Effects of stream network topology on fish assemblage structure and bioassessment sensitivity in the mid-Atlantic highlands, USA

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ABSTRACT

Stream fish assemblages exist within stream networks defined by the size and proximity of connected streams (i.e., stream network topology). The spatial position of sites within stream networks may therefore regulate opportunities for fish dispersal to access distant resources or colonize "new" habitats. Such inter-stream dispersal dynamics will influence local fish assemblage structure and the vulnerability of local assemblages to anthropogenic stressors. In this dissertation, I explored the effects of stream network topology on fish assemblage structure in the mid-Atlantic highlands, USA and tested the hypothesis that dispersal would affect the sensitivity of fish-based environmental quality assessments (i.e., bioassessments).

In chapter 1, I evaluated the effects of stream networks by comparing fish assemblages between sites with and without large downstream confluences (>3rd order) in western Virginia, USA (i.e., mainstem tributaries and headwater tributaries, respectively). I found that local species richness was higher in mainstem tributaries than headwater tributaries and that these effects could not be explained by variation in local environmental habitat conditions. In chapter 2, I developed and applied a continuous model of stream network topology to explore the effects of downstream size and

proximity on local fish assemblage structure within the mid-Atlantic highlands. I found that fish assemblage structure (i.e., Bray-Curtis distances in species abundance) was significantly related to variation in stream network topology up to approximately 9 fluvial km from sites.

Chapters 3 and 4 explored the implications of inter-stream dispersal for fish bioassessments. In Chapter 3, I identified 10 fish metrics that corresponded predictably to environmental stressors in the mid-Atlantic highlands. However, headwater tributary assemblages showed stronger relations to local environmental quality than mainstem tributaries, consistent with the hypothesis of riverine dispersal. In Chapter 4, I compared the effects of stream network topology on fish and benthic macroinvertebrate assemblages. Fish metrics were influenced by the size and proximity of connected streams but benthic macroinvertebrate metrics were not. This finding suggests that stream fishes may complement benthic macroinvertebrate bioassessments by indicating environmental conditions at larger spatial grains.

Dedication

I dedicate this dissertation to the students and faculty members killed during the tragic events on our campus on Monday, April 16, 2007. *Ut prosim!*

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Attribution

Paul L. Angermeier co-authored Chapter 1. This paper was published in volume 48 of the American Fisheries Society Symposia (Hughes, Wang, and Seelbach, eds). Dr. Angermeier's contribution to this chapter includes assistance in developing the testing framework, evaluation of the statistical analysis, and editing the manuscript for publication.

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General Introduction

Landscape ecology posits a fundamental question for community analysis: how does the spatial *context* of a sample site influence the *content* of the sample (Turner 1989)? Stated differently, are ecological communities structured primarily by locally-determined processes of biotic interactions and habitat suitability or by regionally-determined processes of dispersal and connectivity? If the former, ecological communities may be understood as a collection of organisms that are able to persist under local environmental conditions (i.e., species-sorting model, see Liebold *et al*. 2004). If the latter, communities may be understood as a random collection of organisms that were able to disperse into a local site (i.e., neutral theory, Hubbell 2001). A key distinction of these models is that the effects of local habitat conditions would be more important in locally-structured communities than in communities regulated by regional processes of dispersal and connectivity. Several authors have suggested that both local and regional factors affect community structure (Angermeier and Winston 1998; Tuomisto *et al*. 2003; Cottenie 2005), but the relative importance of these factors remains poorly understood in large part because we know so little about dispersal.

Competing models of community organization influence biological management and conservation practices by designating the spatial scale for analysis and actions. If communities are regulated primarily by local processes, management and conservation actions may justifiably focus on local conditions (i.e., biotic interactions and local habitat quality). However, if regional processes dominate community structure, management

and conservation actions that focus on local habitat conditions may or may not be successful, depending on regional processes of dispersal and connectivity. In many cases, management and conservation programs operate under a tacit assumption that local processes regulate community structure (Liebold *et al*. 2004). However, previous studies have demonstrated an important role of regional connectivity and dispersal in ecological restoration (Lepori *et al*. 2005; Jansson *et al*. 2007; Hughes 2007), conservation reserve design (Simberloff and Abele 1975; Pringle 2001; Fagan and Lutscher 2006; Mumby 2006), and biological assessments of environmental quality (i.e., bioassessments; Osborne *et al*. 1992; McBride and Booth 2005).

The study of dispersal is therefore necessary to understand the multiple spatial scales at which environmental conditions influence communities and to design biological management and conservation programs accordingly. In its most general sense, dispersal refers to the movement of individual organisms across a landscape to access resources or to colonize "new" habitats (Wiens 2001). If local conditions are unsuitable for reproduction or survival, dispersal may increase individual fitness by promoting reproductive success elsewhere. However, dispersal has energetic costs and exposes organisms to increased predation risk (e.g., Fraser *et al*. 1995; Fraser *et al*. 2006; Roberts and Angermeier 2006). Fitness costs and benefits of dispersal are therefore influenced by the environmental conditions through which organisms move.

The spatial scales germane to dispersal may be understood based on organismal behavior and the spatial distribution of habitat patches and source populations. Dispersal to access resources may encompass relatively frequent movements over short distances, depending on the spatial proximity of non-substitutable habitat patches (i.e.,

complementary habitats; Dunning *et al*. 1992). For example, local habitat heterogeneity has been hypothesized to reduce the probability of dispersal because local environmental conditions can provide the habitat patches necessary for life history expression (Albanese *et al*. 2004). By comparison, dispersal to colonize "new" habitats may require movements over much longer distances (Van Dyke and Baguette 2005). Under this scenario, the spatial distribution of immigrant-source populations will influence the probability that a local assemblage is regulated by dispersal (Hanksi 1998; Harrison 1991). Although short-distance dispersal is typically more common than long-distance dispersal (i.e., leptokurtic dispersal distance frequencies; Wiens 2001), rare long-distance dispersal events can have important consequences for the geographic distribution of species (e.g., Suarez *et al*. 2001) and for the genetic composition of component populations (e.g., Hitt et al. 2003).

Stream ecosystems provide a distinct model system to study dispersal due to their unique spatial structure. Unlike terrestrial environments, dispersal in lotic environments is constrained to the stream channel (excluding adult dispersal of benthic macroinvertebrates) in upstream-downstream vectors and among connected streams (Wiens 2002). The dendritic branching pattern of stream networks also provides a distinct form of landscape connectivity where small geographic distances (i.e., Euclidean distances) may be very distant in terms of hydrological connectivity (Fagan 2002; Cambell-Grant *et al*. 2006). For example, Hitt *et al*. (2003) found that fluvial distances among sites explained population genetic structure of westslope cutthroat trout (*Oncorhynchus clarki lewisi*) whereas straight-line distances did not. The confined

pathways of dispersal within stream networks also will produce distinct responses to disturbance events, compared to terrestrial environments (Fagan 2002).

The size and proximity of connected streams (i.e., stream network topology) provides a spatial framework to evaluate lotic dispersal. Whereas the River Continuum Concept (RCC; Vannote *et al*. 1980) emphasizes local conditions based on longitudinal variation in stream size, stream network topology emphasizes regional conditions based on the size and proximity of connected streams (Benda *et al*. 2004a; Lowe *et al*. 2006). For example, a network perspective would posit that the biotic community in a stream flowing into a river would be distinct from the community in a similarly-sized stream that lacks a river confluence within a given distance. Furthermore, differences between these two communities would stem from movement by species adapted to live in either stream or river habitats. In contrast, the RCC would predict that the communities in these streams are similar due to their similar size and that species composition largely reflects adaptations to local environmental conditions.

Studies of stream fish movements and distributions suggest that stream networks may have important consequences for fish dispersal dynamics. Empirical studies have documented long-distance movements in non-diadromous stream fishes (i.e., >1 fluvial km [fkm]; Karr and Gorman 1975; Gatz and Adams 1994; Albanese *et al*. 2004; Roghair and Dolloff 2005). Within North America, river systems flowing directly into the ocean generally contain fewer fish species than rivers of equal size connected to other river systems (Sheldon 1988), suggesting that connected streams and rivers provide an important source of immigrants. Analogous patterns have been detected within watersheds: stream sites near large rivers tend to support more species than similarly

sized streams that lack riverine connections (Gorman 1986; Osborne and Wiley 1992; Schaefer and Kerfoot 2004; Smith and Kraft 2005; Hitt and Angermeier 2006).

Studies of stream connectivity also suggest an important role of inter-stream dispersal in freshwater fishes. Matthews and Robison (1998) found that stream fish assemblages of the Ouachita mountains tended to be more similar among sites separated by fewer confluences (i.e., less fluvial distance). Angermeier and Winston (1998) demonstrated that local stream fish species richness was better predicted by fish diversity within relatively small regions (i.e., physiographic region-drainage combinations) than by larger regions (i.e., river drainages), suggesting that inter-stream connectivity and dispersal affect local assemblage structure. Spatial autocorrelation in stream fish assemblages (Wilkinson and Edds 2001; Hitt *et al*. 2003; Grenouillet *et al*. 2004) also provides evidence that inter-stream dispersal affects local assemblage structure. However, previous studies have been largely limited to single zoogeographic regions and therefore may not provide general inferences about stream fish dispersal dynamics (but see Sheldon 1988).

Empirical measurements of fish movements (e.g., radio telemetry) are unlikely to capture long-distance dispersal events because such events are typically rare (see Fraser *et al*. 2001). Fish movement studies therefore may have a low probability of detecting the maximal distance at which dispersal influences stream fish populations and assemblages. As an alternative, spatial analysis of fish distributions and abundances may permit inferences about dispersal distances by evaluating the relative importance of local and regional habitat conditions. In such analyses, local conditions may indicate habitat suitability and regional conditions may indicate colonist availability (*sensu* Angermeier et

al. 2002). A critical limitation of this approach is that spatial analysis cannot identify the temporal scale of movements, which would be necessary to understand organismal behavior related to dispersal (i.e., life history expression or colonizing "new" habitats). However, spatial analyses permit assessments of dispersal over the large geographic extents which are necessary to test concepts of landscape ecology (Wiens 2001).

In this dissertation, I explored the spatial scale of stream fish dispersal dynamics by evaluating fish assemblage structure as a function of stream network topology. In chapter 1, I evaluated the effects of stream networks by comparing fish assemblages between sites with and without large downstream confluences (>3rd order) in western Virginia, USA (i.e., mainstem tributaries and headwater tributaries, respectively). I found that local species richness was higher in mainstem tributaries than headwater tributaries and that these effects could not be explained by variation in local environmental habitat conditions. In chapter 2, I developed and applied a continuous model of stream network topology to explore the effects of downstream habitat size and proximity on local fish assemblage structure within the mid-Atlantic highlands, USA. I found that fish assemblage structure (i.e., Bray-Curtis distances in species abundance) was significantly related to variation in stream network topology up to approximately 9 fluvial km from sites. This assemblage-level effect was explained by total species richness, catostomid species richness and abundance, cyprinid species richness, and riverine species richness.

Chapters 3 and 4 explored the implications of inter-stream dispersal for fish bioassessments. In Chapter 3, I identified 10 fish metrics that corresponded predictably to environmental stressors in the mid-Atlantic highlands, encompassing taxonomic,

reproductive, trophic, and tolerance measures of fish assemblage structure. Headwater and mainstem tributaries were not significantly different in local environmental conditions, but showed important differences in biotic responses to environmental quality gradients. Stream sites flowing into mainstem channels within 5 fluvial km showed consistently weaker relations to local environmental conditions than stream sites that lacked mainstem river connections, consistent with the hypothesis of riverine dispersal.

In Chapter 4, I also compared the effects of stream network topology on fish and benthic macroinvertebrate assemblages in west-central Virginia, USA. I identified five fish metrics and six benthic macroinvertebrate metrics that corresponded predictably to variation in environmental quality within the study area. Fish metrics showed greater sensitivity to the presence and absence of large rivers than benthic macroinvertebrate metrics, consistent with the hypothesis of riverine dispersal. These results suggest that stream fishes may be useful for stream quality assessments in Virginia (e.g., Clean Water Act §305b) and that stream fish metrics may complement current benthic macroinvertebrate metrics by indicating environmental conditions at larger spatial grains.

Chapter 1.

Effects of adjacent streams on local fish assemblage structure in western Virginia: implications for biomonitoring[*]

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INTRODUCTION

Dispersal is a landscape-scale process that influences the local distribution and abundance of organisms (Wiens 2001). Stream fishes may disperse to access remote resources, escape local habitat conditions, or colonize adjacent habitats (Schlosser 1990; Fagan *et al*. 2002, 2005). As such, local fish assemblage structure is regulated not only by local environmental conditions but also by the regional distribution of source populations (Angermeier and Schlosser 1989; Schlosser and Angermeier 1995; Angermeier *et al*. 2002). In this chapter, I evaluate the effects of adjacent streams as potential sources of fish dispersal and explore the implications for fish biomonitoring.

Fish biomonitoring requires an understanding of how dispersal from adjacent areas influences local assemblage structure. First, immigrating fishes may not be exposed to the full range of environmental conditions occurring at the sample site. For

instance, immigration of gravel-spawning fishes from adjacent areas would not necessarily indicate high substrate quality in the sample site, although their presence would be interpreted as such in most biomonitoring studies. Second, dispersal of "new" species from adjacent habitats would inflate local species richness. Third, disproportionate immigration of intolerant and tolerant fishes may bias biomonitoring scores towards overestimating or underestimating site quality. As a result, dispersal from adjacent areas can bias biomonitoring assessments towards either false identification of local degradation (i.e., type I errors) or failure to detect local degradation (i.e., type II errors).

The spatial distributions of stream fishes suggest that dispersal among adjacent streams is an important determinant of local fish assemblage structure. Within North America, river systems flowing directly into the ocean generally contain fewer fish species than rivers of equal size connected to other river systems (Sheldon 1988), suggesting that dispersal from adjacent streams and rivers provides an important source of immigrants. Analogous patterns have been reported within smaller watersheds: headwater streams typically support fewer fish species than streams of equal size connected to larger rivers (Gorman 1986; Osborne and Wiley 1992; Schaeffer and Kerfoot 2004). Local fish assemblages often exhibit positive spatial autocorrelation among stream sites (Matthews and Robison 1998; Wilkinson and Edds 2001; Hitt *et al*. 2003; Grenouillet *et al*. 2004), suggesting dispersal-mediated distributions. Empirical fish movement studies have also documented inter-stream dispersal events (e.g., Albanese *et al*. 2004; Gresswell *et al*. 2006).

To predict the effects of inter-stream dispersal on local fish assemblage structure, it will be necessary to consider how conditions in adjacent streams regulate the composition of potential immigrants (i.e., regional species pool; Tonn *et al*. 1990) and the suitability of local sites for immigrating fishes. Stream size provides one framework within which to develop such predictions. Several assemblage attributes are known to vary with stream size. First, fish species richness tends to increase with stream width and volume (Shelford 1911; Burton and Odum 1945; Sheldon 1968; Angermeier and Schlosser 1989; Goldstein and Meador 2004). Second, fish life histories tend to vary with stream size, in that headwater fishes tend to have shorter life spans, smaller adult body sizes, and earlier reproductive ages than riverine fishes (Schlosser 1990). Longitudinal patterns in fish assemblage structure therefore suggest two predictions based on the size of a site's adjacent streams. First, dispersal from large streams should tend to increase species richness, mean adult body size, mean reproductive age, and mean life span in assemblages of smaller receiving streams. Second, the relative importance of dispersal from adjacent streams should increase with site stream size, because larger sites would tend to have fewer "filters" (sensu Tonn *et al*. 1990, Poff 1997) operating on immigrants. I tested these predictions by comparing stream fish assemblages from sites with similar local habitat conditions but different regional habitat conditions (i.e., adjacent stream sizes) in western Virginia.

METHODS

Data source

I used fish and physical habitat data from the Environmental Monitoring and Assessment Program (EMAP) of the U.S. Environmental Protection Agency (USEPA). EMAP site locations were established using a systematic random methodology (Herlihy *et al*. 2000). USEPA personnel sampled stream sites using standardized methods during base flow conditions during the summers of 1993, 1994, 1997, and 1998. We evaluated sites in second-, third-, and fourth-order streams (Strahler 1957) in western Virginia containing both fish and physical habitat data (n=55; Figure 1.1). This area includes portions of the Blue Ridge and Ridge and Valley physiographic provinces and portions of the Potomac, James, New, Tennessee, and Big Sandy river basins. I chose this study area because it represents a region that has been recommended for fish biomonitoring development in Virginia (Smogor and Angermeier 2001). Raw data are available at http://www.epa.gov/emap.

Physical habitat data included quantitative measures of substrate size, woody debris volume, fish cover, riparian vegetation, thalweg depths, and mesohabitat dimensions (Lazorchak *et al*. 1998). Data were collected with a systematic, randomized protocol that encompassed the entire sampling reach; reach lengths were 40 times mean wetted width (Lazorchak *et al*. 1998; see Figure 3.2). I used these data to characterize local site conditions because they exhibit significant variation among sites in our study area (Yuan and Norton 2003) and because they are known correlates of fish distribution and abundance (McCormick *et al*. 2001). I assumed that local physical habitat conditions

would covary with other local physicochemical conditions that were not measured. For sites sampled more than once, I calculated mean values for all variables and used these in our analyses.

Fish assemblage data were collected with single-pass backpack electrofishing methods at each site following McCormick and Hughes (1998). Fishes were identified to species, counted, and returned to the sampling reach. I rejected twelve EMAP fish records based on Jenkins and Burkhead (1994) and replaced rejected records with the probable species or used mean values for adult body size, reproductive age, and life span calculated from congeners occurring in Virginia (Appendix A). Previous electrofishing surveys in the study area using the EMAP sampling reach length (i.e., 40 times mean stream width) detected over 70% of the fish species present in local sites (Angermeier and Smogor 1995).

Adjacent stream classification

In this analysis, I defined "adjacent streams" as streams confluent to sampling sites within 3 river km (rkm) downstream from sample site locations. I evaluated only downstream habitats to permit comparisons among large and small stream sites without confounding the effects of upstream flow variability. I chose this spatial extent because some common stream fishes can disperse this distance (Logan 1963; Gorman 1986; Osborne and Wiley 1992; Gatz and Adams 1994; Albanese *et al*. 2004) and exploratory analyses revealed sufficient numbers of sites in small and large adjacent stream categories to permit comparisons.

First, stream channels were mapped from the 1:24,000-scale National Hydrography Dataset (http://nhd.usgs.gov) and converted to raster data (30-m^2 cells). Second, we calculated Strahler (1957) stream orders for each grid cell using a geographic information system. Third, I classified sites based on the size of adjacent stream habitats in the analysis zone. We defined first- to third-order streams as "small" and streams larger than third-order as "large" following Jenkins and Burkhead (1994). This stream size criterion typically distinguishes wadeable from non-wadeable streams in the mid-Atlantic highlands region (Herlihy *et al*. 2000). Site categories therefore indicate whether or not large or small adjacent streams were available to provide immigrants into each sample site. Sites with large and small adjacent streams encompassed a wide range of catchment areas, but large adjacent streams tended to occur more frequently at lower elevations than small adjacent streams (Figure 1.2).

I characterized fish assemblage structure with four metrics: species richness, mean body size, mean reproductive age, and mean life span. I chose these metrics because they typically increase from small to large stream sites (Schlosser 1990) and they provide a framework to test hypothesized patterns of dispersal from adjacent streams. Data on adult body size (total length [TL]), reproductive age (years), and life span (years) were taken from Jenkins and Burkhead (1994) and Smogor (1996). Congeneric surrogate species or family averages calculated for Virginia taxa were used where primary data were not available. Of 109 species identified, 25 (23%) required surrogates or family averages for at least one metric.

I also categorized species according to the stream size they tended to occupy based on distribution data in Jenkins and Burkhead (1994). To identify large and small

stream specialists, we excluded species that were reported to inhabit all stream sizes (i.e., stream size generalists) by Jenkins and Burkhead (1994). "River" and "creek" species therefore represent obligate habitat associations in large and small streams. Of the 109 species in EMAP sites, 32 (29%) were classified as "river" species and 20 (18%) were classified as "creek" species. I compared local richness of river and creek species between sites with large and small adjacent streams to assess the potential role of dispersal.

Statistical analysis

The primary challenge in this analysis was to control for local environmental variability while evaluating the regional effect of adjacent stream size. First, I used principal components (PC) analysis from correlation matrices to characterize physical habitat conditions among sites. PC scores encompassed 68% of the variance in physical habitat conditions in two principal components for all eigenvalues >1. Eight variables loaded strongly (i.e., variable loadings >0.5) into the first two components (Table 1.1). PC I represented a gradient of channel size and shape and PC II represented a gradient of structural complexity (woody debris volume, substrate size).

Second, I grouped sites by stream order (i.e., second, third, and fourth) and compared 95% confidence intervals of fish metrics among sites with small and large adjacent streams (Pearson 2002). I assumed that by evaluating the effects of adjacent streams among sites of the same stream order, we would control for some local environmental variability. I also used nonparametric techniques to compare fish metrics in sites with small and large adjacent stream sizes (Mann-Whitney tests). I chose

nonparametric methods because the sample sizes did not permit me to test the assumption that the data were normally distributed.

Third, I used Mantel tests (Mantel 1967) to evaluate associations between adjacent stream size and fish metrics while controlling for the potentially confounding effects of local physical habitat. I calculated three categories of site-by-site dissimilarity matrices for these tests: (1) fish metrics, (2) adjacent stream size, and (3) local physical habitat (i.e., PC I and PC II scores). All matrices were calculated from Euclidean distances and standardized from 0 to 1 (SPSS 10.0). When significant associations between adjacent stream size and fish metrics were observed, we used partial Mantel tests with local habitat data as a blocking matrix to test for covariation. All Mantel statistics were calculated from matrix correlations with Mantel *zt* (MS-DOS program by E. Bonnet, Ghent University, Belgium) using 10,000 randomized resampling iterations.

RESULTS

EMAP surveys reported 109 fish species in western Virginia streams, representing 52% of the species occurring in freshwaters of Virginia (Jenkins and Burkhead 1994). Fish metrics varied consistently along a stream order gradient. Species richness, adult body size, and life span increased with increasing stream order (Figure 1.3). River species richness changed in the expected direction, but creek species richness was not monotonically related to stream order (Figure 1.3). Mean reproductive age

increased slightly from second- to third-order sites but did not increase from third- to fourth-order sites (Figure 1.3).

I found significant effects of adjacent stream size on species richness, mean body size, mean reproductive age, and riverine species richness in local assemblages. Second-order sites with large adjacent streams tended to have more species than similar-order sites with small adjacent streams (Table 1.2; Figure 1.4a). In fourth-order sites, large adjacent streams were associated with younger mean reproductive age and increased river species richness (Table 1.2; Figure 1.4c,e). Large adjacent streams were negatively associated with mean body size and life span in fourth-order sites (Figures 1.4b,d) but these effects were not statistically significant. I detected no significant relationships between fish metrics and adjacent stream size among third-order sites. Among all sites, adjacent stream size influenced mean body size (Table 1.2) but this effect was not observed for comparisons within the same stream order. Only 4 of 24 tests showed significance at the $P<0.05$ level, suggesting weak experiment-wise effects of adjacent stream size on local fish assemblage structure.

Mantel tests provided additional evidence for the effect of large adjacent streams on mean reproductive age and river species richness in fourth-order sites (Table 1.3). However, Mantel tests did not identify significant effects of adjacent stream size in second- or third-order sites. These tests also revealed effects of local physical habitat on some fish metrics. Channel size and shape (i.e., PC I) were significantly associated with species richness in second- and fourth-order sites (Table 1.3). Physical habitat complexity (i.e., PC II) was significantly associated with species richness in fourth-order sites. However, partial Mantel tests using physical habitat data as blocking matrices did

not diminish the significance of adjacent stream size effects on mean reproductive age or river species in fourth-order sites (Table 1.3). Among all sites, Mantel tests showed no significant effects of adjacent stream size on fish metrics but did show effects of channel size and shape on species richness and riverine species (Table 1.3). Overall, I detected the greatest number of correlations with species richness and fewest with body size and creek species richness; these correlations were most common in fourth-order sites and least common in small stream sites (Table 1.3).

DISCUSSION

These results suggest that (1) dispersal from adjacent streams can affect local fish assemblage structure, (2) dispersers from large adjacent streams tend to be smaller-bodied fishes with earlier ages of reproduction, and (3) local site conditions may mediate the influence of dispersal from adjacent streams. My first prediction was that dispersal from large adjacent streams would tend to increase local species richness, mean adult body size, mean reproductive age, and mean life span in local assemblages. I found that large adjacent streams tended to increase local species richness, consistent with previous studies (Osborne and Wiley 1992; Shaeffer and Kerfoot 2004). However, large adjacent streams tended to decrease mean reproductive age, contrary to my expectations. This suggests that large nearby streams may act as sources of dispersal of early-maturing fishes into adjacent stream sites. Gorman (1986) speculated that adventitious stream

effects might be driven by dispersal of small-bodied schooling fishes from large rivers. The current analysis supports Gorman's (1986) notion.

My second prediction was that the effects of dispersal would be greatest in larger sites because these areas would tend to support fewer environmental "filters" (Tonn *et al*. 1990; Poff 1997) for immigrant fishes. My results provide some support for this prediction. Pooled analysis of second-, third-, and fourth-order sites revealed inconsistent effects of adjacent stream size on fish metrics, but several significant effects were detected when comparisons were partitioned into sites of the same stream order. Fourth-order sites supported a greater number of significant adjacent stream effects than did second- or third-order sites. Moreover, the effects of large adjacent streams on fourth-order sites were not explained by channel size and shape (i.e., PC I) or physical habitat complexity (i.e., PC II). However, second-order sites also showed an effect of large adjacent streams on species richness, in contrast to our expectations.

These results have important consequences for stream fish biomonitoring development and interpretation. In cold- and cool-water streams, low levels of enrichment increase fish species richness by addition of more tolerant species (McCormick *et al*. 2001; Scott and Helfman 2001; Hughes *et al*. 2004). However, fish bioassessments typically evaluate species richness, given that degraded streams tend to support fewer species than less disturbed streams (Fausch *et al*. 1984). On average, the presence of large adjacent streams increased local richness by five species in second- and fourth-order sites. These additional species would have inflated metric scores in previous stream biomonitoring studies in the mid-Atlantic highlands region (Angermeier *et al*. 2000) and midwestern U.S. (Angermeier and Schlosser 1989; Karr *et al*. 1987). Smogor

and Angermeier (2001) recommended several metrics for fish biomonitoring in western Virginia streams, including the number of cyprinid species in a sample. In the current study, cyprinids constituted the majority of the additional species associated with large adjacent streams. As a result, the presence of large adjacent streams may tend to mask local habitat degradation and contribute to Type II errors. Additional research is necessary to understand how inter-stream dispersal influences the overall tolerance of local assemblages to environmental stressors. I would expect that increasing dispersal from adjacent areas would decrease metric sensitivity to local stressors, and increase sensitivity to distal stressors like migration barriers but these hypotheses remain to be tested empirically.

Osborne *et al*. (1992) recommended that fish biomonitoring studies calibrate metrics for adventitious and headwater streams due to the potential effects of dispersal from adjacent source populations. My findings suggest that the size of the stream site may provide a useful framework to develop expectations for the influence of inter-stream dispersal. Analogous methods were developed by Fausch *et al*. (1984) to account for natural variation in local species richness as a function of stream size. However, calibrating for adjacent stream sizes will require additional consideration of the spatial scale at which dispersal influences local assemblages. The spatial extent of such influence in my analysis was limited to 3 rkm downstream from sample sites. Future studies should evaluate whether inter-stream dispersal is limited to confluence zones or has more extensive upstream effects.

This study provides new insight into ways that inter-stream dispersal may influence local assemblage structure. More mechanistic models will require a clearer

understanding of the factors that regulate the distribution and proximity of immigrant sources within and among streams (Schlosser and Angermeier 1995). Future studies aiming to resolve local and regional influences on fish assemblage structure may benefit by considering how stream network shape constrains the configuration and connectivity of source population habitats (Fagan 2002). For instance, trellis-shaped stream systems are characterized by a relatively high proportion of adventitious streams, whereas dendritic-shaped stream systems contain more confluences of larger streams (Zernitz 1932; Shreve 1966; Benda *et al*. 2004a). Watershed shape may thereby provide a spatial framework to predict dispersal dynamics at smaller spatial scales.

Table 1.1. Principal components (PC) analysis loadings for physical habitat data at EMAP sites (n=55). The first two components (PC I and PC II) explained 68% of the total variance for all eigenvalues >1. Values represent loadings of variables onto components. Variable loadings >0.5 are indicated in bold.

Variable	PC I	PC II
Standard deviation of stream depth	**0.909**	0.204
Residual pool mean depth	**0.768**	0.068
Sinuosity	**0.528**	0.097
Mean volume fine woody debris	-0.399	**0.851**
Mean volume coarse woody debris	-0.353	**0.856**
Mean stream depth	**0.924**	0.175
Mean stream width	**0.813**	0.278
Log of mean substrate diameter	-0.063	**0.564**
Variance explained (%)	43.7	24.2

Table 1.2. Effects of adjacent stream size on fish assemblage metrics. Mann-Whitney chi-square statistics are presented for each fish metric (column) for comparisons of small and large adjacent streams within stream size categories. Asterisks indicate significance at $P<0.05$. Significant adjacent stream size effects are shown in Figure 1.4.

Site stream order	Species richness	Body size	Reproductive age	Life span	River species richness	Creek species richness
2,3,4	0.01	5.95*	0.35	2.26	0.00	0.11
2	5.24*	0.43	0.85	2.04	1.83	3.63
3	0.56	0.82	0.80	1.86	0.09	0.06
4	0.80	1.72	4.81*	2.55	4.26*	0.23

Table 1.3. Effects of adjacent stream size and local physical habitat on fish assemblage metrics. Mantel test statistics (Mantel *r*) are presented for site-by-site matrix correlations of adjacent stream size ("adjacent") and physical habitat (PC I and PC II) within four stream size categories. Single asterisks indicate significance at $P<0.05$. Double asterisks indicate significance of partial Mantel tests (using PC I and PC II as blocking matrices) at $P<0.05$.

Variable	Site stream order	Species richness	Body size	Reproductive age	Life span	"River" species richness	"Creek" species richness
Adjacent	2,3,4	-0.01	0.01	0.00	-0.01	0.01	0.00
PC I	2,3,4	0.34*	0.07	-0.07	0.10	0.19*	0.00
PC II	2,3,4	0.04	-0.11	-0.09	-0.09	-0.05	-0.03
Adjacent	2	0.07	0.16	0.06	0.00	0.15	0.03
PC I	2	0.46*	0.09	-0.15	-0.10	-0.11	0.44
PC II	2	-0.07	0.07	-0.09	-0.18	0.06	-0.04
Adjacent	3	0.00	-0.12	-0.04	-0.07	-0.10	-0.03
PC I	3	-0.06	-0.18	-0.16	-0.08	-0.10	0.02
PC II	3	0.03	-0.18	-0.09	-0.17*	0.08	0.07
Adjacent	4	0.06	-0.02	0.34**	0.00	0.25**	0.15
PC I	4	0.26*	0.15	0.00	0.03	0.01	-0.11
PC II	4	0.21*	-0.11	-0.10	-0.04	0.04	-0.04

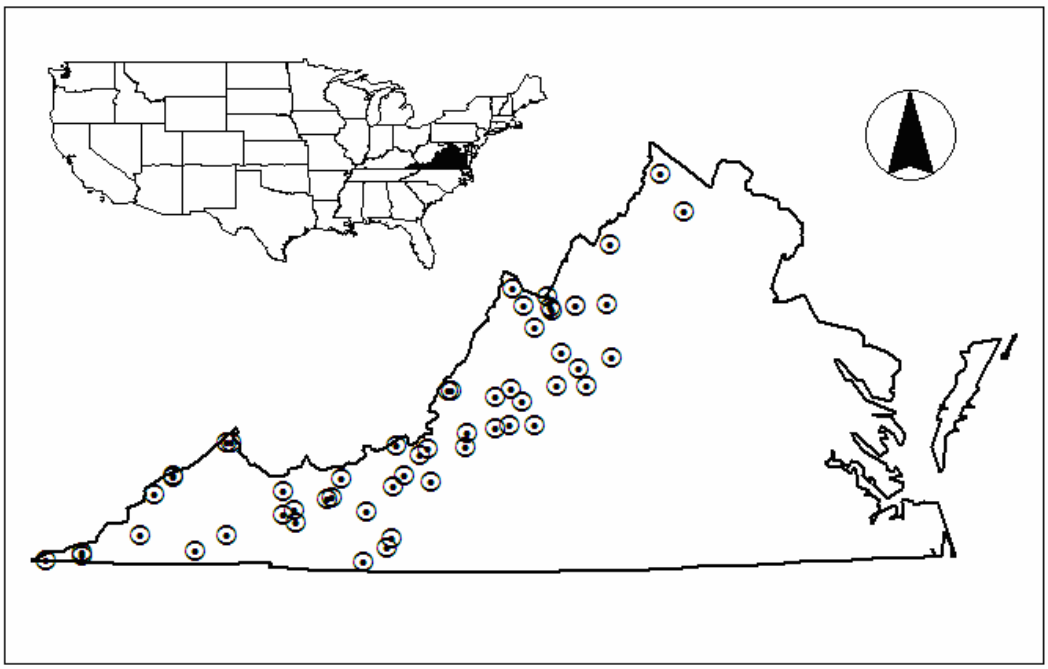

Figure 1.1. USEPA Environmental Monitoring and Assessment Program (EMAP) stream sites evaluated in Chapter 1 (n=55). Sites were represented by fish and physical habitat data from second-, third-, and fourth-order streams.

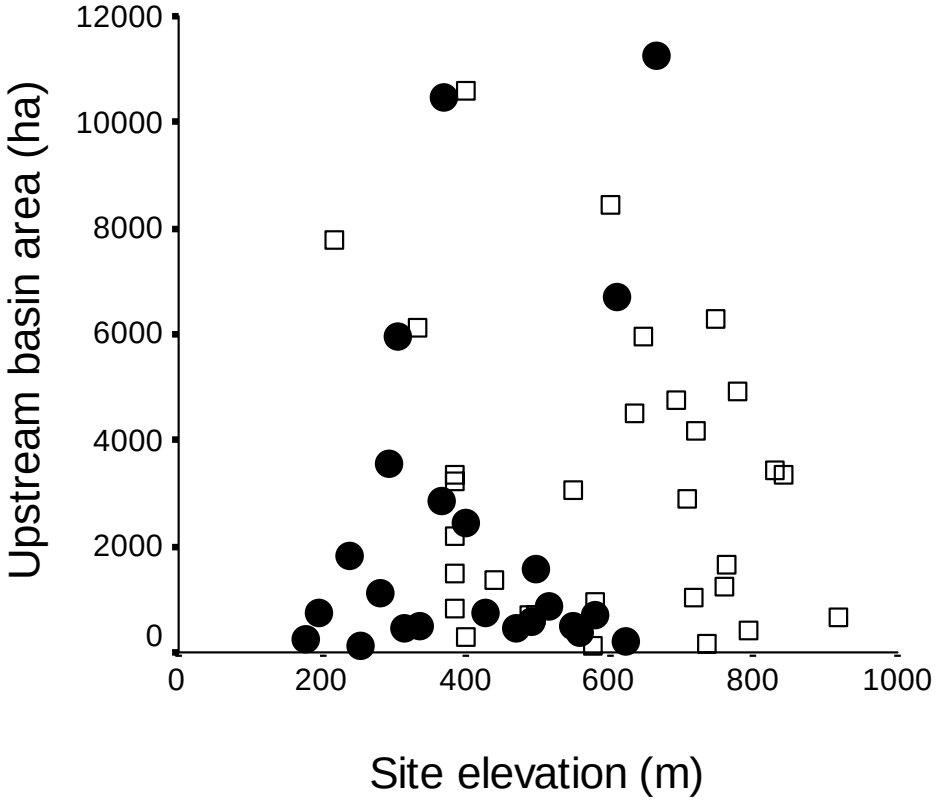

Figure 1.2. Distribution of EMAP sites with small (≤ third order, squares) and large (> third order, circles) adjacent streams along gradients of catchment area and site elevation.

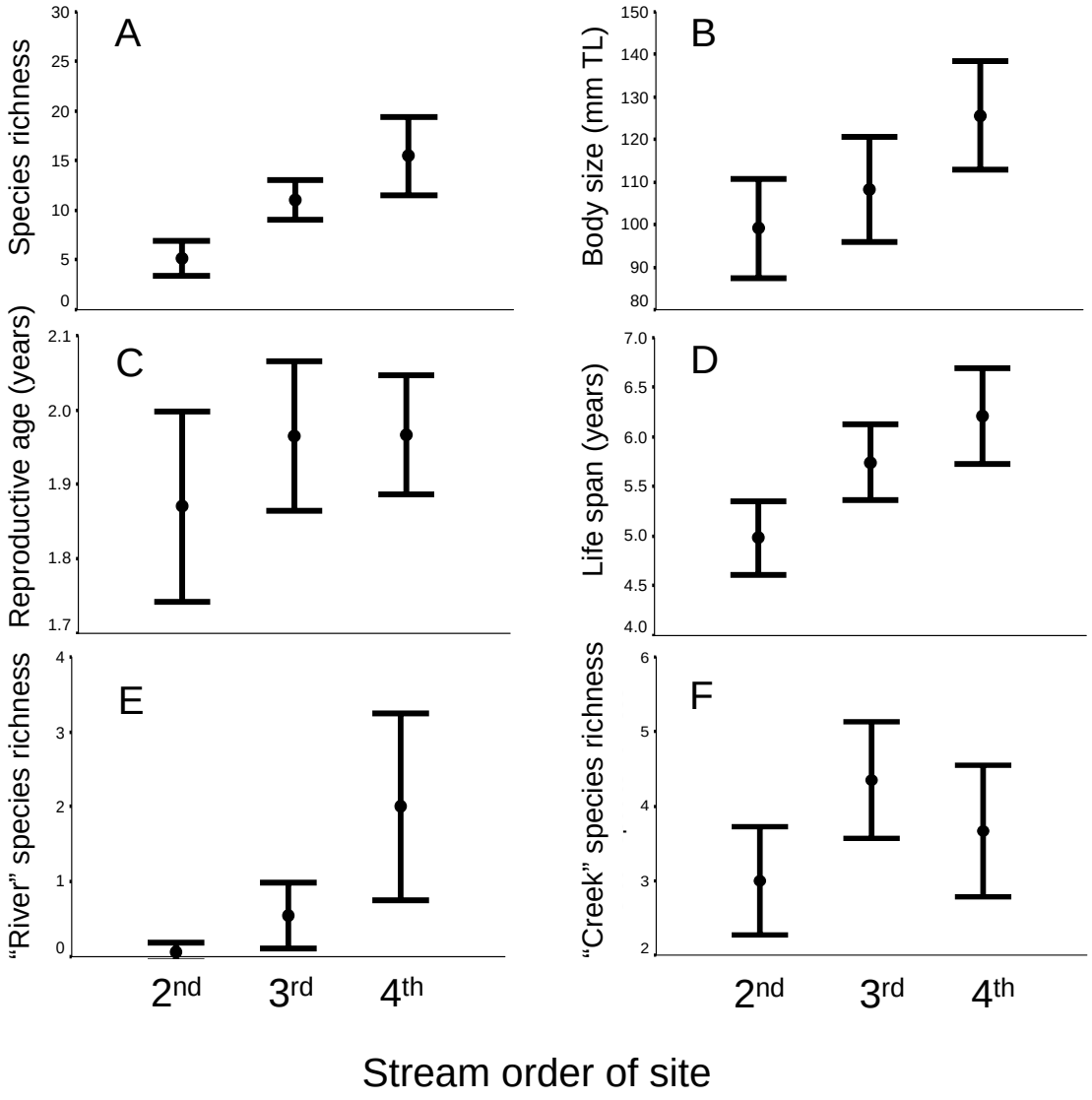

Figure 1.3. Effects of site stream order on local species richness (A), mean adult body size (B), mean reproductive age (C), mean life span (D), "river" species richness (E), and "creek" species richness (F). See text for definitions of river and creek species. Mean values (circles) and 95% confidence intervals (whiskers) are shown. TL refers to total length of fishes.

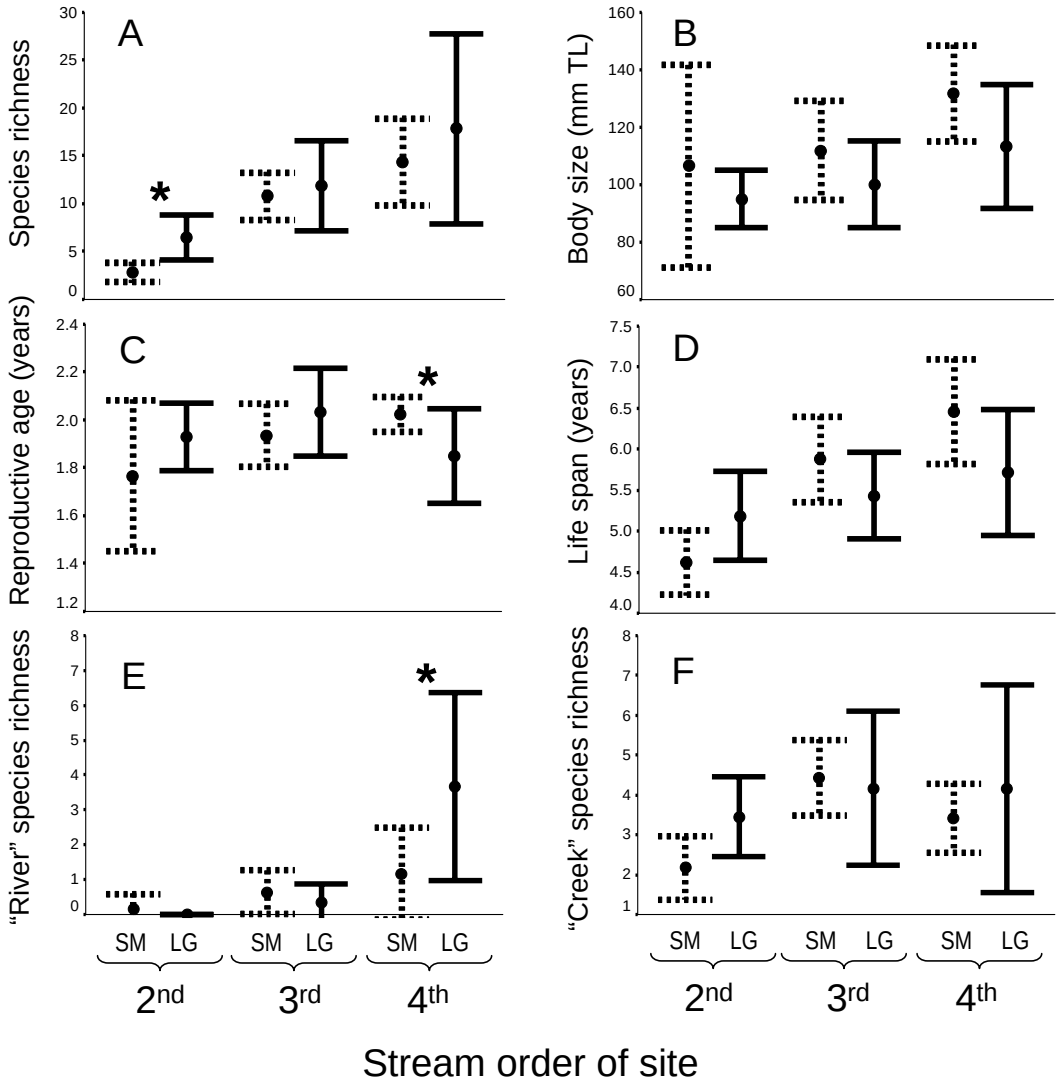

Figure 1.4. Effects of adjacent stream size on local species richness (A), mean adult body size (B), mean reproductive age (C), mean life span (D), "river" species richness (E), and "creek" species richness (F) across stream orders. Mean values (circles) and 95% confidence intervals (error bars) are shown. Small adjacent streams (SM) are indicated with dashed lines; solid lines indicate the presence of large adjacent streams (LG). See text for definitions of river and creek species and adjacent stream size categories. Asterisks indicate significant differences (P<0.05) between large and small adjacent streams (Table 1.2).

Chapter 2.

Effects of stream network topology on fish assemblage structure in the mid-Atlantic highlands, USA

INTRODUCTION

Dispersal is a landscape-scale process that influences the distribution and abundance of organisms (Wiens 2001). Stream fishes may disperse to access remote resources, escape local environmental conditions, or re-establish extirpated populations (Schlosser 1990; Fagan 2002; Fagan *et al*. 2005). As such, local assemblages are influenced not only by local environmental conditions but also by connectivity to habitat and source populations at the landscape-level (MacArthur and Wilson 1967; Angermeier and Schlosser 1989; Schlosser and Angermeier 1995; Angermeier *et al*. 2002). In lotic environments, the size and proximity of connected streams (i.e., stream network topology) influences the landscape-level distribution of habitat (Frissell *et al*. 1986; Fausch *et al*. 2002), yet the effects of inter-stream dispersal on assemblage structure remain poorly understood. In this chapter, I evaluate how stream networks influence local fish assemblage structure through dispersal and explore the potentially mitigating effects of local habitat conditions.

The study of dispersal may improve our understanding of the mechanisms by which local and regional factors influence fish assemblage structure. Previous studies have identified local determinants of species composition (i.e., predation, competition,

habitat availability; see Matthews 1998) but rarely have evaluated the potential influences of dispersal at larger spatial scales (i.e., metapopulation dynamics; Schlosser and Angermeier 1995). An understanding of dispersal is therefore necessary to test competing hypotheses about local and regional influences on fish assemblages. By extension, an understanding of dispersal could improve fish bioassessment methods (i.e., metric development and calibration; Osborne *et al*. 1992) and freshwater reserve design (i.e., reserve size and configuration; Abell *et al* 2007).

Studies of stream fish movements and distributions suggest that inter-stream dispersal has important consequences for local assemblage structure. Empirical studies have documented long-distance movements in non-diadromous stream fishes (i.e., >1 fluvial km [fkm]; Karr and Gorman 1975; Gatz and Adams 1994; Albanese *et al*. 2004; Roghair and Dolloff 2005). Within North America, river systems flowing directly into the ocean generally contain fewer fish species than rivers of equal size connected to other river systems (Sheldon 1988), suggesting that connected streams and rivers provide an important source of immigrants. Analogous patterns have been detected within watersheds: stream sites near large rivers tend to support more species than similarly sized streams that lack riverine connections (Gorman 1986; Osborne and Wiley 1992; Schaefer and Kerfoot 2004; Smith and Kraft 2005; Hitt and Angermeier 2006).

Studies of stream connectivity also suggest an important role of inter-stream dispersal in freshwater fishes. Matthews and Robison (1998) found that stream fish assemblages of the Ouachita range tended to be more similar among sites separated by fewer confluences (i.e., least distance). Angermeier and Winston (1998) demonstrated that local stream fish species richness was better predicted by fish diversity within

relatively small regions (i.e., physiographic region-drainage combinations) than by larger regions (i.e., river drainages), suggesting that inter-stream connectivity and dispersal may affect local assemblage structure. Spatial autocorrelation in stream fish assemblages (Wilkinson and Edds 2001; Hitt *et al*. 2003; Grenouillet *et al*. 2004) also provides evidence that inter-stream dispersal affects local assemblage structure. However, previous studies have been largely limited to single zoogeographic regions and therefore may not provide general inferences about stream fish dispersal dynamics (but see Sheldon 1988).

Stream network topology provides a spatial framework to evaluate the potential effects of inter-stream dispersal across large geographic extents. In this context, stream networks are defined by the size and proximity of connected streams and confluence types (Benda *et al*. 2004a). Stream networks provide a spatial framework for analysis distinct from the River Continuum Concept (RCC, Vannote *et al*. 1980). Whereas the RCC emphasizes local conditions based on longitudinal variation in stream size, stream networks emphasize regional conditions based on size and proximity of connected streams (Benda *et al*. 2004a). For example, a network perspective would posit that the biotic community in a stream flowing into a river would be distinct from the community in a similarly-sized stream that lacks a river confluence within a given distance (i.e., mainstem tributary and headwater tributary, respectively). Furthermore, differences between these two communities would stem from movement by species adapted to live in either stream or river habitats. In contrast, the RCC would predict that the communities in these streams are similar due to their similar size and that species composition largely reflects adaptations to local conditions.

In this chapter, I tested the hypotheses that (a) stream networks influence dispersal through the distribution of source populations or remote resources, and (b) local stream size mitigates the effects of regional dispersal. Longitudinal patterns of fish assemblage structure provide three testable predictions. First, mainstem tributaries should support more species than headwater tributaries because fish species richness increases with stream volume (Shelford 1911; Burton and Odum 1945; Sheldon 1968; Angermeier and Schlosser 1989; Goldstein and Meador 2004). Second, the effects of riverine dispersal will be weakest in the smallest streams because extreme environmental conditions (i.e., disturbance regimes) may exclude potential immigrants (Schlosser 1990). Third, the effects of riverine dispersal will be more evident in percid, catostomid, cyprinid, ictalurid, and centrarchid fishes, which commonly inhabit riverine habitats, than in salmonid or cottid fishes which rarely inhabit riverine habitats in the study area (Jenkins and Burkhead 1994).

To test these predictions, I developed a stream network model based on the rate of flow accumulation downstream from sampled sites. I used this measure of stream network structure to characterize the size and proximity of connected streams and to distinguish among sites that flow into rivers and streams (i.e., rapid and slow accumulation of volume per unit distance, respectively). First, I quantified a surrogate of flow volume at 1-fluvial km (fkm) intervals downstream from sites to a total distance of 20 fkm. Second, I classified streams as mainstem tributaries and headwater tributaries based on the presence or absence of large river confluences at multiple distances. This discrete analysis permitted me to contrast sites based on their relative proximity to riverine areas.

The current analysis does not measure movement empirically but instead infers movement patterns through the evaluation of potential source populations within stream networks. Key limitations of this approach are that it cannot assess potential dispersal from connected streams of the same size and that it cannot identify the temporal scale of dispersal. Instead, the spatial models in this analysis quantify heterogeneity in the stream network to examine expected differences between small and large stream interactions. An advantage of this approach is that it permits an analysis of dispersal among zoogeographic and physiographic regions over a large geographic extent. Such a spatially-extensive analysis is necessary to test concepts of landscape ecology (Wiens 2001). The current analysis provides the most spatially-extensive analysis of stream fish dispersal dynamics to date.

METHODS

Data source

I used data from the U.S. Environmental Protection Agency's (USEPA) Environmental Monitoring and Assessment Program (EMAP) in the mid-Atlantic highlands region, USA (n=308 sites; Figure 2.1). This region encompasses 205,000 km^2 throughout the Appalachian mountains of New York, Pennsylvania, Maryland, West Virginia, and Virginia. EMAP site locations were designated using a systematic random methodology (Herlihy *et al*. 2000) across several physiographic regions (blue ridge, ridge

and valley, central Appalachians, and Allegheny plateau; Omernik 1987) and zoogeographic regions (Atlantic slope drainages, Ohio River basin, New River drainage [of the Ohio River basin], and Tennessee River basin) (Figure 2.2; Table 2.1). The large spatial extent of this study area permits an analysis of stream network effects across zoogeographic regions and ecoregions.

In each site, USEPA personnel sampled stream fishes using single-pass backpack electrofishing (McCormick and Hughes 1998). Sample reach lengths were 40-times the average stream width for each site. Fishes were identified in the field and released. Some problematic specimens were preserved and identified later by USEPA personnel. I used fish samples collected during summer base-flow conditions from 1993 to 1998 (n=104, 100, 2, 4, 61, and 37, annually). I made 78 changes to the EMAP data based on published species accounts, under the assumption that EMAP surveys did not detect new inter-basin range expansions by species (Appendix B). Raw data are available from http://www.epa.gov/emap.

I selected EMAP sites for this analysis using several criteria. First, I restricted sites to wadeable streams (i.e., backpack electrofishing methods) because of decreased sampling efficiencies at boat-electrofishing sites (see Cyterski and Barber 2006). Second, I removed sites that flow into reservoirs within 20 fkm to remove potential effects of downstream reservoirs on upstream assemblages (Winston *et al*. 1991; Guenther and Spacie 2006). When sites were sampled repeatedly, I used the sample with the greatest species richness or, in the case of ties, the more recent sample. Although some EMAP sites included extensive physical habitat and water quality surveys, many of the sites with fish data lacked environmental data. To maximize the use of existing fish

data, I chose to include these sites in my analysis and to develop a stream size surrogate of local physical habitat conditions (see "stream network topology" below).

I calculated taxonomic and habitat size metrics to characterize fish assemblage structure (Table 2.2). Taxonomic metrics included family-level species richness and abundance. The rarest families were excluded from this analysis and cottid species richness was not calculated because most sites lacked multiple cottid species. Habitat size metrics distinguished between "river" and "creek" specialists (Table 2 in Jenkins and Burkhead 1994). Assignments of river and creek specialists excluded species reported to inhabit both large and small streams and therefore denote associations with large and small stream habitats. However, it was necessary to include species with intermediate stream size associations (i.e., "stream" species in Jenkins and Burkhead 1994) in assignments of river and creek specialists. Of 130 total species, 41 (32%) were classified as "river" specialists and 25 (19%) were classified as "creek" specialists (Appendix C).

Stream network topology

Stream network topology refers to the size and proximity of connected streams within watersheds (Benda *et al*. 2004a) and is a property of the "stream system" scale of Frissell *et al*. (1986). Properties of stream network topology therefore are not physical features of individual streams but instead are emergent properties of multiple connected streams. The first studies of stream network topology addressed geomorphic causes of erosion and drainage network evolution (e.g., Shreve 1966). Recent applications of this concept have explored biological issues of dispersal and recolonization dynamics (Fagan 2002; Fausch *et al*. 2002; Ganio *et al*. 2005; Lowe *et al*. 2006).

I characterized stream networks based on the rate of downstream flow accumulation within a 20-fkm analysis window. I chose this analysis grain size because previous work has demonstrated significant effects of large river source populations within this distance (Osborne and Wiley 1992). The large spatial extent of the study area and limited computing capacity precluded the use of digital elevation models to derive flow networks. Consequently, I developed a surrogate of upstream basin area utilizing the relationship between watershed area and stream length (Hack 1957). I used this modeling approach instead of stream order to capture continuous variation in stream networks (Hughes and Omernik 1981; Matthews 1986; Fausch *et al*. 2002).

I calculated upstream cell counts (UCCs) from stream network raster data as a surrogate of upstream basin size and flow volume (C. D. Heatwole, Biological Systems Engineering, Virginia Tech, personal communication). First, I downloaded National Hydrological Data medium-resolution data (1:100,000 scale) and converted stream paths from vector to raster data (30-m^2 cells). Raw data are available at http://nhd.usgs.gov. Second, I used the flow network raster to calculate distances from each cell to the outlet pour-point of each watershed. Third, I converted cost-weighted distances to UCCs and then combined inflows from confluent watersheds. I then sampled the UCC raster at EMAP sites and at every kilometer for 20 fkm downstream. All calculations were preformed in ARCGIS 9.1.

I compared the UCC data against independent measures of upstream basin area to validate UCCs as a surrogate for stream flow volume. I used simple linear regression to relate UCCs to upstream basin areas from EMAP calculations in a subset of sites where physical habitat data were available (n=198). Log-log plots revealed bivariate linearity

and good fit (R^2=0.919, Figure 2.3), suggesting that UCC data provide a reasonable surrogate of stream flow volume (Hack 1957). Based on this relationship, I used a UCC value of 5000 to indicate the presence a large river confluence (i.e., upstream basin area >250 km^2, Osborne and Wiley 1992).

Statistical analysis

I used three approaches to test the effects of stream network topology on fish assemblage structure. First, I evaluated how continuous variation in stream network structure explained local fish assemblage metrics and how these relationships changed as a function of downstream distances from sample sites (i.e., analysis grain sizes). Second, I tested the prediction that the smallest streams would contribute least to fish assemblage-stream network relationships. In each case, the presence of significant relationships between stream network position (i.e., UCC variation) and fish assemblage structure would be consistent with the hypothesis of dispersal from regional source populations. Third, I tested for effects of large river confluences by comparing fish metrics between mainstem tributaries and headwater tributaries (*sensu* Osborne and Wiley 1992).

I used simple and partial Mantel tests to assess the relations between fish metrics and stream network structure (Mantel 1967). Mantel tests are distance-based matrix correlations that use permutation procedures to calculate the probability that observed correlations are random. Simple Mantel tests evaluate bivariate relations while partial Mantel tests incorporate blocking factors to control for potentially confounding variables. These tests are useful for assessing correspondence in variables from unknown statistical distributions and for partitioning the spatial and temporal components of ecological data

(e.g., King *et al*. 2003, 2005). In this analysis, I developed Euclidean dissimilarity matrices from UCC data arrays to characterize stream network structure (Figure 2.4).

I used the R programming language with the ECODIST library (Goslee and Urban 2006) to calculate distance matrices and perform simple and partial Mantel tests. First, I used simple Mantel tests to assess potentially confounding effects of ecoregion, zoogeographic region, sampling month, sampling year, and spatial autocorrelation on fish metrics. Second, I used partial Mantel tests to evaluate UCC relations to fish metrics at increasing analysis grain sizes while controlling for significant variables identified from simple Mantel tests. I used Euclidean distances for all variables except fish assemblage structure (FISH, Table 2.2) for which I used Bray-Curtis distances (Bray and Curtis 1957). To reduce Type I error probabilities, I chose a high alpha level (α=0.10) for inclusion of blocking factors in partial Mantel tests and a low alpha level (Bonferroni-corrected α'=0.05/21 tests=0.0024) to assess significance of stream network effects. All Mantel tests used 10,000 resampling iterations.

I then evaluated the role of local stream size in regulating regional dispersal processes, as inferred from patterns of fish distribution and abundance. I evaluated six fish metrics that demonstrated significant relations with stream network structure (α'=0.0024 at distances <6 fkm). First, I categorized sites into one of three size classes (Table 2.3). Second, I used simple Mantel tests to evaluate potentially confounding effects of ecoregion, zoogeographic region, sampling month, sampling year, and spatial autocorrelation on fish metrics. Third, I conducted partial Mantel tests to evaluate the relationships between stream network structure (i.e., UCC variation) and fish metrics across the site size classes while controlling for potentially confounding variables. I

hypothesized that immigration from downstream source populations of river specialists would be less important in the smallest streams because small streams do not provide suitable habitat for riverine specialists.

I then evaluated the effects of large downstream rivers on local fish assemblage structure. Following Osborne and Wiley (1992), I categorized sites based on the presence or absence of "mainstem" river confluences within 20 fkm downstream from sites (i.e., UCC >5000, see Figure 2.3) as mainstem tributaries and headwater tributaries, respectively. I used Mann-Whitney tests to compare fish metrics among headwater and mainstem tributaries. I chose non-parametric methods because exploratory analyses revealed significant non-normality in fish metrics data (i.e., all Wilks-Shapiro tests $p>0.05$) and the failure of transformations to normalize the data. I identified 248 mainstem tributary sites and 60 headwater tributary sites in the study area. To account for the unbalanced group sizes, I used bootstrap resampling methods (Howell 2000) to compare group means with 90% confidence intervals. I used 10,000 resampling iterations for each bootstrap resample.

RESULTS

Spatial and temporal variation in fish assemblage structure

The 101,457 individual fish documented in the mid-Atlantic highlands dataset included 17 families, 53 genera, and 130 species. By abundance, cyprinids were dominant, comprising 65% of the total individuals. Cottid, catostomid, centrarchid,

percid, and salmonid fishes encompassed 1% to 12% of the total individuals. Other families contributed <1% of the total abundance (Lepisosteidae, Umbridae, Clupeidae, Atherinidae, Percopsidae, Sciaenidae, Esocidae, Petromyzontidae, Anguillidae, Fundulidae, and Ictaluridae).

Variation in fish assemblage structure (Bray-Curtis dissimilarities in species x abundance) was significantly related to zoogeographic regions and ecoregions (Mantel $r=0.203$ and 0.112, $p<0.005$, respectively; Table 2.4). Total species richness was more strongly related to zoogeographic regions (Mantel $r=0.040$, $p<0.10$) than ecoregions whereas total abundance showed the inverse pattern (ecoregion Mantel $r=0.061$, $p<0.10$). Among families, percid species richness was most strongly related to zoogeographic regions and ecoregions (Mantel $r=0.167$ and 0.148, $p<0.005$, respectively). Creek and river species richness were related to zoogeographic regions and ecoregions (Mantel $r>0.04$, $p<0.10$, respectively), but river species richness showed marginally stronger relations to regional classifications than creek species richness (zoogeographic Mantel $r=0.077$ and 0.044, respectively; ecoregion Mantel $r=0.062$ and 0.045, respectively; Table 2.4).

Variation in the month and year of sample collections was related to taxonomic and functional variation in fish assemblage structure (Table 2.4). Variation among sample years (1993-1998) was related to species richness and total abundance (Mantel $r=0.041$ and 0.042, $p<0.10$, respectively), centrarchid species richness (Mantel $r=0.054$, $p<0.10$), and percid species richness and abundance (Mantel $r=0.092$ and 0.042, $p<0.005$, respectively). River species richness and abundance showed significant inter-annual variation (Mantel $r=0.064$ and 0.058, $p<0.01$, respectively) whereas creek species

richness and abundance did not (Table 2.4). For example, samples taken in 1997-1998 tended to contain more river species than samples from 1993-1994 (means = 1.4 and 0.9, respectively), but creek species richness showed less variation between these sampling intervals (means = 2.9 and 3.1, respectively).

EMAP fish data were collected during base-flow conditions from April to September (n=31, 81, 90, 44, 52, and 10, by month). Variation in sample month was positively related to variation in percid species richness and abundance (Mantel $r=0.063$ and 0.049, $p<0.10$, respectively), catostomid abundance (Mantel $r=0.032$, $p<0.10$), and centrarchid species richness (Mantel $r= 0.042$, $p<0.10$; Table 2.4). In contrast, inter-monthly variation was negatively related to variation in salmonid species richness and abundance (Mantel $r=-0.069$ and -0.049, $p<0.10$, respectively). As a result, large differences in sample month (e.g., April versus September) were related to large differences in percid and centrarchid species richness and catostomid abundances. In contrast, small differences in sample month (e.g., consecutive months) were related to large differences in salmonid species richness and abundance (Table 2.4).

Fish assemblage structure exhibited positive spatial autocorrelation in the study area (Mantel $r=0.113$, $p<0.005$; Table 2.4). Cottid abundances, catostomid abundances, ictalurid richness and abundances, salmonid richness and abundances showed positive spatial autocorrelation (Mantel $r>0.035$, $p<0.01$), demonstrating that nearby sites tended to support similar taxa. However, centrarchid species richness showed negative spatial autocorrelation in the study area (Mantel $r=-0.054$, $p<0.005$), suggesting that nearby sites tended to support different numbers of centrarchid species.

Effects of stream network topology

Stream network topology was significantly related to fish assemblage structure up to a distance of approximately 9 fkm downstream from sites (partial Mantel $r>0.095$, $p<0.0024$; Figure 2.5). I detected similar spatial patterns in total species richness, catostomid richness and abundance, and cyprinid richness (Figure 2.6). However, variation in the richness and abundance of ictalurid, percid, salmonid, and cottid fishes was not related to stream network structure (Figure 2.6). Addition of blocking variables in partial Mantel tests (from Table 2.4) had negligable effects on the observed relations between fish and stream network topology.

River species richness was significantly related to stream network topology but creek species richness and abundance was not (Figure 2.6). Unlike other metrics, river species richness was more strongly related to downstream conditions in the stream network than to site-level conditions, resulting in a "peak" pattern of Mantel r coefficients (Figure 2.6). River species richness also showed the strongest relations to stream network topology at the furthest distances from sites (i.e., 20 fkm; Figure 2.6). I retained fish metrics with the strongest observed relations to stream network topology (i.e., $\alpha'=0.0024$ at distances <6 fkm) for subsequent analysis of site size effects (FISH, S, CAT_S, CAT_N, CYP_S, and RIV_S; Table 2.5).

Site size had important consequences for stream network effects on fish metrics and assemblage structure. For the smallest sites (i.e., watershed areas <1000 ha; Table 2.3) fluvial distance accounted for little variation in topology (i.e., all partial Mantel $r\approx0$). In contrast, for larger sites (i.e., watershed areas 1000-5000 ha and 5000-10,000 ha; Table 2.3) fluvial distance showed significant relations to stream network structure (Figure 2.7).

Mid-sized sites (i.e., watershed areas 1000-5000 ha; Table 2.3) showed the most significant stream network effects on fish assemblage structure (Bray-Curtis dissimilarities), total species richness, catostomid richness, and cyprinid richness (Figure 2.7). However, mid-size and large sites both showed significant stream network effects on catostomid abundances and river species richness (partial Mantel $r>0.095$, $p<0.0024$; Figure 2.7). Overall, river species richness showed the strongest relationship to stream network topology (Mantel $r>0.30$; Figure 2.7), and showed the most significant Mantel r values at the largest spatial grain (i.e., 20 fkm; Figure 2.7).

Analysis of stream network effects based on site size revealed spatial patterns distinct from analysis among all sites. When analyzed in discrete site size classes, correlations between fish metrics and downstream topology exhibited peak-shaped patterns in fish assemblage structure, species richness, catostomid richness and abundance, cyprinid richness, as well as river species richness (Figure 2.7). Mid-sized streams showed peak-shaped patterns for all metrics. Large-sized streams showed similar peak-shaped patterns, but correlations were significant ($\alpha'=0.0024$) only for catostomid abundance, cyprinid richness, and river species richness (Figure 2.7). However, all fish metrics except river species richness showed diminishing effects of stream networks with increasing downstream distances when analyzed among all sites (i.e., decreasing Mantel r coefficients; Figures 2.5 and 2.6).

The presence of large river confluences (i.e., watershed areas >250 km^2) had important consequences for local fish assemblage structure. Mainstem tributaries supported greater richness and abundance of catostomid, cyprinid, and ictalurid fishes than did headwater tributaries (Mann-Whitney chi-square >2.78, $p<0.10$ for all tests;

Table 2.6; Appendix D). Total abundance was greater in mainstem than headwater tributaries (Mann-Whitney chi-square=2.78, $p<0.10$; Table 2.6; Appendix D). Total richness tended to be greater in mainstem than headwater tributaries (Appendix D) but this difference was not statistically significant in non-parametric tests at an alpha level of 0.10. River species were more abundant in mainstem tributaries than in headwater tributaries (Mann-Whitney chi-square=2.85, $p<0.10$; Table 2.6; Appendix D). In contrast, the richness and abundance of creek species did not differ between mainstem and headwater tributaries.

DISCUSSION

In this chapter, I tested the hypotheses that the size and proximity of connected streams influences fish assemblage structure through regional dispersal processes, and that local stream size regulates the effects of regional dispersal. I reasoned that if downstream areas provided important source populations or remote resources for local assemblages, variability in downstream conditions would correspond to variability in local fish assemblage structure. I predicted that (a) the smallest streams would be least likely to exhibit effects of riverine dispersal due to locally unsuitable habitats and (b) fish families containing many riverine-adapted species would show stronger effects of dispersal than families with few riverine species. In support of these predictions, I observed the weakest effects of stream network structure among the smallest sites and

detected the strongest stream network effects among families with more riverine-adapted species. My results suggest that fish dispersal from riverine habitats may influence connected streams, and that these effects vary among fish taxa, local habitat sizes, and distance from potential source populations.

Stream network effects vary among taxa

Fish families showed distinct relations to stream network topology. Catostomid and cyprinid fishes were significantly related to stream network topology whereas percid, ictalurid, cottid, and salmonid fishes were not (Figure 2.6). Moreover, catostomid and cyprinid fishes were greater in richness and abundance within mainstem tributaries than in headwater tributaries (Table 2.6; Appendix D). The concordance among continuous and discrete analyses suggests that dispersal from riverine sources may be more common for catostomid and cyprinid species than for other species in the study area, even though these families comprised a minority of the designated "river" specialist species (i.e., 17/41=41% of river species; Appendix C).

Previous studies have demonstrated extensive dispersal distances in catostomid fishes. Osborne and Wiley (1992) found that catostomid species richness was greater in mainstem tributaries than in headwater tributaries. However, it remains unclear whether or not observed patterns are due to seasonal spawning movements or metapopulation dynamics. Based on seasonal variation in catostomid abundances, Curry and Spacie (1984) concluded that *Moxostoma duquesnei* (black redhorse) and *M. erythrurum* (golden redhorse) make extensive upstream migrations for spawning (i.e., >15 fkm) but that *Catostomus commersoni* (white sucker) and *Hypentelium nigricans* (northern hog

sucker) were less mobile. In the current study, *C. commersoni* and *H. nigricans* were the most abundant catostomids, occupying 60% and 32% of sites, respectively. In contrast, M. *duquesnei* and *M. erythrurum* occupied only 3% and 5% of the sample sites. Although catostomid abundances were weakly related to month-to-month variation in the current study (Mantel $r=0.032$, $p<0.10$; Table 2.4), variation among catostomid species was not evaluated.

Cyprinid relations to stream network structure are consistent with previous studies. Matthews and Robison (1998) found that cyprinid distributions were explained by stream network connectivity (i.e., fewest number of connecting nodes), suggesting inter-stream dispersal. Several species of cyprinids are known to disperse from riverine habitats into smaller adjacent streams (e.g., Gorman 1986). In my analysis, the majority of "river species" were cyprinids (Appendix C). I found that mainstem tributaries contained greater cyprinid species richness and abundance than headwater tributaries (Table 2.6; Appendix D). However, Osborne *et al*. (1992) reported no effects of stream network structure on the percent of insectivorous cyprinids observed in sample streams.

My results supported the prediction that salmonid and cottid species would show relatively weak effects of stream network topology due to their relatively low species richness and abundance in riverine habitats within the study area. However, inter-stream metapopulation structure in salmonid fishes may be more prevalent in watersheds connected by coldwater rivers (i.e., suitable dispersal corridors). For example, occurrences of *Salvelinus confluentus* (bull trout) tend to occur within landscape-level patches, suggesting inter-stream recolonization events (Rieman and McIntyre 1995; Dunham and Rieman 1999). However, long-distance movements (i.e., > 1 fkm) have

been reported for *S. fontinalis* (brook trout) in Virginia (Roghair and Dolloff 2005). The current study did not evaluate potential dispersal among connected streams of the same size and therefore may not reflect inter-stream dispersal patterns relevant for coldwater species in the study area.

Stream network effects are mediated by local stream size

Fish assemblage relations to stream network topology varied among local stream sizes. Stream network effects were weakest in the smallest sites (i.e., <1000 ha upstream basin area, Table 2.3) and were greater at larger stream sites (i.e., 1000-10,000 ha upstream basin size; Table 2.3; Figure 2.8). Presumably, harsh conditions in small streams excluded potential immigrants or caused rapid local extirpations (Schlosser 1990; Gotelli and Taylor 1999). In such cases, riverine dispersal had little apparent influence on local assemblage structure. However, in larger streams, local conditions were apparently more suitable for immigrants from downstream areas. This result supports the hypothesis that local assemblage structure is regulated by the interaction of site suitability and colonist availability (Angermeier *et al*. 2002).

The observed differences between small and large stream sites are consistent with the predictions of the RCC (Vannote *et al*. 1980). However, my results also suggest that RCC predictions for fish assemblage structure may be improved by considering site position within stream networks. The original development of the RCC recognized that tributaries may act as discontinuities in the river continuum (Vannote *et al*. 1980) and subsequent work has demonstrated such effects in longitudinal patterns of fine particulate organic matter (Bruns *et al*. 1984) and sediment transport (Benda *et al*. 2004b; Rice *et al*.

2006). However, modifications to the RCC have predominantly focused on the localized effects of connected streams at confluences. In contrast, a stream network perspective recognizes that habitat conditions within connected streams may also provide source populations for dispersal (Fausch *et al*. 2002). My results suggest that fish dispersal dynamics may be understood in part by the spatial position of sites within stream networks and RCC predictions may be improved by incorporating a spatially-explicit framework of stream network topology.

Stream network effects vary with distance

The absence of information about stream network distance effects presents a critical gap for understanding landscape influences on stream biota (Wang *et al*. 2006). Previous studies have used *a priori* distances within which to test for stream network effects (e.g., 20 fkm; Osborne and Wiley 1992; Osborne *et al*. 1992) and were therefore unable to assess the effects of distance from potential source populations. In developing a distance-based model of stream network topology, the current study provides new insights about the continuous nature of stream network effects on fish assemblage structure.

I found that variation in stream network topology was significantly related to fish assemblage structure up to a distance of approximately 9 fkm downstream from sites (Figure 2.5). Similar distance effects were observed for total species richness (10 fkm; Figure 2.6), catostomid abundance (10 fkm; Figure 2.6), and cyprinid species richness (8 fkm, Figure 2.6). The longest distance effects of stream network topology were detected for river species richness (>20 fkm, Figure 2.6). However, analysis within stream size

categories revealed longer distances of stream network effects (Figure 2.7). In sites on mid-sized and large streams (see Table 2.3), stream network effects extended past 20 fkm in fish assemblage structure (Bray-Curtis distances from species abundance), total species richness, catostomid species richness and abundance, cyprinid species richness, and river species richness (Figure 2.7).

Spatially-explicit analysis of distance provided some insights that were not evident from categorical analyses of stream network effects. For example, total species richness was related to continuous variability in stream network structure up to a distance of approximately 10 fkm (Figure 2.6). However, sites categorized as mainstem tributaries and headwater tributaries were not significantly different in total species richness (α=0.10; Table 2.6). Bootstrapped means indicated that mainstem tributaries tended to support approximately two more species than headwater tributaries (Appendix D) but Osborne and Wiley (1992) detected greater differences in species richness between mainstem and headwater tributaries. This discrepancy may be explained in part because Osborne and Wiley (1992) selected sample sites in order to detect mainstem influences whereas the current study evaluated sites that were located randomly with respect to large river confluences.

Interpretation of dispersal distances from this study requires three caveats. First, I did not evaluate potential dispersal among connected streams of the same size or from upstream sources. Instead, stream network topology was quantified as variation in the rate of flow accumulation downstream from sites. This approach allowed me to quantify continuous variation in stream network structure (i.e., size and proximity of connected streams) but may have not detected significant dispersal from all potential source

populations. Second, the significance of distance relations to stream network structure was assessed with statistical thresholds, not ecological thresholds. I chose a conservative alpha-level to assess statistical significance of stream network effects (Bonferroni-corrected $\alpha'=0.05/21$ comparisons$=0.0024$), but different criteria for significance would yield different interpretations of distance effects. Third, this study cannot distinguish between temporary movements to access resources (i.e., life history expression) and inter-population dispersal (i.e., metapopulation dynamics). I would expect movements for life history expression to encompass smaller distances than movements for inter-population dispersal, but was unable to test this notion.

Implications for stream fish bioassessment

Fish bioassessment studies are based on the premise that variation in assemblage structure indicates variation in local environmental quality among sample sites (Karr *et al*. 1986). However, my results suggest that dispersal may influence fish assemblages across much larger areas than are typically sampled in stream-fish bioassessment studies (e.g., 40x mean stream width, McCormick and Hughes 1998). As such, the choice of sample site location may affect fish assemblage data in ways that could not be perceived from site-level observations. Typically, stream bioassessment studies identify sample site locations using systematic-random methods (e.g., EMAP, Herlihy *et al*. 2000). In this case, riverine dispersal would tend to decrease the precision of fish metrics as a random factor among sites. However, systematic biases may occur when stream bioassessment sites are intentionally located near large river confluences (e.g., West Virginia Regional

Environmental Monitoring and Assessment Program, R-EMAP; see Detenbeck *et al*. 2005).

My results suggest fish dispersal from riverine areas may affect the spatial grain at which bioassessment metrics indicate environmental conditions. Stream bioassessment studies often treat the spatial grain of analysis implicitly by stratifying sites according to Strahler stream-order (e.g., EMAP, Virginia Department of Environmental Quality Probabilistic Monitoring Program). Such methods permit extrapolations to unsampled areas under the assumption that sample sites of a given stream-order are representative of other streams of the same stream-order. However, my results suggest that dispersal of riverine fishes may influence similarly-sized streams differently, based on the proximity of sites to riverine source areas. The implications of this finding are that (1) pooling data between mainstem tributaries and headwater tributaries may decrease the precision of cumulative distribution functions for fish metrics (i.e., increasing confidence intervals) and (2) excluding or including river species from metric calculations may enable local or regional assessments of environmental quality, respectively.

Regional dispersal may also affect the sensitivity of specific fish bioassessment metrics. The stream network effects observed in the current study suggest that immigration from downstream areas may decrease the relative sensitivity to local environmental stressors (i.e., bioassessment type II errors) relative to sites that lack these regional connections. For example, I found that fish species richness and cyprinid richness were explained by variation in stream network structure. These metrics were identified for use in fish bioassessment studies within the mid-Atlantic highlands (Angermeier *et al*. 2000; McCormick *et al*. 2001) but it remains unknown whether or not

regional dispersal dynamics affect the sensitivity of these metrics. Additional research is necessary to test the prediction that dispersal from connected streams would decrease the sensitivity of fish metrics to local environmental conditions (see Chapter 3).

Table 2.1. Numbers of EMAP sites within zoogeographic regions and ecoregions in the mid-Atlantic highlands study area (see text). Regions are mapped in Figure 2.2.

		Zoogeographic regions				
		Atlantic slope	New River basin	Ohio River basin	Tennessee River basin	**Totals**
Ecoregions	Appalachian Plateau	19	0	36	0	**55**
	Blue Ridge	12	6	0	0	**18**
	Central Appalachians	2	4	25	1	**32**
	Ridge and Valley	150	24	8	21	**203**
	Totals	**183**	**34**	**69**	**22**	**308**

Table 2.2. Stream fish metrics, variable codes, and transformations used in the current analysis of the mid-Atlantic highlands region. Metrics were calculated with modifications to EMAP data described in Appendix B. "Fish assemblage" was computed from Bray-Curtis distances of species abundance.

Metric	Code	Transformation
Fish assemblage	FISH	Abundances: $\log_{10}(x+1)$
Species richness	S	none
Abundance	N	$\log_{10}(x+1)$
Cottid abundance	COT_N	$\log_{10}(x+1)$
Salmonid richness	SAL_S	none
Salmonid abundance	SAL_N	$\log_{10}(x+1)$
Percid richness	PER_S	none
Percid abundance	PER_N	$\log_{10}(x+1)$
Centrarchid richness	CEN_S	none
Centrarchid abundance	CEN_N	$\log_{10}(x+1)$
Catostomid richness	CAT_S	none
Catostomid abundance	CAT_N	$\log_{10}(x+1)$
Cyprinid richness	CYP_S	none
Cyprinid abundance	CYP_N	$\log_{10}(x+1)$
Ictalurid richness	ICT_S	none
Ictalurid abundance	ICT_N	$\log_{10}(x+1)$
"Creek" species richness	CRK_S	none
"Creek" species abundance	CRK_N	$\log_{10}(x+1)$
"River" species richness	RIV_S	none
"River" species abundance	RIV_N	$\log_{10}(x+1)$

Table 2.3. Site size classes used in the current analysis. Approximate watershed areas are based on the upstream cell count (UCC) relationship to upstream basin area in Figure 2.3.

Site size class	n	UCC range	Watershed area range (ha)
1	102	1 – 192	1 – 1000
2	103	193 – 777	1000 – 5000
3	103	790 – 4780	5000 – 10000

Table 2.4. Fish metric relations to spatial and temporal variation in the mid-Atlantic highlands study area. Cell values are Mantel r correlation coefficients. Significance is indicated as: *p<0.10; **p<0.01; ***p<0.005. Positive coefficients indicate that large differences in one variable are associated with large differences in a second variable. Negative coefficients indicate that large differences in one variable are associated with small differences in a second variable. Variable codes are presented in Table 2.2. Cartesian distances among sites are indicated by "Space". Non-significance (p>0.10) is indicated by "ns".

Variable	Zoogeographic region	Ecoregion	Sample year	Sample month	Space
FISH	0.203***	0.112***	ns	ns	0.113***
S	0.040*	ns	0.041*	ns	ns
N	ns	0.061*	0.042*	ns	ns
COT_N	ns	ns	ns	ns	0.057***
CAT_S	0.041*	0.043*	ns	ns	ns
CAT_N	ns	ns	ns	0.032*	0.036**
CEN_S	ns	ns	0.054*	0.042*	-0.054***
CEN_N	ns	ns	ns	ns	-0.038*
CYP_S	0.039*	ns	ns	ns	ns
CYP_N	0.052*	0.072*	ns	ns	ns
ICT_S	-0.069*	ns	ns	ns	0.080***
ICT_N	ns	ns	ns	ns	0.082***
PER_S	0.167***	0.148***	0.092***	0.063*	ns
PER_N	ns	ns	0.042***	0.049***	ns
SAL_S	-0.067**	ns	ns	-0.069***	0.167***
SAL_N	-0.083**	ns	ns	-0.049*	0.191***
CRK_S	0.044*	0.045*	ns	ns	ns
CRK_N	0.044*	0.055*	ns	ns	ns
RIV_S	0.077*	0.062*	0.064**	ns	ns
RIV_N	ns	ns	0.058**	ns	ns

Table 2.5. Fish metric relations, by stream size class, to spatial and temporal variation in the mid-Atlantic highlands study area. Stream size information is presented in Table 2.3. Cell values are simple Mantel r correlation coefficients. Significance is indicated as: *p<0.10; **p<0.01; ***p<0.005. Positive coefficients indicate that large differences in one variable are associated with large differences in a second variable. Negative coefficients indicate that large differences in one variable are associated with small differences in a second variable. Metric codes are presented in Table 2.2. Non-significance (p>0.10) is indicated by "ns".

Metric	Size class	Zoogeographic region	Ecoregion	Sample year	Sample month	Space
FISH	1	0.091**	ns	ns	ns	0.085**
	2	0.309***	0.158**	ns	ns	0.173***
	3	0.339***	0.108**	ns	0.050*	0.243***
S	1	ns	-0.081*	ns	ns	ns
	2	ns	ns	ns	ns	ns
	3	ns	ns	ns	ns	ns
CAT_S	1	ns	ns	ns	ns	ns
	2	0.123*	0.131*	0.097*	ns	ns
	3	ns	ns	ns	ns	ns
CAT_N	1	-0.079*	ns	ns	ns	ns
	2	0.142***	ns	ns	ns	0.040*
	3	ns	ns	ns	0.089**	ns
CYP_S	1	ns	ns	ns	ns	ns
	2	ns	ns	ns	ns	ns
	3	ns	ns	ns	ns	ns
RIV_S	1	ns	ns	ns	ns	ns
	2	ns	ns	ns	ns	-0.061*
	3	0.156**	0.088*	0.059*	ns	ns

Table 2.6. Mann-Whitney tests for differences in fish metrics between mainstem tributaries (MT) and headwater tributaries (HT) in the mid-Atlantic highlands study area. See text for methods used to assign sites to MT and HT classes. Significance is indicated as: *p<0.10; **p<0.05. Bootstrapped mean values for each comparison are presented in Appendix D.

Variable	Chi-square	P-value	Direction
S	2.12	0.146	
N	2.78*	0.095	MT > HT
CAT_S	2.90*	0.088	MT > HT
CAT_N	2.81*	0.094	MT > HT
CEN_S	0.00	0.988	
CEN_N	0.03	0.871	
COT_N	0.45	0.504	
CYP_S	3.90**	0.048	MT > HT
CYP_N	3.17*	0.075	MT > HT
ICT_S	3.29*	0.070	MT > HT
ICT_N	3.74*	0.053	MT > HT
PER_S	1.17	0.280	
PER_N	0.77	0.381	
SAL_S	0.38	0.537	
SAL_N	0.48	0.490	
CRK_S	0.02	0.896	
CRK_N	0.02	0.882	
RIV_S	2.31	0.129	
RIV_N	2.85*	0.091	MT > HT

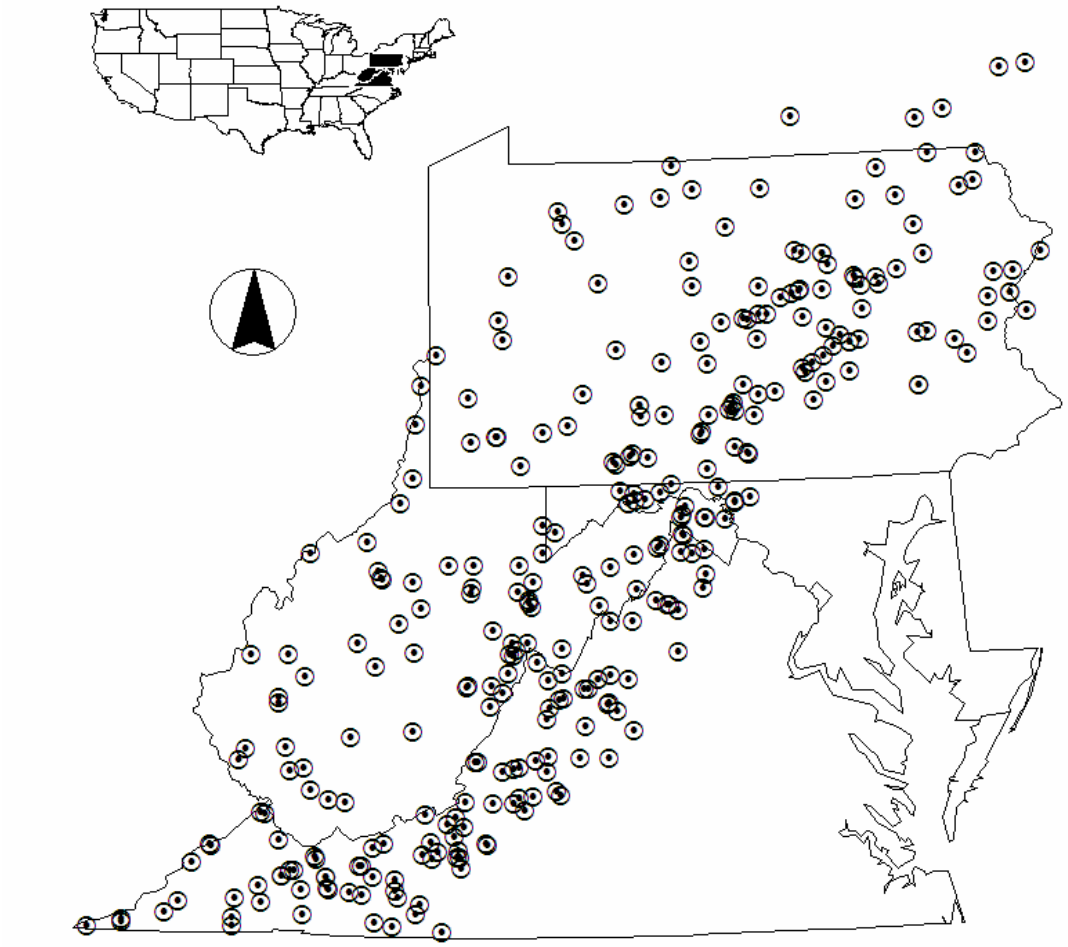

Figure 2.1. U.S. Environmental Protection Agency EMAP study site locations within the mid-Atlantic highlands study area, USA (n=308).

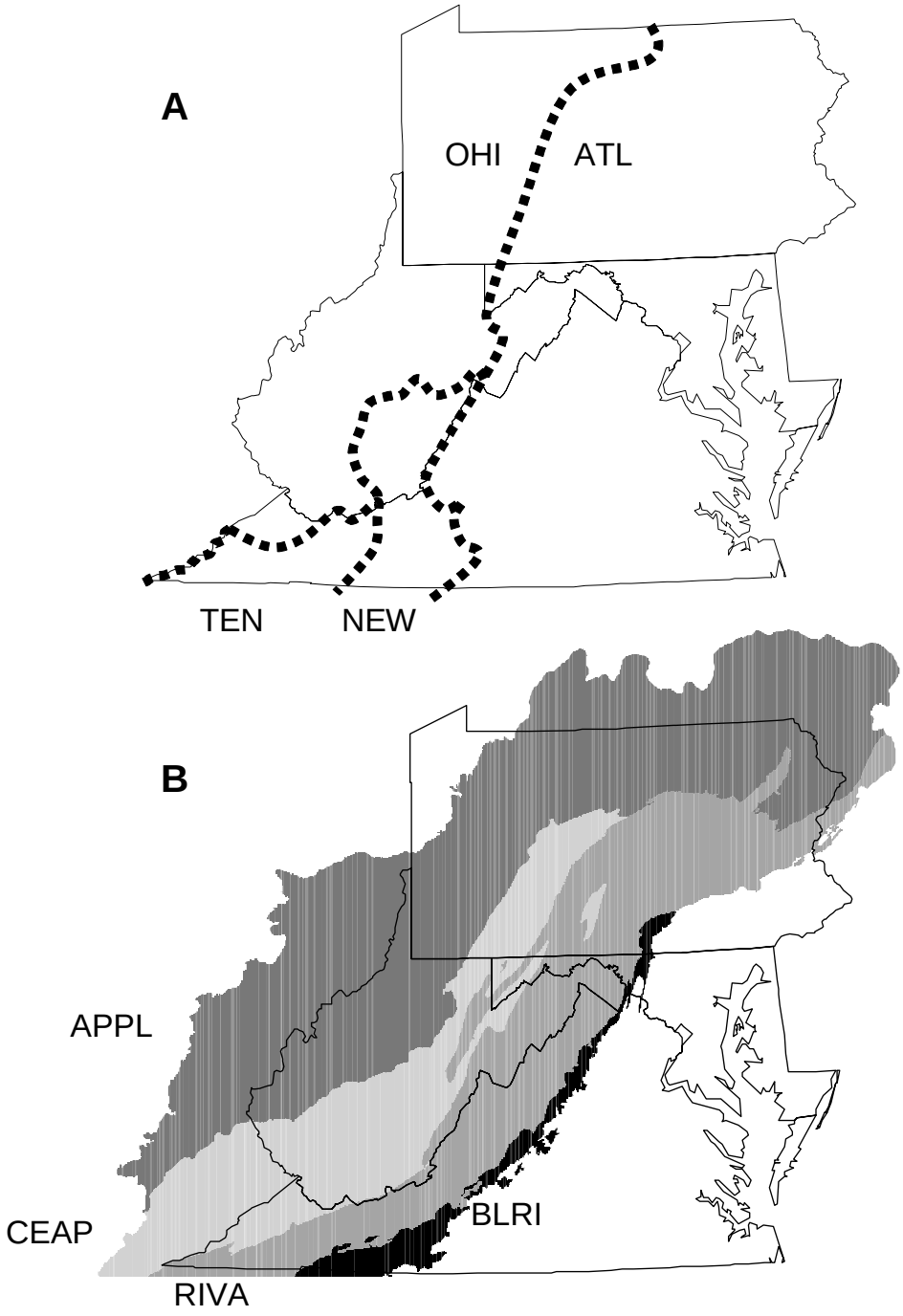

Figure 2.2. Zoogeographic regions and ecoregions within the study area. (A) Zoogeographic regions include the Ohio River basin (OHI), Atlantic slope drainages (ATL), the New River basin (NEW), and the Tennessee River basin (TEN). (B) Ecoregions include the Appalachian Plateau (APPL), the Central Appalachians (CEAP), the Ridge and Valley (RIVA), and the Blue Ridge (BLRI) (see text).

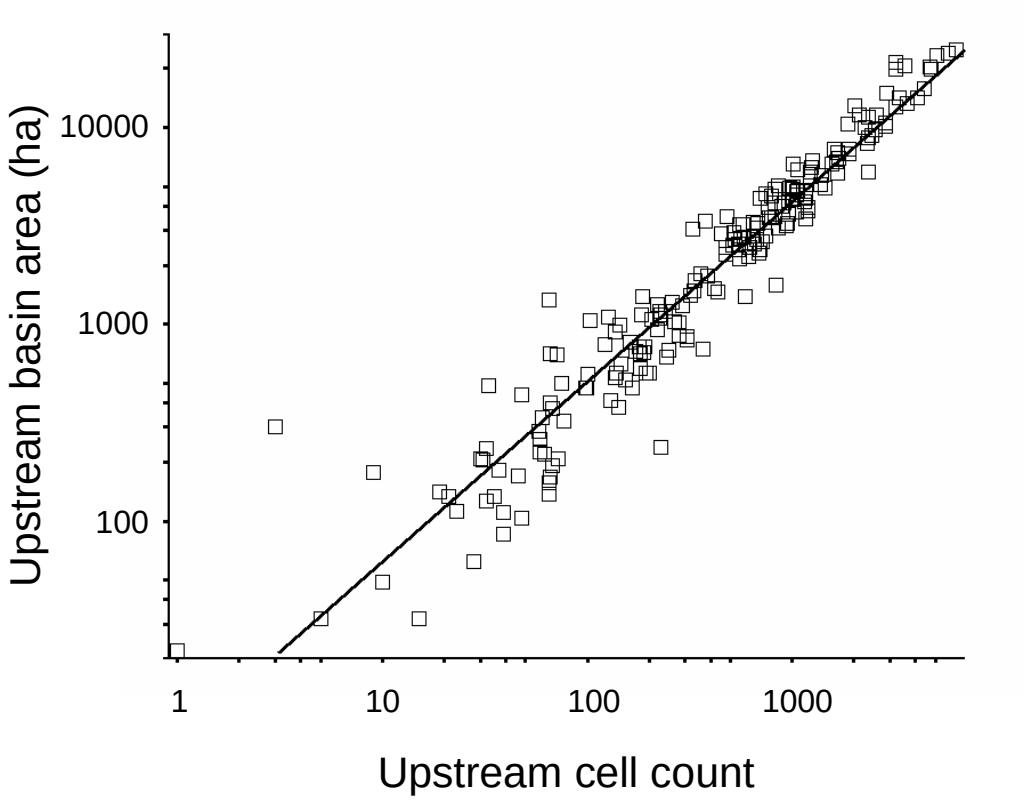

Figure 2.3. Simple linear regression of upstream basin areas (EMAP data) and calculated upstream cell counts (UCCs) for a subset of EMAP sites in the mid-Atlantic highlands study area (n=198). R^2=0.912.

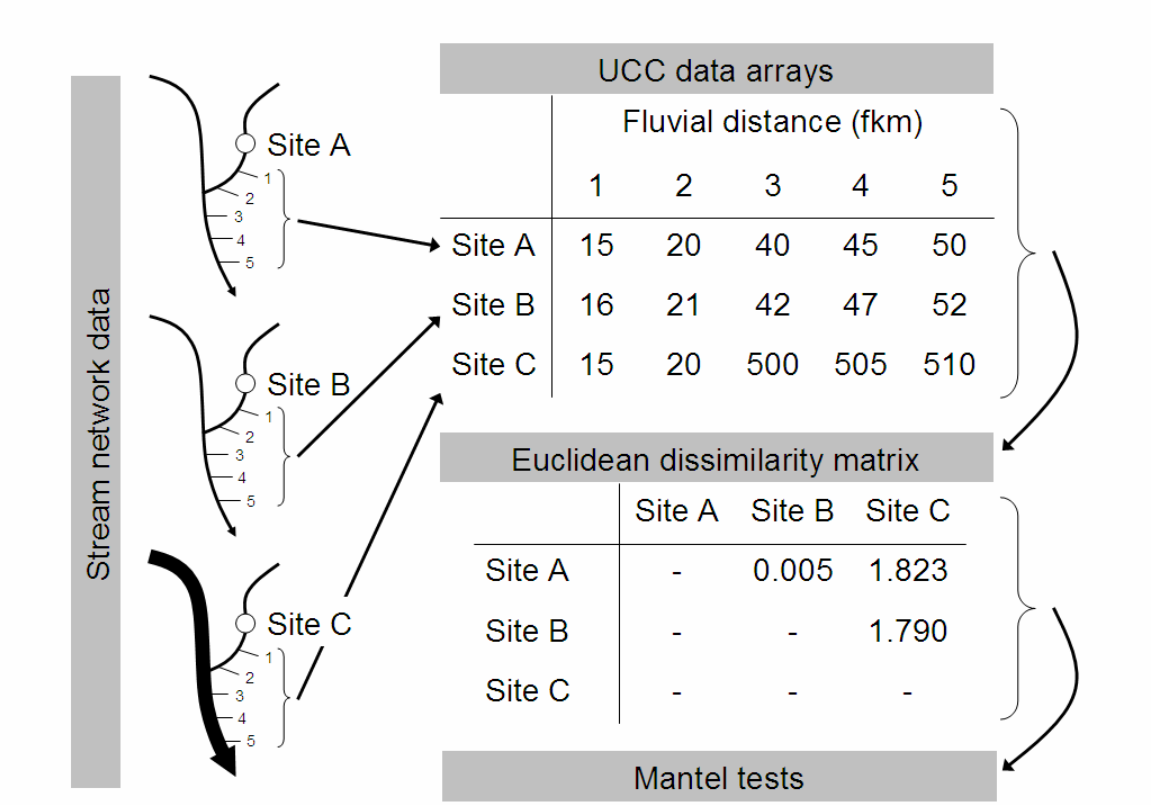

Figure 2.4. Conceptual diagram for calculation of Euclidean dissimilarity matrices to characterize stream networks. UCC=upstream cell count (see text). UCC data are hypothetical in this example.

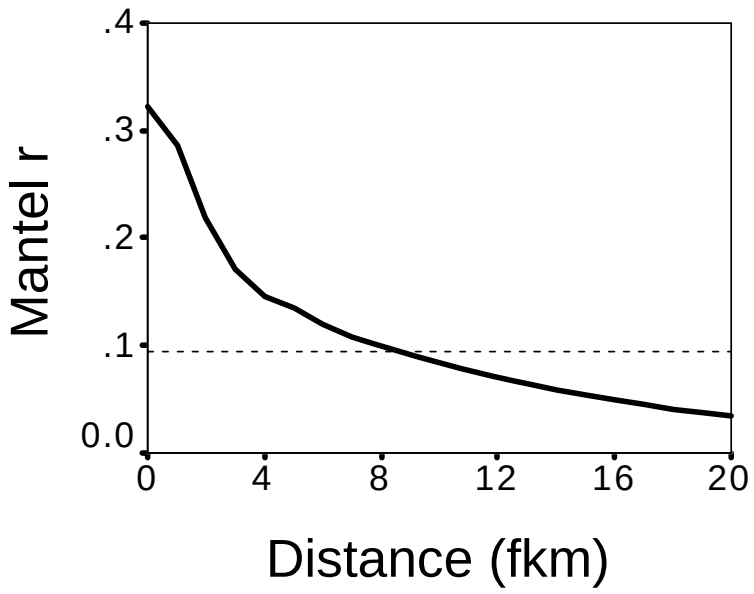

Figure 2.5. Partial Mantel correlations between fish assemblage structure (Bray-Curtis dissimilarities) and upstream cell counts with increasing downstream distances from sites (fluvial km; fkm). All correlations controlled for potentially confounding effects of zoogeography, ecoregion, and spatial autocorrelation with partial correlation matrices (see Table 2.4). Correlations above the dotted line indicate significance at a Bonferroni-corrected error rate, $\alpha'=0.05/21$ (21 distance classes)$=0.0024$. All correlations used 10,000 resampling iterations to establish the significance level.

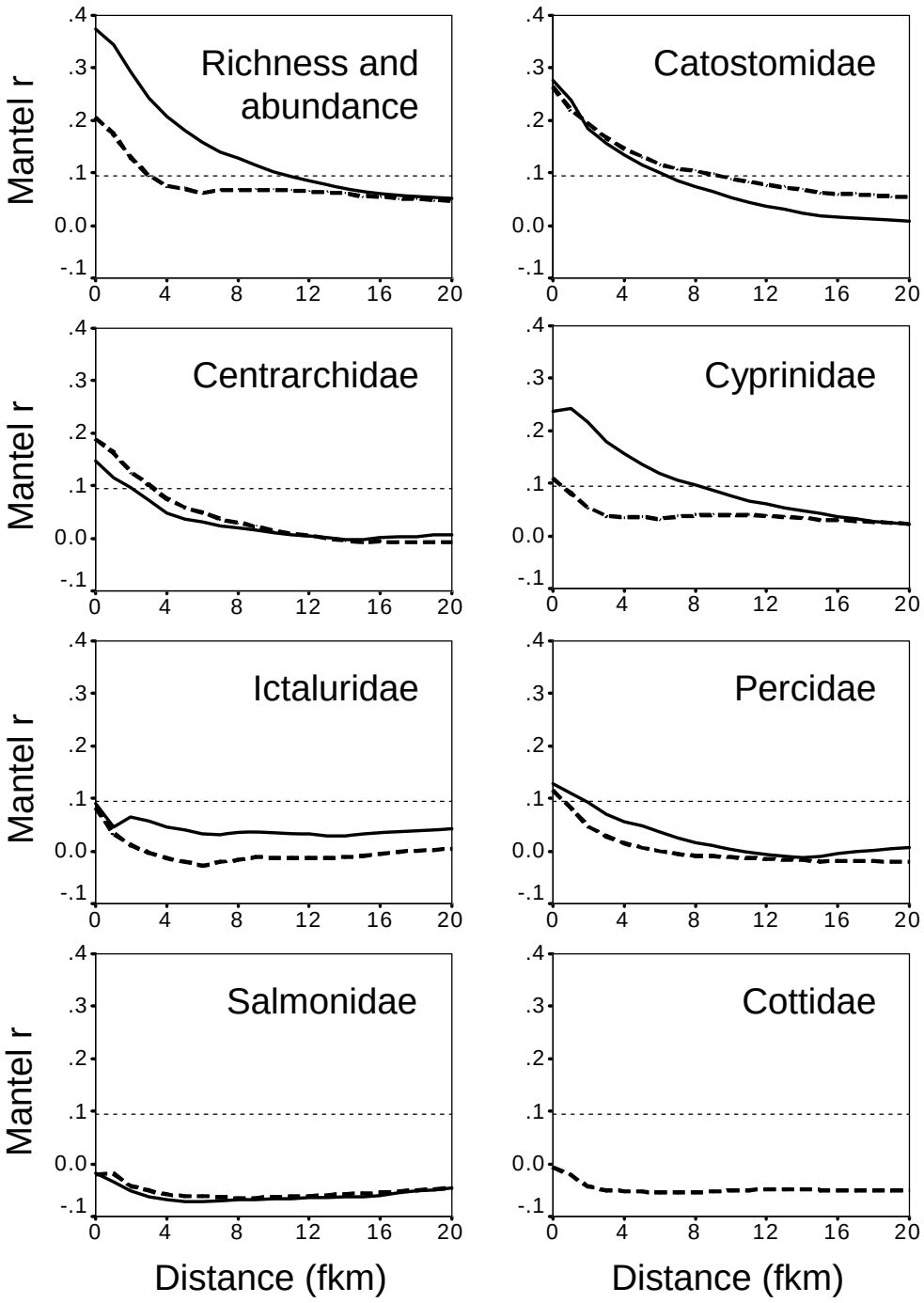

Figure 2.6. Partial Mantel correlations between taxonomic fish metrics and upstream cell counts with increasing downstream distances from sites (fluvial km; fkm). Solid lines indicate species richness and dashed lines indicate abundances. All correlations controlled for potentially confounding effects of zoogeography, ecoregion, sampling year, sampling month, and spatial autocorrelation with partial correlation matrices (see Table 2.4). Correlations above the dotted line indicate significance at a Bonferroni-corrected error rate, $\alpha'=0.05/21$ (21 distance classes)=0.0024. All correlations used 10,000 resampling iterations to establish a significance level. "Creek" and "river" species were defined from Jenkins and Burkhead (1994; see Appendix C).

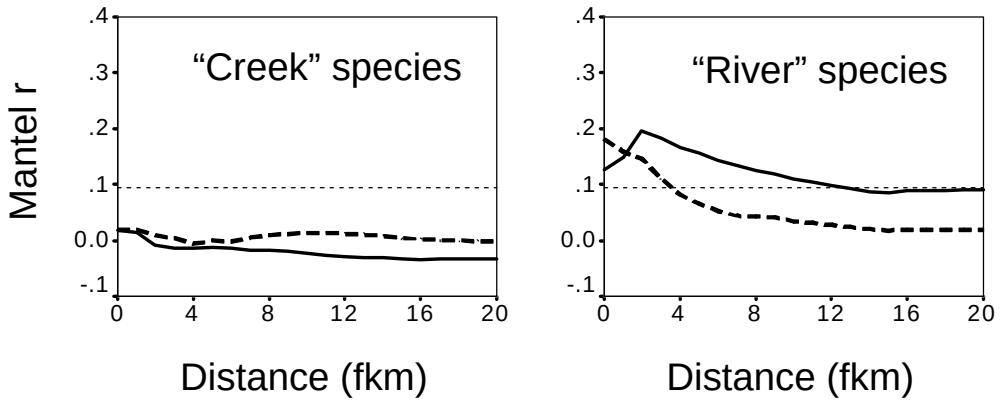

Figure 2.6., continued. Partial Mantel correlations between taxonomic fish metrics and upstream cell counts with increasing downstream distances from sites (fluvial km; fkm). Solid lines indicate species richness and dashed lines indicate abundances. All correlations controlled for potentially confounding effects of zoogeography, ecoregion, sampling year, sampling month, and spatial autocorrelation with partial correlation matrices (see Table 2.4). Correlations above the dotted line indicate significance at a Bonferroni-corrected error rate, $\alpha'=0.05/21$ (21 distance classes)$=0.0024$. All correlations used 10,000 resampling iterations to establish a significance level. "Creek" and "river" species were defined from Jenkins and Burkhead (1994; see Appendix C).

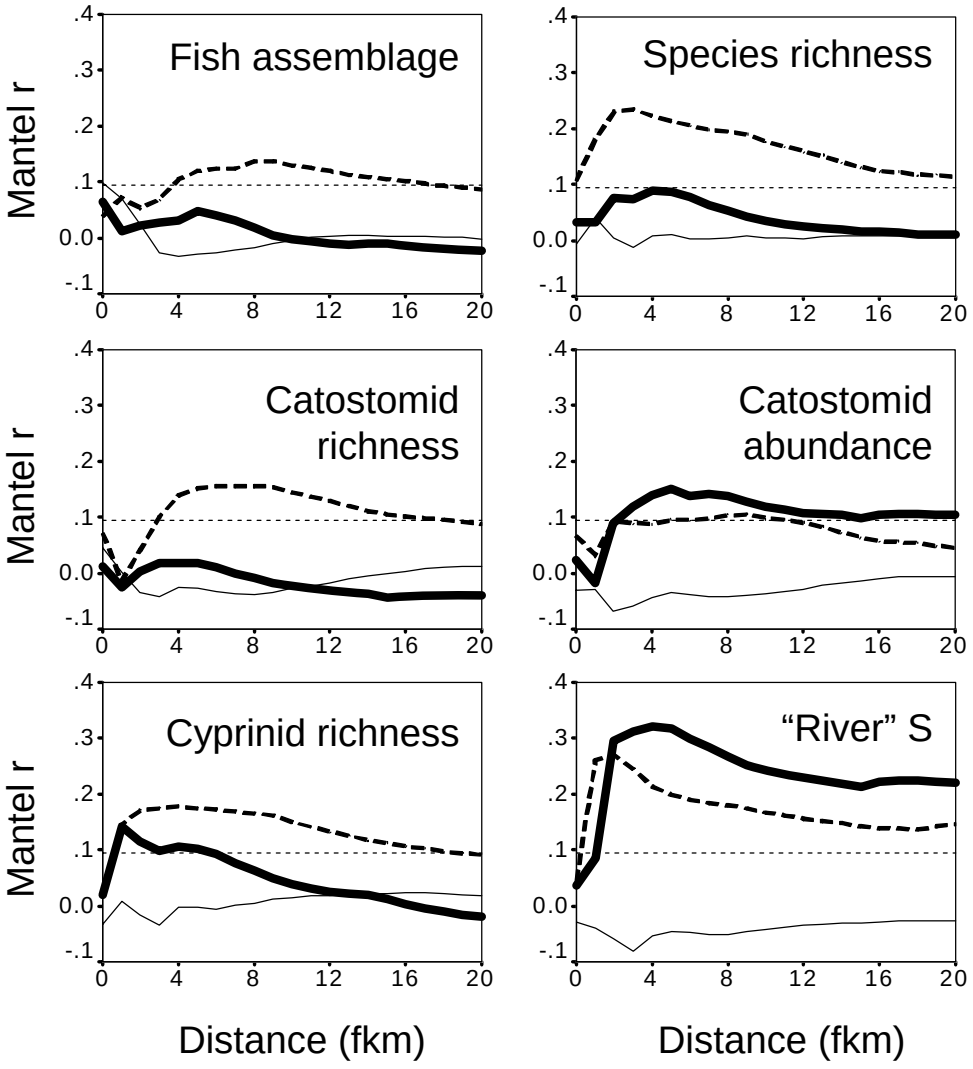

Figure 2.7. Site size variation in partial Mantel correlations between fish metrics and upstream cell counts with increasing downstream distances from sites (fluvial km; fkm). River species richness (River S) was calculated from Jenkins and Burkhead (1994, see Appendix C). "Fish assemblage" indicates species abundances in Bray-Curtis distances. Small stream sites are shown with thin, solid lines, medium-sized stream sites are shown with dashed lines, and the largest stream sites are shown with thick solid lines. See Table 2.3 for stream size data. All correlations controlled for potentially confounding effects of zoogeography, ecoregion, and spatial autocorrelation with partial correlation matrices (see Table 2.5). Correlations above the dotted line indicate are significant at a Bonferroni-corrected error rate, $\alpha'=0.05/21$ (21 distance classes)=0.0024. All correlations used 10,000 resampling iterations to establish the significance level.

Chapter 3.

Effects of stream network topology on fish bioassessment sensitivity in the mid-Atlantic highlands, USA

INTRODUCTION

Over the last 25 years, biological assessments of freshwater ecosystems (i.e., bioassessments) have become more common on a global scale (Karr and Chu 2000). Unlike chemical or physical assessment of water quality, bioassessment evaluates organismal-, population-, and assemblage-level responses to environmental degradation *in situ*. Bioassessment methods have provided insights that were not available from chemical and physical assessments (e.g., Yoder and Rankin 1998) because biota respond to multiple physical and chemical conditions simultaneously. However, bioassessment methods may be subject to biases because metrics describing local communities may be influenced not only by local site quality but also by organismal dispersal at larger spatial scales. If dispersal is an important determinant of local assemblage structure, the apparent local biotic "signals" of environmental quality could be obscured or confounded by the "noise" of dispersing individuals. Interpretation of local bioassessments therefore requires an understanding of the spatial scale of dispersal and of habitat conditions within dispersal distances for the indicator taxa. In this chapter, I explore how the spatial position of sites within stream networks (i.e., stream network topology) affects the

sensitivity of fish bioassessment metrics in wadeable streams of the mid-Atlantic highlands, USA.

Dispersal is a landscape-level process that influences the local distribution and abundance of organisms (Wiens 2001). Stream fish may disperse to access remote resources (e.g., spawning areas, feeding areas), escape locally unsuitable conditions (i.e., access refugia), or colonize distant areas (Schlosser 1990; Fagan 2002; Fagan *et al*. 2005). Several lines of evidence suggest that inter-stream dispersal may influence local fish assemblage structure. First, some stream fishes are capable of dispersing long distances (>1 fluvial km [fkm], Karr and Gorman 1975; Gatz and Adams 1994; Albanese *et al*. 2004; Popoff and Neumann 2005; Roghair and Dolloff 2005). Second, positive spatial autocorrelation in fish assemblage structure (Matthews and Robison 1998; Wilkinson and Edds 2001; Hitt *et al*. 2003; Grenouillet *et al*. 2004) also suggests dispersal-mediated distributions at large spatial scales (i.e., > 1 fkm). Third, local fish species richness is non-randomly associated with adjacent stream sizes (Osborne and Wiley 1992; Wilkinson and Edds 2001; Hitt and Angermeier 2006), in support of the hypothesis that fish disperse among connected streams. Interpretations of the factors that regulate fish assemblage structure should consider the landscape in which sites are embedded (Fausch *et al*. 2002).

Stream fish bioassessment studies therefore require an understanding of how dispersal among adjacent streams may influence local responses to environmental conditions. Fish dispersal from connected streams could bias local fish metrics through recolonization by intolerant fishes or immigration of tolerant fishes from outside the study site. Recolonization events could cause an underestimation of local degradation

(i.e., type II errors). Conversely, immigration of tolerant fishes from outside the study area could cause an overestimation of local degradation (i.e., type I errors). Therefore, to enhance the accuracy of fish bioassessments, protocols should consider how the spatial configuration of streams influences local fish metric sensitivity.

The size and proximity of connected streams (i.e., stream network topology) provides a spatial framework to evaluate the effects of dispersal on local fish bioassessment metrics. In this chapter, I explore the hypothesis that stream networks influence dispersal by regulating the spatial distribution of source populations and remote resources at the landscape scale. I tested the predictions that (1) biotic attributes of streams flowing into large rivers (mainstem tributaries) will show less correspondence to local environmental variation than will biotic attributes of tributaries that lack mainstem connections (i.e., headwater tributaries) and (2) the effects of dispersal from riverine habitats will diminish at a site as distance from mainstem rivers increases. I reasoned that riverine immigrants would not be exposed to the full range of environmental variation within destination streams (i.e., temporal variation) and therefore would decrease the relative sensitivity of fish metric responses to local environmental conditions. I tested these predictions in wadeable streams of the mid-Atlantic highlands by ordinating sample sites along environmental quality gradients, assessing fish metric responses to these gradients, and comparing fish-environment relations among headwater and mainstem tributary sites.

METHODS

Data source

I used data from the U.S. Environmental Protection Agency's (USEPA) Environmental Monitoring and Assessment Program (EMAP) in the mid-Atlantic highlands region, USA (n=157; Figure 3.1). This region encompasses 205,000 km^2 throughout the Appalachian mountains of New York, Pennsylvania, Maryland, West Virginia, and Virginia. EMAP site locations were designated using a systematic random methodology (Herlihy *et al*. 2000). I chose to use the EMAP dataset because it provides a consistent methodology in sampling stream fishes and environmental conditions across a large study area.

In each site, USEPA personnel sampled stream fishes using single-pass backpack electrofishing methods (McCormick and Hughes 1998). Sample reach lengths were 40-times the average stream width for each site. Fishes were identified to species in the field and released but problematic specimens were preserved and identified later by USEPA personnel. Samples were collected during summer base-flow conditions in 1993, 1994, 1997, and 1998. I incorporated many changes to the EMAP data based on published species accounts, under the assumption that EMAP surveys did not capture species outside their known ranges (Appendix B). Raw data are available from http://www.epa.gov/emap.

I selected EMAP sites for this analysis using several criteria. First, I restricted sites to wadeable streams (i.e., backpack electrofishing methods) because of decreased sampling efficiencies at boat electrofishing sites in EMAP data (see Cyterski and Barber

2006). Second, I removed EMAP sites that flow into reservoir systems within 20 fkm to remove potential effects of downstream reservoirs on upstream assemblages (Winston *et al*. 1991; Guenther and Spacie 2006). For sites sampled repeatedly, I used the sample with the greatest species richness or, in the case of ties, the more recent sample. Third, I excluded sites that lacked environmental data.

I selected 36 environmental variables from the EMAP dataset to characterize variation in environmental quality among sites. Variables included measures of land use, mesohabitat structure, periphyton biomass, riparian vegetation, substrate size, stream volume, water quality, and woody debris (Table 3.1). I focused on these categories of environmental conditions because they are known correlates of fish distribution and abundance and were sampled in each site. Environmental data collection methods included GIS analysis, physical habitat measurements, and water quality sampling (Lazorchak *et al*. 1998).

Land use variables were calculated from GIS analysis of U.S. Geological Survey data (Land Use Data Analysis, LUDA) upstream of sample locations (Davis and Scott 2000). All land cover data were aggregated to a raster resolution of 30 m^2. Road densities (RD_DEN) and human population densities (POPDEN_KM) were calculated from U.S. Census Bureau data (Davis and Scott 2000). All other variables were calculated from data collected at sample sites.

Measures of stream volume, mesohabitat structure, substrate size, and woody debris were collected at each site using a systematic-random sampling design (Figure 3.2). Eleven transects were placed at even intervals perpendicular to the sample reach. Within each transect, five sample points were positioned at equal distances across the

width of the stream. At each sample point, stream depth, mesohabitat type (i.e., pool, riffle, or run) and substrate size were recorded. Stream widths were also recorded between transects. Substrate sizes were categorized following Wolman (1954), Bain *et al*. (1985) and Platts *et al*. (1983). Between transects, the proportion of small and large woody debris cover was visually estimated within a distance of 5 m upstream and downstream from each transect. Large woody debris was defined as pieces of wood measuring at least 10 cm in diameter at the small end and at least 1.5 m in length (Lazorchak *et al*. 1998). Each site therefore contains 55 measurements of substrate size and stream depth and 21 measurements of stream width and woody debris.

Attributes of riparian vegetation were visually estimated within the sample reach. At each transect, a sampling zone in the riparian area was delineated as a 10 m x 10 m square centered on the transect (i.e., 5 m upstream and downstream from the transect). Within this zone, USEPA personnel recorded the proportion of canopy cover (i.e., woody vegetation >15 m in height), proportion of large-tree canopy cover (i.e., >0.3 m DBH), and proportion of cover by three layers of riparian vegetation (i.e., ground cover, understory, and canopy cover). The proportion of canopy cover over the mid-channel was also visually estimated at each transect. Riparian vegetation data therefore included 22 measurements of bank conditions and 11 measures of mid-channel canopy cover.

Periphyton data were collected from a subset of sample points in nine of the 11 transects at each sample site (i.e., one sample per transect). From riffles, periphyton was scrubbed from a 12-cm^2 area on the upper surface of a substrate particle. From pools, a sediment sample was collected using a 60-mL syringe at a depth of approximately 1 cm and area of 12 cm^2. Samples were combined among riffles and pools at each site (Hill

1998). Laboratory analyses calculated chlorophyll *a* concentrations and ash-free dry biomass for all pool and riffle samples. To permit comparisons among sites, I used riffle data unless only pool data were available (*sensu* Yuan and Norton 2003). In all sites, I only used area-adjusted data to account for sampling effort. I adjusted the EMAP data for years 1997 and 1998 by multiplying biomass and chlorophyll variables by a factor of 10 to account for a likely conversion error in the raw data (B. Hill, USEPA, personal communication).

Water quality variables were calculated from laboratory analyses of water samples taken from the middle of the channel at each site (Herlihy *et al*. 1998). Measures of cation and nutrient concentrations were calculated from one 4-L sample of water at each site. Dissolved organic carbon, pH, and aluminum were calculated from one 60-mL syringe sample of water at each site. The syringe samples were taken and capped underwater to prevent CO_2 equilibration. All water quality samples were shipped within 24 hours for laboratory chemical analysis (Herlihy *et al*. 1998).

Fish metric calculations

I calculated fish metrics from modified EMAP data to characterize taxonomic and functional attributes of fish assemblage structure among sites (Appendices E and F). Taxonomic metrics assessed species richness and abundance at the family-level as well as native and introduced species. Functional metrics characterized fish assemblage structure based on trophic strategies, reproductive strategies, vertical position (i.e., benthic or pelagic), and overall tolerance to environmental degradation. I chose these metrics because they have demonstrated significant relations to environmental quality in previous

studies of the mid-Atlantic highlands region (Angermeier *et al*. 2000; McCormick *et al*. 2001) or because expected responses to degradation are known (Appendix F). *A priori* predictions for fish metric responses to degradation were based on previous studies of the mid-Atlantic highlands region (Table 3.2).

Trophic metrics characterized adult feeding strategies in four groups: invertivory, piscivory, invertivory/piscivory, and herbivory/omnivory. I used species-level trophic assignments from Smogor and Angermeier (1999a) but combined categories of detritivore/algivore/herbivore and algivore/herbivore/invertivore into a single herbivory/omnivory category *sensu* McCormick *et al*. (2001). I also calculated metrics to characterize specialist and generalist feeding strategies. Following Smogor and Angermeier (1999a), I classified trophic specialists and generalists based on four trophic categories: detritus, algae or vascular plants, invertebrates, or fish/fish blood. Generalist and specialists feeders were defined as species that use >2 or <3 food types, respectively (Smogor and Angermeier 1999a). I predicted that metrics of invertivory, piscivory, invertivory/piscivory, and trophic specialization would respond negatively to degradation and that omnivore/herbivore and trophic generalization metrics would respond positively to degradation (Table 3.2; Appendix F).

Reproductive metrics characterized fish assemblages based on spawning substrates, nest preparation/parental care, and age of female reproduction. I classified species as simple lithophils, complex lithophils, or non-lithophils based on a combination of spawning substrates and parental care attributes. Following Balon (1981), lithophils were designated as species that scatter or deposit eggs among hard substrates (i.e., pebble or gravel) whereas non-lithophils use vegetation or detritus for spawning. I further

separated simple lithophils from "non-simple" lithophils based on the absence or presence of nest preparation (e.g., redd construction) and/or parental care, respectively. I predicted that simple lithophils and late maturing species would respond negatively to degradation and that non-simple, non-lithophils would respond positively to degradation (Table 3.2; Appendix F).

I calculated "vertical position" metrics based on fish behavior and body morphology (Appendix E). These metrics distinguished between species that typically inhabit benthic or water-column strata. Benthic fishes are dorsoventrally compressed (e.g., sculpins) and typically feed on benthic invertebrates or periphyton whereas pelagic fishes are more cylindrical or laterally compressed (Moyle and Cech 1996). Following Angermeier *et al*. (2000), I also calculated metrics that combined trophic specialist strategies (i.e., one food type) and benthic vertical positions. I predicted that benthic fishes would respond negatively to degradation whereas water-column species would not (Table 3.2; Appendix F).

"Tolerance" metrics were calculated from published reports of species' responses to environmental degradation (Appendix E). For example, species with increasing abundances or distributions in degraded habitats are classified as tolerant. Conversely, species with diminishing ranges in degraded habitats are classified as intolerant. I based my classifications on agreement between Smogor and Angermeier (1999a) and McCormick *et al*. (2001). However, if species were not evaluated in either source, I classified species according to Barbour *et al*. (1999). Of the 119 species in the dataset, 13 (11%) were classified as "tolerant" and 8 (7%) were classified as "intolerant." The relatively low percentages of tolerant and intolerant species suggest that the resultant

metrics will reflect lower and upper ends, respectively, of the biotic integrity spectrum (Karr *et al*. 1986; Smogor and Angermeier 1999b).

Stream network topology

I classified sites as "headwater tributaries" and "mainstem tributaries" (*sensu* Osborne and Wiley 1992) to test the hypothesis that the spatial position of sites within a watershed influences the sensitivity of fish metrics to local environmental quality. The distinction between headwater and mainstem tributaries reflects the rate of stream volume accumulation per unit distance downstream from sites. Mainstem tributaries flow into "large" rivers at a given distance whereas headwater streams do not. To permit a spatially-explicit analysis, I categorized sites as headwater and mainstem tributaries at 5-, 10-, 15-, and 20-fkm spatial grain sizes. I chose the upper extent of these grain sizes because previous work has demonstrated significant effects of large river source populations within this distance (Osborne and Wiley 1992).

I used a GIS methodology to standardize the classification of headwater and mainstem streams. To estimate stream volume downstream from sample sites, I calculated upstream cell counts (UCCs) from stream network raster data as a surrogate of upstream basin size and flow volume (C. D. Heatwole, Biological Systems Engineering, Virginia Tech, personal communication). First, I downloaded National Hydrological Data medium-resolution data (1:100,000 scale) and converted stream paths from vector to raster data (30-m^2 cells). Raw data are available at http://nhd.usgs.gov. Second, I used the flow network raster to calculate distances from each cell to the outlet pour-point of each watershed. Third, I converted cost-weighted distances into UCCs and then

combined inflows from confluent watersheds. I then sampled the UCC raster at EMAP sites and at every kilometer for 20 fkm downstream. All calculations were preformed in ARCGIS 9.1.

I compared the UCC data against independent measures of upstream basin area to validate UCCs as a surrogate for stream flow volume. I used simple linear regression to relate UCCs to upstream basin areas from EMAP calculations in sites where basin area data were available (n=198). Log-log plots revealed bivariate linearity and good fit (R^2=0.919, see Figure 2.3), suggesting that UCC data provide a reasonable surrogate of stream flow volume (Hack 1957). Based on this relationship, I used a UCC value of 5000 to indicate the presence a large river confluence (i.e., upstream basin area >250 km^2, *sensu* Osborne and Wiley 1992). Headwater and mainstem streams were not significantly different for most environmental variables (Appendices G and H), thereby permitting an analysis of stream network effects without confounding local factors.

Statistical analysis

I used ordination and randomization procedures to assess fish metric correspondence to environmental quality gradients and to test the role of stream networks in metric sensitivity. I used non-metric multidimensional scaling (NMS) to ordinate sites along environmental quality gradients and to reduce covariation among environmental variables (Mather 1976; Kruskal 1964; Minchin 1987). I chose to use NMS instead of principal components analysis (PCA) because NMS does not assume linear relationships among variables (McCune and Grace 2002) and has produced better representations of simulated ecological gradients than PCA (Fasham 1977). Prior to NMS ordinations, I

transformed all environmental variables to improve linearity and reduce heteroscedasticity ($\log_{10}x+1$ transformations for continuous variables and arcsine square-root transformations for percentage variables; Table 3.1). I used Bray-Curtis distances (Bray and Curtis 1957) for all ordinations in order to minimize the effects of outliers (McCune and Grace 2002). I used PC-ORD (version 5.0) for all NMS ordinations with 50 randomized starting configuration runs and an instability criterion of 0.00001.

To interpret the ordination results in terms of environmental quality gradients, I evaluated the correspondence of NMS scores and land use variables. I plotted land use variables as vectors in the ordination space and calculated nonparametric correlations (i.e., Spearman *rho*) to quantify the direction and magnitude of these relationships (Stewart *et al*. 2001). In subsequent analyses, I used NMS scores from the environmental ordination that showed interpretable relations with land use variation. I assumed that variation in watershed-level land use corresponded to variation in environmental quality at the site-level (Herlihy *et al*. 1998; Jones *et al*. 2001; Vondracek *et al*. 2005; Mugodo *et al*. 2006; Dow *et al*. 2006). I chose to partition land use variables from the environmental ordination to permit a direct analysis of land use – environment relationships.

I then used nonparametric correlations (Spearman *rho*) to evaluate fish metric correspondence to derived environmental quality gradients (i.e., NMS scores). I used nonparametric methods for this analysis because exploratory analyses revealed non-normality in metric data across sites (i.e., all Shapiro-Wilk tests, $p>0.05$). To reduce metric colinearity for subsequent analyses, I selected surrogate metrics as those with the greatest correlation coefficient magnitude from significant metrics that measured intrinsically related quantities (e.g., salmonid richness, abundance, and proportional

abundance). Recent studies have used this ordination-correlation approach to assess community responses to environmental gradients (Paller 2002; Hering *et al*. 2006; Linke *et al.* 2005; Freeman and Marcinek 2006; Lamoroux *et al*. 2006). Although stream fish metrics show important variation among ecoregions and basins in the mid-Atlantic highland streams (e.g., Angermeier *et al*. 2000), I analyzed fish metric variability among all ecoregions and basins simultaneously to facilitate tests of stream network effects and because previous analysis demonstrated that fish relations to stream network structure were not explained by zoogeographic regions or ecoregions (see Figure 2.5).

I developed a Mantel path analysis model (Mantel 1967) to visualize the relationships among environmental variables and fish assemblage structure (King *et al*. 2005). Mantel tests are distance-based matrix correlations that use permutation procedures to calculate the probability that observed correlations were random. Accordingly, these tests are appropriate for assessing correspondence in variables from unknown statistical distributions (Mantel 1967). I summarized environmental variables in categories of land use, mesohabitat structure, periphyton biomass, riparian vegetation, substrate size, stream volume, water quality, and woody debris (Table 3.1). I also characterized spatial autocorrelation among sites using Universal Transverse Mercator coordinates. I used Euclidean distances for all environmental variables and Bray-Curtis distances (Bray and Curtis 1957) to characterize fish assemblage structure from a matrix of sites x species-level abundances (157 sites x 119 species). I used the R programming language with the ECODIST library (Goslee and Urban 2006) to calculate distance matrices and perform all Mantel tests.

I then tested the role of stream network topology by comparing fish assemblage responses to environmental quality gradients among headwater and mainstem sites at multiple distances (i.e., spatial grain sizes). First, I used Mantel tests to assess assemblage-level correspondence to environmental and land use gradients. I predicted that assemblages at mainstem streams would show less correspondence to gradients of local environmental quality than headwater streams if dispersal from downstream areas influences local (i.e., site-level) fish assemblage structure. I used 10,000 Monte Carlo randomization iterations and 1000 bootstrap resamples to generate 95% confidence intervals from Mantel test results. Second, I calculated environmental NMS ordinations for headwater and mainstem tributary sites at 5-, 10-, 15-, and 20-fkm distance classes (see Appendix I). I chose to calculate NMS scores for headwater and mainstem tributaries separately in order to standardize environmental gradients among these site types. I then calculated non-parametric correlations (i.e., Spearman *rho*) between NMS scores and fish metrics to test the prediction that assemblages at mainstem streams would show less correspondence to local environmental variability than assemblages at headwater streams. For this analysis, I included only fish metrics that showed significant correspondence to NMS scores across all sites (n=157).

RESULTS

Environmental quality ordination

A two-dimensional NMS ordination separated stream sites based on mesohabitat, periphyton biomass, riparian vegetation, substrate size, water quality, and woody debris (Table 3.3). NMS axis I primarily represented variation among sites in riparian vegetation, substrate size, and woody debris (i.e., variable loadings >|0.10|, Table 3.3). Riparian vegetation was positively associated with increased woody debris and negatively associated with small substrates and embeddedness (Table 3.3). The second NMS axis primarily represented variation in water quality, periphyton biomass, and mesohabitat structure among sites (i.e., variable loadings >|0.10|, Table 3.3). Ionic concentrations (i.e. NH_4, Mg, Na, conductivity, sum of cations) were positively related to periphyton biomass (ash-free dry mass) and negatively related to the percent of the site with riffle habitat (Table 3.3).

Variation among sites in NH_4, percent sand and fine substrates (PCT_SAFN), and large woody debris (XFC_LWD) contributed most strongly to the ordination scores (i.e., variable loadings >|0.20|, Table 3.3). Conversely, several measures of stream volume (XWIDTH, AREA_WS, XDEPTH) and water quality (ALTD, DOC, NTL, and PHSTVL) contributed least to ordination scores (i.e., variable loadings <|0.05|, Table 3.3). Among environmental categories, riparian vegetation variables were most consistently related to the ordination scores (i.e., all variable loadings >|0.10|, Table 3.3). Conversely, measures of stream volume contributed least to the ordination in either axis (i.e., all variable loadings <|0.05|, Table 3.3).

Land use variability was significantly related to the environmental NMS ordination (Figure 3.3, Table 3.4). Non-parametric correlations revealed significant correspondence among all land use variables and NMS axes (i.e., all Spearman *rho* >0.35, all $p<0.0001$, Table 3.4). Percent agriculture (AG_TOT) and forest cover (FOR_TOT) represented opposite ends of a gradient along NMS axis I (Figure 3.3). Increasing agricultural cover (and corresponding decreasing forest cover) was related to less riparian vegetation and woody debris, smaller substrates, and greater embeddedness (Tables 3.3 and 3.4). Urbanization, human population density, and road density (URB_TOT, POPDENKM, and RD_DENKM) corresponded to a similar gradient along NMS axis II (Figure 3.3). These variables were positively related to increased cation concentrations, increased periphyton biomass, increased percent sand and fine substrates, and decreased percent of the sample reach with riffle mesohabitats (Tables 3.3 and 3.4).

Overall, land use intensity was negatively related to variation in NMS axis I and positively related to variation in NMS axis II (Figure 3.3, Table 3.4). The strong correspondence between land uses and NMS ordination axes permits the use of ordination scores as environmental quality gradients (i.e., inverse of "stressor gradients"). Moreover, the absence of strong correlations with stream volume in the ordination permits subsequent analysis without the confounding effects of stream size.

Fish assemblage and metric relations to environmental quality

Mantel path analysis revealed significant relationships between fish assemblage structure (Bray-Curtis distances) and environmental variables (Table 3.5, Figure 3.5). Variation in land use, mesohabitat structure, water quality, and stream volume was

positively related to variation in fish assemblage structure (Mantel $r>0.119$, $p<0.005$, respectively; Table 3.5). Substrate size and riparian condition were also significantly but less strongly related to fish assemblage structure (Mantel $r>0.073$, $p<0.05$, respectively; Table 3.5). A spatial component of fish assemblage structure was also evident from significant positive spatial autocorrelation (Mantel $r=0.140$, $p<0.005$, Table 3.5). Of all variables, stream volume showed the strongest correspondence with fish assemblage structure (Mantel $r=0.265$, $p<0.005$, Table 3.5). Woody debris and periphyton were not significantly related to fish assemblage structure in this analysis (Mantel $r<|0.05|$, $p>0.10$, Table 3.5).

Taxonomic and functional fish metrics corresponded to environmental quality gradients as represented by NMS ordination scores (Appendix F). Among taxonomic metrics, the proportion of cottid individuals and salmonid species richness and abundance corresponded negatively to environmental degradation gradients (Spearman *rho* > |0.180|, $p<0.05$, respectively). However, species richness and abundance of percids, catostomids, and centrarchids responded positively to increasing degradation (Spearman *rho* > |0.158|, $p<0.05$, respectively). Excluding "tolerant" species from cyprinid metrics (*sensu* McCormick *et al*. 2001) had a negligible effect on cyprinid responses to environmental quality gradients. Native and non-native species richness and abundance were not significantly related to NMS scores (Appendix F).

Several trophic metrics were associated with environmental quality gradients (Appendix F). Invertivore-piscivore proportional abundances decreased with increasing degradation (Spearman *rho* = 0.233, $p<0.005$,). Removing *Semotilus atromaculatus* from this metric (*sensu* McCormick *et al*. 2001) provided marginally increased correspondence

with the environmental quality gradient (Spearman *rho* = 0.250, p<0.005). Conversely, the species richness and abundance of omnivore-herbivores were positively related to increasing degradation (Spearman *rho* > |0.162|, p<0.05, respectively). The abundance of water-column generalist feeders (>2 food types, Smogor and Angermeier 1999a) increased with increasing degradation (Spearman *rho* > |0.174|, p<0.05, respectively), as did the species richness and abundance of piscivorous specialists (Spearman *rho* > |0.158|, p<0.05, respectively).

Reproductive metrics showed consistent associations with environmental quality (Appendix F). Increasing degradation in NMS axis II was negatively associated with the proportion of simple lithophils (Spearman *rho* = |0.181|, p<0.05) and positively associated with the richness and abundance of non-lithophils (Spearman *rho* > |0.174|, p<0.05, respectively). Mean reproductive age was negatively associated with increasing degradation in NMS axis II (Spearman *rho* = |0.171|, p<0.05). However, metrics characterizing the species richness and abundance of early and late spawning species were not significantly related to NMS scores. Excluding "tolerant" species from reproductive metrics (*sensu* Angermeier *et al*. 2000) did not significantly improve metric relations to environmental quality gradients (Appendix F).

Of all fish metric categories, tolerance metrics showed the strongest correspondence to environmental quality gradients (Appendix F). As predicted, tolerant species metrics increased with degradation (Spearman *rho* > |.192|, p<0.05) and intolerant species metrics decreased with degradation (Spearman *rho* > |.251|, p<0.005). However, intolerant species metrics showed stronger associations with environmental quality than tolerant species metrics (i.e., see correlation coefficient magnitudes in Appendix F and

vector lengths in Figure 3.4). Of all fish metrics in this analysis, intolerant species proportional abundance (INTOL_P) showed the strongest association with environmental quality gradients (Appendix F).

Overall, 10 taxonomic and functional metrics were significantly related to environmental quality gradients and conformed with *a priori* predictions about the directionality of these relationships (Tables 3.2 and 3.6). Among taxonomic metrics, the proportional abundances of cottid and salmonid fishes showed the strongest relations to environmental quality (COT_P, SAL_P, respectively). Among trophic metrics, the proportional abundance of invertivore-piscivores (excluding *S. atromaculatus*), proportional abundance of omnivore-herbivores, and the abundance of water-column generalist feeders (IP2_P, OH_P, GEN_COL_N, respectively) showed the strongest relations to environmental quality (Table 3.6). Among reproductive metrics, I observed the strongest patterns in the proportional abundance of simple lithophils, non-lithophil abundances, and mean reproductive age of the assemblage (SL_P, NL_S, REPROAGE, respectively).

Plots of fish metric vectors in NMS ordination space revealed the directionality of metric responses to environmental quality gradients and patterns of intercorrelation among metrics (Figure 3.4). Five of the six most significant fish metrics (i.e., Spearman *rho*, $p<0.005$, Table 3.6) were associated most strongly with variation in NMS axis I, corresponding to a land use gradient of forest and agricultural land cover (Table 3.4; Figure 3.3). Specifically, the vector of intolerant species proportional abundance (INTOL_P) corresponded closely with the proportion of salmonid proportional abundance (SAL_P) as well as invertivore-piscivore proportional abundance (IP2_P)

along a gradient of increasing forest cover (Figure 3.4; see Figure 3.3 and Table 3.4 for land use relations). Conversely, the vector of omnivore-herbivore proportional abundance (OH_P) corresponded closely with tolerant species abundance (TOL_N) along a gradient of increasing agricultural land cover (Figure 3.4; see Figure 3.3 and Table 3.4 for land use relations). In contrast, the vector of non-lithophil species richness (NL_S) represented significant variation in NMS axis II, characterized by increasing urbanization and agricultural land cover (Figure 3.4; see Figure 3.3 and Table 3.4 for land use relations). The observed relations between fish metrics and environmental quality suggest that these metrics will be useful for stream fish bioassessments. In the subsequent section, I evaluated how the sensitivity of these metrics varies with a site's spatial position within stream networks.

Effects of stream network topology

The effects of local environmental conditions on fish assemblage structure and bioassessment metrics varied significantly as a function of site position within stream networks. In comparisons among all sites, fish assemblage structure (i.e., Bray-Curtis distances) was significantly related to variation in land use, riparian vegetation, stream volume, mesohabitat structure, and water quality (Figure 3.5; Table 3.5). However, these effects were not randomly distributed between headwater tributaries and mainstem tributaries. When classified at a distance of 5 fkm, headwater tributaries showed stronger relations to land use, stream volume, riparian vegetation, mesohabitat structure, and water quality than mainstem streams (Figure 3.6). Moreover, land use, riparian vegetation, mesohabitat structure, and water quality were significantly related to fish assemblages in

headwater tributaries but not mainstem tributaries defined at 5 fkm (i.e., Mantel r values with $p<0.05$ and $p>0.05$, respectively; Figure 3.6). Stream volume and substrate size were significantly related to fish assemblages in headwater and mainstem tributaries but stream volume was affected by spatial position whereas substrate size was not (Figure 3.6). Overall, fish assemblage relations to stream volume and riparian vegetation showed the greatest differences between headwater tributaries and mainstem tributaries at 5 fkm (i.e., non-overlapping 95% confidence intervals; Figure 3.6).

Classifications of headwater and mainstem tributaries at distances greater than 5 fkm yielded variable patterns of environmental relations to fish assemblage structure. At 10, 15, and 20 fkm, fish assemblage relations to stream volume followed the pattern observed at 5 fkm (i.e., Mantel r for headwater tributaries > mainstem tributaries; Figure 3.6). Variation in water quality maintained the 5-fkm pattern at 10 and 15 fkm but not at 20 fkm (Figure 3.6). Conversely, riparian vegetation and substrate size showed stronger relations to fish assemblage structure in mainstem streams, rather than headwater streams defined at 10, 15, and 20 fkm (Figure 3.6). As observed among all sites (Table 3.5, Figure 3.5), periphyton biomass and woody debris were not significantly related to fish assemblage structure at any distance or stream type category (Figure 3.6).

Several fish metrics showed distinct relations to environmental quality gradients among headwater and mainstem tributaries. NMS ordinations of environmental variables revealed 2-dimensional environmental quality gradients among headwater and mainstem tributaries at 5, 10, 15, and 20 fkm (Appendix I). When classified at a distance of 5 fkm, headwater tributaries showed stronger correspondence among fish metrics and environmental gradients than observed among mainstem tributaries: invertivore-piscivore

proportional abundance (IP2_P), water-column generalist feeder abundance (GEN_COL_N), tolerant species abundance (TOL_N), and mean reproductive age (REPROAGE) were significantly related to environmental variation in headwater sites but not mainstem sites (Table 3.7). At 10 fkm, omnivore-herbivore proportional abundance (OH_P) and tolerant species abundance (TOL_N) were significantly related to environmental variation among headwater streams but not mainstem tributaries (Table 3.7).

However, when classified at distances greater than 10 fkm, the pattern reversed. At 15 fkm, variation in mean reproductive age (REPROAGE) was significantly related to environmental variation in mainstem tributaries but not in headwater tributaries. Similarly, at 20 fkm, cottid proportional abundance (COT_P), invertivore-piscivore proportional abundance (IP2_P), and non-lithophil richness (NL_S) were significantly related to environmental variation in mainstem sites but not in headwater sites. Overall, the direction of fish metric relations to NMS scores among headwater and mainstem tributaries was consistent with patterns observed in analyses of all sites together (n=157; Table 3.6).

DISCUSSION

Environmental quality ordination

Analysis of candidate bioassessment metrics requires the development of environmental quality gradients against which metric performance may be quantified

(Karr *et al*. 1986). Three lines of evidence suggest that the NMS ordination in the current study revealed ecologically significant variation in environmental quality. First, NMS axis scores were significantly related to land use patterns (Table 3.4, Figure 3.3). Second, environmental variable relations to NMS axes (i.e., loading coefficients) were consistent with previous studies of land use effects on stream ecosystems. Third, the observed range of variation in environmental and land use variables was consistent with previous studies that captured important differences in site quality within the study area.

NMS axes represented gradients in land use intensity among EMAP sites (Table 3.4; Figure 3.3). Increasing agricultural and urban land use was associated with smaller substrates, increased nutrient and ionic concentrations, and diminished riparian vegetation and in-stream woody debris. Mantel test results also detected significant correspondence among land uses and environmental variation in water quality and substrate size (Table 3.5; Figure 3.5). Similar patterns of sedimentation and nutrient loading have been reported from urbanized watersheds (e.g., Walters *et al*. 2003; Freeman and Schorr 2004; Clinton and Vose 2006) and agricultural watersheds (e.g., Wang *et al*. 1997; Sutherland *et al*. 2002; Snyder *et al*. 2003; Dow *et al*. 2006).

The observed environmental gradients are consistent with previous studies of the mid-Atlantic highland streams. Herlihy *et al*. (1998) and Bryce *et al*. (1999) identified gradients in ionic strength and nutrient concentrations and demonstrated that these gradients were related to urban and agricultural land use intensity. Yuan and Norton (2003) concluded that conductivity and chloride are useful surrogates of land use intensity in the study area. I also found that conductivity and chloride were strongly related to the final ordination gradient (i.e., NMS axis II loadings, Table 3.3). However,

my analysis revealed a more significant influence of ammonium ion concentrations than in Bryce *et al*. (1999) and a less significant influence of pH than in Yuan and Norton (2003). Although the current sample sites did not include many extensively urbanized watersheds (only 10 sites [6%] contained more than 7% urbanized watershed area, *sensu* Snyder *et al*. [2003]), the ordination method minimizes this potential problem by evaluating gradients instead of reference versus non-reference comparisons (Bailey *et al*. in press).

The absence of overriding effects of stream volume on local environmental conditions (Tables 3.3 and 3.5; Figure 3.5) permitted the subsequent analysis of fish metrics and environmental quality gradients (see Fausch *et al*. 1984). Of seven environmental variable categories (Table 3.1), only riparian vegetation was significantly related to stream volume (Mantel $r = 0.120$, $p < 0.01$; Table 3.5). However, stream volume variables were weakly related to NMS axes (i.e., variable loadings $< |0.05|$) whereas riparian vegetation variables showed consistently stronger relations to NMS axis I (i.e., variable loadings $> |.10|$; Table 3.3). The absence of stream volume effects was surprising, given the importance of longitudinal environmental gradients in streams (Vannote *et al*. 1980; Hughes and Gammon 1987; Rahel and Hubert 1991; Wright and Li 2002). I excluded EMAP sites >20 m in mean stream width to avoid fish sampling bias (see Cyterski and Barbor 2006) which limited the range of stream volumes measured. However, the stream sites in this analysis showed significant variability in mean width (0.4-20.8 m), mean depth (1.6-75.2 cm), and upstream basin areas (16-21,588 ha), suggesting that the absence of stream volume effects cannot be explained simply by the absence of stream size variability among sites.

Fish assemblage and metric responses to environmental quality

Fish metric relations to environmental quality (Table 3.6) were generally consistent with *a priori* expectations (Table 3.2). I detected significant effects of environmental quality on taxonomic metrics (cottid proportional abundance, COT_P), trophic metrics (invertivore-piscivore proportional abundance, IP2_P; omnivore-herbivore proportional abundance, OH_P), reproductive metrics (simple lithophil proportional abundance, SL_P), and tolerance metrics (proportional abundance of intolerant fishes, INTOL_P; total abundance of tolerant fishes, TOL_N), consistent with previous studies of the mid-Atlantic highlands region (Angermeier *et al*. 2000; McCormick *et al*. 2001). The general correspondence with McCormick *et al*. (2001) is expected because their study used a subset of the EMAP sites in the current analysis (i.e., 69 of the 157 sites [44%]). However, because I evaluated "new" EMAP sites and used different methods to characterize stream quality, my results provided confirmatory evidence for fish bioassessment metric performance within the mid-Atlantic highlands.

My analysis also provided new inferences about fish metric relations to environmental quality. I found that the mean reproductive age of fish assemblages (REPROAGE) tended to decrease with increasing degradation (Table 3.6), consistent with the hypothesis that harsh environmental conditions favor fish species with early reproductive ages (Winemiller and Rose 1992). Schlosser (1990) reported that fish species associated with small streams tended to exhibit earlier reproductive ages than fish species associated with large riverine habitats. However, I cannot explain the observed patterns in mean reproductive age as being due to longitudinal variation because the

environmental quality gradient (i.e., NMS ordination) was not significantly explained by stream volume (Table 3.3). Smogor and Angermeier (1999b) found that species richness of late-maturing fishes (i.e., age >2 years) increased with stream quality in western Virginia, but previous studies have not evaluated the mean reproductive age at the assemblage-level. My results suggest that mean reproductive age in fish assemblages may be useful for assessing environmental quality.

My results also revealed new insights about fish assemblage responses to environmental quality in the study area. I found that fish assemblage structure (Bray-Curtis distances for species abundances) was significantly related to land use, substrate size, water quality, and riparian cover (Table 3.5). Previous studies have developed distance-based multivariate approaches for stream fish bioassessment (e.g., Linke *et al*. 2005) but these methods have not been applied in the mid-Atlantic highlands. My results suggest the feasibility of such applications within the study area. However, the analysis of species x abundance matrices can only weakly indicate functional responses to environmental quality because all species are treated equally (and therefore functionally-different species will be considered as different as functionally-similar species). For this reason, fish assemblage structure in the current study is expected to be less responsive to environmental quality than some metrics. For example, periphyton biomass was not significantly related to fish assemblage structure (Table 3.5; Figure 3.5) but did contribute to the NMS ordinations that explained spatial variation in fish metrics (Table 3.6), suggesting direct or indirect effects of periphyton on fishes. Although fish bioassessment studies commonly utilize either distance-based or metric-based

multivariate analyses, my results suggest that such studies may benefit from simultaneous analyses.

Overall, taxonomic metrics generally did not conform to predicted relations with environmental quality. Of the 33 taxonomic metrics, only four (12%) were related to the environmental gradient in the expected direction whereas nine (27%) showed significant relations to the environmental gradient in the opposite direction (Appendix F). Contrary to expectations, percid, centrarchid, and catostomid fishes increased in species richness and abundance with increasing degradation as reflected by the NMS axes. Moreover, previous studies have detected significant responses to environmental quality for metrics characterizing fish species richness and non-native fishes (Angermeier *et al*. 2000; Wang *et al*. 2001; Hughes *et al*. 2004; Bramblett *et al*. 2005; Kennard *et al*. 2005) but these metrics were not significant in the current study. These observations suggest two possibilities: (1) the NMS gradient did not reflect extreme gradients in environmental quality that would be necessary to see predicted taxonomic effects, or (2) the conceptual models of fish metric responses to degradation (see Table 3.2; Appendix F) are flawed. If the latter scenario is true, the observed "misbehaving" metrics could be useful for bioassessments if conceptual models could relate site quality and metric responses.

The generally weak environmental relations with taxonomic fish metrics in my study may also reflect unmeasured ecoregion and basin effects. For example, Angermeier *et al*. (2000) showed that cyprinid species richness (CYP_S) and darter or sculpin species richness (DOS_S) were significantly related to environmental quality in the mid-Atlantic highlands, but that the strength of these relations varied across ecoregions and basins. These metrics were not significant in the current analysis and

may have been obscured through combining sites across ecoregion and basins. Furthermore, Stancil (2000) found significant environmental relations to catostomid species richness (CAT_S) whereas I did not. This discrepancy may also be due to zoogeographic effects: Stancil's (2000) study area encompassed the upper Roanoke River basin which supports high numbers of native catostomids relative to the rest of the mid-Atlantic highlands region (Jenkins and Burkhead 1994). Moreover, the unexpected direction of relations between taxonomic metrics and environmental quality supports Angermeier *et al*.'s (2000) conclusion that different regions require empirical testing of candidate fish metrics before combining them into a multi-metric index.

In contrast, functional metrics showed more consistent relations to environmental quality gradients, suggesting that fish metric responses to stressor gradients may transcend zoogeographic and physiographic regions. Such patterns may permit applications of fish metrics over large spatial extents. Tolerance metrics exhibited the most promising results in this regard. I found that each of the six metrics characterizing fish tolerance was significantly related to the environmental gradient in the expected direction. These results support previous conclusions that tolerance metrics respond predictably to environmental stressor gradients (Karr 1981; Stancil 2000; Roth *et al*. 2000, Fitzpatrick *et al*. 2001; Wang *et al*. 2001; Snyder *et al*. 2003; Angermeier and Davideanu 2004; Hughes *et al*. 2004; Bramblett *et al*. 2005; Habit *et al*. 2006). Moreover, fish tolerance metrics have responded consistently to stressor gradients across very large spatial extents in France (Oberdorff *et al*. 2001) and western Europe (Pont *et al*. 2006). The utility of tolerance metrics therefore may transcend physiographic and zoogeographic boundaries.

Reproductive metrics also showed consistent patterns of correspondence to the stressor gradients, suggesting some functional convergences in reproductive responses to degradation. Of the 18 reproductive metrics, six were significantly related to the NMS gradients ($p<0.05$) and corresponded to environmental conditions in the expected direction. The response of simple lithophils (SL_P) to stressor gradients was consistent with previous studies of central and southern Appalachian mountain streams (Smogor and Angermeier 1999b; Roth *et al*. 2000; Stancil 2000; Sutherland *et al*. 2002). Conversely, I found that non-lithophilic spawners were positively related to stressor gradients, consistent with Pont *et al*.'s (2006) fish bioassessment study of western Europe.

Trophic metrics showed mixed responses to stressor gradients. Seven of 19 trophic metrics (37%) were significantly correlated with degradation gradients as expected, but three metrics (16%) showed significant responses in the unexpected direction (Appendix F). The proportional abundance of omnivore-herbivores (OH_P) and generalist water-column feeders (GEN_COL_N) increased with increasing percent agriculture in the watershed as observed in other studies (Stancil 2000; McCormick *et al*. 2001; Pont *et al*. 2006; but see Leonard and Orth 1986). In contrast, the proportional abundance of invertivore-piscivores (IP2_P) decreased with increasing percent agriculture in the watershed and increased with increased forest cover in the watershed. Removing *Semotilus atromaculatus* increased the correspondence with environmental gradients (i.e., see IP_P vs. IP2_P, Appendix F). This finding supports Leonard and Orth's (1986) conclusion that *S. atromaculatus* abundance indicates site degradation and that this species should be classified as "tolerant" (Barbour *et al*. 1999; Smogor and

Angermeier 1999a; McCormick *et al*. 2001). In contrast to my predictions, invertivore metrics were not significantly related to site quality (e.g., Roth *et al*. 2000; Pont *et al*. 2006). Welcomme *et al*. (2006) recognized the complications of trophic fish metrics due to ontogenetic shifts in feeding as well as opportunism in most species.

Fish metrics characterizing vertical position within the water column (i.e., benthic or pelagic) were not related to stressor gradients, but information about vertical position influenced trophic metric responses. In contrast to previous studies within the mid-Atlantic highlands, benthic species richness (BEN_S) was not significantly related to stressor gradients (Roth *et al*. 2000; Stancil 2000; McCormick *et al*. 2001). The absence of an effect in the current study may be explained by diverse patterns of family-level responses to stressors (e.g., cottids and percids). However, generalist water-column feeders (GEN_COL_N) increased with stressor gradients, consistent with Angermeier *et al*. (2000). It is possible that zoogeographic or physiographic effects obscured the relations of "pure" vertical position metrics. However, Pont *et al*. (2006) found significant stressor responses in benthic species richness across several zoogeographic and physiographic regions in western Europe.

Effects of stream network topology

The spatial position of streams within watershed networks is known to affect fish assemblage structure (Whiteside and McNatt 1972; Gorman 1986; Osborne and Wiley 1992; Wilkinson and Edds 2001; Shaeffer and Kerfoot 2004; Hitt and Angermeier 2006) presumably as a function of fish dispersal or opportunistic movements to use resources. Osborne *et al*. (1992) showed that these differences in fish assemblage structure among

headwater and mainstem streams affects fish IBI scores. However, their study lacked environmental data and therefore could not test the competing hypothesis that local environmental factors regulate fish assemblage structure (Osborne *et al*. 1992). Further, no previous studies have explored the effects of stream network topology on fish metric sensitivity to stressor gradients. I predicted that, if fish dispersal from riverine source areas influences local fish assemblage structure, fish metrics in streams directly connected to mainstems would be less responsive to local environmental conditions than similar fish metrics in headwater streams. I found strong evidence to support this prediction within 5 fkm of study sites and inconclusive support at greater distances.

Results from assemblage-level and metric-level analyses were concordant for headwater and mainstem streams defined at 5 fkm. Mantel tests revealed that fish assemblage structure (i.e., Bray-Curtis distances) in mainstem tributaries corresponded less strongly to local environmental conditions than assemblage structure in headwater tributaries, as predicted. NMS ordinations further revealed metric-level responses in the predicted direction. Although the Mantel tests and NMS ordinations evaluated different aspects of fish-environment relations, their concordant results at 5 fkm are consistent with the hypothesis that fish dispersal from riverine source areas may influence fish bioassessment metrics within this spatial grain. Long-distance movements (> 1 fkm) of inland freshwater fishes have been reported previously (Karr and Gorman 1975; Gatz and Adams 1994; Albanese *et al*. 2004; Popoff and Neumann 2005; Roghair and Dolloff 2005), suggesting that dispersal is possible at this spatial grain.

Metric-level patterns were generally consistent with analyses from previous chapters. In Chapter 1, I found that mean reproductive age tended to be lower in sites

connected to large streams (i.e., > 4th Strahler order) within 3 fluvial km (Hitt and Angermeier 2006). In this chapter, I found that the sensitivity of this metric varied among headwater and mainstem streams, which further supports the hypothesis that early-maturing fishes may disperse from riverine source areas (Gorman 1986). In Chapter 2, I found that catostomids, cyprinids, and centrarchids were significantly related to variation in stream network structure whereas other families were not. In this chapter, I found that metrics dominated by centrarchids (i.e., invertivore/piscivores) and cyprinids (i.e., generalist water-column feeders) varied in their sensitivity to environmental stressors between headwater and mainstem tributaries. Conversely, the sensitivity of cottid and salmonid fish metrics did not vary as a function of stream network topology, which is also consistent with the spatial analysis in Chapter 2 (see Figure 2.6).

These findings have important implications for stream fish bioassessments in the study area. I found that four metrics were less sensitive to local environmental conditions in mainstem tributaries than in headwater tributaries: tolerant species abundance (TOL_N), invertivore-piscivore proportional abundance (IP2_P), generalist water-column feeder abundance (GEN_COL_N), and mean reproductive age (REPROAGE) (Table 3.7). Accordingly, the spatial location of fish bioassessment sample sites may affect the probability of detecting local degradation when using these metrics. Previous stream fish bioassessment studies in the mid-Atlantic highlands have recommended tolerant species metrics (Angermeier *et al*. 2000; Roth *et al*. 2000; McCormick *et al*. 2001) and invertivore-piscivore metrics (McCormick *et al*. 2001). The results of this chapter suggest that scoring criteria for several metrics may need to be developed for mainstem tributaries and headwater tributaries separately.

This study provides new insights about the relative importance of local and regional factors regulating stream fish assemblages. The river continuum concept (RCC) emphasizes the importance of longitudinal patterns of physical and biotic variation in streams (Vannote *et al*. 1980) and is recognized as one of the most important concepts in freshwater ecology (Resh and Zobzina 2003). In this analysis, stream volume was significantly related to fish assemblage structure for headwater and mainstem tributaries at all distance categories, consistent with predictions from the RCC and observations from other studies (Shelford 1911; Burton and Odum 1945; Sheldon 1968; Angermeier and Schlosser 1989; Goldstein and Meador 2004). However, I also detected significant differences in the relative importance of stream volume as a function of site spatial position: fish assemblage structure in headwater streams showed greater correspondence to stream volume than in mainstem tributaries. This result suggests that fishes in headwater tributaries may be more limited by local habitat size than fishes in mainstem tributaries due to opportunistic use of connected riverine habitats. These results would not be apparent from an analysis based solely on site-level data and therefore underscore the importance of network-based analysis of fish assemblage structure (Fagan 2002; Fausch *et al*. 2002; Ganio *et al*. 2005; Gresswell *et al*. 2006; Campbell-Grant *et al*. 2007).

I recognize several fundamental limitations in the current study. First, I chose to analyze fish metric responses to stressors over a large spatial extent encompassing several physiographic and zoogeographic regions. This analysis extent was necessary to test stream network effects, but may have obscured some of the fish-environment relations that would be apparent within smaller regions (e.g., Angermeier and Winston 1998). Second, I did not measure fish assemblages in mainstem rivers, but instead inferred

patterns of dispersal based on correspondence with spatial variation in stream size. Third, I assumed a space-for-time substitution in characterizing stressor gradients, but did not test this assumption with temporal data. However, the spatial patterns in degraded streams are well-known and were detected in streams associated with intensive agricultural and urban development.

Future research is necessary to test empirically the riverine dispersal patterns inferred from this study. The distribution of EMAP sites in this study presented a random sample of stream conditions throughout the mid-Atlantic highlands. However, sampling designs based on adjacent stream size and proximity could more precisely evaluate the potential effects of regional dispersal. For example, sampling adjacent streams in trellised-shaped watersheds (i.e., Ridge and Valley physiographic region) would permit a direct comparison of regional influences if sample sites represented a gradient in proximity to large rivers but were similar in local habitat conditions. Alternatively, adjacent streams flowing into reservoirs may provide a fragmentation "treatment" to examine the influences of regional dispersal. Such research could contribute not only to fish bioassessment development, but also to the design of freshwater conservation reserves based on landscape-scale criteria for connectivity (see Abell *et al*. 2007).

Table 3.1. Environmental variables used in the Chapter 3 analysis (n=157 sites). Raw data were collected by USEPA's Environmental Monitoring and Assessment Program (EMAP) during 1993-1998. I modified the periphyton raw data (ash free dry mass and chlorophyll *a*) following B. Hill (USEPA, personal communication). See text for details.

Category	Variable	Units	EMAP code	Transformation
Land use	Agricultural land cover	Proportion of watershed	AG_TOT	arcsine square-root
	Forest cover	Proportion of watershed	FOR_TOT	arcsine square-root
	Urban land cover	Proportion of watershed	URB_TOT	arcsine square-root
	Human population density	individuals/km^2	POPDENKM	$\log_{10}(x+1)$
	Road density	m/ha	RD_DEN	$\log_{10}(x+1)$
Mesohabitat	Riffle habitat	Proportion of sample reach area	PCT_FAST	arcsine square-root
	Pool habitat	Proportion of sample reach area	PCT_POOL	arcsine square-root
Periphyton	Ash-free dry mass	g/m^2	ADFM_M2	$\log_{10}(x+1)$
	Chlorophyll *a*	mg/m^2	CHL_M2	$\log_{10}(x+1)$
Riparian	Mean bank canopy density	Proportion of sample reach	XCDENBK	arcsine square-root
	Mean mid-channel canopy density	Proportion of sample reach	XCDENMID	arcsine square-root
	Canopy >0.3m DBH	Proportion of sample reach	XCL	arcsine square-root
	Ground cover, understory, and canopy present	Proportion of sample reach	XPCMG	arcsine square-root
Substrate	Substrate larger than coarse gravel (>16 mm diameter)	Proportion of sample reach	PCT_BIGR	arcsine square-root
	Sand and fines (<2 mm diameter)	Proportion of sample reach	PCT_SAFN	arcsine square-root

Table 3.1., continued. Environmental variables used in the Chapter 3 analysis (n=157 sites). Raw data were collected by USEPA's Environmental Monitoring and Assessment Program (EMAP) during 1993-1998. I modified the periphyton raw data (ash free dry mass and chlorophyll *a*) following B. Hill (USEPA, personal communication). See text for details.

	Mean embeddedness	Percent of substrate area	XEMBED	arcsine square-root
Volume	Mean stream width	m	XWIDTH	$\log_{10}(x+1)$
	Upstream watershed area	ha	AREA_WS	$\log_{10}(x+1)$
	Mean thalweg depth	cm	XDEPTH	$\log_{10}(x+1)$
Water quality	Total dissolved aluminum	µg/L	ALTD	$\log_{10}(x+1)$
	Calcium	µeg/L	CA	$\log_{10}(x+1)$
	Sum of cations	µeg/L	CATSUM	$\log_{10}(x+1)$
	Chloride	µeg/L	CL	$\log_{10}(x+1)$
	Conductivity	µS	COND	$\log_{10}(x+1)$
	Dissolved organic carbon	mg/L	DOC	$\log_{10}(x+1)$
	Potassium	µeg/L	K	$\log_{10}(x+1)$
	Magnesium	µeg/L	MG	$\log_{10}(x+1)$
	Sodium	µeg/L	NA	$\log_{10}(x+1)$
	Ammonium	µeg/L	NH4	$\log_{10}(x+1)$
	Nitrate	µeg/L	NO3	$\log_{10}(x+1)$
	Total nitrogen	µg/L	NTL	$\log_{10}(x+1)$
	pH		PHSTVL	$\log_{10}(x+1)$
	Total phosphorous	µg/L	PTL	$\log_{10}(x+1)$
	Sulfate	µeg/L	SO4	$\log_{10}(x+1)$
Woody debris	Brush cover	Proportion of sample reach	XFC_BRS	arcsine square-root
	Large woody debris	Proportion of sample reach	XFC_LWD	arcsine square-root

Table 3.2. Expected responses to degradation for stream fish bioassessment metrics. Superscripts denote the source for the expected responses: a=Angermeier *et al*. (2000); b=McCormick *et al*. (2001). Appendix F provides additional information about metrics evaluated in Chapter 3.

Category	Metric	Expected response to degradation
Taxonomic	Species richness	Negative[a]
	Non-native species richness	Positive[a,b]
	Cottid proportional abundance	Negative[b]
	Centrarchid species richness	Negative[a]
	Catostomid species richness	Negative[a]
	Cyprinid species richness	Negative[a,b]
	Darter or sculpin species richness	Negative[a]
Trophic	Invertivore proportional abundance	Negative[a,b]
	Invertivore benthic specialist proportional abundance	Negative[a]
	Piscivore proportional abundance	Negative[a]
	Generalist feeders, column species abundance	Positive[a]
	Generalist feeders, column species proportional abundance	Positive[a]
Reproductive	Simple lithophil proportional abundance, excluding tolerant species	Negative[a]
	Non-simple non-lithophil species proportional abundance	Positive[a]
	Late maturing species richness (age >2)	Negative[a]
Tolerance	Tolerant species proportional abundance	Positive[a,b]
	Intolerant species richness	Negative[b]
Vertical position	Benthic species richness, excluding tolerant species	Negative[b]

Table 3.3. Environmental variable loadings on non-metric multidimensional scaling (NMS) axes I and II. Environmental codes are given in Table 3.1. Bray-Curtis distances were used in NMS and the final instability of the ordination was 0.00001. The ordination is plotted in Figures 3.3 and 3.4. Land use variables are omitted to permit subsequent comparisons with NMS axes.

Category	Variable	NMS axis I	NMS axis II
Mesohabitat	PCT_FAST	0.041	-0.165
	PCT_POOL	-0.017	0.089
Periphyton	ADFM_M2	-0.046	0.103
	CHL_M2	-0.085	0.040
Riparian	XCDENBK	0.140	0.005
	XCDENMID	0.146	-0.012
	XCL	0.192	0.031
	XPCMG	0.179	0.020
Substrate	PCT_BIGR	0.110	-0.049
	PCT_SAFN	-0.200	0.116
	XEMBED	-0.110	0.091
Volume	XWIDTH	0.037	0.028
	AREA_WS	0.008	0.047
	XDEPTH	-0.018	0.016
Water quality	ALTD	0.033	-0.002
	CA	-0.066	0.116
	CATSUM	-0.057	0.102
	CL	-0.089	0.138
	COND	-0.078	0.139
	DOC	-0.049	0.048
	K	-0.043	0.088
	MG	-0.069	0.125
	NA	-0.056	0.127
	NH4	-0.176	0.271
	NO3	-0.104	0.092
	NTL	-0.047	0.045
	PHSTVL	-0.007	0.010
	PTL	-0.097	0.041
	SO4	-0.040	0.126
Woody debris	XFC_BRS	0.113	0.038
	XFC_LWD	0.208	0.087

Table 3.4. Spearman correlations among land use variables and non-metric multidimensional scaling (NMS) environmental axes. Land use codes are given in Table 3.1. All correlations were significant at $p<0.0001$.

Variable	NMS axis I	NMS axis II
AG_TOT	-0.566	0.410
FOR_TOT	0.565	-0.456
URB_TOT	-0.373	0.544
POPDENKM	-0.526	0.612
RD_DEN	-0.399	0.515

Table 3.5. Mantel correlations between fish assemblage (fish) and environmental variables (*p<0.05, **p<0.005). Variable relations are plotted in Figure 3.5. Mantel *r* values are presented above the diagonal. Corresponding p-values are presented below the diagonal. 10,000 resampling iterations were used to calculate p-values. See text for descriptions of variables and distance measures used in Mantel r calculations.

	Fish	**Space**	**Land use**	**Meso-habitat**	**Substrate**	**Water quality**	**Riparian**	**Woody debris**	**Peri-phyton**	**Volume**
Fish	–	0.140**	0.122**	0.119**	0.091*	0.149**	0.073*	-0.046	0.058	0.265**
Space	<0.0005	–	0.031	-0.074*	-0.021	-0.001	-0.002	0.013	-0.064*	0.000
Land use	0.001	0.285	–	-0.013	0.174**	0.487**	0.075	-0.005	0.046	0.059
Mesohabitat	0.001	0.010	0.752	–	0.236**	0.032	-0.044	0.045	0.116*	0.060
Substrate	0.014	0.487	<0.0005	<0.0005	–	0.112*	0.110*	0.071	0.124*	0.005
Water quality	<0.0005	0.985	<0.0005	0.409	0.012	–	0.043	-0.033	0.117**	0.022
Riparian	0.043	0.948	0.074	0.290	0.017	0.279	–	0.167**	0.002	0.120*
Woody debris	0.163	0.634	0.906	0.242	0.079	0.367	0.001	–	0.065	0.021
Periphyton	0.105	0.031	0.282	0.008	0.007	0.005	0.965	0.102	–	0.027
Volume	<0.0005	0.995	0.118	0.113	0.908	0.543	0.006	0.557	0.493	–

Table 3.6. Spearman correlations among selected fish metrics and non-metric multidimensional scaling (NMS) axes I and II (*p<0.05, **p<0.005). NMS axis I increased with environmental quality; NMS axis II decreased with environmental quality. NMS gradients are plotted in Figures 3.3 and 3.4 and coefficients are presented in Tables 3.3 and 3.3. Fish metric codes are presented in Appendix F. Fish metrics with p<0.005 are plotted in Figure 3.4.

Variable	NMS axis I	NMS axis II	Response to degradation
COT_P	0.182*	ns	Negative
SAL_P	0.345**	-0.248**	Negative
IP2_P	0.250**	ns	Negative
OH_P	-0.317**	0.165*	Positive
GEN_COL_N	-0.187*	ns	Positive
SL_P	ns	-0.181*	Negative
NL_S	ns	0.339**	Positive
TOL_N	-0.260**	ns	Positive
INTOL_P	0.292**	-0.303**	Negative
REPROAGE	ns	-0.171*	Negative

Table 3.7. Spearman correlations among selected fish metrics and environmental ordinations (non-metric multidimensional scaling [NMS] axes I and II) for stream network topology classes at multiple distances (*p<0.05, **p<0.005, ***p<0.0005). HT indicates "headwater tributaries" and MT indicates "mainstem tributaries." Fish metric codes are presented in Appendix F. Fish metrics were selected based on significant relations to environmental ordinations (Table 3.6). NMS axis variable loadings are presented in Appendix I. Correlation coefficients with p>0.05 are not shown.

		5		10		15		20	
Variable	Group	NMS axis I	NMS axis II	NMS axis I	NMS axis II	NMS axis I	NMS axis II	NMS axis I	NMS axis II
COT_P	HT		0.204**		0.248**	-0.270*			
	MT	0.306**			-0.200*	-0.193*		0.239**	
SAL_P	HT		0.368**		0.482**	-0.459**	-0.359**	0.529**	0.499**
	MT	0.352**			-0.266**	-0.274**		0.273**	
IP2_P	HT		0.197**		0.244**		-0.258*		
	MT				-0.390**		-0.316**		0.191**
OH_P	HT	-0.332**	-0.216**		-0.302**	0.231*	0.405**		-0.338*
	MT	-0.283**				0.203**		-0.210**	0.232**
GEN_COL_N	HT	-0.193**							
	MT								
SL_P	HT	-0.233**		-0.241**			0.429**		-0.372**
	MT		-0.317**	-0.218*			0.279**	0.149*	
NL_S	HT		-0.295**		-0.299**	0.296**			
	MT		0.410**	0.348**	-0.392**	0.213**	-0.455**	-0.272**	0.384**
TOL_N	HT	-0.272**	-0.218**		-0.289**	0.255*	0.231*		
	MT						0.166*		
INTOL_P	HT		0.329**		0.316**	-0.323**	-0.266*	0.330*	
	MT	0.437**	-0.276*	-0.363**	-0.246**	-0.345**		0.370**	
REPROAGE	HT		0.266**		0.189*			0.475**	
	MT				-0.248**	-0.220**		0.152*	0.173*

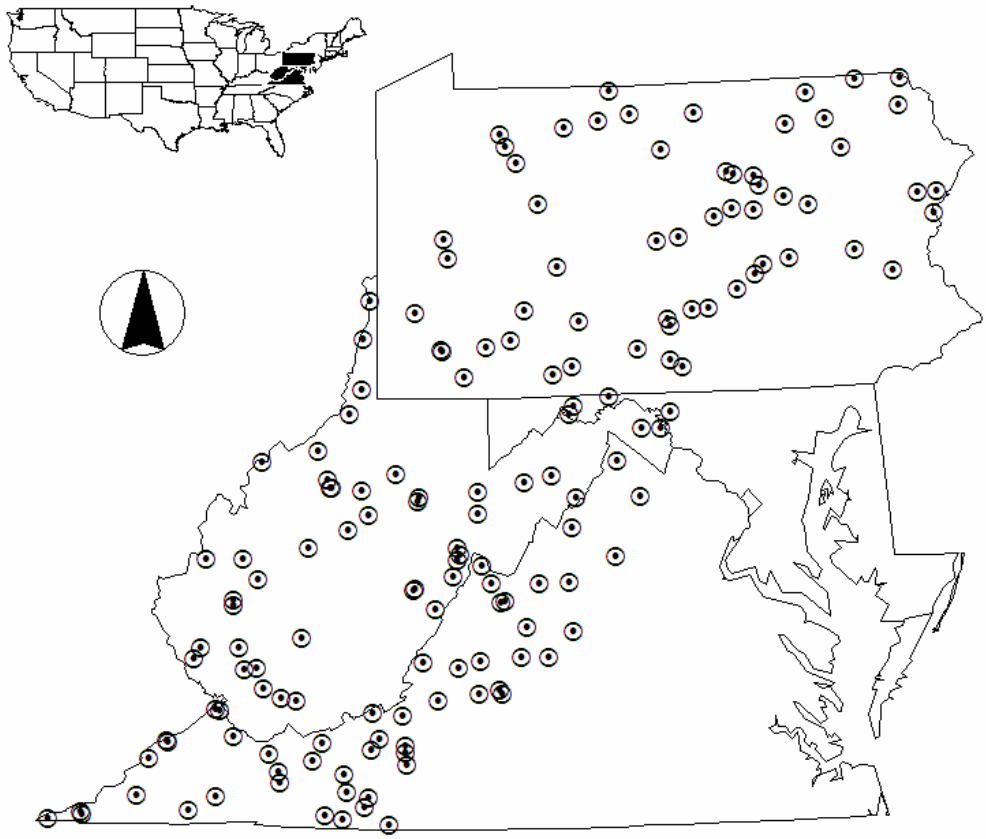

Figure 3.1. EMAP study site locations within the mid-Atlantic highlands region evaluated in Chapter 3 (n=157). Stream sites were located using a systematic-random methodology (Herlihy *et al*. 2000) and were represented by fish and environmental data (Table 3.1). These sites represent a subset of the entire EMAP database (see text).

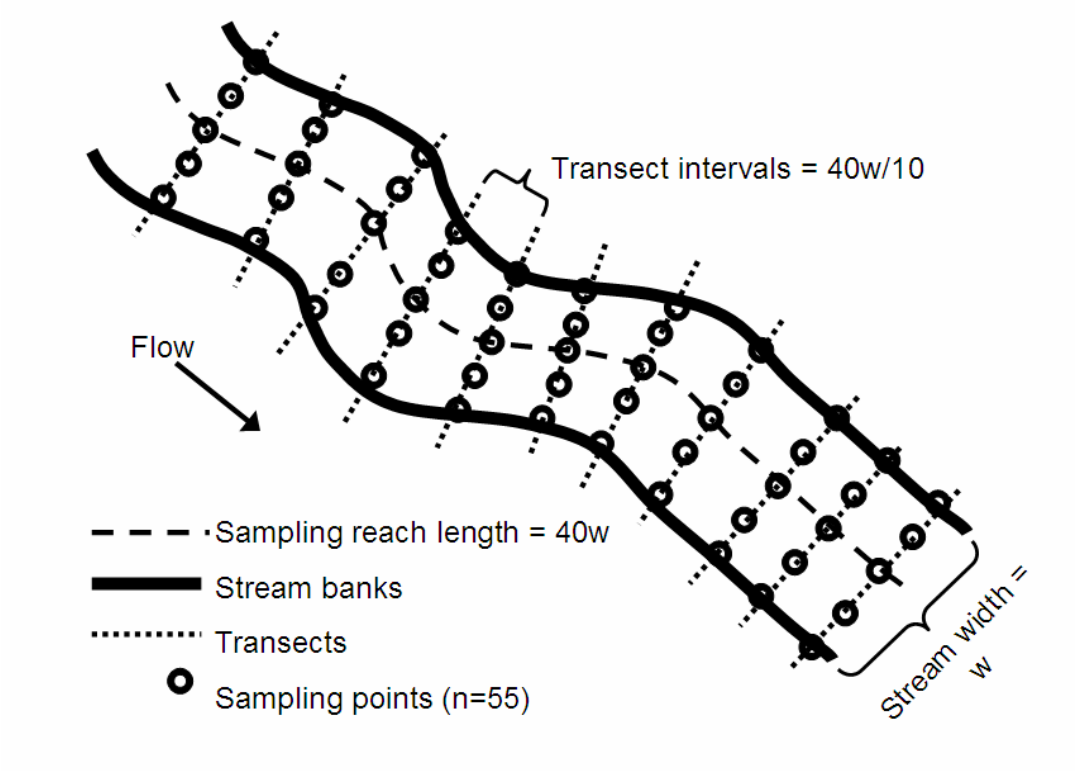

Figure 3.2. EMAP sampling design for physical habitat data. Sample reach lengths were calibrated to mean stream width of the site (see text).

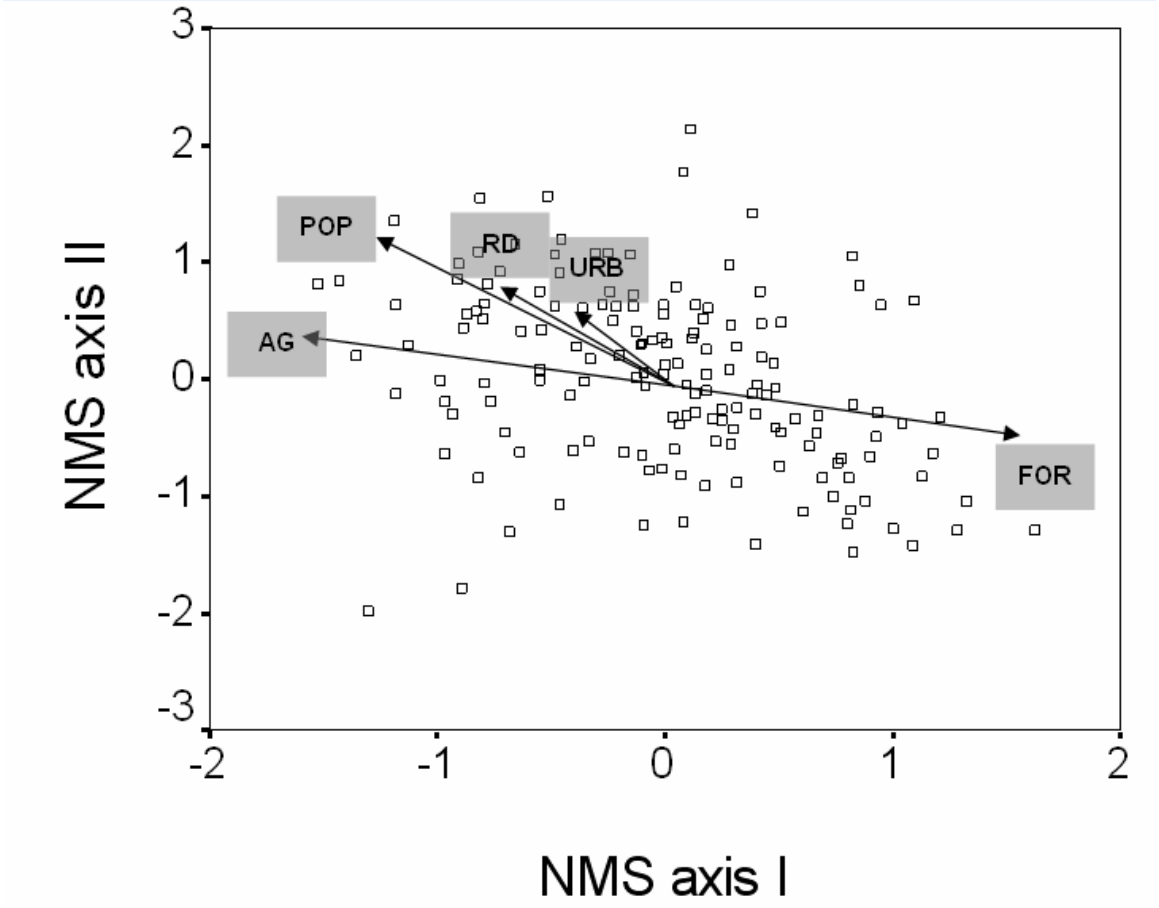

Figure 3.3. Land use relations to environmental non-metric multidimensional scaling (NMS) ordination. Vector length corresponds to the strength of land use relations to NMS axes. Each land use variable was significantly related to both NMS axes (Table 3.4). Environmental variable loadings in the NMS ordination are presented in Table 3.3.

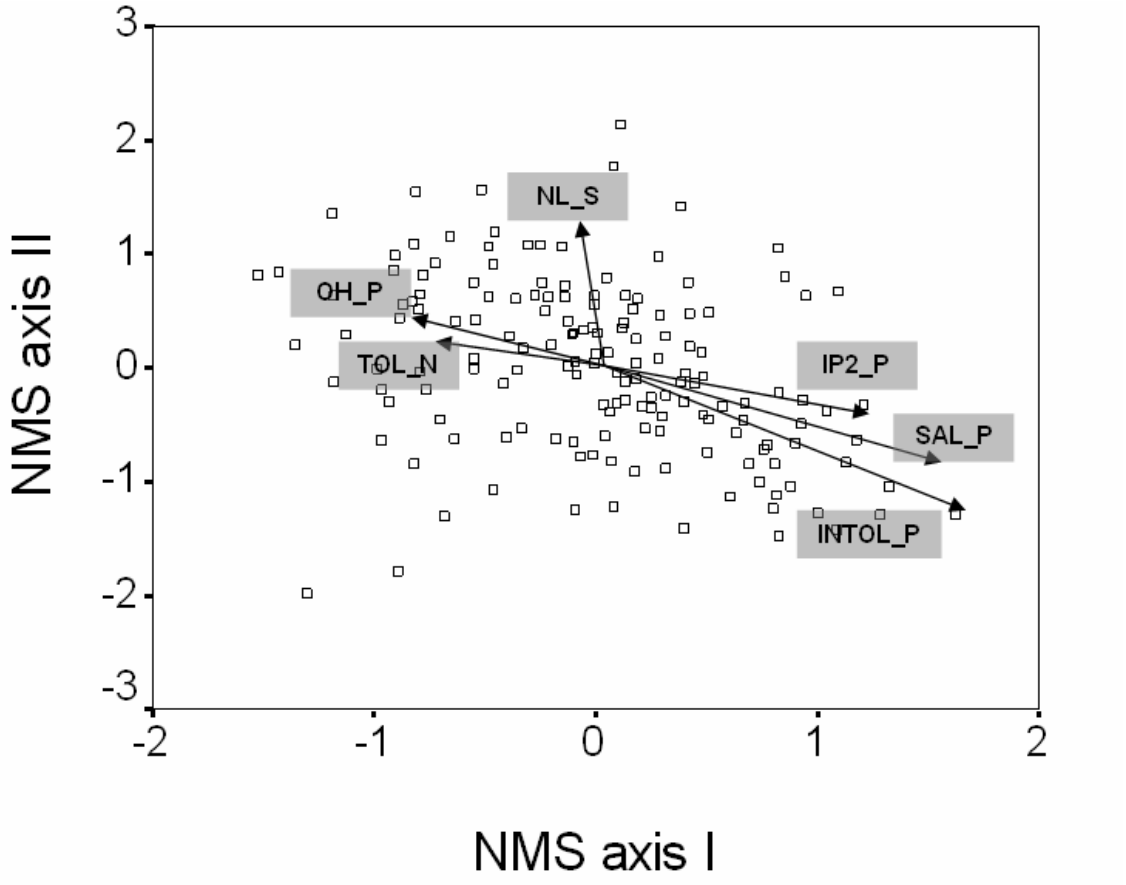

Figure 3.4. Fish metric relations to environmental non-metric multidimensional scaling (NMS) ordination. Vector length corresponds to the strength of fish metric relations to NMS axes. NMS axis I was positively related to site quality; NMS axis II was negatively related to site quality (Table 3.3). Fish metric codes are presented in Appendix F. For clarity, only fish metrics with significant Spearman correlations to NMS axes ($p<0.005$) are presented (see Table 3.6).

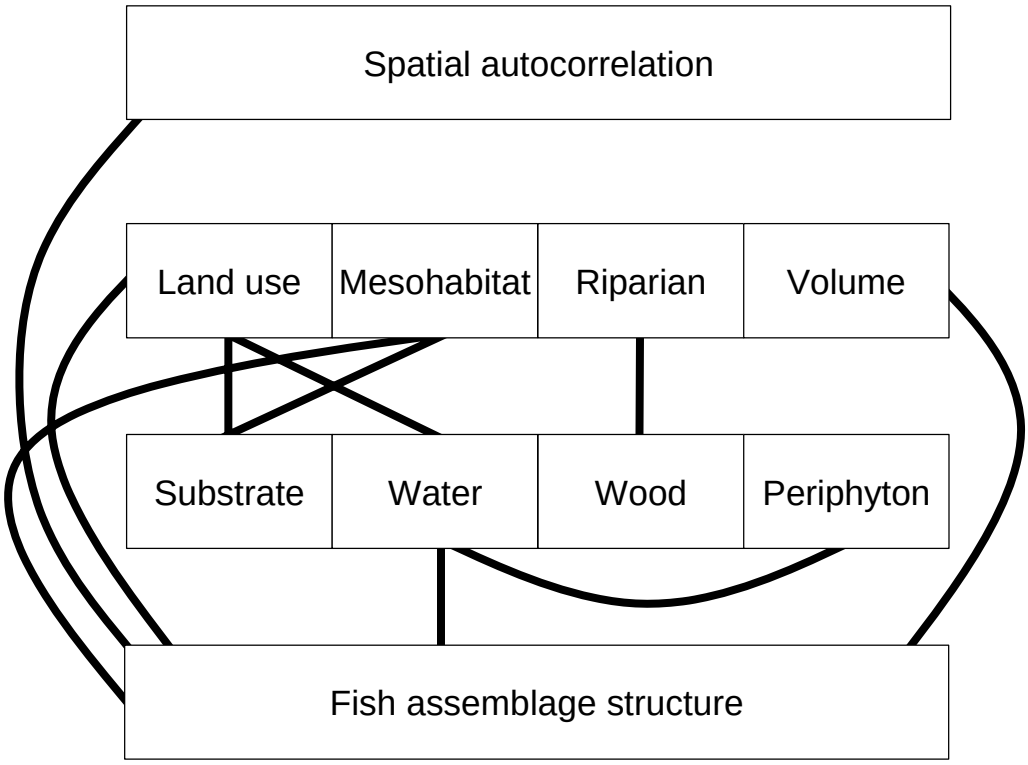

Figure 3.5. Mantel path analysis for fish assemblage structure and environmental factors. Vertical layers represent approximate spatial resolution of environmental variables (top = course-scale; bottom=fine-scale). Bray-Curtis distances were used to quantify fish assemblage structure. Euclidean distances were used for all other variables. Lines indicate Mantel r correlation coefficients with $p<0.005$. 10,000 resampling iterations were used to assess significance.

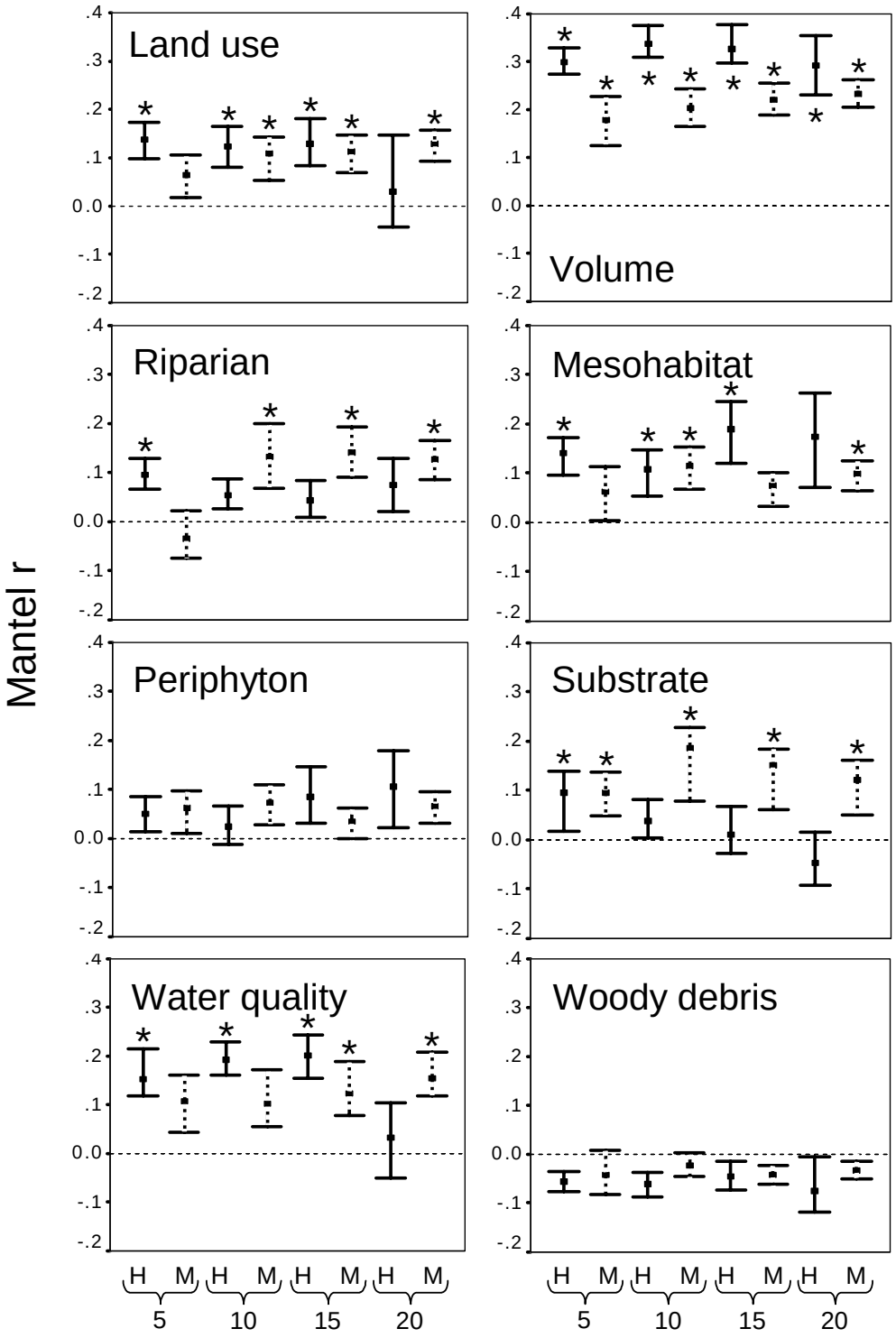

Figure 3.6. Mantel tests for fish assemblage structure and environmental factors as a function of stream network topology classes at increasing distances. H=headwater tributary (solid lines) and M=mainstem tributary (dashed lines) within fluvial distances from sites (fkm). Error bars indicate 95% confidence intervals. Stars indicate where Mantel r correlation coefficients are significantly different from zero ($p<0.05$). 10,000 resampling iterations were used to assess Mantel significance and 1000 bootstrap resamples were used to develop confidence intervals.

Chapter 4.

Contrasting fish and benthic macroinvertebrate relations to stream network topology in western Virginia, USA

INTRODUCTION

Freshwater ecosystems provide vital services for humans and critical habitat for aquatic species, yet many of these ecosystems have been severely degraded by human actions. A prerequisite for assessing or restoring freshwater ecosystems is the development of biological standards for measuring environmental quality, especially standards that pertain to viable populations and communities of native species. Biological assessment (bioassessment) methods provide a quantitative approach to this task by measuring biotic responses to degradation *in situ*. Freshwater bioassessment methods have been adopted by many governmental agencies worldwide (Karr and Chu 2000), including the U.S. Environmental Protection Agency (USEPA) and the Virginia Department of Environmental Quality (VDEQ).

A fundamental challenge in bioassessment is to understand the spatial scale at which biotic elements (organisms, populations, and assemblages) indicate environmental quality. Dispersal is a landscape-scale process that affects organismal distribution and abundance (Wiens 2001) and may also influence the spatial structure of populations used in bioassessments. Organisms may disperse to access remote resources, escape local habitat conditions, or colonize distant areas (Schlosser 1990). Accordingly, dispersal

from adjacent areas could influence bioassessment metrics in several possible ways: (1) immigrating individuals may not require the full range of local environmental conditions (e.g., spawning habitat) needed to sustain a population and therefore may not accurately indicate local habitat conditions; (2) immigration of "new" species would inflate local species richness; and (3) disproportionate immigration of intolerant and tolerant species would bias bioassessments towards overestimating or underestimating site quality, respectively. As such, regional dispersal may result in either false identification of local degradation (i.e., type I errors) or failure to detect local degradation (i.e., type II errors).

In stream environments, dispersal dynamics may be influenced by the size and proximity of connecting streams (i.e., stream network topology) through the provision of habitat and immigrant-source populations (Campbell-Grant *et al*. 2007). Several lines of evidence suggest that inter-stream dispersal influences local assemblage structure. First, river systems flowing directly into the ocean typically contain fewer fish species than rivers of equal size flowing into other rivers (Sheldon 1988). Second, headwater streams typically support fewer fish species than streams of equal size connected to larger rivers (Gorman 1986; Osborne and Wiley 1992; Hitt and Angermeier 2006). Third, spatial autocorrelation in stream fish assemblages (Matthews and Robison 1998; Wilkinson and Edds 2001; Hitt *et al*. 2003; Grenouillet *et al*. 2004) and stream benthic macroinvertebrate assemblages (Sanderson *et al*. 2005; Lloyd *et al*. 2006) suggests that inter-stream dispersal influences local population dynamics. These effects on assemblage composition and population dynamics are likely to be expressed in many metrics used in bioassessments. However, little is known about the relative importance of dispersal for

fishes and benthic macroinvertebrate assemblages and the potential influence of dispersal on bioassessment metrics.

I investigated the effects of inter-stream dispersal on fish and benthic macroinvertebrate bioassessment metrics in west-central Virginia streams. This study area is appropriate for several reasons. First, this region supports a high diversity of fishes and benthic macroinvertebrates (Jenkins and Burkhead 1994; Smith and Voshell 1997). Second, streams in this region exhibit a variety of stream size confluences (i.e., stream network topology) due to diverse watershed shapes (i.e., dendritic- and trellis-shaped watersheds; Benda *et al*. 2004a). Third, VDEQ currently employs stream bioassessment methods in this region. Fourth, study area streams exhibit a wide range of environmental quality (VDEQ 2006a).

Currently, VDEQ uses benthic macroinvertebrate assemblages for bioassessment of surface waters but is considering incorporating stream fish bioassessment methods. To evaluate the potential utility of stream fish bioassessment in Virginia, it will be necessary to compare fish and benthic macroinvertebrate metric responses to degradation and explore the spatial scale of metric responses. To address these topics, I developed a stream fish bioassessment pilot project in west-central Virginia using data from the USEPA Environmental Monitoring and Assessment Program (EMAP) and the VDEQ Probabilistic Monitoring Program (ProbMon).

First, I sampled fishes and physical habitat conditions in a subset of ProbMon sites. Second, I combined data from ProbMon and EMAP sites and tested the comparability of these data sources. Third, I quantified a gradient of environmental quality among stream sites and evaluated the ability of fish and benthic macroinvertebrate

metrics to reflect this environmental gradient. Fourth, I explored the relative effects of stream network topology on fish and benthic macroinvertebrate assemblages and selected bioassessment metrics. I compared stream sites that were similar in local physical habitat conditions but different in regional connectivity to large rivers (i.e., headwater tributaries versus mainstem tributaries). I hypothesized that riverine dispersal would be more important for stream fish assemblages than benthic macroinvertebrate assemblages. I tested the predictions that (a) fish metrics would be more sensitive to environmental conditions among headwater tributaries than mainstem tributaries, and (b) benthic macroinvertebrate metrics would be equally sensitive to environmental conditions among headwater and mainstem tributary sites.

METHODS

Data sources

I used data from EMAP and ProbMon sites in west-central Virginia (n=43; Figure 4.1; Appendix J). This region encompasses 20,000 km^2 within the New River and upper James River watersheds in the ridge and valley physiographic region. Sandstone ridges and limestone valleys typify the geological structure in this physiographic region (Hack 1957).

I selected EMAP sites for this analysis using several criteria. First, I restricted sites to wadeable streams (i.e., backpack electrofishing methods) because of decreased sampling efficiencies at boat electrofishing sites in EMAP data (see Cyterski and Barber

2006). Second, I removed EMAP sites that flow into reservoir systems within 20 fluvial km (fkm) to remove potential effects of downstream reservoirs on upstream assemblages (Winston *et al*. 1991; Guenther and Spacie 2006). When sites were sampled repeatedly, I used the sample with the greatest species richness or, in the case of ties, the more recent sample. Third, I excluded EMAP sites that lacked environmental data. ProbMon sites were selected within the study area based on the availability of water quality and benthic macroinvertebrate data and were restricted to wadeable streams to permit backpack electrofishing methods.

Stream fishes were sampled in each site using single-pass backpack electrofishing methods following McCormick and Hughes (1998). Sample reach lengths were delineated at 40-times the average stream width for each site. Fishes were identified in the field and released. In EMAP sites, problematic specimens were preserved in 10% formalin and identified later by USEPA personnel. For ProbMon sites, problematic specimens were preserved in a 10% formalin solution for subsequent identification. Species identifications for a subset of voucher specimens were confirmed by R. E. Jenkins (Roanoke College). EMAP samples were collected during summer base flow conditions in 1993, 1994, 1997, and 1998. I incorporated several changes to the EMAP data based on published species accounts, under the assumption that EMAP surveys did not detect inter-basin range expansions (Appendix T). Raw data are available from http://www.epa.gov/emap. I collected ProbMon fish samples during baseflow conditions during the summers of 2004 and 2005 (Appendix J).

Benthic macroinvertebrates were sampled by USEPA and VDEQ personnel using methods adapted from USEPA Rapid Bioassessment Protocols (Plafkin *et al*. 1989;

Barbour *et al*. 1999). In EMAP and ProbMon sites, samples were collected by physically disturbing the substrate upstream from a 0.3-m-wide D-frame net (595 μm mesh). Samples were preserved in 70% ethanol or isopropanol for subsequent taxonomic identifications. EMAP protocols used a systematic random methodology to collect samples among a subset of transect stations (see Figure 3.2) and combine samples among riffles and pools (Lazorchak *et al*. 1998). However, ProbMon protocols included only riffle samples (VDEQ 2003). To enable comparisons among sites, I therefore evaluated only data collected from riffles in EMAP sites.

I selected 19 environmental variables from the EMAP dataset to characterize variation in environmental quality among sites. Variables included measures of land use, mesohabitat structure, riparian vegetation, substrate size, stream volume, water quality, and woody debris (Table 4.1). I focused on these categories of environmental conditions because they are known to correspond to environmental quality and were sampled in each site. Environmental data collection methods included GIS analysis, physical habitat measurements, and water quality sampling (Lazorchak *et al*. 1998).

Land use variables were calculated from GIS land cover data upstream of sample locations. EMAP land use data were calculated from U.S. Geological Survey data (Land Use Data Analysis, LUDA) (Davis and Scott 2000). ProbMon land use data were calculated from the National Land Cover Dataset by J. Hill (VDEQ). Both EMAP and ProbMon methods used a raster resolution of $30m^2$. All other variables were calculated from data collected at sample sites.

In EMAP and ProbMon sites, measures of stream volume, mesohabitat structure, substrate size, and woody debris were collected at each site using a systematic-random

sampling design (see Figure 3.2). Eleven transects were placed at regular intervals perpendicular to the sample reach. Within each transect, 5 sample points were positioned at equal distances across the width of the stream. At each sample point, stream depth, mesohabitat type (i.e., pool, riffle, or run) and substrate size was recorded. Stream widths were also recorded between transects. Substrate sizes were categorized following Wolman (1954), Bain *et al*. (1985) and Platts *et al*. (1983). Between transects, the proportion of small and large woody debris cover was visually estimated within a distance of 5 m upstream and downstream from each transect. Large woody debris was defined as pieces of wood measuring at least 10 cm in diameter at the small end and at least 1.5 m in length (Lazorchak *et al*. 1998). Each site therefore contains 55 measurements of substrate size and stream depth and 21 measurements of stream width and woody debris.

Attributes of riparian vegetation were visually estimated within the sample reach. At each transect, a sampling zone in the riparian area was visually estimated for a 10 m x 10 m square (i.e., 5 m upstream and downstream from transects). Within this zone, the proportions of total canopy cover (i.e., woody vegetation >15 m in height) and large-tree canopy cover (i.e., >0.3 m diameter at breast-height) were recorded. Riparian vegetation data therefore included 22 measurements in each site.

Water quality variables included total nitrogen (TN), total phosphorous (TP), conductivity, and pH (Table 4.1). Nutrient data were collected from water samples taken from the middle of the channel at each site (Herlihy *et al*. 1998). All water quality samples were shipped within 24 hours for laboratory chemical analysis (Herlihy *et al*. 1998). In ProbMon sites, pH was measured *in situ* from mid-channel flow at

approximately 0.3 m below the water surface (VDEQ 2003). In EMAP sites, pH was analyzed from stream samples in laboratory analyses. Conductivity was measured *in situ* for both ProbMon and EMAP sites.

Exploratory analyses revealed that most environmental variables were not significantly different between EMAP and ProbMon sites (i.e., Mann-Whitney, $p>0.05$ for 16/19 variables; Appendix N). However, the percent riffle habitat (PCT_FAST) and substrate sizes (PCT_BIGR and PCT_SAFN) varied significantly among sampling teams (i.e., USEPA and Virginia Tech; Mann-Whitney, $p<0.05$, respectively; Appendix N). EMAP data showed more riffle habitat, larger substrates, and more large woody debris than ProbMon sites (Appendix M). It is possible that these differences do not indicate sampling error among teams because EMAP sites also tended to be somewhat smaller in upstream basin area and mean stream width than ProbMon sites (Appendix M). However, this pattern is inconsistent with the observed differences in percent sand and fines (PCT_SAFN) among sites, suggesting the possibility of systematic measurement error. I chose to incorporate these variables (PCT_FAST, PCT_BIGR, PCT_SAFN) in analyses because I assumed that they contained useful information and because statistical methods examined multivariate patterns of environmental quality (i.e., robust to sampling error in individual metrics). Laboratory-based analyses (i.e., TN and TP) were not significantly different from EMAP data (Appendices M and N), suggesting the absence of systematic biases in these variables.

Fish metric calculations

I calculated fish metrics to characterize taxonomic and functional attributes of fish assemblage structure among sites (Appendices K and L). Taxonomic metrics assessed species richness and abundance at the family-level as well as native and introduced species. Functional metrics characterized fish assemblage structure based on trophic strategies, reproductive strategies, vertical position (i.e., benthic or pelagic), and overall tolerance to environmental degradation. I chose these metrics because they have demonstrated significant relations to environmental quality in previous studies of the mid-Atlantic highlands region (Angermeier *et al*. 2000; McCormick *et al*. 2001) and western Virginia (Smogor and Angermeier 1999b; Smogor and Angermeier 2001), or because expected responses to degradation are known (Appendix L). I calculated each metric in terms of total abundance (of individuals), proportional abundance, and species richness. In these analyses, "abundance" indicates catch per unit effort.

Trophic metrics characterized adult feeding strategies in 4 groups: invertivory, piscivory, invertivory/piscivory, and herbivory/omnivory. I used species-level trophic assignments from Smogor and Angermeier (1999a) but combined categories of detritivore/algivore/herbivore and algivore/herbivore/invertivore into a single herbivory/omnivory category *sensu* McCormick *et al*. (2001). I also calculated metrics to characterize specialist and generalist feeding strategies. Following Smogor and Angermeier (1999a), I classified trophic specialists and generalists based on four trophic categories: detritus, algae or vascular plants, invertebrates, or fish/fish blood. I predicted that invertivory, piscivory, invertivory/piscivory, and trophic specialists would respond

negatively to degradation and that omnivore/herbivore and trophic generalist metrics would respond positively to degradation (Appendix L).

Reproductive metrics characterized fish assemblages based on spawning substrates, nest preparation/parental care, and age of female reproduction. I classified species as simple lithophils, complex lithophils, or non-lithophils based on a combination of spawning substrates and parental care attributes. Following Balon (1981), lithophils were designated as species which scatter or deposit eggs among hard substrates (i.e., gravel) whereas non-lithophils use vegetation or detritus for spawning. I further separated simple lithophils from "non-simple" lithophils based on the absence or presence of nest preparation (e.g., redd construction) and/or parental care, respectively. I predicted that lithophils and the mean age of reproduction would respond negatively to degradation and that non-lithophils would respond positively to degradation (Appendix L).

I calculated vertical-position metrics based on fish behavior and body morphology (Appendix K). These metrics distinguished between species that typically inhabit benthic or water-column environments. Benthic fishes are dorsoventrally compressed (e.g., sculpins) and typically feed on benthic invertebrates or periphyton whereas pelagic fishes are more cylindrical or laterally compressed (Moyle and Cech 1996). Following Angermeier *et al*. (2000), I also calculated metrics that combined trophic specialist strategies (i.e., 1 food type) and benthic vertical positions. I predicted that benthic fishes would respond negatively to degradation whereas water-column species would not (Appendix L).

Tolerance metrics were calculated from published reports of species' responses to environmental degradation (Appendix K). For example, species known to have increasing distributions in degraded habitats are classified as tolerant. I based classifications on agreement between Smogor and Angermeier (1999a) and McCormick *et al*. (2001). However, if species were not evaluated in either source, I classified species according to Barbour *et al*. (1999). Of the 53 species in the dataset, 8 (15%) were classified as "tolerant" and 3 (6%) were classified as "intolerant." The relatively low percentages of classified species suggest that the resultant tolerance metrics will reflect lower and upper ends of the biotic integrity spectrum (Karr *et al*. 1986).

Exploratory analyses revealed that ProbMon sites supported greater fish species richness than EMAP sites in the study area (Mann-Whitney, $p<0.05$; Appendices O and P). Accordingly, several metrics relating to species richness (e.g., centrarchid richness, invertivore-piscivore richness, early-maturing species richness) were also significantly greater in ProbMon sites (Appendices O and P). However, two lines of evidence suggest that these differences were not due to systematic measurement error between sampling teams. First, none of the metrics quantifying proportional abundance were significantly different between EMAP and ProbMon sites (Appendix P), suggesting equivalent capture efficiencies between site types. Second, ProbMon sites tended to be somewhat larger than EMAP sites on average (Appendix M), consistent with the direction of observed differences between sites types. Further, I calculated EMAP fish metrics from our modifications of EMAP data (Appendix T), thereby avoiding the possibility of distinct metric calculation methods between ProbMon and EMAP sites.

Benthic macroinvertebrate metric calculations

Benthic macroinvertebrate metrics characterized taxonomic, trophic, and tolerance measures of assemblage structure (Appendix L). Taxonomic metrics included family richness, and proportional abundances and richness of Ephemeroptera, Plecoptera, and Trichoptera larvae. Trophic metrics characterized the proportional abundances of functional feeding groups (Cummins and Klug 1979), including scrapers, shredders, predators, collector-gatherers, and collector-filterers. Tolerance metrics included the proportional abundance of tolerant individuals and an index of organic pollution (Hilsenhoff Biotic Index, HBI; Hilsenhoff 1987). I chose these metrics because they have known responses to environmental quality in the mid-Atlantic highlands (Smith and Voshell 1997; Klemm *et al*. 2002; Yuan and Norton 2003) and Virginia (Burton and Gerritsen 2003).

I used metrics calculated by VDEQ personnel for all ProbMon sites. For EMAP sites, I calculated taxonomic metrics from riffle-based count data and I used USEPA calculations of trophic and tolerance metrics. In ProbMon sites, HBI scores were calculated from a modified family-level version of the HBI which was calibrated for Virginia streams (Burton and Garritsen 2003). In contrast, HBI scores for EMAP sites were calculated from genus- or species-level versions of the HBI. Despite this difference in taxonomic resolution, Klemm *et al*. (2002) found that the VDEQ HBI scores were comparable to EMAP HBI scores in the mid-Atlantic highlands. Moreover, HBI scores were not significantly different between ProbMon and EMAP sites in the current analysis (Mann-Whitney, $p>0.50$; Appendix P).

Exploratory analyses revealed that EMAP and ProbMon sites were not different in family richness (Mann-Whitney, $p>0.20$; Appendix P) but were significantly different in the proportional abundances of Trichoptera, predators, and tolerant taxa (Mann-Whitney, $p<0.05$, respectively; Appendix P). ProbMon sites tended to have greater proportional abundances of Trichoptera and lower proportional abundances of predators and tolerant species than EMAP sites (Appendix O). These metric differences cannot be explained by differences in stream volume between EMAP and ProbMon sites because these metrics do not necessarily change along stream-size gradients (Vannote *et al*. 1980). It is possible that differences in taxonomic resolution between EMAP and ProbMon sites could have affected tolerance and predator metrics without affecting proportional abundances of Trichoptera.

Stream network topology

I classified sites as "headwater tributaries" and "mainstem tributaries" (*sensu* Osborne and Wiley 1992) to test the hypothesis that the spatial position of sites within watersheds influences the relative sensitivity of fish and benthic macroinvertebrate metrics to local environmental conditions. The distinction between headwater and mainstem tributaries reflects the rate of stream volume accumulation per unit distance downstream from sites. Mainstem tributaries flow into "large" rivers at a given distance whereas headwater streams do not. To permit a spatially-explicit analysis, I categorized sites as headwater and mainstem tributaries at 5, 10, 15, and 20 fkm grain sizes. I chose the upper extent of this analysis grain size because previous work has demonstrated

significant effects of large river source populations within 20 fkm (Osborne and Wiley 1992).

I used a GIS methodology to standardize the classification of headwater and mainstem streams. To estimate stream volume downstream from sample sites, I calculated upstream cell counts (UCCs) from stream network raster data as a surrogate of upstream basin size and flow volume (C. D. Heatwole, Biological Systems Engineering, Virginia Tech, personal communication). First, I downloaded National Hydrological Data medium-resolution data (1:100,000 scale) and converted stream paths from vector to raster data (30-m^2 cells). Raw data are available at http://nhd.usgs.gov. Second, I used the flow network raster to calculate distances from each cell to the outlet pour-point of each watershed (5^{th} hydrologic unit code). Third, I converted distances into UCCs and then combined inflows from confluent watersheds. I then sampled the UCC raster at EMAP sites and at every kilometer for 20 fkm downstream. All calculations were preformed in ARCGIS 9.1. Methodological details are provided in Betz *et al*. (in prep).

I compared the UCC data against independent measures of upstream basin area to validate UCCs as a surrogate for stream flow volume. I used simple linear regression to relate UCCs to upstream basin areas from EMAP calculations in sites where basin area data were available throughout the mid-Atlantic highlands region (n=198). Log-log plots revealed bivariate linearity and good fit (R^2=0.919; see Figure 2.3), suggesting that UCC data provide a reasonable surrogate of stream flow volume (Hack 1957). Based on this relationship, I used a UCC value of 5000 to indicate the presence of a large river confluence (i.e., upstream basin area >250 km^2, *sensu* Osborne and Wiley 1992). HT and MT sites were not significantly different for most environmental variables (Appendices Q

and R), thereby permitting an analysis of stream network effects that was not confounded by local factors.

Statistical analysis

I used ordination and randomization procedures to assess biotic metric correspondence to environmental quality gradients and to explore the role of stream network topology in metric sensitivity. I used non-metric multidimensional scaling (NMS) to ordinate sites along environmental quality gradients and to reduce covariation among environmental variables (Mather 1976; Kruskal 1964; Minchin 1987). I chose to use NMS instead of principal components analysis (PCA) because NMS does not assume linear relationships among variables (McCune and Grace 2002) and has produced better representations of simulated ecological gradients than PCA (Fasham 1977). Prior to NMS ordinations, I transformed all environmental variables to improve linearity and reduce heteroscedasticity ($\log_{10}x+1$ transformations for continuous variables and arcsine square root transformations for percentage variables; Table 4.1). I used Bray-Curtis distances (Bray and Curtis 1957) for all ordinations in order to minimize the effects of outliers (McCune and Grace 2002). I used PC-ORD (version 5.0) for all NMS ordinations with 50 randomized starting configuration runs and an instability criterion of 0.00001.

To interpret the ordination results in terms of environmental quality gradients, I evaluated the correspondence of NMS scores and land use variables. I plotted land use variables as vectors in the ordination space and calculated nonparametric correlations to quantify the direction and magnitude of these relationships (Stewart *et al*. 2001). In

subsequent analyses, I used NMS scores from the environmental ordination that showed interpretable relations with land use variation. I assumed that variation in watershed-level land use corresponded to variation in environmental quality at the site-level (Herlihy *et al*. 1998; Jones *et al*. 2001; Vondracek *et al*. 2005; Mugodo *et al*. 2006; Dow *et al*. 2006). I chose to partition land use variables from the environmental ordination to permit a direct analysis of land use – environment relationships.

I then used nonparametric correlations (Spearman *rho*) to evaluate fish metric correspondence to derived environmental quality gradients (i.e., NMS scores). I used nonparametric methods for this analysis because exploratory analyses revealed non-normal in metric data across sites (i.e., all Shapiro-Wilk tests, $p>0.05$). To reduce metric colinearity for subsequent analyses, I selected surrogate metrics as those with the greatest correlation coefficient magnitude from a suite of significant metrics that measured intrinsically related quantities (e.g., salmonid richness, abundance, and proportional abundance). Other recent studies have used this ordination-correlation approach to assess community responses to environmental gradients (Paller 2002; Hering *et al*. 2006; Linke *et al.* 2005; Freeman and Marcinek 2006; Lamoroux *et al*. 2006).

I developed Mantel path-analysis models (Mantel 1967) to visualize the relationships among environmental variables and biotic assemblage structure (King *et al*. 2005). Mantel tests are distance-based matrix correlations that use permutation procedures to calculate the probability that observed correlations were random. Accordingly, these tests are appropriate for assessing correspondence in variables from unknown statistical distributions (Mantel 1967). I summarized environmental variables in categories of land use, mesohabitat structure, riparian vegetation, substrate size, stream

volume, water quality, and woody debris (Table 4.1). I also characterized spatial autocorrelation among sites using Universal Transverse Mercator coordinates. I used Euclidean distances for all environmental variables. I used Bray-Curtis distances (Bray and Curtis 1957) to characterize fish and benthic macroinvertebrate assemblage structure from site x species-abundance matrices (43 sites x 53 species and 85 families, respectively). I used the R programming language with the ECODIST library (Goslee and Urban 2006) to calculate distance matrices and perform all Mantel tests.

I then tested the role of stream network topology by comparing fish and benthic macroinvertebrate assemblage responses to environmental quality gradients among headwater and mainstem sites at multiple distances (i.e., grain sizes). First, I evaluated the assemblage-level correspondence among headwater and mainstem tributaries using Mantel tests (Mantel 1967) and Multiple Response Permutation Procedures (MRPP). MRPP methods provide an assessment of group membership strength using randomization procedures (McCune and Grace 2002). I used Euclidean distances for all MRPP tests. Second, I calculated environmental NMS ordinations for headwater and mainstem tributary sites at 5-, 10-, 15-, and 20-fkm distance classes (see Appendix S). I chose to calculate NMS scores for headwater and mainstem tributaries separately in order to standardize environmental gradients between these site types. I then calculated non-parametric correlations (i.e., Spearman *rho*) between NMS scores and fish metrics to test the prediction that mainstem streams would show less correspondence to local environmental variability than headwater streams. For this analysis, I included only the fish and benthic macroinvertebrate metrics that showed significant correspondence to NMS scores across all sites.

RESULTS

Environmental quality ordination

A 2-dimensional NMS ordination produced interpretable gradients of physical and chemical variation among sites (Table 4.2). NMS axis I scores were positively related to stream volume, large-diameter riparian canopy cover, in-stream woody debris cover, and negatively related to the percent of riffle habitat (variable loadings >|.10|, Table 4.2). NMS axis II scores were positively related to riparian vegetation and in-stream woody debris cover and negatively related to nutrient concentrations and fine substrates (variable loadings >|0.15|, Table 4.2). NMS score distributions were approximately equal among New and James river sites (Figure 4.2a). However, ProbMon sites tended to exhibit less overall diversity in NMS scores than EMAP sites (Figure 4.2b).

In NMS axis I, the strongest environmental variables in the ordination were stream width (XWIDTH), total phosphorous (PTL), and large woody debris cover (XFC_LWD) (variable loadings >|0.20|, Table 4.2). In contrast, total nitrogen (NTL), conductivity (COND), pH (PHSTVL), large substrates (PCT_BIGR), and substrate embeddedness (XEMBED) showed the weakest relations in NMS axis I (variable loadings <|0.05|, Table 4.2). The strongest variables loading into NMS axis II measured riparian vegetation (XCDENBK, XCL), percent sand and fines (PCT_SAFN), total nitrogen (NTL), and in-stream large woody debris cover (XFC_LWD) (variable loadings >|0.20|, Table 4.2). The weakest environmental variables in NMS axis II measured

mesohabitat structure (PCT_POOL), pH (PHSTVL) and stream volume (AREA_WS, XDEPTH) (variable loadings <|0.05|, Table 4.2).

Watershed-level land use patterns were strongly related to variation in the environmental ordination. Forest cover and agricultural land cover were significantly related to NMS axis II scores (Spearman *rho*, $p<0.0001$; Table 4.3; Figure 4.3). As expected, agricultural cover and forest land cover were inversely related to variation in NMS axis scores: forest cover was related to increased riparian vegetation and large woody debris cover whereas agricultural land cover was related to increased nutrient concentrations and fine substrates (Tables 2 and 3). Urbanization was significantly related to variation in both NMS axis I and II (Spearman *rho*, $p<0.05$, Table 4.3), but the urbanization vector length suggests relatively weak overall effects (Figure 4.3). Agricultural and forest land uses were not significantly related to variation in NMS axis I (Table 4.3).

NMS environmental variable relations were concordant with Mantel path analyses. Land use was significantly related to riparian vegetation and substrate composition (Mantel $r>0.25$, $p<0.005$; Table 4.5). In turn, riparian vegetation was significantly related to in-stream woody debris cover (Mantel $r=0.300$, $p<0.005$; Table 4.5) and substrate composition (Mantel $r=0.161$, $p<0.05$; Table 4.5). Stream volume was related to riparian vegetation (Mantel $r=0.186$, $p<0.05$, Table 4.5) but not to other environmental variables. Mesohabitat conditions and water quality were not significantly related to other environmental variables in this analysis (Table 4.5), suggesting that the spatial resolution of NMS ordination was greater than that observed in the Mantel path analyses.

I retained NMS axis II for subsequent analyses of biotic responses to environmental quality gradients. I excluded NMS axis I from subsequent analyses because (1) agricultural and forest land cover were not significantly related to variation in this axis and (2) stream volume variables were significantly related to NMS axis I. Importantly, NMS axis II was not influenced strongly by stream volume (variable loadings <|0.05|, Table 4.2), permitting subsequent analysis of land use and biotic relations without the confounding effects of stream size.

Fish assemblage and metric relations to environmental quality

Several taxonomic, reproductive, and tolerance fish metrics showed significant relations to the environmental quality gradient (Appendix L). Among taxonomic metrics, the number of non-native individuals (NNAT_N) increased with degradation (i.e., negatively related to NMS II, Spearman *rho* = -0.325, $p<0.05$, Table 4.4) and the proportional abundance of cottid individuals (COT_P) and salmonid total abundance (SAL_N) increased with environmental quality (i.e., positively related to NMS II, Spearman *rho* >0.30, $p<0.05$, respectively). Among reproductive metrics, the mean reproductive age (REPROAGE) was positively related to the environmental quality gradient represented by NMS II (Spearman *rho* = 0.331, $p<0.05$). However, tolerance metrics (INTOL_S, INTOL_N, INTOL_P) showed the strongest and most consistent relations to the environmental quality gradient (Spearman *rho* >0.40, $p<0.05$, respectively; Table 4.4).

Fish metrics characterizing trophic structure and vertical position were not significantly related to the environmental quality gradient in this analysis (Spearman *rho*

$p>0.05$ for all comparisons; Appendix L). Removing tolerant species from computations of reproductive and trophic metrics did not significantly improve the performance of reproductive and trophic metrics (i.e., SL_S versus SL2_S; IP_P versus IP2_P, Appendix L) but did improve the strength of environmental quality relations in benthic species metrics (i.e., BEN_S versus BEN2_S; BEN_P versus BEN2_P; Appendix L). Several metrics were not significant at $\alpha = 0.05$ but were related to the environmental quality gradient in the predicted direction, including water column species richness (COL_N), the abundance of tolerant individuals (TOL_N), the proportional abundance of invertivore-piscivores (IP_P), the abundance of generalist water column feeders (GENCOL_N), and the proportional abundance of omnivore-herbivores (OH_P). All significant fish metrics were related to the NMS axis in the predicted direction (Appendix L).

NMS vector plots revealed the relative importance of individual metrics and their correspondence to one another (Figure 4.4a). Intolerant species richness (INTOL_S) and salmonid abundance (SAL_N) vectors were very similar in NMS space and showed very little correspondence to NMS I (i.e., vertical orientation). Vectors of mean reproductive age (REPROAGE) and the proportional abundance of cottids (COT_P) tended to be less strongly related to NMS scores in axis II (i.e., vector lengths) and were oriented at approximately 45-degree angles, suggesting an equal influence of variation from NMS axis I. The vector for abundance of non-native individuals (NNAT_N) maintained a distinct position in the NMS ordination as this was the only metric with significant negative relations to NMS II. However, NNAT_N was also influenced by NMS I to some degree, given the non-vertical orientation of the vector (Figure 4.4a).

Mantel tests also revealed significant relations between land use patterns and fish assemblages (Mantel r=0.195, p<0.005; Table 4.5; Figure 4.5a). Fish assemblage structure (i.e., Bray-Curtis distances from a site x species-abundance matrix) was also significantly related to variation in substrate size (Mantel r=0.241, p<0.005) and stream volume (Mantel r=0.273, p<0.005). Significant spatial autocorrelation was not detected in this analysis (i.e., Mantel r=0.095, p>0.05) nor were significant fish assemblage relations to mesohabitat, riparian vegetation, water quality, or woody debris cover (Table 4.5). However, all environmental variables were positively related to fish assemblage structure, although not significant at $\alpha = 0.05$ (Table 4.5).

Benthic macroinvertebrate assemblage and metric relations to environmental quality

Benthic macroinvertebrate metrics characterizing taxonomic, trophic, and tolerance were significantly related to the observed environmental quality gradient. Among taxonomic metrics, the proportional abundance and richness of Ephemeroptera, Plecoptera, and Trichoptera (EPT_P and EPT_S) were significantly related to NMS II (Spearman *rho* >0.45, p<0.005; Appendix L). Proportional abundances of Plecoptera (PLE_P) and Ephemeroptera (EPH_P) were also significantly related to NMS II (Spearman *rho* >0.35, p<0.05) but the proportional abundance of Trichoptera was not (Appendix L). Among trophic metrics, the proportional abundance of shedders (SHR_P) increased with increasing environmental quality (Spearman *rho*=.336, p<0.05, Table 4.5) but metrics characterizing scrapers, predators, collector-gatherers, and filter-gatherers were not significantly related to NMS II (Appendix L). Among tolerance metrics, HBI

and the proportional abundance of tolerant individuals were negatively related to the environmental quality gradient (Spearman *rho* >|.35|, $p<0.05$, respectively, Table 4.5).

Vector analysis revealed patterns of metric correspondence in NMS space (Figure 4.4b). The proportional abundance of EPT taxa (EPT_P) was closely related to Plecoptera proportional abundance (PLE_P) but was not redundant with the variation in Ephemeroptera proportional abundances (EPH_P). The proportional abundance of tolerant taxa (TOL_P) was closely related to variation in HBI (Figure 4.4b). Shredder proportional abundances (SHR_P) and Ephemeroptera proportional abundances (EPH_P) showed non-redundant patterns in NMS space (Figure 4.4b), although the overall relations to NMS II were weaker than for other metrics (i.e., shorter vector lengths).

Mantel tests revealed significant relations between land use and benthic macroinvertebrate assemblage structure at the family-level (Mantel $r=0.295$, $p<0.005$, Table 4.5; Figure 4.5). Benthic macroinvertebrate assemblage structure was also directly related to substrate composition, water quality, and mesohabitat structure (Mantel $r>0.20$, $p<0.05$, respectively; Table 4.5). No significant relationships were detected between benthic macroinvertebrate assemblage structure and woody debris, stream volume, or riparian vegetation (Table 4.5, Figure 4.5).

Effects of stream network topology

Fish assemblage structure tended to exhibit stronger relations to local environmental quality gradients among headwater tributaries than among mainstem tributaries. Within a 5-fkm analysis zone, fish assemblage structure (i.e., Bray-Curtis distances in species abundance) was significantly related to land use, riparian vegetation,

substrate composition, and stream volume among headwater tributaries but not among mainstem tributaries (Figure 4.6). Within 10-fkm and 15-fkm analysis zones, headwater tributary assemblages were significantly related to land use, mesohabitat, and water quality, but these variables were not related to fish assemblage structure among mainstem streams (Figure 4.6). Headwater tributary assemblages also showed stronger relations to substrate composition and stream volume than mainstem tributary assemblages within 10 and 15 fkm, although all site categories were statistically significant (Mantel r, $p<0.05$, respectively; Figure 4.6). In no analysis zone did mainstem tributary assemblages show significantly greater correspondence to local environmental conditions than in headwater tributaries (Figure 4.6).

Several fish metrics showed distinct relations to environmental quality gradients among headwater and mainstem tributaries. NMS ordinations of environmental variables revealed two-dimensional environmental quality gradients among headwater and mainstem tributaries within 5, 10, 15, and 20 fkm (Appendix S). When classified at a distance of 10 fkm, salmonid abundance (SAL_N) and mean reproductive age (REPROAGE) were significantly related to NMS ordination scores in headwater tributaries (Spearman *rho*$>|0.48|$, $p<0.05$, respectively) but not in mainstem tributaries (Table 4.7). Similar patterns were observed at a 15-fkm analysis zone: salmonid abundance (SAL_N) and cottid abundance (COT_P) were related to NMS ordination scores among headwater tributaries (Spearman *rho*$>|0.55|$, $p<0.05$, respectively) but not among mainstem tributaries (Table 4.7). However, non-native fish abundance (NNAT_N) and intolerant species richness (INTOL_S) were significantly related to NMS

ordination scores for both headwater tributaries and mainstem tributaries in 5 and 10 fkm analysis zones (Table 4.7).

Benthic macroinvertebrate assemblages were not uniformly influenced by site position among headwater tributaries and mainstem tributaries. Within 5 and 10 fkm, headwater and mainstem tributary assemblages did not exhibit significantly different responses to 5 of the 7 environmental variables analyzed (Figure 4.6). However, headwater tributary assemblages were more strongly related to mesohabitat structure than mainstem assemblages within 5 fkm (Figure 4.6). Conversely, mainstem tributary assemblages were more strongly related to woody debris and water quality than headwater tributary sites within 10 fkm (Figure 4.6). At larger spatial grain sizes (15 and 20 fkm), mainstem tributary assemblages were related to land use, mesohabitat and water quality whereas significant relations were not detected among headwater tributary assemblages (Figure 4.6). Conversely, headwater tributary assemblages showed stronger relations to stream volume within 15 and 20 fkm than mainstem tributary assemblages (Figure 4.6).

Benthic macroinvertebrate metrics showed variable responses to environmental gradients among mainstem and headwater tributary sites. Within 5- and 10-fkm analysis zones, Plectoptera proportional abundance (PLE_P) showed greater correlations with NMS scores among mainstem tributaries than among headwater tributaries (Spearman *rho* >|0.57|, $p<0.005$, respectively; Table 4.7). However, Plecoptera proportional abundance (PLE_P) was related to NMS scores in both mainstem tributaries and headwater tributaries within 20 fkm (Spearman *rho* >|0.46|, $p<0.05$ respectively; Table 4.7). Tolerant taxa proportional abundance (TOL_P) and Hilsenhoff Biotic Index scores

(HBI) showed stronger relations to NMS scores among mainstem sites than among headwater sites (Table 4.7). Ephemeroptera, Plecoptera, and Trichoptera collective proportional abundance (EPT_P) and Ephemeroptera proportional abundance (EPH_P) also showed greater correspondence to NMS scores among mainstem tributaries than headwater tributaries. However, these metrics were related to NMS scores in both headwater tributaries and mainstem tributaries within 5 fkm and 20 fkm, respectively (Table 4.7).

MRPP tests revealed some differences among fish and benthic macroinvertebrate assemblages based on the spatial position of stream sites within watershed networks. In 10- and 15-fkm analysis zones, fish assemblages showed stronger group differences between headwater tributaries and mainstem tributaries than did benthic macroinvertebrate assemblages (Table 4.6). However, headwater tributaries and mainstem tributaries had relatively weak overall effects on fish and benthic macroinvertebrate structure. Furthermore, no significant differences between headwater tributaries and mainstem tributaries were detected within 5 fkm and 20 fkm for either fish or benthic macroinvertebrate assemblages (Table 4.6).

DISCUSSION

Environmental quality ordination

A prerequisite for evaluating the utility of candidate bioassessment metrics is the development of an environmental quality gradient among sites (Karr *et al*. 1986). In the

current study, two lines of evidence suggest that the NMS ordination revealed a gradient of environmental quality. First, NMS II scores were significantly related to land use patterns upstream of sample sites (Table 4.3, Figure 4.3). Second, environmental variable relations to NMS II were consistent with previous studies of land use effects on stream ecosystems.

NMS II scores corresponded to variation in agricultural and forested land uses among study sites (Table 4.3, Figure 4.3). Increasing agricultural land use was associated with decreasing substrate size and increasing embeddedness, and increasing concentrations of total nitrogen and total phosphorous. Conversely, increasing forest cover was related to increasing riparian vegetation and in-stream large woody debris (Tables 4.2 and 4.3). Mantel test results also detected significant correspondence among land uses and substrate size (Table 4.5; Figure 4.5). Similar patterns of sedimentation and nutrient enrichment have been reported from urbanized watersheds (e.g., Walters *et al*. 2003; Freeman and Schorr 2004; Clinton and Vose 2006) and agricultural watersheds (e.g., Wang *et al*. 1997; Sutherland *et al*. 2002; Snyder *et al*. 2003; Dow *et al*. 2006). Van Sickle *et al*. (2006) also found similar patterns of environmental covariation among EMAP sites in the mid-Atlantic highlands.

Although the NMS ordination revealed differences in environmental quality among sites, most sites did not deviate substantially from reference conditions identified by VDEQ (2006) for mountain streams in Virginia. Reference thresholds for total nitrogen and total phosphorous (1.5 mg/L and 0.005 mg/L, respectively) were achieved in all but three sites (7%). Reference thresholds for pH (6-9) were obtained in all sites. However, eight sites (19%) exhibited conductivity measures greater than the reference

threshold of 250 μS (VDEQ 2006b) and previous studies recognized conductivity as a good indicator of human influences in the mid-Atlantic highlands (Yuan and Norton 2003).

The site selection protocol used to delineate sample site locations may explain why most sites did not deviate from reference conditions. Both EMAP and ProbMon sites were located using a systematic-random site selection methodology (see Herlihy *et al*. 2000). This design is useful for extrapolating findings from sample sites to unsampled space (i.e., cumulative distribution functions) but tends to capture the central tendency in environmental conditions, not the extreme conditions most useful for bioassessment metric development. In the current study, EMAP sites exhibited a greater range of agricultural and urban land uses than ProbMon sites and also occupied a larger proportion of the environmental NMS ordination space (Figure 4.2b). As a result, the addition of the EMAP data provided important environmental variation for the current analysis.

Comparisons of environmental variables among EMAP and ProbMon sites suggest that sampling methods were generally robust to different sampling dates and sampling teams. Measures of stream volume, upstream land use, riparian vegetation, water quality, and in-stream woody debris were not significant at $\alpha = 0.05$ (Appendix N). However, differences in substrate size classifications present the possibility that systematic biases may have influenced the comparability of results among sampling teams (i.e., USEPA and Virginia Tech). For example, sampling teams may have used different criteria for counting (and excluding) thin layers of fine sediment on substrates. Sennatt *et al*. (2006) demonstrated that EMAP embeddedness measures are robust to

sampling error, but Yuan (2007) showed that measurement error in assessing fine substrates may affect bioassessment metric precision.

Fish and benthic macroinvertebrate responses to environmental quality

Fish and benthic macroinvertebrate metrics corresponded reliably with known environmental quality, suggesting that both types of metrics will be useful for stream bioassessments in the study area. However, differences in metric performance suggest that fishes and benthic macroinvertebrates provide distinctive information about environmental conditions. Benthic macroinvertebrate metrics tended to reflect degraded conditions (i.e., low NMS II scores) more readily than fish metrics (Figure 4.4). In addition, benthic macroinvertebrate assemblage structure corresponded to water quality variation whereas fish assemblage structure did not (Table 4.5, Figure 4.5). These differences suggest that fish and benthic macroinvertebrate assemblages may respond to environmental conditions over distinct spatial and temporal scales.

Fish and benthic macroinvertebrate assemblages exhibited some distinct relations to environmental conditions. Land use patterns were significantly related to fish and benthic macroinvertebrate assemblages (Table 4.5; Figure 4.5), but were more strongly related to benthic macroinvertebrates than to fishes (Spearman *rho* = 0.295 versus 0.195, respectively; Table 4.5). Water quality was also more strongly linked to benthic macroinvertebrate assemblages than to fish assemblages (Table 4.5; Figure 4.5). However, both fish and benthic macroinvertebrate assemblages were significantly related to substrate size (Table 4.5; Figure 4.5). In contrast, Longing (2005) found that the systematic-random sampling protocol for benthic macroinvertebrates used in the current

study (Lazorchak *et al*. 1998) was unable to detect sedimentation in piedmont streams. Our results suggest the sampling protocol used by USEPA and VDEQ can detect anthropogenic shifts in substrate size within mountain streams.

Taxonomic metrics generally showed stronger relations to environmental quality among benthic macroinvertebrates than among stream fishes. Only three of the 33 taxonomic fish metrics (9%) were significantly related to environmental quality whereas four of the six taxonomic metrics for benthic macroinvertebrates were significant (67%). The proportional abundances of EPT taxa (EPT_P) and Plecoptera (PLE_P) showed the strongest relations to site quality (i.e., highest Spearman *rho* values, Table 4.4). These results confirm previous work demonstrating utility of EPT taxa for stream bioassessments within the mid-Atlantic highlands (Smith and Voshell 1997; Bryce *et al*. 1999; Yuan and Norton 2003) and Virginia (Moeykens 2002; Burton and Gerritsen 2003; Braccia 2005; VDEQ 2006b). However, our study was the first to demonstrate a negative relationship between non-native fish abundance (NNAT_N) and site quality within western Virginia (cf. Smogor and Angermeier 1999b). It is possible that I detected an effect of non-native fishes because the density of sample sites in the New River basin was greater than in previous efforts, and because the New River system supports a relatively high proportion of non-native fishes (Jenkins and Burkhead 1994).

Tolerance metrics corresponded strongly with environmental quality in both fish and benthic macroinvertebrates. However, intolerant fish species richness (INTOL_S) showed somewhat greater correspondence to environmental quality than benthic macroinvertebrate tolerance metrics of HBI and the proportional abundance of tolerant taxa (TOL_P) (Spearman *rho* = |0.496|, |0.353|, |0.373|, respectively; Table 4.4). In

previous studies, tolerance metrics also responded to stream quality in the mid-Atlantic highlands among fishes (Angermeier *et al*. 2000; McCormick *et al*. 2001) and benthic macroinvertebrates (Smith and Voshell 1997; Yuan and Norton 2003). Moreover, the absence of significant differences in HBI between EMAP and ProbMon sites (Appendix P) supports Klemm *et al*.'s (2002) conclusion that taxonomic resolution (i.e., genus versus family) does not impair HBI metric performance.

In contrast to our expectations, most trophic and reproductive metrics were not significantly related to site quality in either fish or benthic macroinvertebrate assemblages. This result was particularly surprising for fish reproductive metrics (e.g., simple lithophils), given that previous studies have detected reproductive metric responses in western Virginia (Smogor and Angermeier 1999b), within the Ridge and Valley physiographic region (Angermeier *et al*. 2000), and across the mid-Atlantic highlands (McCormick *et al*. 2001). However, mean reproductive age (REPROAGE) was positively related to environmental quality, consistent with the hypothesis that harsh environmental conditions exhibit directional selection for early reproductive ages (Winemiller and Rose 1992). Smogor and Angermeier (1999b) found that the richness of late-maturing species (i.e., > age 2) increased with site quality in western Virginia streams, but this metric (LATE_S) was not significantly related to site quality in the current analysis. My study provides the first evidence that site quality may influence the mean age of reproduction (across species) in stream fish assemblages.

Among benthic macroinvertebrate metrics, I found that shredder proportional abundance (SHR_P) was significantly related to site quality, but other functional feeding groups were not (Appendix L). Shredders utilize allochthonous coarse organic matter

(e.g., leaves) as food sources (Cummins and Klug 1979) and therefore are expected to decrease with decreasing riparian vegetation and allochthonous inputs (Delong and Brusven 1998; Cummins *et al*. 1989; Braccia 2005). The absence of significant functional feeding group metrics in our study may be attributable to functional variation within the taxonomic resolution of ProbMon data (family-level) and because predator, filter-gatherer, and collector-gatherer functional feeding groups do not necessarily respond uniformly to degradation gradients (Voshell 2002). For example, Burcher and Benfield (2006) also failed to detect significant land use effects on the structure of benthic macroinvertebrate functional feeding groups, even though some assemblage-level differences were observed among land uses in their study.

Stream network topology in bioassessment

The spatial position of streams within watershed networks is known to affect fish assemblage structure (Whiteside and McNatt 1972; Gorman 1986; Osborne and Wiley 1992; Wilkinson and Edds 2001; Schaeffer and Kerfoot 2004; Hitt and Angermeier 2006) and benthic macroinvertebrate assemblage structure (Sanderson *et al*. 2005; Lloyd *et al*. 2006). Osborne *et al*. (1992) showed that these differences in fish assemblage structure among headwater and mainstem streams may affect fish bioassessment scores. However, their study lacked environmental data and therefore could not test the competing hypothesis that local environmental factors regulate fish assemblage structure (Osborne *et al*. 1992). Further, no previous studies have explored the effects of stream network topology on fish metric sensitivity to stressor gradients. I predicted that dispersal from riverine habitats would be more important in organizing stream fish assemblages than

benthic macroinvertebrate assemblages, thereby decreasing the sensitivity of fish metrics to local stressors in mainstem tributary sites relative to benthic macroinvertebrate metrics. My results generally support this prediction.

Distance- and metric-based multivariate tests showed that stream fishes were influenced more by the proximity of riverine habitats than benthic macroinvertebrates. Classifying sites as headwater tributaries and mainstem tributaries had greater effects on fish assemblages than benthic macroinvertebrate assemblages (Table 4.6). Differences were also seen in fish and benthic macroinvertebrate assemblage relations to environmental factors: fish assemblage responses to environmental gradients were consistently greater in headwater tributaries than mainstem tributaries, but benthic macroinvertebrate assemblages were equally likely to respond to environmental gradients between these site classifications (Figure 4.6). Equivalent patterns were detected in metric comparisons: spatial position (headwater tributary versus mainstem tributary) had more consistent effects on fish metrics than on benthic macroinvertebrate metrics (Table 4.7). Because most local environmental variables were not significantly different between headwater and mainstem tributaries (Appendices Q and R), I cannot explain differences in metric performance based on local environmental factors.

Differences in fish and benthic macroinvertebrate dispersal dynamics may help explain why fishes were more sensitive to adjacent riverine habitats than benthic macroinvertebrates. Spatial autocorrelation in benthic macroinvertebrate assemblage structure in streams (Sanderson *et al*. 2005; Lloyd *et al*. 2006) and lakes (Rundle *et al*. 2002; Hrabik *et al*. 2005) suggests that overland adult dispersal is important for some taxa. However, I know of no studies documenting benthic macroinvertebrate dispersal

from riverine source populations. In contrast, fish dispersal from riverine sources has been identified previously (e.g., Gorman 1986; Osborne and Wiley 1992; Hitt and Angermeier 2006), consistent with our current findings.

Our results suggest that fish and benthic macroinvertebrate bioassessment metrics may provide distinct measures of environmental quality, reflecting differences in the spatial grain over which these taxa respond to environmental conditions. Previous studies have suggested that organismal mobility complicates bioassessment efforts (Barbour *et al*. 1999; Osborne *et al*. 1992; Lloyd *et al*. 2006). However, my study is the first to provide a spatially-explicit analysis of how dispersal may affect local metric sensitivities. I found that the performance of fish metrics was influenced by the proximity of large river confluences but the performance of benthic macroinvertebrate metrics was not. This suggests that fish metrics may integrate information from larger areas (i.e., spatial grains) than do benthic macroinvertebrate metrics. Stream bioassessment programs using fish and benthic macroinvertebrates may therefore provide spatially hierarchical inferences regarding local and regional environmental quality.

Table 4.1. Environmental variables used in Chapter 4 analysis of EMAP and ProbMon sites in west-central Virginia, USA.

Category	Variable	Units	EMAP code	Transformation
Land use	Agricultural land cover	Proportion of watershed	AG_TOT	arcsin-sqrt
	Forest cover	Proportion of watershed	FOR_TOT	arcsin-sqrt
	Urban land cover	Proportion of watershed	URB_TOT	arcsin-sqrt
Mesohabitat	Riffle habitat	Proportion of sample reach area	PCT_FAST	arcsin-sqrt
	Pool habitat	Proportion of sample reach area	PCT_POOL	arcsin-sqrt
Riparian	Mean bank canopy density	Proportion of sample reach	XCDENBK	arcsin-sqrt
	Canopy >0.3m DBH	Proportion of sample reach	XCL	arcsin-sqrt
Substrate	Substrate larger than coarse gravel (>16 mm diameter)	Proportion of sample reach	PCT_BIGR	arcsin-sqrt
	Sand and fines (<2 mm diameter)	Proportion of sample reach	PCT_SAFN	arcsin-sqrt
	Mean embeddedness	Percent of substrate area	XEMBED	arcsin-sqrt
Volume	Mean stream width	m	XWIDTH	log10(x+1)
	Upstream watershed area	ha	AREA_WS	log10(x+1)
	Mean thalweg depth	cm	XDEPTH	log10(x+1)
Water quality	Conductivity	µS	COND	log10(x+1)
	Total nitrogen	mg/L	NTL	log10(x+1)
	pH		PHSTVL	log10(x+1)
	Total phosphorous	mg/L	PTL	log10(x+1)
Woody debris	Brush cover	Proportion of sample reach	XFC_BRS	arcsin-sqrt
	Large woody debris	Proportion of sample reach	XFC_LWD	arcsin-sqrt

Table 4.2. Environmental variable loadings into a two-dimensional non-metric multidimensional scaling (NMS) ordination. Variable codes are presented in Table 4.1.

Category	Variable	NMS1	NMS2
Mesohabitat	PCT_FAST	-0.137	0.033
	PCT_POOL	0.074	0.014
Riparian vegetation	XCDENBK	0.092	0.259
	XCL	0.157	0.296
Substrate size	PCT_BIGR	0.035	0.087
	PCT_SAFN	-0.060	-0.288
	XEMBED	0.043	-0.128
Volume	XWIDTH	0.244	0.038
	AREA_WS	0.155	0.011
	XDEPTH	0.098	-0.018
Water quality	COND	0.028	-0.091
	NTL	0.028	-0.277
	PHSTVL	0.005	-0.013
	PTL	-0.225	-0.179
Woody debris	XFC_BRS	0.156	0.158
	XFC_LWD	0.289	0.353

Table 4.3. Spearman's *rho* correlations between land use variables and environmental ordination axes. *p<0.05, **p<0.0001. Environmental variable loadings into the non-metric multidimensional scaling (NMS) ordination are presented in Table 4.2. Variable codes are presented in Table 4.1.

Variable	NMS 1	NMS 2
AG_TOT	0.105	-0.708**
FOR_TOT	-0.101	0.731**
URB_TOT	0.377*	-0.329*

Table 4.4. Summary of significant fish and benthic macroinvertebrate (BMI) metric relations to NMS 2 (an environmental quality gradient) in the west-central Virginia study area. *p<0.05; **p<0.005. Positive correlations indicate that the metrics increase with increasing environmental quality (see land use relations in Table 4.3). The non-metric multidimensional scaling (NMS) ordination is plotted in Figures 4.3 and 4.4. Variable codes are presented in Table 4.1.

Biotic category	Metric category	Variable	Expected response to degradation	Spearman *rho*
Fish	Taxonomic	NNAT_N	Positive	-0.325*
		COT_P	Negative	0.323*
		SAL_N	Negative	0.308*
	Reproductive	REPROAGE	Negative	0.331*
	Tolerance	INTOL_S	Negative	0.496**
BMI	Taxonomic	EPT_P	Negative	0.504**
		EPH_P	Negative	0.358*
		PLE_P	Negative	0.518**
	Trophic	SHR_P	Negative	0.336*
	Tolerance	TOL_P	Positive	-0.370*
		HBI	Positive	-0.353*

Table 4.5. Mantel test correlation coefficients for fish and benthic macroinvertebrate (BMI) assemblage relations to environmental variables. Upper-right half of table contains Mantel r values. Lower-left half of table contains associated p-values. 10,000 Monte Carlo randomization resampling iterations were used to develop test distributions. Variable codes are presented in Table 4.1. *p<0.05; **p<0.005. Significant relations (p<0.05) are plotted in Figure 4.5.

	FISH	**BMI**	**Space**	**Land use**	**Meso-habitat**	**Riparian**	**Substrate**	**Volume**	**Water**	Woody
FISH	—	0.184	0.095	0.195**	0.044	0.099	0.241**	0.273**	0.101	0.085
BMI	0.032	—	-0.053	0.295**	0.203*	0.035	0.267**	0.097	0.245**	-0.012
Space	0.091	0.453	—	0.147*	0.006	0.032	0.117*	0.026	-0.069	0.057
Land use	0.002	0.000	0.006	—	-0.005	0.266**	0.337**	0.092	0.186	0.076
Meso-habitat	0.493	0.005	0.909	0.904	—	0.019	0.085	0.061	-0.046	0.077
Riparian	0.146	0.682	0.570	0.000	0.755	—	0.161*	0.186*	-0.025	0.300**
Substrate	0.000	0.002	0.038	0.000	0.173	0.017	—	0.090	0.056	0.088
Volume	0.001	0.236	0.644	0.068	0.332	0.005	0.195	—	-0.075	0.029
Water	0.098	0.002	0.166	0.003	0.419	0.682	0.375	0.200	—	0.043

Table 4.6. Multiple response permutation procedure (MRPP) tests among fish and benthic macroinvertebrate (BMI) assemblages in two stream network groups at four spatial grain sizes. MRPP tests were calculated from rank-transformed Euclidean distances. Chance-corrected within-group agreement (*A*) indicates the group membership strength within network groups. MT= mainstem tributary, HT= headwater tributary.

Analysis grain	Biotic category	Network groups	No. sites	Chance-corrected within-group agreement, *A*	p-value
5 fkm	FISH	MT vs. HT	12, 31	0.00026	0.411
	BMI	MT vs. HT	12, 31	0.00598	0.272
10 fkm	FISH	MT vs. HT	24, 19	0.01920	0.082
	BMI	MT vs. HT	24, 19	0.00882	0.205
15 fkm	FISH	MT vs. HT	29, 14	0.01769	0.096
	BMI	MT vs. HT	29, 14	-0.00003	0.449
20 fkm	FISH	MT vs. HT	35, 8	0.00856	0.220
	BMI	MT vs. HT	35, 8	-0.01151	0.835

Table 4.7. Fish and benthic macroinvertebrate (BMI) metric relations to environmental conditions among headwater tributaries (HT) and mainstem tributaries (MT) within multiple distances from collection sites. Cell values are Spearman's *rho* correlation coefficients. Metric codes are presented in Appendix L. Fish and BMI metrics were selected based on significant relations to environmental quality gradients (NMS I and NMS II; Table 4.5). Non-metric multidimensional scaling (NMS) axis loadings are in Appendix S. *p<0.05, **p<0.005, ***p<0.0005. Spearman's *rho* correlation coefficients with p>0.05 are not shown.

			5		10		15		20	
Biotic category	**Metric**	**Group**	**NMS I**	**NMS II**	**NMS I**	**NMS II**	**NMS I**	**NMS II**	**NMS I**	**NMS II**
BMI	EPT_P	HT	0.447*							
		MT		0.601*		0.630**		0.549**	-0.478**	
BMI	EPH_P	HT								0.743*
		MT		0.629*		0.663***		0.505*	-0.395*	
BMI	PLE_P	HT	0.575**			0.763***			-0.762*	
		MT							-0.466**	
BMI	SHR_P	HT				0.604*		0.582*		
		MT		0.650*						
BMI	TOL_P	HT								
		MT		-0.627*				-0.417*	0.386*	
BMI	HBI	HT								
		MT		-0.769**				-0.384*		
FISH	NNAT_N	HT	-0.620***			-0.791***			0.874**	
		MT	0.623*		-0.407*					0.512**
FISH	COT_P	HT					0.559*			
		MT								
FISH	SAL_N	HT			0.652**		0.768**			0.770*
		MT							-0.411*	
FISH	REPROAGE	HT				0.487*				
		MT								
FISH	INTOL_S	HT	0.431*		0.562*					
		MT		0.611*		0.510*			-0.419*	

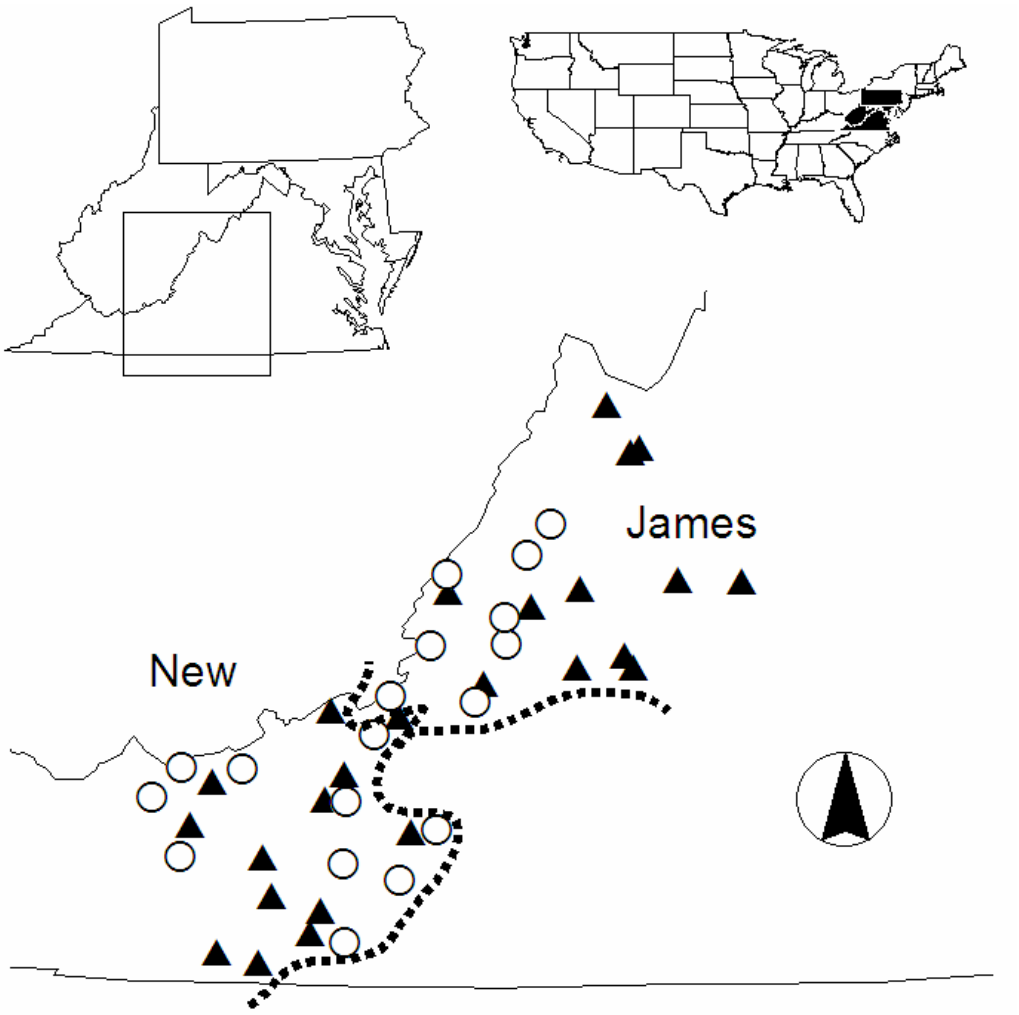

Figure 4.1. Study sites within west-central Virginia including USEPA EMAP sites (triangles) and VDEQ ProbMon sites (circles) within the New river and James river watersheds (n=43).

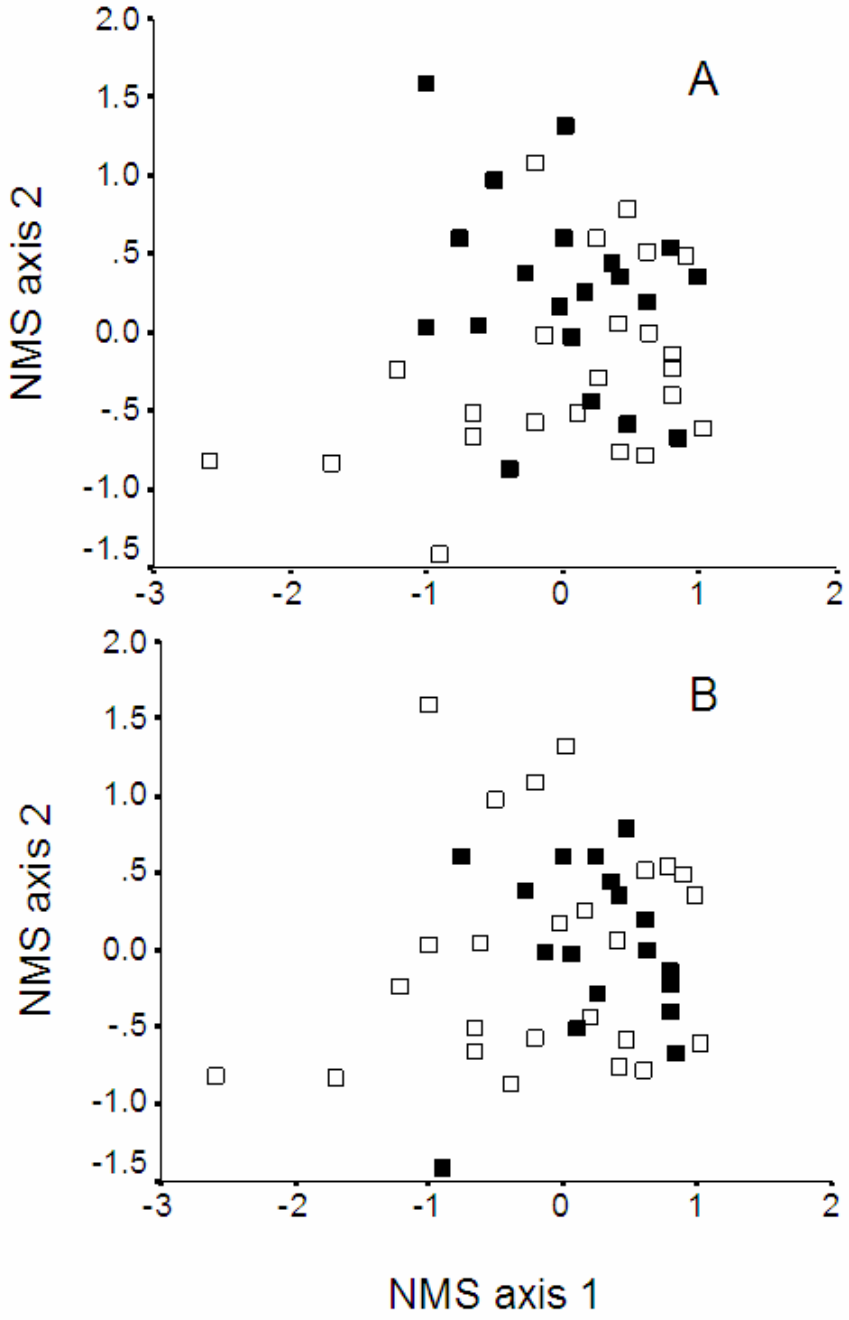

Figure 4.2. Plots of study sites in environmental-quality space based on non-metric multidimensional scaling (NMS) ordination. (A) James and New river basin sites (solid and open, respectively) and (B) ProbMon and EMAP sites (solid and open, respectively). Variable loadings are presented in Table 4.2.

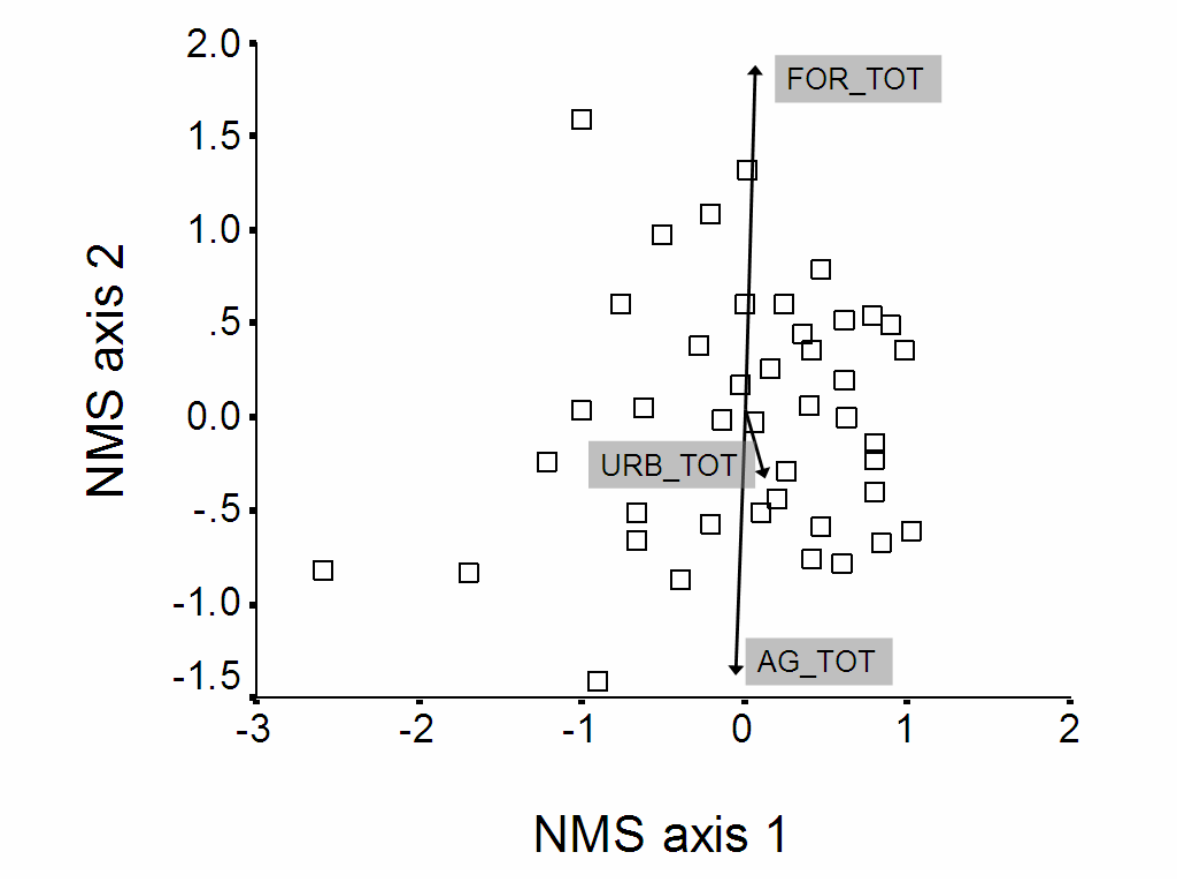

Figure 4.3. Plots of study sites based on non-metric multidimensional scaling (NMS) ordination with land use vectors. Vectors indicate the strength and direction of variable correspondence to NMS ordination axes (length and orientation of vector, respectively). Variable codes are presented in Table 4.1. Environmental variable loadings are reported in Table 4.2.

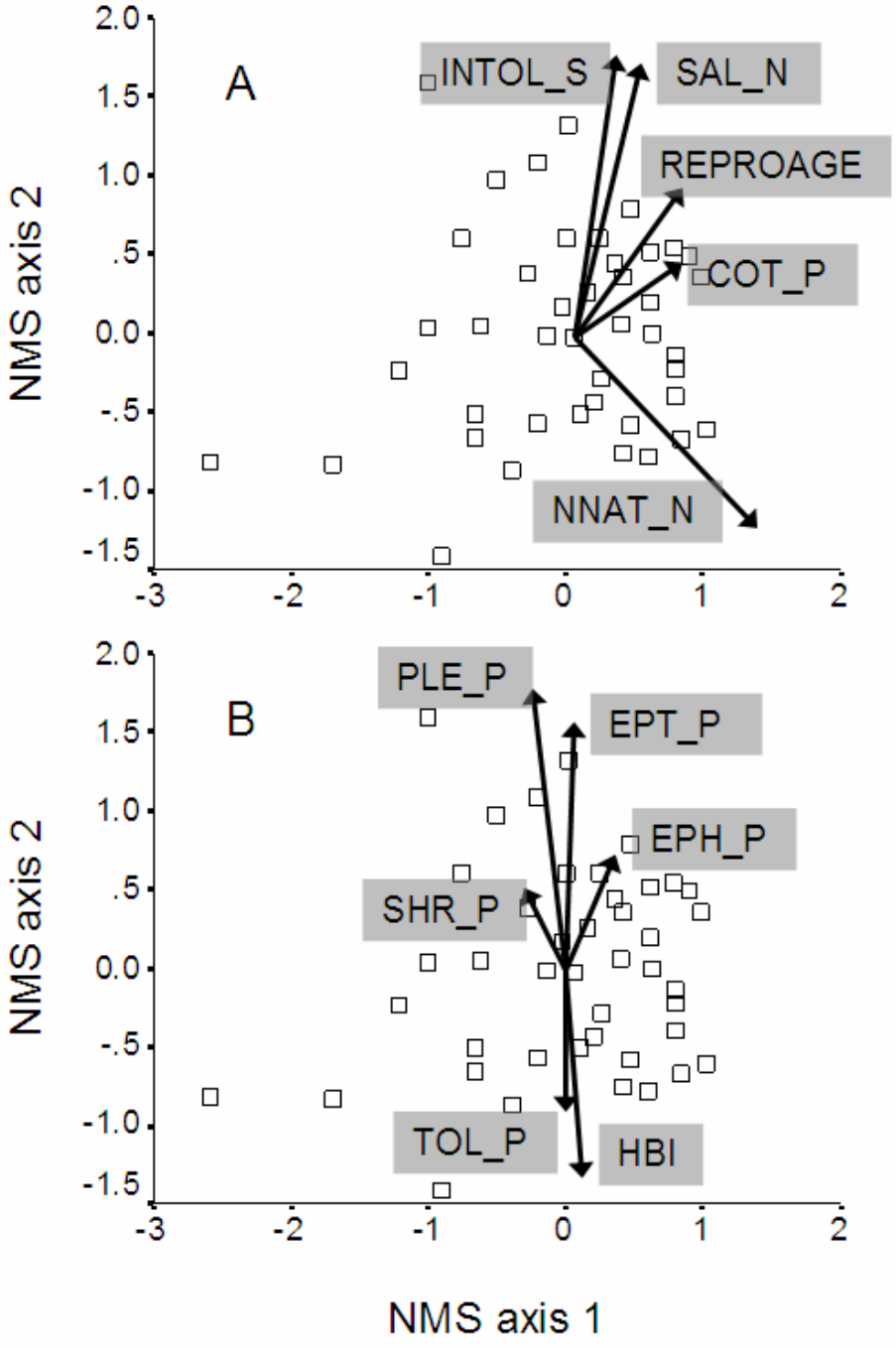

Figure 4.4. Plots of study sites in environmental-quality space based on non-metric multidimensional scaling (NMS) ordinations with (A) fish metric vectors and (B) benthic macroinvertebrate metric vectors. Vectors indicate the strength and direction of variable correspondence to NMS ordination axes (length and orientation of vector, respectively). Correlations between biotic metrics and NMS II are in Appendix L. Variable codes are in Table 4.1. Environmental variables loading into NMS axes are in Table 4.2.

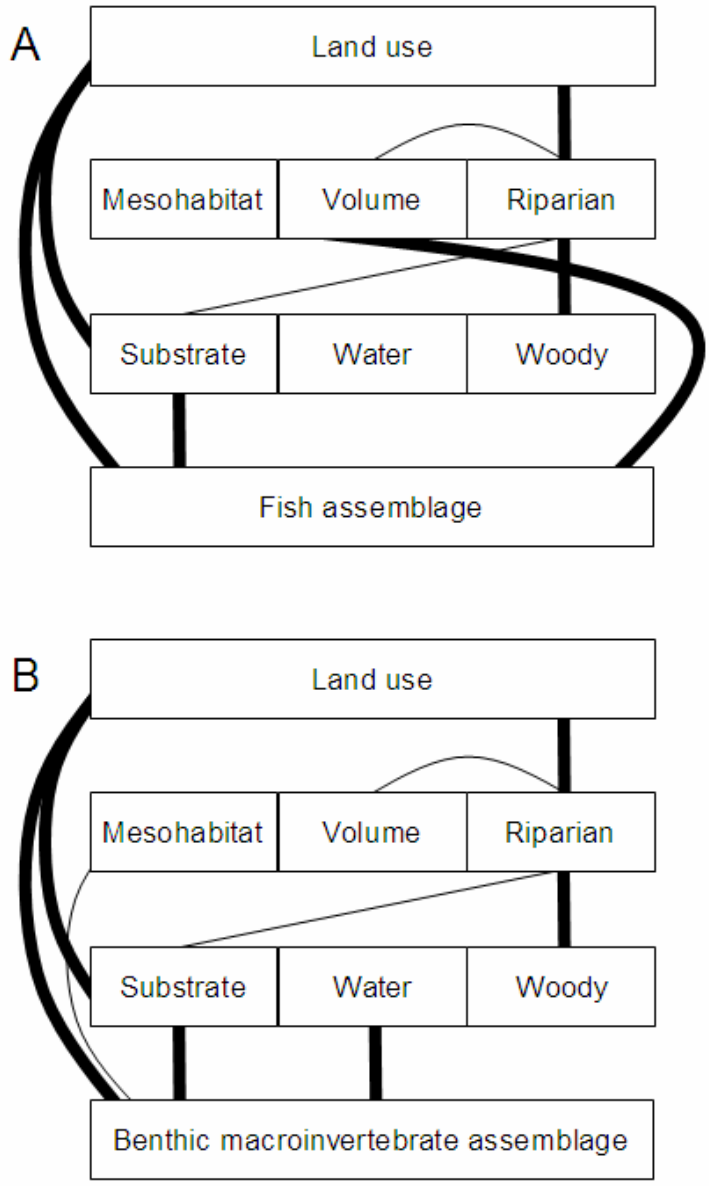

Figure 4.5. Mantel test path analyses for fish assemblages (A) and benthic macroinvertebrate assemblages (B). Vertical layers represent approximate spatial resolution of environmental variables (top = course-scale; bottom=fine-scale). Thick lines indicate $p<0.005$; thin lines indicate $p<0.05$. Variables are described in Table 4.1 (Water = water quality; Woody = woody debris in Table 4.1). Mantel correlations coefficients are in Table 4.5.

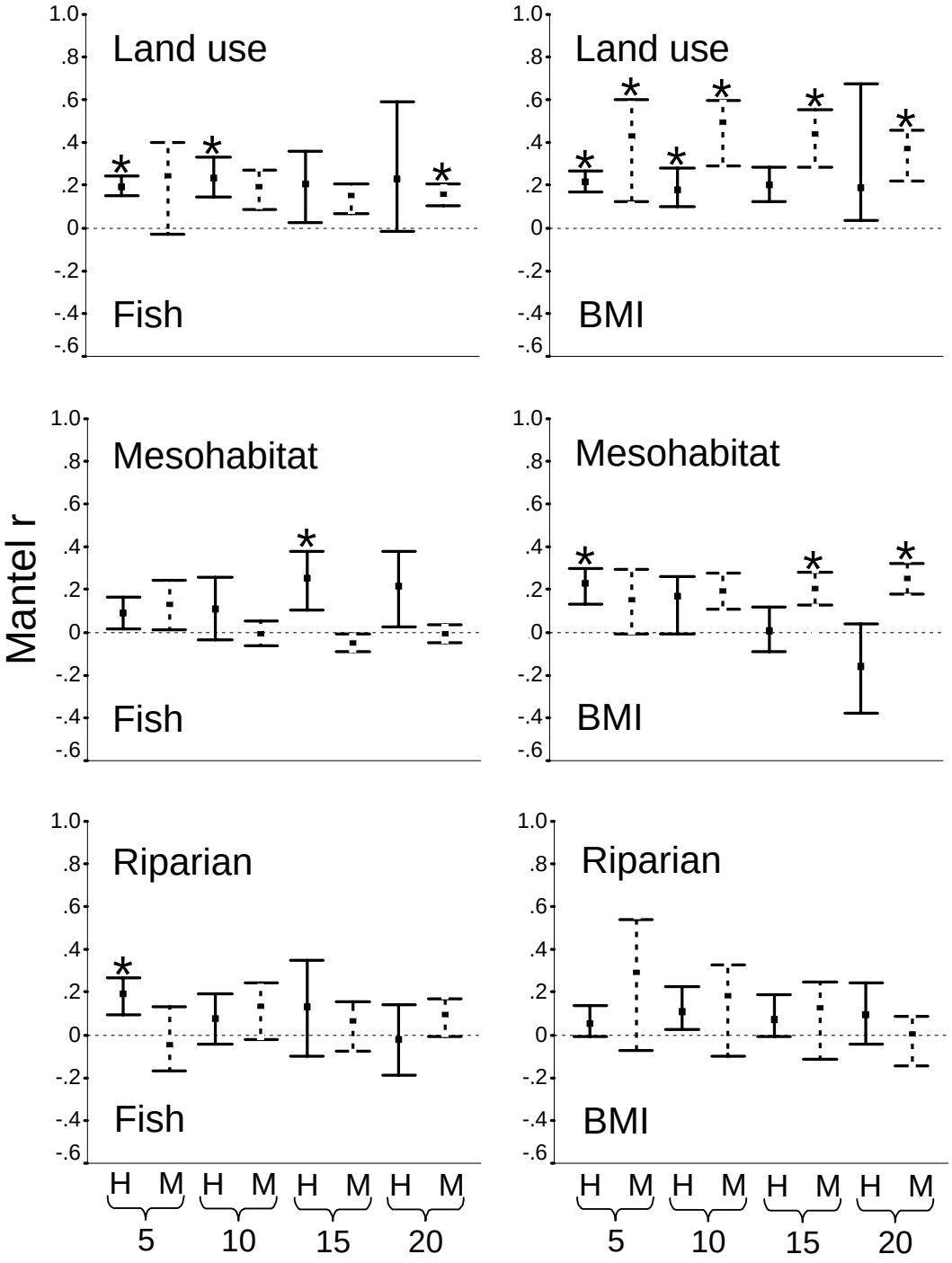

Figure 4.6. Mantel tests for fish and benthic macroinvertebrate (BMI) responses to seven environmental variables, as a function of stream network topology classes at increasing distances from collection sites. H=headwater tributary (solid lines) and M=mainstem tributary (dashed lines) within fluvial distances from sites (fkm). Error bars indicate 95% confidence intervals. Stars indicate where Mantel r correlation coefficients are significantly different from zero ($p<0.05$). 10,000 resampling iterations were used to assess Mantel significance and 10,000 bootstrap resamples were used to develop confidence intervals.

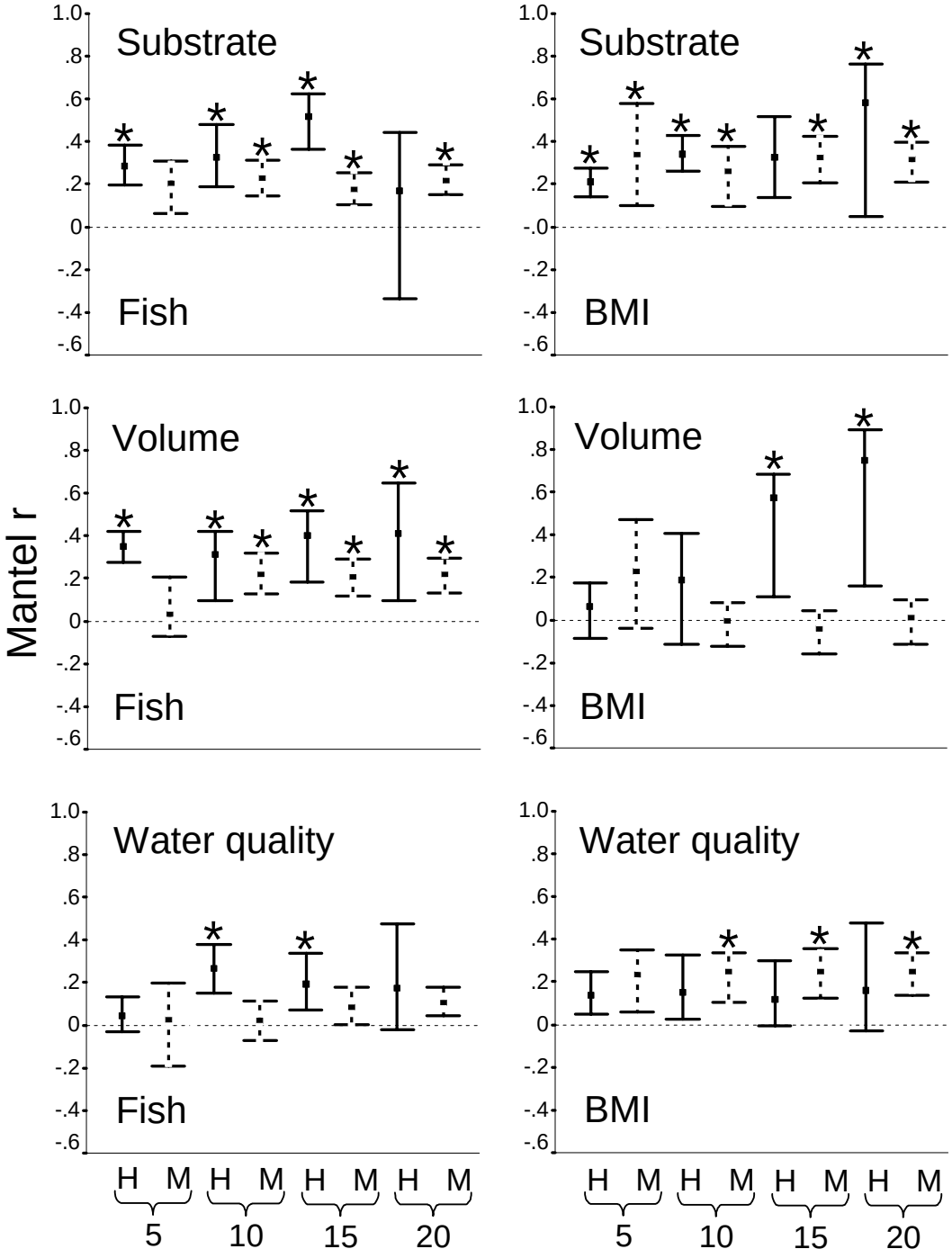

Figure 4.6., continued. Mantel tests for fish and benthic macroinvertebrate (BMI) responses to seven environmental variables, as a function of stream network topology classes at increasing distances from collection sites. H=headwater tributary (solid lines) and M=mainstem tributary (dashed lines) within fluvial distances from sites (fkm). Error bars indicate 95% confidence intervals. Stars indicate where Mantel r correlation coefficients are significantly different from zero ($p<0.05$). 10,000 resampling iterations were used to assess Mantel significance and 10,000 bootstrap resamples were used to develop confidence intervals.

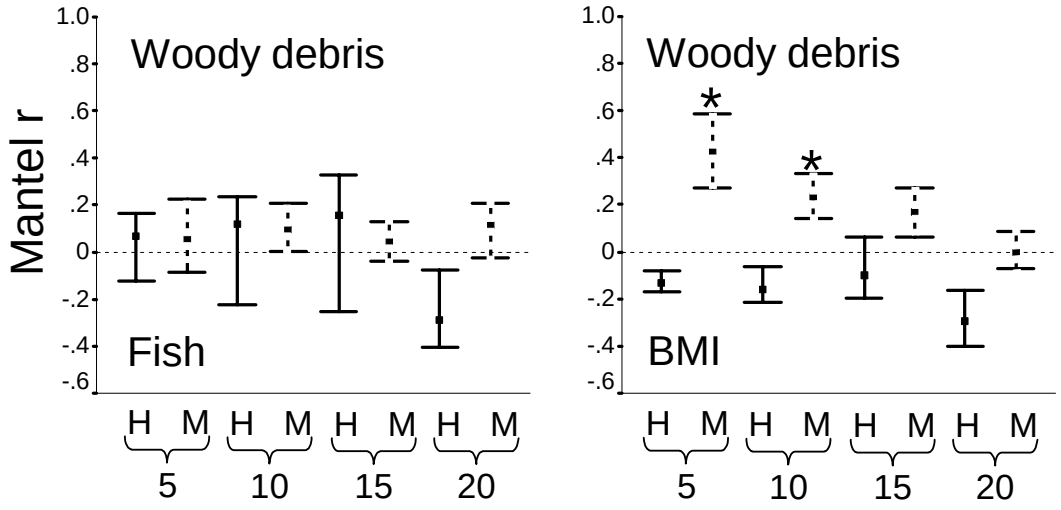

Figure 4.6., continued. Mantel tests for fish and benthic macroinvertebrate (BMI) responses to seven environmental variables, as a function of stream network topology classes at increasing distances from collection sites. H=headwater tributary (solid lines) and M=mainstem tributary (dashed lines) within fluvial distances from sites (fkm). Error bars indicate 95% confidence intervals. Stars indicate where Mantel r correlation coefficients are significantly different from zero ($p<0.05$). 10,000 resampling iterations were used to assess Mantel significance and 10,000 bootstrap resamples were used to develop confidence intervals.

Summary and Conclusions

CHAPTER 1.

A key challenge in stream fish ecology and biomonitoring is to partition local and regional influences on assemblage structure. Numerous studies have identified local determinants of species composition (i.e., competition, predation, habitat availability), but regional influences remain poorly understood. In this chapter, I test the hypotheses that (1) fish dispersal from adjacent streams influences local fish assemblage structure; and (2) the effects of inter-stream dispersal are mediated by local environmental conditions. I evaluated fish and physical habitat data from the U.S. EPA's Environmental Monitoring and Assessment Program in western Virginia streams (n=55). I found significant effects of adjacent stream size on local species richness, mean reproductive age, and riverine species richness. Large adjacent streams (>3rd-order) were associated with increased species richness in second-order sites. Fourth-order sites showed increased riverine species richness and decreased mean reproductive age in the presence of large adjacent streams. The nonrandom effects of adjacent stream size among sites of various stream orders suggests that local environmental conditions mediate the effects of dispersal from adjacent streams. Measures of channel shape (i.e., depth, width, and sinuosity) and microhabitat complexity (i.e., mean substrate size and woody debris) were associated with local assemblage structure in some cases, but did not account for significant variation in fish metrics explained by adjacent stream size. These results

indicate that the ability of fish biomonitoring metrics to detect anthropogenic impacts may be improved by calibrating scoring criteria based on the size of adjacent streams.

CHAPTER 2.

A fundamental challenge in ecology is to understand how local and regional processes influence species distributions and abundances. In stream environments, many studies have addressed local factors that affect fish assemblages (e.g., predation and competition) but regional influences remain poorly understood. In this chapter, I tested the hypotheses that (a) the size and proximity of connected streams (i.e., stream network topology) influences fish assemblage structure through dispersal processes, and (b) local stream size regulates the effects of dispersal. I used data from the USEPA's Environmental Monitoring and Assessment Program (EMAP) in wadeable streams of the mid-Atlantic highlands region (n=308). I evaluated site position in stream networks with a continuous analysis based on the rate of downstream flow accumulation from sites and with a discrete analysis based on the presence of large river confluences (i.e., upstream basin area >250km^2) within 20 fluvial km (fkm). Continuous variation in stream network topology was significantly related to fish assemblage structure (Bray-Curtis dissimilarities of species abundances) within a distance of approximately 9 fkm. This overall effect was explained by total species richness, catostomid species richness and abundance, cyprinid species richness, and riverine species richness. This effect was not explained by zoogeography, ecoregions, sampling month, sampling year, or spatial

autocorrelation. Sites near large river confluences supported greater species richness and abundance of catostomid, cyprinid, and ictalurid fishes than streams further than 20 fkm from large river confluences. The smallest sites were not affected by stream network position, consistent with the hypothesis that local stream size regulates the influence of regional dispersal. These results suggest that understanding local (i.e., reach-scale) fish assemblage organization may be improved by considering the size and proximity of connected streams.

CHAPTER 3.

Stream fish bioassessment methods assume that fish assemblages observed in a sample site reflect responses to local stressors, but previous studies suggest that fish assemblages are influenced not only by local factors, but also dispersal from and to connected streams. Interpretation of bioassessment studies therefore requires an understanding of the spatial scale of dispersal and how distant environmental conditions may constrain local responses to stressors. In this chapter, I explored the hypothesis that the size and proximity of connected streams (i.e., stream network topology) influences the sensitivity of local fish assemblages by regulating the spatial distribution of source populations and remote resources. I tested the predictions that (1) biotic attributes at stream sites connected to mainstem rivers (i.e., mainstem tributaries) would correspond less strongly to local environmental conditions than biotic attributes at stream sites lacking mainstem river confluences (i.e., headwater tributaries) and (2) stream network

effects on local biotic attributes would diminish with increasing distance from mainstem confluences. I used data from the U.S. EPA's Environmental Monitoring and Assessment Program (EMAP) for 157 stream sites in the mid-Atlantic highlands to characterize fish assemblage structure and local environmental conditions (i.e., stream volume, substrate size, water quality, mesohabitat, riparian vegetation, woody debris, and periphyton biomass). First, I used ordination methods to quantify gradients of environmental quality among sites. Second, I assessed the relationships among candidate fish metrics (biotic attributes) and the derived environmental quality gradients. Third, I compared fish metric correspondence to environmental gradients among mainstem tributaries and among headwater tributaries using ordination methods and randomization procedures. I identified 10 fish metrics that corresponded predictably to variation in environmental quality among study sites, encompassing taxonomic, reproductive, trophic, and tolerance measures of fish assemblage structure. Headwater and mainstem tributaries were not significantly different in local environmental conditions, but showed important differences in biotic responses to environmental quality gradients. Stream sites flowing into mainstem channels within 5 fluvial km showed consistently weaker relations to local environmental conditions than stream sites that lacked such mainstem river connections. Moreover, these patterns diminished at longer distances, in support of the dispersal hypothesis. These results suggest that the precision of fish bioassessment metrics may be improved by calibrating scoring criteria based on the spatial position of sites within stream networks.

CHAPTER 4.

Biological assessments (bioassessments) of freshwater ecosystems provide important tools for environmental quality analysis but require an understanding of the spatial scale at which biotic elements (organisms, populations, and assemblages) indicate environmental quality. In Virginia, the Department of Environmental Quality (VDEQ) currently uses benthic macroinvertebrates to assess stream quality but is evaluating the feasibility and utility of stream fish bioassessments. In this chapter, I (1) evaluated fish and benthic macroinvertebrate metric responses to an environmental quality gradient and (2) tested the hypothesis that the spatial position of stream sites would affect fish metrics more than benthic macroinvertebrate metrics due to fish dispersal from riverine sources. First, I collected stream fish and physical habitat data from a subset of VDEQ probabilistic monitoring (ProbMon) sites in the New and James river basins in west-central Virginia. Second, I combined datasets from ProbMon and U.S. Environmental Protection Agency (USEPA) Environmental Monitoring and Assessment Program (EMAP) sites and tested the comparability of these data sources. Third, I used ordination and randomization techniques to test metric responses to environmental quality gradients in the study area. Fourth, I explored the relative performance of biotic metrics among sites with and without large river confluences. I identified five fish metrics and six benthic macroinvertebrate metrics that corresponded predictably to variation in environmental quality within the study area. Metrics included taxonomic, trophic, reproductive, and tolerance measures of assemblage structure. Fish metrics showed greater sensitivity to the presence and absence of large rivers than benthic

macroinvertebrate metrics, consistent with the riverine dispersal hypothesis. These results suggest that stream fishes may be useful for stream quality assessments in Virginia (e.g., Clean Water Act §305b) and that stream fish metrics may complement current benthic macroinvertebrate metrics by indicating environmental conditions at larger spatial grains. However, additional research will be necessary to test empirically the inferences from the current study.

SYNTHESIS

Although it has long been recognized that streams are connected within watersheds (e.g., Shreve 1966), our understanding of the biological implications of stream connectivity is in its nascent stages (Fausch *et al*. 2002; Lowe *et al*. 2006). This dissertation demonstrates that (a) the size and proximity of connected streams (i.e., stream network topology) influences fish assemblage structure as a function of inter-stream dispersal and (b) the use of stream fish data to assess environmental quality requires consideration of stream network topology. Although previous studies have recognized the importance of inter-stream dispersal for fish populations and assemblages, my dissertation contributes the first analysis of inter-stream fish dispersal over a large geographic extent (i.e., mid-Atlantic highlands region) and as a function of distance from potential source populations. In addition, the current research presents the first test of the hypothesis that fish assemblage correspondence to local environmental conditions will be mediated by dispersal dynamics at larger spatial scales.

The results of this dissertation have several implications for stream fish ecology. I found evidence for riverine fish dispersal into wadeable streams up to a distance of 20 fkm (i.e., maximum spatial grain in the analysis) (Chapter 2). Moreover, dispersing individuals appear to be a non-random subset of the riverine fauna. Schlosser (1990) reported that riverine fishes tended to have greater mean body size and age of reproduction than species associated with smaller habitats (i.e., creeks). In contrast, I found evidence for riverine dispersal of small-bodied fishes with early ages of reproduction (Chapter 1). This finding is concordant with the notion that local stream size mediates the effects of regional dispersal (Chapter 2) as an interaction of local habitat suitability and regional colonist availability (Angermeier *et al*. 2002).

My results also have implications for the River Continuum Concept (RCC; Vannote *et al*. 1980). As predicted by the RCC, I found that stream volume was an important determinant of fish assemblage structure (Chapter 3). However, I also found that the relative importance of stream volume varied between streams that flow into rivers and streams those that do not (i.e., mainstem tributaties and headwater tributaries; respectively). Fish assemblages in mainstem tributaries showed consistently weaker relations to stream volume than fish assemblages in headwater tributaries (Chapter 3). This finding suggests that stream fish in mainstem tributaries are less contrained by local stream volume than fish assemblages in headwater tributaries due to the use of riverine areas as temporary habitats (e.g., feeding, refugia) and/or immigration from source populations (e.g., metapopulation dynamics). As a result, stream network topology provides a spatial framework for improving RCC predictions for stream fishes.

The results of this dissertation also have important implications for fish-based biological assessments of environmental quality (i.e., bioassessments). Although stream bioassessment studies typically assume that all sample sites are equally likely to detect all forms of environmental degradation, my results demonstrate that this assumption is incorrect. I found that stream fish assemblages showed consistent responses to environmental quality, but that the strength of these relationships varied based on the size and proximity of connected streams (Chapters 3 and 4). For example, fish assemblage structure was related to variation in water quality (i.e., nutrient concentrations) but fish assemblages in mainstem tributaries showed weaker relations to local water quality than fish assemblages in sites which lacked riverine confluences (i.e., headwater tributaries) (Chapter 3). Because these effects were not explained by differences in local habitat conditions between headwater tributaries and mainstem tributaries, the observed effects are consistent with the hypothesis of regional dispersal. Furthermore, stream network topology did not influence benthic macroinvertebrates (Chapter 4), suggesting that stream fishes may perceive environmental conditions at larger spatial grains.

The results of this dissertation suggest that stream bioassessments may be improved in two ways. First, fish bioassessment studies may benefit by developing separate metrics for headwater tributaries and mainstem tributaries. I found that, in sites connected to riverine habitats, dispersal will tend to diminish the sensitivity of fish metrics to local environmental conditions. Moreover, sample sites selected based on stream size criteria would not capture such effects of stream network topology. Second, fish bioassessment studies may be able to develop spatially-hierarchical analyses of environmental quality by selectively excluding or including riverine-dispersing fishes

from metric calculations. As a result, fish assemblages may provide information about local as well as regional environmental quality based on an understanding of fish dispersal within stream networks.

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Appendix A. Changes to EMAP fish data incorporated in Chapter 1 analyses. We replaced problematic records with the likely species or calculated mean metric values from congeners in Virginia (Jenkins and Burkhead 1994). Our rationale for all changes was based on data in Jenkins and Burkhead (1994).

EMAP site code	EMAP record	Current analysis	Rationale
MAIA97-052	*Micropterus punctulatus*	*M. dolomieu*	*M. punctulatus* is not reported in northern Virginia.
MAIA97-052	*Notropis amnis*	*Notropis* spp.	*Hybopsis amnis* is not reported in Virginia.
MAIA97-178	*Notropis photogenis*	*Notropis* spp.	*N. photogenis* is not reported in the James River basin.
MAIA97-179	*Esox americanus*	*E. niger*	*E. americanus* is not reported in the upper Roanoke River basin.
MAIA97-186	*Stizostedion canadense*	*Sander vitreum*	*S. canadense* is not reported in upper Big Sandy River basin; *Sander vitreum* is native to this basin.
VA526S	*Rhinichthys cataractae*	*Rhinichthys atratulus*	*R. cataractae* is unlikely in Big Sandy River basin.
VA770S	*Notropis atherinoides*	*Notropis* spp.	*N. atherinoides* is not reported in the Big Sandy River basin in Virginia.
VAR01S	*Etheostoma kennicotti*	*E. flabellare*	*E. kennicotti* is not reported in Virginia.
VAR09S	*Percina shumardi*	*Percina* spp.	*P. shumardi* is not reported in Virginia.
VAR09S	*Semotilus corporalis*	*S. atromaculatus*	*S. corporalis* is not reported in the New River basin in Virginia.
VAR09S	*Phoxinus erythryogaster*	*P. oreas*	*P. erythrogaster* is not reported in Virginia.
VAR12S	*Phoxinus erythryogaster*	*P. oreas*	*P. erythrogaster* is not reported in Virginia.

Appendix B. Changes to EMAP data incorporated in Chapter 2 analysis. I replaced problematic records with the likely species or deleted records where multiple similar species occur.

EMAP site code	EMAP record	Current analysis	Rationale
PA015S	Black bullhead, *Ameiurus melas*	Brown bullhead, *A. nebulosus*	*A. melas* is not reported in eastern Pennsylvania but *A. nebulosus* is.[1]
PA565S	Redside dace, *Clinostomus elongatus*	Rosyside dace, *C. funduloides*	*C. elongatus* is not reported in central Pennsylvania but *C. funduloides* is.[1]
PA792S	Rainbow darter, *E. caeruleum*	Fantail darter, *E. flabellare*	*E. caeruleum* is not reported in eastern Pennsylvania but *E. flabellare* is.[1]
PA808S	Mimic shiner, *Notropis volucellus*	Swallowtail shiner, *N. procne*	*N. volucellus* is not reported in eastern Pennsylvania but *N. procne* is.[1]
PA811S	River carpsucker, *Carpiodes carpio*	Quillback, *C. cyprinus*	*C. carpiodes* is not reported in south-central Pennsylvania but *C. cyprinus* is.[1]
PAR06S	Mimic shiner, *Notropis volucellus*	Swallowtail shiner, *N. procne*	*N. volucellus* is not reported in central Pennsylvania but *N. procne* is.[1]
VA507S	Roanoke hogsucker, *Hypentelium roanokense*	Northern hogsucker, *H. nigricans*	*H. roanokense* is not reported in James River basin but *H. nigricans* is.[2]
VA515S	New River shiner, *Notropis scabriceps*	Deleted	*N. scabriceps* is not reported in the Dan River basin in Virginia and the sample could be one of several other *Notropis* species.
VA522S	Roanoke logperch, *Percina rex*	Deleted	*P. rex* not reported in the New River basin and *P. caprodes* is very unlikely in this site.[2]
VA526S	Longnose dace, *Rhinichthys cataractae*	Blacknose dace, *R. atratulus*	*R. cataractae* is unlikely in the Big Sandy River basin but *R. atratulus* is common.[2]
VA536S	Steelcolor shiner, *C. whipplei*	Satinfin shiner, *C. analostana*	*C. whipplei* is not reported in the Potomac River basin but *C. analostana* is.[2]
VA542S	Striped shiner, *L. chrysocephalus*	Common shiner, *L. cornutus*	*L. chrysocephalus* is not reported in the Potomac River basin but *L. cornutus*

			is.[2]
VA543S	Striped shiner, *L. chrysocephalus*	Common shiner, *L. cornutus*	*L. chrysocephalus* is not reported in the Potomac River basin but *L. cornutus* is.[2]
VA558S	Rustyside sucker, *Moxostoma hamiltoni*	Torrent sucker, *M. rhothoeca*	*M. hamiltoni* is not reported in the Roanoke River basin but *M. rhothoeca* is.[2]
VA560S	Saffron shiner, *Notropis rubricroceus*	Swallowtail shiner, *N. procne*	N. *rubricroceus* is not reported in the Roanoke River basin and the stream size makes *N. volucellus* very unlikely.[2]
VA565S	Tennessee dace, *Phoxinus tennessensis*	Mountain redbelly dace, *P. oreas*	*P. tennessensis* is not reported in the New River basin but *P. oreas* is.[2]
VA569S	Longnose dace, *Rhinichthys cataractae*	Blacknose dace, *R. atratulus*	*R. cataractae* is not reported in the Tennessee River system in Virginia but *R. atratulus* is.[2]
VA571S	River shiner, *Notropis blennius*	Deleted	*N. blennius* is not reported in Virginia and the sample could be one of several other *Notropis* species.[2]
VA571S	Slimy sculpin, *Cottus cognatus*	Banded sculpin, *C. carolinae*	*C. cognatus* is not reported in the Tennessee River basin in Virginia but *C. carolinae* is.[2]
VA571S	Johnny darter, *Etheostoma nigrum*	Deleted	*E. nigrum* is not reported in the Tennessee River basin in Virginia and the sample could be one of several other *Etheostoma* species.[2]
VA571S	Riverweed darter, *Etheostoma podostomone*	Deleted	*E. podostomone* is not reported in the Tennessee River basin in Virginia and the sample could be one of several other *Etheostoma* species.[2]
VA571S	Cutlips minnow, *Exoglossum maxillingua*	Deleted	Neither *E. maxillingua* nor *E. laurae* is reported in the Tennessee River basin in Virginia.[2]
VA571S	Longnose dace, *Rhinichthys cataractae*	Blacknose dace, *R. atratulus*	*R. cataractae* is not reported in the Tennessee River basin in Virginia and *R. atratulus*

			is.[2]
VA572S	Eastern mudminnow, *Umbra limi*	Deleted	Neither *U. limi* nor *U. pygmaea* is reported in the Tennessee River basin in Virginia.[2]
VA572S	Slimy sculpin, *Cottus cognatus*	Banded sculpin, *C. carolinae*	*C. cognatus* is not reported in the Tennessee River basin in Virginia but *C. carolinae* is.[2]
VA572S	Riverweed darter, *Etheostoma podostomone*	Deleted	*E. podostomone* is not reported in the Tennessee River basin in Virginia and the sample could be one of several other *Etheostoma* species.[2]
VA574S	Longnose dace, *Rhinichthys cataractae*	Blacknose dace, *R. atratulus*	*R. cataractae* is not reported in the Tennessee River basin in Virginia and *R. atratulus* is.[2]
VA770S	Emeralid shiner, *Notropis atherinoides*	Deleted	*N. atherinoides* is not reported in the Big Sandy River basin in Virginia and the sample could be one of several other *Notropis* species.[2]
VA800S	Tennessee dace, *Phoxinus tennessensis*	Mountain redbelly dace, *P. oreas*	*P. tennessensis* is not reported in the New River basin and *P. oreas* is.[2]
VA805S	Chestnut lamprey, *Ichthyomyzon castenatus*	Ohio lamprey, *I. bdellium*	*I. castenatus* is not reported in the Tennessee River basin in Virginia and *I. bdellium* is.[2]
VA806S	Longnose dace, *Rhinichthys cataractae*	Blacknose dace, *R. atratulus*	*R. cataractae* is not reported in the Tennessee River basin in Virginia and *R. atratulus* is.[2]
VA810S	Bluehead chub, *Nocomis leptocephalus*	River chub, *N. micropogon*	*N. leptocephalus* is not reported in the Tennessee River basin in Virginia and *N. micropogon* is.[2]
VA810S	Longnose dace, *Rhinichthys cataractae*	Blacknose dace, *R. atratulus*	*R. cataractae* is not reported in the Tennessee River basin in Virginia and *R. atratulus* is.[2]
VA811S	Longnose dace, *Rhinichthys*	Blacknose dace, *R. atratulus*	*R. cataractae* is not reported in the Tennessee River basin

	cataractae		in Virginia and *R. atratulus* is.[2]
VA812S	Longnose dace, *Rhinichthys cataractae*	Blacknose dace, *R. atratulus*	*R. cataractae* is not reported in the Tennessee River basin in Virginia and *R. atratulus* is.[2]
VA813S	Longnose dace, *Rhinichthys cataractae*	Blacknose dace, *R. atratulus*	*R. cataractae* is not reported in the Tennessee River basin in Virginia and *R. atratulus* is.[2]
VAR01S	Potomac sculpin, *Cottus girardi*	Mottled sculpin, *C. bairdi*	*C. girardi* is not reported in the New River basin and *C. bairdi* is.[2]
VAR01S	Cutlips minnow, *Exoglossum maxillingua*	Tonguetied minnow, *E. laurae*	*E. laurae* is considered to be native in Big Walker Creek (New River basin) and *E. maxillingua* has not been reported this far upstream.[2]
VAR01S	Stripetail darter, *Etheostoma kennicotti*	Fantail darter, *E. flabellare*	*E. kennicotti* is not reported in the New River basin in Virginia but *E. flabellare* is.[2]
VAR06	Yellowfin madtom, *Noturus flavipinnis*	Orangefin madtom, *N. gilberti*	*N. flavipinnis* is not reported in the Roanoke River basin but *N. gilberti* is endemic in this basin.[2]
VAR06S	Southern redbelly dace, *Phoxinus erythrogaster*	Mountain redbelly dace, *P. oreas*	*P. erythrogaster* is not reported in the Roanoke River basin but *P. oreas* is.[2]
VAR06S	Fallfish, *Semotilus corporalis*	Creek chub, *S. atromaculatus*	*S. corporalis* is not reported in the upper Roanoke River basin but *S. atromaculatus* is.[2]
VAR09S	Fallfish, *Semotilus corporalis*	Creek chub, *S. atromaculatus*	*S. corporalis* is not reported in the New River basin in Virginia but *S. atromaculatus* is.[2]
VAR09S	Southern redbelly dace, *Phoxinus erythrogaster*	Mountain redbelly dace, *P. oreas*	*P. erythrogaster* is not reported in the New River basin in Virginia but *P. oreas* is.[2]
VAR09S	River darter, *Percina shumardi*	Deleted	*P. shumardi* is not reported in Virginia and the sample could be one of several other percid species.[2]
VAR12S	Southern redbelly dace, *Phoxinus*	Mountain redbelly dace, *P. oreas*	P. erythrogaster is not reported in the James River

	erythrogaster		basin but P. oreas is.[2]
VAR14S	Black sculpin, *C. baileyi*	Mottled sculpin, *C. bairdi*	C. baileyi is not reported in the New River basin. Morphological traits suggest *C. bairdi* instead of *C. carolinae*.[2]
VAR14S	Cutlips minnow, *Exoglossum maxillingua*	Tonguetied minnow, *E. laurae*	*E. laurae* is considered to be native in Big Walker Creek (New River basin) and *E. maxillingua* has not been reported this far upstream.[2]
VAR17S	Fallfish, *Semotilus corporalis*	Creek chub, S. atromaculatus	*S. corporalis* is not reported in the upper Roanoke River basin but *S. atromaculatus* is.[2]
VAR25S	Tessellated darter, *Etheostoma olmstedi*	Johnny darter, *E. nigrum*	*E. olmstedi* is not reported in the James River basin but *E. nigrum* is.[2]
VAT02S	Striped shiner, *Luxilus chrysocephalus*	White shiner, *L. albeolus*	*L. chrysocephalus* is not reported in the New River basin in Virginia but *L. albeolus* is.[2]
WV514S	Common shiner, *Luxilus cornutus*	Striped shiner, *Luxilus chrysocephalus*	*L. cornutus* is not reported in western West Virginia and *L. chrysocephalus* is.[3]
WV520S	Black redhorse, *Moxostoma duquesnei*	Golden redhorse, *M. erythrurum*	*M. duquesnei* is not reported in the Potomac River basin in West Virginia but *M. erythrurum* is.[3]
WV779S	Whitetail shiner, *Cyprinella galactura*	Spotfin shiner, *C. spiloptera*	*C. galactura* is not reported in the Greenbrier River basin but *C. spiloptera* is.[3]
WVR02S	Rosefin shiner, *Lythrurus ardens*	Deleted	*L. ardens* is not reported in the Potomac River basin in West Virginia and the sample could be one of several other *Notropis* species.[3]
WVR02S	Mirror shiner, *Notropis spectrunculus*	Deleted	*N. spectrunculus* is not reported in the Potomac River basin in West Virginia and the sample could be one of several other *Notropis* species.[3]
WVR03S	Rosefin shiner, *Lythrurus ardens*	Deleted	*L. ardens* is not reported in the Potomac River basin in

			West Virginia and the sample could be one of several other *Notropis* species.[3]
MAIA97-016	Creek chubsucker, *Erimyzon oblongus*	Deleted	*E. oblongus* is not reported in western West Virginia.[3]
MAIA97-016	Common shiner, *Luxilus cornutus*	Striped shiner, *L. chrysocephalus*	*L. cornutus* is not reported in western West Virginia but *L. chrysocephalus* is.[3]
MAIA97-028	Cresent shiner, *Luxilus cerasinus*	Common shiner, *Luxilus cornutus*	*L. cerasinus* is not reported in southwest Pennsylvania but *L. cornutus* is.[1]
MAIA97-046	Johnny darter, *Etheostoma nigrum*	Tessellated darter, *E. olmstedi*	*E. nigrum* is not reported in south-central Pennsylvania. Morphological traits are similar to *E. olmstedi*.[1]
MAIA97-052	Pallid shiner, *Hybopsis amnis*	Deleted	*H. amnis* is not reported in Virginia and the sample could be one of several *Notropis* species.[2]
MAIA97-052	Spotted bass, *Micropterus punctulatus*	Smallmouth bass, *M. dolomieu*	*M. punctulatus* is not reported in northern Virginia but *M. dolomieu* is.[2]
MAIA97-069	Creek chubsucker, *Erimyzon oblongus*	Deleted	*E. oblongus* is not reported in central Pennsylvania.[1]
MAIA97-074	Longnose dace, *Rhinichthys cataractae*	Blacknose dace, *R. atratulus*	*R. cataractae* is not reported in western West Virginia but *R. atratulus* is.[3]
MAIA97-083	Mountain redbelly dace, *Phoxinus oreas*	Deleted	Site is outside the known range of *P. oreas*, *P. eos*, and *P. erythrogaster*.
MAIA97-084	Johnny darter, *Etheostoma nigrum*	Tessellated darter, *E. olmstedi*	*E. nigrum* is not reported in eastern Pennsylvania. Morphological traits are similar to *E. olmstedi*.[1]
MAIA97-085	Johnny darter, *Etheostoma nigrum*	Tessellated darter, *E. olmstedi*	*E. nigrum* is not reported in eastern Pennsylvania. Morphological traits are similar to *E. olmstedi*.[1]
MAIA97-085	Cresent shiner, *Luxilus cerasinus*	Common shiner, *Luxilus cornutus*	*L. cerasinus* is not reported in eastern Pennsylvania but *L. cornutus* is.[1]
MAIA97-086	Johnny darter, *Etheostoma nigrum*	Tessellated darter, *E. olmstedi*	*E. nigrum* is not reported in eastern Pennsylvania. Morphological traits are similar to *E. olmstedi*.[1]

MAIA97-087	Black bullhead, *Ameiurus melas*	Brown bullhead, *A. nebulosus*	*A. melas* is not reported in western Deleware but *A. nebulosus* is.[1]
MAIA97-087	Johnny darter, *Etheostoma nigrum*	Tessellated darter, *E. olmstedi*	*E. nigrum* is not reported in western Deleware. Morphological traits are similar to *E. olmstedi*.[1]
MAIA97-092	Johnny darter, *Etheostoma nigrum*	Tessellated darter, *E. olmstedi*	*E. nigrum* is not reported in eastern Pennsylvania. Morphological traits are similar to *E. olmstedi*.[1]
MAIA97-093	Sand shiner, *Notropis stramineus*	Swallowtail shiner, *N. procne*	*N. stramineus* is not reported in eastern Pennsylvania but *N. procne* is.[1]
MAIA97-133	Blackside dace, *Phoxinus cumberlandensis*	Mountain redbelly dace, *P. oreas*	*P. cumberlandensis* is not reported in the New River basin but *P. oreas* is.[3]
MAIA97-186	Sauger, *Sander canadensis*	Walleye, *S. vitreus*	*S. canadensis* not reported in upper Big Sandy River basin; *S. vitreus* is native in this basin.[2]
MAIA98-070	Sand shiner, *Notropis stramineus*	Swallowtail shiner, *N. procne*	*N. stramineus* is not reported in southeastern New York but *N. procne* is.[1]
MAIA98-177	Longnose dace, *Rhinichthys cataractae*	Blacknose dace, *R. atratulus*	*R. cataractae* is not reported in western West Virginia but *R. atratulus* is.[3]

[1]Page and Burr (1991)
[2]Jenkins and Burkhead (1994)
[3]Stauffer *et al*. (1995)

Appendix C. List of species included in the EMAP dataset and the calculations of "river" and "creek" species richness and abundance in Chapter 2. Habitat size assignments are from Jenkins and Burkhead (1994) unless indicated by a superscript. Modifications to EMAP raw data are described in Appendix B.

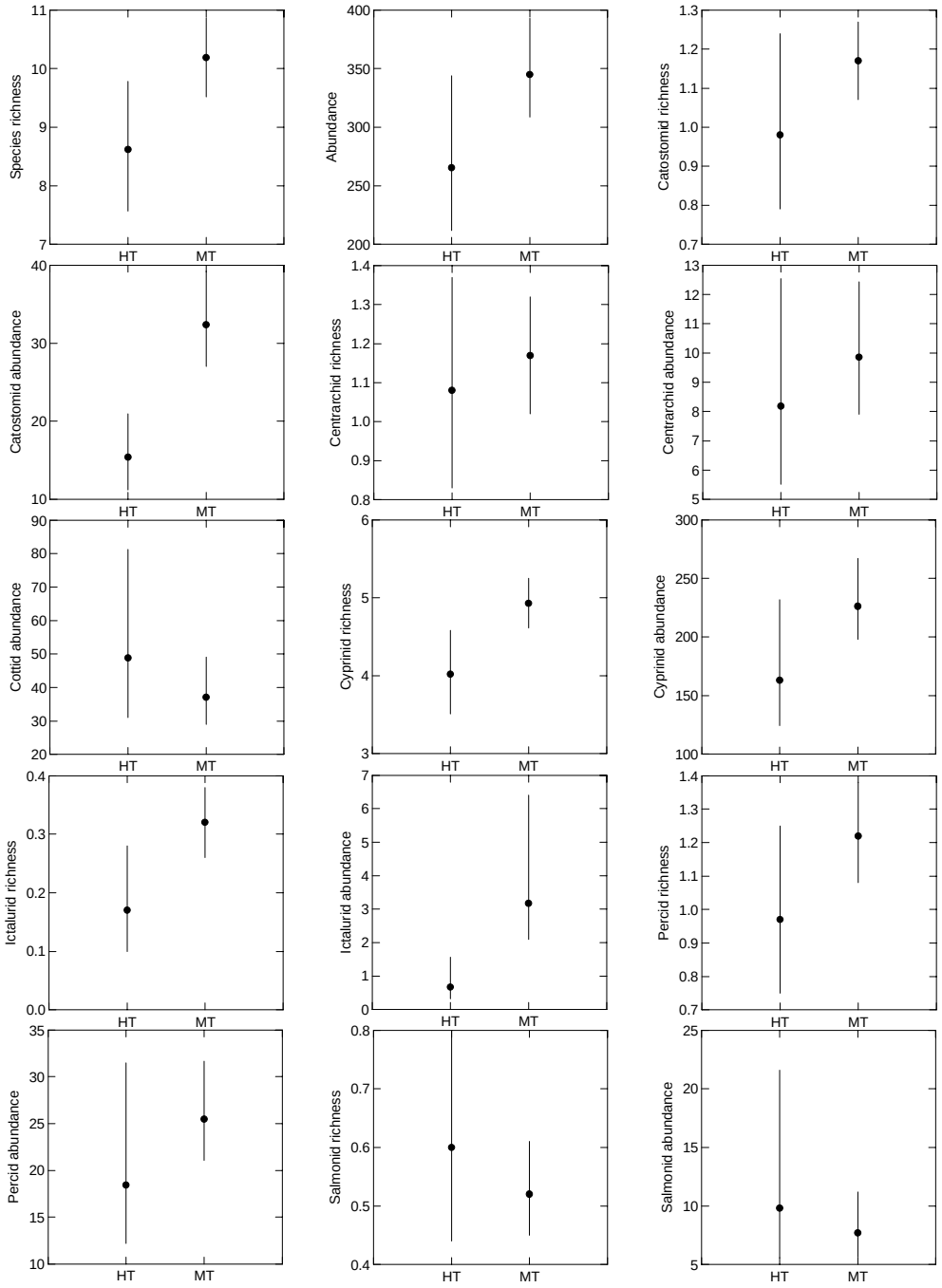
Family	Scientific name	Common name	Habitat size
Anguillidae	*Anguilla rostrata*	American eel	
Atherinidae	*Labidesthes sicculus*	Brook silverside	River
Catostomidae	*Carpiodes cyprinus*	Quillback	River
	Catostomus commersoni	White sucker	
	Erimyzon oblongus	Creek chubsucker	
	Hypentelium nigricans	Northern hog sucker	
	Hypentelium roanokense	Roanoke hog sucker	Creek
	Ictiobus bubalus	Smallmouth buffalo	River[1]
	Moxostoma cervinum	Black jumprock	
	Moxostoma duquesnei	Black redhorse	
	Moxostoma erythrurum	Golden redhorse	
	Moxostoma macrolepidotum	Shorthead redhorse	River
	Thoburnia rhothoeca	Torrent sucker	Creek
Centrarchidae	*Ambloplites rupestris*	Rock bass	
	Lepomis macrochirus	Bluegill	
	Lepomis cyanellus	Green sunfish	
	Lepomis megalotis	Longear sunfish	River
	Lepomis auritus	Redbreast sunfish	
	Lepomis gulosus	Warmouth	
	Lepomis gibbosus	Pumpkinseed	
	Micropterus salmoides	Largemouth bass	
	Micropterus dolomieu	Smallmouth bass	
	Micropterus punctulatus	Spotted bass	River
	Pomoxis nigromaculatus	Black crappie	
	Pomoxis annularis	White crappie	River
Clupeidae	*Dorosoma cepedianum*	Gizzard shad	River
Cottidae	*Cottus carolinae*	Banded sculpin	
	Cottus baileyi	Black sculpin	Creek
	Cottus bairdi	Mottled sculpin	Creek
	Cottus girardi	Potomac sculpin	
	Cottus cognatus	Slimy sculpin	Creek
Cyprinidae	*Campostoma anomalum*	Central stoneroller	
	Clinostomus elongatus	Redside dace	Creek[2]
	Clinostomus funduloides	Rosyside dace	Creek
	Cyprinella analostana	Satinfin shiner	River
	Cyprinella spiloptera	Spotfin shiner	River
	Cyprinella galactura	Whitetail shiner	
	Cyprinus carpio	Common carp	River
	Erimystax insignis	Blotched chub	
	Exoglossum maxillingua	Cutlips minnow	

	Exoglossum laurae	Tonguetied minnow	Creek
	Hybognathus regius	Eastern silvery minnow	
	Hybopsis amblops	Bigeye chub	
	Luxilus cornutus	Common shiner	
	Luxilus cerasinus	Crescent shiner	Creek
	Luxilus chrysocephalus	Striped shiner	
	Luxilus coccogenis	Warpaint shiner	
	Luxilus albeolus	White shiner	
	Lythrurus lirus	Mountain shiner	Creek
	Lythrurus ardens	Rosefin shiner	
	Margariscus margarita	Pearl dace	Creek
	Nocomis platyrhynchus	Bigmouth chub	River
	Nocomis leptocephalus	Bluehead chub	
	Nocomis raneyi	Bull chub	River
	Nocomis micropogon	River chub	River
	Notemigonus crysoleucas	Golden shiner	
	Notropis bifrenatus	Bridle shiner	Creek
	Notropis amoenus	Comely shiner	River
	Notropis atherinoides	Emerald shiner	River
	Notropis volucellus	Mimic shiner	River
	Notropis scabriceps	New River shiner	
	Notropis rubellus	Rosyface shiner	River
	Notropis rubricroceus	Saffron shiner	Creek
	Notropis stramineus	Sand shiner	River
	Notropis photogenis	Silver shiner	River
	Notropis buccatus	Silverjaw minnow	
	Notropis hudsonius	Spottail shiner	
	Notropis procne	Swallowtail shiner	
	Notropis telescopus	Telescope shiner	
	Notropis leuciodus	Tennessee shiner	
	Phenacobius teretulus	Kanawha minnow	
	Phenacobius uranops	Stargazing minnow	River
	Phoxinus oreas	Mountain redbelly dace	Creek
	Phoxinus erythrogaster	Southern redbelly dace	Creek[1]
	Phoxinus tennesseensis	Tennessee dace	Creek
	Pimephales notatus	Bluntnose minnow	
	Pimephales promelas	Fathead minnow	
	Rhinichthys atratulus	Blacknose dace	Creek
	Rhinichthys cataractae	Longnose dace	
	Semotilus atromaculatus	Creek chub	Creek
	Semotilus corporalis	Fallfish	River
Esocidae	*Esox niger*	Chain pickerel	
	Esox americanus	Redfin pickerel	
Fundulidae	*Fundulus diaphanus*	Banded killifish	
	Fundulus catenatus	Northern studfish	River
Ictaluridae	*Ameiurus nebulosus*	Brown bullhead	

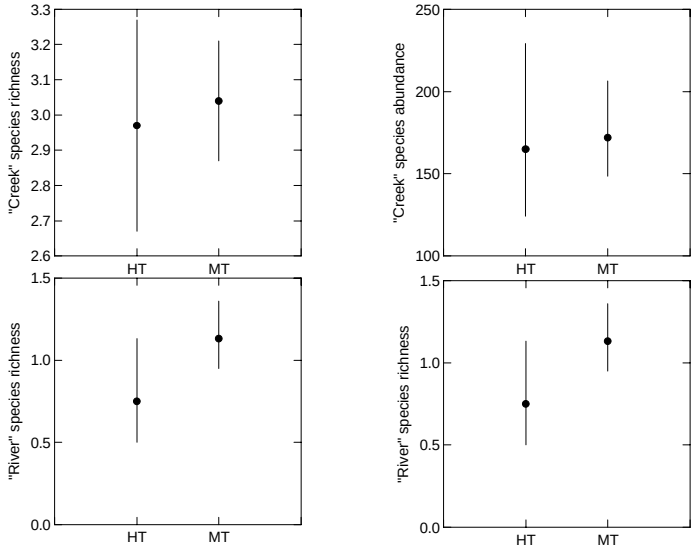
	Ameiurus natalis	Yellow bullhead	
	Ictalurus punctatus	Channel catfish	River
	Noturus insignis	Margined madtom	
	Noturus flavus	Stonecat	River
	Noturus gilberti	Orangefin madtom	
	Pylodictis olivaris	Flathead catfish	River
Lepisosteidae	*Lepisosteus osseus*	Longnose gar	River
Percidae	*Etheostoma zonale*	Banded darter	River
	Etheostoma camurum	Bluebreast darter	River
	Etheostoma osburni	Candy darter	
	Etheostoma flabellare	Fantail darter	
	Etheostoma blennioides	Greenside darter	River
	Etheostoma nigrum	Johnny darter	
	Etheostoma kanawhae	Kanawha darter	
	Etheostoma longimanum	Longfin darter	Creek
	Etheostoma caeruleum	Rainbow darter	
	Etheostoma rufilineatum	Redline darter	
	Etheostoma podostemone	Riverweed darter	
	Etheostoma simoterum	Snubnose darter	
	Etheostoma stigmaeum	Speckled darter	River
	Etheostoma swannanoa	Swannanoa darter	
	Etheostoma olmstedi	Tesselated darter	
	Etheostoma variatum	Variegate darter	Creek
	Perca flavescens	Yellow perch	
	Percina macrocephala	Longhead darter	
	Percina gymnocephala	Appalachia darter	River
	Percina maculata	Blackside darter	
	Percina burtoni	Blotchside logperch	River
	Percina evides	Gilt darter	River
	Percina caprodes	Logperch	River
	Percina rex	Roanoke logperch	River
	Percina peltata	Shield darter	
	Sander vitreus	Walleye	River
	Sander canadense	Sauger	River
Percopsidae	*Percopsis omiscomaycus*	Trout perch	River
Petromyzontidae	*Ichthyomyzon bdellium*	Ohio lamprey	River
	Ichthyomyzon greeleyi	Mountain brook lamprey	Creek
	Lampetra appendix	American brook lamprey	Creek
	Lampetra aepyptera	Least brook lamprey	Creek
Salmonidae	*Oncorhynchus mykiss*	Rainbow trout	Creek
	Salmo trutta	Brown trout	Creek
	Salvelinus fontinalis	Brook trout	Creek
Sciaenidae	*Aplodinotus grunniens*	Freshwater drum	River
Umbridae	*Umbra pygmaea*	Eastern mudminnow	Creek

[1]Etnier and Starnes (1993)
[2]Lee *et al*. (1980)

Appendix D. Bootstrapped mean values (circles) and 90% confidence intervals (whiskers) for fish metrics in headwater streams (HT) and mainstem streams (MT). 10,000 resampling iterations were used for bootstrapping. See text for methods used to classify HT and MT sites.



Appendix D., continued. Bootstrapped mean values (circles) and 90% confidence intervals (whiskers) for fish metrics in headwater streams (HT) and mainstem streams (MT). 10,000 resampling iterations were used for bootstrapping. See text for methods used to classify HT and MT sites.



Appendix E. Fish species classifications for metric calculations in Chapter 3. Families are separated by horizontal lines. Species introductions are designated by river basins as: A = Atlantic slopes, J = James, P = Potomac, R = Roanoke, H = Rappahannock, S = Susquehanna, Y = York, G = Allegheny, K = Kanawha, M = Monongahela, N = New, T = Tennessee, ALL = introduced throughout study area. Reproductive guilds are designed as: NL = non-lithophil, SL = simple lithophil, CL = complex lithophil (i.e., mineral substrate spawning with nest preparation and/or parental care). Spawning substrates are designed as: MIN = mineral (excluding fine substrates), VAR = various substrates, and VEG = vegetation. Vertical positions are indicated as: BE = benthic species and CO = water column species. Trophic guilds are indicated as: INV = invertivore, IP = invertivore/piscivore, OH = omnivore/herbivore, and PIS = piscivore. Tolerance levels are indicated as: TOL = tolerant species and INT = intolerant species. In all cases, "NA" indicates that the variable is not applicable for the given species.

Scientific name	Introductions[a]	Repro. guild[b]	Nest prep. and/or parental care[c]	Spawning substrate[d]	Female repro. age (yr)[e]	Vertical position[f]	Trophic guild[g]	Number of food types[h]	Tolerance[i]
Anguilla rostrata		NA	NA	NA	5	CO	IP	2	
Labidesthes sicculus	ANK	NL	NO[1]	VAR	1	CO	INV	1[1]	
Catostomus commersoni		SL	NO	MIN	3	BE	OH	3	TOL
Erimyzon oblongus		CL	NO	VAR	2	BE	INV	3	
Hypentelium nigricans		SL	NO	MIN	3	BE	INV	2	
Ictiobus bubalus		NL[5]	NO[5]	VAR[5]	2[5]	BE[5]	INV[5]	2[5]	
Moxostoma cervinum		SL	NO	MIN	2	BE	INV	3	
M. duquesnei		SL	NO	MIN	3	BE	INV	3	
M. erythrurum	NP	SL	NO	MIN	4	BE	INV	3	
M. macrolepidotum		SL	NO	MIN	4	BE	INV	3	
Thoburnia rhothoeca		SL	NO	MIN	3	BE	OH	3	INT
Ambloplites rupestris	AN	CL	YES	MIN	2	CO	IP	2	
Lepomis auritus	NT	CL	YES	MIN	2	CO	IP	2	
L. cyanellus	A	NL	YES	VAR	1	CO	IP	2	TOL

L. gibbosus	NKT	NL	YES	VAR	1	CO	INV	1	
L. gulosus	ST	NL	YES	VAR	1	CO	IP	2	
L. macrochirus	ANT	NL	YES	VAR	1	CO	INV	1	TOL
L. megalotis	AN	CL	YES	MIN	2	CO	INV	1	
Micropterus dolomieu	AN	CL	YES	MIN	2	CO	IP	2	
M. punctulatus	AN	NL	YES	VAR	2	CO	IP	2	
M. salmoides	AN	NL	YES	VAR	2	CO	PIS	1	
Pomoxis annularis	AN	NL	YES	VAR	2	CO	IP	2	
P. nigromaculatus	AN	NL	YES	VAR	2	CO	IP	2	
Dorosoma cepedianum		NL	NO	VAR	2	CO	OH	2	TOL
Cottus baileyi		NL	YES	VAR	2	BE	INV	1	
C. bairdi		NL	YES	VAR	2	BE	INV	1	
C. cognatus		NL	YES	VAR	2	BE	INV	1	
C. carolinae		NL	YES	VAR	2	BE	INV	1	
C. girardi		NL	YES	VAR	2	BE	INV	1	
Campostoma anomalum		CL	YES	MIN	2	BE	OH	2	
Clinostomus elongatus		SL	NO[6]	MIN[6]	2[6]	CO	INV[6]	1[1]	
C. funduloides		SL	NO	MIN	2	CO	INV	1	
Cyprinella galactura	K	NL	NO	VAR	2	CO	INV	2	
C. spiloptera		NL	NO	VAR	1	CO	INV	3	
Cyprinus carpio	ALL	NL	NO	VAR	3	CO	OH	4	TOL
Erimystax insignis		SL	NO	MIN	1	BE	OH	3	
Exoglossum laurae		CL	YES	MIN	2	CO	INV	1	
E. maxillingua	N	CL	YES	MIN	2	CO	INV	1	
Hybopsis amblops		SL	NO	MIN	1	CO	INV	1	INT
Luxilus albeolus		SL	NO	MIN	1	CO	INV	1	
L. cerasinus	J	SL	NO	MIN	2	CO	INV	2	
L. chrysocephalus		SL	NO	MIN	2	CO	INV	4	
L. coccogenis	K	SL	NO	MIN	2	CO	INV	1	

L. cornutus		SL	NO	MIN	2	CO	INV	4	
Lythrurus ardens	Y	SL	NO	MIN	1	CO	INV	3	
L. lirus		SL	NO	MIN	1	CO	INV	1	
Margariscus margarita		SL	YES	MIN	1	CO	INV	3	
Nocomis leptocephalus	H	CL	YES	MIN	3	CO	OH	3	
N. micropogon		CL	YES	MIN	3	CO	INV	3	
N. platyrhynchus		CL	YES	MIN	3	CO	INV	3	
N. raneyi		CL	YES	MIN	3	CO	INV	3	
Notemigonus crysoleucas		NL	NO	VAR	2	CO	OH	2	TOL
Notropis amoenus		SL	NO	MIN	1	CO	INV	1	
N. atherinoides		NL	NO[1]	VAR	2	CO	INV	2[1]	
N. bifrenatus		NL	NO[1]	VAR	1	CO	INV	2[1]	
N. buccatus		SL	NO	MIN	1	CO	OH	3	
N. hudsonius	N	NL	NO	VAR	2	CO	INV	2	
N. leuciodus	N	SL	NO	MIN	1	CO	INV	1	
N. photogenis		SL	NO	MIN	1	CO	INV	2	
N. procne	N	SL	NO	MIN	2	CO	INV	2	
N. rubellus		SL	NO	MIN	1	CO	INV	1	
N. rubricroceus	N	SL	NO	MIN	1	CO	INV	2	
N. scabriceps		SL	NO	MIN	2	CO	INV	1	
N. stramineus		SL	NO	MIN	1	CO	INV	3	
N. telescopus	NKJ	SL	NO	MIN	2	CO	INV	1	
N. volucellus		NL	NO	VAR	1	CO	INV	3	
Phenacobius teretulus		SL	NO	MIN	2	BE	INV	1	
P. uranops		SL	NO	MIN	1	BE	INV	2	
Phoxinus erythrogaster		SL[5]	NO[5]	MIN[5]	1[5]	CO	OH[5]	3[5]	
P. oreas	PH	SL	NO	MIN	1	CO	OH	3	
P. tennesseensis		SL	NO[1]	MIN	1	CO	OH	2[7]	
Pimephales notatus	YRHJ	NL	YES	VAR	1	CO	OH	3	TOL
P. promelas	ANK	NL	YES	VAR	1	CO	OH	3	TOL
Rhinichthys atratulus		SL	NO	MIN	2	BE	INV	3	TOL

R. cataractae		SL	NO	MIN	2	BE	INV	2	
Semotilus atromaculatus		CL	YES	MIN	1	CO	IP	4	TOL
S. corporalis		CL	YES	MIN	2	CO	IP	4	
Esox americanus	M	NL	NO	VEG	2	CO	PIS	1	
E. niger	MK	NL	NO	VEG	2	CO	PIS	1	
Fundulus catenatus		SL	NO	MIN	1	CO	INV	1	
F. diaphanus	M	NL	NO	VAR	1	CO	INV	1	
Ameiurus natalis		NL	YES	VAR	2	BE	IP	3	
A. nebulosus	N	NL	YES	VAR	3	BE	OH	3	
Ictalurus punctatus	A	NL	YES	VAR	3	BE	IP	3	
Noturus flavus		CL	YES	MIN	3	BE	INV	2	
N. gilberti	J[1]	CL	YES[1]	MIN	2	BE[1]	INV	1[1]	
N. insignis	T	CL	YES	MIN	3	BE	INV	2	
Pylodictis olivaris	PJR[1,2]	CL	YES[1]	MIN	4	BE[1]	IP	2[1]	
Lepisosteus osseus		NL	YES[1]	VAR	6	CO[1]	PIS	1[1]	
Etheostoma caeruleum		SL	NO	MIN	1	BE	INV	1	
E. camurum		CL	NO[1]	MIN	1[4]	BE[1]	INV	1[1]	INT[8]
E. blennioides		NL	NO	VAR	2	BE	INV	1	
E. flabellare		CL	YES	MIN	2	BE	INV	1	TOL
E. kanawhae		SL	NO	MIN	2	BE[1]	INV	1	
E. longimanum		CL	YES	MIN	1	BE	INV	1	
E. nigrum		NL	YES	VAR	1	BE	INV	1	TOL
E. olmstedi		NL	YES	VAR	1	BE	INV	1	TOL
E. osburni		SL	NO[1]	MIN	2	BE	INV	1[1]	INT
E. rufilineatum		SL	NO	MIN	1	BE	INV	1	
E. simoterum		NL	NO	VAR	1	BE	INV	1	
E. stigmaeum		CL	NO[1]	MIN	1[4]	BE[1]	INV	1[1]	
E. swannanoa		SL	NO[1]	MIN	2	BE[1]	INV	1[1]	
E. variatum		SL	NO[1]	MIN	2	BE	INV	1[1]	INT
E. zonale	A	NL	NO	VAR	1	BE	INV	1	
Percina burtoni		SL	NO[1]	MIN	2	BE[1]	INV	1[1]	
P. caprodes		SL	NO	MIN	2	BE	INV	1	
P. evides		CL	NO[1]	MIN	2	BE	INV	1[1]	INT

P. gymnocephala		SL	NO	MIN	2	BE	INV	1	
P. macrocephala		SL[3]	NO[1]	MIN	2	BE[1]	INV	1[1]	INT[8]
P. peltata		SL	NO[1]	MIN	2	BE	INV	1	
Sander canadense		SL	NO[1]	MIN	4	CO	PIS	2[1]	
S. vitreus	A[1,2]	SL	NO[1]	MIN	2	CO[1]	PIS	2[1]	
Percopsis omiscomaycus		SL	NO[1]	MIN	2	CO[1]	OH	3[1]	
Lampetra aepyptera		SL	YES	MIN	6	BE	OH	2	
L. appendix		SL	YES	MIN	5	BE	OH	2	
Oncorhynchus mykiss	ALL	CL	YES	MIN	1	CO	IP	2	
Salmo trutta	ALL	CL	YES	MIN	1	CO	IP	2	
Salvelinus fontinalis		CL	YES	MIN	2	CO	IP	2	INT
Aplodinotus grunniens		NL	NO[1]	NA	4	BE	INV	2[1]	

[a]Data from McCormick *et al*. (2001) unless otherwise noted.
[b]Data from Jenkins and Burkhead (1994) unless otherwise noted.
[c]Data from Smogor and Angermeier (1999a) unless otherwise noted.
[d]Data from Jenkins and Burkhead (1994) and Smogor and Angermeier (1999a) unless otherwise noted.
[e]Data from Jenkins and Burkhead (1994) and Smogor and Angermeier (1999a) unless otherwise noted.
[f]Data from McCormick *et al*. (2001) unless otherwise noted.
[g]Data from Smogor and Angermeier (1999a) but AHI and DAH combined into "omnivore-herbivore" sensu McCormick *et al*. (2001)
[h]Data from Smogor and Angermeier (1999a) includes four possible food types: (a) detritus, (b) algae/vascular plants, (c) invertebrates, and (d) fish/fish blood
[i]Classifications from agreement between Smogor and Angermeier (1999a) and McCormick *et al*.(2001). If a species is listed in one source but not the other and it has calls, I included those calls here. If the species was not listed in either source, classifications (or lack thereof) are attributed to Barbour *et al*. (1999).

[1]Jenkins and Burkhead (1994)
[2]Page and Burr (1991)
[3]Froese and Pauly (2006)
[4]Congeneric mean value
[5]Etnier and Starnes (1993)
[6]Lee *et al*. (1980)
[7]Starnes and Jenkins (1988)
[8]Barbour *et al*. (1999)

Appendix F. Calculated fish metrics and Spearman correlations with environmental NMS axes (*p<0.05, **p<0.005). Positive correlations with NMS axis I corresponds to increasing environmental quality. Positive relations to NMS axis II correspond to decreasing environmental quality (Tables 2 and 3; Figure 3.3).

Category	Code	Variable	Expected response to degradation	Reference	NMS axis I	NMS axis II
Taxonomic	S	Species richness	Negative	Angermeier *et al*. (2000) [TOTSP]	-0.051	0.097
	NAT_S	Native species richness	Negative		-0.036	0.102
	NAT_N	Native species abundance	Negative		-0.135	0.087
	NAT_P	Native species proportional abundance	Negative		-0.010	0.048
	NNAT_S	Non-native species richness	Positive	Angermeier *et al*. (2000) [INTSP]	-0.023	-0.035
	NNAT_N	Non-native species abundance	Positive		-0.029	-0.046
	NNAT_P	Non-native species proportional abundance	Positive	McCormick *et al*. (2001) [PEXOT]	-0.010	-0.042
	COT_N	Cottid abundance	Negative		0.129	-0.119
	COT_P	Cottid proportional abundance	Negative	McCormick *et al*. (2001) [PCOTTID]	0.182*	-0.150
	SAL_S	Salmonid species richness	Negative		0.278**	-0.218**
	SAL_N	Salmonid species abundance	Negative		0.332**	-0.247**
	SAL_P	Salmonid species proportional abundance	Negative		0.345**	-0.248**
	PER_S	Percid species richness	Negative		-0.103	0.188*
	PER_N	Percid species abundance	Negative		-0.184*	0.166*
	PER_P	Percid species proportional abundance	Negative		-0.173*	0.155
	CEN_S	Centrarchid species richness	Negative	Angermeier *et al*. (2000) [SUNSP]	-0.135	0.289**
	CEN_N	Centrarchid species abundance	Negative		-0.146	0.304**
	CEN_P	Centrarchid species proportional abundance	Negative		-0.134	0.311**
	CAT_S	Catostomid species richness	Negative	Angermeier *et al*. (2000) [SUCSP]	-0.048	0.173*
	CAT_N	Catostomid species abundance	Negative		-0.158*	0.220*
	CAT_P	Catostomid species proportional	Negative		-0.170*	0.242**

		abundance				
	CYP_S	Cyprinid species richness	Negative	Angermeier *et al*. (2000) [MINSP]	-0.081	-0.023
	CYP2_S	Native cyprinid species richness, excluding tolerant species	Negative	McCormick *et al*. (2001) [NSCYPR2]	-0.087	-0.084
	CYP_N	Cyprinid species abundance	Negative		-0.146	0.027
	CYP2_N	Native cyprinid species abundance, excluding tolerant species	Negative		-0.133	-0.006
	CYP_P	Cyprinid species proportional abundance	Negative		-0.103	-0.088
	CYP2_P	Native cyprinid species proportional abundance, excluding tolerant species	Negative		-0.072	-0.116
	ICT_S	Ictalurid species richness	Negative		0.053	-0.074
	ICT_N	Ictalurid species abundance	Negative		0.075	-0.094
	ICT_P	Ictalurid species proportional abundance	Negative		0.086	-0.099
	DOS_S	Darter or sculpin species richness	Negative	Angermeier *et al*. (2000) [DOSSP]	-0.020	0.129
	DOS_N	Darter or sculpin species abundance	Negative		-0.056	0.068
	DOS_P	Darter or sculpin species proportional abundance	Negative		0.063	0.008
Trophic	IP_S	Invertivore-piscivore richness	Negative		0.097	0.119
	IP_N	Invertivore-piscivore abundance	Negative		0.082	0.084
	IP_P	Invertivore-piscivore proportional abundance	Negative		0.233**	-0.009
	IP2_P	Invertivore-piscivore proportional abundance, excluding *S. atromaculatus*	Negative	McCormick *et al*. (2001) [PPISCINV2]	0.250**	-0.038
	OH_S	Omnivore-herbivore richnesss	Positive		-0.205*	0.070
	OH_N	Omnivore-herbivore abundance	Positive		-0.290**	0.162*
	OH_P	Omnivore-	Positive		-0.317**	0.165*

		herbivore relative abundance				
	INV_S	Invertivore richness	Negative		-0.026	0.078
	INV_N	Invertivore abundance	Negative		-0.073	0.033
	INV_P	Invertivore proportional abundance	Negative	Angermeier *et al*. (2000) [INVPR]	0.054	-0.068
	INV_BEN_S	Invertivore benthic specialist richness	Negative	Food type number=1; Angermeier *et al*. (2000)	-0.001	0.106
	INV_BEN_N	Invertivore benthic specialist abundance	Negative	Food type number=1; See Angermeier *et al*. (2000)	-0.053	0.067
	INV_BEN_P	Invertivore benthic specialist proportional abundance	Negative	Food type number=1; Angermeier *et al*. (2000) [BSIPR]	0.068	0.004
	PIS_S	Piscivore richness	Negative		-0.103	0.163*
	PIS_N	Piscivore abundance	Negative		-0.108	0.158*
	PIS_P	Piscivore proportional abundance	Negative	Angermeier *et al*. (2000) [CARPR]	-0.106	0.165*
	GEN_COL_S	Generalist feeders, column species richness	Positive	Food type number >2; Angermeier *et al*. (2000)	-0.140	0.057
	GEN_COL_N	Generalist feeders, column species abundance	Positive	Food type number >2; Angermeier *et al*. (2000)	-0.187*	0.082
	GEN_COL_P	Generalist feeders, column species proportional abundance	Positive	Food type number >2; Angermeier *et al*. (2000) [GENPR]	-0.174*	0.083
Reproductive	SL_S	Simple lithophil richness	Negative		-0.090	0.018
	SL2_S	Simple lithophil richness, excluding tolerant species	Negative		-0.086	-0.016
	SL_N	Simple lithophil abundance	Negative		-0.192	0.034
	SL2_N	Simple lithophil abundance, excluding tolerant species	Negative		-0.146	-0.039
	SL_P	Simple lithophil proportional abundance	Negative		-0.118	-0.181*
	SL2_P	Simple lithophil proportional abundance, excluding tolerant species	Negative	Angermeier *et al*. (2000) [SLTPR]	-0.119	-0.127
	NL_S	Non-lithophil	Positive		-0.135	0.339**

		richness				
	NL_N	Non-lithophil abundance	Positive		-0.119	0.186*
	NL_P	Non-lithophil proportional abundance	Positive		-0.058	0.174*
	NS_NL_S	Non-simple non-lithophil species richness	Positive		-0.097	0.226**
	NS_NL_N	Non-simple non-lithophil species abundance	Positive		-0.101	0.127
	NS_NL_P	Non-simple non-lithophil species proportional abundance	Positive	Angermeier *et al*. (2000) [NNLPR]	-0.139	0.338**
	LATE_S	Late maturing species richness (age >2)	Negative	Angermeier *et al*. (2000) [LAMSP]	-0.037	0.063
	LATE_N	Late maturing species abundance (age >2)	Negative		-0.133	0.102
	LATE_P	Late maturing species proportional abundance (age >2)	Negative		-0.123	0.118
	EARLY_S	Early maturing species richness (age <3)	Positive		-0.054	0.106
	EARLY_N	Early maturing species abundance (age <3)	Positive		-0.144	0.060
	EARLY_P	Early maturing species proportional abundance (age <3)	Positive		0.123	-0.118
	REPROAGE	Mean age of first female reproduction	Negative		0.105	-0.171*
Tolerance	TOL_S	Tolerant species richness	Positive		-0.122	0.225**
	TOL_N	Tolerant species abundance	Positive		-0.260**	0.117
	TOL_P	Tolerant species proportional abundance	Positive	Angermeier *et al*. (2000) [TOLPR] and McCormick *et al*. (2001) [PTOLE]	-0.192*	0.156
	INTOL_S	Intolerant species richness	Negative	McCormick *et al*. (2001) [NSINTOL]	0.251**	-0.261**
	INTOL_N	Intolerant species abundance	Negative		0.273**	-0.299**
	INTOL_P	Intolerant species proportional abundance	Negative		0.292**	-0.303**
Vertical position	COL_S	Column species richness	Positive		-0.079	0.089
	COL_N	Column species	Positive		-0.116	-0.002

	abundance				
COL_P	Column species proportional abundance	Positive		-0.011	-0.088
BEN_S	Benthic species richness	Negative		-0.003	0.104
BEN2_S	Benthic species richness, excluding tolerant species	Negative	McCormick *et al*. (2001) [NSBHAB2]	0.052	0.092
BEN_N	Benthic species abundance	Negative		-0.119	0.109
BEN2_N	Benthic species abundance, excluding tolerant species	Negative		-0.027	0.104
BEN_P	Benthic species proportional abundance	Negative		0.011	0.088
BEN2_P	Benthic species proportional abundance, excluding tolerant species	Negative		0.113	0.089

Appendix G. Mann-Whitney tests for environmental variable differences among headwater tributaries (HT) and mainstem tributaries (MT) at multiple distances from sites. P-values >0.05 are omitted.

		5			10			15			20		
Category	**Variable**	**Mann-Whitney U**	**Z**	**p-value**	**Mann-Whitney U**	**Z**	**p-value**	**Mann-Whitney U**	**Z**	**p-value**	**Mann-Whitney U**	**Z**	**p-value**
Land use	AG_TOT	2643.0	-0.121	ns	2984.5	-0.329	ns	2657.5	-0.366	ns	1743.5	-0.509	ns
	FOR_TOT	2624.0	-0.192	ns	2989.0	-0.313	ns	2633.0	-0.457	ns	1755.0	-0.457	ns
	URB_TOT	2672.0	-0.012	ns	2690.5	-1.397	ns	2516.5	-0.912	ns	1522.5	-1.548	ns
	POPDENKM	2524.0	-0.569	ns	2560.0	-1.819	ns	2331.0	-1.578	ns	1508.5	-1.572	ns
	RD_DEN	2555.0	-0.452	ns	2746.0	-1.166	ns	2409.0	-1.288	ns	1422.5	-1.961	ns
Mesohabitat	PCT_FAST	2472.0	-0.765	ns	3052.0	-0.091	ns	2629.0	-0.471	ns	1596.5	-1.174	ns
	PCT_POOL	2274.5	-1.519	ns	2904.5	-0.614	ns	2424.0	-1.241	ns	1608.0	-1.129	ns
Periphyton	NAFDM_M2	2669.5	-0.021	ns	2821.5	-0.901	ns	2558.0	-0.735	ns	1792.0	-0.289	ns
	NCHL_M2	2526.5	-0.560	ns	2927.0	-0.530	ns	2409.5	-1.286	ns	1786.0	-0.317	ns
Riparian	XCDENBK	2183.5	-1.852	ns	2951.5	-0.444	ns	2477.5	-1.034	ns	1399.5	-2.065	0.039
	XCDENMID	2113.5	-2.116	0.034	2831.5	-0.866	ns	2503.5	-0.937	ns	1572.5	-1.282	ns
	XCL	2177.0	-1.876	ns	2683.0	-1.387	ns	2392.0	-1.351	ns	1440.0	-1.882	ns
	XPCMG	2507.5	-0.640	ns	2868.5	-0.746	ns	2494.5	-0.985	ns	1576.0	-1.285	ns
Substrate	PCT_BIGR	2618.0	-0.215	ns	2751.0	-1.149	ns	2425.5	-1.227	ns	1794.5	-0.278	ns
	PCT_SAFN	2538.5	-0.515	ns	2519.5	-1.963	ns	2317.0	-1.631	ns	1709.5	-0.663	ns
	XEMBED	2506.0	-0.637	ns	2737.5	-1.196	ns	2601.5	-0.574	ns	1792.5	-0.287	ns
Volume	XWIDTH	2601.0	-0.279	ns	2961.5	-0.409	ns	2569.5	-0.692	ns	1486.5	-1.671	ns
	AREA_WS	2633.5	-0.156	ns	3029.5	-0.170	ns	2530.5	-0.837	ns	1330.0	-2.379	0.017
	XDEPTH	2628.5	-0.175	ns	2952.5	-0.441	ns	2621.0	-0.501	ns	1582.5	-1.237	ns
Water quality	ALTD	2631.5	-0.165	ns	3032.5	-0.161	ns	2610.0	-0.545	ns	1741.5	-0.521	ns
	CA	2572.0	-0.388	ns	2713.5	-1.280	ns	2513.5	-0.900	ns	1657.0	-0.900	ns
	CATSUM	3650.0	-0.083	ns	2734.0	-1.208	ns	2453.0	-1.125	ns	1599.0	-1.162	ns
	CL	2512.5	-0.612	ns	2447.0	-2.216	ns	2089.5	-2.474	0.013	1324.5	-2.404	0.016
	COND	2649.0	-0.098	ns	2701.5	-1.322	ns	2403.5	-1.308	ns	1564.5	-1.319	ns
	DOC	2569.5	-0.398	ns	2818.5	-0.912	ns	2735.0	-0.078	ns	1780.0	-0.344	ns
	K	2610.0	-0.245	ns	2893.0	-0.650	ns	2347.0	-1.518	ns	1419.5	-1.974	0.048
	MG	2622.5	-0.198	ns	2759.0	-1.120	ns	2451.0	-1.132	ns	1618.5	-1.074	ns
	NA	2605.0	-0.264	ns	2797.0	-0.987	ns	2323.0	-1.607	ns	1513.5	-1.549	ns
	NH4	2371.5	-1.181	ns	3011.5	-0.241	ns	2722.5	-0.128	ns	1606.0	-1.168	ns
	NO3	2345.0	-1.243	ns	2621.0	-1.605	ns	2306.5	-1.669	ns	1759.0	-0.439	ns
	NTL	2324.5	-1.321	ns	2614.0	-1.630	ns	2286.5	-1.743	ns	1807.0	-0.222	ns
	PHSTVL	2479.0	-0.739	ns	2752.0	-1.145	ns	2674.5	-0.303	ns	1830.0	-0.118	ns
	PTL	2465.5	-0.790	ns	2993.5	-0.297	ns	2670.0	-0.320	ns	1793.0	-0.285	ns
	SO4	2646.0	-0.109	ns	2765.0	-1.099	ns	2165.0	-2.194	0.028	1329.5	-2.382	0.017
Woody debris	XFC_BRS	2089.0	-2.210	0.027	2632.0	-1.568	ns	2412.0	-1.278	ns	1560.5	-1.338	ns
	XFC_LWD	2417.0	-0.988	ns	2817.5	-0.930	ns	2427.5	-1.239	ns	1398.0	-2.105	ns

Appendix H. Summary of environmental variables among all sites, headwater tributaries (HT), and mainstem tributaries (MT) at multiple distances from sites.

Category	Variable	Units	Distance	Group	Mean	Standard deviation	Minimum	Maximum
Land use	AG_TOT	% watershed	All	All	22.81	24.04	0.00	97.54
			5	HT	23.00	23.70	0.00	97.54
				MT	22.42	24.97	0.00	84.11
			10	HT	22.71	24.20	0.00	83.66
				MT	22.91	24.03	0.00	97.54
			15	HT	22.33	23.68	0.00	80.05
				MT	23.06	24.32	0.00	97.54
			20	HT	23.17	26.25	0.00	80.05
				MT	22.73	23.61	0.00	97.54
	FOR_TOT	% watershed	All	All	74.64	25.55	0.00	100.00
			5	HT	74.63	24.97	0.00	100.00
				MT	74.65	27.00	11.58	100.00
			10	HT	75.34	24.74	10.72	100.00
				MT	73.98	26.42	0.00	100.00
			15	HT	75.78	24.37	19.89	100.00
				MT	74.06	26.23	0.00	100.00
			20	HT	75.33	25.94	19.89	100.00
				MT	74.48	25.56	0.00	100.00
	URB_TOT	% watershed	All	All	1.65	4.86	0.00	40.27
			5	HT	1.53	4.11	0.00	24.62
				MT	1.92	6.21	0.00	40.27
			10	HT	1.02	2.49	0.00	12.44
				MT	2.25	6.29	0.00	40.27
			15	HT	1.04	2.59	0.00	12.44
				MT	1.96	5.66	0.00	40.27
			20	HT	0.52	1.48	0.00	7.58
				MT	1.91	5.31	0.00	40.27
	POPDENKM	individuals/km^2	All	All	26.55	58.10	0.00	437.71
			5	HT	28.48	67.33	0.00	437.71
				MT	22.42	30.23	0.66	161.75
			10	HT	19.46	43.97	0.00	332.66
				MT	33.20	68.39	0.66	437.71
			15	HT	20.58	50.02	0.00	332.66
				MT	29.59	61.82	0.66	437.71
			20	HT	10.33	8.33	0.00	31.38
				MT	30.23	63.70	0.12	437.71
	RD_DEN	m/ha	All	All	15.22	8.94	0.00	57.21
			5	HT	14.92	8.81	0.00	55.50
				MT	15.85	9.25	5.08	57.21
			10	HT	14.00	7.61	0.00	35.22
				MT	16.36	9.93	0.00	57.21
			15	HT	13.73	7.50	0.00	35.22
				MT	15.98	9.53	0.00	57.21
			20	HT	11.89	5.90	0.00	23.35
				MT	15.97	9.34	0.00	57.21
Mesohabitat	PCT_FAST	% sample reach	All	All	43.94	24.18	0.00	100.00
			5	HT	44.97	24.00	0.00	100.00
				MT	41.73	24.67	0.00	97.00
			10	HT	44.18	24.07	0.00	100.00
				MT	43.72	24.43	0.00	97.00
			15	HT	45.18	25.00	0.00	100.00
				MT	43.31	23.85	0.00	97.00
			20	HT	48.70	24.55	0.00	100.00
				MT	42.86	24.06	0.00	97.00
	PCT_POOL	% sample reach	All	All	17.19	20.44	0.00	99.00
			5	HT	15.84	19.25	0.00	84.00
				MT	20.09	22.72	0.00	99.00

			10	HT	16.97	20.87	0.00	84.00
				MT	17.41	20.16	0.00	99.00
			15	HT	14.60	18.78	0.00	84.00
				MT	18.52	21.21	0.00	99.00
			20	HT	13.83	18.29	0.00	83.33
				MT	17.96	20.89	0.00	99.00
Periphyton	AFDM_M2[1]	g/m^2	All	All	14.12	21.21	0.29	157.50
			5	HT	14.99	24.36	0.58	157.50
				MT	12.24	11.99	0.29	57.67
			10	HT	14.52	23.64	0.58	157.50
				MT	13.74	18.80	0.29	150.67
			15	HT	14.60	25.13	0.58	157.50
				MT	13.87	19.04	0.29	150.67
			20	HT	18.88	32.35	1.04	157.50
				MT	13.04	17.77	0.29	150.67
	CHL_M2[1]	mg/m^2	All	All	53.83	64.93	0.00	500.00
			5	HT	54.74	67.37	0.00	500.00
				MT	51.86	59.99	0.00	287.80
			10	HT	47.64	50.72	0.00	268.24
				MT	59.63	75.76	0.00	500.00
			15	HT	43.85	50.27	0.00	268.24
				MT	58.91	70.94	0.00	500.00
			20	HT	47.49	42.76	0.00	210.02
				MT	55.26	69.03	0.00	500.00
Riparian	XCDENBK	% sample reach	All	All	77.18	25.21	0.00	100.00
			5	HT	74.64	26.71	0.00	100.00
				MT	82.63	20.86	0.54	100.00
			10	HT	75.05	28.07	0.00	100.00
				MT	79.18	22.18	0.54	100.00
			15	HT	71.89	30.73	0.00	100.00
				MT	79.88	21.54	0.54	100.00
			20	HT	63.20	34.98	0.00	100.00
				MT	80.35	21.35	0.54	100.00
	XCDENMID	% sample reach	All	All	65.12	26.87	0.00	100.00
			5	HT	61.74	28.00	0.00	100.00
				MT	72.34	22.89	0.00	100.00
			10	HT	62.55	28.38	0.00	100.00
				MT	67.52	25.32	0.00	100.00
			15	HT	60.59	30.91	0.00	100.00
				MT	67.42	24.40	0.00	100.00
			20	HT	55.80	34.24	0.00	97.59
				MT	67.23	24.58	0.00	100.00
	XCL	% sample reach	All	All	0.21	0.17	0.00	0.75
			5	HT	0.20	0.17	0.00	0.75
				MT	0.23	0.14	0.00	0.61
			10	HT	0.20	0.17	0.00	0.73
				MT	0.22	0.16	0.00	0.75
			15	HT	0.20	0.19	0.00	0.73
				MT	0.22	0.15	0.00	0.75
			20	HT	0.17	0.17	0.00	0.63
				MT	0.22	0.16	0.00	0.75
	XPCMG	% sample reach	All	All	0.76	0.29	0.00	1.00
			5	HT	0.74	0.31	0.00	1.00
				MT	0.79	0.26	0.00	1.00
			10	HT	0.72	0.33	0.00	1.00
				MT	0.79	0.25	0.00	1.00
			15	HT	0.69	0.36	0.00	1.00
				MT	0.79	0.25	0.00	1.00
			20	HT	0.63	0.39	0.00	1.00
				MT	0.79	0.26	0.00	1.00
Substrate	PCT_BIGR	% sample reach	All	All	63.07	24.38	0.00	100.00
			5	HT	62.61	24.70	0.00	100.00

				MT	64.04	23.90	16.98	100.00
			10	HT	60.65	24.80	0.00	100.00
				MT	65.33	23.90	0.00	100.00
			15	HT	59.51	25.14	0.00	100.00
				MT	64.88	23.90	0.00	100.00
			20	HT	62.32	23.65	7.27	100.00
				MT	63.24	24.62	0.00	100.00
	PCT_SAFN	% sample reach	All	All	24.29	20.67	0.00	100.00
			5	HT	24.39	20.05	0.00	100.00
				MT	24.10	22.16	0.00	74.55
			10	HT	26.38	19.06	0.00	100.00
				MT	22.34	22.01	0.00	100.00
			15	HT	27.01	19.71	0.00	100.00
				MT	22.91	21.10	0.00	100.00
			20	HT	25.27	18.20	0.00	72.73
				MT	24.07	21.25	0.00	100.00
	XEMBED	% substrate area	All	All	48.34	22.14	3.35	100.00
			5	HT	48.87	22.66	3.35	100.00
				MT	47.20	21.14	10.09	90.73
			10	HT	50.01	22.29	3.82	100.00
				MT	46.77	22.02	3.35	100.00
			15	HT	49.72	22.07	3.82	100.00
				MT	47.63	22.24	3.35	100.00
			20	HT	47.14	24.26	3.82	94.63
				MT	48.61	21.72	3.35	100.00
Volume	XWIDTH	m	All	All	6.24	4.42	0.43	20.80
			5	HT	6.20	4.45	0.45	20.80
				MT	6.32	4.41	0.43	18.55
			10	HT	6.30	4.41	0.45	20.80
				MT	6.18	4.46	0.43	18.55
			15	HT	5.88	4.33	0.45	20.80
				MT	6.42	4.48	0.43	19.71
			20	HT	5.13	4.28	0.45	20.80
				MT	6.49	4.43	0.43	19.71
	AREA_WS	ha	All	All	3905.00	4681.15	16.00	21588.00
			5	HT	3912.91	4749.63	16.00	21588.00
				MT	3888.08	4578.53	62.00	20697.00
			10	HT	3836.89	4632.69	16.00	21588.00
				MT	3968.90	4754.12	62.00	20697.00
			15	HT	3505.60	4219.53	16.00	19894.00
				MT	4108.54	4906.76	62.00	21588.00
			20	HT	2366.97	3047.16	16.00	11655.00
				MT	4253.46	4920.09	62.00	21588.00
	XDEPTH	cm	All	All	29.36	15.96	1.61	75.20
			5	HT	29.05	15.50	1.61	75.20
				MT	30.01	17.04	1.99	74.29
			10	HT	29.64	15.22	1.61	71.25
				MT	29.09	16.71	1.99	75.20
			15	HT	28.24	15.43	1.61	71.25
				MT	29.92	16.27	1.99	75.20
			20	HT	25.46	13.20	1.61	49.08
				MT	30.24	16.44	1.99	75.20
Water quality	ALTD	µg/L	All	All	18.18	26.51	0.00	184.00
			5	HT	19.02	29.41	0.00	184.00
				MT	16.40	19.02	0.00	101.00
			10	HT	19.84	28.57	1.00	169.00
				MT	16.63	24.49	0.00	184.00
			15	HT	22.11	32.74	1.00	169.00
				MT	16.18	22.61	0.00	184.00
			20	HT	19.28	26.13	3.00	140.00

			MT	17.94	26.69	0.00	184.00
CA	µeg/L	All	All	1297.66	1349.82	52.60	7394.30
		5	HT	1276.14	1332.78	52.60	7394.30
			MT	1343.71	1398.17	111.60	5249.60
		10	HT	1106.29	1067.46	64.90	4119.80
			MT	1477.22	1554.74	52.60	7394.30
		15	HT	1143.02	1130.36	64.90	4119.80
			MT	1376.47	1447.73	52.60	7394.30
		20	HT	1045.69	1023.33	64.90	3353.50
			MT	1354.75	1410.46	52.60	7394.30
CATSUM	µeg/L	All	All	2496.22	3848.49	184.17	41304.64
		5	HT	2586.18	4374.12	184.17	41304.64
			MT	2303.71	2389.13	255.86	10534.81
		10	HT	2007.16	1927.39	184.17	8591.02
			MT	2955.09	4995.81	219.00	41304.64
		15	HT	2031.28	2094.43	184.17	8591.02
			MT	2733.16	4477.66	190.53	41304.64
		20	HT	1776.33	1672.39	184.17	6868.67
			MT	2659.32	4175.06	189.52	41304.64
CL	µeg/L	All	All	255.99	595.46	11.90	6470.70
		5	HT	281.96	707.29	12.00	6470.70
			MT	200.42	205.01	11.90	817.60
		10	HT	198.56	374.11	12.00	2527.00
			MT	309.87	744.41	11.90	6470.70
		15	HT	161.17	265.14	12.00	1480.00
			MT	304.31	703.25	11.90	6470.70
		20	HT	144.64	278.77	14.00	1480.00
			MT	281.22	644.16	11.90	6470.70
COND	µS	All	All	236.98	337.60	20.00	3590.00
		5	HT	244.68	382.76	20.00	3590.00
			MT	220.51	213.35	29.30	973.00
		10	HT	192.70	172.56	20.00	714.00
			MT	278.52	436.72	24.00	3590.00
		15	HT	190.63	183.11	20.00	714.00
			MT	260.60	392.46	22.00	3590.00
		20	HT	169.33	151.99	22.80	643.00
			MT	252.31	365.55	20.00	3590.00
DOC	mg/L	All	All	1.90	1.03	0.57	8.13
		5	HT	1.93	1.14	0.57	8.13
			MT	1.85	0.74	0.63	3.84
		10	HT	1.97	1.02	0.57	5.57
			MT	1.84	1.03	0.63	8.13
		15	HT	1.95	1.10	0.60	5.57
			MT	1.88	0.99	0.57	8.13
		20	HT	1.98	1.06	0.74	5.57
			MT	1.89	1.02	0.57	8.13
K	µeg/L	All	All	44.54	26.10	8.30	178.50
		5	HT	45.36	27.68	8.40	178.50
			MT	42.79	22.48	8.30	96.00
		10	HT	42.62	23.86	8.40	141.40
			MT	46.35	28.06	8.30	178.50
		15	HT	41.01	25.64	11.90	141.40
			MT	46.34	26.27	8.30	178.50
		20	HT	36.19	20.34	12.30	91.30
			MT	46.44	26.94	8.30	178.50
MG	µeg/L	All	All	645.17	846.93	29.80	6066.00
		5	HT	655.72	898.18	29.80	6066.00
			MT	622.59	733.27	42.90	3232.80
		10	HT	546.13	659.83	29.80	3402.40
			MT	738.10	986.14	42.90	6066.00
		15	HT	566.55	724.42	29.80	3402.40
			MT	685.24	903.67	42.90	6066.00

		20	HT	495.42	640.63	48.50	3198.10
			MT	679.10	885.61	29.80	6066.00
NA	µeg/L	All	All	506.66	2266.44	17.90	27664.50
		5	HT	606.24	2727.96	20.90	27664.50
			MT	293.55	432.78	17.90	2233.40
		10	HT	309.45	495.71	23.10	2782.90
			MT	691.69	3116.85	17.90	27664.50
		15	HT	277.61	464.66	23.10	2782.90
			MT	623.38	2762.28	17.90	27664.50
		20	HT	197.98	252.44	23.90	1144.10
			MT	576.59	2503.80	17.90	27664.50
NH4	µeg/L	All	All	2.17	7.56	0.00	85.70
		5	HT	2.70	9.06	0.00	85.70
			MT	1.04	1.47	0.00	6.40
		10	HT	2.66	10.03	0.00	85.70
			MT	1.72	4.08	0.00	32.80
		15	HT	3.10	11.89	0.00	85.70
			MT	1.70	3.79	0.00	32.80
		20	HT	1.02	1.81	0.00	8.90
			MT	2.43	8.31	0.00	85.70
NO3	µeg/L	All	All	50.48	95.10	0.00	703.10
		5	HT	54.31	105.23	0.00	703.10
			MT	42.28	68.82	1.30	351.80
		10	HT	54.43	96.18	0.30	573.00
			MT	46.78	94.52	0.00	703.10
		15	HT	52.53	84.18	1.40	397.20
			MT	49.44	100.58	0.00	703.10
		20	HT	36.30	45.24	1.50	207.50
			MT	53.69	102.96	0.00	703.10
NTL	µg/L	All	All	921.72	1517.21	76.00	12630.00
		5	HT	992.95	1702.06	76.00	12630.00
			MT	769.28	1013.32	81.00	5388.00
		10	HT	967.66	1434.34	96.00	8993.00
			MT	878.62	1598.78	76.00	12630.00
		15	HT	956.13	1234.17	96.00	5750.00
			MT	904.18	1648.18	76.00	12630.00
		20	HT	686.21	692.24	96.00	3318.00
			MT	975.08	1645.11	76.00	12630.00
PHSTVL		All	All	7.55	0.51	6.36	8.94
		5	HT	7.51	0.49	6.36	8.39
			MT	7.62	0.55	6.61	8.94
		10	HT	7.49	0.50	6.36	8.39
			MT	7.60	0.52	6.55	8.94
		15	HT	7.52	0.52	6.38	8.39
			MT	7.56	0.51	6.36	8.94
		20	HT	7.55	0.52	6.57	8.37
			MT	7.55	0.51	6.36	8.94
PTL	µg/L	All	All	21.79	41.22	1.00	470.00
		5	HT	23.77	49.07	1.00	470.00
			MT	17.54	13.18	2.00	72.00
		10	HT	19.67	22.68	2.00	132.00
			MT	23.78	53.12	1.00	470.00
		15	HT	20.96	24.22	2.00	132.00
			MT	22.21	47.71	1.00	470.00
		20	HT	21.48	22.83	3.00	104.00
			MT	21.86	44.41	1.00	470.00
SO4	µeg/L	All	All	786.65	2581.85	18.40	28569.20
		5	HT	897.01	3023.93	18.40	28569.20
			MT	550.49	1165.10	30.00	6096.00
		10	HT	600.42	1217.51	18.40	6237.30
			MT	961.39	3397.80	30.00	28569.20
		15	HT	580.81	1320.05	18.40	6237.30

				MT	891.55	3030.40	22.20	28569.20
			20	HT	489.32	1182.75	18.40	6237.30
				MT	854.02	2802.66	21.00	28569.20
Woody debris	XFC_BRS	% sample reach	All					
				All	0.06	0.06	0.00	0.29
			5	HT	0.06	0.05	0.00	0.27
				MT	0.08	0.07	0.00	0.29
			10	HT	0.06	0.06	0.00	0.27
				MT	0.07	0.06	0.00	0.29
			15	HT	0.06	0.06	0.00	0.27
				MT	0.07	0.06	0.00	0.29
			20	HT	0.05	0.06	0.00	0.27
				MT	0.07	0.06	0.00	0.29
	XFC_LWD	% sample reach	All	All	0.03	0.05	0.00	0.26
			5	HT	0.03	0.05	0.00	0.26
				MT	0.04	0.05	0.00	0.22
			10	HT	0.03	0.05	0.00	0.26
				MT	0.03	0.05	0.00	0.22
			15	HT	0.03	0.06	0.00	0.26
				MT	0.03	0.04	0.00	0.22
			20	HT	0.03	0.06	0.00	0.26
				MT	0.03	0.05	0.00	0.25

[1]Periphyton raw data were modified following B. Hill (USEPA, personal communication); see text.

Appendix I. Environmental variable loadings into two non-metric multidimensional scaling (NMS) ordination axes for headwater tributaries (HT) and mainstem tributaries (MT) at multiple distance classes.

		5				10				15				20			
		HT		MT		HT		MT		HT		MT		HT		MT	
Category	**Variable**	**Axis I**	**Axis II**	**Axis I**	**Axis II**	**Axis I**	**Axis II**	**Axis I**	**Axis II**	**Axis I**	**Axis II**	**Axis I**	**Axis II**	**Axis I**	**Axis II**	**Axis I**	**Axis II**
Mesohabitat	PCT_FAST	-0.01	0.13	0.09	-0.22	-0.07	0.11	-0.20	0.01	-0.15	0.06	-0.16	0.08	0.11	-0.05	0.18	-0.04
	PCT_POOL	0.02	0.03	-0.19	0.22	0.11	-0.07	0.05	0.03	0.17	-0.13	0.07	0.04	-0.19	0.04	-0.08	-0.05
Periphyton	ADFM_M2	0.00	-0.10	-0.09	0.11	0.04	-0.06	0.16	0.00	0.05	-0.03	0.13	-0.06	-0.08	0.03	-0.12	0.05
	CHL_M2	-0.07	-0.08	-0.03	0.06	-0.09	-0.08	0.09	0.01	0.02	0.11	0.09	-0.03	-0.03	-0.10	-0.06	0.11
Riparian	XCDENBK	0.13	0.09	0.07	0.05	0.10	0.13	-0.03	-0.07	-0.07	-0.18	-0.07	-0.04	0.07	0.28	0.05	-0.04
	XCDENMID	0.15	0.12	0.03	0.05	0.11	0.18	-0.02	-0.04	-0.12	-0.21	-0.07	-0.02	0.06	0.29	0.06	-0.06
	XCL	0.14	0.11	0.07	0.08	0.11	0.18	-0.01	-0.10	-0.08	-0.22	-0.09	-0.06	0.08	0.30	0.05	-0.05
	XPCMG	0.16	0.11	0.09	0.08	0.14	0.18	-0.01	-0.11	-0.11	-0.23	-0.08	-0.07	0.11	0.32	0.04	-0.04
Substrate	PCT_BIGR	0.08	0.08	0.13	-0.05	0.02	0.11	-0.08	-0.09	-0.10	-0.04	-0.12	-0.04	0.06	0.06	0.10	-0.03
	PCT_SAFN	-0.15	-0.17	-0.22	0.13	-0.03	-0.18	0.22	0.16	0.14	0.05	0.27	0.01	-0.10	-0.12	-0.21	0.08
	XEMBED	-0.06	-0.14	-0.10	0.05	0.00	-0.12	0.14	0.08	0.10	0.01	0.16	-0.01	-0.12	-0.05	-0.13	0.04
Volume	XWIDTH	-0.03	0.00	0.09	0.08	-0.04	0.01	0.05	-0.12	-0.05	0.02	-0.02	-0.12	0.10	0.05	0.01	0.12
	AREA_WS	-0.01	-0.04	0.04	0.07	-0.01	-0.03	0.05	-0.07	0.02	0.01	0.01	-0.08	0.01	0.00	-0.03	0.06
	XDEPTH	-0.04	-0.01	0.01	0.05	-0.04	-0.01	0.05	-0.04	-0.02	0.04	0.02	-0.05	0.04	-0.02	-0.01	0.07
Water quality	ALTD	0.03	-0.03	0.07	-0.11	0.02	-0.02	-0.05	-0.05	0.03	0.00	-0.06	-0.02	0.00	-0.03	0.02	-0.04
	CA	0.00	-0.14	-0.07	0.10	0.04	-0.13	0.13	0.02	0.13	0.02	0.12	-0.05	-0.11	-0.05	-0.13	0.03
	CATSUM	0.00	-0.12	-0.07	0.09	0.03	-0.11	0.12	0.02	0.11	0.02	0.11	-0.05	-0.10	-0.04	-0.12	0.02
	CL	-0.02	-0.17	-0.11	0.12	0.02	-0.15	0.17	0.03	0.14	0.06	0.16	-0.06	-0.11	-0.10	-0.17	0.02
	COND	0.00	-0.16	-0.09	0.12	0.05	-0.15	0.16	0.02	0.16	0.02	0.14	-0.06	-0.13	-0.06	-0.16	0.03
	DOC	-0.04	-0.08	-0.03	0.02	-0.01	-0.05	0.08	0.03	0.05	0.00	0.08	-0.02	-0.03	0.03	-0.08	0.02
	K	0.01	-0.10	-0.06	0.07	0.04	-0.09	0.10	0.01	0.10	0.00	0.09	-0.04	-0.09	-0.02	-0.10	0.00
	MG	0.01	-0.14	-0.08	0.11	0.04	-0.14	0.14	0.02	0.15	0.02	0.12	-0.06	-0.12	-0.05	-0.14	0.03
	NA	0.02	-0.14	-0.10	0.09	0.05	-0.12	0.14	0.02	0.13	0.02	0.12	-0.06	-0.10	-0.04	-0.15	0.00
	NH4	-0.07	-0.32	-0.18	0.17	-0.02	-0.28	0.38	0.06	0.30	0.11	0.30	-0.13	-0.05	-0.10	-0.38	0.04
	NO3	-0.09	-0.11	-0.08	0.17	-0.04	-0.10	0.18	0.04	0.06	0.06	0.17	-0.07	-0.03	-0.11	-0.13	0.08
	NTL	-0.03	-0.06	-0.04	0.06	-0.01	-0.05	0.08	0.02	0.04	0.02	0.07	-0.03	-0.02	-0.04	-0.07	0.03
	PHSTVL	0.00	-0.01	-0.01	0.01	0.00	-0.01	0.01	0.00	0.01	0.00	0.01	0.00	-0.01	-0.01	-0.01	0.00
	PTL	-0.08	-0.09	-0.09	0.02	-0.06	-0.09	0.08	0.08	0.06	0.08	0.11	0.02	-0.01	-0.08	-0.10	0.02
	SO4	0.04	-0.14	-0.07	0.07	0.08	-0.11	0.12	0.00	0.14	-0.02	0.10	-0.06	-0.11	0.01	-0.13	0.01
Woody debris	XFC_BRS	0.09	0.10	-0.05	0.14	0.08	0.19	0.09	-0.05	-0.19	-0.20	0.04	-0.08	0.17	0.28	-0.02	-0.02
	XFC_LWD	0.12	0.16	0.01	0.31	0.03	0.34	0.22	-0.14	-0.42	-0.26	0.10	-0.22	0.40	0.38	-0.04	0.02

Appendix J. Sample site information for Chapter 4. Site codes are from EMAP and ProbMon data sources. BMI = benthic macroinvertebrates.

Source	Site code	Stream name	Latitude (decimal degrees)	Longitude (decimal degrees)	Basin	BMI sample	Fish sample	Physical habitat	Water quality
EMAP	VA507S	Rocky Branch	37.497260	-79.978430	James	6/18/1993	6/18/1993	6/18/1993	6/18/1993
	VA509S	Hunting Creek	37.540250	-79.390010	James	6/17/1993	6/17/1993	6/17/1993	6/17/1993
	VA522S	Brush Creek	37.033380	-80.269990	New	4/27/1993	4/27/1993	4/27/1993	4/27/1993
	VA523S	McGavock Creek	36.959700	-80.844410	New	5/11/1993	6/16/1994	6/16/1994	6/16/1994
	VA754S	Calfpasture River	38.227300	-79.354500	James	5/19/1994	5/19/1994	5/19/1994	5/19/1994
	VA755S	Colliers Creek	37.790700	-79.597500	James	5/11/1994	5/11/1994	5/11/1994	5/11/1994
	VA756S	Holloway Draft	38.218100	-79.388500	James	5/17/1994	5/17/1994	5/17/1994	5/17/1994
	VA757S	Unnamed tributary to Hat Creek	37.803900	-78.954200	James	5/4/1994	5/4/1994	5/4/1994	5/4/1994
	VA767S	Burks Fork	36.787900	-80.627500	New	6/15/1995	8/27/1996	8/27/1996	8/27/1996
	VA769S	North Fork of Kimberling Creek	37.189500	-81.045600	New	5/22/1996	8/26/1996	8/26/1996	8/26/1996
	VAR01S	Walker Creek	37.059720	-81.138610	New	4/26/1993	4/26/1993	4/26/1993	4/26/1993
	VAR02S	Sinking Creek	37.395280	-80.312220	New	6/21/1993	6/21/1993	6/21/1993	6/21/1993
	VAR07S	Big Creek	37.736670	-79.788890	New	6/16/1993	6/16/1993	6/16/1993	6/16/1993
	VAR09S	Stony Creek	37.413890	-80.584170	New	6/24/1993	6/24/1993	6/24/1993	6/24/1993
	VAR10S	Bullpasture River	38.361110	-79.476940	James	6/15/1993	6/15/1993	6/15/1993	6/15/1993
	VAR12S	Moss Run	37.786940	-80.113890	James	5/24/1993	5/24/1993	5/24/1993	5/24/1993
	MAIA97-131	Nettle Creek	37.810494	-79.206539	James	8/27/1997	8/27/1997	8/27/1997	8/27/1997
	MAIA97-136	Peters Creek	37.578690	-79.424669	James	7/28/1997	7/28/1997	7/28/1997	7/28/1997
	MAIA97-178	Jennings Creek	37.542762	-79.616376	James	9/1/1997	9/1/1997	9/1/1997	9/1/1997
	MAIA97-189	Unnamed tributary to New River	37.136284	-80.604763	New	7/17/1997	7/17/1997	7/17/1997	7/17/1997
	MAIA97-190	Little Snake Creek	36.719798	-80.665832	New	8/10/1997	8/10/1997	8/10/1997	8/10/1997
	MAIA97-191	Unnamed tributary to	36.834379	-80.820510	New	8/9/1997	8/9/1997	8/9/1997	8/9/1997

		Shorts Creek							
	MAIA98-013	Big Run	37.218098	-80.527703	New	7/15/1998	7/15/1998	7/15/1998	7/15/1998
	MAIA98-123	Coal Creek	36.625027	-80.869029	New	7/16/1998	7/16/1998	7/16/1998	7/16/1998
	MAIA98-154	Moore Creek	36.655833	-81.027693	New	7/17/1998	7/17/1998	7/17/1998	7/17/1998
		Little Indian							
ProbMon	9-LIC004.73	Creek	36.938597	-80.538003	New	4/18/2001	7/20/2004[a]	7/22/2004[a]	11/5/2001
	9-MDR003.60	Meadow Run	37.043899	-80.171561	New	4/18/2001	8/6/2004[a]	6/23/2004[a]	11/1/2001
	9-WFC044.15	Wolf Creek	37.147858	-81.288497	New	4/26/2001	8/11/2004[a]	7/8/2004[a]	11/1/2001
	9-SFK001.10	Stony Fork	36.963496	-81.179709	New	4/24/2002	8/4/2004[a]	7/5/2004[a]	4/24/2002
	9-DDD006.61	Dodd Creek	36.885443	-80.318703	New	3/6/2003	7/28/2004[a]	6/23/2004[a]	3/6/2003
		Nobusiness							
	9-NBS006.58	Creek	37.239376	-80.930012	New	4/21/2003	7/29/2004[a]	6/22/2004[a]	4/21/2003
	9-PLM000.35	Plum Creek	37.131652	-80.523953	New	3/10/2003	7/27/2004[a]	6/17/2004[a]	3/10/2003
	2-DCK003.94	Dicks Creek	37.463344	-80.348271	James	6/1/2004	6/12/2005[a]	6/13/2005[a]	6/1/2004
	2-MIW003.45	Mill Creek	37.996557	-79.711857	James	4/28/2004	7/1/2005[a]	7/1/2005[a]	4/28/2004
	2-STV000.48	Shawvers Run	37.620492	-80.186796	James	4/15/2004	6/14/2005[a]	6/14/2005[a]	4/5/2004
		Big Reed							
	9-RIC051.80	Island Creek	36.688988	-80.531314	New	5/6/2004	6/30/2005[a]	6/30/2005[a]	5/6/2004
	2-OGL005.53	Ogle Creek	37.839865	-80.122457	James	5/1/2001	8/2/2005[a]	8/25/2004[a]	10/9/2001
	2-WLN006.90	Wilson Creek	37.897411	-79.803740	James	5/15/2002	7/2/2005[a]	8/26/2004[a]	5/15/2002
	2-CAT026.55	Catawba River	37.439488	-80.016356	James	4/3/2003	7/12/2005[a]	7/12/2005[a]	4/3/2003
		Patterson							
	2-PTR005.13	Creek	37.622641	-79.890103	James	4/3/2003	8/9/2005[a]	8/9/2005[a]	4/3/2003
	9-SNK019.59	Sinking Creek	37.341549	-80.410891	New	3/18/2003	7/18/2005[a]	6/28/2005[a]	3/18/2003
	2-RGR001.11	Roaring Run	37.705150	-79.892596	James	4/14/2005	7/25/2005[a]	7/13/2005[a]	4/14/2005
	9-LFK005.39	Laurel Creek	37.243087	-81.171301	New	4/7/2005	7/19/2005[a]	7/19/2005[a]	4/7/2005

[a]Data collected by N.P. Hitt *et al*.

Appendix K. Fish species classifications for metric calculations in Chapter 4. Families are separated by horizontal lines. Species introductions are designated by river basins as: J=Native in James river basin, N=Native in New river basin, JX=introduced in James river basin, NX=introduced in New river basin. Reproductive guilds are designed as: NL = non-lithophil, SL = simple lithophil, CL = complex lithophil (i.e., mineral substrate spawning with nest preparation and/or parental care). Spawning substrates are designed as: MIN = mineral (excluding fine substrates), VAR = various substrates, and VEG = vegetation. Vertical positions are indicated as: BE = benthic species and CO = water column species. Trophic guilds are indicated as: INV = invertivore, IP = invertivore/piscivore, OH = omnivore/herbivore, and PIS = piscivore. Tolerance levels are indicated as: TOL = tolerant species and INT = intolerant species. In all cases, "NA" indicates that the variable is not applicable for the given species.

Scientific name	Introductions[a]	Repro. guild[a]	Nest prep. and/or parental care[b]	Spawning substrate[c]	Female repro. age (yr)[c]	Vertical position[a]	Trophic guild[d]	Number of food types[e]	Tolerance[f]
Catostomus commersoni	N, J	SL	NO	MIN	3	BE	OH	3	TOL
Hypentelium nigricans	N, J	SL	NO	MIN	3	BE	INV	2	
Moxostoma cervinum	NX[1], JX[1]	SL	NO	MIN	2	BE	INV	3	
Moxostoma macrolepidotum	J	SL	NO	MIN	5	BE	INV	3	
Thoburnia rhothoeca	N, J	SL	NO	MIN	3	BE	OH	3	INT
Ambloplites rupestris	NX, JX	CL	YES	MIN	2	CO	IP	2	
Lepomis auritus	NX, J	CL	YES	MIN	2	CO	IP	2	
L. cyanellus	N, JX	NL	YES	VAR	1	CO	IP	2	TOL
L. gibbosus	NX, J	NL	YES	VAR	1	CO	INV	1	
L. macrochirus	NX, JX	NL	YES	VAR	1	CO	INV	1	TOL
Micropterus dolomieu	NX, JX	CL	YES	MIN	2	CO	IP	2	
M. salmoides	NX, JX[1]	NL	YES	VAR	2	CO	PIS	1	
Cottus bairdi	N, J	NL	YES	VAR	2	BE	INV	1	
C. caeruleomentum[2]	J	NL	YES	VAR	2	BE	INV	1	
C. carolinae	N	NL	YES	VAR	2	BE	INV	1	
C. girardi	J	NL	YES	VAR	2	BE	INV	1	
Campostoma anomalum	N, J	CL	YES	MIN	2	BE	OH	2	

C. funduloides	N, J	SL	NO	MIN	2	CO	INV	1	
Cyprinella galactura	NX[1]	NL	NO	VAR	2	CO	INV	2	
Exoglossum laurae	N	CL	YES	MIN	2	CO	INV	1	
E. maxillingua	NX[1], J	CL	YES	MIN	2	CO	INV	1	
Luxilus albeolus	N	SL	NO	MIN	1	CO	INV	1	
L. cerasinus	NX[1], JX[1]	SL	NO	MIN	2	CO	INV	2	
L. cornutus	J	SL	NO	MIN	2	CO	INV	4	
Lythrurus ardens	N, J	SL	NO	MIN	1	CO	INV	3	
Nocomis leptocephalus	N, J	CL	YES	MIN	3	CO	OH	3	
N. micropogon	J	CL	YES	MIN	3	CO	INV	3	
N. platyrhynchus	N	CL	YES	MIN	3	CO	INV	3	
Notropis rubellus	N, J	SL	NO	MIN	1	CO	INV	1	
N. rubricroceus	NX[1]	SL	NO	MIN	1	CO	INV	2	
N. scabriceps	N	SL	NO	MIN	2	CO	INV	1	
N. telescopus	NX[1], JX[1]	SL	NO	MIN	2	CO	INV	1	
Phenacobius teretulus	N	SL	NO	MIN	2	BE	INV	1	
Phoxinus oreas	N, J	SL	NO	MIN	1	CO	OH	3	
Pimephales notatus	N, JX[1]	NL	YES	VAR	1	CO	OH	3	TOL
Rhinichthys atratulus	N, J	SL	NO	MIN	2	BE	INV	3	TOL
R. cataractae	N, J	SL	NO	MIN	2	BE	INV	2	
Semotilus atromaculatus	N, J	CL	YES	MIN	1	CO	IP	4	TOL
S. corporalis	J	CL	YES	MIN	2	CO	IP	4	
Esox niger	J	NL	NO	VEG	2	CO	PIS	1	
Ameiurus nebulosus	NX[1], J	NL	YES	VAR	3	BE	OH	3	
Noturus insignis	N, J	CL	YES	MIN	3	BE	INV	2	
Etheostoma blennioides	N	NL	NO	VAR	2	BE	INV	1	
E. flabellare	N, J	CL	YES	MIN	2	BE	INV	1	TOL
E. kanawhae	N	SL	NO	MIN	2	BE	INV	1	
E. longimanum	J	CL	YES	MIN	1	BE	INV	1	
E. nigrum	N, J	NL	YES	VAR	1	BE	INV	1	TOL
E. osburni	N	SL	NO[a]	MIN	2	BE	INV	1[a]	INT

E. simoterum	N	NL	NO	VAR	1	BE	INV	1	
Percina gymnocephala	N	SL	NO	MIN	2	BE	INV	1	
Oncorhynchus mykiss	NX, JX	CL	YES	MIN	1	CO	IP	2	
Salmo trutta	NX, JX	CL	YES	MIN	1	CO	IP	2	
Salvelinus fontinalis	N, J	CL	YES	MIN	2	CO	IP	2	INT

[a]Data from Jenkins and Burkhead (1994).
[b]Data from Smogor and Angermeier (1999a) unless otherwise noted.
[c]Data from Jenkins and Burkhead (1994) and Smogor and Angermeier (1999a) unless otherwise noted.
[d]Data from Smogor and Angermeier (1999a) but AHI and DAH combined into "omnivore-herbivore" *sensu* McCormick *et al*. (2001).
[e]Data from Smogor and Angermeier (1999a) includes four possible food types: detritus, algae/vascular plants, invertebrates, and fish/fish blood.
[f]Classifications from agreement between Smogor and Angermeier (1999a) and McCormick *et al*.(2001). If a species is listed in one source but not the other and it has calls, I included those calls here. If the species was not listed in either source, classifications (or lack thereof) are attributed to Barbour *et al*. (1999).

[1]Considered introduced but possibly native (Jenkins and Burkhead 1994).
[2]Data from *C. bairdi*.

Appendix L. Fish and benthic macroinvertebrate (BMI) metrics, expected responses to degradation, and non-parametric correlations (Spearman *rho*) with an environmental quality gradient, NMS II. Environmental variable loadings into NMS II are presented in Table 3.2 (See Table 3.3 for land use relations to NMS gradients). Positive correlation coefficients indicate that the metric increases with increasing environmental quality. *p<0.05; **p<0.005.

Biotic group	Category	Metric code	Definition	Expected response to degradation	Reference	NMS II
Fish	Taxonomic	S	Species richness	Negative	Angermeier *et al*. (2000) [TOTSP]	-0.050
		NAT_S	Native species richness	Negative	Smogor and Angermeier (1999b) [NATSP]	0.015
		NAT_N	Native species abundance	Negative		-0.100
		NAT_P	Native species proportional abundance	Negative		0.300
		NNAT_S	Non-native species richness	Positive	Smogor and Angermeier (1999b) [NONNATSP]; Angermeier *et al*. (2000) [INTSP]	-0.200
		NNAT_N	Non-native species abundance	Positive		-0.325*
		NNAT_P	Non-native species proportional abundance	Positive	McCormick *et al*. (2001) [PEXOT]	-0.300
		COT_N	Cottid abundance	Negative		0.202
		COT_P	Cottid proportional abundance	Negative	McCormick *et al*. (2001) [PCOTTID]	0.323*
		SAL_S	Salmonid species richness	Negative		0.192
		SAL_N	Salmonid species abundance	Negative		0.308*
		SAL_P	Salmonid species proportional abundance	Negative		0.297
		PER_S	Percid species richness	Negative		0.020
		PER_N	Percid species abundance	Negative		-0.200
		PER_P	Percid species proportional abundance	Negative		-0.190
		CEN_S	Centrarchid species richness	Negative	Smogor and Angermeier (1999b) [SUNSP];	-0.190

			Angermeier *et al*. (2000) [SUNSP]	
CEN_N	Centrarchid species abundance	Negative		-0.270
CEN_P	Centrarchid species proportional abundance	Negative		-0.260
CAT_S	Catostomid species richness	Negative	Angermeier *et al*. (2000) [SUCSP]	0.062
CAT_N	Catostomid species abundance	Negative		0.006
CAT_P	Catostomid species proportional abundance	Negative		0.032
CYP_S	Cyprinid species richness	Negative	Smogor and Angermeier (1999b) [MINSP]; Angermeier *et al*. (2000) [MINSP]	-0.180
CYP2_S	Native cyprinid species richness, excluding tolerant species	Negative	McCormick *et al*. (2001) [NSCYPR2]	-0.170
CYP_N	Cyprinid species abundance	Negative		-0.220
CYP2_N	Native cyprinid species abundance, excluding tolerant species	Negative		-0.160
CYP_P	Cyprinid species proportional abundance	Negative		-0.140
CYP2_P	Native cyprinid species proportional abundance, excluding tolerant species	Negative		-0.120
ICT_S	Ictalurid species richness	Negative		0.080
ICT_N	Ictalurid species abundance	Negative		0.114
ICT_P	Ictalurid species proportional abundance	Negative		0.128
DOS_S	Darter or sculpin species richness	Negative	Smogor and Angermeier (1999b) [DARSCLSP]; Angermeier *et al*. (2000) [DOSSP]	0.115
DOS_N	Darter or sculpin species	Negative		-0.050

		abundance			
	DOS_P	Darter or sculpin species proportional abundance	Negative		0.103
Trophic	IP_S	Invertivore-piscivore richness	Negative		0.091
	IP_N	Invertivore-piscivore abundance	Negative		0.136
	IP_P	Invertivore-piscivore proportional abundance	Negative		0.238
	IP2_P	Invertivore-piscivore proportional abundance, excluding *S. atromaculatus*	Negative	McCormick *et al*. (2001) [PPISCINV2]	0.216
	OH_S	Omnivore-herbivore richnesss	Positive		-0.210
	OH_N	Omnivore-herbivore abundance	Positive		-0.220
	OH_P	Omnivore-herbivore relative abundance	Positive		-0.230
	INV_S	Invertivore richness	Negative		-0.030
	INV_N	Invertivore abundance	Negative		-0.160
	INV_P	Invertivore proportional abundance	Negative	Smogor and Angermeier (1999b) [INVPRP]; Angermeier *et al*. (2001) [INVPR]	0.162
	INV_BEN_S	Invertivore benthic specialist richness	Negative	Food type number=1; Angermeier *et al*. (2000)	0.146
	INV_BEN_N	Invertivore benthic specialist abundance	Negative	Food type number=1; See Angermeier *et al*. (2000)	-0.050
	INV_BEN_P	Invertivore benthic specialist proportional abundance	Negative	Food type number=1; Smogor and Angermeier (1999b) [BINVPRP]; Angermeier *et al*. (2000) [BSIPR]	0.108

	PIS_S	Piscivore richness	Negative		-0.210
	PIS_N	Piscivore abundance	Negative		-0.230
	PIS_P	Piscivore proportional abundance	Negative	Angermeier *et al*. (2000) [CARPR]	-0.230
	GEN_COL_S	Generalist feeders, column species richness	Positive	Food type number >2; Smogor and Angermeier (1999b) [GENPRP]; Angermeier *et al*. (2000) [GENPR]	-0.140
	GEN_COL_N	Generalist feeders, column species abundance	Positive	Food type number >2; Angermeier *et al*. (2000) [GENPR]	-0.240
	GEN_COL_P	Generalist feeders, column species proportional abundance	Positive	Food type number >2; Angermeier *et al*. (2000) [GENPR]	-0.230
Reproductive	SL_S	Simple lithophil richness	Negative		-0.080
	SL2_S	Simple lithophil richness, excluding tolerant species	Negative		-0.040
	SL_N	Simple lithophil abundance	Negative		-0.190
	SL2_N	Simple lithophil abundance, excluding tolerant species	Negative		-0.190
	SL_P	Simple lithophil proportional abundance	Negative	Smogor and Angermeier (1999b) [SLITHPR]	-0.030
	SL2_P	Simple lithophil proportional abundance, excluding tolerant species	Negative	Angermeier *et al*. (2000) [SLTPR]	-0.130
	NL_S	Non-lithophil richness	Positive		-0.040
	NL_N	Non-lithophil abundance	Positive		0.040
	NL_P	Non-lithophil proportional abundance	Positive		0.188
	NS_NL_S	Non-simple non-lithophil species richness	Positive		0.000
	NS_NL_N	Non-simple non-lithophil species abundance	Positive		0.081
	NS_NL_P	Non-simple non-lithophil species proportional abundance	Positive	Smogor and Angermeier (1999b) [VMANPRP];	0.050

				Angermeier *et al*. (2000) [NNLPR]	
	LATE_S	Late maturing species richness (age >2)	Negative	Smogor and Angermeier (1999b) [AGE3SP]; Angermeier *et al*. (2000) [LAMSP]	-0.030
	LATE_N	Late maturing species abundance (age >2)	Negative		-0.130
	LATE_P	Late maturing species proportional abundance (age >2)	Negative		-0.070
	EARLY_S	Early maturing species richness (age <3)	Positive		-0.070
	EARLY_N	Early maturing species abundance (age <3)	Positive		-0.200
	EARLY_P	Early maturing species proportional abundance (age <3)	Positive		0.070
	REPROAGE	Mean age of first female reproduction	Negative		0.331*
Tolerance	TOL_S	Tolerant species richness	Positive		-0.160
	TOL_N	Tolerant species abundance	Positive		-0.250
	TOL_P	Tolerant species proportional abundance	Positive	Smogor and Angermeier (1999b) [TOLPRP]; Angermeier *et al*. (2000) [TOLPR]; McCormick *et al*. (2001) [PTOLE]	-0.030
	INTOL_S	Intolerant species richness	Negative	McCormick *et al*. (2001) [NSINTOL]	0.496**
	INTOL_N	Intolerant species abundance	Negative		0.406*
	INTOL_P	Intolerant species proportional abundance	Negative		0.477**
Vertical position	COL_S	Column species richness	Positive		-0.160
	COL_N	Column species abundance	Positive		-0.160
	COL_P	Column species	Positive		-0.090

			proportional abundance			
		BEN_S	Benthic species richness	Negative		0.078
		BEN2_S	Benthic species richness, excluding tolerant species	Negative	McCormick *et al*. (2001) [NSBHAB2]	0.162
		BEN_N	Benthic species abundance	Negative		-0.080
		BEN2_N	Benthic species abundance, excluding tolerant species	Negative		0.008
		BEN_P	Benthic species proportional abundance	Negative		0.088
		BEN2_P	Benthic species proportional abundance, excluding tolerant species	Negative		0.122
BMI	Taxonomic	FAM_S	Family-level richness	Negative	Smith and Voshell (1997); Burton and Gerritsen (2003)	-0.048
		EPT_S	Ephemeroptera, trichoptera, ephemeroptera richness	Negative	Smith and Voshell (1997); Burton and Gerritsen (2003)	0.445**
		EPT_P	Percent individuals ephemeroptera, trichoptera, or ephemeroptera	Negative	Smith and Voshell (1997); Burton and Gerritsen (2003)	0.504**
		EPH_P	Percent individuals ephemeroptera	Negative	Smith and Voshell (1997); Burton and Gerritsen (2003)	0.358*
		PLE_P	Percent individuals plecoptera	Negative	Smith and Voshell (1997)	0.518**
		TRI_P	Percent individuals trichoptera	Negative	Smith and Voshell (1997)	0.031
	Trophic	SCR_P	Percent individuals scrapers	Decrease	Smith and Voshell (1997); Burton and Gerritsen (2003)	-0.133
		SHR_P	Percent individuals shredders	Decrease	Delong and Brusven (1998)	0.336*
		PRE_P	Percent individuals predators	Variable	Voshell (2002)	0.230
		COL_P	Percent individuals collectors/gatherers	Variable	Voshell (2002)	0.054
		FIL_P	Percent individuals	Variable	Smith and Voshell	-0.021

		filterers/collectors		(1997)	
Tolerance	TOL_P	Percent individuals "tolerant"	Positive	Burton and Gerritsen (2003); Yuan and Norton (2003)	-0.370*
	HBI	Hilsenhoff Biotic Index	Positive	Burton and Gerritsen (2003)	-0.353*

Appendix M. Descriptive statistics for environmental variables among all sites, EMAP sites, and ProbMon sites. Variable codes are presented in Table 1.

Category	Variable	Group	Minimum	Maximum	Mean	Std. dev.
Land use	AG_TOT	ALL	0.00	80.05	23.25	25.07
		EMAP	0.00	80.05	26.09	26.16
		ProbMon	0.00	64.85	19.29	23.64
	FOR_TOT	ALL	11.58	100.00	75.58	26.18
		EMAP	11.58	100.00	72.51	27.97
		ProbMon	34.87	99.70	79.85	23.58
	URB_TOT	ALL	0.00	14.85	0.91	2.88
		EMAP	0.00	14.85	1.33	3.67
		ProbMon	0.00	3.88	0.34	0.93
Mesohabitat	PCT_FAST	ALL	8.00	88.00	46.14	23.51
		EMAP	9.00	88.00	56.58	23.16
		ProbMon	8.00	61.30	31.66	15.06
	PCT_POOL	ALL	0.00	64.65	15.81	14.93
		EMAP	0.00	64.65	16.07	17.65
		ProbMon	0.00	35.30	15.44	10.53
Riparian vegetation	XCDENBK	ALL	0.00	100.00	53.45	32.10
		EMAP	0.00	100.00	58.77	35.43
		ProbMon	0.00	77.14	46.05	25.96
	XCL	ALL	0.00	75.20	19.17	17.78
		EMAP	0.00	75.20	17.95	20.38
		ProbMon	0.00	47.60	20.86	13.77
Substrate	PCT_BIGR	ALL	18.20	92.73	53.58	20.78
		EMAP	20.00	92.73	60.15	22.40
		ProbMon	18.20	68.00	44.46	14.39
	PCT_SAFN	ALL	0.00	70.91	22.24	18.38
		EMAP	0.00	70.91	27.85	20.19
		ProbMon	0.00	40.00	14.44	12.17
	XEMBED	ALL	3.35	90.73	44.11	21.53
		EMAP	3.35	90.73	48.17	23.35
		ProbMon	8.73	71.55	38.47	17.82
Volume	XWIDTH	ALL	0.43	16.85	5.95	4.19
		EMAP	0.43	16.85	5.48	4.85
		ProbMon	1.98	13.79	6.60	3.06
	AREA_WS	ALL	16.00	14202.00	2986.04	3425.13
		EMAP	16.00	14202.00	2791.80	3691.23
		ProbMon	169.46	10018.06	3255.81	3100.92
	XDEPTH	ALL	7.31	66.32	30.12	13.65
		EMAP	7.73	66.32	27.47	14.73
		ProbMon	7.31	50.32	33.80	11.36
Water quality	COND	ALL	9.80	647.00	140.13	158.83
		EMAP	20.00	647.00	151.16	175.42

		ProbMon	9.80	492.00	124.81	135.85
	NTL	ALL	0.08	3.23	0.55	0.57
		EMAP	0.08	3.23	0.55	0.64
		ProbMon	0.10	1.91	0.55	0.49
	PHSTVL	ALL	4.86	8.94	7.43	0.80
		EMAP	6.36	8.94	7.48	0.61
		ProbMon	4.86	8.64	7.37	1.02
	PTL	ALL	0.00	0.10	0.02	0.02
		EMAP	0.00	0.10	0.02	0.02
		ProbMon	0.01	0.05	0.02	0.01
Woody debris	XFC_BRS	ALL	0.00	25.00	5.41	4.95
		EMAP	0.00	25.00	5.44	6.19
		ProbMon	0.46	10.45	5.37	2.51
	XFC_LWD	ALL	0.00	25.00	1.93	4.27
		EMAP	0.00	25.00	1.97	5.32
		ProbMon	0.00	8.45	1.87	2.24

Appendix N. Mann-Whitney tests for environmental variables among EMAP and ProbMon sites.

Category	Variable	Mann-Whitney U	Z	p-value
Land use	AG_TOT	218.0	-0.173	0.863
	FOR_TOT	221.0	-0.099	0.921
	URB_TOT	205.5	-0.506	0.613
Mesohabitat	PCT_FAST	87.0	-3.397	0.001
	PCT_POOL	195.0	-0.739	0.460
Riparian vegetation	XCDENBK	161.5	-1.563	0.118
	XCL	178.0	-1.158	0.247
Substrate	PCT_BIGR	129.0	-2.364	0.018
	PCT_SAFN	141.0	-2.072	0.038
	XEMBED	165.0	-1.477	0.140
Volume	XWIDTH	163.0	-1.526	0.127
	AREA_WS	178.0	-1.157	0.247
	XDEPTH	159.0	-1.625	0.104
Water quality	COND	208.5	-0.406	0.685
	NTL	223.5	-0.037	0.971
	PHSTVL	220.0	-0.123	0.902
	PTL	207.0	-0.451	0.652
Woody debris	XFC_BRS	181.0	-1.086	0.277
	XFC_LWD	153.0	-1.842	0.065

Appendix O. Descriptive statistics for fish and benthic macroinvertebrate (BMI) metrics among all sites, EMAP sites, and ProbMon sites in the study area. Metric codes are presented in Appendix C.

Biotic group	Category	Metric	Class	Min	Max	Mean	Std dev.
Fish	Taxonomic	S	All	2.00	24.00	11.47	5.91
			EMAP	2.00	23.00	9.68	5.71
			ProbMon	5.00	24.00	13.94	5.38
		NAT_S	All	2.00	21.00	9.72	4.65
			EMAP	2.00	21.00	8.60	4.82
			ProbMon	3.00	17.00	11.28	4.01
		NAT_N	All	27.00	2740.00	469.00	563.84
			EMAP	27.00	1232.00	365.80	370.20
			ProbMon	44.00	2740.00	612.33	744.67
		NAT_P	All	0.66	1.00	0.93	0.10
			EMAP	0.66	1.00	0.95	0.10
			ProbMon	0.71	1.00	0.92	0.10
		NNAT_S	All	0.00	7.00	1.74	1.72
			EMAP	0.00	4.00	1.08	1.29
			ProbMon	0.00	7.00	2.67	1.85
		NNAT_N	All	0.00	220.00	29.33	49.53
			EMAP	0.00	102.00	18.32	30.03
			ProbMon	0.00	220.00	44.61	66.04
		NNAT_P	All	0.00	0.34	0.07	0.10
			EMAP	0.00	0.34	0.05	0.10
			ProbMon	0.00	0.29	0.08	0.10
		COT_N	All	0.00	610.00	45.51	104.05
			EMAP	0.00	279.00	36.56	66.74
			ProbMon	0.00	610.00	57.94	142.06
		COT_P	All	0.00	0.90	0.10	0.16
			EMAP	0.00	0.90	0.11	0.20
			ProbMon	0.00	0.34	0.08	0.10
		SAL_S	All	0.00	2.00	0.44	0.63
			EMAP	0.00	2.00	0.40	0.65
			ProbMon	0.00	2.00	0.50	0.62
		SAL_N	All	0.00	113.00	5.35	18.03
			EMAP	0.00	113.00	7.28	23.27
			ProbMon	0.00	19.00	2.67	5.05
		SAL_P	All	0.00	0.48	0.03	0.11
			EMAP	0.00	0.48	0.04	0.12
			ProbMon	0.00	0.43	0.03	0.10
		PER_S	All	0.00	4.00	1.28	1.03
			EMAP	0.00	4.00	1.04	0.93
			ProbMon	0.00	4.00	1.61	1.09
		PER_N	All	0.00	926.00	54.07	145.74
			EMAP	0.00	160.00	25.36	41.71

	ProbMon	0.00	926.00	93.94	217.09
PER_P	All	0.00	0.42	0.07	0.08
	EMAP	0.00	0.29	0.05	0.07
	ProbMon	0.00	0.42	0.09	0.10
CEN_S	All	0.00	4.00	1.19	1.31
	EMAP	0.00	3.00	0.64	0.91
	ProbMon	0.00	4.00	1.94	1.43
CEN_N	All	0.00	71.00	11.00	17.76
	EMAP	0.00	71.00	8.00	18.96
	ProbMon	0.00	61.00	15.17	15.49
CEN_P	All	0.00	0.34	0.04	0.07
	EMAP	0.00	0.34	0.03	0.09
	ProbMon	0.00	0.15	0.04	0.05
CAT_S	All	0.00	3.00	1.30	0.89
	EMAP	0.00	3.00	1.12	1.01
	ProbMon	0.00	2.00	1.56	0.62
CAT_N	All	0.00	415.00	45.70	94.10
	EMAP	0.00	415.00	46.56	96.49
	ProbMon	0.00	411.00	44.50	93.43
CAT_P	All	0.00	0.38	0.08	0.10
	EMAP	0.00	0.37	0.07	0.11
	ProbMon	0.00	0.38	0.08	0.09
CYP_S	All	1.00	14.00	6.12	2.95
	EMAP	1.00	14.00	5.48	2.99
	ProbMon	2.00	12.00	7.00	2.72
CYP2_S	All	0.00	13.00	4.44	2.57
	EMAP	0.00	13.00	4.20	2.96
	ProbMon	1.00	8.00	4.78	1.93
CYP_N	All	18.00	1925.00	332.23	382.62
	EMAP	18.00	1015.00	256.52	266.03
	ProbMon	22.00	1925.00	437.39	491.58
CYP2_N	All	0.00	1589.00	244.72	340.96
	EMAP	0.00	858.00	174.44	238.87
	ProbMon	1.00	1589.00	342.33	435.09
CYP_P	All	0.10	1.00	0.67	0.20
	EMAP	0.10	1.00	0.67	0.23
	ProbMon	0.38	0.93	0.67	0.15
CYP2_P	All	0.00	0.91	0.41	0.23
	EMAP	0.00	0.73	0.36	0.23
	ProbMon	0.00	0.91	0.47	0.21
ICT_S	All	0.00	1.00	0.33	0.47
	EMAP	0.00	1.00	0.24	0.44
	ProbMon	0.00	1.00	0.44	0.51
ICT_N	All	0.00	29.00	4.16	7.66
	EMAP	0.00	29.00	3.36	7.62
	ProbMon	0.00	23.00	5.28	7.78

	ICT_P	All	0.00	0.13	0.01	0.03
		EMAP	0.00	0.13	0.01	0.03
		ProbMon	0.00	0.07	0.01	0.02
	DOS_S	All	0.00	5.00	2.02	1.24
		EMAP	0.00	4.00	1.72	1.10
		ProbMon	0.00	5.00	2.44	1.34
	DOS_N	All	0.00	926.00	99.58	192.56
		EMAP	0.00	318.00	61.92	80.25
		ProbMon	0.00	926.00	151.89	278.45
	DOS_P	All	0.00	0.90	0.17	0.16
		EMAP	0.00	0.90	0.17	0.19
		ProbMon	0.00	0.42	0.17	0.13
Trophic	IP_S	All	0.00	5.00	1.77	1.27
		EMAP	0.00	5.00	1.32	1.22
		ProbMon	0.00	4.00	2.39	1.09
	IP_N	All	0.00	113.00	20.33	25.39
		EMAP	0.00	113.00	21.08	31.00
		ProbMon	0.00	54.00	19.28	15.30
	IP_P	All	0.00	0.48	0.08	0.12
		EMAP	0.00	0.48	0.09	0.14
		ProbMon	0.00	0.43	0.07	0.10
	IP2_P	All	0.00	0.48	0.07	0.13
		EMAP	0.00	0.48	0.08	0.14
		ProbMon	0.00	0.43	0.06	0.10
	OH_S	All	0.00	6.00	3.37	1.56
		EMAP	0.00	6.00	3.00	1.73
		ProbMon	2.00	6.00	3.89	1.13
	OH_N	All	0.00	1310.00	235.58	340.91
		EMAP	0.00	1056.00	176.52	284.78
		ProbMon	3.00	1310.00	317.61	400.47
	OH_P	All	0.00	0.89	0.35	0.23
		EMAP	0.00	0.89	0.31	0.26
		ProbMon	0.07	0.86	0.42	0.19
	INV_S	All	1.00	17.00	6.21	3.87
		EMAP	1.00	17.00	5.28	3.70
		ProbMon	1.00	16.00	7.50	3.82
	INV_N	All	10.00	1525.00	240.88	293.26
		EMAP	10.00	690.00	186.04	157.37
		ProbMon	22.00	1525.00	317.06	408.59
	INV_P	All	0.09	1.00	0.55	0.24
		EMAP	0.09	1.00	0.59	0.27
		ProbMon	0.14	0.77	0.50	0.17
	INV_BEN_S	All	0.00	5.00	2.09	1.34
		EMAP	0.00	5.00	1.80	1.22
		ProbMon	0.00	5.00	2.50	1.42
	INV_BEN_N	All	0.00	926.00	99.84	192.49

		EMAP	0.00	318.00	62.16	80.11
		ProbMon	0.00	926.00	152.17	278.38
	INV_BEN_P	All	0.00	0.90	0.17	0.16
		EMAP	0.00	0.90	0.17	0.19
		ProbMon	0.00	0.42	0.17	0.13
	PIS_S	All	0.00	1.00	0.12	0.32
		EMAP	0.00	1.00	0.08	0.28
		ProbMon	0.00	1.00	0.17	0.38
	PIS_N	All	0.00	50.00	1.53	7.76
		EMAP	0.00	11.00	0.48	2.20
		ProbMon	0.00	50.00	3.00	11.75
	PIS_P	All	0.00	0.20	0.01	0.03
		EMAP	0.00	0.20	0.01	0.04
		ProbMon	0.00	0.08	0.01	0.02
	GEN_COL_S	All	0.00	5.00	2.44	1.39
		EMAP	0.00	5.00	2.16	1.40
		ProbMon	0.00	5.00	2.83	1.29
	GEN_COL_N	All	0.00	1093.00	116.26	217.07
		EMAP	0.00	685.00	95.08	176.08
		ProbMon	0.00	1093.00	145.67	266.58
	GEN_COL_P	All	0.00	0.81	0.20	0.20
		EMAP	0.00	0.70	0.19	0.20
		ProbMon	0.00	0.81	0.21	0.20
Reproductive	SL_S	All	1.00	13.00	5.33	2.73
		EMAP	1.00	13.00	4.64	2.74
		ProbMon	2.00	10.00	6.28	2.49
	SL2_S	All	0.00	11.00	3.93	2.47
		EMAP	0.00	11.00	3.32	2.48
		ProbMon	1.00	8.00	4.78	2.26
	SL_N	All	18.00	1006.00	255.51	268.09
		EMAP	18.00	919.00	227.32	253.75
		ProbMon	19.00	1006.00	294.67	289.61
	SL2_N	All	0.00	974.00	182.47	235.16
		EMAP	0.00	735.00	150.28	206.89
		ProbMon	13.00	974.00	227.17	269.35
	SL_P	All	0.10	0.98	0.54	0.23
		EMAP	0.10	0.94	0.58	0.25
		ProbMon	0.19	0.98	0.50	0.20
	SL2_P	All	0.00	0.73	0.31	0.19
		EMAP	0.00	0.69	0.29	0.22
		ProbMon	0.16	0.73	0.33	0.14
	NL_S	All	0.00	6.00	1.51	1.47
		EMAP	0.00	4.00	1.08	1.00
		ProbMon	0.00	6.00	2.11	1.81
	NL_N	All	0.00	689.00	48.19	113.00
		EMAP	0.00	279.00	38.20	66.04

		ProbMon	0.00	689.00	62.06	158.24
	NL_P	All	0.00	0.90	0.11	0.16
		EMAP	0.00	0.90	0.13	0.20
		ProbMon	0.00	0.34	0.09	0.10
	NS_NL_S	All	0.00	6.00	1.35	1.29
		EMAP	0.00	4.00	1.00	1.00
		ProbMon	0.00	6.00	1.83	1.50
	NS_NL_N	All	0.00	585.00	45.37	99.35
		EMAP	0.00	279.00	37.72	66.29
		ProbMon	0.00	585.00	56.00	134.08
	NS_NL_P	All	0.00	0.34	0.04	0.08
		EMAP	0.00	0.20	0.01	0.04
		ProbMon	0.00	0.34	0.09	0.09
	LATE_S	All	0.00	6.00	2.44	1.56
		EMAP	0.00	6.00	2.04	1.67
		ProbMon	1.00	5.00	3.00	1.24
	LATE_N	All	0.00	600.00	83.16	123.63
		EMAP	0.00	447.00	74.12	114.29
		ProbMon	2.00	600.00	95.72	137.96
	LATE_P	All	0.00	0.49	0.16	0.14
		EMAP	0.00	0.49	0.14	0.14
		ProbMon	0.02	0.45	0.19	0.14
	EARLY_S	All	2.00	19.00	9.02	4.51
		EMAP	2.00	18.00	7.64	4.21
		ProbMon	4.00	19.00	10.94	4.30
	EARLY_N	All	27.00	2817.00	415.16	510.16
		EMAP	27.00	1096.00	310.00	284.09
		ProbMon	42.00	2817.00	561.22	700.15
	EARLY_P	All	0.51	1.00	0.84	0.14
		EMAP	0.51	1.00	0.86	0.14
		ProbMon	0.55	0.98	0.81	0.14
	REPROAGE	All	1.70	2.25	2.00	0.13
		EMAP	1.70	2.20	2.00	0.13
		ProbMon	1.80	2.25	1.99	0.12
Tolerance	TOL_S	All	1.00	6.00	3.05	1.51
		EMAP	1.00	5.00	2.52	1.16
		ProbMon	1.00	6.00	3.78	1.66
	TOL_N	All	4.00	542.00	117.19	120.29
		EMAP	17.00	367.00	104.80	104.43
		ProbMon	4.00	542.00	134.39	140.76
	TOL_P	All	0.03	0.98	0.31	0.22
		EMAP	0.03	0.98	0.35	0.24
		ProbMon	0.04	0.60	0.25	0.17
	INTOL_S	All	0.00	2.00	0.58	0.70
		EMAP	0.00	2.00	0.60	0.76
		ProbMon	0.00	2.00	0.56	0.62

		INTOL_N	All	0.00	414.00	38.72	91.33
			EMAP	0.00	414.00	42.48	92.42
			ProbMon	0.00	394.00	33.50	92.18
		INTOL_P	All	0.00	0.48	0.08	0.14
			EMAP	0.00	0.48	0.09	0.15
			ProbMon	0.00	0.43	0.08	0.13
	Vertical position	COL_S	All	0.00	13.00	5.70	3.47
			EMAP	0.00	12.00	4.56	3.32
			ProbMon	2.00	13.00	7.28	3.10
		COL_N	All	0.00	1173.00	187.07	245.59
			EMAP	0.00	841.00	152.28	215.64
			ProbMon	3.00	1173.00	235.39	281.27
		COL_P	All	0.00	0.87	0.39	0.24
			EMAP	0.00	0.76	0.37	0.24
			ProbMon	0.01	0.87	0.42	0.24
		BEN_S	All	1.00	11.00	5.77	2.76
			EMAP	1.00	11.00	5.12	2.80
			ProbMon	3.00	11.00	6.67	2.50
		BEN2_S	All	0.00	8.00	3.47	2.24
			EMAP	0.00	8.00	3.04	2.30
			ProbMon	1.00	8.00	4.06	2.07
		BEN_N	All	10.00	2661.00	311.26	476.22
			EMAP	17.00	1109.00	231.84	257.54
			ProbMon	10.00	2661.00	421.56	666.71
		BEN2_N	All	0.00	2301.00	200.26	405.51
			EMAP	0.00	1072.00	131.44	227.96
			ProbMon	3.00	2301.00	295.83	562.37
		BEN_P	All	0.13	1.00	0.61	0.24
			EMAP	0.24	1.00	0.63	0.24
			ProbMon	0.13	0.99	0.58	0.24
		BEN2_P	All	0.00	0.91	0.31	0.23
			EMAP	0.00	0.91	0.29	0.25
			ProbMon	0.02	0.80	0.34	0.21
BMI	Taxonomic	FAM_S	ALL	6.00	27.00	17.77	4.40
			EMAP	6.00	27.00	18.48	4.50
			ProbMon	10.00	22.00	16.78	4.18
		EPT_S	ALL	1.00	15.00	9.81	3.26
			EMAP	1.00	15.00	9.64	3.64
			ProbMon	3.00	14.00	10.06	2.73
		EPT_P	ALL	1.36	91.67	49.93	23.70
			EMAP	1.36	86.27	45.51	25.27
			ProbMon	15.45	91.67	56.07	20.43
		EPH_P	ALL	0.00	85.61	29.59	20.93
			EMAP	0.00	66.67	25.97	19.89
			ProbMon	6.60	85.61	34.62	21.85

	PLE_P	ALL	0.00	61.64	10.89	12.23
		EMAP	0.00	61.64	11.95	14.30
		ProbMon	0.00	36.79	9.42	8.76
	TRI_P	ALL	0.00	30.82	9.45	7.82
		EMAP	0.00	30.82	7.58	7.57
		ProbMon	2.27	29.01	12.03	7.60
Trophic	SCR_P	ALL	0.00	64.31	18.12	14.13
		EMAP	0.00	64.31	15.53	13.50
		ProbMon	3.85	53.33	21.70	14.58
	SHR_P	ALL	0.00	59.57	9.80	12.45
		EMAP	0.00	59.57	10.40	13.52
		ProbMon	1.09	46.23	8.97	11.13
	PRE_P	ALL	0.76	36.95	10.19	8.02
		EMAP	2.41	36.95	12.56	9.33
		ProbMon	0.76	16.11	6.91	4.05
	COL_P	ALL	5.66	79.41	40.67	20.16
		EMAP	6.91	79.41	37.51	18.92
		ProbMon	5.66	76.92	45.06	21.52
	FIL_P	ALL	0.77	45.65	14.61	11.32
		EMAP	0.98	42.65	12.64	11.26
		ProbMon	0.77	45.65	17.36	11.12
Tolerance	TOL_P	ALL	0.00	21.36	4.55	5.93
		EMAP	0.00	21.36	6.91	6.64
		ProbMon	0.00	8.46	1.28	2.26
	HBI	ALL	2.94	5.62	4.25	0.64
		EMAP	2.94	5.14	4.21	0.61

Appendix P. Mann-Whitney tests of fish and benthic macroinvertebrate (BMI) metrics among EMAP and ProbMon sites. Metric codes are presented in Appendix C.

Biotic group	Category	Metric	Mann-Whitney U	Z	p-value
Fish	Taxonomic	S	131.0	-2.323	0.020
		NAT_S	143.5	-2.014	0.044
		NAT_N	182.0	-1.059	0.290
		NAT_P	156.0	-1.736	0.083
		NNAT_S	109.5	-2.939	0.003
		NNAT_N	150.0	-1.887	0.059
		NNAT_P	156.0	-1.736	0.083
		COT_N	219.5	-0.138	0.890
		COT_P	219.0	-0.151	0.880
		SAL_S	201.0	-0.694	0.488
		SAL_N	205.5	-0.554	0.580
		SAL_P	203.0	-0.624	0.532
		PER_S	156.5	-1.797	0.072
		PER_N	155.5	-1.719	0.086
		PER_P	166.0	-1.459	0.145
		CEN_S	110.0	-3.007	0.003
		CEN_N	128.5	-2.505	0.012
		CEN_P	143.5	-2.115	0.034
		CAT_S	164.5	-1.588	0.112
		CAT_N	153.0	-1.784	0.074
		CAT_P	184.5	-1.003	0.316
		CYP_S	156.0	-1.713	0.087
		CYP2_S	189.0	-0.898	0.369
		CYP_N	175.0	-1.231	0.218
		CYP2_N	154.5	-1.736	0.083
		CYP_P	216.0	-0.222	0.825
		CYP2_P	174.0	-1.256	0.209
		ICT_S	179.0	-1.395	0.163
		ICT_N	182.0	-1.271	0.204
		ICT_P	181.0	-1.301	0.193
		DOS_S	156.5	-1.74	0.082
		DOS_N	192.0	-0.813	0.416
		DOS_P	199.5	-0.628	0.530
	Trophic	IP_S	107.5	-2.971	0.003
		IP_N	173.5	-1.273	0.203
		IP_P	190.5	-0.852	0.394
		IP2_P	154.0	-1.763	0.078
		OH_S	161.5	-1.599	0.110
		OH_N	148.5	-1.884	0.060
		OH_P	157.0	-1.674	0.094
		INV_S	141.0	-2.077	0.038
		INV_N	196.5	-0.702	0.483

		INV_P	176.0	-1.206	0.228
		INV_BEN_S	163.0	-1.575	0.115
		INV_BEN_N	192.0	-0.813	0.416
		INV_BEN_P	200.5	-0.603	0.546
		PIS_S	205.5	-0.864	0.387
		PIS_N	205.0	-0.884	0.376
		PIS_P	205.5	-0.862	0.389
		GEN_COL_S	168.5	-1.437	0.151
		GEN_COL_N	184.0	-1.011	0.312
		GEN_COL_P	191.5	-0.826	0.409
	Reproductive	SL_S	138.0	-2.166	0.030
		SL2_S	138.0	-2.16	0.031
		SL_N	184.5	-0.997	0.319
		SL2_N	159.5	-1.613	0.107
		SL_P	162.0	-1.551	0.121
		SL2_P	198.0	-0.665	0.506
		NL_S	145.5	-2.065	0.039
		NL_N	195.5	-0.73	0.466
		NL_P	218.0	-0.173	0.863
		NS_NL_S	145.5	-2.082	0.037
		NS_NL_N	188.5	-0.904	0.366
		NS_NL_P	41.0	-5.053	0.000
		LATE_S	140.0	-2.128	0.033
		LATE_N	164.5	-1.491	0.136
		LATE_P	171.0	-1.330	0.183
		EARLY_S	129.0	-2.373	0.018
		EARLY_N	197.0	-0.689	0.491
		EARLY_P	171.0	-1.330	0.183
		REPROAGE	206.0	-0.475	0.635
	Tolerance	TOL_S	124.5	-2.524	0.012
		TOL_N	194.0	-0.763	0.445
		TOL_P	175.0	-1.231	0.218
		INTOL_S	224.0	-0.027	0.978
		INTOL_N	224.0	-0.027	0.979
		INTOL_P	223.0	-0.053	0.957
	Vertical position	COL_S	121.0	-2.574	0.010
		COL_N	164.0	-1.502	0.133
		COL_P	202.0	-0.566	0.571
		BEN_S	153.5	-1.776	0.076
		BEN2_S	164.5	-1.503	0.133
		BEN_N	203.0	-0.542	0.588
		BEN2_N	173.5	-1.268	0.205
		BEN_P	202.0	-0.566	0.571
		BEN2_P	185.0	-0.985	0.325
BMI	Taxonomic	FAM_S	178.0	-1.163	0.245

	EPT_S	220.5	-0.112	0.911
	EPT_P	169.0	-1.379	0.168
	EPH_P	174.5	-1.243	0.214
	PLE_P	220.0	-0.123	0.902
	TRI_P	144.0	-1.994	0.046
Trophic	SCR_P	166.0	-1.452	0.146
	SHR_P	216.0	-0.222	0.825
	PRE_P	133.0	-2.265	0.024
	COL_P	174.0	-1.256	0.209
	FIL_P	159.0	-1.625	0.104
Tolerance	TOL_P	76.0	-3.699	<0.0005
	HBI	219.0	-0.148	0.883

Appendix Q. Descriptive statistics for environmental variables among all sites, headwater tributaries (HT), and mainstem tributaries (MT) at multiple distances from sites.

Category	Variable	Distance	Group	Minimum	Maximum	Mean	St. dev.
Land use	AG_TOT	All	All	0.00	80.05	23.25	25.07
		5	HT	0.00	80.05	23.71	25.47
			MT	0.00	76.65	22.05	25.09
		10	HT	0.00	80.05	32.69	27.21
			MT	0.00	76.65	15.77	20.90
		15	HT	0.00	80.05	35.70	25.52
			MT	0.00	76.65	17.23	22.92
		20	HT	0.14	80.05	47.09	22.96
			MT	0.00	76.65	17.80	22.45
	FOR_TOT	All	All	11.58	100.00	75.58	26.18
		5	HT	19.89	100.00	75.74	25.41
			MT	11.58	100.00	75.19	29.27
		10	HT	19.89	100.00	66.74	27.07
			MT	11.58	100.00	82.58	23.73
		15	HT	19.89	100.00	63.69	25.37
			MT	11.58	100.00	81.32	25.00
		20	HT	19.89	98.04	52.17	22.37
			MT	11.58	100.00	80.93	24.20
	URB_TOT	All	All	0.00	14.85	0.91	2.88
		5	HT	0.00	2.49	0.28	0.62
			MT	0.00	14.85	2.57	5.15
		10	HT	0.00	2.49	0.27	0.61
			MT	0.00	14.85	1.43	3.77
		15	HT	0.00	2.49	0.27	0.66
			MT	0.00	14.85	1.23	3.45
		20	HT	0.00	2.49	0.37	0.87
			MT	0.00	14.85	1.04	3.16
Mesohabitat	PCT_FAST	All	All	8.00	88.00	46.14	23.51
		5	HT	8.00	88.00	45.77	23.94
			MT	14.00	84.00	47.11	23.40
		10	HT	9.00	73.00	40.13	19.75
			MT	8.00	88.00	50.91	25.51
		15	HT	9.00	73.00	43.02	22.04
			MT	8.00	88.00	47.65	24.43
		20	HT	9.00	61.39	40.88	22.97
			MT	8.00	88.00	47.35	23.80
	PCT_POOL	All	All	0.00	64.65	15.81	14.93
		5	HT	0.00	60.00	15.37	13.98
			MT	0.00	64.65	16.94	17.78
		10	HT	0.00	60.00	17.35	15.43
			MT	0.00	64.65	14.59	14.74
		15	HT	0.00	32.70	12.64	11.25
			MT	0.00	64.65	17.34	16.38
		20	HT	0.00	32.00	13.05	12.36
			MT	0.00	64.65	16.44	15.55
Riparian vegetation	XCDENBK	All	All	0.00	100.00	53.45	32.10
		5	HT	0.00	100.00	50.54	34.29
			MT	0.54	98.66	60.96	25.36
		10	HT	0.00	100.00	40.83	37.42
			MT	0.54	98.93	63.43	23.45
		15	HT	0.00	100.00	30.19	35.46
			MT	0.54	99.73	64.67	23.68
		20	HT	0.00	71.25	23.97	25.89
			MT	0.54	100.00	60.19	29.73
	XCL	All	All	0.00	75.20	19.17	17.78
		5	HT	0.00	75.20	18.89	19.32

			MT	0.00	37.75	19.88	13.70
		10	HT	0.00	72.60	16.11	18.64
			MT	0.00	75.20	21.59	17.08
		15	HT	0.00	72.60	13.86	21.13
			MT	0.00	75.20	21.73	15.68
		20	HT	0.00	40.75	10.85	15.26
			MT	0.00	75.20	21.07	17.96
Substrate	PCT_BIGR	All	All	18.20	92.73	53.58	20.78
		5	HT	18.20	92.73	53.52	21.16
			MT	20.00	92.73	53.74	20.67
		10	HT	18.20	92.73	50.79	20.86
			MT	20.00	92.73	55.78	20.89
		15	HT	18.20	92.73	49.50	20.95
			MT	20.00	92.73	55.55	20.78
		20	HT	18.20	69.09	39.57	17.76
			MT	20.00	92.73	56.78	20.29
	PCT_SAFN	All	All	0.00	70.91	22.24	18.38
		5	HT	0.00	56.36	20.63	16.71
			MT	2.00	70.91	26.39	22.40
		10	HT	0.00	56.36	24.16	17.32
			MT	0.00	70.91	20.72	19.40
		15	HT	0.00	56.36	26.68	14.91
			MT	0.00	70.91	20.10	19.72
		20	HT	12.70	56.36	33.61	13.88
			MT	0.00	70.91	19.64	18.44
	XEMBED	All	All	3.35	90.73	44.11	21.53
		5	HT	3.35	76.11	42.54	21.09
			MT	19.35	90.73	48.16	23.06
		10	HT	10.61	76.11	46.62	21.11
			MT	3.35	90.73	42.12	22.10
		15	HT	10.61	76.11	51.94	18.11
			MT	3.35	90.73	40.33	22.30
		20	HT	10.61	76.11	52.44	21.68
			MT	3.35	90.73	42.20	21.35
Volume	XWIDTH	All	All	0.43	16.85	5.95	4.19
		5	HT	0.68	16.38	5.78	3.81
			MT	0.43	16.85	6.41	5.21
		10	HT	0.68	13.79	6.40	3.34
			MT	0.43	16.85	5.60	4.80
		15	HT	0.68	13.79	6.86	3.69
			MT	0.43	16.85	5.51	4.40
		20	HT	0.68	10.68	5.31	3.42
			MT	0.43	16.85	6.10	4.37
	AREA_WS	All	All	16.00	14202.00	2986.04	3425.13
		5	HT	16.00	14202.00	3064.13	3534.91
			MT	86.00	11248.00	2784.29	3263.85
		10	HT	16.00	10018.06	3609.12	3221.11
			MT	86.00	14202.00	2492.76	3567.66
		15	HT	16.00	10018.06	4182.12	3498.05
			MT	86.00	14202.00	2408.62	3294.77
		20	HT	16.00	9916.71	3350.11	3497.71
			MT	86.00	14202.00	2902.82	3454.68
	XDEPTH	All	All	7.31	66.32	30.12	13.65
		5	HT	7.31	50.17	28.68	13.15
			MT	14.87	66.32	33.84	14.79
		10	HT	7.73	50.17	33.18	12.38
			MT	7.31	66.32	27.70	14.37
		15	HT	7.73	50.17	34.62	13.00
			MT	7.31	66.32	27.95	13.64
		20	HT	7.73	50.17	34.25	15.20
			MT	7.31	66.32	29.18	13.33
Water	COND	All	All	9.80	647.00	140.13	158.83

quality							
		5	HT	9.80	647.00	112.44	134.77
			MT	29.30	533.00	211.66	197.60
		10	HT	9.80	323.15	96.79	92.47
			MT	19.00	647.00	174.44	191.30
		15	HT	20.00	323.15	119.81	98.06
			MT	9.80	647.00	149.94	181.85
		20	HT	33.50	233.00	110.66	89.45
			MT	9.80	647.00	146.86	171.07
	NTL	All	All	0.08	3.23	0.55	0.57
		5	HT	0.08	1.91	0.47	0.41
			MT	0.10	3.23	0.76	0.85
		10	HT	0.16	1.91	0.58	0.41
			MT	0.08	3.23	0.52	0.68
		15	HT	0.16	1.91	0.64	0.46
			MT	0.08	3.23	0.50	0.62
		20	HT	0.16	1.91	0.79	0.50
			MT	0.08	3.23	0.49	0.58
	PHSTVL	All	All	4.86	8.94	7.43	0.80
		5	HT	4.86	8.64	7.38	0.85
			MT	6.75	8.94	7.58	0.66
		10	HT	4.86	8.64	7.39	0.92
			MT	6.10	8.94	7.46	0.70
		15	HT	6.16	8.64	7.62	0.69
			MT	4.86	8.94	7.34	0.84
		20	HT	6.16	8.44	7.50	0.74
			MT	4.86	8.94	7.42	0.82
	PTL	All	All	0.00	0.10	0.02	0.02
		5	HT	0.00	0.10	0.02	0.02
			MT	0.01	0.05	0.02	0.01
		10	HT	0.00	0.04	0.02	0.01
			MT	0.00	0.10	0.02	0.02
		15	HT	0.01	0.04	0.02	0.01
			MT	0.00	0.10	0.02	0.02
		20	HT	0.01	0.04	0.03	0.01
			MT	0.00	0.10	0.02	0.02
Woody debris	XFC_BRS	All	All	0.00	25.00	5.41	4.95
		5	HT	0.00	25.00	4.89	4.93
			MT	0.00	15.70	6.76	4.95
		10	HT	0.00	25.00	5.48	5.70
			MT	0.00	15.70	5.35	4.38
		15	HT	0.00	25.00	5.42	6.64
			MT	0.00	15.70	5.40	4.03
		20	HT	0.00	10.45	3.94	4.20
			MT	0.00	25.00	5.75	5.10
	XFC_LWD	All	All	0.00	25.00	1.93	4.27
		5	HT	0.00	25.00	2.27	4.94
			MT	0.00	3.60	1.06	1.30
		10	HT	0.00	25.00	3.15	6.08
			MT	0.00	5.50	0.97	1.46
		15	HT	0.00	25.00	3.71	7.02
			MT	0.00	5.50	1.07	1.43
		20	HT	0.00	8.45	1.62	2.90
			MT	0.00	25.00	2.00	4.55

Appendix R. Mann-Whitney tests for environmental variables among headwater tributaries (HT) and mainstem tributaries (MT) at multiple distances from sites. All p-values were >0.05.

		5			10			15			20		
Category	**Variable**	**Mann-Whitney U**	**Z**	**p-value**	**Mann-Whitney U**	**Z**	**p-value**	**Mann-Whitney U**	**Z**	**p-value**	**Mann-Whitney U**	**Z**	**p-value**
Land use	AG_TOT	147.5	-1.044	ns	166.0	-1.518	ns	171.0	-0.830	ns	110.5	-0.922	ns
	FOR_TOT	149.5	-0.990	ns	173.0	-1.347	ns	174.0	-0.753	ns	112.5	-0.859	ns
	URB_TOT	180.0	-0.171	ns	223.0	-0.129	ns	182.0	-0.574	ns	106.0	-1.119	ns
Mesohabitat	PCT_FAST	146.5	-1.070	ns	183.0	-1.101	ns	148.0	-1.426	ns	108.0	-0.999	ns
	PCT_POOL	151.0	-0.949	ns	172.5	-1.359	ns	173.5	-0.765	ns	99.5	-1.265	ns
Riparian vegetation	XCDENBK	150.5	-0.961	ns	218.5	-0.232	ns	200.0	-0.078	ns	140.0	0.000	ns
	XCL	163.0	-0.623	ns	198.0	-0.734	ns	186.0	-0.441	ns	124.0	-0.500	ns
Substrate	PCT_BIGR	158.5	-0.745	ns	221.0	-0.171	ns	166.0	-0.959	ns	101.0	-1.218	ns
	PCT_SAFN	178.5	-0.203	ns	228.0	0.000	ns	160.0	-1.117	ns	117.5	-0.704	ns
	XEMBED	144.0	-1.137	ns	228.0	0.000	ns	197.0	-0.156	ns	120.0	-0.624	ns
Volume	XWIDTH	162.0	-0.650	ns	219.0	-0.220	ns	141.0	-1.607	ns	100.0	-1.248	ns
	AREA_WS	162.0	-0.650	ns	210.0	-0.440	ns	141.0	-1.607	ns	105.0	-1.092	ns

Appendix S. Environmental variable loadings into 2-dimensional NMS ordination axes for headwater tributaries (HT) and mainstem tributaries (MT) at multiple distance classes.

		5				10				15				20			
		HT		MT		HT		MT		HT		MT		HT		MT	
Category	Variable	NMS I	NMS II	NMS I	NMS II	NMS I	NMS II	NMS I	NMS II	NMS I	NMS II	NMS I	NMS II	NMS I	NMS II	NMS I	NMS II
Mesohabitat	PCT_FAST	0.03	0.15	-0.15	0.09	-0.07	0.07	0.15	-0.02	0.02	0.15	0.14	0.06	-0.02	-0.20	0.00	-0.14
	PCT_POOL	0.11	-0.13	-0.07	-0.28	0.10	0.06	-0.05	-0.06	0.06	-0.16	-0.05	-0.06	-0.17	0.19	0.04	0.06
Riparian	XCDENBK	0.32	-0.03	0.23	0.10	0.30	0.39	-0.09	0.10	0.54	0.02	-0.13	0.04	-0.22	0.43	-0.18	-0.03
	XCL	0.33	-0.09	0.36	0.17	0.33	0.31	-0.12	0.21	0.54	-0.03	-0.20	0.08	-0.32	0.47	-0.23	0.02
Substrate	PCT_BIGR	0.08	-0.03	0.05	0.11	0.08	0.08	-0.02	0.09	0.11	-0.03	-0.06	0.07	0.02	0.06	-0.09	0.00
	PCT_SAFN	-0.31	0.06	-0.18	-0.16	-0.18	-0.20	0.01	-0.35	-0.19	0.05	0.21	-0.31	0.02	-0.11	0.34	0.05
	XEMBED	-0.13	-0.08	-0.10	-0.11	0.06	-0.10	-0.02	-0.19	0.04	-0.09	0.10	-0.18	0.06	0.18	0.15	0.04
Volume	XWIDTH	-0.01	-0.22	0.29	0.13	0.17	-0.05	-0.27	0.13	0.13	-0.18	-0.26	-0.06	0.00	0.31	-0.10	0.23
	AREA_WS	-0.01	-0.16	0.14	0.05	0.14	-0.04	-0.15	0.04	0.09	-0.16	-0.13	-0.05	0.01	0.26	-0.04	0.13
	XDEPTH	-0.04	-0.10	0.08	0.03	0.06	-0.05	-0.11	0.01	0.03	-0.09	-0.08	-0.05	0.02	0.14	0.00	0.09
Water quality	COND	-0.10	-0.01	0.06	-0.08	0.01	-0.11	-0.03	-0.10	-0.03	-0.10	0.06	-0.10	0.04	0.08	0.12	0.04
	NTL	-0.28	0.02	0.02	-0.28	-0.12	-0.20	-0.11	-0.34	-0.18	-0.05	0.13	-0.31	0.15	0.02	0.28	0.09
	PHSTVL	-0.02	0.00	0.01	0.00	0.00	-0.02	0.00	-0.01	0.00	-0.02	0.01	-0.01	0.01	0.02	0.01	0.01
	PTL	-0.22	0.23	-0.22	0.03	-0.27	-0.14	0.25	-0.15	-0.20	0.15	0.32	0.00	0.06	-0.20	0.19	-0.19
Woody debris	XFC_BRS	0.23	-0.11	0.26	-0.03	0.22	0.25	-0.20	0.03	0.39	0.01	-0.17	-0.07	-0.19	0.31	-0.13	0.08
	XFC_LWD	0.37	-0.26	0.44	0.23	0.30	0.40	-0.43	0.28	0.66	0.15	-0.43	-0.04	-0.36	0.36	-0.42	0.19

Appendix T. Changes to EMAP data incorporated in Chapter 4 analysis. I replaced problematic records with the likely species or deleted records where multiple similar species occur. My rationale for all changes was based on data in Jenkins and Burkhead (1994).

EMAP site code	EMAP record	Current analysis	Rationale
VA507S	Roanoke hogsucker, *Hypentelium roanokense*	Northern hogsucker, *H. nigricans*	*H. roanokense* is not reported in James River basin but *H. nigricans* is.
VA522S	Roanoke logperch, *Percina rex*	Deleted	*P. rex* not reported in the New River basin and *P. caprodes* is very unlikely in this site.
VAR01S	Potomac sculpin, *Cottus girardi*	Mottled sculpin, *C. bairdi*	*C. girardi* is not reported in the New River basin and *C. bairdi* is.
VAR01S	Cutlips minnow, *Exoglossum maxillingua*	Tonguetied minnow, *E. laurae*	*E. laurae* is considered to be native in Big Walker Creek (New River basin) and *E. maxillingua* has not been reported this far upstream.
VAR01S	Stripetail darter, *Etheostoma kennicotti*	Fantail darter, *E. flabellare*	*E. kennicotti* is not reported in the New River basin in Virginia but *E. flabellare* is.
VAR09S	Fallfish, *Semotilus corporalis*	Creek chub, *S. atromaculatus*	*S. corporalis* is not reported in the New River basin in Virginia but *S. atromaculatus* is.
VAR09S	Southern redbelly dace, *Phoxinus erythrogaster*	Mountain redbelly dace, *P. oreas*	*P. erythrogaster* is not reported in the New River basin in Virginia but *P. oreas* is.
VAR09S	River darter, *Percina shumardi*	Deleted	*P. shumardi* is not reported in Virginia and the sample could be one of several other percid species.
VAR12S	Southern redbelly dace, *Phoxinus erythrogaster*	Mountain redbelly dace, *P. oreas*	*P. erythrogaster* is not reported in the James River basin but P. oreas is.

Vita

Nathaniel "Than" Hitt was born in Morgantown, West Virginia in 1972. After graduating from Morgantown High School in 1990, Than spent a year as a Rotary International Exchange Student in Aarhus, Denmark. During his undergraduate studies at the College of Wooster in Ohio, Than designed a major in Environmental Science and conducted research on bioremediation of stripmined soils. Than graduated from the College of Wooster with Honors in Biology in 1996. From 1996 to 1998, Than served as the executive director of the Appalachian Restoration Campaign, a non-profit organization dedicated to landscape conservation and restoration in Appalachia. In 2002, Than received a Master of Science degree from the Division of Biological Sciences at the University of Montana. His Masters research addressed genetic introgression and conservation of westslope cutthroat trout (*Oncorhynchus clarki lewisi*).