Environ Monit Assess (2007) 135:409–422
DOI 10.1007/s10661-007-9660-z

Effects of subsample size on seasonal and spatial comparisons of stream macroinvertebrate communities

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Received: 25 September 2006 / Accepted: 12 February 2007 / Published online: 15 March 2007

Abstract We examined the effect of subsample size on the accuracy of information obtained from aquatic macroinvertebrate assemblage samples. Subsamples containing 100 organisms or 300 organisms were compared on the bases of processing time and the ability to discern ecological differences among samples. Independently of subsample size, assemblages differed between study streams, primarily reflecting an intermittent vs. permanent stream difference, and between seasons at most streams. It required, on average, two additional hours to process the larger subsamples. Larger subsamples gave significantly higher estimates of total richness and Ephemeroptera, Plecoptera, and Trichoptera (EPT) richness, but the relative abundances of many assemblage subsets (e.g., EPT organisms and most functional feeding groups) were similar using both subsample sizes. Larger subsamples did not typically enhance the ability to discriminate between samples from different seasons, but did more accurately distinguish among streams when differences were subtle. They also appeared to avoid Type I error in comparisons of compositionally similar reaches within a study stream.

Keywords Fixed count subsampling · Functional feeding groups · Seasonal variation · Stream macroinvertebrates

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Introduction

Macroinvertebrate assemblages are increasingly used to assess the condition of aquatic systems, in accordance with the objectives of the Clean Water Act (ADEQ 2002; MDNR 2002; OEPA 1987; USEPA 2002). Advantages of using this group of organisms include: (1) they are ubiquitous and may therefore be affected by many types of environmental disturbances (Lenat et al. 1980); (2) communities typically contain moderate to large numbers of species, which offers a wide spectrum of responses to stress (Hellawell 1986); (3) they are not very mobile in the aquatic life stage, which allows for spatial examinations of disturbance effects (Abel 1989); and (4) they have relatively long life cycles, which allow for temporal examination of disturbance effects (Gaufin 1973).

But a challenge in examining benthic macroinvertebrate communities is managing the time and monetary cost required to process and analyze samples (Barbour and Gerritsen 1996; Lorenz et al. 2004). To reduce this effort, fixed-count subsampling has frequently been used (Barbour et al. 1996; Somers

et al. 1998; Vinson and Hawkins 1996) although there is some disagreement as to its appropriateness (Courtemanch 1996; Doberstein et al. 2000). Some researchers concluded that a 100-organism endpoint can provide adequate information (Barbour and Gerritsen 1996; Davidson and Clem 2002; Somers et al. 1998;), whereas others recommended larger (e.g., 300-organism) subsamples (Lorenz et al. 2004) and/or a supplemental search for large and/or rare taxa (Vinson and Hawkins 1996). Rapid bioassessment protocols outlined for wadeable streams by the U.S. Environmental Protection Agency recommend the use of fixed-count subsamples, using a 200-organism subsample as a procedural example, while not specifically recommending that number as an endpoint (Barbour et al. 1999). Courtemanch (1996) and Doberstein et al. (2000) maintained that subsamples, particularly those with low endpoints, cannot obtain accurate estimates of taxonomic richness. Smaller subsamples are more likely to exclude rare species, making it more difficult to distinguish between community samples (Cao et al. 1998). Richness metrics that consider either the total macroinvertebrate community or subsets of the community, e.g., the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT), are widely used in biomonitoring studies (DeShon 1995; Kerans and Karr 1994). However, other frequently used characteristics are those that represent relative abundances of various community subsets, e.g., EPT organisms or functional feeding groups (Barbour et al. 1999; DeShon 1995; Lenat 1984). These metrics should be less influenced by rare taxa, and may not differ as widely between fixed-count subsamples of varying sizes.

The principal objective of this study was to compare macroinvertebrate community information obtained from 100-organism subsamples against that from 300-organism subsamples that further included a search for large and rare taxa. We examined whether means of community metrics changed with subsample size, and whether variability of metrics decreased with increased subsample size. We also compared the ability of the two subsample sizes to discern statistically significant differences in community metrics between seasons, as well as within and between geographically separate unimpaired streams. With this information, we examined whether the additional cost of using 300-organisms subsamples was clearly outweighed by the benefit of additional, or more accurate, ecological information.

Materials and methods

Field

Benthic macroinvertebrate samples were collected from five streams in three of the five Level III ecoregions (Omernik 1987) of Arkansas, USA (Table 1). Study streams differed in terms of drainage area, gradient, and flow permanence. We sampled in early winter (mid-December through mid-January) and early spring (mid-March through mid-April) from 2003 through 2005. Nine samples (3 stations × 3 replicates per station) were collected from riffle habitats in each stream, using a 23 × 46 cm kick net with 500-μm mesh. The three stations represented separate, but typically adjacent, reaches of the stream; each station was 150–200 m long. Three samples (replicates) were collected from each station during each survey period. These were not randomly assigned, but represented separate samples from different locations within the

Table 1 General characteristics of the five study streams

Stream	Ecoregion	Permanent or temporary	Gradient (m/km)	Drainage area (ha)	Maximum elevation in survey area (m above msl)
Bailey Creek (BLC)	Ozark Highlands	Temporary	10.1	2,233	105
Big Creek (BGC)	Arkansas Valley	Permanent	4.3	8,951	184
Caney Creek (CNC)	Ouachita Highlands	Temporary	16.7	684	276
Harris Creek (HRC)	Ouachita Highlands	Permanent	12.2	2,424	308
Thompson Creek (TMC)	Ozark Highlands	Temporary	15.8	1,028	197

Gradient, drainage area and elevation data were obtained from USGS 1:24,000 topographic maps.

station. Although these can be considered pseudoreplicates they were intended to address the issue of variability within stream reaches. We used a semi-quantitative sampling method; we disturbed stream substrates by kicking, and the current carried organisms into the net. Each replicate sample consisted of six separate kicks covering approximately 4 m^2 of substrate. Samples, which contained moderate to large amounts of organic and inorganic debris as well as the benthic macroinvertebrates, were placed in 1-l plastic jars, preserved with a 5% formaldehyde solution, and transported back to the laboratory.

Laboratory procedures

In the laboratory, large debris was rinsed and removed prior to subsampling and sorting. Samples were mixed thoroughly, then reduced in size by randomly selecting a 25% portion of the original sample from a 500 μm sieve. We then sorted through this subsample under 10× magnification to a 100-organism endpoint. After 100 organisms were obtained, the remaining 75% of the sample and any remainder of the subsample were remixed. Then, more sample material was examined until an additional 200 organisms were obtained by a two-step process. First, a 10 to 15-min search through the entire remaining sample for large and/or rare taxa was conducted. Second, the remaining sample was sorted as described above, i.e., random 25% portions were selected and picked until an additional 200 organisms were obtained. During the sorting process, organisms were separated on the basis of whether or not they needed to be slide-mounted for taxonomic identification. After slide mounting chironomid larvae and oligochaetes using CMC-10 media, organisms were identified using a Zeiss Axiostar compound microscope. Non-slide mounted specimens were identified using a Zeiss SV-11 stereomicroscope. Benthic macroinvertebrates were identified to the lowest practical taxonomic level (typically genus) using a variety of keys, most frequently those included in Merritt and Cummins (1996) and Thorp and Covich (1991).

Data analysis

Invertebrate community information from each subsample was summarized in two ways: (1) using 2 measures of taxonomic richness – total richness and EPT richness; and (2) using 11 relative abundance-based community characteristics. The relative abundance metrics reflected both taxonomic composition (percent EPT, Plecoptera, Trichoptera, and Diptera), functional feeding group composition (percent collectors, filterers, predators, scrapers, and shredders), and a biotic index based on pollution tolerance. Tolerance values for taxa, ranging from 0 (intolerant) to 10 (most tolerant), were obtained from Hilsenhoff (1987), Lenat (1993), and Bode et al. (1996). The resulting index was the sum of tolerance values, weighted by relative abundance, of all taxa in the sample. The relative abundance of the dominant, i.e., most numerous, taxon was also examined. Additional relative abundance variables – percent Ephemeroptera, percent Chironomidae, and percent non-insects – were initially considered for this examination, but were eliminated because their strong correlations with other variables led us to conclude that they would be redundant (Herbst and Silldorff 2006).

Biological data consisted of macroinvertebrate assemblage metrics described above for each 100- and 300-organism subsample. These data were separated by study stream (Bailey Creek = BLC, Big Creek = BGC, Caney Creek = CNC, Harris Creek = HRC and Thompson Creek = TMC) and by season (winter and spring). We examined small-scale spatial variability by comparing the three stations within each study stream. Statistical tests were performed using Minitab (Version 14) software. We used paired *t* tests to examine whether metrics differed between 100-organism and 300-organism subsamples of the same sample. For the total richness metric, where increases in larger subsamples were inevitable, we used regression analysis to test whether gains were more related to the large/rare taxa search or simply to the greater number of organisms. Two regressions were performed, each using the gain in total richness between the 100-organism subsample and the 300-organism subsample as the response variable. Two predictor variables were used – the number of large/rare taxa (considered only in the larger subsamples) obtained and the number of additional taxa in the larger subsample that were not found in the large/rare search. We assessed the importance of these variables by comparing the amount of variation explained by the respective regression equations. Paired *t* tests were also used to compare coefficients of variation of each metric, i.e., to examine whether metric variability

decreased with increasing subsample size. To evaluate the ability of differing subsample sizes to distinguish seasonal differences, differences between streams, and differences between stations within streams, we used one-way analysis of variance, unless equal variance or normality assumptions were violated. In these cases, we used the non-parametric Kruskal–Wallis test. Only two factor levels – winter and spring – existed for the seasonal comparison, but for the comparisons of study streams, we used the Tukey multiple comparisons test or the non-parametric Mann–Whitney multiple comparisons test. In all cases, we used a Bonferroni adjustment of the α level to prevent error inflation. The potential range of separation was five groups (all streams separated) to one group (no separation). For the comparison of stations within study streams, we tested for a general significant difference, but did not perform the multiple comparisons test.

Results

We compared the field and laboratory effort between subsample sizes by recording the time required to complete each processing step in the 2004 study year. Time spent per subsample type was measured by separate processing/identification tasks. The mean total time spent in collection, processing, and identification was 117 min greater for 300-organism subsamples than for 100-organism subsamples (Fig. 1). Factoring out field effort and sample reduction times, which were equal for both subsample sizes, it took approximately double the amount of time to complete the larger subsample (mean = 201 min) as the smaller subsample (84 min). The majority of the difference was due to extra time spent sorting (all organisms) and identifying the unmounted organisms. This pattern was consistent among the five study streams and between the two survey seasons.

Variation between seasons and between study streams

Total richness increased from winter to spring at all streams (Table 2). Similarly, EPT richness was greater in the spring at four of five streams. Seasonal patterns in composition were similar among the three intermittent study streams (BLC, CNC, and TMC). At these streams, means of 97 to 99% of the organisms in samples were either EPT organisms, dipterans, or non-insects. Spring samples reflected greater relative abundances of EPT individuals, combined with reduced numbers of non-insects as compared to winter. As a result, biotic index values were greater in the winter at these streams. At the two permanent streams, biotic index and percent non-insects did not differ seasonally, but decreased presence of EPT

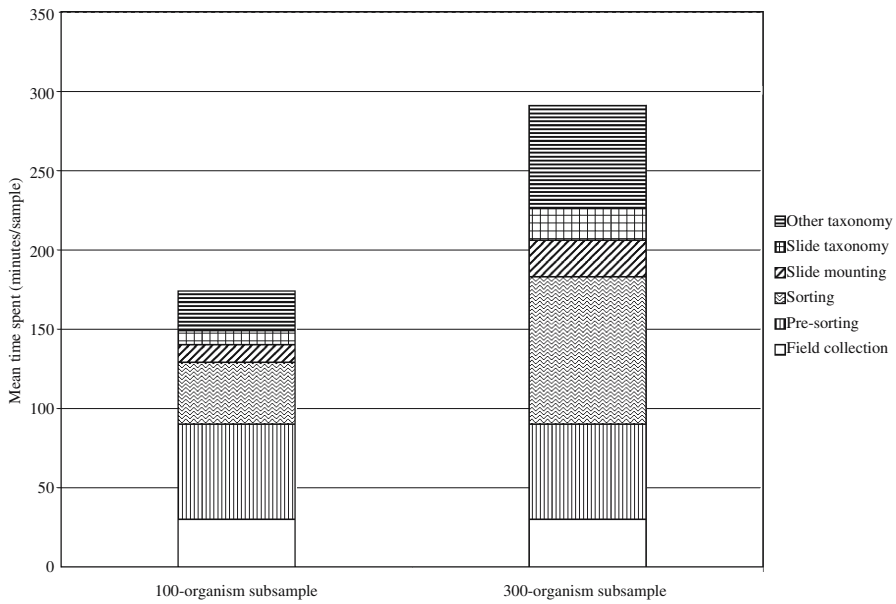

Fig. 1 Mean time spent (minutes) of various sample processing tasks for 100– and 300–organism subsamples (n = 90)

Table 2 Assemblage metrics (mean ± standard deviation) in winter and spring samples from each study stream, 2003–2005

	BLC		BGC		CNC		HRC		TMC	
	Winter	Spring	Winter	Spring	Winter	Spring	Winter	Spring	Winter	Spring
Total richness	36.2 ± 8.1	39.2 ± 3.8	40.2 ± 7.3	45.0 ± 3.7	29.9 ± 7.5	35.6 ± 6.0	44.6 ± 6.0	48.6 ± 3.1	37.5 ± 7.6	38.7 ± 5.6
EPT richness	12.1 ± 5.9	15.9 ± 1.9	18.4 ± 3.5	20.0 ± 2.6	11.8 ± 4.2	13.4 ± 2.0	25.6 ± 2.8	23.4 ± 1.7	15.1 ± 4.7	16.7 ± 2.1
Biotic index	5.6 ± 1.2	4.1 ± 0.4	4.7 ± 0.5	4.6 ± 0.3	6.0 ± 1.1	3.6 ± 0.8	3.9 ± 0.3	3.7 ± 0.2	5.8 ± 0.5	4.8 ± 0.6
Percent EPT	43 ± 17	62 ± 6	72 ± 6	55 ± 8	38 ± 11	67 ± 9	79 ± 5	70 ± 5	39 ± 10	46 ± 5
	Stenonema femoratum	*Amphinemura*	*Stenonema pulchellum*	*S. pulchellum*	*Allocapnia*	*Leucrocuta*	*Stenonema mediopunc.*	*S. mediopunc.*	*Allocapnia*	*Strophopteryx*
	Allocapnia	*Isoperla*	*Isonychia*		*Clioperla*		*Isonychia*	*Isoperla*	*S. femoratum*	*Isoperla*
	Agapetus		*S. femoratum*		*Strophopteryx*		*Cheumatopsyche*	*Isonychia*	*Strophopteryx*	
	Clioperla		*Chimarra*							
Percent	15 ± 8	23 ± 7	17 ± 6	28 ± 10	17 ± 10	14 ± 3	10 ± 4	14 ± 4	14 ± 5	19 ± 7
Diptera	*Prosimulium*	*Prosimulium*	*Prosimulium*	*Polypedilum aviceps*	*Tipula*	*Tipula*	*Prosimulium*	*P. aviceps*	*Prosimulium*	*Prosimulium*
	Tipula	*Parametriocnemus*			*Prosimulium*	*Zavrelimyia*		*Simulium*	*Tipula*	
		Tipula			*Simulium*					
Percent non-insects	40 ± 23	14 ± 4	5 ± 3	4 ± 2	42 ± 14	16 ± 9	2 ± 1	3 ± 1	44 ± 10	33 ± 8
	Lirceus	*Lirceus*	*Lirceus*	*Lirceus*	*Caecidotea*	*Caecidotea*	*Elimia*	*Elimia*	*Lirceus*	*Lirceus*

Values are from 300-organism subsamples (n = 27).

Table 3 Results of paired *t* tests comparing total richness and EPT richness between 100- and 300-organism subsamples at each of five study streams

Variable/ stream	Number	100-Organism mean	300-Organism mean	Mean richness gain	95% CI	p-value	Mean percent change
Total Richness							
BLC	54	25.3	37.7	12.4	11.5, 13.3	<0.001	49.0
BGC	54	28.9	42.6	13.7	12.7, 14.8	<0.001	47.4
CNC	54	21.6	32.7	11.1	9.9, 12.4	<0.001	51.4
HRC	54	32.0	46.6	14.6	13.6, 15.7	<0.001	45.6
TMC	54	25.6	38.1	12.6	11.6, 13.5	<0.001	49.2
EPT Richness							
BLC	54	10.7	14.0	3.3	2.8, 3.8	<0.001	30.8
BGC	54	14.3	19.2	4.9	4.3, 5.5	<0.001	34.3
CNC	54	9.2	12.6	3.4	2.9, 3.9	<0.001	37.0
HRC	54	18.6	24.5	5.9	5.2, 6.6	<0.001	31.7
TMC	54	11.7	15.9	4.2	3.6, 4.8	<0.001	35.9

Richness gains represent the mean of the values obtained by subtracting each smaller subsample from its corresponding larger subsample. Mean percent change represents the percent difference between the subsample values divided by the 100-organism subsample mean.

organisms and increased presence of dipterans were evident in the spring at these sites.

Total and EPT richness levels were consistently greater at BGC and HRC than at the three intermittent streams (Table 2). Numerically dominant EPT organisms at BGC and HRC were primarily mayflies, e.g., *Stenonema* spp., and *Isonychia*, and caddisflies, e.g., *Chimarra* and *Cheumatopsyche*. Stoneflies were the predominant EPT representatives at BLC, CNC, and TMC, particularly *Allocapnia* and *Strophopteryx* in the winter, and *Amphinemura* and *Isoperla* in the spring. *Prosimulium* was a major dipteran taxon at all five streams; the primary differences among streams with regard to dipterans were the greater numbers of *Tipula* in the intermittent streams and the increased spring abundance of the chironomid *Polypedilum aviceps* in the permanent streams. Non-insects were primarily represented by a single taxon, usually an isopod, in both seasons at each of the five streams.

Differences in macroinvertebrate metrics

Total richness values were, on average, 11.1–14.6 taxa greater in 300-organism than 100-organism subsamples at the five study streams (Table 3). Likewise, EPT richness values of 300-organism subsamples significantly exceeded those of 100-organism subsamples at all study sites, with mean levels in the larger subsamples 3.3–5.9 taxa higher than in the smaller ones. In contrast, most relative abundance metrics differed between subsample sizes less frequently (Table 4). Only biotic index, percent Diptera and percent collectors differed significantly at

Table 4 Results of paired *t* tests comparing community characteristics between 100- and 300-organism subsamples at each of the five study streams

	Frequency of significant difference	Mean percent change
Percent EPT	1 different out of 5 streams tested	3.4
Biotic index	4/5	3.0
Percent Plecoptera	2/5	4.1
Percent Trichoptera	2/5	8.6
Percent dominant taxon	2/5	7.0
Percent Diptera	4/5	12.3
Percent collectors	4/5	7.8
Percent filterers	0/5	7.0
Percent predators	1/5	5.2
Percent scrapers	2/5	4.9
Percent shredders	1/5	5.2

Mean percent change represents percent difference between the 100- and 300-organism subsample values divided by the 100-organism subsample mean, then averaged across the five study streams.

more than two of the five study streams. In addition, differences in relative abundance metrics were of smaller magnitudes – generally less than 10% – than were the total richness (~50%) and EPT richness (~33%) metrics. Results of the regression analysis indicated that differences in richness metrics were more related to the greater number of organisms counted as opposed to the large/rare taxa search (Fig. 2). The amount of variation in the response variable – total richness gain between the 100- and 300-organism subsamples – that was explained was substantially higher when the number of additional non-large/rare taxa was used as the predictor variable (72.1%) than when number of large/rare taxa was used (30.2%). Metric variability decreased as subsample size increased, as coefficient of variation

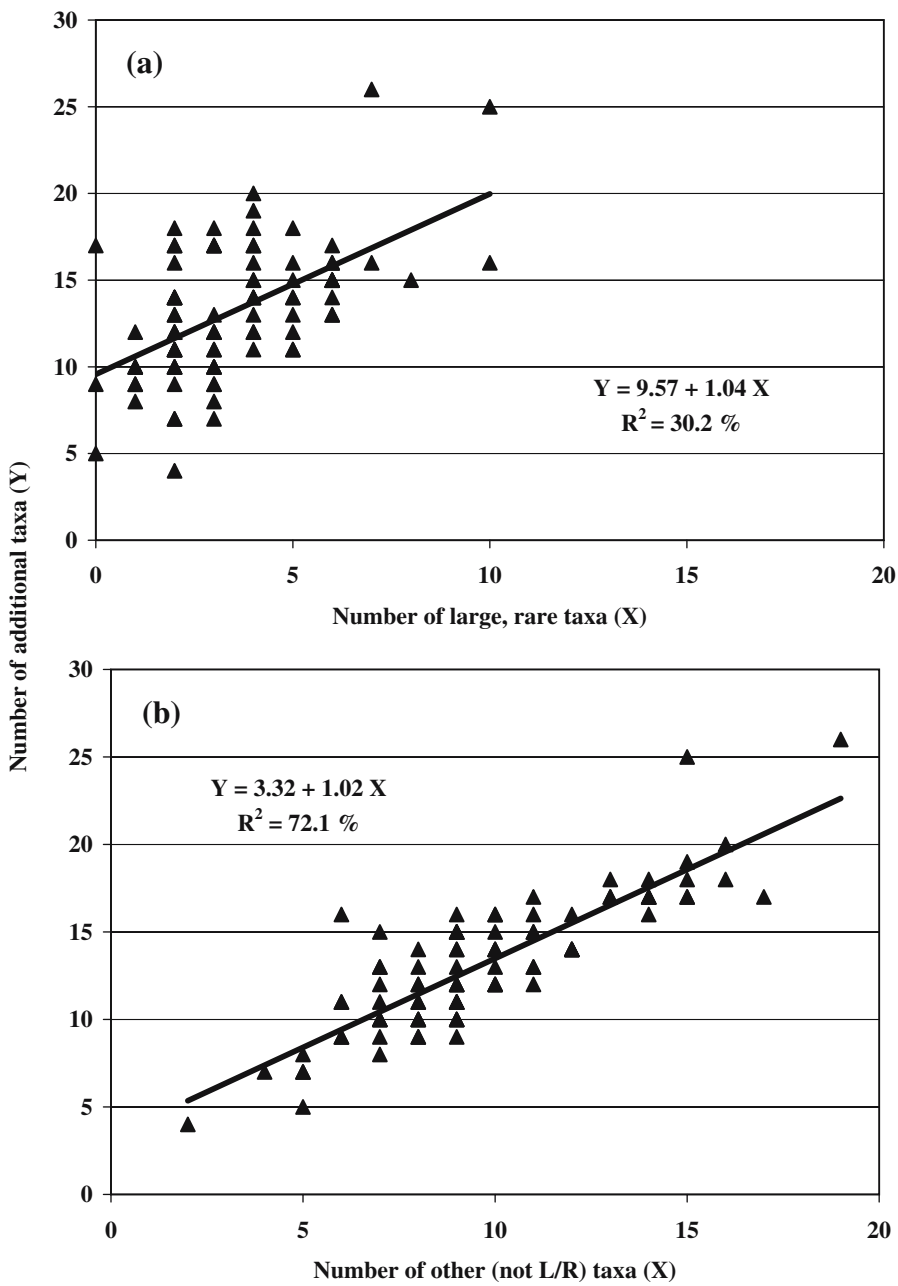

Fig. 2 Regression lines for the relationships between the number of additional taxa in the 300–organism subsample as compared to the 100–organism subsample, and (a) the number of additional taxa found in the large/rare search, and (b) the number of additional taxa (excluding large/rare taxa) found in the larger subsample

Table 5 Results of paired *t* tests comparing mean coefficient of variation between 100- and 300-organism subsamples for selected benthic macroinvertebrate community variables (n = 270)

Variable	Mean CV for 100 subsample	Mean CV for 300 subsample	Significance
Total richness	18.0	15.5	**
EPT richness	24.4	20.4	**
Biotic index	14.0	12.0	**
Percent EPT	20.6	16.4	*
Percent Plecoptera	42.8	39.0	ns
Percent Trichoptera	54.2	44.9	**
Percent dominant taxon	30.8	25.1	**
Percent Diptera	43.8	37.2	**
Percent collectors	28.8	27.8	ns
Percent filterers	74.0	64.8	*
Percent predators	44.0	37.6	**
Percent scrapers	44.1	41.3	ns
Percent shredders	51.0	44.6	*

An * indicates difference at $p < 0.05$; ** indicates difference at $p < 0.01$; *ns* indicates no significant difference.

means were significantly greater in the 100-organism subsamples for 10 of 13 metrics (Table 5).

Differences in discriminatory ability

Sensitivity to seasonal variation in invertebrate community structure was not frequently greater in the 300-organism subsamples as compared to the 100-organism subsamples (Table 6). The same statistical outcome was typically reached using data from both subsample sizes. Differences were only noted in nine cases out of a possible 65 (13 community variables × 5 study streams). Evidence of increased sensitivity, i.e., detection of significant difference with larger subsamples when no difference was observed in the smaller subsamples, occurred in six cases. These were total richness and percent shredders at CNC, EPT richness, biotic index and percent scrapers at HRC, and percent Trichoptera at TMC.

Sensitivity to variations between stations within study streams was also similar using data generated from both subsample sizes. Of a total of 130 comparisons (13

Table 6 Comparison of ability to detect seasonal variation in data from 100- and 300-organism subsamples using analysis of variance and Kruskal–Wallis tests (n = 27; alpha = 0.05)

Variable	BLC		BGC		CNC		HRC		TMC	
	100	300	100	300	100	300	100	300	100	300
Total richness	*0.34*	*0.18*	*<0.01*	*<0.01*	*0.33*	*<0.01*	*<0.01*	*<0.01*	*0.84*	*0.75*
EPT richness	*<0.01*	*0.10*	0.19	0.06	*0.38*	*0.08*	*0.71*	*<0.01*	*0.35*	*0.25*
Biotic index	*<0.01*	*<0.01*	*0.06*	*0.06*	*<0.01*	*<0.01*	0.58	<0.01	<0.01	<0.01
Percent EPT	*<0.01*	*<0.01*	<0.01	<0.01	*<0.01*	*<0.01*	<0.01	<0.01	*<0.01*	*<0.01*
Percent Plecoptera	<0.01	<0.01	0.48	0.78	*0.04*	*0.04*	0.79	0.11	0.02	<0.01
Percent Trichoptera	*0.01*	*0.14*	0.02	*<0.01*	0.16	0.22	*<0.01*	*<0.01*	*0.15*	*0.01*
Percent dominant taxon	*<0.01*	*<0.01*	*0.04*	*0.03*	*<0.01*	*<0.01*	0.03	0.03	*<0.01*	*<0.01*
Percent Diptera	<0.01	<0.01	<0.01	<0.01	*0.19*	*0.23*	*<0.01*	*<0.01*	<0.01	<0.01
Percent collectors	*<0.01*	*<0.01*	0.02	<0.01	*<0.01*	*<0.01*	<0.01	<0.01	<0.01	<0.01
Percent filterers	*0.88*	*0.95*	*<0.01*	*<0.01*	*0.27*	*0.13*	<0.01	<0.01	*0.89*	*0.60*
Percent predators	*<0.01*	*<0.01*	<0.01	<0.01	*<0.01*	*<0.01*	<0.01	<0.01	*<0.01*	*<0.01*
Percent scrapers	*0.95*	*0.79*	0.53	0.46	<0.01	<0.01	0.81	0.01	0.71	0.81
Percent shredders	*<0.01*	*<0.01*	*0.01*	*0.09*	0.30	0.04	0.01	<0.01	*0.97*	*0.35*

Values are *p*-values of the respective test statistics. Italics indicate that the non-parametric test was used.

Table 7 Analysis of variance results for small-scale spatial (among stations) differences; *p*-values in bold are significant at alpha = 0.05 (n = 27)

Stream/season		BLC		BGC		CNC		HRC		TMC	
		Winter	Spring	Winter	Spring	Winter	Spring	Winter	Spring	Winter	Spring
Total richness –	100	0.62	0.44	0.13	0.36	0.80	0.37	0.58	0.22	0.20	**0.01**
	300	0.70	0.08	0.47	**0.01**	0.64	0.22	0.63	0.18	0.21	**<0.01**
EPT richness –	100	0.93	0.14	0.47	0.08	0.57	0.88	0.68	0.66	0.84	0.72
	300	0.84	**0.01**	0.23	**<0.01**	0.49	0.44	0.95	0.47	0.95	0.86
Biotic index –	100	0.78	0.39	0.23	0.06	**<0.01**	**0.01**	0.21	0.74	**0.02**	**<0.01**
	300	0.79	0.26	0.13	0.34	**0.02**	**<0.01**	0.32	0.65	0.08	**<0.01**
Percent EPT –	100	0.81	0.27	0.07	**0.01**	0.14	0.35	**0.04**	0.59	0.55	**<0.01**
	300	0.86	**<0.01**	**0.03**	**<0.01**	0.38	**0.04**	0.33	0.76	0.87	**<0.01**
Percent Plecoptera –	100	0.95	0.12	0.12	0.61	**<0.01**	**0.02**	**0.03**	0.12	0.06	**<0.01**
	300	0.91	**<0.01**	0.16	0.50	**<0.01**	**0.01**	0.09	0.18	0.10	**0.01**
Percent Trichoptera –	100	0.33	0.97	0.98	0.41	0.85	0.40	0.08	**0.01**	0.15	0.49
	300	0.36	0.38	0.96	0.58	0.94	0.19	0.20	**<0.01**	**0.02**	0.82
Percent Dominant –	100	0.75	0.40	0.11	0.29	0.16	0.89	0.08	**0.04**	0.23	0.56
	300	0.54	0.63	0.08	0.64	0.23	0.76	0.23	0.41	0.44	0.95
Percent Diptera –	100	0.22	0.66	0.47	0.07	0.28	0.12	0.13	0.94	**<0.01**	**0.01**
	300	0.25	0.72	0.20	**0.01**	0.36	0.07	0.68	0.77	**<0.01**	0.07
Percent Collectors –	100	0.88	0.54	0.82	0.66	**0.01**	**0.01**	0.91	**0.04**	0.14	**0.01**
	300	0.78	0.06	0.47	0.61	**0.02**	**<0.01**	0.47	0.21	0.51	**0.04**
Percent Filterers –	100	0.62	0.42	0.62	0.76	0.08	0.08	0.11	0.57	**<0.01**	0.38
	300	0.75	0.31	0.15	0.20	0.08	0.16	**0.02**	0.84	**<0.01**	0.19
Percent Predators –	100	0.36	**<0.01**	0.64	0.83	0.55	0.26	**0.01**	**0.04**	0.76	0.87
	300	0.46	**<0.01**	0.17	0.11	0.57	0.06	0.27	0.11	0.97	0.91
Percent Scrapers –	100	0.93	0.57	0.49	0.36	0.89	**0.03**	0.54	**0.04**	0.81	0.67
	300	0.89	0.33	0.07	0.15	0.84	0.07	0.06	0.24	0.32	0.56
Percent Shredders –	100	0.24	0.34	0.05	0.37	**0.02**	0.10	**0.01**	0.86	0.18	0.16
	300	0.08	0.24	0.27	0.17	**0.02**	0.06	**<0.01**	0.93	0.33	0.26

variables × 5 study streams × 2 seasons), differences in test results were only noted for 19 (Table 7). Greater sensitivity and reduced sensitivity in the larger subsample occurred in nearly equal numbers of cases. If the "true" assemblage characteristics are assumed to be those obtained from the larger subsamples, 10 Type I (rejecting a true H_o) errors and 9 Type II (retaining a false H_o) errors occurred. Among study streams, disagreements and potential Type I errors most frequently occurred at HRC, where the greatest compositional similarity among stations was found (McCord 2006).

Sensitivity to variation between streams did not typically increase with subsample size. For total richness, nearly identical separation of streams was evident in both winter (3 groups) and spring (4 groups) (Fig. 3). The pattern of difference in EPT richness was similar between subsample sizes in terms of the degree of separation of streams in the spring, but greater separation was observed using the 300-organism subsample data in the winter (Fig. 4). For the 11 relative abundance metrics, increased discriminatory ability was only observed in the spring (Table 8). In winter samples, the number of distinguishable groups either remained the same, or decreased, using data from 300-organism subsamples. In the spring, however, discriminatory ability was enhanced using data from the larger subsamples for percent EPT, percent Plecoptera, biotic index, percent dominant taxon, percent collectors, percent predators, and percent scrapers.

Discussion

Subsample size had a substantial effect on both richness metrics in our samples. These metrics are widely used by researchers as an estimate of

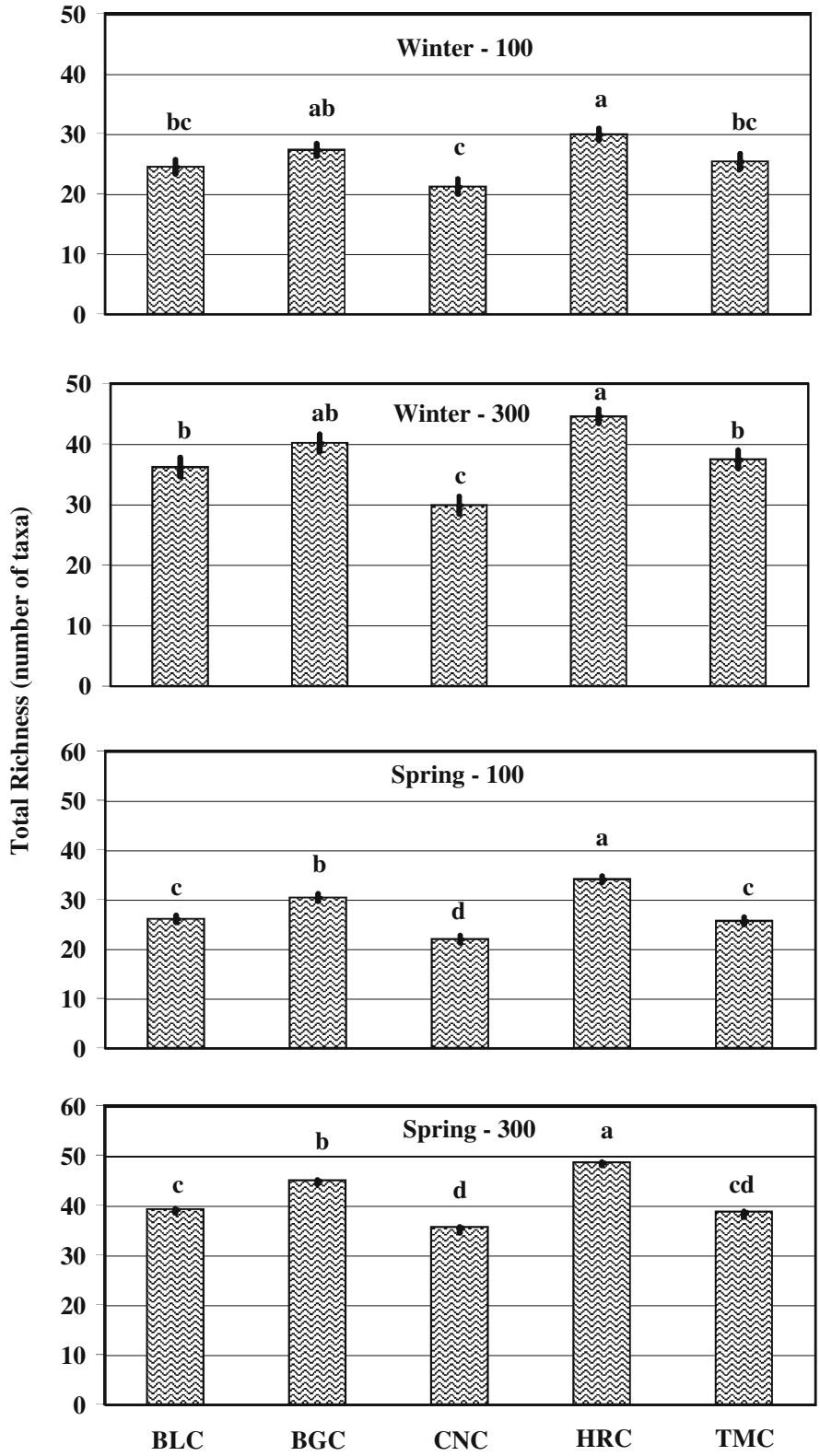

Fig. 3 Differences in total richness among study streams. Values are means calculated from non-parametric Kruskal–Wallis test (n = 27; Bonferroni adjusted $\alpha = 0.0065$). Vertical bars represent standard error values. Letters represent separate groups from Mann–Whitney multiple comparisons test

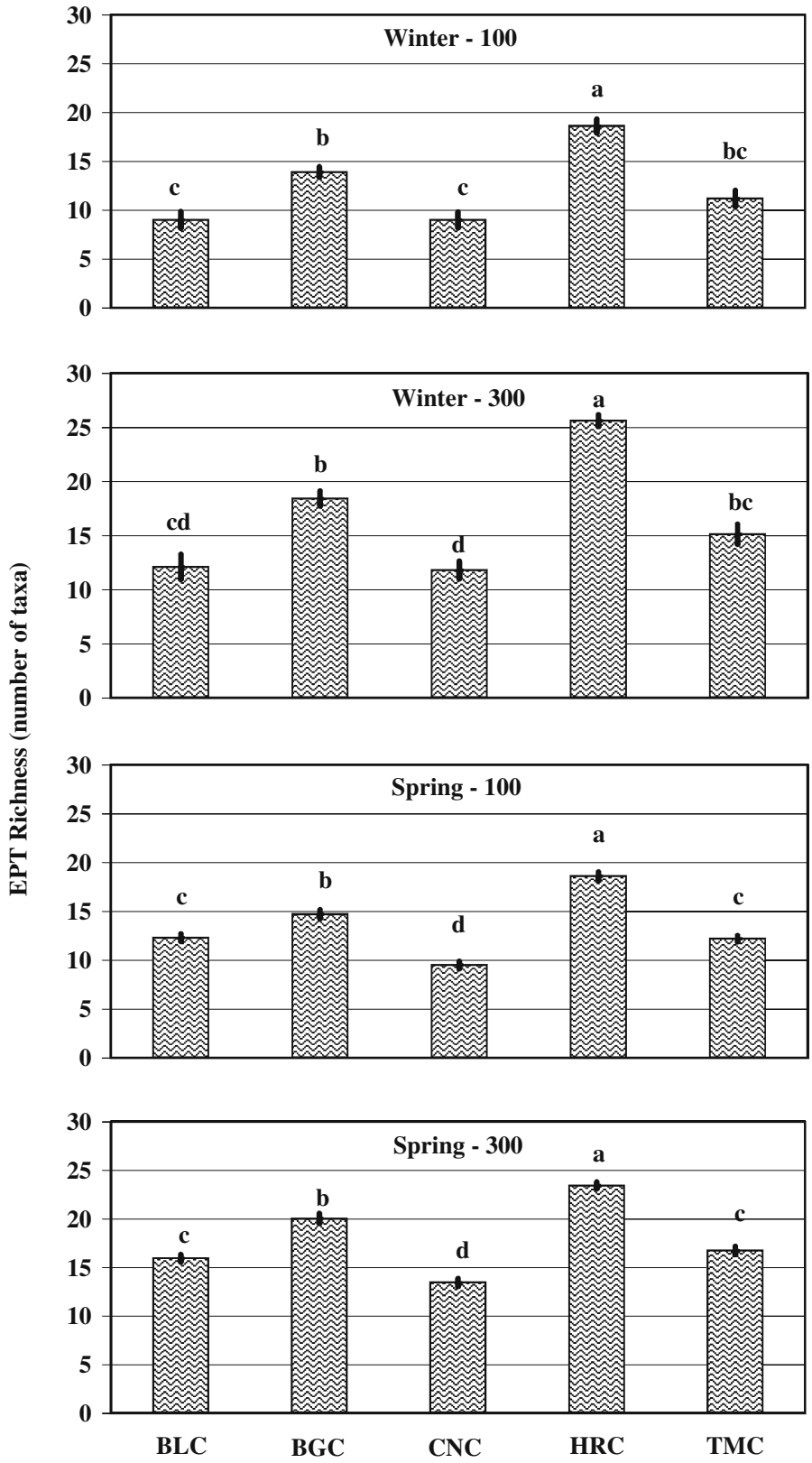

Fig. 4 Differences in EPT richness among study streams. Values are means calculated from non-parametric Kruskal–Wallis test (n = 27; Bonferroni adjusted $\alpha = 0.0065$). Vertical bars represent standard error values. Letters represent separate groups from Mann–Whitney multiple comparisons test

Table 8 Results of Kruskal–Wallis and Mann–Whitney tests of relative abundance variables (n = 27; Bonferroni adjusted alpha = 0.0065)

Variable	Season	Subsample size	BLC	BGC	CNC	HRC	TMC	Groups
Percent EPT	Winter	100	44^{c}	69^{b}	35^{c}	78^{a}	42^{c}	3
		300	43^{c}	72^{b}	38^{c}	79^{a}	39^{c}	3
	Spring	100	65^{b}	52^{c}	68^{ab}	71^{a}	48^{c}	3
		300	62^{bc}	55^{c}	67^{ab}	70^{a}	46^{d}	4
Biotic index	Winter	100	5.3^{abc}	4.7^{c}	6.1^{a}	3.9^{d}	5.7^{ab}	4
		300	5.6^{abc}	4.7^{c}	6.0^{a}	3.9^{d}	5.8^{ab}	4
	Spring	100	3.8^{b}	4.4^{a}	3.5^{b}	3.9^{b}	4.6^{a}	2
		300	4.1^{b}	4.6^{a}	3.6^{bc}	3.7^{c}	4.8^{a}	3
Percent Plecoptera	Winter	100	21^{a}	10^{c}	19^{abc}	14^{bc}	21^{ab}	3
		300	20^{a}	10^{b}	20^{a}	16^{a}	20^{a}	2
	Spring	100	33^{a}	11^{b}	27^{a}	14^{b}	28^{a}	2
		300	34^{a}	10^{d}	26^{ab}	15^{c}	28^{ab}	4
Percent Trichoptera	Winter	100	9^{bc}	16^{ab}	4^{c}	16^{a}	7^{c}	3
		300	8^{b}	19^{a}	5^{b}	18^{a}	8^{b}	2
	Spring	100	14^{a}	11^{ab}	6^{c}	9^{b}	5^{c}	3
		300	11^{a}	11^{a}	6^{b}	8^{a}	5^{b}	2
Percent dominant taxon	Winter	100	27^{abc}	22^{c}	41^{a}	15^{d}	32^{ab}	4
		300	30^{abc}	19^{c}	39^{a}	15^{d}	37^{ab}	4
	Spring	100	15^{b}	18^{b}	27^{a}	13^{b}	25^{a}	2
		300	14^{bc}	17^{b}	27^{a}	13^{c}	29^{a}	3
Percent Diptera	Winter	100	17^{ab}	21^{a}	19^{ab}	13^{b}	16^{ab}	2
		300	15^{ab}	17^{a}	17^{a}	10^{b}	14^{a}	2
	Spring	100	25^{ab}	33^{a}	14^{d}	18^{cd}	22^{bc}	4
		300	23^{ab}	28^{a}	14^{d}	14^{cd}	19^{bc}	4
Percent collectors	Winter	100	49^{a}	13^{c}	56^{a}	18^{b}	51^{a}	3
		300	48^{a}	10^{c}	54^{a}	14^{b}	52^{a}	3
	Spring	100	29^{b}	16^{c}	31^{ab}	24^{b}	40^{a}	3
		300	30^{b}	14^{d}	31^{b}	20^{c}	42^{a}	4
Percent filterers	Winter	100	7^{bc}	35^{a}	6^{c}	29^{a}	10^{b}	3
		300	6^{bc}	35^{a}	5^{c}	30^{a}	9^{b}	3
	Spring	100	6^{bc}	15^{a}	4^{c}	16^{a}	11^{ab}	3
		300	6^{bc}	17^{a}	4^{c}	17^{a}	10^{b}	3
Percent predators	Winter	100	14	8	11	9	10	1
		300	12	9	11	11	10	1
	Spring	100	25^{a}	18^{b}	20^{ab}	19^{ab}	19^{ab}	2
		300	27^{a}	18^{c}	21^{bc}	21^{b}	18^{bc}	3
Percent scrapers	Winter	100	15^{b}	34^{a}	8^{b}	32^{a}	9^{b}	2
		300	16^{b}	34^{a}	9^{b}	32^{a}	9^{b}	2
	Spring	100	16^{b}	33^{a}	29^{a}	33^{a}	10^{c}	3
		300	15^{c}	36^{a}	29^{b}	35^{a}	10^{d}	4
Percent shredders	Winter	100	16^{ab}	11^{c}	19^{ab}	12^{c}	20^{a}	3
		300	17^{ab}	11^{c}	21^{a}	13^{bc}	19^{ab}	3
	Spring	100	24^{a}	18^{abc}	16^{bc}	8^{d}	20^{ab}	4
		300	22^{a}	14^{bc}	15^{abc}	7^{d}	21^{ab}	4

Values are variable means at each study stream, by season. Letters indicate statistically different groups; numbers of separate groups were compared between 100- and 300-organism subsamples.

community quality (Resh and McElravy 1993). Specifically, greater values of total richness and EPT richness indicate a higher quality macroinvertebrate community. Thus, the potential exists to erroneously interpret low richness obtained from lower organism counts as evidence of impairment (Houston et al.

2002). But richness may also vary for natural reasons, such as seasonal differences (e.g., McCord and Lambrecht 2006; Minshall 1981; Williams 1980) or differences associated with flow permanence (Feminella 1996). Variations corresponding to both these factors were noted in the present study. So additional community characteristics, such as relative abundances of compositional or functional subsets, are also valuable in distinguishing macroinvertebrate samples. In contrast to the trend noted for richness variables, subsample size did not strongly or consistently affect most relative abundance metrics in this study. Even so, metric variabilities were lower when data from larger subsamples were used, suggesting increased accuracy.

Despite expanding the scale for richness metrics and reducing the variability of both richness and compositional metrics, our results did not clearly indicate that larger subsamples enhanced the ability to discriminate between samples taken from different seasons, or between streams of different sizes and flow conditions. Larger subsamples may reduce the possibility of interpretation error, but evidence of this was relatively uncommon in this study. Potential Type I and Type II errors were noted in similar frequencies, but occurred in different situations. When richness and compositional variables were very similar – as among the 3 HRC stations – the 300-organism subsamples may have avoided Type I error by not discriminating between stations. In contrast, when "real" variation was present, as it was among study streams for several variables, the larger subsamples added discriminatory power and may have avoided Type II error. Since all our samples were collected from unimpaired stream segments, we were not able to test sensitivity differences along a disturbance gradient. In a similar study, Davidson and Clem (2002) found no enhanced ability to distinguish between reference and nutrient-impaired stream sites using 300-organism subsamples as compared to 100-organism subsamples.

Considering the approximately 2-h additional commitment per sample, our data indicated that it would be more cost effective to use the smaller subsample size in macroinvertebrate studies where substantial seasonal or geographical variation is anticipated. This conclusion is in agreement with that of Davidson and Clem (2002), but for a different reason. Their results indicated no significant differences in metric values that estimated benthic community characteristics whereas in the present study the differences, particularly for richness variables, were considerable. Both studies noted the lack of a major effect of subsample size on the ability to discern between samples collected under clearly varying environmental conditions. However, detection of subtle changes in macroinvertebrate characteristics, whether naturally occurring or human-mediated, would likely require the greater accuracy afforded by higher organism counts. To address both cost effectiveness and the potential for additional information, the unprocessed portions of samples could be retained for additional analysis if needed.

Acknowledgements We thank Paula Lambrecht and Dennis Eagle for assistance in the field. Amanda Smith and Jeff Hall provided laboratory assistance. The research was supported by Grant No. F0455C999610309-30 from the U.S. Environmental Protection Agency and Grant No. 2RG-04 from the Arkansas Soil and Water Conservation Commission.

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