

**PROTOCOL MANUAL FOR LONG-TERM MONITORING OF
AMPHIBIANS IN GRSM-BLRI-CUGA-MACA**

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PART I-POND SAMPLING PROTOCOL

GENERAL INFORMATION: The following protocol was written to allow future researchers to duplicate procedures as precisely as possible when sampling vernal pond amphibians. We have concentrated our efforts on censusing two focal species (*Rana sylvatica*; *Ambystoma maculatum*) and have used total counts of egg masses laid during each breeding season to census relative changes in breeding population sizes.

Our basic sampling scheme involves making repeated censuses of ponds (3-4 times per season) and counting the cumulative number of egg masses laid each year. Spotted salamanders normally have 2-3 major breeding bouts each year. Eggs counted during an initial survey can be distinguished from new eggs laid after the site visit by the developmental stages of the eggs. We visit ponds at regular intervals (every 2-3 weeks) and score any new egg masses that are present along with the frequency of clear versus milky egg masses (this species lays both clear and whitish egg masses). We continue censusing until all breeding for the year has ceased. Because spotted salamander embryos have long developmental periods and the eggs are dispersed throughout ponds, the timing of the initial sampling is not critical. We normally begin our seasonal sampling soon after wood frogs have their major breeding bout (see below), and begin sampling spotted salamanders during the second census of each pond. We also take data on embryonic survival and a variety of other parameters as outlined below.

Wood frogs breed before spotted salamanders and the adults are explosive breeders. Most eggs will be laid in a 2-4 day period anytime from late January to late February depending on the location and elevation of ponds. Most egg masses are laid in large rafts that may contain hundreds of individual egg masses (each female produces one mass). One problem in sampling this species is that the egg masses swell and the jelly tends to fuse with time. As a result, individual egg masses are difficult or impossible to distinguish from one another. When sampling wood frogs, it is essential that an initial census be conducted prior to the time that egg masses fuse. This requires that care be taken to monitor breeding activity and breeding congresses. Wood frogs typically begin breeding when air temperatures exceed 60-65 F and will often engage in one major breeding bout following relatively warm weather during the first 3 weeks of February- be on the lookout for breeding activity during this critical time. Park personnel have been very helpful in providing information concerning calling frogs or frogs observed moving on roads. In addition, we check previous records on data sheets to get a feel for the typical time of breeding at each pond. This varies from year to year, but is reasonably predictable. If eggs are sampled after fusion, an estimate of breeding activity can be obtained by measuring the area of rafts and dividing by the average size of egg masses. This method is far less reliable than direct counts and should be avoided whenever possible.

Rafts of eggs should be sampled within 5-10 days after the first breeding bout. After the major breeding bout, small numbers of females will breed in the ponds at later dates; the eggs of these late arrivers are often scattered about and easy to count. The key to sampling wood frogs is to catch the major breeding bout.

We use a standard data sheet to record data (see sample on the next page). We use Orion meters (model 230A) and electrodes (triode combination electrode) for measuring pH, and Corning meters (Checkmate modular meter system) for measuring conductivity. Mean values of these and other measurements are entered into computers for statistical analyses. Three permanent PVC pipes are placed in each pond and are used as markers for measuring pond depth and for taking water samples each year. We estimate embryonic mortality based on subjective estimates since counting

the number of eggs in each mass is not feasible. We use four categories of mortality (0-25%, 26-50%, etc.) and in blind studies have found high agreement among researchers in categorizing mortality. We score eggs into three embryonic stages (preneural fold, neural fold to pre-tail bud: tailbud and beyond). We selected 'early', 'mid-', and 'late' developmental categories because they are easy to identify under field conditions. We will use mortality of embryos in late developmental stages to determine if there are any long-term changes in embryonic survival at the sites. We sample a minimum of 20 masses per pond for each species, but often sample > 20. We survey ponds by lining up 2-4 researchers and making a sweep around the pond. We take care to assure that all areas of each pond are searched. Polarizing sunglasses are useful for reducing glare on the water surface and for finding eggs. When necessary we mark egg masses with flagging to compare developmental stages of old and new masses that have been laid since the previous visit. Although we use Rana sylvatica and Ambystoma maculatum as focal species, we record observations and counts of any other species observed while moving through ponds and censusing eggs.

We have provided an exact description of our procedures for sampling each site to allow future researchers an opportunity to duplicate procedures as closely as possible. For the sake on continuity and consistency, we encourage researchers to follow these steps and to resist the tendency to change protocols.

DATA SHEET: LONG TERM MONITORING OF TEMPORARY POND BREEDING AMPHIBIANS

Focal species: *Ambystoma maculatum*, *A. jeffersonianum*, *Rana sylvatica*

REGION: Cumberland Gap National Historic Park

TRANSCRIBED FIELD DATA

RESEARCHERS: Petranka and Smith

ASSISTANTS: Beth Smith, Becky Elkin

POND CODE: CUGA - PG

DATE: 6 Mar. 1994

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TIME START: 1:55 P.M.

TIME END: 2:30 P.M.

CURRENT WEATHER:

AIR TEMP. (c) 14.0 14.0 AVG. = 14.0

PRECIPITATION: NONE

SKY: CLEAR

CURRENT WEATHER

NOTES: _____

PREVIOUS WEATHER

NOTES: SEE PA

POND P-CHEM:

SAMPLE	DEPTH	pH (paper)	pH (meter)		CONDUCTIVITY	TEMPERATURE	
	(m)					(ms)	(C)
1	0.39	5.2-5.5		5.42		37.60	8.0
2	0.42	5.2-5.5		5.73		51.20	7.0
3	0.38	5.2-5.5		5.24		34.60	7.0
AVG.	0.40			5.46		41.13	7.33

GENERAL NOTES:

1. *Rana sylvatica*:

1. one new mass (see data sheet)

2. Scoring of old masses

9 masses L - A

1 mass L - B

10 masses M - A

2. *Ambystoma maculatum*

1. new masses see data sheets

OTHER SPECIES SEEN:

1. Fresh beaver signs (not seen last time) within pond

2. *Pseudacris triseriata* calling near pond.

TEMPORARY PONDS - TRANSCRIBED FIELD DATA

Ambystoma maculatum

REGION: Cumberland Gap National Historic Park

DATE: 6 Mar. 1994

POND CODE: CUGA - PG

PAGE: 1 OF 1

MASS #	STAGE CLASS	MORTALITY CLASS	EGG MASS		EGG MASS COLOR MORPH	COMMENTS
			LENGTH (cm.)	DIA. (cm.)		
1	E	A	8.0	8.0	C	
2	E	A	9.0	6.0	C	
3	E	A	7.0	6.0	C	
4	E	A	4.0	3.0	C	
5	E	A	12.0	7.0	C	
6	E	A	9.0	5.0	C	
7	E	A	11.0	7.0	C	
8	E	A	4.0	3.0	C	
9	M	A	10.0	8.0	C	
10	E	A	8.0	6.0	C	
11	E	A	10.0	7.0	C	
12	E	A	7.0	5.0	C	
13	E	A	7.0	9.0	C	
14	E	A	8.0	10.0	C	
15	E	A	12.0	9.0	C	
16	E	A	10.0	8.0	C	
17	E	A	15.0	8.0	C	
18	E	A	9.0	5.0	C	
19	E	A	12.0	6.0	C	
20	E	A	11.0	7.0	C	
20		= TOTAL MEASURED			MORTALITY CLASSES: A=0-25%, B=26-50%, C=51-75%, D=75-100%	
20		= CLEAR			STAGE CLASSES: E = Gosner stage 1-12; M = 13-17; L = > 17	
0		= WHITE				
ADDITIONAL EGG MASS COUNTS:						
86		= CLEAR				
0		= WHITE				
86		= TOTAL OF ADDITIONAL TALLY				
106		= GRAND TOTAL NUMBER OF EGG MASSES (measured masses + additional tally)				
106		= CLEAR				
0		= WHITE				
SPERMATOPHORE CLUSTERS:						
0		= TOTAL OF TALLY				
<i>Ambystoma opacum</i>						
46		= TOTAL OF LARVAL TALLY				
NOTES:						

TEMPORARY PONDS-TRANSCRIBED FIELD DATA

RANA SYLVATICA

DATE: 27 Feb. 1994

REGION: CUGA

POND CODE: CUGA - PG

PAGE: 1 of 2

RAFT #	# MASSES IN RAFT	RAFT SIZE L (m)	RAFT SIZE W (m)	SAMPLE FROM RAFT OR INDIVIDUAL MASS		COMMENTS			
				# MASSES IN SQ. m	MASS #				
				STAGE	MORTALITY CLASS	MASS SIZE (gm.) DIA. 1 DIA. 2			
1	119	2.40	0.75	1	E	A	18	13	
1				2	E	A	16	12	
1				3	M	A	12	10	
1				4	E	A	13	9	
1				5	M	A	13	11	
1				6	M	A	16	14	
1				7	M	A	15	11	
1				8	M	A	14	10	
1				9	M	B	12	10	
1				10	E	A	16	13	
1				11	M	A	15	10	
1				12	E	A	14	12	
1				13	E	A	12	8	
1				14	E	A	9	10	
1				15	E	A	13	11	
1				16	M	A	16	12	
1				17	E	A	13	12	
1				18	E	A	14	8	
1				19	E	A	12	11	
1				20	E	A	12	11	
2	59	0.70	0.90	1	E	A	14	9	
2				2	E	A	14	11	
2				3	E	A	13	11	
2				4	E	A	12	10	
2				5	E	A	12	9	
2				6	E	A	13	9	
2				7	E	A	14	10	
2				8	E	A	15	10	
2				9	E	A	15	11	
2				10	E	A	12	12	
3				1	E	A	15	11	
				= TOTAL MEASURED MASSES		178		MORTALITY CLASSES: A=0-25%, B=26-50%, C=51-75%, D=75-100%	
				= ADDITIONAL ECG MASS TALLY		49		STAGE CLASSES: E = Gosner stage 1-12; M = 13-17; L = > 17	
				= TOTAL FOR PAGE:		227		< = totals from previous pages	
				GRAND TOTAL FOR POND =>				CONTINUED	

P-CHEM DATA:

GENERAL:

1. The following physical and chemical parameters should be estimated on each pond visit.

- a) pond depth (average depth along long axis)
- b) water temperature
- c) pH
- d) conductivity

Replicates: Mean values for each estimate should be based on 3 samples

Sample Point Locations: Depth and temperature should be determined and water samples for pH and conductivity should be taken at three sample points along the long axis of the pond. The locations should be at 0.25, 0.5 and 0.75% of the total length (long axis) of the pond. If the location of the pond permits, the sample points should be permanently marked with a white PBC pipe driven into the substrate.

Depth

1. Measure from the pond bottom to the surface of the water and record in cm.

Temperature

1. Use a standard metal case mercury field thermometer.
2. Measure at 10 cm below the surface or at mid-depth if depth is less than 10 cm.
3. Measure to the nearest 0.5 C

pH

1. **Meter:** Use portable field meter with pH probe as directed.
2. **Calibration of Meter:** At first site each day the meter should be calibrate as directed.
3. **Samples:**
 - a) Use 50 ml graduated tubes with caps to collect samples.
 - b) Collect 3 separated 50 ml water samples, one at each sample point.
 - c) Collect water from a depth of 10 cm or at mid depth if depth is less than 10 cm.
 - d) Collect by inserting inverted tube to depth and then turning upright.
 - e) Cap samples and return to shore.
4. **Standardized pH determination:**
 - a) Haphazardly pick sample tube.
 - b) Rinse probe with distilled water and pat dry with KEM-WIPE.
 - c) Take repeated pH readings until three consecutive values are identical or the values fluctuate about an equilibrium point (6.52; 6.56; 6.48). If the later occurs, take a fourth reading and record.
 - d) Repeat 1 and 2 for each remaining sample.

Conductivity

1. **Meter:** Use portable meter with conductivity probe as directed.
2. **Calibration of Meter:** At first site each day the meter should be calibrated as directed.
3. **Samples:**
 - a) Use 50 ml graduated tubes with caps to collect samples.
 - b) Collect 3 separated 50 ml water samples, one at each sample point.
 - c) Collect water from a depth of 10 cm or at mid-depth if depth is less than 10 cm.
 - d) Collect by inserting inverted tube to depth and then turning upright.
 - e) Cap samples and return to shore.
4. **Standardization of conductivity measurements:**
 - a) Haphazardly pick sample tube.
 - b) Rinse probe with distilled water and pat dry with KEM-WIPE.
 - c) Take repeated pH readings until three consecutive values are identical or the values fluctuate about an equilibrium point. If the later occurs, take a fourth reading and record.
 - d) Repeat 1 and 2 for each remaining sample.

AMBYSTOMA MACULATUM

Egg mass counts:

1. count all masses in the pond on each visit and record whether masses are white or clear.

Sampling for mass size, developmental stage and mortality:

1. 20 masses should be measured for size and scored for developmental stage and mortality.
2. If the pond contains more than 20 masses, the sample should be a haphazard sample.
3. Make an initial survey of the pond from the edge to get an idea of how many masses are present.
 - a) If there are clearly more than 20, then haphazardly pick masses to measure as they are encountered so that those sampled come from all areas of the pond.
 - b) If there appears to be < 20 masses, then simply record data for all masses.

Mass size Estimates

measure maximum length and diameter of each egg mass (record in cm)

Developmental Stage Estimates:

1. Score each egg mass as belonging to one of the following developmental stage classes based on the stage of the majority of the embryos.

class E (early) = Harrison Stage 1-12; before neural folds develop
class M (mid) = Harrison stage 13-28
class L (late) = Harrison Stage > 29; tail bud and post tailbud stages.

We selected these groups because the presence of a neural fold or tail bud is easily recognized in the field.

Mortality Estimates

1. Score egg mass as belong to one of the following mortality classes based on a qualitative estimate of the percentage of embryo that are dead. This requires that a subjective interpretation of the percent of eggs that are dead in each mass be made (counting is not practical under field conditions).

class A = 0-25%

class B = 26%-50%

class C = 51%-75%

class D = 76%-100%

RANA SYLVATICA

RAFT: On the data sheets a 'raft' is considered to be a single egg mass or a clump of egg masses ranging in number from 2 to any number of eggs that are touching one another.

Note: It is critical for this species that egg masses be censused as soon as possible after they are deposited. As they age it becomes difficult to distinguish individual masses in a clump and to observe individual embryos within a mass.

Egg mass counts:

1. count "new" masses on each visit
new masses are those that were deposited since the previous visit as judged by developmental stage. This needs to be done, because egg masses fuse with age and become indistinguishable from others.

2. count masses by counting rafts as they are encountered; then counting the number of masses in each raft

Sampling for mass size, developmental stage and mortality:

1. A minimum of 20 masses should be measured for size and scored for developmental stage and mortality.

2. If the pond contains more than 20 masses, the sample should be a haphazard sample.

3. Make an initial survey of the pond from the edge to get an idea of how many masses are present.

a) If there are appears to 20 or less, then simply record data for all masses as encountered.

b) If there are more than 20 masses then haphazardly pick masses to measure as they are encounter so that those sampled come from all areas of the pond and from different rafts. This can be accomplished by picking no more than 5-10 masses from any one raft, depending on how many rafts there are. It is okay to record data for more than 20 masses so long as the minimum of 20 is met.

Mass size Estimates

1. Measure egg mass diameter (maximum length x width in cm)

Developmental Stage Estimates:

1. Score each egg mass as belonging to one of the following developmental stage classes based on the stage of the majority of the embryos.

class E (early) = Harrison Stage 1-12; stages before neural folds develop

class M (mid) = Harrison stage 13-28

class L (late) = Harrison Stage > 29; tail bud or post tailbud stages

Mortality Estimates

1. Score egg mass as belong to one of the following mortality classes based on a qualitative estimate of the percentage of embryo that are dead.

- class A = 0-25% dead
- class B = 26%-50% dead
- class C = 51%-75% dead
- class D = 76%-100% dead

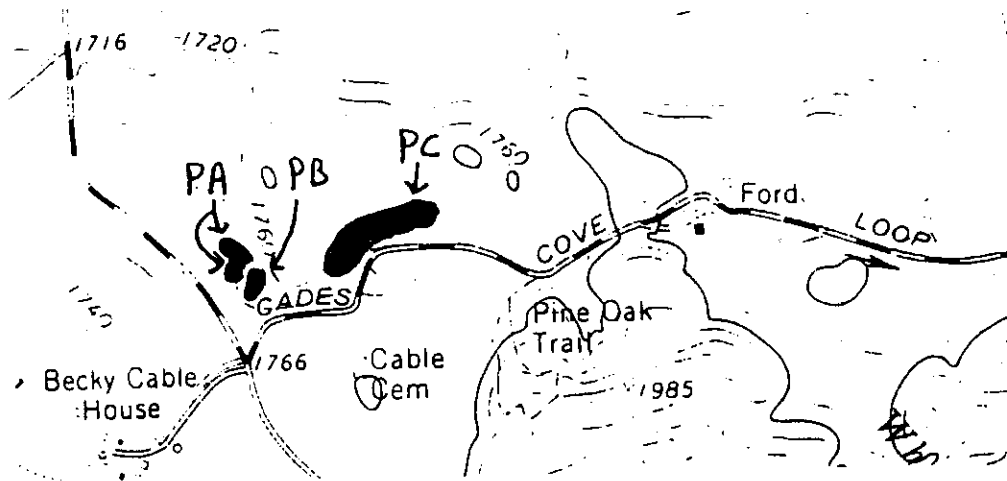
Park: GRSM

Pond code: GRSM-PB ✓

Dimensions (l x w in meters): 20.4 x 7.9 (161 m²)

Location: Immediately north of the junction of Cades Cove Loop Road and Forge Creek Road. This and GRSM-PA are two small ponds that are part of the Gum Swamp system. To reach these sites, proceed to the westernmost end of Gum Swamp and cross the hog fence. Proceed about 300-400 m west and search in the forest for GRSM-PA and GRSM-PB. GRSM-PA is the site farthest from Gum Swamp, while GRSM-PB is between GRSM-PA and Gum Swamp and within 40' of GRSM-PA.

Map:



Park: GRSM ✓

Pond code: GRSM-PC (Gum Swamp)

Dimensions (l x w in meters): 161 x 41 (6601 m²)

Location: About 0.2-0.3 miles east of the junction of Cades Cove Loop Road and Forge Creek Road. Park on pulloff and walk about 60-70 m into the woods to the left (north) of the Loop Road.

Map:

SEE MAP ABOVE

Park: GRSM

Pond code: GRSM-PD (sinkhole pond #1)

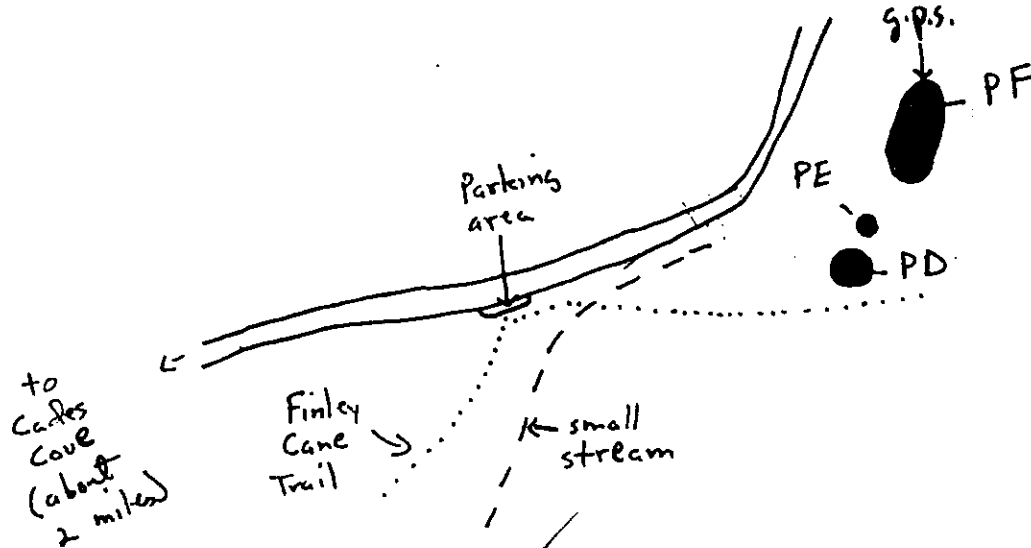
✓ Finley Cane

Dimensions (l x w in meters): 12.2 x 11.9 (145 m²)

Location: About 0.7 miles east of Crib Gap along the Cades Cove Road. Park at the Finley Cane Trailhead and proceed northeast (downhill) along the trail to a small creek. Cross the creek and proceed about 250-300 m. A large sinkhole pond (GRSM-PD) should be visible to the left of the trail. GRSM-PE is located immediately behind GRSM-PD and is smaller. A third pond (GRSM-PF) is located about 50 m northeast of GRSM-PE.

Note: this site is a deep sinkhole pond with maximum depth of > 4 m. Because it difficult to search when full, we are only censusing egg masses of spotted salamanders during years when water levels are low and masses can be accurately counted.

Map:



Park: GRSM

Pond code: GRSM-PE (sinkhole pond #2)

Dimensions (l x w in meters): 8.5 x 8.5 (72 m²)

Location: About 0.7 miles east of Crib Gap along the Cades Cove Road. Park at the Finley Cane Trailhead and proceed northeast (downhill) along the trail to a small creek. Cross the creek and proceed about 250-300 m. A large sinkhole pond (GRSM-PD) should be visible to the left of the trail. GRSM-PE is located immediately behind GRSM-PD and is smaller. A third pond (GRSM-PF) is located about 50 m northeast of GRSM-PE.

Map:

↑
SEE MAP ABOVE

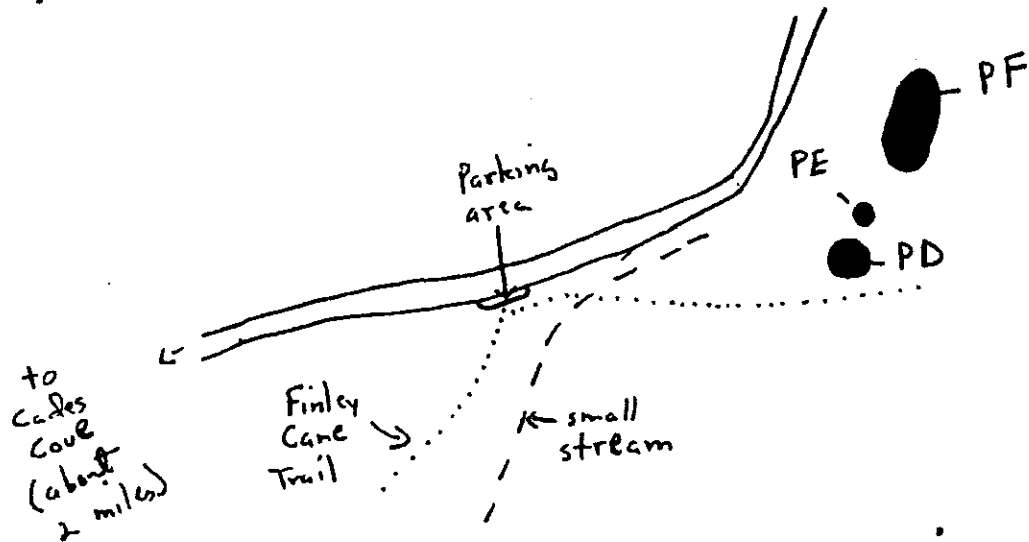
Park: GRSM

Pond code: GRSM-PF (hog pen pond) ✓

Dimensions (l x w in meters): 20.4 x 16.8 (343 m²)

Location: About 0.7 miles east of Crib Gap along the Cades Cove Road. Park at the Finley Cane Trailhead and proceed northeast (downhill) along the trail to a small creek. Cross the creek and proceed about 250-300 m. A large sinkhole pond (GRSM-PD) should be visible to the left of the trail. GRSM-PE is located immediately behind GRSM-PD and is smaller. GRSM-PF is located about 60 m beyond and northeast of GRSM-PE.

Map:



Park: GRSM

Pond code: GRSM-PG (Pond at The Sinks) ✓

Dimensions (l x w in meters): not measured because of road washout.

Location: Pond located at The Sinks parking lot along the Cades Cove Road west of the Metcalf Bottoms picnic area. Pull into parking lot at The Sinks and park at the far end. The pond is immediately to the left and within sight of the parking lot.

Map:

