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

Sixth Annual Report

2016

Ryan C. Means[1], Rebecca P.M. Means[1], Mark Beshel[2], Robert Mendyk[2], Pierson Hill[3], Steve Reichling[4], Bryan Summerford[4], Abbey Elkert[4], Matthew J. Gray[5] , Debra L. Miller[5]

[1] Coastal Plains Institute, Inc., Tallahassee, FL
[2] Jacksonville Zoo and Gardens, Jacksonville, FL
[3] Florida Fish and Wildlife Conservation Commission
[4] Memphis Zoo, Memphis, TN
[5] University of Tennessee, Knoxville, TN

EXECUTIVE SUMMARY

This report summarizes work conducted in Year 6 (January 2016-September 2016) of the striped newt repatriation project within the Apalachicola National Forest (ANF).

A total of 139 western striped newt larvae and adults were released into four recipient wetlands during the 2016 project year. This brings the total number of western striped newts released in this project to 1327.

At least two very significant study benchmarks were reached this year. First, we observed multiple terrestrial adults (three individuals) returning to two natal repatriation ponds with intent to breed. One returner was observed at Pond 16 and two were observed at Pond 18. These numbers are up from the previous year (one returner at one pond). This indicated that repatriated individuals are successfully returning to their release sites and are attempting to breed.

Second, we documented this project's first known breeding by repatriated individuals. Additionally, one of these Fl larvae successfully metamorphosed into an eft and exited its natal pond (Pond 18). Such results indicated that repatriated newts are reproducing at Pond 18 and the subsequent capture of an emigrating eft shows that a member of an F1 generation can survive through metamorphosis and reach the uplands.

We have obtained results from 12 out of 41 striped newt swab samples taken in 2015-2016. Out of 12 samples analyzed thus far, zero have tested positive for *Ranavirus*, *Batrachochytrium dendrobatidis* (*Bd*), or *Batrachochytrium salamandrivorans* (*Bsal*). Six of these samples were from striped newts that exhibited noticeable "sore spots" on the body. These swabbing results may be a preliminary indication that no known pathogens were prevalent in our study area at this time. This evidence also failed to support the hypothesis that known pathogens were all or partly to blame for causing western striped newt decline in the ANF.

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

Due to the instatement of the U.S. Lacey Act in response to the recent *Bsal* fungal pathogen emergence in Europe, the transportation of any species listed as potentially "injurious" to said pathogen became highly regulated. No transport of injurious species into the U.S. or across state lines was allowed unless specifically permitted to do so by the U.S. Fish and Wildlife Service. The striped newt was a species listed as injurious. As a result, the Coastal Plains Institute sought and successfully acquired its USFWS Lacey Act permit to receive striped newts from out of state (Memphis Zoo).

This year, we found a tiny, barely detectable population of presumed wild ANF striped newts in a single historical striped newt wetland within the ANF. We await results from DNA analysis to confirm their identity. If wild, then this was welcome news both for striped newts and potentially for the future of our ANF striped newt conservation efforts. Despite repeated sampling in the area, no striped newts had been detected since 2006.

We believe that the continued repatriation 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 evolves to meet the conservation needs of the western striped newt within the ANF.

ACKNOWLEDGMENTS

We would like to thank the U.S. Forest Service and the Apalachicola National Forest (ANF) for providing permission to conduct this worthwhile conservation project on national forest lands, within the ANF. The striped newt project is the latest in a long list of proud partnerships and conservation collaborations between the Coastal Plains Institute (CPI) and the ANF. John Dunlap, Jeff Gainey, and Marcus Beard of the ANF are greatly thanked.

Numerous colleagues have been involved with the striped newt repatriation project over the 6-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, Jeremy Breland, Jacob Heitsman, Amanda Lankenau, Sean Wallace, Stefanie Jackson, Emily Mikus, and Steve Gott from Jacksonville Zoo and Gardens, and Steve Bogardy from the Memphis Zoo. The work of these individuals and zoological institutions is vital to this project and, therefore, to the conservation of the striped newt.

Numerous colleagues from the Florida Fish and Wildlife Conservation Commission (FWC) collaborated on sampling efforts within our study region, including Kevin Enge, Anna Farmer, Bess Harris, Mike Sisson, Jennylyn Redner, Brad Ohanlon. Dr. Brooke Talley, Amphibian and Reptile Conservation Coordinator for FWC, has worked tirelessly within her agency and in the field to provide assistance and support for the striped newt repatriation project, and those efforts are greatly appreciated.

We would like to give very special recognition to Lydia Eldredge for her outstanding volunteer efforts to the striped newt project in 2016. Lydia spent an exceptional amount of time checking drift fences, entering scientific data, and assisting with releases. The project would not have been the same without Lydia's efforts this year.

Numerous other people provided invaluable volunteer assistance with drift fence checking efforts, including: Gayle Muenchow and Richard Hopkins, Thomas and Carolyn Yarbrough, Nancy Thomas and Stephanie Barnes, Faith and Derry and Max Walsh, Laura Kellam and Jon Chandler, Wendy Anzalone and her boys Joey and John, Chris Womack and his girls, Bradley Perry, and Ashley, Spenser, and Juni Hopkins.

We thank Dr. Karen Lips, University of Maryland, for taking on striped newt swab samples for analysis.

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 final author list, and we look forward to collaboration in future years: Steve A. Johnson (University of Florida), D. Bruce Means (Coastal Plains Institute), Roberto Brenes (University of Tennessee).

Finally, we would like to extend a very special thank you to agencies and entities that have provided funding support to the striped newt project for its continuation beyond the original 5-year funding period, including the Florida Fish and Wildlife Conservation Commission, the Felburn Foundation, and Jacksonville Zoo and Gardens. Without such support, this collaborative and worthwhile salamander conservation effort would not be possible.

CONTENTS

INTRODUCTION

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, Means and Means 2005, 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, the ANF population sharply declined in the late 1990's for unknown reasons (Means et al. 2008).

In 2004, the IUCN added the striped newt to the Red List as "Near Threatened" (IUCN 2010). In 2008, 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. Until this year, the last time we observed larvae in the ANF had been in 1998. Our last adult observation was in 2007, despite intensive wetland sampling nearly every year since that year. By 2010 and up until Spring 2016, we believed that the western striped newt in the ANF had likely become extirpated. Based on this year's intensive sampling of historical striped newt wetlands within the ANF, and pending DNA analysis, we now believe there to be a tiny isolated population of wild ANF striped newts holding on in a single historical wetland.

One possible cause of the striped newt decline in the ANF is drought; another is infectious disease. Other possible causes for decline may be off-road vehicular disturbances to breeding ponds, incompatible historical 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.

CPI and the US Forest Service entered a 5-year cost-share agreement in 2010 to create a study to address the apparent extirpation of the striped newt population in the ANF. Striped newt repatriation coupled with precautionary measures to ensure repatriation success and enhance breeding habitat are being conducted as part of the study.

All repatriated newts were sourced from a single wetland in SW Georgia. The first repatriation event occurred in 2013, and has continued annually in up to six wetlands. Testing for *Ranavirus* has been ongoing since the inception of this project, and cooperating partners more recently began swabbing for *Batrachochytrium dendrobatidis* (Bd) and *Batrachochytrium salamandrivorans* (Bsal) in an effort to identify other disease threats for repatriation. Pending results from disease testing within the Apalachicola National Forest will provide information about the extent to which disease may be a contributor to the striped newt decline.

This year, we welcomed the Florida Fish and Wildlife Conservation Commission (FWC) on board the striped newt project. FWC biologist, Pierson Hill, conducted mark-recapture of our released striped newts using visual elastomer implants (VIE). P. Hill also implemented a study to determine detectability of striped newts in their natural habitat. Currently, we know very little about population dynamics and the fate of individual repatriated striped newts in the Munson Sandhills. Mark-recapture has proven to be extremely effective for estimating population parameters 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). Mark-recapture in the current study is expected to improve our understanding of striped newt population ecology as well as provide a greater measure of success of our repatriation efforts.

The goal of our study is to create a self-sustaining population of striped newts within their former western geographic stronghold, the ANF. This study also is expected to generate new and useful management strategies. We also believe that many of our husbandry, repatriation, and habitat enhancement techniques may provide a blueprint for the conservation of similarly imperiled amphibian species.

This report summarizes work conducted in Year 6 (January 2016-September 2016) of the study.

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, as needed, 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 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.

8) Evaluate the prevalence of *Ranavirus*, Bd, Bsal and possibly other as of yet unknown amphibian diseases within our study region.

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

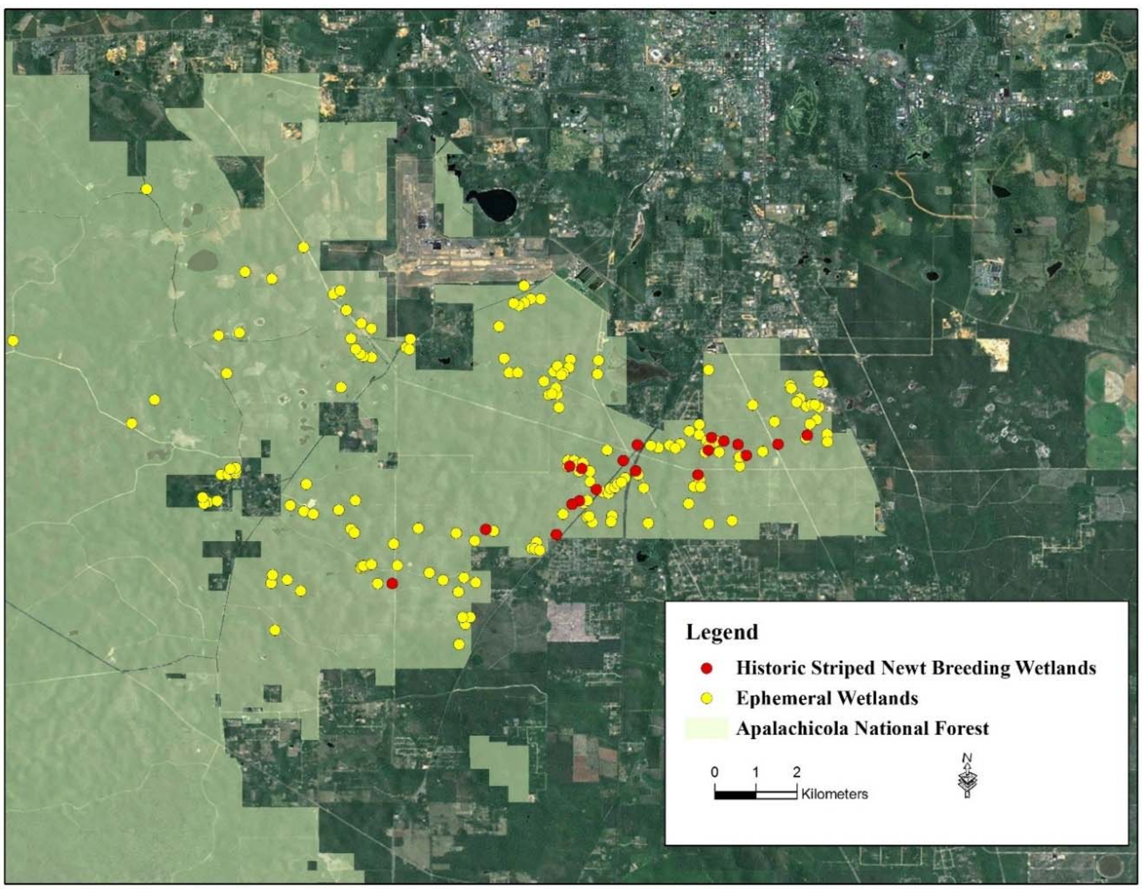

Figure 1. Map of the Munson Sandhills study area. Yellow dots represent the 158 ephemeral wetlands that have been periodically sampled over the last 20 years. Red dots represent the 19 historical striped newt breeding wetlands.

Over the past four 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 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 (001 and 178) that have neither pond liners nor encircling drift fences.

METHODS

Hydrology and Ecology of Repatriation/Liner Wetlands

For almost two decades prior to our study, frequent droughts occurred in our study region and historical striped newt ponds often were dry (R. Means, unpublished data). During the inception of our study, we hypothesized that adding rubber liners into future newt recipient ponds could lengthen hydroperiods and create small pool refugia beneficial for successful development of our future repatriated newt larvae. We installed synthetic rubber EPDM pond liners (40’ 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 observed the lined wetlands for two years prior to the first newt releases. These liners currently increase water residency periods in recipient wetlands by approximately six weeks during a dry-up period (Means et. al 2013, 2014, 2015). Repair is sometimes required due to vandalism.

Striped Newt Assurance Colonies

Jacksonville Zoo and Gardens and Memphis Zoo continued to maintain the captive striped newt colonies (Figure 3). We provide detailed description of assurance colony husbandry and maintenance methodology in prior annual reports (see Means et al. 2014, 2015, 2016).

Figure 3. Striped newt assurance colony housing at the Jacksonville Zoo and Gardens

Striped Newt Repatriation and Monitoring

We monitored the four repatriation wetlands with dipnet and drift fencing to measure repatriation success. Drift fences were installed in winter of 2013 (Pond 16) and winter of 2014 (Ponds 18, 75, 182). Drift fences were composed of 2-ft high galvanized metal flashing, and completely encircled all wetlands. We buried plastic, 2-gallon 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 drilled ten, small-diameter (ca. 2-3mm) holes into each bucket approximately 7 or 8 cm from the bottom. These holes allow accumulated flood water from thunderstorms to percolate out of the buckets while retaining a shallow level of water for captured animals to stay hydrated. 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 from 15 January through 5 September 2016. To close the fences, we removed sections of fencing and filled buckets with sand to prevent undesired captures.

Before this year (2016), drift fences at Pond 16 and Pond 75 flooded annually throughout this project while fences at Pond 18 and 182 flooded at least once. This reduced the efficacy of the drift fences in assessing repatriation success. In 2016 we, along with the help of staff from Jacksonville Zoo and Gardens, moved drift fences at Ponds 16 and 75 farther from the wetland edges. Based on rainfall and flooding frequency observed since this project began, we moved fences of Ponds 16 and 75 far enough away from observed average wetland edges to prevent future flooding except in the most severe cases.

We conducted seasonal dipnet monitoring of the historical striped newt breeding wetlands and nearby, additional MS wetlands using a heavy duty dipnet with 3/16" mesh and/or seine. The number of sweeps per pond varied depending on pond size. For small ponds, we swept the entire pond periphery and the center. We conducted at least 50 dipnet sweeps around the perimeter of large ponds. Sweep efforts were concentrated in submerged or emergent herbaceous vegetation where larvae tend to concentrate. Wetlands were sampled during the winter adult breeding season and during the spring larval season.

Mark-Recapture Study

This year, we initiated a mark-recapture component of our study. In January 2016, Jacksonville Zoo and Gardens sent 113 adult striped newts that were hatched between March and June 2015 to the Coastal Plains Institute for visual implant elastomer (VIE) tagging and subsequent release into recipient wetlands within the ANF. We individually anaesthetized adult 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 a ultra-violet flashlight (Figure 4a). Most of these animals were post-metamorphic and/or exhibiting breeding behaviors at the time of their release. On June 1, Jacksonville Zoo and Gardens sent an additional 26 larval newts for marking and release (Figure 4b). We marked larval newts using two cohort marks that identified them to pond and year. Following marking, we held newts overnight for observation and to allow them to recover from the procedure.

Figure 4. (a) Injecting a colored, flexible VIE tag beneath the skin of an anaesthetized adult striped newt. Adults were marked in four locations on the ventral side near the limbs. (b) Injecting VIE into anaesthetized larval newts.

To estimate baseline detectability of striped newts in a given wetland, we conducted dipnet recaptures on the same day as we released marked newts in Ponds 16 and 18. We allowed newts to disperse to preferred habitats within the pond for six hours before attempting the first recapture. Due to the extremely small amount and sensitivity of inundated habitat during the June 2 larval release, we did not attempt same-day recapture at Ponds 75 and 182. We conducted timed dipnet recaptures monthly through August for a total of seven recapture events at each of Ponds 16 and 18 and one recapture event at each of Ponds 75 and 182.

Because of its large size and extent of available habitat, we dipnetted Pond 16 for a total of one hour per recapture event, while we dipnetted the much smaller and shallower Pond 18 for 30 minutes total. We identified, measured/weighed, noted age class, swabbed for a disease sample, and released recaptured newts on the same day. We held unmarked adult newts overnight for marking and measuring and released the following day. We did not mark captured larval newts until they reached 26 mm SVL as precautionary to not over-stress young, potentially less robust, individuals.

Infectious Disease Investigation

We swabbed striped newts and eastern newts encountered in drift fences or by dipnet to test for presence of *Ranavirus*, *Batrachochytrium dendrobatidis* (Bd), and *Batrachochytrium salamandrivorans* (Bsal). We shipped samples via Fed Ex to study collaborators that specialize in amphibian pathology. Dr. Matt Gray and Dr. Deb Miller, researchers with the Center for Wildlife Health at the University of Tennessee, analyzed samples for presence of *Ranavirus* and Bd. Dr. Karen Lips, University of Maryland, analyzed samples for Bd and Bsal. More details about our swabbing methodology can be found in Means et. al 2015. Detailed methodology for *Ranavirus*, Bd, and Bsal lab analyses will be reported in the next annual report (Means et al. 2017).

RESULTS AND DISCUSSION

Hydrology and Ecology of Repatriation/Liner Wetlands

Pond liners continued to be effective in 2016 for prolonging hydroperiod in recipient wetlands. All lined wetlands became hydrated two to four weeks sooner than corresponding hydrological reference ponds during the transition from dry season to the rainy season. In other words, a first big rain after a long dry period hydrated lined wetlands immediately, whereas unlined reference ponds remained dry until successive rain events became sufficient to raise the local groundwater table enough to create water pools in unlined ponds. During ensuing dry periods following wet periods, Pond 182 retained its central water pool months longer than its unlined reference pond, which remained dry for most of the summer. Lined wetlands nearly went dry twice, but remained at least partially hydrated during the entire 2016 field season. Unlined reference ponds went completely dry at least once during the same period. These observations provide another year's evidence that liners are successfully extending wetland hydroperiods.

Our lined wetlands continue to be significant breeding ponds for a plethora of local pond breeding amphibians, including the rare and imperiled gopher frog (*Lithobates capito*) and ornate chorus frog (*Pseudacris ornata*). Both species either attempted to breed (Pond 75) or successfully bred (Pond 18 and 182) at all lined wetlands. This study's use of liners to create pool refuges in recipient wetlands during periods when other area wetlands remain dry continues to be a beneficial tool to create favorable conditions for developing larvae of repatriated striped newts and other rare amphibians of our study area (Figure 5).

Figure 5. On July 15, we dipnetted 102 gopher frog tadpoles at Pond 182. The pond had just a 20' diameter pool of water residing on top of the lined portion of the wetland. The nearby reference wetland was dry at this time. We also documented 56 metamorphic gopher frogs exiting Pond 182 via drift fence in 2016. Pond liners at 182 and other lined wetlands act to create pool refugia for developing amphibian larvae.

Striped Newt Assurance Colonies

Jacksonville Zoo and Gardens

During the 2015-2016 breeding season, Jacksonville Zoo and Gardens hatched a total of 363 *N. perstriatus* from four pairs of adults. One breeding pair (Group C) produced four fertile eggs in November 2015. Although all of these eggs hatched, the larvae did not survive. This pair produced an additional 247 offspring between February and May of 2016, of which 66 survived. A pair of younger individuals hatched in 2015 (Group D), that was held back to become future breeders, laid four fertile eggs in April. These eggs hatched, but none of the young survived. Another pair (Group B) produced 75 offspring between March and May, of which seven survived. This pair produced an additional clutch of fertile eggs in late July, which resulted in 33 offspring, of which 31 survived. One breeding pair (Group A) failed to reproduce entirely; eggs were produced, but none proved to be viable. Total survivorship of offspring produced in 2016 was 28.7%, with survivorship of offspring from each breeding pair ranging from 0-35.2% (mean= 15.4%). As of December 2016, 78 individuals of undetermined sexes were available and slated for repatriation in winter 2016-2017.

The total number of offspring produced in 2016 (n = 363) was substantially less than the 812 individuals produced in 2015 (Means et al., 2015), and survivorship among offspring this season was also markedly lower than the previous year (29% and 63%, respectively). It is unknown at this time why fecundity and survivorship were substantially lower in 2016 than in the previous year. One possible explanation for the lower reproductive outputs may be that the breeding adults, which are now approaching estimated ages of 12-18 years, may be reaching the ends of their reproductive lifespans, although the age at which reproductive output begins to decline has yet to be studied in wild or captive *N. perstriatus*. Similarly, it may also be possible that the viability, fitness, and survivorship of eggs and larvae decrease as reproductive adults reach a certain age or physical condition. In an effort to combat these possibilities and the inevitability of the current breeding adults reaching the ends of their lifespans, we held back a total of 9 male and 8 female offspring hatched from four different breeding pairs in 2015 to become future breeders. As of December 2016, at approximately 1.5 years in age, several of these individuals have already begun to exhibit courtship and reproductive behaviors.

Survivorship among offspring also may be influenced to some extent by rearing densities within an enclosure, with specimens reared together in greater numbers generally experiencing lower survivorship due to cannibalism. The decision to hold back and provide separate housing for future breeders inadvertently decreased the amount of space available for rearing larval offspring produced in 2016, which led to greater stocking densities and subsequently, lower survivorship. With assistance from an American Association of Zoo Keepers Conservation, Preservation and Restoration Grant, received in late 2015, Jacksonville Zoo and Gardens purchased additional holding tanks and life support equipment. This increase in holding capacity for *N. perstriatus*, allows for more individuals to be reared in lesser densities and should improve survivorship among larval offspring produced in the upcoming 2016-2017 breeding season. Moreover, by adding additional rearing enclosures for larvae, we have repurposed several older enclosures, formerly used for rearing offspring, for accommodating a greater number of breeding adults. This should help improve upon the previous year's marginal reproductive output.

Memphis Zoo

We split our striped newts into three breeding groups. We split the 2011 cohort between five males/three female and four males/three females. The 2013 cohort consisted of three males and five females. We lost two males from the 2013 cohort late this year. As of December 2016, the breeding group census totals 12 males and 12 females.

We also held a number of specimens that were slated for release in early 2017. Currently we have 50 offspring that were produced this year. At one point, we had over 100 larvae that were put into a terrarium and allowed to go terrestrial. We lost many animals over several weeks and so began to transition all the efts back to aquatic forms.

Husbandry did not change during the breeding cycle this year. We offered a greater variety of food items compared to past years. Fruit flies, crickets, calci-worms, and other worm larvae were offered and consumed. We lost one female in November from the 2013 group. This is the first adult we have lost in quite some time. She was too decomposed to submit for necropsy.

We set up the newts for breeding the 2017 in a different way, and will include details in the 2017 report after we have assessed its effect. Both groups 2011 and 2013 have begun producing eggs again. So far none of them have proven fertile but this has been the case in past seasons where later eggs have developed.

Striped Newt Repatriation and Monitoring

We expected to repatriate striped newts into all six wetlands in 2016. Due to captive newt population fluctuations in zoos and low water levels in the spring, we opted to repatriate in only the four original, drift-fenced repatriation wetlands (16, 18, 75, 182). All newts repatriated in 2016 were sourced from Jacksonville Zoo and Gardens. We did not receive any shipments of newts from Memphis Zoo in 2016 because of the Bsal fungal pathogen emergence in Europe and a subsequent U.S. Lacey Act permitting issue (winter release of adults) as well as low water levels in the spring (spring/early summer release of larvae). The Bsal emergence caused enactment of Lacey Act guidelines, which controls the movement of potentially affected or "injurious" species into the U.S. from abroad or across borders of U.S. states. Striped newts are listed as an injurious species under the Lacey Act. By mid-summer 2016, CPI successfully acquired a USFWS permit to receive striped newts across state lines. We are therefore set up to receive striped newts from Memphis Zoo in 2017.

We released a total of 139 total adult and larval striped newts in 2016, spread between the four original recipient wetlands (Ponds 16, 18, 75, and 182). On January 16, we released 90 adult striped newts into Pond 16 and 23 adult striped newts into Pond 18 (Figure 6). Regular monthly dipnet sampling coupled with drift fence data at Pond 16 suggested that some adults persisted in the wetland for at least three months, and then either perished or remained undetectable within the wetland through August 2016. We recorded the presence of one or more adults persisting in Pond 18 each and every month through August. On 12 May, we documented via drift fence, one adult (out of 23 released) that metamorphosed into the terrestrial phase and exited Pond 18.

Figure 6. A male/female pair of aquatic adult western striped newts just released together into Pond 18. The male (above) immediately grasped the female (below) upon careful hand held release.

In February, we observed three terrestrial adults returning to two natal repatriation ponds with intent to breed. One returner was observed at Pond 16 and two were observed at Pond 18. These numbers were up from the previous year (one returner at Pond 18). Incidentally, all individuals were detected by dipnet. We concluded that these returners entered the wetlands just prior to construction of drift fences either in December 2015 or January 2016.

This year, we documented successful breeding by released adults at Pond 18. This was a very significant benchmark for our study. On May 23, we dipnetted a single larval striped newt from Pond 18 and concluded that this individual was produced by adults that were released four months prior in January 2016. Multiple aquatic adults also were present that day. In successive monthly dipnet samplings of Pond 18 June through August, we observed multiple larvae (up to four at a time) present each month. Additionally, one F1 larva was captured in the drift fence in July while exiting Pond 18 as a newly metamorphosed terrestrial eft.

On June 2, we released 10 striped newt larvae into Pond 182. We never encountered any of these individuals again either by dipnet or drift fence. They either remained undetectable or perished. Also on June 2, we released 16 striped newt larvae into Pond 75. None of these larvae ever were detected again. As with Pond 182, they either remained undetectable or perished.

Figure 7. One of 10 marked larvae released into Pond 182. Note the visible orange VIE tag/mark located just anterior of the hind limb.

We conducted dipnet sampling of the four striped newt repatriation wetlands, 19 historical striped newt breeding wetlands, and four additional MS wetlands during the spring larval season (April and May). This sampling is part of our annual efforts to survey our study region for the possible persistence of wild western striped newts. CPI and FWC biologists observed three aquatic adult striped newts (2m, 1f) at one historical striped newt wetland, Pond 37. These animals were presumed by all to represent a tiny isolated hold-out population of wild ANF striped newts that had existed under the intense annual sampling radar since 2007. After observation, these newts were returned into Pond 37.

Knowing that there were breeding-capable striped newts holding on at Pond 37 in April, we returned to Pond 37 with FWC biologists on May 19 to search for possible striped newt larvae. After intensive sampling by four dipnetters, we detected one striped newt aquatic adult male and two medium-sized larvae. The two larvae, also presumed to be wild, were the first striped newt larvae documented within the ANF since 1998.

Incidentally, Pond 37 is the same location where the last known adult had been observed in 2007. Pond 37 is located 2.3 miles Euclidean distance from the nearest repatriation wetland. The wetland is large, and quite isolated in general. Although we believe it likely that Pond 37 striped newts are remnants of the original ANF population, tail clips were taken during the May capture event. DNA analysis will allow us to determine whether these newts are, indeed, wild ANF newts or if they may be the result of our Georgia-sourced repatriation efforts. Either result will be welcome news for striped newts in the ANF. DNA results are pending(A. Farmer, pers. comm.). No other (presumed) wild striped newts were detected in any other wetlands in the ANF study region in 2016.

Mark Recapture Study

Detectability and recapture rates were too low for meaningful statistical analysis using mark-recapture models. At Pond 16 we recaptured only 9 of 89 (10%) newts six hours following release. We recaptured two marked newts in March and 1 in April. Interestingly, we detected a single, unmarked individual from a previous release cohort in February, probably entering the pond in late 2015 before drift fences were erected. We did not recapture any striped newts May-Aug via dipnet or drift fence, although the pond never dried down below 30% of its average volume during the study period.

The baseline recapture rate at Pond 18 was 4%, with only 1 of 26 newts recaptured 6 hours from release. However, recapture rates increased in all following months (Table 1). We recaptured one female newt in Feb, May, and July, surviving at least six months. We dipnetted, marked, and released an adult pair of unmarked newts from a previous years' release cohort in February, presumably having entered the pond before fences were erected January. We captured an adult male in the drift fence while leaving the pond basin on May 12. We detected larval newts in all recaptures May-Aug, presumably the offspring of adult newts released in Jan. Larval newt growth rates were roughly linear (Figure 8), suggesting a single hatching period in March-April.

Table 1. Calendar of newt captures for Pond 18. Cells marked "X" represent a recapture of that individual (X* = drift fence capture). Colored cells represent the period an individual was known to be present in the pond. Green cells represent the two unmarked newts that entered the pond prior to Jan. Minimum detection estimate was calculated assuming no mortality, while maximum detection estimate assumes mortality of all undetected individuals that were not subsequently detected.

Newt ID				Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
Larvae				0	0	0	0	1	4	2	2
R1	O2	Y3	B4								
R1	O2	G3	B4								
R1	O2	Y3	Y4								
R1	O2	Y3	G4		X	X				X	
R1	O2	G3	Y4								
R1	O2	G3	G4		X	X					
R1	B2	O3	O4			X					
R1	Y2	O3	O4		X	X					
R1	G2	O3	O4		X						
R1	B2	O3	B4								
R1	B2	O3	Y4		X						
R1	B2	O3	G4					X			
R1	Y2	O3	B4					X			
R1	G2	O3	B4								
R1	Y2	O3	Y4								
R1	Y2	O3	G4								
R1	G2	O3	Y4								
R1	G2	O3	G4								
R1	B2	B3	O4	X							
R1	B2	Y3	O4								
R1	B2	G3	O4		X						
R1	Y2	B3	O4								
R1	G2	B3	O4								
R1	Y2	Y3	O4					X	X		
R1	G2	G3	G4		X	X		X*			
G1	R2	R3	R4		X						
Det. Est. (min-max)				**4%**	**31-67%**	**19-63%**	**0%**	**12-75%**	**4-50%**	**4-100%**	**na**

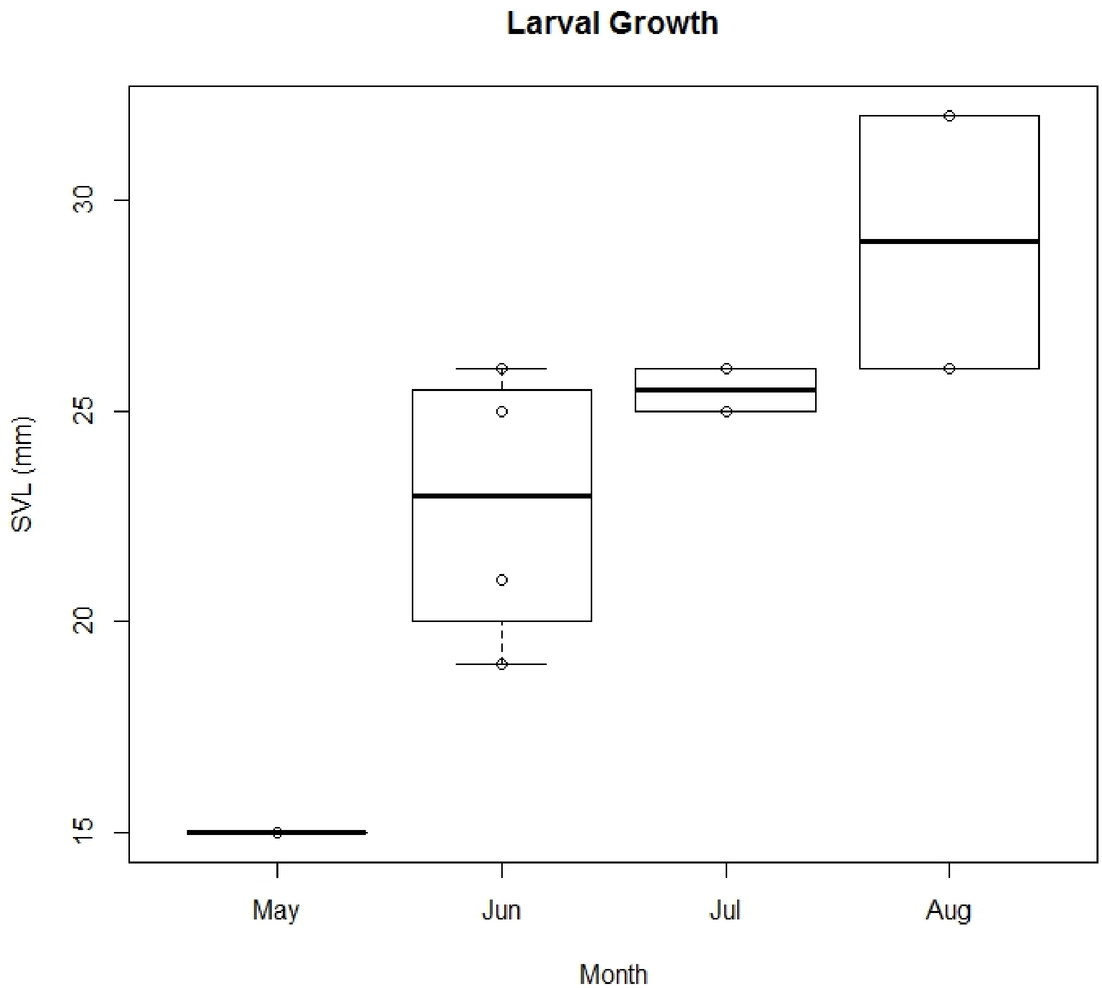

Figure 8. Snout-vent-lengths of larval striped newts captured in Pond 18, May-Aug 2016.

We did not recapture any striped newts at Ponds 75 or 182 via the single dipnet survey nor in a drift fence. These ponds held water through the end of the study period, so emigration of metamorphs could have occurred undetected following the removal of the drift fence or the efts may have remained in the pond basin following dry down.

Although low detectability and recapture rates precluded statistical analysis or inferences regarding mortality, this study was valuable in several ways. We found zoo-reared striped newts to be resilient to anesthesia and the marking procedure, with no deaths or injuries occurring before release. The VIE mark-recapture method promises to be a useful tool for further study of striped newt reintroductions, especially if employed in successive releases and at a larger scale. We expect to have approximately 150 adults on hand by Jan 2017, which will provide additional numbers for next year's markings. The newts marked in this study can serve as a foundation for additional mark-recapture work, as they now can be identified to release location and release cohort should they be recaptured in future sampling. Markings will also allow us to differentiate between repatriated individuals and any more potential wild ANF striped newts.

Infectious Disease Investigations

Striped newt project study collaborators Drs. Matt Gray and Debra Miller at the University of Tennessee, Center for Wildlife Health, tested 12 striped newt swab samples from the ANF study region in 2015 and 2016. Six of the 12 individuals had noticeable sore spots or "lesions" present on the body, and six appeared healthy, with no noticeable sores. All samples were tested via qPCR for *Bd*, *Bsal* and *Ranavirus* DNA as evidence of infection, and no positive results have thus far been detected on any individuals. Although more disease testing is needed, these preliminary results provide some evidence that tested pathogens may not be prevalent in our study region at this time. This evidence also failed to support the hypothesis that known pathogens were all or partly to blame for causing western striped newt decline in the ANF.

Since none of the “lesioned” animals tested positive for known pathogens, the question remains as to what might have caused such sore spots. We suggest that predatory attacks on newly metamorphosed individuals, especially fire ants, may explain the presence of sore spots observed on our repatriated newts. At least one of our newly metamorphosed, repatriated, striped newts was directly observed covered in fire ants while emerging from the edge of Pond 18 in 2015. This animal was taken into captivity, developed open sores all over the body, and eventually died one week later (Means et. al 2015).

We await additional disease testing results from our other amphibian disease expert and study collaborator, Dr. Karen Lips, University of Maryland.

CONCLUSIONS

This year, we documented our project's first known breeding by repatriated individuals. Additionally, we documented one of these Fl larvae successfully metamorphosed into an eft and exiting its natal pond (Pond 18). Such results indicate that reintroduced newts are reproducing at Pond 18 and the subsequent capture of an emigrating eft shows that a member of an F1 generation can survive through metamorphosis and reach the uplands.

We recorded three returning terrestrial adults at two recipient ponds (as opposed to just one last year): two returning to Pond 18 and one returning to Pond 16. These results are clear indications that repatriated individuals are successfully returning to their release sites and are attempting to breed.

Our finding of a tiny hold-out population of presumed wild ANF striped newts is significant news both for striped newts and potentially for the future of our ANF striped newt conservation efforts. If we should be lucky enough to observe these wild striped newts in the future, then some of these individuals could be very useful in developing captive colonies of in-situ striped newts to be available for conservation translocations later in this study.

This year's results represent significant steps toward eventual success of this project. Even though our total release numbers (139) were appreciably down this year relative to the past two years (433 in 2014, 697 in 2015), we still recorded very significant benchmarks, mentioned above. We believe that the continued application of greater and greater numbers of larval and adult striped newts into the prime habitat of the ANF eventually will lead to project success.

The striped newt repatriation project follows closely with the new IUCN guidelines on conservation translocations (IUCN/SSC 2013). As new scientific data are generated by our efforts, our study continually is evolving to meet the conservation needs of the western striped newt within the ANF.

YEAR 7 EXPECTATIONS

Both Jacksonville Zoo and Gardens and Memphis Zoo remain firmly on board to continue captive breeding for this project for as long as it takes to be successful. Memphis Zoo currently estimates that they will have approximately 50 adult striped newts on hand to provide for release in Winter 2017. Jacksonville also estimates they will have between 50-100 adults available by winter 2017, bringing the total number of adults that will be available for release to near 150. We expect to release all these individuals into our study area in early winter 2017. By spring of 2017, we expect to also have plentiful larvae on hand for release.

All released individuals will be marked with VIE implants in 2017. Mark-recapture will continue to provide rare glimpses into population dynamics of a poorly known salamander species as well as a clearer understanding of life histories of individual salamanders in our study.

Intensive drift fence and dipnet monitoring for returning terrestrial adults, recruitment of efts, and presence of remnant larvae/paedomorphic activity in recipient wetlands will resume beginning January, and will last until August 2017.

We expect to incorporate more investigation into the potential for pathogens in our study area next year. We will work closely with Drs. Gray, Miller, and Lips to determine susceptibility of striped newts to known fungal pathogens in the genus *Batrachochytrium*. Newt swabbing testing for prevalence of Bd and Bsal will continue next year, and we expect to report results from prior swabbing efforts.

Because of the finding of a tiny, hold-out population of likely wild striped newts in a single pond within the ANF, we expect to increase our surveillance monitoring of the region to look for potential additional hold-out ponds, as well as continue to monitor the recently discovered small population. If the newts we found this year are determined to be wild ANF newts, this occurrence will present potential opportunities for our repatriation study. If we are lucky enough to find some again, we plan to collect a small number of adults and utilize them to make a captive breeding colony of wild ANF striped newts. This future colony could become an assurance colony of wild ANF newts while possibly providing any future repatriation efforts with in-situ striped newts. In the event that we decide to use wild ANF striped newts for future captive breeding and larval production for this study, then our repatriation study may evolve further into a relocation and/or repatriation study.

LITERATURE CITED

Conant, R. and J. T. Collins. 1998. A field guide to amphibians and reptiles of eastern and central North America. Third ed., expanded. Houghton Mifflin Co. Boston, MA. 616 pages.

Dodd, C. K., Jr., and L. V. LaClaire. 1995. Biogeography and status of the striped newt (*Notophthalmus perstriatus*) in Georgia. Herpetological Natural History 3(1): 37-46.

Franz, R. and L. L. Smith. 1999. Distribution and status of the striped newt and Florida gopher frog in peninsular Florida. Final Report of Project NG90-035 submitted to the Florida Game and Fresh Water Fish Commission, Nongame Wildlife Program.

IUCN/SSC. 2013. Guidelines for Reintroductions and Other Conservation Translocations. Version 1.0. Gland, Switzerland: IUCN Species Survival Commission, viiii + 57 pp.

Johnson, S. A., and R. D. Owen. 2005. Status of historical striped newt (*Notophthalmus perstriatus*) locations in peninsular Florida and some "new" locations. Lakeland, Florida, USA.

Jung, R. E., S. Droege, J. R. Sauer, and R. B. Landy. Evaluation of terrestrial and streamside salamander monitoring techniques at Shenandoah National Park. Environmental Monitoring and Assessment 63:65–79.

May, S. E., K. A. Medley, S. A. Johnson, and E. A. Hoffman. 2011. Combining genetic structure and ecological niche modeling to establish units of conservation: A case study of an imperiled salamander. Biological Conservation (144). pp. 1441-1450.

Means, D. B. 2007. Life cycles, dispersal, and critical habitat utilization of vertebrates dependent upon small isolated water bodies in the Munson Sandhills and Woodville Karst Plain, Leon County, Florida. Final Report submitted to the Florida Department of Transportation, OMNI Project 010562.

Means, D. B. and R. C. Means. 2005. Chapter 7. Effects of sand pine silviculture on pond breeding amphibians in the Woodville Karst Plain of north Florida. Pages 56-61 in W. Meshaka and K. Babbitt, eds. Status and conservation of Florida amphibians and reptiles. Krieger Press,Malabar, Florida.

Means, D.B., R.C. Means, and R.P.M. Means. 2008. Petition to list the striped newt (*Notophthalmus perstriatus*), as a federally threatened species under the Endangered Species Act of 1973. Coastal Plains Institute, Tallahassee, FL.

Means, Ryan C., Means, Rebecca P.M., Miller, D. L., Gray, M. J., Johnson, S. A., Means, D.B., Brenes, R. 2012. A Conservation Strategy for the Imperiled Striped Newt (Notophthalmus perstriatus) in the Apalachicola National Forest, Florida. Second Annual Report submitted to the U.S. Forest Service, Tallahassee, FL.

Means, R. C., R. P. M. Means, D. L. Miller, M. J. Gray, S. Reichling, S. A. Johnson, D. B. Means, and R. Brenes. 2013. A Conservation Strategy for the Imperiled Striped Newt (*Notophthalmus perstriatus*) in the Apalachicola National Forest, Florida. Third Annual Report to the US Forest Service. Coastal Plains Institute, Tallahassee, FL.

Means, R. C., R. P. M. Means, S. Reichling, M. Beshel, B. Summerford, S. A. Johnson, D. B. Means. 2014. A Conservation Strategy for the Imperiled Striped Newt (*Notophthalmus perstriatus*) in the Apalachicola National Forest, Florida. Fourth Annual Report to the US Forest Service. Coastal Plains Institute, Tallahassee, FL.

Means, R. C., R. P. M. Means, M. Beshel, R. Mendyk, S. Riechling, B. Summerford. 2015. A Conservation Strategy for the Imperiled Striped Newt (*Notophthalmus perstriatus*) in the Apalachicola National Forest, Florida. Fifth Annual Report to the US Forest Service. Coastal Plains Institute, Tallahassee, FL.

Phillips, C. T. and J. N. Fries. 2009. An Evaluation of Visible Implant Elastomer for Marking the Federally Listed Fountain Darter and the San Marcos Salamander. North American Journal of Fisheries Management 29 (3):529-532.

Pretlaw, T., M. Huynh, L. Takats, and L. Wilkinson. 2002. Protocol for monitoring long-toed salamander (*Ambystoma macrodactylum*) populations in Alberta. Alberta Sustainable Resource Development, Fish and Wildlife Division, Alberta Species at Risk Report No. 35. Edmonton, Alberta, Canada.

USFWS. 2010. Endangered and Threatened Wildlife and Plants; 90-Day Finding on a Petition to List the Striped Newt as Threatened. 75 Federal Register 75 (55) 23 March 2010, pp. 13720-13726.

USFWS. 2011. Endangered and Threatened Wildlife and Plants; 12-Month Finding on a Petition to List the Striped Newt as Threatened. Federal Register 76(109) June 7 2011, pp. 32911-32929.