Quantifying the environmental factors that determine benthic macroinvertebrate assemblages in streams by analyzing stressors associated with a gradient of cattle grazing

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ABSTRACT

Relationships between macroinvertebrate assemblages and environmental stressors were assessed from fall 2002 through spring 2004 in five small streams that represented a study design that involved a gradient of increasing stress (increased cattle density). Macroinvertebrate assemblages were related to environmental factors that were quantified at the sample scale. Environmental factors and macroinvertebrates were concurrently collected so that assemblage structure could be directly related to environmental variables and so that the relative importance of stressors associated with cattle grazing in structuring assemblages could be assessed. Macroinvertebrate metrics showed significant and strong responses to cattle density during most sampling periods. The majority of metrics responded negatively to the grazing gradient, while a few (total taxa richness, number of sensitive taxa, and % collector filterers) increased along the gradient before declining at the most heavily grazed sites. Total number of sensitive taxa and % Coleoptera had the strongest relationship with cattle density throughout the study period. Based on sample-scale, quantitative measures of environmental variables, measures of physical habitat (% fines and substrate homogeneity) were most important in structuring assemblages. Detrital food variables (coarse benthic and fine benthic organic matter) were secondarily important while autochthonous food variables (chlorophyll *a* and epilithic biomass) were not as important in influencing assemblage structure. Based on a comparative analysis of reach-scale habitat measures and estimates, quantitative measures of % fines, collected from within an enclosed sampler concurrently with macroinvertebrates, were the best predictor of macroinvertebrate assemblages. Quantitative measures and visual estimates of riparian and channel characteristics had strong relationships with macroinvertebrate metrics but the relationships were never as strong as those detected with instream measurements of % fines. The channel characteristic, bank height, was the best predictor of % fines.

For my mother and father, Una E. Braccia and Joseph C. Braccia

in gratitude of their unconditional love and support

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INTRODUCTION

The foundation for today's water quality programs lies in the Clean Water Act (CWA) of 1972 and its amendments of 1987. The overall purpose of the CWA is to protect the quality of the nation's waters. The specific objective is "to restore and maintain the physical, chemical, and biological integrity of our nation's waters." Biological integrity is defined as "the ability of an aquatic ecosystem to support and maintain a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of the natural habitats of a region" (Karr and Dudley 1981). Although largely ignored for about two decades, the eventual recognition of biological integrity in the CWA has led to widespread adoption of bioassessment programs by state regulatory agencies. Bioassessment is defined as an evaluation of the condition of a water body using biological surveys and other direct measurements of the resident biota in surface waters (Gibson et al. 1996). These are usually done for regulatory purposes to determine if human activities have impaired a water body. A review of 48 state and territory regulatory agencies with biomonitoring programs found that benthic macroinvertebrates were used in 47, fish in 25, and periphyton in 3 (Southerland and Stribling 1995). The wide range of natural habitat preferences and pollution tolerances among benthic macroinvertebrates, as well as being relatively easy to collect and identify, makes them excellent organisms for freshwater bioassessment.

Currently in the U.S., most states conduct the required bioassessments by means of the Rapid Bioassessment Protocols (RBPs) developed by the U.S. Environmental Protection Agency (Barbour et al. 1999). Carter and Resh (2001) summarized methods used by state agencies across the U.S. and reported that most agencies sample a single habitat (mostly riffles) with a D-frame kick net and if more than one sample is collected it is composited. 'Rapid' habitat assessments are conducted at the same time and place where the benthic macroinvertebrate collections are made. These rapid habitat assessments, which are also part of the RBPs, are based on visual estimates of streambed, channel, and local riparian characteristics. Upon completion of the RBP macroinvertebrate and habitat survey, the ecological condition of a stream is determined. These methods are so rapid that, if necessary, the ecological condition of a stream can be determined within a day.

If stream bioassessment results indicate impairment (i.e., an unnatural benthic macroinvertebrate assemblage), streams are subject to regulatory actions and enter the Total Maximum Daily Load (TMDL) program. Currently in the U.S., the TMDL Program is being used to restore the condition of the nation's water bodies (U.S. Environmental Protection Agency 1997). The TMDL process involves detailed analysis of land use and point and nonpoint sources of pollution, then development of a management plan for eliminating the impaired condition of a stream. An essential part of the TMDL process is identifying sources (or stressors) causing biological impairment. After site-specific causes of impairment are identified, stressor loads need to be reduced and monitoring must continue during the TMDL implementation and anticipated stream restoration.

State agencies are often resource limited and overwhelmed with impaired streams. Given the need to quickly improve water quality, causal factors, such as degraded habitat, are made by using best professional judgment to relate benthic macroinvertebrate data that were obtained by RBPs to ambient water quality data and RBP habitat assessments. Although RBPs for macroinvertebrates and habitat are useful tools for state agencies to monitor the quality of a large number of waterbodies and initially determine impaired streams, the current approach to stressor identification and quantification is not scientifically sound (NRC 2001). RBPs are screening tools that do not generate data that are adequate for reliable determination of cause-and-effect relationships between stressors and the resident biota. Stream ecosystems are complex, and establishing cause requires thorough, statistically rigorous, quantitative field studies.

At the request of Congress, the National Research Council (NRC) convened a special committee to evaluate the TMDL program. The NRC's 2001 report endorsed the overall TMDL model, but emphasized some shortcomings. In general, the report indicated that the TMDL program does not have a sound scientific basis. Two of the committee's specific recommendations were:

1) "*EPA should promote the development of models that can more effectively link environmental stressors (and control actions) to biological responses.*"

2) "*Monitoring and data collection programs need to be coordinated with anticipated water quality and TMDL modeling requirements.*"

The lack of scientifically valid stressor identification protocols noted by the NRC committee is problematic for TMDL development and the overall protection of freshwater resources. In Virginia (and other states), bioassessment data clearly indicate impairment, but there are no scientifically valid methods for establishing causes or identifying the stressors of degraded benthic macroinvertebrate assemblages. Unfortunately, empirical relationships between stream biota and stressors have not been established.

Agriculture is one of the primary land uses that can impair the ecological condition of streams and trigger the development of TMDLs (U.S. Environmental Protection Agency 2000). There are approximately 1.5 million cattle in Virginia, and in the western part of the state, beef and dairy cattle operations are a predominant land use (Virginia Agricultural Statistics Service 2002). However, there is strong evidence that cattle grazing in and around pasture streams leads to deteriorated habitat and water quality. The Virginia Department of Environmental Quality (VA DEQ) maintains that agriculture is a leading cause of river and stream impairment in Virginia (VA DEQ 2002a). States throughout the country have reported similar findings, so degraded water quality as a result of agricultural practices is considered to be a national trend (U.S. Environmental Protection Agency 2000).

The need for empirical data for the development of predictive models was the impetus for this research study. The overall goal of this study was to quantify and link environmental factors to benthic macroinvertebrates to generate data that could be used to support the development of models that accurately predict assemblages in streams that receive stressors from human activity. I conducted extensive, quantitative benthic sampling and habitat measurements within five streams that represented a gradient of habitat quality as a result of various levels of cattle grazing (Fig 1). The gradient of habitat quality presented by the stream reaches that were selected for this study offered an experimental design to investigate which environmental variables are most important in structuring assemblages. Specific questions that were addressed in this study were (1) What is the composition of the macroinvertebrate fauna in small cattle-impacted streams? (2) Does macroinvertebrate assemblage structure change predictably in response to different levels of cattle grazing? (3) Which environmental factors are most important in structuring macroinvertebrate assemblages?, and (4) Which method of habitat characterization best explains macroinvertebrate assemblage structure: benthic measurements or RBP estimates?

Specific objectives of this study were:

1. Describe the taxa that can be expected to occur in small streams used for cattle production and demonstrate how taxon-specific ecological information can be used to identify primary stressors in streams where there are multiple potential stressors to the benthic macroinvertebrate fauna.
2. Establish relationships between cattle grazing intensity and benthic macroinvertebrate and to identify the level of grazing intensity where benthic macroinvertebrate assemblages begin to change along the habitat gradient.

3. Relate benthic assemblages to environmental factors at the scale that is most relevant to macroinvertebrates (the sample scale) and to determine how much of the variation in benthic macroinvertebrate assemblage structure can be explained by environmental factors.
4. Identify which environmental factors, at the sample scale, are most important in structuring the benthic macroinvertebrate assemblage.
5. Assess the suitability of relating benthic macroinvertebrates to habitat data collected with rapid field methods.

In Chapter 1 of this dissertation, a list of taxa that were encountered during this study is provided. The extensive taxa list, presence/absence patterns and frequency of occurrence and natural history information is used to identify potential stressors associated with cattle grazing. In Chapter 2, macroinvertebrate data were condensed into metrics, i.e., numerical representation of the data, to determine if macroinvertebrate assemblage structure changed predictably in response to the cattle grazing gradient. After establishing that cattle grazing intensity is a determinant of macroinvertebrate assemblage structure, multivariate ordination methods were used in Chapter 3 to identify environmental factors, i.e., sediment and food resources, that best explain macroinvertebrate assemblage structure. Environmental data used in Chapter 3 were collected with quantitative, rigorous field methods. These quantitative methods were compared to more rapid measures of habitat in Chapter4.

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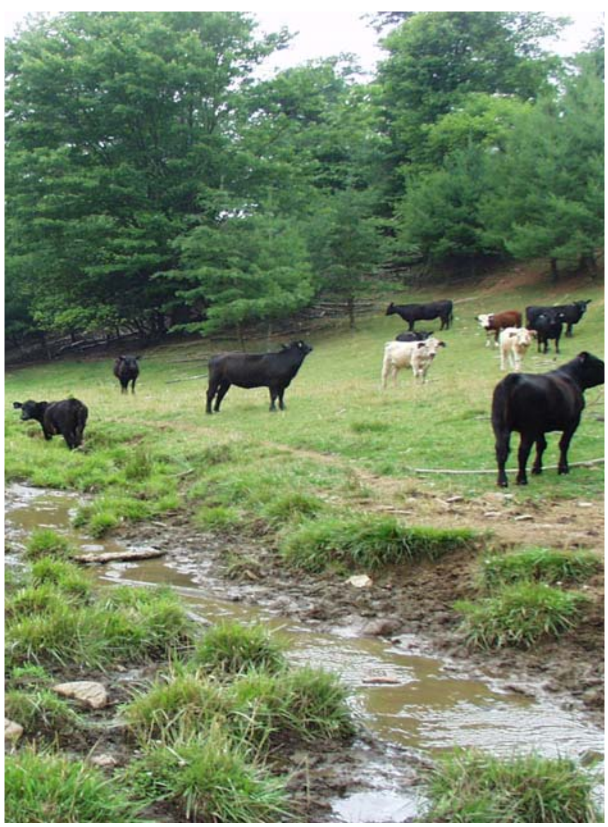
Fig. 1 Study sites. (a) site 1 (b) site 2 (c) site 3 (d) site 4 (e) site 4

A

B

C

D



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CHAPTER 1

Benthic Macroinvertebrate Fauna In Small Streams Used By Cattle In the Blue Ridge Mountains, Virginia

Amy Braccia and J. Reese Voshell, Jr.

Abstract

Cattle production is a common land use, and the adverse effects of cattle grazing on stream habitat and macroinvertebrates has been well documented. The purpose of our study was to provide a list of taxa that can be expected to occur in small streams impacted by cattle in the southern Blue Ridge Mountains and to demonstrate how taxon-specific natural history information can be used to gain insight about benthic habitat condition. We identified 97 benthic macroinvertebrate taxa from five cattle-impacted streams that differed in cattle grazing intensity. Our findings suggest that some macroinvertebrate taxa can sustain low levels of cattle grazing and that sedimentation is a major stressor to the macroinvertebrate fauna.

Introduction

Cattle production is a common use of land throughout the U. S. 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. Production of cattle in pastures with unrestricted access to streams causes multiple changes to stream environments. Trampled stream banks cause increased erosion and sedimentation. 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 (Armour et al. 1991, Cooper 1993, Fleischner 1994, Kauffman and Krueger 1984, Owens 1996, Trimble and Mendel 1995). These cattle-induced changes degrade water quality and habitat, which in turn alter the resident benthic macroinvertebrate fauna (Cook 2003, Dance and Hynes 1980, Delong and Brusven 1998, Harding et al. 1999, Scrimgeor and Kendall 2003, Strand and Merritt 1999, Wohl and Carline 1996).

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. Benthic habitat consists of multiple variables but water current, substrate, and food resources have been shown to be especially important in structuring macroinvertebrate assemblages at small spatial scales (Bouckaert and Davis 1998, Edington 1968, Egglishaw 1964, Eriksen 1968, Palmer et al. 2000, Rabeni and Minshall 1977, Reice 1980, Ulfstrand 1967). Substrate characteristics that influence macroinvertebrate microdistribution include mineral versus plant material, living versus decomposing plants, particle size of mineral substrate, food retention ability, heterogeneity, and stability (Allan 1975, Boyero 2003, Cobb et al. 1992, Cummins and Lauf 1969, DeMarch 1976, Erman and Erman 1984, Minshall and Minshall 1977, Trush 1979, Williams and Mundie 1978). For benthic macroinvertebrates, differences in assemblage structure often manifest themselves within 1 m, and sometimes within a few cm.

The wide range of natural habitat preferences and pollution tolerances among benthic macroinvertebrates makes them excellent organisms for freshwater bioassessment. Bioassessment is defined as an evaluation of the condition of a water body using biological surveys and other direct measurements of the resident biota in surface waters (Gibson et al. 1996). Bioassessments are usually done for regulatory purposes to determine if human activities have impaired a water body. Currently in the U.S., most states conduct the required bioassessments by means of the Rapid Bioassessment Protocols (RBPs) developed by the U.S. Environmental Protection Agency (Barbour et al. 1999). Some important features of the RBPs include qualitative sampling rather than a fixed area scheme, subsampling to manageable numbers (100-200 organisms), and data analysis based on metrics and multimetric indices (Voshell et al. 1997). These features make RBPs cost effective but also result in the loss of taxon-specific ecological information, especially for rare taxa.

Rare taxa are an important component of the benthic macroinvertebrate fauna. They are often sensitive to changes in their environment and are usually among the first taxa to be eliminated following anthropogenic disturbance (Lenat and Resh 2001). However, the features of RBPs mentioned above are not conducive to the collection of rare taxa. Field ecologists have long recognized that the number of taxa, especially rare taxa, increase with sampling effort (see Vinson and Hawkins 1996). Furthermore, even if field and laboratory methods allow for the collection of rare taxa, information about individual taxa, especially rare taxa, is lost when taxonomic data are condensed into metrics, as recommended in RBPs.

In this study, we used extensive sampling, identification of all taxa to the lowest practical level of taxonomy, and taxon frequency to describe changes in the benthic macroinvertebrate fauna that occurred along a predetermined gradient of habitat quality resulting from different levels of cattle grazing. The purpose of this study was to provide a record of taxa that can be expected to occur in small streams used for cattle production. In addition, we demonstrate how taxon-specific ecological information can be used to identify primary stressors in streams where there are multiple potential stressors to the benthic macroinvertebrate fauna.

Methods

Study sites and the habitat quality gradient

Five, first-order stream reaches in the Little River drainage basin, Floyd Co., Virginia were selected as study sites. These study sites were selected because they were similar in size, gradient, underlying geology, and vegetative cover, and were subjected to a gradient of cattle grazing (Table 1-1). 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. All study sites are within the Blue Ridge Interior Plateau ecoregion (Woods et al. 1996). Site 1 was recovering from cattle grazing (cattle were removed 12-15 years ago) and represented the best habitat quality. Cattle were rotationally grazed at Site 2 where there were 1.04 cattle per ha. Cattle had unlimited stream access at Sites 3, 4, and 5, where there were 1.54, 2.13, and 2.85 cattle per ha, respectively. Based on conversations with the private landowners, all pastures have been in operation for at least 50 years.

Prior to macroinvertebrate sampling, reach-scale habitat quality was determined at each study site following EPA's visually based RBP habitat assessment (Barbour et al. 1999). Following this methodology, stream reaches receive an overall habitat score based on stream reach 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. Habitat scores indicated that the study sites represented a gradient of decreasing habitat quality (Table 1-1).

Benthic sampling

Benthic macroinvertebrate samples were taken in fall 2002 and late winter 2003. We used a stratified systematic sample design and restricted collections to three areas with the swiftest current at each study site. Current velocity within the sampling areas ranged from 0.01 to 0.74 m/s. Within each sampling area we collected three or four benthic samples that were evenly spaced at least 2 m apart. A total of 115 benthic samples were collected, 59 in fall 2002, and 56 in late winter 2003.

Benthic macroinvertebrates were collected by inserting a modified stovepipe corer approximately 10 cm into streambed substrates. All material within the core was removed with the aid of a hand pump, preserved with ethanol, and transported to the laboratory for analysis. In the laboratory, benthic samples were rinsed through a 250-µm sieve and macroinvertebrates were hand sorted from stream material under a dissecting microscope, enumerated, and identified. Most insect taxa were identified to genus; other invertebrate taxa were identified to class, order, or family.

Data analysis

The frequency of occurrence of each taxon was reported for each study site. A taxon's frequency was obtained by summing the number of samples at each study site in which the taxon occurred. The sum of the samples that contained the taxon was then divided by that total number of samples collected at the study site. A taxon's frequency at each study sites was reported as a percent. Each taxon was assigned a pollution tolerance value (PTV), functional feeding group (FFG) (a mode of acquiring food based on morphology and behavior), and habit (a mode of existence or how the organism maintains its position in its environment). Assignments to these categories were made based on a synthesis of published literature (e.g., Barbour et al. 1999 and Brigham et al. 1982) 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 to 10, with 0 indicating very sensitive and 10 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. Taxa that occurred in less than 5% of all samples were categorized as rare; all other taxa were considered to be common.

Results

Ninety-seven taxa were identified during this study (Table 1-2). The total numbers of taxa at each study site were 68, 75, 69, 51, and 46 at Sites 1, 2, 3, 4, and 5, respectively. The rotational grazing site (Site 2) supported the most taxa, while the study sites with the most degraded habitat (Sites 4 and 5) had the fewest taxa. The majority (86%) of taxa were insects, represented by six orders. Diptera and Trichoptera had the greatest taxa richness with 27 and 18 taxa, respectively. Taxa richness was similar among the Ephemeroptera, Plecoptera, and Coleoptera with 10, 10, and 11 taxa, respectively. The Odonata were represented by four taxa.

We identified 44 taxa that declined in frequency or became absent along the gradient of cattle grazing. These included Ancylidae, Hydracarina, *Baetis*, *Baetisca, Ephemerella*, *Eurylophella*, *Serratella, Stenonema,* *Habrophlebia vibrans, Paraleptophlebia, Cordulegaster, Gomphus*, *Lanthus*, *Allocapnia*, *Suwalia, Sweltsa*, *Leuctra*, *Amphinemura*, *Tallaperla*, *Acroneuria, Isoperla*, *Remenus bilobatus*, *Yugus*, *Diplectrona, Lepidostoma, Setodes, Pycnopsyche, Psilotreta*, *Wormaldia*, *Polycentropus*, *Lype diversa, Rhyacophila, Helichus*, *Optioservus, Oulimnius latiusculus*, *Promoresia*, *Stenelmis*, *Psephenus herricki, Anchytarsus,* Ceratopogonidae, *Oreogeton*, *Antocha*, *Dicranota*, and *Hexatoma* (higher taxonomic classification categories are given in Table 1-2). Conversely, 11 taxa increased in frequency along the gradient and included *Gammarus*, *Corbicula fluminea*, Sphaeriidae, *Oligostomis*, *Ptilostomis*, Ephydridae, *Limnophora, Pericoma, Psychoda*, *Eristalis*, and *Tipula*.

Taxa that showed no response to the gradient consisted of the non-insect taxa Planariidae, Nematoda, Oligochaeta, Cambaridae, Copepoda, and Pleuroceridae, and the insect taxa *Epeorus*, *Nigronia fasciatus*, *Glossosoma nigrior*, *Goera*, *Agarodes*, *Neophylax*, *Ectopria*, Chironomidae, *Hemerodromia, Simulium*, *Prosimulium*, *Chrysops*, and *Pseudolimnophila*. Thirty-one of the taxa (32%) encountered during this study were pollution sensitive. The total number of pollution sensitive taxa at each study site declined along the gradient; there were 25, 26, 20, 17, and 13 sensitive taxa at Sites 1, 2, 3, 4, and 5, respectively. Twenty-two pollution sensitive taxa (*Ephemerella*, *Serratella*, *Habrophlebia vibrans*, *Paraleptophlebia*, *Lanthus*, *Allocapnia*, *Suwalia*, *Sweltsa*, *Leuctra*, *Tallaperla*, *Acroneuria*, *Isoperla*, *Remenus bilobatus*, *Yugus*, *Diplectrona*, *Lepidostoma*, *Setodes*, *Psilotreta*, *Wormaldia*, *Rhyacophila*, *Oulimnius latiusculus*, and *Promoresia*) declined along the grazing gradient, while 10 pollution sensitive taxa (Pleuroceridae, Epeorus, *Stylogomphus albistylus*, *Glossosoma nigrior*, *Goera*, *Oligostomis*, *Agarodes*, *Fattigia pele*, *Neophylax*, and *Blephariceera*) showed no response to the gradient. We encountered 10 pollution tolerant taxa (Planariidae, Nematoda, Oligochaeta, Hirudinea, Lymnaeidae, Sphaeriidae, *Limnophora*, *Pericoma*, *Psychoda*, and *Eristalis*), and the total number of pollution tolerant taxa at each study site showed a slight increase along the gradient. There were 5, 8, 10, 7, and 8 tolerant taxa at Sites 1, 2, 3, 4, and 5, respectively.

Discussion

Taxa list

We have recorded a fairly long list of taxa that exist in five small streams where cattle currently have direct access to the channel or where there is a history of using the streams for cattle production. It should be noted that our list would probably be much longer if all of the immature specimens could be identified to species. The high number of taxa that we report is also noteworthy because none of the streams are in pristine ecological condition. Even at Site 1, where there are no cattle grazing at the present time, the stream flows through land that was cleared of all trees and converted to agricultural use at least 50 years ago and was subjected to cattle grazing until 12-15 years ago. The habitat score of 155 at Site 1 is barely in the upper quartile of possible scores (151-200), which is considered to be representative of optimal conditions. However, we found 68 taxa at Site 1 with more than one-third of them (37%) considered sensitive to pollution or other environmental stress.

Agriculture, specifically livestock grazing, is recognized to cause degraded water quality and habitat conditions, which lead to reduced biodiversity of macroinvertebrates. However, in our study, the highest number of taxa, 75 out of 97, occurred at the site with light rotational grazing (Site 2). Of the 75 taxa, 25 **(**33%**)** are considered to be sensitive taxa. The habitat score of 142 at Site 2 was at the upper end of the third quartile (101-150), which is considered to be representative of suboptimal conditions that are still satisfactory and nearly as good as Site 1. These findings suggest that in some stream settings, habitat quality and benthic macroinvertebrate biodiversity can be sustained at low levels of grazing.

Where grazing pressure increased to 40 cattle present all of the time (Site 3), the habitat score dropped considerably to 117. Although we found 69 taxa at Site 3, there were fewer sensitive ones (20 taxa or 29%). Even where grazing intensity was high at Sites 4 and 5, the benthic macroinvertebrate fauna was not completely decimated. Habitat scores did not drop much lower, and a moderate number of taxa, including some sensitive taxa, still occurred, although at lower frequency. The complex nature of fluvial geomorphology probably allows for the presence of infrequent patches of suitable habitat for some sensitive taxa, even in the most degraded streams. This is useful information for predicting stream recovery. If cattle are excluded from streams, such as at Site 1, or they are given limited access to streams, such as at Site 2, the infrequent patches of suitable habitat will serve as sources for colonization by sensitive macroinvertebrates. Successional recovery can be expected to take place, including restoration of habitat quality and macroinvertebrate biodiversity.

The above information is not meant to suggest that cattle grazing does not negatively affect the ecological condition of small streams, especially when higher densities of cattle have unrestricted access to the stream channel. For example, at Sites 4 and 5, there were appreciably fewer taxa. The second purpose of this research was to determine if compiling an extensive list of the taxa occurring in the streams and examining the natural history of those macroinvertebrates could explain which of the specific stressors from cattle grazing are most responsible for differences in the fauna.

Identification of potential stressors

Cattle urine, either through direct inputs to water or leaching from pasture, is a source of inorganic nutrients to streams. Excessive nutrient loads stimulate primary production causing streambeds to become covered with algae, especially cyanobacteria. Organic loads, in the form of manure, can also be excessive in livestock-impacted streams. Nutrients and dissolved organic compounds from organic loading cause the growth of various microorganisms, especially fungus, on benthic substrate and biota (see Hynes 1971 and Mason 1996). Excessive algal growth and fungus on streambeds eliminates physical habitat for taxa that need clean substrates for attachment, such as clingers. During the decomposition of excessive algae and organic matter, oxygen is consumed by heterotrophic organisms. Ultimately, dissolved oxygen concentrations are lowered for benthic macroinvertebrates. Oxygen-sensitive macroinvertebrate taxa, such as mayflies and stoneflies, become absent while taxa more tolerant of low dissolved oxygen (e.g., *Eristalis*, *Psychoda*) persist. Furthermore, when fungus colonizes macroinvertebrate bodies and gills, taxa cannot obtain their oxygen requirements. Lemly (1998) attributed increased macroinvertebrate mortality to attached fungus in Appalachian streams that received pasture runoff.

Altered temperature regime is another possible stressor to the macroinvertebrate fauna in cattle-impacted streams. Trees and shrubs are often reduced along streams in agricultural settings, especially in pasture streams. The resulting lack of shade causes warmer, more variable water temperatures and taxa that have narrow temperature requirements, especially stoneflies, are often eliminated.

Because low dissolved oxygen, deteriorated physical habitat from excessive growth of algae or fungus, and temperature do not vary within stream reaches, we expected to find an absence of cold-adapted, oxygen-sensitive, and clinging-scraping taxa at the most degraded study sites if these are the major stressors from cattle grazing; however, this was not the case. We encountered taxa with narrow temperature requirements and high oxygen requirements, such as mayflies and stoneflies, as well as sensitive clinging-scraping taxa, such as Pleuroceridae, *Glossosoma nigrior*, *Goera*, *Psilotreta*, *Neophylax*, *Oulimnius latiusculus*, *Promoresia*, and *Blepharicera*, at the most degraded sites. The presence of these taxa at the most degraded study sites suggests that temperature alterations, decreased dissolved oxygen, and habitat alterations associated with nutrient and organic loading may not have been major stressors to the macroinvertebrate fauna in these streams.

Cattle-impacted streams often have unstable, trampled stream banks which become significant sources of inorganic sediments when they erode. When bedload sediments are excessive, the undersides of cobble and streambed interstices become embedded and clogged with fine sand and silt; clean, interstitial patches are less frequent while the frequency of sand and silt patches increases. These benthic habitat alterations have been shown to alter macroinvertebrate fauna and displace taxa that crawl among streambed interstices (Chutter 1969, Lenat 1984, Lenat et al. 1981, Cordone and Kelley 1961, Wood and Armitage 1997).

Many of the taxa that declined in frequency, or became absent along the grazing gradient, crawl among interstitial spaces formed from mixes of cobble and pebble (often called rubble) and coarse gravel, the undersides of cobble, and hyporheic zones (Godbout and Hynes 1982, Hynes 1970, Hynes 1974, Mackay 1969, Pennak and Van Gerpen 1947, Percival and Whitehead 1929, Sprules 1947, Ward 1975, Williams and Hynes 1974). For example, during daylight hours, several interstitial taxa, including *Baetis* spp. and *Ephemerella* sp., occur on the undersides of stones but migrate to stone surfaces at night to avoid visual predators such as fish (Elliott 1968). Late instar caddisflies, including *Psilotreta*, *Pycnopsyche* spp., and *Rhyacophila*, attach to the undersides of stones or burrow into coarse gravel prior to pupation (Anderson 1967, Lloyd 1921, Mackay and Wiggins 1979). Stoneflies are particularly selective in their substrate choices and Harper and Hynes (1970) found the winter stoneflies, *Capnia*, *Allocapnia*, and *Taeniopteryx,* in diapause at depths as far as 10 to 20 cm beneath a streambed in Canada. Diapause is a genetically encoded state of arrested development that likely evolved so that cold-adapted stoneflies can avoid high summer temperatures and drought. We attribute the decreased frequency of crawling taxa to physical habitat alteration, i.e., clogged streambed pore space, as a result of excessive sediments.

Further evidence for alterations to the physical nature of the streambed were increased frequency and occurrence of soft-bodied taxa that are adapted for burrowing into soft streambed substrates along the grazing gradient. The majority of taxa that responded positively to the grazing gradient, such as *Corbicula fluminea*, Sphaeriidae, Ephydridae, *Limnophora*, *Pericoma*, *Psychoda*, *Eristalis*, and *Tipula*, burrow into or sprawl on substrate composed of densely packed sand and silt, or organic matter, such as detritus, cattle manure, or decaying vegetation. For example, the rat-tailed maggot, *Eristalis*, burrows into soft substrates and is morphologically adapted to exist in environments devoid of oxygen, such as sewage lagoons, because it obtains atmospheric oxygen by means of a long, retractable breathing tube. The occurrence of this rare taxon at Site 3 suggests that patches of manure or decaying vegetation were present but infrequent. *Psychoda* and *Pericoma* occur most frequently in household and sewage drainpipes and are typically not abundant in streams. When they do occur in streams, they burrow into soft sediments and collect fine organic matter for food. Increased frequency of *Psychoda* and *Pericoma*, and the presence of the rare taxon *Eristalis* at Site 3, imply that habitat patches composed of fine sediments enriched with organic matter increased along the grazing gradient. Further evidence that organic matter changed along the gradient, was the occurrence of phryganeid caddisflies at the most degraded study sites. Phryganeid caddisflies (*Oligostomis* and *Ptilostomis*) are often associated with aquatic vascular plants and accumulations of coarse detritus because of their food and case building requirements. When cattle trample the margins of small streams, the channels become braided with small hummocks of pasture grasses. The hummocks, and their associated grasses, provide ideal habitat for taxa such as phryganeid caddisflies. We attribute the presence and increased frequency of *Oligostomis*, *Ptilostomis*, *Eristalis*, *Pericoma*, and *Psychoda* to elevated benthic organic loads in the form of cow patties, pasture vegetation, and vegetation hummocks.

It is noteworthy that several sensitive taxa that did not respond to the grazing gradient, particularly *Glossosoma nigrior*, *Goera*, *Neophylax*, and *Blephariceга*, are clingers that are associated with the exposed surfaces of stable rocks. These taxa have rarely been reported from the undersides of substrates (Frutiger 2002, Kovalak 1976, Scott 1958). Clinging taxa are morphologically or behaviorally adapted to exist on the surface of clean, stable substrate in swift water. 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 portable cases formed from rock fragments. Their case making behavior is unique among the Trichoptera because the larvae attach ballast stones on the sides of the case to help anchor the larvae in swift current. It is possible that these clinging taxa are able to persist because the surfaces of rock and cobble in the swift currents were not altered by sedimentation.

Implications for management and monitoring

Considering the presence or absence of individual taxa in conjunction with taxon-specific natural history provides a great deal of useful information about the ecological condition of water bodies and probable causes of impairment. The absence and decreased frequency of taxa that require clean streambeds along the grazing gradient led us to conclude that excessive sedimentation was the major stressor to the benthic macroinvertebrate assemblage. It is questionable whether we would have reached the same conclusions and findings if we used less rigorous field sampling methods with subsampling (the RBP approach). With limited field sampling it is unlikely that the rare taxa, *Glossosoma nigrior*, *Oligostomis*, *Ptilostomis*, *Blephariceria*, *Eristalis*, all of which provided useful information about habitat, would have been encountered. Furthermore, if rare taxa had been collected, they would have been lost if our data were condensed into metrics. Recent studies have shown that taxonomic determinations beyond family and the inclusion of rare taxa, provide further insight into the status of water quality and may be necessary to determine specific stressors (Nijboer and Schmidt-Kloiber 2004, Waite et al. 2004).

In summary, increased sampling effort resulted in the collection of a rich macroinvertebrate fauna that provided a large amount of ecological information. We did not rely on rigorous statistical methods to determine: (1) that the primary stressor to the macroinvertebrate fauna is likely sediment from eroded stream banks and (2) that low to moderate cattle grazing around these small streams does not deteriorate the macroinvertebrate fauna relative to recovery conditions.

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Table 1-1. 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 per Ha	0	1.04*	1.54	2.13	2.85
Percentage of bank exposed**	0	33	36	49	40
RBP habitat score***	155	142	117	111	113
	(optimal)	(suboptimal)	(suboptimal)	(suboptimal)	(suboptimal)
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)†					
Minimum	10	8	8	14	2
Maximum	62	47	25	77	10
Average	25	25	15	30	5
Mean wetted width (m)‡	0.88	0.72	1.11	0.76	0.60
Mean depth (m) ‡	0.08	0.13	0.13	0.09	0.10

*rotational grazing

**The percent of total stream length composed of bare soil was determined by direct measurement.

***RBP habitat scores and corresponding categories are as follows: optimal, 200 – 150; suboptimal, 149-100; marginal, 99-50; poor, 0-49.

† Baseflow discharge was measured on 8 separate occasions between July 2002 and August 2003.

‡ 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).

Table 1-2. Taxon frequency at each study site. Values represent the percent of benthic samples at each study site that contained the taxon (*n* = the total number of benthic samples collected at each study site). The superscript, R, indicates a taxon was rare. S = pollution sensitive, T = pollution tolerant, CG = collector-gatherer, PR = predator, GN = generalist, SC = scraper, CF = collector-filterer, SH = shredder, SP = sprawler, BU = burrower, CR = crawler, CL = clinger, CI = climber.

						Study sites				
						1	2	3	4	5
			PTV	FFG	Habit	*n=24*	*n=23*	*n=23*	*n=22*	*n=23*
NON-INSECTA										
	Planariidae		T	CG	SP	21	13	57	23	22
Nematoda			T	CG	BU	63	78	74	50	61
Oligochaeta			T	CG	BU	100	100	96	95	100
Hirudinea[R]			T	PR	SP	0	0	9	0	0
Copepoda				-	-	83	91	74	64	78
Amphipoda	Gammaridae	*Gammarus*[R]		CG	CR	0	0	4	0	9
Decapoda	Cambaridae			GN	GN	21	9	17	9	13
Gastropoda	Ancylidae			SC	CL	4	83	0	0	0

	Lymnaeidae[R,]		T	CG	GN	4	0	4	0	0
	Planorbidae[R]			CG	SP	0	0	9	0	0
	Pleuroceridae		S	SC	CL	100	100	78	100	39
Bivalvia	Corbiculidae[R]	*Corbicula fluminea*[R]		CF	BU	0	13	4	0	4
	Sphaeriidae		T	CF	BU	63	74	91	73	70
Hydracarina				PR	CR	88	91	96	41	52
INSECTA										
Ephemeroptera	Baetidae	*Baetis*		CG	CL	33	39	39	5	17
	Baetiscidae	*Baetisca*		CG	SP	4	17	13	0	0
	Ephemeridae	*Ephemera*[R]		CG	BU	0	4	0	0	0
	Ephemerellidae	*Ephemerella*	S	CG	CR	46	57	48	27	22
		Eurylophella		CG	CR	42	52	70	18	0
		Serratella[R]	S	CG	CR	0	4	0	0	0
	Heptageniidae	*Epeorus*	S	CG	CL	25	17	0	14	4
		Stenonema		SC	CL	42	39	17	0	0
	Leptophlebiidae	*Habrophlebia vibrans*[R]	S	CG	CR	0	4	4	0	0
		Paraleptophlebia	S	CG	CR	83	70	30	5	0

Odonata	Cordulegastridae	*Cordulegaster*[R]		PR	BU	4	0	0	0	0
	Gomphidae	*Gomphus*[R]		PR	BU	4	4	9	0	0
		Lanthus	S	PR	BU	33	26	0	0	9
		Stylogomphus albistylus[R]		PR	BU	4	0	4	0	0
Plecoptera	Capniidae	*Allocapnia*	S	SH	CR	75	74	43	18	39
	Chloroperlidae	*Suwalia*	S	PR	CR	33	48	35	0	9
		Sweltsa[R]	S	PR	CR	4	0	0	0	0
	Leuctridae	*Leuctra*	S	SH	CR	71	83	43	14	22
	Nemouridae	*Amphinemura*		SH	CR	25	17	9	5	13
	Peltoperlidae	*Tallaperla*	S	SH	CR	42	30	9	0	9
	Perlidae	*Acroneuria*[R]	S	PR	CR	8	4	0	0	0
	Perlodidae	*Isoperla*	S	PR	CR	42	61	35	9	26
		Remenus bilobatus[R]	S	PR	CR	8	0	0	0	0
		Yugus[R]	S	PR	CR	4	0	0	0	0
	Taeniopterygidae	*Taeniopteryx*[R]		SH	CR	4	0	0	0	4
Megaloptera	Corydalidae	*Nigronia fasciatus*[R]		PR	CR	4	0	0	5	0
Trichoptera	Glossosomatidae	*Glossosoma nigrior*[R]	S	SC	CL	0	9	0	5	0
	Hydropsychidae	*Diplectrona*	S	CF	CL	42	83	30	9	4

		Hydropsyche		CF	CL	0	17	65	0	9
	Lepidostomatidae	*Lepidostoma*	S	SH	CR	13	48	9	0	0
	Leptoceridae	*Oecetis*[R]		PR	CR	0	4	4	0	0
		Setodes	S	CG	CR	29	4	9	0	0
	Limnephilidae	*Goera*	S	SC	CR	13	17	65	9	0
		Pycnopsyche		SH	CR	13	9	0	0	4
	Odontoceridae	*Psilotreta*	S	SC	CL	63	22	22	5	0
	Philopotamidae	*Chimarra*[R]		CF	CL	0	0	9	0	0
		Wormaldia[R]	S	CF	CL	4	9	0	0	0
	Phryganeidae	*Oligostomis*[R]	S	PR	CI	0	0	17	5	0
		Ptilostomis[R]		SH	CI	0	4	9	9	0
	Polycentropodidae	*Polycentropus*[R]		PR	CL	8	0	0	0	0
	Psychomyiidae	*Lype diversa*		SC	CL	13	13	4	0	0
	Rhyacophilidae	*Rhyacophila*	S	PR	CR	21	26	9	0	4
	Sericostomatidae	*Agarodes*	S	SH	SP	4	26	65	5	0
		Fattigia pele[R]	S	SH	SP	0	4	0	0	0
	Uenoidae	*Neophylax*	S	SC	CL	13	9	17	9	9
Coleoptera	Dryopidae	*Helichus*[R]		SC	CL	4	0	0	0	0

	Dytiscidae[R]			PR	GN	0	0	9	0	0
	Elmidae	*Dubiraphia*[R]		SC	CL	0	0	4	0	0
		Optioservus		SC	CL	83	83	52	9	0
		Oulimnius latiusculus	S	SC	CL	88	100	87	27	22
		Promoresia	S	SC	CL	21	39	9	9	0
		Stenelmis		SC	CL	29	9	35	0	0
	Hydrophilidae	*Tropisternus*[R]		PR	GN	4	9	9	0	0
	Psephenidae	*Psephenus herricki*		SC	CL	29	4	43	0	0
		Ectopria		SC	CL	88	43	4	23	0
	Ptilodactylidae	*Anchytarsus*		SH	CL	29	39	22	0	0
Diptera	Blephariceridae	*Blepharicera*[R]	S	SC	CL	0	0	0	23	0
	Ceratopogonidae			-	-	100	100	91	73	83
	Chironomidae			-	-	100	100	100	100	100
	Dixidae	*Dixa*[R]		CG	CR	8	4	0	0	9
	Dolichopodidae[R]			PR	SP	4	9	0	0	4
	Empididae	*Hemerodromia*		PR	CR	21	22	83	18	13
		Oreogeton[R]		PR	SP	0	9	0	0	0
	Ephydridae			CG	SP	0	9	22	18	13

Muscidae[R]		T	PR	SP	0	13	4	0	4
	Limnophora	T	PR	BU	0	4	4	23	22
Psychodidae	*Pericoma*	T	CG	BU	0	26	17	27	57
	Psychoda	T	CG	BU	0	4	0	36	22
Ptychopteridae	*Bittacomorpha*[R]		CG	BU	0	0	9	0	0
Simuliidae	*Simulium*		CF	CL	42	70	74	64	57
	Prosimulium		CF	CL	17	35	0	14	30
Syrphidae	*Eristalis*[R]	T	CF	BU	0	0	4	0	0
Tabanidae	*Chrysops*		CG	BU	25	57	43	9	22
Tipulidae	*Antocha*		CG	CL	79	91	43	32	4
	Dicranota		PR	CR	38	48	0	9	9
	Hexatoma		PR	CR	67	78	61	9	17
	Molophilus		CG	BU	4	13	13	9	22
	Ormosia		CG	BU	4	13	17	0	4
	Pedicia		PR	BU	0	0	4	0	0
	Pilaria[R]		PR	BU	0	0	0	0	4
	Pseudolimnophila		PR	BU	17	4	39	0	26
	Rhabdomastix[R]		-	-	0	9	4	0	4

Tipula	SH	BU	8	57	26	41	48

CHAPTER 2

Predicting changes in benthic macroinvertebrate assemblage structure in response to increasing levels of cattle grazing in Blue Ridge mountain streams, Virginia, USA

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Abstract

The relationship between macroinvertebrate assemblages and cattle density was assessed from fall 2002 through spring 2004 in five small streams that represented a gradient of cattle grazing intensity. All study stream reaches were in pasture with no woody riparian vegetation, but varied in the intensity of cattle grazing (0 cattle ha^{-1} at site 1 to 2.85 cattle ha^{-1} at site 5). Regression analysis was used to determine if grazing intensity (cattle ha^{-1}) was an important determinant of macroinvertebrate metrics and taxa. Metric similarity and taxon composition was examined among study sites to determine the point along the cattle grazing gradient where macroinvertebrate assemblages change appreciably from reference conditions. Regression analysis indicated highly significant and strong macroinvertebrate metric responses to cattle density during most sampling periods. The majority of metrics responded negatively to the grazing gradient, while a few (total taxa richness, number of sensitive taxa, and % collector filterers) increased along the gradient before declining at the most heavily grazed sites. Total number of sensitive taxa and % Coleoptera had the strongest relationship with cattle density throughout the study period. During some sampling periods, nearly 80% of the variation in these metrics was explained by cattle density. The elmid beetle, *Oulimnius*, had a particularly strong negative response to the grazing gradient. Study site groupings from Tukey-Kramer post hoc tests and detrended correspondence analysis (DCA) indicated that benthic samples collected from the reference site and light rotational grazing site were more similar in macroinvertebrate composition than samples collected from the intermediate grazing and heavy grazing sites. Our findings demonstrate that cattle density alone is a good predictor of benthic macroinvertebrate assemblages and that small streams in the Blue Ridge with light rotational cattle grazing (1 cattle ha^{-1}) can sustain benthic macroinvertebrate assemblages relative to reference conditions.

Introduction

In southwestern Virginia, cattle are commonly grazed in pasture where they have unrestricted access to small fist and second order streams. Results from past research studies clearly demonstrate that allowing cattle unlimited access to streams alters stream habitat and water quality which in turn negatively impacts biological integrity. Cattle impacted streams typically lack woody riparian vegetation so sunlight and temperatures are increased while inputs of coarse organic matter are reduced (Cook, 2003). Nutrient and organic matter loads increase as a result of cattle urine and feces (Dance & Hynes 1980; Strand & Merritt, 1999). Cattle grazing around streams physically destructs stream channels. Stream banks erode and become a significant source of fine inorganic sediments (Trimble & Mendel, 1995; Wohl & Carline, 1995; Owens, Edwards & Van Keuren, 1996). These cattle-induced changes to stream habitat and water quality have negative impacts on biological integrity. (Kauffman & Krueger, 1984; Armour *et al*., 1991).

Most research studies that have addressed the effects of livestock grazing on benthic macroinvertebrates have used study designs that only compare streams where cattle have access to streams where cattle are absent (i.e., grazing vs. no grazing). Streams without cattle grazing, which serve as a reference for comparison, have typically been relatively undisturbed, forested streams. On the contrary, the cattle impacted streams inherently have had little to no woody riparian vegetation and, thus, received much more sunlight. Research findings from these study designs have consistently reported drastic shifts in benthic macroinvertebrate assemblage structure and function. As a result of the differences in riparian vegetation, taxa that graze algae and collect fine particles of detritus often dominate assemblages in cattle impacted streams while shredding taxa that prefer terrestrial organic inputs, i.e., leaves and wood, are reduced (Delong & Brusven, 1998; Cook 2003). Sensitive Ephemeroptera, Plecoptera, and Trichoptera taxa are replaced by taxa more tolerant of harsh environmental conditions, e.g., Chironomidae, and benthic macroinvertebrates that require substrates free of fine sediment are eliminated and replaced by burrowing taxa (Dance & Hynes, 1980; Harding & Winterbourn, 1995; Quinn *et al*., 1997; Collier, Wilcock & Meredith, 1998; Delong & Brusven, 1998). Overall, the total number of taxa decline and benthic macroinvertebrate assemblages become more homogenous.

It has been well documented that cattle-impacted streams have altered benthic habitat and macroinvertebrate assemblages relative to forested, reference streams. However, there have been far fewer studies that address the more subtle changes that may occur in streams with increasing levels of cattle grazing on lands that have been previously deforested to establish pastures. Not knowing the level at which grazing intensity begins to alter benthic macroinvertebrate assemblages and what the structure of those assemblages will be makes it difficult to develop agricultural best management practices that maintain acceptable water quality and biological integrity in streams.

Although cattle grazing intensity is likely an important determinant of benthic macroinvertebrate assemblages, we are aware of only a few published studies that have attempted to address this subject. In studying agriculture intensity along a river continuum in New Zealand, Harding *et al*. (1999) suggested that the intensity of agriculture may be more useful in assessing river health than the percent of agricultural land use in a watershed. Sovell *et al*. (2000) compared five macroinvertebrate metrics among streams subjected to rotational and continuous cattle grazing in Minnesota, U.S. and found inconsistent macroinvertebrate metric responses among sampling periods and within streams. In Alberta, Canada, Scrimgeour & Kendall (2003) used a 2-year, cattle-enclosure study to examine the benthic macroinvertebrate response to four livestock grazing treatment levels. They found little response by the benthic macroinvertebrates, which they attributed to the short time frame of their study.

In this study, we directly assess the relationship between livestock grazing intensity and benthic macroinvertebrates. Our study design is unique in that all study stream reaches were in pasture, with no woody riparian vegetation, but varied in the intensity of cattle grazing. Using this study design, we attempted to answer two research questions: (1) do benthic macroinvertebrates change predictably in response to different levels of cattle grazing and (2) if there are predictable responses, at what level of grazing intensity do benthic macroinvertebrate assemblages begin to change?

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, U.S. The Blue Ridge physiographic province is characterized by deeply dissected valleys and ravines that are primarily composed of metamorphosed igneous rocks (granites, granidiorite, 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 (Virginia Agricultural Statistics Service, 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 (Virginia Agricultural Statistics Service, 2004).

Five, first-order stream reaches in the Little River drainage basin, Floyd Co., Virginia were selected as study sites (Fig. 2-1). 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 differed in the density of cattle (Table 2-1). 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 2-1, Barbour et al. 1999). Following this protocol, 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 2005), reference sites rarely have habitat scores below 140, and scores less than 120 generally indicate impaired conditions.

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 habitat score at site 1 (159) also supported the use of this site as a reference.

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

Benthic samples were collected by inserting a modified stovepipe corer (0.30 m diameter) approximately 10 cm into streambed substrates. Within the core, macroinvertebrates, organic detritus, and inorganic substrate were removed by hand and with the aid of a hand pump. All material was preserved with 95% ethanol and transported to the laboratory for analysis. Benthic samples were elutriated and rinsed through a 250-µm sieve in the laboratory. Macroinvertebrates were hand sorted from organic detritus under a dissecting microscope (10x magnification); benthic samples were sorted in their entirety and were not subsampled. Macroinvertebrates were 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.

Data analyses

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., Barbour et al. 1999 and Brigham et al. 1982) 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 to 10, with 0 indicating very sensitive and 10 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 (see Table 2-3).

Regression analysis was used to explore whether there were predictable relationships between benthic macroinvertebrate metrics and the grazing gradient. With the exception of total taxa richness and the number of sensitive taxa, metrics were either arc sin or $\log_{10}(x+1)$ transformed and taxa abundances were $\log_{10}(x+1)$ transformed, to meet the equal variance assumption of analysis of variance (ANOVA) tests. Individual regression tests were performed for each metric in every sampling period resulting in 52 individual tests. A quadratic model was selected only if the quadratic term differed significantly from zero.

To determine the point along the grazing gradient where macroinvertebrate assemblages changed appreciably, we grouped study sites based on metric and taxa composition similarity. Groups were established using two methods. First, study sites which represented different levels of grazing were considered as treatments with macroinvertebrate metrics as responses. Data were analyzed using one-way ANOVA followed by Tukey-Kramer post hoc tests. Study sites were grouped if the majority of metrics were similar between them. Our second grouping method involved multivariate ordination. Detrended correspondence analysis (DCA) using PC-ORD (McCune & Mefford, 1999), without downweighting or axis rescaling, was used to ordinate benthic samples in species space using the relative abundance of 60 taxa. Rare taxa (those that made up less than 0.02% of total macroinvertebrate abundance) were removed prior to DCA to reduce variability in the dataset (Gauch, 1982). Following DCA, regression analysis was used to statistically test the relationship between the 60 individual taxa that were used in the ordination and the grazing gradient.

Throughout our statistical analyses, we performed multiple independent statistical tests, which may increase the probability of making a Type I error (procedure wise error). Bonferroni adjustments were made to control for procedure wise error by adjusting the test significance level (alpha) by the number of repeated tests. Results were considered significant only if *P* was less than the adjusted alpha; however the original *P* values are also reported so readers can interpret the results without Bonferroni adjustments if desired (Perneger 1998).

Results

A total of 204,916 individuals, belonging to 112 macroinvertebrate taxa, were identified during this study (Table 2-2). Fifty-two taxa, or 46% of the assemblage (0.3% of total assemblage abundance), were rare. Chironomidae (68 – 46,186 individuals m^{-2}), Oligochaeta (0 – 9,218 individuals m^{-2}), and Copepoda (0 – 8,641 individuals m^{-2}) were the most numerically abundant, respectively, and comprised approximately 75% of the total individuals collected. Other common taxa, which comprised an additional 20% of the assemblage composition, included *Ephemerella*, *Oulimnius*, *Leuctra*, *Baetis*, Ceratopogonidae, *Allocapnia*, Pleuroceridae, Nematoda, Hydracarina, *Diplectrona*, Sphaeriidae, *Paraleptophlebia*, *Simulium*, and *Antocha*, respectively.

Predicting benthic macroinvertebrates responses to the grazing gradient

Following Bonferroni adjustments, 46 out of 52 regression analyses were statistically significant (Table 2-3). Eleven of the 13 metrics showed a negative response to the grazing gradient while 2 metrics had a positive response to the grazing gradient. Although % Coleoptera, Simpson's diversity, % scrapers, % sensitive taxa, % clingers, and % crawlers always declined along the grazing gradient, the rates of decline (whether models were quadratic or linear) varied among sampling period (Table 2-3, Fig. 3-2a). Three metrics (total taxa richness, % collector-filterers, number of sensitive taxa) had concave responses to the grazing gradient; metric values increased slightly before declining along the gradient (Table 2-3, Fig. 2-2b.). Four metrics had consistent linear responses throughout the study period; % Plecoptera and % shredders always had a negative linear relationship with cattle density while % collector-gatherers and % burrowers always had positive linear responses to the grazing gradient (Table 2-3).

The responses between most metrics and the grazing gradient were relatively weak during fall 2002 and spring 2004. A severe drought occurred in the southeastern U.S. between 1998-2001 and streams during the fall 2002 sampling period were well below base flow. On the other hand, spring 2004 was an abnormally wet year and streams were above base flow during this sampling period. Streams were sampled at normal base flow during the spring 2003 and fall 2003 sampling periods. We attribute the weak relationships between the metrics and the grazing gradient in fall 2002 and spring 2004 to extreme flow conditions and did not include data from these sampling periods in further analyses.

Eight metrics (% Coleoptera, % collector-gatherers, % scrapers, % sensitive taxa, number of sensitive taxa, % clingers, % crawlers, % burrowers) had strong relationships ($r^2 \geq 0.5$) with the grazing gradient under base flow conditions in spring and fall 2003. Simpson's diversity showed a strong response to the grazing gradient only during spring 2003, while total taxa richness, % Plecoptera, and % shredders showed strong responses to the gradient only during fall 2003. Percent Coleoptera and number of sensitive taxa had exceptionally strong and consistent relationships with cattle density, even during the extreme flow conditions (fall 2002 and spring 2004). Cattle density explained approximately 80% of the variation in the number of sensitive taxa during fall 2003 and 78% of the variation in the relative abundance of beetles in spring 2003 (Table 2-3, Figs. 2-2a and 2-2b).

Cattle grazing thresholds

Based on results of ANOVA and Turkey-Kramer post hoc tests, two groups were formed from metric similarity among sites. Sites 1 and 2 shared the most metric similarity; 11 of the 13 test metrics were not statistically different (Table 2-4). Therefore, sites 1 and 2 formed group 1. Eight of the 13 metrics were not statistically different between sites 3 and 4, so these sites were combined to form group 2. Site 5 was placed in group 2 because 7 of the 13 metrics did not differ significantly from site 4.

The study site grouping pattern that emerged from the ordination graphic was the same pattern that formed from the ANOVA grouping method. DCA separated benthic samples collected from sites 1 and 2 from benthic samples collected from sites 3, 4, and 5 (Fig. 2-3a). The majority of benthic samples collected from sites 1 and 2 grouped together toward the left of the ordination. Benthic samples from sites 3, 4, and 5 formed a second group on the right side of the ordination.

Macroinvertebrate taxa associated with benthic samples are shown in Fig. 2-3b. Taxa associated with group 1 were mostly insects: mayflies (*Baetis*, *Paraleptophlebia*, *Epeorus*, *Stenonema*), stoneflies (*Allocapnia*, *Isoperla*, *Amphinemura*, *Tallaperla*, *Suwalia*, *Leuctra*), caddisflies (*Diplectrona*, *Glossosoma*, *Psilotreta*, *Wormaldia*, *Rhyacophila*), beetles (*Oulimnius*, *Optioservus*, *Stenelmis*, *Ectopria*), and flies (*Tipula*, *Hexatoma*, *Antocha*, *Dicranota*, *Ormosia*). In addition, the non-insect taxon, Pleuroceridae was associated with group 1. Taxa associated with benthic samples that formed group 2 included fewer insects: mayflies (*Ephemerella*, *Eurylophella*), caddisflies (*Neophylax*, *Agarodes*), and flies (Chironomidae, *Pericoma*, *Limnophora*, *Hemerodromia*, *Ormosia*, *Pseudolimnophila*). Several non-insect taxa (Nematoda, Oligochaeta, Sphaeriidae, Ancylidae) were associated with group 2.

Eighteen of the 60 taxa used in the ordination had a statistically significant relationship with the cattle grazing gradient (Table 2-5). *Oulimnius* and *Ephemerella* slightly increased along the gradient before declining at cattle densities > 0.1 cattle ha^{-1} (Fig. 2-4). Pleuroceridae and *Optioservus* declined at a slower rate than taxa that had a negative linear relationship with the grazing gradient. Elmid beetles (*Oulimnius* and *Optioservus*) showed particularly strong responses to the grazing gradient; 57% of the variation in *Oulimnius* densities was explained by cattle density. Chironomidae was the only taxon that had a significant positive response to the grazing gradient.

Discussion

Our intensive field sampling through space (12 replicates per study site) and time (4 sampling periods) and lack of laboratory subsampling resulted in a large proportion of rare taxa and exceptionally high abundances of Oligochaetes, Copepods, and Chironomidae. If we had used a sampler fitted with mesh, e.g., Surber or Hess sampler, or a larger sieve size (500-600 µm) we would not have collected benthic copepods, nematodes, and early instars of midges and elmids and, thus, may not have been able to detect patterns we report here. Due to our detailed field and laboratory methods, we feel that our results closely represent the true benthic macroinvertebrate assemblages.

Most of the observed metric responses that were explained by cattle density are probably caused by alterations to food resources and physical habitat associated with cattle grazing. Low numbers of shredders and scrapers and high numbers of collector-gatherers and filterers were likely the result of direct or indirect cattle-induced changes to food resources. Although none of our study sites were forested, other types of non-woody riparian vegetation were abundant in the riparian zone of the reference site but were absent at other sites along the gradient because cattle grazed in riparian areas. Absence of riparian vegetation reduces loads of coarse particulate organic matter inputs into the streams and could account for the decline in shredders. The increase in collector-gatherers along the gradient is likely the result of elevated deposits of fine particulate organic matter (FPOM) in streams with high cattle density. Benthic FPOM has several sources related to cattle: decomposition of organic solids in feces, excess algae production and subsequent decomposition of senescent cells, and excessive production of heterotrophic microbes (fungus, bacteria) caused by increased nutrients and dissolved organic compounds from cattle feces and urine (see Hynes, 1971 and Mason, 1996). The decline in scrapers was possibly the result of altered periphyton food resources. Where nutrient and organic matter loading is elevated, periphyton can contain less palatable species (e.g., cyanobacteria), more dead and senescent algae cells, and more fungi and bacteria, all of which reduces the nutritional value of the food that scrapers must consume.

Macroinvertebrate habits also changed predictably with the cattle grazing gradient. Cattle-impacted streams usually have unstable, trampled stream banks, which become significant sources of inorganic sediments when they erode. When bedload sediments are excessive, the undersides of large stable stones, i.e., boulders and cobble, become embedded, so clingers and crawlers no longer have access to stable substrate and interstitial spaces beneath streambeds. Under natural conditions in erosional zones, interstitial spaces of varying sizes usually extend at least 10 - 20 cm down in the stream bottom. These spaces are essential habitat for crawlers 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 be washed downstream. Sand and silt that erodes from unstable stream banks clog the interstitial spaces and physical habitat becomes unsuitable for crawlers. However, the influx of sand and silt creates additional habitat for burrowers, which require loose fine particles for their movement. The net effect of cattle grazing is that patches of clean, firm, stable stones and open interstitial spaces are less frequent while patches of soft, unstable sand and silt are more frequent; hence, clingers and crawlers decrease while burrowers increase (Cordone & Kelley, 1961; Chutter, 1969; Lenat, Penrose & Eagleson, 1981; Lenat, 1984; Wood & Armitage, 1997). The physical habitat on the upper surfaces of large stable substrate also changes to the detriment of clingers and crawlers. These taxa have highly evolved morphological and behavioral adaptations, such as suction, silk, claws, etc., that allow them to maintain their positions on clean substrate in fast current. Where suspended fine particles settle or where excessive growths of algae and heterotrophic microbes cover streambed substrates, clingers and crawlers are eliminated because they can no longer maintain their positions.

The concave relationship between total taxa richness, % collector-filterers, total number of sensitive taxa and the grazing gradient is a pattern that has not been reported for benthic macroinvertebrates in relation to cattle grazing. It is possible that the fauna at the reference site (site 1) has not completely recovered from previous cattle grazing. However, it is also possible that low levels of disturbance caused by cattle grazing, e.g., at site 2, enhances food resources by providing nutrients and organic matter that stimulates algae production and elevates FPOM but does not decimate physical habitat for clinging and crawling taxa. Cattle grazing at low densities probably increased benthic habitat heterogeneity by creating patches of fine sand and silt while patches of clean substrate and interstitial spaces also remained available. Therefore, the availability of different substrate patches provided habitat for a larger number of taxa. These patterns, of increased macroinvertebrate metric values at low levels of grazing, support the intermediate disturbance hypothesis (Connell, 1978), which suggests that low levels of disturbance has a positive effect on diversity. Increased density of clinging, scraping taxa such as *Oulimnius*, Pleuroceridae, *Optioservus* and collector-filterers at the light rotational level of grazing (site 2) is evidence that physical habitat was suitable for clinging taxa. Taxa shifts within collector-filterers provided further evidence for altered physical habitat along the grazing gradient. Pollution sensitive, clinging, collector-filterers, e.g., *Diplectrona* and *Wormaldia*, were associated with samples from the reference and rotational grazing streams, while the burrowing, pollution tolerant, fingernail clams (Sphaeriidae) were more abundant in samples from the high grazing streams. These shifts from clinging collector-filterers to burrowing collector-filterers suggest that FBOM food resources were always abundant but physical habitat changed along the grazing gradient.

The strong relationships between the cattle grazing gradient and the number of sensitive taxa and % Coleoptera during nearly all sampling periods suggests they are robust metrics for assessing impacts of cattle grazing in these small, first order streams, even during extreme flows. Larvae of riffle beetles (Elmidae) were the most abundant Coleoptera taxa in this study (0 – 2,291 individuals m^{-2}). The response of elmids, especially *Oulimnius*, to the cattle grazing gradient is particularly noteworthy. Elmids occur in shallow, fast flowing riffles where they cling to substrate and feed by scraping hard surfaces for algae and detritus (Brown, 1987). In European studies, distributions of aquatic Coleoptera have been related to land cover and coal mining pollution (García-Criado & Fernández-Aláez, 2001; Eyre, Foster & Luff, 2005). Eyre *et al*. (1993) reported that silt was an important determinant of beetle distribution, especially elmids, in streams in northern England. Miyake & Nakano (2002) clearly demonstrated that the abundance of *Optioservus kubotai* was strongly influenced by deposited sediment in a Japanese stream. On the contrary, using aquatic Coleoptera to assess anthropogenic disturbance has not been well studied in the U.S. The only published work we found that addresses Coleoptera distributions in relation to water pollution in the U.S. is a study by Sinclair (1964). He provided a thorough description of elmid species distributions in western Tennessee in relation to a variety of water pollutants and suggested elmids as useful indicators of a wide variety of water pollutants. Our findings demonstrate that elmids respond predictably to cattle grazing intensity, and we believe that aquatic Coleoptera, especially riffle beetles, have great potential as tools for assessing water pollution.

The large amount of variation in macroinvertebrate metrics that was explained by cattle density clearly demonstrates that grazing intensity is an important determinant of the structure and function of benthic macroinvertebrate assemblages. Despite not knowing the specific mechanisms by which stress from cattle grazing is imposed upon benthic macroinvertebrates, e.g., physical habitat versus trophic, cattle density alone is a good predictor of benthic macroinvertebrate assemblages in small streams. Furthermore, the stream groupings that emerged from ANOVA and ordination analyses provide strong statistical and biological evidence that small streams with light rotational cattle grazing with 1 cattle ha^{-1} can sustain benthic macroinvertebrate assemblages relative to reference conditions. Because sites 1 and 2 were similar and sites 3, 4, and 5 were never grouped with sites 1 and 2, we conclude that benthic macroinvertebrate assemblages in these small Blue Ridge streams change appreciably from reference conditions when the level of continuous grazing reaches 1.5 cattle ha^{-1}.

The inconsistency in the strength of most metric-grazing gradient relationships among seasons demonstrates the importance of sampling effort through time. Variation in the strength of relationships among seasons also suggests that natural disturbance, e.g., drought and flood, may override the effects of cattle grazing on benthic macroinvertebrate assemblages. Our results only apply to small streams in the Blue Ridge ecoregion. Other factors that may influence benthic macroinvertebrate responses to cattle grazing intensity include geographic region, slope, soil type, stream elevation, and size. We suggest that future research on the effects of cattle grazing will be most informative if conducted with a study design that includes a gradient of grazing intensity.

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Table 2-1 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*	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})†					
Minimum	10	8	8	14	2
Maximum	62	47	25	77	10
Average	25	25	15	30	5
Mean wetted width (m)‡	0.88	0.72	1.11	0.76	0.60
Mean depth (m) ‡	0.08	0.13	0.13	0.09	0.10
Conductivity (µS cm^{-1})£	16 - 22	18 - 23	54 - 63	19 - 22	54 – 57
Maximum temperature (°C)£	18.5	20.0	20.5	21.5	21.5

* 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).

† Baseflow discharge was measured on 8 separate occasions between July 2002 and August 2003.

‡ 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).

£ Conductivity and temperature values are based on 15 spot measures taken throughout the study period.

Table 2-2 Mean density of taxa at each study site. The superscript, R, indicates a taxon was rare. Data are from all 4 sampling periods; *n* = total number of benthic samples collected at each study site.

			1 *n=45*	2 *n=48*	3 *n=45*	4 *n=45*	5 *n=47*
NON-INSECTA							
	Planariidae		9.45	6.57	149.67	4.57	27.14
Nematoda			56.39	122.03	203.02	24.39	267.05
Oligochaeta			319.77	985.65	1,987.80	672.76	1,789.68
Hirudinea[R]			0	0	3.35	0	0
Copepoda			399.02	775.89	886.14	199.97	1,343.43
Amphipoda	Gammaridae	*Gammarus*	0	0	7.93	0	4.38
Decapoda	Cambaridae		2.44	2.86	2.13	1.52	1.46
Gastropoda	Ancylidae		0.30	132.03	0	0	0
	Lymnaeidae[R]		0.30	0	0.30	0	0
	Planorbidae[R]		0	0	0.91	0	0
	Pleuroceridae		265.20	118.60	142.05	218.56	4.96
	PHYSIDAE[R]		0	0.57	0.61	0	0
Bivalvia	Corbiculidae	*Corbicula fluminea*	0	2.00	10.67	0	0.29

	Sphaeriidae		12.50	40.29	362.44	110.04	69.46
Hydracarina			100.59	233.48	236.24	30.79	25.68
INSECTA							
Ephemeroptera	Ameletidae	*Ameletus*[R]	0	0.86	1.22	0	0.29
	Baetidae	*Baetis*	219.17	99.74	247.83	210.33	145.64
	Baetiscidae	*Baetisca*[R]	0.61	1.14	1.52	0	0
	Ephemeridae	*Ephemera*[R]	0	20.29	0.61	0.30	0
	Ephemerellidae	*Drunella*[R]	0	1.71	0	0	0
		Ephemerella	102.42	208.33	876.10	44.51	25.01
		Eurylophella	65.84	52.01	106.69	3.05	2.04
		Serratella[R]	0	0.57	0	0	0
	Heptageniidae	*Cinygmula subaequalis*[R]	0	1.14	0	0	0
		Epeorus	20.12	8.00	7.01	8.23	4.38
		Stenonema	12.80	7.72	9.14	3.05	0
	Leptophlebiidae	*Habrophlebia vibrans*[R]	0	0.29	2.74	0	0
		Habrophlebiodes[R]	0.61	1.14	2.74	0	0
		Paraleptophlebia	139.92	267.78	123.46	42.68	2.92
Odonata	Coenagrionidae	*Argia*[R]	0	0	0.30	0	0
	Cordulegastridae	*Cordulegaster*[R]	1.22	0	0	0	0

	Gomphidae	*Gomphus*[R]	0.30	0.86	0.61	0	0
		Lanthus	5.79	7.43	0	0	0.58
		Stylogomphus albistylus[R]	0.30	0	0.30	0	0
	Libellulidae	*Libellula*[R]	0.30	0	0	0	0
Plecoptera	Capniidae	*Allocapnia*	303.00	353.22	31.09	46.33	133.96
	Chloroperlidae	*Suwalia*	16.16	26.01	12.80	2.13	1.17
		Sweltsa[R]	0.61	0	0	0	0
	Leuctridae	*Leuctra*	254.84	470.68	151.81	149.98	50.78
	Nemouridae	*Amphinemura*	23.78	17.72	2.44	4.57	4.96
	Peltoperlidae	*Tallaperla*	44.51	58.58	14.02	9.45	1.17
	Perlidae	*Acroneuria*[R]	2.13	0.29	0.61	0	0
		Eccoptura xanthenes[R]	0	0	0.30	0	0
	Perlodidae	*Cultus*[R]	1.83	0	0	0	0
		Isoperla	54.56	158.32	59.14	28.96	30.35
		Remenus bilobatus[R]	0.91	0	0	0.61	0
		Yugus	5.18	11.72	0.91	1.52	0
	Pteronarcyidae	*Pteronarcys*	0	12.57	0	0	0
	Taeniopterygidae	*Taeniopteryx*[R]	2.74	0	0	0	0.29
Megaloptera	Corydalidae	*Nigronia fasciatus*[R]	0.61	0	0	0.61	0
	Sialidae	*Sialis*[R]	0.30	0	0.30	0	0

Trichoptera	Glossosomatidae	*Agapetus*[R]	0.91	0	0.91	1.52	0
		Glossosoma nigrior	13.41	66.87	7.32	16.46	0
	Hydropsychidae	*Diplectrona*	49.99	455.82	85.66	2.74	25.01
		Hydropsyche	0	19.15	200.58	0.30	3.21
	Lepidostomatidae	*Lepidostoma*	5.49	11.72	5.49	2.44	0.58
		Theliopsyche[R]	0	0.57	0	0.30	0
	Leptoceridae	*Oecetis*[R]	1.22	1.71	1.22	0	0
		Setodes[R]	4.57	0.57	0.91	0	0
	Limnephilidae	*Goera*	1.22	11.72	37.80	1.52	0
		Pycnopsyche[R]	2.44	1.14	0.30	0	0.58
	Odontoceridae	*Psilotreta*	15.85	4.57	3.35	0.91	0
	Philopotamidae	*Chimarra*[R]	0	0	2.74	0	0
		Dolophilodes[R]	0	0	0.30	0	0
		Wormaldia	3.66	2.57	8.54	2.13	0
	Phryganeidae	*Oligostomis*[R]	0	1.14	5.18	0.61	0
		Ptilostomis[R]	0	0.29	0.61	0.61	0
	Polycentropodidae	*Polycentropus*[R]	0.61	0	0	0	0
	Psychomyiidae	*Lype diversa*[R]	4.27	1.43	0.30	0.30	0
	Rhyacophilidae	*Rhyacophila*	7.93	14.86	0.61	1.22	0.29
	Sericostomatidae	*Agarodes*	0.91	9.72	24.08	0.30	0

		Fattigia pele[R]	0	0.29	0	0	0
	Uenoidae	*Neophylax*	11.28	4.57	35.36	8.84	3.79
Coleoptera	Dryopidae	*Helichus*[R]	0.30	0	0	0	0
	Dytiscidae[R]		0	0	0	0	0.88
	Elmidae	*Dubiraphia*[R]	0	0	0.61	0	0
		Optioservus	63.40	78.88	41.46	1.83	0
		Oulimnius latiusculus	302.39	634.43	233.81	25.91	6.42
		Promoresia	27.13	80.88	2.13	1.52	0
		Stenelmis	6.71	0.86	6.10	0	0
	Hydrophilidae	*Tropisternus*[R]	0	0.29	0.61	0.30	1.46
	Psephenidae	*Psephenus herricki*	9.14	0.29	11.89	0	0
		Ectopria	31.70	7.72	3.05	4.57	0.29
	Ptilodactylidae	*Anchytarsus*	5.18	8.57	2.74	0	0
Diptera	Blephariceridae	*Blepharicera*[R]	0	2.57	0	2.74	0
	Ceratopogonidae		183.20	218.34	301.48	54.56	132.80
	Chironomidae		4,725.49	5,788.744	10,695.00	3,932.32	10,925.76
	Dixidae	*Dixa*[R]	1.52	0.29	0	0	1.46
	Dolichopodidae[R]		0.30	0.86	0	0	0.58
	Empididae	*Chelifera*[R]	0	0.29	0	0.30	0
		Clinocera[R]	0	2.86	0.30	0.30	0

	Hemerodromia	4.57	12.29	224.97	3.35	13.72
	Oreogeton[R]	0	0.57	0	0	0
Ephydridae		0	1.14	1.83	4.27	3.21
Muscidae[R]		0	0.86	0.30	0	0.29
	Limnophora	0	2.00	0.61	3.96	17.51
Psychodidae	*Pericoma*	0	3.43	4.27	2.13	7.88
	Psychoda	0	0.57	0	17.99	2.92
Ptychopteridae	*Bittacomorpha*[R]	0.30	0	1.83	0	0
Simuliidae	*Simulium*	196.01	90.59	112.48	39.02	89.60
	Prosimulium	4.57	10.57	1.52	0.91	7.30
Syrphidae	*Eristalis*[R]	0	0	0.30	0	0
Tabanidae	*Chrysops*	3.05	8.86	10.67	1.22	2.63
Tipulidae	*Antocha*	67.98	72.87	266.42	81.69	13.13
	Dicranota	24.08	24.01	2.74	4.27	2.04
	Hexatoma	21.34	35.15	17.99	1.52	1.46
	Molophilus[R]	0.30	1.43	0.91	0.61	3.21
	Ormosia	0.61	1.71	2.74	0	1.46
	Pedicia	0	0	0	0	0
	Pilaria[R]	0	0	0	0	0.29
	Pseudolimnophila	3.96	0.86	13.41	2.44	6.13

Rhabdomastix[R]	0	0.57	0.30	0	0.29
Tipula	1.83	16.00	5.79	10.97	12.55

Table 2-3 Results from regression analyses for macroinvertebrate metrics versus cattle density for each sampling period. N = the total number of benthic samples that were collected during each sampling period. Signs in front of coefficient of determination (r^2) indicate the direction of the relationship; q indicates the relationship was quadratic. The superscript NS indicates results were not stastically significant following Bonferroni adjustments.

	Fall 2002		Spring 2003		Fall 2003		Spring 2004	
	n = 59		*n* = 57		*n* = 56		*n* = 58	
	r^2	*P*	r^2	*P*	r^2	*P*	r^2	*P*
Richness	q 0.3330	<0.0001	q 0.4392	<0.0001	q 0.5620	<0.0001	q 0.4734	0.0033 ^{NS}
% Plecoptera	- 0.2633	<0.0001	- 0.3637	<0.0001	- 0.6026	<0.0001	- 0.2438	<0.0001
% Coleoptera	- 0.6391	<0.0001	- 0.7772	<0.0001	q 0.7292	<0.0001	q 0.5387	<0.0001
Simpson's Diversity	q 0.4691	<0.0001	- 0.6622	<0.0001	q 0.3913	<0.0001	- 0.0013	0.7860 ^{NS}
% collector-gatherers	+ 0.1098	0.0103 ^{NS}	+ 0.7279	<0.0001	+ 0.5349	<0.0001	+ 0.1127	0.0100 ^{NS}
% collector-filterers	q 0.5247	<0.0001	q 0.2664	<0.0001	q 0.2556	0.0004	- 0.4403	<0.0001
% scrapers	q 0.3298	<0.0001	- 0.7111	<0.0001	q 0.7117	<0.0001	q 0.5005	<0.0001
% shredders	- 0.0727	0.0388 ^{NS}	- 0.4282	<0.0001	- 0.6207	<0.0001	- 0.2366	<0.0001
%sensitive taxa	q 0.3502	<0.0001	- 0.6906	<0.0001	q 0.7367	<0.0001	q 0.5015	<0.0001
Number of sensitive taxa	- 0.5130	<0.0001	- 0.5275	<0.0001	q 0.7949	<0.0001	q 0.5446	<0.0001
% clingers	q 0.2417	0.0004	- 0.7174	<0.0001	q 0.5901	<0.0001	- 0.1364	0.0043 ^{NS}
% crawlers	- 0.3849	<0.0001	- 0.5513	<0.0001	q 0.7497	<0.0001	q 0.4601	<0.0001
% burrowers	+ 0.2924	<0.0001	+ 0.7221	<0.0001	+ 0.5476	<0.0001	+ 0.0620	<0.0001

Table 2-4 Average values of macroinvertebrate metrics (±SE) within study sites. Metrics with different letters indicate significant differences based on Tukey-Kramer post hoc tests. Data are from the spring 2003 and fall 2003 sampling periods. N = the total number of benthic samples that were collected at each study site.

	P	Site 1 *n* = 23	Site 2 *n* = 24	Site 3 *n* = 21	Site 4 *n* = 22	Site 5 *n* = 23
Richness	<0.0001	27.17 ± 1.32 a	31.42 ± 1.28 a	28.81 ± 1.52 a	15.50 ± 1.25 b	15.39 ± 1.23 b
% Plecoptera	<0.0001	10.04 ± 1.23 a	6.29 ± 0.95 a	0.81 ± 0.20 b	1.64 ± 0.38 b	0.48 ± 0.19 b
% Coleoptera	<0.0001	5.78 ± 0.72 a	5.21 ± 0.66 a	1.81± 0.32 b	0.45 ± 0.11 c	0 d
Simpson's Diversity	<0.0001	0.70 ± 0.02 a	0.67 ± 0.04 a	0.61 ± 0.03 a	0.41 ± 0.04 b	0.33 ± 0.04 b
% collector-gatherers	<0.0001	65.00 ± 2.18 a	67.67 ± 2.11 a	82.67 ± 1.74 b	88.14 ± 1.27 bc	92.26 ± 1.10 c
% collector-filterers	<0.0001	2.61± 0.60 cb	5.5 ± 1.02 a	3.86 ± 0.91 ab	2.27 ± 0.45 cb	1.04 ± 0.25 c
% scrapers	<0.0001	11.91 ± 1.39 a	8.54 ± 0.81 a	3.24 ± 0.48 b	3.95 ± 0.60 b	0 c
% shredders	<0.0001	9.13 ± 1.17 a	4.89 ± 0.74 b	0.86 ± 0.27 c	1.05 ± 0.31 c	0.48 ± 0.19 c
% sensitive	<0.0001	23.87 ± 1.70a	21.04 ± 2.74 a	9.95 ± 1.27 b	6.45 ± 0.87 b	0.78 ± 0.19 c
Number of sensitive taxa	<0.0001	11.57 ± 0.73 ab	12.83 ± 0.69 a	10.29 ± 0.62 b	6.05 ± 0.66 c	3.17 ± 0.43 d
% clingers	<0.0001	19.04 ± 1.76 a	15.29 ± 1.46 ab	8.38 ± 1.64 c	11.18 ± 1.93 bc	1.00 ± 0.24 d
% crawlers	<0.0001	15.74 ± 1.26 a	12.96 ± 1.64 ab	10.10 ± 1.32 b	3.36 ± 0.56 c	1.00 ± 0.18 c
% burrowers	<0.0001	58.17 ± 2.54 a	63.63 ± 2.76 a	75.81± 2.62 b	82.68 ± 2.24 b	92.96 ± 1.03 c

Table 2-5 Results from regression analysis for macroinvertebrate taxa versus cattle density. Relationships were quadratic (q), positive linear (+), or negative linear (no symbol). Date are from spring 2003 and fall 2002 (*n* = 113). All relationships were significant following Bonferroni adjustments.

Taxon	r^2	*P* value
Oulimnius	q 0.5685	< 0.0001
Pleuroceridae	q 0.4585	< 0.0001
Optioservus	q 0.4283	< 0.0001
Ephemerella	q 0.3851	< 0.0001
Paraleptophlebia	0.3860	< 0.0001
Leuctra	0.3622	< 0.0001
Hexatoma	0.3118	< 0.0001
Ectopria	0.3070	< 0.0001
Psilotreta	0.2957	< 0.0001
Tallaperla	0.2624	< 0.0001
Allocapnia	0.2480	< 0.0001
Rhyacophila	0.2208	< 0.0001
Suwalia	0.1836	< 0.0001
Stenonema	0.1771	< 0.0001
Chironomidae	+ 0.1586	< 0.0001
Hydracarina	0.1364	< 0.0001
Lanthus	0.1274	0.0001
Epeorus	0.1121	0.0003

Fig. 2-1 Study site locations in Floyd Co., Virginia.

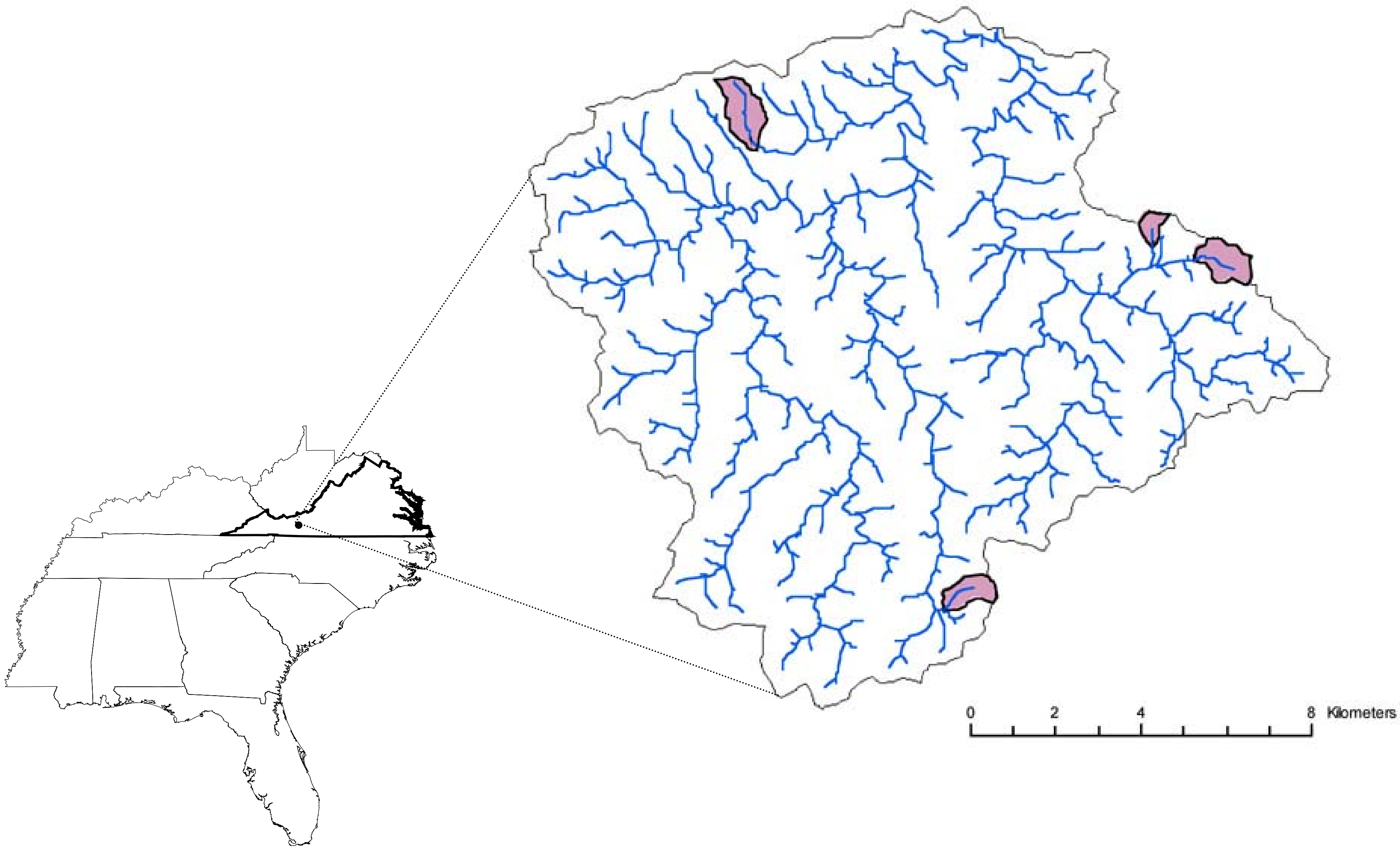


Fig. 2-2 Examples of strong metric responses to the cattle grazing gradient from regression analysis. All relationships were highly significant ($P < 0.0001$). Values in the graphs are coefficients of determination (r^2) from Table 3. (a) Examples of linear and quadratic relationships with cattle density. Data for % Coleoptera and % burrowers are from the spring 2003 sampling period; data for % scrapers are from the fall 2003 sampling period. (b) Examples of concave relationships to cattle density. Data for total taxa richness and number of sensitive taxa are from the fall 2003 sampling period; data for % collector-filterers are from the fall 2002 sampling period.

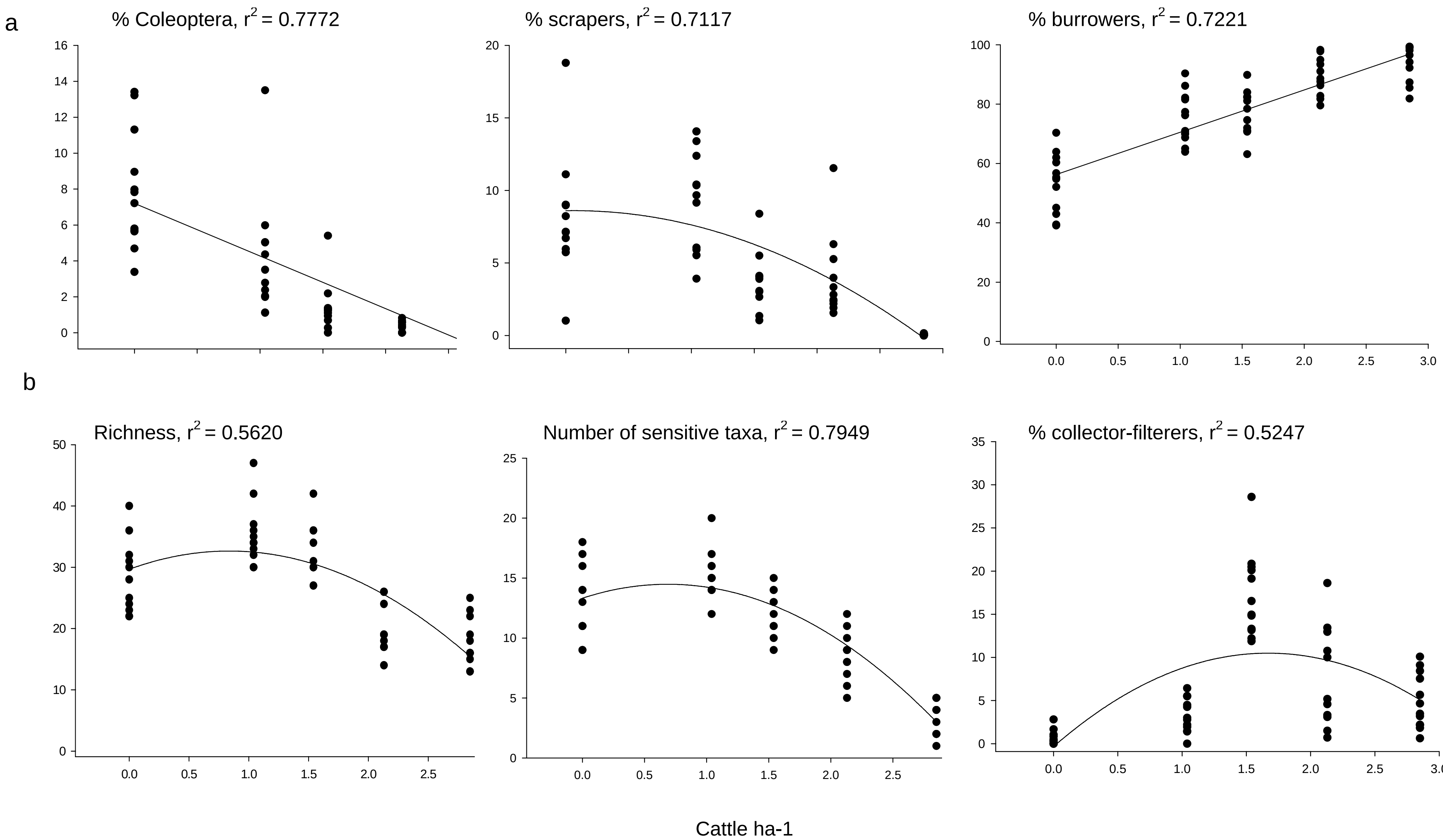

a
% Coleoptera, r² = 0.7772
% scrapers, r² = 0.7117
% burrowers, r² = 0.7221
b
Richness, r² = 0.5620
Number of sensitive taxa, r² = 0.7949
% collector-filterers, r² = 0.5247
Cattle ha-1

Fig. 2-3 Results from detrended correspondence analysis for 113 benthic samples and 60 taxa. (a) symbols represent benthic samples from site 1 (○), site 2 (●), site 3 (●), site 4 (×), and site 5 (*). (b) Taxa associated with benthic samples. Taxa codes are the first eight letters of a taxon. See Table 2 for taxon names.

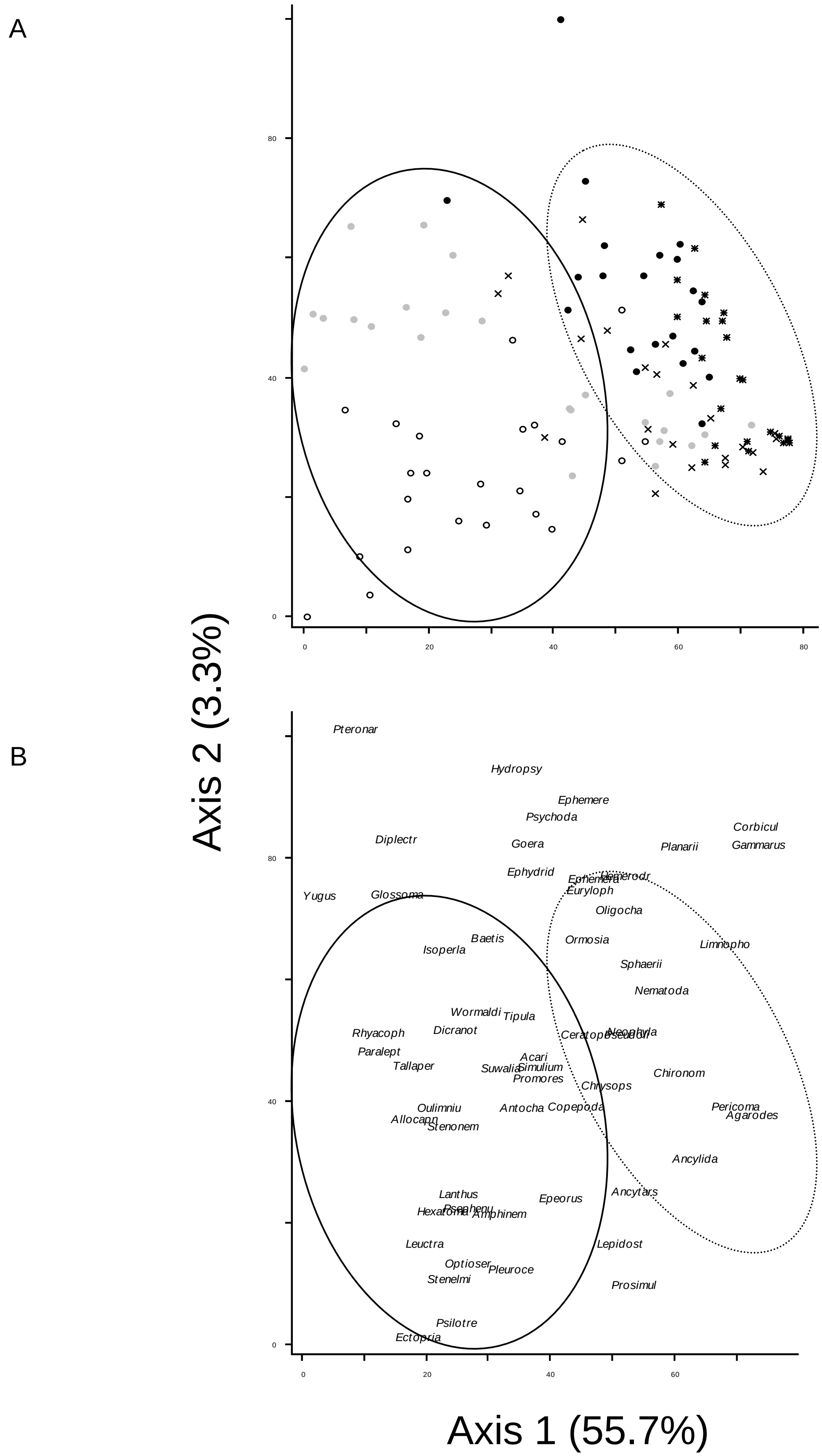
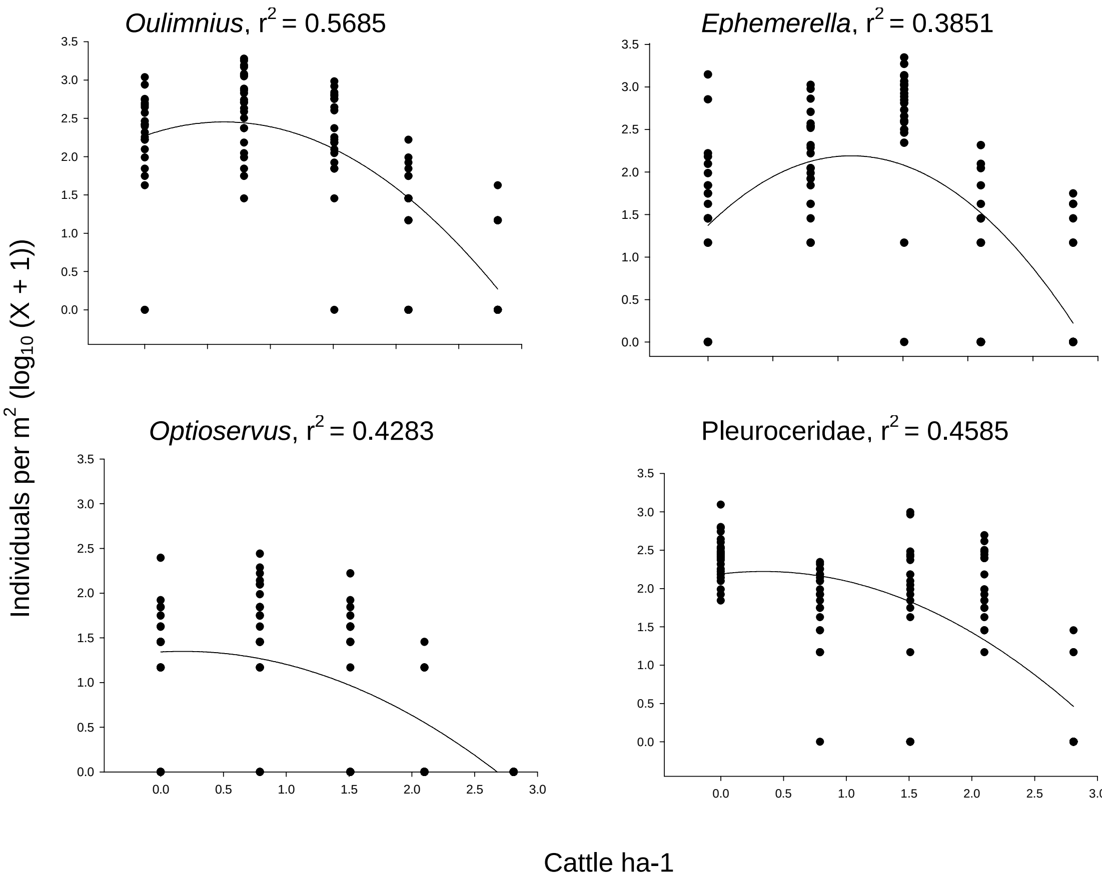

A
B
Axis 2 (3.3%)
Axis 1 (55.7%)
Pteronar
Hydropsy
Ephemere
Psychoda
Diplectr
Goera
Planarii
Corbicul
Gammarus
Ephydrid
Eurylop
Yugus
Glossoma
Oligocha
Baetis
Isoperla
Ormosia
Limnopho
Sphaerii
Nematoda
Wormaldi
Tipula
Rhyacoph
Dicranot
Paralept
Acari
Tallaper
Suwalia
Simulium
Promores
Chironom
Chrysops
Oulimniu
Antocha
Copepoda
Pericoma
Agarodes
Allocapn
Stenonem
Ancylida
Lanthus
Epeorus
Ancytars
Hexatoma
Amphinem
Leuctra
Lepidost
Optioser
Pleuroce
Stenelmi
Prosimul
Psilotre
Ectopria

Fig. 2-4 Examples of individual taxa responses to the cattle grazing gradient from regression analysis. Data are from spring 2003 and fall 2003 (n = 113). Values in the graphs are coefficients of determination (r^2) from Table 5.



CHAPTER 3

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

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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 that assemblage structure could be directly related to environmental variables and so that 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) were most important in structuring assemblages. Detrital food variables (coarse benthic and fine benthic organic matter) were secondarily important while autochthonous food variables (chlorophyll *a* and epilithic biomass) were not as important in influencing assemblage structure. Results from this study design will be useful in the development of models that predict biotic responses to stressors in small streams impacted by cattle. Findings from this study also demonstrate the importance of quantitative sampling through time when research goals are to identify stressors causing biological impairment.

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, Ruttner 1964). Findings from detailed benthic studies have demonstrated that small areas (approximately 0.1 m^2) of similar benthic habitat have similar macroinvertebrate faunas (Allen 1959, Mackay 1969, Pennak and VanGerpen 1947, Percival and Whitehead 1929, Sprules 1947). 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 (Benke et al. 1984, Bouckaert and Davis 1998, Clements 1987, Cummins and Lauff 1969, Dudley et al. 1986, Edington 1968, Egglishaw 1964, Erman and Erman 1984, Minshall and Minshall 1977, Palmer et al. 2000, Poff and Ward 1992, Rabeni and Minshall 1977, Trush 1979, Williams and Mundie 1978). In ecological literature, the terms “patch scale” and “microscale” have been used synonymously with “sample scale”, which we use in this paper (Palmer et al. 2000, Evans and Norris 1997). It is possible to explain macroinvertebrate distributions and assemblage structure by environmental factors measured at the sample scale (Boyero 2003, Downes et al. 1995, Evans and Norris 1997, Gee 1978).

Although there is much evidence that substrate, flow, and food influence assemblages at small spatial scales, few studies have quantified these factors simultaneously to establish their relative importance in structuring assemblages. This is a difficult question because benthic habitat is complex and many environmental factors are interrelated (Rabeni and Minshall 1977, Williams and Smith 1996). However, there is evidence that inorganic substrate characteristics such as composition, complexity, and heterogeneity, are primary factors that influence assemblage structure at the sample scale or smaller scales of study (Downes et al. 1995, Reice 1980). For example, Evans and 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.

Previous studies that examined relationships between benthic habitat variables and macroinvertebrates were conducted in relatively undisturbed streams. Streams affected by multiple stressors from human activities in a watershed offer an experimental design to investigate the role of environmental variables in structuring assemblages at the sample scale. This information is needed to help solve the important problems that society faces about the diminishing quality of freshwater resources. 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). The TMDL process involves detailed analysis of land use and point and nonpoint sources of pollution, and the development of a management plan for eliminating the impaired condition of a stream. An essential part of the process is quantifying pollution sources and in-stream conditions, such as benthic macroinvertebrate assemblages. Freshwater resources are increasingly under stress from a variety of human activities (e.g., urban development, timber harvest, agriculture) (Naiman and Turner 2000), and benthic macroinvertebrates are used more than any other organism to assess the condition of streams (Barbour et al. 1999). Therefore, it is important to link environmental variables and the resulting assemblage structure in order to isolate the effects of specific stressors. Unfortunately, this information is not available, so best professional judgment is often used to relate environmental stressors with biocriteria (US EPA 2000 a). 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.

Cattle grazing is a particular type of agricultural land use that can impair ecological conditions in streams, including changes to benthic macroinvertebrate assemblages. 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. Grazing cattle in pastures and allowing unrestricted access to the streams causes multiple changes to stream environments. Trampled stream banks cause increased erosion and sedimentation. 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 (Armour et al. 1991, Cooper 1993, Fleischner, 1994, Kauffman and Krueger 1984, Owens 1996, Trimble and Mendel 1995). These cattle-induced environmental changes degrade water quality and habitat, which in turn alter the resident benthic macroinvertebrate fauna (Dance and Hynes 1980, Delong and Brusven 1998, Quinn et al. 1997, Scrimgeor and Kendall 2003, Strand and Merritt 1999, Wohl and Carline 1996).

In a previous study, we determined that the intensity of cattle grazing (cattle ha^{-1}) was a good predictor of macroinvertebrate assemblages in small, Blue Ridge streams (Figure 3-1, regression analysis $r^2 = 0.738$, $P < 0.0001$). Although cattle density alone is a good predictor of macroinvertebrate assemblages, the specific environmental factors related to cattle that cause the alterations in the 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) how much of the variation in benthic macroinvertebrate assemblage structure can be explained by environmental factors that were measured at the sample scale and (2) which environmental factors are most important in structuring the benthic macroinvertebrate assemblage?

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, U.S. The Blue Ridge physiographic province is characterized by deeply dissected valleys and ravines that are primarily composed of metamorphosed igneous rocks (granites, granidiorite, 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 (Virginia Agricultural Statistics Service 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 (Virginia Agricultural Statistics Service, 2004).

Five, first-order stream reaches in the Little River drainage basin, Floyd Co., Virginia were selected as study sites (Fig. 3-2). 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 3-1). 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 3-1, 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 scores less than 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 least 2 m apart, which resulted in the collection of 230 benthic samples for the entire study. Benthic samples were collected by inserting a modified stovepipe corer (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 core, inorganic substrates that were ≥ 64mm 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 core were agitated and a 250-ml container was used to subsample water within the core. 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 core were removed by hand and placed in large sample containers. The rest of the water within the core 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) were 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 to 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

Eight environmental factors (FBOM, CBOM, % pasture vegetation, % wood, % deciduous leaf, % miscellaneous, chlorophyll *a*, and epilithic biomass) that were thought to affect benthic macroinvertebrates primarily as food resources were measured from the samples (see Table 3-2 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 ^{o}C for 24 hours, desiccated, and reweighed to obtain ash free dry mass (AFDM). Epilithic subsamples were split and analyzed for periphyton biomass and epilithic biomass. The periphyton 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 ^{o}C for 24 hours, desiccated, and reweighed to obtain ash free dry mass (AFDM). Chlorophyll *a* was extracted with 90% acetone and then analyzed with a spectrophotometer after correcting for pheophytin *a* 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

Ten environmental factors that were thought to affect macroinvertebrates primarily through habitat suitability were measured from the benthic samples (see Table 3-2 for explanations). Organic matter and macroinvertebrates were elutriated from sediments and remaining inorganic substrates were separated into standard Wentworth size classes (Wentworth 1922). Particles greater than 8 mm were manually separated into size classes and weighed. Particles smaller than 8mm 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

Following the field and laboratory procedures described above, measurements of 17 benthic environmental factors were available for statistical analyses (Table 3-2). These variables 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 and 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 reduce noise in the data set (Gauch 1982). To avoid the possibility of collinearity among environmental variables, we performed Pearson product-moment correlations and removed environmental variables that were highly redundant. After removing rare taxa and redundancy among environmental variables, 17 environmental variables (see Table 3-2) 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., Barbour et al. 1999 and Brigham et al. 1982) 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 to 10, with 0 indicating very sensitive and 10 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 correlations greater than or equal to 0.5 on each axis. 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. Bonferroni adjustments were made to control for procedure wise error by adjusting the test significance level (alpha) by the number of repeated tests. Results were considered significant only if *P* was less than the adjusted alpha; however the original *P* values are also reported so readers can interpret the results without Bonferroni adjustments if desired (Perneger 1998).

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 3-3). 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 a gradient of benthic samples that increased in % fines, FBOM, and substrate homogeneity (Table 3-4, Fig. 3-3a). Taxa with high negative scores on the first CCA axis during fall 2002 included limpets, (Ancylidae), stoneflies (*Tallaperla*, *Isoperla*, *Suwalia*, *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. 3-3a). No benthic habitat variables had high correlations with axis 2 and only depth was correlated with axis 3 (Table 3-4).

The fall 2003 ordination showed the strongest taxa-environment relationship of all sampling periods; 25% of the taxa-environment relationship was explained (Table 3-3). 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 benthic samples with CBOM composed of pasture vegetation but less wood (Table 3-4). Taxa with high positive scores on axis 1 included *Pteronarcys*, *Eurylophella*, *Hydropsyche*, *Wormaldia*, *Goera*, *Agarodes*, *Psephenus*, *Promoresia*, *Anchytarsus*, and *Prosimulium* (Fig. 3-3b). Few taxa had high negative scores with axis 1. Axis 2 explained 8.6% of the variance in taxa-environment relations (Table 3-3). Trask's sorting coefficient and % fines were positively correlated with axis 2, so this axis was interpreted as a gradient of benthic samples with increased inorganic fines and homogenized substrate (Table 4-3). 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. 3-3b).

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 3-5). 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 3-5). 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 a gradient of benthic samples with increasing FBOM (Table 3-6). 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. 3-4a). Axis 2, of spring 2003, was interpreted as a gradient of benthic samples composed of increased CBOM and with a lower proportion of pebble sized particles (Table 3-6). 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. 3-4a).

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 3-5) 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 benthic samples with increased fine sediments (Table 3-6). 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 (Fig. 3-4b). Axis 2 was interpreted as a gradient of benthic samples with increased FBOM and CBOM (Table 3-6). 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. 3-4b)

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 and were selected as worthy of further study. However, only four of the nine environmental variables (FBOM, CBOM, Trask's sorting coefficient, % fines) showed statistically significant relationships with macroinvertebrate metrics (Tables 3-7 and 3-8). Richness, Simpson's diversity, and % collector-gatherers showed significant relationships with environmental variables only during spring, while % Plecoptera, % Coleoptera, % collector-filterer, % sensitive taxa, and % crawler showed significant relationships with environmental variables only during the fall. Four metrics (% scrapers, % shredders, % clingers, % burrowers) never had a significant relationship with any of the environmental variables during any sampling period. Habitat variables (Trask's sorting coefficient, % fines) were significant during fall sampling periods, but not during spring sampling periods. On the other hand, there were more significant relationships between metrics and organic matter (CBOM, FBOM) during spring sampling periods. Overall, the strength of relationships between metrics and habitat variables were always stronger than the relationships between metrics and organic matter.

There were 13 statistically significant relationships between metrics and environmental variables during the fall sampling periods (Table 3-7). The majority of significant macroinvertebrate metric relationships were with % fines or Trask's sorting coefficient. Percent Coleoptera consistently showed significant relationships with % fines and Trask's sorting coefficient during both fall sampling periods. Furthermore, the strongest relationship that was observed during this study occurred during fall 2002 where 50 % of the variation in the relative abundance of Coleoptera was explained by % fines. Percent crawlers also had significant relationships with % fines during both fall sampling periods but had a significant relationship with Trask's sorting coefficient only during fall 2003.

Six significant relationships occurred during the spring sampling periods (Table 3-8) and spring, 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 richness.

Discussion

Results from this study demonstrate that macroinvertebrate assemblages can be explained by the environmental variables that were quantified at the sample scale in these small streams. Given the many interacting environmental variables that can influence the spatial distribution of macroinvertebrates, it is understandable that the individual environmental variables measured in this study rarely explained more than 25-30 % of the variance in taxa composition or metrics. If streams with higher cattle densities had been included in our study design, the relationships between metrics and environmental variables would likely have been stronger. For example, we never encountered benthic samples where % fines were greater than 70% of the substrate composition. If our data set had included benthic samples in which the proportion of fines was 100%, which does occur in streams subjected to exceptionally high cattle densities, stronger relationships would likely have been detected. Regardless of the amount of variation explained, 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 the most important stressor in these cattle-impacted streams.

At the sample scale of measurement in these cattle-impacted streams, the physical nature of benthic habitat, as indicated by % fines and Trask's sorting coefficient, seems most important in structuring the macroinvertebrate assemblages. The significant response 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 in streams. Several crawler taxa that were encountered during this study declined in samples with increased % fines. Larvae of the elmid beetle, *Oulimnius*, showed a particularly strong, negative response to % fines (regression analysis, $r^2 = -0.4139$, $P < 0.0001$) during this study. 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.

Although measures of the physical nature of habitat (% fines and Trask's sorting coefficient) were most important in structuring the macroinvertebrate assemblage, 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 showed that % fines and substrate homogeneity, e.g., Trask's sorting coefficient, in benthic samples were always less at the most heavily grazed sites during spring sampling periods (Fig. 3-5 a-d). Higher flows during spring likely keep larger substrate and interstitial spaces free from fine sediments. During fall, when baseflow is low relative to spring, fine sediments settle on stream bottoms, and interstitial spaces become clogged with fine sediments, thus eliminating habitat for crawler taxa.

Although less variation was explained during spring sampling periods, the variation that could be explained 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 (Fig. 3-6 a-d). 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. Futhermore, CBOM may serve as a habitat resource 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 relationships with macroinvertebrate taxa or metrics during any sampling period. A significant difference in periphyton biomass was detected among our study sites during only one of the four sampling periods (Fig 3-7 a-d). All study 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 associated with the exposed surfaces of stable rocks and have rarely been reported from the undersides of substrates (Frutiger 2002, Kovalak 1976, Scott 1958). Thus fine sediments deposited around and beneath stable stones may not be a stressor to these taxa. Clinging taxa are morphologically or behaviorally adapted to exist on the surface of clean, stable substrate in swift water. 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 and the similarity in autochthonous food resources among study sites suggests that the elevated nutrients that are often associated with cattle grazing did not alter the autochthonous food resources to the point of stressing the macroinvertebrate assemblage in these small streams.

Our findings demonstrate that relating macroinvertebrate assemblages to environmental factors or stressors requires significant effort to establish the relationships at the scale that is most relevant to macroinvertebrates, the sample scale. Differences in the relative importance of benthic environmental factors that structured assemblages between spring and fall sampling periods demonstrate that these factors are dynamic. To ensure a full understanding of the variables that alter assemblages, sampling should occur over time and through seasons. Furthermore, if environmental factors had been quantified or estimated over a larger spatial scale, e.g. stream reach, as is commonly done in most regulatory biomonitoring protocols, it seems unlikely that the relationships we report here would have been detected. Using an enclosed sampler to concurrently collect macroinvertebrates and measure benthic environmental factors provides a more reliable measure of the true nature of the ecological condition in streams. If we had used a sampler fitted with a net (e.g., surber, d-frame dip net), fine sediments would have been washed through the mesh netting and the patterns we report here would not have been detected.

Using a study design that involved a gradient of increasing stress (increased cattle density) allowed us to quantify and rank the relative importance of stressors associated with cattle grazing. It appears that fine sediment is the primary stressor to benthic macroinvertebrate assemblages in small, Blue Ridge streams. Establishing these relationships is necessary for the development of models that link environmental stressors to biocriteria in a scientifically sound manner. Our results indicate that fine sediment should be emphasized in ecological risk models that predict the effects of pollutants on biota, e.g., AQUATOX (US EPA 2000 b). However, our results may only apply to small streams in the Blue Ridge ecoregion. The relative importance of stressors to macroinvertebrate assemblages likely varies according to geographic region, slope, soil type, stream elevation, and size. We suggest that future research on the effects of stressors associated with agricultural land use will be most informative if conducted with a study design that includes a gradient of cattle grazing intensity.

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Table 3-1 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*	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})†					
Minimum	10	8	8	14	2
Maximum	62	47	25	77	10
Average	25	25	15	30	5
Mean wetted width (m)‡	0.88	0.72	1.11	0.76	0.60
Mean depth (m) ‡	0.08	0.13	0.13	0.09	0.10
Conductivity (µS cm^{-1})£	16 - 22	18 - 23	54 - 63	19 – 22	54 – 57
Maximum temperature (°C)£	18.5	20.0	20.5	21.5	21.5

* 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).

† Baseflow discharge was measured on 8 separate occasions between July 2002 and August 2003.

‡ 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).

£ Conductivity and temperature values are based on 15 spot measures taken throughout the study period.

Table 3-2 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 < 1mm 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 and 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 1962). $\left[D_{84}/D_{16} \right]^{-0.5}$
Surface cobble to subsurface cobble ratio	none	Ratio of surface cobble to subsurface cobble within each benthic sample

Table 3-3 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**			
	Axis1	Axis 2	Axis 3	Total variance	Axis1	Axis 2	Axis 3	Total variance
Eigenvalue	0.176	0.076	0.058	1.3930	0.072	0.068	0.055	0.7926
%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 3-4 Intraset correlation coefficients between environmental variables and axes derived from CCA for fall sampling periods. Values in bold were considered as important in structuring the macroinvertebrate assemblage.

Variable	Fall 2002			Fall 2003		
	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
Periphyton	0.014	-0.130	0.110	0.208	-0.175	0.131
D50	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

Table 3-5 Summary of CCA results for spring sampling periods for the abundance of macroinvertebrate taxa and 17 environmental variables. The superscript, NS, indicates axes were not significant following Monte Carlo permutation procedures.

	Spring 2003				Spring 2004			
	Axis1	Axis 2	Axis 3	Total variance	Axis1	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	

Table 3-6 Intraset correlation coefficients between environmental variables and axes derived from CCA for spring sampling periods. Values in bold were considered important in structuring the macroinvertebrate assemblage. The superscript, NS, indicates axes were not significant following Monte Carlo permutation procedures.

Variable	Spring 2003			Spring 2004		
	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
Periphyton	0.234	0.447	-0.274	-0.136	0.071	0.349
D50	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

Table 3-7 Results from regression analysis for macroinvertebrate metrics versus environmental variables during fall sampling periods (F02 = fall 2003, F03 = fall 2003). Values are coefficients of determination and signs in front of values indicate the direction of the relationship. *P* values are in parentheses. Results in bold indicate relationships were significant following Bonferroni adjustments.

		Richness	% Plecoptera	% Coleoptera	Simpson's Diversity	% collector-gatherer	% collector-filterer	% scraper	% shredder
Depth	F02								
	F03								
FBOM	F02		-0.1872 (0.0006)	-0.1299 (0.0051)			**+0.3386 (<0.0001)**		
	F03								
CBOM	F02		-0.2016 (0.0004)						
	F03								
% wood	F02								
	F03	-0.1966 (0.0006)					-0.1548 (0.0027)		
% p.veg	F02						+0.1587 (0.0018)		
	F03	+0.1599 (0.0023)							
Trask's	F02		-0.1168 (0.0081)	**-0.2398 (<0.0001)**			+0.1759 (0.0009)	-0.1661 (0.0014)	
	F03		-0.1240 (0.0078)	**-0.2289 (0.0002)**	-0.1352 (0.0053)	+0.1914 (0.0007)		-0.1772 (0.0012)	-0.1614 (0.0021)
% pebble	F02								
	F03								
% fines	F02		**-0.3217 (<0.0001)**	**-0.5095 (<0.0001)**			**+0.2457 (<0.0001)**	-0.1258 (0.0059)	-0.1576 (0.0019)
	F03		-0.1593 (0.0023)	**-0.2487 (<0.0001)**	-0.1434 (0.0040)	+0.1877 (0.0009)		-0.1949 (0.0007)	-0.1800 (0.0011)

Table 7 (continued).

		% sensitive taxa	Number of sensitive taxa	% clinger	% crawler	%burrower
Depth	F02				-0.2009 (0.0004)	
	F03					
FBOM	F02					
	F03					
CBOM	F02					
	F03					
% wood	F02					
	F03					
% p.veg	F02					
	F03					
Trask's	F02	-0.1767 (0.0009)		-0.1476 (0.0027)	-0.1261 (0.0058)	+0.1530 (0.0022)
	F03	**-0.2325 (0.0002)**	**-0.2662 (0.0002)**		**-0.2802 (<0.0001)**	+0.1577 (0.0024)
% pebble	F02					
	F03					
% fines	F02	-0.1514 (0.0023)		-0.1184 (0.0076)	**-0.2959 (<0.0001)**	+0.1833 (0.0007)
	F03	**-0.2641 (<0.0001)**	-0.2006 (0.0005)		**-0.2756 (<0.0001)**	+0.1780 (0.0012)

Table 3-8 Regression results from important benthic environmental variables and benthic macroinvertebrate metrics during spring sampling periods (S03 = spring 2003, S04 = spring 2004). Values are coefficients of determination and signs in front of values indicate the direction of the relationship; q indicates the relationship was quadratic. *P* values are in parentheses. Results in bold indicate relationships were significant following Bonferroni adjustments.

		Richness	% Plecoptera	% Coleoptera	Simpson's Diversity	% collector-gatherer	% collector-filterer	% scraper	% shredder
Depth	S03						+0.1371 (0.0050)	+0.1226 (0.0081)	
	S04								
FBOM	S03								
	S04	**+0.1827 (<0.0001)**							
CBOM	S03	**+ 0.3922 (<0.0001)**			**+0.2616 (0.0003)**	**-0.3067 (<0.0001)**	+0.1754 (0.0012)		
	S04	**q 0.2513 (0.0003)**					+0.1130 (0.0099)		
% wood	S03								
	S04								
% p.veg	S03								
	S04			+0.1916 (0.0006)					
Trask's	S03								
	S04								
% pebble	S03	q 0.2126 (0.0016)			q 0.2366 (0.0007)				
	S04								
% fines	S03	q 0.2104 (0.0017)			q 0.1642 (0.0079)				
	S04								

Table 8. (continued)

		% sensitive taxa	Number of sensitive taxa	% clinger	% crawler	%burrower
Depth	S03			+0.1420 (0.0042)		
	S04					
FBOM	S03					
	S04		**+0.2860 (<0.0001)**			+0.2022 (0.0004)
CBOM	S03		+0.1548 (0.0022)			
	S04					
% wood	S03					
	S04					
% p.veg	S03					
	S04					
Trask's	S03			-0.1762 (0.0012)		
	S04				q 0.1764 (0.0053)	
% pebble	S03					
	S04				q 0.1764 (0.0053)	
% fines	S03			-0.1315 (0.0056)		
	S04					

Fig. 3-1 The relationship between Stream Condition Index (SCI) (Burton and 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 during spring 2003 (closed circles) and fall 2003 (open circles).

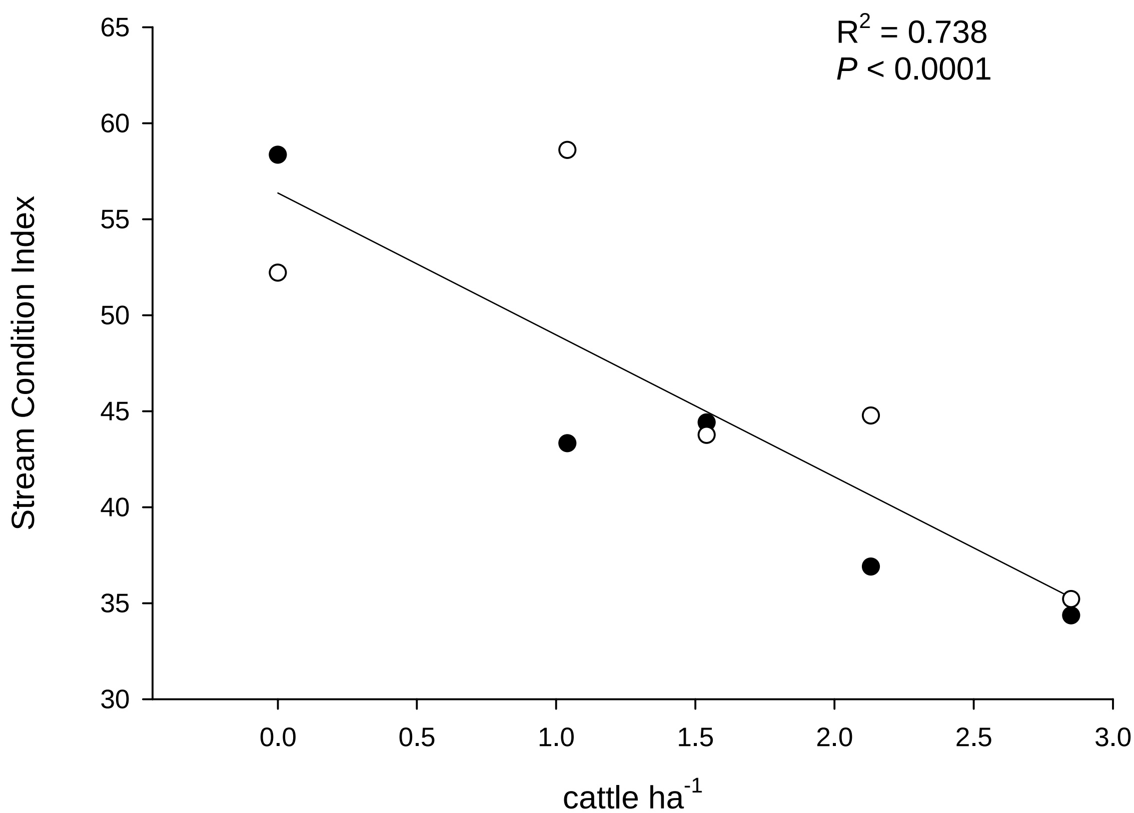


Fig. 3-2 Study site locations in Floyd Co., Virginia.

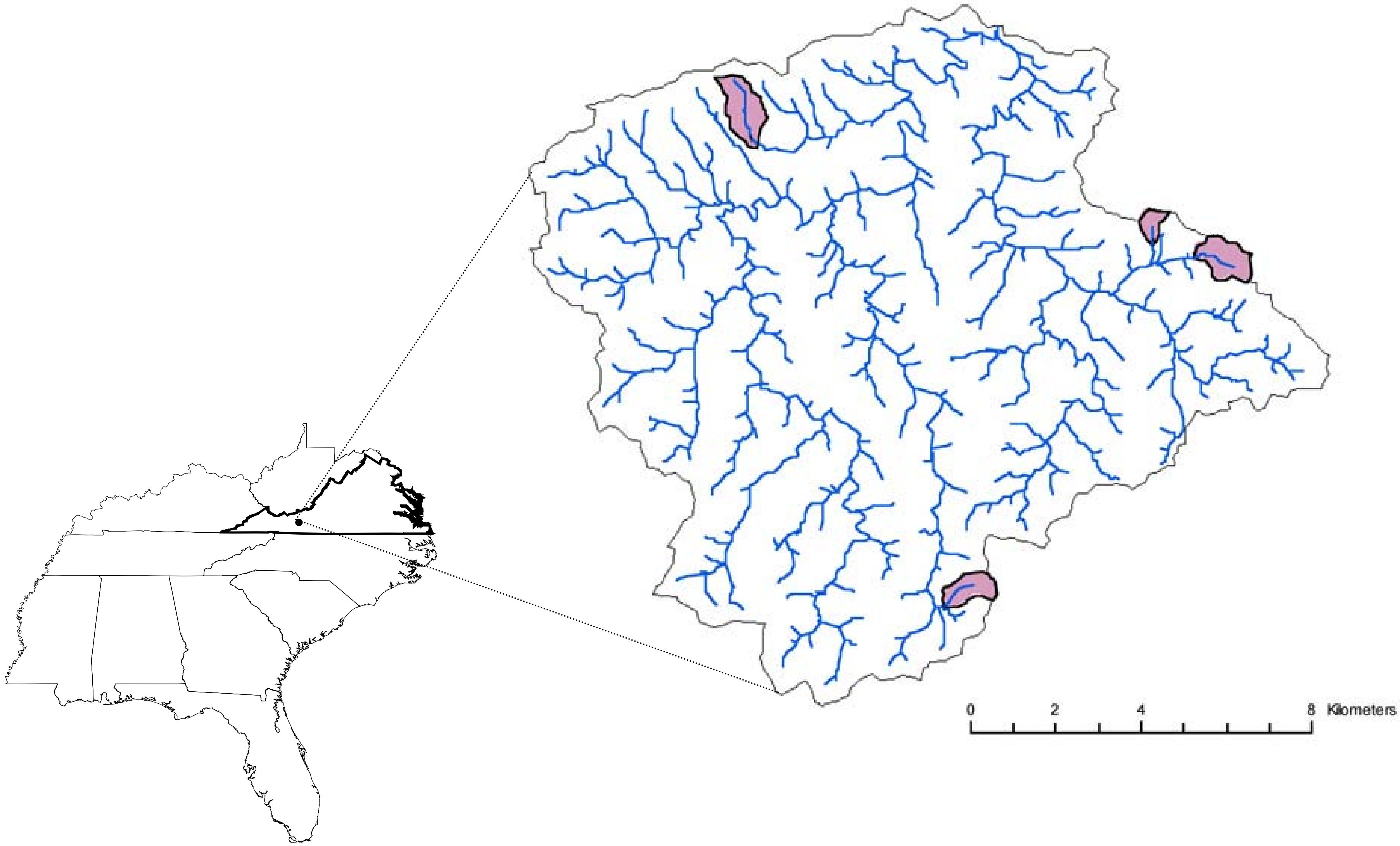


Fig. 3-3 a Results from CCA for the fall 2002 sampling period

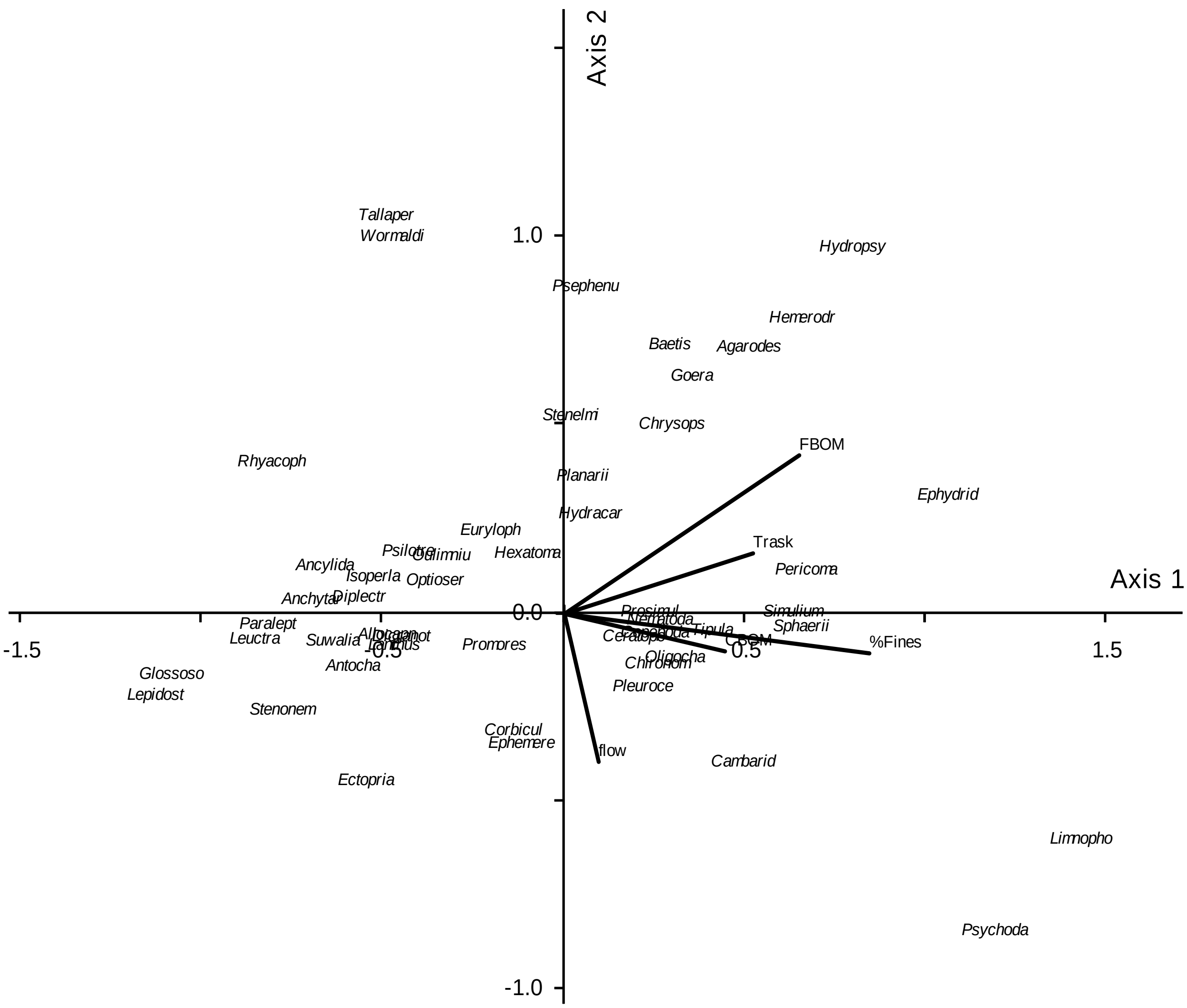


Fig. 3-3 b Results from CCA for the fall 2003 sampling period

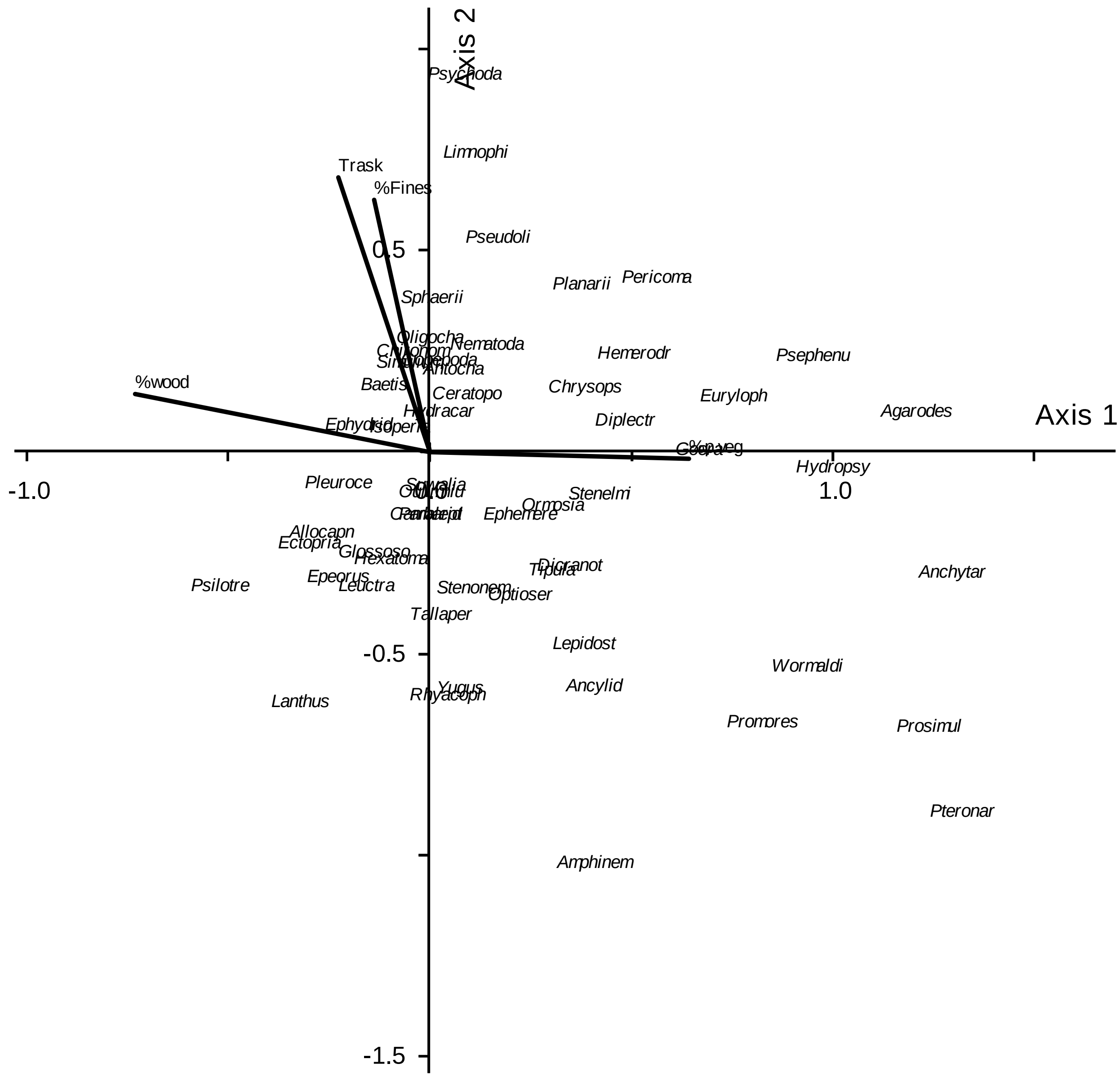


Fig. 3-4 a Results from CCA for the spring 2003 sampling period

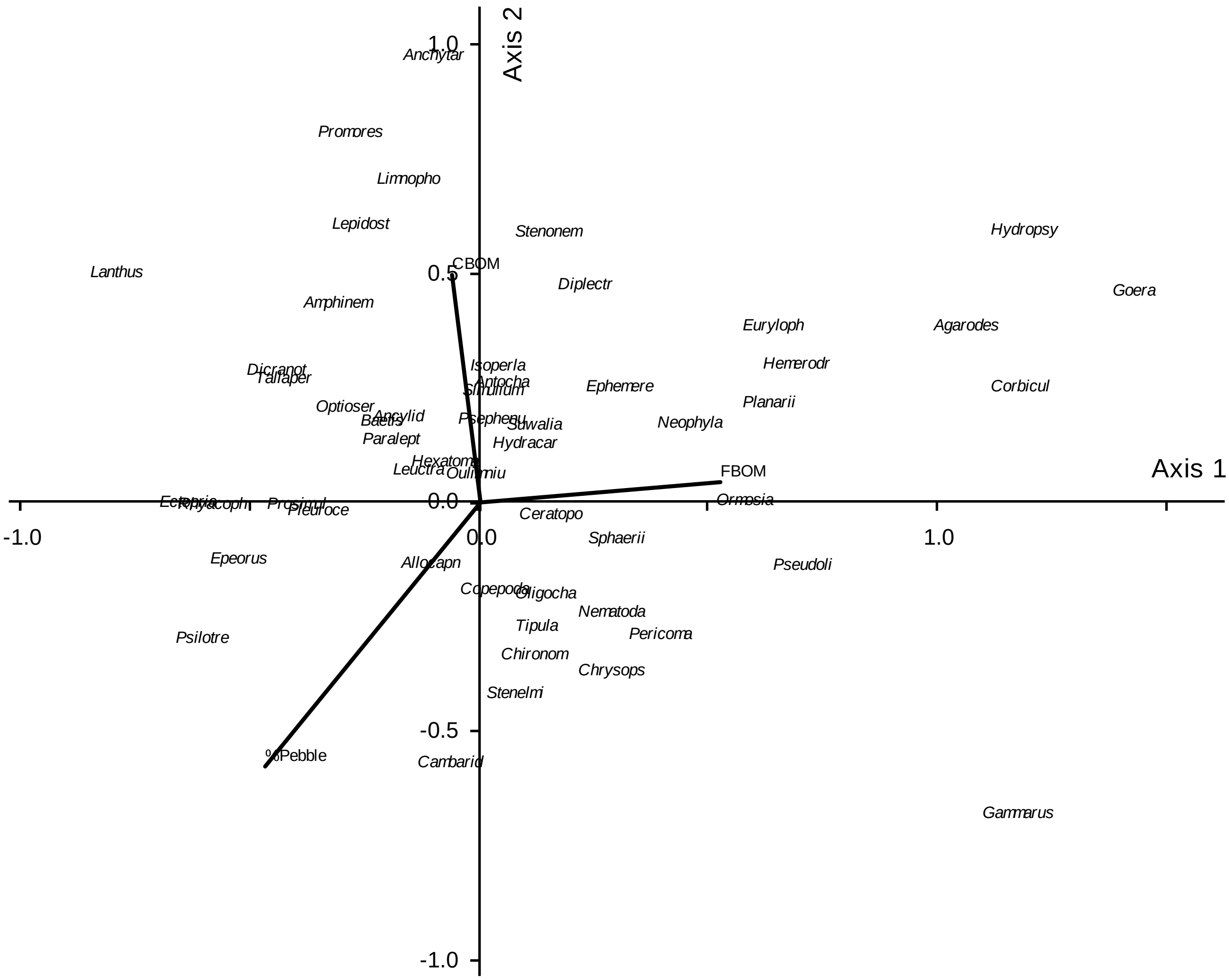


Fig. 3-4 b Results from CCA for the spring 2003 sampling period

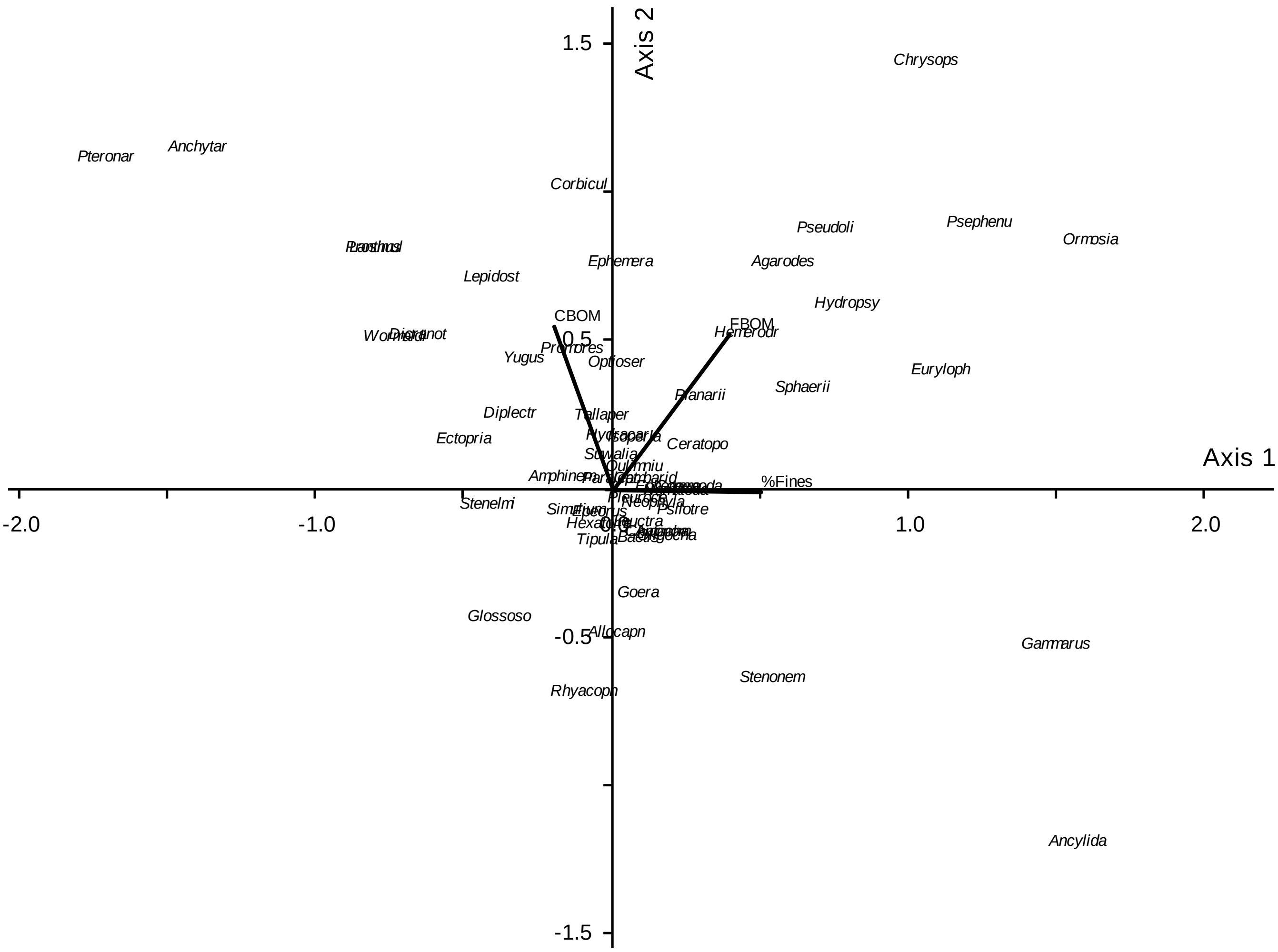


Fig. 3-5 Mean % fines (± SE) and cattle density during (a) spring and (b) fall sampling periods. Metrics with different letters indicate significant differences based on Tukey-Kramer post hoc tests, alpha = 0.05.

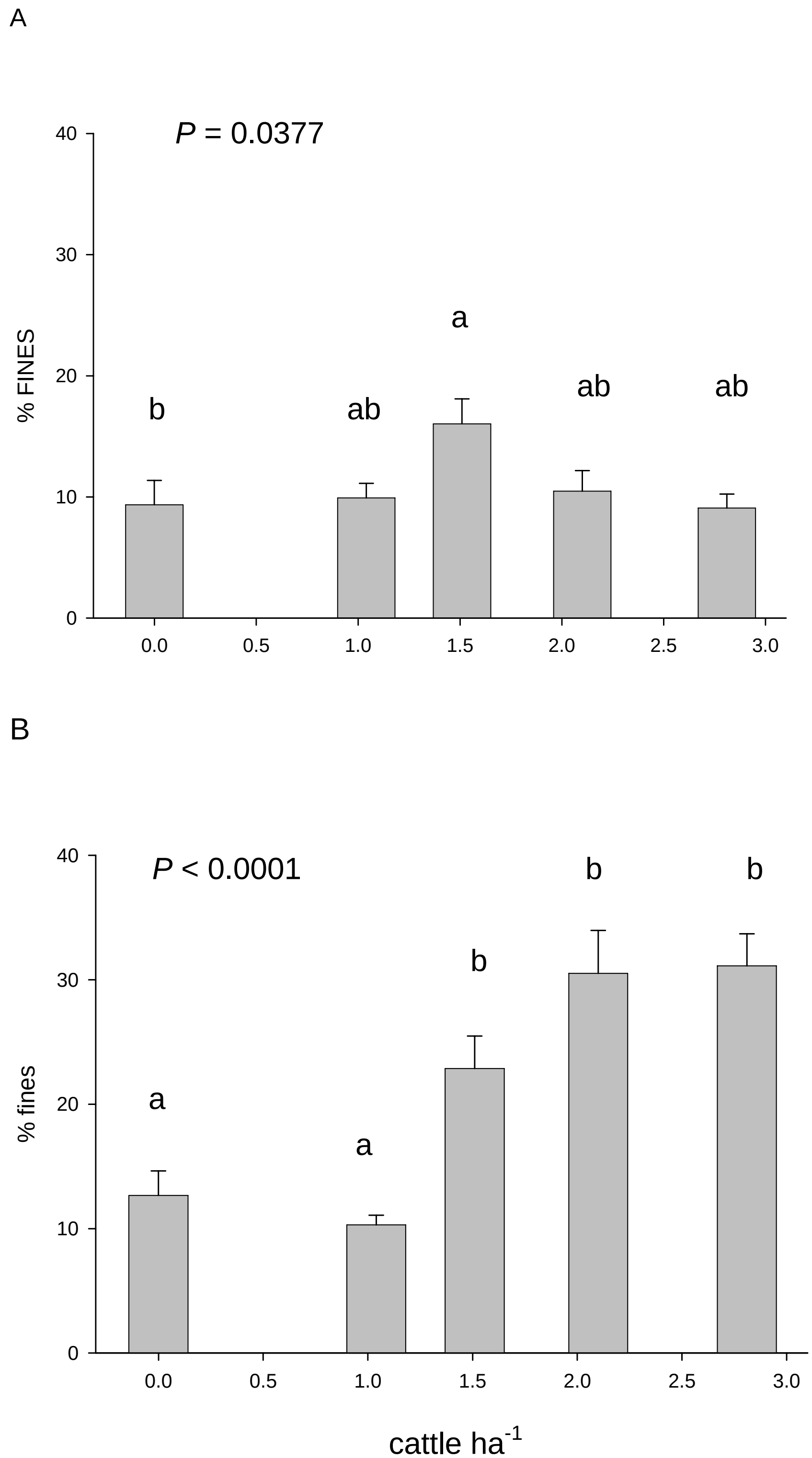
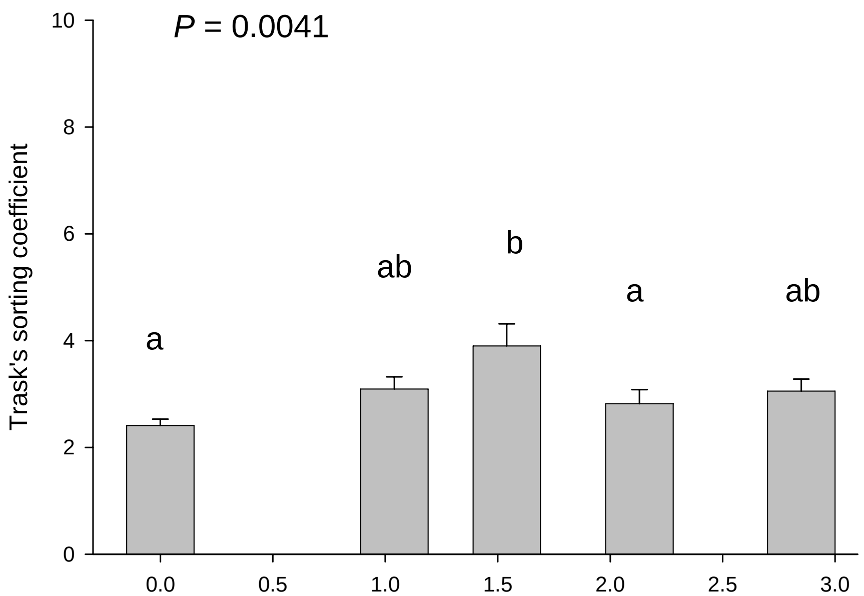


Fig. 3-5 (continued) Mean Trask's sorting coefficient (± SE) and cattle density during (c) spring and (d) fall sampling periods. Metrics with different letters indicate significant differences based on Tukey-Kramer post hoc tests, alpha = 0.05.

C



D

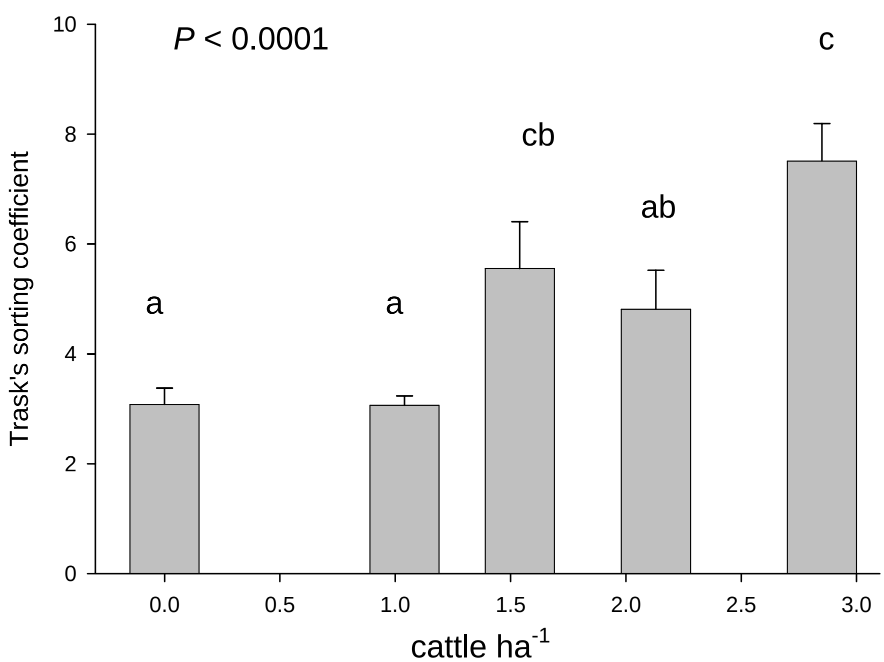
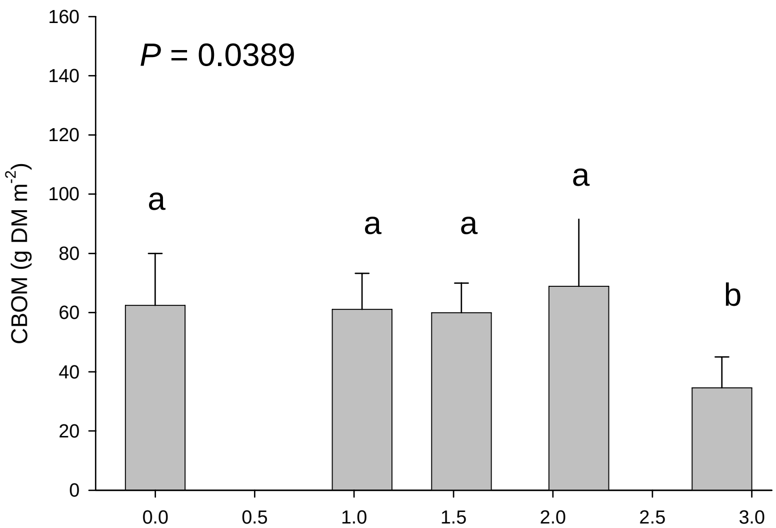


Fig. 3-6 Mean CBOM (± SE) and cattle density during (a) spring and (b) fall sampling periods. Metrics with different letters indicate significant differences based on Tukey-Kramer post hoc tests, alpha = 0.05.

A



B

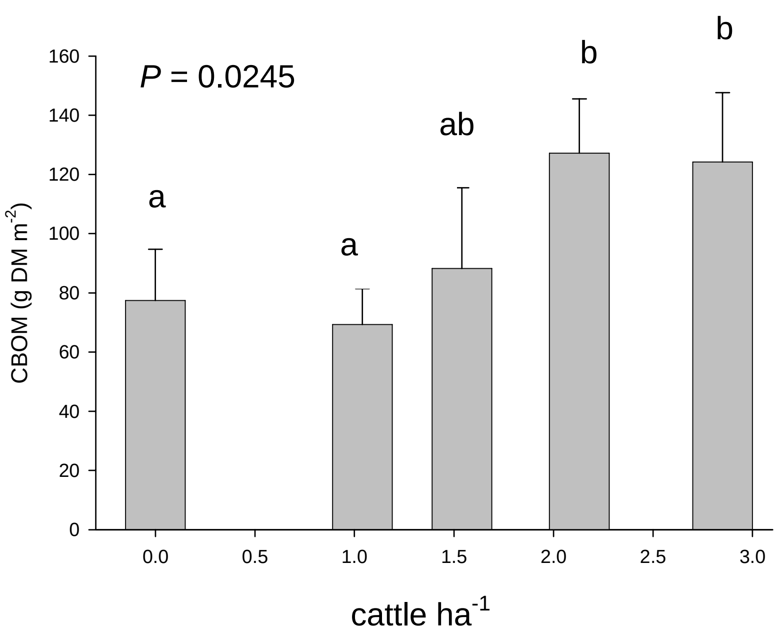
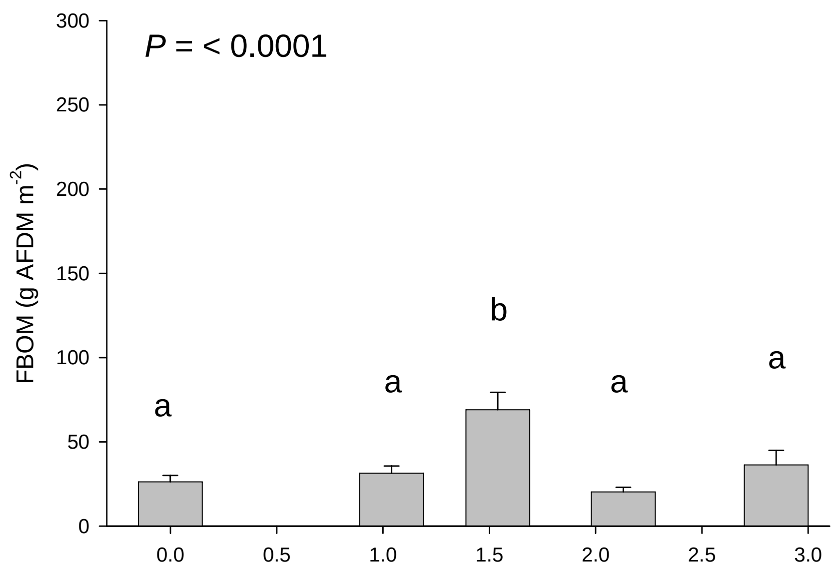


Fig. 3-6 (continued) Mean FBOM (± SE) and cattle density during (c) spring and (d) fall sampling periods. Metrics with different letters indicate significant differences based on Tukey-Kramer post hoc tests, alpha = 0.05.

C



D

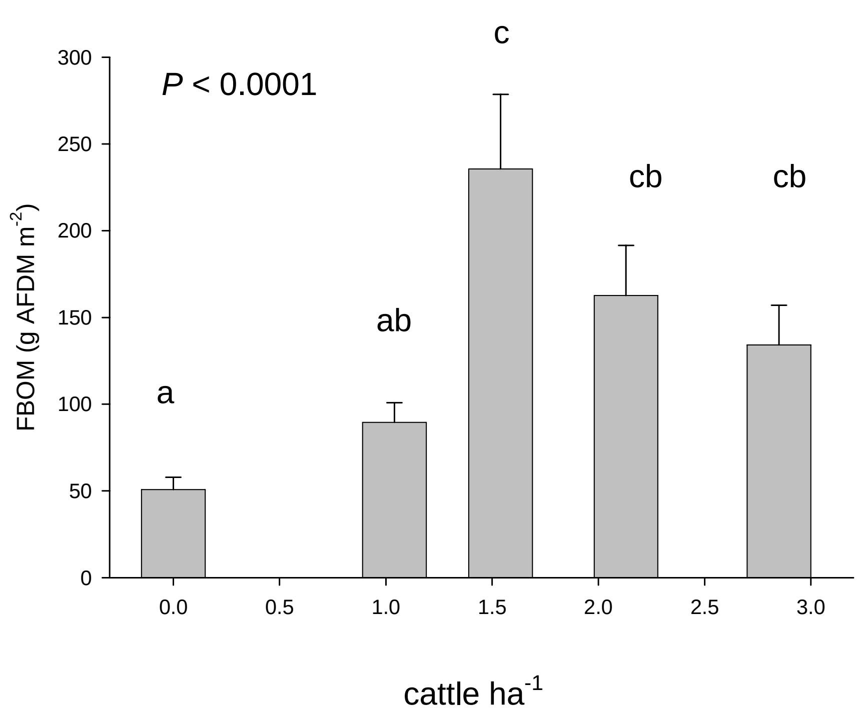
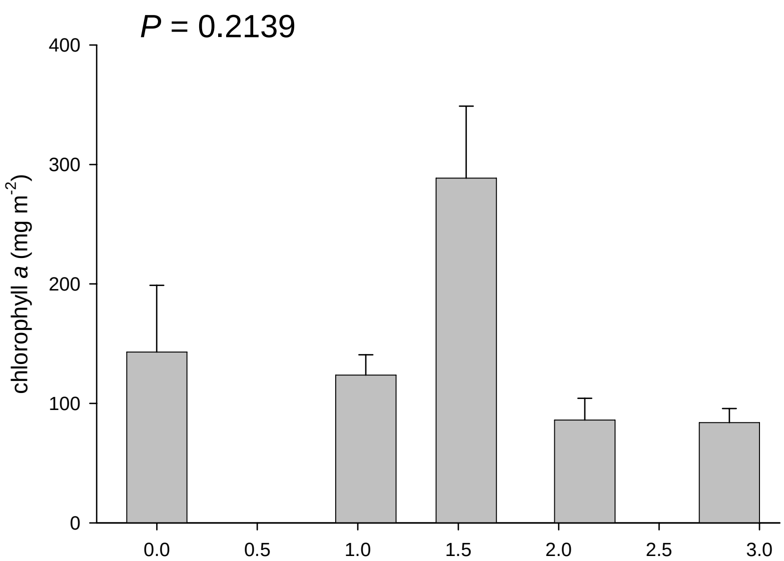


Fig. 3-7 Mean chlorophyll *a* (± SE) and cattle density during (a) spring and (b) fall sampling periods. Metrics with different letters indicate significant differences based on Tukey-Kramer post hoc tests, alpha = 0.05.

A



B

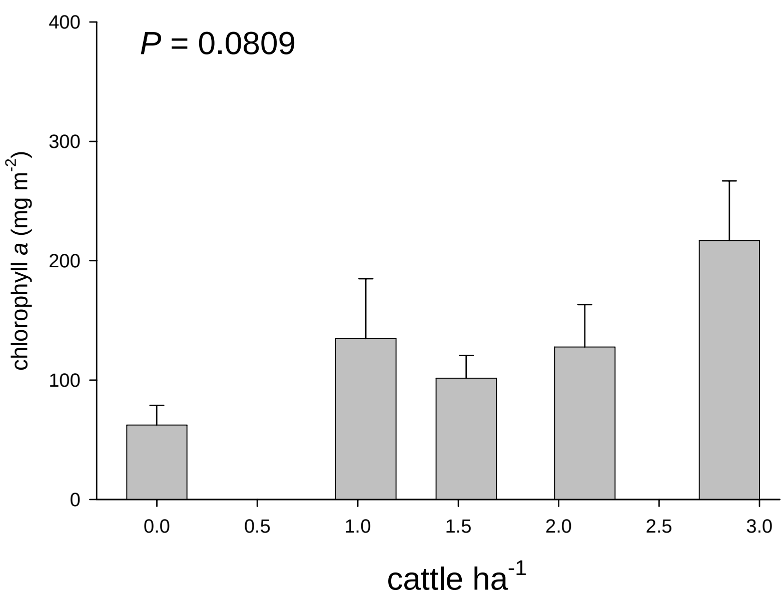
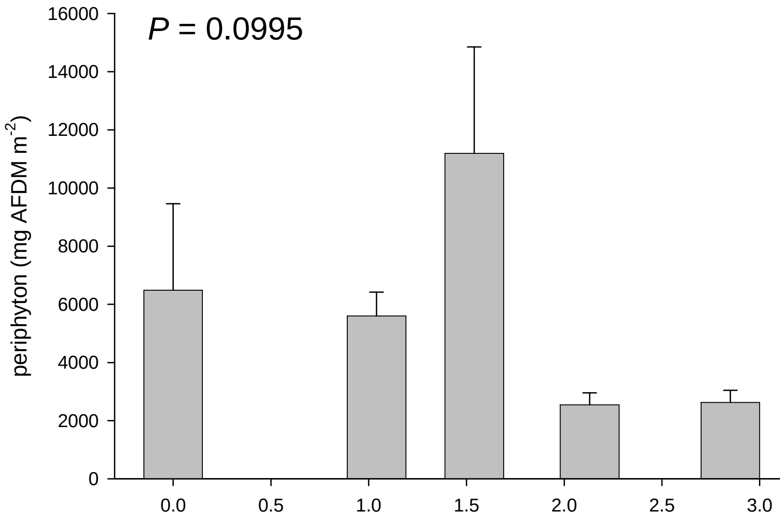
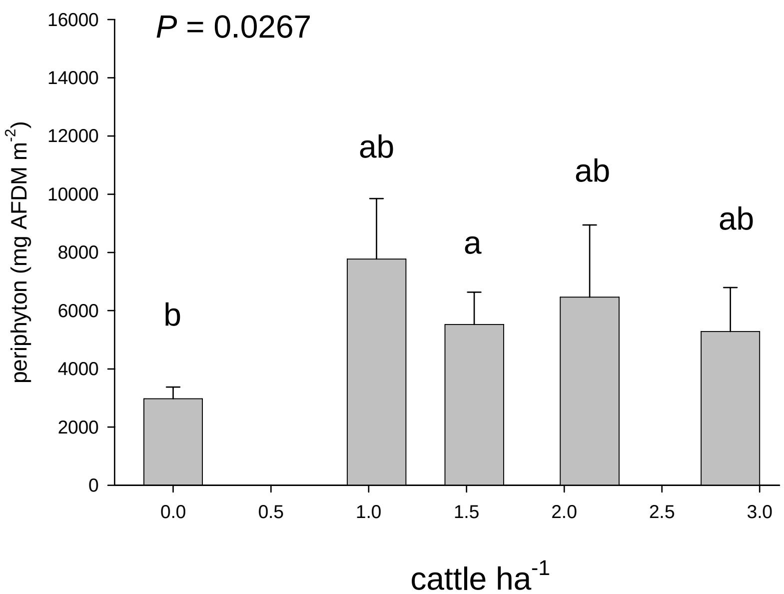


Fig. 3-7 (continued) Mean epilithic biomass (± SE) and cattle density during (c) spring and (d) fall sampling periods. Metrics with different letters indicate significant differences based on Tukey-Kramer post hoc tests, alpha = 0.05.

C



D



CHAPTER 4

Evaluation of different methods of assessing habitat for benthic macroinvertebrates in streams subjected to a gradient of cattle grazing.

Amy Braccia and J. Reese Voshell, Jr.

Abstract

Given the immediate need to improve water quality, data collected using 'rapid' methodologies for benthic macroinvertebrate and habitat are often used in attempts to identify stressors causing biological impairment. This approach, which involves unreplicated qualitative sampling and visual estimates, to identifying stressors is questionable. During this study, benthic macroinvertebrates, benthic habitat, channel, and riparian characteristics were quantified through replicated measurements. The predictive capability of quantitative measurements were compared to the predictive capability of data obtained from 'rapid' habitat measures and estimates (pebble counts and visual estimates) of stream habitat. Results indicate that % fines measured from within the benthic sampler was the best predictor of macroinvertebrate metrics. Quantitative measurements and visual estimates of riparian and channel characteristics had strong relationships with macroinvertebrate metrics but the relationships were never as strong as those detected with instream measurements of % fines. The channel characteristic, bank height, was the best predictor of % fines. Our findings demonstrate the usefulness of quantitative repeated measurements of instream habitat when research goals are to explain macroinvertebrate assemblages.

Introduction

The recognition that 'biological integrity' is included in the Clean Water Act (1972) has led to the development of bioassessment programs where biota and habitat surveys are used to judge the quality of surface water and to determine whether streams are impaired from human activity. Benthic macroinvertebrates are the most frequently used organisms in biomonitoring programs. Methods commonly used by state agencies for stream bioassessment were adopted from U.S. EPA's Rapid Bioassessment Protocols (RBPs) (Barbour et al. 1999). Carter and Resh (2001) summarized methods used by state agencies across the U.S. and reported that most agencies sample a single habitat (mostly riffles) with a D-frame kick net, and if more than one sample is collected it is composited. At the time of the benthic macroinvertebrate collection, 'rapid' habitat assessments are conducted along a 100-m reach where benthic macroinvertebrate collections are made. These habitat assessments are based on visual estimates of streambed, channel, and local riparian characteristics. The status of a stream is then determined based on results of these rapid surveys. These methods are so rapid that, if necessary, biological integrity of a stream can be determined within a day. Bioassessment programs, when well developed and validated, are useful tools for state agencies to monitor the quality of a large number of waterbodies.

If stream bioassessment results indicate impairment (i.e., an unnatural benthic macroinvertebrate assemblage), streams are subject to regulatory actions and enter the Total Maximum Daily Load (TMDL) program. Currently in the U.S., the TMDL Program is being used to restore the condition of the nation's water bodies (U.S. Environmental Protection Agency 1997). The TMDL process involves detailed analysis of land use and point and nonpoint sources of pollution, then development of a management plan for eliminating the impaired condition of a stream. An essential part of the TMDL process is identifying sources (or stressors) causing biological impairment. RBP visual estimates are commonly used to identify stressors associated with habitat, such as sedimentation, and to relate that information to the macroinvertebrate assemblage. Ecological systems are complex, so establishing cause-and-effect relationships may require statistically sound, quantitative data. In the case of benthic habitat, quantitative measurements might need to involve techniques such as pebble counts or granular sieve analysis. RBP protocols are 'rapid screening tools' and were developed to detect impairment - not to identify cause. However, state agencies are often resource limited and overwhelmed with stream reaches that need to be surveyed, thus, conducting detailed quantitative studies is not feasible. However, there is no strong evidence that RBP habitat estimates accurately predict benthic macroinvertebrate metrics (Rabeni 2000). In the state of Virginia, it was recently shown that there is little relationship between rapid habitat assessments and benthic macroinvertebrate data (Frondorf 2001). 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.

If RBP data are going to be used to identify the stressors responsible for impaired water quality, the ability of these rapid measurements of habitat in predicting macroinvertebrate assemblages needs to be evaluated. To address this research question, the 'true' nature of habitat needs to be established and related to reliable estimates of the macroinvertebrate assemblage. We use the term habitat in a narrow sense to mean the physical space where an organism lives and grows (Odum 1983, Ruttner 1964). The best way to estimate the 'true' state is through replicated, quantitative sampling of habitat and macroinvertebrates. Once relationships have been established through replicated, quantitative measurements, results can be compared to less quantitative, rapid approaches.

During this study, we conducted intensive sampling of benthic macroinvertebrates and their habitat in streams subjected to a gradient of cattle grazing. The cattle grazing gradient provided a suitable field experiment because cattle grazing has been shown to affect benthic macroinvertebrate assemblages by habitat alteration, primarily through sedimentation and eutrophication (Armour et al. 1991, Cooper 1993, Fleischner, 1994, Kauffman and Krueger 1984, Owens 1996, Strand and Merritt 1999, Trimble and Mendel 1995). We compared results of our quantitative measurements to results from rapid estimates of habitat to address four questions: (1) which instream quantitative measures of benthic habitat best predict macroinvertebrates as reflected by assemblage metrics? (2) do visual estimates of instream habitat adequately predict benthic macroinvertebrate assemblages? (3) can quantitative measures of riparian habitat predict benthic macroinvertebrate assemblages?, and (4) how well do visual estimates of riparian habitat predict macroinvertebrates?

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, U.S. The Blue Ridge physiographic province is characterized by deeply dissected valleys and ravines that are primarily composed of metamorphosed igneous rocks (granites, granidiorite, 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 (Virginia Agricultural Statistics Service 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 (Virginia Agricultural Statistics Service, 2004).

Five, first-order stream reaches in the Little River drainage basin, Floyd Co., Virginia were selected as study sites (Fig. 4-1). 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 4-1). 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 4-1, Barbour et al. 1999). 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 scores less than 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 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 county 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.

Field methods

Benthic sampling occurred during fall 2002, spring 2003, fall 2003, and spring 2004. However, only data collected during the two fall sampling periods were used in this study because previous studies showed that relationships between habitat and macroinvertebrate metrics were better detected during fall rather than spring when stream baseflow is higher. There were five types of habitat samples:
(1) benthic sampler measurements, (2) pebble count measurements, (3) RBP benthic estimates, (4) quantitative out-of-stream measurements, and (5) RBP out-of-stream estimates.

Benthic sampler measurements. These were instream quantitative measurements that were made inside individual macroinvertebrate samples, or immediately adjacent to them, within study reaches. 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 least 2 m apart, which resulted in the collection of 230 benthic samples for the entire study. Benthic samples were collected by inserting a modified stovepipe corer (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 stovepipe corer, 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 corer were agitated and a 250-ml container was used to subsample water within the sampling device. 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) were 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® meter (see Table 4-2 for further explanations).

<u>Pebble count measurements</u>. These were instream quantitative measurements that were made over entire study reaches. Modified Wolman pebble counts were used to determine the substrate composition of benthic habitat. Using this methodology, particles within high velocity areas were selected at random as the field personnel walked upstream in a zig-zag pattern with heel-to-toe steps and blindly picked up the first particle at the tip of the toe. Each particle was sized with a gravelometer. Heel-to-toe steps continued until 100 particles were selected and sized. When cobble sized particles were encountered during the pebble count, the proportion of cobble buried by fine sediments was measured for an estimate of cobble embeddedness (see Table 4-2 for further explanations).

<u>RBP benthic estimates</u>. These were instream visual estimates that were made over entire study reaches. Visual habitat assessments were independently conducted by two field personnel following U.S. EPA's RBP visual habitat assessment protocols. 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. Results from each independent survey were averaged for an overall study site score. Categories on the form that were relevant to our instream benthic sampler measurements and pebble counts and were considered estimates of instream habitat that could possibly predict macroinvertebrate metrics included velocity/depth regime, sediment deposition, and embeddedness (see Table 4-2 for further explanations).

Quantitative out-of-stream measurements. These were measurements of channel and riparian areas that were made over entire study reaches. Bank and riparian characteristics were measured over a 60-m reach at 12 transects that were spaced 5 m apart. At each transect overhanging vegetation, bank height, and length of exposed bank were measured (see Table 4-2 for further description of methods).

RBP out-of-stream estimates. These were visual estimates of bank and riparian areas that were made over entire study reaches. The same two people made visual estimates of out-of-stream habitat by EPA RBP methods. Categories on the RBP habitat form that were relevant to quantitative measures of bank and riparian characteristics and could possibly predict macroinvertebrate metrics included vegetative protection and bank stability (see Table 4-2 for further explanations).

Laboratory analyses

Benthic macroinvertebrates

In the laboratory, benthic samples were rinsed through a series of stacked sieves (16-mm to 63-µm). Organic materials on sieves ≥ 250-µm were elutriated to separate macroinvertebrates and organic matter from inorganic substrate. All inorganic substrate were 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.

Benthic sampler measurements

Inorganic substrates that remained after organic matter and macroinvertebrate elutriations were separated into standard Wentworth size classes (Wentworth 1922). Particles greater than 8 mm diameter were sized with a gravelometer and weighed. Particles smaller than 8mm were dried to a constant weight, separated into standard

Wentworth particle size classes with a series of stacked sieves and a sieve shaker (e.g., granular sieve analysis), and weighed. Surface cobble weights that were obtained in the field were consolidated 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 Trask's sorting coefficient (see Table 4-2 for explanations).

Data analyses

Particle size data that were obtained from pebble counts were summarized into standard Wentworth particle size classes so that the proportions of cobble, pebble, gravel, and fines were determined for each study site. Embeddedness for each reach was established by computing the mean cobble embeddedness from pebble counts. So that our quantitative, sample scale measures of benthic habitat could be compared to data obtained through pebble counts and visual estimates of habitat at the reach scale, measures of habitat from within the benthic sampler were collapsed by taking an average based on the replicated benthic samples. Following our quantitative field collections and laboratory analyses, 14 benthic environmental variables were available for analyses. See Table 4-2 for a complete list of variables.

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., Barbour et al. 1999 and Brigham et al. 1982) 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 to 10, with 0 indicating very sensitive and 10 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.

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 analysis of variance tests. Following transformations, a series of regression tests were performed strictly on benthic habitat variables that were measured from within the benthic sampler and macroinvertebrate metrics. The variables with the strong relationships with a macroinvertebrate metric were considered most important. Relationships were considered strong if more than 50% of the variation in a metric could be explained by a predictor variable. Although we had quantitative measures of food resources (CBOM, FBOM, chlorophyll *a*, epilithic biomass) they were not included in our analyses because there was no relevant RBP habitat category for comparison.

After establishing which quantitative variables from within the benthic sampler were important in structuring assemblages, the predictive capability of data obtained from pebble counts, stream bank and riparian measures, and visual estimates of habitat were assessed. The category on the RBP habitat survey that was most closely related to important variables measured from within the benthic sampler were selected for comparative purposes. For example, if r^2 values indicated that % fines were important then regressions were rerun using results from the sediment deposition and embeddedness categories on the RBP habitat survey. After identifying the instream habitat variable that best predicted macroinvertebrate metrics, the ability of bank and riparian characteristics in predicting instream habitat was assessed. Linear relationships between environmental variables and macroinvertebrate metrics were assessed first, but a quadratic model was selected if the quadratic term differed significantly from zero.

Individual regression tests were performed for each metric in every sampling period resulting. Multiple individual statistical tests may increase the likelihood of Type I error. Bonferroni adjustments were made to control for procedure wise error by adjusting the test significance level (alpha) by the number of repeated tests. Results were considered significant only if P was less than the adjusted alpha ($\alpha < 0.0001$); however the original P values are also reported so readers can interpret the results without Bonferroni adjustments if desired (Perneger 1998).

Results

Instream measurements and estimates

During fall 2002, % fines from benthic sampler measurements was the best explanatory variable of macroinvertebrate metrics; % fines had a strong relationships with 4 macroinvertebrate metrics and explained the maximum amount of variation (73%) for any individual metric (% Coleoptera) during this sampling period (Table 4-3). Percent fines and embeddedness measurements obtained from pebble counts each had only one strong relationship with a macroinvertebrate metric. The maximum amount of variation in macroinvertebrate metrics that was explained with pebble count measurements was 67% (% collector-filterer) (Table 4-3). RBP visual estimates of sediment deposition and embeddedness each had strong relationships with one macroinvertebrate metric, and the maximum amount of variation explained in macroinvertebrate metrics was 61% (% collector-filterer) (Table 4-3).

During fall 2003, benthic sampler measurements of water depth, was the best explanatory variable of macroinvertebrate metrics (Table 4-4). Water depth measurements associated with the benthic sampler had strong relationships with nine macroinvertebrate metrics. The maximum amount of variation explained during the entire study occurred during this sampling period when water depth measurements explained 83% of the variation in % sensitive taxa (Table 4-4). No strong relationships were detected between RBP visual estimates of the velocity/depth regime and macroinvertebrate metrics.

Trask's sorting coefficient and % fines were other measurements from within the benthic sampler that showed strong relationships with macroinvertebrate metrics during fall 2003; each of these measures had strong relationships with eight macroinvertebrate metrics (Table 4-4). The maximum amount of variation explained by Trask's sorting coefficient and % fines from measurements within the benthic sampler was 73% (% Coleoptera). Percent fines and embeddedness data obtained from pebble counts had fewer strong relationships with macroinvertebrate metrics than substrate measurements from within the benthic sampler, but in most cases these relationships were nearly as strong, or stronger, than relationships that were established with measurements from within the benthic sampler (Table 4-4). RBP visual estimates of sediment deposition and embeddedness had a similar number of strong relationships with macroinvertebrate metrics as quantitative measures from the benthic sampler and pebble counts. In some cases these relationships between RBP visual estimates and macroinvertebrate metrics were nearly as strong, or stronger, as relationships between substrate measurements from within the benthic sampler (Table 4-4).

Three macroinvertebrate metrics (% Coleoptera, number of sensitive taxa, % crawler) had strong relationships with % fines measurements from within the benthic sampler during both years (Figs 4-2 and 4-3). Only one macroinvertebrate metric (% collector-filterer) had strong relationships with % fines and embeddedness measures obtained from pebble counts in both years. Similarly, only one macroinvertebrate metric (% Coleoptera) had a strong relationship with visual estimates of sediment deposition in both years. The remaining habitat measurements and estimates had no consistent, strong relationships with macroinvertebrate metrics.

Out-of-stream characteristics

Relationships between quantitative measurements and visual estimates of bank and riparian characteristics explained less variation in macroinvertebrate metrics than the relationships that were established with instream characteristics (Tables 4-3 through 4-6). The maximum amount of variation explained in macroinvertebrate metrics by measurements or estimates of bank and riparian characteristics never exceeded 69% in either year. During both years, quantitative measurements of overhanging vegetation and exposed bank did not have as many strong relationships with macroinvertebrate metrics as did quantitative measurements of bank height and visual estimates of bank vegetation and bank stability.

During fall 2002, quantitative measurements of bank height had the greatest number of significant relationships with macroinvertebrate metrics. The strongest relationship detected during this sampling period was between bank height and % Coleoptera ($r^2 = 0.6691$, $P < 0.0001$, Table 4-5). Visual estimates of bank stability and vegetative protection had the same number of strong relationships with macroinvertebrate metrics as quantitative measurements of bank height, and the maximum amount of variation in macroinvertebrate metrics that was explained by visual estimates was 61% (% Coleoptera).

During fall 2003, measurement of bank height was the best quantitative predictor of benthic macroinvertebrate metrics; bank height had a strong relationship with seven macroinvertebrate metrics. Visual estimates of vegetative protection and bank stability had strong relationships with 4 and 5 macroinvertebrate metrics, respectively. In some cases, visual estimates had relationships with macroinvertebrate metrics that were as strong, or stronger than quantitative measurements of bank height.

Out-of-stream characteristics and instream % fines

During fall 2002, bank height and exposed bank measurements and visual estimates of bank stability had strong relationships with % fines that were measured from within the benthic sampler. However, bank height was the best predictor of % fines in fall 2002 ($r^2 = +0.8435$, $P < 0.0001$, Table 4-7). During fall 2003, only bank height had a strong relationship with % fines measurements from within the benthic sampler ($r^2 = +0.7684$, $P < 0.0001$, Table 4-7).

Discussion

In this study, macroinvertebrate assemblages were best explained by quantitative measurements of % fines that were collected concurrently with macroinvertebrates from within an enclosed sampler. Although pebble count measurements of instream habitat and RBP visual estimates of instream habitat showed some strong relationships with some macroinvertebrate metrics, the relationships were not as consistent for both years. Out-of-stream habitat characteristics (bank height measurements and visual estimates of bank stability) can adequately predict macroinvertebrate metrics but not as well as instream quantitative measurements. These out-of-stream characterisics are better used for predicting instream habitat characteristic (% fines) rather than macroinvertebrate assemblages.

Other research on RBP habitat estimates has shown them to be much less effective at predicting macroinvertebrate assemblages. Hannaford and Resh (1995) reported that the results of RBP habitat estimates were inconsistent with RBP macroinvertebrate results in three California stream reaches. Similarly, Frondorf (2001) analyzed a dataset from many widespread streams in Virginia over multiple years and found that the best relationship between RBP visual estimates and macroinvertebrates was with the RBP estimate for embeddedness, but the relationship was always weak ($r^2 < 0.50$). In a recent study of another large dataset in Virginia, the Department of Environmental Quality (2005) found that three RBP habitat estimates (embeddedness, riparian vegetation, total score) were the best predictors of macroinvertebrate assemblages, but the relationships were always weak. Our more positive evaluation of RBP habitat estimates may have been influenced by two factors. The estimates were always an average of two people, one of whom was always an experienced aquatic biologist (the senior author) and the other was one of several experienced undergraduate students who had taken courses such as freshwater ecology, aquatic entomology, and freshwater biomonitoring. This special circumstance would not apply to the benthic sample measurements and pebble count measurements because those techniques involve random sampling and standardized analytical techniques that remove the possibility of human error and bias.

There are other aspects of RBP habitat estimates that call into question their validity and usefulness for meeting the needs of the TMDL program, as described by NRC (2001). RBP habitat estimates do not produce a unit of measurement that can be incorporated into models of ecological interactions, either within streams or between land and streams. RBP habitat estimates are unitless values, whereas benthic sample measurements and pebble count measurements generate empirical values that describe the actual stream environment. In the case of sediment, which is a primary stressor from agricultural land use, there are models for overland transport into waterbodies but to relate these model outputs to benthic conditions requires data on exactly how much deposited sediment there is (e.g., mg of fines per m^3 of stream bottom).

Benthic habitat assessments are usually done for a study reach without replication in order to reduce costs. Hannaford and Resh (1995) have pointed out that the cost-saving advantages come at the price of losing information on spatial variability and not being able to report the probability of sites being different due to chance versus the effect of the stressor in question. Even if RBP habitat estimates are replicated, we question the reliability of the estimates of variance. RBP habitat estimates are categorical discrete variables that can only be whole numbers from 0 to 20, in contrast to benthic habitat measurements that are absolute continuous variables that can range from 0 to infinity with fractions of units in decimal places that are only limited by the sensitivity of the measuring devices. Thus, the variance of RBP habitat estimates may be artificially low. Unitless values and artificially low variance likely produce a situation in which statistical analyses of relationships are significant but not ecologically meaningful.

In summary, we concur with others who have suggested that RBPs are only intended to serve as screening tools to rank sites according to their ecological condition and to prioritize which sites need further study (Hannaford and Resh 1995). To develop TMDLs and to implement them for stream restoration requires identifying and linking stressors to macroinvertebrate assemblages. In the case of sedimentation, we suggest that this is best done with benthic sampler measurements of % fines and bank height measurements out of the stream.

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Table 4-1 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*	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})†					
Minimum	10	8	8	14	2
Maximum	62	47	25	77	10
Average	25	25	15	30	5
Mean wetted width (m)‡	0.88	0.72	1.11	0.76	0.60
Mean depth (m) ‡	0.08	0.13	0.13	0.09	0.10
Conductivity (µS cm^{-1})£	16 - 22	18 - 23	54 - 63	19 - 22	54 – 57
Maximum temperature (°C)£	18.5	20.0	20.5	21.5	21.5

* 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).

† Baseflow discharge was measured on 8 separate occasions between July 2002 and August 2003.

‡ 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).

£ Conductivity and temperature values are based on 15 spot measures taken throughout the study period.

Table 4-2 Explanations of instream and out-of-stream environmental variables.

	Environmental variables	Units	Description
INSTREAM CHARACTERISTICS	**Variables from within the benthic sampler**		
	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
	% fines	%	Proportion (by weight) of substrate sized < 2 mm within each benthic sample
	Trask's sorting coefficient	none	Substrate size homogeneity within each benthic sample (heterogeneity >1) (Inman 1962) $\left[D_{84}/D_{16}\right]^{-0.5}$
	Pebble count variables		
	% fines	%	Proportion of fine particles (by frequency) of substrate sized < 2mm from modified Wolman pebble counts (Wolman 1954)
	embeddedness	%	Mean proportion of cobble embeddedness from modified

			Wolman pebble counts
	Visual estimates		
	Velocity/Depth Regime	none	Heterogeneity of velocity/depth regimes (slow-deep, slow-shallow, fast-deep, fast-shallow); category 3 on RBP habitat survey
	Deposited Sediment	None	Proportion of stream bottom affected by sediment deposition; category 4 on RBP habitat survey
	Embeddedness	None	Proportion of cobble, pebble, and gravel surrounded by fine sediment; category 2 on RBP habitat survey
STREAM BANK/RIPARIAN CHARACTERISTICS	**Quantitative measures**		
	Overhanging vegetation	%	The amount of bank vegetation providing stream shade; measured at the water surface with a spherical densitometer at evenly spaced transects.
	Exposed Bank	%	Length of riparian composed of bare, exposed soil determined through direct measures at transects
	Bank height	cm	Height of stream bank measured as a vertical distance from the

		within the stream channel at water surface determined through direct measures at transects
Visual estimates		
Vegetative Protection	None	Proportion of stream bank surfaces coverer by vegetation; category 9 on RBP habitat survey
Bank Stability	None	Proportion of stable and eroded bank; category 8 on RBP habitat survey

Table 4-3. Relationships between measures and estimates of instream benthic habitat and macroinvertebrate metrics from data collected during fall 2002. Coefficients of determination (r^2) and *P* values are provided for each relationship; ns indicates relationships were not significant at alpha = 0.05. Following Bonferroni adjustments, results were considered significant if $P < 0.0001$. Relationships were considered strong if r^2 was greater than 0.50. Strong relationships are indicated in bold.

	Benthic sampler measurements		RBP benthic esimates	Benthic sampler measurements		Pebble count variables		RBP benthic esimates	
	Flow	Depth	Velocity-depth regime	Trask's	% fines	% fines	Embeddedness	Sediment Deposition	Embeddedness
Richness	q0.1908 0.0027	q0.2496 0.0003	ns	-0.1238 0.0063	q0.4251 <0.0001	ns	-0.0794 0.0306	q 0.2490 <0.0001	+0.1412 0.0034
%Plecoptera	q0.4078 <0.0001	q0.2320 0.0006	ns	-0.1930 0.0005	-0.3936 <0.0001	-0.3507 <0.0001	-0.3078 <0.0001	+0.2085 0.0003	+ 0.2408 <0.0001
%Coleoptera	q0.4530 <0.0001	q0.4784 <0.0001	ns	-0.3410 <0.0001	**q0.7305 <0.0001**	ns	ns	**+0.5910 <0.0001**	+0.3701 <0.0001
Simpson's Diversity	ns	q0.3193 <0.0001	ns	q0.3434 <0.0001	q0.4757 <0.0001	ns	ns	q 0.4500 <0.0001	q 0.1748 0.0046
% collector-gatherer	ns	-0.1407 0.0034	ns	ns	ns	ns	ns	-0.1479 0.0100	-0.0904 0.0207
% collector-filterer	ns	+0.2254 0.0008	ns	**q0.7179 <0.0001**	**q0.7148 <0.0001**	**+0.6710 <0.0001**	**+0.5794 <0.0001**	q0.4607 <0.0001	**q0.6145 <0.0001**
% scraper	q0.2427 0.0004	q0.1179 0.0298	+0.1154 0.0085	q0.4977 <0.0001	-0.1712 0.0011	ns	ns	q0.3586 <0.0001	+0.4313 <0.0001
% shredder	ns	ns	ns	ns	-0.1097	ns	ns	+0.0725	ns

					0.0104			0.0391	
%sensitive taxa	q0.2246 0.0008	q0.1265 0.0227	+0.1115 0.0098	q0.4998 <0.0001	-0.1786 0.0009	ns	ns	q0.3769 <0.0001	+0.4912 <0.0001
Number of sensitive taxa	q0.3559 <0.0001	q0.3611 <0.0001	ns	-0.3037 <0.0001	**q0.6310** **<0.0001**	-0.2144 0.0002	-0.2181 0.0002	+ 0.4707 <0.0001	+ 0.3351 <0.0001
% clinger	q0.1248 0.0004	ns	+0.1227 0.0065	q0.3968 <0.0001	-0.1666 0.0013	-0.0839 0.0261	ns	q 0.2515 0.0003	q 0.4025 <0.0001
% crawler	q0.3175 <0.0001	**q0.5254** **<0.0001**	ns	-0.0753 0.0354	**q0.5635** **<0.0001**	ns	-0.1224 0.0066	+0.3683 <0.0001	+0.0803 0.0297
% burrower	ns	ns	q0.3875 <0.0001	+0.3424 <0.0001	+0.2760 <0.0001	ns	ns	-0.3035 <0.0001	-0.3401 <0.0001
Number of significant relationships	4	4	1	8	9	2	2	9	8
Number of strong relationships	0	1	0	1	4	1	1	1	1

Table 4-4. Relationships between measures and estimates of instream benthic habitat and macroinvertebrate metrics from data collected during fall 2003. Coefficients of determination (r^2) and P values are provided for each relationship; ns indicates relationships were not significant at alpha = 0.05. Following Bonferroni adjustments, results were considered significant if $P < 0.0001$. Relationships were considered strong if r^2 was greater than 0.50. Strong relationships are indicated in bold.

	Benthic sampler measurements		RBP benthic esimates	Benthic sampler measurements		Pebble count measurements		RBP benthic estimates	
	Flow	Depth	Velocity-depth regime	Trask's	% fines	% fines	Embeddedness	Sediment Deposition	Embeddedness
Richness	ns	**q0.5665** **<0.0001**	q 0.4873 <0.0001	**-0.6704** **<0.0001**	**q0.6956** **<0.0001**	-0.3515 <0.0001	**-0.6419** **<0.0001**	q0.4873 <0.0001	+0.1585 0.0024
%Plecoptera	+0.3948 <0.0001	**q0.6356** **<0.0001**	ns	-0.3974 <0.0001	-0.3719 <0.0001	-0.4741 <0.0001	**q0.6142** **<0.0001**	**+0.5507** **<0.0001**	**+0.6879** **<0.0001**
%Coleoptera	ns	**q0.7927** **<0.0001**	ns	**-0.7304** **<0.0001**	**-0.7262** **<0.0001**	**0.6081** **<0.0001**	**q0.7053** **<0.0001**	**q0.5844** **<0.0001**	+0.4695 <0.0001
Simpson's Diversity	ns	q0.4861 <0.0001	ns	-0.4117 <0.0001	**-0.5026** **<0.0001**	-0.3436 <0.0001	q0.3579 <0.0001	q0.3375 <0.0001	+0.2957 <0.0001
% collector-gatherer	q0.2890 0.0001	ns	ns	**q0.7052** **<0.0001**	**+0.6603** **<0.0001**	**q0.6284** **<0.0001**	+0.4490 <0.0001	-0.4939 <0.0001	q 0.4768 <0.0001
% collector-filterer	ns	q0.2435 0.0006	ns	-0.2495 <0.0001	-0.3480 <0.0001	0.1601 0.0022	0.2239 0.0022	q 0.2155 0.0016	+0.0802 0.0344
% scraper	ns	**q0.7237** **<0.0001**	**ns**	**-0.5149** **<0.0001**	-0.4496 <0.0001	**-0.7277** **<0.0001**	**q0.7389** **<0.0001**	**+0.6299** **<0.0001**	**+0.6280** **<0.0001**
% shredder	+0.3777 <0.0001	**q0.6229** **<0.0001**	ns	-0.4028 <0.0001	-0.3535 <0.0001	-0.4491 <0.0001	**q0.5960** **0.0005**	**+0.5642** **<0.0001**	**+0.6457** **<0.0001**

%sensitive taxa	+0.1535 0.0028	**q0.8365** **<0.0001**	+0.1031 0.0158	**-0.6528** **<0.0001**	**-0.6719** **<0.0001**	**-0.6908** **<0.0001**	**-0.7246** **<0.0001**	**+0.6360** **<0.0001**	**+0.6867** **<0.0001**
Number of sensitive taxa	ns	**q0.7750** **<0.0001**	ns	**-0.7017** **<0.0001**	**q0.6836** **<0.0001**	**-0.6743** **<0.0001**	**q0.7794** **<0.0001**	**q0.7566** **<0.0001**	+0.4481 <0.0001
% clinger	ns	**q0.5792** **<0.0001**	q0.0811 0.0334	-0.3198 <0.0001	-0.2803 <0.0001	**-0.6785** **<0.0001**	**q0.6222** **<0.0001**	**q0.6110** **<0.0001**	**q0.6166** **<0.0001**
% crawler	ns	**q0.8023** **<0.0001**	ns	**-0.7002** **<0.0001**	**q0.6751** **<0.0001**	**-0.6318** **<0.0001**	**q0.7237** **<0.0001**	**q0.6974** **<0.0001**	**-0.5333** **<0.0001**
% burrower	q0.2054 0.0023	ns	ns	**+0.5724** **<0.0001**	**+0.5354** **<0.0001**	**q0.7060** **<0.0001**	+0.4431 <0.0001	**-0.5452** **<0.0001**	**-0.5612** **<0.0001**
Number of significant relationships	2	10	1	13	13	12	11	12	11
Number of strong relationships	0	9	0	8	8	8	10	9	7

Table 4-5. Relationships between measurements and estimates of out-of-stream characteristics during fall 2002. Coefficients of determination (r^2) and *P* values are provided for each relationship; ns indicates relationships were not significant at alpha = 0.05. Following Bonferroni adjustments, results were considered significant if *P* < 0.0001. Relationships were considered strong if r^2 was greater than 0.50. Strong relationships are indicated in bold.

	Quantitative measurements	RBP estimates	Quantitative measurements		RBP estimates
	Overhanging vegetation	Vegetative protection	Bank height	Exposed bank	Bank stability
Richness	ns	q0.3959 <0.0001	-0.3806 <0.0001	q0.2986 <0.0001	q0.3941 <0.0001
% Plecoptera	q0.2339 0.0006	q0.1994 0.0020	-0.2881 <0.0001	q0.2399 0.0005	+0.1883 0.0006
% Coleoptera	ns	**q0.5045** **<0.0001**	**0.6691** **<0.0001**	**q0.5060** **<0.0001**	**q0.6089** **<0.0001**
Simpson's Diversity	-0.2678 <0.0001	**q0.5144** **<0.0001**	-0.1940 0.0005	q0.1209 0.0271	q0.4249 <0.0001
% collector-gatherer	0.2476 <0.0001	+0.1865 0.0006	+0.0848 0.0252	ns	ns
% collector-filterer	**0.5011** **<0.0001**	0.1690 0.0012	q0.3804 <0.0001	q0.3361 <0.0001	q0.3717 <0.0001
% scraper	q0.2828 <0.0001	q0.2577 0.0002	ns	ns	+0.0840 0.0260
% shredder	ns	q0.1723 0.0050	0.1486 0.0026	q0.1417 0.0138	q0.1742 0.0047
% sensitive taxa	q0.2815 <0.0001	0.2723 0.0001	ns	ns	0.0918 0.0197
Number of sensitive taxa	q0.1109 0.0372	q0.4652 <0.0001	**-0.5662** **<0.0001**	-0.3890 <0.0001	**+0.5067** **<0.0001**
% clinger	q0.2621 0.0002	0.1516 0.0100	0.0708 0.0417	ns	ns
% crawler	ns	**q0.5756** **<0.0001**	**q0.5707** **<0.0001**	q0.4931 <0.0001	**q0.5817** **<0.0001**
% burrower	ns	ns	q0.3346 <0.0001	ns	-0.1703 0.00
Number of significant	5	5	7	5	6

relationships					
Number of strong relationships	1	3	3	1	3

Table 4-6. Relationships between measurements and estimates of out-of-stream characteristics during fall 2003. Coefficients of determination (r^2) and *P* values are provided for each relationship; ns indicates relationships were not significant at alpha = 0.05. Following Bonferroni adjustments, results were considered significant if *P* < 0.0001. Relationships were considered strong if r^2 was greater than 0.50. Strong relationships are indicated in bold.

	Quantitative measurements	RBP estimates	Quantitative measurements		RBP estimates
	Overhanging vegetation	VP	Bank height	Exposed bank	BS
Richness	**q0.6358 <0.0001**	**q0.6928 <0.0001**	**-0.5738 <0.0001**	q0.4165 <0.0001	**q0.6901 <0.0001**
% Plecoptera	0.3530 <0.0001	q0.2007 0.0026	0.4167 <0.0001	q0.3280 <0.0001	0.3931 <0.0001
% Coleoptera	**q0.5465 <0.0001**	**q0.6376 <0.0001**	**-0.6222 <0.0001**	q0.3365 <0.0001	**q0.6370 <0.0001**
Simpson's Diversity	q0.2987 <0.0001	q0.3528 <0.0001	-0.3361 <0.0001	q0.1950 0.0032	q0.3461 <0.0001
% collector-gatherer	0.2261 0.0002	ns	**q0.6993 <0.0001**	0.1369 0.0050	-0.3900 <0.0001
% collector-filterer	q0.2870 0.0001	q0.2822 0.0002	0.1780 0.0012	q0.1957 0.0031	q0.3197 <0.0001
% scraper	q0.3443 <0.0001	q0.4714 <0.0001	0.4121 <0.0001	-0.1579 0.0024	q0.4497 <0.0001
% shredder	0.3907 <0.0001	q0.2097 0.0020	0.4292 <0.0001	q0.3653 <0.0001	+0.4277 <0.0001
% sensitive taxa	q0.4366 <0.0001	q0.4891 <0.0001	**0.5750 <0.0001**	q0.2819 0.0002	**q0.5208 <0.0001**
Number of sensitive taxa	**q0.5260 <0.0001**	**q0.6835 <0.0001**	**0.5718 <0.0001**	q0.2709 0.0002	**q0.6496 <0.0001**
% clinger	+0.1259 0.0073	q0.4440 <0.0001	0.2161 0.0003	ns	+0.2262 0.0002
% crawler	**q0.5245 <0.0001**	**q0.5716 <0.0001**	**-0.6166 <0.0001**	q0.3361 <0.0001	**q0.6108 <0.0001**
% burrower	-0.1952 0.0007	ns	**q0.6416 <0.0001**	+0.1117 0.0118	-0.3462 <0.0001
Number of significant	9	8	11	5	12

relationships					
Number of strong relationships	4	4	7	0	5

Table 4-7. Relationships from regression analysis between measures of % fines (from benthic sampler measurements) and bank characteristics from fall 2002 (F02) and fall 2003 (F03). Coefficients of determination (r^2) and *P* values are provided for each relationship; ns indicates relationships were not significant at alpha = 0.05. Following Bonferroni adjustments, results were considered significant if *P* < 0.0001. Relationships were considered strong if r^2 was greater than 0.50. Strong relationships are indicated in bold.

		Quantitative measures		RBP estimates
		Bank height	Exposed bank	Bank Stability
% fines	F02	**+ 0.8435** **<0.0001**	**+ 0.5617** **<0.0001**	**- 0.7848** **<0.0001**
% fines	F03	**q 0.7684** **<0.0001**	ns	-0.3573 <0.0001

Fig. 4-1 Study site locations in Floyd Co., Virginia.

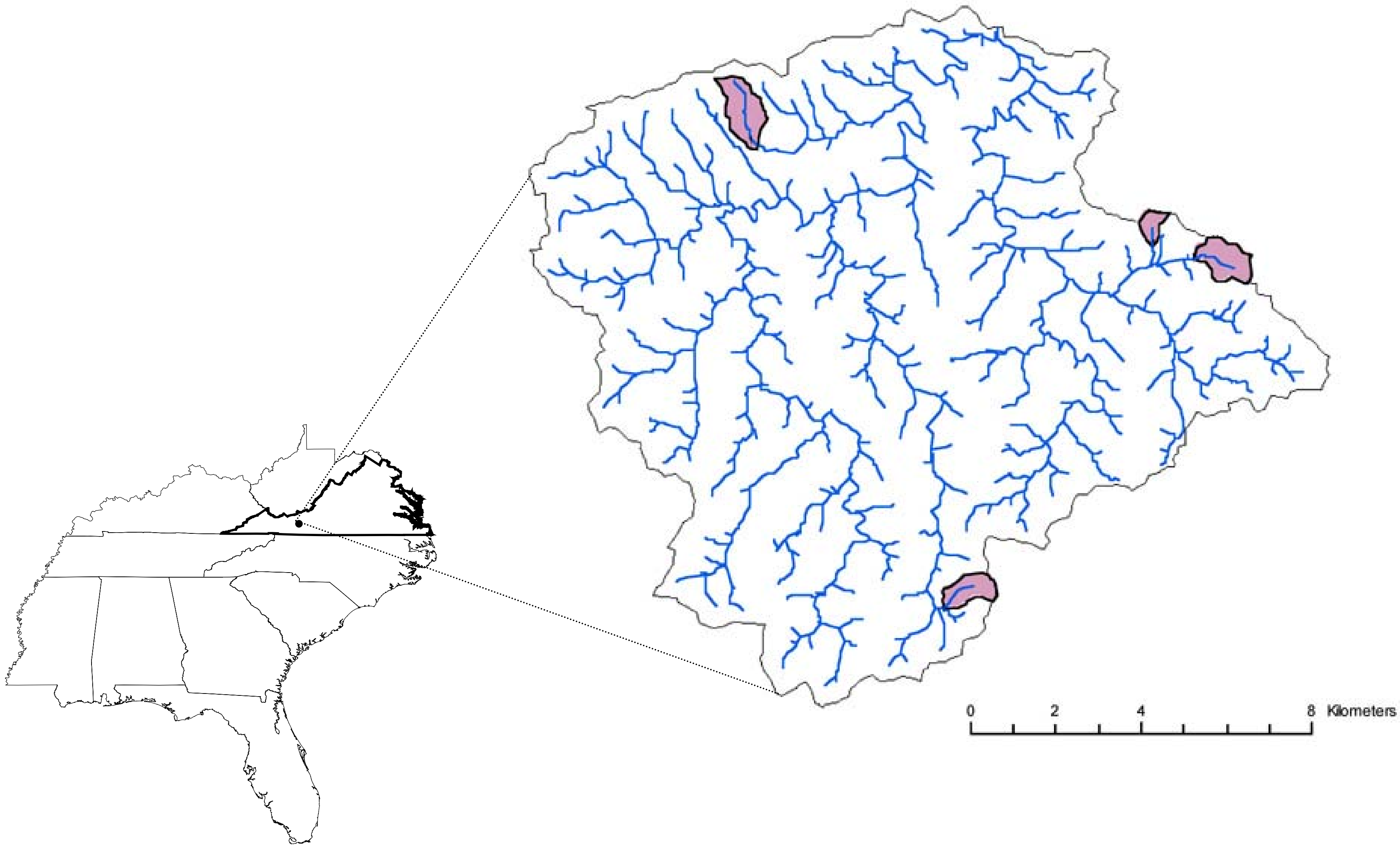
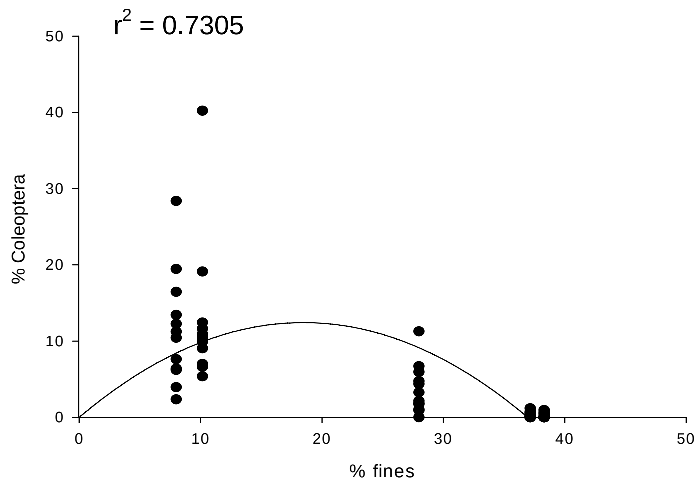
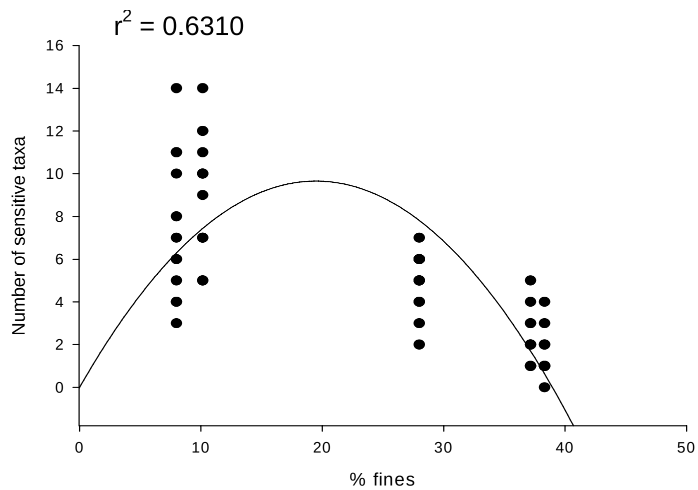


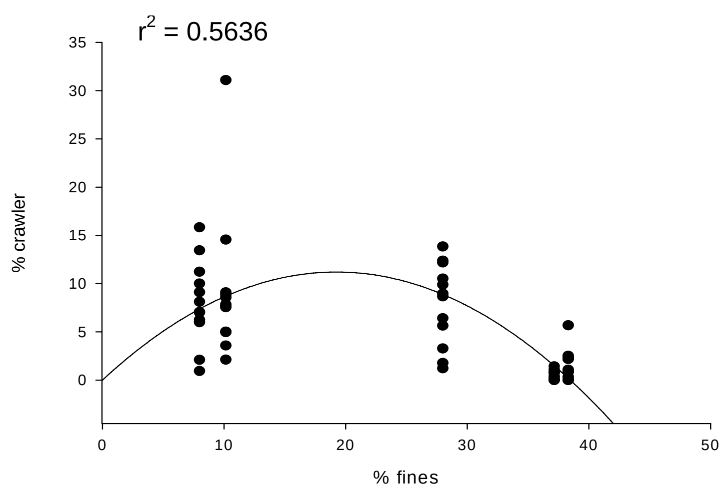
Fig. 4-2 Relationships between % fines (obtained from within the benthic sampler) and macroinvertebrate metrics from fall 2002. All relationships were highly significant ($P < 0.0001$). Coefficients of determination (r^2) are from Table 3.



$r^2 = 0.7305$
% Coleoptera
0
10
20
30
40
50
% fines

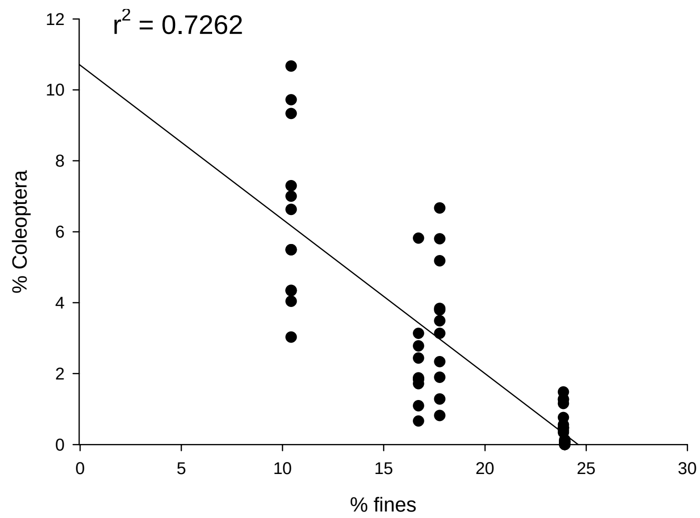


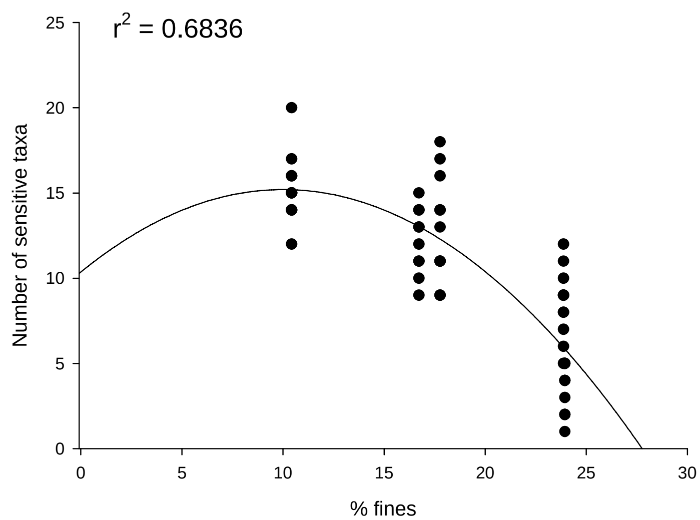
$r^2 = 0.6310$
Number of sensitive taxa
0
2
4
6
8
10
12
14
16
% fines

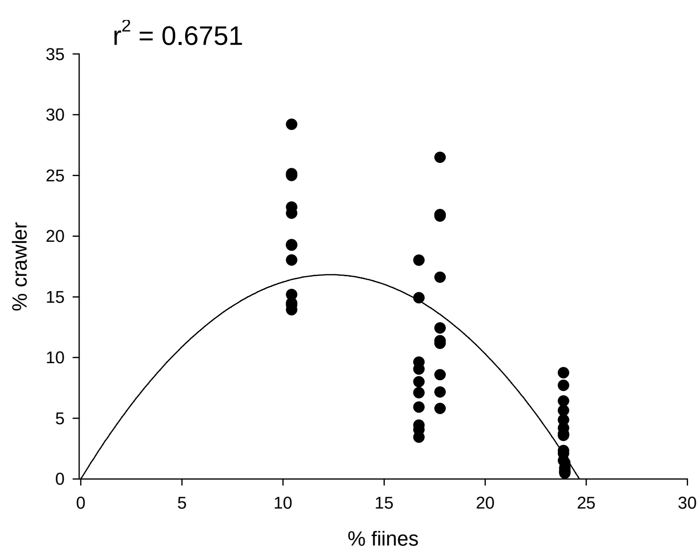


$r^2 = 0.5636$
% crawler
0
5
10
15
20
25
30
35
% fines

Fig. 4-3. Relationships between % fines (obtained from within the benthic sampler) and macroinvertebrate metrics from fall 2003. All relationships were highly significant ($P < 0.0001$). Coefficients of determination (r^2) are from Table 4.


r² = 0.7262
% Coleoptera
12
10
8
6
4
2
0
0
5
10
15
20
25
30
% fines


r² = 0.6836
Number of sensitive taxa
25
20
15
10
5
0
0
5
10
15
20
25
30
% fines


r² = 0.6751
% crawler
35
30
25
20
15
10
5
0
0
5
10
15
20
25
30
% fiines

CONCLUSIONS

Using a study design that involved a gradient of increasing stress (increased cattle density) allowed us to link environmental stressors associated with cattle grazing to macroinvertebrates. The empirical relationships that are presented in this dissertation will facilitate the development of computer models that more accurately predict biological responses to stressors in streams (e.g., U.S. EPA's AQUATOX) associated with cattle grazing. The results will also be useful in establishing habitat thresholds and in the design of surveys that include components of habitat that are most relevant to macroinvertebrates. In relation to the original research questions that were posed, our findings can be summarized as follows:

(1) What is the composition of the macroinvertebrate fauna in small cattle-impacted streams?

> During this study a fairly large number of taxa were identified from streams impacted by cattle. Additionally, there are likely many more taxa in our study streams because macroinvertebrates were rarely identified beyond genus. Our findings demonstrate that taxon frequency and presence/absence patterns, in conjunction with taxon-specific natural history information, are useful information for identifying potential stressors associated with cattle grazing.

(2) Does macroinvertebrate assemblage structure change predictably in response to different levels of cattle grazing?

> Macroinvertebrate assemblages changed predictably in response to cattle grazing intensity and responses were best detected under base flow. Most macroinvertebrates showed a negative response to the grazing gradient, and % Coleoptera and % sensitive taxa showed particularly strong responses. Slight increases in some metrics (richness, number of sensitive taxa, % collector-filterer) before declining along the gradient suggested that light levels of cattle grazing created conditions suitable for many taxa.

(3) Which environmental factors are most important in structuring macroinvertebrate assemblages?

Alteration of benthic habitat, especially % fines, as a result of cattle grazing, rather than alterations to detrital and autochthonous food resources, were the most important stressor of the macroinvertebrate assemblages in these small streams. It is recommended that when research goals are to relate macroinvertebrates to environmental factors or stressors, a significant level of effort should be exerted to establish relationships at the scale that is most relevant to macroinvertebrates, the sample scale. To ensure a full understanding of the variables that alters macroinvertebrate assemblages: (1) replicated sampling should occur within a study design that includes a gradient of stress, (2) an enclosed sampler should be used so that macroinvertebrates and measures of benthic environmental factors can be collected concurrently and, (3) sampling should continue through time.

(4) Which method of habitat characterization best explains macroinvertebrate assemblage structure: benthic measurements or RBP estimates?

Quantitative measures of % fines, collected from within an enclosed sampler concurrently with macroinvertebrates, were the best predictor of macroinvertebrate assemblages. Quantitative measures and visual estimates of riparian and channel characteristics had strong relationships with macroinvertebrate metrics, but the relationships were never as strong as those detected with instream measurements of % fines. The channel characteristic, bank height, was the best predictor of % fines.

VITA

Amy Braccia was born in Newport News, Virginia, USA in 1972. She completed her Bachelor of Science in Biology at Virginia Tech in 1994. Before beginning graduate studies, she worked as an Environmental Scientist for a private consulting firm in Arlington, VA. In fall 1997, Ms. Braccia began her master's thesis under the direction of Dr. Darold Batzer in the Department of Entomology at the University of Georgia. She remained at the University of Georgia and worked as a research coordinator after completing her Master of Science in Entomology. In 2001, she began her Ph.D. in Entomology at Virginia Tech under the guidance of Dr. J. Reese Voshell, Jr.