

Evaluating methods for sampling stream salamanders across multiple observers and habitat types

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Abstract

While techniques for sampling pond-breeding amphibians are relatively well-established, comparable methods for stream amphibians are still being developed. Uncertainty about sampling techniques is particularly acute for approaches that involve multiple observers. I evaluated three techniques for sampling stream salamanders with multiple observers—time-constrained surveys, leaf-litter bags, and controlled-cobble added to the stream. I tested for differences among techniques, differences among habitats, and technique by habitat interactions. I also asked whether coefficients of variation across observers and sample dates differed among methods and habitats. For all species and life-stages, time-constrained surveys produced higher counts of stream salamanders and greater richness estimates than did leaf-litter bags or controlled-cobble surveys. However, interactions between techniques and habitat were never significant, indicating that all the methods detected similar patterns in counts among stream habitats. Coefficients of variation in single-species counts tended to be higher for time-constrained surveys, whereas CVs for species richness were significantly lower for time-constrained surveys. Overall, these results suggest that: (1) time-constrained surveys for stream salamanders work surprisingly well even with multiple observers, (2) leaf-litter bags may be quite useful for single-species studies, but are less effective for multi-species sampling, and (3) controlled-cobble added to streams is not particularly effective for sampling salamanders in rocky streams.

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Key words

Amphibians, *Desmognathus*, *Eurycea*, *Gyrinophilus*, lotic, monitoring, population index, survey, techniques.

Introduction

Salamanders are important components of headwater stream ecosystems. Stream salamanders are often the dominant predators in headwater streams because they can move past rocks and waterfalls that are barriers to fish (Murphy and Hall, 1981; Huang and Sih, 1991; Resetarits, 1997). Stream salamanders may also be useful indicators of ecosystem health (Welsh and Olliver, 1998). Salamanders are adversely affected by deforestation and physical disturbance (Orser and Shure, 1972; Willson and Dorcas, 2003a; Petranka and Smith, 2005), siltation (Welsh and Ol-

liver, 1998; Lowe et al., 2004), and stream acidification (Kucken et al., 1994, but see Grant et al., 2005). For these reasons, reliable sampling methods for stream salamanders are crucial (Jung et al., 2000; Chalmers and Droege, 2002). Whereas sampling approaches for pond-breeding species are comparatively well established (Gibbons and Semlitsch, 1981; Crouch and Paton, 2000, 2002; Bailey et al., 2004; Nelson and Graves, 2004), viable methods for sampling stream salamanders are still being developed.

A few recent studies have compared multiple sampling techniques for stream salamanders. Barr and Babbitt (2001) examined the relationship between *Eurycea bislineata* counts from 0.5 m² quadrat surveys of the stream bed and counts from time-constrained searches. They reported that time-constrained surveys were effective at low salamander densities but appeared to be less sensitive than quadrats at higher densities. Jung et al. (2000) used quadrat surveys, transect counts, electroshocking, and leaf-litter bags to sample salamanders in Shenandoah National Park, Virginia. They found that, in general, all of the techniques gave similar estimates of population abundance for *Eurycea bislineata* and that electroshocking and leaf litter bags gave estimates that were more strongly related to environmental variables. More recently, Quinn et al. (2007) compared thorough searches of the stream substrate to “lighter”, less invasive sampling of rocks and logs in a Washington streambed. They reported moderate correlations in results between these approaches for Columbia Torrent Salamanders (*Rhyacotriton kezeri*) but only weak correlations for Coastal Tailed Frogs (*Ascaphus truei*), suggesting that the utility of these approaches might vary among species.

Other studies have critically evaluated salamander sampling techniques one at a time. Chalmers and Droege (2002) used experimental arenas to examine the relationship between leaf-litter samples and the true abundance of *Eurycea bislineata*. They concluded that these quantities were not significantly related, though their sample sizes were small ($n = 8$ arenas) and they did not report the actual correlations obtained. Waldron et al. (2003) also assessed the suitability of leaf-litter bags for sampling stream salamanders. They concluded that leaf-litter bags were ineffective for estimating abundance but could play a role in species inventory programs.

I add to this growing literature on methods for sampling stream salamanders with tests of multiple techniques in an intermittent stream in the Appalachian mountains of Virginia. However, my study differs from previous studies in two principle respects. First, I examine sampling methods across multiple teams of observers and analyze the resulting variation in counts. While methodological comparisons with single observers (or single teams) may be entirely relevant for short-term research studies, longer-term studies and monitoring programs generally involve multiple observers, so it is important to take inter-observer variability into account. In particular, one might expect that active search techniques would work fine with single observers, but would give highly variable results with multiple observers who differ in search ability. Second, the current study differs from previous work in that

sampling was explicitly stratified across variable types of channel units, allowing a comparison of sampling techniques across different stream habitats.

Materials and methods

Study site and species

All sampling was carried out in Benson's Run, an intermittent stream in George Washington National Forest in Highland County, VA. Benson's Run is a second-order stream formed by the confluence of two headwater streams; from this confluence, it flows approximately 9 km to the Cowpasture River. The forest surrounding the stream is composed of mixed hardwood and coniferous forest dominated by mature Eastern hemlock (*Tsuga Canadensis*). The habitat composition of Benson's Run includes isolated pools, cascades, runs, riffles, and numerous side channels (*sensu* Bisson et al., 1996), and the fish community of the stream is dominated by Brook Trout (*Salvelinus fontinalis*), cottids (*Cottus* spp.), and cyprinids (primarily *Rhinichthys atratulus*).

We commonly found four species of stream salamanders within channel units at Benson's Run: Southern Two-lined Salamanders (*Eurycea cirrigera*), Spring Salamanders (*Gyrinophilus porphyriticus*), Northern Dusky Salamanders (*Desmognathus fuscus*), and Seal Salamanders (*D. monticola*). *Desmognathus* larvae are highly variable morphologically and can be difficult to distinguish in the field (Waldron et al., 2003), so we grouped these larvae into a single category, as have previous authors (Grant et al., 2005). We occasionally encountered individuals of the more terrestrial Northern Red-backed Salamander (*Plethodon cinereus*) and of the Northern Red Salamander (*Pseudotriton ruber*) near the stream margin. Data from these latter species were not included in the analyses.

Stream surveys

We sampled Benson's Run for stream salamanders in May 2005. We selected 12 stream sections that contained at least one distinct pool, one riffle, and one side channel. Within each stream section, the pool, riffle, and side channel were separated by less than 20 m. Stream sections themselves were at least 50 m away from each other and were spread over 5 km of Benson's Run.

Stream pools were defined as areas that contained still water and that were at least 2 m wide and 5 m² in total area. Riffles were areas in which shallow water flowed consistently over cobble and boulders. Riffles, like pools, were at least 2 m wide. Side channels were areas where flows or seeps met the primary stream channel. Most side channels were primarily lentic and were areas where water appeared to flow after heavy rainfall. Side channels contained at least 8 m² of surface water and were at least 1 m wide. To maximize the number of species encountered, pools, riffles, and side channels were each defined to include a one meter-wide strip of dry streambed.

The 36 total channel units (12 pools, 12 riffles, and 12 side channels) were sampled by six teams of two observers. These six teams were rotated through the sites so that each team surveyed 18 of the 36 channel units once over the course of the study. Each channel unit was thus surveyed a total of three times, once each by three different teams, and the three survey dates for any given channel unit were separated by approximately one week. This design allowed me to assign separate random error terms for sites, teams, and survey dates.

Within the individual channel units, three techniques were used to sample stream salamanders. The first technique was active, time-constrained sampling (Crump and Scott, 1994; Barr and Babbitt, 2001). With this technique, observers turned over rocks in the channel unit for precisely 20 min (i.e. 40 person-min of search effort). During a time-constrained search, observers attempted to capture all salamanders using aquarium fish nets and turkey basters (Barr and Babbitt, 2001). At the end of the 20-minute search period, observers identified salamanders to species (where possible) and to life-stage. Observers then returned each salamander to its precise location of capture. The 20-minute time period was chosen to be long enough to result in some salamanders being captured, but not so long that all possible rocks were searched. A 20-minute search period was within the range of active surveys in other published studies (e.g. Jung et al., 2000; Barr and Babbitt, 2001, 2002; Grant et al., 2005).

The second sampling technique consisted of searches of three leaf-litter bags placed within each channel unit. Litter bags were constructed with a 70 cm × 70 cm mesh bag (1.5 cm gauge deer mesh) secured with cable ties (Pauley and Little, 1998; Jung et al., 2000; Waldron et al., 2003). Bags contained approximately 1.5 liter of leaf-litter from the forest floor and between one and three rocks to keep them submerged below the surface of the stream. We used fishing line to attach bags to overhanging logs, vegetation, or to re-bar driven into the side of the streambed. We established these leaf-litter bags two weeks before the first surveys to allow time for them to be colonized by salamanders (Jung et al., 2000). To carry out sampling, bags were removed from the water and gently shaken over a plastic basin for one minute. Initial trials suggested that most salamanders dropped from the bags into the basin within the first 30 s and that few salamanders were likely to drop beyond the one-minute time period. After one minute, salamanders were captured from the tub and identified. The bags were then returned to the stream and the salamanders were returned to the bags. Over the course of the study, 11 of the 108 bags disappeared—most of these after a single large rainfall early in the study. Missing bags were immediately replaced and the new bags were surveyed the following week.

The third technique employed marked cobble that we placed within each channel unit. Two weeks before the first surveys, we collected mid-sized cobble (64–256 mm wide, Cummins, 1962) from dry ground on the side of each stream and marked one face with orange, waterproof paint. We placed 10 of these rocks within each channel unit, flush with the streambed so that the area beneath them could be easily searched. During each sampling occasion, we overturned the 10 pieces of

cobble and attempted to capture and identify all salamanders beneath them. These controlled-cobble surveys were somewhat similar to the time-constrained surveys. However, they ensured that each observer team was searching exactly the same numbers and types of rocks within every channel unit.

One week prior to initiating surveys, we carried out a “trial run” of all the search techniques, so that each team would have some experience prior to formal data collection. On a separate date, observers were trained and tested in species identification and capture techniques in an attempt to minimize differences in methodology among groups.

Data analysis

Data collected from each survey included: counts of all species and all life-stages captured, the total number of species seen, and the total number of groups seen (i.e., the total number of life-stages of all species). Across the three sampling dates for each channel unit, I also calculated the coefficient of variation in counts for each species and for each richness measure. I used these CVs to examine the consistency of the different techniques across observers/sampling dates. With three sampling techniques carried out three times each in 36 channel units, individual species counts and richness data yielded 324 data points, whereas CVs calculated across three sampling dates resulted in 108 data points.

I used generalized linear mixed-models (Venables and Ripley, 2002) to test for fixed effects and to account for the various sources of error affecting each data point. Three fixed effects were of interest: overall differences among sampling techniques in counts for each species, in measures of richness, and in measures of variation across sampling dates; overall differences among channel units (pools vs. riffles vs. side channels) in individual species counts and richness measures; interactions between sampling technique and channel unit type that would indicate that the relative efficacy of each technique depended on the type of channel unit sampled. Models also incorporated up to three random effects: a random effect for site, a random effect for observation team, and a random effect for sampling date. Models for CVs included only a random effect for site, as CVs were calculated across observers and sampling dates.

Count data are often overdispersed, and overdispersion can seriously bias results when counts are treated as Poisson-distributed (Link and Sauer, 1998; Burnham and Anderson, 2001). To account for overdispersion, I modeled single-species counts as Poisson with a fixed overdispersion factor (\hat{c}) estimated from the global model (Burnham and Anderson, 2002). Species and group richness, unlike single-species counts, did not diverge significantly from a Poisson ($p > 0.5$ for each), so no overdispersion was incorporated into the analysis of these response variables (i.e. $\hat{c} = 1$). CVs in counts and richness measures (i.e. variance to mean ratio) were treated as lognormal response variables.

Overdispersion renders likelihood calculations suspect and thus does not permit likelihood ratio tests for the statistical significance of each variable (Burnham and Anderson, 2002; Venables and Ripley, 2002). However, Akaike's Information Criterion (AIC; Akaike, 1973) can be adjusted for overdispersion. Using AIC, one can therefore test the importance of each explanatory variable by examining the difference in quasi-AIC values (AIC adjusted for overdispersion) between a model that includes or excludes each parameter (Burnham and Anderson, 2002).

For each response variable, I began by examining each of the three potential random effects. I dropped each random effect from the global model and used quasi-AIC differences ($\Delta\text{AIC}/\hat{c}$) to determine whether the dropped random effect contributed substantially to the fit of the model. I conservatively used a $\Delta\text{qAIC} > 1$ to indicate that the model was improved by inclusion of the random effect, as there's little harm in including plausible random effects for which there's only moderate evidence. Random effects for which there was little or no support (i.e. $\Delta\text{qAIC} < 1$) were not incorporated into the analysis of the fixed effects.

After I decided which random effects to include in an initial model, I used sequential dropping of parameters to evaluate the three fixed effects: differences among techniques, differences among channel units, and technique by habitat interactions. For overdispersed response variables (i.e. single-species counts), I used the standard benchmark of $\Delta\text{AIC} > 2$ (in this case quasi-AIC > 2) to indicate that a parameter contributed substantially to the fit of the model. For Poisson or log-normal response variables (species and group richness and CVs), I used likelihood ratio tests to evaluate the contribution of each parameter to the model. Tests for overdispersion and estimates of \hat{c} used `odTest` in the `pscl` package and `glm.nb` in the `MASS` package of R, version 2.2.1 (R Development Core Team, 2005). Mixed-models were developed using the `lmer` function for GLMMs in the `lme4` package of R.

Results

Summary capture results

We captured 574 *Eurycea cirrigera* (300 larvae, 274 adults), 45 adult *Desmognathus fuscus*, 32 *D. monticola*, 36 *Desmognathus* larvae, and 135 *Gyrinophilus porphyriticus* (34 adults, 101 larvae). Thirty-eight percent of all captures were in side channels, 32% were in riffles, and 30% were in stream pools (fig. 1). With respect to sampling techniques, time-constrained surveys accounted for 68% of all captures, leaf-litter bags accounted for 19% of captures, and controlled-cobble surveys accounted for 13% of captures (fig. 2).

Individual species results

For *E. cirrigera*, adults were more or less evenly distributed across channel unit classes (30% in pools, 38% in riffles, and 32% in side channels, table 1). There was

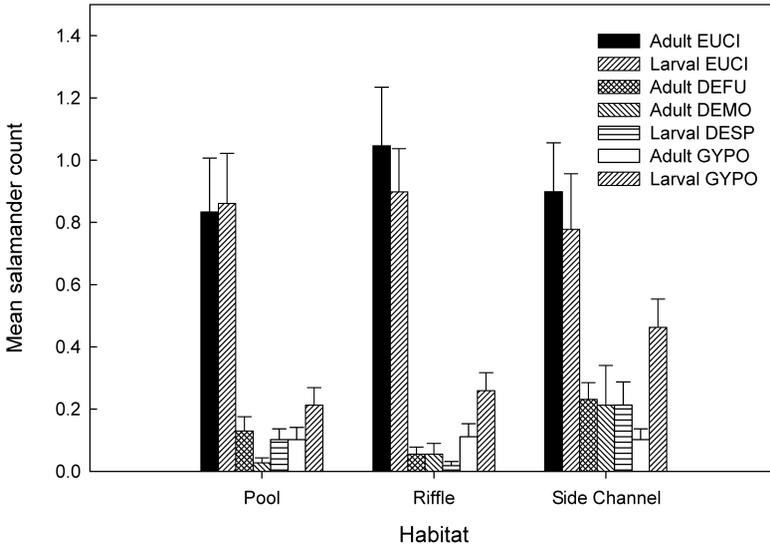


Figure 1. Salamander counts in different stream habitats. EUCI = *Eurycea cirrigera*, DEMO = *Desmognathus monticola*, DEFU = *Desmognathus fuscus*, DESP = *Desmognathus* spp., GYPO = *Gyrinophilus porphyriticus*. Error bars represent one standard error.

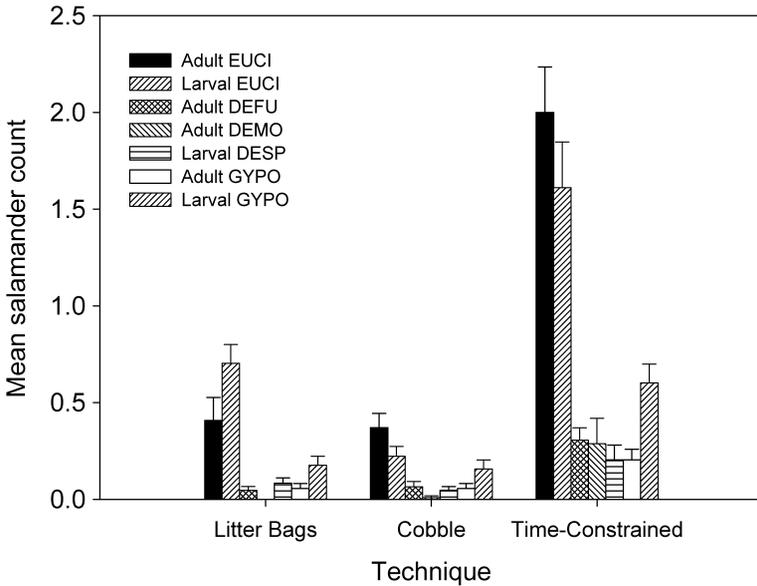


Figure 2. Salamander counts from different sampling techniques. EUCI = *Eurycea cirrigera*, DEMO = *Desmognathus monticola*, DEFU = *Desmognathus fuscus*, DESP = *Desmognathus* spp., GYPO = *Gyrinophilus porphyriticus*. Error bars represent one standard error.

no interaction between habitat and sampling technique—each technique yielded a proportional distribution of counts across habitats (table 1). However, the fixed

Table 1.

Quasi-AIC analysis of the effects of sampling technique, habitat type, and their interaction on individual species counts. AIC differences were adjusted for over-dispersion in counts (c-hat); c-hat values are shown as one for counts that displayed no evidence of overdispersion. Negative values for Δ qAIC indicate that the model performed more poorly when the term was dropped, i.e. the term was important to the overall explanatory model. Values from -2 to -5 are generally taken to be moderately supported and values less than -5 indicate strong support for the variable.

Response variable	Variable dropped	Δ loglik	Δ par	c-hat	Δ qAIC
Adult <i>Eurycea</i>	Technique*Habitat	3.9	4	4.14	6.12
Adult <i>Eurycea</i>	Technique	93.56	2	4.14	-41.20
Adult <i>Eurycea</i>	Habitat	1.62	2	4.14	3.22
Larval <i>Eurycea</i>	Technique*Habitat	1.39	4	1.74	6.40
Larval <i>Eurycea</i>	Technique	66.59	2	1.74	-72.54
Larval <i>Eurycea</i>	Habitat	0.49	2	1.74	3.44
Adult <i>D. fuscus</i>	Technique*Habitat	4.38	4	2.37	4.30
Adult <i>D. fuscus</i>	Technique	30.38	2	2.37	-21.64
Adult <i>D. fuscus</i>	Habitat	12.61	2	2.37	-6.64
Adult <i>D. monticola</i>	Technique*Habitat	2.54	4	1	2.92
Adult <i>D. monticola</i>	Technique	30.73	2	1	-57.46
Adult <i>D. monticola</i>	Habitat	7.38	2	1	-10.76
Larval <i>Desmognathus</i>	Technique*Habitat	2.18	4	1	3.64
Larval <i>Desmognathus</i>	Technique	6.37	2	1	-8.74
Larval <i>Desmognathus</i>	Habitat	10.42	2	1	-16.84
Adult <i>Gyrinophilus</i>	Technique*Habitat	2.07	4	1.22	4.61
Adult <i>Gyrinophilus</i>	Technique	6.96	2	1.22	-7.41
Adult <i>Gyrinophilus</i>	Habitat	0.09	2	1.22	3.85
Larval <i>Gyrinophilus</i>	Technique*Habitat	4.56	4	1.71	2.67
Larval <i>Gyrinophilus</i>	Technique	20.28	2	1.71	-19.72
Larval <i>Gyrinophilus</i>	Habitat	5.89	2	1.71	-2.89

effect for sampling method was strongly supported by Δ qAIC (table 1). Time-constrained surveys yielded the majority of adult *E. cirrigera* (72%), whereas both leaf-litter bags and controlled cobble yielded low numbers (13% and 15% respectively).

Results for *E. cirrigera* larvae were similar. Neither habitat effects nor interactions between sampling technique and habitat were supported by Δ qAIC (table 1). Once again, sampling technique was strongly supported, with time constrained surveys yielding substantially more captures than litter bags (63% vs. 28%) and litter bags yielding more than controlled cobble surveys (9%).

For adult *D. fuscus* there was no suggestion that the various techniques yielded different results in different types of channel units (i.e. no technique by habitat interaction; table 1). However, there were overall differences in the numbers of captures among habitats, with the greatest number of captures in side channels (56%), fewer

captures in pools (31%), and fewer still in riffles (14%). There were also overall differences in captures among the different methods. Time-constrained surveys yielded most of the *D. fuscus* captures (73%) with comparatively small numbers from leaf-litter bags (11%) and controlled cobble surveys (16%).

Patterns for *D. monticola* adults generally mirrored those for *D. fuscus*. There was no evidence for a habitat by technique interaction, but the main effects for both habitat and technique were supported by the models (table 1). Side channels accounted for most (72%) of the *D. monticola* captures, and all but one of the *D. monticola* captures (97%) were found with time-constrained surveys.

Models for larval *Desmognathus* yielded weak support for a habitat by technique interaction and very strong support for main effects of habitat and technique (table 1). The majority were captured in side channels (64%), moderate numbers were collected from pools (31%), and only 2 captures (5%) were within riffles. With respect to sampling techniques, once again time-constrained surveys yielded the greatest number of individuals (61%), followed by leaf-litter bags (25%), and controlled cobble surveys (14%).

For adult *G. porphyriticus*, models including an interaction between habitat and sampling technique were inferior to models including no such interaction (table 1). Differences in counts among channel units were weakly supported ($\Delta qAIC = 3.85$): 45% of counts were in side channels, 30% were in riffles, and 25% were in stream pools. Differences in counts among methods were much more strongly supported: 65% of captures were with time-constrained surveys and 17.5% were made with leaf-litter bags and with controlled cobble surveys (table 1).

Finally, larval *G. porphyriticus* counts were influenced strongly by habitat type and by method, but not by their interaction (table 1). Of all *G. porphyriticus* captures, 49% were in side channels, 29% were in riffles, and 22% were in stream pools. With respect to sampling techniques, 64% of captures were with time-constrained surveys, 19% were with leaf-litter bags, and 17% were with controlled-cobble (table 1).

Species and group richness

Species richness differed somewhat among habitats (likelihood ratio test, $\chi^2 = 6.54$, $df = 2$, $p = 0.038$). Richness was highest in side channels (2.25 ± 0.21 SE) versus pools (1.81 ± 0.17 SE) and riffles (1.61 ± 0.18 SE), due mainly to larval and adult desmognathines that were largely restricted to these habitats. Species richness was even more strongly influenced by sampling method ($\chi^2 = 72.15$, $df = 2$, $p < 0.001$). Time-constrained surveys produced a mean richness of 2.78 ± 0.17 SE, compared to 1.56 ± 0.16 SE for leaf-litter samples, and 1.33 ± 0.15 SE for controlled cobble surveys. There was, however, no significant interaction between sampling method and habitat ($\chi^2 = 2.82$, $df = 4$, $p = 0.59$)—methods tended to differ in similar ways across habitat types.

Results for life-stage group richness were largely similar, which was not surprising since group and species richness were highly correlated ($r = 0.92$). Differences in group richness across habitat types were marginally significant ($\chi^2 = 4.79$, $df = 2$, $p = 0.09$). Once again richness was higher in side channels (3.0 ± 0.28 SE) than it was in either pools (2.33 ± 0.23 SE) or riffles (2.33 ± 0.25 SE). Differences in observed group richness among techniques were stronger ($\chi^2 = 96.79$, $df = 2$, $p < 0.0001$). Time constrained-surveys yielded a mean of 3.86 groups ± 0.20 SE, whereas leaf-litter bags yielded 2.08 ± 0.20 SE and controlled cobble surveys found 1.72 ± 0.21 SE. The interaction between sampling method and habitat type did not approach significance ($\chi^2 = 3.64$, $df = 4$, $p = 0.48$).

Variability across observers and sample dates

In models for single-species counts, the random effect for observer team was supported in 6 out of 7 cases, implying that differences among observers can be a detectable source of error. However, this inter-observer variation (coupled with inter-date variation) was not generally large. It accounted for less than 15% of the overall variation for all single-species counts with the exception of time-constrained counts of adult *Eurycea*, where it accounted for 38% of the overall variation. Nevertheless, coefficients of variation across observers never differed significantly among sampling techniques or among channel units (table 2), implying that (with the possible exception of adult *Eurycea*) these inter-observer differences were relatively consistent. In contrast, for species and group richness, CV did differ significantly among methods (table 2). CVs were highest in controlled-cobble surveys, lowest in time-constrained surveys, and intermediate in leaf-litter surveys. There was no significant interaction between sampling method and habitat type for any of the response variables.

Discussion

Time-constrained surveys for stream salamanders do not control for the way that observers sample different microhabitats or for observers' searching ability. Given this, one might expect that time-constrained surveys would work fine for a single observer, but would perform poorly when multiple observers were involved. The surprising result from the current study is that time-constrained surveys appeared to work at least as well as more rigorously-controlled strategies for sampling stream salamanders. For single-species counts, coefficients of variation for time-constrained surveys were moderately (though not significantly) higher for time-constrained surveys than for the other techniques, whereas CVs for species and life-stage richness were significantly lower for time-constrained surveys. The one notable exception to this was adult *Eurycea*, where inter-observer variation during time-constrained surveys accounted for almost 40% of the total variation in counts. The high inter-observer variation for adult *Eurycea* was probably due to the fact

Table 2.

Analysis of coefficients of variation (CV) across observer teams and sample dates. Means and standard errors on CVs are shown for each variable, and *p*-values reflect likelihood ratio tests with each either the technique or the habitat variable dropped from the global model.

Response variable	Techniques (Mean ± SE)				Habitats (Mean ± SE)				<i>p</i> -value
	Time-constrained	Litter bags	Cobble	<i>p</i> -value	Side-channels	Pools	Riffles		
Adult <i>Eurycea</i>	2.30 ± 0.32	1.92 ± 0.44	1.36 ± 0.16	0.59	2.05 ± 0.32	1.75 ± 0.47	2.02 ± 0.26	0.19	
Larval <i>Eurycea</i>	2.27 ± 0.46	1.11 ± 0.21	1.14 ± 0.10	0.19	1.86 ± 0.55	1.59 ± 0.26	1.31 ± 0.16	0.13	
Larval <i>Gyrinophilus</i>	1.52 ± 0.20	0.99 ± 0.30	1.23 ± 0.21	0.34	1.37 ± 0.23	1.38 ± 0.27	1.26 ± 0.21	0.09	
Species Richness	0.31 ± 0.04	0.61 ± 0.06	0.88 ± 0.08	< 0.0001**	0.58 ± 0.06	0.62 ± 0.10	0.53 ± 0.09	0.02*	
Group Richness	0.43 ± 0.08	0.74 ± 0.07	1.23 ± 0.21	0.0001**	0.66 ± 0.07	0.80 ± 0.11	0.64 ± 0.14	0.04*	

that most of these salamanders were found along the margin of the stream, and survey teams likely varied in the extent to which they sampled the margin versus the stream interior. More definitive instructions regarding sampling of these different habitats during time-constrained surveys could likely have reduced some of these inter-observer differences.

Counts for four species, seven life-stage groups, and two aggregate measures of richness never supported an interaction between sampling technique and channel unit type. Thus, where differences in salamander counts among habitats were present, these differences were consistent across the different sampling techniques. Put another way, the relative usefulness of the techniques was not strongly dependent on the types of habitat surveyed. In addition, each of the three techniques gave a similar picture of the habitat affinities of the different species. All techniques found *Eurycea cirrigera* to be similarly common in pools, riffles, and side channels, and all techniques showed *Desmognathus fuscus* and *D. monticola* to be more common in side channels than in the main channel of the stream. These results generally confirm habitat affinities that have been demonstrated in previous work (e.g. Grover, 2000; Barr and Babbitt, 2002; Grover and Wilbur, 2002). But they do suggest that most sampling techniques will pick up basic patterns in stream salamander communities, as long as sampling is sufficiently well-replicated (see Jung et al., 2000 for a similar conclusion).

That said, there were some differences in the data collected with the different techniques. First, leaf-litter bags appeared to be far more effective for sampling some species than for sampling others. Leaf-litter bags resulted in the capture of many larval *Desmognathus*, but only a single adult *D. monticola*. In different seasons and in other geographic regions, there are likely to be other salamanders that do not readily use leaf-litter bags. For example, Waldron et al. (2003) captured a number of adult *D. monticola*, but had no larvae in litter-bag samples collected during the summer. Second, controlled-cobble resulted in lower counts than the other techniques. As a result, species richness estimates obtained from controlled-cobble surveys were typically one to two species lower for each channel unit than estimates obtained from time-constrained surveys. These data suggest that both controlled-cobble and leaf-litter bags are probably better suited for research on single species or life-stages than for multi-species surveys.

In our surveys, we made no attempt to equalize search effort among the different sampling techniques. While time-constrained surveys always took 20 minutes, litter-bag sampling generally took 5-10 minutes and controlled cobble surveys usually required just 3-5 minutes. This implies that larger numbers of litter bags or marked cobble could possibly have yielded higher counts with less sampling error. If there were no saturation in occupancy of leaf litter bags, approximately 10 litter bags per channel unit would have yielded roughly the same number of total salamanders as time-constrained surveys, and would have required 20-30 minutes to sample. For controlled cobble surveys, 50-60 pieces of cobble would have been necessary to achieve parity in numbers with time-constrained surveys. For litter

bags, this increase in effort would have been feasible—there were certainly plenty of other sites within channel units where more litter bags could have been installed. In contrast, for controlled-cobble surveys, we had limited ability to increase sampling effort. Because Benson's Run is a rocky stream, there were few additional sites in which we could have added more cobble. Furthermore, in such a rocky stream, it would be surprising if many salamanders were actively seeking new rocks for cover. Controlled-cobble surveys might be better suited for sandy and muddy-bottom streams where these cover sites may be more limited.

I was not able to evaluate all techniques that have been used to sample stream salamanders. For example, researchers commonly use area-constrained surveys in which small plots (often, 1 m²) or transects (often 1 m wide) are searched thoroughly (e.g. Resetarits, 1997; Welsh and Olliver, 1998; Grant et al., 2005). Other researchers have sampled stream salamanders by searching beneath one cover object per unit area of stream (Lowe and Bolger, 2002; Lowe et al., 2004). All of these techniques are forms of active search, and thus may be similar to the time-constrained surveys that we employed. However, future comparisons of time-constrained versus area-constrained approaches to salamander sampling would certainly be useful (e.g. Barr and Babbitt, 2001). An additional sampling technique that we did not test employs funnel traps to catch salamanders that are drifting downstream (Willson and Dorcas, 2003b). We initially installed funnel traps at each site but found that we were unable to keep them properly anchored to the rocky substrate. We were also concerned that funnel traps, unlike leaf-litter bags or controlled cobble, needed to be checked at frequent intervals during the sampling period to avoid harming salamanders. Nevertheless, funnel traps may be useful in soft-bottomed streams where they can be more easily anchored, and may be the best technique for sampling salamanders that are dispersing via downstream drift (Willson and Dorcas, 2003b).

In summary, these results support several recommendations with respect to sampling methods for stream salamanders. First, time-constrained surveys for stream salamanders can be surprisingly effective even with multiple observers, as long as observers are rotated through the different sampling sites. Although time-constrained surveys do not tightly control search effort, these surveys reliably identified the same patterns of abundance and habitat use as the more rigorous sampling techniques. Furthermore, time-constrained surveys were more likely to pick up uncommon species than were the other techniques. As a result, time-constrained surveys gave more complete and less variable snapshots of salamander species richness. More precise instructions for observers about sampling strategies for different habitat types within channel units could probably have made time-constrained counts even more reliable. The second recommendation arising from this study is that leaf litter bags appear to be better suited for single-species studies than for multi-species surveys. Leaf-litter bags are frequently used by some salamander species, but rarely used by others (see also Jung et al., 2000; Waldron et al., 2003). However, leaf-litter bags have quite a bit of untapped potential for the

study of the ecology of the species that do use them. Almost nothing is known about the importance of leaf litter for stream salamander ecology, and litter bags provide a practical approach for studying these relationships. Finally, based on the results of this study, use of controlled-cobble surveys is not highly recommended. A greater variety of cobble sizes might lead to increased use by stream salamanders, but one could just as well control the number and sizes of rocks searched during a time-constrained survey. Other types of sampling units that could be added to streams and that might be more readily used by salamanders (e.g. wood cover objects) remain a valid area of investigation.

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