

Annual Surveys of Larval *Ambystoma cingulatum* Reveal Large Differences in Dates of Pond Residency

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Abstract - Effective sampling of pond-dwelling larval stages of the federally listed *Ambystoma cingulatum* (Flatwoods Salamander) requires sufficient knowledge of when larvae are present and how best to sample them. Through systematic sampling with active and passive sampling techniques, we found dipnetting to be significantly more effective than three types of passive traps. During surveys for Flatwoods Salamander larvae at Fort Stewart Military Installation, GA in 2005 and 2006, we found that pond residency varied by at least 1.5 months between the 2 years due to the timing of pond filling. In addition, our latest capture on 23 May 2005 was about 2 weeks later than previously recorded at any site range-wide. A simple growth model was used to evaluate likely hatching dates based on significant rain events, observed sizes at capture, and likely growth rates. This analysis suggested that the primary dates of hatching occurred in late February 2005 and early January 2006, a difference that corresponds to that seen in the residency of the latest larval stages. A review of the survey records for Fort Stewart for the past 13 years shows a steep decline in the number of occupied ponds from near 20 to a single pond for the past two years (the only documented breeding success in a natural pond since 1999).

Introduction

Ambystoma cingulatum Cope (Flatwoods Salamander) was listed as federally threatened in 1999 due to range-wide population declines attributable to habitat loss and habitat conversion for silviculture, agriculture, and residential and commercial development (USFWS 1999). Restricted to northern Florida and the Coastal Plain of South Carolina, Georgia, and Alabama, this species is endemic to mesic flatwoods and savannahs dominated by *Pinus palustris* Mill (longleaf pine) and *Aristida stricta* Michx. (wiregrass), where it breeds in small (1.5-ha mean size), isolated depressional wetlands (Palis 1997). Wetlands used by breeding Flatwoods Salamanders are ephemeral and usually dry on an annual basis (Anderson and Williamson 1976, Palis 1997). The basins of breeding ponds are usually abundantly vegetated with graminaceous vegetation and are often partially forested with *Taxodium ascendens* Brongn (pond cypress), *Nyssa sylvatica* Marsh (black gum), and *Ilex myrtifolia* Walt (myrtle-leaf holly) (Jensen 1999; Palis 1996, 1997).

During the nonbreeding season, adult Flatwoods Salamanders are fossorial and inhabit crayfish burrows and other ground cavities within mesic flatwoods and savannah habitats located near breeding ponds (Palis 1996). At the time

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when adults migrate to breeding sites (mid-October–mid-December), the basins of breeding ponds are typically dry (Anderson and Williamson 1976; D.J. Stevenson, unpubl. data; Palis 1997). Females deposit eggs terrestrially in moist microhabitats (e.g., at entrances to crayfish burrows or under sphagnum moss, leaf litter, or dead grass) (Anderson and Williamson 1976). *Ambystoma opacum* Gravenhorst (Marbled Salamander), also a fall breeder, is the only other ambystomatid salamander species that deposits eggs terrestrially (Petranka 1998). Flatwoods Salamander eggs begin to develop immediately after they are laid, but do not hatch until inundated by rising pond levels, which might occur weeks or even months after deposition (Anderson and Williamson 1976; Palis 1995, 1997), typically December to February. The aquatic larvae of Flatwoods Salamanders inhabit a specific microhabitat—graminaceous vegetation of linear growth form (Palis 1996, Sekerak et al. 1996)—that is likely maintained by occasional fires burning into or through the dry pond basins (Bishop and Haas 2005, Palis 1997, Sekerak et al. 1996). Larval development is completed in 11 to 18 weeks, and timing of metamorphosis may be influenced by pond drying (Palis 1995). More detailed life-history and ecology information can be found in USFWS (1999) and Palis (1996, 1997).

The current distribution of the Flatwoods Salamander is mostly restricted to large tracts of public lands, such as national forests, state parks, and military bases. One such sanctuary is Fort Stewart Military Installation, which is home to many rare and imperiled species of reptiles and amphibians (Stevenson 1999, USFWS 1999). Herpetofaunal inventories conducted at Fort Stewart by the Savannah Science Museum during the mid- to late 1970s (Williamson and Moulis 1979), The Nature Conservancy from 1992–1995 (Gawin et al. 1995), and the Fort Stewart Wildlife Branch Office from 1996 to present confirmed Flatwoods Salamander breeding on at least one occasion at 25 ponds (henceforth referred to as “known” ponds).

Successful management and conservation of Flatwoods Salamander habitat at Fort Stewart and elsewhere will depend on successfully identifying active and potential breeding sites among hundreds of potential ponds. Because the only viable field method for locating adults (monitoring drift fences during adults’ migrations to and from breeding sites) is labor and time intensive, aquatic sampling of breeding sites for Flatwoods Salamander larvae is the preferred method to assess presence (Bishop et al. 2006, Palis 1996). Sampling for larvae is also challenging because they typically reside in dense vegetation that is difficult to sample, densities are often low, and abundance and period of pond residency varies annually and among ponds (Bishop et al. 2006, Palis 1997, Sekerak et al. 1996). For these reasons, it is crucial that biologists use the most effective sampling methodology and clearly understand the factors that affect salamander presence and detection.

The objectives of this study were to 1) systematically sample larval Flatwoods Salamanders using different sampling methods to compare method effectiveness, 2) survey throughout the larval development period for two years to determine the period of larval residency at Fort Stewart, 3) relate

hatching to rain events using a simple growth model, and 4) summarize survey success at Fort Stewart over the past 13 years.

Methods

Fort Stewart (113,064 ha) is located in the Atlantic Coastal Plain of southeastern Georgia and supports significant areas of intact, fire-maintained longleaf pine ecosystems that contain embedded depressional wetlands (Carlile 1995, Gawin et al. 1995). Within these longleaf pine ecosystems are nearly 500 ponds that have been identified as potential Flatwoods Salamander breeding habitat.

In 2005, we systematically sampled eight known breeding ponds intermittently from late February to early May with traps and dipnetting to test the relative effectiveness of different capture methods. The ponds ranged in size from about 1.3 to 4 ha. Three of the eight ponds were located on the west side of Fort Stewart, and five on the east side about 34 km away; all are located in Liberty County. Within a pond, we identified and marked with numbered flagging tape at least 16 sample sites based on a qualitative judgment of what we considered suitable larval habitat with adequate water depth for sampling. These sites were vegetated with moderate to profuse herbaceous cover and had an average water depth of about 15–30 cm.

We sampled larvae with either long-handled 40-cm diameter dipnets (5-mm mesh) or passive traps. The trap types included commercially-made plastic (5-mm mesh) or metal (3-mm mesh), double-opening, funnel traps and a 61- x 61- x 46-cm wood-framed box trap with 2 vertical funnel entrances (3-mm mesh). Traps were placed at a depth or perched on debris such that air-breathing organisms had access to the water surface. For each typical day of effort within a pond, we randomly assigned: 1) to four sites, a standard effort of dipnetting, which was usually 2 surveyors dipnetting for 5 min each; 2) to four sites, a combination of 4 plastic and 4 metal minnow traps deployed for 24 hours; and 3) to two sites, a box trap deployed for 24 hours. We rotated dipnetting and trapping efforts daily among the sites within a pond for 2–4 days. For example, during 3 days of sampling at one pond we would typically dipnet a total of 120 minutes (3 days x 4 sites x 2 netters x 5 minutes), trap for 96 trap nights with both metal and plastic funnel traps (3 nights x 4 sites x 4 traps x 2 trap types), and trap for 6 box-trap nights (3 nights x 2 traps). We measured all Flatwoods Salamander larvae captured (snout–vent length [SVL] and total length [TL] in mm) and released them shortly after processing to their original site of capture. The amount of time required to set and check traps was also documented. Additional dipnetting without trapping was performed at these ponds and two other known ponds as part of a general survey.

In 2006, we modified our sampling strategy based on 2005 results and designed a sampling plan that included more ponds but less effort per pond (i.e., fewer trips) and sampling primarily with dipnets. We sampled a total of 60 ponds (21 known breeding ponds and 39 potential breeding ponds)

that ranged in size from about 0.14 to 7.5 ha. Most ponds (46) were sampled only once, 13 were sampled two to three times, and the single pond where Flatwoods Salamander larvae were found during this study was sampled on eight occasions.

We evaluated possible hatching dates by matching observed sizes at capture with projected size at age based on modeled growth, which was initiated on dates of rain events that caused pond levels to rise and could have triggered hatching. Hatching of Flatwoods Salamander larvae on multiple dates within a season is not unusual (Palis 1995, Sekarek et al. 1996) and was considered in our analysis. We obtained daily rainfall data from two meteorological stations located equidistant (10 km) to the east and west of the single pond where larvae were found during this study and averaged the daily values. For modeling purposes we considered rain events in January and February that resulted in a daily total >20 mm or a running weekly total >25 mm as sufficient to result in a rise in pond levels.

Size at hatching information used in our model was based on total lengths reported by Anderson and Williamson (1976), which we converted to SVL based on the ratio of SVL to TL calculated for the larvae captured in this study ($SVL = 0.55 * [TL]$). They reported average lengths at hatching based on laboratory observations of 6.5 mm (SVL) on 25 November, 6.2 mm on 2 December, and 8.5 mm on 24 January after conversion. They also reported an average length of 7.0 mm (SVL) for newly hatched larvae captured in the field on 14 December. From these data, we derived a relationship between date of hatching and mean SVL at hatching:

$$\text{Mean } SVL_{\text{hatch}} = 0.0446 * \text{Julian day} + 7.658$$

We then subtracted and added 1 mm to include a measure of natural variation in size at hatching, which became the origin of the minimum and maximum growth trajectories for a hatching date.

We also recognize that individual variation in growth exists, and, therefore, we used the range of growth rates calculated by Palis (1995) for two breeding sites in the Florida panhandle as minimum (1.78 mm/week; 0.254 mm/day) and maximum (2.54 mm/week; 0.363 mm/day) rates for the model. Although larval growth rates vary due to several factors, such as temperature, food availability, and densities of conspecifics and competitors, because we lacked any other growth data for this species, we assumed that larval growth at Fort Stewart was within the range reported by Palis (1995).

The growth trajectory modeled from a particular date was comprised of both minimum and maximum trajectories that created an envelope or cone of likely size at age. The maximum and minimum size-at-age lines are described by the following equations:

$$\text{Minimum trajectory } SVL_t \text{ (mm)} = (SVL_{\text{hatch}} - 1) + t * 0.254,$$

and

$$\text{Maximum trajectory } SVL_t \text{ (mm)} = (SVL_{\text{hatch}} + 1) + t * 0.363,$$

where t is time in days from hatching.

Lastly, we obtained survey results from the Fort Stewart Wildlife Branch Office of all the Flatwoods Salamander surveys at Fort Stewart since 1994 that included the dates and types (larval or adult) of surveys and the number and sizes of Flatwoods Salamanders found. These data were summarized for each of the 22 confirmed breeding sites.

Results

Table 1 summarizes the temporal distribution of pond sampling in 2005 and 2006 and the capture of Flatwoods Salamander larvae. In mid-February 2005, when we first visited six known ponds for sampling, they were dry except for a few small (2- x 2-m) shallow pools. By late February, these ponds had filled to a depth sufficient for sampling (maximum ≈ 30 cm). No Flatwoods Salamander larvae were captured during February and March after sampling an average of 7 days each at six ponds with dipnets and traps. Sampling effort was reduced in April due to concerns that earlier dry conditions had resulted in

Table 1. Number of ponds sampled and number and mean snout-vent length (SVL) of *Ambystoma cingulatum* (Flatwoods Salamander) larvae captured at Fort Stewart on a weekly basis during 2005 and 2006. Some ponds were sampled more than once during a week. All larvae were captured from a single pond, Alpha Pond.

Sampling period (week of)	2005			2006		
	No. of ponds sampled	<i>A. c.</i> larvae	Mean SVL (mm)	No. of ponds sampled	<i>A. c.</i> larvae	Mean SVL (mm)
January						
19	-			6	2	15.5
26	-			4	1	16.0
February						
2	-			10	0	-
9	-			5	11	16.9
16	-			12	0	-
23	3	0	-	14	0	-
March						
2	4	0	-	12	0	-
9	-			10	7	29.4
16	3	0	-	-		
23	3	0	-	-		
30	-			6	0	-
April						
6	-			2	6	31.7
13	-			8	0	-
20	1	15	32.0	4	0	-
27	5	0	-	-		
May						
4	3	15	34.3	-		
11	3	5	32.0	-		
18	1	2	33.5	-		
25	1	1	32.0	-		
June						
1	2	0	-	-		

reproductive failure, but on 21 April, we captured a larva at a known breeding pond (Alpha Pond) in a trap followed by 14 more individuals by dipnetting. From 21 April to 23 May, we captured a total of 38 larvae from Alpha Pond during seven visits (Table 1). No other larvae were found in Alpha Pond during sampling from 31 May–3 June, and none were found in follow-up surveys of other ponds during late April and May.

In 2006, the ponds had filled when sampling began in the middle of January. We captured two Flatwoods Salamander larvae at Alpha Pond during the first visit on 18 January. On four subsequent visits (27 January, 7–8 February, 8 March, and 4 April), 27 additional larvae were captured by dipnetting. Ponds dried during March, and larvae captured on 4 April were dipnetted from among the few remaining shallow pools located within the Alpha Pond basin. We found no larvae in the other 59 ponds sampled in 2006, including one that is within 100 m of Alpha Pond.

Dipnetting produced significantly more larvae than any of the traps we tested (Table 2). Our method comparison in 2005 included 1744 minutes of dipnetting coincident with 1794 total trapnights (838 metal traps, 840 plastic traps, and 116 box traps) at eight ponds. Because the typical unit of effort for trapping is number of nights set and the unit of effort for dipnetting is minutes netted, we chose to standardize effort for comparison based on actual hands-on investigator time needed to use each method. After standardization, the amount of effort at each site within a pond was similar for all methods. Our typical dipnetting effort per site was 5 minutes of netting by two people for a total of 10 minutes. It also took about 10 minutes for two people to set and check eight minnow traps (plastic or metal), which was the number normally placed at a site overnight. A single box trap took about 5 minutes to set and check because it often captured more individual organisms and took longer to empty because of its design. When standardized to hours of investigator effort, we found that dipnetting was roughly 5–10 times more effective than the traps. Since Flatwoods Salamander larvae were only captured in one pond, we limited our analysis to only those dates when we were certain larvae were in the pond. Based on data from 20–21 April and 2–4 May, metal traps captured two

Table 2. Trapping and dipnetting effort in Alpha Pond in 2005 with number of Flatwoods Salamander larvae captured in parentheses.

Sampling dates	Metal traps (trapnights)	Plastic traps (trapnights)	Box traps (trapnights)	Dipnet (minutes)
March 1–3	44	44	6	122
March 21–23	48	48	6	60
April 20–21	49	48 (1)	-	220 (14)
May 2–4	68 (2)	68	7	211 (13)
May 11–12	-	-	-	244 (5)
May 17	-	-	-	89 (2)
May 23–24	-	-	-	85 (1)
May 31–Jun 3	12	14	-	165
Total	221 (2)	222 (1)	19	787 (35)

larvae in the equivalent of 146 minutes (0.8/hour), plastic traps captured one larva in the equivalent of 145 minutes (0.4/hour), and dipnetting captured 27 larvae in 431 minutes (3.8/hour).

Our analysis of the rainfall data revealed that in 2005 three dates from 1 January to 28 February met the criteria we established for what was necessary to raise pond levels (14 January, 3 and 28 February), and four dates (2 and 24 January, and 3 and 26 February) met the criteria in 2006. We produced size-at-age envelopes (i.e., minimum and maximum growth trajectories) for each of these dates and evaluated how well the size envelopes encompassed the observed larval sizes. Figure 1 shows an envelope from the most likely hatching date defined as the first day with daily rainfall of >25 mm and another from the remaining hatching dates that encompassed the greatest number of the remaining larval sizes.

Biologists at Fort Stewart have maintained comprehensive records of Flatwoods Salamander sampling effort and captures at 22 confirmed breeding sites over the past 13 years (Fig. 2). Prior to the 2 years of sampling reported here, nearly all of the sampling occurred in February to early April. Of 86 sampling

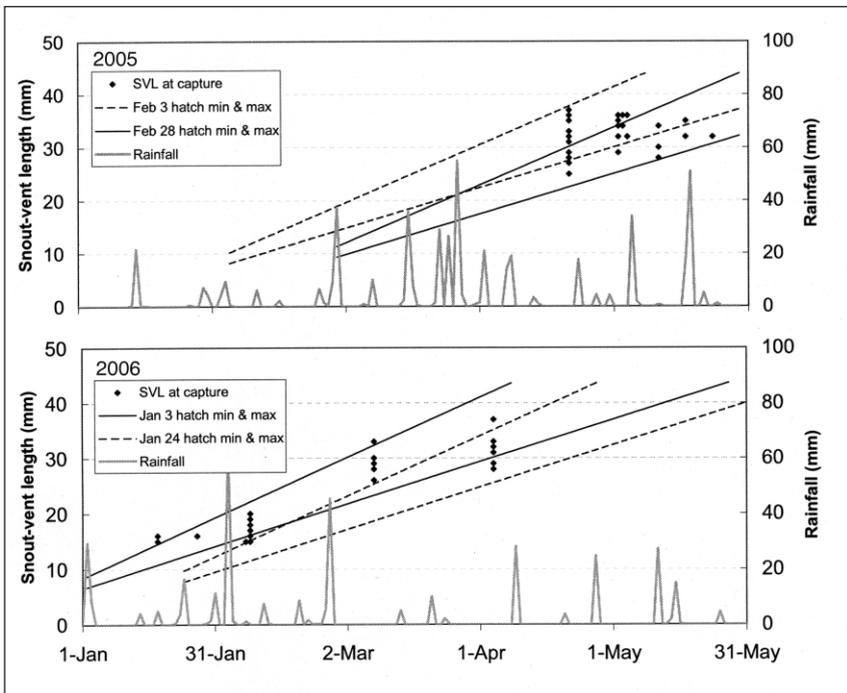


Figure 1. Snout-vent length (mm) for larval Flatwoods Salamander by date of capture from Alpha Pond at Fort Stewart, GA. Envelopes of modeled size-at-age for two possible hatching dates for 2005 and 2006 are shown as solid lines (most likely date of hatching) and dashed lines (second most likely). Daily rainfall (mm) is indicated by the gray line.

trips to known ponds from 1994 to 2004, only three ponds were sampled in January and none after 13 April. These records show a decline since 1994 in the proportion of confirmed ponds surveyed in a given year that contained Flatwoods Salamanders. Nearly every previously confirmed pond that was sampled in 1994 (18 of 19 sampled) produced larvae. In the late 1990s, about half of the confirmed ponds that were sampled each year had larvae present. A protracted drought (1999–2002) experienced throughout the Coastal Plain of Georgia and South Carolina resulted in four consecutive years of potentially complete reproductive failure at most breeding sites. Although some pond basins on Fort Stewart were partially inundated in 1999, pond hydroperiods were of insufficient duration to allow larval development through metamorphosis (D.J. Stevenson, unpubl. data). In the past 2 years, we only found larvae in 1 of 21 known ponds sampled, even though survey effort (based on pond visits) was as high or higher than ever.

Discussion

The difference in dates of initial hatching and latest occupancy of Flatwoods Salamanders in 2005 and 2006 at Alpha Pond at Fort Stewart was roughly 1.5 months. Of particular significance is the presence of larvae in a breeding pond until at least 23 May. Previously, the latest date that larvae had been observed at Fort Stewart was 13 April (1994), and that observation along with those from a day earlier were of larvae nearing metamorphosis

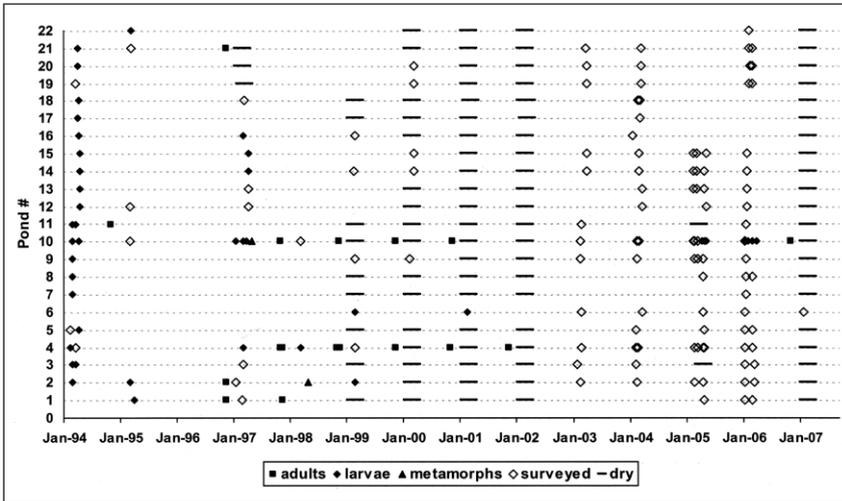


Figure 2. Compiled survey history for Flatwoods Salamander on Fort Stewart from 1994–2006 showing unsuccessful and successful surveys for larval salamanders (open and filled diamonds), observations of adults or metamorphs outside of the ponds (squares and triangles), and periods when ponds were dry and could not be sampled (bars). Pond #10 is Alpha Pond where larvae were found in 2005 and 2006. Sources of data were Fort Stewart Wildlife Branch Office records, Safer (2001), and this study.

(Gawin et al. 1995). The latest larvae capture dates of which we are aware are 1 May (1974) and 12 May (1972) (Williamson and Moulis 1979); these collections were made in Jasper County, SC. Although rarely observed, late occupancy is not necessarily a rare event; Williamson and Moulis (1979) captured larvae in the latter half of April or later in 4 consecutive years in South Carolina.

We do not know when the eggs were deposited that likely hatched 28 February 2005 because several rain events occurred in November and December of 2004 that could have triggered breeding migrations. The early January hatching date estimated for 2006 is probably not the earliest possible for this study site, because Anderson and Williamson (1976) observed eggs hatching as early as 4 December in southeastern South Carolina and southeastern Georgia. Little has been published regarding how long Flatwoods Salamander eggs (located in dry pond basins) remain viable after deposition before inundation; however, it is possible that larvae that hatched in late February 2005 were from eggs deposited 3 months earlier. Anderson and Williamson (1976) reported that advanced eggs taken from the field hatched in the laboratory approximately 74 days later. The terrestrially deposited eggs of Marbled Salamanders, a related species, may remain viable 3–4 months post-oviposition (Noble and Brady 1933, Petranka and Petranka 1981). Flexibility in this aspect of reproduction is critical to Flatwoods Salamanders if rainfall during the reproductive season is below normal as it has been in southeastern Georgia for many of the last 10 years.

Our simple modeling of larval growth suggests that for the 2 years of our study a single date of hatching does not account for all the larval sizes observed. In 2005, we observed nearly dry ponds in mid-February and presumed that the larvae captured later in the spring hatched following significant rain events in late February or early March. However, the growth envelope initiated on 28 February does not include the largest larvae captured on 21 April, which suggests that hatching also occurred on an earlier date. Four days of rain from 29 January to 3 February that totaled 25 mm was probably enough to partially fill the pond and could have inundated eggs at low pond elevations. Some larvae may have hatched during this partial pond filling and survived the following 3 weeks of minimal rain by taking refuge in small pools that remained. Alternatively, it is possible that we underestimated the actual larval growth rate in our model; however, if that were the case, some larvae hatched on 28 February would have had to grow at a rate about 36% greater than the maximum rate estimated by Palis (1995). In 2006, the most likely date for hatching was in early January. However, hatching at that time does not account for all the sizes of larvae observed. We believe that some larvae must have also hatched in late January to account for the smaller individuals captured in early April.

Although we illustrated the two most-likely hatch dates based on our analysis, we do not rule out the possibility that larvae hatched on more than two dates given the uncertainty in size at hatching and known variation in growth rates. It is quite possible that multiple hatching dates within a population

during a season is the rule and not the exception. Since eggs are laid individually and not in large egg masses, multiple females would likely deposit their eggs at a variety of elevations within a dry depression. Gradual or incremental pond filling would therefore result in multiple hatching dates.

Of over 2000 isolated depressional ponds on Fort Stewart, approximately 500 have been identified as potential breeding habitat for Flatwoods Salamanders (Palis 2002). Less than half of these ponds have been sampled to date, and most of those have not been sampled enough to conclude that they do not support breeding. Better knowledge of when larvae are present and most susceptible to specific sampling methods is crucial to successful monitoring and to maximize likelihood of detection. Sampling methods limit detectability during the first few weeks of larval residency because larval size at that stage is smaller than the mesh size of nets and traps. For example, in 2005, we thoroughly sampled Alpha pond twice during March without finding any larvae, but we found larvae in late April, and we are certain, based on the size of the larvae, that they were present throughout March.

Our study demonstrates that dipnetting is far more effective for surveying Flatwoods Salamander larvae than passive traps. Although checking a single trap for larvae can be done in less than a minute, the time it takes to transport the traps to the pond, distribute them throughout the pond, and locate the proper depth for deployment accumulates to significant time expenditure for minimal return.

We suggest that the most opportune time to sample is during the second and third months after a pond fills to at least half full. In Florida, metamorphosis of Flatwoods Salamander larvae is usually complete by April (Sekarek et al. 1996), but is likely later on average at more northern latitudes. In the Fort Stewart region, April–May is a period of reduced rainfall and pronounced evapotranspiration; thus, pond water levels recede rapidly during this time (Palis 1997). Bishop et al. (2006) recommended that surveys for Flatwoods Salamander larvae be conducted primarily from February to early April, but depending on various weather-related factors, sampling in other months could be fruitful. The results of our study demonstrate that, during some years and for some locations, sampling outside of the recommended months is certainly productive. Thus, we recommend extending surveys through April and into May in years when breeding ponds do not fill until late winter (February–March).

Flatwoods Salamander larvae captured in 2005 were the first captured at Fort Stewart from a natural wetland since 1999 (larvae were found in a former borrow pit in 2001) (Fig. 2). Since 2002, repeated surveys at known breeding sites elsewhere in Georgia and in South Carolina have found larvae at just one site in South Carolina (a single larva found in 2003 at a site on Francis Marion National Forest; S. Bennett, South Carolina Department of Natural Resources, Columbia, SC, pers. comm.) and at one site in Georgia (single larva found in each of 2001 and 2003 from adjacent wetlands on Townsend Bombing Range; J. Jensen, Georgia Department of Natural

Resources, Forsyth, GA, and W. Seyle, US Army Corps of Engineers, Savannah, GA, pers. comm.). Biologists have found Flatwoods Salamanders on a more frequent basis at many sites in Florida during this period (K. Enge, Florida Fish and Wildlife Conservation Commission, Tallahassee, FL, pers. comm.) including high numbers observed at St. Marks National Wildlife Refuge in late February 2007 (M.S. Bevelhimer, unpubl. data).

Lastly, we do not know whether the high level of occurrence reported in 1994 was the result of ideal hydrologic conditions, a peak in a cyclic pattern of natural population fluctuation, a result of greater survey effort, or a combination of these and other environmental factors. Palis et al. (2006) observed a decline in the number of breeding adult Flatwoods Salamanders over 4 consecutive years at a breeding pond in Florida and attributed this decline to adult attrition, lack of juvenile recruitment, and lack of rain or abnormally low rain during the period of breeding migrations. Similarly, we suspect that adult attrition and lack of juvenile recruitment due to the drought are responsible for the putative decline of Flatwoods Salamanders on Fort Stewart and elsewhere in Georgia and South Carolina. If the conservation and preservation of this and other rare amphibian species is to be successful, biologists must identify and utilize which survey methods are most effective and should maximize the likelihood of detection through a better understanding of the relationship between pond residency and various environmental factors.

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