Frosted flatwoods salamander
(Ambystoma cingulatum)

5-Year Review:
Summary and Evaluation

Photo credit: Mark Mandica (The Amphibian Foundation)

U.S. Fish and Wildlife Service
Southeast Region
Panama City Field Office
Panama City, Florida

5-YEAR REVIEW
Frosted flatwoods salamander/*Ambystoma cingulatum*

I. GENERAL INFORMATION

A. Methodology used to complete the review:

In conducting this 5-year review, we relied on the best available information pertaining to historical and current distributions, life history, threats to, and habitats of this salamander species. Our sources included the original final rule listing this species under the Endangered Species Act (Act) and the revised listing final rule issued in 2009; peer reviewed scientific publications; unpublished field observations by Service, State and other experienced biologists; unpublished survey reports; and notes and communications from other qualified biologists or experts. We announced initiation of this review and requested information in a published Federal Register notice with a 60-day comment period on September 23, 2014 (79 FR 56821). The completed draft review was sent to the other affected Service Field Offices and peer reviewers for their review. Comments were evaluated and incorporated where appropriate into this final document (see Appendix A). We did not receive any public comments during the open comment period. No part of this review was contracted to an outside party. This review was completed by the Service's lead Recovery biologist in the Panama City Field Office, Panama City, Florida.

B. Reviewers:

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C. Background

1. Federal Register Notice citation announcing initiation of this review:

79 FR 56821(September 23, 2014)

2. Species status:

Overall decreasing. The number of individuals per population, and number of populations throughout the historic range have declined. Recent surveys demonstrate significantly fewer extant populations of *Ambystoma cingulatum*. Out of the original 25 populations described in the final rule (74 FR 6700, April 2015), only nine are currently known to still exist, based on surveys conducted on public lands in 2014/2015 (W. Barichivich, USGS, pers. comm.). In Florida, there are five populations in Apalachicola National Forest, two at St. Marks National Wildlife Refuge, and one at Fort Stewart in Georgia. A ninth possible population, located in the Francis Marion National Forest in South Carolina, has not had a detection of this species since 2010 (J. Palis, Palis Consulting, pers. comm.). We and our partners have had limited opportunity to evaluate private land populations since the final rule in 2009.

3. Recovery achieved: 1(0 - 25% species recovery objectives achieved); recovery actions are ongoing

4. Listing history

Original Listing **(of the flatwoods salamander)**
FR notice: 64 FR 15691
Date listed: April 1, 1999
Entity listed: species; *Ambystoma cingulatum*
Classification: threatened

Revised Listing:
Ambystoma cingulatum was split into two distinct species in 2009 (74 FR 6700), the reticulated flatwoods salamander (*A. bishopi*) was listed as endangered, and the frosted flatwoods salamander (*A. cingulatum*) retained threatened status.

5. Associated rulemakings: not applicable

6. Review History:
Each year, the Service reviews and updates listed species information for inclusion in the required Recovery Report to Congress. Through 2013, we did a recovery data call that included status recommendations such as "declining" for this amphibian. We continue to show that species status recommendation as part of our 5-year reviews. The most recent evaluation for this salamander was completed in 2017.

5-year review: We initiated and announced a 5-year review to the public for flatwoods salamander in 2005 (70 FR 34492). Our evaluation of data ultimately resulted in us as the outcome of the review proposing the split into two distinct species and ultimately listing the both the reticulated and frosted flatwoods salamander in 2009.

7. Species' Recovery Priority Number at start of review (48 FR 43098):
5. In 1999, when this species was listed as threatened, it was given a RPN of 5, which indicates there is a high degree of threat, and the recovery potential of this species is low. This RPN is being revised (see Section III, Results for the updated RPN discussion) to reflect the increased management, attention, funding, and new techniques geared to improve the recovery potential.

8. Recovery Plan or Outline
A recovery plan is being drafted at the time of this writing.

II. REVIEW ANALYSIS

A. Application of the 1996 Distinct Population Segment (DPS) policy

1. Is the species under review listed as a DPS? No.

2. Is there relevant new information that would lead you to consider listing this species as a DPS in accordance with the 1996 policy? No.

B. Recovery Criteria

1. Does the species have a final, approved recovery plan containing objective, measurable criteria?

No. At present, this species does not have an approved recovery plan. However, the recovery planning process is underway and we expect to be announcing a draft recovery plan soon.

C. Updated Information and Current Species Status

1. Biology and Habitat:

Frosted flatwoods salamanders are moderately-sized (76 mm snout-to-vent length, 135 mm total length), slender salamanders with relatively short, pointed snouts and stout tails (Martof and Gerhardt, 1965; Palis, 1996; J. Palis, Palis Consulting, unpubl. data). Their heads are small and only about as wide as the neck and shoulder region (Petranka, 1998). They weigh from 4.5 to 11 grams (adult males and adult gravid [containing mature eggs] females), respectively (Palis, 1996; J. Palis, Palis Consulting, unpublished data). Their bodies (see photo at start) are black to chocolate-black with fine, irregular, light gray lines or specks that form a reticulate or cross-banded pattern across the back. In some individuals, the gray pigment is widely scattered and "lichen-like." Melanistic, uniformly black individuals have been reported (Carr, 1940). The venter (underside) is dark gray to black with a scattering of gray spots or flecks. Frosted flatwoods salamanders are difficult to discern from reticulated flatwood salamanders visually, and can only be identified with genetic or blood tests.

The frosted flatwoods salamander is a pond-breeding amphibian with a complex life cycle; i.e., there is an aquatic egg and larval life history stage, as well as a terrestrial metamorphosed juvenile and adult stage. As adults, flatwoods salamanders migrate to ephemeral (seasonally-flooded) wetlands to breed in the fall, where females lay eggs singly on bare mineral soil in small depressions that later fill with water (Anderson and Williamson, 1976; Palis, 1995a, 1997). Once inundated, well-developed embryos hatch into larvae in the winter and metamorphose between March and May after an 11 to 18-week larval period (Palis, 1995a). Juveniles normally disperse from ponds shortly after metamorphosing, but may stay near ponds during seasonal droughts (Palis, 1997). Juveniles, along with adults, are highly fossorial and spend much of their time in crayfish burrows or root channels until they reach sexual maturity (1 year for males; 2 years for females) and return to their natal pond to breed during the fall months (Petranka, 1998).

Breeding wetlands are located within mesic (moderate moisture) to intermediate-mesic pine-dominated flatwoods/savanna communities where adults and metamorphosed juveniles spend the rest of their life outside of the breeding season. The dominant species of pine differs in some localities. For example, in Florida, longleaf pine (*Pinus palustris*) is dominant in the Apalachicola National Forest, where it is associated with wiregrass (*Aristida stricta* [= *A. beyrichiana*]). In contrast, at St. Marks National Wildlife Refuge (SMNWR), slash pine (*Pinus elliotti*) dominates the uplands that surround salamander breeding sites which, in turn, are currently dominated by sawgrass (*Cladium jamaicense*) in the basin. Although flatwoods salamanders persist in both aforementioned areas, these conditions may not reflect the habitats of past decades. There are other variations in vegetation, geology, and soils among geographic areas within the range of the salamander; however, basic characteristics are otherwise fairly similar throughout.

Pine flatwoods/savannas are characterized by low flat topography and relatively poorly drained, acidic, sandy soil that becomes seasonally saturated. In the past, this ecosystem was characterized by open pine woodlands maintained by frequent fires. Naturally ignited by lightning during spring and early summer, these flatwoods historically burned at intervals ranging from 1 to 4 years (Clewell, 1989). The topography can vary from nearly flat to gently rolling hills.

The groundcover of longleaf pine flatwoods/savanna ecosystem is typically dominated by wiregrass (*Aristida stricta* [= *A. beyrichiana*]) (Kesler et al., 2003). Other herbaceous plants often found in the groundcover include toothache grass (*Ctenium aromaticum*), bluestems (*Andropogon* spp.), beakrushes (*Rhynchospora* spp.), pitcherplants (*Sarracenia* spp.), meadowbeauties (*Rhexia* spp.), and a variety of legumes. Low-growing shrubs, such as saw palmetto (*Serenoa repens*), gallberry (*Ilex glabra*), blueberries (*Vaccinium* spp.), and huckleberries (*Gaylussacia* spp.) co-exist with a highly diverse suite of grasses and forbs in the groundcover.

Flatwoods salamanders breed and deposit eggs in wetlands that are not yet inundated with water (Anderson and Williamson, 1976; Hill, 2013; Powell et al., 2013; Gorman et al., 2014. Females select egg deposition sites that have high amounts of herbaceous vegetation and concave depressions (Gorman et al., 2014). Dr. Gorman's work has primarily been with *A. bishopi*; many data points, techniques and methods are applicable to both species. Adults select areas of complex and diverse stands of herbaceous vegetation within breeding wetlands for egg deposition. In this microhabitat, eggs are typically located in small depressions that likely minimize desiccation of developing embryos in the otherwise dry wetland. (e.g., Gorman et al., 2009; Jones et al., 2012). As noted, management of breeding wetlands for this species should include a suite of management actions that increase the cover of herbaceous vegetation (Gorman et al., 2014).

Larval frosted flatwoods salamanders generally occur in acidic (pH 3.4 to 5.6), tannin-stained ephemeral wetlands (swamps and marshes) that typically range in size from <1 to10 acres (ac) (0.4 to 4.0 hectares [ha]), but may reach or exceed 30 ac (12 ha) (Palis, 1997; Safer, 2001). Ponds are often round or oval, but larger breeding sites may be quite irregular in shape. The basins are bowl- or plate-shaped in profile and often perched above the normal water table on clay lenses (Wolfe et al., 1988). Pond depth fluctuates greatly, but is usually less than 0.5 meters (m) (Palis, 1997) in areas where larval salamanders are found. Ponds typically fill in late fall or early winter, and dry in late spring or early summer.

Summer thunderstorms may refill some ponds, but most of these dry again by early fall.

Under current conditions, the overstory within breeding ponds is typically dominated by pond cypress (*Taxodium ascendens* [=*T. distichum* var. *imbricarium*; Lickey and Walker, 2002)], blackgum (*Nyssa sylvatica* var. *biflora*) longleaf and slash pine, but can also include red maple (*Acer rubrum*), sweetgum (*Liquidambar styraciflua*), sweetbay (*Magnolia virginiana*), and loblolly bay (*Gordonia lasianthus*). Canopy cover of occupied sites is typically moderate and ranges from near zero to almost 100% (Palis, 1997) The midstory, which is sometimes very dense, is most often composed of young of the aforementioned species, myrtle-leaved holly (*Ilex myrtifolia*), St. John's-worts (especially *Hypericum chapmanii* and *H. fasciculatum*), titi (*Cyrilla racemiflora*), sweet pepperbush (*Clethra alnifolia*), fetterbush (*Lyonia lucida*), vine-wicky (*Pieris phillyreifolius*), and bamboo-vine (*Smilax laurifolia*). When dry, breeding ponds burn naturally due to periodic wildfires (especially during late spring and summer), thus fire scars are frequent on live trees within the basin, and smaller trees and shrubs are often killed or top-killed. Depending on canopy cover and midstory, the herbaceous groundcover of breeding sites can vary widely, although larvae are most often associated with higher amounts of herbaceous cover (Gorman et al., 2009; Gorman et al., 2013) which, on average, is greater than 40% coverage of the wetland (Gorman et al., 2009; Gorman et al., 2013). Most, but not all, breeding sites exhibit distinct vegetative zonation, with bands of different herbaceous plant assemblages in shallow versus deeper portions of the pond. The groundcover is dominated by graminaceous species, including beakrushes, sedges (*Carex* spp.), panic grasses (*Panicum* spp.), bluestems (*Andropogon* spp.), jointtails (*Coelorachis* spp.), longleaf three-awned grass (*Aristida palustris*), plumegrasses (Erianthus spp.), nutrush (*Scleria baldwinii*), hatpins (*Eriocaulon* spp.), Characteristic forbs may include milkworts (*Polygala* spp.), meadowbeauties (*Rhexia* spp.), marsh pinks (*Sabatia* spp.), bladderworts (*Utricularia* spp.) and seedboxes (*Ludwigia* spp.). The basin of breeding sites generally consists of relatively firm mud with little or no peat. Burrows of crayfish (genus *Procambarus*, principally) are a common feature of flatwoods salamander breeding sites, which are typically encircled by a bunchgrass (wiregrass and/or dropseed [*Sporobolus*])-dominated graminaceous ecotone.

These ponds often harbor small fishes; the most typical species include pygmy sunfishes (*Elassoma* spp.), Eastern mosquitofish (*Gambusia holbrookii*), and *Enneacanthus* (Palis, 1997). Typical amphibian associates of flatwoods salamander larvae include southern leopard frog (*Rana sphenocephala*), ornate chorus frog (*Pseudacris ornata;* but not at SMNWR), and dwarf salamander (*Eurycea quadridigitata*) larvae, as well as larval and adult newts (*Notophthalmus viridescens*) (Palis, 1997).

Historically, flatwoods salamander adult and larval habitats represented high quality conditions. However, many populations persist in less than ideal habitat which may differ from what is presented above. Appropriate management will be needed at most of these sites to prevent populations from disappearing as habitat conditions worsen. For example, fire suppression at many sites has led to greater canopy closure in the overstory of both the flatwoods uplands and ephemeral ponds (Bishop and Haas, 2005; Gorman et al., 2009; Gorman et al., 2013) and the shrub layers of both habitats have similarly increased (Gorman et al., 2013). This has resulted in a lower cover of herbaceous groundcover that is less diverse. Further, the ecotone between the breeding wetland and associated flatwoods may be obscured or non-existent, replaced with a dense layer of shrubs, such as titi, fetterbush, gallberry, saw palmetto, wax myrtle (*Myrica cerifera*) and/or dog hobble (*Leucothoe* spp.) due to fire suppression or exclusion (Gorman et al., 2013). Additionally, prescribed burns across the range of the species are conducted most often in winter and early spring when ponds would typically be flooded and less likely to burn (Bishop and Haas, 2005). To increase the opportunity for flatwoods salamander habitat to burn more effectively, land managers should diversify burning strategies (Bishop and Haas, 2005). For example, if a growing season fire cannot be performed another option may include burning uplands during the dormant season and return in the growing season to burn wetlands when they are dry (Gorman et al., 2009). Additionally, mechanical treatments can be coupled with fire to restore sites that have become too overgrown for fire alone to restore the site (Gorman et al., 2013). Other types of suboptimal habitat, such as roadside ditches and borrow pits, have the physical and biotic characteristics of natural breeding sites and may be used by flatwoods salamanders, especially when located near natural breeding ponds (Anderson and Williamson, 1976; Palis, 1995b; Stevenson, 1999; T. Gorman and C. Haas, Virginia Tech. Univ., unpubl. data).

a. Abundance, population trends (e.g., increasing, decreasing, stable), demographic features (e.g., age structure, sex ratio, family size, birth rate, age at mortality, mortality rate, etc.), or demographic trends:

A more detailed description of the historic and current range of *A. cingulatum* (Figure 2) is addressed in paragraph (d) below. However, historically this species occurred in Alachua, Baker, Bradford, Columbia, Duval, Franklin, Jefferson, Liberty, Marion, Nassau, Suwannee, and Wakulla counties in Florida; Atkinson, Ben Hill, Berrien, Brantley, Brooks, Bryan, Bulloch, Camden, Candler, Charlton, Clinch, Colquitt, Cook, Echols, Effingham, Emanual, Evans, Glynn, Grady, Irwin, Jeff Davis, Jenkins, Lanier, Liberty, Long, Lowndes, McIntosh, Mitchell, Screven, Tattnal, Thomas, Tift, Ware, Wayne, and Worth counties in Georgia; and Allendale, Bamberg, Beaufort, Berkeley, Charleston, Colleton, Dorchester, Hampton, Jasper, and Orangeburg counties in South Carolina. (W.J. Barichivich, USGS, pers. comm.)

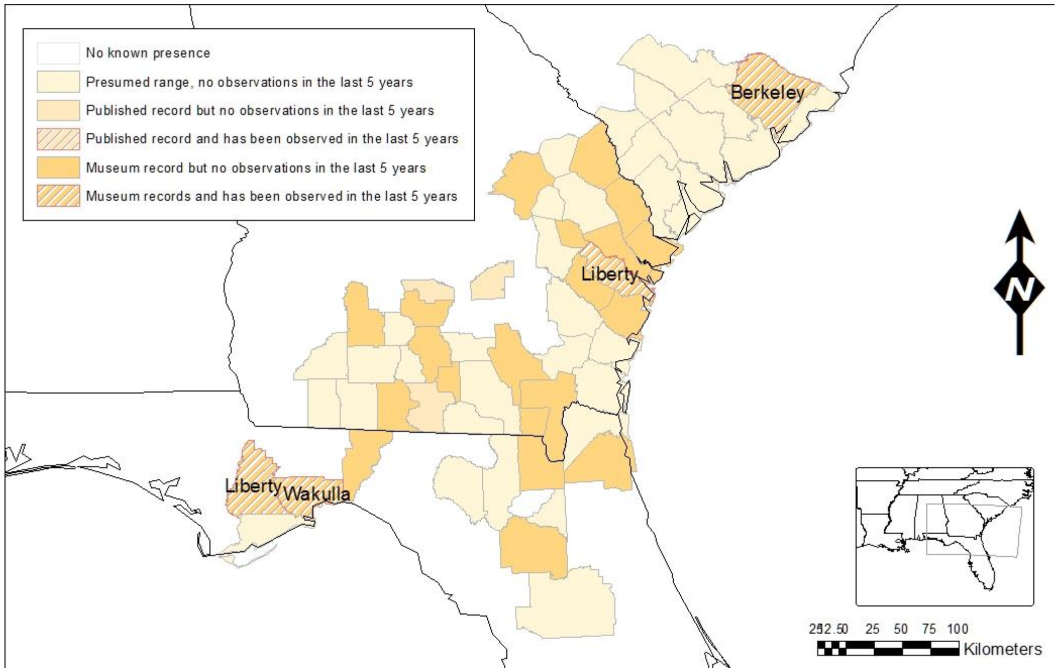

Figure 2. Geographic range of frosted flatwoods salamanders (W.J. Barichivich, USGS, pers. comm.)

Abundance:
There are currently no estimates of abundance or other data on population demographics of *A. cingulatum*. The larval surveys that have been conducted generally show detection/non-detection and the number of animals captured during a given survey period. Though surveys have occurred in some years, not enough of the historic range has been covered in a given year to make an inference about the total population abundance of the species. Drought has also prevented breeding in several years, with little or no water in ponds or ponds filling too late in the season to allow successful breeding.

Trend:
Historically, flatwoods salamanders (both species) occurred throughout the Coastal Plain of the southeastern U.S., across South Carolina, Georgia, Alabama, and the panhandle of Florida (Palis and Means, 2005). Over time and despite recently increased efforts to survey historical locations and find new populations, the combined range of *A. bishopi* and *A. cingulatum* has dwindled from 458 historical locations (i.e., mostly individual breeding sites) prior to 1999 to only 49 locations over the last five years (89.3% loss; Semlitsch et al., 2017; noting we have not been able to collaboratively visit all private locations). When the final rule was published (74 FR 6700) in 2009, there were 25 existing populations (some including multiple breeding sites) of *A. cingulatum*. These populations were defined as those salamanders using breeding sites within 3.2 km of each other, barring an impassable barrier such as a perennial stream (64 FR 15692). Ecologically, this definition best describes a metapopulation.

As of the end of the 2014/15 breeding season, there were nine known and currently occupied breeding populations (based on unpublished data from W.J. Barichivich, USGS; J. Mott, TNC; K. Enge, FFWCC; J. Jensen, GADNR; and J. Palis, Palis Environmental Consulting). The seven largest and most resilient of these nine populations occur at Apalachicola National Forest and St Marks National Wildlife Refuge (Table 1). A small population (one known breeding pond) remains on Fort Stewart, GA. Despite considerable sampling effort the status of another small population on the Francis Marion National Forest in SC remains uncertain as no observations of flatwoods salamanders has been made since 2010. In table 1 below, we include FMNF as it is of paramount importance in the recovery of this species, and the last known population was found 1 year prior to the surveys presented in the table. We do not have returning documented records for half the Apalachicola National Forest sites. There were a couple private locations we have not been able to resurvey. In the following table below, it is important to note that while Townsend Bombing Range and Santee Coastal Preserve were counted, no surveys were done in the 5 previous years. They are included as placeholders for future reference.

Table 1. The number of populations of *A. cingulatum* from the 2009 USFWS final rule (74 FR 6700) and the status of those populations in the last five years (Based on unpublished data from W.J. Barichivich, USGS; Jana Mott, TNC; K. Enge, FWC; J. Jensen, GADNR; J. Palis, Palis Environmental Consulting; and R. King, Ft. Stewart and W. Dillman, SCDNR, pers. comm.). Numbers in parentheses represent the total number of populations in each area; ? = uncertainty regarding whether locations have been surveyed in last five years; NF = National Forest; NWR = National Wildlife Refuge; WMA = Wildlife Management Area.

	Populations according to 2009 final rule (74 FR 6700 6774)	**Observations in the last 5 yrs[a]**
Florida (15)		
Apalachicola NF	10	5/10
St. Marks NWR	2	2/2
Osceola NF	1	0/1
Aucilla WMA/private	1	0/1
Private property, Baker Co.	1	?
Georgia (6)		
Fort Stewart	5	1[b]/5
Townsend Bombing Range	1	1
South Carolina (4)		
Private Properties, Jasper Co.	2	?
Francis Marion NF	1	1[b, c]/1
Santee Coastal Preserve, Charleston Co.	1	1
Total	25	9/22

[a] number of populations where *A. cingulatum* was observed /number of populations sampled (2016 – 2011)
[b] Population appears to be reduced to a single breeding pond
[c] The last observation of flatwoods salamanders within this population was 2010

b. Genetics, genetic variation, or trends in genetic variation (e.g., loss of genetic variation, genetic drift, inbreeding, etc.):

In addition to identifying *A. bishopi* as a genetically distinct species from *A. cingulatum*, Pauly et al. (2007) also documented two distinctive clades within *A. cingulatum*, with one occurring in the eastern panhandle on the Gulf Coastal Plain and the other occurring on the Atlantic Coastal Plain. The separation of these two clades coincides with the Suwannee River (Pauly et al., 2007). Subsequent genetic analyses (using both mitochondrial and nuclear genes) provided strong support for the existence of two lineages within *A. cingulatum*: the Atlantic coastal plain populations were distinct from *A. cingulatum* populations immediately east of the Apalachicola-Flint Rivers in FL (e.g., the region of the Apalachicola National Forest) and that the South Carolina specimens were genetically similar to other *A. cingulatum* from the Atlantic Coastal Plain of Georgia and Florida (Pauly et al., 2012). Current lack of demographic connectivity due to habitat loss and fragmentation, artificial barriers (e.g., agriculture, roads, and hydrological alteration), and distance may now further prevent gene flow among remaining isolated populations, which are separated by distances that likely exceed the dispersal capabilities of the species, based on known dispersal distances of other ambystomatid salamanders (Semlitch et al., 2017). The two clades are similar in habitat needs, and the management and recovery needs for both clades are very similar. In appearance the two salamander species are very similar as well, and visual identification is not reliable enough to tell them apart consistently without knowledge of where they were captured.

c. Taxonomic classification or changes in nomenclature:

The flatwoods salamander was split into two distinct species in 2009, reticulated flatwoods salamander (*Ambystoma bishop*) and frosted flatwoods salamander (*Ambystoma cingulatum*), based on findings of Pauley et al. (2007) (74 FR 6700, 2009). Since that time, there have been no additional taxonomic classification changes or changes in nomenclature.

d. Spatial distribution, trends in spatial distribution (e.g., increasingly fragmented, increased numbers of corridors, etc.), or historic range:

The frosted flatwoods salamander historically occurred east of the Apalachicola/Flint River system in Florida and Georgia and inland and as far north as the coastal plain in Georgia and South Carolina (Figure 1). Comparison of historical locations with records since 2000 demonstrate that the distributions of both species of flatwoods salamanders have been significantly reduced

(Semlitsch et al., 2017). This decline is occurring at multiple spatial scales; (i.e., there has been a reduction in the number of populations (as defined), along with a loss of individual breeding ponds within populations), which has diminished the probability of long-term persistence of this species. The potential for metapopulation dynamics (i.e., the natural exchange of individuals among discrete populations [via migration or dispersal] in the same general geographical area: Akçakaya et al., 2007) is now extremely limited. For example, using locality data, Semlitsch et al. (2017) calculated that the average distance between neighboring flatwoods salamander (for both species combined) breeding ponds was 8.9 km prior to the species' listing in 1999, 12.7 km between 2000-2009, and 28.3 km from 2010 to present. Because individual salamanders probably do not disperse more than 1-2 km within a generation and multi-generation gene flow likely is limited to 5-10 km or less for most species (Semlitsch, 2008; Peterman et al., 2015), loss of flatwoods salamander populations over time, even prior to 1999, has evidently created severe isolation that is a critical component of extinction (Semlitsch et al., 2017). The reasons for these declines are not yet fully understood.

The combined data from all survey work completed from 1990 to 2009 in Florida, Georgia, and South Carolina indicated that there were 25 recognized populations of the frosted flatwoods salamander remaining (74 FR 6700). Fifteen of these populations were known from Baker, Franklin, Jefferson, Liberty, and Wakulla Counties in Florida. In Georgia, six populations occurred in Bryan, Evans, Liberty, and McIntosh Counties, Georgia, all on Department of Defense lands (five on Fort Stewart Military Installation and one on the Townsend Bombing Range). Notably not all sites on Fort Stewart are accessible by surveyors per base policy. It is possible that salamanders occur in more than the one known remaining pond, but this is unlikely to be confirmed in the foreseeable future. In South Carolina, four populations were known from Berkeley, Charleston, and Jasper Counties.

With some exceptions (e.g., Apalachicola National Forest), *A. cingulatum* populations have become increasingly isolated and are currently so spatially separated that it is unlikely, if not impossible, for animals to share any genetic material. Because of this genetic distinctiveness between the Gulf Coastal Plain clade and the Atlantic Coastal Plain clade, Pauly et al. (2012) advised against the use of eastern panhandle populations as a source for future reintroduction on the Atlantic Coastal Plain, assuming that source populations from within the Atlantic Coastal Plain were available. Moreover, the remaining populations in South Carolina and Georgia are extremely important from a conservation perspective as they represent the only known extant populations of *A. cingulatum* in the entire Atlantic Coastal Plain (Pauly et al., 2012). Yet only 8 adults and approximately 12 larvae have been captured on the Francis Marion National Forest in South Carolina in the past 20 years (M. Danaher, USFS, pers. comm.). Recently in 2019, the Amphibian Foundation working with Fort Stewart biologists found 20 larvae on station in ponds where detections had occurred before (https://www.amphibianfoundation.org/index.php/frog-blog-2/109-2019-status-update-the-atlantic-clade-of-the-frosted-flatwoods-salamander). The lack of populations of *A. cingulatum* in Georgia and South Carolina will require consideration of where recovery populations may come from, if the Service determines that those populations are not recoverable by natural means.

Apalachicola National Forest and St. Marks National Wildlife Refuge harbor the greatest number of remaining populations, as well as number of breeding sites within populations. Some ponds are more isolated than others and some are clustered in areas near known occupied sites. It should be noted that there may still be sites that could potentially harbor salamanders within the historical range that have not yet been adequately sampled. We recommend increased survey effort in areas outside historically known ponds (see Recommendations for Future Conservation actions section).

e. Habitat:

Habitat conditions have continued to decline, primarily from suppression or exclusion of prescribed fire, but also because of an increase in drought conditions (Chandler, 2015). Increased cover from woody vegetation in breeding ponds and some uplands can contribute to a decrease in both herbaceous vegetation (shading) and hydroperiod (increased evapotranspiration) (Bishop and Haas, 2005; Gorman et al., 2013). Unfortunately, prescribed burns are often conducted during the dormant season, rather than the lightning season (Bishop and Haas, 2005). Dormant season fire may not be ideal for these salamanders as the breeding season occurs during this time. Dormant season fire can remove cover used by salamanders during ingress and egress from breeding ponds, expose areas that would provide cover for egg deposition sites, and may even cause direct or indirect mortality when it coincides with salamander movements. Prescribed fire should occur during the lightning season or if drought conditions allow, during the dormant season when wetlands are dry and fire will successfully carry through the dry wetland. Under this scenario, the conditions needed for successful salamander egg and/or larval development are not adequate and fire will pose minimal threat to survival of these developmental stages. Further, to increase herbaceous vegetation and open the canopy, burning of the uplands during the dormant season to create a ‘‘safety’’ strip to prepare for a growing season fire that targets the basin of the wetland may be necessary at some sites (Gorman et al., 2009). Mechanical treatments with handheld equipment (e.g., brush saws and chainsaws) may also be used to successfully reduce canopy cover and facilitate herbaceous vegetation growth (Gorman et al., 2013).

Between October 2014 and January 2015, St. Marks NWR burned over five thousand acres of the St Marks Unit containing all of the known and potential breeding ponds of the refuge. This had both positive and negative effects as the fire occurred mostly during the dormant season, when salamanders are moving. The fire program is getting more aggressive toward achieving maintenance condition, growing season fire, but this will require fuel reduction burns in dormant season to reduce the possibility of wildfire escape. Progress is being monitored going forward. Prescribed fire is generally applied to large sections of upland pine habitats, and while there is a trend toward more lightning/growing season fire use, the great majority still relies on fuel reduction burns in the dormant season (H. Mitchell, USFWS, pers. comm.).

f. Other: In August 2014, a structured decision making workshop was held specifically to discuss the potential for developing captive assurance colonies and a captive breeding program and, if done, how to implement such a program (Walls et al., 2015). This workshop addressed the very important need to dramatically increase reproductive success (from egg to metamorphosed individuals). Animals have been brought into captivity to figure out the most efficient husbandry techniques so that salamanders can be reared to sexual maturity to become part of an assurance colony. Only one species of the genus *Ambystoma* has been bred successfully in captivity, but it is necessary to try to work out captive methods in case it becomes an option of last resort to prevent extinction. If these techniques are successful, it was deemed the most likely approach to acquire sufficient numbers of salamanders to conduct recovery efforts beyond habitat restoration. It is unlikely, given the current disjunct distribution of populations, that any meaningful progress toward recovery can happen without some form of reintroduction, or translocation. The common denominator in all potential recovery actions is having a sufficient number of animals that can be used for these purposes.

A subsequent structured decision making workshop was held in February 2015 in which participants discussed specific management actions in the near term for *A. cingulatum* at St. Marks National Wildlife Refuge (O'Donnell et al., 2015). The techniques developed for the refuge will be applicable to restoration efforts in other areas and for the sibling species, *A. bishopi*.

Given the long-term nature and challenge of developing a captive breeding program for both reticulated and frosted flatwoods salamanders (Fenolio et al., 2014), the participants of this second workshop explored additional *in situ* methods to significantly increase survival of eggs and larvae of flatwoods salamanders to metamorphosis. Existing methodologies that are known to improve larval survival in other ambystomatid salamanders will be used in this effort. By rearing amphibian larvae in mesocosms (cattle tanks), the extremely low survival that has been observed in nature (e.g., from egg to metamorphosed juvenile, less than 1.0% survival for *A. annulatum* and *A. maculatum* [Semlitsch et al., 2014; Anderson et al., 2015] and as low as 1.0-3.3% for *A. maculatum* [Shoop, 1974]) can be increased to as high as 90% (in the absence of predators) in mesocosms (R. D. Semlitsch, pers. comm.; T. L. Anderson, pers. comm.; Anderson and Semlitsch, 2014; Anderson and Whiteman, 2015).

Effects of Hurricane Michael

On October 10, 2018, Hurricane Michael made landfall along the Florida Panhandle as a massive Category 5 hurricane with maximum sustained winds of 161 mph and a pressure of 919 mega bars (https://www.weather.gov/tae/HurricaneMichael2018). All residents including wildlife on the northern Gulf of Mexico's coast experienced a storm surge of 9 to 14 feet. This storm continued inland all the way to interior southern Georgia as a high Category hurricane resulting in excessive wind damage and destruction to an area from Panama City Beach to Mexico Beach to Cape San Blas (https://www.weather.gov/tae/HurricaneMichael2018). USGS partners working with the Service and the Florida Fish and Wildlife Conservation Commission through personal observations, unpublished data, and that summarized by Walls et al. (2019) have indicated:

- Storm surge pushed seawater into some ephemeral freshwater ponds used for breeding by the frosted flatwoods salamander.
- Not all ephemeral wetlands were inundated, but salt concentrations in inundated ponds ranged from 11 to over 200 times the normal salt concentrations.
- Populations at St Marks NWR took the most immediate damage. The majority of occupied ponds were inundated with salt water and all ponds were deeply flooded for several months after Hurricane Michael.
- 80 to 90 percent of standing trees were blown over or knocked down at St. Marks. Frosted flatwoods salamander individuals observed are underweight. The 2019 breeding season appears to be a near complete failure.
- Effects of Hurricane Michael were less severe, but still extremely bad on Apalachicola National Forest. This area is still being investigated.

2. Five-Factor Analysis:

a. Present or threatened destruction, modification or curtailment of its habitat or range:

The main threat to the flatwoods salamander is loss of both its longleaf pine/slash pine flatwoods terrestrial habitat and its isolated, seasonally inundated breeding habitat. The combined pine flatwoods (longleaf pine-wiregrass flatwoods and slash pine flatwoods) historical acreage was approximately 32 million ac (12.8 million ha) (Wolfe et al., 1988; Outcalt, 1997). The combined flatwoods acreage has been reduced to 5.6 million ac (2.27 million ha) or approximately 18% of its original extent (Outcalt, 1997). These remaining pine flatwoods (non-plantation forests) areas are typically fragmented and degraded, with second-growth forests.

Many ecologists consider fire suppression to be the primary reason for the degradation of remaining longleaf pine forests. The disruption of the natural fire cycle has resulted in an increase in hardwood midstory and understory and a decrease in herbaceous ground cover (Wolfe et al., 1988; Gorman et al., 2013). Ponds surrounded by pine plantations and protected from the natural fire regime may become unsuitable flatwoods salamander breeding sites due to canopy closure and the resultant reduction in emergent herbaceous vegetation needed for egg deposition and larval development sites (Palis, 1993). In addition, lack of fire within the pond during periods of dry-down may result in chemical and physical (vegetative) changes that are unsuitable for the salamander (Bishop and Haas, 2005; Gorman et al., 2013). Large scale prescribed fire is often accomplished in the dormant season, and can have negative effects on salamander habitat (Bishop and Haas, 2005). However, these burns can be important for reducing woody fuels and decreasing wildfire danger, but more emphasis should be placed on burning the sites when they are dry while avoiding burning when salamanders may be migrating to and from the pond. Follow-up burns should be used to ensure wetlands benefit from fire even when prescribed fires are incomplete or do not pass through the basin.

Fragmentation of the longleaf pine ecosystem, resulting from habitat conversion, threatens the survival of the remaining flatwoods salamander populations. Large tracts of intact longleaf pine flatwoods habitat are fragmented by roads and pine plantations. Most flatwoods salamander populations are widely separated from each other by unsuitable habitat. General ecological studies have shown that the loss of fragmented populations is common, and recolonization is critical for their regional survival (Fahrig and Merriam, 1994; Burkey, 1995). After local extirpation, amphibian populations may be unable to recolonize areas due to their physiological constraints, relatively low mobility, and site fidelity (Blaustein et al., 1994).

Road construction in the last two decades destroyed an historic breeding pond (of *A. bishopi*) in Escambia County, Florida. Roads also contribute to habitat fragmentation by isolating blocks of remaining contiguous habitat. They may disrupt migration routes and dispersal of individuals to and from breeding sites. In addition, vehicles may also cause the death of flatwoods salamanders during migrations across roads (Means, 1996). Road construction is also a recurring threat in the remaining flatwoods salamander habitats. Roads generally can cause disruptions to groundwater and sheetflow, and have serious direct and indirect impacts on the breeding ponds.

Conversion of natural pine flatwoods to intensively managed (i.e., impacted by heavy mechanical site preparation, high stocking rates, and low fire frequencies) slash or loblolly pine plantations often degrades flatwoods salamander habitat by creating well-shaded, closed-canopied forests with an understory dominated by shrubs or pine needles (Means et al., 1996). According to Enge et al. (2014), commercial forestry using silvicultural Best Management Practices (Florida Forest Service, 2012) will likely extirpate flatwoods salamander populations over time. More favorable practices for ephemeral pond-breeding amphibians are provided by Calhoun and deMaynadier (2004) and Bailey et al. (2006). Disturbance-sensitive groundcover species, such as wiregrass, dropseed, and perennial forbs are either greatly reduced in extent or are replaced by weedy pioneering species (Schultz and White, 1974; Moore et al., 1982; Outcalt and

Lewis, 1988; Hardin and White, 1989). Wiregrass is an herbaceous species often lost in habitat conversion and considered an indicator of site degradation from fire suppression and/or soil disturbance (Clewell, 1989). It also appears to be absent from areas where flatwoods salamanders no longer occur (Palis, 1997). Past pine plantations were created on natural pine sites, whereas future pine plantations will increasingly be created on former agricultural land (Wear and Greis, 2002); thus this type of habitat conversion is not considered an on-going threat to flatwoods salamanders.

Land use conversions to urban development and agriculture eliminated large acreages of pine flatwoods in the past (Schultz, 1983; Stout and Marion, 1993; Outcalt and Sheffield, 1996; Outcalt, 1997). State forest inventories completed between 1989 and 1995 indicate that flatwood salamander habitat losses through land use conversion are still occurring (Outcalt, 1997). Urbanization, especially in the panhandle of Florida and around major cities, is reducing the available pine forest habitat. Wear and Greis (2002) identified conversion of forests to urban land uses as the most significant threat to southern forests. These authors predicted that the South could lose about 12 million forest acres (about 8% of its current forest land) to urbanization between 1992 and 2020.

Forestry management which includes intensive site preparation may adversely affect flatwoods salamanders both directly and indirectly (Means et al., 1996). Bedding (a technique in which a small ridge of surface soil is elevated as a planting bed) alters the surface soil layers, disrupts the site hydrology and often eliminates the native herbaceous groundcover. This can have a cascading effect of reducing the invertebrate community that serves as a food source for flatwoods salamander adults. Intensive site preparation also destroys subterranean voids such as crayfish burrows, root channels, etc. that are the probable fossorial habitats of adult salamanders and may result in entombing, injuring, or crushing individuals.

Flatwoods salamander wetland breeding sites have also been degraded or altered. The number and diversity of these often small wetlands have been reduced by alterations in hydrology, agricultural and urban development, incompatible silvicultural practices, shrub encroachment, dumping in or filling of ponds, conversion of wetlands to fish ponds, domestic animal grazing, and soil disturbance (Vickers et al., 1985; Ashton, 1992). Hydrological alterations, such as those resulting from ditches created to drain flatwoods sites or fire breaks and plow lines, for example, represent one of the most serious threats to flatwoods salamander breeding sites. Lowered water levels and shortened hydroperiods at these sites may prevent successful flatwoods salamander recruitment.

Off-road vehicle (ORV) use within flatwoods salamander breeding ponds and their margins severely degrades wetland habitat. Continued use of sites by ORVs can completely degrade the integrity of breeding sites by killing herbaceous vegetation and rutting the substrate, which can alter hydrology. There is also the potential for direct injury and/or mortality of flatwoods salamanders by ORVs at breeding sites.

b. Overutilization for commercial, recreational, scientific, or educational purposes:

As in the listing rule for both of these salamander species, overcollecting continues to not appear to be a threat to the frosted flatwoods salamander at this time. The pet trade still deals in wild caught amphibians. Rarity drives up the value and price of these creatures, so that as these animals become rarer, they become more valuable in the pet trade market. With the relatively few populations currently known, this is a potential threat that we will continue to closely monitor. At this time, though, we continue to have no evidence of collection of this species. We will also closely work with partners and monitor intensive survey work to ensure it has no negative effect on small populations of this species. We have determined though that overutilization for commercial, recreational, scientific, or educational purposes is not a threat to the frosted flatwoods salamander at this time. Although overcollecting does not appear to be a threat at this time, it could become a threat if the location of known breeding sites were made available to the general public. Larval flatwoods salamanders are threatened in some wetlands by the harvest of crayfish as fish bait (Palis, 1996). Some individuals are likely killed, but this practice is not considered a significant threat to the species as a whole, mostly because it does not happen on a large enough scale.

c. Disease or predation:

Disease is currently unknown in natural populations of flatwoods salamanders. However, Whiles et al. (2004) found a parasitic nematode (*Hedruris siredonis*, family Hedruridae) in larvae from South Carolina and Florida. This parasite has been found in other ambystomatids and can cause individuals to be become undersized and thin, thus reducing their fitness (Whiles et al., 2004). The infestations were not considered heavy and were probably not having a negative impact on the larvae studied (Whiles et al., 2004). However, environmental degradation may change the dynamics between salamander populations and normally innocuous parasites (Whiles et al., 2004).

Ranaviruses in the family Iridoviridae and chytrid fungus may be other potential threats, although the susceptibility of the frosted flatwoods salamander to these diseases is unknown. Ranaviruses have been responsible for die-offs of tiger salamanders throughout western North America and spotted salamanders (*A. maculatum*) in Maine (Daszak et al., 1999). The chytrid fungus *Batrachochytrium dendrobatidis,* or Bd), which causes chytridiomycosis in many amphibians, has been discovered and associated with mass mortality in tiger salamanders in southern Arizona and California, and the Santa Cruz long-toed salamander (*A. macrodactylum croceum*) (Vredenburg and Summers, 2001; Davidson et al., 2003; Padgett-Flohr and Longcore, 2005). This chytrid fungus has been found at an *A. bishopi* breeding wetland on Eglin Air Force Base and at a site near occupied breeding wetlands but, to date, no impact has been observed on *A. bishopi* (T. Gorman, Virginia Tech, pers. comm.). Moreover, Bd has been detected at known flatwoods salamander breeding sites at St. Marks NWR. (Barichivich Pers comm (2015). The detection of this pathogen from individual *A. cingulatum*, however, has not yet been documented (W.J. Barichivich and D. Calhoun, pers. comm., 30 July 2015). Recently, a new species of chytrid fungus, *Batrachochytrium salamandrivorans* (Bsal), was isolated from a mortality event that caused the near extinction of a population of fire salamanders (*Salamandra salamandra*) in Europe (Martel et al., 2013; Spitzen-van der Sluijs et al., 2013). Currently, it is not known whether any amphibian mortality events in the U.S. are attributable to this pathogen, or whether this new species even occurs in this country. Efforts to begin sampling for Bsal in the U.S. are currently underway by the USGS Amphibian Research and Monitoring Initiative (ARMI) (S. Walls, USGS; pers. comm.).

Predation
Exposure to increased predation by fish is a potential threat to the frosted flatwoods salamanders when ephemeral wetland breeding sites are changed to, or connected to, more permanent wetlands inhabited by fishes that are not typically found in ephemeral wetlands. Wetlands may be modified specifically to serve as fish ponds or sites may be altered because of drainage ditches, firebreaks, or vehicle tracks which can all provide avenues for fish to enter the wetlands from other water bodies. Studies of other ambystomatid species have demonstrated a decline in larval survival in the presence of predatory fish (Semlitsch, 1987; 1988).

Red imported fire ants (*Solenopsis invicta*) are potential predators of frosted flatwoods salamanders, especially in disturbed areas. This species has been seen in areas disturbed by the installation of drift fences at known breeding sites (T. Gorman, pers. comm.). Controlling fire ants in areas with a high degree of disturbance can be accomplished by using hot water rather than pesticides (Tschinkel and King, 2007), so on a small scale fire ants can be controlled around breeding sites. Further study on the effects of fire ants on flatwoods salamanders is recommended. Because the severity and magnitude, as well as the long term effect of fire ants on frosted flatwoods salamander populations is currently unknown. We consider predation to be a threat to the frosted flatwoods salamander at this time.

d. Inadequacy of existing regulatory mechanisms:

The Clean Water Act (CWA) covers ephemeral wetlands, when they impact downstream waters and, in many cases, wetlands used by flatwoods salamanders are connected to downstream waters. On April 21, 2014, the U.S. Environmental Protection Agency and the U.S. Army Corps of Engineers, in response to the SWANCC and Rapanos decisions, proposed clarifications to the CWA that would affect which types of waters would be considered jurisdictional under the Act (see

U.S. Army Corps of Engineers and U.S. Environmental Protection Agency "Definition of Waters of the United States under the Clean Water Act," CFR Docket ID No. 79 FR 22188). The clarifications (as of this writing) include reasserting CWA jurisdiction to wetlands adjacent to (i.e., bordering, contiguous, and neighboring) jurisdictional lakes, rivers, and streams. Furthermore, wetlands that are other waters, or those that are nonadjacent to waters of the United States, will have jurisdiction assessed on a case-by-case basis. However, it is unclear how these newly released proposed regulations will aid in recovery of flatwoods salamanders. The proposed regulations also allow the evaluation of other waters either alone or in combination with other similarly situated waters in the region to determine whether they significantly affect the chemical, physical, or biological integrity of traditional navigable waters, interstate waters, or the territorial seas. Other waters are similarly situated when they perform similar functions and are located sufficiently close together or sufficiently close to a water of the United States. The fact that CWA jurisdiction may be extended to geographically isolated wetlands (GIWs) on the basis of a watershed assessment of connectivity and the effect of GIWs on downstream waters suggests that watersheds in regions with large amounts of functioning GIWs (such as the prairie pothole region of the Upper Midwest and Canada, California vernal pools, Carolina bays and cypress ponds of the southeastern United States and other GIWs) may gain CWA protections under these new rules should they be finalized.

There are few, if any, mechanisms in place to adequately protect ephemeral wetlands, like those necessary for successful flatwoods salamander breeding. This includes the breeding ponds themselves as well as the surrounding upland habitat. The exceptions to this are the federally designated Critical Habitat Units for *A. cingulatum* but this only applies to actions that involve a federal nexus, not to state or private sector actions.

e. Other natural or manmade factors affecting its continued existence:

Nonindigenous feral swine can significantly impact frosted flatwoods salamander breeding sites by rooting of critical complex herbaceous vegetation, so intensive approaches (e.g., control measures and fencing) may be needed to avoid degradation to occupied sites and sites going through restoration.

Invasive and exotic plant species threaten to further degrade existing habitat. Invasive and exotic vegetation can change the structure of the habitat by creating thick midstory and displacing native vegetation. Flatwoods salamander habitat management plans will need to address threats posed by these plants and develop strategies to control them.

Climate change, especially in combination with other stressors, is a daunting challenge for the persistence of amphibians (Walls et al., 2013). Sea level rise is becoming and will likely continue to increase as a threat to the extant populations of both species of flatwoods salamanders. Some of the remaining populations occur in very low lying areas within a short distance of the Gulf Coast (St. Marks NWR). These populations are already vulnerable to high tide storm-influenced saltwater intrusion, and these threats will likely increase as sea level rise from global climate change continues. As described earlier, Hurricane Michael is a clear indication of a massive storm with strong storm surge that can impact this animal. We are still assessing the storm's impact with partners but the 2019 breeding season appears to be a near complete failure. High salinity, extreme water levels, and damage to the cover of plants in the ponds are likely strong indicators of the season's failure as we continue to collaborate with partners from USGS and FWC. Climate change models predict the occurrence of more variable patterns of precipitation in the future, with longer droughts and larger (but fewer) rainfall events, in addition to increased temperatures (Heisler-White et al., 2008; Lucas et al., 2008). Increases in the occurrence of drought and heavy precipitation events are known to be impacting a variety of amphibians, including those that breed in ephemeral wetlands (Walls et al., 2013). In addition to rainfall amounts, the timing of precipitation events is an important stimulus for reproduction in many pond-breeding amphibians (Walls et al., 2013). Thus, climate change may have an impact on frosted flatwoods salamanders by altering the timing of fall and winter rains, as well as creating drier winters than historically would have occurred (Chandler, 2015). This impact has been observed for *A. bishopi* at Eglin Air Force Base, where the adult population steadily declined after the first year of the study, likely due to drought (Gorman et al., 2014). Similarly, this same decline in the adult population was observed in an *A. cingulatum* breeding wetland over the course of a three-year winter drought (Palis et al., 2006).

Small populations, especially concentrated in small areas, are more susceptible to stochastic events that could negatively impact the entire population. Examples include salt water intrusion from storm surge (for those areas near enough to the coastline) extended drought, introduced contaminants, fire exclusion, among others.

Pesticides and herbicides may pose a threat to amphibians such as the frosted flatwoods salamander, because their permeable eggs and skin readily absorb substances from the surrounding aquatic or terrestrial environment (Duellman and Trueb, 1986). Negative effects on amphibians may include delayed metamorphosis, paralysis, reduced growth rates, and mortality (Bishop, 1992). Herbicides used in the vicinity of flatwoods salamander breeding ponds may alter the density and species composition of vegetation surrounding a breeding site and reduce the number of potential sites for egg deposition, larval development, or shelter for migrating salamanders. However, the potential for negative effects from pesticide and herbicide use can be reduced by following label directions for application and avoiding aerial spraying over areas adjacent to breeding ponds (Tatum, 2004). Aerial spraying of herbicides over outdoor ponds has been shown to reduce zooplankton diversity, a food source for larval frosted flatwoods salamanders and cause very high (68-100 percent) mortality in tadpoles and juvenile frogs (Relyea, 2005). Additionally, herbicides, if used according to the label and used in specific applications, may aid in restoration of upland and wetland habitat that have been altered by fire suppression and/or exclusion.

Another natural threat in relation to the presence of predatory fish mentioned under Factor C is these fish have a marked effect on invertebrate communities and alters prey availability for larval salamanders with the potential for negative effects on larval fitness and survival (Semlitsch, 1987).

D. Synthesis

In summary, given the low detectability of this species, intermittent or even sustained multi-year drought, and inconsistent monitoring of breeding wetlands in many areas, it is difficult to make a strong inference of its status. However, despite these challenges, we believe the status of this species has continued to decline based on the best available data we have been able to gather since our 2009 rule.

Since 2010, survey data indicate that there has been a 59% decline in the total number of populations that have had some sampling between 2010-2015 (i.e., excluding those three populations annotated by Table 1). In some years, a portion of the range was sampled (except for those private landowners that declined access), but surveys were not comprehensive across the entire range until 2014 and 2015. Recent surveys have occurred mostly (but not exclusively) on public lands, which reflects the majority of the landownership of critical habitat units for this species (74% of the area designated as critical habitat occurs on public lands, with 26% on privately-owned lands). Regardless of ownership, as of 2014 a significant percentage (nearly 40%) of the land area categorized as critical habitat was not suitable breeding habitat for frosted flatwoods salamanders (W.J. Barichivich, USGS, pers. comm.) and provides additional support to the continued decline. Much of the Critical Habitat on both the ANF and SMNWR has not had the frequency or intensity of fire in the growing season has gradually degraded from lack of proper growing season fire. Lastly, survey data from 2014-2015 indicates that occupancy of breeding wetlands within extant population is also in decline as compared with survey data collected from 2002 to 2006 (surveys were conducted by FWC and J. Palis on both public and private lands [K. Enge, FWC and J. Palis, Palis Consulting, pers. comm.]).

Many of the threats (mentioned above) continue to plague this species and it is quite possible that one or two stochastic events (hurricane and/or drought), as Hurricane Michael has done to the St. Marks area populations, could push the remaining populations to extirpation given their current locations, vulnerability, and low numbers. Everything reasonably possible is being explored and implemented to prevent extinction and promote recovery, including using *in situ* techniques to increase survival of individuals, *ex situ* approaches to develop captive breeding, and habitat management of both existing and potential habitat.

III. RESULTS

A. Recommended Classification:

____ Downlist to Threatened
_x__ Uplist to Endangered
____ Delist (*Indicate reasons for delisting per 50 CFR 424.11):*

____ No change is needed

Based on the best available data we have been able to gather since our 2009 rule, we believe that *A. cingulatum* has experienced dramatic population declines in the last five years. Evidence for this conclusion is as follows:

1. Based on the number of populations (as defined in this document), there has been a 59% decline of the 22 populations of frosted flatwoods salamanders that were sampled between 2010 and 2015 (Table 1). This is more severe than losses of its sibling species, *A. bishopi* (already listed as endangered) during the last 5 years. We attribute this difference between the two species to the greater focus on population monitoring and restoration of breeding habitat for *A. bishopi* in recent years (Gorman et al., 2013). Moreover, although the 2009 ruling noted the declining trend in *A. bishopi*, it did not include recent evidence of further losses for *A. cingulatum*. For example, in a review of survey records for Fort Stewart, GA, Bevelhimer et al. (2008) demonstrated a steep decline in the number of ponds occupied by *A. cingulatum*, from near 20 sites to a single pond, which has become the only documented breeding success in a natural pond at Fort Stewart since 1999. *A. cingulatum* was not evaluated for status change after the 2009 rule split the species. *A. bishopi* was reclassified as endangered. Since 2009, examination of both species through the same lens with respect to classification strongly suggests that uplisting *A cingulatum* is prudent.

2. Both species of flatwoods salamanders have experienced severe losses of individual historic breeding sites (overall loss of 89.3% for both species combined; Semlitsch et al., 2017). When losses are analyzed separately for each species, however, they have been more extensive for *A. cingulatum* (a reduction from 333 unique localities to 31 [91% loss]) than for *A. bishopi* (loss of from 106 locations to 21, an 80% loss; W.J. Barichivich, USGS, unpubl. data).

3. Based on a recent GIS analysis by USGS (W.J. Barichivich, USGS, pers. comm.), nearly 40% of the land area categorized as critical habitat is not suitable breeding habitat for frosted flatwoods salamanders. Critical Habitat within the range of *A. cingulatum* has not received the growing season fire of sufficient frequency or intensity to maintain good breeding habitat. This provides additional support to the likelihood of a continued decline since listing.

4. The most recent survey data available (from 2014-2015; W.J. Barichivich [USGS], J. Mott [TNC], K. Enge, and E. Hill [FWC], unpubl. data) indicate that occupancy of breeding wetlands within extant populations is also in decline, compared to survey data collected from 2002 to 2006 (earlier surveys conducted by FWC and J. Palis on both public and private lands [K. Enge, FWC, and J. Palis, Palis Consulting, pers. comm.]). According to these latest data, even Apalachicola National Forest and St. Marks National Wildlife Refuge, historically touted as the "strongholds" for populations of *A. cingulatum*, have experienced a 53% (37 out of 79 known sites are still occupied) and 80% (10 out of 51 sites confirmed to still be occupied) loss of occupied breeding sites, respectively, in recent years (since 2009 for Apalachicola National Forest). Moreover, captures per unit effort (CPUE) at St. Marks National Wildlife Refuge were extremely low in 2015: out of 4960 trap/nights, only 13 individual larvae were caught in a total of 8 ponds (W.J. Barichivich, USGS, unpubl. data). In addition to drought that may impact many sites across a large spatial scale, the remaining populations in low lying coastal areas (e.g., those at St. Marks) are highly vulnerable to other stochastic events associated with climate change, such as sea level rise, saltwater inundation and flooding due to hurricane-associated storm surge, as indicated by the recent results following Hurricane Michael in 2018.

5. Populations belonging to this species' eastern clade (Pauly et al., 2012) have dwindled to a single breeding pond in each of SC and GA. Thus, the eastern clade of *A. cingulatum* is severely imperiled, almost to the point of extirpation. Loss of this clade would result in loss of one half of the genetic representation of this species.

6. The primary threats to this species are the loss of its flatwoods habitat and the resulting habitat fragmentation, which further isolates populations and disrupts metapopulation dynamics. Fortunately, remaining populations are predominantly on public lands where its habitat may be protected from further loss. However, habitat deterioration (via fire suppression, conversion to pine plantations, and hydrological alteration) has rendered much of the remaining habitat unsuitable for long-term persistence of this species. Recovery will require natural resource managers to make long-term commitments to habitat restoration and management for this species. Commercial forestry using common state-recommended silvicultural Best Management Practices will likely extirpate flatwoods salamander populations over time (Enge et al., 2014). The severity of these threats is compounded by environmental stochasticity (e.g., drought) and the enhanced vulnerability of small populations to such events.

   Given the decline and resulting population isolation over the last few years, the threats faced by *A. cingulatum*, low numbers, the current state of breeding habitats, we recommend the status of *A. cingulatum* be changed to endangered. This would be consistent with the threats and status in comparison with its close relative, *A. bishopi*, and other species listed as endangered.

B. New Recovery Priority Number __2___

We recommend that the recovery priority number (RPN) be revised from 5 to 2. An RPN of 2 indicates this is a species with high threat yet high recovery potential. Several threats still face this species, including among others, loss of high-quality habitat, prolonged droughts impacting breeding wetlands, and occurrence in a reduced geographic area.

Restoration of frosted flatwoods salamander habitat has been a long-term focus for Apalachicola National Forest, the biggest stronghold for frosted flatwoods salamanders. In 2015, personnel at St. Marks National Wildlife Refuge made habitat restoration for this species (via an increased frequency of prescribed burning and other methods), along with *in situ* "assisted metamorphosis", a top priority in their management actions. The process of restoring habitat takes time, but combining habitat restoration with *in-situ* assistance techniques (i.e., cattle-watering tanks to increase survival to metamorphosis) shows strong promise to both restore habitat, and stabilize and then increase the number of individuals within a population (O'Donnell et al., 2015). The approach of using cattle-watering tanks as mesocosms is a well-established technique in the field of amphibian ecology which, when salamanders are reared at low densities in the absence of predators with an ample food supply, has repeatedly been demonstrated to increase overall survival of individuals from the egg to the metamorphic life history stage, including for *A. annulatum*, a close relative of *A. cingulatm* with a similar breeding strategy (Shoop, 1974; Anderson and Semlitsch, 2014; Semlitsch et al., 2014; Anderson et al., 2015; Anderson and Whiteman, 2015; R. D. Semlitsch, pers. comm.; T. L. Anderson, pers. comm.). Moreover, under these circumstances, individuals can metamorphose at a larger body size, which has been demonstrated empirically to be related to correlates of adult fitness in at least two other species of *Ambystoma* (*A. talpoideum* and *A. opacum*; Semlitsch et al., 1988; Scott, 1994). Specifically, for both of these species, larger juveniles at metamorphosis were also larger adults at first reproduction which, in turn, produced larger clutches of eggs at a younger age (Semlitsch et al., 1988; Scott, 1994). Further, this *in situ* approach has successfully been employed for the conservation and recovery of another federally endangered amphibian, *Rana sevosa* (USFWS, 2014). Last, methods to rear this species in captivity are underway (M. Mandica, The Amphibian Foundation, pers. comm.) and if this process is successful, it may provide a captive source population for future repatriations of formerly occupied sites that have been properly restored (Walls et al., 2015). This new potential, along with the implementation of methods to boost production of metamorphosing individuals (undertaken since the 2009 final rule), will offer a suite of approaches for more rapid population recovery by increasing the survivability of larvae and improving habitat conditions. These promising new developments improve the recovery potential for this species and justifies the upgrade from an RPN of 5 to an RPN of 2.

C. If a reclassification is recommended, indicate the Listing and Reclassification Priority Number (FWS only):

Reclassification (from Threatened to Endangered) Priority Number 2

IV. RECOMMENDATIONS FOR FUTURE ACTIONS

Habitat Restoration
Having suitable, available, breeding and upland habitat is of utmost importance in any restoration/recovery work for a species. Every effort should be made to first maintain suitable wetland breeding and upland habitat where it occurs and expand /restore such habitat to suitable and preferred condition as soon as possible. Keeping habitat from being degraded by lack of fire, changes in hydrology, and potential invasion of exotic and normally fire excluded plants will be a significant challenge. Frequent (1-3 years) lightning season prescribed fire, as well as careful use of dormant season fire in instances where ponds remained dry and reproduction was not successful. Maintenance of hydrological function is also one of the most important tools for restoring habitats and keeping them functional for salamander persistence. Additionally, restoration and management for flatwoods salamanders could be more effectively focused on the need for demographic connectivity and recolonization that are essential for metapopulation stability (Semlitsch et al., 2017).

Surveys/inventories
The surveys conducted for both species of flatwoods salamander in the Winter/Spring of 2014 and 2015 constitute the most comprehensive survey efforts since at least the 1993-1994 surveys conducted by John Palis (Palis, 1995b). Surveys have occurred by various agencies (e.g., FWC) and individuals each year but rarely is there comprehensive information collected in a single season that gives an adequate 'snapshot' of the range – wide status of the species. In short, annual surveys that adequately allow us to evaluate status should be completed in the near term. The precarious situation of both species of flatwoods salamanders is such that these data may be critical to preventing extinction.

Monitoring
Monitoring programs need to be refined so that data will be compatible among all participating partners and to develop a better understanding of the current status of the species with particular emphasis on private lands and other populations that have not been sampled as intensively. Additionally, multiple surveys should be conducted at known/historic sites each year for a minimum of 3-5 years to develop a base-line for the current status. Relying on single year surveys may provide misleading results, because weather conditions and other variables can obscure detectability or prevent sampling altogether (e.g., sampling of aquatic larvae, the most widely recommended sampling technique for range-wide surveys [Bishop et al., 2006], is compromised during drought years with dry winters).

The continued decline of numbers of individual breeding sites and populations, as well as continued habitat degradation, will require much more intensive monitoring to remain well informed on the status of the species. Annual status updates are recommended for the coming years until the next status review. Specific attention and effort to gain access to currently inaccessible private lands that historically are known to have flatwoods salamanders present needs to be initiated.

Research
Capture-mark-recapture studies that provide information on vital rates (e.g., individual growth and survival) and demographic parameters of populations are needed to develop a Population Viability Analysis (PVA), which is essential for recovery planning (Walls et al., 2015). Very little is currently known about the ecology of juveniles and adults during the portion of the year that they are using habitat in the uplands surrounding breeding ponds.

Population Management
Head starting, "assisted metamorphosis", is recommended to increase larval survival to the metamorph life-stage and to help increase the numbers of individuals more rapidly than is possible under current natural circumstances (O'Donnell et al., 2015; Walls et al., 2015). Implementation of these options is particularly essential because one stochastic event, such as recent results from Hurricane Michael have indicated for the St. Marks NWR area, could extirpate many coastal breeding populations.

V. REFERENCES:

Akçakaya, H.R., Mills, G. and Doncaster, C.P. (2007) The role of metapopulations in conservation. Key Topics in Conservation Biology (eds. D. Macdonald and K. Service), pp. 64–84. Blackwell Publishing, Oxford, UK.

Anderson, J. D. and G. K. Williamson. 1976. Terrestrial mode of reproduction in *Ambystoma cingulatum*. Herpetologica 32:214-221.

Anderson, T. L., D. J. Hocking, C. A. Conner, J. E. Earl, E. B. Harper, M. S. Osbourn, W. E. Peterman, T. A. G. Rittenhouse, and R. D. Semlitsch. 2015. Abundance and phenology patterns of two pond-breeding salamanders determine species interactions in natural populations. Oecologia 177:761-773.

Anderson, T. L., and R. D. Semlitsch. 2014. High intraguild predator density induces thinning effects on and increases temporal overlap with prey populations. Population Ecology

56:265-273.

Anderson, T. L., and H. Whiteman. 2015. Non-additive effects of intra- and interspecific competition between two larval salamanders. Journal of Animal Ecology: 10.1111/1365-2656.12335.

Ashton, R. E., Jr. 1992. Flatwoods salamander, *Ambystoma cingulatum*. Pp. 39-43 *in*: P. E. Moler, editor. Rare and Endangered Biota of Florida, Volume Three, Amphibians and Reptiles. University of Florida Press, Gainesville, Florida. 291 pp.

Bailey, M. A., J. N. Holmes, K. A. Buhlmann, and J. C. Mitchell. 2006. Habitat management guidelines for amphibians and reptiles of the southeastern United States. Partners in Amphibian and Reptile Conservation Technical Publication HMG-2, Montgomery, Alabama, USA. 88pp.

Bevelhimer, M. S., Stevenson, D. J., Giffen, N. R., & Ravenscroft, K. (2008). Annual surveys of larval *Ambystoma cingulatum* reveal large differences in dates of pond residency. Southeastern Naturalist, 7(2), 311-322.

Bishop, C. A. 1992. The effects of pesticides on amphibians and the implications for determining causes of declines in amphibian populations. Pgs. 67-70 *in*: C. A. Bishop and K. E. Pettit (eds.). Declines in Canadian amphibian populations: designing a national monitoring strategy. Occasional Paper Number 76, Canadian Wildlife Service, Ottawa, Ontario.

Bishop, D. C. and C. A. Haas. 2005. Burning trends and potential negative effects of suppressing wetland fires on flatwoods salamanders. Natural Areas Journal 25:290-294.

Blaustein, A. R., D. B. Wake, and W. P. Sousa. 1994. Amphibian declines: judging stability, persistence, and susceptibility of populations to local and global extinctions. Conservation Biology 8:60-71.

Burkey, T. V. 1995. Extinction rates in archipelagoes: implications for populations in fragmented habitats. Conservation Biology 9:527-541.

Carr, A. F., Jr. 1940. A contribution to the herpetology of Florida. University of Florida Publication, Biological Science Series 3:1-118.

Calhoun, A. J. K., and P. G. deMaynadier. 2004. Forestry habitat management guidelines for vernal pool wildlife. MCA Technical Paper No. 6, Metropolitan Conservation Alliance, Wildlife Conservation Society, Bronx, New York, USA. 32pp.

Chandler, H. C. 2015. The effects of climate change and long-term fire suppression on ephemeral pond communities in the southeastern United States. Thesis, Virginia Tech, Blacksburg, VA, USA.

Clewell, A.F. 1989. Natural history of wiregrass (*Aristida stricta* Michx., Gramineae). Natural Areas Journal 9:223-233.

Daszak, P., L. Berger, A.A. Cunningham, A.D. Hyatt, D.E. Green, and R. Speare. 1999. Emerging infectious diseases and amphibian population declines. Emerging Infectious Diseases 5:735-748.

Davidson, E.W., M. Parris, J.P. Collins, J.E. Longcore, A.P. Pessier, and J. Brunner. 2003. Pathogenicity and transmission of chytridiomycosis in tiger salamanders (*Ambystoma tigrinum*). Copeia 2003:601-607.

Duellman, W. E. and L. Trueb. 1986. Biology of amphibians. McGraw-Hill, New York, New York. 670 pp.

Enge, K. M., A. L. Farmer, J. D. Mays, T. D. Castellón, E. P. Hill, and P. E. Moler. 2014. Survey of winter-breeding amphibian species. Final Report. Florida Fish and Wildlife Conservation Commission, Florida State Wildlife Grants Project No. 92412216399. 31 December 2014. 136 pp.

Fahrig, L. and G. Merriam. 1994. Conservation of fragmented populations. Conservation Biology 8:50-59.

Fenolio, D., T. A. Gorman, K. C. Jones, M. Mandica, L. Phillips, L. Melde, H. Mitchell, and C. A. Haas. 2014. Rearing the federally endangered Reticulated Flatwoods Salamander, *Ambystoma bishopi*, from eggs through metamorphosis. Herpetological Review 45:62-65.

Florida Forest Service. 2012. Silviculture Best Management Practices. Florida Department of Agriculture and Consumer Services, Tallahassee, Florida, USA. 116pp.

Gorman, T. A., C. A. Haas, and D. C. Bishop. 2009. Factors related to occupancy of breeding wetlands by flatwoods salamander larvae. Wetlands 29:323-329.

Gorman, T. A., C. A. Haas, and J. G. Himes. 2013. Evaluating methods to restore amphibian habitat in fire-suppressed pine flatwoods wetlands. Fire Ecology 9:96-109.

Gorman, T. A., S. D. Powell, K. C. Jones, and C. A. Haas. 2014. Microhabitat characteristics of egg deposition sites used by Reticulated Flatwoods Salamanders. Herpetological Conservation and Biology. 9:543-550.

Hardin, E. D. and D. L. White. 1989. Rare vascular plant taxa associated with wiregrass (*Aristida stricta*) in the Southeastern United States. Natural Areas Journal 9:234-245.

Hill, E.P. 2013. *Ambystoma cingulatum*, courtship and oviposition. Herpetological Review

44:112–113.

Jones, K. C., P. Hill, T. A. Gorman, and C. A. Haas. 2012. Climbing behavior of flatwoods salamanders (*Ambystoma bishopi /A. cingulatum*). Southeastern Naturalist 11:537-542.

Lucas, R.W.; Forseth, I.N.; Casper, B.B. 2008. Using rainout shelters to evaluate climate change effects on the demography of *Cryptantha flava*. Journal of Ecology 96, 514-522.

Lickey, E. B. and G. L. Walker. 2002. Population genetic structure of bald cypress (*Taxodium distichum* [L.] Rich. Var. *distichum*) and pond cypress (*T. distichum* var. *imbricarium* [Nuttall] Croom): biogeographic and taxonomic implications. Southeastern Naturalist 1:131-148.

Martel A, Spitzen-van der Sluijs A, Blooi M, Bert W, Ducatelle R, Fisher MC, Woeltjes A, Bosman W, Chiers K, Bossuyt F, Pasmans F. 2013. *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. Proc. Natl. Acad. Sci. U. S. A. 110:15325–15329

Martof, B. S. and H. C. Gerhardt. 1965. Observations on the geographic variation in *Ambystoma cingulatum*. Copeia 1965:342-346.

Means, D. B. 1996. A preliminary consideration of highway impacts on herpetofauna inhabiting small isolated wetlands in the southeastern U.S. coastal plain. Pgs. 1-11 *in*: G. L. Evink, P. Garrett, D. Zeigler, and J. Berry (eds.). Trends in addressing transportation related wildlife mortality. Proceedings of the transportation related wildlife mortality seminar. State of Florida, Department of Transportation, Tallahassee, Florida.

Means, D. B., J. G. Palis, and M. Baggett. 1996. Effects of slash pine silviculture on a Florida panhandle population of flatwoods salamander (*Ambystoma cingulatum*). Conservation Biology 10:426–437.

Moore, W. H., B. F. Swindell, and W. S. Terry. 1982. Vegetative response to clearcutting and chopping in a north Florida flatwoods forest. Journal of Range Management 35:214-218.

O'Donnell, K. M., S. C. Walls, F. A. Johnson, J. Martin, S. S. Romañach , T. A. Gorman, A. F. Messerman, H. G. Mitchell, J. Mott, T. Peacock, J. Reinman, R. D. Semlitsch, G. Titus, and D. R. Smith. 2015. An Adaptive Management Approach to Habitat Restoration and Population Recovery of Flatwoods Salamanders. A Case Study from the Structured Decision Making Workshop. 8-13 February 2015, National Conservation Training Center, Shepherdstown, WV.

Outcalt K. W. 1997. Decline of the pine flatwoods of the southern Coastal Plain. Unpublished report, USDA Forest Service, Athens, Georgia. 10 pp.

Outcalt, K. W. and C. E. Lewis. 1988. Response of wiregrass (*Aristida stricta*) to mechanical site preparation. Pp. 1-12 in: L. C. Duever and R. F. Noss, editors. Proceedings of the symposium of wiregrass biology and management: maintaining groundcover integrity in longleaf pine ecosystems. KBN Engineering and Applied Sciences, Inc., Gainesville, Florida.

Outcalt, K. W. and R. M. Sheffield. 1996. The longleaf pine forest: trends and current conditions. Resource Bulletin SRS-9. U.S. Forest Service, Southern Research Station, Asheville, North Carolina.

Padgett-Flohr G. E. and J. E. Longcore. 2005. *Ambystoma californiense*. Fungal infection. Herpetological Review 36:50-51.

Palis, J. G. 1993. A status survey of the flatwoods salamander, *Ambystoma cingulatum*, in Florida. Unpublished report submitted to the U.S. Fish and Wildlife Service.

Palis, J. G. 1995a. Larval growth, development, and metamorphosis of *Ambystoma cingulatum* on the Gulf Coastal Plain of Florida. Florida Scientist 58:352-358.

Palis, J. G. 1995b. A survey of flatwoods salamander (*Ambystoma cingulatum*) breeding sites east of the Apalachicola River, Florida. Unpublished report submitted to the U.S. Fish and Wildlife Service.

Palis, J. G. 1996. Element stewardship abstract: flatwoods salamander (*Ambystoma cingulatum* Cope). Natural Areas Journal 16:49–54.

Palis, J. G. 1997. Distribution, habitat, and status of the flatwoods salamander (*Ambystoma cingulatum*) in Florida, USA. Herpetological Natural History 5:53-65.

Palis, J. G., M. J. Aresco, and S. Kilpatrick. 2006. Breeding biology of a Florida population of *Ambystoma cingulatum* (Flatwoods Salamander) during a drought. Southeastern Naturalist 5:1–8.

Palis, J. G. and D. B. Means. 2005. *Ambystoma cingulatum*. Pp. 608-609 in: Status and conservation of U.S. amphibians. Vol. 2, Species Accounts. M. J. Lannoo, ed. University of California Press, Berkeley, California.

Pauly, Gregory, Oliver Piskurek, and Bradley Shaffer. 2007. Phylogeographic concordance in the southeastern United States: the flatwoods salamander, *Ambystoma cingulatum*, as a test case. Molecular Ecology 16: 415-429.

Pauly, G. B., S. H. Bennett, J. G. Palis, and H. B. Shaffer. 2012. Conservation and genetics of the frosted flatwoods salamander (*Ambystoma cingulatum*) on the Atlantic coastal plain. Conservation Genetics 13:1-7.

Peterman, W. E., T. L. Anderson, B. H. Ousterhout, D. L. Drake, R. D. Semlitsch, and L. S. Eggert. 2015. Differential dispersal shapes population structure and patterns of genetic differentiation in two sympatric pond breeding salamanders. Conservation Genetics

16:59-69.

Petranka, J.W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington, D.C. 587 pp.

Powell, S. D., K. C. Jones, T. A. Gorman, and C. A. Haas. 2013. *Ambystoma bishopi* (Reticulated Flatwoods Salamander) egg survival after fire. Herpetological Review 44:290-291.

Safer, A. 2001. Natural history and ecology of the flatwoods salamander, *Ambystoma cingulatum*, on the Atlantic Coastal Plain. Unpublished Master's Thesis. Georgia Southern University. 64 pp.

Schultz, R. P. 1983. The original slash pine forest -- an historical view. Pp. 24-47 *in*: Proceedings of the managed slash pine ecosystem symposium, University of Florida, Gainesville, Florida.

Schultz, R. P. and L. P. White. 1974. Changes in a flatwoods site following intensive preparation. Forest Science 20:230–237.

Scott, D. E. 1994. The effect of larval density on adult demographic traits in *Ambystoma opacum*. Ecology 75:1383-1396.

Semlitsch, R. D. 1987. Interactions between fish and salamander larvae. Oecologia 72:481-486.

Semlitsch, R. D. 1988. Allotropic distribution of two salamanders: effects of fish predation and competitive interactions. Copeia 1988:290-298.

Semlitsch, R. D. 2008. Differentiating migration and dispersal processes for pond-breeding amphibians. Journal of Wildlife Management 72:260-267.

Semlitsch, R. D., T. L. Anderson, M. S. Osbourn, and B. H. Ousterhout. 2014. Structure and dynamics of ringed salamander (*Ambystoma annulatum*) populations in Missouri. Herpetologica 70:14-22.

Semlitsch, R. D., W. J. Barichivich, and S. C. Walls. A comprehensive multi-level approach to preventing amphibian extinction. The Journal of Wildlife Management: in review.

Semlitsch, R. D., and M. D. Boone. 2009. Aquatic mesocosms. Pp. 87-104 in: Dodd, C. K., Jr. (ed.). Amphibian ecology and conservation: a handbook of techniques. Oxford University Press, Oxford, UK.

Semlitsch, R. D., D. E. Scott, and J. H. K. Pechmann. 1988. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. Ecology 69:184-192.

Shoop, C.R. 1974. Yearly variation in larval survival of *Ambystoma maculatum*. Ecology 55:440–444.

Spitzen-van der Sluijs, A., F. Spikmans, W. Bosman, De Zeeuw M, van der Meij T, Goverse E, Kik M, Pasmans F, Martel A. 2013. Rapid enigmatic decline drives the fire salamander (*Salamandra salamandra*) to the edge of extinction in the Netherlands. Amphibia-Reptilia 34:233–239.

Stevenson, D. J. 1999. The herpetofauna of Fort Stewart, Georgia: habitat occurrence, status of protected and rare species, and species diversity. Fort Stewart Fish and Wildlife Branch, Hinesville, Georgia. 58 pp. + appendices.

Stout, I. J. and W. R. Marion. 1993. Pine flatwoods and xeric pine forests of the southern (lower) Coastal Plain. Pgs. 373-446 *in*: W. H. Martin, S. G. Boyce, A. C. and Echternacht (eds.). Biodiversity of the southeastern United States. Lowland Terrestrial Communities. John Wiley and Sons, Inc., New York, New York.

Tatum, V. L. 2004. Toxicity, transport, and fate of forest herbicides. Wildlife Society Bulletin 32:1042-1048.

Telford, S. R., Jr. 1954. A description of the larvae of *Ambystoma cingulatum bishopi* Goin, including an extension of the range. Quarterly Journal of the Florida Academy of Sciences 17:233-236.

Tschinkel, W.R., and J. R. King. 2007. Targeted removal of ant colonies in ecological experiments, using hot water. Journal of Insect Science 7:1-12.

U.S. Fish and Wildlife Service (USFWS). 2015. Recovery Plan for the Dusky Gopher Frog (*Rana sevosa*). Atlanta, Georgia. 90pp.

Vredenburg, V.T. and A.P. Summers. 2001. Field identification of chytridiomycosis in *Rana muscosa* Camp 1915). Herpetological Review 32:151-152.

Vickers, C. R., L. D. Harris, and B. F. Swindell. 1985. Changes in herpetofauna resulting from ditching of cypress ponds in coastal plains flatwoods. Forest Ecology and Management 11:17-29.

Walls, S., N. Allan, A. Green, A. Keever, A. Messerman, D. Fenolio, T. Gorman, M. Mandica, H. Mitchell, R. Semlitsch, and C. McGowan, 2015. Adaptive Management for Population Supplementation of Imperiled Flatwoods Salamanders. A Case Study from the Structured Decision Making Workshop, August 18-22, 2014, High Performance Computing Laboratory, Mississippi State University, Starkville, MS.

Walls, S.C., W.J. Barichivich, J. Chandler, A.M. Mead, M. Milinichik, K.M. O'Donnell, M.E. Owens, T. Peacock, J. Reinman, R.C. Watling, and O.E. Wetsch. 2019. Seeking shelter from the storm: Conservation and management of imperiled species in a changing climate. Ecol. Evol. 2019;00:1–12. https ://doi.org/10.1002/ece3.5277

Walls, S. C., W. J. Barichivich, and M. E. Brown. 2013. Drought, deluge and declines: the impact of precipitation extremes on amphibians in a changing climate. Biology 2:399-418.

Wear, D. N. and J. G. Greis. 2002. Southern forest resource assessment: summary report. Gen. Tech. Rep. SRS-54. U.S. Department of Agriculture, Forest Service, Southern Research Station, Asheville, North Carolina. 103 pp.

Whiles, M. R., J.B. Jensen, J.G. Palis, and W.G. Dyer. 2004. Diets of larval flatwoods salamander, *Ambystoma cingulatum*, from Florida and South Carolina. Journal of Herpetology 38:208-214.

Wolfe, S. H., J. A. Reidenauer, and D. B. Means. 1988. An ecological characterization of the Florida Panhandle. U.S. Fish and Wildlife Service Biological Report 88(12); Minerals Management Service, OCS Study\MMS 88-0063. 277 pp.

U.S. FISH AND WILDLIFE SERVICE
5-YEAR REVIEW of
Frosted flatwoods salamander/*Ambystoma cingulatum*

Current Classification: Threatened
Recommendation resulting from the 5-Year Review:

____ **Downlist to Threatened**
X__ **Uplist to Endangered**
____ **Delist**
____ **No change is needed**

Appropriate Listing/Reclassification Priority Number, if applicable: 2

Review Conducted By: Harold Mitchell, Panama City Field Office

FIELD OFFICE APPROVAL:

Lead Field Supervisor, U.S. Fish and Wildlife Service

Approve: ______________________________ Date: 7/23/2019

The lead Field Office must ensure that other offices within the range of the species have been provided adequate opportunity to review and comment prior to the review's completion. The lead field office should document this coordination in the agency record.

REGIONAL OFFICE APPROVAL:

Lead Assistant Regional Director, U.S. Fish and Wildlife Service

Acting Approve: Franklin J. Arnold III Date: 9/13/19

APPENDIX A: Summary of peer review for the 5-year review of
Frosted flatwoods salamander/*Ambystoma cingulatum*

A. Peer Review Method:

Upon completion of the draft of this document, the Service chose three peer reviewers (see I.B.) for their close evaluation of this 5-year review document. The reviewers used the markup review feature in MS Word and returned their corrections, comments and edits to the recovery lead to evaluate and consider making the appropriate changes. The peer reviewers allowed us to use this review method twice to improve this document further. Upon receipt, all comments were evaluated and changes were made where appropriate and then the document was submitted forward for supervisor approval with final edits.

B. Peer Review Charge:

We provided all reviewers with the draft document via email. We asked them to review all provided materials by the Service; to identify, review, and provide other relevant data that appears to not have been used by us; do not provide recommendations on the ESA classification; and provide comments on the validity of data; adequacy of the data; scientific uncertainties; strengths and weaknesses; and reasonableness of the assessment. We specifically asked for updated scientific data on recent findings of occupancy, numbers of individuals, and habitat information. All reviewers provided comment.

C. Summary of Peer Review Comments/Report:

Drafts were sent to and returned by each of the peer reviewers. All reviewers submitted grammatical edits. Those corrections were made.

The following are significant comments made by each of the reviewers.

Dr. Susan Walls: (with input from Dr. Jamie Barichivich, and Dr. Katie O'Donnell) Throughout the draft process, we worked closely with USGS Amphibian Research Initiative. During this time, comments were submitted by the USGS team, and incorporated accordingly. The vast majority of edits focused on updating the biological information to reflect the most recent data available. The core edits originate from the taxonomic split of flatwoods salamanders in 2009. Populations and survey data had to be teased apart to more accurately reflect the relative situations of each species.

Dr. Brook Talley: Submitted comments about disease in salamanders, and made some usage and grammatical edits.

Dr. Thomas Gorman: Submitted comments in conjunction with Dr. Walls, on the same subjects: clarifying and updating recent biological information on *A. cingulatum*.

D. Response to Peer Review:

Throughout the review process, communication was frequent. The great majority of comments and edits were accepted, particularly because nearly all were simply updates to our body of biological knowledge about this species. There were no controversial items or comments that were rejected based on disagreement. Virtually all grammatical and style edits were accepted.

There were a few items that required clarification between the reviewers, reflected in comments that were settled and common language agreed upon. Differences mainly centered on characteristic habitat plants occurring prominently in one of the species range, but not prominently in the other. Sawgrass was the primary issue. This was clarified in the five-year review.