ECOLOGICAL UNITS AND SPATIAL PATTERN IN RIVER ECOSYSTEMS

by

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A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Natural Resources and Environment)
in the University of Michigan
2015

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Acknowledgements

Three grants have partially funded this research: 1) Great Lakes Fisheries Trust Grant 2001.0068, An ecological assessment of the Muskegon River watershed to solve and prevent environmental problems, 2) Great Lakes Fisheries Trust Grant 2001.0071, A Collaborative Approach to Understanding the Dynamics of the Muskegon River Watershed, and 3) EPA Star Grant R830596, Ecological classification of rivers for environmental assessment: Demonstration, validation, and application to regional risk assessment across Illinois, Michigan, and Wisconsin.

To my advisor and dissertation chair Michael J. Wiley, thank you for helping cultivate my fascination with rivers. I look forward to a career where I can follow your example by continuing to learn about river systems while sharing my enthusiasm and knowledge with others.

To my dissertation committee members Michael J. Wiley, Paul W. Seelbach, J. David Allan, and George W. Kling, thanks for your patience, encouragement, thoughtful questions, and critical reviews. And an extra thank you for not retiring before I finished this dissertation.

To my unofficial advisor and mentor Bobbi S. Low, thank you for your support, encouragement, and grammar, career, and life advice. Your attention to grammatical accuracy, as well as your natural ability to make me explain the “big picture,” greatly improved this dissertation.

Thanks to Catherine Riseng, a mentor and colleague who always seems to have my best interest in mind.

Many thanks to Lori Ivan for helping me work with the fish data, Solomon David for help in the field electrofishing and identifying fish, and Ethan Bright, for his help identifying benthic macroinvertebrates. Thank you Lacey Mason for sharing your extraordinary GIS expertise. Thank-you Shaw Lacy for insightful dissertation discussions and GIS help.

A thunderous “woohoo” to my two lab groups, the Wiley Lab and the BobbiLabbers who guided and supported me as I struggled to organize and disseminate the ideas in this dissertation. You all sat through some “challenging” presentations on the road to a great final defense.

The field data analyzed in this dissertation could never have been collected without an army of field crews. My sincere thanks to every one of you who donned the waders before sunrise, survived blisters and sunburn, reeked of wader stank, and ended the day identifying fish by car headlights. A special thanks to my family who “volunteered” to help with field work while on vacation. This amazing dataset would not have been possible without you.

Love and gratitude to my Mother, Father, and Sister whose encouragement (and especially the motherly nagging) kept me moving forward and helped me feel celebrated when I finished.

To my husband Dan, a simple thank-you doesn’t cut it, but I can’t find other words to convey how grateful I am for your love, support, and encouragement. You will always be my go-to Sherpa.

To my children, Helen and Cyrus who, when asked what Mommy does all day, answered decisively with a crayon-drawn illustration of their mother at the computer with a caption that read “Mommy does data.”

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Abstract

Rivers are advective, largely unidirectional ecological networks whose spatial patterning reflects both catchment and network structural characteristics. I used a headwaters-to-mouth, longitudinal, high-frequency-spatial sampling design to facilitate analyses of the extent and variability of spatial patterning in Midwestern river systems and to test alternate hypotheses about the underlying causes of biological spatial autocorrelation. These analyses establish the conceptual validity of channel segment based classifications used in management settings, and provide guidance for appropriate survey sampling design and statistical analyses in river systems.

In the first study I tested the theoretical assumptions underlying the mapping and practical application of riverine ecological units (EUs) within a river mainstem. EUs require concordance between fish and invertebrate assemblage composition and between biological assemblages and environmental variables. Along the Lower Muskegon River mainstem, fish/invertebrate concordances and many environment/biology concordances were strong, resulting in distinct, homogeneous biological assemblages that persisted through time.

In the second study I tested the same theoretical assumptions of EUs in a variety of disjunct river tributary systems in Michigan and Ohio. Although fish/invertebrate and environment/biology concordances were very strong in all of the tributaries, downstream tributary channels with substantial stream flow were the only contiguous stream segments with similar environmental and biological character. This suggests a better understanding of spatial

pattern and processes in headwater streams is needed to guide effective EU delineation in tributaries.

In the third study I explored a common feature of spatial patterning, positive spatial autocorrelation (SAC). SAC was common in both environmental variables and fish assemblage composition, although the magnitude of SAC varied by measure and spatial extent. Strong environment/biology associations accounted for most or all of the SAC in biological assemblages, offering strong support for niche processes as the origin of biotic SAC in these river systems. Likewise, proximity effects on biological assemblages were largely mediated through similarity in the environment.

My work here suggests that EUs do provide realistic units to map, inventory, and classify river segments for practical management, and provide a way to abstract and communicate the complex ecological processes and patterns that are characteristic of river ecosystems.

Chapter 1 : Dissertation introduction and overview

River ecosystems

Rivers are diverse ecosystems and unique in that they are strongly directional, transporting both water and material downstream. Although rivers are frequently described as linear systems, growing from small, ephemeral trickles to comparatively gigantic flows at the river mouth, viewing rivers as "linear" ignores the network aspect of river structure. The specific arrangement and shape of a river network can shape ecological pattern and processes by restricting movement and creating patches of physical habitat (Benda et al. 2004, Grant et al. 2007). River networks also flow from, and are shaped by, their landscape catchments. Hydrological, chemical, and biological characteristics of a stream or river reflect the climate, geology, landforms, and land use/land cover of its drainage basin (Hynes 1970, Oglesby1972, Likens et al. 1977, Newsom 1994, Johnson et al. 1997). Therefore, it is widely acknowledged that riverine environments and organisms are influenced by both local- and landscape-scale factors (Wang et al. 2006). In addition to perennial channel systems, rivers can have floodplains shaped by variation in disturbance frequency, and characterized by a high degree of habitat heterogeneity, spatial-temporal fluxes of materials, and complex biological associations (Tockner and Stanford 2002, Baker and Wiley 2009). In total, these distinctive riverine characteristics have encouraged a holistic view of rivers as complex "Landscapes" or "Riverscapes" (Fausch et al. 2002, Wiens 2002).

Because river catchments house humans, rivers are also often imperiled ecosystems (Warren and Burr 1994, Abromovitz 1996, Graf 1999, Allan 2004). This recognition that human actions can negatively impact river ecosystems drives my personal desire to conduct research that contributes to advances in fluvial ecosystem management. Ecosystem managers seek to protect the environment from anthropogenic pollution, maintain healthy ecosystems, permit sustainable development, and preserve biodiversity (Brussard et al. 1998). As such, ecosystem managers focus on ecological systems as a whole rather than on just some of their parts. If ecosystem management principals are applied to river ecosystems, this holistic focus requires an operational understanding of the hydrologic, geomorphic, chemical, and biological characteristics of rivers. My dissertation takes such an approach and explicitly addresses three (of ten) dominant themes in ecosystem management identified by Grumbine (1994): 1) Recognition of ecological boundaries with a "systems" perspective starting with consideration of spatial and temporal scales and hierarchical relationships, 2) Maintenance of ecological integrity to protect diversity as well as the ecological patterns and processes that maintain diversity, and 3) Use of empirical data to understand pattern and process in ecosystems and to monitor ecological change.

Spatial pattern in rivers

The over-arching theme for my dissertation is spatial pattern in river ecosystems. I build on a large body of literature describing the physical and ecological structure of rivers; a literature that has included numerous debates as to how to best describe and explain the spatial patterns observed in rivers and the universality of described spatial patterns.

In the context of the physical features of rivers, geomorphologists described self-similarity and variety in river networks by developing and analyzing hierarchical organization of

channel systems, developing the now familiar stream ordering systems and empirical "laws" (Horton 1945, Strahler 1952). The configuration of a river network, as structured by topography and geology, can also be described by various drainage pattern types (e.g., dendritic, trellis, radial, etc.; Howard 1967). Frissell (1986) developed a hierarchical classification system based on geomorphic stream habitat. More recently, Benda et al. (2004) proposed a geomorphic framework, the "Network Dynamics Hypothesis," and developed testable predictions of how the spatial arrangement of tributaries in a river network interacts with watershed processes to influence spatiotemporal patterns of habitat heterogeneity.

Ecologists have also described spatial pattern in rivers and have conceptualized rivers as longitudinal gradients, mosaics of patches, or patchy gradients. Early European stream biologists described longitudinal faunal zones of fishes and invertebrates and related these to changes in habitat, elevation and temperature as one moved from the headwaters to river mouth (Huet 1959, Illies and Botosaneanu 1963). The River Continuum Concept (RCC; Vannote et al. 1980) described common spatial and temporal downstream changes in channel morphology, biota, and ecosystem processes over many stream orders. In response to the RCC's disregard for tributaries, Perry and Schaeffer (1984) and Minshall et al. (1985) proposed adjustments to the RCC that accounted for tributaries, while others eschewed the RCC completely in favor of a view of the river "discontinuum" (Perry and Schaeffer 1987, Townsend 1998, Montgomery 1999, and Rice et al. 2001). Poole's "Fluvial Landscape Ecology" (2002) and Thorp et al.'s (2006, 2008) "Riverine Ecosystem Synthesis" recognize rivers as patchy gradients, based on longitudinal hydrogeomorphic patterning.

Although documenting spatial pattern in rivers is itself interesting, maintaining river diversity requires knowledge about the origin of those spatial patterns. Most of the

aforementioned descriptions of spatial pattern in rivers are likely to produce positive spatial autocorrelation (SAC) within river ecosystems. A common statistical property, SAC is the tendency for more proximal locations to have more similar characteristics. Ecologists have developed two opposing theories about the origin of SAC in biological assemblage composition: 1) environmental control and species sorting (Whittaker 1956, Hutchinson 1957) and 2) neutral theory (Hubbell 2001). Although these two theories can produce similar spatial patterns in assemblage composition, understanding the relative contribution of these sources of SAC has important consequences for understanding the functioning of ecosystems, for the conservation of biodiversity, and for ecosystem management (Legendre 2005).

An example of ecosystem management based on riverine spatial pattern

One particular ecosystem management approach that has been applied in both terrestrial and aquatic systems is the delineation of ecological units (EUs) and subsequent classification of these units into ecological "types" that can be related to management options/actions. EUs are defined as spatially contiguous areas with relatively homogeneous environmental and biological features. In theory, they are holistic, in the sense that they represent both environmental patterns and the biota's "perception" of the environment. EUs are conceptualized as real, persistent, and map-able places that may be repeated across a larger landscape (Rowe 1961).

If riverine EUs exist, attributed maps of such units would be a valuable asset for aquatic resource management activities. EUs could provide a way to abstract and communicate complex ecological processes and resultant patterns we see in river ecosystems (Rowe 1961, Levin 1992). Because EUs explicitly acknowledge river systems to be composed of many discrete habitat units, delineating and attributing EUs with measures of influential environmental characteristics (e.g. size, temperature, flow regime, and LULC), could help illustrate important "places" within

river systems. Maps of these "places" can then support practical classifications such as "valued trout streams" or can designate specific units where particular fisheries management tools, such as special fishing regulations or stocking, can be applied.

Mapping, inventory, and classification are important steps in the river conservation planning process; and EUs provide fundamental units for these activities (Seelbach et al. 1997, Seelbach et al. 2006). For example, the MI-VSEC ecological unit system was used by The Nature Conservancy to assure representative coverage of Michigan's stream resource types as land conservation priorities were developed (Higgins et al. 1999). The MI-VSEC system has also been used by resource and fisheries managers as the organizing framework for basin-wide assessments of Lake Michigan tributaries (e.g. 'Special Management Reports' for the Jordan, Manistee, Muskegon, Kalamazoo, and St. Joseph Rivers; http://www.michigan.gov/dnr).

The existence of EUs within rivers would also have utility for sampling design and model application. Homogeneity within an EU and subsequent classification of EUs into types would also allow extrapolation of information from sampled river reaches to the larger EUs and to un-sampled EUs of similar ecological type. For example, a basin-wide ecological assessment of the Muskegon River in Michigan used the MI-VSEC framework as statistical strata to ensure comprehensive sampling (Riseng et al. 2006, Stevenson et al. 2009). Building on assessment studies of the Muskegon River, GIS summaries of driving variables and model runs on the MI-VSEC framework were used to generate climate and landscape change models of hydrologic, chemical loading, and biological response (Wiley et al. 2010).

Despite the current and expanding use of EUs for river ecosystem management, little explicit testing of either the theoretical assumptions of ecological units or the performance of delineated ecological units has been performed. Neither 1) testing for the existence of

ecologically distinct, homogeneous river segments independent of any particular EU delineation; nor 2) testing of the ability of a particular EU delineation to partition independently observed variation in a real river system, have been rigorously undertaken. Testing the theoretical basis of riverine EUs and the performance of an existing EU delineation is limited by the requirement of *longitudinal, high-spatial-frequency empirical* datasets*;* a sampling design not common in existing data sources or in governmental agency data-collecting regimes.

Dissertation research objectives

The analyses in all three research chapters of this dissertation are based on empirical ecological data. I used a headwaters-to-mouth, longitudinal, high-frequency-spatial sampling design to facilitate analyses of the extent and variability of spatial patterning in Midwestern river systems. At all sites, I collected environmental data including measures of stream size, network position, in-stream habitat, water chemistry, and temperature, and assessed fish and benthic macroinvertebrate assemblage composition using occurrence, abundance, and biomass measures.

In Chapter 2, I test the validity of underlying assumptions of ecological classification (e.g., existence of EUs) using an 80 km portion of the mainstem of the Muskegon River in Michigan and analogous sampling on four confluent tributaries. Based on five combinations of spatial and temporal extents, I test the validity of the underlying assumptions of ecological units: 1) concordance between biological assemblages (e.g., different biological assemblages exhibit similar spatial variation in assemblage composition), 2) environment/biology concordance (e.g., the pattern of spatial variation is similar for environmental variables and biological assemblages), and 3) the occurrence of ecologically distinct, homogeneous river segment units. I also evaluate an existing ecological valley-segment scale delineation (VSEC version 1.0,

Seelbach et al. 1997) in terms of its ability to partition observed spatial heterogeneity. One conclusion of this study was a need for more detailed analysis of EUs in river tributary systems.

In Chapter 3, I again test the validity underlying assumptions of ecological units, this time in five small Midwestern tributaries of varying physical and ecological character. Analyses also considered whether longitudinal network position could help explain spatial patterning in biological assemblage similarity. As in Chapter 2, I also evaluated an existing ecological valley-segment scale delineation (VSEC version 1.0, Seelbach et al. 1997) in terms of its ability to partition observed spatial heterogeneity in these small systems.

In Chapter 4, I first ask how concordances between environmental and biological compositions arise, and then how network structure itself might influence patterns of biophysical concordance, spatial autocorrelation, and distance decay rates. I approach these questions by comparing rates of longitudinal change in both environmental variables and biological assemblages along network trajectories. Finally, I use a path analytic approach to assess the extent to which 1) observed SAC in biological assemblage composition in these systems arises from environmental controls, and 2) effects of proximity are mediated through environmental similarity. The variety of tributary systems and range of spatial extents I examined allows for a broad exploration of spatial autocorrelation in Midwestern riverine ecosystems.

In Chapter 5, I summarize the findings of each research chapter and discuss the implications of the four major conclusions of this dissertation. These conclusions are 1) observed spatial patterns were consistent regardless of the measure of the biological assemblage, 2) spatial extent and sampling regime can affect study conclusions, 3) The physical environment provides the template that creates within-basin biological spatial pattern, and 4) fluvial ecological units are real and therefore classification should be an effective tool for river management.

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Chapter 2 : Ecological units, concordance, and spatial patterns along a river mainstem and confluent tributaries

Abstract

The delineation of Ecological Units (EUs; contiguous river segments with homogeneous biological and physical features) in rivers provides an important tool for resource management. However, little independent testing of either the theoretical assumptions of such units or the practical performance of extant delineations has been reported. The existence of ecologically distinct and homogenous river units requires that strong concordance occur between biological assemblages, and between biological assemblages and environmental variables. This study used a longitudinal, high-spatial-frequency sampling design along a river mainstem and confluent tributaries to test these and related assumptions of EUs across temporal and spatial extents. Along the river mainstem, fish/invertebrate concordances and many environment/biology concordances were strong, and distinct, homogeneous biological assemblages were observed at both within- and across-season temporal extents. Including confluent tributaries emphasized large differences between the biological character of the mainstem and its tributaries. Although fish and invertebrates had common concordances with size-related environmental variables (catchment area, link, discharge, and channel shape), this study does suggest spatial pattern in substrate may be more important for invertebrates than fish. Both the theoretical assumptions and performance of a specific EU delineation were well supported by spatial pattern in biological and environmental measures.

Introduction

Two central tasks in ecology are to identify spatial pattern in biological communities and to understand the mechanisms that create them (Wiens 1989, Levin 1992). One approach to these tasks is classification, the identification and grouping of similar items or patterns. Ecological classification studies often begin by delineating spatially homogeneous units, proceed by characterizing the defined units, and finally group units with similar characteristics into ecological classes or "types (Cormack 1971, Barnes et al. 1982, stream classification reviewed in Melles et al. 2014).

Although the specifics of such a process vary widely, most useful ecological classifications incorporate knowledge about both biological patterns and processes and include some form of hierarchical organization (Gauch and Whittaker 1981, Klijn and Haes 1994, Maxwell et al. 1995, Snelder and Biggs 2002, Higgins et al. 2005, Dunn and Majer 2007). River classification has an extensive, though often discipline-specific history (Naiman et al. 1992, Hawkins and Norris 2000, Makaske 2001, Melles et al. 2012). Here I focus on the development of ecological classifications built on meso-scale (e.g., 1 to 50 km) segments of river channel, often referred to as "valley segments" (Frissell et al. 1986, Cupp 1989, Maxwell et al. 1995, Brierley and Fryirs 2000).

Early European stream biologists described longitudinal faunal zones of fishes and invertebrates and related these to changes in habitat, elevation and temperature as one moved from the headwaters to river mouth (Huet 1959, Illies and Botosaneanu 1963). Geomorphologists described self-similarity and variety in river networks by analyzing hierarchical patterns of organization in channel systems and developed the now familiar stream ordering systems and basin and hydraulic geometry "laws" (Horton 1945, Strahler 1952) used by present-day

hydrologists, geomorphologists, and ecologists (e.g. RCC, Vannote et al. 1980). Geomorphic analyses also led to classifications of river planform patterns, and to valley segment classifications that delimited contiguous river valley segments where floodplain character, river planform, and other geomorphic features could be organized into types and sub-categories (Frissell et al. 1986, Cupp 1989, Frissell and Liss 1993, Bisson et al. 1996, Montgomery and Buffington 1997, 1998). By linking the river biologists' traditional view of faunal zonation to geomorphic valley segment characteristics, workers in North America and Australia began to identify and make use of ecologically-defined mesoscale habitat units (Maxwell et al. 1995, Seelbach et al. 1997, Brierley et al. 2002, Baker 2006, Seelbach et al. 2006, Thorp et al. 2008). In rivers, it is not unusual to have spatially concurrent change in hydrologic, thermal, geomorphic, and biological character (Poff et al. 1997, Frissell et al. 2001, Jensen et al. 2001, Zorn et al. 2002, Wehrly et al. 2003, Benda et al. 2004a, 2004b, Seelbach et al. 2006, Thorp et al. 2006, Thorp et al. 2008). Therefore, unit boundaries in large rivers are often placed at observable shifts in river energy balance (e.g. observed as changes in sinuosity, slope, and valley form, and at major tributary confluences) while unit boundaries in smaller rivers and tributaries are often placed at tributary junctures, and shifts in land use/land cover (LULC) and surficial geology. The development of mesoscale habitat units supported a move in fisheries and watershed management towards a more holistic and landscape-oriented perspective (Maxwell et al. 1995, Bryce and Clarke 1996, Seelbach et al. 1997, Higgins et al. 2005, Seelbach et al. 2006, Baker 2006, Brenden et al. 2006, Sowa et al. 2007, Brenden et al. 2008b, Melles et al. 2012, McKenna et al. 2014).

The delineation of river channel spatial units and subsequent classifications in Great Lakes watersheds was heavily influenced by the work of terrestrial ecologists interested in

mapping forests and classifying *integrated ecological landscape units* (also referred to as landscape ecosystems; Rowe 1980, 1992, Forman & Godron 1986, Cleland et al. 1997). Ecological units (EUs) were defined as spatially contiguous areas with relatively homogeneous environmental and biological features. In theory, EUs are holistic in the sense that they represent both environmental patterns and the biota's "perception" of the environment. EUs are defined as real, persistent, and map-able places that may be repeated across the landscape (Rowe 1961) and therefore lend themselves to classification. In delineating homogeneous units, emphasis is given to regions of rapid changes in characteristics known to determine patterns of biological organization and ecosystem functioning. The EUs, therefore, are not arbitrarily bounded and the length of an EU should correspond to the physical template on which biological assemblages are arranged.

In practical application then, valley segment EUs in rivers will have two defining properties: 1) Relatively homogeneous biological and environmental composition and/or patterning; and 2) Boundary areas of rapid change at unit transitions. For these properties to exist, some significant degree of biological concordance (i.e., concurrent change in multiple biological assemblages) and concordance between biological structure and the physical environment (i.e., concurrent change in biological assemblages and environmental variables) must be present. In other words, the existence of EUs entails significant *ecological* concordance within a river network. Because it is a logical requirement of the existence of ecological units, concordance is a property that should exist independently of any specific unit delineation or classification scheme.

I argue here that because concordance is a necessary condition for EUs to exist, the conceptual utility of ecological units can be tested with appropriate empirical data. However,

little *targeted and comprehensive* (i.e., across multiple measures such as habitat, multiple biological assemblages) testing of either the theoretical basis of ecological units or the efficacy of delineated ecological units has been performed. Seelbach et al. (2006) and Boys and Thoms (2006) found fish assemblages within delineated units (e.g. valley segments and fluvial process zones respectively) were typically more similar as compared to assemblages in different units and compared to fish assemblages in units of different ecological "type." Thomson et al. (2004) found that three geomorphic-based river "style" classes had distinct macroinvertebrate assemblages and Warrner et al. (2010) found fairly homogeneous stream habitat within valley segment units, but differences in habitat between abutting units. Melles et al. (2014) conclude a review of stream classification by arguing the testing of proposed classifications is one of the most important steps in the creation of ecosystem classifications. However, such testing of riverine ecological units is limited by the requirement of *comprehensive, longitudinal, high-spatial-frequency empirical* datasets*;* a sampling design not common in existing data sources or in governmental agency data-collecting regimes.

This study tests the underlying assumptions of ecological classification using empirical, longitudinal, high-spatial-frequency sampling in a detailed examination of an 80 km portion of the mainstem of the Muskegon River in Michigan. Based on five combinations of spatial and temporal extents, I examine: 1) fish/benthic invertebrate concordance, 2) environmental/biological concordance, and 3) the existence of ecologically distinct, homogeneous river segment units. I also evaluate an existing ecological valley-segment scale delineation (VSEC version 1.0, Seelbach et al. 1997) in terms of its ability to partition observed spatial heterogeneity.

If the concept of EUs is valid, the implications are extensive. EUs would provide real units to both inventory and classify river segments for practical management (Seelbach et al. 2006), and provide a way to abstract and communicate complex ecological processes and resultant patterns we see in river ecosystems (Rowe 1961, Levin 1992). Further, the existence of EUs within rivers should have substantial implications for study design. Homogeneity within an EU and subsequent classification of EUs into types would allow extrapolation of representative samples or models to larger-scale units and to EUs of similar type. Conservation of rivers may also be aided because the mapping and inventory of functional units (cf. valley segments in Seelbach et al. 1997, 2006, Sowa et al. 2007, and McKenna et al. 2014; macrohabitats in Higgins et al. 2005, and functional process zones in Thorp et al. 2008) are important steps in conservation planning.

Methods

Study area and study sites

The Muskegon River is the second largest tributary to Lake Michigan, draining a basin of 682,200 hectares in west-central Michigan (Figure 2.1 inset, O'Neal 1997). The Muskegon is well known for its recreational fishery, including smallmouth bass (*Micropterus dolomieu*) and walleye (*Sander vitreus*), and numerous resident and migratory salmonids. The study area is in the lower third of the basin and is the 80 km long section between Croton Dam and Muskegon Lake, a drowned river mouth that connects to Lake Michigan (connection is 10 km downstream of the lowermost study site). Croton Dam is one of three major dams on the Muskegon River and serves as the lower-most barrier to upstream-migrating fishes from the Lake Michigan and Muskegon Lake. The Muskegon River mainstem receives four major tributaries (Bigelow,

Brooks, Cedar, and Mosquito Creeks) within the study area. The upper part of the mainstem study area is high gradient with shallow riffles and runs; the middle part includes transition zones and deep U-shaped channels; and the lower part flows through a low gradient wetland complex and splits into a north and south branch with numerous side- and cross-channels. Based on shifts in river planform and valley confinement, the river mainstem in the study area was delineated into five EUs that are expected to differ in physical, chemical, and biological character (see Figure 2.1 upper map, Seelbach et al. 1997 and the delineation process is explained in more detail in the delineated EU methods subsection). Additionally, all mainstem EUs have designated subsections, four in EU1 and two each in the other EUs. For the purpose of this study, tributary EUs are not recognized, and analyses simply identify the tributary (i.e., T1, T2, T3, or T4) in which a study site occurs.

The study data are from 114 sites on the Muskegon River mainstem and 15 sites on the four major tributaries confluent in the study area (Figure 2.1). The sites along the mainstem represent stratified random locations allocated within subsections of each delineated EU as well as specifically targeted locations designed to sample known notable habitats not captured by the random sampling of EUs. Targeted locations included confluences of tributaries, unique riffle habitats, edge wetlands, mid-channel island shorelines, and larger side- and cross-channels. The distance between sites varied, but on average, sites along the mainstem were about 0.75 km apart. Sites were spaced further apart in the tributaries and were located near road crossings, and in lower Mosquito Creek, at random locations and in notable side-channel and confluence-influenced habitats. Six of the 114 sites were visited only during synoptic chemistry runs, while biological assemblages (fish, invertebrate, or both) were sampled at the remaining 98 sites. Fish

were more intensively sampled (both spatially and temporally) than invertebrates (Figure 2.1 lower map).

Delineated EUs

The delineated EUs used in this study (Figure 2.1 upper map) were developed prior to sampling and data analyses by Seelbach et al. 1997 (VSEC version 1.0, available from the Michigan Geographic Data Library, MiGDL). The goal was to identify valley segment-scale ecological units with relative homogeneity in hydrologic, geomorphic, and water quality characteristics, and in likely biological assemblages. EUs were delineated "from above"; two experienced aquatic ecologists worked together, interpreting map information on catchment, river network, and valley characteristics, using their combined knowledge of ecological processes and interactions. EUs were delineated beginning at the mouth of the river working upstream, and boundaries of units were placed at important stream junctures, slope breaks, changes in river planform and valley form, and boundaries of local landforms. Delineations and biological interpretations were reviewed and adjusted as necessary after consultation with regional Michigan Department of Natural Resources biologists (see Seelbach et al. 1997, Seelbach and Wiley 2005, and Seelbach et al. 2006 for more details).

Biological data collection and dataset development

Fish assemblages at a site were characterized through a combination of DC boat boom and tow barge electrofishing. Boom electrofishing targeted large fish from the center of the river channel and non-wadeable habitats while barge electrofishing targeted smaller fish in shallow areas, usually along the river's edge. Boom shocking runs varied in length from 0.2 to 1.9 km in length and occasionally included multiple sites in one boom run. Barge shocking runs were

always site-specific and were usually two-pass depletion-type runs on 100 meter unblocked reaches. Fish were sampled seasonally in Spring (May & June), Summer (July& August) and/or Fall (September & October) of 2003 and/or 2004. In both sample types, all fish were identified to species, measured, and weighed. To characterize the fish assemblage at each site, boom and barge samples should be weighted by the proportion of boom and barge suitable habitat at a site. However, because the boom runs were much larger than the 100 meter reach of a barge sample and a single boom sample sometimes spanned several sites, I could not use site-based proportions of habitat to weight the fish samples. Instead, I used the proportion of boom and barge habitat in a 500 meter buffer around each site (e.g. a compromise between barge and boom run lengths) to weight boom and barge samples (see Appendix 2.1 and discussion of habitat maps in environmental data section for details).

Invertebrates were collected at fewer sites and with less frequency than were fish. Collections occurred during spring (May & June) of 2003 and 2004, and at a handful of sites in summer (Aug) 2003. Sampling of invertebrates was quantitative and targeted both common and rare habitats at a site. The specific sampling method (e.g., Hess, rock cluster, ponar grab, core, kickscreen, and wood and leaf debris grabs) was dictated by a sample location's depth and particular substrate. Samples with large amounts of organic and non-organic debris were elutriated in the field. All samples were preserved in 95% ETOH and samples were processed in the laboratory under dissecting microscopes. Organisms were identified to the lowest taxonomic resolution possible with moderate effort. Most organisms were identified to genus, while some organisms (such as Chironomidae, flatworms, mites, Branchiobdellidae and very early instar insects) remained at higher taxonomic resolution. Invertebrates identifiable only to order were excluded from analyses as well as oligochaetes, for which accurate density and biomass

measures were not possible. The length of each organism was measured and converted to dry biomass (mg) following length-biomass equations in Benke et al. (1999) and unpublished conversions developed in our laboratory (M.J. Wiley, University of Michigan, personal communication). Habitat-specific samples were combined and "scaled up" to represent the invertebrate assemblage at a site by weighting samples according to represented local habitat proportions in a 100m buffer around sites (see Appendix 2.1 and discussion of habitat maps in environmental data section for more details).

The biological assemblage was characterized in three ways: Occurrence (presence or absence), abundance (density in #/m^2), and biomass (total dry biomass in mg/m^2). Although excluding rare taxa from multivariate analyses can be warranted (reviewed in Cao et al. 2001), including rare taxa had little effect on the identification of major structure in the data and values of test statistics in this study and are therefore included in the dataset.

Environmental data

Environmental data for the mainstem and tributary sites were developed from field measurements, quantitative models, aerial photography, and GIS maps. Although different methods were occasionally necessary to develop data for sites on the mainstem and the tributaries, comparable data were developed for all study sites. Channel width was estimated at each mainstem and non-wadeable site from winter aerial imagery (Google Earth 2013) and averaged from multiple field-measured cross-sections for tributary sites. Slope and sinuosity were estimated from aerial imagery for sites on the river mainstem using a river length of 50x the river width (Google Earth 2013). For sites on tributaries, slope was measured in the field using a tripod and sinuosity was estimated in ArcGIS (ESRI 2011) from network maps using a river

length of 50x the river width. Catchment area and link number for all sites were also developed from network and basin maps in ArcGIS (ESRI 2011).

In-stream Geomorphic Units (IGUs), substrate, and depth measures were developed from direct field observations and a habitat map developed from field observations. After thorough inspection of a sampling reach for each site in a tributary, the percentage of IGUs (i.e., riffle, run, pool, edge, bar, and backwater) and substrate types (i.e., cobble, gravel, sand, claybed, wood, and fine and coarse organic matter) within a sampling reach were recorded. The average depth of a site on a tributary was calculated as the average depth from five cross sections distributed within the sampling reach. IGUs, substrate, and depth measures for sites on the mainstem were developed from a highly-detailed, continuous habitat map for the river channel based on extensive direct field observations and indirect observations using a Sontek Acoustic Doppler Profiler. This digital map of the 80 km mainstem study area included substrate (11 classes; most frequent classes included cobble, gravel, sand, clay, POM, wood and emergent and submerged vegetation), IGUs (19 types; most frequent types included riffle, run, edge, backwater, and point bar), and water depth. Around each site, I used a 100 meter buffer to clip the habitat map and calculated the percent of each substrate class, percent of each IGU type, and average depth for each site.

Daily temperature (max, min, and average), nutrients (concentrations of nitrogen, total phosphorus, and soluble reactive phosphorus) and average discharge were estimated for the mainstem and tributary sites using “The Muskegon River Ecological Modeling System” (MREMS; Wiley et al. 2010). MREMS consists of a set of integrated component models targeting aspects of the Muskegon River ecosystem such as hydrology, temperature, climate, and LULC. Model output was selected to correspond to biological sampling seasons (i.e., May values

for Narrow Temporal (NT) datasets and an average or min/max of May, August, and October for Wide Temporal (WT) datasets). The spatial framework of MREMS was adapted from the VSEC system of Seelbach et al. (1997) and includes 1-2 modeling units for each delineated EU along the mainstem and each tributary. Because of this difference in scale between model units and sampling sites, variability in temperature, nutrients, and discharge variables largely reflects the delineated EU structure. However, as evidenced by excellent fit of model results to field data, this imposed spatial structure may in fact reflect real patterns in river flow, temperature, and nutrient loads. In addition to field data used to develop and test MREMS models, an independent, synoptic sampling of water temperature and conductivity occurred at about 35 sites along the river mainstem between Croton Dam and Muskegon Lake during the spring and summer of 2003. Average velocity was calculated from the MREMS discharge estimates measure and channel cross-section measures (i.e., average velocity = Discharge/(width*average depth)).

Data Analyses

My analyses had three primary objectives: 1) To measure the degree of concordance between fish and invertebrate assemblage composition; 2) to measure the degree of concordance between environmental variables and biological assemblage composition; 3) to identify patterns of environmental and biological change to assess the homogeneity and boundary assumptions of generalized and delineated ecological units. All dissimilarity matrices and statistical tests were performed with PC-ORD version 6.08 (McCune & Mefford 2011).

To address these objectives at multiple spatial and temporal extents I developed five different datasets (Table 2.1). The dataset naming convention used here reflects the spatial and temporal extent of the dataset. Consider the WSPNT dataset for example: The first two letters

"WS" indicate a dataset with a wide spatial extent (i.e., the entire study mainstem), the "P" (for Plus) indicates tributary sites are added to the dataset, and the last two letters "NT" indicate a narrow temporal extent (i.e., spring of 2003, the season and year with the most samples). The datasets used in the concordance analyses were smaller than those used in NMDS analyses because there are fewer sites with both fish and invertebrate samples than for fish or invertebrates samples alone (Table 2.1).

Many of the analyses required use of a similarity, dissimilarity, or distance matrix. For the biological occurrence measure, I quantified differences in biological assemblages using the Sorenson dissimilarity index and when necessary, converted dissimilarity to similarity by subtracting from one. The Sorenson measure of similarity is desirable since it ignores joint absences, a combination that dominates many assemblage matrices (Faith et al. 1987). Because of the extreme right skew in abundance and biomass values and the undue influence of unusually large values on distance calculations, I natural log transformed abundance and biomass measures prior to distance matrix calculations. This transformation results in negative values for absent and rare taxa, thus preventing the use of Sorenson dissimilarity for abundance and biomass measures. For transformed abundance and biomass measures I used Euclidean distance instead. Euclidean distance was also calculated to represent environmental distance between sites for each environmental variable. Environmental variables with many small values and a few large values, (e.g., discharge, catchment area, link, nutrients, velocity, width and depth) were natural log transformed prior to analyses.

Using analyses based on distance matrices allowed flexibility in the types of environmental variables developed for this study. Some environmental variables were single measures (e.g., catchment area, link, slope, sinuosity, discharge, depth and width) while others

were based on a multivariate suite of related measures (e.g., proportions of 11 substrate classes, proportions of 19 IGU types, concentrations of three nutrient measures, and min, max and average water temperatures). The ability to use multivariate data was critical, as dissimilarity matrices calculated from suites of measures, such as the three measures of water temperature, typically performed better than any one measure.

I used simple Mantel tests to investigate the degree of concordance between a variety datasets. These included concordance between 1) different measures of a biological assemblage (i.e., fish occurrence vs. fish abundance, fish occurrence vs. fish biomass, etc.), 2) fish and invertebrate assemblages, and 3) environmental variables and biological assemblages. A simple Mantel test is extremely flexible and is used to test the null hypothesis of "no relationship" between two square symmetric matrices. It is an alternative to regressing one matrix against the other, and avoids the problem of partial dependence within each matrix. The standardized Mantel test statistic (r) ranges from -1 to 1, with 1 indicating perfect congruence between the two matrices. For all Mantel tests, the significance of r was assessed with a Monte Carlo randomization method using a maximum of 3000 permutations. In concordance analyses a large positive r indicates strong agreement between distance matrices for assemblage measures, fish and invertebrate assemblages, or biological and environmental character. Because the number of sites affects the power and significance of statistical tests, the number of sites (Table 2.1) should be considered when interpreting the magnitude and significance of a Mantel test statistics (r).

I used Non-metric Multidimensional Scaling (NMDS) to illustrate concordance and patterns of spatial heterogeneity for fish and invertebrate assemblages. NMDS is an ordination method based on ranked distances; it is well-suited to non-normal data with many zero values (Minchin 1987, McCune and Grace 2002). A successful NMDS procedure produces a low-

dimension ordination where the distances between pairs of sites are in rank-order agreement with their dissimilarities in species composition. The distance between sites in NMDS plots can be directly interpreted, for example, sites closer together have more similar assemblages. Each NMDS was run in the autopilot mode “medium” setting, (a balance of speed and thoroughness), with a maximum of 500 runs with random starting seed and a stability criterion of 0.00001. In most runs a 2D solution was suggested, although rarely a 3D solution was suggested. All of the NMDS ordinations performed well: Either two or three axes explained more than 80% of the variability in the original data sets, the ordination was significant ($p=0.0196$), and final stress was good or acceptable (usually between 7 and 15). However, after examination of the few 3D solutions, the additional axis explained little additional variance and interpretation of the results was the same regardless of dimensionality. For ease and consistency of viewing, NMDS plots present the two axes that explained the most variation. Figure 2.2 demonstrates how NMDS plots can be used to assess homogeneity and boundary assumptions of EUs under hypothetical situations of valid and null EUs.

I used trajectory plots and longitudinal plots to explore environmental and biological transitions in the study systems, facilitating assessments independent of and in context of delineated EUs. Sites on side and cross channels were excluded from trajectory analyses. To assess differences in rates of biological change along the river mainstem, I created trajectory plots by plotting cumulative dissimilarity in fish and invertebrate assemblages for neighboring sites against distance from Muskegon Lake. Similarly, I also created trajectory plots for a suite of environmental variables with spatial patterns similar to the biology (e.g., Catchment area, link, discharge, width, hard substrate, temperature and nutrient regime). Temperature and nutrient regimes were first summarized by the first PCA axis (temperature: 98% variance explained;

Nutrients: 99% explained) and all variables were Z-score normalized. Therefore, all seven environmental variables contributed equally to dissimilarity values. In trajectory plots, a steep slope indicates rapid change in the biological assemblages or environmental features. I also created longitudinal plots by plotting several individual environmental variables against distance from Lake Muskegon to illustrate spatial pattern in specific environmental variables.

This study also investigated the utility of an existing EU delineation. To investigate how well an existing EU delineation (i.e., VSEC 1.0, Seelbach et al. 1997) captured patterning in the environmental and biological data developed in this study, I used Mantel tests, Multi-response Permutation Procedures (MRPP), and longitudinal graphical analyses. I used simple Mantel tests with a design matrix with 1 for sites within the same delineated EU or tributary and 0 for sites in different EUs or tributaries. In this test a large Mantel r indicates sites within the same delineated EU/tributary are associated with higher biological similarity than those in different EUs/tributaries. Pairwise comparisons after significant MRPPs were used to determine which subEus/EUs/tributaries had different biological fish and invertebrate assemblages. MRPP is akin to ANOVA in that it is designed to assess whether there is greater difference within predetermined groups or among predetermined groups, but it is a data-dependent permutation procedure based on pairwise distance measures. It is ideally suited to ecological data because it makes few assumptions about the distributional structure of the data (Zimmerman et al. 1985) and can be used on multivariate arrays such as biological assemblage data. As with ANOVA, pairwise comparisons after a significant MRPP test determine which groups differed.

Results

Biological measure concordance

Preliminary analyses showed there was strong and highly significant correlation (and concordance) between occurrence, abundance, and biomass measures for both invertebrate and fish assemblages. This was true at all spatial and temporal extents (Table 2.2). Concordance (r) between these different measures of biological abundance ranged from 0.70 to 0.97 for invertebrates and from 0.77 to 0.96 for fish. This strong concordance indicates similar patterns of faunal transition and homogeneity are found regardless of the specific measure employed. Furthermore, NMDS ordinations for the three fish assemblage measures showed the same overall arrangement of sites (Figure 2.3 1st column). NMDS plots for invertebrates differed more between measures than they did for fish (Figure 2.3, 2nd column). Because invertebrate sampling effort was not always in proportion to available habitats and weighting of samples was by proportion of habitat in quantitative measures, rare taxa appear to have had a larger effect on occurrence measures than on abundance or biomass measures. As compared with occurrence, quantitative measures of the invertebrate assemblage generally increased variability of invertebrate assemblages and frequency of outliers within EU1, and decreased variability in EU2. However, as with fish, the overall spatial pattern of invertebrate assemblages was consistent regardless of measure. Since results for analyses based on occurrence, abundance, and biomass were highly correlated, I will only discuss results for occurrence data in biological/environmental concordance tests, NMDS plots, trajectory plots, and tests of the efficacy of delineated EUs.

Fish and invertebrate assemblage concordance

The overall degree of concordance (as measured by the Mantel r) between fish and invertebrate assemblage composition varied with spatial and temporal perspective (Table 2.3). At the narrowest spatial extent (Table 2.3 row 1), there was no or weak concordance between fish and invertebrate assemblages. In contrast, for the wide and wide plus tributary data sets (Table 2.3 lines 2-5), there was statistically significant concordance between fish and invertebrates ranging in strength from moderate to strong. The magnitude of fish/invertebrate concordance was typically higher within a single season and year (NT) than across seasons and years (WT) (Table 2.3 rows 2 vs. 4 and 3 vs. 5). The concordance between fish and invertebrate assemblages was also observed in the similarity of patterning of sites in NMDS ordinations (Figures 2.5 and 2.6) and patterns of change in trajectory plots (top two plots in Figure 2.7).

Environment and biology concordance

The degree of concordance between the environment and biological assemblages and environmental variables varied with spatial and temporal extent. As required by the second EU assumption, there were many strong concordances between spatial pattern in environmental variables and biological assemblage composition at wide and wide plus tributary spatial extents (Columns 3-6 in Table 2.4). Because of concordance of the fish and invertebrate assemblage, spatial patterns of both fish and invertebrates were usually concordant with the same environmental variables. Typically, fish/environment concordances were stronger than invertebrate/environment concordances for the same environmental variable; however, invertebrate/substrate concordance was usually higher than fish/substrate concordance. Except for the substrate measure, expanding spatial extent to include tributaries typically strengthened fish/environment concordance. Expanding the spatial extent to include tributaries in

invertebrate/environment concordance did not have a consistent effect on the strength of concordance. Combining data from multiple seasons and years had no consistent effect on the strength of biological/environment concordance.

The strongest environmental/biology concordances (Table 2.4) included those environmental variables representing aspects of size (e.g., catchment area, link, river discharge) or environmental variables whose spatial patterning was similar to the delineated ecological units (i.e., discharge, nutrients, and temperature). The importance of such longitudinally changing environmental variables on biological spatial pattern is also evident in NMDS ordinations. Especially for fish assemblages, the primary or secondary gradient arrangement of sites by biological assemblages is the longitudinal upstream to downstream gradient within the study area (Figures 2.5 & 2.6). Other strong environment/biology concordances included water velocity, channel shape, and substrate. Spatial pattern in slope, sinuosity, and IGUs were only weakly, rarely, or never associated with spatial pattern in biological assemblages.

At the narrowest spatial extent (i.e., limited to sites in EU1), spatial pattern in fish assemblages were not related to spatial pattern in any environmental variables, and spatial pattern of invertebrates was related to only a few environmental variables (2^{nd} column in Table 2.4). These invertebrate/environment relationships were weak (r from 0.18 to 0.32) and included invertebrates/link, invertebrates/slope, and invertebrates/ave depth. Because of the parallel of model units in MREMS and delineated EUs, river discharge, nutrients, and temperature measures hardly varied within EU1 and were not analyzed.

Distinct, homogeneous EUs

Environmental and biological data strongly paralleled the delineated EU structure, thus results support both the theoretical assumption of ecologically distinct, homogeneous EUs and

the particular EU delineation developed for the Muskegon River (i.e., VSEC version 1.0; Seelbach et al. 1997). Although distinct and homogeneous biological assemblages emerged at all spatial extents, these units were weakly defined at the narrow spatial extent. In the NSWT datasets, both fish and invertebrate assemblages typically differed by EU subsections (Figure 2.4 and Table 2.5), but this partitioning is weak and there is considerable similarity in biological assemblages between sites in different delineated subsections of the same EU.

At the wide spatial extent, spatial patterning in fish assemblages strongly reflected delineated EUs. The wide spatial, narrow temporal (WSNT) NMDS ordination for fish (Figure 2.5a) showed distinct and separate clusters of sites within each proposed ecological (the EU-based clusters were statistically verified with analyses presented in Table 2.5). These clusters of sites are arrayed along the primary explanatory axis in upstream to downstream longitudinal order. Despite close proximity to sites in EU5S, the study site on a cross-channel connected to EU5s had a fish assemblage distinct from the rest of the sites within EU5s. Increasing the temporal extent to across seasons/years did modestly increase compositional overlap in the downstream EUs, but correlation with delineated EUs remains (Figure 2.5c). Fish assemblages at sites on the side channel of EU5S were similar to those in EU5S, although side-channel sites proximal to EU4N and EU3 were distinct from sites in the adjacent river mainstem. Statistical analyses of this pattern indicated higher fish assemblage similarity was associated with being in the same EU and that each delineated EU had a distinct fish assemblage (Table 2.5 WSNT and WSWT columns).

At the wide spatial extent, spatial pattern in invertebrate assemblages strongly supported existence of ecological units and provided moderate support for the efficacy of delineated EUs. The wide spatial, narrow temporal (WSNT) NMDS ordination for invertebrates (Figure 2.6a)

shows distinct clusters of sites within all proposed EUs although the amount of variability differed. EU1 had the most distinct and homogeneous invertebrate assemblage while sites in EU2 were the most variable and sometimes had invertebrate assemblages that resembled sites in other EUs. As with fish, sites on side and cross channels often had different invertebrate assemblages than those in the adjacent mainstem. Although invertebrate assemblages generally differed between delineated EUs, small sample size and large variability resulted in low statistical power and made it difficult to successfully assess differences in invertebrate assemblages between delineated EUs (Table 2.5 WSNT and WSWT columns).

When the spatial perspective was widened to include sites on tributaries, previously described spatial patterns along the mainstem persisted and clear differences between the biotic assemblages in tributaries and the mainstem became evident (Figures 2.5b and 2.6b). Including tributary data changed the meaning of the primary axes in fish NMDS ordinations; the principal explanatory axis for fish assemblages distinguished sites on tributaries versus the mainstem and the secondary axis reflected the upstream-downstream arrangement of sites along the mainstem. For invertebrates, tributary sites were distinguished from mainstem sites by position along both explanatory axes. Expanding the temporal extent to include samples from different seasons and years had little effect on the invertebrate ordination (Figure 2.6d), and minimal effects on the fish ordination (figure 2.5d). The most obvious difference was the addition of four sites in Mosquito Creek (T4) that had fish assemblages similar to those in the mainstem. These four sites are located downstream in a non-wadeable, wetland complex section of Mosquito Creek (T4) and are unlike the upstream, wadeable part of Mosquito Creek (represented by two sites). Again, higher fish assemblage similarity was associated with being in the same delineated EU or tributary (Table 2.5 WSPNT and WSPWT columns). Individual EUs and Bigelow Creek had

distinct fish assemblages, and fish assemblages in tributaries differed from those in the mainstem. Because of limited sampling on the tributaries, it was difficult to assess whether biological assemblages differed between tributaries.

Trajectory and longitudinal plots

Trajectory plots (Figure 2.7) suggested strong concordance between rates of change in fish assemblages, invertebrate assemblages, and environmental variables. Much of the upper half of the study mainstem was dominated by consistent and gradual rates of change in biological and environmental character. However, noticeable changes in the slope of the trajectory line occurred at about 40 km and 20 km from Muskegon Lake and steep slopes between 15 and 20 km from Muskegon Lake indicate rapid change in fish assemblages, invertebrate assemblages, and environmental features. Rates of change were also slightly higher downstream of the channel split and the north and south branches had similar patterns of rates of change. Including biological data from multiple seasons and years smoothed some transitions, but the overall pattern in slopes persists (Figure 2.7 plots 3 & 4).

Major transitions in the environmental and biological character of the mainstem of the Muskegon River largely coincided with tributary confluences and were near boundaries of delineated EUs. Although the confluence with Bigelow Creek (most upstream C in Figure 2.7) did not coincide with an appreciable alteration in the rate of change, the downstream confluences were associated with rapid change in biological assemblages (Figure 2.7). Boundaries between delineated EUs were near transitions in biological assemblages, especially within a single season and year (i.e., WSNT dataset, Figure 2.7).

The delineated EUs also successfully represented real differences in typical values or variability in values of many environmental variables (Figure 2.8). Water temperature measured

during both spring and summer synoptic chemistry sampling demonstrate clear homogeneity of temperature within a delineated EU and marked differences in temperature between EUs (Figure 2.8a). As compared with the south channel (5S), groundwater contributions to the north channel (4N) result in warmer water in the spring and cooler water in the summer. With the exception of one site (which is near outflow of a wastewater treatment plant), conductivity measure during a synoptic study also corresponded well with delineated EUs (Figure 2.8b). Delineated EUs also differ in the proportion of hard substrate, sinuosity, and channel width (Figure 2.8 c-e). Areas of hard substrate dominated sites in the wide and sinuous upper EU1, while soft substrate dominated the straight and narrow downstream EUs. Average water depth differed both in typical values and in variability of values between EUs (Figure 2.8f).

Discussion

I used a variety of analytical techniques to test the validity of three basic assumptions underlying the practical mapping of riverine ecological units: 1) concordance between fish and invertebrate assemblages, 2) concordance between environment and biological assemblage composition, and 3) the existence of environmentally and biologically homogeneous river lengths. In the lower Muskegon River, these assumptions were well supported by the empirical data, and delineated ecological units reflected real spatial patterns in biological assemblages and key environmental variables. This is the first study to test these theoretical assumptions in the context of ecological units, although each assumption has an individual history of study. Additionally, this is one of only a few attempts (Thomson et al. 2004, Seelbach et al. 2006, Boys and Thoms 2006, Warrner et al. 2010) to evaluate the efficacy of *existing ecological unit delineations* in a river classification context. It is also the only study I am aware of that jointly

explores physical, chemical, fish, and invertebrate spatial pattern in the context of river classification.

Fish/Invertebrate Concordance

The existence of *ecological* units requires concordance between biological assemblages and between biological assemblages and environmental variables (Rowe 1961, 1980, 1992). Both requirements were met in the lower Muskegon River. The strength of fish/invertebrate concordance varied with the spatial extent of sampling, generally increasing in strength as more of the river basin was included and sampled variance increased. Measured biological concordance was negligible within an ecological unit, moderate across multiple reaches along the mainstem, and stronger as sampling approached basin-scale. This study used a longitudinal sampling design that was at the same time both spatially frequent enough to sample local variability and spatially extensive enough to include major physical transition zones in the fluvial system. Expanding the spatial extent of samples captured more abrupt transitions in size, hydraulic habitat, and temperature, that were then reflected in assemblage composition as we might expect (Hawkins et al. 1993, Rosgen 1994, Poff et al. 1997, Montgomery and Buffington 1997, 1998, Biesel et al. 2000, Rice et al. 2001, Benda et al. 2004a, 2004b, Gordon et al. 2004, Kiffney et al. 2006, Rice et al. 2006). This suggests a large spatial perspective that transcends scales at which environmental transitions are organized in rivers is essential for detecting and evaluating concordant biological transitions.

Previously reported strengths of within-basin fish/invertebrate concordance varies greatly, from no/weak concordance (Paavola 2003, Paavola et al. 2006, Infante et al. 2009), to moderate/strong concordance (Grenouillet et al. 2008, Dolph et al. 2011). Although Paavola et al. (2006) suggest biological concordance may be stronger at larger spatial scales (e.g., across

basins and ecoregions), studies focused at these scales have also yielded a range of concordance strengths (Reviewed in Heino 2010, Johnson and Hering 2010, Yates and Bailey 2010, Dolph et al. 2011, Larsen et al. 2012).

Does the lack of invertebrate/fish concordance reported in some other studies limit the potential utility of the concept of ecological unit mapping in other river systems? Many instances of no/weak biological concordance arise from differences in study context, some from differences in sampling design, and in a few cases, both. This study frames questions of concordance in the context of longitudinal change in the assemblage composition of different riverine taxa. In contrast, many studies that have measured concordance between different taxa have done so within a biological assessment context, and therefore pose different questions concerning community concordance. For example, assessment-based questions included whether biodiversity in indicator taxa can be used to predict variation in biodiversity in other taxa (Heino 2010), whether different taxa exhibit similar trends in human-induced taxa loss (Dolph et al. 2011), and whether there is concordant variation along a gradient of anthropogenic stressors (Infante et al. 2009, Yates and Bailey 2010, Larsen et al. 2012) within regional datasets.

To target the specific effects of humans on riverine taxa, some of these assessment studies have used sampling designs based on spatially random sample locations or restricted sites to a narrow range in size (e.g., streams of the same order, the same width and depth, or only small headwaters). Studies with these sampling designs typically found little or no concordance between different taxa (Paavola et al. 2003, 2006, Infante et al.2009, Larsen et al. 2012). These and similar studies (Lammert and Allan 1999, Johnson and Hering 2010, Dolph et al 2011) have led to the suggestion that strong concordance between fish and invertebrates does not occur because the two assemblages respond to different environmental variables operating at different

spatial scales. Although this may have been observed in context of assessment sampling, the sampling design of many of these studies simply precludes accounting for the principal component of spatial variation in the fluvial environment: the hydraulic gradient which shapes geomorphic, thermal, and many chemical and biological processes (Vannote et al. 1980, Snow and Slingerland 1987, Ensign and Doyle 2006).

Biology/Environment Concordance

Spatial concordance between fish and invertebrate assemblages within a river basin could arise from several mechanisms: 1) response of assemblages to the same environmental gradients; 2) response to correlated but different gradients; 3) substantial biological interactions between the assemblages (leading to strong covariances); or 4) similar limitations in dispersal or reproductive capabilities of the assemblages (adapted from Gaston and Williams 1996). Because the study area does not contain major barriers to fish or invertebrate distribution, the fourth mechanism is likely irrelevant in the lower Muskegon River. Because of its purely observational design, this study cannot directly address whether biological interactions between fish and macroinvertebrate assemblages could create concordant spatial patterns; however, many strong environment/biology concordances (discussed below) suggest that environmental gradients may largely control the organization of biological assemblages in the lower Muskegon River basin.

Observed changes in assemblage structure for both fish and invertebrates were associated with changes in the same environmental variables. Thus, it seems likely that the fish/invertebrate concordance I observed in this study arises from fish and invertebrates responding to the same environmental gradients. The most important environmental variables included measures of size (catchment area, discharge and depth/width), link number, temperature, and nutrient regime. Longitudinal gradients in river size/temperature have often been associated with changes in fish

(Huet 1959, Illies and Botosaneanu 1963, Hawkins and Sedell 1981, Schlosser 1991, Duncan and Kubecka 1996, Matthews 1998, Zorn et al. 2002, Wehrly et al. 2003) and, likewise, invertebrates (Statzner and Higler 1986, Perry and Schaeffer 1987, Statzner et al. 1988, Hawkins et al. 1997). Local longitudinal and spatial effects of tributary junctures on biological assemblages have also been recognized (Osborne and Wiley 1992, Benda et al. 2004a, 2004b, Ferguson et al. 2006, Kiffney et al. 2006, Rice et al. 2006).

Despite strong overall environment/biology concordance, differences in fish and invertebrate distribution coupled with varying strength of environment/biological concordance suggest substrate may have more influence on invertebrate assemblages and temperature may have more influence on fish assemblages. Aside from associations with measures of size, changes in invertebrate assemblages were most strongly associated with changes in substrate. Substrate in the lower portion of the study area was largely uniform shifting sand, a habitat suited to few invertebrate taxa (Soluk 1985, Palmer 1990) and thus only minor differences between sparse and low diversity invertebrate assemblages in these river sections might be expected. Accordingly, invertebrate assemblages in the lower three ecological units were often similar and could not consistently be distinguished.

In contrast, despite proximity and extensive fluvial interconnection, fish assemblages in the north and south channels were notably different. Although similar in substrate and IGUs, the north and south channel differed in hydrologic, and thus temperature, regime. The northern channel receives more groundwater influx and is warmer in the spring and cooler in the summer than the south channel. These faunal differences and strong associations with temperature regime variables suggest that fish may be responding strongly to even minor differences in temperature

regime, a commonly recognized variable controlling lotic fish distribution (Schuller et al. 1999, Zorn et al. 2002, Wehrly et al. 2003, Buisson et al. 2008).

Distinct, homogeneous ecological units

My empirical data provided strong support for the existence of ecologically distinct and homogeneous river channel segments. Transitions in environment character and biological assemblages included both gradual and sharp adjustments. Transitions in many environment features coincided (e.g., changes in flow rate, planform, temperature, and substrate) and typically occurred at certain network junctures (confluences or distributary channel splits). The influence of individual environment characteristics on biological assemblages has already been noted, so the view that emerges from my study is consistent with views of the river as a patchy continuum (Duncan and Kubecka 1996, Wiens 2002, Poole 2002, Thorp et al. 2006, 2008) where transitions between patches reflect changes in geomorphic process domains (Montgomery 1999).

The extant ecological unit delineation (VSEC version 1.0; Seelbach et al. 1997) performed well; it captured much of the large-scale patchiness in the lower Muskegon River. This suggests that riverine ecological boundaries can be successfully predicted from map features, especially when changes in river energy balance (observed as changes in sinuosity, channel slope, and valley form) and network confluences correspond to changes in biological assemblages. The strong association between river temperature and fish assemblage composition certainly helped in this regard, and suggests inclusion of specific regional models of river fauna (e.g., Zorn et al. 2002, Steen 2008, McKenna et al. 2014) would be critical when important ecological drivers cannot be developed simply from maps. As suggested by Benda et al. (2004a, 2004b) the relative effect of a tributary juncture on mainstem ecological pattern was predictable

based on character (i.e., water temperature, nutrient and substrate load) and relative size of the tributary.

Spatial and temporal extent considerations

I addressed the research questions at two temporal and three spatial extents. As in Hawkins and Sedell (1981) and Ostrand and Wilde (2002), longitudinal changes in biological assemblages were much more dramatic than seasonal changes. Given large seasonal migrations of anadromous fishes upstream into tributaries and downstream to Lake Michigan (O'Neal 1997), this was somewhat surprising for fish assemblages. Although combining fish data from several seasons and years did somewhat blur spatial transitions between fish assemblages, distinct fish assemblages persisted in all delineated ecological units. It seems reasonable to assume that local movements of fish between seasons may fuzz the boundaries between ecological units when sampled over extended periods of time.

This study suggests lengths of ecological units within a basin are not arbitrary and must correspond to physical scaling laws in rivers, even if this results in EUs of varying size. Within the largest ecological unit there was no fish/invertebrate concordance, a small number of weak biology/environment associations, and small differences between biological assemblages in each subunit. This suggests shorter ecological units within this portion of the Muskegon River, although easily delineated, would not usefully reflect observed patterns in environment and biological change in this section of river. Although confluence to confluence river segments (e.g., arcs or node to node river segments in GIS stream networks) have been proposed as the smallest aquatic unit to which environmental data should be attributed (Wang et al. 2011, Melles et al. 2014), these units should not be conceptualized as ecological units or confused with ecological units. Instead, these arcs should be lumped (or split) as necessary based on

environmental features known to control biological distributions within a basin (Seelbach et al. 1997, Brenden et al. 2008a). Only then should classification of the ecological units proceed (Seelbach et al. 1997, Brenden et al. 2008b, Melles et al. 2014).

This study also suggests ecological unit delineation should proceed at the basin scale to recognize the influence of network structure on environmental and biological pattern in rivers. A basin-wide perspective implicitly incorporates strong discontinuities between tributaries and the river mainstem, while also recognizing spatial pattern within the river mainstem (Benda et al. 2004a, 2004b). Taking a basin-wide perspective can also facilitate incorporation of differences in rates of change along a river network and allow adjustment of the size of ecological units based on location in the river network. This study suggests biological assemblages on tributaries may be more variable than assemblages along the river mainstem. This highlights a need to examine longitudinal patterns in tributaries in even more spatial detail, and to test the assumptions of ecological units in tributary systems.

Ecosystem management implications

In summary, recurring strong fish/invertebrate and environment/biology concordances and marked transitions in ecological character (e.g. channel shape, substrate, temperature, fish, and invertebrates) between units provide strong empirical support for the validity of EUs in the Muskegon River. Becasue delineated EUs do usefully summarize the environmental and biological heterogeneity of the Muskegon River, such map-based EUs can be used to guide practical management decisions, simplify large-scale modeling efforts, and can be used as strata in biological sampling designs. My analyses suggest that this utility rests on a secure conceptual foundation.

Table 2.1: Spatial and temporal descriptions of the five datasets and the number of sites/taxa in the three main analyses. The number of sites is mainstem sites with the exception of datasets beginning WSP where it is # mainstem sites + # tributary sites. Since the number of sites affects the power and significance of statistical tests, the number of sites should be considered when interpreting the magnitude and significance of statistical tests in this chapter.

Dataset	Spatial (S) Extent	Temporal (T) Extent	Fish/Invert concordance analyses	Environ/Biology concordance analyses	NMDS analyses
NSWT	Narrow (NS): 35 river km section of mainstem in upstream most delineated ecological unit	Wide (WT): Across three seasons and two years	26 sites 65 fish taxa 137 inv taxa	43 fish sites 70 fish taxa 29 inv sites 138 inv taxa	43 fish sites 70 fish taxa 29 inv sites 138 inv taxa
WSNT	Wide (WS): Mainstem from Croton Dam to Muskegon Lake (80 river km)	Narrow (NT): Within a single season and year	24 sites 56 fish taxa 94 inv taxa	36 fish sites 66 fish taxa 27 inv sites 103 inv taxa	37 fish sites 66 fish taxa 32 inv sites 115 inv taxa
WSPNT	Wide PLUS tributaries (WSP): Mainstem from Croton Dam to Muskegon Lake plus sites on four tributaries	Narrow (NT): Within a single season and year	24 + 7 sites 63 fish taxa 134 inv taxa	36 + 11 fish sites 70 fish taxa 27 + 7 inv sites 141 inv taxa	37 + 11 fish sites 70 fish taxa 32 + 7 inv sites 148 inv taxa
WSWT	Wide (WS): Mainstem from Croton Dam to Muskegon Lake (80 river km)	Wide (WT): Across three seasons and two years	40 sites 80 fish taxa 151 inv taxa	92 fish sites 90 fish taxa 43 inv sites 150 inv taxa	96 fish sites 90 fish taxa 49 inv sites 161 inv taxa
WSPWT	Wide PLUS tributaries (WSP): Mainstem from Croton Dam to Muskegon Lake plus sites on four tributaries	Wide (WT): Across three seasons and two years	40 + 8 sites 84 fish taxa 181 inv taxa	92 + 15 fish sites 91 fish taxa 43 + 8 inv sites 182 inv taxa	96 + 15 fish sites 91 fish taxa 49 + 8 inv sites 189 inv taxa

Table 2.2: Biological measure concordance: Significant Mantel tests with large test statistics (r) indicate strong concordance between occurrence, abundance, and biomass measures of biological assemblage. Dataset classes are described in Table 2.1 and all distance measures are Euclidean. Abundance and biomass measures were LN transformed.

Variables or Dataset Extent	NSWT	WSNT	WSPNT	WSWT	WSPWT
Fish Occurrence vs. Abundance	0.81***	0.79***	0.90***	0.86***	0.84***
Fish Occurrence vs. Biomass	0.94***	0.92***	0.96***	0.93***	0.93***
Fish Abundance vs. Biomass	0.81***	0.77***	0.93***	0.85***	0.88***
Invert Occurrence vs. Abundance	0.88***	0.97***	0.95***	0.93***	0.94***
Invert Occurrence vs. Biomass	0.85***	0.94***	0.90***	0.87***	0.88***
Invert Abundance vs. Biomass	0.70***	0.94***	0.96***	0.81***	0.83***

*p<0.05, **p<0.01, *** p<0.001

Table 2.3: Biological concordance: Mantel concordance r values for the five datasets and three measures of assemblage structure. Large positive r values indicate strong concordance between fish and invertebrate assemblages. Datasets are described in Table 2.1 and abundance and biomass are LN transformed. Sorenson distance is used for occurrence measure and Euclidean distance is used for abundance and biomass measures.

Dataset extent	Occurrence	Abundance	Biomass
1) NSWT	0.06	0.22*	0.10
2) WSNT	0.40***	0.06	0.20
3) WSPNT	0.36***	0.41***	0.58***
4) WSWT	0.33***	0.26***	0.10
5) WSPWT	0.29**	0.40***	0.30***

*p<0.05, **p<0.01, *** p<0.001

Table 2.4: Environment/Biology concordance: Significant Mantel tests with large test statistics (r) indicate strong concordance between fish or invertebrate assemblages (occurrence measure) and environmental characteristics. Fish and invertebrate distance matrices are Sorenson and environmental is Euclidean distance. Some variables were not tested with the NSWT dataset due to minimal variance in values at this spatial extent. NS=No significant association between biological occurrence and environmental characteristic.

Variables or Extent	NSWT	WSNT	WSPNT	WSWT	WSPWT
Size/Geomorphic					
Fish/CatchArea	NS	0.58***	0.87***	0.40***	0.57***
Invert/CatchArea	NS	0.47***	0.24**	0.43***	0.17*
Fish/Link	NS	0.60***	0.88***	0.44***	0.57***
Invert/Link	0.22**	0.48***	0.24**	0.54***	0.17*
Fish/Slope	NS	NS	0.42***	NS	0.34***
Invert/Slope	0.32**	NS	NS	NS	NS
Fish/Sinuosity	NS	NS	NS	0.11*	NS
Invert/Sinuosity	NS	NS	NS	NS	NS
Fish/QAve	No test[1]	0.65***	0.88***	0.49***	0.57***
Invert/QAve	No test[1]	0.31**	0.23**	0.34**	0.18*
Fish/AveVelocity	NS	0.16*	0.43***	0.21***	0.23**
Invert/AveVelocity	NS	0.17**	NS	0.17*	NS
Fish/AveDepth	NS	0.31***	0.60***	NS	0.35***
Invert/AveDepth	0.18*	0.20**	0.37***	NS	0.25**
Fish/Width	NS	0.15*	0.82***	0.30***	0.62***
Invert/Width	NS	0.16*	0.25**	0.17*	0.18*
Habitat					
Fish/Substrate	NS	0.43***	NS	0.25***	0.14**
Invert/Substrate	NS	0.41***	0.25***	0.41***	0.31***
Fish/IGUs	NS	NS	NS	NS	NS
Invert/IGUs	NS	NS	NS	NS	NS
Chemistry/Temp					
Fish/Nutrients	No test[1]	0.65***	0.80***	0.49***	0.60***
Invert/Nutrients	No test[1]	0.29**	0.27**	0.31**	0.21*
Fish/Temp	No test[1]	0.59***	0.74***	0.45***	0.57***
Invert/Temp	No test[1]	0.33***	0.23**	0.32**	0.17*

*p<0.05, **p<0.01, ***p<0.001; [1]Did not test: Variable is based on modeled data with only two possible values.

Table 2.5: Performance of delineated EUs: Mantel tests (r) indicate higher biological similarity is associated with sites in the same delineated EU subunit (subEUs), EU, or tributary. Although statistically significant at all spatial extents, this association is weak at narrow spatial extents and strong at wide spatial extents. Mantel tests used a design matrix with one for sites in the same subEU, EU, or tributary and zero for all other comparisons. Pairwise comparisons from MRPP tests determined if specific subEUs, EUs, or tributaries differed in biological character. If subunits, EUs, or tributaries did not differ in biological character, the comparison is indicated by a line under the similar subunits, EUs, or tributaries. For fish assemblages at all spatial and temporal extents, all subEUs, EUs, and Bigelow Creek (T1) had distinct fish assemblages while other tributaries had fish assemblages distinct from the mainstem, but not from each other. For invertebrates, results varied somewhat with spatial extent. EU1 had a consistently unique invertebrate assemblage, the north (4N) and south branch (5S) often had similar invertebrate assemblages, and tributaries had different invertebrate assemblages than the mainstem. All analyses are based on occurrence measure and Sorenson similarity.

Taxa or Dataset Extent	NSWT	WSNT	WSPNT	WSWT	WSPWT
Fish	r=0.15**	r=0.64***	r=0.53***	r=0.41***	r=0.42***
Differences between delineated subEUs, EUs and/or tribs	s1 s2 s3 s4	1 2 4N 5S	1 2 3 4N 5S T1 T2 T3 T4	1 2 3 4N 5S	1 2 3 4N 5S T1 T2 T3 T4
Invertebrates	r=0.14*	r=0.53***	r=0.44***	r=0.65***	r=0.57***
Differences between delineated subEUs, EUs and/or tribs	s1 s2 s3[1] s4	1 2 4N 5S[2, 3]	1 2 4N 5S T2 T3[4,5]	1 2 4N 5S[3]	1 2 4N 5S T1 T2 T3[45]

*p<0.05, **p<0.01, ***p<0.001; [1]Comparison s2 to s3 borderline significant at p=0.06; [2]Comparison 4N and 5S borderline significant at p=0.08; [3]differences in EUs 2, 4N and 5S not required for validity of delineated EUs since river segments are not adjacent. [4]Some tributaries are not included in analysis because of single samples; [5]Included tributaries differ from the mainstem, but between tributary comparisons were limited by small number of sites on the tributaries.

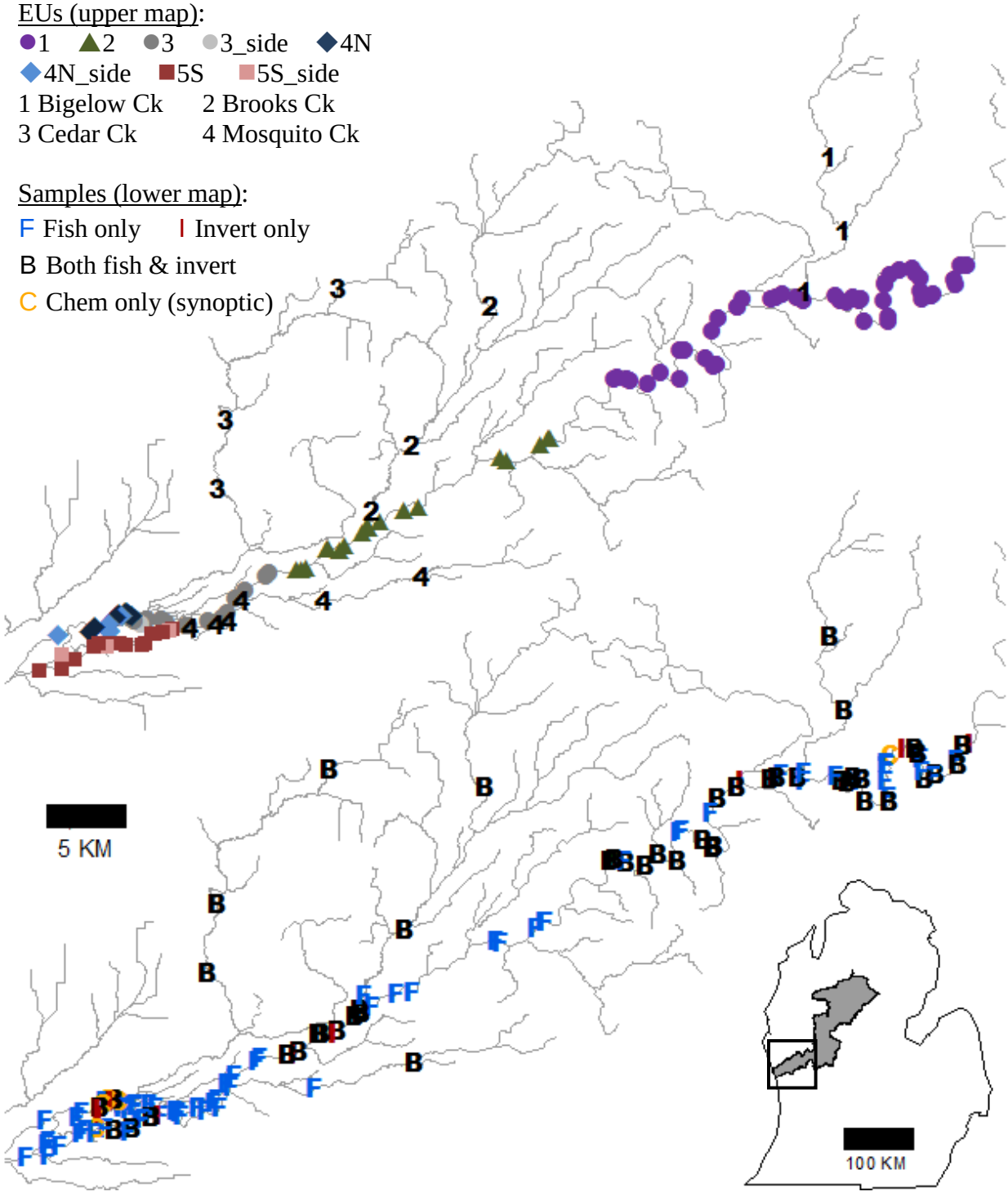
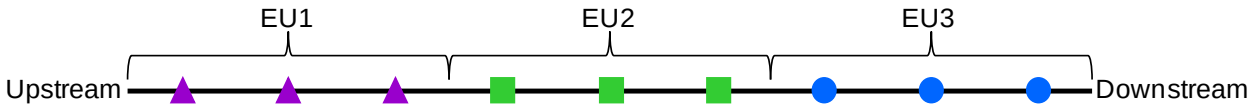
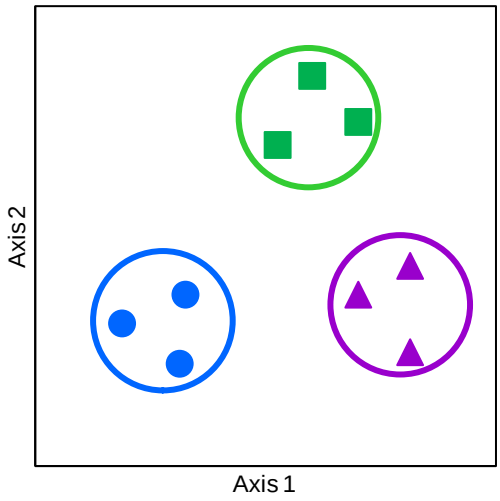


Figure 2.1: The lower Muskegon River study area. Lower right inset shows the location of the study area in the Muskegon River basin in Michigan's lower peninsula and the two network maps show the location of sites within the study area. Symbols marking sites in the top map specify the tributary, the assigned delineated Ecological Unit (EU), and if a site is on a side or cross channel adjacent to the mainstem. Symbols in the bottom diagram illustrate the type of samples collected at a site.

a) Hypothetical river mainstem, sampling sites, and delineated ecological units (EUs).



b) Situation 1: NMDS plot of study sites



c) Situation 2: NMDS plot of study sites

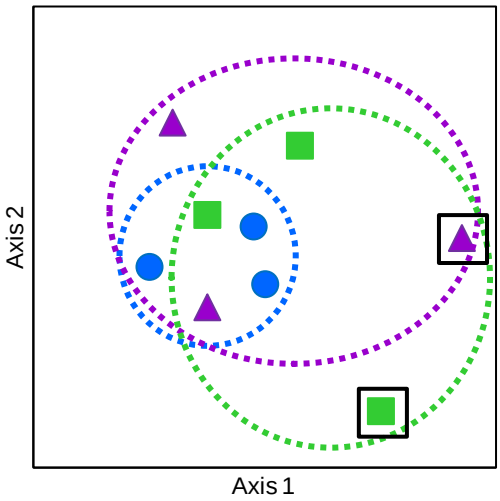


Figure 2.2: Ecological units and NonMetric Multidimensional Scaling (NMDS) ordinations: Overview: Demonstration of a hypothetical river mainstem with delineated ecological units (EUs), two biotic assemblage spatial patterns, and interpretation of corresponding NMDS ordinations. Proximity between sites in NMDS ordinations equates to assemblage similarity; closer sites have more similar assemblages than distant sites. If the underlying assumption of biological/biological concordance is met, plots for both fish and invertebrates will have a similar arrangement of sites. If the concordance assumption is met, these demonstration plots can guide interpretation of NMDS ordinations for three additional factors relevant to my research questions: 1) Within unit assemblage variability, 2) within unit assemblage variability compared to between unit assemblage variability, and 3) outliers. Details: a) Simple river mainstem with three delineated EUs (three stream segments) and nine sampling sites (three sites per delineated EU with unit assignment indicated by symbol). b) In situation 1 the delineated EUs are valid since sites within an EU have similar assemblages that are distinct from the assemblages in other EUs. Although not an assumption for valid EUs, the variability of assemblages is the same for all units and none of the sites have particularly unusual assemblages. c) In situation 2 the delineated EUs are null since sites within an EU are arrayed across ordination space regardless of EU assignment (i.e. no homogeneity within and EU and sites in different EUs do not have distinct assemblages). In contrast to situation 1, within EU variability differs between EUs (less variable assemblage in EU3) and two sites have unusual assemblages as compared with other sites along the mainstem (indicated by the outline squares).

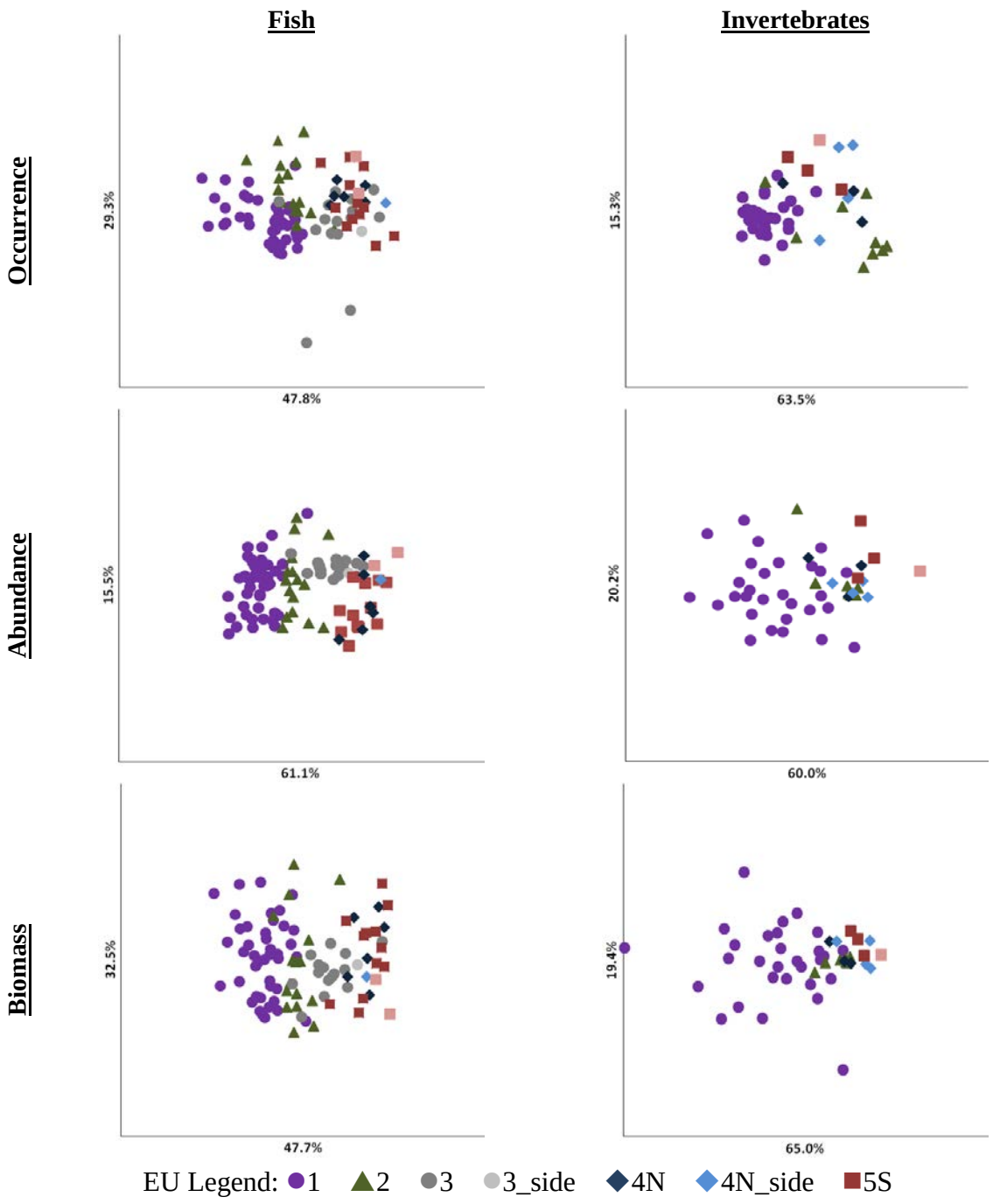
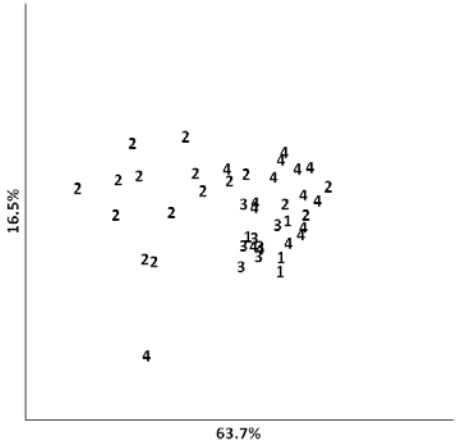


Figure 2.3: NMDS ordinations for biological assemblage measures and Wide Spatial, Wide Temporal (WSWT) datasets. Ordinations are displayed with symbols in Figure 1 upper map, identical axes extents, and include variance explained by each axis. Different assemblage measures had no effect on overall interpretation of spatial pattern. Different measures did not affect fish ordinations, but did affect invertebrate assemblage variability. Compared with occurrence, quantitative measures of the invertebrate assemblage increased variability and frequency of outliers in EU1 and decreased variability in EU2. These effects were consistent across all datasets except NSWT where there were no effects of different measures.

a) Fish



b) Invertebrates

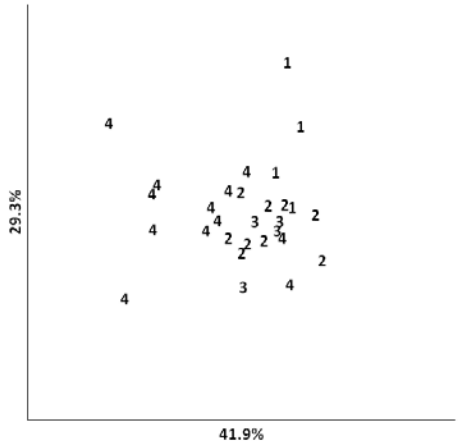


Figure 2.4: NMDS ordinations for Narrow Spatial, Wide Temporal data sets based on a) fish and b) invertebrate occurrence. Variance explained is displayed on each axis, plots have identical axes extents, and number markers are subsections in EU1, the longest and most upstream of delineated EUs in the study area. Subsections are numbered upstream (1) to downstream (4) and vary in size and number of samples. Biological assemblages typically differed by EU subsections (also see Table 2.5), but this partitioning is weak and there is also considerable overlap between sites in different subsections. There is no concordance between fish and invertebrate assemblages.

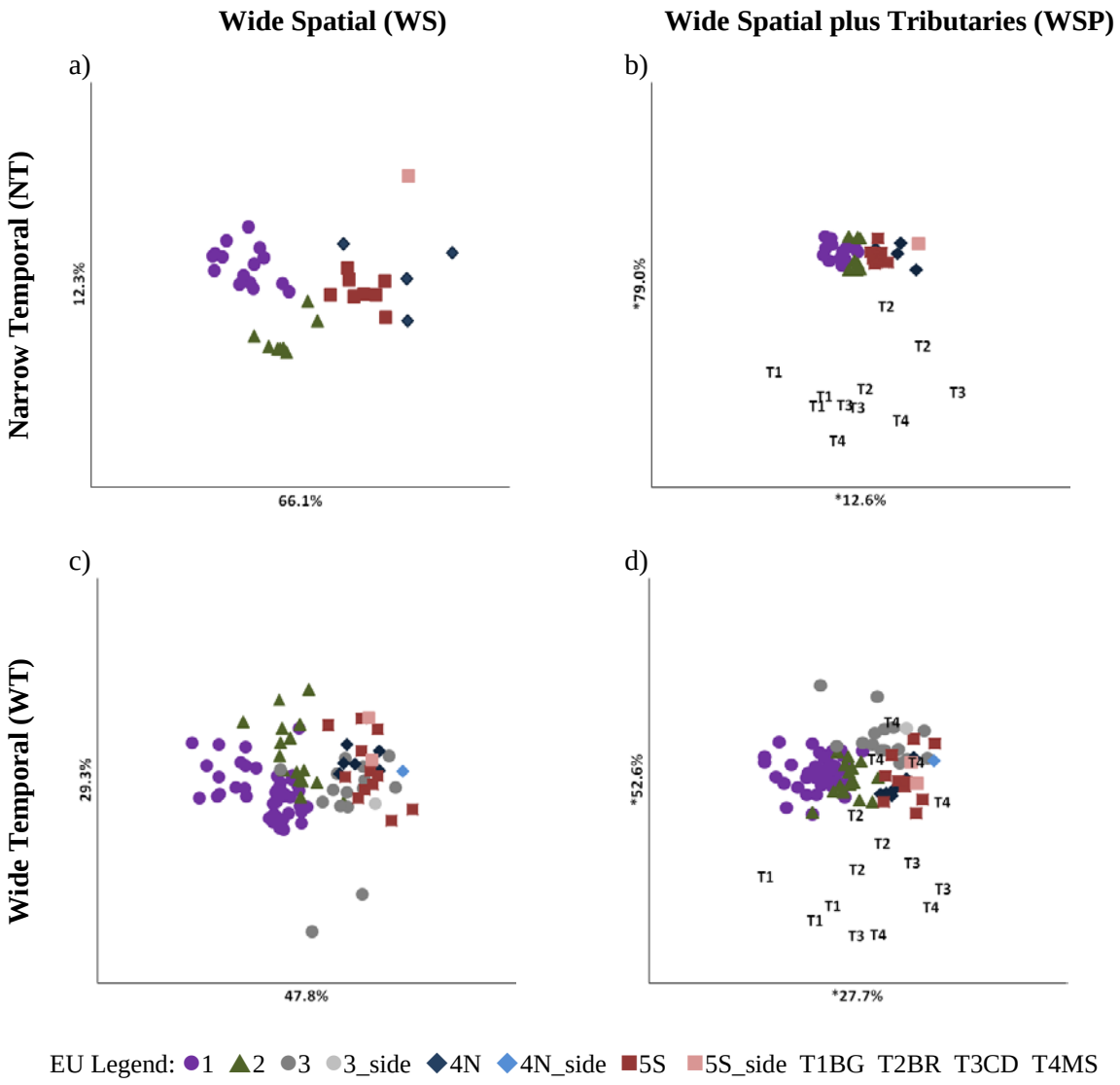


Figure 2.5: NMDS ordinations for fish assemblages at a) WSNT, b) WSPNT, c) WSWT, and d) WSPWT spatial and temporal extents. Ordinations based on occurrence measure, and displayed with symbols in Figure 1 upper map, identical axes extents, and include variance explained by each axis (*primary axis is displayed on the y-axis for consistency in spatial arrangement of mainstem sites). For WS ordinations (a, c) the primary axis reflects the longitudinal arrangement of sites along the mainstem while for WSP (b, d) ordinations, the primary axis differentiates sites on the mainstem sites in the tributaries and the secondary axis reflects the longitudinal arrangement of sites along the mainstem. Within a single sampling season and year (a, b), sites on the mainstem within the same delineated EU have similar and distinct fish assemblages, fish assemblages in side channels were different from proximal mainstem sites, and the tributaries have mixed fish assemblages that were distinct from those in the mainstem. Spatial patterns are similar at the wide temporal extent (c, d) except side channel assemblages are not consistently as different from those in the mainstem and downstream sites in Mosquito Creek (T4) have fish assemblages similar to those in the mainstem. See Table 2.5 for an explicit test of differences in fish assemblages between delineated EUs/tributaries.

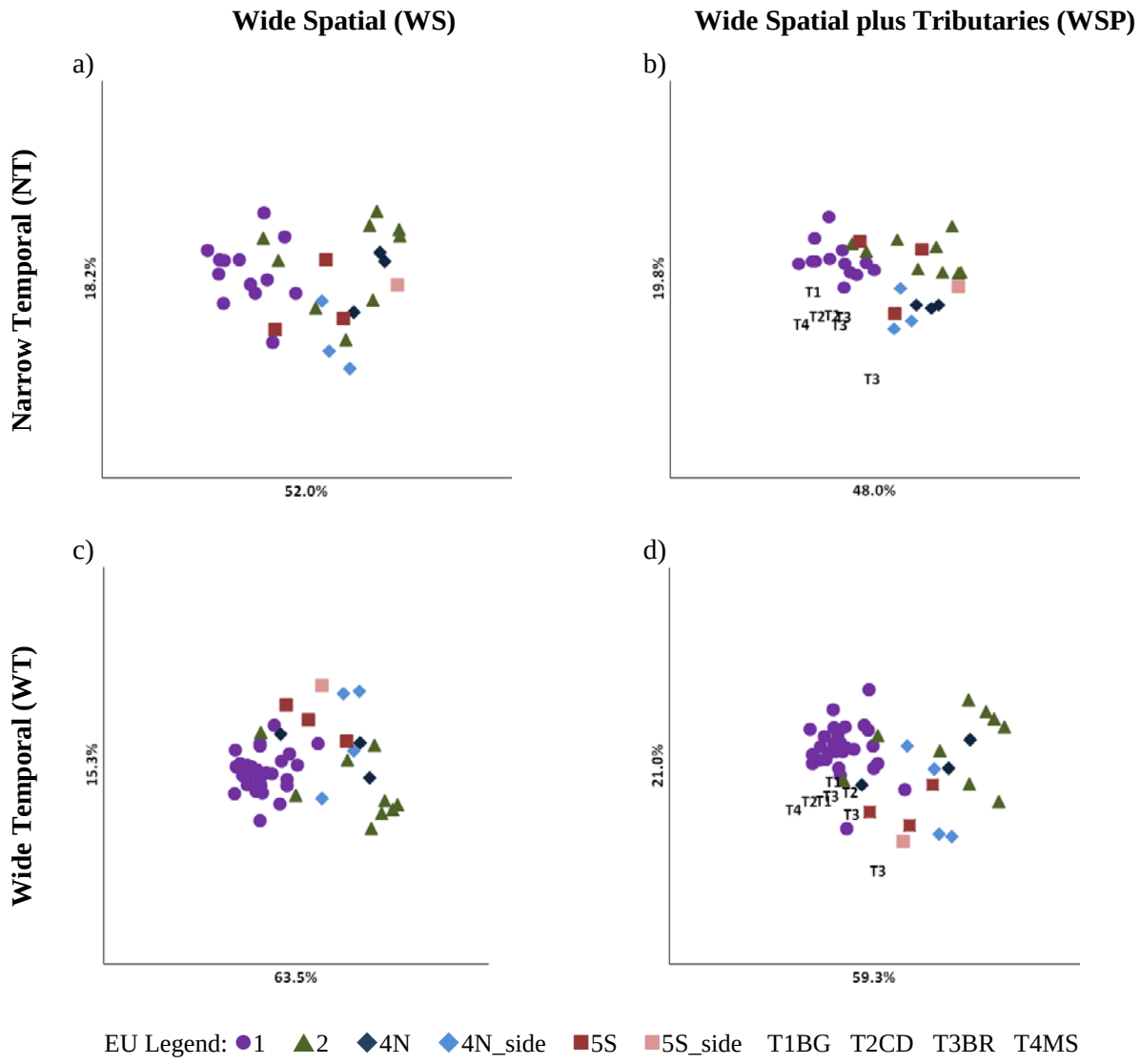


Figure 2.6: NMDS ordinations for invertebrate assemblages at a) WSNT, b) WSPNT, c) WSWT, and d) WSPWT spatial and temporal extents. Ordinations based on occurrence measure, are displayed with symbols in Figure 1 upper map, identical axes extents, and include variance explained by each axis. Unlike ordinations for fish, the primary axis only loosely reflects the longitudinal arrangement of sites along the mainstem for invertebrates. Within a single sampling season and year (a, b), sites on the mainstem within the same delineated EU often have similar but not always distinct invertebrate assemblages, invertebrate assemblages in side channels are often quite different from proximal mainstem sites, and the tributaries have mixed invertebrate assemblages that are distinct from those in the mainstem. These spatial patterns are also evident at the wide temporal extent (c, d). See Table 2.5 for an explicit test of differences in invertebrate assemblages between delineated EUs/tributaries.

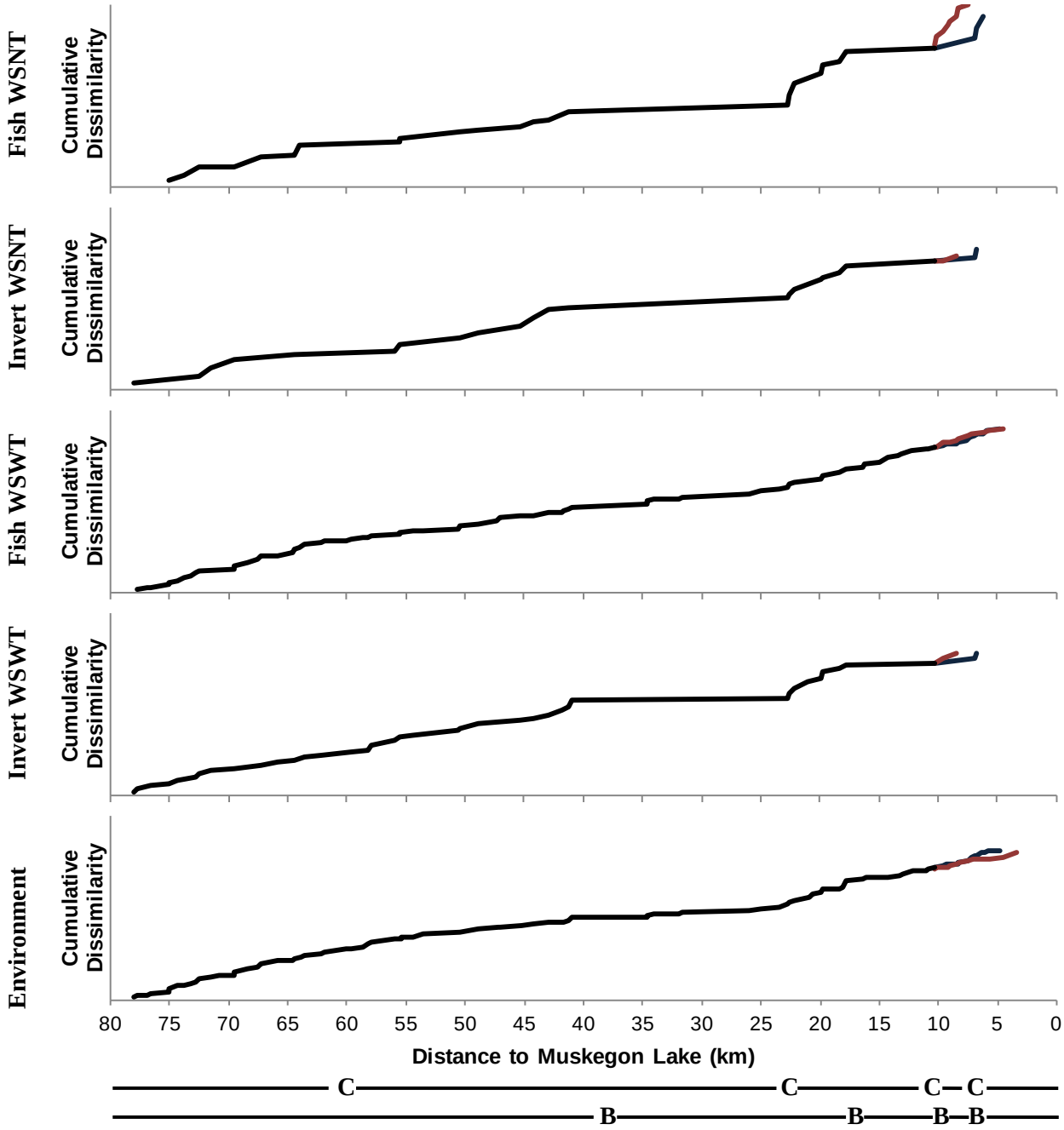


Figure 2.7: Trajectory analyses: Cumulative dissimilarity versus distance from Muskegon Lake for fish, invertebrates, and a suite of environmental variables (see methods for details). Slope at any position on the line indicates rate of change in biological assemblages or environmental features (e.g., steep slope indicates rapid change). After the main channel splits into two branches, the north branch is red and the south branch is dark blue. Diagrams below the plots show location of four major confluences (C) and boundaries between delineated EUs (B). All plots show similar pattern in slopes, with rapid changes in biological and environmental character downstream of the confluence of Brooks Creek (at 23 km). Including biological data from multiple seasons and years smoothed some transitions, but the overall pattern in slopes persists.

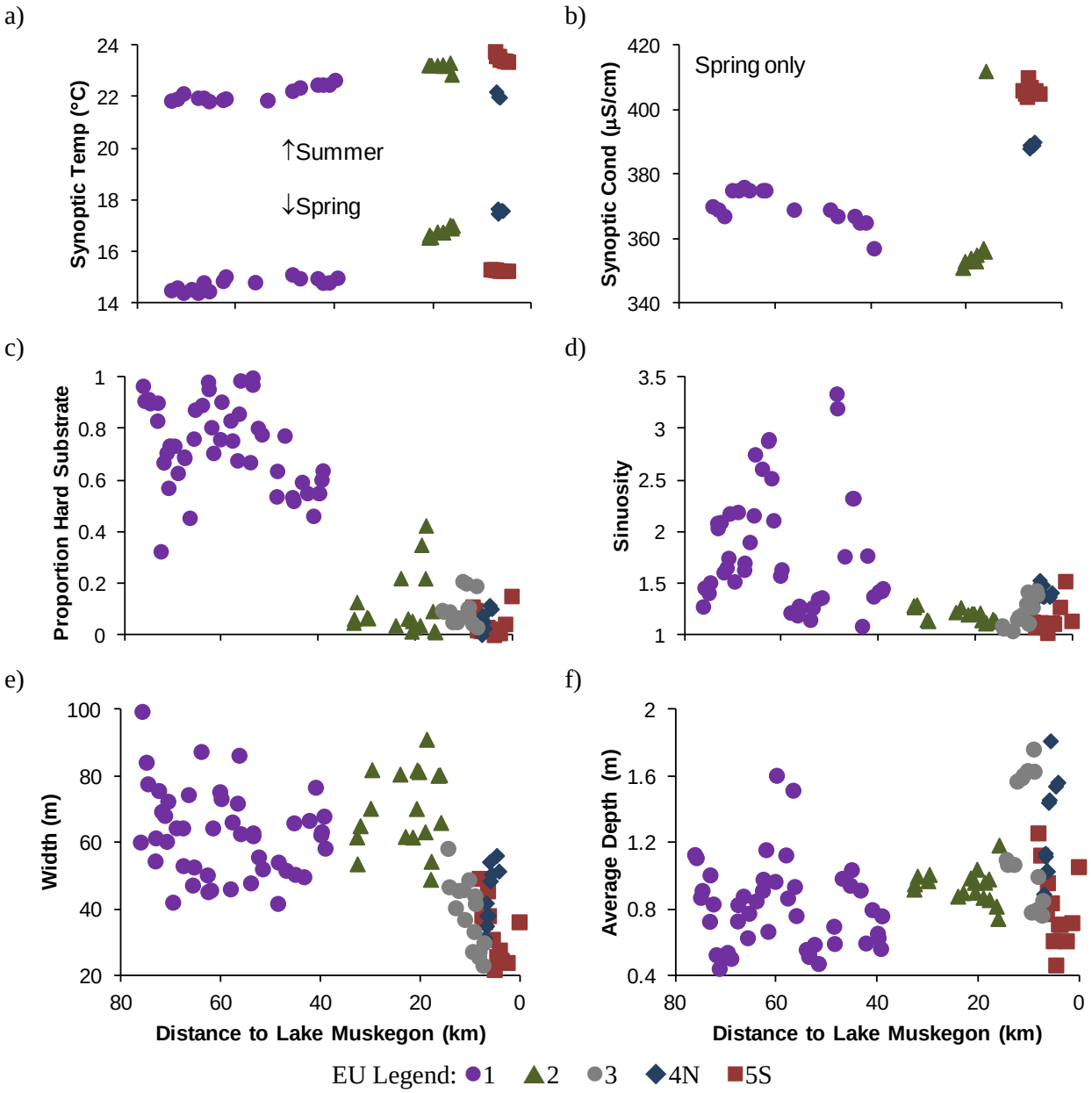


Figure 2.8: Values of six environmental variables plotted against distance from Lake Muskegon. Most environmental variables show strong differences in average value or variability between some or all EUs. a) Water temperature is consistent within but varies between delineated EUs in both the spring and summer. As compared with the south channel (5S), groundwater contributions to the north channel (4N) result in warmer water in the spring and cooler water in the summer. b) With the exception of one site in EU2, conductivity is consistent within and varies between delineated EUs. The odd site is near wastewater treatment plant outflow. c) Hard substrate dominates EU1, substrates are mixed in EU2, and soft substrate dominates EUs 3-5. d) Channel sinuosity is highest and most variable in EU1 and lowest and least variable in EU2. e) The channel is wide in EUs 1 & 2 and narrow in EUs 3-5. f) Although average water depth is on average the same in EUs 1, 2, & 5 and in EUs 3 & 4, variability in water depth differs between EUs.

Appendix 2.1

From samples to sites in the Muskegon River mainstem: Considerable processing was required to move from sample-specific data to site-based summaries of fish and invertebrate assemblage composition across narrow and wide spatial and temporal extents. The specific process differed between fish and invertebrates and is summarized here.

Fish:

Quantitative measures of the fish assemblage at each site were based on both towbarge and boom sampling. Site-based assemblage measures required conflation of towbarge samples that included small and juvenile fish in wadeable portions of the river and boom samples that focused on large fish in non-wadeable portions of the river. These calculations required an estimation of the amount of towbarge and boom habitat at each site. Barge samples were from 100 meter reaches of river while boom samples varied in length from 0.2 to 1.9 km and sometimes spanned multiple study sites.

Step 1: Determine the percentages of boom and towbarge habitat at each site: A 500 meter buffer was created for each georeferenced site and the underlying digital habitat map was clipped. Because of the mismatch in the scale of sampling by barge and boom, an intermediary scale, a 500 meter buffer, was used to estimate the proportion of each sampling habitat for each site. The proportion of each of 22 habitat variables (i.e., riffle, pool, edge, backwater, etc.) was calculated for each site and each habitat was assigned a towbarge or boom designation based on depth, location of habitat, and personal observations of aquatic habitat in the Muskegon River. The proportion of towbarge and boom habitat for every site was summed and checked for unity (i.e., proportion towbarge habitat + proportion of boom habitat = 1).

Step 2: Associate towbarge and boom samples: Based on river network proximity and start/end coordinates of boom runs, all but a few barge samples were associated with boom samples. Sometimes multiple barge samples were associated with the same boom sample.

Step 3: Refine taxa database: Exclude undesired taxa (e.g., young of the year minnows and the rare unidentified fish).

Step 4: Calculate fish densities for each taxa for "Narrow Temporal" data sets: Narrow temporal extent only included samples from the spring of 2003. Observed densities for each fish taxon at each site and type of sample were multiplied by the proportion of towbarge or boom habitat. Densities for each fish species were summed a at site. Rarely, usually because of depth restrictions (e.g., too shallow for the boom shocker or too deep for the barge shocker), a site only had one type of fish sample and the sample was given full weight.

Step 4: Calculate densities for "Wide Temporal" data sets: Many sites were sampled on multiple occasions (across seasons and two years) and these sampling occasions were conflated to produce the wide temporal datasets. Within each sample type (i.e., boom or barge), density or biomass were summed by taxon and divided by the number of samples for a "typical" fish

sample. These typical samples were then multiplied by the proportion of barge or boom habitat as appropriate and boom and barge contributions were combined for a summary of biomass/m^2 or density by site.

Invertebrates:

At each site, sampling was designed to account for invertebrate densities in all major habitats and in notable habitats. The number of samples from each habitat, and thus also the number of samples within a site, varied. Samples are from two seasons in two years (although many sites were only sampled once).

Step 1: Calculate the proportions of sampled habitats: Using a 100 meter buffer around each site, calculate the total amount of each habitat type. For each site and season, include only habitats that were sampled for invertebrates and calculate the proportion of each *sampled* habitat.

Step 2: Refine taxa database: Exclude undesired taxa (oligochaetes, taxa identified only to order, etc.) and conflate different life stages (i.e., larvae, pupae, adult) for each taxon.

Step 3: Develop sampling information: Calculate the number of samples per habitat per site per season. Create a file with the number of samples per habitat per site per season, e.g., Site A, season 1, 2 samples in scour pool, 1 sample in edge, 4 samples in run; Site A season 2, 1 sample in scour pool, 4 samples in run, etc.). Include the total area sampled per habitat per site per season for per m^2 measure.

Step 4: Calculate average biomass or density per taxa per habitat at each site/season: Sum the total biomass or counts for each taxon per habitat per site per season. Join the samples table and the sum by taxon table and calculate the average dry biomass/m^2 or density per habitat per site per season.

Step 6: Weight samples by proportions of habitats sampled: Multiply average biomass/m^2 or density by sampled habitat proportions per site per season.

Step 7: Characterize the invertebrate assemblages at a site: Sum weighted biomass/m^2 and densities per site per season for each taxon.

Step 8: Organize a sites by taxon file and adjust for number of seasons per year with samples: For all narrow temporal datasets the data is simply formatted using a crosstabs query. For all wide temporal datasets the cross-tabbed file is adjusted for the number of times a site was sampled by dividing all sums of biomass/m^2 and density by the number of times the site was sampled.

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Chapter 3 : Ecological units, concordance, and spatial patterns analyses from the headwaters to the mouth of river tributaries

Abstract

The delineation of Ecological Units (EUs; contiguous river segments with homogeneous biological and environmental features) in rivers may provide an important tool for resource management, especially in numerous and varied headwater tributary systems. However, little independent testing of either the theoretical assumptions or practical performance of extant delineations has been reported. Ecologically distinct and homogenous river segments can only occur where there is strong concordance between biological assemblages and between biological assemblages and environmental variables. This study used a longitudinal, high-spatial-frequency sampling design in a variety of Midwestern stream systems to test these and related assumptions of EUs.

Across and within-system fish/invertebrate concordances were very strong, as were environment/biology concordances. Strong concordance between biological assemblage compositions and measures of stream size suggest weak concordances between fish and invertebrates observed in other studies may result from sampling designs that exclude the stream size gradient. Location in the network influenced patterns of spatial homogeneity, with decreasing rates of change in environmental variables and increased biological similarity moving from headwaters to the mouth. High order/main channels with substantial stream discharge were the only contiguous areas of environmental and biological homogeneity. This suggests a need for better understanding of spatial pattern and processes in headwater streams, the delineation of

many, small EUs in the headwaters, and the need for classification of EUs into ecological "types" following delineation.

Introduction

Lotic systems begin as small, ephemeral rivulets and grow to become comparatively enormous bodies of water near the rivermouth. Although images of the larger channels are most often associated with the word "river," headwaters dominate river systems, sometimes accounting for more than 75% of total channel length (Leopold et al. 1964, Benda et al. 2004a). With the exception of a handful of headwater systems (such as Hubbard Brook in New Hampshire), ecosystem processes and resulting ecological spatial pattern in headwaters are poorly understood (Lowe and Likens 2005, Bishop et al. 2008, McGuire et al. 2014). Bishop et al. (2008) called headwaters the "aqua incognita" after recognizing management agencies tasked with maintaining the ecological health of Swedish streams didn't even know the length of all perennial streams in Sweden, let alone their ecological condition. Lowe and Likens (2005) proposed "moving headwaters to the head of the class" and called for increased study in headwater systems.

Although a standard definition of a "headwater" does not exist, most headwaters are recognized by low stream order, small channel width, and strong coupling with their catchment. Headwaters are also widely recognized for their contribution to biodiversity of river networks (Richardson and Danehy 2007, Meyer et al. 2007, Clarke et al. 2008, Finn et al. 2011). As Matthews (p. 31, 1998) stated, "overall, there are probably more environmental, biological and ichthyological differences among different kinds of 1st order streams than among stream reaches in higher orders." Because of their small size and strong coupling with the landscape, headwater streams are also vulnerable to a variety of threats including groundwater extraction, developed

land use, and channel modification (Elmore et al. 2008, Waco and Taylor 2010, Rasmussen et al. 2013). Such changes can produce local effects and have profound consequences downstream (Alexander et al. 2007, Freeman et al. 2007, Wipfli et al. 2007).

Given the unique character of headwater streams and their potential degradation, it is reasonable to have concerns about how can we efficiently increase our knowledge of headwater systems, describe spatial pattern in headwater systems, and enact appropriate ecological management actions. One tool may be the use of ecological units (EUs). EUs are spatially contiguous areas with relatively homogeneous environmental and biological features. In theory, they are holistic, in the sense that they represent both environmental patterns and the biota's "perception" of the environment. EUs are conceptualized as real, persistent, and map-able places that may be repeated across the landscape (Rowe 1961) and therefore lend themselves to classification. In delineating homogeneous units, emphasis is given to regions of rapid changes in characteristics known to determine patterns of biological composition and functioning of ecosystems. In rivers, it is not unusual to have spatially concurrent change in hydrologic, thermal, geomorphic, and biological character (Poff et al. 1997, Frissell et al. 2001, Jensen et al. 2001, Zorn et al. 2002, Wehrly et al. 2003, Benda et al. 2004a, 2004b, Seelbach et al. 2006, Thorp et al. 2006, Thorp et al. 2008). Therefore, unit boundaries in large rivers are often placed at observable shifts in river energy balance (e.g. observed as changes in sinuosity, slope, and valley form, and at major tributary confluences) while unit boundaries in smaller rivers and tributaries are often placed at tributary junctures, and shifts in land use/land cover (LULC) and surficial geology.

In practical application then, valley segment-scale EUs in headwater tributaries will have two defining properties: 1) Relatively homogeneous biological and environmental composition

and/or patterning; and 2) Areas of rapid change across unit boundaries. For these properties to exist, some significant degree of biological concordance (i.e., concordance between different types of organisms) and concordance between biological structure and the physical environment must be present. Because it is a logical requirement of the existence of ecological units, concordance is a property that should exist independently of any specific unit delineation or classification scheme.

If effective EUs can be delineated in headwater tributary systems, the implications are extensive. EUs would provide real units to both inventory and classify tributary stream segments for practical management, providing a crucial tool to better understand and manage the plethora of headwater streams in river catchments. Homogeneity within an EU and subsequent classification of EUs into types would allow extrapolation of representative samples or models to larger-scale units and to EUs of similar ecological "type". Effective EUs would also provide a way to communicate complex ecological processes and resultant patterns we see in river tributaries (Rowe 1961, Levin 1992). Conservation of river headwaters may also be aided since the mapping and inventory of functional units (cf. valley segments in Seelbach et al. 1997, 2006, Sowa et al. 2007, McKenna et al. 2014; macrohabitats in Higgins et al. 2005, and functional process zones in Thorp et al. 2008) are important steps in conservation planning.

Despite the potential utility of EUs in tributary systems, little *targeted and comprehensive* (i.e., across multiple measures such as habitat, multiple biological assemblages) testing of either the theoretical basis of ecological units or the efficacy of delineated ecological units has been performed. Four studies which have addressed the effectiveness of ecological units have done so for fish (Boys and Thoms 2006, Seelbach et al. 2006), macroinvertebrates (Thompson et al. 2004), or habitat (Warrner et al. 2010). Melles et al. (2014) conclude a review

of stream classification by arguing the testing of proposed classifications is one of the most important steps in the creation of ecosystem classifications. However, such testing of riverine ecological units is limited by the requirement of *comprehensive, longitudinal, high-spatial-frequency empirical* datasets*;* a sampling design not common in existing data sources or in governmental agency data-collecting regimes. Longitudinal sampling in small tributaries is especially rare, as most bioassessment-oriented sampling programs prioritize sampling a variety of tributary systems more than targeted study of particular systems (US Environmental Protection Agency 2006).

Because concordance is a necessary condition for EUs to exist, the conceptual utility of ecological units can be tested with appropriate empirical data. I applied a headwaters-to-mouth, high-spatial-frequency sampling design to five Midwestern tributaries of varying ecological character to test the validity of the underlying assumptions of EUs: 1) fish/benthic invertebrate assemblage composition concordance, 2) environment/biology concordance, and 3) the existence of ecologically distinct, homogeneous river segment units. This sampling design also allows investigation of ecological transition zones and rates of change within a river tributary. I also examined an existing ecological, valley-segment-scale delineation (VSEC version 1.0 with extension into previously unmapped headwater stream segments; Seelbach et al. 1997) in terms of its ability to partition spatial homogeneity in tributary systems.

Methods

Study systems and sites

Five tributary systems were chosen to represent a range of network complexity, drainage density, hydrologic regime, anthropogenic impairment, and biological assemblages. Fifty-seven

study sites were assigned across the five systems with the goal of characterizing each system from its smallest, permanent streams to its mouth (Figure 3.1). Sampling sites were classed by position in the network and by delineated EU. Network position classes include Extreme headwaters ("E"; 1st or 2nd order sites with low-flow discharge <0.1cms and catchment area <25km^2), Creek main channel ("C"; Highest order sites after a large jump in link number), and Headwaters ("H"; all other sites). The H and C classes are similar to those Hitt and Angermeier (2011) used when predicting effects of stream network position on fish community composition and bioassessment. Sites also spanned delineated EUs with the goal of including maximum variability within the delineated EUs. Most sites within a system were spaced 3-4 km apart, but a few sites were less than a km apart and one set was more than six km apart.

Three of the five study systems are in the Muskegon River watershed, a cool- and cold-water tributary of Lake Michigan. Bigelow Creek (BG) is the smallest study system (catchment≈80 km^2) with the fewest sites and delineated EUs. This catchment is dominated by forest and wetland with minimal agricultural (Ag) and urban (Ur) development (5.8% Ag, 1.2% Ur). The main branch is a cold-water trout stream, while an intermittently connected side branch originates from a small warm water lake and flows through a wetland complex before joining the main branch. BG terminates at the Muskegon River <1 km downstream of site BG5. Cedar Creek (CD) is larger (wadeable portion of catchment≈160 km^2) and includes warm-water agricultural headwaters and a cold, groundwater-dominated mainstem. The eastern branch of CD originates in a warm water lake and flows through a large wetland complex. Overall, this system is minimally impacted (14.7% Ag, 3.5% Ur) and becomes non-wadeable downstream of site CD9, and then becomes the north branch of the Muskegon River. Brooks Creek (BR) drains primarily till plain topography (catchment≈160 km^2) and therefore has a dense, highly bifurcated

stream network which includes flashy warm-water agricultural ditches, stable cold-water segments, and lake outflows. Agriculture is widespread in this system (44.8%) and urban development is moderate (4.9%). Within BR there is a large warm-water lake immediately upstream of site BR8 and a small in-line lake upstream of site BR9. BR terminates at the mainstem of the Muskegon River <0.5 km downstream of site BR14.

In contrast, the other two study tributary systems, Mill Creek (ML) in SE Michigan and Crane Creek (CR) in NW Ohio are far removed (Figure 3.1 inset) and comparatively more impaired. ML, a highly dendritic tributary of the Huron River, has a large catchment (≈370 km^2) draining mixed land use/land cover (LULC) and surficial geologies. The northern and western headwaters are largely groundwater sourced while southern headwaters originate as agricultural ditches. This catchment is largely agricultural (48.3%) with a moderate amount of urban development (5.7 %) and extensive channelization. At the time of sampling, a small man made impoundment existed just upstream of ML16, preventing movement of fish between sites ML15 and ML16, strongly affecting physical conditions at ML16, and producing lentic habitat at ML15. ML terminates about 2 km downstream of site ML16 at the Huron River which drains into the western basin of Lake Erie. CR is smaller (riverine portion of catchment≈115 km^2), dominated by agriculture and clay soils and flows into an estuary complex which terminates in Lake Erie (Fig 1). Crane Creek has highly degraded water quality, and is a flow-gradient, flashy, run-off driven highly channelized system. CR's headwaters are largely drainage ditches. The most downstream site (CR13) is strongly affected by estuary and lake seiches, having little discharge except under high flow conditions or during falling levels in western Lake Erie. This catchment is dominated by agriculture (84.1%) and urban land cover (6.3%) and has little riparian cover upstream of site CR7.

Delineated EUs

The delineated EUs used in this study (Figure 3.1) were developed prior to sampling and data analyses (For tributaries in MI: VSEC version 1.0; Seelbach et al. 1997). Their goal was to identify valley segment-scale ecological units with relative homogeneity in hydrologic, geomorphic, limnologic, and water quality characteristics, and in likely biological assemblages. EUs were delineated "from above"; two experienced aquatic ecologists worked together, interpreted map information on catchment and valley characteristics, using their combined knowledge of ecological processes and interactions. Delineations and biological interpretations were reviewed with regional MDNR biologists to ensure consistency with general experience.

EUs were delineated beginning at the mouth of the river and working upstream. Boundaries of units were placed at important stream junctures, slope breaks, changes in river planform, and boundaries of local landforms. Seelbach et al (1997) recognized that very small, 1st order streams were likely ecologically different than adjacent stream segments and terminated EU delineation prior to the mapped end of the smallest stream segments. However, in practical application of EUs, it is common to simply extend these upstream-most EUs to include all mapped stream segments. In the MI study tributaries, three upstream EU boundaries were extended to include three study sites. A similar approach was used to delineate EUs for Crane Creek, with emphasis on stream junctures since LULC, surficial geology, and slope varied little within the catchment.

Environmental data collection and development

I described the environmental character of each study site based on field measurements and network and landscape features derived from GIS maps. At each site I established a sampling reach of length approximately 20x stream width (with a minimum length of 50 meters

and a maximum length of 250 meters). This sampling reach was used for all field measures and biological collections. With the exception of spring chemistry and temperature measures, sampling occurred during typical summer low-flow conditions. GIS-derived characteristics were developed for each site based on field-recorded GPS coordinates at the midpoint of each sampling reach and snapped to the 1:100,000 scale National Hydrography Dataset (NHD) network lines.

Channel characterizations were made using visual habitat and cross-sectional surveys. After thorough inspection of each sampling reach, the percentage of erosional and depositional habitat, specific In-stream Geomorphic Unit types (IGUs; i.e., riffle, run, pool, edge, bar and backwater) and substrate types (i.e., cobble, gravel, sand, claybed, wood, fine and coarse organic matter) within a sampling reach were recorded. Five cross-sections were equally spaced across the sampling reach and described by line transect methods (width, depth, and water velocity). The cross-sections were used to summarize channel shape by averaging or maximizing values resulting in width, depth, maximum depth, depth and width at bankfull, ratio of width to depth at bankfull, cross-sectional area, wetted perimeter, and hydraulic radius measures for each site. Additionally, bankfull shear stress and channel average power (Gordon et al. 2004) were calculated based on field measures.

Hydrologic and chemical character was measured in the summer of 2004 and the spring of 2005 through a combination of field and laboratory techniques. To ensure temporal variation contributed little to differences between measured values, sites within a tributary system were all sampled within a 24-hour period without a precipitation event immediately prior to or during sampling. Stream discharge was measured using the velocity-area method with a pressure-induced Marsh-McBirney flowmeter. Conductivity, dissolved oxygen, pH, and temperature were

measured in the field with handheld meters (YSI-brand) while nutrients (nitrate, ammonia, and soluble reactive phosphorous), and alkalinity were measured in the laboratory following standard HACH procedures.

GIS-derived landscape and network characteristics were developed from the 1:100,000 scale NHD. For each site, network link number was determined. Catchment area, low-flow discharge, bankfull shear, and average power were LN transformed to reduce the influence of extremely large values.

Biotic assemblages

To assess fish assemblage abundance and composition, sites were electrofished during low-flow condition between July and August 2004. Based on a stream's size and accessibility, either a backpack or a towbarge electroshocking unit was used, with the exception of one site, CR13, whose unusual width required a boat-mounted boom electroshocker. At all sites except CR13, we used two-pass depletion with upper and lower block nets; at CR13 we shocked a 100-meter reach without blocknets. All fish were identified to species and were counted, with the exception of some young-of-year minnows and immature native lamprey. Abundances were estimated as follows:

$$\textit{Estimated abundance} = (\#\ \textit{in pass 1})^2/(\#\ \textit{in pass 1} - \#\ \textit{in pass 2}).$$

To account for differences in estimated abundance due to stream size and reach length, abundances were converted to densities (#/m^2) based on shocked areas calculated from reach length and average stream depth.

The standardized, semi-quantitative procedure used to collect macroinvertebrates in this study attempts to detect all taxa within a sampling reach and quantify their relative abundance. This procedure is more comprehensive than most rapid bioassessment protocols, although this

procedure can also be used for bioassessment (Barbour et al. 1995, Park 2007). Invertebrates were collected prior to emergence during the spring of 2005. At each sampling reach, two individuals trained in collection and identification techniques used D-nets, kickscreens, and wood grabs to collect organisms from either erosional or depositional habitats throughout the sampling reach. Usually each individual collected for one hour, rarely less than one hour if 20 minutes of sampling yielded no additional taxa and the reach was fully explored. Organisms were identified to family or genus while in the field and relative abundance was recorded on a scale of 1 (extremely rare) to 5 (dominant) for each taxon for each habitat. Field identifications were verified, or corrected as necessary, with voucher specimens examined in the laboratory. Erosional and depositional habitat subsamples were combined, and relative abundances were weighted according to the proportional abundance of erosional and depositional habitat across the sampling reach. The resulting composite relative abundance score was our best estimate of relative abundance of the taxon across the entire sampling reach.

Data analyses

My analyses had four primary objectives: 1) To measure the degree of concordance between fish and invertebrate assemblages in tributary systems; 2) to measure the degree of concordance between biological and environmental variables in tributary systems; 3) to identify patterns of biological and environmental change in tributaries from their headwaters to mouths, and 4) to assess the homogeneity and boundary assumptions of generalized and delineated ecological units. Analyses at the within- and across-system spatial extents provide both system-specific results and more generalized results. All analyses were performed with PC-ORD version 6.08 (McCune & Mefford 2011).

For concordance analyses, I used simple Mantel tests to investigate the degree of concordance between occurrence and abundance measures of the fish and macroinvertebrate assemblages, concordance between fish and invertebrate assemblage composition, and concordance between biological and environmental spatial variability. A simple Mantel test is extremely flexible in utility and is used to test the null hypothesis of no relationship between two square symmetric matrices. It is an alternative to regressing one matrix against the other, avoiding the problem of partial dependence within each matrix. The standardized Mantel test statistic (r) ranges from -1 to 1, with 1 indicating perfect positive congruence between the two matrices. For all Mantel tests, the significance of r was assessed with a Monte Carlo randomization method using the maximum number of possible data permutations or a maximum of 3000 permutations. In concordance analyses a large positive r indicates strong agreement between distance matrices for assemblage measures, fish and invertebrate assemblages, or biological and environmental variables.

For all biological variables I calculated Sorenson similarity between sites within each stream system and across all sites and systems. This measure of similarity is desirable since it ignores joint absences, a combination that dominates many assemblage matrices (Faith et al. 1987) and gives less weight to rare taxa (McCune and Grace 2002). I used the square root transform on fish abundance to allow influence, but not dominance of, extremely abundant fish taxa (Zar 2010). I excluded extremely rare taxa (only one individual total or only a few individuals at one site) and YOY minnows and lamprey that were not identified to species, resulting in 141 macroinvertebrate taxa (out of 168) and 53 fish species (out of 65) used in the analyses.

Analyses based on similarity matrices required fish to be present at all sites, but at the three headwater-most sites in Crane Creek (Figure 1) fish were completely absent from the sampling reach. Sampling occurred during extremely hot and dry conditions, and I believe fish that would normally be at these sites moved downstream to escape toxic chemical and thermal conditions. Therefore I added one Fathead Minnow, (*Pimephales promelas*), to the data matrix for the three fishless sites in Crane Creek; Fathead minnow was the most abundant fish in Crane Creek and present downstream of the fishless sites. These three sites were excluded if the analysis permitted.

I also used simple Mantel tests to assess concordance between biological and environmental dissimilarity matrices. Euclidean distance was used to measure differences between environmental character. Some distance matrices were calculated based on a single variable (e.g., catchment area, low-flow discharge, link, shear, and power) while others were composite measures calculated on multiple variables (e.g., nine measures of channel shape, proportions of seven substrate classes, proportions of six IGU types, concentrations of three nutrient measures across seasons, alkalinity and conductivity across seasons, and water temperature across seasons) Because of the difference in units, Alkalinity and conductivity measures were Z-score normalized prior to distance matrix calculation.

I used Non-metric Multidimensional Scaling (NMDS) to illustrate concordance and patterns of spatial homogeneity for fish and invertebrate assemblages both across- and within-study systems. NMDS is an ordination method based on ranked distances and it is well-suited to non-normal data with many zero values (Minchin 1987). A successful NMDS produces a low-dimension ordination where the distances between pairs of sites are in rank-order agreement with their dissimilarities in species composition. The distance between sites in NMDS plots can be

directly interpreted, for example, sites closer together have more similar assemblages. Each NMDS was run in the autopilot mode "medium" setting, (a balance of speed and thoroughness), with a maximum of 500 runs with random starting seed and a stability criterion of 0.00001. In most runs a 2D solution was suggested although rarely a 3D or 4D solution was suggested. However, after careful examination of the 3D+ solutions, the additional axis explained little additional variance and interpretation of the results was the same regardless of the additional dimensionality. For ease and consistency of viewing, the NMDS plots include the two axes that explained the most variation and are oriented similarly for fish and invertebrates. Figure 3.2 demonstrates how NMDS plots can be used to assess homogeneity and boundary assumptions of EUs under hypothetical situations of valid and null EUs.

I used Multi-response Permutation Procedures (MRPP) and simple Mantel tests to test the ability of network position classes to partition differences in biological assemblages. MRPP is akin to ANOVA in that it is designed to assess whether there is greater difference within predetermined groups or among predetermined groups, but it is a data-dependent permutation procedure based on pairwise distance measures. It is ideally suited to ecological data because it makes few assumptions about the distributional structure of the data (Zimmerman et al. 1985) and can be used on multivariate arrays. The null distribution of the test statistic, chance-corrected within-group agreement (A), is based on the collection of all possible permutations of sites into groups of specified size. A is maximized at 1 when sites within predefined groups are identical, and A is 0 when the within-group heterogeneity exceeds that expected by chance (McCune and Grace 2002). Because MRPP requires replicates within a group, it could not be used to analyze location class in Bigelow Creek; Instead, I used a simple Mantel test to test the relationship between similarity matrices and a design matrix, a matrix with ones for comparisons of sites

within the same location class and zeros for sites in different location classes. In these analyses, r is large if sites within the same location class are associated with higher similarity than those in different location classes.

Trajectory analyses and NMDS plots explored biological and environmental transitions in the study systems. These analyses allowed assessment of transitions independent of EUs and assessment of transitions across delineated EU boundaries. For each study system I calculated the average Sorensen assemblage dissimilarity per km separation for fish and invertebrates from neighboring sites. To assess differences in rates of change from the headwaters to the river mouth, I created trajectory plots by plotting cumulative dissimilarity in fish and invertebrate assemblages for each headwater to mouth flow route against distance from the downstream-most site. In these plots, a steep slope indicates rapid change in the biological assemblages.

Boundaries between delineated EUs are illustrated with arrows in the NMDS plots (Figures 3.3, 3.4). Within the five systems, there are a total of 14 proposed ecological boundaries, one in Bigelow, two in Cedar, four in Brooks, four in Mill, and three in Crane. These proposed boundaries were examined individually and compiled for an overall assessment of boundary performance. The similarity of each biotic assemblage across each boundary was calculated and compared to the similarity of the fish assemblage of the upstream site and its upstream neighbor in the same EU. For example, in Cedar Creek there is a proposed boundary between sites 5 and 9. The similarity of the fish assemblage between sites 5 and 9 was compared to the similarity of the fish assemblage at sites 4 and 5. If the proposed boundaries are realized in the data, the similarity of fish assemblages should be lower across the boundary than between upstream sites. This hypothesis was tested using a one-sided Sign test, whose null hypothesis is that there should be equal numbers of differences in each direction.

I used two sample t-tests to evaluate the ability of delineated EUs to partition sites into biologically and environmentally similar groups. For the t-test I grouped biological and environmental similarities into three categories: 1) Same, where the similarity is a comparison of sites in the same delineated EU; 2) Adjacent, where the similarity is a comparison of sites in adjacent but different EUs; and 3) Neither, where the similarity is a comparison of sites that are not in the same or adjacent EUs. If EUs are effective, we would expect higher average similarity for sites within the same unit than for comparisons with sites in adjacent units. Analyses do not include the neither category since there is no directional prediction of similarity in this category; similarities could be high between sites if species pools and environmental conditions are the same or low if species pools differ and/or environmental conditions vary. These expectations for the "neither" comparisons also preclude the use of more rigorous statistical tests (e.g. MRPP) tests comparing within and between group variances. Either the standard t-test or Welch's t-test for unequal variance was used (Zar 2010). Significance of t was determined for degrees of freedom limited to the number of sites (not the number of similarities) included in the analyses minus two.

Results

Differences between tributary systems

The five study systems differed considerably in physical, chemical, and biological character, achieving my intention of including a spectrum of systems in this study. The systems can be usefully arrayed along a nutrient/water source gradient (Table 3.1). At one extreme, Bigelow and Cedar Creeks were largely cold, nutrient-poor, mineral-rich systems (Table 3.1) with low fish taxa richness and intolerant fish and invertebrate species (with the exception of site

BG3, Table 3.2). In contrast, Crane Creek was highly impacted by agricultural practices within the watershed. Crane Creek experienced large fluctuations in temperature, dissolved oxygen, and flow, and consistently experienced extremely high nutrient concentrations (Table 3.1). The biotic assemblage in Crane Creek was largely pollution-tolerant fish and invertebrate taxa (Table 3.2). The environmental and biological character of Brooks and Mill Creeks fell between these other tributary systems.

Fish and invertebrate assemblages generally differed between study systems and at sites within study systems, although within-system variability was typically lower than between-system variability, especially when extreme outliers were excluded (Table 3.2 and Figures 3.3a, 3.4a). Fish assemblages in Bigelow and Cedar Creek were similar to each other, but different from those in Brooks and Mill Creeks, which were also similar to each other. The fish assemblage in Crane Creek was distinct from the other four systems. Two extreme differences in fish assemblages within a stream system were highlighted by the outlying sites BG3 and ML10 (Figure 3.3a). Both sites had extremely small basins resulting in little to no flow except in storm events, small standing pools, and limited physical connectivity to downstream sections. As compared with fish, most study systems had more distinct invertebrate assemblages. Brooks Creek was the exception with invertebrate assemblages similar to sites in other study systems, although the typical assemblage (indicated by the centroid of the distribution of sites) was unique to Brooks Creek (Figure 3.4a). Again BG3 and ML10 were outliers for their respective stream systems, although the invertebrate assemblage at ML10 was not as extreme as the fish assemblage (Figures 3.3a & 3.4a).

Occurrence and abundance measure concordance

There was very strong and statistically significant concordance between occurrence and abundance measures at across- and within-system spatial extents (Table 3.3 section a). This concordance implies similar patterns of faunal transition and homogeneity using either measure of the assemblage. For simplicity, NMDS and biological/environmental concordance analyses presented in subsequent sections of this study are based on the occurrence measure of the assemblage.

Fish and invertebrate concordance

There was very strong and statistically significant concordance between fish and invertebrate assemblages at across- and within-system spatial extents (Table 3.3 section b). By including sites from all study systems, faunal differences across sites within the same system *and* faunal differences between systems contribute to strong across-system concordance ($r > 0.6$). Within-system concordances varied (Table 3.3 section b, r ranged from 0.40 to 0.93), but were generally quite strong and statistically significant for all study systems. This concordance implies similar patterns of faunal transition and patterned biological homogeneity, supporting the first underlying assumption required in ecological unit delineation. Likewise, the similar configuration of sites in corresponding fish and invertebrate NMDS plots (e.g., Figures 3.3a and 3.4a, 3.3b and 3.4b, etc.) implies strong concordance of fish and invertebrate assemblages at the across- and within-system spatial extents. As with Mantel tests (Table 3.3 section b), NMDS interpretations were similar whether analyses used occurrence or abundance assemblage measures.

Environment/Biology concordance

As required by the second EU assumption, there were many strong concordances between environmental variables and biological assemblage composition. However, the strength varied with environmental variable, study system, and spatial extent of analysis (Table 3.4). Because of strong concordance of fish and invertebrate assemblages, spatial patterns of both assemblages were usually associated with patterns of the same environmental variables. At the across-system extent, temperature, water chemistry, substrate, IGUs, channel shape, link, low-flow discharge and catchment area were strongly associated with biological assemblages. These associations reflect across- and within-system differences in size, network typology, hydrologic regime, surficial geology, and LULC. With the exception of Crane Creek, spatial pattern in bankfull shear and average power were rarely associated with patterns in biological assemblages at the across- or within-system spatial extent.

At the within-tributary system spatial extent, repeated environment/biology concordances suggest common associations regardless of tributary system and a few associations specific to particular systems (Table 3.4). With a few exceptions, there was strong concordance between biological assemblages and the environmental variables temperature, nutrient chemistry, habitat, channel shape, and link number. In all systems except Crane Creek, which has a largely homogeneous catchment, concordance involving nutrient chemistry was much stronger than concordance for the alkalinity and conductivity composite variable. In Bigelow Creek, low-flow discharge, link, and temperature were very strongly concordant with the biological assemblage, although this may primarily reflect differences between the wetland-influenced site BG3 from the other four sites. There was especially strong concordance between biological assemblages and catchment area and channel shape in the three drainage-dense systems, Brooks, Mill and Crane Creeks. Cedar Creek was unusual in that, especially for fish, it had fewer strong

environmental/biological concordances than the other four systems. In contrast, Crane Creek was unusual in that there were significant concordances between biological assemblage composition and all non-habitat environmental variables except fish and substrate.

Distinct, homogeneous EUs

Distinct and fairly homogenous biological assemblages were only observed downstream in the network along the creek mainstem; these river segments are indicated in the NMDS plots by distinct, clumped blue letters (Figures 3.3 & 3.4 b-f). In Mill Creek, the tributary with the largest catchment and highest number or links, some high-discharge, but headwater-classified sites also had similar fish assemblages to sites in the downstream-most channel section (Figure 3.3e and likely represent errors in the a priori network position classification). Two exceptions to the rule of homogeneity in downstream/main channel sections are estuary-influenced site 13 in Crane Creek (Figures 3.3f & 3.4f, upper blue C) and the big river confluence site 9 in Cedar Creek (Figures 3.3c & 3.4c green C). Both of these sites had divergent fish and invertebrate assemblages. Assemblage similarity was quite high in downstream/mainstem channel sections, as high as 91% for fish and 78% for invertebrates, and averaging about 55-60% within the creek main channel (C) class sites (Table 3.5).

In contrast, many extreme headwaters (E) and headwater (H) sites not on the tributary mainstem had biological assemblages that differed from those at spatially-contiguous sites. Instead, position in the network appeared to be as important a factor in partitioning biological similarity as longitudinal proximity. Extreme headwater sites on different network branches (as indicated by different color markers) often shared ordination space and headwater sites on different network branches often shared ordination space, but the ordination space for E and H class sites rarely overlapped (Figures 3.3 & 3.4 b-f). Although the EHC location classes did

partition more biological heterogeneity than was expected by chance, within group variability was generally large and between group variability was generally small (Table 3.5). The small, but significant test statistics may result from differing variability within site classes. The variability of assemblages at sites within a stream system generally decreased as location in the network transitioned from E to H to C (i.e., transitioned downstream; Table 3.5).

Extreme headwater sites consistently had comparatively unusual biological assemblages as indicated by their location on the edge of NMDS ordination space and frequent separation from longitudinally-adjacent sites (Figures 3.3 & 3.4 b-f). The uniqueness of fish assemblages at extreme headwater sites usually resulted from a paucity of taxa, rather than the presence of unique species. The average percent increase in fish taxa richness between an E site to the next downstream non-E site (i.e., [Closest downstream H or C Richness - E Richness]/Closest H or C Richness*100) varied by system (BG:56%, CD:0% , BR:48%, ML:47%, CR: 88%). Only three E sites had fish unique to the site within their respective systems, (CD1: *Cyprinus carpio* (Common Carp); CD6: *Semotilus atromaculatus* (Creek Chub) and *Notropis cornutus* (Common Shiner); and ML7: *Etheostoma flabellare* (Fantail Darter).

The reasons for divergent extreme headwaters are not as obvious for invertebrates, as they are for fish. Although taxa richness tended to be lower at extreme headwaters, this was not always the case in Mill and Crane Creeks, and average percent increases in taxa richness were generally much smaller than for fish (BG:15%, CD:17%, BR:26%, ML:2%, CR:9%). Of the 15 E sites, six did not have any unique invertebrate taxa, six had one or two unique taxa representing less than 10% of the taxa at the site, and one had four unique taxa representing 11% of the taxa at the site. The two remaining extreme headwater sites had considerably more unique species. Site CD1 had 7 unique invertebrate taxa representing 30% of the taxa at the site and site

BG3 had 15 unique invertebrate taxa representing 44% of the taxa richness at the site. At both sites, the invertebrate assemblage included taxa commonly associated with marsh and lentic systems rather than lotic systems (e.g., *Hyallela azteca*, *Notonecta*, and *Peltodytes*).

My observation of strong fish/invertebrate concordance and differences in rates of change along the network are supported by trajectory plots (Figure 3.5a-e). Average rates of biological change (i.e., average dissimilarity/km) were quite consistent across study systems (Fish: 0.11 to 0.20 and Invert: 0.14 to 0.25; Figure 3.5). The slightly higher values for Brooks Creek result exclusively from the unusual proximity of sites BR3 and BR7 and BR6 and BR7. Excluding these transitions, Brooks Creek actually had the lowest average dissimilarity per km of all the systems (i.e., Fish: 0.09 and Invert: 0.11). The average rate of change for invertebrates was slightly higher than for fish in all systems except Crane Creek, although these increases could be an artifact of 3-4 fold increase in the number of invertebrate taxa as compared to fish taxa included in the analyses. Strong concordance between fish and invertebrates within a system is evident in similar pattern in slopes of the corresponding fish and invertebrate trajectories (Figure 3.5 a-e). The highest rates of change in biological assemblages were in a route's headwaters or on trajectories where a small stream joined a much larger channel.

Performance of delineated EUs

NMDS plots (Figures 3.3 & 3.4 b-f) and t-tests (Table 3.6) suggest the performance of the delineated EUs lay somewhere between the two hypothesized extreme situations illustrated in Figure 3.2; delineated EUs varied between and within systems in their ability to identify distinct, biologically and environmentally homogeneous river segments. As summary measures of EU performance within a system, average biological similarity within delineated EUs was usually higher than the average similarity of comparisons of sites in adjacent EUs (Table 3.6 section a).

The delineated EUs performed slightly better for fish than for invertebrates, and with the exception of Brooks Creek, differences in average similarities between occurrence measures were larger than differences between abundance measures.

While t-tests provide statistical and system-wide assessments of delineated EUs, NMDS ordinations suggest the success of delineated EUs in partitioning homogeneous river segments depends on the location of the EU in the network. NMDS ordination plots indicated only mainstem/downstream-most EUs had fairly homogeneous biological assemblages (as indicated by distinct groups of blue letters in Figures 3.3 & 3.4 b-f). In most systems, these sites were distinct from adjacent headwaters. The biological assemblages in EUs containing E and H classed sites were not homogeneous and sites within these EUs were often more similar to sites from different EUs (as indicated by scattering of same colored sites in Figures 3.3 & 3.4 b-f).

The delineated EUs successfully partitioned large differences in most environmental variables measuring aspects of stream size (e.g., link, catchment area, low-flow discharge, and channel shape; Table 3.6 section b). Performance varied by system for water chemistry, temperature, and average power. Delineated EUs never or rarely partitioned distinct, homogeneous river segments for the environmental variables bankfull shear, average power, substrate, and IGUs.

Overall, boundaries between delineated EUs did not correspond with strong transitions in biological assemblages and only corresponded with transitions in physical characters measuring some measures of stream size. Sign Test analyses based on the compiled 14 unit boundary transitions from all systems suggest moderate rather than abrupt faunal transitions for both assemblage measures at delineated boundaries (sign test probabilities of observed number of successes, i.e., Similarity across EU boundary < Similarity between upstream sites: fish

occurrence and abundance p=0.2, invert occurrence p=0.4, and invert abundance p=0.6). However strong individual discontinuities in biological assemblages are evident in NMDS plots and in specific site to site comparisons. Strong biological discontinuities did correspond to boundaries between delineated EUs for transitions BG3 to BG4, CD5 to CD 9, BR 12 to BR13, ML11 to ML12, and CR10 to CR11. All of these transitions correspond to large differences in stream size (e.g., large increases in catchment area, link, flow, and channel width) in the system.

In contrast to biological community transitions, boundaries between delineated EUs were particularly effective at capturing some environmental discontinuities (i.e., Similarity across EU boundary < Similarity between upstream sites). These include link number (13 of 14 transitions, p=<0.001), channel shape and low-flow discharge (12 of 14 transitions, p=<0.01), and the composite alkalinity and conductivity chemistry measure (11 out of 14 transitions, p<0.05). Boundaries between EUs did not represent strong transitions in power, bankfull shear, substrate, habitat, temperature, and nutrient chemistry.

Discussion

Biological concordance

The biological concordances in this study, the degree to which patterns in biological assemblage compositions are similar, were high and perhaps surprisingly so. Both across- and within-tributary systems, faunal transitions in fish and macroinvertebrate assemblages coincided, as did areas of faunal continuity. Other analyses from large spatial extents indicate moderate to strong fish-invertebrate concordances (defined as r>0.5 for Mantel tests as reviewed in Heino 2010) are common. An exception is Larsen et al. (2012) which found weak concordance between fish and invertebrates across 13 wadeable basins. Smaller within-basin studies have even more

ambiguous results, with many reporting no or weak concordance. This study joins two recent exceptions (Grenouillet et al. 2008 and Dolph et al. 2011) in finding significant concordance at both large and smaller scales.

Strong fish/invertebrate assemblage concordance could arise from several mechanisms: 1) response of assemblages to the same environmental gradients; 2) response to correlated but different gradients; 3) substantial biological interactions between the assemblages; and/or 4) similar limitations in dispersal or reproductive capabilities of the assemblages (adapted from Gaston and Williams 1996). Because of its purely observational design, this study cannot directly address whether biological interactions between fish and macroinvertebrate assemblages could create concordant spatial patterns; however, many strong environmental and biological concordances suggest environmental gradients may largely control the organization of biological assemblages in these tributary systems.

In this study, it is likely that some portion of the across-system concordance results from spatial and hydrologic isolation of the five stream systems. However, large differences in the natural character (e.g. flow and temperature regime) and degree of anthropogenic impact in the stream systems necessarily lead to relatively distinct environmental and chemical environments in each system, and these differences likely account for much of the across-basin concordance. In Michigan, it is well established that biological communities respond to strong gradients in size and hydrologic regime (Zorn et al. 2002), surficial geology (Johnson et al. 1997, McRae et al. 2004), temperature (Wehrly et al. 2003, 2006), and anthropogenic stressors (Riseng et al. 2010). Stream fish and macroinvertebrate assemblages clearly respond similarly to many natural and human-driven landscape patterns (Paavola et al. 2006, Johnson et al. 2007, Yates and Bailey 2010), creating strong biological concordance at large spatial scales. One across-system study

which restricted streams to similar depth, velocity and width found weak concordance between fish and macroinvertebrates (Larsen et al. 2012).

Within-tributary system fish/invertebrate concordances observed in this study are unusually large, even compared to other studies documenting strong within-system concordance. I believe concordance was especially strong because assemblage composition was responding to the same principal longitudinal gradient in stream systems: the necessary increase in channel hydraulic geometries (e.g., catchment area, discharge, velocity, depth, and width; Leopold and Maddock 1953) as one travels downstream (Vannote et al. 1980, Ward 1998). Given the context of this study, this longitudinal gradient and probable discontinuities within tributary systems were specifically targeted using a high-spatial frequency, longitudinal sampling design. Two recent studies that show strong within-system concordance also either sampled longitudinally (Grenouillet et al. 2008) or included a range of stream sizes (Dolph et al. 2011). Although longitudinal hydraulic gradients are an inherent feature of every river system, their influence on stream assemblages can only be detected if the scale of analyses is large enough (Wilkenson and Edds 2001), and if a sampling regime adequately targets the size gradient (as in this study and Dolph et al. 2011).

In contrast, studies showing little or no concordance within a river basin (Paavola 2003, Paavola et al. 2006; Infante et al. 2009), have typically used randomly selected locations or restricted sites to a narrow size range (e.g., streams of the same order, the same width, or only small headwaters). Differing study contexts and corresponding differences in sampling design may preclude incorporating, and thus recognizing, a strong size-related hydraulic gradient to which both assemblages respond.

It has been suggested that degree of human impact influences within-basin concordance. Paavola et al. (2006) suggest that stronger concordances between fish and macroinvertebrates are expected when sites are "variously modified by human activities." However, concordances in the present study were consistently strong, regardless of the degree of human impact within a system's basin. In fact, the highest concordances were in the least-impacted systems, and the lowest concordances were in the system with the widest variety of impact. However, it is alternately possible that this trend is simply an effect of the varying sample sizes among the basins. This suggests a need for more research to understand how sampling design, spatial intensity, and LULC variation affect measures of biological concordance.

Patterns of spatial homogeneity

Location in the network had a strong influence on patterns of spatial homogeneity, with decreasing rates of change and variability of biological assemblages as one moves from the headwaters to the mouth. Despite high-spatial-frequency sampling, I was not able to identify contiguous, distinct environmentally and biologically homogenous river lengths within river headwaters. However, if the spatial pattern in river headwaters is a patchy gradient like the rest of the river system, then headwater EUs would exist, although they would necessarily be many and short. Ascertaining whether spatial pattern in headwaters is best described by a gradient or a patchy gradient requires even higher-spatial-frequency sampling than I used in this study; it would require multiple sites within stream segments and bracketing of network junctures (as in Sparks-Jackson 2000), or continuous sampling (as in Torgersen et al. 2006). These two studies suggest tributary junctures are associated with biological discontinuities.

The observed patterns in spatial heterogeneity should be expected because of the physical structure of a river. Empirical "laws" predicting declines in the number of streams segments of a

given order and increasing stream segment length as stream order increases (Horton 1945, Strahler 1952) suggest that, the very structure of the network will help shape biological heterogeneity in rivers, a suggestion borne out in recent modeling studies (e.g., Padgham and Webb 2010, Neeson et al. 2012, Webb and Padgham 2013). In the headwaters, rapid accumulation of discharge through increases in catchment size and frequent junctures of low-order streams create rapid change in physical character over short distances. In contrast, downstream in the network, many junctures between small and large channels occur with little distinguishable effect on the character of the large channel (Benda et al. 2004a, 2004b; Kiffney et al. 2006). Although the joining of a small stream to a large stream may have minimal effect on the physical or biological character of the large stream, such junctures do create sharp boundaries between tributary and mainstem EUs. Similarly, sharp biological transitions observed in this study corresponded to abrupt changes in size, flow, and temperature.

Because rivers are aggregating systems, downstream reaches of river networks are also defined and confined by the character of upstream reaches. While upstream sections of rivers can theoretically exhibit an infinite variety of biological and environmental conditions as shaped by an infinite variety of possible catchments, the character of a downstream reach is limited by upstream contributions. For example, in Michigan, differences in catchment LULC and surficial geology can produce both cold, stable, nutrient-poor groundwater-sourced stream segments and flashy, nutrient-rich, runoff-sourced stream segments within the headwaters of a tributary system, but downstream segments necessarily reflect a homogenization of upstream influences.

Extreme headwaters

Extreme headwater sites had extremely varied biological and environmental character. Meyer et al. (2007) suggest that because headwater streams are characterized by small

catchments that are easily influenced by small-scale differences in local condition, they are the most varied of all running-water habitats. As would be expected for most low order streams in the Midwest, the headwaters within and between systems in this study varied greatly in catchment and riparian land cover, hydrologic and temperature regimes, degree of channelization, slope, and chemical load regime (Beugly and Pyron 2010, Holmes et al. 2011). For example, headwaters in Crane Creek were open, channelized agricultural ditches while headwaters in Brooks Creek were often well-shaded and non-channelized. This variety in headwater stream "types" within and between study systems contrasts with considerable similarity of headwater streams in the Pacific Northwest (e.g., consistently characterized as steep, forested, and often fishless; Gomi et al. 2002, Richardson and Danehy 2007).

Much of the recent discussion around the biology of headwater streams concerns their limited contribution to within-assemblage diversity (α), but potentially large contribution to among-assemblage diversity (β) and regional diversity (γ) (Heino et al. 2003; Meyer et al. 2007; Clarke et al. 2008; Finn et al. 2011). However, headwater sites in these tributary systems rarely contributed ecologically meaningful within-system β diversity; instead, headwater biological assemblages was mainly subsets of taxa found downstream and usually distinguished by a paucity of taxa (low α diversity). As compared with other regions, the recent glaciation of the Great Lakes (approximately 12,000 years ago, Bay 1938) and the young geological age of streams may also contribute to the lack speciation in the headwaters.

All of the headwater sites in this study were permanent streams although in Brooks, Mill and Crane Creeks these sites could have no flow or be reduced to a series of pools during low flow conditions. Reductions in macroinvertebrate diversity (Mackie et al. 2012) and fish diversity (Beugly and Pyron 2010) are associated with stream intermittency, and taxa found in

intermittent streams were a subset of those found in perennial reaches. Although lower fish and invertebrate diversity in the headwaters can be partially attributed to small catchment area (Horowitz 1978, Matthews 1998), harsh conditions in the extreme headwaters may also contribute to low diversity. Although shading effects of forested headwater streams in the temperate Pacific Northwest create thermal stability (Richardson and Danehy 2007), headwater streams in the Midwest are often agricultural ditches with no shading resulting in large diel temperature and dissolved oxygen swings. Such harsh conditions likely contributed to the absence of fish in three extreme headwater sites in Crane Creek.

Performance of delineated EUs

Delineated EUs successfully defined distinct, environmentally homogeneous stream segments based on several measures of stream size, but these same units did not contain sites with similar substrates or habitats. In all study systems, only sites within the most downstream EU or along the main channel EU were of distinct and similar biological character. The defined EUs in low-order sections of the tributaries often contained sites with disparate biological character. Because sampling sites were specifically chosen to span the entire length of proposed EUs, and thus quantify the maximum variability within an EU, this is not entirely unexpected.

This failure to delineate biologically homogenous ecological units in low-order streams suggests a more detailed delineation of headwater ecological units may be warranted. The high rates of change in biological assemblages in these stream headwaters suggest that delineated ecological units in these areas need to be broken into more and smaller EUs. Consistently observed discontinuities in biological assemblages in this study suggest utility in adding additional ecological unit boundaries between extreme headwaters (defined here as 1-2 order and catchment of <25 km^2 with little to no low-flow discharge) and adjacent headwaters.

Although the division of headwater streams into many, small ecological units more closely reflects ecological heterogeneity, verifying appropriate boundaries many require even more intense sampling than in this study. The required intensity of sampling is extremely rare in most sampling regimes. Continuous sampling has been used to quantify overall fish abundance (Duncan and Kubecka 1996), single-species abundance (Kanno et al. 2012), and multi-species abundance (Torgersen et al. 2006). However, this type of sampling is impractical in species-rich Midwestern streams where access is often limited to road crossings by impenetrable riparian vegetation.

Developers of the original EU delineation for rivers in Michigan (Seelbach et al. 1997; Wiley, personal communication) terminated EU delineations prior to the end of mapped stream lines in 1st order stream. Although it is common practice to simply extend VSEC version 1.0 EUs into all currently mapped stream segments, the results of this study question the utility of such extensions. A second ecological unit delineation for MI was developed using an improved hydrography data (1:100,000 NHD) and a computer-driven, automated process that grouped stream arcs into EUs based on environmental features (Brenden et al. 2008a, 2008b). Although this delineation successfully divided the headwaters into many, small EUs it also divided tributary mainstems and river mainstems into many more ecological units than is supported by the results of this and the previous study (Chapter 2). This problem suggests that the rules governing ecological unit delineation may need to differ with location in a river network. Acknowledging such limitations of the EU delineation, MIDNR personnel combined many delineated units, greatly reducing the number of delineated EUs to reflect their own knowledge of biological spatial pattern (Paul Seelbach, personal communication 4/2014).

The results of this study also suggest the need for nested, hierarchical classification of EUs after their delineation. Each study system had a distinct biological assemblage, and within a study system, location (i.e., E, H and C) had a strong effect on the biological similarity of sites. Although this study suggests the need for many, small EUs in the headwaters of stream tributaries, scores of headwater EUs present challenges for sampling, modeling, and assessment if EUs are not subsequently classed by type.

Ecological management implications

Recurring strong biological/biological and environmental/biological concordance in a variety of tributaries provide empirical support for the theoretical existence of EUs in Midwestern tributary systems. However, tributary EUs should be scaled to river network geometries, and thus the length of EUs should change with position in the network. If EUs in river tributaries can be delineated to accurately reflect the environmental and biological homogeneity in river tributaries, these EUs should aid in river ecosystem management. EUs are real units that can be used to describe the holistic character of river segments, should guide modeling efforts and sampling regimes, and certainly can provide a foundation on which ecological classifications can be built. These capabilities would be particularity useful because of the abundance and considerable variety of stream headwaters.

Table 3.1: Study systems differed in chemical and physical characteristics. For all variables except link, values in table are average (minimum, maximum) of all sites within designated system. For link, values are median (minimum, maximum).

	Bigelow	Cedar	Brooks	Mill	Crane
Spring Chemistry					
Alkalinity (mg/L $CaCO^3$)	124 (97,128)	131 (103, 196)	158 (103, 222)	164 (117, 213)	156 (136, 178)
Conductivity (µS/cm^3)	324 (299, 344)	375 (300, 644)	488 (375, 550)	674 (441, 213)	824 (714, 977)
Nitrate (µg/L)	100 (30, 180)	210 (30, 630)	530 (10, 1810)	310 (10, 1910)	940 (480, 1490)
SRP (µg/L)	20 (0, 40)	40 (10, 130)	30 (0, 80)	30 (10, 70)	50 (10, 110)
Link	3 (1,5)	6 (2,11)	2 (1,18)	8 (1,31)	2 (1,6)
Catchment Area (km^2)	36 (18,73)	52 (16,143)	48 (8,158)	147 (9,368)	41 (3,142)
Q (cms) at low flow	0.40 (<0.01, 0.81)	0.53 (0.04, 1.53)	0.31 (0.02, 1.07)	0.19 (<0.01, 0.53)	0.07 (0.01, 0.20)
Summer Temp (°C)	18.9 (16.7, 24.4)	19.4 (15.2, 24.1)	18.8 (16.3, 24.6)	15.9 (12.5, 21.2)	22.8 (19.6, 27.6)
Habitat/Substrate					
Erosional habitat (% site)	77 (35, 97)	73 (40, 85)	79 (60, 94)	75 (0, 90)	66 (20, 90)
Cobble or gravel (% site)	20 (0, 59)	5 (0, 31)	6 (0, 31)	28 (0, 82)	21 (0, 60)
Wood (% site)	9 (0, 15)	11 (2, 19)	4 (0, 16)	6 (0, 15)	4 (0, 12)
Channel shape					
Bankfull Width/Depth	13.3 (7.5, 17.8)	16.2 (7, 32.3)	7.6 (5.1, 11.4)	10.5 (5.9, 19.1)	8.6 (4.3, 22)
Hydraulic Radius (m)	0.25 (0.15, 0.36)	0.30 (0.19, 0.42)	0.20 (0.07, 0.39)	0.24 (0.04, 0.46)	0.16 (0.03, 0.50)

Table 3.2: Study systems differed in fish and invertebrate assemblages. Site richness is presented as average (min, max). Taxa are listed in order from most abundant, including the five most abundant fish and eight most abundant invertebrate taxa (excluding Chironomidae which were very common in all systems).

	Bigelow	Cedar	Brooks	Mill	Crane
Fish Assemblage					
Richness (system)	20	24	31	31	23
Richness (per site)	6.8 (4, 11)	8.0 (6, 13)	11.1 (2, 19)	12.7 (5, 24)	6.4 (0, 18)
Most common taxa	Mottled Sculpin (*C. bairdii*)	Mottled Sculpin (*C. bairdii*)	Blacknose Dace (*R. atratulus*)	Fathead Minnow (*P. promelas*)	Bluntnose Minnow (*P. notatus*)
	Blacknose Dace (*R. atratulus*)	Creek Chub (*S. atromaculatus*)	Cental Mudminow (*U. limi*)	Brook Stickleback (*C. inconstans*)	Creek Chub (*S. atromaculatus*)
	Rainbow Trout (*O. mykiss*)	Cent. Mudminnow (*U. limi*)	Creek Chub (*S. atromaculatus*)	Johnny Darter (*E. nigrum*)	Fathead Minnow (*P. promelas*)
	Pumpkinseed (*L. gibbosus*)	Johnny Darter (*E. nigrum*)	Mottled Sculpin (*C. bairdii*)	Creek Chub (*S. atromaculatus*)	Common Shiner (*N. cornutus*)
	Brown Trout (S. trutta)	Brook Trout (*S. fontinalis*)	Brook Stickleback (*C. inconstans*)	Mottled Sculpin (*C. bairdii*)	Green Sunfish (*L. cyanellus*)
Invert Assemblage					
Richness (system)	78	84	92	97	55
Richness (per site)	33.2 (27, 41)	27.2 (18, 32)	27.1 (17, 36)	29.1 (16, 44)	18.2 (10, 27)
Most common taxa	*Gammarus*	*Gammarus*	*Gammarus*	Pisidiidae	Caecidotea
	Baetis	*Baetis*	*Pycnopsyche*	*Stenonema*	Pisidiidae
	Pisidiidae	*Ceratopsyche*	Physidae	*Pycnopsyche*	Oligochaeta
	Stenonema	*Anabolia*	Pisidiidae	*Ceratopsyche*	Hirudinea
	Ephemerella	*Stenonema*	*Baetis*	Oligochaeta	*Ischnura*
	Pycnopsyche	Oligochaeta	*Sigara*	*Chrysops*	*Peltodytes*
	Ceratopsyche	Physidae	Caecidotea	*Calopteryx*	*Sigara*
	Amphinemura	*Simulium*	*Simulium*	*Corbicula*	*Stenacron*

Table 3.3: Biological measure and assemblage composition concordance: Significant Mantel tests with large test statistics indicate strong concordance between a) occurrence (Occur) and abundance (Abun) measures of biological assemblage or between b) fish and invertebrate (Invert) biological assemblages.

Variables or Study System	All	Bigelow	Cedar	Brooks	Mill	Crane
a) Measure Concordance						
Fish Occur/Abun	0.62****	0.71	0.73****	0.51****	0.74****	0.47***
Invert Occur/Abun	0.90****	0.98***	0.85****	0.88****	0.87****	0.87****
b) Fish/Invert Concordance						
Fish/Invert Occur	0.61****	0.85*	0.56***	0.61****	0.40**	0.53***
Fish/Invert Abun	0.62****	0.93*	0.67****	0.57****	0.58***	0.49***

*p<0.10 **p<0.05, ***p<0.01, ****p<0.001

Table 3.4: Environment/Biology concordance: Significant Mantel tests with large test statistics indicate strong concordance between fish or invertebrate assemblages and environmental characteristics. Fish and invertebrate distance matrices based on occurrence measure. Fishless sites excluded from Crane fish-based analyses. NS=No significant association between biological occurrence and environmental characteristic distance matrices.

Variables or System	All	Bigelow	Cedar	Brooks	Mill	Crane
Size/Geomorphic						
Fish/CatchArea	0.15****	NS	NS	0.42***	0.51****	0.42**
Invert/CatchArea	0.17****	NS	NS	0.27**	0.41****	0.50****
Fish/Qlowflow	0.24****	0.92!	NS	0.68****	0.42***	0.33**
Invert/Qlowflow	0.21***	0.87*	NS	0.48***	NS	0.52****
Fish/Link	0.13***	0.82!	NS	0.45***	0.42****	0.45**
Invert/Link	0.16***	0.82*	0.41***	0.23**	0.37****	0.49****
Fish/BFShear	NS	NS	0.24**	NS	NS	0.22*
Invert/BFShear	NS	NS	NS	0.29*	NS	0.27**
Fish/AvePower	NS	NS	NS	NS	NS	0.53***
Invert/AvePower	NS	NS	NS	0.14*	NS	0.38***
Fish/Chan Shape	0.30****	0.56!	NS	0.64****	0.41***	0.40****
Invert/Chan Shape	0.23****	0.64*	NS	0.44****	0.28**	0.46***
Habitat						
Fish/Substrate	0.34****	NS	NS	NS	0.40**	NS
Invert/Substrate	0.50****	NS	0.47**	NS	0.32**	0.44***
Fish/Habitat	0.16**	NS	0.28*	0.31**	0.66****	0.39**
Invert/Habitat	0.31****	NS	0.62***	NS	0.40**	0.34***
Chem/Temp						
Fish/Nutrients	0.21****	0.56!	0.37**	0.32*	0.53****	NS
Invert/Nutrients	0.30****	0.32	0.52***	0.41**	0.30**	NS
Fish/AlkCond	0.44****	NS	NS	NS	0.32**	NS
Invert/AlkCond	0.41****	NS	NS	NS	NS	NS
Fish/Temp	0.41****	0.91*	0.34**	0.36**	0.35**	0.25**
Invert/Temp	0.42****	0.95**	0.44***	0.22*	0.24*	0.31**

*p<0.10 **p<0.05, ***p<0.01, ****p<0.001; !Likely not significant because of low power of test due to small number of sites.

Table 3.5: Analyses of network position classes: Statistical tests show some ability of network position classes (E=Extreme headwater, H=Headwater, C=Creek main channel) to partition biological variability. If the test statistic (r or A) is large or significant, sites within a class are more similar to each other and distinct from sites in different classes. The average similarity within a group is a rough measure of variability within a group. With the exception of Bigelow and Crane Creeks, group similarity increases as location moves down the stream network.

Variable or System	Bigelow	Cedar	Brooks	Mill	Crane	Ave.
Fish Occurrence	r=0.37[1]	A=0.07	A=0.18****	A=0.14	A=0.28**[3]	
E ave similarity	N/A[2]	N/A[2]	0.50	0.36	0.40	0.42
H ave similarity	0.60	0.50	0.57	0.65	0.39	0.54
C ave similarity	0.50	0.55	0.72	0.77	0.61	0.63
Fish Abundance	r=0.37[1]	A=0.12*	A=0.15****	A=0.10***	A=0.14**[3]	
E ave similarity	N/A[2]	N/A[2]	0.39	0.26	0.23	0.29
H ave similarity	0.45	0.33	0.41	0.52	0.36	0.41
C ave similarity	0.63	0.53	0.60	0.56	0.33	0.53
Invert Occurrence	r=0.43[1]	A=0.09**	A=0.10****	A=0.07****	A=0.12**	
E ave similarity	N/A[2]	N/A[2]	0.47	0.45	0.60	0.51
H ave similarity	0.62	0.41	0.52	0.53	0.58	0.53
C ave similarity	0.54	0.62	0.69	0.50	0.48	0.57
Invert Abundance	r=0.38[1]	A=0.05*	A=0.10***	A=0.07****	A=0.10**	
E ave similarity	N/A[2]	N/A[2]	0.39	0.40	0.52	0.44
H ave similarity	0.60	0.41	0.50	0.45	0.61	0.51
C ave similarity	0.50	0.60	0.70	0.49	0.44	0.55

p<0.10 **p<0.05, ***p<0.01, ****p<0.001; [1]Since MRPP requires one group to have 3 or more members I substituted the MRPP analyses with a Mantel test with distance matrix with within-class comparisons coded 0 and between-class comparisons coded 1. [2]Excluded from analysis: only one site in the class. [3]Test statistic is artificially inflated because analysis required addition of one Fathead Minnow to three E class fishless sites.

Table 3.6: Two-sample t-tests indicate sites within the same Ecological Unit (EU) had, on average, more similar biological assemblages as compared to sites in adjacent EUs. The same is true for many environmental characteristics, although the significant environmental characteristics varied with study system. Table values are average Bray-Curtis similarity for biological measures and average normalized Euclidean similarity for environmental measures. Statistical comparisons compare the average similarity within EUs vs. average similarity of comparisons between sites in adjacent EUs. Significance of differences between means was determined with degrees of freedom limited to the number of included sites minus two. Fishless sites in Crane Creek are excluded from fish analyses. NS=No significant difference between the means.

Variable or System	Bigelow	Cedar	Brooks	Mill	Crane
a) Biological:					
Fish Occurrence	0.46 vs. 0.04***	0.58 vs. 0.46**	0.59 vs. 0.51*	0.65 vs. 0.57*	0.66 vs. 0.21****
Fish Abundance	0.46 vs. 0.01***	NS	0.51 vs. 0.31***	NS	0.45 vs. 0.19***
Invert Occurrence	0.56 vs. 0.34**	0.56 vs. 0.46**	0.54 vs. 0.49*	0.52 vs. 0.45***	0.57 vs. 0.44***
Invert Abundance	NS	NS	0.54 vs. 0.45**	0.47 vs. 0.42**	0.54 vs. 0.42***
b) Environmental:					
Catchment Area	NS	0.67 vs. 0.35**	0.78 vs. 0.52****	0.80 vs. 0.63***	0.83 vs. 0.53****
Qlowflow	0.9 vs. 6.7****	NS	0.79 vs. 0.64**	0.89 vs. 0.63****	0.74 vs. 0.52***
Link	0.79 vs. 0.16***	0.80 vs. 0.41**	0.81 vs. 0.50****	0.79 vs. 0.57***	0.84 vs. 0.22****
Bankfull Shear	NS	NS	NS	NS	NS
Ave Power	NS	NS	NS	NS	0.76 vs. 0.49****
Channel Shape	0.56 vs. 0.27**	0.54 vs. 0.29**	0.71 vs. 0.61*	0.78 vs. 0.65***	0.76 vs. 0.62***
Substrate	NS	NS	NS	NS	NS
Habitat	NS	NS	NS	NS	NS
Nutrients	0.52 vs. 0.25*	NS	0.67 vs. 0.58*	NS	NS
Alka/Cond	NS	NS	0.74 vs. 0.67**	0.84 vs.0.64****	0.81 vs. 0.66**
Temperature	0.79 vs. 0.13***	NS	0.67 vs. 0.59*	NS	0.73 vs. 0.58**

*p<0.10 **p<0.05, ***p<0.01, ****p<0.001

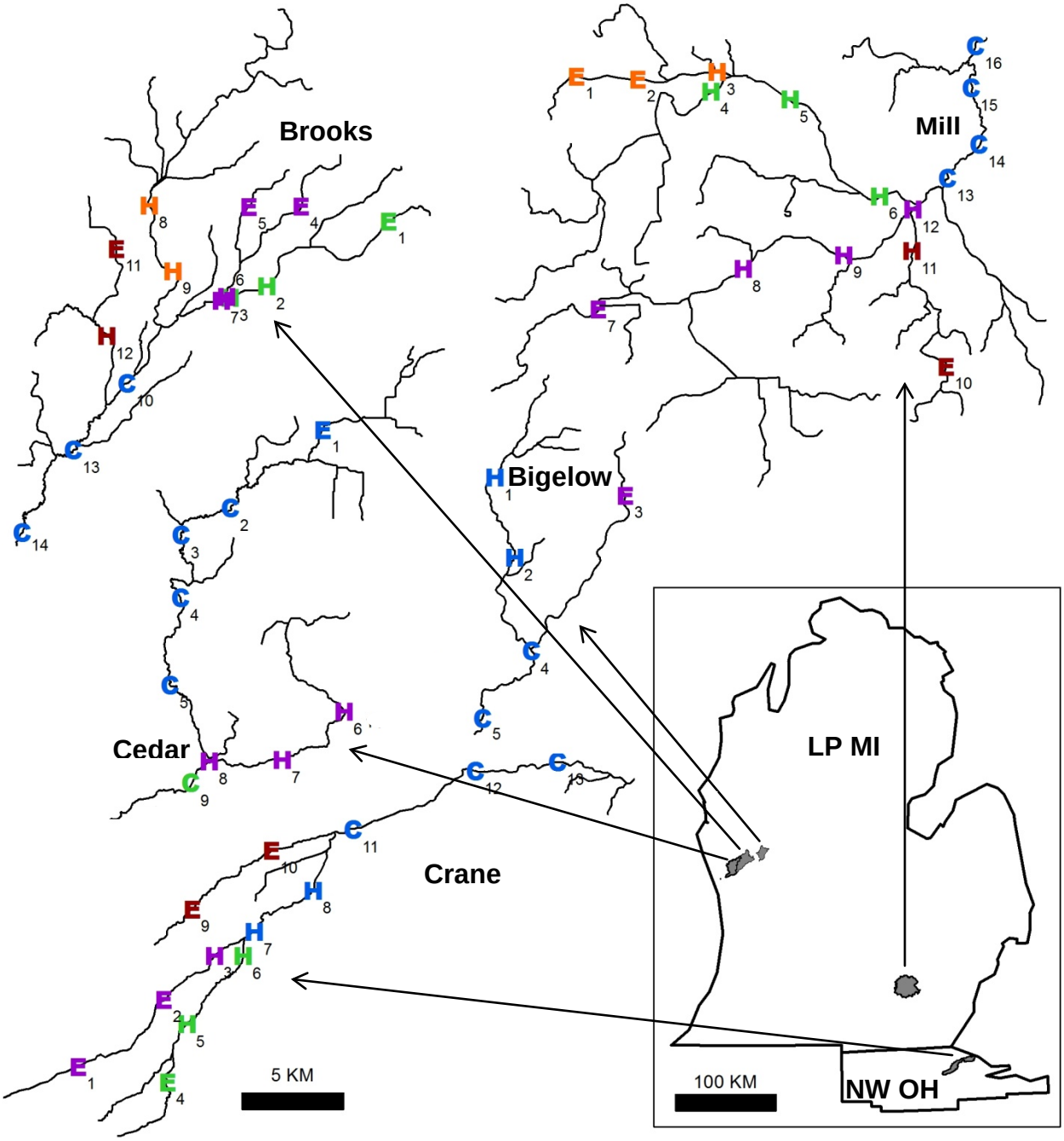
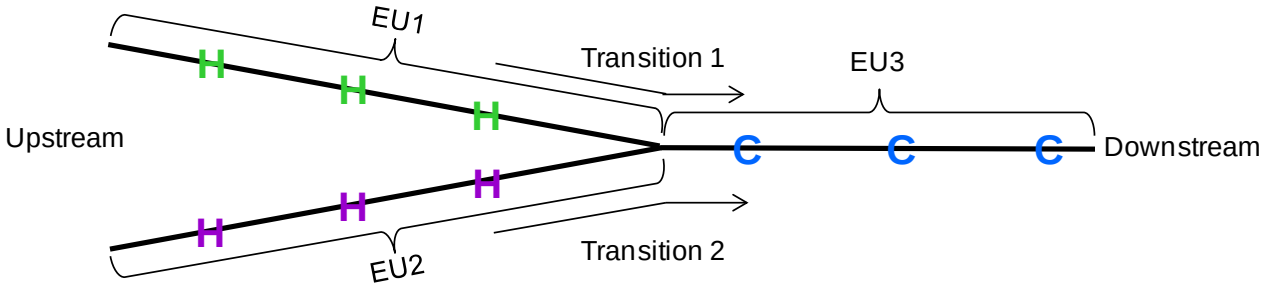
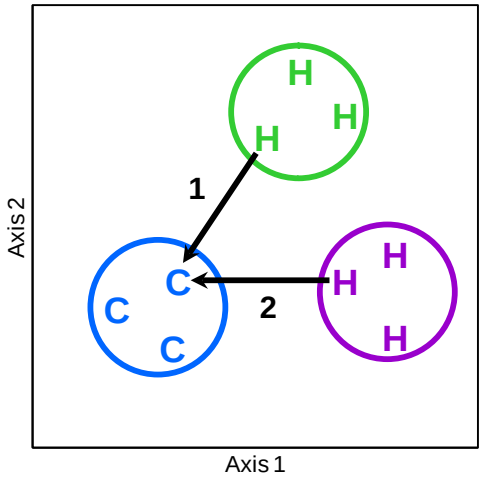


Figure 3.1: Study systems and sites: Four of the five study systems are in Michigan's Lower Peninsula; one is in northwestern Ohio. For simplicity, not all 1st order streams are illustrated. Sampled locations (sites) are shown by letter markers indicating location in the network (E=Extreme headwater, H= Headwater, and C=Creek main channel; see text for specific definitions). Sites within the same proposed ecological unit are indicated by the same color. The repetition of markers across systems does not indicate sites from different systems are in the same ecological unit (EU). The site markers in this figure are used consistently throughout the manuscript. Within each system, sites are numbered consecutively from the most distant headwater site moving downstream.

a) Hypothetical stream system, sampling sites, and delineated ecological units (EUs).



b) Situation 1: NMDS ordination



c) Situation 2: NMDS ordination

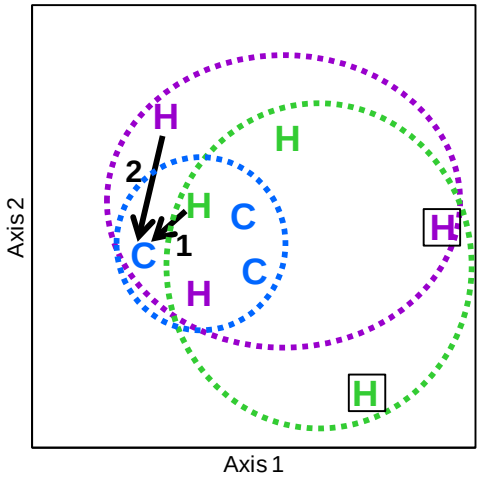


Figure 3.2: Ecological Units and Non-metric Multidimensional Scaling (NMDS) ordinations. Overview: Demonstration of a hypothetical stream system with delineated ecological units (EUs), two biotic assemblage spatial patterns, and interpretation of corresponding NMDS ordinations. Proximity between sites in NMDS ordinations equates to assemblage similarity; closer sites have more similar assemblages than distant sites. If the underlying assumption of biological/biological concordance is met, ordinations for both fish and invertebrates will have a similar configuration of sites. If the concordance assumption is met, these demonstration plots can guide interpretation of NMDS plots for four additional factors relevant to my research questions: 1) Within unit assemblage variability, 2) within unit assemblage variability compared to between unit assemblage variability, 3) outliers, and 4) transitions between adjacent EUs. Details: a) Simple stream system with three delineated EUs (the three stream segments), nine sampling sites, (letters indicate location class, headwater (H) and creek mainstem (C), and colors indicate EU membership), and two transitions between EUs. b) In situation 1 the delineated EUs are valid since sites within an EU have similar assemblages that are distinct from the assemblages in other EUs. Although not an assumption for valid EUs, the variability of assemblages is the same for all units and none of the sites have particularly unusual assemblages. c) In situation 2 the delineated EUs are null since sites within an EU are arrayed across ordination space regardless of EU assignment (i.e. no homogeneity within and EU and sites in different EUs do not have distinct assemblages). In contrast to situation 1, within EU variability differs between EUs (less variable assemblage in EU3) and two sites have unusual assemblages as compared with other sites along the mainstem (indicated by the outline squares). Transitions 1 and 2 are “typical” changes in assemblage for the system.

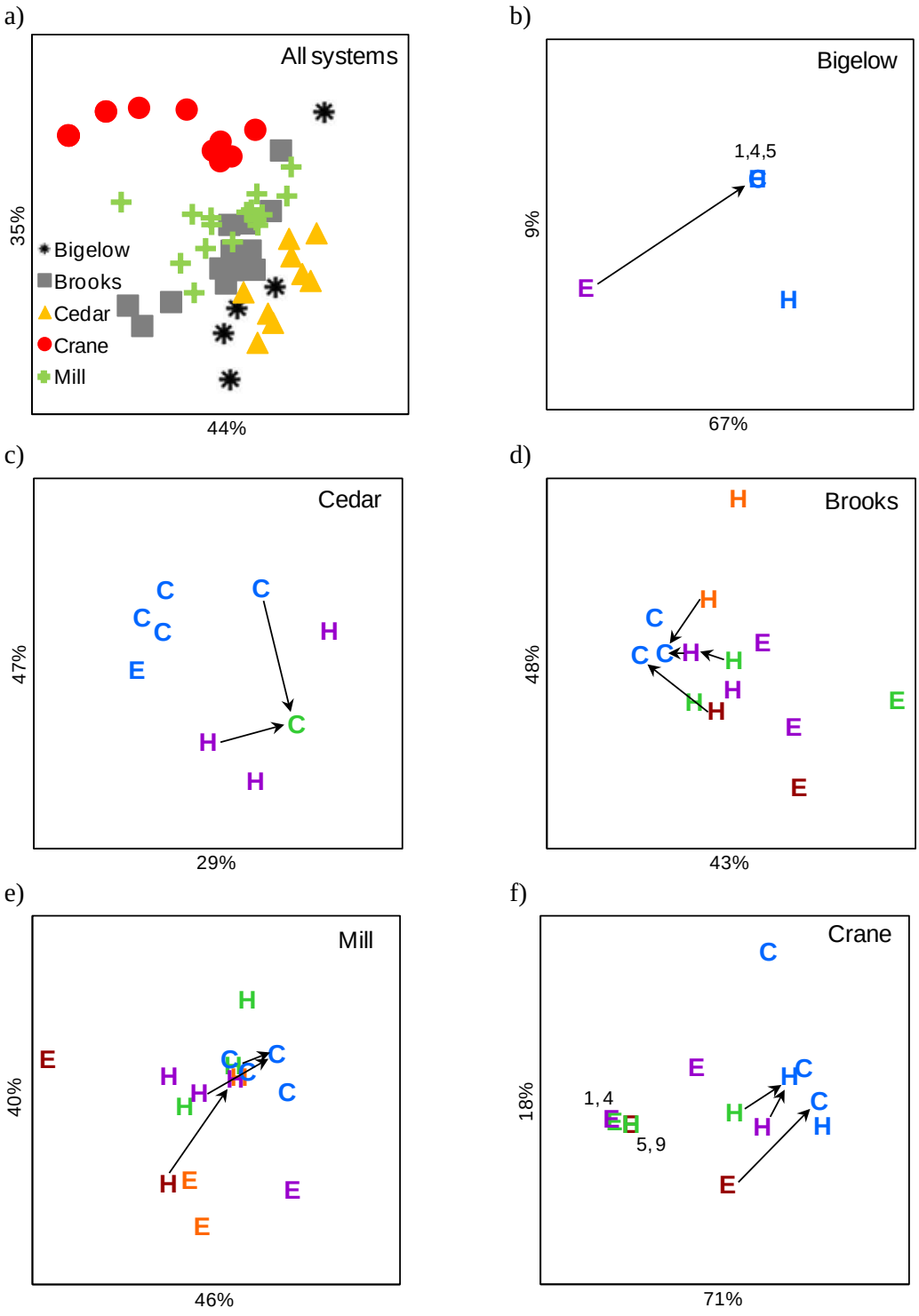


Figure 3.3: NMDS ordinations (with % variability explained by each axis) for fish assemblages for sites across systems (a) and within each study system (b-f). Fish occurrence is used in all plots except for Crane Creek where abundance is used. Symbols in system-specific analyses are those in Figure 1. Letters denote network location class and colors denote shared EU. Numbers indicate sites with the same NMDS coordinates. Arrows illustrate transitions between delineated EUs (one transition in Mill is not shown because the distance is tiny).

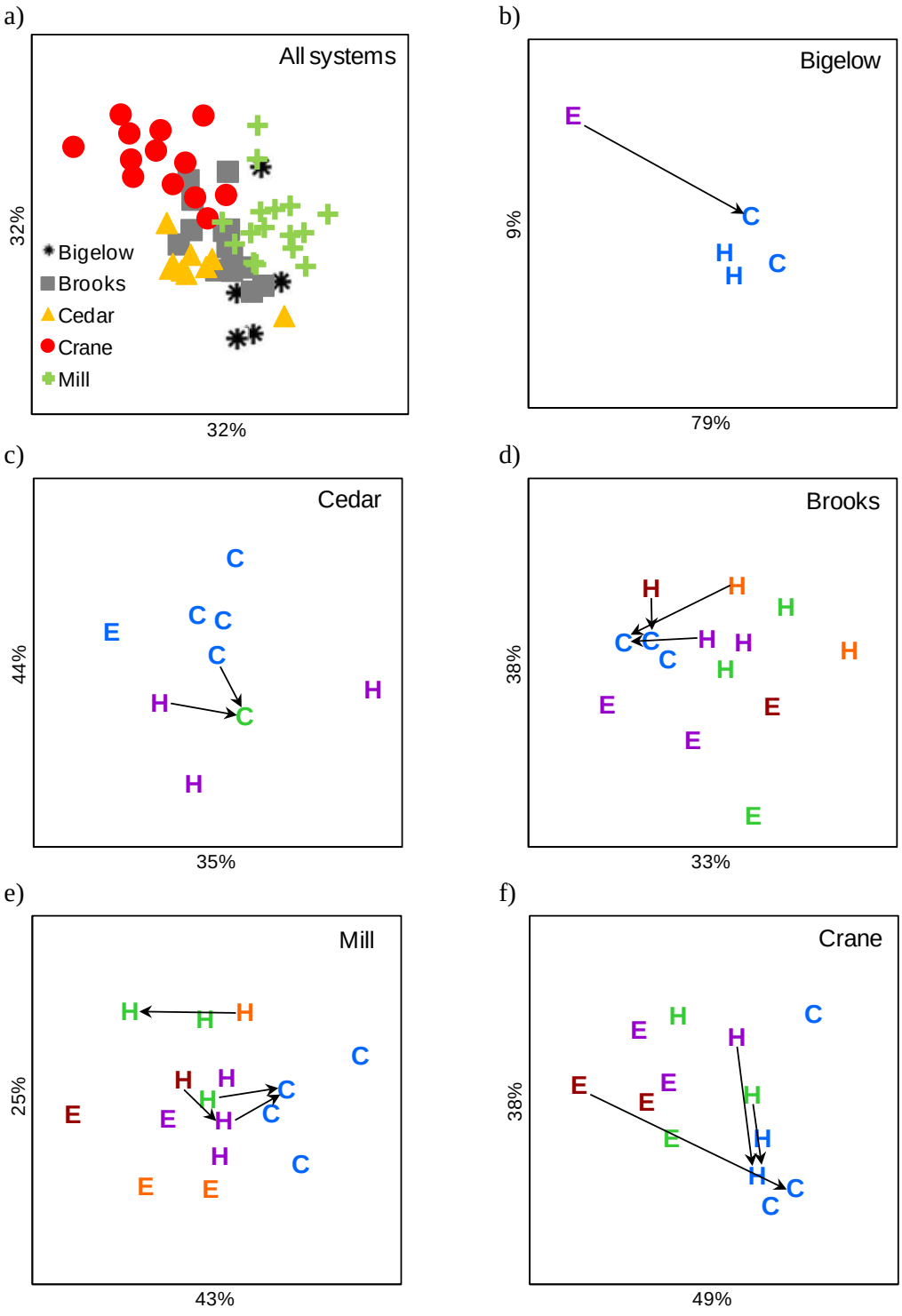


Figure 3.4: NMDS ordinations (with % variability explained by each axis) for invertebrate assemblages (occurrence measure) for sites across systems (a) and within each study system (b-f). Symbols in system-specific analyses are those in Figure 1. Letters denote network location class and colors denote shared EU. Arrows illustrate transitions between delineated EUs.

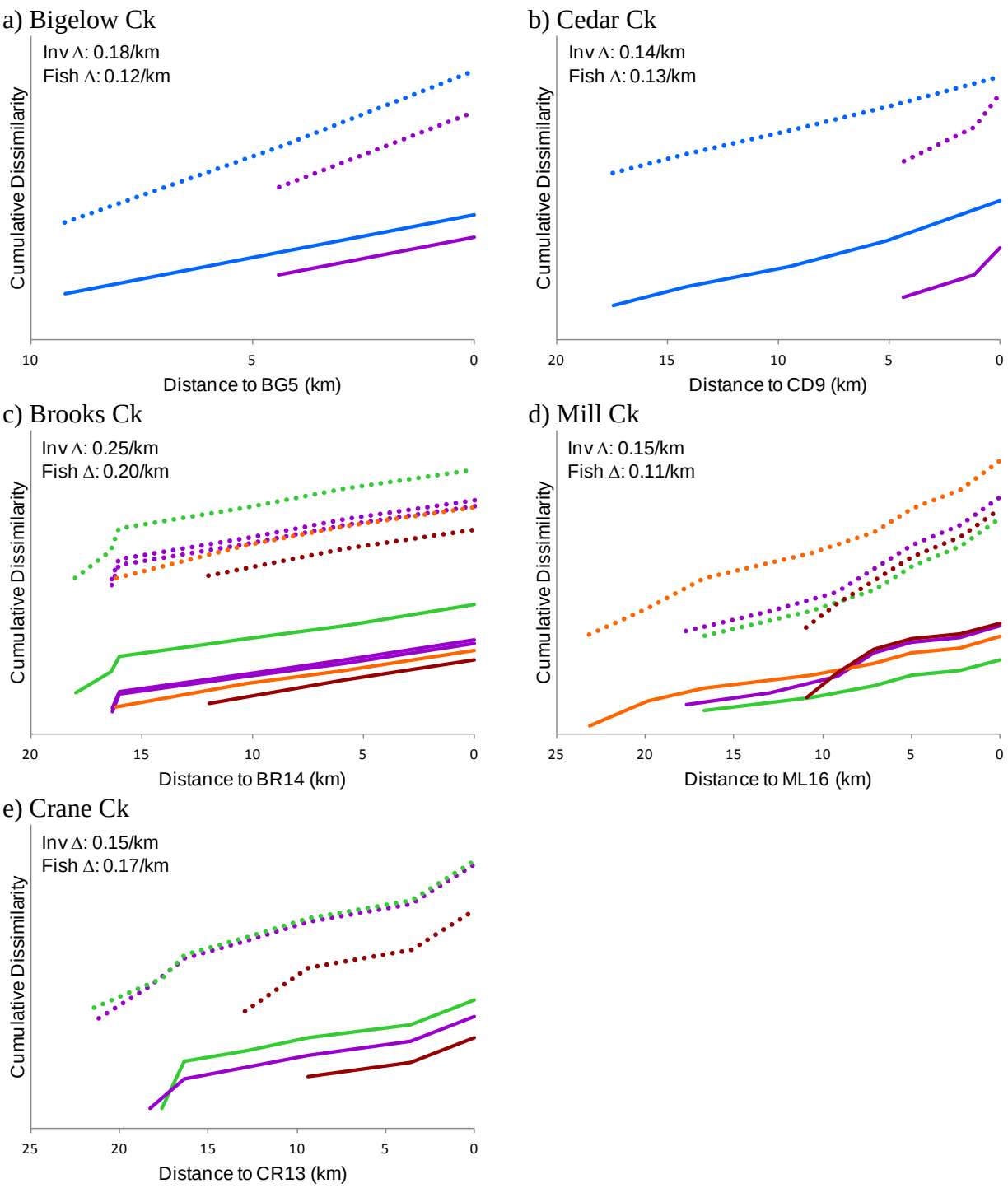


Figure 3.5: Headwaters to mouth trajectory analyses for each study system (a-e): Slope at any location on a line indicates rate of change in biological assemblage occurrence (e.g., steep slope indicates rapid change in taxa) and is summarized by Δ (average biological dissimilarity per km) for neighboring sites in each system. Distance is network distance. The color of each line corresponds to the EU of the headwater-most site and specifies trajectory route. Solid lines are for fish and dotted lines are for invertebrates (vertically offset for easier interpretation). Fishless sites in Crane Creek are excluded.

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Chapter 4 : Rates of change and spatial dependence in river ecosystems

Abstract

The particular spatial pattern of a river system has both ecological and statistical implications. Positive spatial autocorrelation (SAC) and distance decay are common in river ecosystems because rivers are characterized by patchy, longitudinal gradients in physical, chemical, and biological attributes. Despite the ubiquity of longitudinal ecological patterning in rivers, studies that measure within basin SAC for multiple ecological measures are surprisingly rare. Nor have studies that have explored SAC in riverine biology composition led to any consensus about the origin of said SAC. In this study I use a within basin, high-spatial-frequency, longitudinal sampling regime to explore spatial patterning in a set of seven Midwestern river ecosystems. The channel systems explored include five headwater tributaries, and a lower mainstem river mouth system, considered both with and without its confluent tributaries. I measured SAC and characterized distance decay rates in each of seven systems, and examined the degree of concordance between physical environments and biological assemblages. Using path analyses, I assessed how spatial separation directly and indirectly (through environment) affects biological assemblage composition, and the implications of these results for neutral and niche theory explanations of the source of SAC.

Rates of change in most environmental variables and fish assemblage composition decreased downstream, suggesting the assumption of stationarity in many common spatial statistics is violated. I observed strong SAC in many environmental variables and in both fish and invertebrate assemblage composition. Strong environment/biology assemblage interactions

accounted for most or all of the SAC in biological assemblages, and much of the association between proximity and biological assemblage similarity is mediated through similarity in environment. This offers strong support for niche processes as the primary origin of SAC in these river systems. This also suggests that river ecologists may be able to avoid lack of independence statistical problems caused by SAC by developing appropriate non-spatial models.

Introduction

The rapid development of landscape perspectives in ecology (Risser et al. 1984, Forman and Godron 1986, Wiens 1992, Turner 2005) has fostered the explicit study of spatial structure and spatio-temporal interactions of ecological processes (Liebhold et al. 1993, Liebhold and Gurevitch 2002, Wagner and Fortin 2005). Legendre (1993) has argued "spatial heterogeneity is … *functional* in ecosystems, and not the result of some random, noise-generating process, so it becomes important to study it for its own sake." Positive spatial autocorrelation (SAC) and distance decay are common spatial patterns in ecosystems (Legendre 1993, Koenig 1999, Nekola and White 1999). SAC can be broadly defined as a statistical property in which closer locations are typically more similar than locations further apart (Legendre 1993). As a result, measured similarity of samples decreases as geographic distance increases, a distance decay relationship that can be explicitly described by mathematical equations and/or as rates of change in similarity (Nekola and White 1999, Soininen et al. 2007).

Strong, SAC is expected in systems organized as gradients or patchy mosaics. Rivers are often described as both gradients (i.e., longitudinal changes in chemical, physical, and biological assemblages; Huet 1959, Illies and Botosaneanu 1963, Vannote et al. 1980; Statzner and Borchardt 1994), and as longitudinally arrayed patches, i.e., patchy gradients (Poole 2002, Thorp et al. 2006, 2008). Rivers are accumulating, advective network systems, moving water and

material largely in one direction (downstream), and flux rates are strongly influenced by both network typology (Benda et al. 2004a, 2004b) and characteristics of the catchment landscape (Horton 1945, Hynes 1970). It is not surprising, therefore, that SAC is a commonly observed property of samples in studies of riverine water chemistry (Jager et al. 1990, Peterson et al. 2006, Isaak et al. 2014, McGuire et al. 2014), benthic invertebrates (Parsons et al. 2003, Lloyd et al. 2006, Mac Nally et al. 2006, Marshall et al. 2006, Mykrä et al.2007, Maloney and Munguia 2011, Bonada et al. 2012, Heino et al. 2012), and fish (Wilkinson and Edds 2001, Magalhães et al. 2002, Grenouillet et al. 2004, 2008, Stewart-Koster et al. 2007, Maloney and Munguia 2011).

The origin of SAC in ecological systems determines what is appropriate with regard to ecological interpretation and statistical treatment (Figure 4.1). For example, ecologists acknowledge two mechanistic hypotheses that can produce positive SAC in biological composition data: 1) *environmental control*, where species distributions are caused by niches and species sorting rules and spatially-structured environmental conditions (Whittaker 1956, Hutchinson 1957, and supported by many authors) and 2) *neutral control*, where random mortality and dispersal limitations create SAC in biological assemblages (Bell 2001, Hubbell 2001, He 2005). Although these hypotheses are commonly presented as competing either-or hypotheses (as will done in this introduction for illustrative purposes), SAC in an ecological system can be created through the actions of both mechanisms (Cottenie 2005). Understanding the *relative influence* of the two mechanisms on SAC, therefore, is likely more important than simply providing evidence for or against each hypothesis.

The environmental and neutral control hypotheses lead to different expectations for the strength of relationships between geographic distance, environmental variables, and biological assemblages (see Figure 4.1). Appreciation of the relative influence of these sources of SAC has

important consequences for understanding the functioning of ecosystems, for the conservation of biodiversity, and for ecosystem management (Legendre et al. 2005). For example, if environmental control is the dominant mechanism creating SAC in biological assemblage composition, all locations within an ecosystem are not equal and a mosaic of connected habitats is likely important in maintaining diversity. In contrast, if dispersal limitation is the dominant mechanism creating SAC in biological assemblage composition, maintaining high rates of dispersal and connectivity is of utmost importance in maintaining diversity.

An understanding of the relative importance of environmental and neutral control sourced SAC in ecological systems is also required to appropriately handle statistical issues including pseudoreplication and overly liberal hypothesis tests when SAC is present (Legendre 1993; Figure 4.1). For example, if a spatially-structured environment controls community composition, *and sufficient environmental variables are included in statistical models*, many statistical and geostatistical tools developed to remedy spatial autocorrelation (e.g., Rossi et al. 1992, Perry et al. 2002) are unnecessary. In fact, removing the spatial structure of such an ecosystem prior to association with environmental variables could mask relationships with the most important environmental variables and stress only secondary relationships (i.e., only relationships that exist once the dominant gradient of SAC is removed). However, if spatial autocorrelation is present, but not accounted for, traditional statistical tests are too liberal, type-1 errors likely occur more frequently than the specified α-level, and analysis of ecological patterns may produce misleading results (Lennon 2000, Lichstein et al. 2002, Keitt et al. 2002).

To clarify the use of the term SAC and these ecological and statistical distinctions, ecologists well-versed in statistics have encouraged limiting the use of the term “spatial autocorrelation” to spatial patterns that arise from dispersal limitation and using “spatial

dependence" to describe spatial autocorrelation that arises from dependence on spatially-autocorrelated environmental variables (Legendre et al. 2005, Tuomist and Ruokolainen 2006). In reality, this terminology is not consistently applied. Perhaps this is because ecological interpretation of such spatial pattern requires a priori knowledge of the primary mechanism that creates spatial pattern in a particular ecosystem, but naming the statistical property that closer sites are typically more similar does not. Many studies (including this one) necessarily first measure the strength of the relationship between proximity and similarity, using the term SAC as a descriptor of a statistical property, and then refine and interpret the use of the term by comparing the likelihood of the two hypotheses concerning the source of SAC.

Despite the frequency of SAC observed in physical, chemical, and biological components of river ecosystems, most studies do not approach SAC from an ecological perspective (but see Grenouillet et al. 2008 who jointly analyzed diatoms, benthic invertebrates, fish, and environmental variables). Likewise, despite an abundance of studies addressing the relative influence of environment and spatial proximity on biological assemblage composition, there is little consensus concerning the origin of SAC in the biology of rivers. Detailed comparison between studies is hindered by different spatial extents, varied sampling design, disparate environmental variables, and competing analytical approaches (see Legendre et al. 2005, Tuomisto and Ruokolainen 2006, and subsequent discussion by Pélissier et al. 2008, Laliberté 2008, Legendre et al. 2008, and Tuomista and Ruokolainen 2008). Equally problematic are results where SAC in biological assemblages is *not* fully accounted for by included environmental variables. In such analyses, the question remains as to whether remaining SAC is the result of biotic processes or because important explanatory environmental variables were missing from the analyses.

In this study, I first ask 1) how concordance between different stream biota and between environmental and biological compositions may arise, and then 2) how network structure itself might influence patterns of biophysical concordance, SAC, and distance decay rates. I approach these questions by comparing rates of longitudinal change in both environmental variables and biological assemblages along network trajectories. Finally, 3) I use path analyses to evaluate relative support for environmental and neutral control hypotheses as the source/s of SAC in biological assemblage composition in these systems. In related analyses, I also calculate the portion of the effect of geographic distance on biological assemblage structure that is mediated through the spatial structuring of the riverine environment. The variety of network systems and range of spatial extents examined here allows a broad exploration of the prevalence of, the strength of, and the mechanisms that create SAC and the effects of geographic distance in Midwestern riverine ecosystems.

Methods

Sampling design and study systems

This study used longitudinal, high-spatial-frequency, within-basin spatial sampling in five tributary systems across Michigan and Ohio and the lower mainstem of the Muskegon River (Michigan) to develop a set of comparable environmental and biological datasets. The five tributary systems were chosen to represent a range in network complexity, drainage density, hydrologic regime, anthropogenic impairment, and biological community composition. Fifty-seven study sites, spaced 3-4 km apart on average, were assigned across the five tributary systems with the goal of characterizing each tributary system from its smallest permanent streams (1:24,000 scale NHD) to its mouth (Bigelow, Cedar, Brooks, Mill, and Crane Creeks;

Figure 4.1, Table 4.2). The Muskegon River has been the focus of recent, intense ecological study (e.g., Riseng et al. 2006, Steen et al. 2010, Wiley at al. 2010), and my study area includes 104 sites on an 80 km length of the lower river mainstem (Figure 4.2, Muskegon River). Fish were more intensively sampled (both spatially and temporally) in the mainstem than were invertebrates, although invertebrates were collected from all distinct ecological segments (as determined by VSEC 1.0, Seelbach et al. 1997) of the river (Figure 4.2, Table 4.2). The distance between sites varied, but on average, sites along the mainstem were about 0.75 km apart.

The Muskegon River in west-central Michigan is the second largest tributary system of Lake Michigan, draining a basin of 682,200 hectares with mixed land use/land cover (LULC) (Figure 1 inset; O'Neal 1997). The Muskegon is in good ecological condition (Riseng et al. 2006) and is well known for its recreational fishery. The 80 km study area lies between a drowned river mouth known as Muskegon Lake and Croton Dam (lower-most barrier to migrating fishes). Although the Muskegon River upstream of Croton Dam has also received extensive study, I limited my study area to river segments where dispersal is not limited by dams. The upper part of the mainstem study area is high gradient with shallow riffles and runs; the middle part includes transition zones and deep U-shaped channels; and the lower part flows though a low gradient wetland complex and splits into a north and south anabranch with numerous small side- and cross-connected channels.

Three of the five tributary systems I used in this study are located in the Muskegon River watershed and confluence in the Muskegon mainstem study reach (Figure 4.2). Bigelow Creek has a small catchment (≈80 km^2) dominated by forest and wetland and is primarily a cold-water trout stream. Cedar Creek is larger (catchment of wadeable portion ≈150 km^2) and includes warm-water agricultural headwaters and a cool, groundwater-dominated mainstem. Brooks

Creek drains primarily till plain topography (catchment ≈160 km^2) and therefore has a dense, highly bifurcated stream network which includes flashy warm-water agricultural ditches, stable cold-water segments, and lake outflows. LULC in Brooks Creek's catchment is similar to that in Cedar Creek, with mixed LULC including some urban development.

The other two study tributary systems are far removed both spatially and ecologically (Figure 4.2); Mill Creek in SE Michigan and Crane Creek in NW Ohio are both comparatively more impaired systems. ML, a highly dendritic tributary of the Huron River, has a large catchment (≈370 km^2) draining mixed landcovers and surficial geologies, resulting in a variety of stream types. Mill Creek joins the Huron River, a tributary of the western basin of Lake Erie. Crane Creek is smaller (catchment ≈115 km^2), dominated by agriculture and clay soils. It is a low-gradient, flashy, run-off driven, highly channelized system. Crane Creek flows into an estuary complex which terminates in Western Lake Erie. Downstream sections of Crane Creek are strongly affected by estuary and lake seiches, having little flow discharge except under high flow conditions or during falling water levels in Western Lake Erie.

Environmental data collection

Comparable environmental measures were developed for all study sites, although occasionally different field methods were necessary to collect these data and some measures differed slightly for sites in tributaries and the mainstem (Table 4.1). Environmental variables include catchment area, low-flow discharge, network link, channel shape, substrate, In-stream Geomorphic Units (IGUs), nutrients, and water temperature. Environmental data for the mainstem and tributary sites were developed from a combination of field measurements, quantitative models (i.e., Muskegon River Ecological Modeling System (MREMS), Wiley et al. 2010), aerial photography, and/or GIS maps. Some additional variables were developed

specifically for tributary sites (i.e., field slope, bankfull shear, average power, alkalinity and conductivity, and additional channel shape measures) and mainstem sites (i.e., map slope, sinuosity, and average velocity). In previous analyses (Chapters 2 and 3), these variables were not associated with biological assemblages and are excluded from analyses in this study. Two measures of substrate are used in this study, proportions of all substrate types and proportion of hard substrate (Table 4.1). Preliminary analyses (Chapters 3 and this study) at the tributary spatial extent indicated strong associations between substrate types and biological assemblages were lost if the substrate measure was simplified to the proportion of hard substrate. However, along the mainstem, associations between biological assemblages and substrate types and proportion hard substrate were equivalent. Thus, except where notes, analyses on mainstem sites used the proportion of hard substrate measure while analyses on tributary sites used the proportion of substrate types.

Biological data collection

Benthic invertebrate assemblages were characterized using a standardized, semi-quantitative Rapid Assessment Procedure (RAP) in the tributaries and quantitative sampling in the river mainstem. The semi-quantitative procedure attempts to detect all taxa within a sampling reach and is more comprehensive than most rapid bioassessment protocols (Barbour et al. 1995, Park 2007). Invertebrates in tributaries were collected using dip nets, kickscreens, and manual collection during the spring of 2005. Invertebrates were collected on the Muskegon mainstem during spring (May & June) of 2003 and 2004, and at a handful of sites in summer (Aug) 2003. Sampling of invertebrates was quantitative and targeted both common and rare habitats at a site. The specific sampling method (i.e., Hess, rock cluster, ponar grab, core, kickscreen, and wood and leaf debris grabs) was dictated by a sample location's depth and particular substrate.

Although abundance-based measures were developed for both tributary and mainstem sites, these measures were not comparable; thus macroinvertebrate data were simplified to occurrence (presence-absence) for these analyses. For both tributary and mainstem sites, invertebrates were identified to the lowest taxonomic resolution possible with moderate effort. Most organisms were identified to genus, while some organisms (such as Chironomidae, flatworms, mites, Branchiobdellidae and very early instar insects) remained at higher taxonomic resolution. Insect taxa that could only be identified to order were removed from the dataset prior to analyses.

The fish assemblage at each site was sampled using DC electrofishing gear. Depending on channel size and accessibility, backpack and barge electrofishing units targeted fish in wadeable areas, e.g., tributaries and along mainstem river edges, and boom electrofishing from boats targeted large fish from the center channel of the river mainstem. Fish in tributaries sites were assessed once in summer 2004 and fish in the Muskegon mainstem were electroshocked seasonally in spring, summer, and/or fall of 2003 and/or 2004. Young of year fish and fish which could not be identified to species were excluded from analyses and abundance data were simplified to presence/absence.

Biological assemblage data for mainstem sites were summarized at two temporal scales; 1) a more spatially and temporally restricted dataset limited to a single season/year (Spring 2003), and 2) a more temporally and spatially comprehensive dataset including multiple seasons across two years. Previous analyses (Chapter 2) indicate species occurrence remained quite stable between seasons and years, justifying the across season/year compilation of biological assemblages.

Distance calculations

Analyses in this study required the calculation of biological dissimilarity, environmental dissimilarity, and a measure of geographic distance for between site comparisons. Variability in fish and invertebrate assemblages was summarized in a Sorensen dissimilarity matrix based on occurrence of taxa. Use of Sorensen dissimilarity is desirable because it ignores joint absences, gives less weight to rare taxa, and takes values between zero (all species in common) and one (no species in common)(McCune and Grace 2002). Variability in environmental condition was summarized by a Euclidean distance matrix. Euclidean distance is strongly influenced by large outliers; thus environmental variables with many small values and a few large values, (i.e., low-flow discharge, catchment area, link, nutrients, and channel shape) were natural log transformed prior to analyses.

Because this study includes fish, whose dispersal is limited to in-stream routes, and invertebrates, whose dispersal can include both in-stream and terrestrial routes, both overland and watercourse distance might be reasonable measures of geographic distance. However, I chose watercourse distance (i.e. network-restricted distance between sites or "swim" distance) for three reasons: 1) It is a more relevant measure of geographic distance for most physical and chemical environmental variables, 2) preliminary analyses suggested stronger biological associations with watercourse distance than overland distance, and 3) Landeiro et al. (2011) showed watercourse distances provided better representations of the spatial patterns generated by fish and invertebrate dispersal along a dendritic network. The shortest watercourse distance between sites was calculated using the Network Analyst extension in ArcGIS (ESRI 2011).

Rates of change analyses

Trajectory plots were developed to illustrate spatial variability in rates of change as well as concordance between fish, invertebrate, and environmental character. Trajectory plots were

confined to the longest watercourse distance path within a study system. For Mill and Crane Creeks, cumulative dissimilarity (beginning upstream) in biological assemblages and environmental character of neighboring sites was plotted against watercourse distance to the site nearest the river mouth (i.e., longest route from the headwaters to the downstream-most site). To assess differences in rates of change in the biology along the river mainstem and confluent tributaries, I created the trajectory plots by plotting cumulative dissimilarity in fish and invertebrate assemblages for neighboring sites against distance from Muskegon Lake. Slope at any position on the trajectory line reflects rate of change in biological assemblages or environmental features (e.g., steep slope indicates area of rapid change; conversely, mild slope indicates areas of little change). Since only the slope of the line is important in these plots and the actual values on the Y-axis are irrelevant, trajectory lines were sometimes shifted up or down on the y-axis for ease of interpretation and visualization. Biological data were restricted to one sampling time (Spring 2003) to avoid introducing temporal differences in the trajectories. Prior to distance calculations, environmental variables based on multiple measures were summarized by the primary Principal Components Analysis axis (average percent variation explained: Ch Shape = 93%, Substrate = 66%, IGUs=68%, Nutrients = 73%, and Temperature=82%). Each environmental variable was also normalized by Z-score, accounting for differences in scale between variables and allowing each variable to contribute equally to the overall environmental distance matrix.

These trajectory plots and prior analyses (Chapters 2 & 3) suggest a more detailed comparison of rates in different river network positions was warranted. Comparison of distance decay rates for different positions in a river network required grouping sites into position classes, calculating biological and environmental dissimilarity, and creating comparable proximity

between sites. Prior analyses (Chapters 2 & 3) suggested that considerable biological differences existed between mainstem sites and confluent tributaries, and between a sites on tributary mainstem and sites in its headwaters. Accordingly, all study sites were assigned a position class: "River Mainstem," sites on the mainstem of the Muskegon River; "Creek Mainstem," highest order sites along tributary systems after a large jump in link number; and "Headwaters," all other upstream sites in tributary systems. Sites within the same position class from the five tributary systems were pooled for analysis.

Longitudinal trajectories along the longest water route largely ignore the network aspects of a river, so I also developed a very simple measure of standardized distance decay, the percent change in similarity per km of watercourse distance. Biological and environmental similarities were calculated with Sorensen and Jaccard dissimilarity and Euclidean distance, respectively. Jaccard dissimilarities are used in my rate calculations because Jaccard dissimilarities are adjusted for species richness, which differs by network position (i.e., species richness increases as position advances downstream in a river network). Because Euclidean distance does not have an upper bound on the maximum value and the magnitude of distance depends on the scaling of original variables, raw Euclidean distances were normalized to a range of zero to one using the formula $D_{norm}=D/D_{max}$ where D_{norm} is the normalized Euclidean distance, D is the observed Euclidean distance, and D_{max} is the maximum observed Euclidean distance.

Although the average and variability of spacing of sites was similar for Headwater and Creek Mainstem sites (with the exception of two sites in Brooks Creek that were unusually close and excluded from rate analyses), each tributary differed in overall watercourse length and River Mainstem sites were on average much closer and the distances between sites more variable. These differences introduced problems when calculating rates of change; spacing of sites has a

large effect on rates since the denominator of the rate of change measure can vary, but the numerator is restricted to values between 0 and 1. To remedy these issues, rate calculations were limited to adjacent sites within a tributary, and a subsample of sites on the River Mainstem. Subsampled sites on the River Mainstem were chosen to replicate the average separation and variability in separation of sites in Headwater and Creek Mainstem groups. Likewise, biological data were restricted to a single season and year to avoid introducing temporal difference in River Mainstem rate calculations. Statistical significance of differences between average rates for the three position classes were calculated with the SumF permutation procedure (Edgington 1995) in PC-ORD version 6.08 (McCune & Mefford 2011).

Environment, biology, and proximity associations

Because of its flexibility (Urban 2003), simple Mantel tests were used to answer a variety of questions in this study. A simple Mantel test is used to test the null hypothesis of "no relationship" between two square symmetric matrices and is an alternative to regressing one matrix against the other, avoiding the problem of partial dependence within each matrix. The standardized Mantel test statistic (r) ranges from -1 to 1, with -1 indicating negative positive correlation between the two matrices, 0 no correlation between matrices, and 1 perfect positive correlation between matrices. For all Mantel tests, the significance of r was assessed with a Monte Carlo randomization method using the maximum number of possible permutations for a given dataset or a maximum of 3000 permutations. All Mantel tests were conducted using PC-ORD version 6.08 (McCune & Mefford 2011).

Mantel tests were performed on seven systems in three spatial extent classes, 1) within a tributary, 2) along a mainstem only, and 3) along a mainstem plus three confluent tributaries. These seven systems include substantially different numbers of sites, and thus sample size (Table

4.2) should be considered when evaluating both the magnitude of r and its statistical significance. The seven systems also differ in spatial extent and watercourse length, network complexity, and physical character (Table 4.2). For Mantel analyses, I used across season and years mainstem biological datasets because a larger spatial coverage was more important than the minimal temporal variability introduced in the dataset.

A simple Mantel test between an environmental variable and watercourse distance or a biological assemblage and watercourse distance is an overall measure of SAC in the dataset. This use of a Mantel test addresses the questions whether proximal sites are more similar in environmental condition or have more similar biological assemblages. Simple Mantel tests between environmental variables and biological assemblages can assess whether spatial patterns are concordant, i.e., changes in biological assemblages co-occur with changes in environmental character.

Path analyses and variance partitioning based on environmental and geographic distances can quantify the direct and indirect effects (through environment) of geographic distance, and determine the likelihood of environmental control as the primary cause of biological spatial autocorrelation (Borcard et al. 1992; Tuomisto and Ruokolainen 2006). I used partial Mantel tests to test for significant patterns between biological dissimilarity and watercourse distance while controlling for environmental effects. This use of partial Mantels addresses the question "can the variation in the difference in community composition between two sites be explained by variation in difference in environmental characteristics or geographic distance?" (Tuomisto and Ruokolainen 2006). I also performed partial Mantel tests between environmental and biological dissimilarity while controlling for watercourse distance. Using these Mantel test coefficients, I also used path analyses to calculate the total effect of the watercourse distance on biological

assemblage composition, partitioning direct and indirect effects (effects mediated through the riverine environment). For these analyses, the composite environmental matrix included only variables with large correlation coefficient (r) or statistically significant environment/biological assemblage associations. As with the trajectory analyses, environmental variables based on multiple measures were summarized by the primary Principal Components Analysis axis and normalized to Z-scores.

Results

Rates of change in river ecosystems

Trajectory plots (Figure 4.3) illustrate differences in rates of change along the headwaters to mouth trajectory, as well as concordance between fish and invertebrate assemblages, and concordance between biological assemblages and environmental character. In many systems, upper headwaters had high rates of change and invertebrate assemblage composition changed more rapidly per km than did the fish assemblage. In the Muskegon tributaries, rates of change of fish assemblages were much higher than in the corresponding mainstem. Within the Muskegon River mainstem, rates of change in fish, invertebrates, and environmental components changed as position along the trajectory shifted downstream; biological and environmental character changed rapidly from 80-53 km from Muskegon Lake, moderately from 53 to 50 km, more slowly from 40 to 20 km, and changed very rapidly between the confluence of Brooks and Cedar Creeks. These trajectory plots (Figure 4.3) also demonstrate that changes in biological assemblages and environmental character often corresponded, and therefore system-by-system analyses associating biological change with environmental change are warranted. These associations are explicitly assessed in the environmental and biological concordance section.

The average rate of distance decay (% change per km) and the variability in the rates of distance decay differed with position in the network (Table 4.3). With the exception of channel shape, all environmental variables and the fish assemblage changed more rapidly within the Headwaters than along a River Mainstem. The rates of fish assemblage and environmental change were also typically more variable in headwaters, although statistical differences in standard deviations were not assessed. The average rates of change for environmental variables in the Creek Mainstem were either the same as the headwaters (i.e., Hard Substrate and Nutrients), the same as the River Mainstem (CatchArea and Link), or because of low statistical power, somewhere in between (Qlow and IGUs). Average rate of change in temperature decreased from 11% to 5.7% to 1.7% as network position shifted from Headwaters to Creek Mainstem to River Mainstem, respectively. For the fish assemblage, the dissimilarity measure used affected which average rates were statistically different; with Sorensen dissimilarity all three rates differed significantly while with Jaccard dissimilarity (which accounts for species richness) only the average rate in the Headwaters was lower. Average rate of change and variability in invertebrate assemblage rates of change were similar in all three network position classes, regardless of dissimilarity measure used.

Spatial autocorrelation

Many environmental variables and both fish and invertebrate assemblage composition displayed some degree of SAC (i.e., proximal sites tend to be more similar than more distant sites), although specific associations varied by spatial extent and system (Table 4.4). Within tributary systems, measures of size (i.e., CatchArea, Link, Qlow, and Ch. Shape) were usually strongly associated with network distance, while in-stream habitat (i.e., Substrate and IGUs) were rarely or never spatially autocorrelated. Significance and magnitude of SAC in Nutrients

and Temperature varied with tributary system. As compared with other tributaries, Cedar and Crane Creeks had fewer variables that exhibited SAC.

Because of the large number of sites, all environmental variables had statistically significant SAC at the Mainstem and Mainstem plus tributaries spatial extents, but the magnitude of the associations varied considerably between variables and between spatial extents. At the mainstem spatial extent, the strongest associations were between watercourse distance and link and CatchArea. Unlike in the tributaries, similarity in hard substrate was strongly associated with proximity, while similarity in IGUs and channel shape were weakly associated with proximity. Qlow, nutrients, and temperature showed moderate SAC at the mainstem spatial extent. When confluent tributaries are include with the mainstem, the association between watercourse distance and CatchArea, Qlow, link, and temperature were substantially reduced (reductions in magnitude of Mantel r's of 0.60, 0.38, 0.67, and 0.30 respectively). Associations between channel shape and watercourse distance and IGUs and watercourse distance increased slightly and associations between substrate and nutrients decreased slightly.

Spatial autocorrelation was observed in fish and invertebrate assemblages in all systems and at all spatial extents (Table 4.4). In all systems except Crane Creek, SAC in fish composition was moderate to strong (Mantel r's from 0.40 to 0.67). In Bigelow, Brooks, Mill, and the Muskegon plus confluent tributaries, SAC in fish assemblages were stronger than SAC in invertebrate assemblages, in Cedar and Crane Creek SAC magnitude was similar and in the Muskegon mainstem there was stronger SAC in invertebrates than fish.

Environment and biology concordance

There were many strong associations between change in environmental variables and change in biological assemblages (Table 4.5). The strongest biological/environmental

concordances included variables representing aspects of size (i.e., CatchArea, Link, Qlow, and Ch. Shape). Temperature regime was concordant with biological assemblages in all seven systems, although the strength of the association varied with study system. Because of concordance between the fish and invertebrate assemblages, spatial patterns of both fish and invertebrates were usually concordant with the same environmental variables. Typically, fish/environment concordances were stronger than or equivalent to invertebrate/environment concordances for the same physical variable; however, invertebrate/substrate concordances were typically stronger than fish/substrate concordance.

Spatial extent also affected the significance and magnitude of environmental/biological associations. While IGUs were associated with biological assemblages in several tributary systems, IGUs were not associated with biological spatial pattern in the river mainstem or the river mainstem plus confluent tributaries spatial extents. Combining the tributary and mainstem sites increased the magnitude of all environmental/biological associations, with the exception of Invertebrates/Link (equal magnitude), and Invertebrates/Substrate (decreased magnitude).

Joint environmental, biological, and spatial proximity concordance

In all study systems, composite environmental variables were spatially structured; that is sites closer on the watercourse typically had more similar environmental character. The SAC in environmental variables was usually similar in magnitude to the SAC for both fish and invertebrate assemblages within a system because strong concordance between fish and invertebrate assemblages resulted in similar or identical composite environmental datasets. SAC in the composite environmental variables was particularly strong in the Muskegon mainstem (Fish $r_{DE} = 0.80$, Invert $r_{DE} = 0.76$) while SAC was weakest when the sites on confluent tributaries were added to those mainstem sites (Fish $r_{DE} = 0.33$, Invert $r_{DE} = 0.45$).

In most study systems there were strong associations between composite environmental variable and biological assemblages (Figure 4.4, r_{EF} and r_{EI}). The magnitude of most of these associations remained strong when partial tests accounted for watercourse distance (Figure 4.4, $r_{EF|D}$ and $r_{EI|D}$). Two exceptions are substantially weaker environment/fish assemblage association in Cedar Creek and weaker association with both biological assemblages in the Muskegon Mainstem once watercourse distance was accounted for. In all systems, the SAC between biological assemblages and watercourse distance (as measured by simple Mantel tests r_{DF} and $r_{DI,}$ Figure 4.4) were reduced or eliminated when environmental associations were accounted for with a partial test ($r_{DF|E}$ and $r_{DI|E}$). For most systems, these reductions in SAC were substantial even if the partial mantel test remained statistically significant because of large sample size (e.g., Muskegon $r_{DF|E} = 0.07$ but $p<0.10$). The SAC in invertebrate assemblages in Cedar Creek was the only association that was minimally reduced once environment had been statistically accounted for.

Averaged across systems, much of the total effect of geographic distance on biological assemblage composition (77% for fish and 84% for invertebrates) was mediated through the environment. However, the total effect of geographic distance (as measured by watercourse distance) varied by system and biological assemblage, as did the relative importance of direct and indirect effects (Table 4.6). In larger river systems with more network confluences (i.e. Brooks, Mill, and Muskegon+Tribs), the direct effects of watercourse distance on fish assemblages was comparatively large (41 to 53% for these three systems versus 0 to 27% for the other four), but statistically nill for invertebrate assemblages. The only systems with large direct effects of watercourse distance on invertebrate assemblages were Cedar Creek and the Muskegon

River mainstem. The invertebrate-based analyses for these two systems included variables with the strongest associations between proximity and environmental similarity.

Summary of results

Average of and variability in standardized distance decay rates were highest in headwater systems and lowest in the large river system; and in almost all cases, confluence points marked important changes for both fauna and key environmental variables. At the system extent, I observed strong SAC in many environmental variables and in both fish and invertebrate assemblage composition. Strong environment/biology associations accounted for most or all of the SAC in biological assemblage composition, offering strong support for the hypothesis that niche processes with species sorting are the origin of most biological SAC within river basins. Similarly, on average, most of the total effect of geographic distance on biological assemblage composition is mediated through the riverine environment.

Discussion

Rates of change in river ecosystems

Trajectory analyses along the Muskegon mainstem demonstrated that ecological spatial pattern in this river is best described as a patchy ecologic gradient (Poole 2002, Thorp et al. 2006, 2008). The confluence of the smallest tributary system, Bigelow Creek, had little effect on the rate of change in the river mainstem while the confluence of much larger Brooks Creek was immediately followed by very rapid change in the environment and biological assemblage composition in the river mainstem. These observations are consistent with Benda et al.'s (2004a, 2004b) predictions of larger confluence effects with a larger ratio of tributary to mainstem size.

Trajectory analyses also suggested there may be differences in rates of change with position in the river network. These were confirmed with calculation of standardized distance decay rates (% change per km)

Both the average and variability in standardized decay rates of many environmental variables decreased from headwaters to mainstem. What underlies these differences in distance decay rates in different portions of the drainage network? Early geomorphic studies of network structure developed empirical "laws" (e.g., the "law of stream numbers", and "law of stream lengths"; Horton 1945) that indicated rates of channel bifurcation (and therefore confluence) generally decline with increasing stream order (and covariates: link magnitude and catchment basin area). In advective networks, confluence points provide the physical opportunity for new hydrologic, chemical and biological inputs and downstream regimes. Thus the local bifurcation rate of the network system itself will influence the frequency of trajectory change points. Since bifurcation rate declines with increasing stream order, longitudinal variation in rates of change as described by decay rates will necessarily vary across the watershed system. Network nodes (confluence points) thereby play an important role in shaping the physical habitat template and potentially the biological community as well (e.g., Osborne and Wiley 1992, Rice et al. 2001, Padgham and Webb 2010, Neeson et al. 2012, Webb and Padgham 2013). In the headwaters of most Midwestern streams, discharge grows rapidly through increases in catchment size and the frequent confluence of similarly sized low-order streams draining different landscapes. The result is rapid change in the environment over short distances. In contrast, downstream in the network, confluences with comparably sized channels are rarer; the frequent entry of smaller channels that do occur have little distinguishable effect on flow, chemistry or the hydraulic character of the larger channel (Benda et al. 2004a, 2004b, Kiffney et al. 2006).

Standardized distance decay rates in fish community composition also generally decreased from headwaters to mainstem systems. Hitt and Angermeier (2011) have reported a similar pattern for riverine fish communities in West Virginia, USA; finding that community heterogeneity was inversely related to stream size, with headwater streams having extremely variable fish assemblages, and the largest streams comparatively little variation in fish assemblages. The observed distance decay rates in invertebrate assemblages in this study did not vary with position in the network, even though the same scaling and network rules discussed above should apply. This failure to observe lower rates of change in the Muskegon mainstem is likely an artifact of the invertebrate sampling methodology. In tributaries, macroinvertebrate assemblages at each site were comprehensively sampled across all existing habitats, while sampling in the non-wadeable portions of the mainstem was necessarily limited to a handful of samples, usually grab samples. Adequately sampling invertebrates in large rivers is notoriously difficult and requires a very large effort to account for the full suite of taxa present (Bady et al. 2004, Flotemersch et al. 2006, Flotemersch et al. 2011). Thus, it is likely a smaller proportion of the invertebrate taxa present were represented in samples from the river mainstem, as compared to samples from tributary sites. In difficult to sample areas, low sampling efficiency and small sample numbers could lead to random fluctuations in measured composition and conflate variability in assemblage composition from sampling methods with true spatial variability, artificially increasing calculated rates of change in the Muskegon mainstream.

Partitioning the sampling sites into Headwater, Creek Mainstem, and River Mainstem classes was based on previous analyses (Chapters 2 & 3) and is very similar to classes Hitt and Angermeier (2011) used to reflect relative network position. However, this gradient in position is likely a continuum suggesting a continuous decline in rates of change from headwaters to river

mouth. This hypothesis could be tested by plotting rates of change against continuous measures of stream size or network position (e.g., average catchment area, discharge, or link). However, such analyses were not possible in this study because the study tributaries join the Muskegon mainstem in the lower third of the watershed. Sites in this study contrast comparatively headwater positions within tributaries (e.g., catchments of 10s to 100s of km^2, discharges around one cms, and links from 1 to 10s of orders of magnitude) to comparatively downstream positions on the lower mainstem of a large river (e.g., catchments of 6000+ km^2, average discharges around 7000 cms, and links of 390+).

Understanding how distance decay varies across a river basin could usefully inform the choice of statistical methods used in river research. For example, incorporating different distance decay rates (or its conceptual inverse, SAC) across a river network could improve spatial regression models, and geostatistical techniques such as kriging used to interpolate sample data to entire river networks (see Gardner et al. 2003, Sauquet 2006, Cressie et al. 2006, and Garreta et al. 2010 for discussion and application of such issues). The observed variation in distance decay rates also raises questions about the applicability of many spatial statistics frequently suggested for use in river systems (Rossie et al. 1992, Cooper et al. 1997, Ganio et al. 2005). Many common spatial analyses are based on variograms and correlograms that assume a single dominant spatial structure exists across the entire study area (i.e., stationarity; Legendre and Fortin 1989). This assumption of stationarity fails if rates of change vary with network position. Solutions might include performing analyses within network position subgroups or scaling SAC parameters by stream order or other network metrics.

SAC in river environment and biological assemblages

Most environmental variables included in this study showed some degree of SAC, although the magnitude of the SAC varied by environmental measure, system, and spatial extent of the analysis. By including a variety of tributary systems, I was able to document both shared and system-specific SAC patterns. In the five tributary systems, there was no SAC observed in instream geomorphic units (i.e., riffles, runs, pools, etc) and only one instance of SAC in substrate. This was surprising given there are well established expectations of gradients in channel slope, power, sediment grain size, and types of IGUs with increasing stream size (Church 2002, Fryirs and Brierley 2013). The within tributary scale of the analyses may have limited analyses to a narrow portion of the expected gradient.

SAC in environmental measures was common and similarity was strongly associated with proximity along the Muskegon River mainstem. SAC in environmental variables was also observed at the mainstem plus confluent tributaries spatial extent, although the magnitude of the strongest associations decreased substantially. This expansion of spatial extent included tributary to mainstem transitions, where environmental conditions change abruptly. Rates of change at major network junctures are unusually large, and including such transitions, in effect, reduces overall associations between proximity and similarity. The marked decreases in the magnitude of overall SAC are also consistent with a view of a river as a patchy gradient (Poole 2002, Thorp et al. 2006, 2008), rather than simply a gradient (Vannote et al. 1980).

SAC has regularly been reported in riverine environments (Wilkinson and Edds 2001, Magalhães et al. 2002, Lloyd et al. 2006, Stewart-Koster et al. 2007, Grenouillet et. al 2008), although differences in sampling regime and spatial extent complicate detailed comparisons. Nevertheless, the physical arrangement of study sites within and across basins does appear to affect whether SAC in riverine environments is observed. Studies with sites of similar stream

size primarily arranged across basins and ecoregions, have found no association between environment and proximity (Thompson and Townsend 2006, Heino and Mykrä 2008). In contrast, this study and many others that used longitudinal sampling designs, observed moderate to strong SAC in many environmental variables (Wilkinson and Edds 2001, Magalhães et al. 2002, Lloyd et al. 2006, Stewart-Koster et al. 2007, Grenouillet et. al 2008).

SAC in fish and invertebrate assemblage composition was observed in all of my study systems and all spatial extents, indicating sites closer together typically had more similar biological assemblages. These results largely agree with studies using within-basin, longitudinal sampling regimes (Fish: Wilkinson and Edds 2001, Grenouillet et al. 2004, Stewart-Koster et al. 2007, Maloney and Munguia 2011; Fish and Invertebrates: Grenouillet et al. 2008). The ubiquity of SAC found here contrasts with Lloyd et al. (2005) who found SAC in macroinvertebrate assemblages in only one of two adjacent river systems and Ganio et al. (2006) who found SAC in abundance data for Cutthroat Trout in only one of two adjacent forks in Hinkle Creek. Rather than assume SAC is universal, these authors suggest that one cannot assume similar SAC patterns even in adjacent rivers because each river may have its own idiosyncratic spatial patterning.

All SAC analyses require a measure of proximity, therefore the measure of proximity chosen could also affect the magnitude of SAC observed in rivers. Efforts to incorporate river network structure, flow direction, and biologic dispersal patterns into more meaningful measures of “proximity” have proliferated over the past decade (Cressie et al. 2006, Ver Hoef et al. 2006). I chose watercourse distance as the measure of proximity because it was a simple measure that could be applied to environment, invertebrate, and fish measures at all spatial extents. Watercourse distance assumes a unit of distance within a river network is constant, i.e., a km

separation in the headwaters is functionally equivalent to a km separation in the mainstem. Analyses in this study indicate this assumption oversimplifies proximity relationships in river ecosystems. Olden et al. (2001) developed a proximity measure where distance was calculated as the number of stream reaches an organism must travel through between any two locations. Since this measure implicitly incorporates river network geometry, it may help resolve issues with changing rates of change within a river network and may work particularly well in dendritic networks (Stewart-Koster et al. 2007).

Effects of environment and distance on biological assemblage composition

This study, and the majority of ecological studies addressing the relative importance of environmental and spatial processes (Cottenie 2005), provides evidence for the dominance of environmental control on taxa sorting. Comparing the results of this study to others riverine studies is difficult, however, because of differences in spatial extent, sampling design, environmental variables, and analytical approaches. In comparable studies that address within-basin SAC using longitudinal sampling, results have varied widely. For fish assemblages, my results agree with Wilkinson and Edds (2001) and Stewart-Koster et al. (2007) who found a spatially structured environment was the primary factors organizing fish communities. In contrast, Maloney and Munguia (2011) found weak SAC in fish assemblage composition that was not accounted for by environmental variables. Likewise Grenouillet et al. (2004) found spatial pattern in local fish species richness remained once stream width and other gradients were accounted for. Grenouillet et al. (2008) was able to account for SAC in invertebrate assemblages with environmental variables, but not for SAC in fish assemblages, while Lloyd et al. (2005) accounted for little of the observed SAC in benthic invertebrates with environmental variables. Studies where environmental variables cannot completely account for SAC in biological

assemblages face the challenge of determining whether such results indicate the importance of biotic processes in structuring biotic assemblages or whether the results arise simply from missing important, spatially-structured environmental variables.

In my analyses, a small number of environmental variables were able to account for most, and in some cases all, of the observed SAC in biological assemblages. The spatial structure of these environmental variables was also responsible for strong indirect effects of geographic distance on biological assemblages. These environmental variables include measures of channel size, channel shape, substrate, IGUs, and nutrient and temperature regimes, measures known to be associated with stream assemblage composition (Cummins and Lauff 1969, Minshall and Minshall 1977, Vannote et al. 1980, Hawkins and Sedell 1984, Matthews 1986, Hawkins et al. 1997, Zorn et al. 2002, Wehrly et al. 2003, 2006). With the exception of substrate and IGUs, this short list of environmental variables includes variables that are widely available (e.g., network structure from the National Hydrography Dataset, NHD; large river channel width estimates from aerial photography), or can be modeled with existing techniques (Wiley et al. 2004, Wehrly et al. 2006, HEC-HMS Anonymous 2010). LULC and surficial geology variables were not included in the analyses, but influences of these variables were indirectly included in water temperature and nutrient measures.

Do these results imply spatial dispersal processes are wholly unimportant in the study river systems? Probably not, but the effects of dispersal limitation do appear to be minimal as compared to the effects of environmental heterogeneity. However, the study area in the Muskegon River was restricted to the lower 1/3rd of the catchment, but dispersal limitations (especially for fish) are likely in upper 2/3 of the Muskegon River because of three major dams on the river mainstem (O'Neal 1997). It also appears that differences in dispersal of fish and

invertebrates may be suggested by observed direct effects of geographic distance; direct effects of dispersal were important for fish in network systems with many confluences, but absent for invertebrates in these same systems. Movement of fish across tributary confluences is common, both in spawning anadromous fishes and in local fish movement that is for non-reproductive reasons (Dames et al. 1989, Wilkinson and Edds 2001, Osborne and Wiley 1992). In contrast, it is unlikely invertebrates can move as easily upstream through large confluences. Instead, the primary effect on invertebrates appears to be changes in assemblage composition in the river mainstem (Rice et al. 2001, Rice et al. 2006).

Several authors (Thompson and Townsend 2006, Bahn and McGill 2007, Currie 2007, Heino and Mykrä 2008, Grönroos et al. 2013) maintain exploring the independent effects of spatial proximity and environment on assemblage structure is only possible if environmental variables are not spatially autocorrelated. Bahn and McGill (2007) argue environmental variables may predict spatial variation in the abundance of organisms because the two have similar spatial structures, and not because environment actually influences abundance. Although independence of riverine spatial structure and environment variables may be the ideal to statistically tease apart the effects of each on biological assemblages, my study suggests data that comply with this ideal are extremely unlikely. Furthermore, an extensive history of laboratory and experimental field studies support the thesis that SAC in biological assemblages is caused by a spatially-structured environment. There are widely documented direct effects of environmental variables on riverine organisms (e.g., temperature on fish survival and metabolic and growth rates in Diana 2004) and biological assemblage composition (e.g., stream flow reduction on riffle species in Wills et al. 2006, substrate/current on benthic invertebrates in Minshall and Minshall 1977, hydraulics in Statzner and Higler 1986, and longitudinal gradients in Statzner and Borchardt 1994).

Statistical, ecological, and management implications

The dominant influence of a river's physical template on biological assemblage composition has statistical, ecological and management implications. Differences in rates of change with river network position should be considered when choosing appropriate statistical and management tools. Since many common spatial analyses are based on variograms and correlograms that assume a single dominant spatial structure exists across the entire study area, these statistical tools may not be appropriate for exploring spatial pattern in river ecosystems. It also suggests that management techniques, such as delineation of homogeneous and distinct ecological units, should recognize changes in spatial pattern, especially in regards to scaling, with position in the network. This study suggests spatial autocorrelation in biological assemblage composition can be accounted for by a relatively small number of environmental variables. The ability of these spatially-structured environmental variables to explain the spatial autocorrelation observed in fish and invertebrate assemblages suggests environmental control determines taxa sorting rules and produces much of the spatial pattern in riverine biology. In rivers, therefore, statistical modeling should first attempt to account for SAC with traditional non-spatial models and appropriate explanatory environmental variables. If necessary, more complex spatial models (discussed in Isaak et al. 2014) can be used to account for residual SAC.

Table 4.1: Comparable environmental variables were developed for sites on tributaries and the mainstem, although methods of collection necessarily differed for some variables. Some variables were based on a single measure for each site, while others were based on multiple related measures. If the distribution of a variable was strongly right-skewed, the variable was LN transformed. MREMS is the Muskegon River Ecological Modeling System; see Wiley at al. 2010 for model description and development.

Variable Name (abbreviation)	**Single or multiple variables**	**Measure**	**Method of acquisition in tributaries**	**Method of acquisition in mainstem**
Catchment Area (CatchArea)	Single	LN Catchment area (km^2)	Calculated for study site basin maps in ArcGIS	Calculated for MREMS model units in ArcGIS
Qlowflow (Qlow)	Single	LN Low-flow discharge (cms)	Estimate from field measured flow cross section	Estimated with MREMS
Link (Link)	Single	LN Number of upstream network junctures	Developed from tributary networks (NHD) in ArcGIS	Developed from river network (modified NHD to include major cross channels) in ArcGIS
Channel Shape (Ch. Shape)	Multiple	LN Channel width, LN water depth, and LN cross-sectional area (m or m^2)	Width (W) & Depth (D): Average of five field measured cross sections Cross-sectional area: W*D	Width (W): Estimated from winter aerial photos Depth (D): Calculated as weighted average from habitat map depths Cross-sectional area: W*D
Substrate (Substrate)	Multiple or Single	Tribs: Proportion of study reach in each of seven substrates (cobble, gravel, sand, silt, claybed, CPOM, wood) Mainstem and Tribs+Mainstem: Proportion of substrate that includes hard substrates	Estimated from field observations	Calculated for 100 meter buffer around site and from comprehensive GIS habitat map. GIS habitat map was developed from field observations of depth, substrate, and IGUs.
Instream Geomorphic units (IGUs)	Multiple	Proportion of study reach in each of six major types (riffle, run, pool, edge, bar, backwater)	Estimated from field observations	Calculated from habitat map for 100 meter buffer around site
Nutrients (Nutrients)	Multiple	Tribs: LN NO3, LN NH4, LN SRP Mainstem: LN N, LN SRP, LN TP Tribs+Mainstem: LN N, LN SRP	Field samples in Spring and Summer; laboratory tested	Estimated with MREMS
Temperature (Temp)	Multiple	Tribs: Synoptic measurements during spring and summer Mainstem: Ave, max, min of daily values Tribs+Mainstem: Ave, max	Synoptic field measures	Estimated with MREMS

Table 4.2: Study systems differed in spatial extent, network complexity, environmental character, and the number of sites. The number of sites also depends on the type of variable. Since the number of sites affects the power and significance of statistical tests, the number of sites should be considered when interpreting the magnitude and significance of Mantel test statistics (r's). In the tributaries, fish and invertebrates were sampled at all sites, while fish were sampled at more sites than invertebrates in the Muskegon mainstem. Three sites in Crane Creek were devoid of fish during summer sampling and are excluded from fish-based analyses.

	Tributaries					Mainstem	Mainstem+Tribs
Variables or Study System	Bigelow	Cedar	Brooks	Mill	Crane	Muskegon	Muskgeon+BG,BR,CD
Number of Sites							
Fish	5	9	14	16	10	92	120
Invertebrates	5	9	14	16	13	43	71
Environmental	5	9	14	16	13	104	132
System Characteristics							
Total catchment area (km^2)	80	150	160	370	115	6761	6761
Max between site watercourse distance (km)	17	30	25	32	26	76	101
Min/Max Link	1/5	1/11	1/18	1/29	1/6	392/465	1/465
Average depth (m)	0.3	0.4	0.2	0.3	0.2	0.9	0.7
Average % hard substrate	30	15	10	25	34	34	32
Average Temp (°C)	14.7	17.1	15.4	12.2	20.1	17.6	17.3

Table 4.3: The average and standard deviation (SD) of standardized distance decay rates (% change/km) with sampling position in the network. Averages indicated with the same letter cannot be distinguished statistically ($p>0.10$). All environmental variables (except channel shape), and the fish assemblage changed more rapidly within the Headwaters than along a River Mainstem. The rates were also usually more variable in headwaters. Rates of change for environmental variables are normalized Euclidean distance per km separation and rates of change for biological assemblages are Sorenson or Jaccard dissimilarity per km watercourse distance.

	Average dissimilarity per km separation			SD dissimilarity per km separation		
Variable/location	Head-waters	Creek Mainstem	River Mainstem	Head-waters	Creek Mainstem	River Mainstem
# of rates included	27	12	20	27	12	20
Environment:						
CatchArea	4.6 (a)	2.3 (b)	1.3 (b)	2.9	2.5	1.6
Qlow	6.3 (a)	3.8 (a,b)	1.3 (b)	9.4	5.7	4.8
Link	5.6 (a)	0.9 (b)	1.3 (b)	7.6	1.3	1.3
Ch. Shape	7.0	4.8	7.0	6.4	3.4	5.8
Substrate (hard)	12.5 (a)	7.0 (a)	0.2 (b)	11.4	8.9	0.2
IGUs	10.8 (a)	7.6 (a,b)	5.3 (b)	7.5	5.9	4.6
Nutrients	11.2 (a)	7.7 (a)	1.6 (b)	8.1	4.6	4.7
Temperature	11.0 (a)	5.7 (b)	1.7 (c)	8.5	2.5	5.4
Biology:						
Fish (Sorensen)	15.4 (a)	8.0 (b)	6.05 (c)	12.0	3.0	3.6
Invert(Sorensen)	14.6	11.6	14.2	6.8	6.6	8.1
Fish (Jaccard)	19.7 (a)	12.0 (b)	14.2 (b)	13.0	3.9	5.2
Invert (Jaccard)	19.8	16.1	19.0	9.3	8.1	9.5

Table 4.4: Mantel tests for overall SAC. Significant Mantel tests with large test statistics indicate sites closer together typically have more similar environmental characteristics and biological assemblages than sites further apart. The strength of these relationships varied by spatial extent, system, and variable of interest. Distance is watercourse distance between sites. NS = not significant

	Tributaries					Mainstem	Mainstem+Tribs
Variables or System	Bigelow	Cedar	Brooks	Mill	Crane	Muskegon	Muskgeon+BG,BR,CD
Environmental:							
CatchArea	NS	NS	0.42***	0.44****	0.51***	0.86****	0.26****
Qlow	0.64**	0.34*	0.47***	0.19*	NS	0.66****	0.28****
Link	0.61*	NS	0.38***	0.44****	0.46***	0.93****	0.26****
Ch. Shape	NS	NS	0.46***	0.27**	0.55***	0.19****	0.27****
Substrate	0.55*	NS	NS	NS	NS	0.80****	0.63****
IGUs	NS	NS	NS	NS	NS	0.12***	0.14***
Nutrients	0.83***	0.65***	0.30*	0.33**	NS	0.63****	0.41****
Temp	0.61*	0.45**	NS	0.28**	NS	0.60****	0.30****
Biological:							
Fish Dissimilarity	0.67*	0.48***	0.52***	0.46***	0.26**	0.40****	0.42****
Invert Dissimilarity	0.57*	0.50**	0.33**	0.33**	0.31**	0.51****	0.30****

*p<0.10 **p<0.05, ***p<0.01, ****p<0.001;

Table 4.5: Mantel tests for environment/biology concordance. Significant Mantel tests with large test statistics indicate strong concordance between fish or invertebrate assemblages and environmental characteristics, i.e., large transitions in the biotic assemblage coincide with large changes in environmental attributes. NS=Not a significant association and a small Mantel r. Italics indicates this variable is included in the environmental matrix used in simple and partial mantel tests in Figure 4.4.

	Tributaries					Mainstem	Mainstem+Tribs
Variables or System	Bigelow	Cedar	Brooks	Mill	Crane	Muskegon	Muskgeon+BG,BR,CD
Size/Geomorphic							
Fish/CatchArea	NS	NS	*0.42***	*0.51****	*0.42***	*0.40****	*0.72****
Invert/CatchArea	NS	NS	*0.27***	*0.41****	*0.50****	*0.43****	*0.53****
Fish/Qlow	*0.92*[!]	NS	*0.68****	*0.42****	*0.33***	*0.48****	*0.75****
Invert/Qlow	*0.87**	NS	*0.48****	NS	0.52***	*0.33****	*0.52****
Fish/Link	*0.82*[!]	NS	*0.45****	*0.42****	*0.45***	*0.44****	*0.72****
Invert/Link	*0.82**	*0.41****	*0.23***	*0.37****	*0.49****	*0.54****	*0.53****
Fish/Ch. Shape	*0.56*[!]	NS	*0.66****	*0.40****	*0.43****	*0.21****	*0.71****
Invert/Ch. Shape	*0.68**	NS	*0.44****	*0.27***	*0.45****	*0.22****	*0.53****
Habitat							
Fish/ Substrate	NS	NS	NS	*0.40***	NS	*0.25****	NS
Invert/ Substrate	NS	*0.47***	NS	*0.32***	*0.44****	*0.51****	*0.31****
Fish/IGUs	NS	*0.28***	*0.31***	*0.66****	*0.39***	NS	NS
Invert/IGUs	NS	*0.62****	NS	*0.39***	*0.34****	NS	NS
Chem/Temp							
Fish/Nutrients	*0.55*[!]	*0.37***	*0.32***	*0.53****	NS	*0.49****	*0.64****
Invert/Nutrients	NS	*0.51****	0.41**	*0.30***	NS	*0.31****	*0.38****
Fish/Temp	*0.90**	*0.34***	*0.36***	*0.35***	*0.25***	*0.45****	*0.69****
Invert/Temp	*0.95**	*0.44****	0.21*	*0.23**	*0.32***	*0.32****	*0.48****

*p<0.10 **p<0.05, ***p<0.01, ****p<0.001; [!]Likely not significant because of low power of test due to small number of sites.

Table 4.6: Direct, indirect, and total effects of watercourse distance on fish and invertebrate (invert) assemblage composition based on path diagrams in Figure 4.4. If a path coefficient in Figure 4.4 was not statistically different than zero, the path coefficient was set to zero for these calculations. Direct effects are the effect of watercourse distance on biological assemblage composition similarity while accounting for similarity of the riverine environment, indirect effects are the effect between watercourse distance and biological assemblage composition similarity mediated through environmental similarity, and total effects is the sum of the direct and indirect effects. Across all systems, on average 77% of the watercourse distance effect on fish and 84% of the watercourse distance effect on invertebrate assemblage composition could be attributed to the environment.

	Tributaries					Mainstem	Mainstem+Tribs
Assemblage or System	Bigelow	Cedar	Brooks	Mill	Crane	Muskegon	Muskgeon+BG,BR,CD
Fish Assemblage							
Direct effect	0 (0%)	0 (0%)	0.24 (43%)	0.21 (41%)	0 (0%)	0.07 (27%)	0.27 (53%)
Indirect effect	0.55 (100%)	0.13 (100%)	0.31 (57%)	0.30 (59%)	0.21 (100%)	0.19 (73%)	0.24 (47%)
Total effect	0.55	0.13	0.55	0.51	0.21	0.26	0.51
Invert Assemblage							
Direct effect	0 (0%)	0.40 (48%)	0 (0%)	0 (0%)	0 (0%)	0.24 (64%)	0 (0%)
Indirect effect	0.56 (100%)	0.43 (52%)	0.23 (100%)	0.24 (100%)	0.38 (100%)	0.14 (36%)	0.25 (100%)
Total effect	0.56	0.83	0.23	0.24	0.38	0.38	0.25

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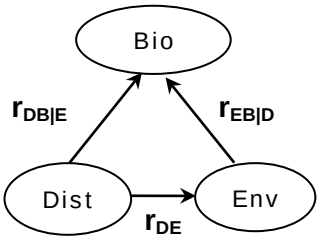
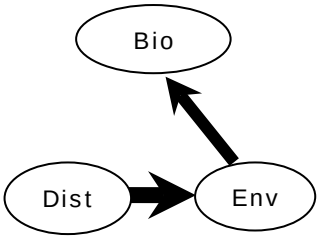
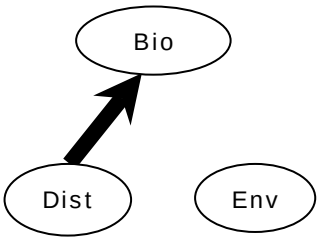
	Path diagram:	Ecological Interpretation and Implications:	Statistical Interpretation and Implications:
Analytical Template:		Diagram represents potential causal associations between dissimilarities in biological assemblage (Bio), environmental variables (Env), and geographic distance (Dist). Heads of arrows indicate assumptions of causal direction. Arrow weight shows magnitude of the association and which control pathways dominate in an ecological system.	Distance approach: Input data are dissimilarity/distance matrices based on raw data $\mathbf{r_{DE}}$ = Simple Mantel test between Dist and Env, $\mathbf{r_{DB\|E}}$ = Partial Mantel test between Dist and Bio, after accounting for Env, and $\mathbf{r_{EB\|D}}$ = Partial Mantel test between Env and Bio, after accounting for Dist. Weight of arrows is proportional to the magnitude of simple and partial mantel correlations.
Environmental Control hypothesis:		Landscapes are mosaics where species composition is controlled by environmental site characteristics. Positive Spatial Autocorrelation (SAC) in biological assemblages is caused by SAC in environmental variables. Dispersal limitations do not contribute to SAC in biology.	Account for SAC in biological variables by including all necessary environmental variables in models. If residual SAC persists after the modeling process, model needs to include additional explanatory variables; dispersal is not important and residual SAC is caused by unaccounted for environmental variables. If spatially structured environmental variables control biological assemblages, methods that control for SAC first will mask important environmental effects.
Neutral Control hypothesis:		All species are demographically and competitively equal. SAC in biological assemblages is caused by ecological processes such as dispersal, and spatial dependence on underlying environmental variables is not present.	Variation in biological assemblages is explained by variation in geographic, but not environmental distances. Use spatial models to account for dispersal affects and then explore residual environmental/biology associations. Neighboring sites are not statistically independent of one another and traditional statistical tests are too liberal.

Figure 4.1: Diagram illustrating simple and partial mantel test results under the extremes of complete environmental versus complete neutral control of biological assemblages. These extremes are presented for illustrative purposes with the acknowledgement that biological assemblages may be controlled by a combination of these processes. Both ecological and statistical interpretation and implications are addressed.

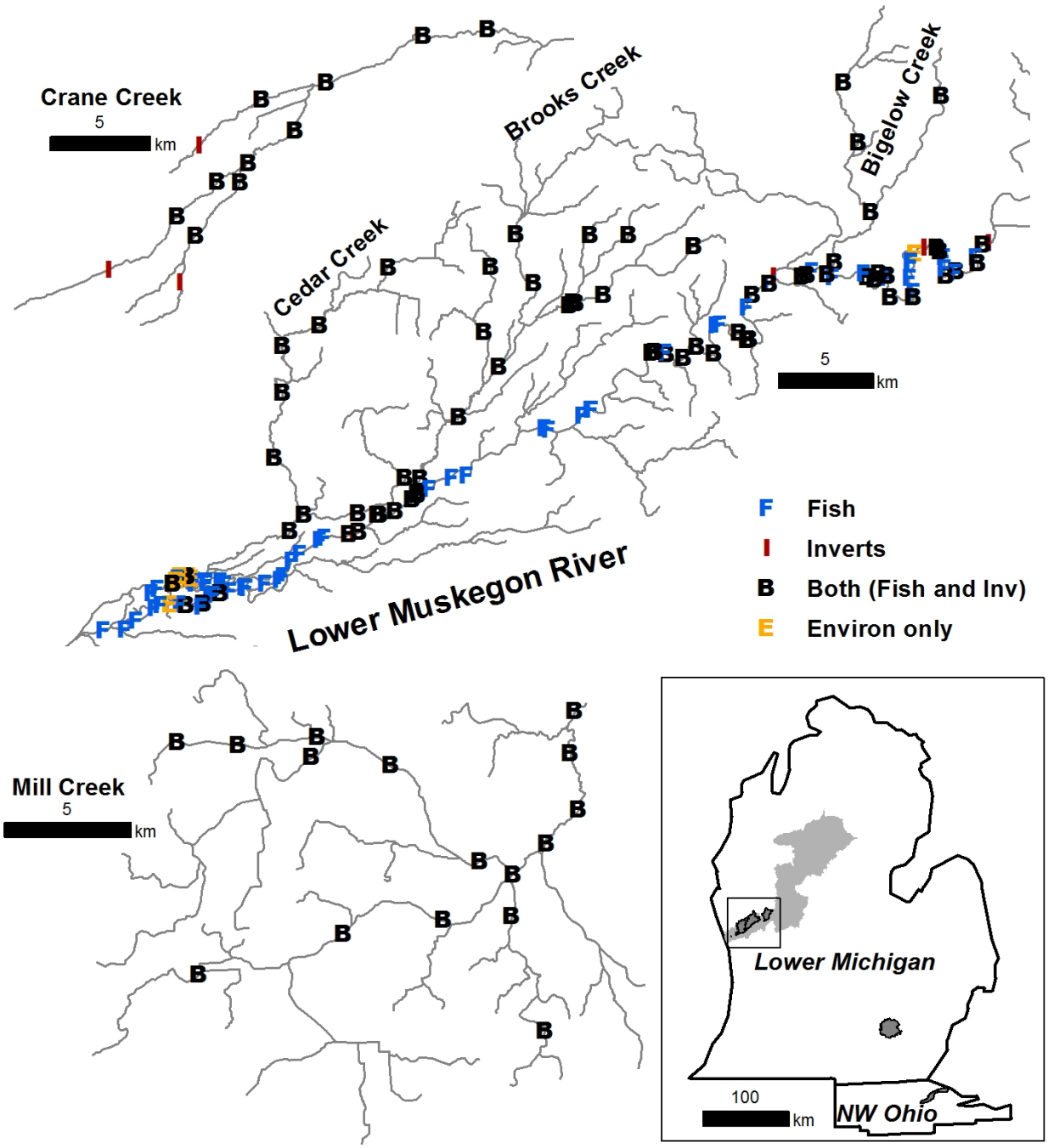


Figure 4.2: The seven study systems and sampling sites. The lower right inset shows the location of the six systems in Michigan's Lower Peninsula and Crane Creek in Northwestern Ohio. The entire Muskegon River basin is also shown, although only the lower third of the basin (indicated with a box in inset) was included in this study. Watercourse maps show the location of sites within a system, with letter markers indicating the type of biological samples collected at a site (i.e., both fish and invertebrates, fish only, invertebrates only, or no biological sample). All sites on Crane Creek were sampled for fish, but fish were not present during sampling at the three sites marked invertebrate only. Environmental data were collected at all sites in all systems. For simplicity, not all 1st order streams are illustrated.

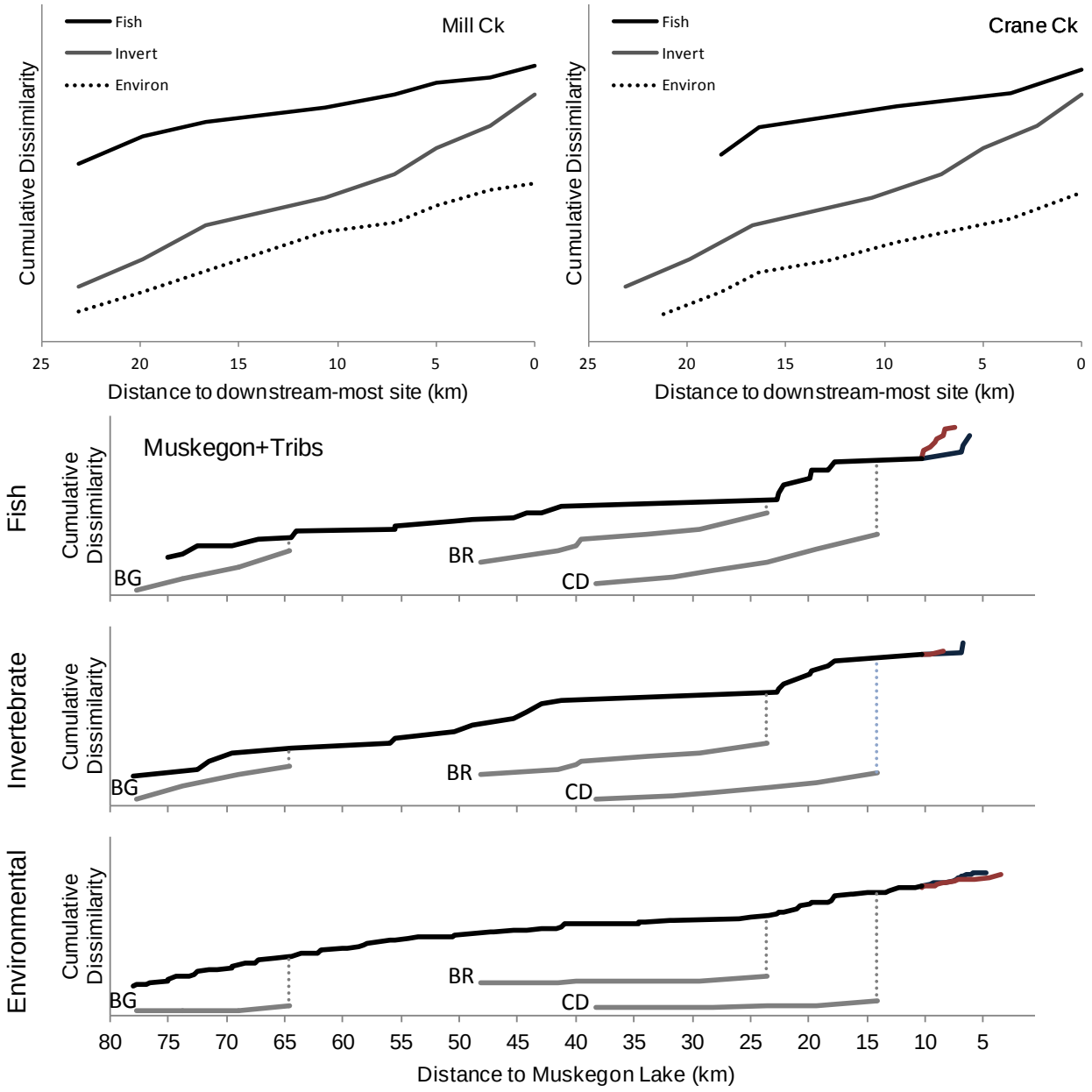
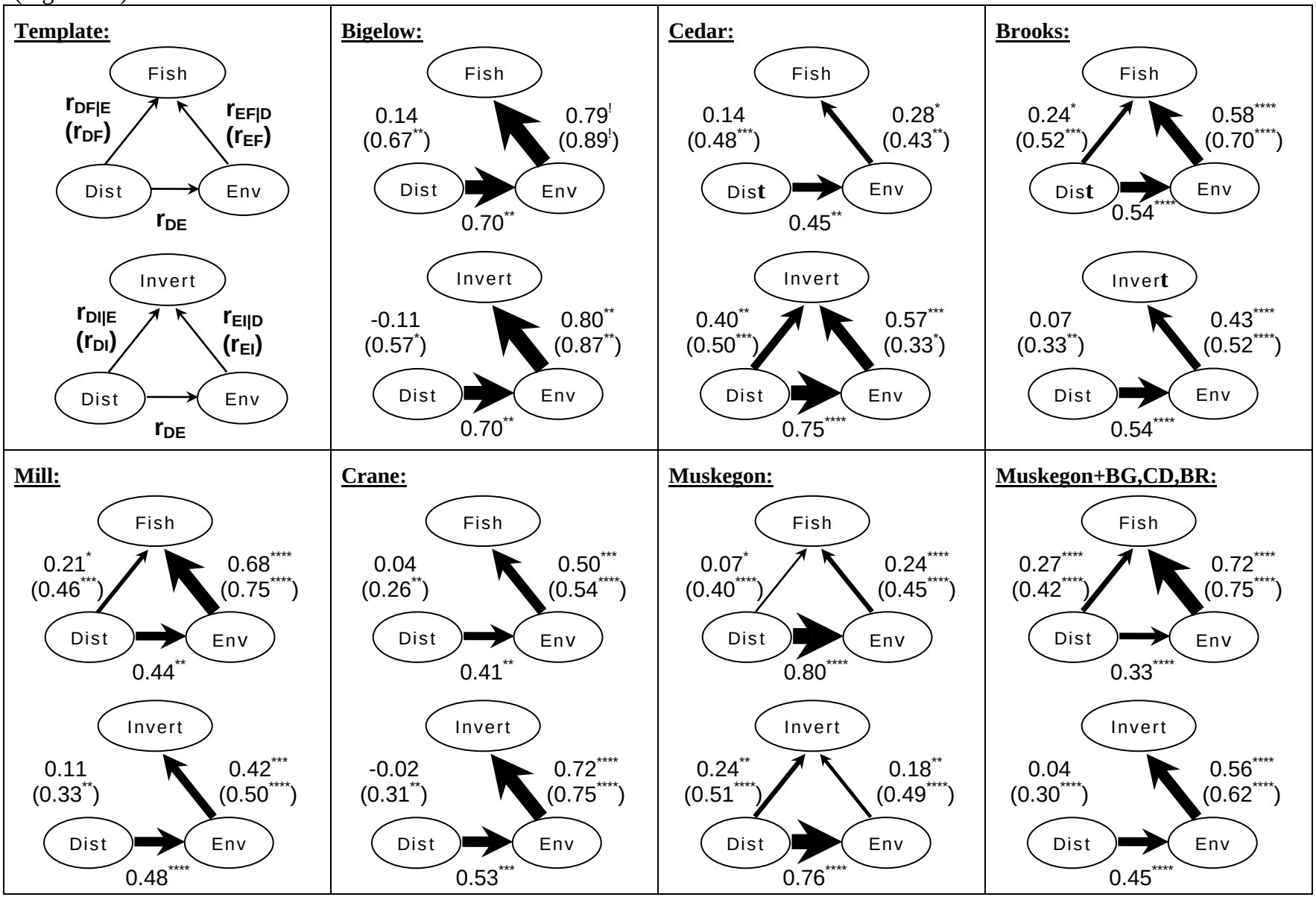


Figure 4.3: Trajectory plots of cumulative dissimilarity along river flow watercourse. Slope at any position on the lines indicates rate of change. These plots illustrate differences in rates of change along the headwaters to mouth trajectory, as well as concordance between fish and invertebrate assemblages, and between biological assemblages and environmental character. The upper plots are for Mill Creek and Crane Creek and include fish, invertebrates, and environmental change in one plot. The bottom three plots show cumulative dissimilarity versus distance from Muskegon Lake for the Muskegon mainstem and confluent tributaries. For clarity, fish, invertebrates, and environmental variables are displayed in separate plots and Bigelow (BG), Brooks (BR), and Cedar (CD) Creeks are offset from the mainstem. After the mainstem splits into two branches, the north branch is shown in red and the south branch in dark blue.

Due to size, figure follows on the next page.

Figure 4.4: Path diagrams of each study system based on simple and partial Mantel tests. In most systems, associations for fish and invertebrates (Invert) are similar within a system; environmental variables (Env) are spatially structured, Env accounts for most or all of the association between watercourse distance (Dist) and biological assemblages, and Dist accounts for little of the association between Env and the biological assemblage. The Env matrix includes only the variables associated with the biological assemblage (i.e., variables in italics in Table 4.4). The weight of an arrow is directly proportional to the magnitude of the Mantel r for statistically significant partial mantels and Dist Env associations. Simple mantels corresponding to partial tests are included in parentheses for comparison purpose, but do not affect the weight of arrows. *$p<0.10$ **$p<0.05$, ***$p<0.01$, ****$p<0.001$.

(Figure 4.4)


Template:
Fish
$r_{DF|E}$ (r_{DF})
$r_{EF|D}$ (r_{EF})
Dist
Env
r_{DE}
Invert
$r_{DI|E}$ (r_{DI})
$r_{EI|D}$ (r_{EI})
Dist
Env
r_{DE}
Bigelow:
Fish
0.14 (0.67**)
0.79^I (0.89^I)
Dist
Env
0.70**
Invert
-0.11 (0.57*)
0.80** (0.87**)
Dist
Env
0.70**
Cedar:
Fish
0.14 (0.48***)
0.28* (0.43**)
Dist
Env
0.45**
Invert
0.40** (0.50***)
0.57*** (0.33*)
Dist
Env
0.75****
Brooks:
Fish
0.24* (0.52***)
0.58**** (0.70****)
Dist
Env
0.54****
Invert
0.07 (0.33**)
0.43**** (0.52****)
Dist
Env
0.54****
Mill:
Fish
0.21* (0.46***)
0.68**** (0.75****)
Dist
Env
0.44**
Invert
0.11 (0.33**)
0.42*** (0.50****)
Dist
Env
0.48****
Crane:
Fish
0.04 (0.26**)
0.50*** (0.54****)
Dist
Env
0.41**
Invert
-0.02 (0.31**)
0.72**** (0.75****)
Dist
Env
0.53***
Muskegon:
Fish
0.07* (0.40****)
0.24**** (0.45****)
Dist
Env
0.80****
Invert
0.24** (0.51****)
0.18** (0.49****)
Dist
Env
0.76****
Muskegon+BG,CD,BR:
Fish
0.27**** (0.42****)
0.72**** (0.75****)
Dist
Env
0.33****
Invert
0.04 (0.30****)
0.56**** (0.62****)
Dist
Env
0.45****

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Chapter 5 : Dissertation conclusions and synthesis

Summary of research chapter conclusions

The common theme of this dissertation is the nature and genesis of ecological spatial pattern in rivers. Each of the three studies contributed to a better understanding of spatial patterning in river ecosystems. By framing much of the exploration of spatial pattern in the context of ecological units (EUs), I was also able to fulfill my personal aspiration of carrying out dissertation research with clear management applications.

In Chapter 2, the theoretical assumptions underlying EUs were validated in a study of the mainstem of the Muskegon River. An extant delineation was evaluated and deemed effective, particularly for fish. I found moderate fish/invertebrate assemblage composition concordance, frequent environment/biology concordance, and distinct, homogeneous biological assemblages in this section of the Muskegon River. These characteristics persisted through time. Although there were many common biological associations with measures of stream size, reflecting the primary longitudinal gradient, there were also some differences in the spatial patterning of fish and benthic invertebrate assemblages. These differences suggest substrate may be more important for invertebrate community structure and temperature more important for fish community structure. Within a delineated EU, concordance assumptions were not met suggesting smaller than valley-segment scale units would be unfounded. Biological assemblages in confluent tributaries were quite distinct from, and more varied than, those in the river mainstem.

In Chapter 3, the theoretical assumptions underlying EUs were only partially supported across a set of five different headwater tributary systems. The effectiveness of existing EU

delineations varied with longitudinal location in the tributary system. There were very strong fish/invertebrate concordances and frequent environment/biology concordances, but distinct homogeneous biological assemblages existed only in higher order/downstream channels with substantial streamflow (Q). Biological assemblages in headwaters varied and noncontiguous sites were just as likely to have similar assemblage composition as were adjacent sites. The sites in extreme headwaters (i.e., the smallest permanent channels) had depauperate biological assemblages composed of taxa also found downstream in the tributary system. This suggests 1) a need for better understanding of spatial pattern and process in headwater units and/or 2) the delineation of shorter EUs in the headwaters to better reflect the scale of observed heterogeneity.

In Chapter 4, I looked more closely at how spatial pattern in rivers arise. Rates of change varied by location in the river network, both along a river mainstem and along a headwaters to river mouth trajectory; rates of change and variability in the rates of change generally decreased with downstream position in the network. I measured strong positive spatial autocorrelation (SAC) in the riverine environment and in biological assemblage composition, but also sharp transitions that reduce overall SAC magnitude if the spatial extent was expanded to include tributary/mainstem transitions. Strong environment/biology associations accounted for most or all of the SAC observed in biological assemblage composition, offering strong support for niche processes and species sorting in a diverse environment as the origin of within basin SAC in river biological assemblage composition. Likewise, proximity effects on biological assemblages were largely mediated through similarity in the environment.

Dissertation synthesis

Four major conclusions can be drawn from this dissertation. These conclusions include 1) observed spatial patterns were consistent regardless of the measure of the biological assemblage,

2) sampling regime and spatial extent can affect study conclusions, 3) environmental pattern in rivers create within-basin biological spatial pattern (i.e., the environmental template dominates), and 4) ecological units are real and mapping them can be an effective tool for river management, especially in downstream river segments. I will address these conclusions sequentially in the following sections.

Different biological assemblage measures, same spatial patterns

In Chapters 2 and 3 I tested the assumptions underlying EU delineation using three different measures of the biological assemblage, 1) occurrence, 2) abundance, and 3) biomass (Note: for brevity, only occurrence based analyses were typically included in the results). The three data measures were highly concordant, and all led to the same conclusions about EU assumptions and longitudinal spatial patterns. This insensitivity to the type of data measure has been previously reported in studies on benthic macroinvertebrates (Marshall et al. 2006) and freshwater mussels (Miller and Payne 1993). Likewise, Gauch (1982) suggested that most of the pattern in assemblages over *large spatial scales* can be represented by differences in the presence/absence of taxa. I concur that rigorously-collected occurrence data are sufficient for understanding spatial pattern in rivers *at all spatial extents*.

While qualitative sampling of rivers assemblages may be sufficient to describe a river's large-scale spatial patterning, there is still need for some caution. In my datasets, fish and invertebrate occurrence measures were developed from quantitative and semiquantitative sampling respectively, so sampling effort was equivalent for both occurrence and abundance measures. This may not always be the case if differences between qualitative and quantitative sampling result in different rates or proportion of taxa detection.

In addition to being quantitative, sampling was also of high-spatial-frequency, helping to identify unusual sites/samples. There were rare occasions where the invertebrate assemblage at a site was more appropriately described by the abundance/biomass measures. For example, downstream in the anabranching channels of the lower Muskegon, shifting sand is the dominate substrate and is associated with a unique, low-diversity benthic assemblage (Soluk 1985, Palmer 1990). Within these channels, however, infrequent snags can catch woody debris/leaves and create uncommon microhabitats that support benthic fauna commonly found upstream on hard substrate. At the site level, the occurrence measure gives these extremely rare taxa equivalent weight in assemblage composition, while the abundance measure properly accounts for their rarity. The high-spatial-frequency sampling design used in these studies contained replicate sites in ecologically homogeneous river segments of similar ecological character. This facilitated quick recognition of unusual microhabitats and provided an explanation for differences in occurrence, abundance, and biomass assemblage measures.

Spatial extent and sampling regime can affect concordance and SAC

This dissertation presents several examples of how a particular sampling regime and/or spatial extent can affect study conclusions. The longitudinal, high-spatial-frequency sampling regime used in this dissertation was necessary to test the hypothesis that river segments had homogeneous ecological character and to evaluate the transitions between unit boundaries. Not surprisingly, the results of statistical analyses of the validity of ecological units varied with spatial extent. Within a single EU, I found no fish/invertebrate concordance nor many strong environment/biology concordances. But delineated EUd did partition a river segment with homogenous ecological condition and this ecological condition was distinct from that in adjacent units. When spatial extent was expanded to include tributaries confluent with the Muskegon

mainstem, large differences between biological assemblages in tributary systems and the entire mainstem were evident, and the mainstem appeared to have more comparatively more homogenous biological assemblages.

I also found spatial extent could affect the strength of observed SAC in ecosystems exhibiting patchy or patchy gradient spatial patterns. Measures of overall SAC in environmental variables in the Muskegon mainstem were very strong, indicating proximity was strongly associated with similarity in environmental condition. However, when the spatial extent was expanded to include confluent tributaries, SAC was still observed, but with greatly reduced magnitude. Does this imply proximity is not as effective a predictor of similarity at large spatial extents? If there was no additional information available to describe spatial pattern in rivers, this may be a logical conclusion. But I also showed there are marked difference between tributaries and the mainstem and the tributary/mainstem confluence marks a rapid change in ecological condition over a very short distance. The reduction of SAC when the tributaries were included, tells us less about the effects of proximity on biological pattern and more about the effects of river network structure.

This dissertation also demonstrated sampling regime may affect the ability to detect concordance between different riverine biological assemblages. In all study systems sampled in this dissertation, there was strong concordance between fish and invertebrates, i.e., shifts in fish and invertebrate assemblage composition occurred jointly. The pervasiveness of this concordance and its strength (especially in tributaries) contrasts with many studies in the literature showing little to no concordance between biological assemblages. Some differences in observed concordance are likely from differences in sampling regimes. High, concordance was typically observed when streams were sampled longitudinally or streams varied substantially in

size. When sampling was restricted to streams of the same size, no concordance was observed. Since longitudinal size gradients were one of the main causes of concordance in my dissertation research, it may be that sampling regimes which exclude this gradient are unlikely to observe strong concordance between biological assemblages.

Physical pattern in rivers create patterns in the biological data

My dissertation offers strong evidence that most of the spatial pattern in biological assemblage composition within a river basin can be explained by longitudinal patterning of the river's physical environment. This patterning affects rates of ecological change within river systems and supports the validity of EU delineations based on hydrogeomorphologic spatial units.

Numerous other studies have also illustrated the importance of confluences in river ecosystems (Osborne and Wiley 1992, Rice et al. 2001, Benda et al. 2004a, 2004b, 2006, Kiffney et al. 2006). The frequency and arrangement of confluences affects rates of change in riverine environments and biological assemblages. In river networks, confluence points provide the physical opportunity for new hydrologic, chemical, and biological inputs and downstream regimes. However, the effect of each particular confluence varies with position in the network and the size ratio of the confluent streams. In the headwaters of most Midwestern streams, discharge grows rapidly through increases in catchment size and frequent additions of similarly sized low-order streams draining different landscapes. The result is rapid change in the environment and corresponding biological assemblage composition over short distances (Horton 1945). In contrast, downstream in the network, confluences with comparably sized channels are rarer; the frequent entry of smaller channels that do occur have little distinguishable effect on flow, chemistry, or the hydraulic character of the larger channel (Benda et al. 2004a, 2004b,

Kiffney et al. 2006). Accordingly, rates of change in environmental character and biological assemblage composition were lower downstream in rivers.

Tributary/mainstem confluences also mark large discontinuities in riverine environments and biological assemblages. By using varied spatial and temporal extents in my analyses, I was able to illustrate that spatial discontinuity at tributary/mainstem confluences persists across seasons and years. Although I expected this for macroinvertebrates, I was surprised strong discontinuities in fish assemblage composition persisted despite anadromous fishes' seasonal use of tributaries for spawning.

Concordant changes in river size, shape, and water temperature and fish and invertebrate assemblage composition were the most frequent and compelling biology/environment associations. These associations support delineation of valley-segment scale EUs based on stream size and hydrogeomorphic patches (Seelbach et al. 1997, 2006, Thorp et al. 2008). Because of its glacial history, the Midwest has tremendous heterogeneity in landform and hydrology that produces spatial variation in biotic assemblages (Seelbach and Wiley 1997, Zorn et al. 1998, 2002). In Michigan streams, measures of stream size including catchment area and low-flow yield link catchment-scale features of the landscape to multiple, site-specific characteristics of stream habitat (e.g., temperature, velocity, and depth) important to fishes. Hydrologic differences create varied temperature regimes, which also contributes to variation in biotic assemblages (Hawkins et al. 1997, Wehrly et al. 2003, 2006). The study systems investigated in this dissertation illustrated both within-system and between-system variation in size, geomorphic character, and hydrologic and thermal regimes.

By including a variety of tributary systems, I was also able to note certain environmental variables can produce distinct ecological units in different ways in different stream systems.

Differences in hydrologic and temperature regime created variation in fish assemblages in both Bigelow Creek, the most pristine tributary system, and Crane Creek, the most degraded tributary system. In Bigelow Creek, an intermittentantly flowing eastern channel originates from a small lake outflow and travels through a wetland complex before joining the main channel, a stable, cold, groundwater stream. Despite being physically close (<5 km overland distance), the diverse warm-water fish assemblage in the eastern channel bore no resemblance to the cold-water fish community in the main channel. This creates a clear EU boundary at this confluence. In Crane Creek, the upper headwaters were extremely harsh aquatic environments of unshaded agricultural ditches with intermittent flow and huge diel oxygen and temperature swings. Immediately following their confluence, the stream was shaded by riparian vegetation and diel oxygen and temperature swings were moderated. Although the available fish species pool was the same in all three stream segments, sites in the upstream-most segments had on average three fish species, while the segment downstream of the confluence had on average nine fish species. This creates a clear EU boundary at the confluence. These system-specific effects of the same environmental variables, suggest personal knowledge of local conditions, biological assemblages, and controls on assemblage composition should supplement computer-driven delineation of EUs (Brenden et al. 2008).

Utility of ecological units (EUs)

In this dissertation I closely examined the validity of two assumptions which lay behind the mapping and classification of EUs: 1) concordance between different biological assemblages and 2) concordance between the biological community and the physical environment. I observed strong concordance within river headwater systems and at transitions between tributaries and the Muskegon River mainstem, and moderate concordance along the Muskegon mainstem. My

results highlight the appropriateness of the term “ecological” when referring to these physically distinctive channel units. Although there was some evidence that invertebrates were more sensitive to substrate, and fish more sensitive to temperature, both assemblages were associated with environmental changes caused by network confluences and longitudinal gradients in rivers.

The validity of third assumption of ecological units, the existence of ecologically distinct, homogeneous river segments, was validated in all but the headwaters of river systems, where EU delineation itself was problematic. Despite high-spatial-frequency sampling, I was not able to identify contiguous, distinct environmentally and biologically homogenous river lengths within river headwater systems. However, if the spatial pattern in river headwaters is a patchy gradient like the rest of the river system, then headwater EUs would exist, although they would necessarily be many and short. Ascertaining whether spatial pattern in headwaters is best described by a gradient or a patchy gradient requires even higher-spatial-frequency sampling than I used in these studies; it would require replication of sites within stream segments and bracketing of each network juncture, or continuous sampling (as in Torgersen et al. 2006).

Therefore, my research supports the contention that EUs really do exist as map-able channel units in river systems. As such, they are useful basic units for mapping, inventory, and classification of river systems (Seelbach et al. 1997, Seelbach et al. 2006, Melles et al. 2014). EUs provide a convenient way to generalize and communicate about recurring ecological processes and resultant patterns we see in river ecosystems (Rowe 1961, Levin 1992). EUs within rivers should function like strata in a statistical design sense, and mapped EUs should be accounted for in sampling regime and study design. Furthermore, classification of EUs into groups with similar characteristics (i.e. typing) might allow extrapolation from representative samples or models to unsampled or under-sampled units, and guide management actions in areas

where data are scare. However, because of their small size, EUs in the headwaters may be of less utility than EUs in lower longitudinal positions in the network.

Management application example

One aspiration of my dissertation was to perform research with clear ecological management applications. To conclude this dissertation, I will illustrate how several findings of this dissertation can be applied to a specific ecosystem management need: effectively assessing the biological condition of a watershed. Let's assume that in this watershed EUs have been delineated on recognized hydrogeomorphic patches and influential stream network junctures, and the delineated EUs have been attributed with network (e.g. stream size, link, order, etc.), flow and temperature regime, and catchment (land use/land cover, surficial geology, groundwater potential, etc.) measures. Also assume that based on these attributed measures and established statistical relationships between these measures and biological assemblages, EUs have subsequently been classified into EU types. To simplify this example I will contrast sampling in the headwaters and the mainstem, although bioassessment sampling should occur across streams of all sizes. Based on decreasing rates of change and reduced variability in rates of change along a river network, in the headwaters there are many, short EUs described by numerous ecological types and in the mainstem there are a few, long EUs described by just a few ecological types. If a watershed bioassessment is to truly represent the condition of the entire catchment, sampling sites should be stratified by ecological type and distributed in proportion to the ecological types within the watershed. This requires a sampling regime with many, possibly proximal sites in the headwaters and few, distant sites along the mainstem.

Once sampling is complete, regional normalization models can be developed to assess variation in biological condition metrics (e.g. species richness, EPT taxa, percent intolerant taxa)

caused by both natural variation within the watershed and human-influenced change in the watershed (Wiley et al. 2003, Baker et al. 2005, Riseng et al. 2010). Normalization is founded on the premise that location-specific reference conditions can be modeled using large-scale features because these features are important controls on the ecological character of a stream. Models predicting biological assemblage condition from large-scale features can include both non-stressors (e.g. catchment area, surficial geology, stream temperature) and stressors (e.g. urban land use, proximity to dams, proximity to NPDES site). When models include anthropogenic controls such as urban or agricultural land uses, these can be set to zero to produce site-specific reference conditions even if "true" reference sites do not exist in the current landscape. Deviations from reference condition are normalized (or scaled) by accounting for natural variation of the metric and a measure of the model fit.

Because each EU is fully attributed with measures that are known to control biological assemblages, EUs serve as the basic unit on which such normalized models are constructed and on which model results can be displayed. For example, impact levels (e.g. levels from not impacted to highly impacted) can be mapped onto the EU system illustrating both local and watershed-wide assessment of a river's ecological condition. Likewise, by using the EU system with normalized models, the spatially-explicit effects of future land development on riverine health can be explored (as in Wiley et al. 2010).

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