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A Comparison of Aquatic Surveying Techniques Used to Sample *Ambystoma opacum* Larvae

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Effective survey methods are needed for the marbled salamander (*Ambystoma opacum*) because of the species' rarity in the northeastern United States (DeGraaf and Rudis 1983) and increasing residential development that is affecting their upland habitats. Terrestrial surveys for adults are either impractical given the species' fossorial lifestyle (Petranka 1998) or costly (e.g., drift fences and pitfall traps; Dodd and Scott 1994). Larval surveys may be optimal because larvae can remain in pools for up to 7–9 months in some years (pers. observ.; Stewart 1956), creating a long window of opportunity for surveys. Aquatic funnel traps have been used successfully to capture other larval amphibians (Adams et al 1997; Bury and Major 1997; Fronzuto and Verrell 2000; Richter 1995; Wilson and Pearman 2000) and nocturnal visual surveys are a standardized transect surveying method commonly used in amphibian studies (Crump and Scott 1994; Thoms et al. 1997). We evaluate these two techniques for larval marbled salamanders. Specifically, we: (1) compare the results of each technique for determining presence and an index of relative abundance of marbled salamander larvae; (2) quantify the effect of time of year and survey intensity on the results of nocturnal visual surveys; and (3) describe the costs and logistical constraints associated with each technique.

We selected a sample of vernal pools in western and central Massachusetts using a hierarchical approach. We used the Massachusetts Natural Heritage Program's database to identify known marbled salamander sites within the study region. From this distribution, we selected five initial sampling areas that were well-distributed throughout the study region. Each initial sampling area consisted of a 3 km radius circular area roughly centered on one or more known marbled salamander sites. Within this 3 km circle, we mapped land cover and delineated all wetlands using aerial photographs (1991 color infrared, 1:40,000). We classified all temporary wetlands (e.g., vernal pools) as potential marbled salamander breeding habitat. Within each 3 km circle, we positioned a 1 km radius circle around the highest density cluster of potential breeding habitat, regardless of whether it contained any known marbled salamander sites. These five 1 km radius areas represented our final sampling areas.

We sampled a total of 97 pools distributed among the five sampling areas (Table 1) using both surveying techniques during the winter and spring of 1998. Each pool represented a separate observation (i.e., experimental unit) in the analyses described below. We sampled pools using bottle traps (Richter 1995) for 13 days between February 1 and March 1, when larvae were approximately 5 to 6 months old. The number of bottle traps placed in each pool was proportional to the surface area of the pool. We placed two traps per 10 m^2 (range 1–5 traps/pool), which represent a more intensive survey than recommended by Adams et al. (1997). Traps were randomly located within the pools at water depths between 0.5 and 0.75 m. If necessary, trap location and height were adjusted in response to fluctuations in water levels during the trapping period. Traps were baited with shrimp-scented salmon eggs and checked approximately every other day. Amphibian captures were recorded and released.

We conducted nocturnal visual surveys between March 1 and May 1. Each pool was sampled once during this period and all pools within a sampling area were surveyed on the same night. Each survey began shortly after dark and continued until all pools within the sampling area were surveyed. Pools were surveyed using transects; two observers walked parallel to one another, roughly 10 m apart, across the deepest section of the pool and then in opposite directions around the entire periphery of the pool approximately 2 m from the shore. Each observer utilized a high-powered flashlight to search and counted larvae within a 4 m wide swath.

After the 1998 season, we chose one of the five sampling areas to conduct more intensive tests evaluating the effect of timing and frequency of nocturnal visual surveys. During 1999, nocturnal

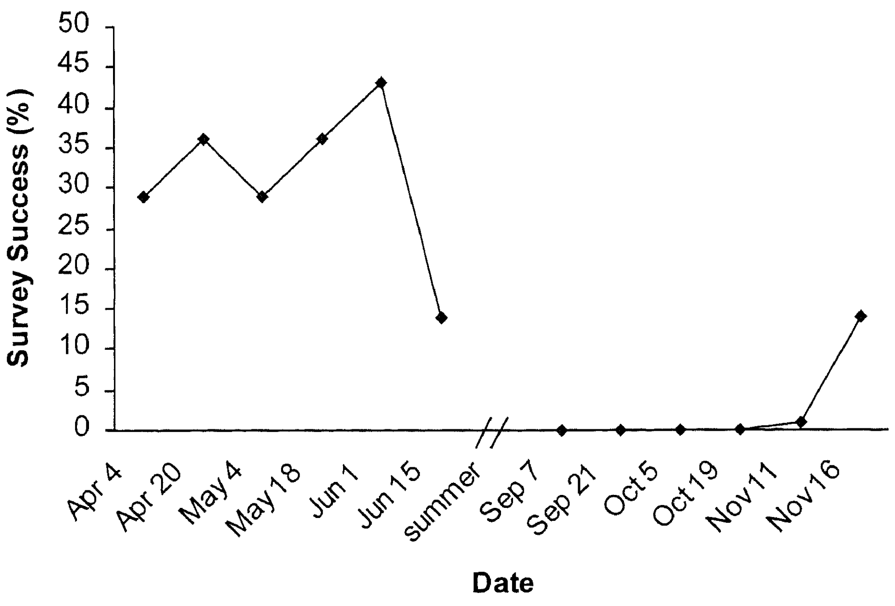

FIG. 1. Success (% occupied pools with detections) of nocturnal visual surveys at detecting marbled salamander larvae in relation to survey date in South Hadley, Massachusetts, during spring and fall sampling seasons, 1999. The break in the horizontal axis represents the summer period when pools were dry.

TABLE 1. Marbled salamander presence and total abundance in vernal pools distributed across 5 sites in Massachusetts during 1998 using two sampling techniques aquatic bottle traps and nocturnal visual surveys. The numbers in 'presence' columns represent the number of pools with marbled salamanders present at a given site. The numbers in 'abundance' columns represent the total abundance across pools at a given site.

	Presence			Abundance	
Site	No. Pools	Bottle Trap	Nocturnal Visual Survey	Bottle Trap	Nocturnal Visual Survey
Charlton	15	0	0	0	0
Holyoke	22	7	7	69	387
Leverett	24	0	2	0	116
South Hadley	19	6	6	22	448
Sutton	17	0	0	0	0
Totals	97	13	15	91	951

visual surveys (as described above) were conducted on a bi-weekly basis at 14 pools within the South Hadley sampling area, except during periods when the pools were iced over or dry.

We quantified the effectiveness of each survey technique at determining presence and an index of relative abundance of marbled salamander larvae. We conducted a Chi-square test of independence using all pools with and without marbled salamanders, testing the null hypothesis that there is independence between techniques in determining presence/absence. Also, we computed a Pearson's product-moment correlation in larval abundance for the two survey methods using pools with marbled salamanders, as determined by either technique, as independent observations. To compare efficacy of the survey techniques, we described the logistical constraints associated with each technique and compared the mortality rates between techniques.

To evaluate the effectiveness of nocturnal visual surveys in relation to the time of year and sampling intensity (i.e., number of surveys), we plotted survey success against time of year and sampling frequency for the 14 pools intensively sampled in the South Hadley sampling area. To construct the latter plot, a sampling intensity of one represented a single survey; success for a single survey was computed as the number of pools in which larvae were detected on that survey divided by the total number of pools with known marbled salamander populations. The mean survey success for a sampling intensity of one was computed as the average success rate for all the surveys (N = 12). A sampling intensity of two represented every possible combination of two consecutive surveys; success for each two consecutive surveys was computed as the number of pools in which larvae were detected by either survey divided by the total number of pools with known marbled salamander populations. The mean survey success for a sampling intensity of two was computed as the average success rate for all the 2-survey combinations (N = 11). This procedure was repeated for each sampling intensity between 1 and 12. Note that this procedure results in decreasing sample sizes as sampling intensity increases.

Nocturnal visual surveys were slightly more effective than bottle traps at determining the presence of marbled salamanders (Table 1), although the detection of presence was not independent between techniques ($\chi^2 = 82.08$; df = 1; $P = < 0.005$). Larvae were detected by both survey techniques at 13 of 97 (13.4%) pools. At an additional two pools (3% of total, 13% of pools with salamanders), larvae were detected by nocturnal visual surveys only. In addition, higher counts of marbled salamander larvae were recorded from nocturnal visual surveys (Table 1). Despite differences in absolute relative abundance, the results from these two survey methods were highly correlated after removing one outlier (N = 14; $R^2 = 68\%$; $P = 0.005$), indicating that both procedures can be used to derive a similar index of relative abundance. The outlier pool was excluded from analysis because thick vegetation throughout the pool prevented us from effectively conducting nocturnal visual surveys.

There were logistic constraints with both methods. Bottle traps caused higher direct mortality (5 adult *Ambystoma maculatum*, 2 adult *Rana sylvatica*, and 8 adult *Notopthalmus v. viridescens*) by exposing individuals to predation and freezing temperatures during cold nights. No mortality was observed with nocturnal visual surveys. Unobserved mortality may have been caused by walking through pools (both methods). Based on the number of days required to conduct each survey method, the effort required to bottle trap one pool was approximately three times greater than that required for visual surveys.

The effectiveness of nocturnal visual surveys varied with time of year. Spring surveys were more successful than autumn surveys (Fig. 1). The highest success occurred immediately prior to metamorphosis into terrestrial juveniles (June 1, Fig. 1), although previous studies found marbled salamanders at intermediate sizes to be more active then older larvae (Anderson and Graham 1967; Hassinger et al. 1970). There are a couple possible explanations for the relatively low effectiveness of autumn surveys. Immediately after hatching in the autumn, larvae have been observed spending proportionately more time in the pond bottom at night where they would be undetectable with nocturnal visual surveys (Anderson and Graham 1967; Hassinger et al. 1970). In addition, early autumn rains associated with Hurricane Floyd caused the pools to completely fill before all adults had migrated to the pools. Consequently, it is possible that reproductive success was partially or totally affected, leading to lower detection rates. Given that detection rates change with time, our earlier results comparing nocturnal visual surveys and bottle traps may be biased because of differences in the timing of surveys (bottle traps February–March, and nocturnal visual surveys March–May).

The effectiveness of nocturnal visual surveys also varied in relation to sampling intensity. Specifically, the success rate increased non-linearly with increasing sampling frequency (Fig. 2). Eight surveys were needed to detect salamanders in 91.6% of the pools where they were known to occur. These trends may reflect the fact that three pools had the presence of larvae detected during only one or two surveys. When these pools were removed the relation-

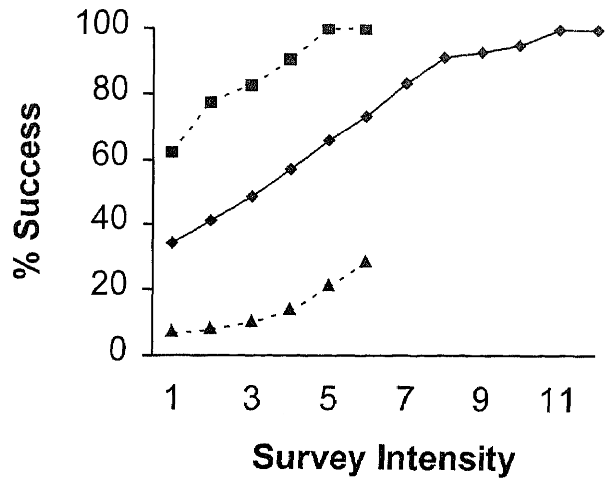

FIG. 2. Success (% occupied pools with detections) of nocturnal visual surveys at detecting marbled salamander larvae in relation to survey intensity (i.e., number of surveys; see text for details) in South Hadley, Massachusetts, during 1999. The diamonds represent the combined spring and fall surveys (N = 12); the squares and triangles represent the spring (N = 6) and fall (N = 6) surveys, respectively.

ship became more linear. Sampling intensity required to achieve the same level of success was much lower in spring than autumn (Fig. 2).

Given the increasing impacts of anthropogenic disturbance (e.g., urban development) on marbled salamander breeding habitat, there is growing interest in methods to identify and protect critical habitat. Determining marbled salamander presence is prerequisite to developing a better understanding of the species' habitat needs and in providing justification for protecting vernal pools from human impacts. Our findings suggest that nocturnal visual surveys were the least intrusive and expensive, and are slightly more accurate at detecting presence. Both methods provide an index of relative abundance, although nocturnal visual surveys may give counts that are closer to the true population abundance. We also show that the timing and intensity of surveys are important considerations; multiple site visits in late spring may be optimal.

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Coleonyx switaki, male (Switak's Banded Gecko). USA: California: San Diego Co. Illustration by Thad A. Howard.