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Research Article

Differences between kick sampling techniques and short-term Hester-Dendy sampling for stream macroinvertebrates

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Abstract. Differences among sampling techniques are of crucial importance when interpreting the results of aquatic biomonitoring studies. We compared two commonly used variations of the kick sampling technique with the Hester-Dendy (multi-plate substrate) technique in an urban impacted stream. The effect of sample pooling on data interpretation was also examined. Hand scrubbing and disrupting substrates yielded more hydropsychid caddisflies than either kick sampling or Hester-Dendy samples. Total macroinvertebrate abundance, species richness, and Shannon's diversity (H') were all lower in Hester-Dendy samples than in samples taken by kick sample or by hand scrubbing substrates. Species richness pooled within sampling technique was highest in kick samples and lowest in Hester-Dendy samples. Richness of Ephemeroptera, Plecoptera, and Trichoptera (EPT) pooled among sampling techniques was higher in Hester-Dendy samples than in either kick samples or substrate-scrubbed samples. Relatively subtle differences among sampling technique and data processing have the potential to influence interpretation of biomonitoring studies. In particular, differential success in sampling hydropsychid caddisflies and other EPT taxa can influence a large number of benthic indices. Finally, samples pooled and rarified can illuminate differences detectable only at higher abundance levels.

Introduction

Aquatic macroinvertebrate sampling is a common component of water quality assessment and monitoring programs. Policy-driven guidelines for macroinvertebrate sampling, however, range from vague (Davies, 2001) to highly specific (Barbour et al., 1999). For instance, the common kick sampling methodology is widely employed by those following policy-driven guidelines (Carter and Resh, 2001), and often includes 1) simply kicking submerged substrates, or 2) hand-scrubbing and removal of larger substrates followed by substrate kicking. In either scenario, dislodged macroinvertebrates are collected in a downstream net. Although exact techniques are often prescribed in monitoring programs, lack of technique specificity under vaguer sampling guidelines for water quality monitoring (Davies, 2001) might falsely lead one to assume all kicking techniques equivalent and to

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be used interchangeably in biomonitoring studies (Sacco et al., 1993).

Although results of kick sampling and Surber sampling have been compared in the context of biomonitoring (e.g. Hornig and Pollard, 1978; Storey et al., 1991) community data from the kick-only and the hand-scrubbing and kicking variations of kick sampling have not to our knowledge been systematically compared. Use of sampling techniques assumed to be equivalent may significantly increase variance within studies, and reduce temporal and spatial consistency and comparability across biomonitoring studies. Technique-driven differences among studies have the potential to artificially influence management decisions.

Artificial substrate samplers have been used as alternative or supplementary sampling techniques, particularly in habitats not easily kick sampled, such as deep or fast-moving water (e.g. Carter and Resh, 2001; Davies, 2001). The selectivity of artificial samplers has received considerable attention in the literature (Rosenberg and Resh, 1982, and references therein). Artificial substrates have been shown to be comparable (De Pauw et al., 1986) or complementary additions (Battegazzore et al., 1994) to other sampling techniques for biomonitoring purposes. However, more comparative studies are needed to evaluate the selectivity and to determine the appropriateness of this potential substitute sampling method in monitoring studies. Understanding key differences between sampling techniques is crucial for data interpretation and formulation of agency guidelines for methodological standardization.

One widely recognized problem with artificial substrate use is lengthy incubation periods (Rosenberg and Resh, 1982). We evaluated the sampling potential of artificial substrates using short (10-day) incubation periods.

We compared macroinvertebrate community data collected with kick samples, hand-scrubbed and kicked samples, and Hester-Dendy samplers from the same sampling points in Munroe Brook in South Burlington Vermont (USA) to determine the selectivity of each method and to examine potential differences between three sampling methods commonly used in biomonitoring programs.

Materials and Methods

Study area and field sampling

This study was conducted in Munroe Brook (Vermont, USA; 44.41° N, 73.22° W) in July 2008. Munroe Brook is a tributary to Shelburne Bay in Lake Champlain, where the surrounding watershed has been subject to increasing development and urbanization. Water quality of Munroe Brook is listed as "impaired" due to contamination from stormwater runoff (VT DEC, 2006). The study was conducted in a third-order stream reach with a bank-full width of approximately 1.8 m.

Hester-Dendy samples and two types of kick samples were collected from each of seven riffles. Sampling locations were laid out using a randomized-block design. There was one block per riffle and sample location within block was randomly assigned. To avoid displacing macroinvertebrates to future sampling locations, sampling began at the downstream end of the study site and progressed upstream. Seven Hester-Dendy multiplate samplers (Hester and Dendy, 1962) had been tied to steel anchors driven into the stream bed at randomly-assigned positions across the width of each riffle ten days in advance of macroinvertebrate sampling. The samplers were tied on short ropes (approximately 10 cm of free rope between anchor and sampler) and laid length-ways on the benthos parallel to the direction of stream flow. The samplers consisted of 14, 75 mm-diameter masonite plates alternating with 25 mm nylon discs (Wildco, Buffalo, New York). Spacing between plates ranged from 3 mm to 12 mm.

All samples were taken on July 30, 2008 using a rectangular (500 μm mesh, 457 mm x 305 mm frame) pond net held immediately downstream of the sample location. Samples were transferred from the net to a plastic tray (280 mm x 375 mm). Large rocks and debris in the tray were rinsed of remaining organisms and discarded. Samples were then drained on a 600 μm sieve, placed in labeled plastic bags, and

preserved in 99% ethanol with 1% glycerin for laboratory analysis.

After ten days of incubation, the Hester-Dendy samplers were lifted from the benthos into a down-stream net and then disassembled in the previously-described plastic tray. Separate sampler components were scraped to recover attached macroinvertebrates. The samples were otherwise processed as described above. One of the seven samplers was not recovered.

Two types of kick samples were taken with equal sampling effort for each. Both of the sampling protocols involved agitating the substratum in a 457 mm x 457 mm area immediately upstream of the sampling net.

We took hand-scrubbing 'kick' samples by first moving larger cobbles and boulders from the sampling area to the net mouth and scrubbing to dislodge attached invertebrates. After discarding larger substrates, smaller substrates in the sample area were agitated by hand to a 5 – 10 cm depth; invertebrates and sediment were swept into the collecting net and the net contents were processed as described above. This procedure emulates that used by the Vermont Department of Environmental Conservation (VT DEC, 2006) with one important exception: VT DEC generates a single composite sample from four representative areas of a sampled riffle (VT DEC, 2006). The sum of four of our samples would thus be comparable to one VT DEC sample. Standard kick samples were taken by vigorously kicking and disrupting the substrate in the 457 mm x 457 mm sampling area to an approximate depth of 5 – 10 cm without prior handling of larger substrates.

Laboratory procedures

In the laboratory, all samples were poured into a 600 μm sieve and rinsed of preservative. Samples were washed from the sieve and spread evenly onto a plastic tray (30 × 40 x 1.5 cm) that had been evenly divided into 12 numbered squares. Beginning with a square selected using 12-sided dice, each sample was counted under 2X magnification until a minimum of 80 macroinvertebrates and 4 complete randomly-selected squares had been picked. Additional squares needed to reach the 80-macroinvertebrate target were completely picked resulting in samples that frequently exceeded 80 individuals, and other samples that were picked entirely and yet fell short of 80 individuals. Macroinvertebrates were counted and identified to the lowest practical taxonomic level, typically genus, using keys in Merritt et al., (2008).

Data analysis

To correct for subsampling, all abundance measures (total macroinvertebrate abundance, Ephemeroptera, Plecoptera, and Trichoptera (EPT) abundance, and abundance of hydropsychid caddisflies) for each sample were corrected by dividing the actual number of invertebrates picked and identified in a sample by the proportion of the sample picked.

Rarefaction was used to estimate the expected number of species present for a given number of macroinvertebrates in each sample. Ecosim software (Gotelli and Entsminger, 2012) was used to randomly sample 79 individuals from each sample, which represented the lowest number of individuals observed in one sample. The mean species richness of 5,000 such simulated subsamples was used as rarefied species richness for each sample.

Rarefaction was also used to estimate the expected number of EPT species present for a given number of EPT individuals in each sample. Because the lowest EPT abundance in any sample was fifteen individuals, we randomly sampled fifteen individuals from the EPT assemblage observed in each sample and replicated this subsampling 5,000 times. The average number of EPT species in the 5000 subsamples was recorded as rarified EPT richness for each sample.

Rarefaction curves for each sampling method (kick, hand-scrubbed, and Hester-Dendy) were created by first pooling the replicate samples within each sampling method and then randomly sampling a given number of individuals from each pooled sample. This was replicated 5,000 times for a sampling group at a series of abundance levels to obtain a mean expected species richness in samples of a given abun-

Table 1. ANOVA of effect of sampling method on abundance, species richness, evenness (PIE; arcsine-transformed), number of Hydropsychidae (log_{10}-transformed), EPT species richness (log_{10}-transformed), and Shannon's diversity index. *P < 0.05, **P < 0.01, *** P < 0.005

Source	d.f.	SS	MS	*F* ratio
(a) Abundance				
Sampling method	2	36331	181665	12.23***
Error	16	237575	14848	
Total	18	600907		
(b) Species richness				
Sampling method	2	91.02	45.5	19.6***
Error	16	37.09	2.32	
Total	18	128.10		
(c) Evenness (PIE)				
Sampling method	2	0.050	0.025	3.56 NSD
Error	16	0.112	0.007	
Total	18	0.162		
(d) No. Hydropsychidae				
Sampling method	2	189764	94882	10.22***
Error	16	148533	9283	
Total	18	338298		
(e) Percentage of Hydropsychidae				
Sampling method	2	3604	1802	12.3***
Error	16	2343	146	
Total	18	5947		
(f) EPT species richness				
Sampling method	2	1.79	0.896	1.35 NSD
Error	16	10.63	0.664	
Total	18	12.42		
(g) Shannon's diversity index				
Sampling method	2	0.974	0.487	10.26***
Error	16	0.759	0.047	
Total	18	1.732		

dance level. Rarefaction curves were also created for EPT species following a similar procedure, using the pooled EPT assemblages from each sampling method.

As a measure of evenness we calculated Hurlbert's (1971) PIE (probability of interspecific encounter) from each sample. Shannon Diversity Index (H'; Shannon and Weaver, 1949) was also calculated for each sample. Both PIE and H' were calculated using Ecosim software (Gotelli and Entsminger, 2005).

Normality of all parameters was confirmed using the Kolmogorov-Smirnov test. One-way ANOVAs followed by Tukey's tests were performed to evaluate the effect of sampling methods on abundance, species richness, PIE, number of hydropsychids, EPT species richness, and Shannon Diversity index of samples.

Results

Abundance

There was a significant effect of sampling technique on the total abundance of macroinvertebrates collected (one-way ANOVA; $p < 0.001$; Table 1). Each of the kick sampling techniques collected significantly more individual macroinvertebrates than did the Hester-Dendy samplers (Table 2; Figure 1), however, there was no difference between abundance in kick and hand-scrubbed samples (Table 2). Of the techniques used, hand scrubbing yielded the highest macroinvertebrate abundance (Figure 1).

Species richness

Sampling technique significantly impacted the number of species per sample (Table 1) with almost double the number of species collected by the kick sampling techniques relative to the

Table 2. Sample metrics differing significantly between sampling methods determined by Tukey's test; significance levels determined by subsequent t-test. *P < 0.05, **P < 0.01, *** P < 0.005

	Hester-Dendy	Kick
Kick	Abundance*	
	Species richness***	
	PIE*	
	Shannon Diversity***	
Hand	Abundance***	
	Species Richness***	No. Hydropsychidae*
	No. Hydropsychidae***	% Hydropsychidae**
	% Hydropsychidae***	
	Shannon Diversity*	

Hester-Dendy samplers. Scrubbing substrates in addition to kicking did not significantly change the number of species collected when compared to simply kick sampling (Table 2) and kick sampling without hand scrubbing yielded the richest samples (Figure 2). Because rarefaction of samples to the abundance level of the smallest sample did not particularly alter the species richness results, we have not presented rarefied data from individual samples. However, rarefaction of samples pooled within treatment revealed that at higher abundance levels, simple kick samples would be significantly richer than samples taken by kicking and scrubbing (Figure 3).

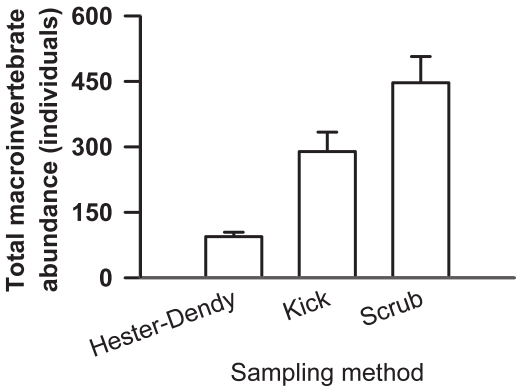

Figure 1. Average (± SE) total abundance of benthic macroinvertebrates in Hester-Dendy, kick, and hand-scrubbed samples. Abundance has been adjusted for proportion of the sample picked for kicked and scrubbed samples. Hester-Dendy multiplate samples were picked and identified in their entirety. 'Kick' refers to 30-second kick samples; 'scrub' refers to samples taken by hand scrubbing larger substrates and then agitating underlying sediments using the hand – total sampling effort was 30 seconds. All sample types were each taken in single locations in riffles.

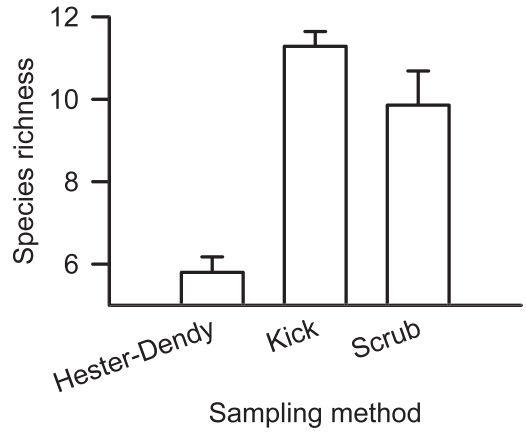

Figure 2. Average (± SE) species richness of benthic macroinvertebrates samples taken using by three different sampling techniques. Terms are as defined in Figure 1.

Evenness (PIE)

Hurlbert's (1971) PIE was highest in kick samples and lowest in Hester-Dendy samples (Figure 4) but these differences were not statistically significant (Table 1). The relatively high variance in the Hester-Dendy treatment may in large part explain this result.

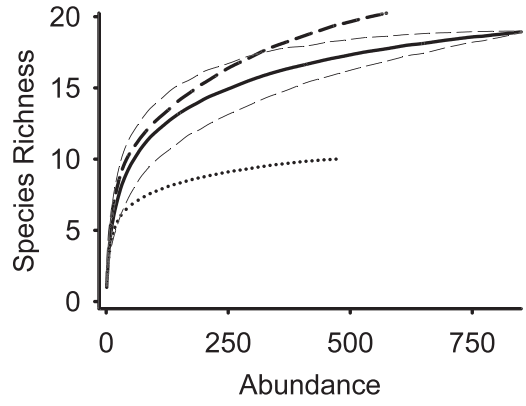

Figure 3. Rarefaction curves for whole-community samples collected using three macroinvertebrate sampling techniques. The solid line represents the pooled hand-scrubbed samples with the light dashed lines representing 95% confidence limits. The upper, thicker dashed line represents kick samples and the lower, dotted line represents Hester-Dendy samples.

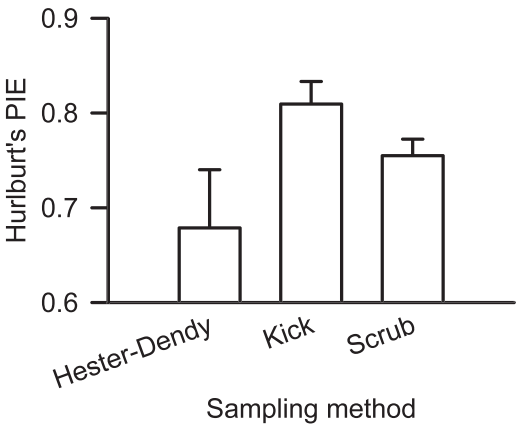

Figure 4. Average (± SE) probability of interspecific encounter (PIE; Hurlbert 1971) in Hester-Dendy, kick, and hand-scrubbed samples. Terms are as defined in Figure 1.

Hydropsychidae (Trichoptera)

Hand-scrubbed samples contained by far the highest number of hydropsychids (Table 1; Figure 5); significantly higher than either Hester-Dendy or kick samples ($p = 0.001$ and 0.013, respectively; Table 2). The result was similar when the data were expressed as a percentage of Hydropsychidae in samples.

EPT species richness

Total EPT richness was similar in samples from all sampling methods (Table 1) with about three species per sample regardless of technique

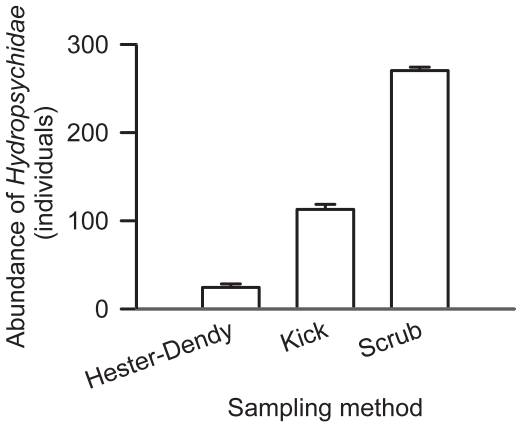

Figure 5. Average (± SE) number of Hydropsychidae identified in Hester-Dendy, kick, and hand-scrubbed samples. Terms are as defined in Figure 1.

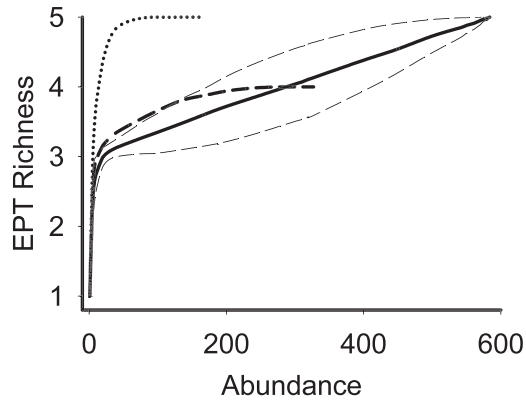

Figure 6. Rarefaction curves for the combined *Ephemeroptera*, *Plecoptera*, and *Trichoptera* assemblages collected using three macroinvertebrate sampling techniques. The solid line represents the pooled hand-scrubbed samples with the light dashed lines representing 95% confidence limits. The thicker dashed line represents kick samples and the higher, dotted line represents Hester-Dendy samples.

used. As was the case with species richness of the total community, differences became apparent with pooled samples. In contrast with whole community richness, EPT richness was highest in Hester-Dendy samples and fell outside of the 95% confidence limit of the scrubbed samples over most of the abundance range (Figure 6). EPT richness of kick samples fell inside the 95% range of the scrubbed samples over most of the abundance range.

Shannon's diversity

Sampling method significantly affected Shannon's diversity index of samples (Table 1). Hester-Dendy samples had significantly lower values of H' than samples taken using either of the kick sample techniques. Although kick samples had the highest average diversity (Figure 7), H' did not differ significantly between of kick and hand-scrubbed samples (Table 1).

Discussion

The largest differences among the sampling techniques tested were between Hester-Dendy multi-plate samplers and either of the two forms of kick samples taken. Abundance, species richness, Hurlbert's PIE, and Shannon's diver-

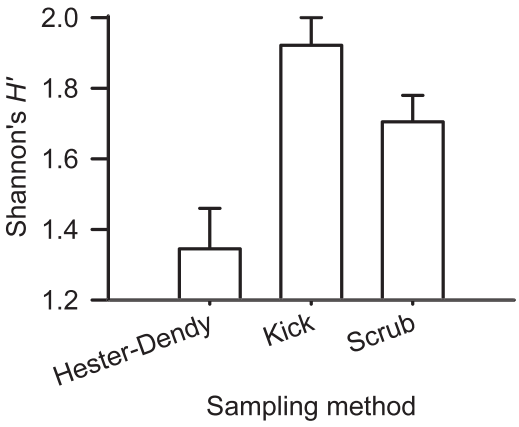

Figure 7. Average (± SE) Shannon's diversity index in Hester-Dendy, kick, and hand-scrubbed samples. Terms are as defined in Figure 1.

sity index were higher for both kick and scrubbed samples than multiplate samplers incubated for just ten days (Figures 1, 2, 4, and 5). This is not surprising and can likely be explained in part because of the small size of the samplers relative to the large area of substrate disturbed by kick sampling and in part because of the short incubation time. Longer incubation is problematic in studies of this nature and increases risk of sampler loss to high-water events. In contrast to other indices, EPT richness pooled across samplers was significantly higher than pooled EPT richness measured from Kick samples or scrubbed samples (Figure 6).

In addition artificial substrates placed directly in water current may have greater exposure to shear stresses during high-water events than would naturally imbedded substrates. Macroinvertebrates seeking refugia may migrate from more current-exposed substrates to benthic interstices (Dole-Olivier et al., 1997) thus further reducing abundance in Hester-Dendy samplers.

Low total abundance in the Hester-Dendy samples does not explain why fewer species were sampled by the Hester-Dendy multiplate substrates. When data from several Hester-Dendy samplers were pooled to achieve an abundance levels comparable to those in kick or hand-scrubbed samples, the number of species was still substantially lower in the pooled Hester-Dendy samples than even in any single kick sample or in typical hand-scrubbed samples. This could be because the Hester-Dendy samplers were selective and fully two thirds of the taxa collected by the kick and hand scrubbed samples were missed by the Hester-Dendy Samplers. Artificial substrates are selective (Rosenberg and Resh, 1982) and Hester-Dendy samplers can select for stoneflies and mayflies (Canton and Chadwick, 1983). Of the eight taxa accumulated by our Hester-Dendy samplers, five were either ephemeropterans or trichopternas. Selectivity for Ephemeroptera and Trichoptera is of significance because of their importance as components of benthic metrics for bioassessment (Barbour et al., 1999). Total abundance of EPT individuals accumulated on Hester-Dendy substrates was less than half of that sampled using either of the kick-net techniques (Figure 1). Importantly, the EPT portion of the community was quite even in the Hester-Dendy samples explaining the steep rise in the rarefaction curve relative to the curves representing the other techniques.

Differences between hand-scrubbed samples and kick samples were more subtle but potentially important because these very similar techniques are more likely to be used interchangeably or compared among studies. Average total abundance and species richness did not differ between the two techniques when comparing raw sample data using ANOVA. However, when samples were pooled and then rarified, the number of taxa sampled by the two techniques diverged significantly at abundance levels exceeding 350 individuals. Vinson and Hawkins (1996) recommended using samples with greater than 300 individuals for drawing inferences regarding benthic communities in rivers. It is clear from our data that kick and hand-scrubbed samples yield significantly different numbers of species at abundance levels in the 300-500 individual range.

It is worth noting that four of our separate samples would be, in combination, equivalent to a single sample taken using the Vermont Department of Environmental Conservation procedures designed to better represent the riffle habitat in a single stream reach (VT DEC,

2006). In Carter and Resh's (2001) survey of macroinvertebrate monitoring approaches used by US state agencies, 74.4% of respondents reported using composite samples from between two and twenty locations. We sub-sampled with an 80-invertebrate or quarter sample minimum from each of four samples so that our combined samples could be compared to a single VT DEC sample picked with a 300 individual or quarter sample target. With our sample pooling approach, or by combining samples in the field, sample sizes would typically fall into a range where kick samples are likely to yield more species than hand-scrubbed samples (Figure 3). Higher richness in kick samples cannot be explained by differences in abundance between techniques because hand-scrubbed samples collected more individuals. If richness differences were strictly a mathematical consequence sampling more individuals, then the more abundant hand-scrubbed samples should have higher richness whereas the opposite is true. Rarefaction confirmed that richness differences were independent of abundance (Figure 3) and are consistent with kick samples having higher evenness than hand-swept samples (Figure 4).

Hand-scrubbed samples were less even (Figure 4) because of increased numerical dominance of hydropsychid caddisflies in samples taken with this technique (Figure 5). Higher evenness together with higher species richness explains the higher Shannon's Diversity values in kick samples (Figure 7). Hydropsychid caddisflies represented 35% of the macroinvertebrates sampled by kick samples but this percentage increased to 60% in hand-scrubbed samples taken side by side with the kick samples in the same riffle. We attribute the larger numbers of hydropsychids in hand-scrubbed samples to tactile detection of the caddisfly nets attached to the substrates lifted by hand from the stream bed. Caddisfly nets on boulders, cobbles, and large gravel were quite obvious to our trained field technicians (IM and AC) and were reliably included in the samples.

Hydropsychid caddisflies are globally distributed and typically important components of benthic communities (Hynes, 1970). We have sampled hydropsychid caddisflies in 68 of the 70 Vermont stream sites we have monitored during the 2008 through 2010 field seasons (McCabe, unpublished). Hydropsychids are easily distinguished from other taxa and are predicted to increase as a percentage of Trichoptera in response to perturbation (Barbour et al., 1999). Natural factors or artifactual consequences of sampling techniques that alter the perceived density, percent composition, or diversity of samples represented by Hydropsychidae, have the potential to influence most of the *best candidate benthic metrics* listed by Barbour et al (1999) for use in rabid bioassessment. Differential success in collecting the Hydropsychidae is thus of critical importance in stream assessment.

Pooling of field samples, while useful at the stream scale to better represent the sum of diverse microhabitats, sacrifices patch-scale information. Keeping microhabitat samples separate as we did is more labor intensive, but it preserves patch-scale information without sacrificing the ability to later pool the data. Variance at the patch scale was sufficiently high in our data set to render differences among techniques statistically undetectable for most metrics examined. However, when samples were pooled and rarefied, differences became apparent.

Macroinvertebrate sampling techniques used in flowing waters vary widely depending upon the objectives of the sampling program, and the availability of resources including time, funding, and expertise (e.g. Lenat, 1988). While EPA's rapid bioassessment protocols (Barbour et al., 1999) provided guidance on standardization of techniques, a wide range of sampling techniques remain in common use with relatively few studies comparing these techniques (Herbst and Silldorff, 2006). Our results suggest that fairly minor differences between techniques can significantly alter the nature of community samples.

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