Development of an Identification Process Using Digital Photographs: Marbled Salamander Migration Study

Jug Bay Wetlands Sanctuary
Prepared by Karyn Molines

INTRODUCTION

Salamanders in the genus *Ambystoma* (in the Ambystomidae family) or mole salamanders, the primary migratory salamanders found throughout the United States (Conant and Collins 1991). During the relatively brief reproductive period they become conspicuous when mature males and females travel to suitable breeding locations. Marbled Salamanders (*Ambystoma opacum*), Spotted Salamander *(A. maculatum*), Jefferson Salamander (*A. jeffersonianum*), and Tiger Salamander (*A. tigrinum tigrinum*) are the mole salamanders found within Maryland.

Many researchers have investigated various aspects of the life history of mole salamanders, especially the spotted and tiger salamanders (Harris 1980; Husting 1965; Semlitsch and Pechmann 1985; Semlitsch et al. 1993; Semlitsch 1981). Prior to 1936 eight papers or notes described the biology of Marbled Salamanders (Brimley 1920; Deckert 1916; Dunn 1917; King 1935; Lantz 1930; Mann 1855; McAtee 1933; Noble and Brady 1933). One of the most thorough studies, by Noble and Brady in 1936, has been the basis of almost all knowledge of the Marbled Salamander lifestyle. More recently, researchers have investigated larval development (Anderson and Graham 1967; Hassinger et al. 1970; Scott 1994; Stenhouse et al. 1983; Stenhouse 1984, 1985a, 1985b) and the evolution of nest-site selection (Jackson et al. 1989; Kaplan and Crump 1978; Petranka and Petranka 1981; Petranka 1990; Semlitsch et al. 1993).

Marbled Salamanders are well known for the unusual timing of their breeding migration. They migrate to vernal pools in the fall in order to mate and lay eggs (Petranka 1998). In general, males arrive first, with females arriving a week or so later (Noble and Brady 1933). Migration is dependent on evening rains and the influx to the pool varies along the species' entire range: from August in Rhode Island to October or November in South Carolina (Petranka 1998). In Maryland, their arrival into the breeding ponds is in mid-September (Molines and Swarth 1999). Shortly after mating males leave the pools. Females lay their eggs and remain in the pools for varying periods of time, sometimes for as long as two months (Noble and Brady 1933).

The migration patterns of Marbled Salamanders have been documented at the Jug Bay Wetlands Sanctuary (Molines and Swarth 1999) during a long-term drift fence and pitfall trap monitoring study. Migration begins in early September, with peak movement between 13-18 September. Migration is dependent on evening rainfall, although only minimal amounts (0.75 mm) are needed to result in salamanders being caught in the traps. The total number of marbled salamanders caught year to year is highly variable and not correlated to the amount of rainfall that fell during the season.

We created a generalized pattern of their breeding season (Fig. 1). A few male Marbled Salamanders entered the vernal pools in early September. The first large wave of salamanders would arrive at the pools after September 13. Females usually arrived a week after males. In October, males and females began leaving the pools, returning to their upland forest habitat. By late November the salamanders returned to the upland forest; their annual breeding migration had come to an end.

In addition to understanding their general breeding biology, we were interested in determining how many individuals were breeding each year. The number of salamanders captured within a season was not necessarily the actual number of salamanders breeding. Animals were not marked via toe-clipping or other permanent mark, so we may have caught the same individual several times during the breeding season. In one study in Virginia, individual marbled salamanders were often caught several times during one season, typically when they entered and again when they left the pools (Bailey pers. comm.)

Concerns regarding potential adverse effects of toe-clipping and other marking techniques have encouraged development of non-invasive photographic identification techniques. Common mark-recapture techniques can be ineffective on amphibians because of their sensitive skin and ability to regenerate limbs and toes. (Davis and Ovaska 2001). Photographs have been used to identify individuals in other species but a systematic method for searching the photographs has not come easy. Sophisticated computer programs have been developed to identify cheetahs (Kelly 2001) and zebras (Peterson 1972 in Krijger 2002) via their patterns. Ravela and Gamble (2003) developed a mathematical algorithm to search digital photographs to identify Marbled Salamanders.

In 2000, I began an effort to photograph every salamander captured in order to identify individuals. Initially, I, along with several volunteers, visually reviewed each photograph looking for recaptures. During this process, it became apparent that the white or gray patterns could be described in systematic terms to facilitate the search system. From my experience comparing photographs, several specific characteristics arose as potential sources for developing a pattern code and computerize search system. I developed a technique to search for recaptures based on their patterns which combined a manual process of describing the pattern and a systematic database search for similar patterns. This report analyzed our effort to identify individuals captured at one vernal pool in the fall breeding seasons of 2001-2003. The effectiveness of this relatively low cost technique for pattern recognition was evaluated.

METHODS

Study Area

Jug Bay Wetlands Sanctuary is a 600 hectare (1400 acre) ecological research station and wetland learning center located on the Patuxent River in southwest Anne Arundel County, Maryland, about 29 km (18 miles) south of Annapolis. The Sanctuary is operated by the County Department of Recreation and Parks, and a portion of the Sanctuary is within Maryland's Chesapeake Bay National Estuarine Research Reserve. Major habitats within the Sanctuary are fresh water tidal wetlands, non-tidal wetlands, upland hardwoods forests and agricultural fields. Four permanent creeks drain the uplands and flow into the Patuxent River. Vernal pools and floodplain wetlands are found throughout the Sanctuary. Several small, man-made ponds which vary in size from 5 to 25 m^2 were created when a railroad levee was built in the 1890s. These wetlands fill with water seasonally and are important habitats used by amphibians.

Drift Fence and Pitfall Traps

Permanent drift fences and pitfall traps were used to monitor salamander populations at three different breeding locations (Fig. 2). Fences were made of aluminum flashing or plastic mesh. The fences extended about one meter above the ground and approximately 10 cm below ground. The fences served as a barrier to movement. When salamanders encountered a fence they were forced to travel along it until the fell into the pitfall traps, where they were unable to climb out. The pit fall traps were 18.9 L buckets buried flush with the surface of the ground. A damp sponge was placed in the trap to provide moisture to prevent the trapped animals from becoming desiccated. Foam floats were placed in buckets which provided refugia for trapped animals when the buckets filled with water. Buckets were covered with lids when not in use.

The status of each bucket trap was always recorded as either 'functioning' or 'non-functioning.' Non-functional traps resulted from several causes: saturated groundwater caused traps to pop out of the ground; flooding may have provided some animals an avenue for escape; or lids were not removed from a bucket.

Description of Study Site Trap Arrays

Most of the data under discussion were collected at the Wet Forest Site. Trapping arrays were also erected at two other breeding locations within the Sanctuary: near a semi-permanent pond (Mark's Pond) and above the Two Run Creek floodplain (Forest Bluff) were Y-shaped fence lines with four pitfall traps. Mark's Pond also had a 10-m straight line fence with two traps.

Wet Forest Site: An Upland Seasonally Flooded Forest

The Wet Forest Site (WF) was a seasonally-flooded forest, dry most of the year but was periodically flooded in late winter and early spring. In dry springs almost no standing water was present, while in very wet years the flooded area approached 1 Ha.

The trap array had five pitfall traps each, arranged along two 50 m long fences perpendicular to the slope of the hillside, identified by their bucket numbers: WF 1-6 and WF 7-11 (note, WF 2 was removed from the array). The buckets were positioned directly under the fences, so salamanders moving both toward and away from the pond were captured in the traps. A third array 'The Hill' (WF 12-17) along a 30 m fence was installed above the WF 1-6 trap line (Fig. 3). Three traps were located on the 'uphill' side of the fence, while three were on the 'downhill' side.

Trapping Schedule

The fall trapping season began around 1 September and continued through November. Traps were checked each day between 8:00 and 10:00 a.m. Traps remained open for as many consecutive nights as possible, depending on the availability of staff and volunteers. Table 1 summarizes the 2001-2003 trapping seasons.

Table 1: Summary of trapping seasons for Marbled Salamanders at the Wet Forest.

Year	Opened	Closed	Trapping Days
2001	1-Sep	9-Dec	98
2002	4-Sep	17-Nov	73
2003	2-Sep	26-Nov	85

Definition of Capture

The number of 'captures' does not represent individuals, since salamanders were not marked and identifiable as individuals. Therefore some animals might have been recaptured several times during the season, resulting in an overstatement of number of individuals at the site. A capture was defined as one animal in one trap.

Sex Identification

We attempted to determine the sex of every animal captured. Animals were captured during the breeding season: individuals with swollen vents were males; swollen abdomens indicated gravid females. Animals that had neither swollen vent nor abdomen were listed as 'unknown" or were sexed by their color compared to a gray scale (Fig. 4). Marbled Salamander males had a whiter pattern (values 1 or 2) compared to the gray banding (values 4 or 5) in the females. All animals were released shortly after capture.

Measurements

Salamanders were weighed in the lab. Most were weighed in the lab using an Ohaus CT1200 electronic balance to the nearest 0.1 gram, while some salamanders were weighed using a hand held Pesola® scale to the closest 0.25 grams. Length was not measured, but photographs of animals included a centimeter grid to determine length.

Photography

Photography was done in the lab using a Canon PowerShot A50. Date and location of capture, sex characteristics, weight, and other information were recorded on data sheet and included in the photo. A centimeter grid provided a scale reference (Fig. 5) Photos were stored on a Macintosh Apple computer and backed up onto CD-ROM.

Identification of Individuals: Finding Recaptures Based on Crossbar Cohort and Pattern Codes

For identification, all characteristics were determined by the patterns formed by the white, silver or gray markings. Patterns formed by the black regions were only used incidentally. The number of crossbars on the head, body, and tail was the primary feature used to group salamanders into 'cohorts.' In addition, a 'pattern code' was developed that described the pattern of crossbars (C), lateral spots (S), and lateral bands(B) and gaps with no pattern (-) on the body. Both the right and left lateral body patterns of crossbars and spots were coded. Figure 6 illustrates this process. The Appendix includes the specific protocol followed in coding the salamander patterns.

Data were entered into a Microsoft® Access database designed specifically for this project. Queries were written to search for varying combinations of crossbar counts and body patterns. Only the right body pattern was used to determine possible recaptures. To account for the variation in crossbar counts and coding, an additional step compared the primary cohort with other cohorts that had one more or one less body crossbar, disregarding the number of head or tail crossbars. Photographs were reviewed to verify identification. A database query provided a report of all salamanders within a specific crossbar cohort along with specific information such as date of capture, sex, pattern codes. Photographs of these salamanders were compared to determine if any were recaptures, then they were assigned a 'Recapture Number.' Table 2 used salamanders in the cohort with 2, 5, and 6 crossbars on their head, body, and tail, respectively, as an example of the process.

Table 2. Example of the result of a Microsoft® Access query for salamanders in the cohort with 2, 5, and 6 crossbars on their head, body, and tail, respectively. The data were sorted by Pattern Code. Notes: (a) Recapture number was assigned after positive identification from the review of photographs. (b) Recapture #44 was identified by searching cohorts of ±1 crossbars for unusual body patterns. See text and Appendix for Pattern Code definitions

Date Captured	Sex	Pattern Code	Recapture Number [a]
21 Sep 2001	Male?	BC-C-C, -C*-C	
24 Nov 2001	Unknown	C*BC-SC-S-CSC	28
26 Oct 2002	Male	CBC-SC-S-CSC	28
13 Sep 2003	Male	CBSBYBC-YC	44[b]
16 Oct 2001	Male	C-C-C-C-C	26
21 Sep 2001	Male	C-C-C-C-C*	
21 Sep 2001	Male?	C-C-C-C-S*-C	4
26 Oct 2002	Male?	C-C-C-C-S*-C	4
15 Oct 2001	Male	C-C-C-C-SC	26
21 Sep 2001	Male	C-S-C-Y-C-C	
21 Sep 2001	Male	C-S-S-C-C-C-C	7
15 Sep 2002	Male	C-S-S-C-C-C-C*	7

Population Estimate

The Jolly-Seber Model was used to calculate a population estimate based on recaptures. This model was designed for population mark-recapture studies where there were multiple sampling dates over time. It also allowed for the assumptions of death, recruitment, immigration and emigration from the population.

$$N = \frac{M}{a} \text{ where } M = \frac{(R+1)z}{r+1} + m \text{ and } a = \frac{(m+1)}{(n+1)}$$

N=population estimate
R=total number of animals released in period i
r=number of animals at period i that are ever captured again in a subsequent sample
m=total number of marked animals in period i
z=number of animals marked before period i which are not caught in the ith sample that are caught subsequently
n=total number of animals caught in period i

RESULTS

During the autumns of 2001, 2002 and 2003, 2,538 Marbled Salamander were captured in the pitfall traps. Results presented were for the 438 salamanders captured at the Wet Forest 7-11 traps during these years. This subset was selected to facilitate the development and analysis of identifying individuals by their patterns. This sample of photographs had to be large enough to provide variations and likelihood of recaptures, yet small enough to verify that all recaptures were identified.

Migration Pattern and Rainfall

Throughout the study, salamanders followed our expected pattern of migration as illustrated in Fig. 7. Salamanders were not necessary captured after every rainfall and the earliest captures were when we had mid-September rains. As has been found in our past studies, the amount of rainfall was not correlated to number of salamanders captured (r^2=0.22).

At all three trapping locations, we captured approximately 53% of all the salamanders before October 1 ($\bar{x}$ =53±13%; 22-77%; N=2,538) (Table 3).

Table 3. Captures before and after October 1 at all trapping sites. Numbers in parentheses were numbers captured in WF 7-11.

	Before Oct 1	After Oct 1	Total Captured	Percent Before
2001	635 (139)	451 (80)	1086 (223)	58% (63%)
2002	423 (53)	416 (52)	839 (105)	50% (50%)
2003	291 (48)	322 (62)	613 (110)	47% (44%)
Total	**1349 (240)**	**1189 (197)**	**2538 (438)**	**53% (55%)**
Mean	**450 (80)**	**397 (66)**	**846 (145)**	**52% (53%)**
St. Dev.	**173.4 (51.2)**	**66.7 (14.0)**	**236.6 (64.4)**	**6% (10%)**

To determine if the October 1 median point was related to the breeding migration, we analyzed the captures from the Wet Forest Hill traps, where three of the traps were on the ‘uphill’ side of the fence, while the other three were on the ‘downhill’ side. Salamanders caught in the ‘uphill’ buckets (Fig. 3) were usually moving into the pond. Those caught in the ‘downhill’ buckets were typically returning to the forest. More than 90% of the salamanders captured in the Wet Forest Hill traps prior to October 1 were in the uphill buckets. After October 1, more than 92% of the salamanders were in the downhill buckets. In 2002 and 2003, the number captured in the uphill buckets was approximately the same as those caught in the downhill buckets (Table 4).

Table 4. Captures before and after October 1 at Wet Forest Hill.

	Before Oct 1	After Oct 1	Percent Before	Percent After	Total
2001 Uphill	184	9	95%		**193**
2001 Downhill	11	122		92%	**133**
2002 Uphill	106	11	91%		**117**
2002 Downhill	6	110		95%	**116**
2003 Uphill	83	9	90%		**92**
2003 Downhill	3	103		97%	**106**

The change in the weight of salamanders over the season was also an indication of their breeding status. After a salamander deposited eggs or spermatophores, they lost mass. Comparing the weights of salamanders caught at different time periods offered insight to the migration pattern (Table 5). Within any one year, the weights of all animals were significantly heavier in September than those caught in either October or November.

Table 5. T-Test comparing the weights of Marbled Salamanders captured in Wet Forest 7-11, regardless of sex or recapture status.

2001	**p=**	**N=**		**2002**	**p=**	**N=**		**2003**	**p=**	**N=**	
Sep-Oct	<0.001	Sep	120	**Sep-Oct**	<0.001	Sep	54	**Sep-Oct**	<0.001	Sep	48
Sep-Nov	<0.001	Oct	42	**Sep-Nov**	<0.001	Oct	42	**Sep-Nov**	<0.001	Oct	37
Oct-Nov	0.61	Nov	39	**Oct-Nov**	0.13	Nov	13	**Oct-Nov**	0.30	Nov	28

Males and Females

We were certain of the sex determination of 332 of the 438 salamanders captured at WF 7-11. On average, 54% were males. (Table 6). Our first captures were typically males. Females were rarely caught early in the season; only 3 of the 109 females were caught prior to September 15. Males arrived first, followed seven to ten days later by females. Figures 11a-c illustrated the proportion of males and females captured each day.

Table 6. Sex ratio of Marbled Salamanders captured at Wet Forest 7-11.

	Males	Female	Unknown	%male
2001	110	38	148	49.3%
2002	60	28	88	57.1%
2003	68	27	95	61.8%
			Mean	**54.1%**

Finding Recaptures Based on Crossbar Cohort and Pattern Codes

Of the 438 captures, we had 389 digital images, of which 57 individuals were identified as recaptures. Five individuals were recaptured a second time, and both recaptures were included when analyzing the search technique.

Through the basic identification procedures (grouping the salamanders by the crossbar cohort and comparing the body code) I found 60 % of the recaptures (40 of 67) (Table 7). Usually the discrepancy in the body pattern was of a single spot (S) (See Recapture #9, Figure # and Appendix for specific data.)

When cohorts were compared to the cohorts with one more or one less body crossbar another 38% of the recaptures (20 of the 67 recaptures) were found. The remaining recaptures were identified by number of body crossbars (regardless of the number of tail or head crossbars) and their body patterns.

Initial investigation of the coding system identified several features of crossbar counts and body patterns useful for identification. Some patterns had crossbars forming letters such as ‘Y’, ‘W,’ or ‘X.’ Recaptures were readily identified using these distinctive features. Some patterns had unusual designs that were difficult to code. Recaptures were readily identified using these distinctive features. Recapture #44 (Fig. 9) was identified by its unique pattern even though the crossbar count and body pattern code were slightly different.

The extent of variability of crossbar counts and pattern codes was primarily due to poor photo quality and the salamander’s position. Several coding and search techniques were instituted to account for these differences.

- Sometimes a crossbar could be mistaken to be a spot, or vice versa. To indicate that this could be a possible variation in the code, they were indicated by an asterisk (C* or S*). Both were considered equivalent to a C when comparing pattern codes. Recapture # (Fig #)
- Crossbars at the junction of the body and the vent, or the neck and body, may be recorded on different sections depending on the position of the salamander in the photo. These were recorded as ‘misplaced’ junction crossbars and were considered equivalent to a body crossbar when comparing pattern codes. Recapture #39 (Fig. 7) had both a head and tail junction crossbar placed on different sections, but was readily identified by comparing its code to the cohorts with one more or one less body crossbar.

- Variations in tail crossbar counts were caused when the tail twisted to the side or when the tail was damaged. Tail crossbar counts and patterns were not necessary for determining individuals, but were used for final verification when reviewing photographs. Recapture #57 (Fig #) had re-grown the tip of his tail, resulting in variation in tail crossbar count; but his body pattern was exact when compared to individuals with the same number of body crossbars.
- Head patterns often were irregular patterns rather than crossbars and made counting crossbars difficult. Head crossbar count was not useful in determining individuals, although the unusual patterns were used in verifying recaptures when reviewing photographs. Recapture # (Fig #)

Table 7. Summary of recaptures based on crossbar count and pattern code. Percentages do not equal 100% due to rounding. Notes: (a) indicates the number of pattern codes that included obvious letters that were used to identify recaptures. (b) five of the 57 salamanders were recaptured twice and both recaptures are included in data. See text for details.

	Total	Body Pattern with Letters (a)	Recapture # (b)
Exact Crossbar Count			
A) Pattern Code Exact	16	4	*See Table #*
B) Pattern Code Almost	21	8	*See Table #*
L) Mistaken Crossbars or Spots, Indicated & Pattern Almost	2	1	8, 41
Mistaken Junction Crossbar			
C) Pattern Code Exact (except junction)	1		*50*
R) Tail or Head ±1 Pattern Code Almost (except junction)	1		*57*
Crossbar Count Tail or Head ± Body Crossbars Exact			
E, G, O) Tail or Head ±1 Pattern Code Exact	9	3	*See Table #*
F, H) Tail or Head ±1 Pattern Code Close	8	1	*See Table #*
I) Head ±1 and Tail ±1; Pattern Code Exact	2	1	46, 47
Q) Head ±1 and Tail ±1; Pattern Code Almost	2		22, 39
J) Tail Crossbars ±2; Pattern Code Exact	2		48, 49
K) Tail Crossbars +-2; Pattern Code Almost	1		42
M) Head ±1 and Tail ±2; Pattern Code Exact	1	1	27
Unusual and Complicated Pattern			
N) Pattern Difficult to Code, But Easy to Recognize	1	1	44

Year-to-Year vs. Within Year Recaptures

We recaptured Marbled Salamanders throughout the three seasons under investigation. Of the 51 salamanders recaptured once during the study, twenty-three (45%) were found during the same year of original capture. The other 29 (55%) were recaptured in a future year (Table 8).

Recaptures rates varied by year of original capture. Of the 202 captures in 2001, 21% (42) were eventually recaptured. Only 8 of the 102 animals from 2002 were recaptures, and only one individual from 2003 was recaptured.

Table 8. Summary of recaptured Marbled Salamanders. The row indicates the year the animal was first captured. The column indicates the year it was recaptured. For example, 13 individuals were first marked in 2001 and subsequently recaptured in 2002. Only the 52 salamanders recaptured once are included, the remaining five are summarized in Table 9.

		Year Recaptured			Number Photo-graphed
		2001	**2002**	**2003**	
Original Capture	**2001**	19	14	10	202
	2002		3	5	102
	2003			1	85

The five salamanders that were recaptured a second time (Table 9) were all originally captured in September, while their recapture patterns varied. Three were captured each year of the study (Recapture #9, 12, and 33) and always in September.

Table 9. Summary of Marbled Salamanders recaptured twice.

Recap #	**First Capture**	**Recapture 1**	**Recapture 2**
9	21 Sep 01	23 Sep 02	13 Sep 03
12	21 Sep 01	15 Sep 02	13 Sep 03
33	21 Sep 01	27 Sep 02	28 Sep 02
30	27 Sep 02	7 Nov 02	6 Nov 03
31	27 Sep 02	26 Oct 02	13 Sep 03

Males and Females

Of those animals recaptured, 79% (N=45) were males. This was a greater proportion than in the general population found at Wet Forest 7-11 where 54% of the captures from 2001-2003 were males. Refer to Table 6.

Migration Pattern

The individuals identified through the pattern recognition system appeared to follow a similar migration pattern found in our previous analysis. Most the salamanders (43 of 57) were originally captured in September. Their month of recaptured varied, with most being recaptured in September, either the same year or a future year (Table 10).

Table 10. Number individuals recaptured based on month of first capture (rows) and month recaptured (Column.) (♂ =Male, ♀ =Female)

	Second Capture					
	September		October		November	
First Captured	Same Year	Future Year	Same Year	Future Year	Same Year	Future Year
September	7 (6♂ , 1♀)	22 (17♂ , 5♀)	7 (7♂)	6 (5♂ , 1♀)	5 (2♂ , 3♀)	1 (1♀)
October		4 (3♂ , 1♀)	5 (4♂ , 1♀)	2 (2♂)	0	0
November		1 (1♀)		1 (1♂)	1 (1♂)	0

Individuals captured in September were entering the pool in breeding condition, and readily identified as male or female, based on sex characteristics. Individuals lost weight between their first capture in September and when they were recaptured later the same year (Table 11).

Table 11. One-tailed T-Test comparing the weights of individuals recaptured within the same year, regardless of sex. Not all of the animals had weights recorded.

Captured Within Same Year			
First Cap.	Recap	p =	N
Sep	Sep	0.003	6
Sep	Oct	0.01	5
Sep	Nov	0.03	3
Oct	Oct	0.13	4
Oct	Nov	na	1
Nov	Nov	na	0

For individuals captured in September of one year, there was a significant decrease in weight when captured in October and November. There was no significant difference in weights when caught in the October and recaptured later that year.

Population Estimate

Based on animals marked in 2001, a population estimate was made for the five dates in 2001 that had more than 10 captures. The mean population estimate was 1,530 salamanders (sd=±1,621; range=387-4374) using the vernal pool during the study period. See Appendix for the calculations.

DISCUSSION

Rainfall and Migration Pattern

In general, if salamanders were found in the traps, we were assured that it had rained the previous 24 hours; yet if there was rain in the previous 24-hours, there was not a guarantee that salamanders would be in the traps. Rainfall was necessary for salamander movement around the vernal pool, but seasonality played a strong role in determining the magnitude of the migration.

During the mid-September period salamanders were primed for migrating *en masse* to the pools. The first pulse of individuals was dominated by males. When females arrived a week or so later (also during an evening rain) males were ready and in place for mating. Later in the season the trigger to return to the upland forests was not a strongly correlated to a specific time frame. Scott (1998) hypothesized that the timing of migration was predictable because they are not dependent on laying eggs in water. The mass migration of most salamanders during the mid-September window may indicate other environmental, behavioral or physiological adaptations not measurable through the trapping study. Once mating is completed, the salamanders were more 'casual' in leaving the ponds, resulting in a more prolonged and less intensive number of salamanders after each rain event. The pattern of captures at the Wet Forest Hill traps and the decline in salamander weights indicated that salamanders had laid eggs and spermatophores sometime in September.

Using Crossbars and Patterns for Identifying Individuals

Using the crossbar cohorts was an effective method for reducing the sample size for using the pattern recognition process. The head and tail crossbar counts were more variable than the body crossbar count, but could be used as a secondary characteristic for confirming identification. Body patterns were easily reduced to a simple code and readily categorized making them an appropriate tool for identifying individuals. Simple improvements can be made so that writing computer code for searching the database would be easier. One could easily eliminate characters such as the asterisks, hyphens and apostrophes in the pattern code, which represent specific functions in some computer languages. The use of numbers rather than letters could facilitate developing a mathematical algorithm approach to searching the data.

Several features of the coding system were prone to discrepancies in the body pattern, specifically features that could vary depending on the twisting and curvature of the salamander's body. Another challenge in the coding was that some crossbars seemed to have an adjacent spot which may or may not be seen in all photos as illustrated by Recapture #26 (Fig. 8).

An improvement to this system would be to sort the body patterns into different groups based on how consistently and easily they code. Each sub-group could have specific coding systems applied and variations on search techniques. For example, patterns with very distinct letters (such as Y or X) made identification of an individual relative easy. Also whether the Y is right-side up or inverted (λ) was another readily identified characteristic. These individuals were the easiest to identify as recapture when comparing body crossbar count and body patterns.

In a similar vein, individuals with complex patterns were easy to recognize but difficult to code consistently. I had the most confidence that all the recaptures of individuals with distinct letters or unusual body patterns were identified.

Another sub-group would be salamanders that had lateral bands or a many lateral spots and only one or two crossbars. Two problems arose when searching for recaptures. Often, the exact number of spots was subject to interpretation or the body position of the salamander. Distinguishing a band and spot, or when a series of spots becomes a band, was often difficult to discern. One solution was to consider spots and bands equivalent. Even then, these patterns were not unique enough to easily decipher with the coding process. A separate coding and/or search system could be developed to account for the similarity of this pattern type.

The most difficult individuals are those with very few, faint, or even no pattern. Fortunately, there are not many individuals with minimal patterns, therefore enabling specific searching within this category of body pattern.

Overall the process was somewhat tedious, yet relatively inexpensive method that causes minimal stress to the animals (and volunteers.) It was a much more 'permanent' mark as compared to toe-clipping. The coding process included many features that were not necessary for identification of individuals. Through this analysis, the number of body crossbars and the body pattern were sufficient to recognize individuals.

Recaptures and Population Estimate

The number of recaptures was lower than expected compared to other sites that monitor Marbled Salamanders. For example, a site in Virginia, over a four year period, of the 33,000 salamanders captured, they identified almost 10,000 individuals. The majority of the recaptures were within year captures (Bailey, pers. comm.)

Our low recapture rate could be an artifact of the sample chosen for testing the search technique. I specifically selected the Wet Forest 7-11 sample because it was a small manageable subset of salamanders for finding recaptures. I felt that I could verify, through a manual review the 389 photographs, whether the animal was recaptured on a future date.

In addition, the drift fence did not completely encircle the pool. Animals could easily bypass the fence if their pathway varied by just a few meters. Therefore, I would expect that our recapture rate and population estimate may not be as precise as if the entire pond were enclosed by the drift fence.

Other factors could influence our recapture rate. Most of these scenarios result in a lower recapture rate and high population estimate.

- Routes into and out of the ponds were different. Shoop and Doty (1972) found that Marbled Salamanders tend to leave the ponds at the approximate location of their entry. Whether our salamanders exhibit the same behavior is unknown.
- Animals remained in ponds longer than the trapping study. During the few year when we did extend the trapping season, salamanders were captured in December. In particular, females are known to remain at their egg nests (Petranka 1990) for prolonged periods. Extending the trapping season into December may produce more recaptures of females.
- Animals avoided traps after having experienced them once. This would reduce the within-year recapture rate, resulting in animals being found only once per season.
- Traps did not capture every animal moving into or out of the ponds. On evenings with heavy rainfall, I have found that salamanders can escape from partially flooded buckets (unpubl. data.)
- Most animals did not breed most years, so each year we may be capturing a different subset of the breeding population. Our population may be composed of individuals on staggered breeding cycles. Continuing this effort may result in recapturing individuals who may be breeding on a four or five year cycle.
- New recruits (first-year breeders) each year. Each year there was a potential that first-year breeders were joining the breeding migration.
- Search system was not accurate and recaptures of individuals were missed.

Migration Pattern

Some of our salamanders appear to breed every year since 24 of the 57 individuals were captured in consecutive years, including the two individuals captured all three years of the study.

Most of our recaptures (47 of 52 salamanders) were captured at least once entering the pond during the peak migration period in September, during the en masse migration into the ponds. Twenty-three Individuals were caught in September, and then again in either October or November. This variable pattern of when salamanders leave the pool reflects the less predictable return to the upland forest. This confirms our original assumption that there is a mass migration into the pool during a brief period, and a more prolonged period for leaving the pool.

Knowing that the period for entering the pond is more concentrated could facilitate the population studies. One could undertake a population estimate, evaluation of the sex ratio, or year-to-year breeding efforts of individuals, based on the peak migration capture data rather than all the captures throughout the entire season.

Weight Decline

The significant weight decline of five of the six individuals caught twice in September of the same year was surprising, considering these each were recaptured the day after their original capture date—September 25-26, 2001 (Recaps #15, 18, & 19); September 27-28, 2001 (Recap #22); and September 27-28, 2002 (Recap #33). Apparently, during the intervening evening, they deposited spermatophores or eggs. The sixth individual (Recap #41) had no weight loss, although he was captured six days later (September 15 and 21, 2001.)

Although this is a small data set, some conjecture about the breeding season could be made. In 2001, a only 5% of that year's capture total (62 of 1086 captures) entered the pond between September 11 and 17, the traditional *en masse* migration period into the ponds. That year the en masse movement was delayed and it was did not begin until September 18. During the following ten day period, ending on September 28, 52% of the year's animals were captured (573 of 1086.) It appeared that the breeding congress occurred at the end of September rather than mid-September. Perhaps if we had caught Recapture #41 in early October he would have shown a weight loss.

RECOMMENDATIONS

This identification technique was a feasible method for identifying individuals based on their body patterns. Sorting the body pattern types into distinct categories would facilitate finding recaptures more easily. In addition, specifically studying individuals with easy to recognize patterns, such as those with letters or unusual designs, could provide insight into their migration patterns.

Determining that the body patterns are a reliable feature for identifying individuals can facilitate the development of other automated techniques to compare body patterns.

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FIGURES

1. Generic Pattern of Migration
10a-Total Number of Captures at All Sites 1998-2006
10b. Maximum day captures at all site
Rainfall-Captures 2001
Rainfall-Captures 2002
Rainfall-Captures 2003

APPENDIX

Map of trap sites
Schematic of trap arrays
Data sheets
Gray scale
Table ##. Population Estimate (N) Using Jolly-Seber

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