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Factors Influencing the Accuracy of a Macroinvertebrate Bioassessment Protocol in South Carolina Coastal Plain Streams

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ABSTRACT

The Multiple Habitat Sampling Protocol (MHSP) is a bioassessment method designed to assess the ecological health of South Carolina streams on the basis of macroinvertebrate samples collected from natural substrates. The MHSP is computed by averaging the EPT (number of Ephemeroptera, Plecoptera, Trichoptera taxa) and BI (a biotic index that reflects the pollution tolerances of individual taxa) to produce a bioclassification score. The MHSP produced low bioclassification scores that could falsely indicate environmental degradation in some undisturbed, high quality streams in the Sandhills ecoregion. This problem had two causes: 1) the metrics (especially EPT) were significantly related to stream size, which confounded stream size effects with environmental impacts, and 2) the scoring criteria for EPT were too high for some Sandhills streams, likely because of unrecognized heterogeneity among the Sandhills streams from which the criteria were derived. We corrected these problems by developing new scoring criteria from ecologically comparable undisturbed streams and by utilizing residuals from regressions of the metrics on stream width to normalize for stream size. The MHSP and related protocols are effective methods for assessing environmental quality but allowances must be made for the effects of stream size and the potential ecological heterogeneity that naturally exists among streams in some ecoregions.

INTRODUCTION

Many state agencies use bioassessment protocols that are based on the diversity and species composition of macroinvertebrates collected from natural substrates to evaluate stream health. An example is the Multiple Habitat Sampling Protocol (MHSP) developed by the South Carolina Department of Health and Environmental Control (SCDHEC 1998). Recent attempts to apply the MHSP in the Sandhills ecoregion (Keys et al. 1995) of the upper coastal plain of South Carolina have shown that it produces unexpectedly low ratings for unpolluted streams with largely undisturbed watersheds. Inaccurate results that falsely indicate environmental degradation can cause a variety of problems for both resource users and regulatory agencies.

The MHSP is a multimetric index composed of two community level variables (i.e., metrics) that are ecologically important and sensitive to environmental disturbances of various types. These metrics are the EPT (number of Ephemeroptera, Plecoptera, Trichoptera taxa) and BI (a biotic index that reflects the relative pollution tolerances of individual taxa; Lenat, 1993). The EPT and BI are measured at assessment sites and assigned scores of 1 to 5 based on expected values in similar but undisturbed reference streams. The two scores are then averaged to produce an overall bioclassification score of 5=Excellent, 4=Good, 3=Good-Fair, 2= Fair, or 1=Poor (SCDHEC 1998). Excellent and Good indicate little or no

modification of biological assemblages from a natural state, Good-Fair and Fair indicate moderate modification, and Poor indicates severe modification.

We postulated three possible reasons for inaccurate MHSP scores in the Sandhills streams we studied:

1. The MHSP does not compensate for differences in the invertebrate fauna associated with differences in stream size. The River Continuum Concept (RCC), an important conceptual framework for understanding biotic changes that occur with progression from headwater to higher order stream reaches (Vannote et al. 1980), predicts that the taxonomic richness of benthic communities increases with stream size, reaching a maximum in mid-order streams (Minshall et al. 1985). Such naturally occurring changes could be confounded with changes resulting from environmental degradation.
2. The MHSP may not measure attributes of the macroinvertebrate community that accurately reflect the health of some Sandhills streams. Although the EPT and BI have proven bioassessment value (Barbour et al. 1999), most multimetric indices include more than two metrics. A recently developed multimetric index for mid-Atlantic coastal plain states included five metrics (total number of taxa, number of EPT, percentage of Ephemeroptera, Hilsenhoff Biotic Index, and percentage of taxa with a clinger mode of existence) (Maxted et al. 2000). Inclusion of more or

different metrics in the MHSP might result in more accurate bioclassifications.

3. The scoring criteria used in the MHSP may be inaccurate. Because scoring criteria are developed by comparing impaired sites to undisturbed reference sites, they are strongly dependent upon choosing appropriate reference sites comparable with potentially impaired sites in all respects except disturbance. This results in the common practice of using different scoring criteria for different ecological regions that support different types of macroinvertebrate communities. The MHSP incorporates different scoring criteria for Mountain, Piedmont, and Sandhills/Coastal Plain ecoregions, but the reference site standards for the Sandhills/Coastal plain ecoregion may not be representative of all Sandhills streams.

The objective of this study was to determine why the MHSP does not produce accurate scores for some Sandhills streams and to find ways to improve its accuracy. Specific objectives were to determine the effects of stream size on the performance of the MHSP, assess whether the inclusion of additional metrics could improve its accuracy, and determine whether the scoring criteria for Sandhills/Coastal Plain streams were accurate. Lessons learned in our Sandhills streams are potentially applicable to any other regions where macroinvertebrate bioassessment protocols are used.

MATERIALS AND METHODS

Study area and field methods

This study included 27 sites in 12 first through fourth order streams on the Savannah River Site (SRS), a 780 km² Department of Energy (DOE) reservation in the Sandhills ecoregion of South Carolina. The streams were 2-16 m wide, 0.6-2.5m/km in average gradient, somewhat acidic (pH 4.5-6.9), and had relatively low conductivity (11-104 uS/cm). Sand was the predominant substrate together with woody material (e.g., snags, logs, twigs, roots, and leaves). Some streams received point discharges or were otherwise affected by industrial activities; but others were largely undisturbed with little or no agriculture, urbanization, or industrialization in their watersheds (because the public is excluded from the SRS).

Sixteen sites were sampled in 1997, 18 in 2000, and 22 in 2003. Some sites were sampled more than once. Sampling was conducted in the fall when most insect larvae were comparatively large. The SCDHEC (1998) MHSP was employed in 2003, in which all natural habitats were qualitatively sampled with a D-frame dip net, kick net, hand sieve, white plastic pan and fine mesh sampler with the objective of collecting as many macroinvertebrate taxa as possible during three man-hours of effort at each site. The last 1.5 hours were directed mainly towards collection of additional species rather than more individuals. Generally similar methods were used in 1997 and 2000 except that sampling was conducted for two man-hours.

Macroinvertebrates were identified to the lowest practical taxonomic level (generally genus or species).

Stream width at the water surface was measured at each sample site before or after macroinvertebrate sampling at seven to 12 evenly spaced transects across the stream perpendicular to the direction of water flow. Habitat quality was assessed using a methodology developed by SCDHEC (1998).

Data analysis

To calculate the MHSP for each site, the number of EPT taxa were tallied, and the BI was calculated by averaging the tolerances of the collected organisms. The EPT and BI metrics were each assigned a score of 1 to 5 using SCDHEC (1998) criteria, and the two scores were averaged to determine the final bioclassification. The MHSP was calculated only with 2003 data because the MHSP field protocol was followed in detail only in 2003. However, data from all three years were used in the other analyses described below.

Metrics evaluated for addition to the MHSP included number of taxa, percent clinger, and percent Ephemeroptera. We investigated these metrics because they were effective in mid-Atlantic coastal plain streams (Maxted et al. 2000) relatively similar to SRS streams, and because preliminary investigations indicated they were useful in SRS streams. Number of taxa and percent Ephemeroptera are included in many bioassessment protocols

(Barbour et al. 1999) further testifying to their effectiveness. Pearson correlation coefficients were used to assess the relationships among metrics, and between metrics and disturbance levels (described below). Multiple regression was used to determine if the addition of metrics to a model containing EPT and BI could significantly increase the ability of the MHSP to predict disturbance level. When “stepwise regression” was used, P for variables entering and exiting the model was 0.05. Stream size related effects on EPT and the BI were investigated by regressing metric values on average stream width. Regression residuals were calculated as shown in Sokal and Rohlf (1995) as were partial correlations of metrics on disturbance level with the effects of stream size removed.

The suitability of MHSP scoring criteria was evaluated by comparing existing and modified criteria with independently assessed levels of disturbance in each stream. Each stream site was assigned an ordinal disturbance level ranging from 0 (undisturbed) to 4 (maximum disturbance) based on the history of the site, proximity to industrial areas and outfalls, habitat evaluations, and the results of fish based bioassessments (IBI, Paller and Dyer 2003). The use of ordinal dependent variables in regression is technically improper, but common, and generally yields acceptable results when the number of ordinal classes is five or more (Berry 1993). The largest impact is on the accuracy of significance tests, which are also compromised by repeated testing in stepwise regression and by the assumption that sites

sampled more than once are independent. Therefore, reported P values should be regarded only as approximations. Kolmogorov-Smirnov tests indicated that all variable were normally distributed except for %Ephemeroptera and stream width. We corrected this problem by arcsine transformation for %Ephemeroptera and log transformation for stream width, but since correlation and regression results were very similar for transformed and untransformed data, we report only the latter.

The ability of the MHSP to accurately classify sites as impaired or unimpaired was summarized by constructing a receiver operating characteristic (ROC) curve. ROC curves are commonly used in the biomedical field to assess the discriminatory power of diagnostic tests (DeCarlo 1998, Motulsky 2005). They express the relationship between sensitivity (the fraction of disturbed sample sites that are correctly identified as disturbed by a low MHSP score) and specificity (the fraction of undisturbed sample sites that are correctly identified as undisturbed by a high MHSP score). The ROC curve was constructed by plotting the true positive rate (sensitivity) against the false positive rate (1-specificity) for each MHSP value. The area under an ROC curve ranges from 0.5 to 1.0, with 0.5 indicating a worthless test and 1.0 indicating a test that perfectly discriminates disturbed from undisturbed sites. The significance of the difference between the area under the curve and 0.5 was tested as shown in Motulsky (2005).

RESULTS AND DISCUSSION

Effects of stream size on bioassessment metrics

Over 225 macroinvertebrate genera were collected from the SRS streams under study, with most in the orders Diptera (primarily Chironomini, Orthoclaadiinae, Tanypodinae, and Tanytarsini), Trichoptera, Ephemeroptera, Odonata, Crustacea, Coleoptera, and Mollusca (see Paller et al. in press for a complete list of genera and their relative abundances).

Analysis of the metrics included in this study indicated that all except percent Ephemeroptera were significantly ($P < 0.001$) related to stream width. This relationship was direct for EPT ($R^2 = 0.46$), taxa richness ($R^2 = 0.38$), and percent clingers ($R^2 = 0.42$) and indirect (and weaker) for the BI ($R^2 = 0.18$). Therefore, these metrics should be normalized to avoid confounding stream size related changes with changes resulting from environmental degradation. There are several ways to make such adjustments. For EPT, Specht and Paller (2004) drew a “maximum species richness line” (following Fausch et al. 1984) through the highest points on a plot of number of EPT taxa versus stream size and divided the area below the line into intervals of equal size corresponding to different bioclassification scores. Alternatively, regression of EPT on stream size can be used to compute residuals that correspond to EPT values with the effects of stream size removed (Figure 1). Positive values indicate more EPT than expected for a stream of given size and negative values indicate fewer. For example, the EPT residual for site “2A” in Figure

1 was negative (i.e., below the regression line) indicating fewer species than expected for a stream of that size and relatively poor ecological health. However, the raw score for this comparatively large stream was above average suggesting it was ecologically healthy. A remaining step is to produce scoring classes (Figure 2) by separating the residuals into equally spaced intervals after excluding the upper 5% (Barbour et al. 1999). For the BI, which increases with organism tolerance, negative BI residuals receive higher bioclassification scores than positive residuals (Figure 3).

Addition of metrics to the MHSP

Pearson correlations between the three metrics evaluated for addition to the MHSP and the disturbance classes for each sites were -0.60 for number of taxa, -0.58 for % Clingers, and -0.30 for % Ephemeroptera (Table 1). However, higher correlations with disturbance class were exhibited by the BI (0.70) and EPT (-0.62), both of which are already included in the MHSP. Correlations were also high among some of the metrics. Partial correlations between the metrics and disturbance class adjusted for the effects of stream size were only slightly smaller (0.00-0.04) than the previous unadjusted correlations. The differences were small because disturbance class was only weakly related to stream width ($r=-0.26$).

Backwards stepwise regression of all five bioassessment metrics on disturbance class showed that only the BI and total number of taxa met the $P=0.05$ criterion for retention in the model. Inclusion of total taxa richness

rather than EPT may seem surprising since the simple correlation was slightly higher between EPT and disturbance (-0.62) than between total taxa and disturbance (-0.60) (Table 1). However, EPT and the BI shared more variation ($r=0.86$) than did total taxa and the BI ($r=-0.61$), indicating that total taxa contributed more unique information to a model that already included the BI (which was the best single predictor). R^2 for the model that included the BI and total taxa was 0.49. Addition of EPT, % Clingers, and %Ephemeroptera increased the R^2 to only 0.51 indicating little change in predictive power.

The simple correlation between EPT and total taxa was 0.85 (Table 1), indicating these two variables contained similar information. Therefore, a regression model with BI and EPT as predictor variables explained almost as much variance in disturbance level ($R^2=0.47$) as a regression model with BI and total taxa as predictor variables ($R^2=0.49$). In other words, EPT and total taxa were interchangeable with little effect on predictive power. An additional consequence of the high correlation between EPT and total taxa was that inclusion of taxa richness in a model containing EPT and the BI only minimally affected the predictive power of the model (R^2 of 0.50 for BI, EPT, and total taxa compared with 0.47 for BI and EPT only). These results show that the ability of the MHSP to predict disturbance is not significantly improved by the inclusion of total taxa, % Ephemeroptera, and % Clinger.

Appropriateness of MHSP scoring criteria

The criteria for assigning bioclassification scores to different values of EPT and the BI have a critical influence on the MHSP rating of a stream site. No SRS streams attain the highest scores of 4 or 5 based on the existing criteria for assigning EPT scores (Figure 1) despite the fact that many received no effluents and had largely undisturbed watersheds. Some of these undisturbed or largely undisturbed sites received EPT scores as low as 1, which indicates “Poor” quality and a “Not Supporting” aquatic life use rating. “Not Supporting” is defined as “severe modification of the biological community compared to the reference condition” (SCDHEC 1998), which inappropriately characterizes these undisturbed sites.

One reason the scoring criteria for EPT did not reflect the condition of SRS streams is that they did not adjust for the effects of stream size as previously mentioned. However, this did not entirely account for the problem because even larger undisturbed streams received low scores. An alternative explanation is that the reference sites from which the EPT criteria were developed were not representative of SRS streams. This problem can arise if there is substantial unrecognized heterogeneity among Sandhills/coastal plain streams. Such heterogeneity could stem from substrate type (SRS streams have sand substrates that typically support fewer EPT taxa than larger substrates, Jowett et al. 1991), amount and type of macrophyte growth, and stream size.

Assuming that the EPT scoring criteria are unsuitable, it is possible to develop new criteria from reference sites that support numbers of EPT taxa expected under undisturbed conditions. We developed such criteria from undisturbed streams and stream reaches on the SRS. Metric scoring is commonly based on the distribution of values in a population of ecologically similar sites (except for the occurrence of disturbance) that includes both disturbed and reference streams. For metrics that decrease with disturbance, the upper 5% of the values are often eliminated to exclude outliers and the remaining values are trisected, quadrisected, etc. to provide a range of scores (although other methods are also possible, Barbour et al. 1999). Figure 2 demonstrates the use of this method with EPT residuals. Figure 2 has the combined effect of controlling for the effects of stream size as well as adjusting the scoring criteria for numbers of EPT taxa characteristic of undisturbed SRS streams. Full validation of these scoring criteria will require future tests with an independent data set derived from Sandhills streams outside the SRS.

Table 2 shows unadjusted MHSP scores and adjusted MHSP scores for SRS streams. Unadjusted scores were calculated using original scoring criteria (SCDHEC 1998), and adjusted scores by correcting for stream size and using undisturbed SRS reference sites to establish criteria (Figures 2 and 3). Streams were divided into disturbed and relatively undisturbed categories for this comparison, with relatively undisturbed including

disturbance categories 0 and 1 and disturbed including disturbance categories 2, 3, and 4. Without adjustment, the average EPT score for undisturbed streams was 2.0 (equal to a bioclassification of “Fair”). Following adjustment, EPT increased to an average of 3.6 (“Good-Fair” to “Good”). In contrast, adjustment resulted in relatively small changes in the BI (3.7 versus 3.9 for undisturbed streams), which had original scoring criteria less strongly affected by stream size and more suitable for SRS streams. The adjustments resulted in an average increase in the MHSP of 0.8 points for undisturbed streams (2.9 versus 3.7) and 0.5 for disturbed streams (1.8 versus 2.3), with most of the increase resulting from the change in EPT.

An ROC curve was constructed to summarize the sensitivity and specificity of the adjusted MHSP, with sensitivity being the proportion of impaired sites identified as impaired and specificity being the proportion of unimpaired sites identified as unimpaired. Sites in disturbance classes 0 and 1 were defined as impaired and sites in disturbance classes 2, 3, and 4 were defined as unimpaired. The area beneath the ROC was 91.1% which was significantly different ($P < 0.0001$) from the 50% indicative of a random classifier (Figure 4). The value, 91.1%, can be interpreted as the probability that a randomly selected impaired site will have a lower MHSP score than a randomly selected unimpaired site. Generally, a classification test is rated as excellent when the area under the curve exceeds 0.90 (Motulsky 2005).

Bioassessment protocols such as the MHSP are efficient and practical for evaluating the ecological health of southeastern coastal plain streams. Our results shows that the two metrics in the MHSP, EPT and the BI, effectively discriminate disturbed from undisturbed conditions, but that EPT (and other taxa richness metrics) should be adjusted for stream size to avoid confounding stream size and disturbance. Our results also indicate the possibility of substantial ecological heterogeneity among streams within ecoregions that must be fully evaluated when developing metric scoring criteria.

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Table 1. Pearson correlation coefficients among bioassessment metrics, stream width, and stream disturbance level. Disturbance level was expressed on a scale from 0 (least disturbed) to 4 (most disturbed).

	BI	No. taxa	EPT	% Ephem- eroptera	% clingers	Width (m)	Distur- bance
BI	1						
No. taxa	-0.61	1					
EPT	-0.75	0.86	1				
% Ephemeroptera	-0.27	0.33	0.34	1			
% clingers	-0.72	0.86	0.90	0.27	1		
Width (m)	-0.43	0.62	0.68	0.24	0.65	1	
Disturbance	0.70	-0.60	-0.62	-0.30	-0.58	-0.26	1

Table 2. Unadjusted and adjusted MHSP scores for Sandhills streams.

Scores are adjusted for stream size and for comparable reference sites.

Stream site	MHSP			Adjusted MHSP		
	EPT	BI	Avg.	EPT	BI	Avg.
Undisturbed streams						
Mcqueens Branch	2	5	3.5	5	5	5.0
Mill Creek 1	1	4	2.5	3	5	4.0
Meyers Branch 1	1	2.4	1.7	3	3	3.0
Pen Branch 1	2	5	3.5	5	5	5.0
Mill Creek 2	2.4	4	3.2	5	5	5.0
Indian Grave Branch	1.4	3	2.2	3	3	3.0
Pen Branch 2	1	4	2.5	2	4	3.0
Fourmile Branch 1	2	3	2.5	4	4	4.0
Fourmile Branch 2	2	3	2.5	3	4	3.5
Tinker Creek 1	3	4	3.5	5	4	4.5
Tinker Creek 2	2.4	3	2.7	4	3	3.5
Pen Branch 3	2	3	2.5	2	3	2.5
Upper Three Runs 1	3	5	4.0	3	4	3.5
Upper Three Runs 2	3.4	3	3.2	3	2	2.5
Average	2.0	3.7	2.9	3.6	3.9	3.7
Disturbed streams						
Meyers Branch 2	1	1	1.0	2	2	2.0
Crouch Branch 1	1	2	1.5	2	2	2.0
Crouch Branch 2	1	3	2.0	3	4	3.5
Fourmile Branch 3	1	2	1.5	3	2	2.5
Tims Branch	1	1	1.0	2	1	1.5
Steel Creek	1.4	4	2.7	1	5	3.0
Lower Three Runs 1	1.4	3	2.2	2	3	2.5
Lower Three Runs 2	2	2.4	2.2	1	2	1.5
Average	1.2	2.3	1.8	2.0	2.6	2.3

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Figure 1. Regression of EPT on stream width. Sample site symbols indicate level of disturbance (0=undisturbed to 4=highly disturbed). Numbers 1-5 on the left indicate unadjusted MHSP scoring intervals for EPT taken from SCDHEC (1998). Dotted line represents the average EPT for all sites.

Figure 2. Distribution of EPT residuals (derived from the regression of EPT on stream width) divided into five scoring intervals after excluding the upper 5% (shown by dotted line). Higher EPT residuals indicate greater than expected EPT and receive higher scores.

Figure 3. Distribution of BI residuals (derived from the regression of BI on stream width) divided into five scoring intervals after excluding the lower 5% (shown by the dotted line). Lower BI residuals indicate a greater than expected BI and receive higher scores.

Figure 4. ROC curve for the MHSP with adjusted scoring criteria. The straight line represents a random classifier.







