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

Fifth Annual Report

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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 wetland enhancement techniques to increase success potential of repatriations. At the end of March 2015, the original cost-share agreement between the CPI and ANF concluded. However, our efforts continued throught September 2015. This report summarizes work conducted in Year 5 (October 2014-September 2015) of the study.

The most profound moment in the study and most significant indication of some study success occurred this year. On 5 February, we captured the first terrestrial adult in a drift fence returning to its natal repatriation wetland with intent to breed. We released this newt as a larva the prior year (2014). It lived seven months in the surrounding uplands before returning to Pond 18.

We surpassed the 1000 newt release milestone this year. In 2015, we released a total of 697 striped newt adult and larvae into six recipient wetlands. Our release total for the entire project by year's end was 1188. This number includes 126 adult striped newts. This year marks our first ever releasing of adult newts for the project.

Measured percent metamorphic yields ranged anywhere from zero to 37% in 2015, depending on the wetland and the life stage (adult versus larvae) repatriated. Measured overall percent yields at Ponds 18 (30%) and 182 (10%) continued to be higher for the second straight year. Overall percent metamorphic yield for 2015, which incorporates adults and larvae repatriated into the four drift-fenced wetlands, was 5.8%. Due to drift fence flooding and trespass potential, our measured percent metamorphic yield likely was an underestimate.

We continued to investigate whether infectious disease was present in our study area and therefore, whether it could be implicated in the mysterious decline of the western striped newt from the ANF. We swab-sampled 23 striped newts this year, captured in drift fence traps after repatriation, to test for either chytrid fungus or *Ranavirus.* Swabbing results will not be available until next year (2016).

Six of the swabbed individuals (26%) had some observed skin spot, wound, or sore. Spots could be the result of many things, including possible disease or predator attack. A direct observation was made of fire ants attacking a newly metamorphosed adult striped newt near the edge of a recipient wetland. Sores and wounds, many reminiscent of the

wounds observed on encountered newts this year, developed all over the body. The newt died after one week of observation.

We re-sampled 17 historical striped newt wetlands in our ANF study region and found zero striped newts. We did find eastern newts (*Notophthalmus viridescens*) present at multiple wetlands, and we observed other pond-breeding amphibians, including the rare ornate chorus (*Pseudacris ornate*) and gopher (*Lithobates capito*) frogs, in relative abundance.

Repatriation ponds underlain with rubber liners continued to boost hydroperiods up to three months longer than hydrological reference ponds. All lined repatriation wetlands, however, did go dry at some point during the calendar year 2015, a good indication that lined wetlands still are ephemeral.

ACKNOWLEDGMENTS

Numerous colleagues have been involved with the striped newt repatriation project over the 5-year history of the project. This year, 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 provided assistance and expertise in producing assurance colonies of both western (and eastern) striped newts used for this project including: Brian Eisele, Amanda Lankenau, Sean Wallace, Stefanie Jackson, Emily Mikus, Steve Gott, and John Lukas 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, therefore, to the conservation of the striped newt.

We want to thank Pierson Hill, Kevin Enge, Anna Farmer, Jonathan Mays, and Andrew Townsend for assistance with dipnetting historical striped newt and other wetlands associated with this project. Wendy, Joey, and John Anazalone and Ashley, Juniper, and Spencer Hopkins provided invaluable assistance with drift fence checking efforts.

We appreciate an important natural history observation involving fire ant predation on newts provided by Scott Davis. Many thanks again to Pierson Hill for providing care and photography of the fire ant newt.

Two new colleagues came on board the striped newt project this year. Dr. Karen Lipps (University of Maryland) and Dr. Brooke Talley (Florida Fish and Wildlife Conservation Commission). We greatly thank these individuals for their colossal assistance and guidance with this year's chytrid fungal investigation and requisite swab sampling efforts. We look forward to continued study collaboration in 2016.

We would like to extend a very special thank you to the Felburn Foundation and to the Jacksonville Zoo and Gardens for providing significant funding assistance to the striped newt project this year. Without such support, this collaborative and worthwhile salamander conservation effort would not be possible.

Finally, we would like to acknowledge former authors who contributed in past years to the striped newt repatriation project. These colleagues remain a part of the repatriation team and we look forward to collaboration in future years: Roberto Brenes (University of Tennessee), Matthew J. Gray (Univerity of Tennessee), Steve A. Johnson (University of Florida), D. Bruce Means (Coastal Plains Institute), and Debra L. Miller (University of Tennessee).

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INTRODUCTION

The Florida Gas Transmission Company has increased the amount of natural gas it transports throughout the U.S. Gulf Coastal region. To accomplish this task, the Company expanded an already existing natural gas pipeline that spanned east/west across the Munson Sandhills region south of Tallahassee. 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). Evidence suggests there may be two 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 more thoroughly documented 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 IUCN added the striped newt to the Red Lis as NT ("near threatened") (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 in the Federal Register in response to the petition (USFWS 2010). A 12-month review followed and found the threatened listing as warranted. However, this action was precluded by higher priority listings (USFWS 2011). The striped newt has been a federal candidate species for listing since 2011.

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. We last observed an adult in 2007, despite intensive wetland sampling nearly every year since that year. 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 infectious disease. 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 infectious disease. 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.

CPI 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 5 (Oct 2014-Sept 2015) of the 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. House, maintain, and grow the assurance colony within collaborating zoological institutions. Use larvae and adults from the assurance colonies as the source for repatriations in the ANF.

2. Continue sampling the ANF for local striped newts. 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. Conduct *Ranavirus* susceptibility tests in striped newts and conduct surveillance testing for *Ranavirus* in sympatric species at repatriation wetlands and in nearby wetlands. Test post-repatriated striped newts encountered in the field for chytrid fungus.
4. Conduct seasonal striped newt repatriation efforts in the ANF in at least four wetlands, using captive-bred larvae from striped newt assurance colonies.
5. Investigate and implement techniques to ensure suitable hydrological conditions at selected repatriation wetlands by repatriation time. Techniques include synthetic liner installation.
6. Enhance striped newt breeding habitat, including prescribe burning of wetlands, and hand-removal of encroaching woody shrubs and trees from the basins of repatriation wetlands. 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.

As we hereby report our study results from Year 5, we also prepare to initiate the next phase of the striped newt project. The next phase will consist of another three years of full-scale repatriations and monitoring to be conducted 2016-2018.

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.

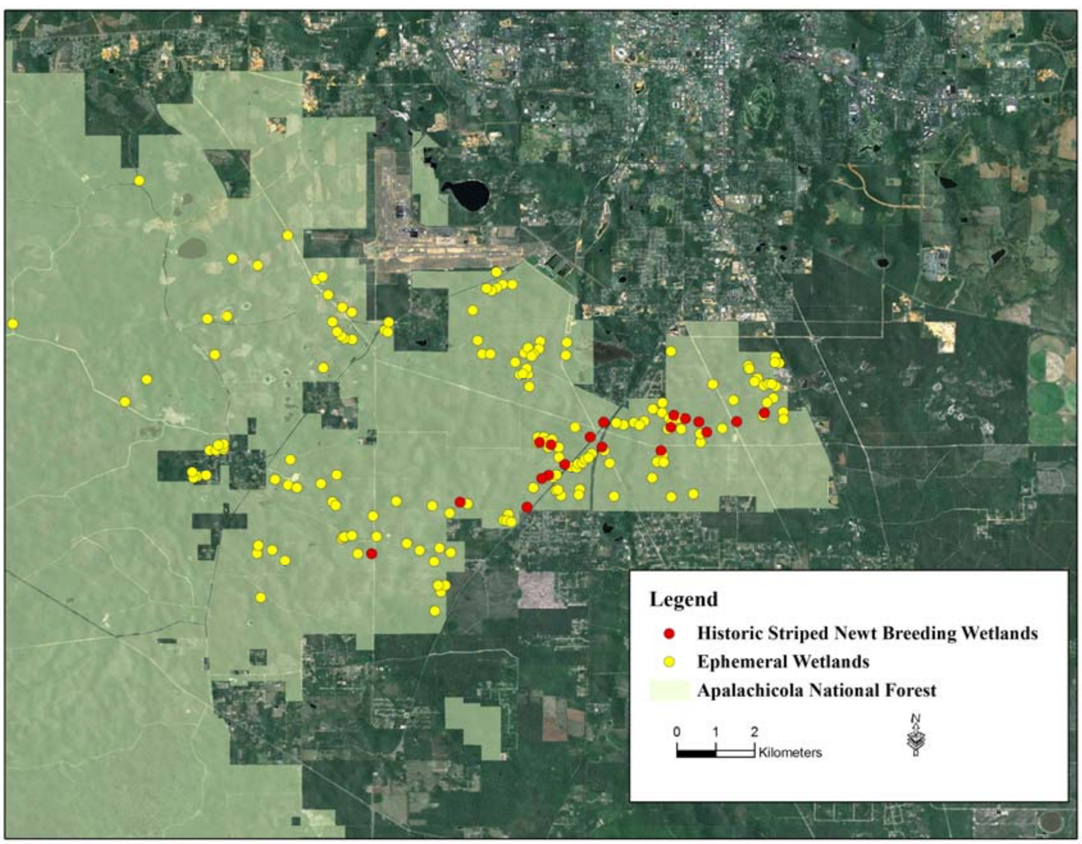

Figure 1. Map of the Munson Sandhills study area. Yellow dots represent the 158 ephemeral wetlands that have been periodically sampled over the last 20 years. Red dots represent the 19 historical striped newt breeding wetlands.

METHODS

Background Monitoring for the Striped Newt

During Year 5, we sampled all 19 historically known striped newt-breeding wetlands in the MS region of the ANF as water levels allowed. The first sampling effort took place during February 2015 winter breeding season to search for presence of striped newt aquatic adults. We sampled 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, we thoroughly swept the entire pond periphery and the center. For larger ponds, we conducted 50 dipnet sweeps, spaced somewhat randomly around the pond perimeter.

The second sampling effort took place on 24 April during the prime larval detection season for striped newts. R. Means was accompanied by a team of colleagues from the Florida Fish and Wildlife Conservation Commission (FWC). The FWC team consisted of K. Enge, P. Hill, A. Farmer, J. Mays, and A. Townsend. Our collective mission was to intensively sample for presence/absence of striped newts. The FWC team additionally conducted occupancy modeling for all encountered pond-breeding amphibians.

We sampled seventeen historical striped newt breeding wetlands throughout the former ANF striped newt range in one day. Water levels and hydroperiod histories were prime for pond-breeding amphibians. Observers used a heavy-duty dipnet (Memphis Net and Twine Co. HDD-2 model) with 3/16” mesh. One FWC team member used a dipnet with 1/8" mesh.

The spring dipnet effort adhered to occupancy modeling standards developed by the FWC team (K. Enge, pers. comm.). We concentrated sweep efforts in submerged or emergent herbaceous vegetation or around shallow perimeter where newt larvae tend to concentrate. We also used a 6' seine net to supplement our dipnetting efforts. Two researchers pulled the seine net through a large swath of water, 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 are based on 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 2015 striped newt repatriation wetlands. The four wetlands with drift fences (16, 18, 75, and 182) are part of the original repatriation methodology. We installed liners under three of these wetlands (18, 75, and 182) during the 2nd year of this project (2012). We repatriated striped newts into two additional wetlands this year (001 and 178). These wetlands have neither pond liners nor encircling drift fences.

We installed synthetic (EPDM) liners 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. We selectively paired four wetlands that were nearby and hydrologically similar wetlands to repatriation wetlands as hydrological reference/control wetlands. Pond 15 is paired with Pond 16, Pond 17 is paired with Pond 18, Pond 73 is paired with 75, and Pond 182 is paired with Pond 182a. We collected observational water presence/absence data monthly at the four repatriation and four reference wetlands since liner installation.

We selected two additional recipient wetlands (Ponds 1 and 178) in 2015, which brings the total number of recipient wetlands to six (Figure 2). We did not modify the new recipient ponds with liners. We also did not construct drift fences to document movement into and out of these wetlands. We selected Pond 1 based on its relatively longer hydroperiod and its having been one of the more productive striped newt wetlands in the historical past. We chose Pond 178 based on its large size, relatively long hydroperiod with ability to resist drought, proximity to Pond 16, and its presumed ability to sustain a striped newt population based on a combination of said factors.

Striped Newt Husbandry

Jacksonville Zoo and Gardens

The Jacksonville Zoo and Gardens has maintained captive specimens of the western clade of the striped newt in its Amphibian Conservation Center since 2012. For the past three years, our breeding group has been comprised of 3.3 adult individuals. These three breeding pairs have been responsible for the production of approximately 1000 larval and adult progeny available for release into the wild. Details for adult and larval striped newt husbandry methodology are included in the Fourth Annual Report (Means et al. 2014).

In 2014, we held back more larvae than in previous years for the purpose of headstarting individuals for release at a larger body size. Due to cold weather conditions, we held these offspring several weeks longer than in the past. Of particular interest, during the winter cool-down period, many of these offspring that had begun to metamorphose into efts returned to an aquatic stage and no longer ventured out of the water. Others bypassed the eft stage altogether, resembling smaller adults, but retaining small, possibly non-functioning gills. At approximately eight months of age, many of these animals began mating and laying fertile eggs in the communal rearing enclosures. We released these individuals, which included several that were still mating or gravid with eggs, in the Apalachicola National Forest in early February 2015.

In addition to these repatriated individuals, we delivered three sub-adult pairs of the striped newt, hatched at the Jacksonville Zoo and Gardens in 2014, to the Central Florida Zoo in April 2015. We provided these animals in order to found an additional ex-situ assurance colony for the species that can produce and contribute further offspring for repatriations.

In 2015, we hatched a total of 808 striped newts, with a single adult pair producing 469 offspring. In contrast, another pair of breeding adults produced only 11 surviving offspring from 13 fertile eggs, after which the female stopped laying altogether. We added four additional larval rearing tanks (up from only five tanks in 2014) in 2015, which reduced the total number of larvae typically maintained in each enclosure by almost half.

Based on the number of 2014 offspring that survived to reach a large enough size for release in February 2015 and the number of 2015 offspring that are currently living at the Jacksonville Zoo and Gardens, we estimate overall survivorship for the 2014 and 2015 seasons combined to be around 65%. Rearing densities within each enclosure apparently influenced survivorship, with specimens reared in greater densities generally having lower survivorship. Including animals held back for headstarting for which survivorship is still being measured, survivorship of offspring in 2015 ranged from 58%-85% (Figure 3). Offspring from Group 3, which had a starting density of 98 individuals and a survivorship of 62%, were produced by the previous year's offspring, who began breeding early at eight months in age. This group's lower survivorship when compared to that of Group 2 (72%), which had a greater density of 228 individuals, may be related to the young age of the parents and the fact that this was their first breeding season.

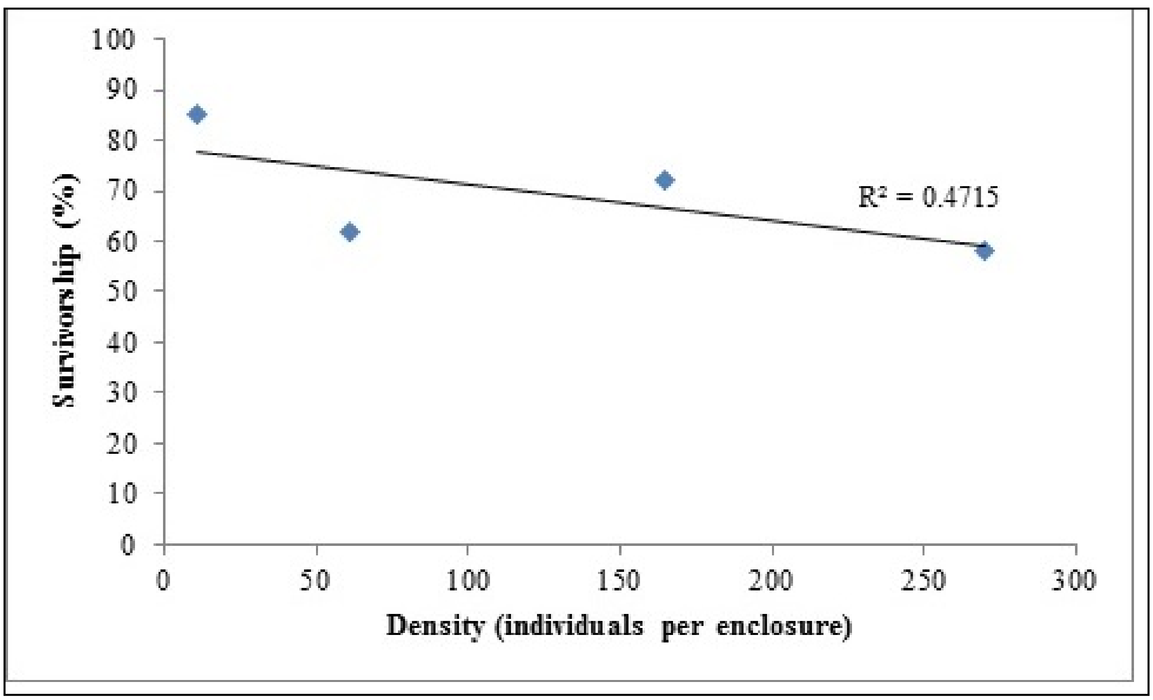

Figure 3. Graph depicting striped newt offspring survivorship as a function of density in enclosures.

The American Association of Zoo Keepers (AAZK) awarded the Jacksonville Zoo and Gardens a Preservation and Restoration Grant in 2015. We will use this grant assistance to acquire additional holding tanks and life support equipment. We also seek to expand on our current holding capacity for the striped newt, allowing for more individuals to be reared in lesser densities. Moreover, additional enclosures will create more space for accommodating breeding adults, enabling the Zoo to increase and maximize its overall reproductive output. The Zoo will seek additional external funding in 2016 for continued expansion and refinement of the Jacksonville Zoo and Gardens' striped newts rearing and reproductive efforts.

Memphis Zoo

By the end of 2015, the breeding group census stood at 9.6. Individuals were collected and obtained in 2011 from the Georgia source pond, and 3.7 were collected in 2013, for a total of 25. They were distributed among seven 10 gallon aquariums in groups of 1.1, 3.2, 2.1, 1.1, and 2.1 (2011 cohort) and 1.3 and 2.4 (2013 cohort). We held no larvae; all newts were sexually mature.

We maintained aquaria with 4 inches of water, java moss, a bubble filter and a piece of floating cork bark. We stimulated breeding activity 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, we reduced the artificial rains, then terminated upon the onset of oviposition. We collected eggs and put them in a hatching tank to avoid predation by adults, or live zooplankton, which the adults feed on ad lib. As eggs hatched, we removed the larvae and put them into 10-gallon tanks set up in the same way as the adults. We limited the number of larvae per aquarium to 25. As larvae grew we moved larger animals to prevent predation on smaller individuals. We reared larvae on a diet of chopped blackworms and daphnia.

We shipped ~50 adult newts from the 2014 cohort for winter release early in 2015. We subsequently shipped 110 larval newts for release, 35 of which did not survive shipping or shortly thereafter, and 75 of which were successfully released.

By the time of this report, breeding activity had again begun, but no eggs had been laid.

Striped Newt Repatriation and Monitoring

Drift Fence Operation

Drift fencing is this study's greatest method of measuring repatriation success at various life history stages of our released newts. It allows us to estimate percent metamorphic yield (i.e. the percentage of released newts (larvae and aquatic adults) captured exiting a wetland), and the timing of such events. It also allows us to ascertain the number and timing of returning adults back to natal wetlands with intent to breed.

This year, we re-activated galvanized metal drift fences at Ponds 16, 18, 75 and 182. Each wetland's drift fence was operated from the time of first newt release, water conditions permitting, through the first week of August in order to measure potentially returning striped newt terrestrial adults or potentially emigrating efts that leave the wetland after metamorphosis. We describe general drift fence construction and operation in Means et al. (2013).

We operated Pond 16 drift fence from 23 Dec (2014) through 7 Aug (2015). However, significant fence flooding occurred in late January lasting until the end of March. By 1 April, most buckets (32 out of 34) had emerged from floodwaters and remained so for the rest of the operation period. Total number of full operation days for Pond 16 drift fence in 2015 was 167.

We operated Pond 18 drift fence from 19 Jan through 2 Aug. No flooding of the fence or bucket pitfall traps occurred here this year. Total number of full operation days for Pond 18 drift fence was 183.

We operated Pond 75 drift fence from 19 May through 30 July. The drift fence and all pitfall traps were totally inundated from January 2015 until operation began in May 2015. Total number of full operation days for Pond 75 drift fence was 78.

We operated Pond 182 drift fence from 1 Feb through 31 May. No significant flooding of this fence occurred in 2015. We terminated fence operation here early because the wetland went dry in April and did not refill (see Appendix A). Total number of full operation days for Pond 182 drift fence was 119.

<u>Newt Repatriation</u>

This year marked the first year of adult striped newt release (Figure 4). We believed that releasing adults, in addition to larvae, could only increase our chances to establish self-sustaining populations. We decided, by the end of the prior (2014) field season, to hold a substantial number of larvae in captivity, raise them into adulthood, and release them during winter 2015. Memphis Zoo and Jacksonville Zoo and Gardens rasied the newts into adulthood over an approximately seven-month period. Memphis Zoo carefully packaged the adult newts in coolers and sent them periodically via overnight Fed Ex. Jacksonville Zoo and Gardens personally transported the newts and participated in their release. This year (2015) was the first year of this study that we released adult newts.

Figure 4. Release of aquatic adult striped newts into repatriation wetlands.

We released adult newts during the winter, which is the natural time in the life cycle that adults move into wetlands to breed (Figure 4). Adults reared by both zoos were in the 6-7 month-old age range. Snout to vent lengths of all released adults ranged from 27-33 mm. We partitioned animals into recipient wetlands based on suitability of water levels. We provided a 15-minute acclimation period for individuals by mixing transport water with pond water and letting the temperature slowly become that of ambient. We released individuals at large (i.e. not into holding boxes), and spaced roughly evenly around the pond perimeter. We abandoned releasing any newts into holding boxes in 2015 because evidence from past years suggested that holding boxes were not particularly favorable in the release process. As best as possible, we deteremined sexes and released equal numbers of sexes per pond. We released male-female pairs together (Figure 4). We spread out pairs around the pond in favorable areas providing plenty of cover. All adults arrived healthy this year.

We have not yet employed mark-recapture techniques. The extensive sampling data we generated over the past 20 years strongly suggest that the local historical populations were extirpated. We presumed all individuals encountered in drift fences are a result of our repatriation efforts. Up to this point in the study, we were able to adequately ascertain percent metamorphic yields and the occurrence of returning adults back to natal wetlands with drift fencing alone.

We continued to release larval striped newts throughout spring and summer 2015 (Figure 5). Most animals arrived from the zoos in healthy condition. However, in one large shipment from the Memphis Zoo, 35 out of 157 larvae died of asphixiation. This is the first time in our study that animals were lost during the transport process from zoo to recipient pond. All animals in this particular shipment arrived in healthy condition, but they rapidly began to deteriorate within 6 hours after arrival, in hindsight because they remained in small, enclosed water containers and ran out of oxygen. The principal investigator, R. Means, assumed responsibility for this mishap. In the future, we will avoid asphixiation on the receiving end by immediately opening all enclosed newt containers upon arrival to increase oxygen levels.

Figure 5. Release of larval striped newts.

We partitioned newts into recipient wetlands based on suitability of water levels. Releases began in early May, within the historical time of year that larvae naturally would begin to show up in natal wetlands. Before release into a given wetland, we gave larvae a 1/2 hour acclimation time in a mixture of pond water and their transport water. To avoid the release of exotic invertebrate species into recipient wetlands, we carefully removed all remaining blackworms (captive newt food) from transport water. Lastly, after acclimation and blackworm removal, we transferred newts 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 we did not release blackworms into wetlands.

Infectious Disease Investigations

<u>Background</u>

While salamanders can be susceptible to *Batrachochytrium dendrobatidis* (*Bd*), salamander declines in the United States so far appear to be associated more so with *Ranavirus* outbreaks than with *Bd* (Gray et al. 2009, Gray and Miller 2013). In study Years 1-3, we investigated whether *Ranavirus* may have played a significant role in the decline of ANF striped newts by testing the susceptibility of various life stages to the disease, and by testing the prevalence of the disease in our study area. Our study collaborators and experts in amphibian disease from the University of Tennessee, Dr. M. J. Gray, Dr. D. L. Miller, and Roberto Brenes, directed these investigations. Although our results indicated that *Ranaviruses* could significantly impact upland recruitment of striped newts by affecting survival during the juvenile stage, *Ranavirus* was not highly prevalent within our study sites, at least at the time of sampling. (Means et al. 2012, 2013).

Last year (2014), we noticed grayish to whitish “spots” or “sores” on the skin of a few of our newly-metamorphosed, post-repatriated efts (Means et al. 2014). These observations prompted this year’s investigation into the possibility that a chytrid fungal pathogen may be affecting our repatriated newts.

Chytridiomycosis, the infectious disease caused by the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), is responsible for population declines and extinctions of amphibians in many parts of the world, particularly in tropical regions (Lips et al. 2003, 2006). However, *Bd* and its infectious disease are either spreading or being detected worldwide (Whittaker and Vredenburg 2011). Both frogs and, to a lesser degree, salamanders, can be susceptible to *Bd*. Jancovich et al. (2003) determined that some U.S. salamander species are known to be carriers of *Bd* but are not susceptible to its disease.

Recently, another closely retated chytrid fungus that causes chytridiomycosis in salamanders, *Batrachochytrium salamandrivorans (Bsal)*, caused mass die-offs of wild European fire salamanders (*Salamandra salamandra*) in the Netherlands (Yap and Koo 2014). It has since spread to Belgium and Germany. *Bsal* has aquatic zoospores that infect the skin of the animals, leading to skin lesions, anorexia, apathy, ataxia and death (Martel et al. 2014). Martel et al. (2014) assessed 24 species of salamanders from five families and found that species from the families Plethodontidae and Salamandridae, particularly newts (family Salamandridae), were found to be the most susceptible to the disease.

Given both the documented and potential spread worldwide of chytrid fungus, and the observable skin spots on some newts encountered last year, we began a collaboration with Dr. Karen Lipps (University of Maryland) and Dr. Brooke Talley (FWC) to investigate chytrid in our study area.

Additional investigation into the potential for *Ranavirus* also took place this year. As per request by study collaborators Drs. Gray and Miller, we swabbed any post-repatriated striped newts that had observable spots or sores. We sent samples to Drs. Gray and Miller, at the University of Tennessee’s Center for Wildlife Health, to test for *Ranavirus*.

Swab sampling for chytrid/*Ranavirus* took place during the entire 2015 field season as we encountered post-repatriated striped newts, either in drift fences or by dipnetting (Figure 6). Our swab sampling techniques followed closely those reported by Brem et al. (2007), and as per instructions from Drs. Lips and Talley (pers. comm.).

Figure 6. Swabbing an emergent terrestrial adult striped newt in the field.

Results and Discussion

General Weather and Wetland Conditions

Annual monthly rainfall totals were at or above average from January through February 2015. March was drier than average. In April, we experienced three large rains, including a single 4-inch rain that provided more than the average total amount for the whole month. May was hot and extremmly dry. Average monthly rainfall totals were lower than average June through September. By the end of September 2015, the study area was approximately eight inches below its average annual rainfall quota at that time.

All newt recipient wetlands, as well as their hydrological reference ponds, began the study year hydrated and exhibited a filling trend through early March 2015. There was a drying trend during most of March corresponding with less rainfall. The three large April rains boosted water levels at all liner wetlands. Thereafter, rainfall was below average, and all unlined area wetlands began a drying trend that would last until field season's end in August.

This year's hydrological data showed that liners continued to be effective at providing a boost to lined wetland hydroperiods (Appendix A). Liner ponds recharged rapidly in big rains, while their nearby hydrological reference ponds reamained dry. Lined ponds also recharged much more rapidly than reference ponds. For example, a heavy two-inch rain on 23 June boosted all, previously dry, liner ponds by as much as a foot while reference ponds remained completely dry after the event (Figure 7). Pooled water percolated rapidly into the sandy, permeable soils of the reference wetlands, while lined wetlands captured pooled water on top of the impermeable liner. Lined wetlands continued to support small shallow pools throughout mid summer until they all dried again in September. Reference ponds remained dry for the rest of summer into fall. Liner wetlands filled rapidly in response to heavy rains that produced sheet flows of water. Light rains were not observably effective in recharging lined wetlands.

Sandy soils underlying most of the ephemeral pond assemblage in our study region are permeable, much more so than a rubber lined wetland. Study area wetlands (minus lined wetlands) can recharge only when enough rain has fallen on the landscape to raise the underlying water table (e.g. precipitation accumulation surpasses drawdown from evapotranspiration) to an elevation higher than the bottom of wetlands.

All lined recipient wetlands eventually dried at variable times throughout the summer. This observation indicated that lined wetlands still were ephemeral enough to go dry, particularly during severe heat and dryness. It was not our desire to create “permanent” lined recipient wetlands in this study, but rather, to enhance lined recipient wetlands to have longer hydroperiods that would resist drought and allow enough time for newt larval metamorphosis. Results from this year and all prior study years have indicated hydrological success thus far.

Figure 7. Pond 18 (left) and its hydrological reference pond (17) (on right). Photos were taken 12 hours after a heavy, 2-inch rain on 23 June. Sheet flowing water pooled within the central portions of both wetlands rapidly. A foot-deep pool formed on Pond 18, and remained for weeks. The pool remained for two more months, and was perpetuated by additional summer rain showers. Pond 17, however, never developed a pool of water from the June 23 rain, nor from any other throughout the summer. This observation strongly indicated that the underlying rubber liner at Pond 18 effectively boosted hydroperiod relative to its unlined reference wetland. The other two liner wetlands (75, 182) also behaved this way relative to their unlined reference wetlands.

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. In January and February, heavy rains filled area wetlands to relatively high levels. These rains hydrated all newt recipient wetlands and all 19 historical striped newt ponds, and water levels remained prime through the spring larval season. Despite prime breeding conditions we found no striped newts present in the ANF. This observaion adds further evidence to support our ANF extirpation hypothesis.

Many other pond-breeding amphibians were observed in relative abundance at the 19 historical striped newt ponds this year, including rarer species like the gopher frog (*Lithobates capito*) and ornate chorus frog (*Psuedacris ornata*). During the winter and spring background monitoring efforts we detected 14 species of amphibians:

- 3 salamander species: mole salamander (*Ambystoma talpoideum*), central newt (*Notophthalmus viridescens*), and dwarf siren (*Pseudobranchus axanthus*)
- 11 frog species: southern cricket frog (*Acris gryllus*), southern toad (*Anaxyrus terrestris*), oak toad (*Anaxyrus quercicus*), pine woods treefrog (*Hyla femoralis*), barking tree frog

(*Hyla gratiosa*), gopher frog, bullfrog (*Lithobates catesbeianus*), southern leopard frog (*Lithobates sphenocephalus*), spring peeper (*Pseudaris crucifer*) southern chorus frog (*Psuedacris nigrita*), and ornate chorus frog

Although numbers were lower than last year (Means et al. 2014), ornate chorus and gopher frogs continued to be well represented in the MS. Out of the 19 wetlands sampled, they were observed at 9 and 11 ponds, respectively.

All newt recipient wetlands had some observed presence of both gopher and ornate chorus frogs, including observed calling after big rains (Figure 8). In Florida, both are Species of Special Concern, and both are regarded to be either rare or in decline by most herpetologists. The MS region of the ANF continues to be a stronghold location for both species.

Figure 8. This photo shows two gopher frogs and an ornate chorus frog captured in one drift fence bucket during an overnight February rain at Pond 18. We detected gopher frogs and ornate chorus frogs at all of our newt recipient wetlands this year.

Striped Newt Repatriation and Monitoring

Below are specific, per pond descriptions of our repatriation results. We report and discuss a percentage figure per wetland called "percent metamorphic yield." This quantity is the percentage of released individuals (larvae and/or aquatic adults) we captured exiting a wetland after metamorphosis. Graphic summaries of results are located in the Repatriation Summary section below.

<u>Pond 16</u>

Adults

On 20 December (2014), we released six striped newt aquatic adults shipped from Jacksonville Zoo. On 29 January (2015), we released ten aquatic adults shipped from Memphis Zoo. We released 30 more aquatic adults from Jacksonville Zoo on 1 February. The total number of aquatic adults released into Pond 16 this year was 46. During this year's drift fence operation, we observed zero terrestrial adults exiting Pond 16 this year. Measured percent metamorphic yield of adults, therefore, was zero.

In April, we dipnet sampled in an effort to detect larvae and ascertain whether breeding had taken place among the prior released aquatic adults. We observed zero striped newt larvae. This was an indication that our released aquatic adults either did not breed or bred so little that their produced larvae were not in sufficient concentration to be detected or all offspring died.

Larvae

On 7 May (2015) we released 116 larvae sourced from Jacksonville Zoo. On 15 May, we released an additional 64 larvae, sourced from Memphis Zoo. The total number of larvae released into Pond 16 this year was 180. Two months later, from July 1 through July 18, we captured five striped newt efts exiting Pond 16. All individuals possessed small gill stubs, a clear indication that they originated as gilled larvae, not air-breathing adults. Snout to vent lengths (SVL) ranged from 26-28 mm. Measured percent metamorphic yield of efts at Pond 16, therefore, was 2.8%.

One of our exiting metamorphic efts was captured in an exterior drift fence bucket at Pond 16 within the time period that the other four efts were being captured in interior buckets while exiting. This animal possessed gill stubs, a clear indication that it had just emerged out of water. We concluded that this metamorphic eft had climbed over the fence, then proceeded to fall into an exterior bucket. This observation alerted us to the presence of trespassing newts in our study. We have suspected this possibility for a while, but not confirmed until now. This year, Pond 16 drift fence developed a heavy rusty coating over most of the fence. A rust surface may be easier to climb than a slicker, non-rusty, metallic surface. It is likely that additional trespassing newts occurred here, and that our capture data therefore are incomplete. We will take action next year to offset the trespassing issue by replacing Pond 16 drift fence with brand new galvanized metal flashing.

Pond 18

Adults

On 29 January, we released ten aquatic adults shipped from Memphis Zoo. We released 20 more aquatic adults from Jacksonville Zoo on 1 February. The total number of adults released into Pond 18 this year was 30.

The most important event thus far in the striped newt project's history occurred on 5 February. We captured a single terrestrial adult male (28 mm SVL) in an exterior drift fence bucket during an overnight winter rain (Figure 9). It had a swollen cloaca with a visible whitish spot inside the vent. We dubbed this is individual "Number One." (Return #1 P-18 2-5-15).

Figure 9. First returning striped newt adult in project history. We captured this terrestrial adult in an exterior bucket of the Pond 18 drift fence. This male newt had a swollen cloaca and we presume it was migrating into the wetland to breed. We originally released the animal into Pond 18 in May 2014 as a young larva. It spent approximately 6 weeks developing in the pond, then metamorphosed into an eft. We captured it in the drift fence for the first time while exiting the wetland during June or July 2014. It subsequently spent the next seven months in the surrounding uplalnds before its return to Pond 18 with intent to breed.

Number One was born in Jacksonville Zoo and Gardens in March or April 2014. He remained in captivity as a small larva for approximately 6-8 weeks. We released him in this pond in a cohort of 55 larvae on 13 June (2014).

Number One spent the next 3-4 weeks in Pond 18 growing toward metamorphosis, then exited the wetland as a terrestrial eft. We captured him interiorly in the drift fence during a period between June 22 and July 25, 2014, along with 18 other emigrating efts from this release cohort. He then spent the next seven months in the longleaf pine uplands before returning and falling into an outside bucket of our drift fence. The swollen nature of his cloaca coupled with the correct natural timing of imigration into a breeding wetland indicated that Number One was sexually mature and had returned to his natal wetland with intent to breed. This event occurred sooner than we expected. We anticipate more such returns this coming winter at Pond 18 because recruitment of both efts and terrestrial adults has been the highest observed here over the past two years among all recipient wetlands.

Number One appeared quite healthy, although there was a small, dark spot on his back (Figure 10). As with all post-repatriated striped newts observed in this study, he was photographically documented. We also swabbed him for the presence/absence of chytrid fungus (swab ID: 18-001). Swab results will be available next year.

Figure 10. A black spot on the back of the first returning terrestrial adult striped newt.

On 22 March, we dipnet sampled Pond 18 in an effort to detect larvae and ascertain whether breeding had taken place among the prior released aquatic adults. We found zero larvae. However, we did capture two transforming aquatic adults, remnants from the prior adult releases.

From 22 March through June 1, the drift fence captured 11 metamorphic terrestrial adults exiting Pond 18. Interestingly, we only detected two of the 11 recruited terrestrial adults by dipnet before they were captured in the drift fence. This indicated that striped newts have the ability to evade capture by dipnet. Alternatively, some of the individuals may have been living terrestrially in the sizeable area between the central pond puddle and the drift fence, therefore evading dipnet capture. Measured percent metamorphic yield of terrestrial adults at Pond 18 was 37%.

Larvae

We released ten larvae, sourced from Jacksonville Zoo on 7 May. This was the only larval release of the 2015 season. On June 24, we captured one emergent eft via drift fence. Measured percent metamorphic yield of larvae at Pond 18, therefore, was 10%.

Other Notes

Interestingly, Pond 18 had been dry exactly 10 days prior to the capture of our lone, exiting eft from this pond. The eft moved out and was captured by drift fence during a heavy afternoon/evening thundershower. This rain re-hydrated the wetland to an estimated 50% of liner capacity, which equated to an approximately 20-foot diameter, 16-inch deep pool on top of the liner. Ensuing summer rains maintained this liner pool for another 2 months until it dried again by late summer. In contrast, the nearby hydrological reference wetland (Pond 17) remained dry all summer (Appendix A). Other liner wetlands behaved similarly with respect to their hydrological reference wetlands. These observations indicate not only that liners are extending pond hydroperiods, but also that emergent metamorphs can spend several days in a dried wetland basin interior to the drift fence before being captured. This also highlights the need to keep drift fences operational for a while after a given pond dries, so we can ensure to capture all potential metamorphs.

Drift fence results from Pond 18 were very promising again this year, and they continue to provide this study's greatest glimpse of success potential to date. For the second year in a row, recruitment at Pond 18 was the highest among all drift fenced recipient wetlands in both absolute numbers and percent yields. We suggest that the relatively high herbaceous plant species diversity, as well as having the least frequently flooded drift fence are important contributing factors. We are hopeful that last year’s observed recruited efts (19), coupled with this year’s observed recruited adults (11) and eft (1), will translate to more returning terrestrial adults in 2016, and beyond.

Pond 75

Adults

On 29 January, we released ten aquatic adults shipped from Memphis Zoo. We released 20 more aquatic adults from Jacksonville Zoo on 1 February. The total number of adults released into Pond 75 this year was 30.

On March 24, we dipnet sampled Pond 75 in an effort to detect larvae and ascertain whether breeding had taken place among the prior released aquatic adults. We found zero larvae. However, we did capture one transforming aquatic adult striped newt. This was a remnant from the prior adult releases. This individual had been in the pond for two months.

We captured no metamorphic terrestrial adults exiting this wetland. Measured percent metamorphic yield of adults at Pond 75, therefore, was 0%.

Larvae

We released 11 larvae, sourced from Jacksonville Zoo on 7 May. This was the only larval release of the 2015 season.

We captured zero metamorphic efts in the drift fence as exiting this wetland. Measured percent metamorphic yield of efts at Pond 75, therefore, was 0%.

Drift fence results at Pond 75 were highly impacted by fence flooding for the second year in a row. Any effort to interpret fence results here would be severely biased by fence flooding, and so we avoid interpretation. It has become quite clear that we need to move Pond 75's drift fence to significantly higher ground, and we will do so at the beginning of next field season (more drift fence flooding discussion below).

Pond 182

Adults

On 29 January, we released ten adults shipped from Memphis Zoo. We released 10 more adults from Jacksonville Zoo on 1 February. The total number of adults released into Pond 182 this year was 20.

On 20 March, we dipnet sampled Pond 182 in an effort to detect larvae and ascertain whether breeding had taken place among the prior released aquatic adults. We found zero larvae. However, we did capture one transforming aquatic adult striped newt. This was a remnant from the prior adult releases. This individual had been in the pond for nearly two months. Interestingly, we also found one transforming aquatic adult eastern newt (*Notophthalmus viridescens*). This individual must have either trespassed over our drift fence or entered the wetland prior to February 1 (the date of drift fence installment) and remained in the pond for at least 50 days until its capture.

On 17 May, we captured two newly metamorphic terrestrial adults exiting Pond 182 during a nighttime rain shower. As with Pond 18, we observed more remnant adults by drift fence (2) than by dipnet sampling (1). Measured percent metamorphic yield of terrestrial adults at Pond 182 was 10%.

Measured metamorphic yield at Pond 182 continues to be the second highest among the four drift fenced recipient wetlands for the second year in a row. We suggest that the relatively high herbaceous plant species diversity, as well as having a less frequently flooded drift fence also may be important contributing factors here.

Larvae

We did not release larval striped newts in Pond 182 in 2015 due to extremely low water levels and subsequent dry-up.

<u>Pond 178</u>

On 2 February, we released 38 aquatic adults from Jacksonville Zoo. This marked the first ever release of striped newts into Pond 178 as part of this project. On 24 April, along with a group of four FWC colleagues, we dipnet sampled Pond 178 in an effort to detect larvae and ascertain whether breeding had taken place among the prior released aquatic adults. We found zero larvae or remnant aquatic adults.

On 15 May, we released 58 larvae, sourced from Memphis Zoo. On 28 May, we released 235 additional larvae also sourced from Jacksonville Zoo. Total number of striped newt larvae released into Pond 178 this year was 293.

Percent metamorphic yield estimates were not available at Pond 178 because of the lack of an encircling drift fence.

<u>Pond 1</u>

On 2 February, we released 39 aquatic adult striped newts from Jacksonville Zoo. This marked the first ever release of striped newts into Pond 1 as part of this project. We did not release any striped newt larvae this year into Pond 1.

On 24 April, along with a group of four FWC colleagues, we dipnet sampled Pond 1 in an effort to detect larvae and ascertain whether breeding had taken place among the prior released aquatic adults. We found zero striped newt larvae or remnant aquatic adults. However, we observed 8 aquatic adults and 5 larval eastern newts.

Drift Fence Flooding

Fence flooding impacted our ability to measure repatriation success at wetlands this year. Pond 75, a lined recipient wetland, filled in Nov (2014) and flooded over the drift fence. Water levels were above the drift fence until the beginning of May 2015. Pond 16 drift fence also was flooded for multiple months, and during a prime period for measuring recruitment.

When this study began, the study area was experiencing a long-term, severe drought. Since the beginning of repatriations, however, the region has received annual rainfall quotas. The rain has fallen rather erratically, with several huge single rain events (i.e. >8 inches), and several extmemely wet periods (i.e. > 20 inches in 1-2 months). This type of rainfall resulted in the flooding of most of our drift fences at least some of the time.

With frequent drift fence flooding now a study concern, we conclude that it is time to reestablish drift fence rings to higher locations at Ponds 16 and 75. We will move fences in December 2015 or January 2016, before the 2016 breeding season begins. With the development of El Niño climatic conditions and predictions for a very wet winter-spring 2015-2016, we expect wintertime flooding to occur again by early 2016.

Repatriation Summary

The biggest news for 2015 is that we observed our first returning adult coming back to its repatriation wetland with intent to breed. Another project first this year was the release of adult striped newts. We released adults during the winter months, when adults typically come down to the wetlands to breed. We were unable, however, to detect any larvae produced by these adults.

One of this study's objectives was to release 1000 striped newts in a given year. Although we have not yet accomplished 1000 releases in a single year, the number of available newts for release in this study continues to dramatically increase from year to year. We released 58 larvae in 2013, 433 larvae in 2014, and 697 larvae and adults in 2015, for a total of 1188 newts released thus far for the entire project (Figure 11). Our capacity to produce newts continues to grow from year to year as our zoo colleagues continue to expand their capacities and perfect their husbandry techniques. Additionally, we expect to have two more zoological institutions involved next year, Central Florida Zoo and Lowry Park Zoo. This should increase our production capacity even further.

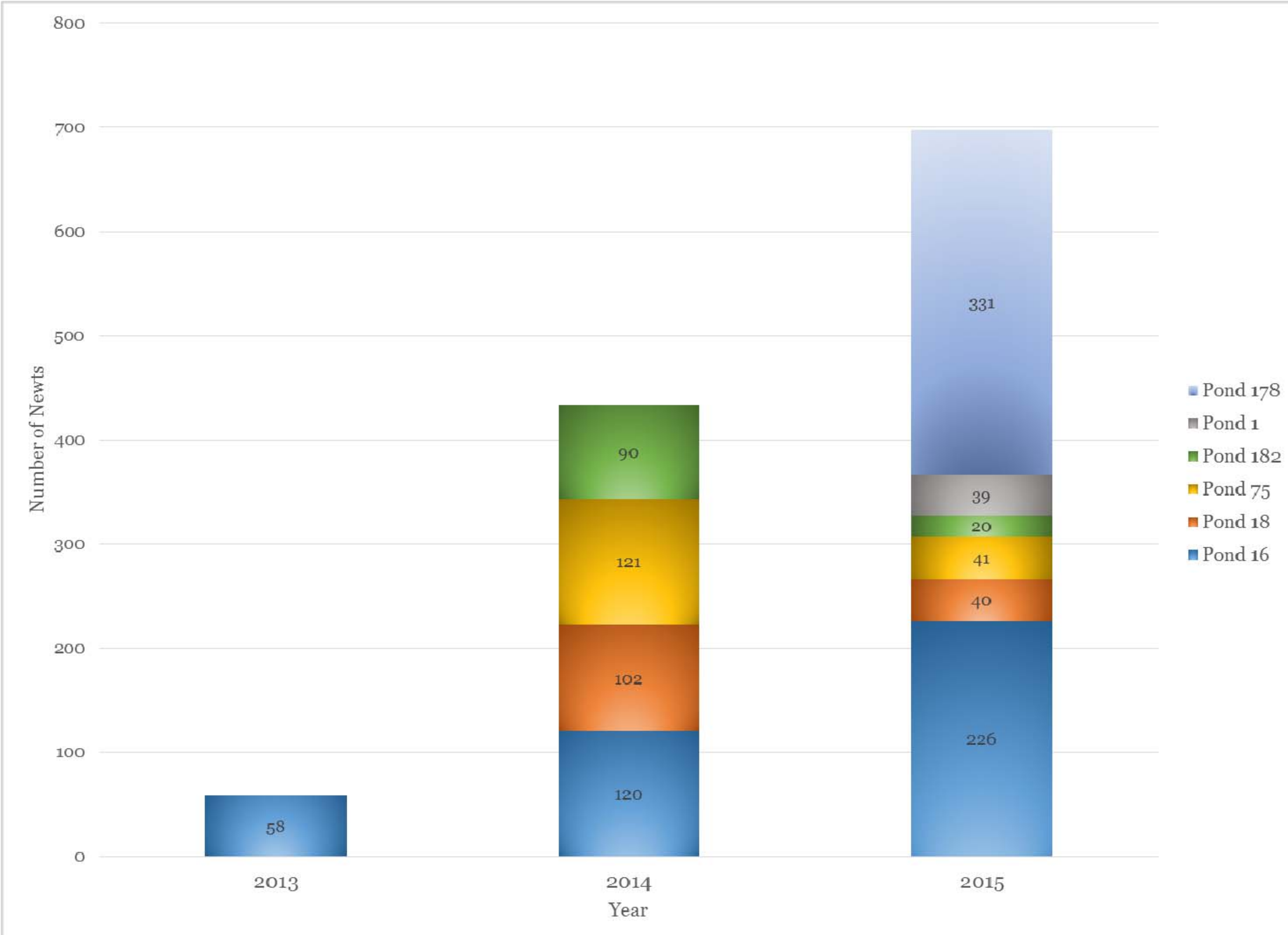

Figure 11. Number of larval and adult striped newts released into each of six ponds during the three-year release period (2013-2015). The total number of released individuals was 58 in 2013, 433 in 2014, and 697 in 2015.

Some time after repatriation of newts, the animals metamorphose and exit a given recipient wetland. Our recipient wetlands are ephemeral, and typically go dry at least once per year, so we do not expect any of our released newts to remain in wetlands indefinitely as paedomorphs. During the three-year repatriation period, we released 818 individuals into the four drift-fenced wetlands, and captured 54 emigrating efts/adults (Figure 12). Total measured percent metamorphic yield thus far summed for the entire project at the four drift fenced recipient ponds was 6.6%.

Throughout the three-year history of repatriations, we have measured percent yields anywhere from zero to 37 %, depending on the wetland and the life stage (adult versus larvae) repatriated. When we combine larvae and adult repatriation numbers for each wetland, metamorphic yields have ranged from zero to 30% (Figure 13). Percent metamorphic yields from Pond 18 and 182 were consistently the highest, and both increased from last year to this year. Percent yields for Pond 16 are at or below 5 % for each of the past three years. Pond 75 exhibited the lowest measured percent yields, presumably because the drift fence here has been the most impacted by frequent flooding. We expect that Pond 18 has the best likelihood to see more returners in the near future, since the measured number of recruits of both efts and adults there was the highest.

We stress that our metamorphic yield percentages were likely underestimates of actual numbers of emigrating/recruiting newts. Drift fence flooding and the timing of such flooding affected our results at every wetland, more or less, for each of our repatriation years thus far. Our strongest percent yields from Ponds 18 and 182, the two least impacted by flooding, may be the result of lesser fence flooding. However, we suspect that high herbaceous plant diversity at these two wetlands also played some kind of unquantified role in generating the higher percent yields. Drift fence trespass also may have reduced our ability to detect newts leaving the wetlands. We documented one trespassing newt who climbed over the fence this year at Pond 16, where fence corrosion was the worst. A rusty fence surface may be easier for newts to climb.

We believe that percent yield results for the study thus far represent success when viewed within an r-selective ecological framework, where parents produce large numbers of offspring in order to overcome many environmental obstacles (e.g. predation) before reaching sexual maturity and reproducing. It is noteworthy that all 1188 released newts have thus far been the offspring of just 31 captive parents in zoos, while just six of the parents produced the majority of the newts released. With 54 post-metamorphic newts observed exiting four of the six repatration wetlands thus far for the project and the likelihood that more recruits have exited while fences were flooded, it is possible that our results could be typical for this kind of study. There are, however, no similar newt studies from North America that can serve as baseline for comparison.

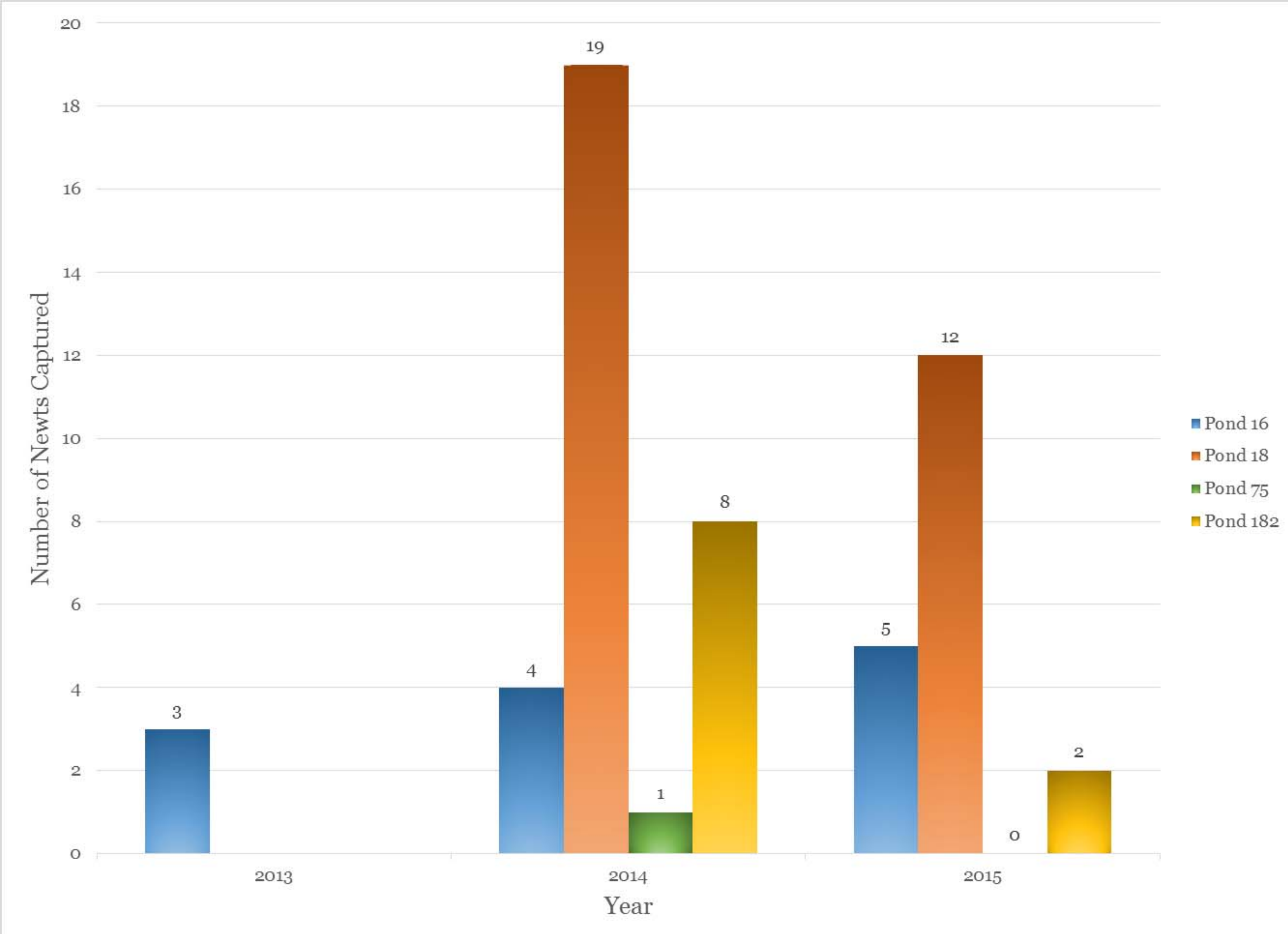

Figure 12. Number of striped newts captured leaving each of the four drift-fenced ponds during the three-year release period. We released 818 indivduals (adult and larvae) into these four wetlands during this period.

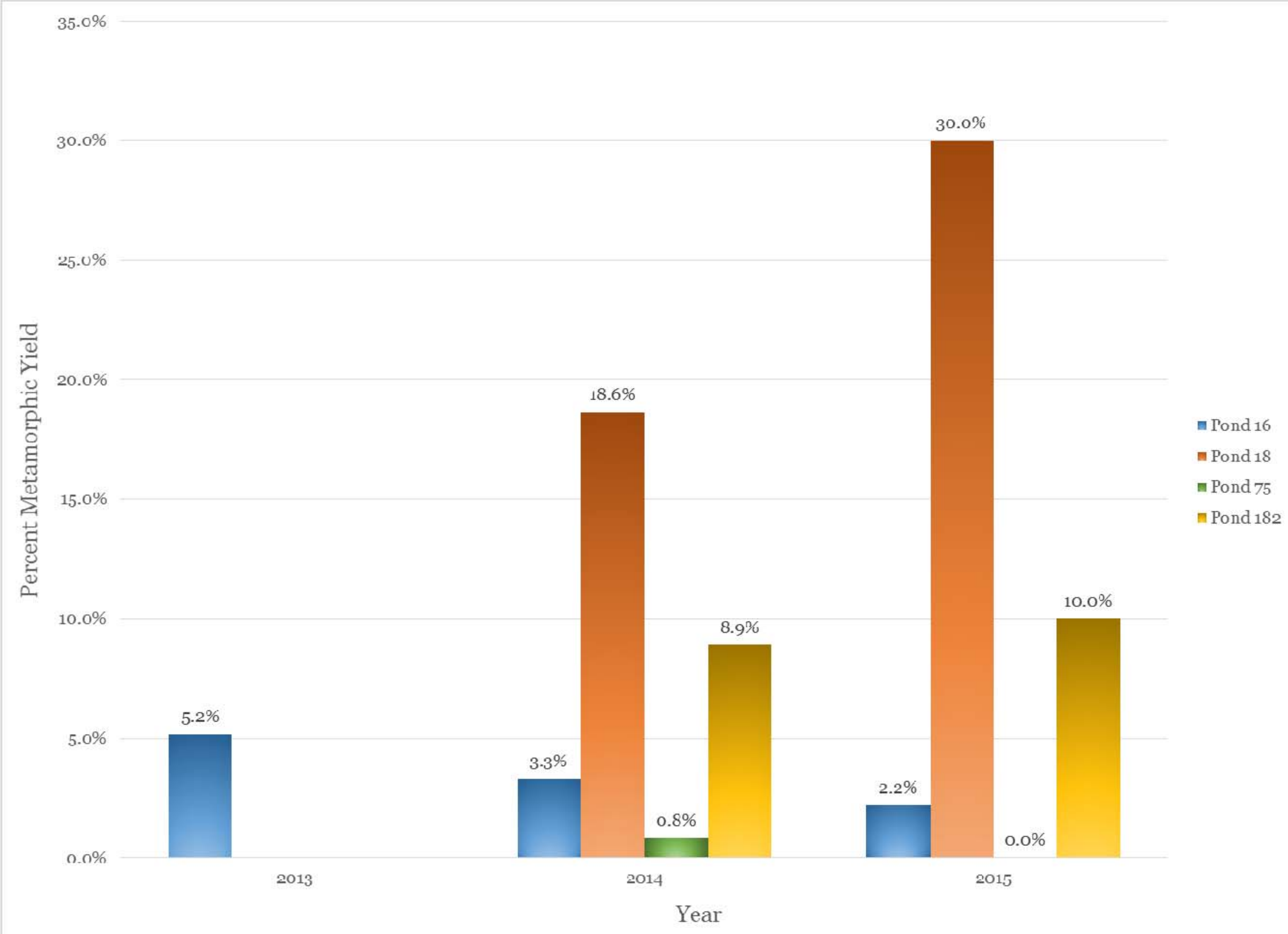

Figure 13. Overall percent metamorphic yield per recipient wetland per year. Metamorphic yield is the percentage of repatriated newts (larvae and aquatic adults) we captured exiting a given wetland by drift fence traps. We did not release adults in 2013 and 2014; metamorphic yield during those years resulted from larval releases only.

Infectious Disease Investigations

We continued to investigate whether some kind of pathogen is the cause for the mysterious striped newt decline in the ANF, and whether it may still be affecting our released individuals. Although prior results indicated that *Ranaviruses* could significantly impact upland recruitment of striped newts by affecting survival during the juvenile stage, *Ranavirus* was not highly prevalent within our study sites, at least at the time of sampling (Mean et al. 2013). 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).

This year (2015), we began investigation of whether chytrid fungus could be playing a role in the decline of ANF striped newts and/or whether repatriated newts may be suffering from infection. We observed conspicuous “sores” or “skin spots” on a few of our newts after metamorphosis. These sore spots have put us in a heightened state of investigative awareness. Spots could be a result of many things, including disease, predator attack, or environmental stress from extreme heat or cold.

We swab sampled 23 post-repatriated newts encountered either in drift fences or during dipnet sampling. Out of the 23 striped newts swabbed, six (26%) possessed visually noticeable sores, wounds, or spots (Figure 14). We currently await laboratory anaylisis of our swab samples and expect to have some results next year as to whether chytrid is present and/or prevalent in our study region.

Figure 14. Open sore, lesion, or wound on the back of one of our emergent terrestrial adults in 2015. This animal was swab sampled for potential disease. Individual ID: P-18 Emergent Terrestrial Adult #3 (swab 006).

On 17 May, at Pond 18, one of this year's outgoing, newly-metamorphosed terrestrial adults (TA#8(009) P18) died in hand after swabbing and photography. It was a male who had been released two months prior as an aquatic adult. The newt had a conspicuous dark spot dorsally and centrally along the left costal region (Figure 15). We preserved the individual in 95% EtOH and sent it to Drs. Gray and Miller for disease investigation. We are awaiting results.

Figure 15. An emergent terrestrial adult who died in hand after swabbing and photography. There was a conspicuous shiny black spot on the right costal side of the animal. ID: TA #8 (swab 009) P-18.

After seeing several newts with skin spots or sores over the course of the season, R. Means took an afflicted newt home to observe it for a period of time. One of the year's outgoing terrestrial adults from Pond 18 (TA#10(011) P18), with an open sore on the back of the neck, was taken home and cared for in a container lined with natural substrate. R. Means observed the newt for five days. Food and water always were available. The open sore healed significantly; however, the animal did not eat during the entire period. It is possible that the shock of transport and captivity played a factor in the lack of appetite. Although R. Means did not weight the animal before or after, it lost some body weight. Not being an expert in caring for a terrestrial striped newt, R. Means released it back at Pond 18 to avoid malnourishment. Photos were taken on the day of capture and 5 days later to show significant healing of the neck sore. On the day of capture, the sore was open, circular, and moist. Five days later, the sore was dry and had shrunk by an estimated 20-30% (Figure 16).

Figure 16. Emergent striped newt terrestrial adult (ID: TA #10 (swab 011) P-18) taken into captivity for five days for observation. There was a large open, circular, moist sore on the back of the neck the day of capture (left photo). The sore had dried over and healed somewhat by observation day five (right photo). The animal was released immediately after the right side photo was taken.

If the sore had remained in place during observation, and the animal either died or did not improve, then such an observation could be consistent with potential disease. If the sore healed during observation, then this result could be consistent with fire ant or other predatory attack. The sore, did, in fact, heal somewhat, but because the newt had to be released early, the cause of the sore remains uncertain. We must await next year's swab results in order to generate more data on this individual. Next year, more such take home observations should be done in order to increase sample sizes and generate more observational data that potentially could help us determine the origin of spots or sores that have been seen on some of our post-repatriated newts.

With the current data we have at this time regarding pathogen investigations, we believe that repatriations must proceed, although cautiously. Disease swabbing also must continue.

Fire Ant Predation on a Striped Newt

On 3 April, local biologist S. Davis of the St. Marks National Wildlife Refuge discovered a striped newt covered in fire ants near the edge of recipient Pond 18. S. Davis picked up the newt and brushed off all the fire ants. The newt was still alive. S. Davis delievered the injured newt to a colleague, P. Hill, who cared for the newt and attempted to nurse it back to health. The newt evenutally died after one week from approximately 20 serious open wounds all over the body (Figure 17). The ant-attacked newt was preserved in 95% EtOH.

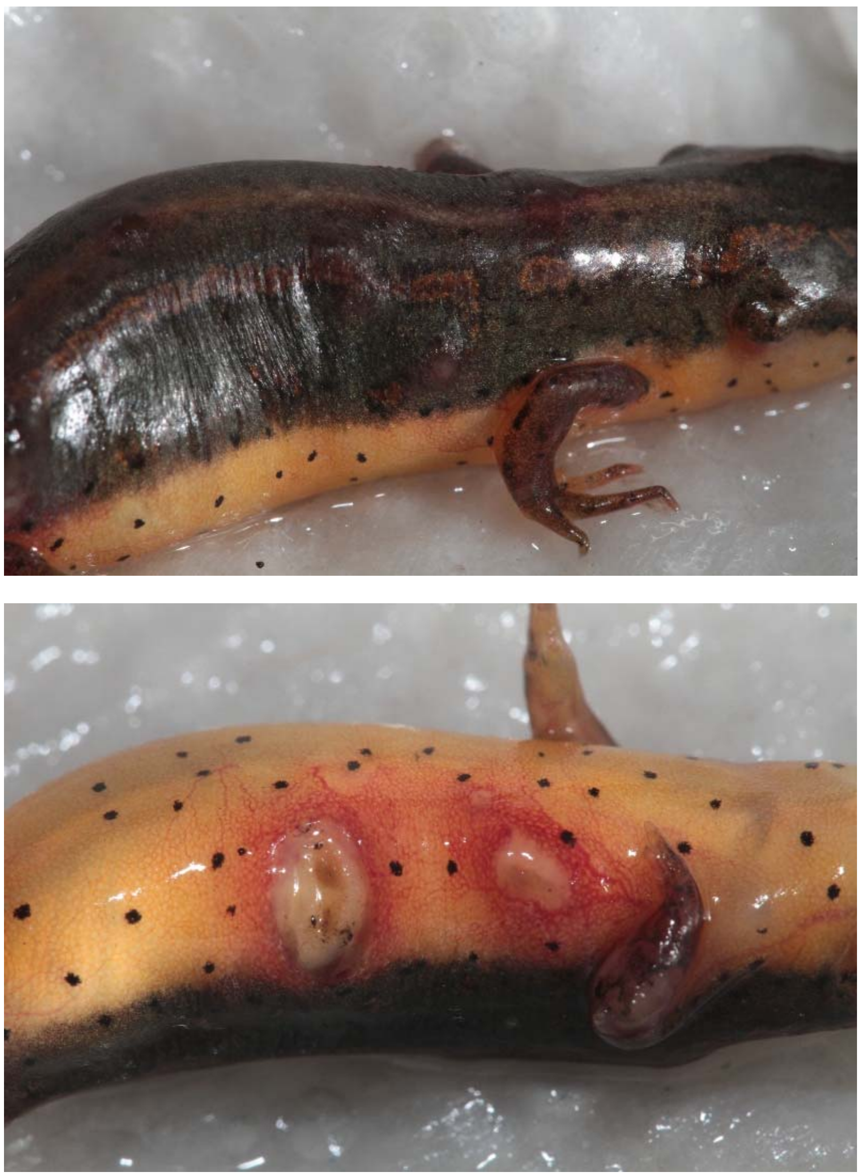

Figure 17. Emergent striped newt terrestrial adult attacked by fire ants at Pond 18. Open sores and skin blisters were present all over the body. The animal was cared for by P. Hill, however, it died after one week. Photos show fire ant attack wounds all over the body. Photos taken by P. Hill.

After the observation of the fire ant attacked newt, R. Means observed fire ant “gauntlets” around the periphery of drying recipient wetlands. They were a common occurrence, especially in the spring and early summer during water level draw-down. Within the first foot of perimeter around Pond 18’s edge, we observed thousands of patrolling fire ants that could attack anything that emerged from the water. Also, after a big rain and subsequent water level rise at Pond 182, we observed a large raft of fire ants floating and clinging to an emergent broomsedge clump (Figure 18). Fire ants are extremely abundant and well-documented from all recipient wetlands. Striped newts do not move rapidly, and may more likely succumb to ant predation than, for example, saltatory frogs. It is uncertain whether the striped newt may produce noxious skin secretions that are unpalatable for fire ants. At least the one ant-attacked striped newt observed this year was unable to avoid fire ant attack.

Figure 18. Fire ant raft observed this year at Pond 182 after a heavy rain filled wetland basin.

As far as we are aware, this is the first unequivocal observation of direct fire ant predation on a striped newt. The open wounds on the ant victim resembled some of the sores or "lesions" encountered on some of this year's newts observed in the field and swabbed. It is possible that fire ant attacks cause some (or even all) sore spots on this year's newts instead of resulting from potential infectious pathogen. We must await results from this year’s swabbing efforts in order to determine the role potentially played by pathogen versus fire ants. This fire ant/newt observation certainly now brings into discussion the possibility that fire ants may have played at least a minor role in causing the mysterious extirpation of the western striped newt in the ANF. Clearly, more research into fire ant predation on the striped newt is needed.

Additional Information

Western Striped Newt Status Update:

In early May 2015, K. Sash (Tall Timbers Research Station) and P. Hill (FWC) led a group on a weekend herpetology field trip to Dixie Plantation in Jefferson County, FL. Early in the trip, the two biologists captured six tiny larval newts that were too small to identify unequivocally. P. Hill raised one at home. Two and half months later, P. Hill confirmed that the tiny newt larva had grown into a striped newt. This is a new site record for the western striped newt.

At the initiation of this study, there was only one breeding wetland known globally to annually produce western striped newts, located within the Fall Line Sandhills Natural Area of SW Georgia (J. Jensen, pers. comm.). This single wetland was the source of all individuals produced in captivity for our repatriation project. It continues to produce western striped newts at relatively low concentrations. The striped newt observation in Jefferson County, FL indicates that more extensive sampling may be needed to ascertain the true global status of wild western striped newts. It also highlights the need to continue a regular sampling effort within the ANF.

Vandalism

Illegal motorized vehicular activity continued to plague the striped newt repatriation project intermittently at nearly all study wetlands. The worst activity occurred at Pond 18, where someone drove a truck through the wetland, creating deep ruts in the wetland interior (Figure 19). This destruction also damaged the liner in two places. We expect to repair the liner when dry conditions permit. It is very unfortunate that Pond 18, thus far our most successful repatriation wetland, also has experienced the worst vehicular damage. Other vandalism included tampering with the drift fence and traps (and suspected removal of animals) at Pond 182 for several days.

Figure 19. Vandalism at Pond 18, with 4x4 truck ruts throughout the wetland basin. Spinning tires created deep ruts and penetrated to the rubber liner, and punctured the liner in two places.

Social media

The striped newt project now can be followed on Twitter (@stripednewts). Important project events or daily natural history notes are posted. Coastal Plains Institute has also createad a Facebook page (https://www.facebook.com/coastalplainsinstitute/) where updates and photos will be published.

YEAR 6 EXPECTATIONS

We are actively pursuing additional funding to continue this study in full operation for the next three years (2016-2018).

In 2016, we plan to continue full-scale repatriations in all six recipient wetlands. Identical to this year, we will release adults in winter and larvae in the middle and late springtime. We expect to release in excess of 1000 newts in next year's repatriation efforts. We also have at least two additional zoos joining in our striped newt husbandry efforts for the upcoming project year: Central Florida Zoo (Orlando, FL) and Lowry Park Zoo (Tampa, FL). Our likelihood for long-term repatriation success will increase with this continued upward trend of animals available for release.

Strong El Nino conditions had developed in the eastern Pacific Ocean by October 2015, and are expected to strengthen throughout the coming wintertime 2015/2016. El Nino refers to the periodic warming of the eastern equatorial Pacific Ocean that brings sea surface temperatures above average. El Nino conditions, which can last 1-2 years, develop concurrently with atmospheric changes leading to a variety of global effects including cooler than average temperatures and wetter than average precipitation for the southern United States. Forecasters predicted the current El Nino to be one of, if not the strongest, ever on record. We expect that winter rains will be heavy and frequent, and our study wetlands will become flooded. This is good news for repatriations and longevity of hydroperiods necessary for metamorphosis.

We will reinstall all drift fences in December/January and we expect them to be operational through July 2016, pending secured funding for this project. We will move drift fences at Ponds 16 and 75, where the most frequent flooding of fences has occurred, to significantly higher ground to avoid flooding from the upcoming El Nino and beyond.

We will resample the 19 historical ANF striped newt wetlands in winter and spring 2016 in order to continue to sample for the possibility that wild striped newts could still exist in the Munson Sandhills. Even though we believe the striped newt likely is extirpated in the ANF, it is still very important to continue a sampling presence to add vital data concerning the status of the striped newt in the ANF.

We expect to welcome aboard biologist P. Hill, of FWC, in 2016. P. Hill will coordinate a mark-recapture aspect to our study. We expect mark-recapture to enhance our ability to interpret the life history, survivorship, movement patterns, and better measure repatriation success. All striped newts we release in 2016 will be uniquely marked using a combination of Visible Implant Elastomer (VIE) tags (Northwest Marine Technology, Inc.). VIEs consist of an inert flexible plastic that is injected beneath the skin and fluoresces when shined with an ultra-violet flashlight. This method has proven to be extremely effective for mark-recapture in small ectotherms and has been used successfully in several studies of salamanders (Jung et al. 2000, Phillips and Fries 2009, Pretlaw et al. 2002). P. Hill has four years of experience using VIEs in prior studies.

We expect to have some results in 2016 from our chytrid fungus swab sampling efforts that took place in 2015. We also will continue to swab post-repatriated striped newts as we encounter them in the field during 2016, in order to increase our swab sample sizes.

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APPENDIX A. Hydroperiod of Recipient Wetlands and Reference Wetlands

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. 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 during dry spells. This drying is desirable as it preserves the ephemeral nature of these wetlands.

	Pond 16 (recipient)	**Pond 15**		**Pond 18 (recipient)**	**Pond 17**		**Pond 75 (recipient)**	**Pond 73**		**Pond 182 (recipient)**	**Pond 182a**
	No Liner	No Liner		Liner	No Liner		Liner	No Liner		Liner	No Liner
Oct 2014											
Nov 2014											
Dec 2014											
Jan 2015											
Feb 2015											
Mar 2015											
Apr 2015											
May 2015											
Jun 2015											
Jul 2015											
Aug 2015											
Sep 2015											