Experimental Determination of Benthic Macroinvertebrate Metric Sensitivity to Fine Sediment in Appalachian Streams

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Abstract: Many fisheries management agencies incorporate benthic macroinvertebrate metrics in stream assessment, yet concern exists over regional variability in metric sensitivity and the reproducibility of results over time. Two field experiments were conducted in Mullenax Run, Pocahontas County, West Virginia during summers 1999 and 2000 to investigate the sensitivity of benthic macroinvertebrate metrics to fine sediment and annual variation. Substrate composition of fine sediment (< 2mm) was manipulated from 0%–40% in 10% increments in 0.3-m^2 circular trays arrayed in 2 sections of the study stream. The trays were allowed to colonize for 5 weeks in each year. In 1999, Ephemeroptera, Plecoptera, and Trichoptera, (EPT) taxa richness (R^2=0.144, P=0.0031) was negatively related to increasing fine sediment while % Climber (R^2=0.217, P=0.0019) was positively related to increasing fine sediment. In 2000, annual flow differences may have clouded relationships between fine sediment and benthic macroinvertebrate metrics in the experiment suggesting that low flow may mask the sensitivity of metrics used in stream bioassessment.

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Many current environmental monitoring approaches utilize benthic macroinvertebrates. Benthic macroinvertebrates are found in a wide variety of habitats, exhibit a gradient of response across taxonomic groups, live a generally sedentary lifestyle that may reflect past perturbations, and live fairly long life cycles (Rosenberg and Resh 1996). Therefore, benthic macroinvertebrates are incorporated in many state and federal environmental monitoring programs, such as the U.S. Environmental Protection Agency's Rapid Bioassessment Program (RBP) and EMAP, the Maryland Biological Stream Survey (Maryland Department of Natural Resources), and index

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of well-being (Ohio Environmental Protection Agency) (Stribling et al. 1998, Karr and Chu 1999). Many other state and federal agencies use benthic macroinvertebrates and fish in combined indices of biotic integrity (IBI) (Karr and Chu 1999).

The use of benthic macroinvertebrates in the assessment of environmental disturbance is hampered by insensitivity to some types of disturbance, the influence of intrinsic habitat and water quality characteristics of the stream, and seasonal and temporal variation in abundance and diversity (Rosenberg and Resh 1996). Some regions and taxonomic groups lack identification keys (Rosenberg and Resh 1996). Furthermore, taxonomic diversity varies by ecoregion (Feminella 2000). Within ecoregions, taxonomic assemblages vary with stream order (Waite et al. 2000). With so many potentially confounding factors, selecting appropriate benthic macroinvertebrates and interpreting their responses can be difficult.

We addressed 2 of the factors influencing the use of benthic macroinvertebrates in monitoring central Appalachian streams. We tested fine sediment (< 2 mm diameter) sensitivity among many commonly used benthic macroinvertebrate metrics to identify metrics useful in detecting excess fine sediment in the substrate and to identify metrics used in the detection of other perturbations (e.g., acid mine drainage or nutrient enrichment) that may be unduly influenced by fine sediment in the substrate. Furthermore, we assessed the reproducibility of experimental determination of metric sensitivity.

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Methods

The field experiment was conducted in Mullenax Run, a second order tributary of the East Fork of the Greenbrier River (3837 N, 7941 W), in the Monongahela National Forest in West Virginia. Habitat and water chemistry data were collected in 1999 and 2000 during July low flows by Representative Reach and Basinwide Visual Estimation Techniques (Hankin and Reeves 1988, Dolloff et al. 1997) (Table 1).

In the summers of 1999 and 2000, we placed trays with known sediment composition into Mullenax Run to examine the influence of sediment levels on macroinvertebrate colonization. Fine sediment (<2-mm diameter) composition within the trays was manipulated from 0% to 40% in increments of 10% similar to the method described by Angradi (1999) encompassing (0%, 10%, 20%, and 30%) and exceeding (40%) the range of fine sediment observed by Angradi and Vinson (1996) in nearby streams. Six riffle sediment samples taken in May 1999 by grain scoop (similar to shovel sampling [Hakala 2000]) and shaken through a Wentworth series (32 mm, 16 mm, 8 mm, 4 mm, 2 mm, 1 mm, 0.5 mm, 0.25 mm, 0.125 mm, 0.63 mm, and

Table 1. Physical and chemical characteristics of Mullenax Run.

	1999	2000
Slope (%)	11.5	11.5
Avg. pH	7.3	6.9
Avg. summer temp (˚C)	15.3	14.9
High yearly temp (˚C)	22.9	21.2
Low yearly temp (˚C)	-0.1	-0.2
Fine sediment (% < 2mm)	9.3	1.1
Ave. discharge (m/second)	<0.01	0.1
DO (mg/liter)	7.1	9.1
Specific conductance	42.8	17.3
Dissolved organic carbon (mg/liter)	NA	2.5
Alkalinity (mg/liter)	NA	4.9
Calcium (mg/liter)	NA	2.5

a pan) generated a mean composition of sediment for riffles in Mullenax Run. Sediment used in the treatment trays was collected in Mullenax Run and nearby streams (Abe's Run, Elleber Run, Lick Run, Little Low Place, Long Run, and Poca Run). Treatment mixtures were based upon manipulating the amount of each sediment size class such that the total weight of the size classes <2 mm was equal to the proportion of the total weight that would fill the trays (2.91 kg) indicated by the treatment level (0%–40%) with the remaining weight needed to reach 2.91 kg comprised of size classes >2 mm. Sediment mixtures were prepared in the lab by drying sediment collected in the streams at 100 C and sorting with the Wentworth sieve series. Sediment mixtures were transported to the stream in individual plastic bags to be placed in circular trays (0.3 m^2) within the stream. Each tray was constructed from 5 cm section of 17.5 cm (inside diameter) plastic pipe fitted to a 30 x 30 cm sheet of 6-mm plastic (Angradi 1999).

Field experimentation began in the first week of June 1999 to take advantage of historically stable flow and weather patterns (Angradi 1999). We placed 8 trays in 10 riffles (stations) distributed equally in 2 stream reaches (8 trays x 5 riffles x 2 reaches). The stream bottom within each riffle was excavated and the trays were placed so their tops were equal to the surrounding substrate. Within each riffle, 5 trays randomly received 1 of the 5 manipulated mixtures such that each station received all 5 mixtures. The other 3 trays received ambient sediment collected at the site. Sediment trays were covered with fine muslin during placement and removal to prevent sediment and macroinvertebrate loss.

Following Shaw and Minshall (1980) and Angradi (1999), 5 weeks were allowed for macroinvertebrate population colonization and stabilization. Three Surber samples were taken at each station to detect any sampling artifacts from tray avoidance or selection based on tray design and material (Mason 1976). Samples were placed into plastic containers and preserved in approximately 70% ethanol.

Field experimentation in 2000 began in the second week of June. Two stations were added (1 in each reach) to bring the total to 12. Placement and removal followed the 1999 methodology except for ambient sediment. Ambient sediment was previously collected and dried to prevent "seeding" trays with pre-existing macroinvertebrates and organic matter. The experimental sites were visited weekly due to concerns over high flows from frequent June storms. Three flow measurements were taken by a Flowmate flow meter (nearest 0.1 m/second) above each tray from left to right perpendicular to stream flow.

Once returned to the lab, tray and Surber samples were dyed with Rose Bengal before washing through 2 sieves of 1 mm and 0.25 mm. All macroinvertebrates collected on the 1 mm sieve were picked, enumerated, weighed, and identified to lowest practical taxa, usually genus. Macroinvertebrates collected on the 0.25 mm sieve were subsampled using a method similar to that described by Feminella (1996) and Angradi (1999). Sediment and macroinvertebrates were diluted to a known 500-ml volume in a 1000-ml beaker, agitated with an air hose system, and 10 10-ml aliquots were sampled with a Hensen-Stemple pipette from the 500-ml volume. Subsamples were completely enumerated and identified under the microscope. After identification and enumeration, a 10% subsample of macroinvertebrates >1 mm was identified and enumerated a second time with a second identification key (either Stewart and Stark 1988, Peckarsky et al. 1990, Merritt and Cummins 1996, Wiggins 1998) for quality control.

Macroinvertebrates were dried at 60 C for 48 hours, then placed in a desiccator for 24 hours, before weighing using an electronic balance. Drying for 48 hours was experimentally determined to yield an unchanging mass (no mass change between 12-hour weighing periods) with increasing time period. Dry mass (DM) was converted to ash free dry mass (AFDM) using conversions reported in Benke et al. (1999).

A random selection of 10% of the trays (12 trays) was tested for changes in sediment composition over the experiment in 2000. Samples were sorted using the same macroinvertebrate method as other trays except sediment was conserved in the original plastic bag. After macroinvertebrate removal, all sediment was dried at 100 C, shaken in a Wentworth sieve series, and weighed by fraction. The compositions before and after the experiment were compared to examine potential changes in sediment composition.

Metrics were selected primarily from the Benthic Index of Biotic Integrity for western Maryland streams (Stribling et al. 1998). Due to their close proximity, we assumed eastern West Virginia streams were similar to western Maryland streams. Additional metrics were added based upon studies in the region (Clayton and Menendez 1996, Angradi 1999, Kaller 2001). All taxonomic richness metrics were based upon generic-level identification. A complete list of metrics can be found in Table 2. In each year, arcsine transformations were used on percentage metrics and non-normal data (Krebs 1999). In 1999, linear regression was used to test metric sensitivity to increasing amounts of fine sediment across the treatments. We used a *t*-test to compare surber samples to ambient treatment trays after standardizing to a common unit of area (m^2) to detect potential tray avoidance or selection that may have lead to sampling artifacts. We suspected greater variability in flows in 2000 may have influenced the experiment.

Table 2. Metric tested against fine sediment (<2 mm), expected response, and reference.

Metric	Expected response	Reference
EPT[a] taxa richness	Decrease	Stribling et al. (1998), Angradi 1999
Ephemeroptera taxa richness	Decrease	Stribling et al. (1998), Angradi 1999
Plecoptera taxa richness	Decrease	Stribling et al. (1998), Angradi 1999
Trichoptera taxa richness	Decrease	Stribling et al. (1998), Angradi 1999
Diptera taxa richness	Decrease	Stribling et al. (1998)
Odonata taxa richness	Decrease	Stribling et al. (1998)
Coleoptera taxa richness	Decrease	Stribling et al. (1998)
%EPT	Decrease	Stribling et al. (1998), Angradi and Vinson 1996
% Baetidae of Ephemeroptera	Decrease	Stribling et al. (1998), Angradi 1999
% Chironomidae of Diptera	Increase	Stribling et al. (1998)
% Chironomidae	Increase	Stribling et al. (1998)
% Ephemeroptera	Decrease	Stribling et al. (1998)
% Trichoptera	Decrease	Stribling et al. (1998)
% Plecoptera	Decrease	Stribling et al. (1998)
% Odonata	Decrease	Stribling et al. (1998)
% Coleoptera	Decrease	Stribling et al. (1998)
% Diptera	Increase	Stribling et al. (1998), Angradi and Vinson 1996
% Oligochaeta	Increase	Stribling et al. (1998)
% non–insect	Increase	Stribling et al. (1998)
% Amphipoda	Decrease	Stribling et al. (1998)
% Hydropsychidae of Trichoptera	Increase	Stribling et al. (1998)
% swimmer	Decrease	Stribling et al. (1998)
% clinger	Decrease	Stribling et al. (1998)
% predator	Decrease	Stribling et al. (1998)
% shredder	Decrease	Stribling et al. (1998)
% scraper	Decrease	Stribling et al. (1998)
% climber	Decrease	Stribling et al. (1998)
% *Cheumatopsyche* spp.	Decrease	Clayton and Menendez (1996)
% Heptageniidae	Decrease	Kaller (2001)
% Baetidae	Decrease	Kaller (2001)
% Corydalidae	Decrease	Kaller (2001)
% Decapoda	Increase	Kaller (2001)
% *Epeorus* spp.	Decrease	Kaller (2001)
% Ephemeridae	Decrease	Kaller (2001)
% Ephemerellidae	Decrease	Kaller (2001)
% Glossosomatidae	Decrease	Kaller (2001)
% Hydropsychidae	Increase	Kaller (2001)
% Letophlebiidae	Decrease	Kaller (2001)
% Leucritidae	Decrease	Kaller (2001)
% *Leucrocuta* spp.	Decrease	Kaller (2001)
% Limnephilidae	Decrease	Kaller (2001)
% Megaloptera	Decrease	Kaller (2001)
% Peltoperiidae	Decrease	Kaller (2001)
% Perlidae	Decrease	Kaller (2001)
% Perlodidae	Decrease	Kaller (2001)
% Philopotamidae	Decrease	Kaller (2001)
% Rhyacophilidae	Decrease	Kaller (2001)
% Sialidae	Decrease	Kaller (2001)
% *Stenocron* spp.	Decrease	Kaller (2001)
% *Stenonema* spp.	Decrease	Kaller (2001)

a. Ephemeropta, Plecoptera, and Trichoptera taxa.

Therefore, in 2000, multiple linear regression was used to examine the sensitivity of macroinvertebrate metrics to sediment in the context of varying current velocities (Dowdy and Wearden 1991). In each year, the Dunn-Sidak method was used to adjust the α-level to 0.01 to reduce increasing experiment wise error rate from performing multiple statistical analyses on the same data set (Sokal and Rohlf 1995).

Results

Due to stable flows during the 1999 experimental period, the sediment composition of trays was assumed to not have changed during the experiment (Natl. Oceanic and Atmos. Admin. 1999, 2000; Ward et al. 2000). Statistical analysis failed to find any significant differences in the sediment composition of the 10% subsample of trays examined for changes in composition during the experiment in 2000. However, 12 trays experienced a partial or complete sediment loss in 2000 during the field experiment. These trays were overturned or swept out of the sites and excluded from the analyses.

We compared Surber samples to ambient trays to detect possible tray selection or avoidance. We found % Elmidae (P=0.02) was higher and Coleoptera taxa richness (P=0.01) was lower in trays than Surber samples. In 2000, macroinvertebrate density (P<0.01), Ephemeroptera taxa richness (P<0.01), EPT taxa richness (P<0.01), Diptera taxa richness (P<0.01), Plecoptera taxa richness (P<0.01), Trichoptera taxa richness (P<0.01), % Baetidae (P<0.01), and % swimmer (P<0.01) were lower in trays than in Surber samples.

We also tested ambient trays to manipulated sediment mixtures of similar composition to examine possible influences in colonization from the handling of the sediment mixtures. Analysis of the 1999 field experiment revealed metrics differing between ambient sediment trays and treatment trays (Table 3). In 2000, only 1 metric,

Table 3. Differences in metrics between ambient trays (%<2 mm= 9.3) and 10% treatment level in 1999.

Metric	*P*–value	Treatment effect
Macroinvertebrate density	<0.0001	(–)[a]
Macroinvertebrate biomass	<0.0001	(–)
Ephemeroptera taxa richness	<0.0001	(–)
EPT taxa richness	<0.0001	(–)
Plecoptera taxa richness	<0.0001	(–)
Trichoptera taxa richness	<0.0001	(–)
Diptera taxa richness	<0.0001	(+)
Odonata taxa richness	0.0263	(+)
% Chironomidae	<0.0001	(+)
% Diptera	<0.0001	(+)
% burrower	<0.0001	(+)
% climber	<0.0001	(+)
% collector	<0.0001	(+)

a. (+) or (–) indicates higher or lower values in treatment trays.

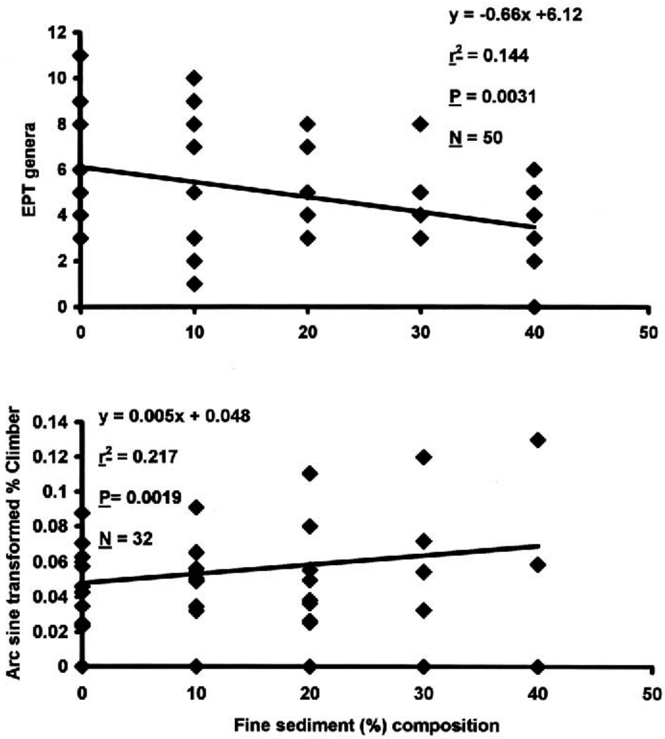

Figure 1. Metric responses to increasing fine sediment treatments in 1999. Individual trays at each treatment level shown.

EPT taxa richness, differed between ambient trays and treatment mixtures ($P<0.01$).

Finally, we tested the metrics against the fine sediment treatments. In 1999, EPT taxa richness declined while % climber increased in response to fine sediment (<2 mm) (Fig. 1). EPT taxa richness declined with increasing sediment treatments (Table 4). The highest sediment treatment had significantly lower EPT taxa richness (P=0.01) than any of the other treatments. The increase in % climber also was observed in concurrent stream surveys (Kaller 2001). The taxa comprising % climber appeared to favor depositional areas possibly explaining the increase in % climber in the experiment. Metric response to fine sediment was not evident in statistical analyses in 2000.

Table 4. Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa richness declines with increasing fine sediment (<2mm) levels in trays during the 1999 field experiment.

Fine sediment level	Mean *N* EPT	Standard deviation
0	8.9	3.73
10	9.3	4.50
20	8.0	2.16
30	6.9	2.85
40	5.7[a]	3.35

a. Significantly different $P<0.01$.

Discussion

We tested benthic macroinvertebrate metrics across the range of sediment treatments to assess sensitivity to fine sediment and experimental reproducibility. Our intention was to provide a wide level of treatments that might influence even taxa tolerant of fine sediment. We observed flow stability and similarity in water chemistry between the stations suggesting these factors would not influence the outcome of the experiment. The 1999 and 2000 field experiments should have been able to validate or refute metrics found to be sensitive in previous research and concurrent stream surveys (see Angradi 1999, Kaller 2001).

Two metrics responded to increasing fine sediment in the 1999 experiment. The metrics EPT taxa richness was negatively related to fine (<2-mm) sediment while % climber was positively related to fine (<2-mm) sediment in 1999. In streams in the nearby Fernow experimental forest, Angradi (1999) found macroinvertebrate density, biomass, EPT richness, and % Chironominae of Chironomidae declined while % Orthocladiinae of Chironomidae and % Baetidae of Ephemeroptera increased with increasing fine (<2-mm) sediment. In Mullenax Run and 6 nearby streams, metrics assessed against ≤2-mm sediment fractions found EPT taxa richness to be negatively related to size classes <2 mm (specifically <0.25 mm [spring 2000] and <0.125 mm [fall 1998]) (Kaller 2001). However, the 1999 experiment only corroborated an effect of fine sediment on EPT taxa richness.

Trays with ambient sediment were significantly different from introduced trays with a similar composition of fine sediment (Table 3). Collecting sediment directly from Mullenax Run probably "seeded" the trays with organic matter, periphyton, and pre-existing populations of benthic macroinvertebrates imparting an advantage in numbers and possibly attracting other macroinvertebrates. The evidence of "seeding" in 1999 was best demonstrated by the absence of obvious "seeding" in 2000. In 2000, when all sediment mixtures were oven dried, differences between ambient and treatment trays were not as apparent. Therefore, in 1999, macroinvertebrate distributions may have been influenced by optimal conditions in ambient trays rather than sediment treatments.

Despite "seeding," EPT taxa richness and % climber did respond to increasing fine sediment treatments in 1999. These are 2 metrics with known relationships to fine sediment within streams (Sandine 1974, Waters 1995, Angradi 1999). The 1999 experiment suggests these 2 metrics may have been sufficiently related to fine sediment to overcome experimental artifacts such as "seeding" of the trays.

The 2000 experiment addressed some of the concerns arising from the 1999 experiment. Precautions were taken against "seeding" and measurements were made to account for flow. However, we failed to detect statistically significant relationships between metrics and fine sediment.

Differences in precipitation between 1999 and 2000 may have prevented the detection of macroinvertebrate-sediment relationships. Precipitation in 1999 over the experimental period was 1/3 the amount in 2000 (Natl. Oceanic and Atmos. Admin. 1999, 2000). The area of available benthic habitat was 50% less during the drought in 1999 than in a more average year of 2000 (Hakala 2000). Macroinvertebrate distribution in the fall of 1999 in concurrent stream surveys suggest macroinvertebrates may have redistributed away from some riffles in response to decreasing wetted habitat (Kaller 2001). Lake (2000) and Boulton et al. (1992) reported the effects of drought may have lengthy recovery periods. The 2000 field experiment may have been influenced by effects of low flows from the previous summer.

Furthermore, several pronounced spates occurred during the experimental period in 2000. Holomuzki and Biggs (2000) found Ephemeroptera and Trichoptera taxa to be vulnerable to displacement during high flow events from unstable substrates (e.g., high fine sediment tray mixtures). Spates early in the 2000 experiment may have dislodged Ephemeroptera and Trichoptera taxa from trays possibly reducing the effectiveness of EPT taxa richness as a metric.

Evidence of the influence of drought, spates, or an interaction of the 2 was observed in lower macroinvertebrate abundance ($P<0.0001$) and diversity in the trays between 1999 and 2000. This suggests that the influence of fine sediment may be a lesser perturbation than high or low flow events or temporal variation in headwater streams may be too great to establish a consistent set of monitoring metrics.

Summary

We tested benthic macroinvertebrate metric sensitivity to fine sediment and temporal variation. In 1999, we found EPT taxa richness and % climber to respond to increasing levels of fine sediment during stable flow conditions. In 2000, we were unable to statistically detect any relationships between metrics and fine sediment. Given the consistent sensitivity of EPT taxa richness to increasing fine sediment in 2 other studies (Angradi 1999, Kaller 2001) in the region, we may be able to consider the 2000 field experiment to be an aberration. Therefore, EPT taxa richness appears to be suitable for the assessment of fine sediment and resilient to temporal variation. When EPT taxa richness is used in stream assessment, consideration should be paid to the potential influence of fine sediment in the substrate upon the performance of the metric.

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