# Capture Rate, Body Size, and Survey Recommendations for Larval Ambystoma cingulatum (Flatwoods Salamanders)

David C. Bishop<sup>1,6,\*</sup>, John G. Palis<sup>2</sup>, Kevin M. Enge<sup>3</sup>, David J. Printiss<sup>4</sup>, and Dirk J. Stevenson<sup>5</sup>

**Abstract** - Recovery of the federally threatened *Ambystoma cingulatum* (Flatwoods Salamander) will require monitoring of known populations, as well as continued searches for additional populations. In an effort to develop recommendations for maximizing efficiency of future surveys of larval Flatwoods Salamanders, we combined data from surveys conducted between 1990 and 2004 in Florida and Georgia. Analysis of these data revealed variation in the number of larvae captured, survey effort, capture rates, and larval body size among years and months. An average of 16 min or 45 one-m long dipnet sweeps was required to catch each larva. For wetlands surveyed twice in a season, results (i.e., larval presence or assumed absence) were consistent in 74% of consecutive surveys. We make recommendations for conducting future surveys and the implementation of a coordinated research and monitoring program for Flatwoods Salamanders.

## Introduction

In response to the apparent decline in many amphibian species (Halliday 1998, Pechmann and Wilbur 1994, Wake 1991), conservation efforts have been focused on the development of survey techniques and monitoring programs (e.g., Heyer et al. 1994, Smith and Petranka 2000, Welsh and Droege 2001). Ideally, monitoring enables land managers to evaluate population status over time and respond to potential threats with timely management decisions. Before a monitoring program can be successful, however, managers must first know the appropriate survey techniques and survey schedule for the species of interest.

Ambystoma cingulatum Cope (Flatwoods Salamander) was listed as federally threatened in 1999 (US Fish and Wildlife Service [USFWS] 1999). Because of its protected status and the general perception that it has declined in abundance over the last few decades (Means et al. 1996), greater effort has been made recently to survey historical and potential sites for breeding populations, primarily using larval dipnet surveys. Recovery of this species will require monitoring of known populations as well as continued searches

<sup>&</sup>lt;sup>1</sup>Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061. <sup>2</sup>PO Box 387, Jonesboro, IL 62952. <sup>3</sup>Florida Fish and Wildlife Conservation Commission, 5300 High Bridge Road, Quincy, FL 32351. <sup>4</sup>The Nature Conservancy, Northwest Florida Program, PO Box 393, Bristol, FL 32321. <sup>5</sup>Fort Stewart Fish and Wildlife Branch, 1557 Frank Cochran Drive, Building 1145, Fort Stewart, GA 31314. <sup>6</sup>Current address - Spring Island Trust, 40 Mobley Oaks Lane, Okatie, SC 29909. \*Correspondening author davidlci@charterinternet.com.

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for additional populations (L. LaClaire, USFWS, Jackson, MS, pers. comm.). However, there has been no coordinated effort to combine data from past surveys to identify trends in capture rates and capture repeatability. This information would prove helpful to future surveyors wishing to maximize monitoring efficiency.

We combined data from surveys of larval Flatwoods Salamanders conducted between 1990 and 2004 in most known breeding wetlands in Florida and Georgia. We report data on capture rates, capture repeatability, and larval body size, and make recommendations for maximizing efficiency of larval surveys for this species. In addition, we make suggestions for future *A*. *cingulatum* monitoring programs.

## Methods

# **Study species**

Flatwoods Salamanders historically inhabited mesic flatwoods and savannas within the *Pinus palustris* Miller (longleaf pine) and *Aristida stricta* Michaux (wiregrass) ecosystem in South Carolina, Georgia, Florida, and Alabama (USFWS 1999). Adults migrate at night to ephemeral breeding wetlands from October to January during rainfall events associated with cold fronts (Anderson and Williamson 1976, Means 1972, Palis 1997a, Safer 2001). Courtship and egg deposition have been observed on land (Anderson and Williamson 1976, 1977; Safer 2001), but it has been suggested that aquatic egg deposition also may occur (Ashton 1992). Eggs that are deposited terrestrially within the wetland basin hatch quickly upon inundation (Anderson and Williamson 1976). The larval period lasts 11–18 weeks from hatching to metamorphosis (Palis 1995), with some individuals reaching sexual maturity and returning to the breeding wetlands the following fall (Palis 1997a).

Migrating adults can be captured in funnel or pitfall traps positioned next to drift fences around the perimeter of breeding wetlands (Palis 1997a, Safer 2001). Because of relative inefficiency of sampling multiple sites for Flatwoods Salamanders by these methods, most presence-absence surveys focus on larval sampling by dipnet. Surveyors typically use 4-mm mesh dipnets (Model SH-2 or SH-2D, Mid–Lakes Corporation, Knoxville, TN). Larvae have been captured November to April (Means 1972, Palis 1996). Larvae typically are captured in submerged herbaceous vegetation during the day (Palis 1997b, Sekerak et al. 1996); therefore, most surveyors focus their dipnetting efforts in vegetated areas (e.g., Palis 1997b).

#### **Data collection and analyses**

We combined data from previous surveys (N = 757) for larval Flatwoods Salamanders at 176 different breeding wetlands in Florida and Georgia (Table 1). We included only wetlands where at least one larva was captured from 1990 through 2004. Each participant provided data on wetland location, capture date, larval body size (total length [TL]), and survey effort. Effort was reported as the total minutes spent dipnetting and/or the number of 1-m long dipnet sweeps (Palis 1997b). Because we compiled data from different sources, not all variables were collected for each individual captured; hence, sample size differs among variables.

We calculated the total number of larvae captured, total effort (min. or sweeps), and mean body size for 2-week intervals throughout the larval period (Nov–Apr). We also calculated capture rates for positive surveys (i.e., those where larvae were captured) by dividing the number of larvae captured in each wetland by the total survey effort (min. or sweeps). Because some surveyors stopped surveying after the first larva was captured, whereas others continued dipnetting, capture rates should be interpreted as the average effort needed to capture each larva. For records where surveyors recorded effort in both minutes and sweeps, we used linear regression to examine the relationship between the two methods.

For wetlands surveyed twice in a single season, we calculated the probability of obtaining consistent results (i.e., larval presence or assumed absence) for consecutive surveys. If a wetland was surveyed more than twice in a season, we used only those data from the first two surveys in that year. We performed a ttest to evaluate whether the mean number of days between consecutive surveys differed between those that had consistent survey results those that did not.

Lastly, we looked at yearly variation in survey success for wetlands dipnetted  $\geq 5$  different years (not necessarily sequential). We calculated the percentage of successful years for each wetland and used these data to estimate the minimum number of years potential or historical wetlands should be surveyed to detect larval *A. cingulatum*. All analyses were conducted using SPSS 11.0.

Location	State	Counties	Total wetlands	Total surveys	Total larvae
Apalachicola NF	FL	Liberty, Franklin	54	351	234
Eglin AFB (includes Hurlburt Field)	FL	Okaloosa, Santa Rosa	28	163	116
Fort Stewart (Army)	GA	Bryan, Evans, Liberty	14	72	142
Holley Field (Navy)	FL	Santa Rosa	1	1	1
Osceola NF	FL	Baker	3	9	7
Pine Log SF	FL	Washington	1	2	2
St. Marks NWR	FL	Wakulla	44	86	54
Private	FL	Baker	1	1	1
Private	FL	Calhoun	4	21	26
Private	FL	Holmes	1	1	1
Private	FL	Jackson	3	4	15
Private	FL	Jefferson	2	4	2
Private	FL	Liberty	4	12	9
Private	FL	Santa Rosa	4	9	8
Private	FL	Wakulla	9	18	10
Private	FL	Walton	1	1	1
Private	FL	Washington	2	2	2
Totals		-	176	757	631

Table 1. Number	of wetlands,	surveys,	and A.	cingulatum	larvae	captured	by location.	NF =
National Forest, A	AFB = Air Fo	rce Base,	SF = S	tate Forest,	NWR =	National	Wildlife Re	fuge.

#### Results

Of the 757 surveys of known *A. cingulatum* breeding wetlands in our data set, 348 (46.0%) resulted in the capture of one or more larvae (total larvae = 631). Larvae were captured from November 28 to April 23. The number of larvae captured and the amount of survey effort varied by month (Table 2). When data from all years and months were combined, larvae were captured at a mean rate of 1 individual per  $16.0 \pm 23.9$  min (range: 1–256, 95<sup>th</sup> percentile = 60.0, N = 296) or 1 individual per  $44.6 \pm 67.2$  1-m long sweeps (range: 1–664, 95<sup>th</sup> percentile = 150.2, N = 231). There was a significant linear relationship between the number of minutes spent surveying and the number of dipnet sweeps (Sweeps = 4.292[min.] - 4.588, R<sup>2</sup> = 0.7155, P < 0.001, N = 183). Mean larval size and the range of sizes differed among months (Fig. 1).

For wetlands surveyed twice in the same season, 107 out of 145 (73.8%) yielded consistent results (i.e., larval presence or assumed absence) in consecutive surveys. The mean number of days ( $\pm$  SD) between consecutive surveys was 29.3  $\pm$  18.1 (range: 1–101, N = 145). When separated, the mean number of days between surveys was 27.2  $\pm$  16.0 (range: 1–81, N = 107) for those with the same results and 34.9  $\pm$  22.3 (range: 3–101, N = 38) for those with different results. This difference was statistically significant (t = -2.279, df = 143, P = 0.024). For the 37 wetlands surveyed  $\geq$  5 years (mean: 6.7  $\pm$  1.5 yrs, range: 5–11), the mean number of years needed to capture larvae was 2.5  $\pm$  1.3 (range: 1–8 yrs; 95<sup>th</sup> percentile = 5.3 yrs).

# Discussion

We made no attempt to account for geographic variation in capture rates, capture repeatability, or larval size associated with latitude, years,

Table 2. Number of surveys, total captures, and capture rates for *A. cingulatum* by month. Period 1 includes days 0-15 (0-14 for Feb). Capture rates were calculated using only data from positive surveys (i.e., at least one larva was captured). Sample sizes vary between capture rate categories because not all records had effort data in both minutes and sweeps. Mo. = month, Per. = period, TS = total surveys, PS = positive surveys, TL = total larvae.

					Capture rate (min/larvae)				Capture rate (sweeps/larvae)				
Mo.	Per.	TS	PS	TL	Mean	SD	Range	Ν	Mean	SD	Range	Ν	
Nov	1	0	0	0	_	_	_	0	_	_	_	0	
	2	2	1	1	2.0	_	_	1	7.0	_	_	1	
Dec	1	2	1	1	25.0	_	_	1	_	_	_	0	
	2	1	1	2	10.0	_	_	1	_	_	_	0	
Jan	1	13	8	8	80.6	86.2	10.0-256.0	7	112.5	10.6	105.0-120.0	2	
	2	78	41	79	30.3	32.0	1.8-150.0	39	222.0	294.2	14.0-430.0	2	
Feb	1	119	59	88	8.8	11.9	1.0 - 70.0	48	23.8	29.6	1.0 - 118.0	45	
	2	92	49	127	9.2	10.8	1.0 - 50.0	33	54.0	109.2	1.0-664.0	40	
Mar	1	243	101	175	12.0	13.5	1.0-75.0	92	46.1	55.0	1.0 - 245.0	79	
	2	136	57	86	13.1	12.3	1.0-52.0	51	46.5	46.8	2.0 - 167.0	45	
Apr	1	52	26	55	21.3	17.3	1.0-60.0	22	41.8	37.1	1.0-118.0	13	
-	2	19	4	9	1.5	NA	NA	1	29.5	29.8	1.5-60.0	4	

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hydroperiod, habitat, or weather. Likewise, we did not control for differences in surveying methodology or larval measurements among individual surveyors. Although surveyors used similar equipment and had comparable techniques, individuals likely differed slightly in dipnetting methods. Despite these problems, combining data sets provides useful information on capture rates, capture repeatability, and body size of larval Flatwoods Salamanders.

When data were combined for all months, an average of 16 minutes or 45 one-m long sweeps was needed to capture each larval salamander. Larvae were captured from November 28 to April 23, with the majority of surveys occurring in February and March. Not surprisingly, the total number of larvae captured increased with the number of surveys, both of which varied among months. Assuming larvae were captured at rates relative to their abundance, we can compare capture rates over time to estimate relative abundance each month. Using this method, the data suggest that capture rates are similar in February, March, and April. However, because the amount of effort spent surveying varied among months, our analysis is limited.

Few surveys were conducted in November and December; hence, the capture-rate data in Table 2 could be misleading if sample size is ignored. Because ephemeral breeding wetlands often are not filled completely in



Figure 1. Size of larval *A. cingulatum* each month. Boxes display interquartile range. The dark line is the median. Open circles are outliers. SVL = snout-vent length.

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November or December, we do not recommend surveying during this time in lieu of later months if the goal is to detect the species. In addition, because larvae are small from November to January, they may be more likely to slip through the netting of dipnets. As expected, mean larval size increased as the larval season progressed, as observed by Whiles et al. (2004). Variation in size reached a maximum in the middle of the larval season (February and March), which likely can be attributed to multiple hatching dates (Palis 1995, Sekerak et al. 1996).

If a wetland was dipnetted twice in the same season, there was a 74% probability of obtaining consistent results in consecutive surveys. Discrepancy in consecutive surveys could result from several factors: 1) larvae may have metamorphosed between surveys, 2) habitat conditions may have changed (e.g., wetland dry-down then refill), 3) differences in survey methodology (e.g., different sections of the wetland were sampled, the amount of effort varied, etc.), 4) differences in larval density between surveys, or 5) larvae may have been too small to catch in the first survey, but large enough in the second. Wetlands that yielded the same results and those that did not had a mean difference of 7.7 days between consecutive surveys. Although this was statistically significant, we believe this has little biological relevance.

Historical or potential A. cingulatum breeding wetlands may need to be dipnetted for several years before larvae are captured. For the 37 wetlands surveyed  $\geq 5$  years, an average of 3 (2.5) years of surveys were needed to document the presence of larvae. However, some wetlands contained larvae every year, whereas others had larvae only once in 8 years. Because we only included those years for which dipnetting occurred, even more site visits may be needed if drought years are included. Larvae were captured in 90% of the wetlands after 4.6 years of sampling; therefore, we suggest a minimum of 5 years of surveys be conducted in potential wetlands to document the presence or assumed absence of A. cingulatum. Historical wetlands that have not produced larvae in 5 years should continue to be dipnetted, especially those located near other active breeding wetlands; Flatwoods Salamanders likely occur in metapopulations, where wetland occupancy varies over time.

Larvae were captured in 46% of the surveys. We do not have sufficient data to determine why some surveys were successful and others were not; however, casual observations indicated that locations and wetlands differed annually in the relative abundance of salamanders. Little information is available on how habitat modification (e.g., forestry operations, fire exclusion and suppression [Bishop and Haas 2005]) or weather conditions (e.g., rain dates, rainfall, hydroperiod) affects the population dynamics of Flatwoods Salamanders. If the species is declining, the scientific community needs to determine the reasons and mitigate any anthropogenic causes. Long-term annual monitoring programs should help identify the primary factors that influence successful reproduction and recruitment; progress will be faster if there is a coordinated effort by all management agencies to

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standardize data collection. By simply evaluating the presence or assumed absence of *A. cingulatum* in each wetland over a long period of time (e.g., 10 years) and coupling this information with data on weather patterns, habitat changes (in uplands, wetlands, and ecotones), and survey efforts, we may begin to understand the population dynamics. Even greater understanding may be achieved through the development of quantitative techniques to relate relative larval abundance to the aforementioned variables.

In summary, we recommend surveying from February to early April to document the presence of *A. cingulatum* larvae. However, surveying in all months of the larval period may be fruitful, and weather patterns may dictate appropriate times. Larvae typically were captured in an average of 16 min or 45 one-m dipnet sweeps; however, there was significant variation in capture rates, and we suggest that surveyors dipnet for longer periods. We recommend each known breeding wetland be surveyed at least twice each season, if the first survey was negative, preferably with several weeks between surveys. Several years of surveys may be needed to document breeding activity in potential wetlands. We encourage all Flatwoods Salamander researchers and land managers to develop a consolidated plan for future monitoring and research efforts.

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