential for repatriation failure.

4. Conduct striped newt repatriation efforts in the ANF (Years 3-4). We will conduct repatriation efforts in four wetlands using captive-bred larvae from striped newt assurance colonies developed in the first 2 years of the study (Objective #1). We believe it is paramount to act as soon as possible to boost the western striped newt before its remaining vestiges in southwest Georgia potentially suffer the same fate as the ANF metapopulation.

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

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

any management recommendations favorable for the long term ecological management of the striped newt.

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

STUDY AREA

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

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

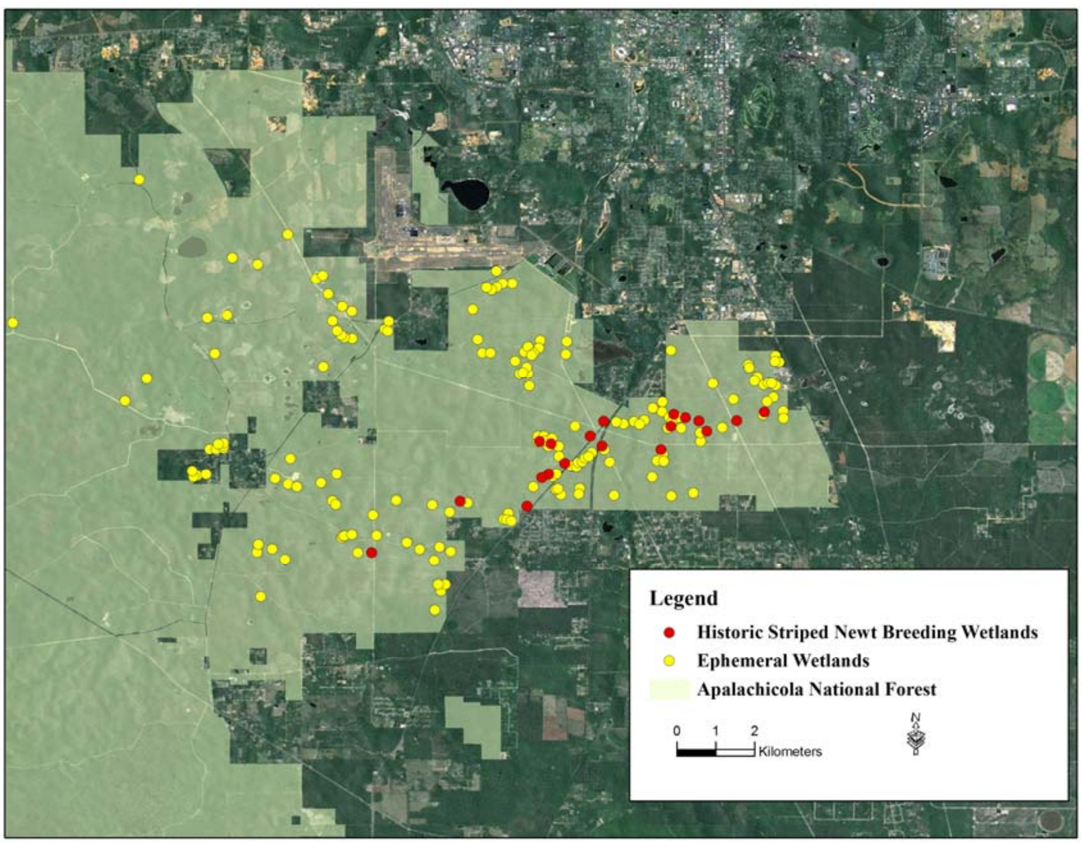

Figure 1. Map of the Munson Sandhills study area. Yellow dots represent the 158 ephemeral wetlands that have been periodically sampled over the last 20 years. Red dots represent the 19 historic striped newt breeding wetlands.

METHODS

Background Monitoring for the Striped Newt

During Year 3, 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 the January-February 2013 winter breeding season to search for presence of striped newt aquatic adults. The second sampling effort took place during March and April 2013, 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.

Ranavirus Surveillance Testing and Estimation of Prevalence

On 25 April 2013, 60 tadpoles of various species were collected from each of the four repatriation ponds (n=240 total tadpoles). Super-spreader species (i.e. gopher frog, ornate chorus frog [*Pseudacris ornata*], pine woods treefrog [*Hyla femoralis*]) were targeted because of their high likelihood of infection when/if the virus is present. Larvae were tested because of their higher probability of infection compared to adults (Gray et al. 2009a). If individuals are infected with ranavirus, there is a 95% chance of detection with n = 60 samples and a pathogen prevalence of 5% (Green et al. 2002).

Individuals were randomly collected with dipnets and put into separate glass jars in the field. Separation of individuals was important to reduce potential pathogen spread during transport to a nearby laboratory for further processing. In the lab, individuals were humanely euthanized individually with benzocaine hydrochloride. Tadpoles were then packaged individually into Whirl-Paks®. They were immediately refrigerated and promptly packed in coolers snugly with ice packs. Whirl-packs were not allowed to touch ice packs directly. Each Whirl-Pak® was labeled with proper species and location information. Coolers were shipped overnight to the University of Tennessee, Center for Wildlife Health.

Individuals were necropsied at the University of Tennessee within eight hours of receipt. A homogenate of liver and kidney tissue were used to test for the presence of ranavirus DNA using quantitative PCR as described previously (Means et al. 2011).

Hydrology and Ecology of Repatriation/Liner Wetlands

During Year 2, CPI 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 (Fig. 2). Numerical designations were carried over from past surveys conducted by CPI on the MS ephemeral pond assemblage over the past 20 years. All wetlands were nearby to the expanded gas pipeline corridor within 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).

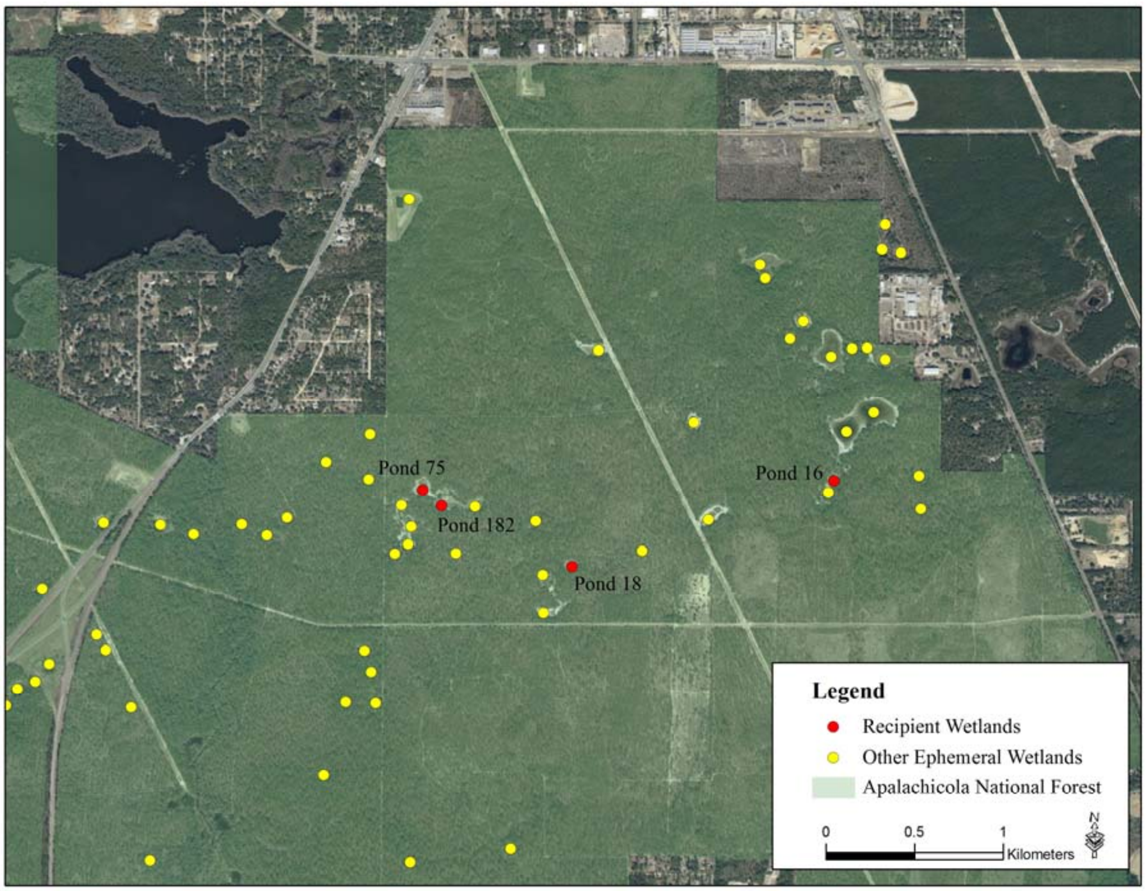

Figure 2. Map depicting location of the four striped newt repatriation/liner wetlands. Only Pond 16, the liner-free repatriation wetland, received larval newts in Year 3.

Synthetic (EPDM) liners were installed last year 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 is scheduled to receive a liner, but conditions there have been too wet and muddy to complete installation. A liner will be installed whenever it dries sufficiently enough to enable installation. Four nearby and hydrologically similar wetlands were selectively paired to repatriation wetlands as 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 Repatriation and Monitoring

Due to the low number of striped newt larvae available for repatriation (n=58), we decided to release larval newts in only one wetland this year. Predator-free enclosures modeled after Scott (1994) were constructed onsite within the one recipient wetland, Pond 16. Enclosures consisted of wooden 2.5cm x 2.5cm square stakes driven into the pond bottom wrapped in fiberglass window screening stapled to the exterior of the stakes. Screen mesh was small enough to keep out larger potential predatory organisms but large enough to allow the free passage of pond zooplankton to provide food for the young larvae. The screen was flared out at the bottom. Masonry bricks were placed onto the flared screen edges all the way around the enclosure to "seal" out unwanted bottom dwelling predacious organisms (Fig 3a). Each completed enclosure measured 2m x 1m x 1m. Four enclosures were constructed on site and spaced evenly around the periphery of the wetland (Fig 3b). Enclosures were placed at various water depths to account for pond water fluxuation. Predatory animals (e.g. macroinvertebrates, mole salamander larvae) were dipnetted out of the enclosures immediately after installment. All other pond-dwelling animals and plants were left intact within enclosures.

a. b.

Figure 3. Photographs of a single striped newt repatriation enclosure (a.) and all four such enclosures within the repatriation wetland (b.) These predator-free enclosures were modeled after Scott (1994).

On 1 May 2013, 58 larvae of four distinct age cohorts were released into the four predator-free enclosures in Pond 16 (Fig. 4). Another single small larva was introduced into 2 weeks later. Larvae were placed into each enclosure based on larval age class. Enclosures were labeled A, B, C, and D to reflect age class differences. Enclosure A received three individuals of the oldest age class, approximately 13 weeks old. Enclosure B received 30 ten-week-old individuals. Enclosure C received 14 nine-week-old individuals. Enclosure D received 8 two-week-old individuals. Enclosures were dipnetted periodically to monitor larval development.

Figure 4. All 58 striped newt larvae released into Pond 16. Cohort A (a.), Cohort B (b.), Cohort C (c.), Cohort D (d.), and their release into a predator-free enclosure (e.)

An encircling drift fence was installed around the repatriation wetland soon after repatriation to monitor for outgoing terrestrial striped newt efts in the ensuing weeks (Fig. 5). This drift fence was designed to maximize potential to capture striped newt efts, while minimizing impact to the local ecology.

Figure 5. Repatriation wetland (Pond 16) with encircling drift fence.

Approximately 120 m of rolled, 0.6 m (2 ft) high tin fencing was buried in a shallow trench excavated around the wetland. Tin material was selected because of its ability to resist corrosion, UV light damage, and burning during prescribed fires. Our drift fence was placed slightly above the long-term intermediate high water line, above a prominent ring of buttonbushes (*Cephalanthus occidentalis*) that have established around the wetland. This placement was done so as to not have the fence too far away to capture potential efts that may remain close to the wetland after metamorphosis, but not too close so as to become flooded too frequently. As with most drift fencing studies, fence placement around the wetland was ultimately an educated judgment call. After installment, the fence measured about 0.3-0.4 m tall, while being buried to a depth of about 0.1 m.

Plastic, 2-gallon buckets (U.S. Plastics Corp.) were buried flush with the ground surface and taut against the drift fence on each side of the fence at an interval of approximately 7 m. Many drift fencing studies have utilized 5-gallon buckets in the past, including our own (Means 2007). But thinking in terms of maximizing potential to capture newts while minimizing impacts led us to conclude that 2-gallon buckets would be adequate to use as pitfall traps along the drift fence. A total of 34 buckets were installed around the fence (17 interior, 17 exterior).

Ten small diameter (ca. 2-3mm) holes were drilled into each bucket approximately 7 or 8 cm from the bottom such that accumulated flood water from thunderstorms would percolate out of the buckets down to a shallow level that would retain some water for captured animals to stay hydrated within. To reduce potential for drowning of captured animals in hydrated buckets, we placed small sponges inside the buckets. Sponges had two distinctive, important functions. First, they floated in the bucket water providing an island for animals to climb onto so as to not drown (Fig. 6). Second, they retained moisture if buckets went dry during dry spells. During extreme cases of heat and dryness, we shuttled water from the pond and poured it into dried buckets for rehydration.

Figure 6. Sponges were used within bucket traps as safeguards to prevent drowning as well as to retain moisture if buckets went dry. One of three captured emigrating striped newt efts sits atop a sponge in a pit fall trap.

The drift fence was checked daily in the early morning to reduce stress on captured and released animals found in pitfall traps. Animals captured within exterior buckets were released inside the fence, and animals captured within interior buckets were released outside the fence in areas containing sufficient cover. Captured, outgoing striped newt efts were released less ≤ 30 m from the wetland, within areas containing sufficient ground cover. Fence maintenance was performed as needed.

Incoming turtles, snakes, and crayfish were not allowed to enter the repatriation wetland during the 3 month drift fencing period so as to reduce potential for predation on repatriated striped newt larvae. Undesired predators were transported to another ephemeral pond (Pond 15) located 100 m south of Pond 16.

Results and Discussion

Background Monitoring for the Striped Newt

Last year, we reported that the striped newt had become extirpated in the ANF (Means et al. 2012). Substantial data in supported our extirpation hypothesis. To recapitulate, since 1998, despite repeated regionwide sampling of 158 ephemeral wetlands, zero striped newt larvae and less than 10 adults were observed in the ANF (Means and Means 1998a, 1998b, 2005, Means 2007, Means et al. 2008, 2011, 2012). The last two adults were observed in 2007, one from each of two of the 19 historical breeding wetlands. All other pond breeding obligate species, including the rare gopher frog and ornate chorus frog, have remained in relative abundance throughout the same time (Means and Means 1998a, 1998b, 2005, Means 2007, Means et al. 2008, 2011, 2012) .

Intensive additional sampling of the 158 wetlands in the first two years of our current study also had failed to turn up any striped newts in the Munson Sandhills. These findings, coupled with all past surveys, resulted in the formulation of our extirpation hypothesis. Even though the striped newt is likely extirpated in the ANF, it is very important to continue a sampling presence now and in the future to continue to add vital data in support (or not) of our extirpation hypothesis.

This year, our monitoring efforts were focused only on the 19 historical striped newt breeding wetlands in our study area. Below average rain fell during winter 2012-2013 and most ponds remained dry. Only 5 out of the 19 wetlands contained small central water pools. No larval amphibians were observed.

In late February, after winter sampling had taken place, a frontal passage produced 10-15 inches of rainfall over 3 days. Many area wetlands, including 16 out of the 19 historical newt ponds, hydrated. Such large rains in the past were good signs that striped newts may breed. However, we note that the striped newt in the ANF tended to breed earlier, in late fall or early winter. Also, whereas spring was much wetter than winter, by no means did the study area experience "exceptional" pond flooding. Area wetlands were intermediately hydrated by the heavy rains. We waited a month and a half after the big rain to begin sampling to give any potential newt larvae a chance to become large enough to detect. This year's results were the same as all year's since 2007, no striped newts were observed.

All other local pond-breeding amphibians were observed in relative abundance at the 19 historical striped newt ponds in the spring (late March-early April) (Appendix A). Ten species of larval amphibians were observed. Another species, the cricket frog (*Acris gryllus*), was observed widespread as adults only around pond edge. This year's sampling data continue to support our striped newt extirpation hypothesis.

The ornate chorus frog and gopher frog were the most widespread species detected during springtime dipnetting efforts. Out of the 19 wetlands sampled, they were abundantly observed at 13 and 10 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.

Ranavirus Surveillance Testing and Estimation of Prevalence

We detected the occurrence of anuran tadpoles infected with ranavirus at all repatriation sites except Pond 75. The most commonly infected species were gopher frog and barking trefrog (*Hyla gratiosa*) tadpoles. Infected ornate chorus frog and pinewoods treefrog tadpoles also were detected. Overall infection prevalence was 5.8%, with the greatest infection occurring at Pond16 (15%) followed by Pond 18 (5%) and Pond 182 (3.3%).

Gray and Miller (2013) suggested that ranavirus hotspots (i.e. locations of outbreaks) were sites with infection prevalence exceeding 40%. Several surveillance studies without outbreaks suggest that ranavirus prevalence between 0 – 5% is typical (Hoverman et al. 2011a). All four study wetlands are well below the 40% outbreak level and three wetlands were at or below the typical prevalence level at non-outbreak sites. Pond 16 contained four ranaviral host species of anuran larvae (gopher frog, barking treefrog, ornate chorus frog, and pine woods treefrog); whereas, the other sites were composed of predominately a single host species (gopher frog). The higher prevalence of ranavirus at Pond 16 may be a consequence of its having a higher species richness (up to 5 species of larvae at one time), and therefore greater number of host species. The other wetlands contained up to two species during the sampling event.

During the single ranavirus surveillance sampling event on a single spring 2013 day at all four study wetlands, we captured whatever tadpole species were readily available. Naturally, since Pond 16 had a higher species richness than the other ponds, we captured more species at Pond 16 than at the other ponds and utilized them for study.

The higher species richness of Pond 16 relative to the other three wetlands resulted from its having had a much longer hydroperiod than the other wetlands, and it contained a larger volume of water at any given time throughout the year. It never went dry this year.

Given the susceptibility of juvenile striped newts to ranavirus (Means et al. 2012) and the ranavirus surveillance results, it is possible, though likely not probable, that ranavirus may be a threat to successful repatriation at all sites except Pond 75 at this time. Depending on the number of larvae available for the 2014 repatriation effort, we will concentrate repatriation efforts in Pond 75, followed by Pond 182, Pond 18, and lastly Pond 16.

We believe that it will be important to continue repatriation efforts within Pond 16 even though we detected a relatively high (although well below outbreak status) ranavirus prevalence. First, Pond 16 has a naturally long hydroperiod and is the most drought-resistant of our selected recipient wetlands. This characteristic will be very beneficial during larval development periods in the typically dry springtime when other area wetlands go dry. Second, Pond 16 is located nearby to a cluster of other long-hydroperiod wetlands. These wetlands are expected to eventually serve as additional breeding habitat for repatriated striped newts within Pond 16 that may wander into nearby wetlands over time. Our vision for this repatriation study included making sure that repatriated striped newts had the capacity to expand their beeding range on their own over time--particularly after this study's conclusion. Pond 16 and nearby wetlands, with their long hydroperiods, provides the best opportunity for population expansion in the future.

We detected ranavirus in larvae of four species: barking treefrog, pine woods treefrog, ornate chorus frog, and gopher frog. This is the first study to report infected individuals in wild populations for these species (Miller et al. 2011). Hoverman et al. (2011b) and Brenes (2013) found that gopher frog and ornate chorus frog tadpoles are highly susceptible to ranavirus. These species could function as amplification hosts in amphibian communities and initiate an outbreak if ranavirus is present.

In this study, we have been progressively investigating whether ranavirus can be implicated as the cause for the mysterious striped newt decline in the Apalachicola National Forest. This is a critical piece of information that can affect the course of repatriation. After this year's ranavirus investigations we may now posess enough data to determine whether ranavirus may have been the cause for decline and how to proceed with repatriation in a potentially detrimental environment.

To recap, in study Year 1 (2011) we determined that striped newt larvae were not susceptible to ranaviral disease, experiencing only 5-10 % mortality when exposed to ranavirus (three unique isolates) in water (Means et al. 2011). In Year 2 (2012), we determined that post-metamorphic juveniles < 1 yr. old were hightly susceptible, experiencing 60 – 100% mortality when exposed. This year (2013) we determined that ranavirus had a low background prevalence within three of our study sites and a moderate (although well below outbreak status) prevalence at the fourth study site.

Although our results indicate that ranaviruses could significantly impact upland recruitment of striped newts by affecting survival during the juvenile stage, ranavirus would have to be prevalent at a given site for this to happen. Ranavirus preliminarily appears to not be highly prevalent within our study sites, at least at this time. Given the high susceptibility of ornate chorus frogs and gopher frogs to ranavirus coupled with their documented relative high abundance in our study area year after year, we hypothesize that ranavirus probably is not the greatest culprit of striped newt decline in the ANF. At best, ranavirus could be a lesser of many factors operating synergistically to cause decline. Other factors include long-term drought and past land management practices (e.g. fire suppression and winter prescribed burning).

With the data we have at this time, we believe that repatriations must proceed, although with caution. For next year's planned repatriations, we believe it best to add the greatest numbers of larvae to Pond 75 (where there is zero ranavirus prevalence), while still introducing fewer numbers of larvae at the other three recipient wetlands.

Status of Captive Western Striped Newt Assurance/Repatriation Colony

The original western striped newt adults collected as larvae in Year 1 from Big Pond in the Fall Line Sandhills Natural Area (FLSNA), Georgia continued to be housed at the Memphis Zoo in Year 3. Currently there are 29 adults within this colony, 15 of which are wild caught from FLSNA in 2010, 2 are F1 captive bred in 2012, 12 are wild collected from FLSNA this year (2013).

In an effort to boost size, genetic robustness, and avoid an "eggs in one basket" scenario, five additional paedomorphic adult western striped newts were donated to the Jacksonville Zoo and Gardens to initiate another captive assurance colony. The five donated individuals belonged to W. James Barichivich for nearly 10 years. They also were originally captured wild from Big Pond in FLSNA, Georgia. Zoo colleagues Dino Ferri, Mark Beshel and staff took in these animals, induced them to breed, and produced 61 larvae of 4 different cohorts that would eventually become the individuals utilized for this year's repatriation.

Our captive western striped newt colony serves two important roles. It is the first assurance colony ever created for the highly imperiled western genetic variant of the striped newt and serves obvious conservation needs. Second, larvae produced from this colony will continue to be utilized for this study's repatriation efforts next year (Year 4).

Hydrology and Ecology of Repatriation/Liner Wetlands

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

Table 1. Monthly presence (blue shading) or absence (white shading) of water within receipient wetlands and selected, nearby similar hydrological reference wetlands. During dry spells, pooled water at liner wetlands persisted 1-2 months longer than at nearby reference wetlands. The recipient pond 16 has no liner, and it hydrologically behaves similarly to its reference pond 15. These data indicate that liners are lenghthening the hydroperiods of recipient wetlands during dry spells. (*) denotes striped newt recipient wetlands. (**) indicates nearby paired hydrological reference 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
Sep 2012											
Oct 2012											
Nov 2012											
Dec 2012											
Jan 2013											
Feb 2013											
Mar 2013											
Apr 2013											
May 2013											
Jun 2013											
Jul 2013											
Aug 2013											

Table 1 shows clearly that liner wetlands hold water approximately 1-2 months longer than nearby similar hydrological reference wetlands during dry-up periods. It also shows that liner ponds fill more readily than reference ponds during rainy periods. In the dry spell of Fall 2012, all three liner wetlands (18, 75, 182) held water approximately 1 month longer than nearby reference wetlands (17, 72, 48). The dry trend continued until December 2012 when several frontal rains began accumulating 6-9 inches ofwinter rainfall on the study region. During this time, liner wetlands developed open water pools only atop the central, lined portion of wetland basins, and these pools persisted for nearly 2 months during mid-winter 2012-2013. Nearby reference wetlands held no water during the same winter period because 1) the local water table still had not risen enough to create pools within these aquifer-driven wetlands, and 2) there was no geological confining layer underneath the sandy bottomed wetlands to hold water. In this study, liners are acting as artificial confining layers. During periods of light to average rainfall, rainfall, alone has become the source for wetland flooding in liner wetlands with respect to reference wetlands.

In late February 2013, a huge single rain event accumulated 10-15 inches of rainfall on our study region over a 3-day period, as previously mentioned. This rain easily filled liner wetlands to liner capacity and well beyond. All reference wetlands also developed sizeable open water pools. Liner wetlands filled rapidly during thi rain event, while reference wetlands took approximately 5 days to develop water pools. This effect clearly indicates that reference wetlands are aquifer/water table-recharged, while liner wetlands are now primarily rainfall-recharged wetlands. During this heavy rain, liner wetlands were filled to well over the liner capacity, indicating that the rising water table (aquifer) became the primary source for wetland flooding past a certain, as of yet unmeasured, threshhold. By the end of April 2013, spring drought had dried two out of three reference wetlands while nearby liner wetlands persisted as very shallow pools for another month into May 2013. The third reference wetland (pond 73) had persisted into May and dried two weeks before its nearby paired liner wetland (pond 75).

During early July 2013, another heavy rainfall pattern developed across the study region and filled all reference and liner wetlands rapidly. Water remained at all liner and reference wetlands throughout the end of this study Year 3 (September 2013).

The Pond 16/Pond 15 wetland pair both stayed hydrated during the entire Year 3 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 sinkhole wetlands adjacent to one another with nearly the same bottom elevation. Both these wetlands nearly dried by late June/early July 2013 until the heavy summer rains replenished their water supplies.

Pond 16 was our chosen, single striped newt release site this year because we had low numbers of available larvae for repatriation, and its deepness makes it more drought-resistant than even the lined recipient wetlands. Even though lined recipient wetlands show some drought-resistance because of liners, they still may not be able to completely withstand lenghthy dry spells within the critical larval repatriation and development periods of late spring/early summer. In the event that recipient wetlands may threaten to go dry during next year's planned repatriations, we have a contingency plan to supplement water levels via water trucks if needed.

All liner-enhanced wetlands currently appear to be ecologically healthy, containing rapidly regenerated and diverse natural vegetation. They also contain the naturally occurring assemblage of pond breeding amphibians that are expected within local ephemeral wetlands (see Appendix A). There have been to five species of larval amphibians observed at any given time within liner wetlands. The amphibian communities of liner wetlands have not changed over time resulting from liners--except that there are now more opportunities for local amphibians to breed because of lengthened hydroperiods induced by the liners.

Both the gopher frog (*Lithobates capito*) and ornate chorus frog were abundantly observed as tadpoles in March and April 2013 within all liner wetlands during a time when most of the other area wetlands were dry and unsuitable as breeding habitat (Fig 7). As previously mentioned, these species are considered by many, including the authors, to be either rare or declining globally. Their relatively abundant presence within our liner wetlands is a good indication of ecological heath of the liner wetlands and 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, particularly in regions that have been impacted by increased drought frequency and longevity, and provide additional breeding habitat for species of concern.

a.

b.

c.

Figure 7. Gopher frog tadpoles observed at liner wetlands Pond 18 (a.), Pond 182 (b.), and Pond 75 (c.).

Vandalism

Recipient/liner Pond 18 was damaged twice by illegal, off-road vehicular traffic, once in October 2012 and another in April 2013 (Fig. 8). The first was the most severe. Deep tire ruts were made, exposing and puncturing a portion of the liner. Fortunately, we were able to successfully repair the puncture and re-cover the liner. Tire spinning created circular doughnuts all over the depression and killed back the grasses within the tire tracks. The vegetation showed some regrowth after several months. If the ORV assaults can be eliminated, we expect that the vegetation will eventually fully recover, although it may take several growing seasons. It will be critically important to keep out vandals during next year's planned repatriations.

Figure 8. Pond 18 was damaged by illegal, off-road vehicle traffic twice during the 2012-2013 study year.

Striped Newt Repatriation and Monitoring

Repatriated larvae remained in enclosures for 2-4 weeks depending upon rapidly lowering pond water levels during a May 2013 dry spell. A total of three larvae were observed while surveillance dipnetting enclosures. Those that were, exhibited rapid growth beginning after release (Fig 9). The first newt captured in an enclosure (enclosure C) 2 weeks after introduction measured 20 mm SVL, while the two captured 4 weeks later (enclosure B) measured 21 mm SVL.

Figure 9. Striped newt larva captured during surveillance dipnetting of enclosure.

Finding only 3 out of 58 released newts while surveillance dipnetting the enclosures may be explained in one some combination of several ways. First, the released larvae may have perished. Second, repatriated larvae may have escaped underneath the weighed down screening along the bottom edges of enclosures, despite efforts to lay bricks down along the edges. The burrowing potential of striped newts is a possible behavioral characteristic that is recognized by the authors but not found in the literature. Third, larval newts in small enclosures within a clearwater pond may be wary of capture and possibly could rapidly swim to avoid being scooped in a dipnet. After 4 weeks of leaving enclosures intact in Pond 16, all were opened because of rapidly drying pond water levels and subsequent exposure of enclosures. Any enclosed newts were at large in Pond 16 by the end of May 2013.

Rapid pond drying continued as a trend throughout June 2013. Pond 16 threatened to go dry by this time and became a small 8 m diameter pool in the center of the depression with all enclosures high and dry. The central puddle teemed with concentrated amphibian and invertebrate larvae. It was during this time that three terrestrial striped newt efts were measured by the drift fence emerging out of the wetland, presumably to avoid pond drying. The first eft was found in a pitfall bucket trap on June 21, 2013 (Fig 10a); the second on June 25, 2013 (Fig 10b); and the third on July 1, 2013 (Fig 10c). Each eft appeared robust and healthy. All three exhibited prominent vestigial gill stubs. Each individual was released outside the drift fence in the nearest patch of vegetative cover to the wetland edge--all approximately 25' away from the drift fence along the ecotone between upland and wetland vegetation. These are the first three terrestrial striped newt efts produced in this repatriation study.

a.

b.

c.

Figure 10. First (a.), second (b.), and third (c.) striped newt efts captured in the drift fence around Pond 16. These three represent the only striped newts detected leaving the wetland this year (2013).

Terrestrial efts were left unmarked. Marking was unnecessary because these are the only terrestrial striped newts within the ANF, that is, until future sampling efforts discover otherwise. 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, all we need do is measure and compare the numbers of released larvae, emerging terrestrial efts, and returning breeding adults over time in order to measure study success. Marking is not necessary at this time.

Thousands of other vertebrates and invertebrates were captured in drift fence pitfall traps, including 271 newly metamorphosed gopher frogs (Figure 11).

a. b.

Figure 11. Thousands of vertebrates and invertebrates were captured in drift fence traps including metamorphic oak toads, eastern narrowmouth toads, and southern toads (a.) and gopher frogs (b.).

May and June were very dry months, and water levels in the repatriation wetland lowered and almost dried. However, heavy and frequent rain fell in July. Pond 16 began to rise after several rains and continued to rise through August. The drift fence became flooded and was deactivated in late July, nearly three months after repatriation. We periodically dipnetted and seined the wetland afterward to continue to measure for newt occupation of the wetland. No striped newt larvae were observed within the flooded wetland after the drift fence flooding. This finding could be explained in several, or a combination of, ways. First, dilution of the wetland due to flooding clearly was a factor affecting the detection probability of striped newt larvae. Second, it is possible that newts avoided capture. Third, individuals may have metamorphosed and exited the wetland in the 20 days following the closure of the drift fence. This year's repatriation monitoring effort concluded in mid-August.

We are pleased with the initial repatriation results in this study. Because most amphibians, including the striped newt, are r-selected species (in which parents produce large numbers of potential offspring of which only a few survive), this year's results are quite promising. Considering that four parents produced three metamorphosed efts exiting the wetland, regardless of the other 55 larvae unaccounted for, these may represent quite successful numbers when viewed within an r-selective ecological framework.

Preparations are underway at the Memphis and Jacksonville Zoos to produce larger numbers of larvae for use in next year's repatriations. We anticipate that the 12 additional wild individuals captured this year will increase our larval production capabilities.

YEAR 4 EXPECTATIONS

We will continue to produce as many western striped newts as possible in the assurance colony such that there will be abundant larvae available by next year's planned repatriations.

With the data we have at this time, we believe that repatriations must proceed, although with caution. For next year's planned repatriations, we believe it best to add the greatest numbers of larvae to Pond 75 (where there is 0% ranavirus prevalence), while still introducing fewer numbers of larvae at the other three recipient wetlands.

Additional repatriations within the four selected recipient wetlands will begin in January 2014. We will conduct repatriation efforts in four wetlands using captive-bred larvae from striped newt assurance colonies developed in the first two years of the study. Multiple repatriations will be conducted through March 2014. Drift fences encircling all recipient wetlands will be installed. Wetlands will be continuously monitored through metamorphosis or through the end of June 2014, whichever comes first, to measure recruitment of striped newts into the uplands.

The 19 historical striped newt wetlands in the ANF will be resampled in winter and in spring 2014. 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.

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APPENDIX A. Amphibian species communities observed as larvae or adults (Agryl only) during 2013 dipnet surveys at the 19 historical striped newt breeding wetlands and one additional reference wetland (Pond 17). Species abbreviations are taken from the first letter of the genus followed by the first four letters of the species epithet. Pond numbers with (*) denotes liner wetlands.

Pond No.	N viri	A talp	A gryl	A quer	A terr	H femo	H grat	L capi	L cate	L sphe	P orna	Total No. Species
1	X		X		X	X	X			X	X	7
2		X	X		X						X	4
3		X				X	X	X			X	5
6		X						X				2
16		X	X		X	X	X	X	X		X	8
17											X	1
18*			X	X		X	X	X			X	6
20			X	X			X	X			X	5
26			X		X			X		X	X	5
33											X	1
37						X	X	X			X	4
42	Dry throughout sampling effort											0
48			X				X					2
50			X							X		2
54			X					X		X	X	4
61	Dry throughout sampling effort											0
71	Dry throughout sampling effort											0
73			X									1
75*			X	X		X	X	X			X	6
182*				X		X		X			X	4
Total	1	4	11	4	4	7	8	10	1	4	13	