Hydrobiologia (2006) 573:55–73
DOI 10.1007/s10750-006-0257-2

Primary Research Paper

Environmental factors accounting for benthic macroinvertebrate assemblage structure at the sample scale in streams subjected to a gradient of cattle grazing

Amy Braccia* & J. Reese Voshell Jr.
Department of Entomology, Virginia Tech, 300A Price Hall, Blacksburg, VA 24061, USA
*(*Author for correspondence: E-mail: abraccia@vt.edu)*

Received 29 November 2005; in revised form 30 March 2006; accepted 22 May 2006; published online 2 August 2006

Key words: agriculture, benthic macroinvertebrates, environmental stress, habitat, sample scale, sediment

Abstract

Macroinvertebrate assemblages were related to environmental factors that were quantified at the sample scale in streams subjected to a gradient of cattle grazing. Environmental factors and macroinvertebrates were concurrently collected so assemblage structure could be directly related to environmental factors and the relative importance of stressors associated with cattle grazing in structuring assemblages could be assessed. Based on multivariate and inferential statistics, measures of physical habitat (% fines and substrate homogeneity) had the strongest relationships with macroinvertebrate assemblage structure. Detrital food variables (coarse benthic and fine benthic organic matter) were also associated with assemblage structure, but the relationships were never as strong as those with physical habitat measures, while autochthonous food variables (chlorophyll *a* and epilithic biomass) appeared to have no association with assemblage structure. The amount of variation explained in taxa composition and macroinvertebrate metrics is within values reported from studies that have examined macroinvertebrate metric–sediment relationships. The % Coleoptera and % crawlers had consistent relationships with % fines during this study, which suggests they may be useful metrics when sediment is a suspected stressor to macroinvertebrate assemblages in Blue Ridge streams. Findings from this study also demonstrate the importance of quantitative sampling through time when research goals are to identify relationships between macroinvertebrates and environmental factors.

Introduction

Benthic macroinvertebrates, especially insects, are a diverse group of animals that are highly adapted to a wide range of natural conditions in freshwater environments. Nowhere is this more evident than in shallow, flowing water bodies, where the complex nature of fluvial geomorphology forms heterogeneous streambeds of unevenly distributed habitats (we use the term habitat in a narrow sense to mean the physical space where an organism lives and grows (Odum, 1983)). Findings from detailed benthic studies have demonstrated that small areas (approximately 0.1 m^2) of similar benthic habitat have similar macroinvertebrate faunas (Sprules, 1947; Ulfstrand, 1967; Mackay, 1969). Although there are multiple environmental factors that influence assemblage structure, habitat (e.g., water current and substrate) and food resources (e.g., detritus and algae) have been shown to be especially important at the sample spatial scale (Egglishaw, 1964; Edington, 1968; Cummins & Lauff, 1969; Dudley et al., 1986; Bouckaert & Davis, 1998). In benthic research studies, the terms "patch scale" and "microscale" have been used synonymously with "sample scale" (Evans & Norris, 1997; Palmer et al., 2000), which we use in this paper.

Benthic habitat is complex and many of the environmental factors that influence macroinvertebrate assemblage structure are interrelated (Rabeni & Minshall, 1977; Williams & Smith, 1996), thus identifying a single environmental factor as most important in structuring assemblages is challenging. Findings from studies that addressed the relative importance of environmental factors in structuring assemblages suggest that inorganic substrate characteristics such as composition, complexity, and heterogeneity, primarily influence assemblage structure at the sample scale or smaller scales of study (Reice, 1980; Downes et al., 1995). For example, Evans & Norris (1997) found that the length, height, and area of rocks, and water velocity were more important than detritus and periphyton in influencing macroinvertebrate distributions and abundance at the sample scale.

Understanding the relative importance of environmental factors in structuring assemblages is important because benthic macroinvertebrates are used more than any other organisms to assess the condition of streams (Carter & Resh, 2001) and identifying the factor(s) that is primarily associated with altered macroinvertebrate assemblages is necessary before restoration action can begin. For example, currently in the U.S., the Total Maximum Daily Load (TMDL) Program is being used to restore the condition of the nation's water bodies (US EPA, 1997). According to the National Research Council (2001), the TMDL program will not have a sound scientific basis unless the links between environmental stressors and biological responses are quantified and modeled. Although numerous research studies have related benthic macroinvertebrates to stressors over various spatial scales (Lenat & Crawford, 1994; Zweig & Rabeni, 2001; Clements et al., 2002; Morse et al., 2003; Stone et al., 2005), research has not progressed to the point where regional-specific models are available to accurately predict macroinvertebrate responses to human-induced changes to streams.

Streams affected by multiple stressors from human activities offer an experimental design to investigate the role of environmental factors in structuring assemblages at the sample scale. Livestock agriculture, such as cattle grazing, is a particular type of agricultural land use that causes multiple changes to stream environments. Trampled stream banks cause increased erosion and sedimentation, while nutrient and organic loads increase from cattle urine and feces. Because of reduced trees and shrubs in the riparian zone, sunlight and water temperature increase while inputs of coarse particulate organic matter decrease (Kauffman & Krueger, 1984; Fleischner, 1994; Trimble & Mendel, 1995). These cattle-induced environmental changes degrade water quality and habitat, which in turn alter the resident benthic macroinvertebrate fauna (Dance & Hynes, 1980; Wohl & Carline, 1996; Delong & Brusven, 1998; Strand & Merritt, 1999).

In the Blue Ridge Mountains, cattle are commonly raised in pastures where there are extensive lengths of first and second order streams. Cattle use these small streams year-round as a source of drinking water and during warm months as a place to cool themselves. In a previous study, we identified a strong relationship between the intensity of cattle grazing (cattle ha^{-1}) and macroinvertebrate assemblages in small, Blue Ridge streams (Fig. 1). Although macroinvertebrate assemblages had a good relationship with cattle density, the in-stream environmental factors associated with alterations to the macroinvertebrate assemblages have not been identified. The purpose of this study was to quantify the relationship of benthic macroinvertebrate assemblages to environmental factors at the sample scale in streams that represented a gradient of ecological condition as a result of different levels of cattle grazing. The research questions that we addressed were: (1) which environmental factors are best related to benthic macroinvertebrate assemblage structure at the sample scale and (2) how much of the variation in benthic macroinvertebrate assemblage structure can be explained by environmental factors measured at the sample scale?

Methods

Study sites and the grazing gradient

All study sites are within the Blue Ridge Interior Plateau ecoregion (Woods et al., 1996), Floyd Co., Virginia, US. The Blue Ridge physiographic province is characterized by deeply dissected

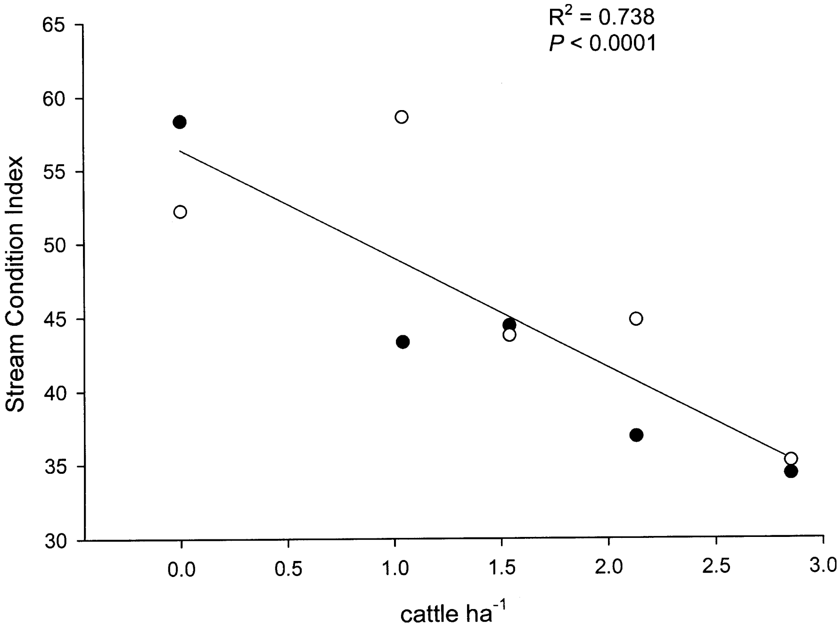

Figure 1. The relationship between Stream Condition Index (SCI) (a macroinvertebrate based multimetric index) (Burton & Gerritsen, 2003) values and cattle density was established with regression analysis. Index values below 61.7 indicate biological impairment. Circles represent SCI values based on macroinvertebrate samples collected from 5 streams that varied in cattle density during spring 2003 (closed circles) and fall 2003 (open circles).

valleys and ravines that are primarily composed of metamorphosed igneous rocks (granites, granodiorite, slates, and green stone) (Hoffman, 1969). Floyd Co. receives an average of 109 cm of precipitation a year, and average air temperatures range from 1.1 °C in January to 21.7 °C in July. Soils in the area consist primarily of clay and sands and are well suited for farming (VASS, 2004). Cattle grazing is a common use of land in the region. Approximately 55% of the total land in Floyd Co. is used for farming, and nearly 30% of farmland is pasture. Beef cattle are an important commodity in the region; 87% of the livestock in Floyd Co. are cattle (VASS, 2004).

Five, first-order stream reaches in the Little River drainage basin, Floyd Co., Virginia were selected as study sites (Fig. 2). Study sites 1, 2, 3, and 5 were on separate streams. Study site 4 was located on the same stream as site 1, about 100 m downstream. These study sites were selected because they were similar in size, gradient, underlying geology, and vegetative cover, but they were subjected to a gradient of cattle grazing (Table 1). Study sites were circumneutral (pH 6.8–7.0), and daytime dissolved oxygen concentrations were never below saturation (9.20–10.27 mg l^{-1}) at any of the study sites. All of the streams originated in forested areas and then flowed into pastures where the sampling reaches were located. The sampling reaches had no woody vegetation in the riparian area, and streambeds consisted mostly of mixes of cobble, pebble, and gravel, except at the heavily grazed sites where patches of sand and silt increased in frequency.

Prior to benthic sampling, reach-scale habitat quality was determined at each study site according to the U.S. Environmental Protection Agency's Rapid Bioassessment Protocol habitat assessment (Table 1, Barbour et al., 1999). Following this methodology, stream reaches receive an overall habitat score based on features that include streambed characteristics, channel morphology, bank structure, and the riparian zone. A stream could receive a habitat score ranging between 200, indicating the optimal condition, and 0, indicating the poorest habitat condition. According to the Virginia Department of Environmental Quality (VA DEQ), reference sites in the part of the state where our study was conducted rarely have habitat scores below 140, and

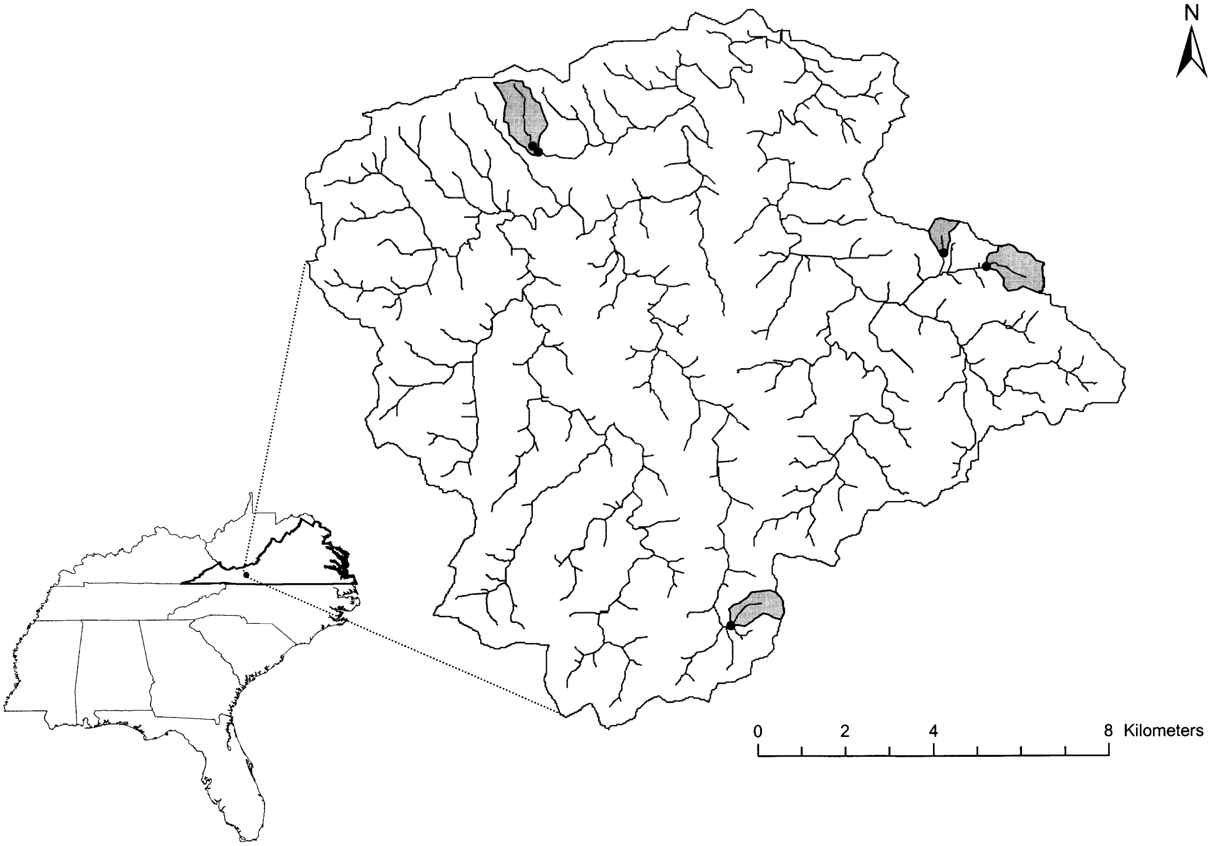

Figure 2. Study site locations in Floyd Co., Virginia.

scores above 120 generally indicate impaired conditions (VA DEQ, 2005).

Site 1 was selected as the reference site because it had not been subjected to cattle grazing for 12–15 years, and surrounding pasture was continuously mowed and managed for hay production. Although site 1 was not pristine, it was a valid reference site for this study because cattle were absent, but the stream lacked forest cover. It was important that the reference site for this study receive sunlight and lack woody vegetation so that it would serve as a valid comparison to streams with cattle grazing, which also lacked woody vegetation. Having an open canopy and no woody vegetation at all sites, including the reference site, ensured that all streams offered the same potential food base for macroinvertebrates. The high habitat score at site 1 (159) also supported the use of this site as a reference.

Cattle were rotationally grazed at site 2 where there were 1.04 cattle ha^{-1} when present. Cattle had continuous stream access at sites 3, 4, and 5, where there were 1.54, 2.13, and 2.85 cattle ha^{-1}, respectively. These study sites, ordered sites 1 through 5, represented the grazing gradient. Based on conversations with state extension agents and private land-owners, all pastures have been in operation for at least 50 years. The stocking densities at sites 2–5 are well within the range of common livestock management practices in Floyd Co., but higher stocking densities are not uncommon.

Benthic sampling

Benthic macroinvertebrate samples were taken in fall 2002, spring 2003, fall 2003, and spring 2004. We used a stratified sampling design and conducted systematic sampling in three areas with swift current at each site. Current velocity within the sampling areas ranged from 0.01 to 0.74 m s^{-1}. Within each sampling area we collected three or four benthic samples that were evenly spaced at

Table 1. Cattle grazing gradient and physical characteristics of study sites in Floyd, Co., Virginia

	Study sites				
	1	2	3	4	5
Grazing/habitat gradient					
Number of cattle ha^{-1}	0	1.04	1.54	2.13	2.85
Grazing category	reference	light rotational	intermediate	heavy	very heavy
Habitat score[a]	159	142	113	116	114
Physical characteristics					
Watershed area (ha)	125	78	109	133	38
Elevation (m a.s.l.)	777	882	755	769	747
Reach slope (%)	3.5	4.3	3.3	3.5	4.1
Discharge (L sec^{-1})[b]					
Minimum	10	8	8	14	2
Maximum	62	47	25	77	10
Average	25	25	15	30	5
Mean wetted width (m)[c]	0.88	0.72	1.11	0.76	0.60
Mean depth (m)[c]	0.08	0.13	0.13	0.09	0.10
Conductivity (μS cm^{-1})[d]	16–22	18–23	54–63	19–22	54–57
Maximum temperature (°C)[d]	18.5	20.0	20.5	21.5	21.5

[a] Habitat scores are averages of three separate assessments that occurred during spring 2003, fall 2003, and spring 2004. Reference sites in Virginia rarely have habitat scores below 140, and scores less than 120 generally indicate impaired conditions (VA DEQ, 2005).
[b] Baseflow discharge was measured on 8 separate occasions between July 2002 and August 2003.
[c] Wetted width and depth are averages of 12 transect measurements taken at each study reach during fall 2002 and spring 2003 ($n = 24$ at each study site).
[d] Conductivity and temperature values are based on 15 spot measures taken throughout the study period.

least 2 m apart, which resulted in the collection of 230 benthic samples for the entire study.

The detailed field and laboratory methods used in this study were slightly modified from those proposed by Cummins (1962) and are more quantitative than rapid methods that are now commonly used in stream bioassessment studies throughout the U.S. (Barbour et al., 1999; Carter & Resh, 2001). Benthic samples were collected by inserting a modified stovepipe sampler (30.48 cm diameter) approximately 10 cm into streambed substrates. Using a fully enclosed sampler (i.e., without a net) facilitated accurate measurements of environmental factors associated with bottom material at the sample scale (epilithic material, benthic organic matter, and inorganic substrates). In addition, this device retained all macroinvertebrates, even the early instars.

Within the sampler, inorganic substrates that were ≥64 mm in size and were located at the substrate-water interface (i.e., surface cobble) were removed, placed in a wash pan with 2 L of water, and scrubbed with wire brushes to remove epilithic material. A 250-ml subsample of the resulting slurry was collected, placed on ice, and transported to the laboratory for chlorophyll *a* and epilithic biomass analyses. Surface cobble were weighed in the field with a portable balance. Following removal of surface cobble, contents within the sampler were agitated and a 250 ml container was used to subsample water within the sampler. Containers with subsamples were packed on ice and transported to the laboratory for fine benthic organic matter (FBOM) analysis. The remaining inorganic substrates and coarse benthic organic matter (CBOM) within the sampler were removed by hand and placed in large sample containers. The rest of the water within the sampler was removed with a hand pump and filtered through a 63 μm sieve. All material retained on the sieve (fine organic and inorganic matter and macroinvertebrates) was added to the sample container. Contents of the sample containers were preserved in 95% ethanol and transported to the laboratory for granular sieve, CBOM, and macroinvertebrate analyses. Mean depth and current velocity were obtained from measurements taken directly adjacent to each benthic sample location. Current was measured at the substrate-water interface with a digital Marsh–McBirney® flow meter.

Laboratory analyses

Benthic macroinvertebrates

In the laboratory, benthic samples were rinsed through a series of stacked sieves (63 μm–16 mm). Organic materials on sieves ≥250 ml were elutriated to separate macroinvertebrates and organic matter from inorganic substrate. All inorganic substrate was set aside and retained for granular sieve analysis. Macroinvertebrates were hand sorted from organic matter under a dissecting microscope, enumerated, and identified to the lowest practical taxonomic level. Most insect taxa were identified to genus; other macroinvertebrate taxa were identified to class, order, or family.

Food resources

Environmental factors that were thought to affect benthic macroinvertebrates primarily as food resources were measured from the samples (see Table 2 for explanations). Organic matter that was hand sorted from benthic macroinvertebrates was rinsed through a 1-mm sieve to obtain CBOM. CBOM was sorted into one of four categories that included wood, deciduous leaves, pasture vegetation, and miscellaneous material that could not be sorted but made up a small proportion of CBOM. All CBOM material was dried to a constant weight and weighed. The subsamples of benthic organic matter were filtered through a 1-mm sieve to obtain FBOM. The filtrate was filtered onto preweighed glass fiber filters (0.45 μm) and dried to a constant weight at 60 °C. After dry weights were obtained, filters were ignited at 550 °C for 24 h, desiccated, and reweighed to obtain ash free dry mass (AFDM). Epilithic subsamples were split and analyzed for chlorophyll *a* and epilithic biomass. The epilithic fraction was filtered onto preweighed glass fiber filters (0.45 μm) and dried to a constant weight at 60 °C. After dry weights were obtained, filters were ignited at 550 °C for 24 h, desiccated, and reweighed to obtain epilithic AFDM. Chlorophyll *a* was extracted with 90% acetone and then analyzed with a spectrophotometer after correcting for pheophytin following the methods of Lorenzen (1967). All food variables were standardized to the surface area covered by the core sampler and converted to m^2 of stream bottom.

Habitat

Environmental factors that were thought to affect macroinvertebrates primarily through habitat suitability were measured from the benthic samples (see Table 2 for explanations). Organic matter and macroinvertebrates were elutriated from sediments and remaining inorganic substrates were separated into standard Wentworth (1922) size classes. Particles ≥8 mm were manually separated into size classes and weighed while particles smaller than 8 mm were dried to a constant weight, separated into standard Wentworth particle size classes with a series of stacked sieves and a sieve shaker (i.e., granular sieve analysis), and weighed. Surface cobble weights that were obtained in the field were combined with weights of particles that were measured in the laboratory and thus provided the complete range of particle sizes in each benthic sample. Weights of each sediment size class were used to calculate particle percentiles, sediment size class proportions, and measures of sorting and skewness.

Data analyses

Measurements of benthic environmental factors were treated as predictors of the macroinvertebrate assemblage throughout our analyses. We used canonical correspondence analysis (CCA) in PCORD to determine if there were relationships between the environmental variables and taxa abundance data and to identify environmental variables that were important in structuring the macroinvertebrate assemblage (McCune & Mefford, 1999). CCA is a constrained ordination method where axes are created through linear combinations of environmental variables, which makes it a useful method for detecting environmental variables that ‘best’ explain variation in species data (ter Braak, 1995). Prior to CCA, rare taxa (those that comprised less than 0.2% of the total assemblage abundance) were removed to

Table 2. Explanations of environmental factors that were measured from within each benthic sample

Environmental variables	Units	Description
Food resources		
FBOM	g AFDM m^{-2}	Deposited benthic organic matter < 1 mm obtained from benthic water subsamples from within each benthic sample.
CBOM	g DM m^{-2}	Deposited benthic organic matter ≥ 1 mm from within each benthic sample.
% pasture vegetation	%	Proportion of CBOM (based on dry weight) composed of decomposing pasture vegetation.
% wood	%	Proportion of CBOM (based on dry weight) composed of decomposing wood.
% deciduous leaf	%	Proportion of CBOM (based on dry weight) composed of decomposing deciduous leaves.
Chlorophyll *a*	mg m^{-2}	Chlorophyll *a* extracted from epilithic material that was collected from surface cobble within each benthic sample.
Epilithic biomass	mg AFDM m^{-2}	Epilithic material extracted from surface cobble within each benthic sample.
Physical habitat		
Flow	m sec^{-1}	Average flow ($n = 3$) at the substrate-water interface of the sample location.
Depth	cm	Average water depth ($n = 3$) at the sample location.
% cobble	%	Proportion (by weight) of substrate sized < 256, ≥ 64 mm within each benthic sample.
% pebble	%	Proportion (by weight) of substrate sized < 64, ≥ 16 mm within each benthic sample.
% gravel	%	Proportion (by weight) of substrate sized < 16, ≥ 2 mm within each benthic sample.
% fines	%	Proportion (by weight) of substrate sized < 2 mm within each benthic sample.
D_{50}	none	Median particle size determined from substrate size class weights obtained through granular sieve analysis.
Fredle index	none	Geometric skewness as the ratio of geometric mean to geometric sorting (Lotspeich & Everest, 1981). $\left(\frac{D_{84} * D_{16}}{\frac{D_{75}}{D_{25}}}\right)^{0.5}$.
Trask's sorting coefficient	none	Substrate size homogeneity within each benthic sample (heterogeneity > 1) (Inman, 1952). $(D_{84}/D_{16})^{-0.5}$.
Surface cobble to subsurface cobble ratio	none	Ratio of surface cobble to subsurface cobble within each benthic sample.

reduce noise in the data set (Gauch, 1982). To avoid the possibility of collinearity, environmental variables were grouped into one of two categories of resources, either food or habitat. Pearson product–moment correlations were performed among variables in each category. If correlation analyses indicated variables were significantly and highly correlated ($p < 0.05$, $r > 0.7$), the redundant variables were removed from analyses. After removing rare taxa and redundancy among environmental variables, 17 environmental variables (Table 2) and 60 taxa were used in CCA. Monte–Carlo procedures, using 200 permutations, were used to test the statistical significance of the first three canonical axes. An axis was not interpreted if it was not statistically significant ($p > 0.05$). Eigenvalues of statistically significant axes were summed to determine the amount of variation in the macroinvertebrate data that was explained by environmental variables. Intraset correlations (ter Braak, 1995) were used to interpret axes and identify variables that were most influential in structuring the macroinvertebrate assemblage.

Following CCA, macroinvertebrate abundance data were condensed into metrics. For metric calculation, each taxon was assigned a pollution tolerance value (PTV), functional feeding group (mode of acquiring food based on morphology and behavior), and habit (how the organism moves or maintains its position in its environment; also called mode of existence). Assignments to these categories were made based on a synthesis of published literature (e.g., Brigham et al., 1982; Barbour et al., 1999) and 30 years of data and professional experience in the aquatic entomology program at Virginia Tech. PTVs are commonly reported on a scale of 0–10, with 0 indicating very tolerant. In this study, taxa with PTVs of 0–2 were considered sensitive while taxa with PTVs of 8–10 were considered tolerant.

Prior to statistical analyses, 27 macroinvertebrate metrics that have been shown to respond to anthropogenic disturbance were selected as candidates for data analysis (Barbour et al., 1999). Candidate metrics were placed into one of the following five categories: taxa richness, community balance, trophic status, pollution tolerance, and habit. To reduce metric redundancy, Pearson product–moment correlations were performed among metrics in each category. If correlation analyses indicated metrics were significantly and highly correlated ($p < 0.05$, $r > 0.7$), the redundant metrics were removed from the list of candidate metrics unless no metrics were left in a category. In that case, a few of the least correlated and most ecologically meaningful metrics were retained for further statistical analyses. Following correlation analyses, 13 'test' metrics were retained for statistical analyses.

Regression analysis was used to determine if there were significant relationships between benthic macroinvertebrate metrics and the benthic habitat variables that were indicated as important in CCA. Important variables were defined as those with intraset correlations ≥ 0.5. With the exception of taxa richness and total number of sensitive taxa, macroinvertebrate metrics and environmental variables were either arc sin or $\log_{10}(x + 1)$ transformed to meet the equal variance assumption of normality. Linear relationships between environmental variables and metrics were assessed first, and then a quadratic model was selected if the quadratic term differed significantly from zero and the coefficient of determination indicated a better fit, i.e., increased coefficient of determination. Individual regression tests were performed for each metric in every sampling period resulting in 117 individual tests per sampling period. Multiple individual statistical tests may increase the likelihood of Type I error so Bonferroni adjustments were made to control for procedure wise error by adjusting the test significance level (α) by the number of repeated tests. Thus, results were considered significant only if p values were less than the adjusted alpha ($\alpha = 0.0004$).

Results

We first used an exploratory approach, with multivariate statistics, to identify the environmental variables that were most important in structuring the assemblage. Following exploratory analysis, inferential statistics were used to test the relationships between macroinvertebrate metrics and environmental variables. Preliminary analyses showed only a few patterns between the benthic macroinvertebrate assemblage and environmental variables when all sampling periods were combined for analysis. After analyzing seasons separately, patterns became clear.

Fall sampling periods

For fall 2002 data, the first three axes generated by CCA explained approximately 22% of the taxa–

environment relationship. Axis 1 explained most of the variation with an eigenvalue of 12.6 (Table 3). Percent fines had the highest correlation with axis 1 followed by FBOM and Trask's sorting coefficient. Because these were positive correlations, axis 1 was interpreted as an environmental gradient of increasing % fines, FBOM, and substrate homogeneity (Table 4, Fig. 3a). Taxa with high negative scores on the first CCA axis during fall 2002 included limpets, (Ancylidae), stoneflies (*Tallaperla*, *Isoperla*, *Suwallia*, *Leuctra*, *Allocapnia*), the mayfly, *Paraleptophlebia*, caddisflies (*Diplectrona*, *Rhyacophila*, *Wormaldia*, *Psilotreta*, *Lepidostoma*, *Glossosoma*), beetles (*Ectopria*, *Anchytarsus*) and two fly taxa (*Antocha*, *Dicranota*). Fingernail clams (Sphaeriidae) and flies (*Psychoda*, *Pericoma*, *Simulium*, *Ephydridae*, *Hemerodromia*, *Limnophora*) had high positive scores on axis 1 (Fig. 3a). No benthic habitat variables had high correlations with axis 2, and only depth was correlated with axis 3 (Table 4).

The fall 2003 ordination showed the strongest taxa–environment relationship of all sampling periods; 25% of the taxa–environment relationship

Table 3. Summary of CCA results for fall sampling periods for the abundance of macroinvertebrate taxa and 17 environmental variables. All axes were significant following Monte–Carlo permutation procedures

	Fall 2002				Fall 2003			
	Axis 1	Axis 2	Axis 3	Total variance	Axis 1	Axis 2	Axis 3	Total variance
				1.3930				0.7926
Eigenvalue	0.176	0.076	0.058		0.072	0.068	0.055	
%variance explained in taxa data	12.6	5.5	4.2		9.0	8.6	6.9	
Cumulative %variance explained	12.6	18.1	22.3		9.0	17.7	24.5	
p value	0.0050	0.0400	0.0050		0.0100	0.0050	0.0050	

Table 4. Intraset correlation coefficients between environmental variables and axes derived from CCA for fall sampling periods

	Fall 2002			Fall 2003		
Variable	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3
Flow	0.101	−0.412	0.022	0.054	−0.182	0.105
Depth	0.404	0.027	**0.559**	−0.014	−0.090	**−0.743**
FBOM	**0.712**	0.438	0.104	0.191	0.292	−0.020
CBOM	0.489	−0.101	0.260	−0.116	−0.179	0.480
% wood	−0.351	−0.308	0.523	**−0.762**	0.148	−0.021
% leaf	−0.093	−0.219	0.135	−0.417	−0.339	−0.160
% pasture vegetation	0.369	0.253	−0.535	**0.666**	−0.015	0.081
Chlorophyll *a*	0.143	−0.315	0.076	0.090	0.263	0.151
Epilithic biomass	0.014	−0.130	0.110	0.208	−0.175	0.131
D_{50}	0.260	−0.062	0.468	−0.012	−0.182	−0.112
Fredle index	0.081	−0.349	0.381	0.056	−0.365	−0.030
Trask's sorting coefficient	**0.575**	0.168	−0.241	−0.238	**0.706**	0.061
Surface to subsurface cobble ratio	0.201	−0.050	0.325	−0.207	−0.253	0.050
% gravel	0.151	0.384	−0.534	0.362	0.204	−0.154
% pebble	0.123	0.391	−0.538	−0.029	−0.128	0.308
% cobble	0.315	−0.211	0.487	−0.166	−0.229	−0.078
% fines	**0.930**	−0.111	−0.109	−0.146	**0.649**	−0.169

Values in bold were considered important in structuring the macroinvertebrate assemblage.

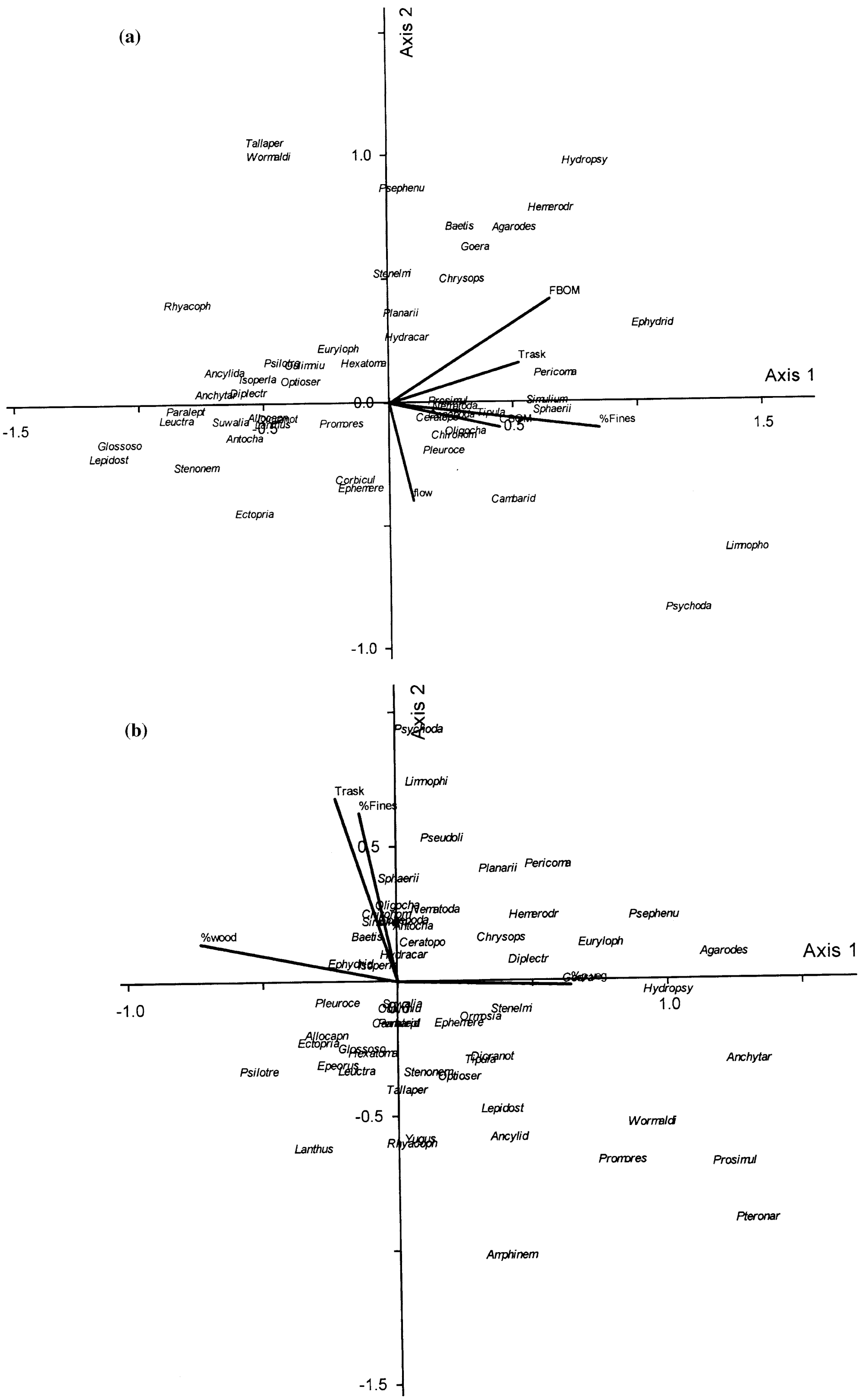

Figure 3. Results from CCA for the (a) fall 2002 sampling period and (b) fall 2003 sampling period.

was explained (Table 3). Axes 1 and 2 explained nearly the same amount of variation, so benthic habitat variables that were correlated with these axes were considered equally important in structuring the assemblage. Axis 1 explained approximately 9.0% of the taxa–environment relationship. Percent wood had a high negative correlation while % pasture vegetation had a high positive correlation with axis 1. Axis 1 was interpreted as an increasing gradient of CBOM composed of pasture vegetation but less wood (Table 4). Taxa with high positive scores on axis 1 included *Pteronarcys*, *Eurylophella*, *Hydropsyche*, *Wormaldia*, *Goera*, *Agarodes*, *Psephenus*, *Promoresia*, *Anchytarsus*, and *Prosimulium* (Fig. 3b). Few taxa had high negative scores with axis 1. Axis 2 explained 8.6% of the variance in taxa–environment relations (Table 3). Trask's sorting coefficient and % fines were positively correlated with axis 2, so this axis was interpreted as a gradient of increasing inorganic fines and homogenized substrate (Table 3). Taxa with strong negative scores on axis 2 included Ancylidae, *Pteronarcys*, *Yugus*, *Lanthus*, *Rhyacophila*, *Wormaldia*, *Lepidostoma*, *Promoresia*, and *Prosimulium*. The fly taxa, *Psychoda* and *Limnophora*, had high positive scores with axis 2 (Fig. 3b).

Spring sampling periods

The overall amount of variation in the taxa–environment relationships explained during the spring sampling periods was never as great as the amount of variation explained during the fall sampling periods (Table 5). Furthermore, intraset correlations between environmental variables and axes were rarely greater than 0.50. During spring 2003, approximately 18% of the taxa–environment relationship was explained by the first 3 axes (Table 5). Axis 1 and 2 explained most of the variation with eigenvalues of 7.6 and 5.2, respectively. FBOM was the only benthic habitat variable with a strong correlation with axis 1 so this axis was interpreted as an increasing gradient of FBOM (Table 6). Taxa with high positive scores on axis 1 included non-insect taxa (Planariidae, *Corbicula*, *Gammarus*), *Eurylophella*, *Hydropsyche*, *Goera*, *Agarodes*, *Pseudolimnophila*, *Ormosia*, and *Hemerodromia*. Taxa with high negative scores on axis 1 were *Epeorus*, *Lanthus*, *Rhyacophila*, *Psilotreta*, *Ectopria*, and *Dicranota* (Fig. 4a). Axis 2, of spring 2003, was interpreted as a gradient of increasing CBOM with a lower proportion of pebble sized particles (Table 6). Taxa with high positive scores on axis 2 were *Stenonema*, *Hydropsyche*, *Lepidostoma*, *Promoresia*, *Tipula*, and *Limnophora*. Cambaridae was the only taxon with a high negative score on axis 2 (Fig. 4a).

The least amount of variation explained by CCA for a sampling period was for spring 2004. Axes 1 and 2 combined explained just 14% of the taxa–environment relationship (Table 5) in that season. Only the first two axes were significant, and each explained approximately 7% of the variation. Axis 1 was interpreted as a gradient of increasing fine sediments (Table 6). Taxa with high positive scores on axis 1 were Ancylidae, *Gammarus*, *Eurylophella*, *Hydropsyche*, *Psephenus*, *Pseudolimnophila*, *Ormosia*, and *Chrysops*. *Pteronarcys*, *Lanthus*, *Wormaldia*, *Lepidostoma*, *Ectopria*, *Stenelmis*, *Anchytarsus*, *Dicranota*, and *Prosimulium* had high negative scores with axis 1

Table 5. Summary of CCA results for spring sampling periods for the abundance of macroinvertebrate taxa and 17 environmental variables

	Spring 2003				Spring 2004			
	Axis 1	Axis 2	Axis 3	Total variance	Axis 1	Axis 2	Axis 3^{NS}	Total variance
				1.2438				1.0155
Eigenvalue	0.094	0.064	0.059		0.071	0.071	0.040	
%variance explained in taxa data	7.6	5.2	4.8		7.0	7.0	3.9	
Cumulative %variance explained	7.6	12.8	17.5		7.0	14.0	17.9	
P value	0.0050	0.0450	0.0050		0.0150	0.0050	0.0600	

The superscript, NS, indicates axes were not significant following Monte Carlo permutation procedures.

Table 6. Intraset correlation coefficients between environmental variables and axes derived from CCA for spring sampling periods

	Spring 2003			Spring 2004		
Variable	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3^{NS}
Flow	0.021	0.046	**0.743**	−0.152	0.259	−0.157
Depth	−0.292	0.462	0.088	−0.055	−0.016	0.843
FBOM	**0.550**	0.047	0.072	0.406	**0.545**	−0.063
CBOM	−0.065	**0.515**	−0.350	−0.208	**0.573**	0.042
% wood	−0.344	−0.192	0.254	−0.216	−0.221	−0.113
% leaf	−0.224	−0.333	−0.171	−0.382	−0.022	−0.157
% pasture vegetation	0.174	0.268	−0.106	0.034	0.290	−0.001
Chlorophyll *a*	0.214	0.284	−0.124	0.066	0.059	0.583
Epilithic biomass	0.234	0.447	−0.274	−0.136	0.071	0.349
D_{50}	0.358	0.334	0.165	0.021	−0.334	0.404
Fredle index	0.014	0.294	0.101	−0.327	−0.098	0.595
Trask's sorting coefficient	0.403	−0.063	0.100	0.384	0.227	−0.304
Surface to subsurface cobble ratio	−0.121	0.222	0.177	−0.150	−0.100	0.290
% gravel	0.285	−0.090	−0.036	0.188	0.309	−0.398
% pebble	−0.493	**−0.597**	−0.158	−0.182	−0.049	−0.427
% cobble	0.051	0.435	0.182	−0.090	−0.145	0.476
% fines	0.492	0.154	−0.088	**0.518**	−0.009	−0.065

Values in bold were considered important in structuring the macroinvertebrate assemblage. The superscript, NS, indicates axes were not significant following Monte Carlo permutation procedures.

(Fig. 4b). Axis 2 was interpreted as a gradient of increasing FBOM and CBOM (Table 6). Taxa with high positive scores on this axis were *Corbicula*, *Pteronarcys*, *Ephemera*, *Lanthus*, *Hydropsyche*, *Lepidostoma*, *Agarodes*, *Psephenus*, *Anchytarsus*, *Pseudolimnophila*, *Ormosia*, *Prosimulium*, and *Chrysops*. Taxa with high negative scores on axis 2 were Ancylidae, *Gammarus*, *Allocapnia*, *Stenonema*, and *Rhyacophila* (Fig. 4b).

Macroinvertebrate metrics

From the exploratory analysis with CCA, nine environmental variables (flow, depth, FBOM, CBOM, % pebble, % wood, % pasture vegetation, Trask's sorting coefficient, % fines) were considered most likely to determine the structure of the macroinvertebrate assemblage (i.e., intraset correlations ≥0.5) and were selected as worthy of further analysis. However, only four of the nine environmental variables (FBOM, CBOM, Trask's sorting coefficient, % fines) showed statistically significant relationships with macroinvertebrate metrics following Bonferroni adjustments (Tables 7 and 8). Six metrics (% Plecoptera, % Coleoptera, % collector-filterer, % sensitive taxa, number of sensitive taxa, % crawler) showed significant relationships with environmental variables only during the fall, while four metrics (richness, Simpson's diversity, % collector-gatherers, number of sensitive taxa) showed significant relationships with environmental variables only during spring. Four metrics (% scrapers, % shredders, % clingers, % burrowers) never had a significant relationship with any of the environmental variables during any sampling period.

Two habitat variables (Trask's sorting coefficient, % fines) had significant relationships with metrics during fall sampling periods, but not during spring sampling periods. Six significant relationships occurred during the spring sampling periods (Table 8), but the only significant metric relationships were with either FBOM or CBOM. Total taxa richness had a significant relationship with CBOM during both spring sampling periods. The strongest relationship observed during the spring occurred during spring 2003 when CBOM explained 39% of the variation in total taxa rich-

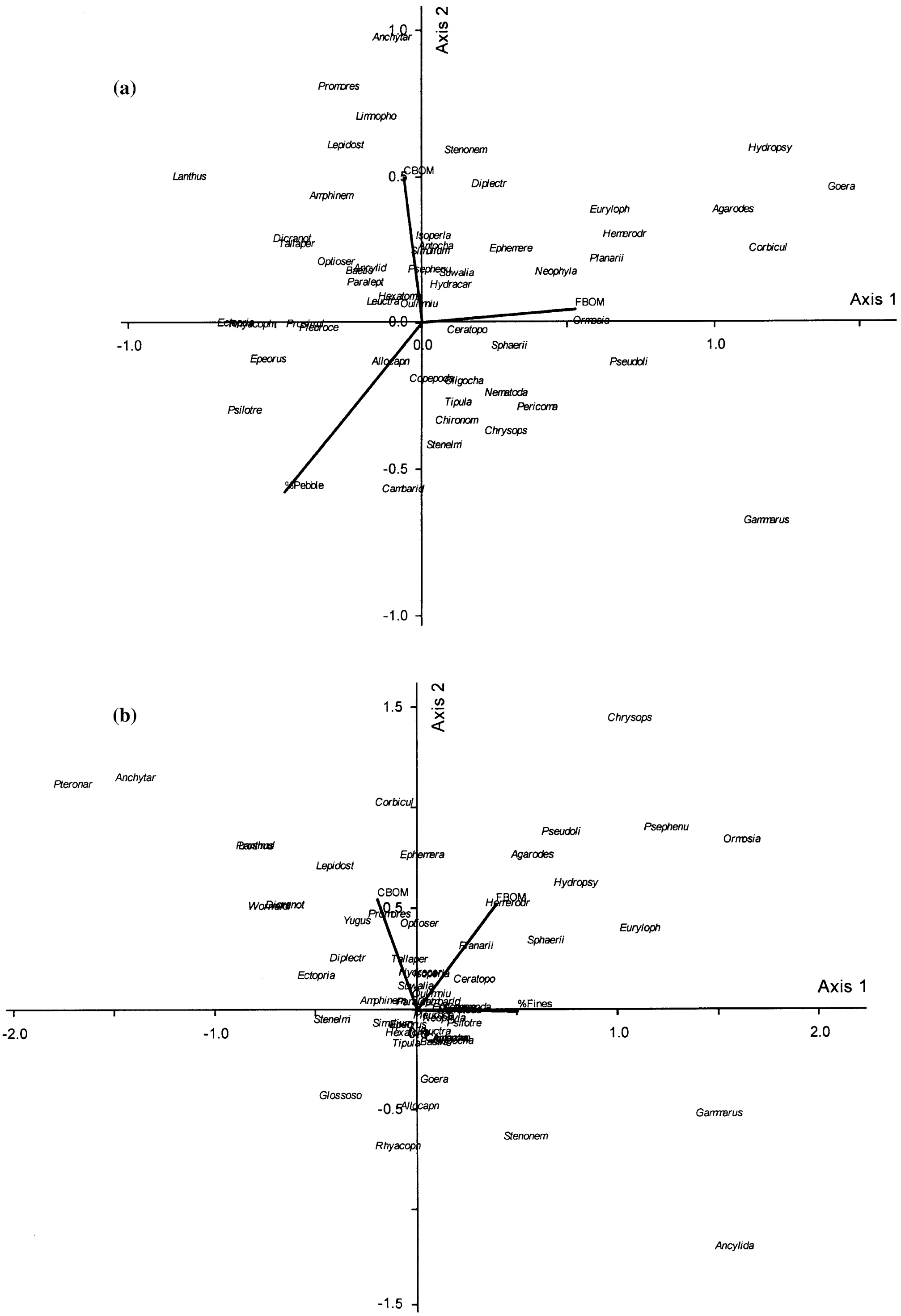

Figure 4. Results from CCA for the (a) spring 2003 sampling period and (b) spring 2004 sampling periods.

Table 7. Results from regression analysis for macroinvertebrate metrics vs. environmental variables during fall sampling periods (F02 = fall 2003, F03 = fall 2003)

		% Plecoptera	% Coleoptera	% collector-filterer	% sensitive taxa	Number of sensitive taxa	% crawler
FBOM	F02			+0.3386			
Trask's	F02		−0.2398				
	F03		−0.2289		−0.2325		−0.2802
% fines	F02	−0.3217	−0.5095	+0.2457			−0.2959
	F03		−0.2487		−0.2641	−0.2662	−0.2756

Values are coefficients of determination and signs in front of values indicate the direction of the relationship. $n = 59$ for fall 2002 and $n = 56$ for fall 2003. Only relationships that were significant following Bonferroni adjustments ($p < 0.0004$) are shown.

Table 8. Results from regression analysis for macroinvertebrate metrics vs. environmental variables during spring sampling periods (S03 = spring 2003, S04 = spring 2004)

		Richness	Simpson's diversity	% collector-gatherer	Number of sensitive taxa
FBOM	S04	+0.1827			+0.2860
	S03	+0.3922	+0.2616	−0.3067	
CBOM	S04	q 0.2513			

Values are coefficients of determination and signs in front of values indicate the direction of the relationship. (q = quadratic) $n = 57$ for spring 2003 and $n = 58$ for spring 2004. Only relationships that were significant following Bonferroni adjustments ($p < 0.0004$) are shown.

ness. Overall, the strength of relationships between metrics and habitat variables during fall sampling periods were always stronger than the relationships between metrics and organic matter.

Discussion

Using a study design that involved a gradient of increasing stress (increased cattle density) allowed us to quantify and rank the influence of environmental factors on macroinvertebrate assemblages in streams impacted by cattle grazing. Based on results of CCA and regression analysis, it appears that at the sample scale of measure, sedimentation (as measured by % fines and Trask's sorting coefficient) is the primary stressor to benthic macroinvertebrate assemblages in these small, Blue Ridge streams. These results are not surprising; streams that support agriculture typically have higher sediment loads compared to relatively undisturbed streams (Lenat & Crawford, 1994; Owens et al., 1996; Wohl & Carline, 1996); excessive sediment loads deteriorate benthic habitat and thus alter biological assemblages, especially macroinvertebrates (Chutter, 1969; Lenat et al., 1981; Waters, 1995; Wood & Armitage, 1997).

Our results are similar to results from other studies that have related fine sediments to macroinvertebrate metrics. We found two published studies that occurred within our geographic region and related macroinvertebrate metrics to fine sediment with correlation (r) or regression (r^2) analyses. Lemley (1982) documented good relationships between % fines and taxa richness ($r = 0.79$), diversity ($r = 0.84$), and benthic invertebrate standing stock biomass ($r = 0.74$) in a human impacted reach of an Appalachian trout stream. Angradi (1999) conducted a detailed field experiment in forested mountain streams of West Virginia, where the range of metric variation explained by % fines from his experimental tray study was $r^2 = 0.10$–0.27. In our study, the amount of variation explained in metrics by % fines ($r^2 = 0.25$–0.51) is within the range of metric variation explained by % fines in Lemley (1982) and Angradi (1999). Taxa richness was the only metric in common between these two studies and ours. Although Lemley (1982) found a good relationship between taxa richness and % fines, there was no significant relationship detected between this metric and % fines in our study or Angradi (1999).

In a different geographic region (Missouri Ozark streams), Zweig & Rabeni (2001) found many good relationships (r = 0.53–0.91) between macroinvertebrate metrics and deposited sediment, including taxa richness (r = 0.53–0.73). They suggested the stronger relationships detected in their study, relative to Angradi (1999), may be a function of the naturally high deposited sediment levels in Missouri streams. But also, the range of % fines in the Appalachian studies were limited (0–30% in Angradi's experimental trays, 5–46% in Lemley's study, and 0–70% in our study) compared to the range of % fines in Zweig & Rabeni (2001) (0–100%). If our data set had included benthic samples in which the proportion of fines was 100%, which do occur in streams subjected to exceptionally high cattle densities, stronger relationships would likely have been detected.

The macroinvertebrate metrics % Coleoptera and % crawlers were not analyzed in any of the studies discussed above. In our study, the significant and consistent responses of % Coleoptera and % crawlers with % fines during fall sampling periods suggests these may be useful metrics for biologically assessing the ecological condition of streams when sedimentation is a suspected stressor. Several crawler taxa that were encountered during this study declined in samples with increased % fines. For example, larvae of the elmid beetle, *Oulimnius*, showed a particularly strong, negative response to % fines (regression analysis, $r^2 = -0.4139$, $p < 0.0001$). Elmids occur in shallow, fast flowing riffles where they cling to substrate and feed by scraping hard surfaces for algae and detritus (Brown, 1987). Spaces between clean substrate are essential habitat for crawlers, such as *Oulimnius*, because the different sizes and depths of the spaces provide a continuum of current velocity, refuge from predators, and a repository for detrital food that would otherwise have been washed downstream. The decline of *Oulimnius* with increased fine sediments is likely the result of habitat elimination.

While measures of the physical nature of habitat (% fines and Trask's sorting coefficient) were highly related to macroinvertebrate assemblage structure, these relationships were only detected during fall sampling periods. Further analyses of environmental variables and the cattle-grazing gradient provided explanations for these important seasonal patterns in environmental factors. Follow-up analyses (ANOVA with Tukey's *post hoc* tests) showed that % fines and substrate homogeneity, i.e., Trask's sorting coefficient, increased significantly along the gradient during fall but did not show a clear pattern with the gradient during spring (Table 9). Higher flows during spring likely keep larger substrate and interstitial spaces free from fine sediments (Waters, 1995). During fall, when baseflow is low relative to spring, fine sediments settle on stream bottoms, and interstitial spaces become clogged, thus eliminating habitat for crawler taxa.

The variation in macroinvertebrate assemblages that could be explained during spring sampling periods was related to decomposing plant matter (CBOM, FBOM). It is important to note that relationships between the macroinvertebrate assemblage and organic matter during spring sampling periods were never as strong as relationships with the habitat variables detected during fall. Although all of our study sites lacked tall tree cover, pasture vegetation was abundant in riparian zones, and this material is a significant source of detritus that can influence the assemblage structure of benthic macroinvertebrates. Based on further analyses, the most heavily grazed sites (sites 4 and 5) had significantly more CBOM during the fall, but there was only a slight difference in CBOM among sites during spring (Table 9). The significant relationships between CBOM and compositional metrics (richness and Simpson's diversity) detected during spring may be a function of macroinvertebrate life histories. Most stream insects are cold adapted and exist as actively growing immatures from late fall to mid-spring thus, spring samples often have more taxa that are of larger size. Furthermore, CBOM may serve as habitat during high flows in the spring.

It is noteworthy that autochthonous forms of plant matter (attached algae as indicated by chlorophyll *a* and epilithic biomass) showed no significant relationships with macroinvertebrate assemblage structure during any sampling period. A significant difference in epilithic biomass was detected among our study sites during fall sampling periods, but the differences were not related to the cattle grazing gradient (Table 9). All study

Table 9. Summary of environmental factors (mean ± 1SE) at each study site during spring and fall sampling periods

	Study sites				
	1	2	3	4	5
Cattle ha^{-1}	0.0	1.04	1.54	2.13	2.85
Spring sampling periods	$n=22$	$n=24$	$n=23$	$n=23$	$n=23$
% fines*	9.3 (2.0) *b*	9.9 (1.2) *ab*	16.0 (2.1) *a*	10.5 (1.7) *ab*	9.1 (1.2) *ab*
Trask's sorting coefficient**	2.4 (0.1) *a*	3.1 (0.2) *ab*	3.9 (0.4) *b*	2.8 (0.3) *a*	3.1 (0.2) *ab*
CBOM (g DM m^{-2})*	62.4 (17.5) *a*	61.0 (12.2) *a*	59.9 (10.0) *a*	68.8 (23.8) *a*	34.6 (10.4) *b*
FBOM (g AFDM m^{-2})***	26.3 (3.8) *a*	31.4 (4.2) *a*	69.0 (10.4) *b*	20.3 (2.7) *a*	36.3 (8.7) *a*
Chlorophyll *a* (mg m^{-2})^{NS}	142.9 (55.9)	123.7 (17.1)	288.5 (60.2)	86.1 (18.2)	84.0 (11.7)
Epilithic biomass (mg AFDM m^{-2})^{NS}	6485.8 (2974.9)	5601.9 (819.7)	11192.9 (3657.5)	2543.4 (410.8)	2624.7 (422.8)
Fall sampling periods	$n=23$	$n=24$	$n=22$	$n=22$	$n=24$
% fines***	12.7 (2.0) *a*	10.3 (0.8) *a*	22.9 (2.6) *b*	30.5 (3.5) *b*	31.1 (2.6) *b*
Trask's sorting coefficient***	3.1 (0.3) *a*	3.1 (0.2) *a*	5.5 (0.9) *cb*	4.8 (0.7) *ab*	7.5 (0.7) *c*
CBOM (g DM m^{-2})*	77.4 (17.3) *a*	69.3 (12.1) *a*	88.2 (27.3) *ab*	127.2 (18.4) *b*	124.2 (23.5) *b*
FBOM (g AFDM m^{-2})***	50.7 (7.1) *a*	89.5 (11.3) *ab*	235.5 (43.1) *c*	162.7 (28.8)*cb*	134.2 (22.8) *cb*
Chlorophyll *a* (mg m^{-2})^{NS}	62.3 (16.4)	134.7 (50.1)	101.5 (19.2)	127.8 (35.3)	216.8 (49.9)
Epilithic biomass (mg AFDM m^{-2})*	2972.5 (405.6) *b*	7769.8 (2079.5) *ab*	5524.6 (1110.2) *a*	6459.8 (2482.9) *ab*	5284.2 (1505.8) *ab*

Values with different letters indicate significant mean differences following Tukey–Kramer *post hoc* tests. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, NS = $p > 0.05$.

sites were exposed to sunlight because there was no riparian woody vegetation to provide shade, so it is unlikely that differences in epilithic biomass and chlorophyll *a* influenced assemblage structure, especially for taxa that cling to stable substrate and scrape epilithic material for food. Several sensitive taxa, including rare ones, did not respond to the grazing gradient and occurred at the most heavily grazed sites, including *Glossosoma nigrior*, *Goera*, *Neophylax*, and *Blepharicera*. These taxa are clingers that are morphologically or behaviorally adapted to exist on the surface of clean, stable substrate in swift water; they are associated with the exposed surfaces of stable rocks and have rarely been reported from the undersides of substrates (Scott, 1958; Kovalak, 1976; Frutiger, 2002). Thus, fine sediments deposited around and beneath stable stones may not be a stressor to these taxa. For instance, the net-winged midge, *Blepharicera*, maintains its position in swift, shallow current by clinging to clean, stable substrate by means of a row of suction discs on its ventral side. The caddisflies, *Neophylax*, and *Goera*, are able to exist on the current-exposed side of stable rocks with the aid of heavy portable cases formed from rock fragments. The occurrence of these taxa at the most heavily grazed study sites suggests that elevated nutrients that are often associated with cattle grazing did not alter autochthonous food resources or habitat to the point of stressing the macroinvertebrate assemblage in these small streams.

Our results demonstrate that macroinvertebrate assemblages can be explained by the environmental variables quantified at the sample scale in these small streams. Given the many interacting environmental variables that influence the spatial distribution of macroinvertebrates at this scale of study and the limited range of % fines (0–70%) in our study, it is understandable that measures of individual environmental variables rarely explained more than 25–30% of the variance in taxa composition or metrics. Results from studies that have used CCA to relate macroinvertebrate assemblages to environmental factors have reported greater amounts of variation explained (40–60%) in the macroinvertebrate assemblages by environmental factors, but these studies were always conducted over larger scales of study (i.e., stream reaches, watersheds) (Richards et al., 1993; Griffith et al., 2001; Riva-Murray et al., 2002). We found CCA to be a very useful exploratory tool because (1) the variables most important in structuring the macroinvertebrate data set were extracted, and (2) it allowed us to relate all environmental variables to changes in taxa composition (taxa counts) simultaneously. A multivariate approach, in conjunction with a univariate-metric approach, provided two lines of evidence that % fines was the most highly related variable to the macroinvertebrate assemblage.

Assessing the relative amount of variation in metrics that could be explained by each environmental variable allowed us to draw ecologically meaningful conclusions and identify stressors to the macroinvertebrate assemblage in these cattle-impacted streams. Differences in the relative importance of benthic environmental factors that structured assemblages between spring and fall sampling periods demonstrates the temporally dynamic nature of the environmental factors that influence benthic macroinvertebrate assemblages. Thus, to ensure a full understanding of the variables that influence assemblages, sampling should occur over time, and at a minimum include seasons with normally high and low base flows. Although measurements over larger spatial scales have also shown relationships between environmental factors and benthic macroinvertebrate assemblages, using an enclosed sampler to concurrently collect benthic macroinvertebrates and measure environmental factors at the sample scale provides additional meaningful insights into the explanations for the ecological condition of streams.

Acknowledgements

We thank all of the private landowners for their hospitality, and we are especially grateful to Kathy Hanna, Stephen Hiner, Brian Jackson, Trisha Voshell, Rachel Wade, and Hillery Warner for their assistance in the field and laboratory. The senior author was supported by a U.S. Department of Agriculture, Food and Agricultural Sciences National Needs Graduate Fellowship. The Department of Entomology and the Virginia Agricultural Experiment Station at Virginia Tech provided further support.

References

Angradi, T. R., 1999. Fine sediment and macroinvertebrate assemblages in Appalachian streams: a field experiment with biomonitoring applications. Journal of the North American Benthological Society 18(1): 49–66.

Barbour, M. T., J. Gerritsen, B. D. Snyder & J. B. Stribling, 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish. Office of Water, U.S. Environmental Protection Agency, Washington DC.

Bouckaert, F. W. & J. Davis, 1998. Microflow regimes and the distribution of macroinvertebrates around stream boulders. Freshwater Biology 40(1): 77–86.

Brigham, A. R., W. U. Brigham & A. Gnilka, 1982. Aquatic Insects and Oligochaetes of North and South Carolina. Midwest Aquatic Enterprises, Mahomet, IL.

Brown, H. P., 1987. Biology of riffle beetles. Annual Review of Ecology and Systematics 32: 253–273.

Burton, J. & J. Gerritsen, 2003. A Stream Condition Index for Virginia Non-coastal Streams. Tetra Tech Inc, Owings Mill, MD.

Carter, J. L. & V. H. Resh, 2001. After site selection and before data analysis: sampling, sorting, and laboratory procedures used in stream benthic macroinvertebrate monitoring programs by USA state agencies. Journal of the North American Benthological Society 20: 658–682.

Chutter, F. M., 1969. The effects of silt and sand on the invertebrate fauna of streams and rivers. Hydrobiologia 34: 57–76.

Clements, W. H., D. M. Carlisle, L. A. Courtney & E. A. Harrahy, 2002. Integrating observational and experimental approaches to demonstrate causation in stream biomonitoring studies. Environmental Toxicology and Chemistry 21(6): 1138–1146.

Cummins, K. W., 1962. An evaluation of some techniques for the collection and analysis of benthic samples with special emphasis on lotic waters. American Midland Naturalist 67: 477–504.

Cummins, K. W. & G. H. Lauff, 1969. The influence of substrate particle size on the microdistribution of stream macrobenthos. Hydrobiologia 34: 145–181.

Dance, K. W. & H. B. N. Hynes, 1980. Some effects of agricultural land use on stream insect communities. Environmental Pollution 22: 19–28.

Delong, M. D. & M. A. Brusven, 1998. Macroinvertebrate community structure along the longitudinal gradient of an agriculturally impacted stream. Environmental Management 22(3): 445–457.

Downes, B. J., P. S. Lake & E. S. G. Schreiber, 1995. Habitat structure and invertebrate assemblages on stream stones: a multivariate view from the riffle. Australian Journal of Ecology 20: 502–514.

Dudley, T. L., S. D. Cooper & N. Hemphill, 1986. Effects of macroalgae on a stream invertebrate community. Journal of the North American Benthological Society 5: 93–106.

Edington, J. M., 1968. Habitat preferences in net-spinning caddis larvae with special reference to the influence of water velocity. Journal of Animal Ecology 37(3): 675–692.

Egglishaw, H. J., 1964. The distributional relationship between the bottom fauna and plant detritus in streams. Journal of Animal Ecology 33: 463–476.

Evans, L. J. & R. H. Norris, 1997. Prediction of benthic macroinvertebrate composition using microhabitat characteristics derived from stereo photography. Freshwater Biology 37: 621–633.

Fleischner, T. L., 1994. Ecological costs of livestock grazing in western North America. Conservation Biology 8(3): 629–644.

Frutiger, A., 2002. The function of the suckers of larval net-winged midges (Diptera: Blephariceridae). Freshwater Biology 47: 293–302.

Gauch, H. G., 1982. Multivariate Analysis in Community Ecology. Cambridge University Press, Cambridge.

Griffith, M. B., P. R. Kaufmann, A. T. Herlihy & B. H. Hill, 2001. Analysis of macroinvertebrate assemblages in relation to environmental gradients in Rocky Mountain streams. Ecological Applications 11(2): 489–505.

Hoffman, R. L., 1969. The Biotic Regions of Virginia. The Insects of Virginia: No.1. Research Division Bulletin 48, Virginia Polytechnic Institute, Blacksburg, VA.

Inman, D. L., 1952. Measures for describing the size distribution of sediments. Journal of Sedimentary Petrology 22: 125–145.

Kauffman, J. B. & W. C. Krueger, 1984. Livestock impacts on riparian ecosystems and streamside management implications...a review. Journal of Range Management 37(5): 430–438.

Kovalak, W. P., 1976. Seasonal and diel changes in the positioning of *Glossosoma nigrior* (Banks) (Trichoptera: Glossosomatidae) on artificial substrates. Canadian Journal of Zoology 54(9): 1585–1594.

Lemley, A. D., 1982. Modification of benthic insect communities in polluted streams: combined effects of sedimentation and nutrient enrichment. Hydrobiologia 87: 229–245.

Lenat, D. R. & J. K. Crawford, 1994. Effects of land use on water quality and aquatic biota of three North Carolina piedmont streams. Hydrobiologia 294: 185–199.

Lenat, D. R., D. L. Penrose & W. Eagleson, 1981. Variable effects of sediment addition on stream benthos. Hydrobiologia 79: 187–194.

Lorenzen, C. J., 1967. Determination of chlorophyll and pheopigments: spectrophotometric equations. Limnology and Oceanography 12: 343–346.

Lotspeich, F. B. & F. H. Everest, 1981. A new method for reporting and interpreting textural composition of spawning gravel. Pacific Northwest Forest and Range Experiment Station, PNW-369.

Mackay, R. J., 1969. Aquatic insect communities of a small stream on Mont St. Hilaire, Quebec. Journal of the Fisheries Research Board of Canada 26(5): 1157–1183.

McCune, B. & M. J. Mefford, 1999. Multivariate Analysis of Ecological Data. MjM Software, Gleneden Beach, OR.

Morse, C. C., A. D. Huryn & C. Cronan, 2003. Impervious surface area as a predictor of the effects of urbanization on stream insect communities in Maine, U.S.A. Environmental Monitoring and Assessment 89: 95–127.

National Research Council, 2001. Assessing the TMDL Approach to Water Quality Management. National Academy Press, Washington, DC.

Odum, E. P., 1983. Basic Ecology. Saunders College Publishing, NY.

Owens, L. B., W. M. Edwards & R. W. Van Keuren, 1996. Sediment losses from a pastured watershed before and after stream fencing. Journal of Soil and Water Conservation 51(1): 90–94.

Palmer, M. A., C. M. Swan, K. Nelson, P. Silver & R. Alvestad, 2000. Streambed landscapes: evidence that stream invertebrates respond to the type and spatial arrangement of patches. Landscape Ecology 15: 563–576.

Rabeni, C. F. & G. W. Minshall, 1977. Factors affecting microdistribution of stream benthic insects. Oikos 29: 33–43.

Reice, S. R., 1980. The role of substratum in benthic macroinvertebrate microdistribution and litter decomposition in a woodland stream. Ecology 61(3): 580–590.

Richards, C., G. E. Host & J. W. Arthur, 1993. Identification of predominant environmental factors structuring stream macroinvertebrate communities within a large agricultural catchment. Freshwater Biology 29: 285–294.

Riva-Murray, K., R. W. Bode, P. J. Phillips & G. L. Wall, 2002. Impact source determination with biomonitoring data in New York state: concordance with environmental data. Northeastern Naturalist 9: 127–162.

Scott, D., 1958. Ecological studies of the Trichoptera of the River Dean, Cheshire. Archiv fur Hydrobiologie 54(3): 340–392.

Sprules, W. M., 1947. An ecological investigation of stream insects in Algonquin Park, Ontario. University of Toronto Studies, Biological Series 56: 1–81.

Strand, M. & R. W. Merritt, 1999. Impacts of livestock grazing activities on stream insect communities and the riverine environment. American Entomologist 45(1): 13–29.

Stone, M. L., M. R. Whiles, J. A. Webber, K. W. J. Williard & J. D. Reeve, 2005. Macroinvertebrate communities in agriculturally impacted southern Illinois streams: patterns with riparian vegetation, water quality, and in-stream habitat quality. Journal of Environmental Quality 34: 907–917.

ter Braak, C. J. F., 1995. Ordination. In Jongman, R. H. C., C. J. F. ter Braak & O. F. R. Tongerenvan (eds), Data Analysis in Community and Landscape Ecology. Cambridge University Press, Cambridge.

Trimble, S. W. & A. C. Mendel, 1995. The cow as a geomorphic agent – a critical review. Geomorphology 13: 233–253.

Ulfstrand, S., 1967. Microdistribution of benthic species (Ephemeroptera, Plecoptera, Trichoptera, Diptera: Simuliidae) in Lapland streams. Oikos 18: 293–310.

U.S. Environmental Protection Agency (US EPA), 1997. Compendium of Tools for Watershed Assessment and TMDL Development. Office of Water, U.S. Environmental Protection Agency, Washington DC.

Virginia Agricultural Statistics Service (VASS), 2004. Historic census county data for Virginia, 1935–1997 census historical data for major commodities. Available at http://www.vass.usda.gov/va. Accessed June 2005.

Virginia Department.of Environmental Quality (VA DEQ), 2005. Associations between Biological Metrics, Physical Habitat, and Water Chemistry in Virginia's Montane Ecoregions. Office of Water quality and Assessments, Richmond, VA.

Waters, T. F., 1995. Sediment in Streams. Sources, Biological Effects and Control. American Fisheries Society Monograph 7. American Fisheries Society, Bethesda, MD.

Wentworth, C. K., 1922. A scale of grade and class terms for clastic sediments. Journal of Geology 30: 377–392.

Williams, D. D. & M. R. Smith, 1996. Colonization dynamics of river benthos in response to local changes in bed characteristics. Freshwater Biology 36: 237–248.

Wohl, N. E. & R. F. Carline, 1996. Relations among riparian grazing, sediment loads, macroinvertebrates, and fishes in three central Pennsylvania streams. Canadian Journal of Fisheries and Aquatic Sciences 53(Suppl. 1): 260–266.

Wood, P. J. & P. D. Armitage, 1997. Biological effects of fine sediment in the lotic environment. Environmental Management 21(2): 203–217.

Woods, A. J., J. M. Omernik, D. D. Brown & C. W. Kilsgaard, 1996. Level III and IV Ecoregions of Pennsylvania and the Blue Ridge Mountains, the Ridge and Valley, and the Central Appalachians of Virginia, West Virginia, and Maryland. Office of Research and Development, U.S. Environmental Protection Agency, Corvallis, OR.

Zweig, L. D. & C. F. Rabeni, 2001. Biomonitoring for deposited sediment using benthic invertebrates: a test on 4 Missouri streams. Journal of the North American Benthological Society 20(4): 643–657.