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Are Two Methods Better than One? Area Constrained Transects and Leaf Litterbags for Sampling Stream Salamanders

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Concern over large-scale amphibian and reptile declines and general acknowledgement of sparse baseline information for most herpetological species has prompted the initiation of several monitoring programs. These programs, as well as ecological studies focused on metapopulation dynamics and habitat associations, often use presence-absence (or probability of occupancy) as the parameter of interest. For example, the long-term monitoring design of the US Geological Survey's Amphibian Research and Monitoring Initiative (ARMI) uses multi-season models developed by MacKenzie et al. (2003) to estimate the changes in the proportion of sites occupied by a species. As a national program, ARMI has chosen occupancy as the state variable of interest (rather than abundance), because of the ease and relative cost efficiency of collecting survey data (i.e., detection or non-detection of each target species), and the ability to incorporate the probability of detecting a species to obtain unbiased estimates of occupancy (MacKenzie et al. 2002). Sampling methods may differ in their effectiveness in detecting a species at an occupied site (Bailey et al. 2004). Because a higher probability of detection means fewer surveys are needed to obtain good precision for the occupancy estimator (MacKenzie and Royle 2005), efficient survey designs should consider detection probabilities in the cost/benefit analysis of sampling methods. The goal of this study was to determine the most efficient method for estimating stream salamander habitat occupancy at a regional scale, as part of the Northeast region of the ARMI program (NE ARMI).

Several methods exist for sampling stream salamanders including area-constrained transects (Grant et al. 2005; Heyer et al. 1994), cover-controlled active searches (Heyer et al. 1994; Lowe and Bolger 2002), time-constrained searches (Barr and Babbitt 2002) and leaf litter refugia bags ('leaf litterbags'; Pauley and Little 1998). Area-constrained transect surveys may give a reliable index of the relative abundance of stream salamanders, and multiple passes can be used to estimate population sizes using removal models (Bruce 1995; Jung et al. 2000). Leaf litterbags are a uniform way to sample the leaf litter habitat and are an effective method for determining species presence, but not abundance (Chalmers and Droege 2002; Waldron et al. 2003). Leaf litterbags have been proposed as an appropriate method for determining site occupancy (Pauley and Little 1998; Waldron et al. 2003), and they may increase detection probabilities of some species or life stages.

During a survey, a species can be present but not detected (i.e., a false absence), causing the site to appear unoccupied. To estimate the true occupancy state of a species, multiple 'surveys' are required, which can be in the form of repeat site visits, multiple observers, replicate surveys, or multiple methods conducted simultaneously. Methods that increase detection probabilities of a species can reduce the optimal number of surveys of a site needed to obtain a precise estimate of occupancy (MacKenzie and Royle 2005). To determine the most efficient survey design, we compared the probabilities of detecting *Desmognathus fuscus*, *Eurycea bislineata*, and *Pseudotriton ruber* using area-constrained transects (hereafter 'transects') and leaf litterbags in twenty-five 30 m stream reaches within 12 first- and second-order streams in the Chesapeake & Ohio Canal National Historic Park, Maryland (38°59'N, 77°14'W) and Rock Creek National Park, District of Columbia (38°57'N, 77°02'W). We surveyed each site twice from 16 June to 29 July 2005. During the sample period, all age classes of *E. bislineata* and *P. ruber* were available for capture, while only adult and juvenile *D. fuscus* were available (as the previous year's larvae had metamorphosed by this time). By sampling with both methods within the same stream reach, we were able to estimate detection probabilities for each species-method combination, and determine the possible bias associated with each sampling method.

Methods.—The transects consisted of two 15 × 3 m areas (1 m in the water and 2 m on the bank), located on opposite banks and separated by 15 m. To survey each transect, one observer proceeded upstream, turning all cover objects greater than 6 cm in diameter. An aquarium net was used to facilitate the capture of salamanders. Three leaf litterbags were placed within each 15 m transect at 0, 7.5, and 15 m. The bags were placed one week prior to sampling to allow colonization by salamanders. Our leaf litterbags were constructed of two layers of 50 × 50 cm Deer Block brand plastic netting, with a mesh size of 15 × 15 mm, and filled with 50-60 grams (dry weight) of leaf litter (Chalmers and Droege 2002; Waldron et al. 2003). To maximize the likelihood of capturing larval salamanders, leaf litterbags were partially submerged (Waldron et al. 2003), using a rock to hold each bag in place. The leaf litterbags were checked after a week and again 3–4 weeks later by placing a net under the bag, and immediately placing the bag into a wash basin with water. We shook the bag in water for 15–20 seconds to loosen salamanders, then drained the contents of the basin into a net, and searched for salamanders.

We defined a site as a 30 m stream reach and used the program PRESENCE (MacKenzie et al. 2002) to estimate the proportion of sites that were occupied. For each of the following analyses we used the detection/non-detection data for each species separately, and estimated the species-specific detection probability (p; defined as the probability of detecting the species at an occupied site) and the proportion of sites occupied (ψ), while accounting for a species not always being detected when present (i.e., $p < 1$).

We conducted three separate analyses. First, we combined detection information from transect and leaf litterbag searches into a single survey event ('combined' dataset). In this dataset, the probability of detection represents the likelihood that the species was detected by either survey method during a survey event, and the resulting estimate of occupancy should provide an unbiased esti-

Table 1. Detection probability (p) and estimates of site occupancy (ψ) for the salamanders *Desmognathus fuscus*, *Eurycea bislineata*, and *Pseudotriton ruber*. The data was analyzed in three ways: using method as a covariate ('Method-covariate'), separately for each method ('Method-specific'), and combined detections from both methods for each survey event ('Combined'). Naïve occupancy estimates for *E. bislineata* (ψ = 0.68), *D. fuscus* (ψ = 0.44) and *P. ruber* (ψ = 0.28) do not account for missed detections. Occupancy could not be estimated for *D. fuscus* under the method-specific leaf litterbag model because there were too few detections for parameter estimation.

		Eurycea bislineata		*Desmognathus fuscus*		*Pseudotriton ruber*	
Survey Method	Dataset	p (SE)	ψ (SE)	p (SE)	ψ (SE)	p (SE)	ψ (SE)
Transect	Method-covariate	0.6206 (0.0879)	0.7153 (0.0992)	0.7869 (0.1052)	0.4602 (0.1052)	0.1127 (0.2979)	0.5412 (0.2979)
	Method-specific	0.6278 (0.1206)	0.7104 (0.1382)	0.8857 (0.0800)	0.4079 (0.1003)	0.6619 (0.3165)	0.0913 (0.0660)
Leaf litterbag	Method-covariate	0.5078 (0.0879)	0.7153 (0.0992)	0.1311 (0.0711)	0.4602 (0.1052)	0.2255 (0.2979)	0.5412 (0.2979)
	Method-specific	0.5476 (0.1410)	0.6669 (0.1678)	0.0612 (0.0342)	—	0.3282 (0.2465)	0.3713 (0.2699)
Both methods	Combined	0.8204 (0.0781)	0.7131 (0.1001)	0.8377 (0.0919)	0.4560 (0.1040)	0.4383 (0.2059)	0.4164 (0.1949)

mate of the true occupancy state of the site. Second, we analyzed a single dataset in which each detection/non-detection observation was separate for the two methods employed during a survey event ('method-covariate'). By modeling "method" as a covariate in the PRESENCE models, we were able to obtain detection estimates for each survey method, using knowledge of sites where the species was detected by the other method. Finally, we analyzed separate datasets ('method-specific') for each method, in which the probability of detection represents the likelihood that the species was detected by only one method. This dataset represents the data that would be collected if only one method was implemented, and thus may reveal a potentially biased estimate of the site occupancy, suggesting that the sampling method itself may be flawed.

These analyses allowed us to investigate possible heterogeneity in detection probabilities caused by sampling bias associated with each survey method. The two methods may differ in their detection probabilities, but if the methods are able to detect a species, then the detection-adjusted estimates of occupancy should be the same among all the analyses. Drastic differences in the occupancy estimates would suggest a bias in the actual sampling method (i.e., if one method was unable to detect, or had very low probability of detecting the target species at occupied sites). Comparing the method-specific estimates of occupancy with the 'method-covariate' and 'combined' datasets gives an assessment of sampling bias for each survey method (Bailey et al. 2004).

Results and Discussion.—For *D. fuscus* and *E. bislineata*, the detection probabilities were higher for transects than leaf litterbags (Table 1). For *D. fuscus* this was expected, because submerged leaf litterbags target the larval life stage (Waldron et al. 2003), which was not present during the survey period. We were therefore unable to estimate a method-specific estimate for leaf litterbags for *D. fuscus* (Table 1). For *E. bislineata* the probability of detection increased slightly when both methods were used. Both methods appear suitable for detecting this species, as the point estimates of site occupancy were similar across all datasets, though transects alone had a slightly higher probability of detection (Table 1). For both *D. fuscus* and *E. bislineata*, incorporating detection probability resulted in an estimate of occupancy that was higher than the naïve estimate (the fraction of sites where the species was detected without accounting for missed detections; naïve $\psi_{E. bislineata}$ = 0.68; naïve $\psi_{D. fuscus}$ = 0.44, Table 1).

For *P. ruber*, leaf litterbags were more effective at detecting salamanders than area constrained transects (Table 1). Using transects, *P. ruber* was detected at 2 of the 25 sites, and at one site it was found during both survey events. This resulted in a high estimate of p, (though with a large SE) for the method-specific transect dataset. However, when method is modeled as a covariate (Table 1; method-covariate dataset), additional information is provided from leaf litterbag detections, which were more efficient at detecting larval salamanders. Including detections from leaf litterbags reduced the estimate of p for the transect method (as *P. ruber* was never detected by both methods at a site). The estimate of occupancy for the method-specific dataset using transects was much lower than the known, naïve estimate of site occupancy (method-specific ψ = 0.09, naïve ψ = 0.28). These data indicate the bias in occupancy estimates which would have resulted from using only the transect sampling method, due to the very low probabilities of detecting *P. ruber* (i.e., p = 0.1127 for the transect survey method in the 'method-covariate' dataset). For this species, sampling with leaf litterbags in combination with transects increases the detection probability, eliminates or reduces bias in occupancy estimates that may result from using just one detection method, and also decreases the number of times a site should be visited to obtain an optimal occupancy estimate (i.e., low SE) from 19 to 4 visits (MacKenzie and Royle 2005; Table 2).

As expected, using two methods to detect the presence of a species provides a more precise estimate of occupancy than a single method alone (Table 1). For example, *P. ruber* was never detected by both methods at the same site, and the estimate of occupancy is more precise for the combined dataset (Table 1).

If one method is superior for detecting a species, then the addition of a second method provides redundant information that does not improve the occupancy estimate. The inferior sampling method can still be used, but the optimal number of visits to a site increases substantially (e.g., Table 1, 2; leaf litterbags are less suitable for detection of *D. fuscus*, and therefore inflate the optimal number of surveys from k = 2 to 17, MacKenzie and Royle 2005). Further, when the detection probability is high, the increase in detection provided by a second method does not change the esti-

TABLE 2. Optimal number of surveys (k) given occupancy (ψ) and detection (p) estimates from the method-covariate dataset and the combined dataset models (from Table 1).

Survey Method	*Eurycea bislineata*	*Desmognathus fuscus*	*Pseudotriton ruber*
Transect	3	2	19
Leaf litterbag	4	17	9
Combined	2	2	4

mate of occupancy or the optimal number of visits to a site (e.g., Table 1, 2; *E. bislineata*).

Sampling methodologies may vary in their effectiveness of sampling different life stages. Leaf litterbags are designed to preferentially capture larval salamanders (Waldron et al. 2003), and our transect surveys are designed to sample all life stages. In our study, leaf litterbags detected adult salamanders of all three species with low probabilities, and thus leaf litterbags are not likely to provide the data necessary to estimate patterns in stream occupancy by adult salamanders with sufficient power. If occupancy of a habitat by a particular life stage is of primary interest, then the sampling program should be designed primarily using methods that target that life stage. Regardless, interpretation of results should consider that a sampling method may detect all life stages, while having different detection probabilities for each life stage.

Conclusions.—In studies designed to assess the status and trends in occupancy of a suite of species across a large area, the allocation of survey effort is a chief concern. For species that are difficult to detect on a given sampling occasion, such as *P. ruber*, the use of an additional method may increase the precision and decrease bias in estimates of occupancy. However, since we found leaf litterbags were expensive to construct (materials cost per bag was US $2.50), difficult to maintain in the field, and had lethal effects on non-target organisms (i.e., two dead snakes were found tangled in the litterbags), we suggest sampling the leaf litter at a set distance interval (i.e., 1 m) using an aquarium net, rather than deploy leaf litterbags. Incorporating leaf litter sampling into the transect surveys may be more effective than using leaf litterbags because of increased detections of species that are more likely to be captured within the leaf litter (Bruce 2003; E. Grant, unpubl. data).

Regardless, when designing a research or monitoring program, assessment of the potential bias in survey methods should be incorporated into the study design (e.g., this study; Bailey et al. 2004; O'Connell et al. 2006). In addition, pilot data can guide optimization of data collection to meet a variety of study objectives (Bailey et al. *in press*; MacKenzie and Royle 2005), and will ultimately yield estimates that facilitate comparisons among studies, provided the state variable estimates account for missed detections.

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