A Conservation Strategy for the Imperiled Western Striped Newt in the Apalachicola National Forest, FL

Seventh Annual Report

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EXECUTIVE SUMMARY

This report summarizes work conducted in Year 7 (January 2017-August 2017) of the striped newt repatriation project within the Apalachicola National Forest (ANF).

A total of 110 western striped newt larvae and adults were released into two recipient wetlands during the 2017 project year. This brings the total number of western striped newts released in this project to 1,437. This year's release numbers were significantly lower than in prior years (139 in 2016, 697 in 2015, 433 in 2014).

Captive newt colonies suffered population crashes at Jacksonville Zoo and Gardens and Memphis Zoo. Testing indicated the presence of a mycobacteria that may have been the cause of captive colony declines. We put crashed colonies on lockdown, and zoo personnel worked tirelessly to clean their systems and restart their colonies. New wild striped newts from the Georgia site, Apalachicola National Forest, and Dixie Plantation were supplied to Jacksonville Zoo and Gardens to help augment parental stocks and increase genetic diversity of captive colonies.

As a result of suffering population crashes at our two most prolific and experienced zoological institutions, we worked to expand our captive rearing efforts in order to offset such potential crashes in the future. We welcomed aboard the striped newt project's captive rearing efforts the following institutions: Orianne Center for Indigo Conservation at the Central Florida Zoo, The Amphibian Foundation, and the National Amphibian Conservation Center at Detroit Zoo. Each institution was supplied with wild striped newts to become parental founders for captive colonies.

Despite declined number of releases, two very significant study benchmarks were reached this year: 1) Pond 18 continued to harbor aquatic, paedomorphic striped newts into a second calendar year. Paedomorphs were the offspring (F1 generation) of aquatic captive-raised adults that were released in January 2016. 2) The F1 paedomorphs produced a second generation (F2) of larvae which were observed by dipnet in April and July 2017. The F2 larvae, therefore, were the grandchildren of aforementioned aquatic adults released back in January 2016. Such results indicated that a persistent, multi-generational, striped newt population, created by our repatriation efforts, continued to be detectable at one of our recipient ponds for 1.5 years.

On 24 March 2017, during a drought, we conducted an emergency water augmentation at Pond 18 to prevent it from drying in order to preserve the ability of F1 striped newt paedomorphs to remain

in the pond and breed. We delivered 4,000 gallons of water from a carefully selected, nearby pond into Pond 18 using a water pump and water tank on a trailer. Augmentation raised water level by nearly a foot, and greatly expanded the diameter of the pond to liner capacity. Pond 18 resisted drying because of our water input. Detection of tiny larvae in July indicated that striped newts were able to breed successfully. Incidentally, augmentation also saved over 300 gopher frog larvae, which metamorphosed and exited the pond. Our experimental augmentation was considered a success.

Newt marking of all repatriated striped newt larvae and adults continued using Visual Implant Elastomer (VIE). We also sampled regularly to determine detectability of striped newts at different times and wetlands post-release. Detectability levels were very low, usually at or below 10%.

There are three presently known sites that harbor small populations of wild western striped newts. Pond 37 continues to support the only known wild western striped newts within the Apalachicola National Forest. Dixie Plantation, FL, part of Tall Timbers Research Station and Land Conservancy, continues to support wild western striped newts in at least two ponds. Wild western striped newts also continue to be observed annually in a single pond within the Fall Line Sandhills of Georgia.

Past data suggest that annual recruitment and returning breeding adult success are directly proportional to the numbers of individuals released per year. In the near future, when our new zoos get their breeding colonies established, we will be capable of producing 1000s of captive striped newts to be released into the ANF in a given year. We believe that the continued release of greater and greater numbers of larval and adult striped newts into the prime habitat of the ANF eventually will lead to project success. As new scientific data are generated by our efforts, our study continually adapts to meet the conservation needs of the western striped newt within the ANF.

ACKNOWLEDGMENTS

We would like to thank John Dunlap, Jeff Gainey, and Marcus Beard of the U.S. Forest Service and the Apalachicola National Forest (ANF) for continued permission to conduct this worthwhile conservation project on National Forest lands. We also would like to remind readers that nowretired ANF biologist, Chuck Hess, provided much of the original vision for this project.

We greatly thank Tall Timbers Research Station and Land Conservancy for their assistance and partnership. A huge thank you to Eric Staller, Andrew Chase and Kim Sash for invaluable assistance while conducting an emergency wetlands augmentation. Tall Timbers also allowed us to acquire wild western striped newts from Dixie Plantation to be used as founders for a captive colony.

Numerous people have provided volunteer assistance with drift fence checking efforts, including Ashley Prow, Nancy Thomas, Peter Kleinhenz, and Stephanie Barnes. We would like to give special recognition to Florida State University doctoral candidate, Lydia Eldredge, for her outstanding volunteer efforts to the striped newt project in 2017. Lydia spent an exceptional amount of time assisting in the field with every aspect of the striped newt project including constructing and checking drift fences, dipnetting, and managing scientific data.

We would like to extend a very special thank you to agencies and entities that have provided funding support and support to the striped newt project for its continuation beyond the original 5year funding period, including: Disney Conservation Fund, Felburn Foundation, Florida Fish and Wildlife Conservation Commission, and Jacksonville Zoo and Gardens.

We thank Dr. Brooke Talley, Amphibian and Reptile Conservation Coordinator, Florida Fish and Wildlife Conservation Commission, for her assistance and support of the striped newt repatriation project.

We would like to recognize the generous support from our zoo partners for maintaining the striped newt assurance colonies and transporting the newts for release this year: Jacksonville Zoo and Gardens, Orianne Center for Indigo Conservation. We greatly thank Georgia Department of Natural Resources (DNR) and John Jensen, senior biologist with the Georgia DNR. Without their cooperation and continued allowance of Georgiasourced wild striped newts to become captive colony founders, this conservation project likely would not be possible!

CONTENTS

| EXECUTIVE SUMMARYii |
|--|
| ACKNOWLEDGMENTSi |
| INTRODUCTION1 |
| STUDY AREA |
| METHODS |
| Hydrology and Ecology of Repatriation/Liner Wetlands9 |
| Striped Newt Assurance Colonies13 |
| Mark-Recapture and Detectability Study14 |
| RESULTS AND DISCUSSION |
| Hydrology and Ecology of Repatriation/Liner Wetlands16 |
| Striped Newt Assurance Colonies19 |
| Mark-Recapture and Detectability Study26 |
| CONCLUSIONS |
| YEAR 8 EXPECTATIONS |
| LITERATURE CITED |

INTRODUCTION

The striped newt (*Notophthalmus perstriatus*) is a small to medium-sized salamander in the family Salamandridae. It is endemic to the Coastal Plain of the southeastern United States. The known global distribution of the striped newt is small and restricted to parts of southern Georgia, the northern half of the Florida peninsula, and the eastern Florida Panhandle (Conant and Collins 1998, Amphibiaweb 2019). Its preferred habitat consists of xeric longleaf pine or scrubby upland ecosystems with an abundance of fishless ephemeral wetlands available for breeding.

Genetic evidence suggests there are two distinct 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 Apalachicola National Forest (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 (Farmer et al. 2017).

According to their genetic analysis, May et al. (2011) propounded that the western and eastern striped newt may represent "distinct population segments" within the species. However, Farmer et al. (2017) suggested that the western and eastern striped newt populations represent "evolutionary significant units." Regardless of what researchers call each group, all agree that genetic divergence between the western and eastern populations is enough to warrant some form of recognition. In this study, we refer to each group as either the "western" or "eastern" striped newt.

Before striped newt repatriations began in the ANF, numerous surveys thoroughly documented the occurrence and distribution of the striped newt in Florida and Georgia (Dodd and LaClaire 1995, Enge 2011, Franz and Smith 1999, Jensen and Klaus 2004, Johnson and Owen 2005, Means and Means 2005, Means 2007). These surveys indicated that the striped newt was rare globally and reliably found in less than 100 ephemeral wetlands, primarily within the eastern group. Striped newt ponds were scattered and distributed primarily within several public conservation lands that were all isolated from one another by agriculture, roads, and development.

The ANF historically was the geographic stronghold for the western striped newt. Western striped newts once had been observed within 20 ephemeral ponds in the ANF (Means 2007, Means and

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Means 1998, Means and Means 2005, Means et al. 1994). However, the ANF population sharply declined in the late 1990's and 2000's enigmatically while all other local pond-breeding amphibian species appeared to remain in relative abundance (Means et al. 2008, Means et al. 2011, Means et al. 2012). Coastal Plains Institute (CPI) researchers believed the ANF decline likely was linked primarily to severe drought from 1999-2003, followed by past altered fire regime, and habitat fragmentation. We also speculated that disease may have been a potential decline factor, although no disease outbreak had ever been documented.

In 2004, the striped newt was added to the IUCN Red List as being "Near Threatened." In 2008, Coastal Plains Institute (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 (USFWS 2011). However, this action was precluded by higher priority listings. It has remained in "warranted but precluded" status ever since.

By 2011, based on many sampling events throughout the range of the western striped newt, many qualified biologists believed that the western striped newt could only be found reliably at a single remaining pond in the world (Enge 2011, J. Jensen, personal communication, Means et al. 2008). In 2011, as a proactive response to the recognized severe imperilment of the western striped newt in the ANF and globally, ANF, CPI, and the Georgia Department of Natural Resources (GaDNR) entered into a partnership to implement a conservation strategy for the western striped newt within its historical stronghold. The conservation strategy would include repatriations if necessary.

Before any striped newt repatriations were made in the ANF, several actions were taken step-wise in 2011 and 2012 prior to releases because we desired to be as certain as possible that repatriations would be necessary. First, four more exhaustive surveys were conducted in the ANF before moving ahead with repatriations. Next, captive assurance colonies were created as soon as possible using wild western striped newts from the closest known location and genetic lineage to the ANF. The GaDNR assisted us in acquiring an initial batch of striped newts from the Georgia western striped newt location. These newts were sent to our zoo partners to become initial founders of assurance colonies. Third, we carefully selected four initial prospective ephemeral wetlands to become future repatriation wetlands. Fourth, the cause of decline was investigated. Since drought was suspected to have been a major decline factor, we created a method to extend shortened hydroperiods of breeding ponds to ameliorate drought conditions. We installed synthetic rubber liners underneath interior portions of three drought-stricken, pre-selected repatriation ponds to extend hydroperiods so that any released larval newts would have a better chance to reach metamorphosis (Means et al. 2012). Finally, after all prior actions were taken, and results supported repatriations, F1 progeny from the wild-caught Georgia striped newts would be released into ANF wetlands (Means et al. 2013).

The four surveys were undertaken within 200 ephemeral wetlands scattered across the seemingly prime historical ANF striped newt habitat. All surveys failed to turn up any striped newts. At that time, no striped newts had been found in the ANF since finding three adults in two wetlands in 2007. In addition, zero larvae had been observed since 1998. CPI researchers concluded that the western striped newt likely had become extirpated (Means et al. 2012). Based on all available data, researchers concluded that repatriations were warranted as the next step to take in our western striped newt conservation strategy (Means et al. 2012).

In 2013 CPI began western striped newt repatriations within the historical habitat of the ANF. Since then, we have partnered with a plethora of agencies and have released 1,437 newts into six repatriation wetlands.

Disease testing results for *Ranavirus*, *Batrachochytrium dendrobatidis* (Bd) and *Batrachochytrium salamandrivorans* (Bsal) have thus far shown no conclusive indication that any of these three pathogens were the cause of decline. However, disease results are preliminary and ongoing, and we continue to investigate disease as a possible cause of decline. We hope that continued disease testing of newts will provide conclusive information about the extent that disease may or may not be a contributor to the striped newt decline.

In 2015 and 2016, major discoveries of two new wild western striped newt populations were made. A new population consisting of two proximal ponds was discovered on Dixie Plantation by FWC and Tall Timbers Research Station (TTRS) colleagues (Hill and Sash 2015). A small hold-out of striped newts was discovered at Pond 37 within the ANF by CPI and Florida Fish and Wildlife Conservation Commission (FWC) researchers (Means et al. 2016). Incidentally, the ANF newts were found in the same pond where they were last observed by CPI in 2007. The number of adults and larvae documented at Pond 37, however, were extremely low and could not be considered a healthy population (three adults in April, two larvae and one adult in May). These new discoveries brought the total known number of reliable locations for the western striped newt up to three. Not only were these discoveries good for wild newts, but they presented additional sourcing options for assurance colonies. Almost immediately, project researchers and partners seized opportunities to acquire wild western striped newts from the two additional sites to boost genetic diversity at repatriation sites and create additional captive assurance colonies of newts from each site.

In response to the finding of this hold-out population of wild western striped newts, the Florida Natural Areas Inventory (FNAI) was contracted to sample the Munson Sandhills striped newt habitat in Spring 2017 (FNAI 2017). FNAI sampled the same pond set that CPI has sampled repeatedly throughout the last 20 years. Zero striped newts were found. These data reconfirm that wild striped newts remain exceedingly rare in the Munson Sandhills and reconfirm that our repatriation efforts continue to be warranted. Incidentally, the survey found no striped newts at Pond 37 where striped newts were rediscovered in the Fall of 2016 (FNAI 2017). This result likely reflects low detectability of striped newts in the wild, especially in large ponds with very small populations.

A recent analysis of striped newt surveys and distribution suggested that the species faces significant and ongoing threats (Farmer et al. 2017). This assessment supports the determination by the U.S. Fish and Wildlife Service that the striped newt warranted federal protection as a threatened species under guidelines of the Endangered Species Act (ESA) of 1973. The striped newt has remained a federal candidate species for listing since 2011. We expect the USFWS list the striped newt as federally "threatened."

The federal listing process for imperiled species under guidelines of the ESA can take a decade or more. In this length of time, imperiled species that are candidates for listing can go extirpated or extinct. We believe that conservation action can be done proactively before and during lengthy USFWS imperiled species listing processes.

The goal of this proactive conservation study is to create a self-sustaining, viable metapopulation of western striped newts within their former western geographic stronghold. We hope to eventually replenish the western striped newt in the ANF back to at least the level observed before decline. Therefore, we define ultimate study success as that point in time when monitoring data show that striped newts are established at 20 ANF breeding ponds, including ANF Pond 37. Our

methodology aligns closely with accepted IUCN guidelines for repatriation and translocation studies (IUCN 2013).

Several repatriation success benchmarks have thus far been reached in our study. Success benchmarks include: release of nearly 1,500 captive reared striped newts into the ANF, documentation of terrestrial recruitment after aquatic release events, documentation of breeding within one repatriation wetland, and documentation of returning, breeding-ready terrestrial adults back to multiple release ponds.

Whereas we have experienced some success benchmarks, most have occurred at only one pond. Although we believe that this study will reach ultimate success, it is still much too early to claim complete success at any pond just yet. It remains imperative that we continue our repatriation and monitoring efforts.

This conservation study already has generated new and useful management techniques for husbandry, repatriation, and habitat enhancements required for similar conservation efforts. We hope that this study may serve as a blueprint for the conservation of similarly imperiled amphibian species.

This report summarizes work conducted in Year 7 (January 2017-August 2017) of the striped newt repatriation project. All prior study results thus far have been reported annually from 2011-2016 within Striped Newt Repatriation Project reports (see Means et al. 2011, 2012, 2013, 2014, 2015, 2016). Reports can be viewed or downloaded from www.coastalplains.org.

STUDY OBJECTIVES

1) Assess potential cause of decline of the western striped newt with emphasis on disease, fire ants, and increasingly frequent droughts in the climate change era.

2) Continue collaboration with zoos to maintain captive assurance colonies for conservation and repatriation needs.

3) Continue repatriation of the western striped newt back into its former western geographic stronghold (ANF).

4) Utilize and maintain liners to enhance repatriation sites to ensure sufficient pond hydroperiod throughout critical larval metamorphosis period.

5) Monitor repatriation success with the use of drift fencing and dipnet sampling.

6) Continue surveillance and monitoring to detect more possible future occurrence of wild western striped newts throughout our study region.

7) Determine survival, recapture, and movement rates among striped newts of different life stages/ages and release sites to evaluate the effectiveness of repatriation as a conservation strategy for striped newts.

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 at least 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-plus years. The wetlands provide breeding habitat for over 20 amphibian species, including the western striped newt, our current study focus.



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.

Over the past five years, we have focused repatriation efforts in six wetlands within the MS (Figure 2). We monitor four of these wetlands (16, 18, 75, 182) with encircling drift fences, the other two wetlands are not fenced and serve as expansion sites. As described in detail in Means et al. (2012), we installed EPDM rubber liners under three of the six repatriation wetlands as a technique to boost recipient pond hydroperiods and make them more drought resistant, particularly during larval repatriation periods.



Figure 2. Detailed map depicting location of the six striped newt repatriation wetlands. The four wetlands with drift fences (016, 018, 075, and 182) are original repatriation ponds. We installed liners under three of these wetlands (18, 75, and 182) during the 2nd year of this project (2012). We expanded repatriation efforts into Ponds 001 and 178 in 2015.

METHODS

Hydrology and Ecology of Repatriation/Liner Wetlands

For almost two decades prior to our study, frequent droughts occurred in our region and historical striped newt ponds often were dry (Means et al. 2008; R. Means, unpublished data). During the inception of our study, we decided to implement a strategy to enhance our future newt recipient ponds such that drying from future dry spells would be mitigated. We hypothesized that adding rubber liners underneath central portions of our future newt recipient ponds might lengthen hydroperiods and create pool refugia to aid in the successful development of our future repatriated newt larvae.

We installed synthetic rubber EPDM pond liners (12m diam.) underneath the central portions of three wetlands targeted for repatriation (Ponds 18, 75, and 182) during Year 2 of this study (see Means et. al 2012 for detailed methodology). We closely observed the lined wetlands for two years prior to the first newt releases and for every study year afterwards.

Wetland Augmentation

In response to extremely dry conditions, on 24 March 2017 we conducted an emergency water augmentation to deliberately avoid drying of Pond 18. The goal of augmentation was to keep breeding conditions relatively stable at our most successful repatriation pond during a critical time early in the process of establishing a viable breeding population. We wanted existing F1 striped newt paedomorphs to breed and potentially produce this study's first F2 generation.

Immediately prior to augmentation, we dipnet sampled both source and recipient ponds. Before adding supplemental water to Pond 18, we considered several factors and took several steps to eliminate unwanted adverse effects to the chemistry, quantity, or ecology of either the source or recipient wetland. Factors included selecting a nearby ephemeral wetland as an augmentation source that had similar water quality and ecology to Pond 18. It was also important that a source pond be of substantial size to ensure that our water extraction would not significantly draw down the source pond. Additionally, we made sure that our water transport and extraction machines

were not contaminated with any unnatural water sources, such as city water that might contain levels of chlorine, fluoride or other undesired pollutants.

We selected Pond 178 to be our augmentation source pond because it satisfied all our source pond qualification criteria. Pond 178 is located approximately 1 mile east of Pond 18 (Figure 2) and is one of our six striped newt recipient ponds. It possesses flora and fauna similar to Pond 18, however Pond 178 is many times larger in size and by volume.

Coastal Plains Institute reached out to TTRS and its fire personnel for assistance with water transport. Eric Staller and Andrew Chase of TTRS eagerly agreed to partner with us to accomplish this experimental conservation endeavor. They brought their water transport trailer, equipped with 1000-gallon water tank, pulled by heavy duty pickup truck. CPI rented a gasoline powered water pump equipped with extraction and delivery hoses utilizing suction to move water from source to tank to recipient as needed during augmentation.

Before water extraction from Pond 178, we set out a water level staff gauge near the extraction point. We hypothesized that our water extraction would not appreciably subtract from its volume. We tested this hypothesis during augmentation by monitoring and recording water level as measured on the staff gauge both before and after water extractions. Two hoses extended from the pump, one for water extraction, one for water delivery into the water tank (Figure 3). To accomplish extraction of water from source pond and simultaneous delivery of water into the water tank, one person set the extraction hose into the water of the source pond, while another hand held the delivery hose inside the opening of the tank. The pump was cranked, and water began flowing from source pond to tank due to suction created by the pump. The person holding the extraction hose in the pond paid careful attention to not suck up any vegetation, debris, or animal life by placing a dipnet over the hose opening. Dipnet mesh acted as a filter that strained out any undesired living organisms or unliving debris. When the tank became filled, the pump was turned off.



Figure 3. Water extraction from source Pond 178. A gas-powered suction pump removed water from the source pond and delivered it into a nearby 1000-gallon water tank.

We used a large pickup truck to pull the tank to Pond 18, and we parked it approximately 50 feet from Pond 18 edge (Figure 4). We operated the pump in the reverse direction, sucking water out of the tank and discharging the tank water into the recipient pond. Hoses were held in place as needed while the pump ran. Immediately before discharging water into Pond 18, we laid a flat piece of plywood, measuring about 1.5m x 1.5m, down along the pond's edge. We directed the water out of the delivery hose onto the flat piece of plywood (Figure 5). Plywood acted to shield the edge of the pond from the creation of a hydraulically blasted hole. We carried out this extraction/delivery process between source and recipient ponds four times. We augmented Pond 18 with as much water as was necessary to raise water level/volume to maximum liner capacity. We believed this amount would provide enough drought resistance such that the pond could last until summer monsoon season when thunderstorms typically maintain liner pools all summer.



Figure 4. A 1000-gallon water tank on a trailer. Pulled by a large 4x4 fire truck, this tank and trailer combination was our water transport medium from source pond to recipient pond.



Figure 5. Water delivery into recipient Pond 18. A gas-powered pump sucked water out of a nearby 1000-gallon tank and delivered it through a hose into our recipient pond. Water discharged through the hose onto a 1.5m x 1.5m flat plyboard. The plyboard acted to shield the pond edge from the formation of a hydraulically blasted out hole.

To make sure we didn't negatively impact the chemistry of either source or recipient wetland, we used a combination of YSI meter and pH strips to test various chemistry parameters of source and recipient ponds before and after augmentation including temperature, pH, specific conductivity, dissolved oxygen (DO), and salinity.

Striped Newt Assurance Colonies

We acquired striped newts for the zoo's captive assurance colonies by dipnetting known western striped newt breeding wetlands. We used a heavy duty dipnet with 3/16" mesh to sweep in submerged or emergent herbaceous vegetation where striped newts tend to concentrate. In 2017, we focused our efforts in three locations: Pond 37 in the ANF, Florida; Dixie Plantation, Florida; and Big Pond in the Fall Line Sandhills Natural Area, Georgia.

Newts from each source location are housed separately ex situ. Newts are housed in a combination of plastic and glass containers (Figure 6). Detailed description of assurance colony husbandry and maintenance methodology can be found in prior annual reports (Means et al. 2014, 2015, 2016) and in Mendyk and Beshel (2017).



Figure 6. Striped newt captive assurance colony at the Orianne Center for Indigo Conservation.

Striped Newt Repatriation and Monitoring

As mentioned above, striped newts for repatriation into ANF wetlands are sourced from zoological institutions. We typically release aquatic adults in winter and larvae in springtime or early summer during the natural seasons when they are known to be present at said life history phases.

We monitored three repatriation wetlands with dipnet (see Mark-Recapture Study section below) and drift fencing to measure repatriation success in 2017. Monitored release ponds this year were lined Ponds 18, 75, and 182. Unlined Pond 16 was not monitored or used as a release pond this year. We believe that Pond 16 has become unsuitable for striped newt population establishment. It has never gone completely dry during the entire course of the striped newt project. It is an annual producer of a large number of bullfrogs and other potential predators. We may elect to use it again as a newt recipient pond only after it potentially dries in the future.

Drift fences were re-activated at Ponds 18, 75, 182 in January of 2017. Drift fences were composed of 0.06m high galvanized metal flashing, and completely encircled all wetlands. We buried plastic, 7.5-liter buckets flush with the ground surface and taut against the drift fence on each side of the fence at an interval of approximately 7 m. We used small sponges to reduce potential for drowning of captured animals in hydrated buckets and to improve moisture retention if buckets dried completely. We operated drift fences and checked the traps daily until the end of field season in August 2017. Upon closing the drift fences, we removed sections of fencing and filled buckets with sand to prevent undesired captures. Fence sections and filled buckets remained in the field until the next year's field season.

Due to low water levels and the dipnet efforts of FNAI, we did not conduct seasonal dipnet monitoring of historical striped newt breeding wetlands and nearby, additional wetlands other than those associated with our repatriation efforts.

Mark-Recapture and Detectability Study

Before marking, we individually anaesthetized newts with a calibrated solution of MS-222 and uniquely marked using a combination of four Visible Implant Elastomer (VIE) tags (Northwest Marine Technology, Inc.). VIEs consisted of an inert flexible plastic that was injected beneath the skin and fluoresced when shined with an ultra-violet flashlight. Marking technique is thoroughly described in Means et al. (2016). We marked half of our larvae and all adult newts using two cohort marks that identified them to pond and year. The smallest larvae (e.g. below 20 mm SVL) were not marked because we suspected that marking stress might harm or kill them.

Following marking, we held newts overnight for observation and allowed them to recover from the procedure. We placed newts into a mixture of 50% natural recipient pond water and 50% transport water. This procedure allowed newts to acclimate to natural water conditions before next-day release.

To determine detectability of striped newts and fulfil monitoring objectives, we conducted frequent dipnet efforts in repatriation and select other wetlands. We conducted dipnet recaptures on the same day as we released a known number of marked newts into a given pond. We allowed newts to disperse to preferred habitats within the pond for six hours before attempting the first recapture. We conducted timed dipnet recaptures monthly through August at each repatriation pond after releases. We used a heavy duty dipnet with 0.5cm mesh, and conducted sampling for one personhour, or less, depending on the small size of some wetlands at the time of sampling. Sweep efforts were concentrated in submerged or emergent herbaceous vegetation where larvae tend to concentrate. Efforts were made to ensure that we did not dipnet too heavily a given recipient pond so as to not adversely affect striped newt repatriation success potential.

RESULTS AND DISCUSSION

Hydrology and Ecology of Repatriation/Liner Wetlands

At the inception of our conservation study, we needed to find a way to lengthen pond hydroperiods in a drought-stricken landscape for the benefit of future larval newt development. Past data strongly show that our pond liners have, indeed, significantly increased pond hydroperiods by several months up to a year or more (Means et. al 2013, 2014, 2015, 2016). Of equal importance, our lined ponds still go dry on occasion. We did not wish to create "permanent" water bodies that may become unsuitable for striped newt establishment. Data have shown that we have succeeded on both counts.

In 2017, water levels of all three monitored newt recipient ponds were generally very low. Water levels were maintained by the existence of installed pond liners until early springtime. In March and April, a combination of rising temperatures and less rainfall caused both Ponds 75 and 182 to go completely dry for a few days apiece. Pond 18 likely would have done so were it not for the water augmentation (see below). Water returned to both dried ponds after heavy rains pooled atop liners, and they remained hydrated only within liner capacity until the end of field season in early August. Nearby unlined, historically hydrologically similar reference ponds all remained dry the entire year. Had liners not existed within our active striped newt study ponds, they likely would have been dry all year. Current data also show that liners are functioning as desired (Means and Means 2017).

In general, this study's liner/striped newt recipient wetlands continue to be significant and perennial breeding ponds for all of the region's expected pond-breeding amphibians, including the ornate chorus frog (*Pseudacris ornata*), and the rare and imperiled gopher frog (*Lithobates capito*) (Figure 7). Incidentally, we documented, via drift fence, 320 metamorphic gopher frogs leaving Pond 18 from 30 April through 19 August. Terrestrial recruitment of newly transformed gopher frogs would not have been possible without the existence of a liner in combination with the March wetland augmentation. This study's use of liners to create pool refuges in recipient wetlands

continues to be a beneficial tool to create conditions favorable for developing larvae of not only repatriated striped newts, but also of other rare amphibians in our study area.



Figure 7. Metamorphic gopher frog. We captured 320 young gopher frogs exiting Pond 18 during the spring and summer of 2017. Successful breeding of gopher frogs and striped newts would not have occurred were it not for the presence of a pond liner underneath Pond 18.

Emergency Wetland Augmentation

Before augmentation, we sampled both Pond 178 and 18, and we concluded that both pond ecologies were very similar. Pond 178 had medium-sized gopher frog (*Lithobates capito*) tadpoles abundantly present. Striped newts and gopher frogs frequently occupy the same ephemeral pond breeding habitat. We observed by dipnet at Pond 18 one large paedomorphic striped newt male present (2.17 grams weight), 100-200 gopher frog larvae of multiple size classes; 10-20 southern leopard frog (*Lithobates shpenocephalus*) larvae; 7 larval dwarf salamanders (*Eurycea quadridigitata*); and many adult cricket frogs (*Acris gryllus*). Also present were similar plant communities around the pond littoral zones.

We delivered a total of 4,000 gallons of water into Pond 18. Immediately before augmentation, water level at Pond 18 measured 1.28 feet on our staff gauge at 9:30 a.m. Water level of Pond 18 rose steadily to 1.54 feet as the first 1000 gallons of water was discharged into the pond. We delivered water about once each hour afterward. Water level of Pond 18 after second 1000 gallons input rose to 1.73 feet. Water level of Pond 18 after third 1000 gallons input measured 1.88 feet. Water level of Pond 18 after fourth 1000 gallons rose to 1.97 feet. The last augmentation event concluded at 1:30 pm. Augmentation raised water level by approx. 0.7 feet total. Augmentation increased volume of Pond 18 approximately to liner capacity. The pond diameter increased from 4m across before augmentation to 12m afterward.

The water level at Pond 178 remained unchanged throughout the entire day. It measured 1.15 feet immediately before and after all water extractions. This indicated that our water extraction never discernably subtracted from our voluminous source pond. Our hypothesis that we would not measurably subtract from source pond was supported by our water level data, and these results were desired at the outset.

Water chemistry results are found in Table 1 below. Measured water quality values did not change at the extraction pond from before to after extractions. We recognized that temperature would rise at both ponds regardless of augmentations due to daytime heating. Some other measured water quality values did change to a small degree at Pond 18 from before to after augmentation. PH remained the same at 18 both before and after augmentation. DO increased, likely the result of increased interaction between delivery water and the atmosphere during water discharge processes. We speculate that rainfall events likely would increase DO similarly at a given pond. Specific conductivity slightly increased at 18 from before to after augmentation. Salinity did not change. None of our water quality results or changes were cause for alarm. Based on available data, we concluded that augmentation did not negatively impact water chemistry at either the extraction or the recipient pond.

| Parameters | Pond 18 | | Pond 178 | |
|-----------------------|---------------------|--------------------|-----------------|----------------|
| | Before Augmentation | After Augmentation | Before Withdraw | After Withdraw |
| Water Level | 1.28 ft | 1.97 ft | 1.15 ft | 1.15 ft |
| РН | 6.5 - 7.0 | 6.5 - 7.0 | 7.0 | 7.0 |
| DO | 1.2 mg/L | 3.95 mg/L | 3.5 mg/L | 3.5 mg/L |
| Specific Conductivity | 11.3 μs | 12.2 μs | 11.4 µs | 11.4 µs |
| Salinity | 0 | 0 | 0 | 0 |
| Temperature | 17.2 °C | 27° C | N/A | N/A |

Table 1. Results of various parameters before and after augmentation (Pond 18) and before and after water withdraw(Pond 178).

Water remained in Pond 18 until the summer rainy season, and beyond until the end of our monitoring and fieldwork season (August 2017). We concluded that augmentation had boosted pond 18 hydroperiod and was the reason for avoidance of pond drying in 2017.

Striped Newt Assurance Colonies

This year, we captured dozens more wild western striped newts from all three currently known and reliable global sources: Pond 37 in the ANF, Florida; Dixie Plantation, Florida; and Big Pond in the Fall Line Sandhills Natural Area, Georgia. These animals were shipped to participating zoos to either boost genetic diversity of captive colonies or become the founders of new captive colonies.

We captured 11 presumed wild ANF striped newts from Pond 37 during multiple sampling attempts February through June. We drove halfway and met staff from Jacksonville Zoo and Gardens to deliver six of these newts (four gravid females, two males). The newts will become founders for this project's first ANF-sourced western striped newt colony. In future study years, we expect to have their progeny available for releases.

On 18 March, partnering with TTRS, we captured 15 wild western striped newt larvae from a single pond on Dixie Plantation. These newts were housed with P. Hill and eventually sent to the Orianne Center for Indigo Conservation (Central Florida Zoo) to begin the first Dixie Plantation-sourced captive newt colony. We expect to obtain more Dixie Plantation newts for both the Amphibian Foundation and the Detroit Zoo. On 17 August, partnering with John Jensen of the GaDNR, we captured 25 large, robust wild western striped newts from the Fall Line Sandhills Natural Area in Georgia. Three larvae died in transit. Eleven larvae are now with the Amphibian Foundation and 10 larvae soon will be shipped to the Detroit Zoo.

We now have a total of six institutions on board the striped newt project in various phases of cultivating captive striped newt breeding colonies. Memphis Zoo has a Georgia colony of striped newts, Jacksonville Zoo and Gardens has both Georgia and ANF colonies, Lowry Park Zoo has a Georgia colony, Central Florida Zoo/Orianne Center for Indigo Conservation has both Georgia and Dixie Plantation colonies, the Amphibian Foundation has a Georgia colony and is waiting to receive Dixie Plantation newts, Detroit Zoo will soon have a Georgia colony and is waiting to receive Dixie Plantation newts.

Jacksonville Zoo and Gardens

Below, we report and describe in detail Jacksonville Zoo's acquisition of wild ANF-sourced striped newts and a subsequent, enigmatic population crash of our existing Georgia-sourced colony.

In February 2017, the Jacksonville Zoo and Gardens (JZG) received 2.5 wild striped newts that were collected from Pond 37 in the Apalachicola National Forest. These individuals represent the first ANF-sourced animals to be incorporated into the captive population, and are of great genetic value. While in quarantine, several hundred eggs were produced by the group, which resulted in a total of 0.0.145 live offspring. Three of the seven adult individuals were lost during quarantine; one of the adult males died as a result of human error, one female succumbed to cellulitis and renal tubular necrosis, and a second female died as a result of granulomatous meningitis. Histopathology showed that the first female had a focal corneal ulcer, renal issues, and cellulitis; edema was present on the fascial mandibular area. The second female showed severe xanthogranulomatous inflammation of the meninges associated with displacement and compression of the cerebellum. This condition is often associated with hypercholesterolemia which can be the result of high-fat diets to amphibians; however, as this animal had only been in captivity for two months, such causation is unlikely. Both females were reportedly in good nutritional status and body condition and were in active folliculogenesis. The remaining 1.3 ANF newts and their offspring were cleared from quarantine following negative chytridiomycosis and *Ranavirus* test results and upon

confirmation from histopathology that the deceased individuals' deaths were not attributable to an infectious agent.

In addition to the offspring produced by the new group of ANF newts in quarantine, the Jacksonville Zoo and Gardens hatched a total of 0.0.165 striped newts from its long-term Falls Line, Georgia locality group during the 2016-2017 breeding season. Although eggs were received from additional females, only two groups produced viable eggs resulting in live offspring. One of these adult pairs has been a consistent producer, with the female consistently laying several hundred eggs each season. Eggs were also received from a group of the previous year's offspring (holdovers from 2016 that had not yet been repatriated); five fertile eggs were collected prior to the release of these individuals.

Eggs were produced from late January through early April. Egg fertility from the single pair of Georgia animals that produced live offspring was 58.6% (n = 273). Egg fertility was not measured for the ANF animals in quarantine. Total survivorship of Georgia and ANF offspring produced in 2017 through 1 December 2017 was 33.9% and 1.4%, respectively. However, while survivorship in previous years has been largely influenced by larval rearing densities (Means et al., 2015, 2016), this year saw the direct effects of a fatal infectious agent that affected several groups of animals in the collection (see below); therefore, direct comparison of survivorship in 2017 to previous breeding seasons is not possible. As of 25 December 2017, a total of 0.0.50 Georgia locality individuals and 0.0.2 individuals of ANF origin were available for repatriation as sub-adult individuals nearing reproductive maturity (i.e. females swollen with eggs) in the upcoming winter 2017-2018 field season.

Excluding the offspring produced by the newly acquired ANF individuals, the total number of offspring produced by Georgia locality adults in 2017 (n= 165) was substantially less than the 0.0.363 offspring produced in 2016 (Means et al., 2016), which was substantially less than the 0.0.812 offspring produced in 2015 (Means et al., 2015), illustrating a continuing decline over the last two years. With estimated ages of 13-19 years, it is possible that these Georgia locality individuals have reached, or are nearing the ends of their reproductive lifespans, which would explain the lack of reproductive output in all but one of the original breeding females. Substitution of these individuals with younger animals, preferably wild-sourced individuals to avoid potential effects from undesirable captivity-selected traits in F1 captive-bred individuals, may be necessary for JZG to improve on its limited reproductive output the last two years.

In 2017, JZG initiated a collaborative endocrinological study with the Southeast Zoo Alliance for Reproduction and Conservation (SEZARC) to assess cyclical patterns in reproductive and stress hormone concentrations in its group of Striped newts. Using non-invasive methods to extracting hormones from standing purified water that newts had been placed in, we seek to characterize hormonal cycles in the species throughout the year. The results of this study can potentially shed light on the declining reproductive output seen in JZG's older breeder animals, as well as the onset of sexual maturity in F1 holdback individuals. Subsequently, this study can help inform important decisions regarding the timing and pairing of individuals for breeding, as well as the effects of environmental changes such as temperature and photoperiod on reproductive activity. This study is ongoing and no results have yet been obtained.

The most significant update to report from 2017 was the loss of 271 individuals (6.8 2015 F1 holdbacks and 0.0.257 2017 offspring) to infectious agents that manifested in two of the three striped newts rearing systems at JZG.

Newt deaths were first observed two weeks after the addition of the recently-acquired ANF newts to a new larval rearing rack and a system housing F1 hold-backs and following a slight spike in the water's pH following carbon filtration replacement. Losses occurred over an eight-week period. Adult mortality often occurred only 1-2 days after becoming symptomatic, which included subdermal hemorrhage, ulcerations on the skin, epidermal sloughing, and swelling of the throat. Affected adults were also seen attempting to leave the water. Symptoms among larvae included resting upside-down, spinning while swimming, buoyancy issues, and uncoordinated movements. Some symptomatic larvae were able to be kept alive for more than two weeks; however, no course of treatment proved to be successful in the long-term.

Gross necropsy revealed that most deceased individuals had erythema on the throat and ventral aspect of the abdomen. Cultures and samples from deceased individuals were sent out for expedited histopathology, which revealed an intracellular ciliated protozoal infection (present in all deceased specimens). Polymerase chain reaction (PCR) assays were performed in an attempt to identify the protozoan but were inconclusive.

Several different treatments were attempted. First, symptomatic individuals (both larvae and adults) were treated episodically with topical medications (Miconazole and Ceftazidine). After no improvement in the situation and the continued loss of individuals, the affected systems were

treated systemically with liquid Metronidazol (50 mg/L for 24 hours) on 02 May. This treatment was performed again on 10 May. Despite these treatments, JZG continued to lose individuals.

After no improvement from these treatments, samples were sent out from deceased individuals for PCR testing for Mycobacterium infection. Two individuals tested positive for Mycobacterium chelonae. Since Mycobacterium can be ubiquitous in aquatic environments, water samples and samples of filtration media were collected from all three newt systems (2 affected and 1 unaffected) to test for the presence of Mycobacterium. Test results were negative.

The last animal lost to apparent infection was on 14 June 2017, approximately eight weeks after the first mortality event. In total, 6.8 F1 Georgia holdbacks from 2015, 0.0.115 2017 Georgia offspring, and 0.0.143 2017 ANF offspring were lost (most of these latter individuals [n = 129] were lost in quarantine due to limited housing facilities). Interestingly, although present in one of the two infected systems, the newly acquired ANF adults did not show any signs of susceptibility to the infectious agents and appeared unaffected. The infectious agent(s) did not appear to infiltrate the adult breeder rack, as none of these animals were lost or appeared symptomatic at any time. Since die-offs were only seen in the two systems in which the new ANF adults or their offspring were housed, we suspect that the adult ANF animals are responsible for introducing one or both of these agents into the collection. It is unclear whether the ciliated protozoa or the Mycobacterium were the primary infectious agent, or if one was opportunistic in nature and affected animals that were already compromised by the other agent, or if they represented separate primary infections.

In July 2017, an additional three wild-sourced ANF newts were delivered to JZG to bolster the current ANF captive population. These animals passed through quarantine without incident, and are currently housed in an aquarium that is separate from the three other newt systems.

Orianne Center for Indigo Conservation/Central Florida Zoo

Animals were first brought in to the Central Florida Zoo from the Jacksonville Zoo's colony in April of 2015. In March of 2016, the Central Florida Zoo transferred their 3.3 animals to the zoo's offsite conservation center, the Orianne Center for Indigo Conservation (OCIC) in Eustis, Florida. Larvae began to hatch from April to June of 2017, marking the first year of successful reproduction from the breeding colony at this facility. In 2017, the OCIC successfully raised 0.0.34 larval striped newts for release on June 16, 2017. In addition to a successful reproductive year at the OCIC in 2017, 7.4 additional striped newts were acquired from Dixie Plantation in October 2017 through partnership with TTRS. This group is being housed separate from the original colony in a 170-gallon glass aquarium with 2/3 water and 1/3 land.

Memphis Zoo

Memphis zoo assurance colony of Georgia-sourced western striped newts suffered population crashes in 2017. Memphis Zoo production of striped newts for the striped newt project currently is in a holding pattern. Colleagues are investigating cause of decline while working to mitigate for losses. Staff departures confound logistical issues related to carrying out complex animal husbandry. Results are pending.

Striped Newt Repatriation and Monitoring

A total of 79 adult striped newts raised at Jacksonville Zoo and Gardens and 31 larvae raised by the Central Florida Zoo-Orianne Center for Indigo Conservation were marked and released into drift fence-encircled ephemeral ponds in 2017. All newts survived the marking procedure.

On 25 January, CPI received 79 aquatic adult striped newts raised by and shipped from the Jacksonville Zoo and Gardens to be released into the ANF (Figure 8). Newts were marked and released in pairs the following day. We released 49 marked adults into Pond 18 and 30 marked adults into Pond 182.



Figure 8. Release of adult striped newts.

On 16 June, the Orianne Center for Indigo Conservation transported 31 small to medium sized striped newt larvae to CPI. Larvae were marked and released the next day. All 31 larvae were released into Pond 182.

To measure repatriation/recruitment success, we operated drift fences from 23 January through 19 August this year. No striped newts were captured in our drift fences in 2017 entering or exiting any recipient wetlands. After eight months of drift fence monitoring both wetlands, zero of the released aquatic adults were detected in drift fences as transforming into terrestrial efts. The adults either had died or, as is equally plausible, may still be living in or around the rather large area between pond and drift fence.

Because of augmentation, water remained present at Pond 18 throughout the rest of the dry spring. The other lined wetlands (182 and 75) both went dry at least once in May. During each monthly dipnet sampling event at Pond 18 after augmentation (April-June), we continued to observe hundreds of healthy gopher frog tadpoles, but we found zero striped newts. Finally, on 26 July, we captured by dipnet one small striped newt larva (photo cover). This larva was too small to mark, but we did photo-document it. Based on a combination of drift fence and monthly dipnet data, we conclude that this larva likely represents the first occurrence of an F2 generation present at any of our recipient wetlands in this study to date. Its grandparents were released as aquatic adults in January 2016. The F2 larva indicates that a persistent, multi-generational population of striped newts has been present for 18 straight months at Pond 18. The presence of this F2 larva indicated that our experimental wetland augmentation had created conditions favorable for striped newt breeding. Therefore, we conclude that augmentation was a success. Also, with this latest study benchmark, Pond 18 is believed to be in the early phases of striped newt repatriation success.

Mark-Recapture and Detectability Study

Before releases, FWC biologist colleague, Pierson Hill, along with CPI biologists, marked all newts with visual implant elastomer (VIE) colored tags. After marking, newts were observed overnight before next-day release into ANF.

Five paedomorphic adult newts were detected in a repatriation pond, ostensibly offspring from adults released the previous year. Six reintroduced larvae were recaptured 1 month following release. Detection and recapture rates were too low to allow meaningful analysis.

During detectability sampling at Pond 182 in late June, we captured 4 larvae out of the 31 larvae released 2 weeks prior (13% detectability). Interestingly, none of them were marked. This observation suggests that our extremely small larvae deemed too small to survive marking can be released into a recipient wetland and survive the initial release process. On 26 July, during a detectability sampling six weeks after the original release, we captured 6 out of the 31 originally released newts (19% detectability). Three were marked and three were unmarked. These data indicate that newts are able to resist capture quite effectively. Unmarked captured newts, which represented the smallest animals in our original larval release group, were taken home, given a VIE cohort mark, and released back into 182 the next day. Water levels at 182 have remained intermediate and roughly stable up until the conclusion of field season, and we believe that at least some striped newts remain within the wetland.

Striped newt detectability remained quite low throughout the year, ranging between zero and 20%. Same-day-as-release detectability was typically the highest (between 10% and 20%), with an

average same-day detectability of about 10%. Detectability each successive month after a release event eventually dropped several months after release, except at pond 18 where a persistent aquatic breeding population has been documented for 1.5 years and counting.

CONCLUSIONS

Pond 18 continued on a path toward striped newt repatriation success throughout the 2017 field season. We documented the persistence of a small multi-generational aquatic population of striped newts present for 18 straight months. This population was created by our repatriation efforts. Documentation of a second generation of newts past original release here is a project benchmark and is the high point of the 2017 field season. Whereas Pond 18 may be in the early phases of repatriation success, it still is much too early to determine whether we have created a viable, selfsustaining population. Substantially more time and continued monitoring will tell.

Liners have been shown to work similarly well at all three lined study ponds. Furthermore, the occurrence of the liner at Pond 18 and its positive effect on repatriation success there cannot be overstated. Without the liner, Pond 18 would have dried at least twice during the preceding 18 months, and the persistent, multi-generational aquatic population of striped newts would have either exited the wetland or perished. Additionally, without the presence of the pond liner at Pond 18, wetland augmentation would not have been possible. We have shown previously that liners act as confining layers in local ephemeral ponds that do not naturally possess them. Lined ponds pool rapidly during single rain events. Study area soils consist of sand on top of limestone/aquifer system. Without liners, rainwater will percolate rapidly into the porous sand that comprises the wetland bottoms. It takes rising of the local aquifer system to fill un-lined local ephemeral wetlands.

Zero striped newt drift fence captures were made at all three drift-fenced study ponds in 2017. This may have reflected that 2017 was not a prime year for striped newt migration and breeding activity. It also could have signified that many of our released individuals died before reaching metamorphosis, or remained in ponds. More time will tell.

Experimental wetland augmentation techniques were tested and considered successful at Pond 18. We learned that a liner pond can respond well and predictably to supplemental water augmentation. Employing wetlands augmentation at rubber-lined Pond 18, we purposefully avoided pond-drying and deliberately prolonged conditions favorable for striped newt breeding. Through this, we successfully avoided a setback to striped newt breeding within our most successful newt repatriation pond to date. Augmentation conducted within a rubber-lined repatriation pond represents a new emergency management technique to extend pond hydroperiod for management purposes. It may serve useful for other similar imperiled amphibian conservation projects.

Although we suffered captive colony declines at two of our most productive zoological institutions in 2017, our partners have taken much action to investigate and ameliorate the situations. Additionally, we welcomed aboard new institutions to begin captive breeding programs, increasing the total number of participating zoos to seven. Our capacity to collectively produce greater numbers of striped newts for future releases expanded despite setbacks.

YEAR 8 EXPECTATIONS

In 2018, we expect to continue all aspects of the striped newt repatriation project at full capacity.

All our zoo partners remain firmly committed to continue captive breeding for this project as long as it takes to be successful. With the addition of three more institutions on board this year, we expect to better absorb any future captive population setbacks. As original participating zoo partners rebound from population crashes, and as new zoos begin cultivation of new captive colonies, we expect to have hundreds of newts to release by next year. That number should increase beyond 1000 in successive study years.

We expect to continue intensive, region-wide sampling for more potential wild ANF striped newts. We also will continue our inquiry into the potential cause(s) of striped newt decline within the ANF next year, including continuation of disease swabbing any striped newts encountered post-release.

Next year and beyond, we expect to see more success at Pond 18. We greatly desire that successes enjoyed at Pond 18 will eventually be duplicated at additional release ponds.

Based on data generated to date, we strongly believe that the continued application of greater and greater numbers of larval and adult western striped newts into the prime habitat of the ANF eventually will reach a tipping point that should lead to overall project success.

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