STREAMS IN A CHANGING LANDSCAPE:

IDENTIFYING CANDIDATE REFERENCE REACHES TO ASSESS THE PHYSICAL AND BIOTIC INTEGRITY OF MISSOURI'S WADEABLE STREAMS

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ABSTRACT

North American freshwater resources have grown increasingly imperiled as a result of human-induced landscape alterations, with many present-day stream fish and aquatic invertebrate communities representing only a fraction of their historic constituents. Consequently, resource managers need the ability to predict areas of high and low biological integrity to inform management decisions and meet conservation needs. Previous efforts to quantify anthropogenic disturbances to aquatic systems have resulted in indices lacking the ability to identify specific stressor impacts, describe the ways stressors alter the physical and chemical condition of receiving waters, and predict biological integrity. Thus, the need exists for a flexible, quantitative approach to characterizing stream impairment and identifying candidate least-disturbed stream reaches to serve as benchmarks for high quality physical habitat and biological integrity. After accounting for natural sources of biological variation (e.g., watershed drainage area, reach gradient, spring density), we used boosted regression trees to model the influence of channel morphology, substrate, cover, and water quality, and watershed-level flow modification and fragmentation, urbanization, agriculture, and point-source pollution on stream fish and aquatic macroinvertebrate community characteristics of wadeable streams of Missouri. Reach-level environmental predictors explained between 8% and 46% of the variation in our ten biotic metrics, with channel morphology and water quality metrics consistently accounting for more variation than substrate or cover. Biotic metrics related to stream health (e.g., Ephemeroptera, Plecoptera, Trichoptera richness, native lithophilic fish species richness) increased with bankfull width/depth ratios and dissolved oxygen, and decreased with increasing total chlorophyll. Watershed-level environmental predictors accounted for between 4% and 51% of the variation in biotic metrics, with stream health metrics increasing with forest cover, and decreasing with increased densities of headwater impoundments, road crossings, and pasture lands. In general, invertebrate metrics showed higher sensitivity to row-cropping and water quality impairment than did fish, particularly in the Ozark Highlands aquatic subregion. We used the results of our watershed-level models to predict biotic metric values to over 28,000 wadeable stream reaches across the state and rescaled and summed individual metric scores to generate an overall estimate of biological integrity at each site. We identified streams scoring in the top 95th percentile of each stream size class and aquatic subregion to serve as regional candidate least-disturbed reference reaches predicted to exhibit relatively high quality habitat and biotic conditions. Our method represents a novel approach to characterizing and forecasting stream impairment, and represents a critical step in refining existing biological indices, developing a companion physical habitat index, and ultimately conserving the diversity and integrity of Missouri's flowing waters.

KEYWORDS: *Streams, Anthropogenic Disturbance, Biological Integrity, Conservation*

DESCRIPTION OF CHAPTERS

The primary chapters of this thesis have been written and formatted as independent manuscripts for submission to peer-reviewed journals, hence the inclusion of chapter-specific abstracts, acknowledgements, and literature cited sections. For this same reason, the reader will encounter a fair amount of redundant introductory information and site descriptions throughout the document. To ensure clarity and continuity, a general introduction, a brief discussion on headwater stream conservation, and overall conclusions and conservation implications have been included.

GENERAL INTRODUCTION

FLOWING WATERS: DIVERSITY AND STATUS

Flowing waters have sustained human civilizations for millennia, and we continue to rely heavily on these water bodies for numerous uses, including food and drinking water supply, crop irrigation, hydroelectricity, freight transport, waste removal, and recreational opportunities (Allan 1995). Beyond these ecosystem services, streams and rivers themselves are incredibly diverse and valuable ecosystems. Despite representing only one-hundredth of a percent of the Earth's total water, approximately one-third of all vertebrate species, including over 40 percent of the nearly 28,000 described fish species, reside in freshwater systems (Shiklomanov 1993; Dudgeon et al. 2006; Nelson 2006; Jelks et al. 2008). The United States, considered a hotbed of temperate freshwater diversity, contains more than ten percent of all freshwater fish species, 30 percent of mussel species, and over 60 percent of the world's freshwater crayfish species (Williams et al. 1993; Warren and Burr 1994; Taylor et al. 1996), with over 210 species of fish and nearly 70 mussel species described in Missouri alone (Oesch 1995; Pflieger 1997).

The diversity of fluvial systems is largely owed to the dynamic, and spatially complex processes occurring in both their immediate channels, and adjacent riparian zones and floodplains (Fausch et al. 2002; Wiens 2002). These "riverine landscapes" are characterized by an intricate, shifting mosaic of successional habitats, and support many obligate terrestrial and aquatic species (Robinson et al. 2002). The critical role of river-floodplain connectivity in maintaining both the structure and function of aquatic ecosystems is well recognized (Ward and Stanford 1995), and increasingly more studies are documenting the importance of reciprocal energy flows from streams to their riparian areas in maintaining terrestrial species diversity and abundance (Baxter et al. 2005).

Over the last several decades, ecologists have pursued investigations regarding the influence of landscape-level processes on flowing waters, and have increasingly viewed stream health within a greater watershed context (Hynes 1975). This intricate coupling of flowing waters with their surrounding landscapes, however, leaves them extremely vulnerable to human activity. It is now apparent that landscape alterations and/or direct modification of stream channels and flows often have negative downstream effects on aquatic biota (Allan 2004). Consequently, North American freshwater resources have grown exceedingly imperiled, and many present-day aquatic communities likely represent only a fraction of their historic constituents (Malmqvist and Rundle 2002). During what is described as a freshwater biodiversity crisis, the last century has witnessed the extinction of over 120 North American freshwater species, a rate more than five times that of terrestrial fauna (Ricciardi and Rasmussen 1999; Abell 2002). Currently, over 700 North American fish species spanning 133 genera and 36 families are listed as either endangered, threatened, or vulnerable, representing a 92 percent increase since 1989 (Jelks et al. 2008). A similar trend exists in Missouri, where nearly a third of fishes and over half of all mussel species are listed as either imperiled or of conservation concern (Missouri Natural Heritage Program 2013). Factor in the pervasive threats of global climate change, invasive species, and the need to balance biodiversity conservation with increasing demand for water and other valuable ecosystem services and it becomes evident that conservation and restoration of flowing waters is a tremendous natural resources challenge (Malmqvist and Rundle 2002; Dudgeon et al. 2006).

A CHANGING LANDSCAPE

Stream habitat and biotic composition result from a series of complex, hierarchical interactions between broad climatic and geological conditions and finer scale physical and ecological processes (Frissell et al. 1986; Montgomery and Buffington 1997; Figure 1). Precipitation, basin morphology, bed composition, and riparian vegetation interact to influence the supply of water, sediment, and woody debris needed for channel formation and maintenance (Montgomery and MacDonald 2002) and ultimately determine the physical habitat template upon which biological communities are built (Rabeni 2000). These natural environmental drivers are major determinants of local stream fish and macroinvertebrate assemblage composition, yet their effect is often disrupted by the many adverse, and potentially synergistic effects of widespread human disturbances (Allan 2004; Figure 1). These anthropogenic landscape alteration can influence the hydrology, geomorphology, and chemical condition of the receiving waters, often to the detriment of their biota (Paul and Meyer 2001).

River regulation has resulted in altered flows and fragmented and degraded stream habitats across the globe (Allan 1995), and is considered one of the primary sources of stream impairment in the world. Alterations to a stream's flow regime, often described as a "master variable" limiting the distribution of species, can result in drastic physical and ecological change (Ward and Stanford 1995; Poff et al. 1997). The reduced magnitude and frequency of high flow events caused by dams and diversions may facilitate the invasion of nonnative taxa (Olden et al. 2006), while increased flow stability and water velocity may disrupt species' life cycles and habitat use, particularly those exhibiting drifting larval stages (Scheidegger and Bain 1995; Dudley and Platania 2007). The maintenance of discharge timing is equally important for conserving biodiversity, as many species' spawning and migrations are adapted to predictable variation in flow, such as snow melt or seasonal precipitation (Montgomery et al. 1983; Ward 1998; Dudgeon 2000).

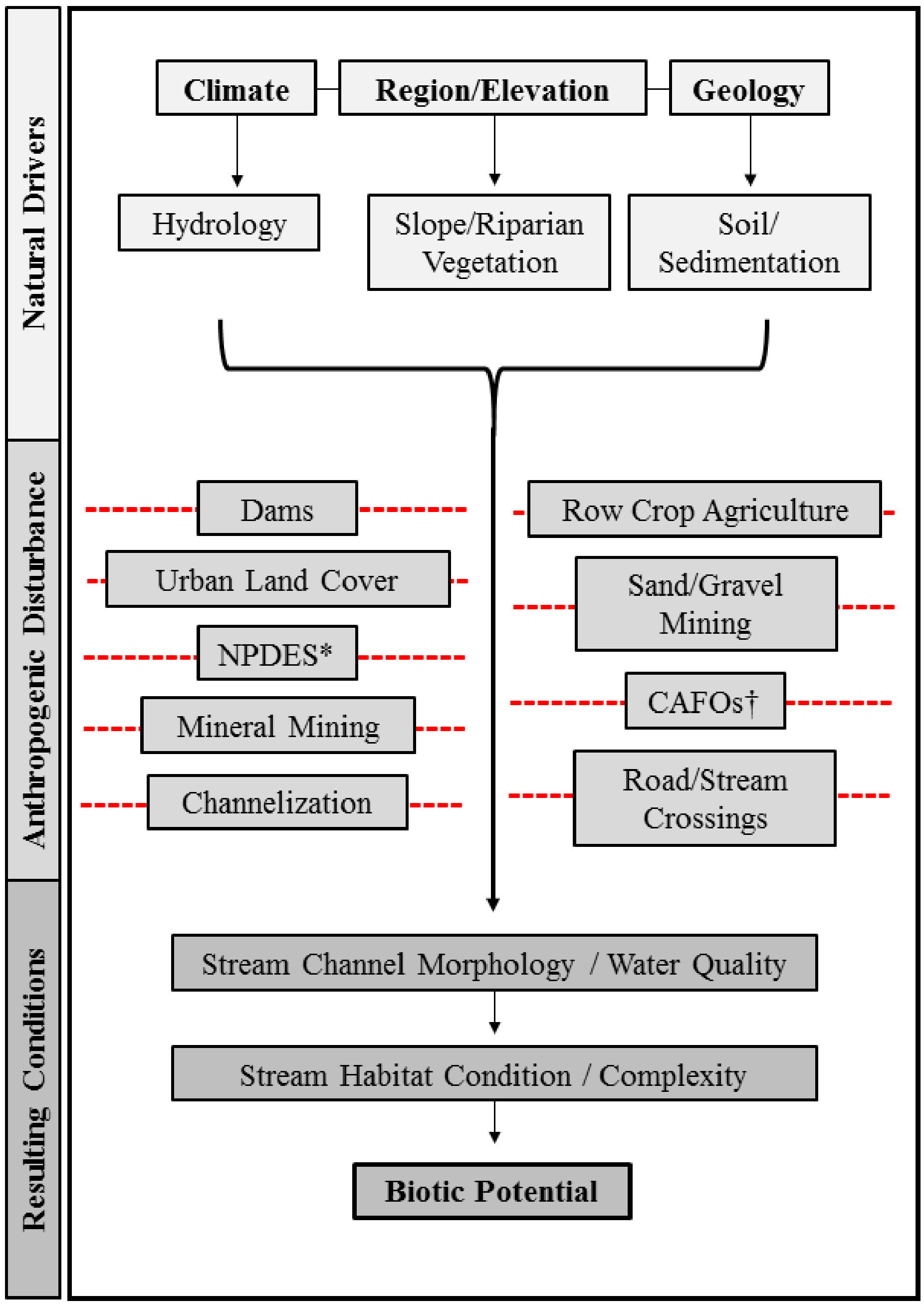

Figure 1. Simplified conceptual framework depicting the natural processes and anthropogenic disturbances contributing to the biotic condition of streams. * National Pollution Discharge Elimination Systems, † Confined Animal Feeding Operations.

Additionally, numerous landscape-level disturbances are capable of altering stream hydrology. Urbanization and watershed imperviousness reduce the infiltration of precipitation, intensifying flood pulses and reducing base stream flows (Arnold and Gibbons 1996; Booth and Jackson 1997; Paul and Meyer 2001). Similarly, streams in agricultural lands may experience reduced base flows due to irrigation withdrawals, and sharper flood peaks resulting from increased runoff due to the lack of ground-cover vegetation (Postel 1998).

Closely linked to these hydrologic processes, stream channel morphology influences species assemblages based on finer-scale habitat requirements (Frissell et al. 1986; Montgomery and Buffington 1997), and much like a stream's flow regime, it can be easily disrupted by anthropogenic landscape alterations (Figure 1). Mining and agricultural activities often remove hillslope and bank stabilizing vegetation and contribute high sediment inputs, resulting in unstable, highly embedded stream channels (Allan 2004). Similarly, stream channelization, instream gravel mining, and riparian deforestation all increase sedimentation while greatly reducing the availability of woody debris critical for channel formation and maintaining habitat heterogeneity (Montgomery and Buffington 1997; Brown et al. 1998). As a result, these degraded channels may be unsuitable for species reliant on clean gravel substrate for feeding and/or spawning (Berkman and Rabeni 1987).

Acting in conjunction with these physical habitat alterations, water quality impairment from urban and agricultural surface runoff, mine waste, wastewater treatment discharge, and other point-source locations has long been recognized as a major threat to aquatic communities (Cairns and Pratt 1993; Figure 1). Excess nutrients, ions, heavy metals, and pesticides associated with urban and agricultural areas often contribute to the homogenization of fish and macroinvertebrate assemblages, shifting toward tolerant taxa (Chutter 1972; Winner et al. 1990). Because physiological tolerances to water temperatures, dissolved oxygen, and ion concentrations are primary factors shaping species distributions, steep fluctuations resulting from anthropogenic impacts may result in the loss of species and/or ecosystem function (Matthews 1998).

STREAM BIOASSESSMENT

The graded and relatively predictable responses of stream habitat and biota to environmental degradation have led to numerous conceptual, and quantitative models for assessing stream health (Davies and Jackson 2006). The development of these bioassessment tools have improved our ability to characterize anthropogenic disturbance, evaluate its effects on biota, and address conservation needs (Davis 1995; Barbour et al. 1999).

The primary objective of many of these efforts has been to assess *biological integrity*, a concept that has evolved over a long history of protective legislation and subsequent refining amendments (Davis 1995). The goal of maintaining or restoring waters to meet human health and recreational needs shifted when the term was first introduced in the Clean Water Act of 1972, later defined as “the capability of supporting and maintaining a balanced, integrated, adaptive community of organisms having species composition, diversity, and functional organization comparable to that of natural habitat of the region” (Karr and Dudley 1981). This new biological endpoint dramatically altered the way scientists monitor and manage aquatic resources (Davis 1995).

Since the introduction of Karr's (1981) index of biotic integrity, many state and federal agencies have constructed regionally-specific fish, macroinvertebrate, and physical habitat indices in attempts to better quantify stream conditions. These multi-metric indices compare observed habitat and/or assemblage characteristics (e.g. substrate embeddedness, residual pool characteristics, taxonomic richness, trophic and reproductive guild representation) to values thought to occur naturally within that ecoregion, commonly referred to as reference conditions (Karr 1981; Karr et al. 1986; Stoddard et al. 2006). Although these indices offer a distinct advantage over water quality-based assessments or species-specific ecological indicators (Hughes et al. 1998), determining appropriate reference values and index-scoring calibrations can be challenging (Dale and Beyeler 2001). Among other things, these efforts are complicated by the covariance of natural and anthropogenic disturbance gradients, potential legacy effects of previous landscape alterations, and uncertainties concerning the relative impact of multiple stressors and possible threshold effects (Allan 2004). Furthermore, issues commonly arise when attempting to delineate ecologically significant classifications within which to compare streams (Hughes et al. 1986; Hughes et al. 1994), and since truly natural reference reaches, or those reflecting pre-settlement conditions, are largely nonexistent in the Midwestern United States, attention has turned instead to the inherently subjective determination of "minimally" or "least-disturbed" sites (Stoddard et al. 2006). Despite these challenges, the increasing availability of in-stream and landscape-level data, along with numerous analytical advancements, are helping researchers address some of these ecological questions and better meet bioassessment goals.

PURPOSE AND OBJECTIVES

Missouri, like much of the Midwestern United States, has experienced tremendous habitat loss and degradation, and pristine habitats that remain exist largely as scattered fragments. Now with nearly 90 percent of its wetland area lost, less than one percent of its historic prairie intact, and lingering effects of historic deforestation, there is great concern over the condition of the state's waters (Schroeder 1981; Dahl 1990; Jacobson and Primm 1997). Thus, Missouri's natural resource agencies are tasked with the development of stream assessment tools and management plans to help identify and mitigate the effects of these disturbances.

Numerous attempts have been made to characterize human threats to aquatic ecosystems and identify minimally disturbed stream reaches throughout Missouri (Rabeni et al. 1997; Sowa et al. 2007; Annis et al. 2010). Initial efforts employed a qualitative 'best professional judgment' technique to compile a list of sites thought to contain relatively intact natural communities (Rabeni et al. 1997), though the efficacy of these and other similarly subjective methods have since been called into question, suggesting the need for a more quantitative, data-based approach (Doisy et al. 2008). However, quantitative landscape-level approaches, such as Missouri's Synoptic Human Threat Index (Annis et al. 2010), often provide an incomplete picture of stream health, with admitted shortcomings including an inability to discern the relative severity of individual threats, the nature of biotic responses to those threats, and the ways in which threats might alter the physical and chemical characteristics of streams (Annis et al. 2010). Moreover, without linking landscape characteristics to local environmental and biotic conditions, managers are typically left without a mechanistic understanding of these relationships, resulting in a limited ability to diagnose impairment and propose restoration actions (Hynes 1994; Rabeni 2000; Infante and Allan 2014).

Given the increasing imperilment of stream biota in Missouri, and the apparent shortcomings of previous efforts to identify candidate reference reaches, the need still exists for a better understanding of physical habitat change and biotic response to both natural and anthropogenic disturbances, and for a regionally-specific, quantitative methodology for determining minimally, or least-disturbed stream reference reaches. Thus, the primary objectives of this thesis are: 1) to quantify the relationship between stream biotic metrics and natural environmental gradients, 2) to evaluate the effects of local environmental variables and landscape-level anthropogenic disturbances on stream fish and macroinvertebrate communities, and, in-turn, develop a stepwise, quantitative approach for identifying reference reaches, and 3) to provide a provisional threat index for Missouri's headwater streams to guide future sampling and assessment efforts of these under-studied systems. Through these steps, we will be able to identify streams of high estimated biological integrity and conservation value, and will have framed a methodology applicable elsewhere. Additionally, this work will allow for both the recalibration of currently employed biotic indices, and the development of a companion physical habitat index for Missouri's wadeable streams.

PHYSIOGRAPHIC REGIONS AND LAND-USE HISTORY OF MISSOURI

Our study was conducted on wadeable streams of Missouri, a physiographically diverse state situated near the center of the conterminous United States (Figure 2). Missouri contains a high diversity of aquatic habitats and fauna, with over 210 species of fish and nearly 70 mussel species described to date (Oesch 1995; Pflieger 1997). The state can be classified into three primary aquatic subregions, all featuring distinct geology, soils, landform, vegetative cover, groundwater influence, and aquatic fauna (Sowa et al. 2007).

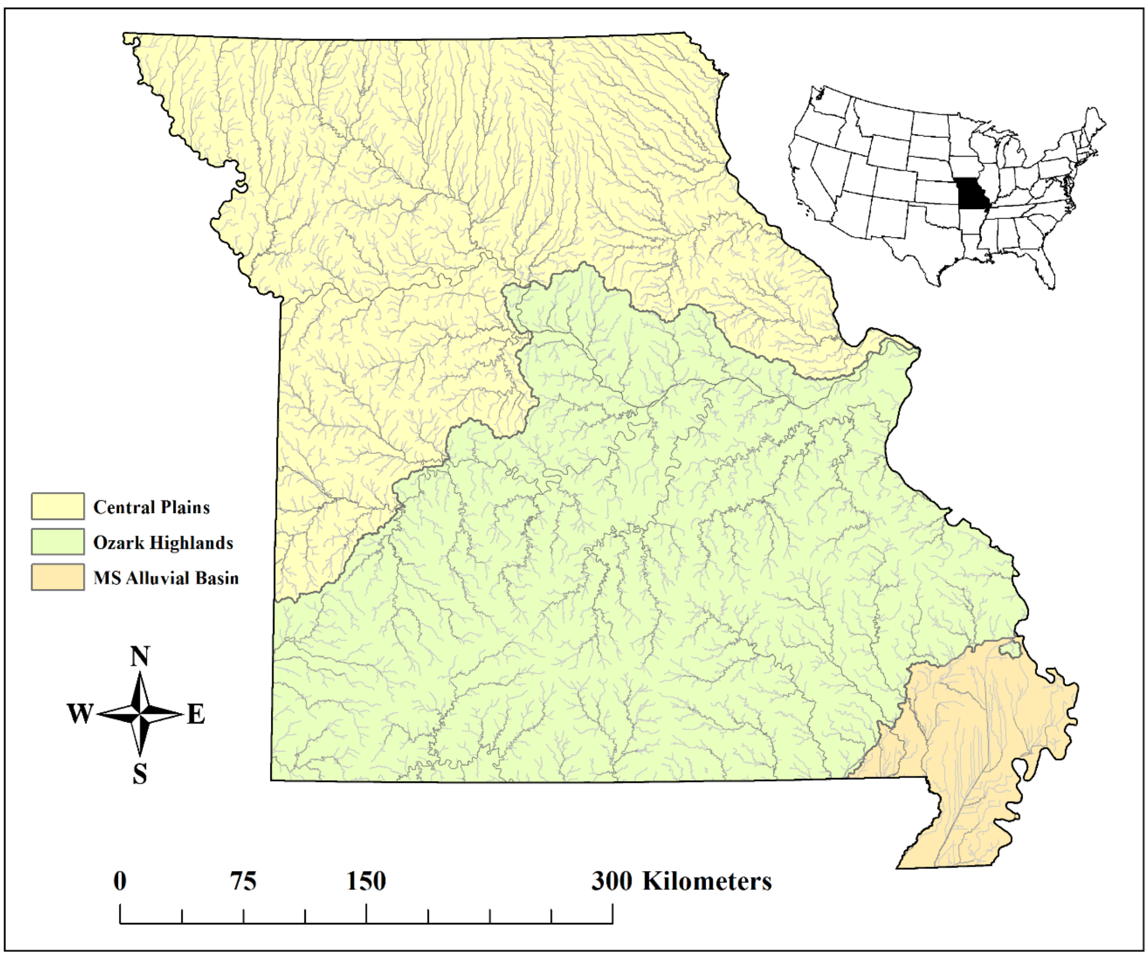

Figure 2. Map depicting Missouri's major aquatic subregions and major river drainages.

The central, or dissected till plains (hereafter, plains region), cover much of northern Missouri, and extend into southern Iowa and the eastern portions of Kansas and Nebraska (Figure 2). This once-glaciated region contains low, rolling hills, broad river valleys, and generally low gradient streams with silty or fine gravel substrates (Pflieger 1971). Though prairie historically covered much of the plains, less than one percent remains, and much of the region has been converted for pasture and agricultural production (Schroeder 1981; Figure 3). As a result, the fish communities of the region generally consist of widespread, tolerant taxa (Pflieger 1997).

To the south, the Ozark highlands (hereafter, Ozark region) encompass much of southern Missouri, and range primarily through northern Arkansas and northeastern Oklahoma (Figure 2). This highly dissected plateau is characterized by high local relief, deep and narrow river valleys, and much higher stream gradients than commonly seen in the plains region (Pflieger 1971). Streams in the Ozark region receive considerable ground-water input, and typically exhibit low turbidity, high dissolved oxygen levels, and coarse gravel substrates. Despite having experienced historic, widespread deforestation, much of the Ozark region is again forested (Jacobson and Primm 1997; Figure 3), and, as a whole, supports a large number of endemic and sensitive aquatic fauna (Sowa et al. 2007).

Missouri's most starkly delineated subregion, the Mississippi Alluvial Basin (hereafter, MS Alluvial Basin), represents the northern extent of the Mississippi embayment, and extends south toward the Gulf of Mexico (Pflieger 1971; Figure 2). Once part of the largest interior swampland in the United States, the region underwent extensive stream channelization and diversion in the early 1900s, and now exists as a nearly homogenous agricultural landscape (Pflieger 1971; Figure 3). Streams in this broad, flat valley are often highly vegetated, exhibit relatively low dissolved oxygen levels, and consist primarily of silty and fine gravel substrates (Sowa 2007). Despite these extreme alterations, the fish fauna of the southeast lowlands remains distinctive and more varied than that of the plains, including more than 20 endemic species (Pflieger 1997; See Appendix 1 for complete species-specific summaries for each subregion).

Together with Missouri's stream size classification system (Pflieger 1989), these three major aquatic subregions served as the base spatial scale for our model development and lest-disturbed reference reach identification.

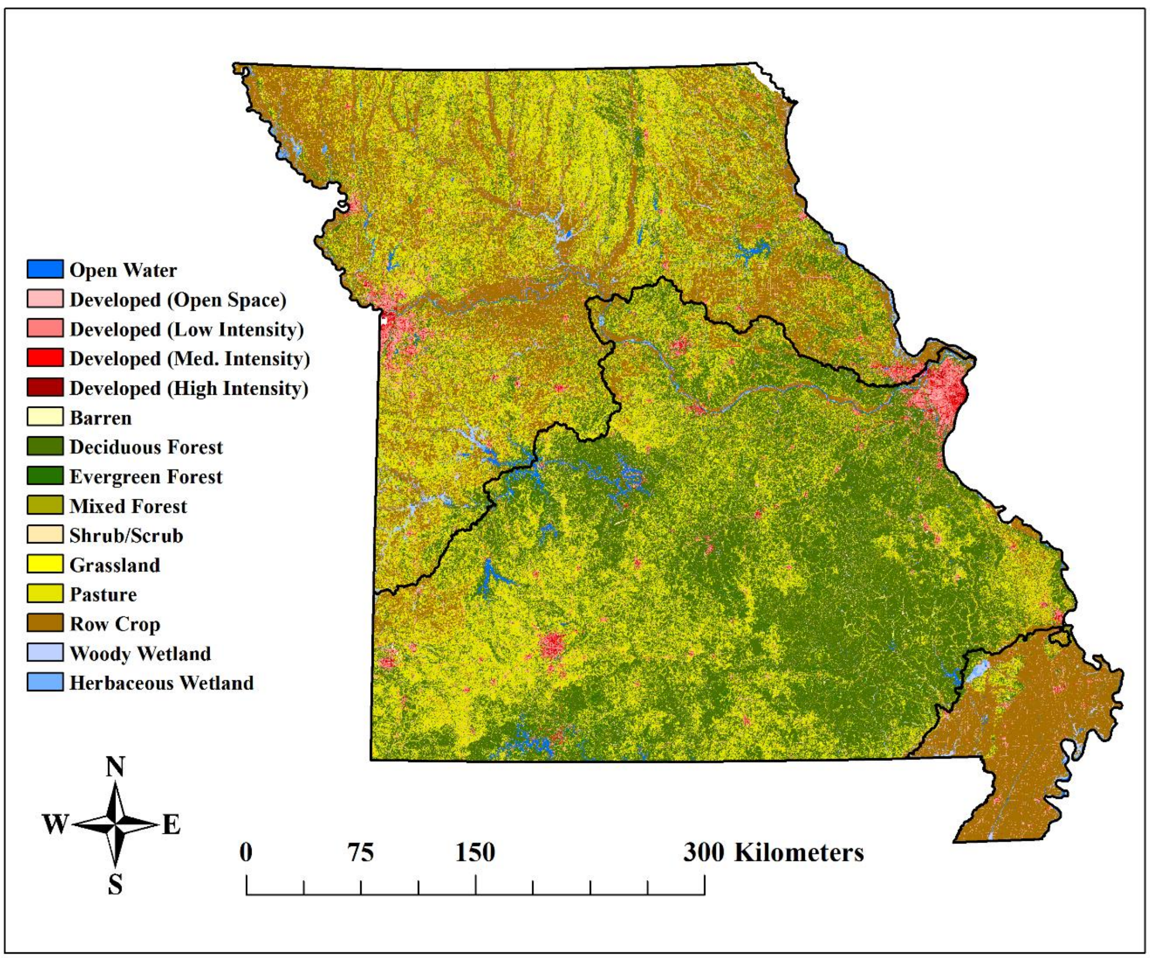

Figure 3. Map depicting the major land-uses of Missouri's aquatic subregions (NLCD 2011).

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CHAPTER ONE

ACCOUNTING FOR NATURAL SOURCES OF BIOLOGICAL VARIATION IN STREAM ASSESSMENTS

Ethan R. Kleekamp

ABSTRACT

Stream habitat and biotic composition result from a series of complex, hierarchical interactions between broad climatic and geological conditions, anthropogenic disturbances, and finer scale physical and ecological processes. Accounting for the influence of these natural environmental gradients is a critical first step in accurately assessing stream condition and addressing basic conservation need. We summarized existing landscape-level natural variables (e.g. watershed area, stream gradient, spring density, distance to mainstem rivers) and used Boosted Regression Trees to model their influence on ten separate fish and invertebrate metrics reflecting various aspects of stream community structure and function. Landscape-level natural environmental variables best predicted measures of fish species richness (e.g. total native species, native benthic species) followed by invertebrate and proportional fish metrics (e.g. proportion of insectivorous cyprinid individuals, proportion of omnivorous/herbivorous individuals). Drainage area and the percentage of fine soils in the watershed were consistently among the most influential variables for fish metrics, both positively associated with total native species, native benthic species, and native lithophilic species). Conversely, increased distance to mainstem river was related to decreases in all three fish richness metrics. Spring density had a stronger influence on invertebrates and proportional fish metrics, with decreased Hilsenhoff Biotic Index values and proportion of tolerant fish individuals, and increased EPT richness and proportion of native insectivorous cyprinids with increased spring

density in both the Plains and Ozark aquatic subregions. Our results indicate that measures of stream size and flow, surficial geology, and network positioning may all significantly influence stream biota, and should be taken into consideration when attempting to assess the influence of anthropogenic disturbance on stream condition, or when developing biological criteria for stream fish and invertebrates.

KEYWORDS: *Stream fish, Invertebrates, Natural environment gradients, Conservation*

INTRODUCTION

Accounting for natural sources of variation in stream habitat and biotic composition is critical for accurately assessing stream condition and addressing conservation needs (Schlosser and Angermeier 1995; Smogor and Angermeier 2001). Studies attempting to identify anthropogenic sources of stream impairment have frequently concluded that natural variables, such as stream size and gradient, better predict biotic assemblage characteristics than do human activities such as urban and agricultural land use (Wang et al. 2003; Infante and Allan 2010). Because stream habitat and biotic composition ultimately result from a series of complex, hierarchical interactions between broad climatic and geologic conditions, anthropogenic disturbances, and finer scale physical and ecological processes (Frissell et al. 1986; Montgomery and Buffington 1997), understanding and accounting for the influence of natural environmental gradients is a key first step in evaluating stream health.

Among the many natural factors driving stream fish and macroinvertebrate assemblage characteristics, biogeographical influences have been given the most consideration (Omernik 1986; Abell et al. 2008). Major physiographic zones exhibit distinct ecological conditions and biotic communities and are largely classified based on climate, geology, landforms, and natural vegetative cover (Bailey 1995). Previous stream assessment studies in Missouri have used the state's three distinct aquatic ecoregions (Central Plains, Ozark Highlands, Mississippi Alluvial Basin) as the base spatial scale for their analyses, and have adjusted biological expectations accordingly (Sarver et al. 2002; Sowa et al. 2007; Doisy et al. 2008). In addition, fish assemblage characteristics change along a stream's longitudinal gradient (Vannote et al. 1980; Schlosser 1982; Oberdorff et al. 1993), and thus, stream size is considered in many studies. In contrast, considerably less attention is given to stream network positioning and the potential contributions of habitat distribution and spatial configuration of source populations to local fish assemblage characteristics (see Gorman 1986; Campbell-Grant et al. 2007; Hitt and Angermeier 2008). Because stream fish are frequently traversing tributary connections in search of refugia and feeding and spawning opportunities (Matthews 1998), population and community dynamics of stream systems may be heavily influenced by immigration and emigration, elevating the importance of network context in successful stream characterization and assessment (Lowe et al. 2006; Brown et al. 2011). Additionally, stream gradient may exert significant influence on stream fish and macroinvertebrate community structure (Schlosser 1982), just as thermal regime and surficial watershed geology have been shown to influence stream communities (Sweeney 1978; Matthews 1987; Infante and Allan 2010). Though ecoregion and stream size help account for large amounts of biotic variation, considering the potential influence of these additional natural environmental variables may improve our ability to describe and accurately forecast stream conditions (Matthews 1998; Brenden et al. 2008).

Numerous biotic and habitat-based hierarchical stream classification systems have been proposed to delineate strata for stream monitoring programs, to identify reference reaches, and to calibrate biotic and habitat health indices (Sowa et al. 2007; Brenden et al. 2008). However, these often-times discrete, reach-level classifications may arbitrarily define natural environmental gradients (Angermeier and Schlosser 1995), and risk delineating an unmanageable level of classes (Sarver et al. 2002). For instance, Missouri's aquatic classification system incorporates numerous large and fine-scale environmental variables, such as climate, soils, temperature, and flow stability, ultimately recognizing over 1,100 unique stream classes (Sowa et al. 2007), which makes designating class-specific reference criteria logistically infeasible. Thus, the primary objective of this study was to use landscape-level natural environmental variables to predict stream fish and macroinvertebrate community metrics to 1) determine the relative importance of natural variables on each fish and macroinvertebrate community characteristic, and 2) to assess the overall ability of natural variables to explain variation in biotic responses unaccounted for when simply using stream size and regional classifications.

METHODS

STUDY REGIONS AND SPATIAL FRAMEWORK

Our study focused on stream fish and macroinvertebrate communities of two distinct size classes of wadeable streams in Missouri, hereafter referred to as "creeks" and "small rivers" (Pflieger 1989). These classes are based on shreve-link magnitude, and exhibit mean watershed areas of approximately 60 km^2 and 480 km^2, respectively. These sizes translate to mean wetted channel widths and depths of 8.6 m and 36 cm for creeks, and 16.4 m and 55 cm for small rivers, respectively. We spatially referenced in-stream biotic data to stream reaches from a modified version of the 1:100,000-scale National Hydrography Dataset (NHDPlus V1, 2008; Annis et al. 2010), and attributed each reach's local and network catchment with landscape-level environmental variables using ArcGIS 10.2. (ESRI, Redlands, CA, USA), and the stream network topology tool, RivEX (Hornby, 2013).

Missouri is a physiographically diverse state situated near the center of the conterminous United States, and exhibits three primary ecoregions, all featuring distinct geology, soils, landform, vegetative cover, groundwater influence, and aquatic fauna (Sowa et al. 2007). The central, or dissected till plains (hereafter, Central Plains), cover much of the northern half of the state, and contain low, rolling hills, broad river valleys, and generally low gradient streams with silty or fine gravel substrates (Pflieger 1971; Figure 1.1). The Ozark highlands (hereafter, Ozarks) is a highly dissected plateau that encompasses much of southern Missouri and features high local relief, deep and narrow river valleys, and much higher stream gradients than commonly seen in the plains region (Figure 1.1). Streams in the Ozarks receive considerable groundwater input, and typically exhibit low turbidity, high dissolved oxygen levels, and coarse gravel substrates (Sowa et al. 2007).

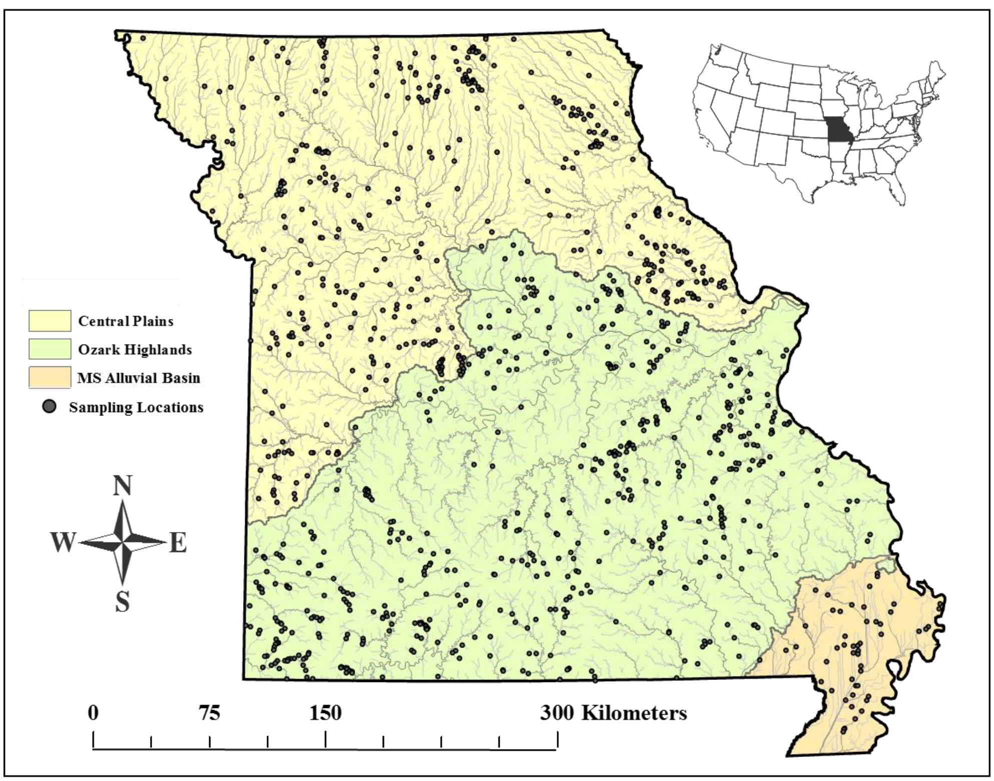

Figure 1.1. Map depicting Missouri's major aquatic subregions and stream sampling locations.

The Mississippi Alluvial Basin (hereafter, MS Alluvial Basin), once part of one of the largest interior swamplands in the United States, now exists as a nearly homogenous agricultural landscape. Streams in this broad, flat valley are often highly vegetated, exhibit relatively low dissolved oxygen levels, and consist primarily of silty and fine gravel substrates (Pflieger 1971; Figure 1.1). Due to sampling limitations and the homogenous nature of the region (e.g. ~ 75 % cultivated crop; see Figure 3, General introduction), we were unable to develop usable models for the MS Alluvial Basin, and no further information will be presented in this chapter. Together with our two-tiered stream size classifications, the Central Plains and Ozark aquatic subregions constituted the base spatial scale for our model development.

DATA COLLECTION AND SUMMARIZATION

We used fish and macroinvertebrate data collected by the Missouri Department of Conservation's (MDC) Resource Assessment and Monitoring (RAM) Program from 2000 to 2014. The RAM program uses the Environmental Protection Agency's Environmental Monitoring and Assessment Program standardized stream fish sampling protocol, and collects macroinvertebrate community data following the Missouri Department of Natural Resource's (MDNR) semi-quantitative macroinvertebrate bioassessment protocol (MDNR 2001; Fischer and Combes 2003). Fish community data were collected at randomly selected stream reaches (n = 944) between late May and early October using backpack and/or tote barge pulsed DC electrofishers and seine nets in single upstream passes. Block nets were placed at in-stream distances 40 times the mean stream width to retain fish and delineate the sampling reach. Fish were either field identified or preserved in formalin for later laboratory identification (Fischer and Combes 2003). Macroinvertebrate community data were collected in return visits (n = 604) to fish collection sites between September and October of the same year. Six kick net (500 x 500 micron mesh bag) samples were taken from each of three primary habitat types; flowing-water coarse substrate, non-flowing water depositional substrate, and rootmat substrate (MDNR 2001). Specimens were returned to the laboratory and identified to the family level, and genus when possible.

We identified ten biological metrics commonly used as indicators of stream health (Karr 1981; Daniel et al. 2014). These included stream fish and invertebrate community characteristics related to richness and diversity, habitat preference, trophic and reproductive ecology, and sensitivity to human disturbance (Table 1.1).

Table 1.1 Mean and (standard deviation) values of fish and invertebrate community characteristics within each aquatic subregion and stream size class.

	Central Plains		**Ozark Highlands**		**MS Alluvial Basin**	
Metric	Creeks	Small River	Creeks	Small River	Creeks	Small River
Richness/Diversity						
Native Fish Species Richness	13.3 (4.9)	19.3 (8.3)	19.3 (7.1)	26.5 (6.7)	19.7 (6.9)	22 (6.5)
Shannon Diversity Index (Invert)	2.8 (0.4)	2.9 (0.4)	3.1 (0.5)	3.3 (0.3)	2.5 (0.7)	2.7 (0.5)
Habitat Preference						
No. Native Benthic Species	2.7 (1.8)	4.9 (3.6)	6.1 (2.5)	9.1 (2.6)	1.9 (1.8)	1.8 (2.1)
Trophic Ecology						
Prop. Native Insectivore Cyprinid	0.13 (0.18)	0.12 (0.13)	0.15 (0.14)	0.28 (0.16)	0.24 (0.23)	0.29 (0.28)
Prop. Native Omnivore/Herbivore	0.29 (0.19)	0.25 (0.18)	0.45 (0.22)	0.31 (0.17)	0.20 (0.19)	0.37 (0.24)
Reproductive Ecology						
No. All Native Lithophilic Species	10.6 (3.8)	15.6 (6.8)	14.5 (5.4)	20 (5.1)	13.6 (4.5)	16.4 (5.9)
Sensitivity						
Prop. Tolerant	0.27 (0.24)	0.44 (0.23)	0.07 (0.12)	0.05 (0.09)	0.37 (0.23)	0.36 (0.27)
Prop. Non-Native	.001 (.006)	.006 (.02)	.001 (.005)	.001 (.003)	.005 (0.01)	.011 (0.03)
EPT Richness (Invertebrate)	10.8 (5.7)	14.9 (6.8)	19.5 (8.8)	24.5 (6.9)	7.1 (6.1)	7 (3.5)
Hilsenhoff Biotic Index (Invert.)	7.1 (0.7)	6.6 (0.8)	5.8 (1.1)	5.6 (0.8)	7.3 (0.8)	7.5 (0.5)
Sampling Localities: Fish/Inverts	**278/175**	**111/90**	**383/229**	**121/85**	**41 / 16**	**10 / 9**

Fish metrics include the total number of native species, the number of native benthic species, the proportion of native insectivorous cyprinid individuals, the proportion of native herbivorous or omnivorous individuals, the number of native lithophilic species, and the proportion of tolerant individuals in the sample. The three invertebrate metrics assessed included Shannon's Diversity Index (SDI), the number of species occupying the orders Ephemeroptera, Trichoptera, or Plecoptera (EPT Index), and the Hilsenhoff Biotic Index (HBI; Table 1.1). Both the SDI and EPT Index can indicate community sensitivity, and are expected to decrease with increasing anthropogenic disturbance, while the HBI is a measure of community tolerance, and thus is likely to increase with heightened disturbance (Hilsenhoff 1988; Sarver et al. 2002).

We attributed each sampling reach's upstream catchment with natural environmental variables pertaining to surficial geology, flow velocity and stability, network positioning, and sampling seasonality (Table 1.2). We included mean watershed level of fine soils based on its influence on stream substrate particle size and water delivery rate, ultimately resulting in varying degrees of hydrologic variability and benthic habitat availability (Infante and Allan 2010).

Table 1.2 Mean and (standard deviation) values of natural environmental variables within each aquatic subregion and stream size class.

	Central Plains		**Ozark Highlands**		**MS Alluvial Basin**	
Metric	Creeks	Small River	Creeks	Small River	Creeks	Small River
Drainage Area (km^2)	48 (37)	507 (466)	67 (49)	486 (322)	70 (63)	308 (85)
Drainage Density (km/km^2)	0.95 (0.13)	0.93 (0.08)	0.98 (0.15)	0.99 (0.09)	0.98 (0.25)	0.95 (0.1)
Reach Gradient (m/km)	2.55 (2.4)	0.89 (1.1)	3.72 (2.6)	1.44 (1.7)	0.79 (2.3)	0.09 (0.1)
Distance to Mainstem (km)	29 (22)	22 (24)	18 (17)	9.2 (15)	34 (18)	20 (16)
Spring Density (no./km^2)	.005 (0.02)	.004 (0.01)	.05 (0.09)	.053 (0.08)	.005 (0.03)	0 (0)
Fine Soils (% of Catchment)	65.9 (41.4)	74.4 (37.9)	52.2 (27.9)	53.4 (22.8)	99.8 (0.15)	99.8 (0.07)
Sampling Month (May - October)	-	-	-	-	-	-

We used watershed area, spring density, and reach gradient as surrogates for stream flow stability, velocity, and habitat heterogeneity (Roberts and Hitt 2010). We also incorporated a measure of network drainage density (network stream length (km)/network catchment area (km^2) and the distance to mainstem river ($\geq 5^{th}$ Order) to account for the influence of network positioning on species richness and composition based on recommendation of Thornbrugh and Gido (2009). Lastly, we included sampling month to account for potential seasonal variation in stream fish community structure (Horwitz 1978; Ostrande and Wilde 2002). Drainage area and reach gradient information were available through the Missouri Resource Assessment Partnership's MoVST stream layer attribution (Sowa et al. 2005), and network positioning metrics were calculated using the stream network topology tool, RivEX (Hornby 2013). Soil data were available through the Natural Resources Conservation Service STATSGO database (http://sdmdataaccess.nrcs.usda.gov/).

DATA ANALYSIS

We developed a suite of boosted regression tree (BRT) models to evaluate the relationship between natural environmental predictor variables and the ten stream fish and invertebrate metrics within each aquatic subregion and stream size classification. BRTs are a non-parametric machine-learning method that uses a boosting algorithm to combine many simple regression trees to enhance predictive performance (De'ath 2007). Specific advantages of BRTs are their ability to fit nonlinear responses, incorporate higher-order predictor interactions, handle missing data, and that they are uninfluenced by extreme outliers (Elith et al. 2008; Soykan et al. 2014). After setting our initial tree complexity to 5 and bag ratio to 0.5, we tailored the models' learning rate to minimize prediction error after completing no fewer than 1000 iterations following recommendations of Elith et al. (2000). We then used ten-fold cross validation to identify the optimal number of trees in each case, and to estimate cross-validated residual deviance. Model performance was evaluated by calculating the proportion of total deviance explained (D^2) based on recommendations of Leathwick et al. (2006). To safeguard against overfitting, we included a randomly generated predictor variable (values ranging from 0-100) to use as stopping criterion once models began incorporating predictors explaining less variation than our random variable (Soykan et al. 2014). Models were fit using the package 'dismo' in the R statistical programming language (R Development Core Team 2015).

RESULTS

Biological metrics exhibited considerable variation within and across aquatic subregions and stream size classifications (Table 1.1). Measures of fish species richness were considerably higher in small rivers than in creeks, and greater in the Ozark region. Though less pronounced than the count-based metrics, the proportion of native omnivorous/herbivorous individuals was higher in creeks than small rivers, while the proportion of native insectivorous cyprinids was higher in Ozark small rivers, though varied little between stream size classes in the Central Plains. The EPT richness was consistently higher in larger streams, and along with Shannon Diversity Index, was noticeably higher in the Ozark region. Additionally, the proportion of tolerant individuals and the Hilsenhoff Biotic Index values were higher in the Central Plains, reflecting the heightened impairment level of these streams compared to those in the Ozarks. While the proportion of non-native individuals appears to be slightly higher in small rivers than in creeks, values were extremely low in both regions (< 1%).

In addition to the noticeable variation in biotic metrics, natural environmental variables differed significantly between aquatic subregion and stream size class (Table 1.2), with reach gradients and spring densities consistently higher in the Ozark region, while higher percentages of fine soils were measured in the Plains.

We successfully constructed models for eight of ten biotic metrics using our suite of natural environmental variables. We were unable to predict the proportion of non-native individuals and Shannon Diversity Index scores within both subregions and stream size classes, as well as Ozark small river values of native benthic species and native lithophilic species, and Central Plains creek values of EPT Richness (i.e. could not reduce model deviance within the first 1000 tree iterations; Table 1.3).

Count-based fish community metrics (i.e. total richness, benthic species richness, lithophilic species richness) were consistently best predicted by natural environmental variables, with the highest amount of variation explained for native benthic species in small rivers of the Central Plains (D^2 = 0.39; Table 1.3). Invertebrate and proportional fish metrics were generally less predictable, though values varied considerably between aquatic subregion and stream size classification. While deviance reduction was higher for count-based metrics in the Central Plains (mean D^2 = 0.26) than in the Ozarks (mean D^2 = .12), invertebrate and proportional fish metrics tended to be better predicted in the Ozarks, with greater amounts of variation explained in HBI, proportion of native insectivorous cyprinids, proportion of native benthic omnivore/herbivores, and proportion of tolerant individuals within the region (Table 1.3).

Table 1.3 Boosted regression tree modeling results. NT – number of trees included in the model, TC – tree complexity, K – model parameters, D^2 – Proportion of deviance explained by each model.

	Plains Creeks				Plains Small Rivers				Ozark Creeks				Ozark Small Rivers			
Response	NT	TC	K	D^2	NT	TC	K	D^2	NT	TC	K	D^2	NT	TC	K	D^2
No. Native Fish Species	2050	5	5	0.18	5500	5	4	0.27	2600	5	5	0.13	2400	4	4	0.05
Shannon Diversity Index (Invertebrate)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
No. Native Benthic Species	4250	5	6	0.24	5100	5	5	0.39	3600	5	6	0.19	-	-	-	-
Prop. Native Insectivorous Cyprinid Individuals	1150	5	5	0.10	6400	5	2	0.03	3800	5	6	0.21	1850	5	2	0.11
Prop. Native Omnivorous / Herbivorous Individuals	1800	4	4	0.06	1600	5	3	0.03	1300	5	7	0.09	1000	4	5	0.05
No. Native Lithophilic Species	1950	5	7	0.19	7550	5	4	0.30	2350	5	5	0.12	-	-	-	-
Prop. Native Tolerant Individuals	2150	5	5	0.14	2550	5	3	0.10	1900	5	5	0.12	4250	5	2	0.18
Prop. Non-Native Individuals	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
EPT Richness (Invertebrate)	4500	5	5	0.20	-	-	-	-	1950	5	5	0.11	3000	5	5	0.17
Hilsenhoff Biotic Index (Invertebrate)	1600	5	5	0.07	1450	5	5	0.08	2850	5	6	0.20	3450	5	6	0.30

Drainage area and the watershed percentage of fine soils were consistently the top two predictors of fish richness measures, and together routinely accounted for over 50% of the explained variation in those metrics (Figure 1.2). The remainder of the explained variation in these metrics was primarily explained by drainage density, distance to mainstem, and reach gradient. Spring density was much more influential on invertebrates and proportional fish metrics than count-based fish metrics. This trend was particularly apparent for Ozark small rivers, where spring density alone accounted for roughly 35-45 % of the overall explained variation in both invertebrate metrics and all three proportional fish metrics (Figure 1.2). Similarly, reach gradient exerted a stronger influence on our sensitivity metrics (EPT, HBI, proportion of native tolerant individuals). Sampling month was detectable in only three of our final 29 models, and at most explained only 2% of the overall variation in any biotic metric.

Fish and invertebrate responses also differed in the directionality of their relationship with natural environmental gradients. The number of native fish species, native benthic species, and native lithophilic species increased with drainage area, and with increased percentages of fine soils in the watershed and decreased as the distance to the nearest mainstem river increased (Figure 1.3B). Proportional fish metrics showed considerably less similarity in their response to environmental gradients than did count metrics. The proportion of native insectivorous cyprinids generally increased with drainage area, fine soils, and spring density, though decreased with increased distance to mainstem, drainage density, and reach gradient. The proportion of native omnivorous/herbivorous individuals decreased with drainage area, increased with fine soil and distance to mainstem, and decreased with spring density.

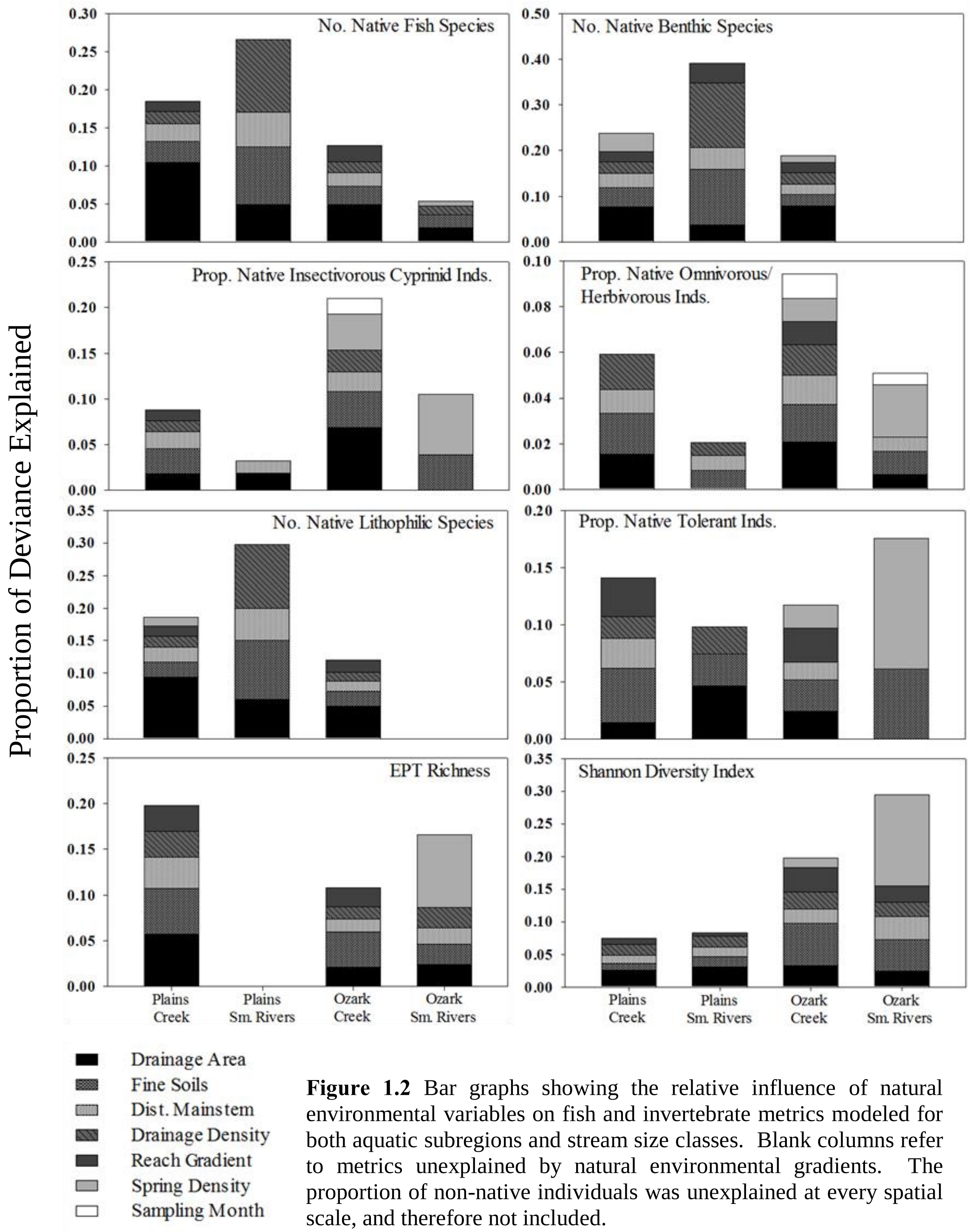

Figure 1.2 Bar graphs showing the relative influence of natural environmental variables on fish and invertebrate metrics modeled for both aquatic subregions and stream size classes. Blank columns refer to metrics unexplained by natural environmental gradients. The proportion of non-native individuals was unexplained at every spatial scale, and therefore not included.

The proportion of native tolerant individuals decreased with distance to mainstem, and increased with drainage area, fine soils, reach gradient, and spring density. Hilsenhoff Biotic Index similarly increased with distance to mainstem, and decreased with spring density and reach gradient, while EPT richness typically increased with drainage area and reach gradient, and decreased with fine soils and distance to mainstem (Figure 1.3A) (See Appendix 2 for full model results).

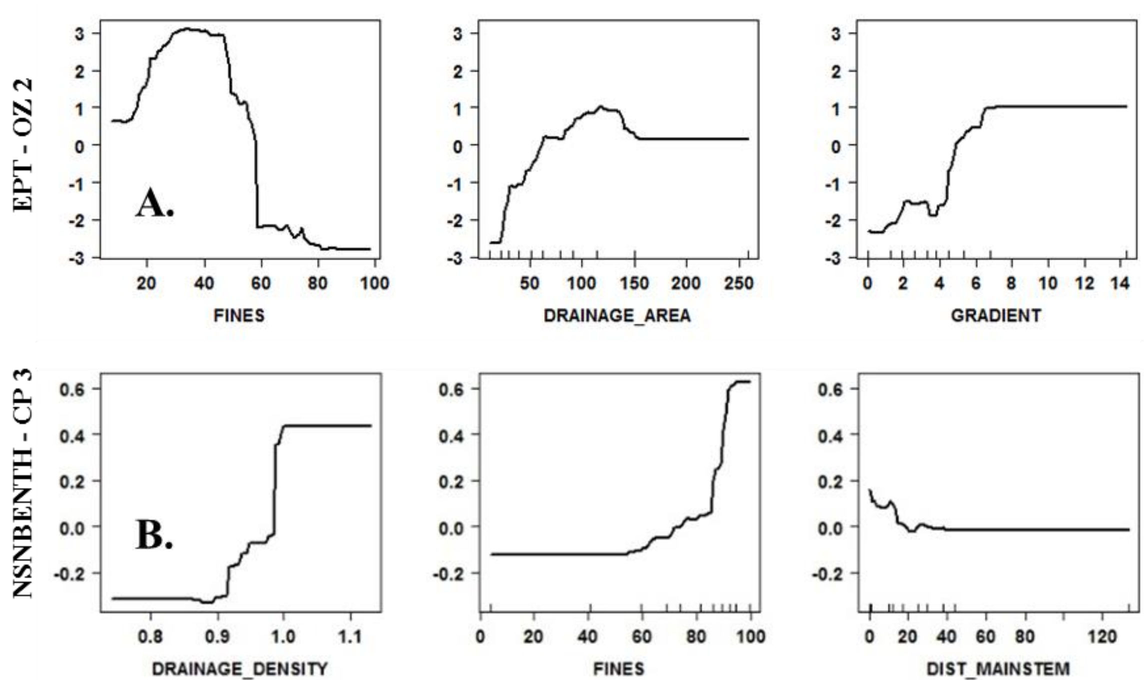

Figure 1.3. Example partial dependence plots of top influential environmental variables for **A.** – EPT Richness of Ozark creeks, and **B.** – The number of native benthic species of Central Plains small rivers. The y axes are presented as dimensionless transformations of each response. Rug plots at inside bottom of plots show the distribution of sites across the given predictor variable.

DISCUSSION

Our study represents one of the first attempts to model the specific influences of natural environmental gradients on both stream fish and invertebrate assemblages in Missouri. Previous studies have classified stream reaches based on differences in watershed characteristics (drainage area, surficial geology, groundwater influence, etc.), though have not specifically quantified their relationships with stream fish and invertebrate community characteristics (Sowa et al. 2007). By using boosted regression tree models, we were able to allow for complex predictor interactions and fit nonlinear responses, noted improvements over previous similar studies using multiple regression techniques (Stoddard et al. 2008; Daniel et al. 2014). Although results varied significantly between ecoregion and among response variables, our models were able to explain up to 39% of the variation in biotic responses unaccounted for using stream size and ecoregional classifications alone.

Our results indicated that categorical fish species richness increased with stream size, similar to Oberdorff et al. (1993), while the proportion of omnivorous/herbivorous individuals was greater in streams with smaller drainage areas, consistent with findings of Vannote et al. (1980) and Schlosser (1982). Although the relationships remained largely consistent, models predicting richness measures performed significantly better in the Central Plains than in the Ozark region. The disparate influence of drainage area between these regions may be due to several factors. Previous studies have concluded that the plains fish communities are largely driven by water availability (Matthews 1988; Dodds et al. 2004; Doisy et al. 2008), and although stream size likely influences Ozark stream biota, drainage area may not be as reflective of flow conditions in the region due to widespread karst geology and the prevalence of losing and spring-fed streams (Orndorff et al. 2001). Additionally, our measure of network positioning (distance to mainstem) accounted for small amounts of variation in each of our eight modeled response variables, with each measure of fish species richness decreasing as distance to mainstem river increases, a finding consistent with previous studies (Hitt and Angermeier 2008; Thornbrugh and Gido 2009).

EPT richness, and fish and invertebrate community sensitivity (decreased HBI and lower proportion of tolerant fish individuals) increased with spring density, likely due to the lower stream temperature and higher dissolved oxygen levels associated with these stream reaches (Westhoff and Paukert 2014). Springs were also influential for small rivers of the Central Plains, despite their low density relative to the Ozark region.

Fish richness metrics increased with increasing percentages of fine soil in the watershed, a finding we did not initially anticipate. A similar counterintuitive result was observed for the proportion of tolerant individuals, which were shown decreasing with increased fine sediment in the Central Plains region. These results may be due to the correlation between fine sediments and drainage area in the region (Pearson r = 0.44), as larger streams may be expected to have wider alluvial valleys with finer surficial geology. Additionally, the percentage of fine sediment in the watershed was associated with cultivated crop in the Ozark region (Pearason r=0.41), a common correlation potentially skewing model results (Allan 2004).

We were unable to predict the proportion of non-native individuals using our suite of natural environmental variables, likely because metric values varied little, and were extremely low in both size classes of each aquatic subregion (<1%). Although more variable than the proportion of non-native fishes, Shannon Diversity Index was also unpredictable, indicating little influence of natural watershed characteristics on metric values.

Our study represent a useful step in assessing stream conditions, highlighting the need to establish correction factors for natural differences in stream biotic composition when utilizing traditional biological index scoring. Our models could be improved by incorporating additional environmental variables contributing to stream conditions. For instance, including measures of precipitation or instream flow and temperature data when available. Additionally, because stream environments are extremely stochastic and biotic communities are known to fluctuate over time, our use of single stream samples to represent reach conditions may provide an incomplete picture (Horwitz 1978; Ostrande and Wilde 2002). Our approach allows for the continuous influence of natural variation, rather than discretely classifying stream reaches, and could be paired with subsequent residual analysis to model anthropogenic disturbances as a means to limit the noise associated with natural variation.

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CHAPTER TWO

PREDICTING STREAM FISH AND MACROINVERTEBRATE COMMUNITY COMPOSITION USING REACH AND WATERSHED-LEVEL ENVIRONMENTAL VARIABLES

Ethan R. Kleekamp

ABSTRACT

Previous efforts to quantify anthropogenic disturbances to aquatic systems have resulted in threat indices lacking the ability to identify specific stressor impacts, describe the ways stressors alter the physical and chemical conditions of receiving waters, and predict of biological integrity. Thus, the need exists for a flexible approach to characterizing stream impairment and identifying candidate least-disturbed stream reaches to serve as benchmarks for high quality physical habitat and biological integrity. We summarized stream fish and macroinvertebrate community characteristics, reach-level physical habitat and water quality metrics, and watershed-level anthropogenic disturbance metrics for 944 wadeable stream reaches in Missouri. We used boosted regression trees to model the influence of reach-level channel morphology, substrate, cover, and water quality, as well as the effect of watershed-level fragmentation and flow modification, urbanization, agriculture, and point-source pollution on 10 fish and macroinvertebrate community metrics commonly used as ecological indicators. Reach-level environmental variables typically accounted for higher percentages of biotic variation than did watershed-level stressors, with measures of channel morphology (e.g. bankfull width/depth ratio, channel incision height) and water quality (e.g. dissolved oxygen, total chlorophyll) consistently among the top predictors. Fish richness values in the agriculturally-dominated Central Plains ecoregion were most negatively associated with measures of fragmentation/flow modification (e.g. headwater impoundment density, road crossing density), while invertebrate metrics responded more strongly to agricultural disturbances, showing signs of impairment once the percentage of cultivated crops within the local riparian zone exceeded 35-40%. Similarly, invertebrate metrics in the heavily forested Ozark Highlands ecoregion were sensitive to row-crop agriculture, with community degradation apparent with cultivated crop coverage as low as 8-10% of the local and network riparian zones. Urban impairment was also best detected using invertebrate indicators of biotic integrity and measures of fish trophic ecology and sensitivity. Fish and invertebrate indicators of high quality habitat were positively associated with the percentage of forested area in both ecoregions. We used watershed-level model results to predict all ten biotic metric values to over 28,000 wadeable stream reaches throughout Missouri. By rescaling and summing our individual predictions, we were able to generate an estimate of overall biological integrity for each reach within the Central Plains and Ozark Highlands ecoregions, reflecting a continuum of stream health from least-disturbed to highly impaired. Our stepwise, objective approach to characterizing stream impairment offers specific advantages, including a reach, and watershed-level evaluation of human disturbance, and an inductive, multi-metric determination of biological integrity.

KEYWORDS: *Streams, Biological Integrity, Anthropogenic Disturbance, Conservation*

INTRODUCTION

Stream habitat and biotic composition result from a series of complex, hierarchical interactions between broad climatic and geological conditions, anthropogenic disturbances, and finer scale physical and ecological processes (Frissell et al. 1986; Montgomery and Buffington 1997; Allan 2004). The habitat template upon which biotic communities are built is naturally determined by the interactions between precipitation, basin morphology, bed composition, and riparian vegetation (Rabeni 2000; Montgomery and MacDonald 2002), though is often altered by the many adverse, and potentially synergistic effects of widespread human disturbances (Allan 2004). These landscape changes can influence the hydrology, geomorphology, and chemical condition of the receiving waters, often to the detriment of their biota (Paul and Meyer 2001). Consequently, North American freshwater resources have grown exceedingly imperiled, and many present-day aquatic communities likely represent only a fraction of their historic constituents (Malmqvist and Rundle 2002).

Alterations to streams' flow regimes can result in drastic physical and ecological change (Ward and Stanford 1995; Poff et al. 1997). Urbanization and watershed imperviousness reduce the infiltration of precipitation, intensifying flood pulses and reducing base stream flows (Arnold and Gibbons 1996; Booth and Jackson 1997; Paul and Meyer 2001). Additionally, the reduced magnitude and frequency of high flow events caused by dams and diversions may facilitate the invasion of nonnative taxa (Olden et al. 2006), while increased flow stability and water velocity may disrupt species' life cycles and habitat use (Scheidegger and Bain 1995; Dudley and Platania 2007). Stream channel morphology too is often disrupted by anthropogenic landscape alterations, as mining and agricultural activities often remove hillslope and bank stabilizing vegetation and contribute high sediment inputs, resulting in unstable, highly embedded stream channels unsuitable for species reliant on clean gravel substrate for feeding and/or spawning (Berkman and Rabeni 1987). Similarly, stream channelization, instream gravel mining, and riparian deforestation all increase sedimentation while greatly reducing the availability of woody debris critical for channel formation and maintaining habitat heterogeneity (Montgomery and Buffington 1997; Brown et al. 1998). In conjunction with these physical habitat alterations, excess nutrients, ions, heavy metals, and pesticides associated with urban and agricultural surface runoff, mine waste, wastewater treatment discharge, and other point-source locations are recognized as major threats to aquatic communities (Cairns and Pratt 1993).

In light of these pervasive threats, there has been growing concern for the condition of flowing waters. Widespread deforestation and increasing agricultural and urban expansion highlight the need to conserve remaining high quality stream reaches and successfully identify and restore those already degraded. Numerous conceptual and quantitative models linking stream health to environmental degradation have been proposed (Davies and Jackson 2006), often with the stated goal of assessing and/or restoring biological integrity, or “the capability of supporting and maintaining a balanced, integrated, adaptive community of organisms having species composition, diversity, and functional organization comparable to that of natural habitat of the region” (Karr and Dudley 1981). Many state and federal agencies currently employ multi-metric fish and invertebrate indices to estimate biological integrity by comparing test site values to those measured at “reference reaches”, or those streams thought to represent minimally, or least disturbed conditions within a particular ecoregion (Karr 1981; Karr et al. 1986; Stoddard et al. 2006). Initial criteria proposed for reference reach determination included the identification of relatively homogenous stream regions, evaluation of regional disturbance types and intensities, and selection of regional candidate sites exhibiting the least amount of anthropogenic disturbances (Hughes et al. 1986). Although these steps are useful as a conceptual guide, successful implementation is challenging. Among other things, these efforts are complicated by the covariance of natural and anthropogenic disturbance gradients, potential legacy effects of previous landscape alterations, and uncertainties concerning the relative impact of multiple stressors and possible threshold effects and other non-linear responses (Harding et al. 1998; Allan 2004).

To improve upon the subjective best-professional judgment techniques initially used to identify reference conditions (Rabeni et al. 1997), researchers have developed landscape-scale multi-metric threat indices to more objectively characterize stream health or impairment (Sowa et al. 2007; Annis et al. 2010; Paukert et al. 2011; Fore et al. 2014). However, because these methods often lack in-stream biological data to train and test their models, they may have limited ability to identify specific stressor impacts and severity, and to describe the specific ways in which stressors alter the physical and chemical characteristics of receiving waters (Annis et al. 2010). Landscape-level indices that have incorporated biotic data, however, have often employed threshold analyses to simplify their characterization of impairment (Wang et al. 2008; Baker and King 2010; Daniel et al. 2014). While the physiological tolerances of biota to environmental degradation may exhibit threshold responses (Davies and Jackson 2006), the complex and dynamic relationship between streams and their landscapes typically renders these forms of analyses unsuccessful in describing true biotic conditions (Daily et al. 2012), likely because landscape-level environmental metrics are often crude surrogates for in-stream environmental conditions, further suggesting that reach-level habitat and water quality data may be a necessary addition in designating candidate reference reaches (Rabeni 2000). Moreover, without linking landscape characteristics to local environmental and biotic conditions, managers are typically left without a mechanistic understanding of these relationships, resulting in a limited ability to diagnose impairment and propose restoration actions (Hynes 1994; Rabeni 2000; Infante and Allan 2014).

Given the increasing imperilment of stream biota in Missouri, and the apparent shortcomings of previous efforts to identify candidate reference reaches, the need exists for a flexible and informative approach to characterizing stream impairment in Missouri. In this study, we propose a novel approach for predicting stream impairment and identifying candidate reference stream reaches. Our specific objectives were to 1) assess the influence and relative importance of reach and watershed-level environmental variables on stream fish and macroinvertebrate community characteristics, 2) determine the relationship between reach-level habitat and water quality and watershed-level environmental characteristics, and 3) predict statewide stream biotic conditions and identify candidate regional stream reference sites.

METHODS

STUDY REGIONS AND SPATIAL FRAMEWORK

Our study focused on stream fish and macroinvetebrate communities of two distinct size classes of wadeable streams in Missouri, hereafter referred to as “creeks” and “small rivers”. These classes were delineated using Shreve-link magnitude ranges (Pflieger 1989), and exhibit mean watershed areas of approximately 60 km^2 and 480 km^2, respectively. These sizes translate to mean wetted channel widths and depths of 8.6 m and 36 cm for creeks, and 16.4 m and 55 cm for small rivers, respectively. We compiled biotic response data and spatially referenced them to stream reaches from a modified version of the 1:100,000-scale National Hydrography Dataset (NHDPlus V1, 2008; Annis et al. 2010), and attributed each reach's local and network catchments and riparian zones with landscape-level environmental variables using ArcGIS 10.2. (ESRI, Redlands CA, USA), and the stream network topology tool, RivEX (Hornby, 2013).

Missouri is a physiographically diverse state situated near the center of the conterminous United States and exhibits three primary ecoregions, all featuring distinct geology, soils, landform, vegetative cover, groundwater influence, and aquatic fauna (Sowa et al. 2007). The central, or dissected, till plains (herafter, Plains), cover much of the northern half of the state, and contain low, rolling hills, broad river valleys, and generally low gradient streams with silty or fine gravel substrates (Pflieger 1971; Figure 2.1). The Ozark Highlands (hereafter, Ozarks), is a highly dissected plateau that encompasses much of southern Missouri and features high local relief, deep and narrow river valleys, and much higher stream gradients than commonly seen in the plains region (Figure 2.1). Streams in the Ozarks receive considerable groundwater input and typically exhibit low turbidity, high dissolved oxygen levels, and coarse gravel substrates (Sowa et al. 2007). The Mississippi Alluvial Basin (hereafter, MS Alluvial Basin), once part of one of the largest interior swamplands in the United States, now exists as a nearly homogenous agricultural landscape. Streams in this broad, flat valley are often highly vegetated, exhibit relatively low dissolved oxygen levels, and consist primarily of silty and fine gravel substrates (Pflieger 1971; Figure 2.1). Due to sampling limitations and the highly homogenous nature of the region (e.g. ~ 75% cultivated crop; Figure 3, general introduction), we were unable to develop usable models for the MS Alluvial Basin, and no further information will be presented in this chapter. Together with our two-tiered stream size classification, the Plains and Ozark aquatic subregions constituted the base spatial scale for our stream comparisons.

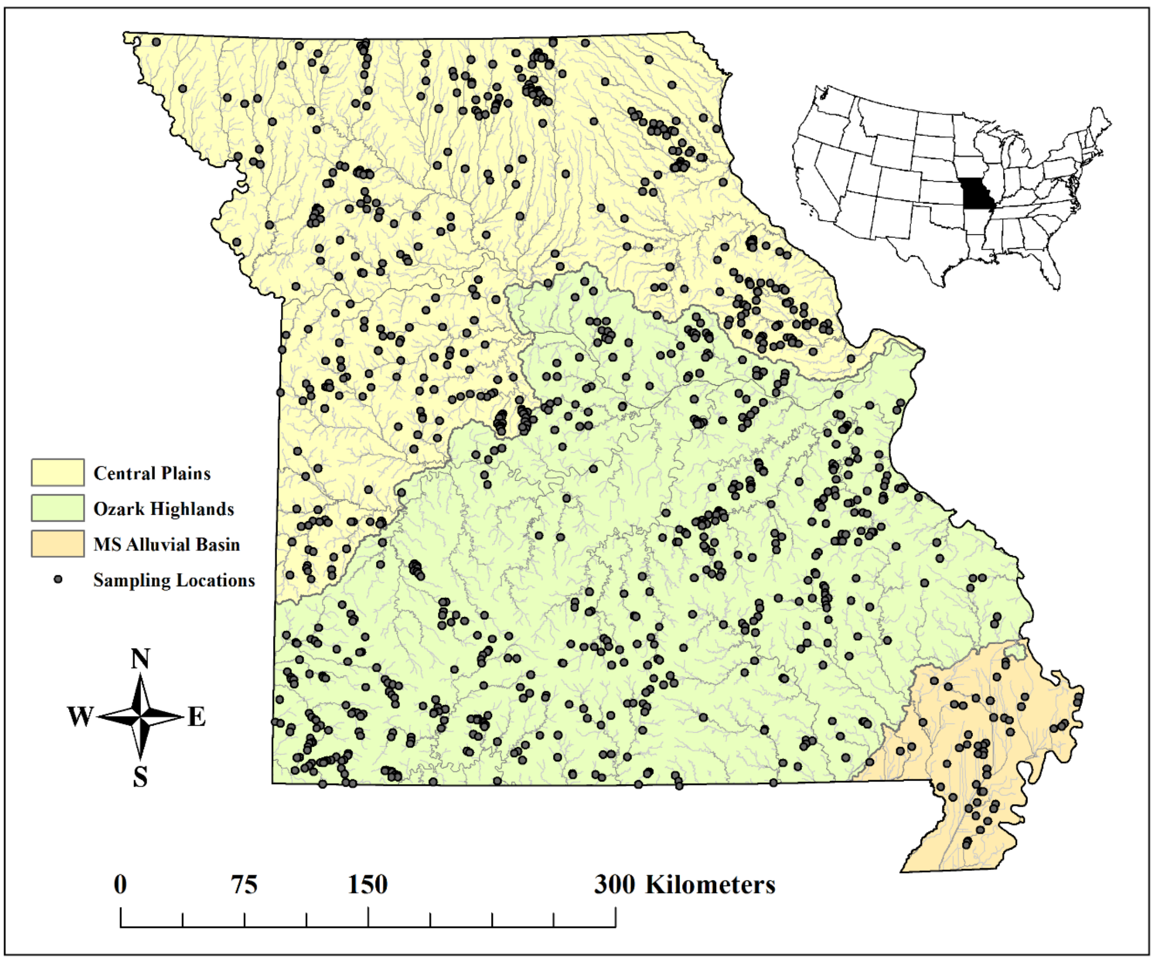

Figure 2.1. Map depicting Missouri's major aquatic subregions and stream sampling locations

BIOLOGICAL DATA

We used fish and macroinvertebrate data collected by the Missouri Department of Conservation's (MDC) Resource Assessment and Monitoring (RAM) Program from 2000 to 2014. The RAM program uses the Environmental Protection Agency's Environmental Monitoring and Assessment Program standardized stream fish sampling protocol, and collects macroinvertebrate community data following the Missouri Department of Natural Resource's (MDNR) semi-quantitative macroinvertebrate bioassessment protocol (MDNR 2001; Fischer and Combes 2003). Fish community data were collected at 944 randomly selected stream reaches between late May and early October using backpack and/or tote barge pulsed DC electrofishers and seine nets in single upstream passes. Block nets were placed at in-stream distances 40 times the mean wetted stream width to retain fish and effectively delineate the sampling reach. Fish were either field-identified or preserved in formalin for later laboratory identification (Fischer and Combes 2003). Macroinvertebrate community data were collected at fish sampling sites during return visits from September and October of the same year (n=604). Six kick net (500 x 500 micron mesh bag) samples were taken from each of three primary habitat types; flowing-water coarse substrate, non-flowing water depositional substrate, and root mat substrate (MDNR 2001). Specimens were returned to the laboratory and identified to the genus level, and species when possible.

Community-level analyses of the impact of land-use on stream health offers distinct advantages over single metric or species-specific approaches (Karr 1981), as fish and macroinvertebrate assemblages are thought to integrate the effects of multiple disturbance sources, types, and pathways (Fausch et al. 1990; Wang et al. 2008). To be practical and effective indicators of disturbance, these community metrics must be easily measured, sensitive to stressors, and be both predictable and consistent in their individual response to disturbance (Smogor and Angermeier 1999; Dale and Beyeler 2001). To meet these needs, we summarized fish and invertebrate data to reflect multiple aspects of stream community structure and function, selecting 10 total metrics related to richness and diversity, habitat preference, trophic and reproductive ecology, and sensitivity to disturbance (Table 2.1). The total number of native fish species and the total number of benthic species typically decrease with increased sedimentation and the loss of large woody debris (Allan 2004). The number of native lithophilic spawning species, including many darters (Percidae), minnows (Cyprinidae), and suckers (Catostomidae), spawn on or in clean gravel or cobble, and are similarly sensitive to substrate embeddedness from heightened sediment inputs (Berkman and Rabeni 1987). Omnivorous/herbivorous species and other trophic generalists increase with anthropogenic disturbances such as riparian clearing and nutrient enrichment (Allan 2004), while trophic specialists, such as insectivorous cyprinids, often decline (Smogor and Angermeier 1999; Table 2.1). Tolerant and non-native fish species also persist in higher proportions in streams draining extensively urbanized landscapes (Paul and Meyer 2001). The three invertebrate metrics assessed included Shannon's Diversity Index (SDI), the number of species occupying the orders Ephemeroptera, Trichoptera, or Plecoptera (EPT richness), and the Hilsenhoff Biotic Index (HBI). Both the SDI and EPT richness indicate community sensitivity, and are known to decrease with increasing disturbance (Paul and Meyer 2001; Sponseller et al. 2001), while the HBI is a measure of community tolerance, and thus is likely to increase with point-source pollution and other sources of water quality impairment (Hilsenhoff 1988; Sarver et al. 2002; Table 2.1).

Table 2.1. Mean and (standard deviation) values of fish and invertebrate community characteristics within each aquatic subregion and stream size class. (±) values refer to the predicted response of each community characteristic to increasing anthropogenic disturbance. EPT Richness – number of species belonging to the orders Ephemeroptera, Trichoptera, or Plecoptera.

		Central Plains		**Ozark Highlands**	
Metric	**Code (±)**	Creek	Small River	Creek	Small River
Richness/Diversity					
Native Fish Species Richness	numnat (-)	13.3 (4.9)	19.3 (8.3)	19.3 (7.1)	26.5 (6.7)
Shannon Diversity Index (Invertebrate)	SDI (-)	2.8 (0.4)	2.9 (0.4)	3.1 (0.5)	3.3 (0.3)
Habitat Preference					
No. Native Benthic Species	nsnben (-)	2.7 (1.8)	4.9 (3.6)	6.1 (2.5)	9.1 (2.6)
Trophic Ecology					
Prop. Native Insectivore Cyprinid Individuals	pincyp (-)	0.13 (0.18)	0.12 (0.13)	0.15 (0.14)	0.28 (0.16)
Prop. Native Omnivore/Herbivore Individuals	pomhb (+)	0.29 (0.19)	0.25 (0.18)	0.45 (0.22)	0.31 (0.17)
Reproductive Ecology					
No. All Native Lithophilic Species	nsnlith (-)	10.6 (3.8)	15.6 (6.8)	14.5 (5.4)	20 (5.1)
Sensitivity					
Prop. Tolerant Individuals	pntole (+)	0.27 (0.24)	0.44 (0.23)	0.07 (0.12)	0.05 (0.09)
Prop. Non-Native Individuals	pintro (+)	.001 (.006)	.006 (.02)	.001 (.005)	.001 (.003)
EPT Richness (Invertebrate)	EPT (-)	10.8 (5.7)	14.9 (6.8)	19.5 (8.8)	24.5 (6.9)
Hilsenhoff Biotic Index (Invertebrate)	HBI (+)	7.1 (0.7)	6.6 (0.8)	5.8 (1.1)	5.6 (0.8)
Sampling Localities: Fish/Invertebrates		**278/175**	**111/90**	**383/229**	**121/85**

PHYSICAL HABITAT AND WATER QUALITY DATA

Physical habitat characteristics were measured at eleven cross-channel transects and at intervals along the stream thalweg. These included measures of channel morphology (e.g. sinuosity, width/depth ratio, channel incision height, etc.), substrate characteristics (e.g. substrate size and variability, embedeness, etc.), habitat complexity and cover (e.g. large woody debris, macrophytic plant cover, etc.), and riparian characteristics, such as vegetation composition and canopy cover (Kaufmann et al. 1999; Fischer and Combes 2003; Table 2.2). Water quality parameters (e.g. dissolved oxygen, pH, conductivity, etc.) were recorded on-site using hand-held water quality meters (Table 2.2).

Table 2.2. Mean and (standard deviation) values of in-stream physical habitat and water quality within each aquatic subregion and stream size class. The standard deviations of xbkf_w, xinc_h, xdepth, and subx_diam were also included in our predictor set as measures of habitat heterogeneity.

		Central Plains		**Ozark Highlands**	
Metric Description	**Code**	Creek	Small River	Creek	Small River
Channel Morphology					
Mean bank-full width (m)	xbkf_w	10.1 (4.3)	19.8 (8.7)	16.8 (7.5)	28.5 (9.9)
Mean channel incision height (m)	xinc_h	2.5 (1.4)	3.4 (1.6)	1.8 (1)	2.3 (1.5)
Glide habitat (%)	pct_gl	37.5 (34.9)	53.6 (36.3)	31 (22.7)	37.4 (24.6)
Riffle habitat (%)	pct_ri	10.5 (12.6)	5.6 (7.5)	18.1 (12.9)	14.4 (11.3)
Pool habitat (%)	pct_pool	49.4 (34.2)	40.2 (34.7)	47.7 (25.4)	47.6 (26.4)
Channel sinuosity (m/m)	sinu	1.3 (0.7)	1.1 (0.2)	1.2 (0.5)	1.1 (0.1)
Mean width/depth ratio (m/m)	bfwd_rat	13 (8)	20.1 (11.9)	15.6 (6.4)	21.4 (7.3)
Mean depth (cm)	xdepth	30.5 (15.9)	49.5 (25.7)	36.3 (16.2)	57.4 (16.8)
Mean residual pool depth (cm)	rpxdep	19.7 (11.2)	24.6 (12.9)	23.3 (11.4)	31.9 (12.9)
Maximum residual pool depth (cm)	rpmxdep	71.3 (35.3)	84.7 (44.1)	82 (36.6)	104.5 (45.4)
Substrate					
Mean mobile substrate diameter (mm)	subx_diam	41.2 (58.5)	38.8 (61)	97.8 (82.9)	79.6 (60.1)
Fine substrate: silt, clay, muck (%)	pct_fn	24.1 (24.8)	22.2 (21.7)	5.8 (9.1)	8.7 (9.4)
Sand and fine substrate (% < 2 mm)	pct_safn	48.2 (30.9)	61.5 (28.5)	12.8 (16.2)	18.8 (15.9)
Fine gravel (% 2 - 16 mm)	pct_gf	11.4 (12)	5.2 (6.7)	12.6 (10)	13.6 (11.2)
Coarse Substrate (% > 16 mm)	pct_crs	26.8 (26.7)	21.5 (24.6)	60.6 (22)	58.8 (20.7)
Bedrock substrate (%)	pct_bdrk	3.8 (10)	3 (8.3)	10.7 (16.9)	6.1 (9.8)
Wood or detrital substrate (%)	pct_org	1.4 (3)	2.3 (4.6)	0.7 (1.8)	0.7 (1.2)
Mean channel embededness (%)	xcembed	63 (28.5)	75.5 (23.5)	27.1 (20.2)	33.5 (21.5)

Table 2.2. *Continued.*

		Central Plains		Ozark Highlands	
Metric Description	**Code**	Creek	Small River	Creek	Small River
Cover and Shading					
Algal cover (Prop: 0-1)	xfc_alg	0.03 (0.06)	0.06 (0.1)	0.06 (0.1)	0.07 (0.1)
Aquatic macrophytes (Prop: 0-1)	xfc_aqm	0.03 (0.07)	0.02 (0.04)	0.08 (0.1)	0.1 (0.1)
Brushy and small debris (Prop: 0-1)	xfc_brs	0.1 (0.1)	0.1 (0.1)	0.07 (0.07)	0.08 (0.07)
Large woody debris (Prop: 0-1)	xfc_lwd	0.06 (0.07)	0.08 (0.1)	0.04 (0.05)	0.06 (0.06)
Large woody debris count (No./100m)	c1wm100	10.2 (13.7)	11.1 (14.9)	8.5 (19.1)	6.4 (6.6)
Large woody debris vol. (m^3/100m)	v1wm100	6.3 (11.8)	11.8 (15.8)	5 (12)	6 (8.6)
Undercut banks (Prop: 0-1)	xfc_ucb	0.03 (0.04)	0.04 (0.06)	0.05 (0.08)	0.04 (0.06)
Riparian canopy presence (%)	xpcan	0.9 (0.1)	0.8 (0.1)	0.8 (0.1)	0.8 (0.1)
Riparian mid-layer presence (%)	xpmid	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)
Riparian ground veg. presence (%)	xpgveg	0.9 (0.02)	0.9 (0.06)	0.9 (0.06)	0.9 (0.07)
Mean bank canopy density (%)	xcdenbk	84.5 (14.5)	74 (19.7)	80.5 (17.4)	74.9 (18)
Mean mid-channel canopy dens. (%)	xcdenmid	72.2 (20.1)	45.1 (22.9)	60.6 (22.4)	38.6 (19.4)
Water Quality					
Dissolved Oxygen (mg/L)	DO	5.5 (2.1)	6.2 (2)	6.7 (2.2)	6.7 (1.7)
pH	pH	7.6 (0.7)	7.7 (0.9)	7.8 (0.5)	7.8 (0.3)
Turbidity (NTU)	turbid	68.4 (163.9)	812.2 (6589.4)	8.8 (9.3)	9 (11.4)
Conductivity (μS/cm)	cond	356.6 (247.5)	410.5 (169.5)	321.8 (222.9)	283.1 (216.1)
Total Chlorophyll (μg/L)	t_chl	15.2 (22)	20.6 (29.3)	4.7 (6.9)	7.8 (16.8)

LANDSCAPE DATA

We summarized landscape-level human disturbance data for local (area draining directly into stream reach) and network catchments (total upstream area) for every creek (n = 19,736) and small river (n = 8,937) segment in Missouri. Additionally, we calculated land-use/ land-cover percentages within a 45 meter riparian buffer for each creek segment, and 110 meter buffer for small rivers (Annis et al. 2010). We selected metrics that represent known environmental stressors that have been linked to stream impairment, including measures of stream fragmentation and flow modification, urban and agricultural impairment, point source pollution, and natural land-cover (Table 2.3). Land-cover metrics were calculated from the 2011 National Land Use/Land Cover dataset (e.g. agricultural cover, imperviousness; Homer et al. 2011) as catchment percentages, and point-stressors (e.g. stream crossings, mining operations, landfills) were converted to watershed densities (no./km^2). Means for network catchment and riparian zones were calculated as area-weighted averages (Table 2.3).

Table 2.3. Mean and (standard deviation) values of 'network-catchment' anthropogenic disturbances within each aquatic subregion. CAFO* - Confined Animal Feeding Operation, NPDES† - National Pollution Discharge Elimination System, MODNR – Missouri Department of Natural Resources, MORAP – Missouri Resource Assessment Partnership, NLCD – National Land Cover Database, EPA – U.S. Environmental Protection Agency.

Metric Description	Code	Central Plains	Ozark Highlands	Source
Fragmentation / Flow Modification				
Dams (No./km^2)	dams	0.06 (0.07)	0.03 (0.07)	MODNR (2010)
Headwater impoundments (No./km^2)	hw_imps	0.64 (0.31)	0.41 (0.28)	MORAP (2004)
Road crossings (No./km^2)	rd_crs	0.55 (0.23)	0.6 (0.31)	MORAP (2008)
Wells (No./km^2)	wells	0.47 (0.75)	1.56 (1.45)	MODNR (2006)
Urbanization				
Developed, open and low intensity (%)	dev_low	6.57 (5.05)	6.29 (7.4)	NLCD (2011)
Developed, medium intensity (%)	dev_med	0.3 (0.95)	0.45 (1.7)	NLCD (2011)
Developed, high intensity (%)	dev_high	0.07 (0.27)	0.15 (0.76)	NLCD (2011)
Total imperviousness (%)	imperv	2.14 (2.75)	2.26 (4.95)	NLCD (2011)
2010 population density (No./km^2)	pop_dens	25.1 (82.2)	37.3 (124.7)	US Census (2010)
2000-2010 pop. change (No./km^2)	pop_chng	+ 5.4 (25)	+ 3.0 (11.7)	US Census (2010)
Agriculture				
Row-crop agriculture (%)	crop	31.2 (21.1)	4 (10.2)	NLCD (2011)
Pasture land (%)	pasture	42.3 (17)	31.8 (22.2)	NLCD (2011)
Point Source Pollution				
Coal mines (No./km^2)	coal	0.014 (0.05)	0.004 (0.018)	MORAP (2008)
Lead mines (No./km^2)	lead	0.001 (0.007)	0.038 (0.2)	MORAP (2007)
CAFO* sites (No./km^2)	cafo	0.013 (0.035)	0.007 (0.022)	MODNR (2012)
NPDES† sites(No./km^2)	npdes	0.011 (0.027)	0.021 (0.043)	EPA (2007)
Landfills (No./km^2)	lndfl	0.001 (0.003)	0.002 (0.008)	EPA (2007)
Hazardous waste sites (No./km^2)	hazard	0.004 (0.013)	0.015 (0.068)	EPA (2007)
Superfund sites (No./km^2)	sprfnd	0.001 (0.006)	0.006 (0.015)	EPA (2007)
Natural Landcover				
Forest (%)	forest	16.2 (12.5)	54.6 (26.8)	NLCD (2011)
Grassland (%)	grass	1.2 (2.1)	1.3 (0.96)	NLCD (2011)
Wetland (%)	wtlnd	0.67 (0.79)	0.28 (0.59)	NLCD (2011)

RELATING REACH AND WATERSHED VARIABLES WITH BIOTIC METRICS

Prior to modeling the influence of reach and watershed-level anthropogenic disturbance, we first accounted for the effect of natural environmental gradients known to influence fish and invertebrate community structure (e.g. watershed area, reach gradient, distance to mainstem river, etc.)(Chapter 1). Using these natural variables to fit boosted regression tree models for each fish and invertebrate response metric, we calculated residual values for each metric, effectively reducing each metric to values relative to other streams occurring under similar natural characteristics, thus allowing us to limit the effect of natural variation and more directly model stream condition (Smogor and Angermeier 1999; Stoddard et al. 2008; Daniel et al. 2014; Figure 2.2).

Additionally, before developing our reach and watershed-level models, we used Pearson's pairwise correlation to examine the correlation structure of both our reach and watershed-level predictors. For variable pairs exhibiting correlations ($r > |0.70|$), we retained the variable exhibiting the strongest relationship with our in-stream biotic metrics. We then developed suites of boosted regression tree models to separately evaluate the relationship between our sets of reach and watershed-level environmental predictors and 10 fish and invertebrate response metrics within each aquatic subregion and stream size class (Figure 2.2). Boosted regression trees are a non-parametric machine-learning method that uses a boosting algorithm to combine many simple regression trees to enhance predictive performance (De'ath 2007). Advantages of the technique include its ability to fit nonlinear responses, incorporate higher-order predictor interactions, handle missing data, and remain largely uninfluenced by extreme outliers (Elith et al. 2008; Soykan et al. 2014). After setting initial tree complexity to 5 and bag ratio to 0.5, we tailored the models' learning rate to minimize prediction error after completing no fewer than 1,000 iterations following recommendations of Elith et al. (2000). We then used ten-fold cross validation to identify the optimal number of trees in each case, and to estimate cross-validated residual deviance. Model performance was evaluated by calculating the proportion of total deviance explained (D^2) (Leathwick et al. 2006).

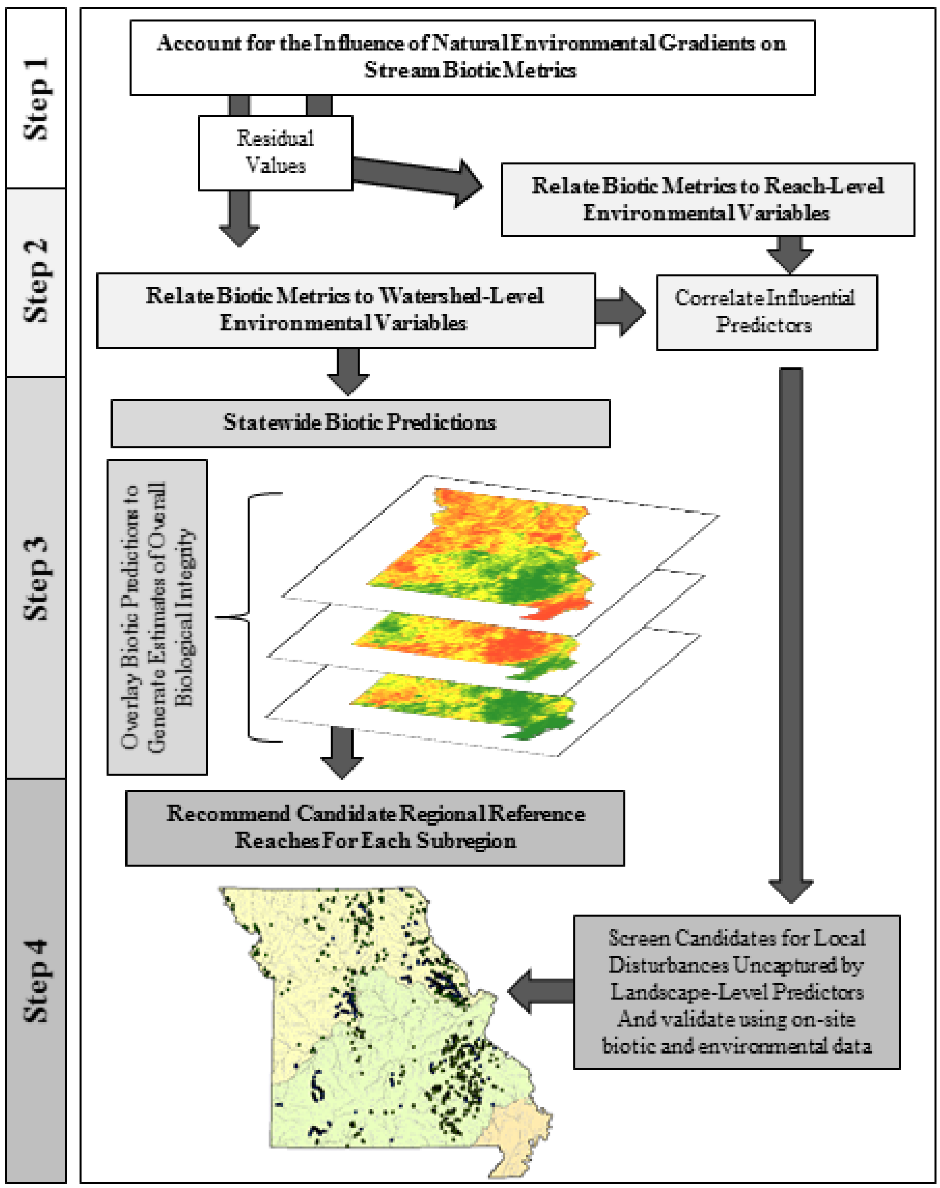

Figure 2.2. Conceptual framework detailing the process of identifying regional candidate reference stream reaches.

To safeguard against overfitting, we included a randomly generated predictor variable (values ranging from 0-100) to use as stopping criterion once models began incorporating predictors explaining less variation than our random variable (Soykan et al. 2014). Models were fit using the '*dismo'* package in the R statistical programming language (R Development Core Team 2015).

After modeling the relationship between stream biota and environmental predictors, we used Spearman's rank correlation to examine the strength and directionality of the relationships between reach-level habitat and water quality characteristics and landscape-level anthropogenic disturbances, allowing us to identify potential pathways of impairment, and assess redundancies and gaps in our predictor sets' abilities to describe stream impairment (Figure 2.2). Next, we used the results from our landscape-based models to extend our biotic predictions to unsampled stream reaches throughout the state. After rescaling each fish and invertebrate metrics' predicted value from 0-10, we summed all values for each stream reach to generate an estimate of overall biological integrity (Figure 2.2). Incorporating multiple ecological indicators into our stream scoring process ensured that our final suite of candidate least-disturbed sites met the habitat and water quality requirements of various components of the fish and invertebrate communities. We then selected streams with the highest predicted biological integrity (overall scores in the top 95th percentile within each aquatic subregion and stream size class) to serve as candidate stream reference reaches (Figure 2.2).

RESULTS

Biological metrics exhibited considerable variation within and across aquatic subregions and stream size classifications (Table 2.1). Measures of fish species richness were considerably higher in small rivers than in creeks, and reached their peak in the Ozark region. Although less pronounced than the count-based metrics, the proportion of native omnivorous/herbivorous individuals was higher in creeks than small rivers, while the proportion of native insectivorous cyprinids was higher in Ozark small rivers, but varied little between stream size classes in the Central Plains. The EPT Richness was consistently higher in larger streams, and, along with Shannon Diversity Index values, was higher in the Ozark region. Additionally, the proportion of tolerant individuals and the invertebrate Hilsenhoff Biotic Index values were higher in the Central Plains, reflecting the heightened impairment level of these streams compared to those in the Ozarks. While the proportion of non-native individuals appeared slightly higher in small rivers than in creeks, values were extremely low in both regions (< 1%).

Landscape-level environmental variables differed greatly between aquatic subregion, and reach-level physical habitat and water quality parameters showed considerable differences between both regions and stream size classes (Tables 2.2, 2.3). Streams within the Plains region consistently showed higher levels of row-crop agriculture ($\bar{x}$=31.2%), slightly higher pastureland ($\bar{x}$=42.3%), and greater densities of headwater impoundments ($\bar{x}$=.64/km^2) and coal mining operations ($\bar{x}$=0.14/km^2) than Ozark streams (Table 2.3). On average, Plains streams consisted of narrower, and more highly incised channels, with higher levels of substrate embeddedness, turbidity, and total chlorophyll relative to streams in the Ozark region (Table 2.2). Conversely, Ozark streams typically had more heavily forested catchments ($\bar{x}$=54.6%) and much sparser row-crop production ($\bar{x}$=4%) than streams of the Plains, although the Ozarks did generally exhibit watersheds with denser human populations, and greater densities of lead mining operations and other point-source pollution sources than present in the Plains region (Table 2.3). Streams within the Ozark region tended to be wider, more riffle-dominated than those in the Plains, exhibited coarser substrate and higher dissolved oxygen levels, but tended to have less mid-channel canopy cover and woody debris than Plains streams (Table 2.2).

REACH-LEVEL ENVIRONMENTAL INFLUENCE ON BIOTIC METRICS

Nine of the 39 initial reach-level predictors were highly correlated and thus were removed from further consideration, resulting in a set of 30 predictors for model development. We successfully constructed models for nine of ten biotic metrics for at least one stream size classification. We were unable to reduce model deviance within the first 1000 tree iterations within both subregions and stream size classes for the proportion of non-native individuals, as well as for Plains creek Shannon Diversity Index (SDI) values and Ozark small river SDI values (Table 2.4). On average, reach-level models explained ~ 25% of the variation in fish and invertebrate metrics in the Plains region, with the proportion of native insectivorous cyprinids in small rivers as the lowest (8%), and the number of native benthic species in small rivers the highest (40%). In the Ozark region, reach-level models on average explained ~ 27% of the variation in biotic metrics, with the proportion of tolerant individuals in creeks as the lowest (13%), and Hilsenhoff Biotic Index (HBI) values in small rivers the highest (46%).

Table 2.4. Reach and watershed-level boosted regression tree model results for each biotic response within creeks and small rivers of the Plains and Ozark aquatic subregions of Missouri. *K* – number of model parameters, D^2 – proportion of deviance explained, 'Top Predictors' refer to those metrics accounting for at least 10% of the explained deviance in each respective model. LC – Local Catchment, NC – Network Catchment, LR – Local Riparian, NR – Network Riparian. Refer to Tables 2.2 and 2.3 for metric abbreviations.

	Plains "Creeks"			***Plains "Small Rivers"***		
Metric	***K***	**D^2**	**Top Predictors**	***K***	**D^2**	**Top Predictors**
numnatsp						
reach	*7*	0.14	xdepth(+) bfwd_rat(+) DO(+) xbkf_w(+)	*12*	0.36	pct_crs(+) cond(-) sdinc_h(+) bfwd_rat(+)
watershed	*16*	0.05	NC_hw_imps(-) LR_forest(+) LC_pasture(-)	*18*	0.29	LR_forest(+) NC_hw_imps(-) LR_pasture(-)
nsnbenth						
reach	*13*	0.15	xdepth(+) pct_fn(-) xbkf_w(+) pct_crs(+)	*15*	0.40	cond(-) pct_crs(+) pct_ri(+) bfwd_rat(+)
watershed	*15*	0.16	LC_pasture(-) LR_forest(+) NC_hw_imps(-)	*18*	0.26	NC_hw_imps(-) LR_forest(+) LC_grass(-)
pnincyp						
reach	*16*	0.32	bfwd_rat(+) DO(+) xdepth(-) rpmx_dep(-)	*15*	0.08	bfwd_rat(+) xdepth(-) xfc_brs(-) pH(-)
watershed	*11*	0.21	LC_pop_den(-) LC_psture(-) NC_hw_imps(-)	*9*	0.14	NC_cafo(+) LC_dev_low(-) LC_grass(-)
pnomhb						
reach	*16*	0.21	cond(+) DO(-) pct_pool(+) rpmx_dep(+)	*13*	0.17	DO(+) pct_crs(+) pH(+) xfc_ucb(+)
watershed	*13*	0.16	LC_pop_den(+) NC_hw_imp(+)LC_forest(+)	*4*	0.10	NC_dams(-) NC_npdes(+) LR_imp(+)
nsnlith						
reach	*11*	0.16	bfwd_rat(+) DO(+) cond(+) xdepth(+)	*17*	0.31	bfwd_rat(+) pct_crs(+) cond(-) xinc_h(-)
watershed	*9*	0.04	NC_hw_imps(-) LR_forest(+) LR_imp(+)	*18*	0.31	NC_hw_imps(-) LR_forest(+) LC_grass(-)
pntole						
reach	*8*	0.29	pct_fn(+) cond(-) rpmx_dep(+) bfwd_rat(-)	*11*	0.34	bfwd_rat(-) DO(+) pct_crs(-) xdepth(+)
watershed	*12*	0.20	NC_hw_imps(+) LC_forest(-) NC_rd_crs(+)	*4*	0.20	LR_forest(-) NC_cafo(-) LC_grass(+)
pintro						
reach	-	-	-	-	-	-
watershed	-	-	-	-	-	-
SDI						
reach	-	-	-	*13*	0.20	v1wm100(+) DO(-) pct_ri(+) pct_crs(+)
watershed	-	-	-	*13*	0.26	LR_crop(-) LC_pasture(-) LC_forest(+)
EPT						
reach	*18*	0.28	xbkf_w(+) xcdenmid(-) t_chl(-) DO(+)	*5*	0.26	bfwd_rat(+) v1wm100(+) xcdenmid(-)
watershed	*13*	0.11	LC_forest(+) LR_crop(+) NR_pasture(-)	*11*	0.14	LR_crop(-) LC_pop_dens(-) NC_hw_imps(-)
HBI						
reach	*13*	0.23	DO(-) rpmxdep(+) pH(-) sinu(-)	*17*	0.17	xbkf_w(-) xinc_h(-) rpmxdep(-) pH(-)
watershed	*9*	0.17	LC_grass(-) LC_forest(-) LC_pop_dens(+)	-	-	-

Table 2.4. *Continued.*

	Ozark "Creeks"			Ozark "Small Rivers"		
Metric	***K***	**D^2**	**Top Predictors**	***K***	**D^2**	**Top Predictors**
numnatsp						
reach	*15*	0.21	xfc_aqm(+) bfwd_rat(+) DO(+) pct_pool(+)	*16*	0.43	bfwd_rat(+) sdinc_h(+) pct_pool(+) DO(+)
watershed	*8*	0.16	NC_forest(+) NC_rd_crs(-) LR_forest(+)	*15*	0.15	NC_hw_imp(+) NR_crop(+) NC_dev_low(-)
nsnbenth						
reach	*17*	0.18	xfc_aqm(+) rpmx_dep(-) xbkf_w(+)	*16*	0.19	bfwd_rat(+) xinc_h(+) sdinc_h(+) xpcan(-)
watershed	*9*	0.15	NC_dev_low(-) NC_rd_crs(-)	*10*	0.20	NC_wells(+) LC_grass(-) NC_npdes(-)
pnincyp						
reach	*16*	0.15	v1wm100(+) pH(+) xfc_ucb(+) xpcan(+)	*11*	0.16	bfwd_rat(+) xcdenmid(-) xfc_aqm(-)
watershed	*11*	0.11	NC_forest(+) NC_lead(+) LC_grass(-)	-	-	-
pnomhb						
reach	*17*	0.17	rpmx_dep(-) cond(+) pH(-) DO(-)	*12*	0.31	pct_gf(-) pH(-) xbkf_w(-) xcembed(-)
watershed	*14*	0.15	NC_forest(-) NC_dams(-) NR_grass(-)	*9*	0.13	NR_grass(-) NC_dev_low(+) NC_forest(-)
nsnlith						
reach	*18*	0.22	xfc_aqm(+) pct_pool(+) bfwd_rat(+)	*14*	0.44	turbid(+) bfwd_rat(+) xcdenmid(-)
watershed	*10*	0.15	NC_forest(+) NC_rd_crs(-) LR_forest(+)	*13*	0.16	NC_hw_imps(+) LR_forest(-) LC_grass(-)
pntole						
reach	*15*	0.13	t_chl(+) xcembed(+) pct_gf(-) cond(+)	*12*	0.24	xcembed(+) pct_fn(+) xinc_h(+)
watershed	*9*	0.08	LR_forest(-) NR_crop(+) LR_crop(+)	*8*	0.16	NR_crop(+) NC_lndfl(+) NC_npdes(+)
pintro						
reach	-	-	-	-	-	-
watershed	-	-	-	-	-	-
SDI						
reach	*6*	0.22	pct_ri(+) t_chl(-) xdepth(+) cond(-)	-	-	-
watershed	*10*	0.07	NR_crop(-) NC_forest(+) NC_dev_low(-)	-	-	-
EPT						
reach	*18*	0.42	t_chl(-) DO(+) cond(-) xfc_aqm(+)	*17*	0.27	t_chl(-) DO(+) pct_ri(+) xbkf_w(+)
watershed	*8*	0.38	NC_forest(+) NR_crop(-) NC_wells(-)	*15*	0.30	NC_wells(+) NC_forest(+) NC_coal(-)
HBI						
reach	*16*	0.37	t_chl(+) xinc_h(+) DO(-) pct_ri(-)	*12*	0.46	t_chl(+) pct_ri(-) xcembed(+) DO(-)
watershed	*9*	0.37	NR_crop(+) NC_forest(-) NC_hw_imps(+)	*9*	0.51	NC_dams(+) NC_dev_low(+) NR_crop(+)

In general, measures of fish species richness (number of total species, benthic species, lithophilic species) were most related to measures of channel morphology, which consistently accounted for between 40 and 50% of the explained variation in each metric (Figure 2.3). Fish richness measures increased with mean depth, bank-full width/depth ratio, and standard deviation of channel incision height (Table 2.4, Appendix 3). Richness values also increased with dissolved oxygen, aquatic macrophyte cover in the Ozark subregion, and coarse gravel substrate in the small rivers of the Plains region (Figure 2.4). Proportional fish metrics (proportion of native insectivorous cyprinids, proportion of native omnivorous/herbivorous, proportion of native tolerant), varied considerably in predictability between subregion and stream size, and in their relationship with reach-level environmental characteristics. The proportion of native insectivorous cyprinids decreased with increased mean depth in the Plains region, and exhibited a positive relationship with bank-full width/depth ratio and dissolved oxygen. In the Ozark region, the proportion of native insectivorous cyprinids was more influenced by available cover and riparian characteristics, as seen by an increase with woody debris, undercut banks, and riparian canopy presence in creeks, and decrease with aquatic macrophyte cover and mid-channel canopy density in small rivers (Table 2.4, Appendix 3). Within both subregions, the proportion of native tolerant individuals increased with fine substrate, channel embeddedness, and total chlorophyll, and decreased with increased gravel substrate (fine and coarse) and bank-full width/depth ratio (Table 2.4, Appendix 3). The proportion of omnivorous/herbivorous individuals generally decreased with dissolved oxygen, and increased with conductivity (Table 2.4).

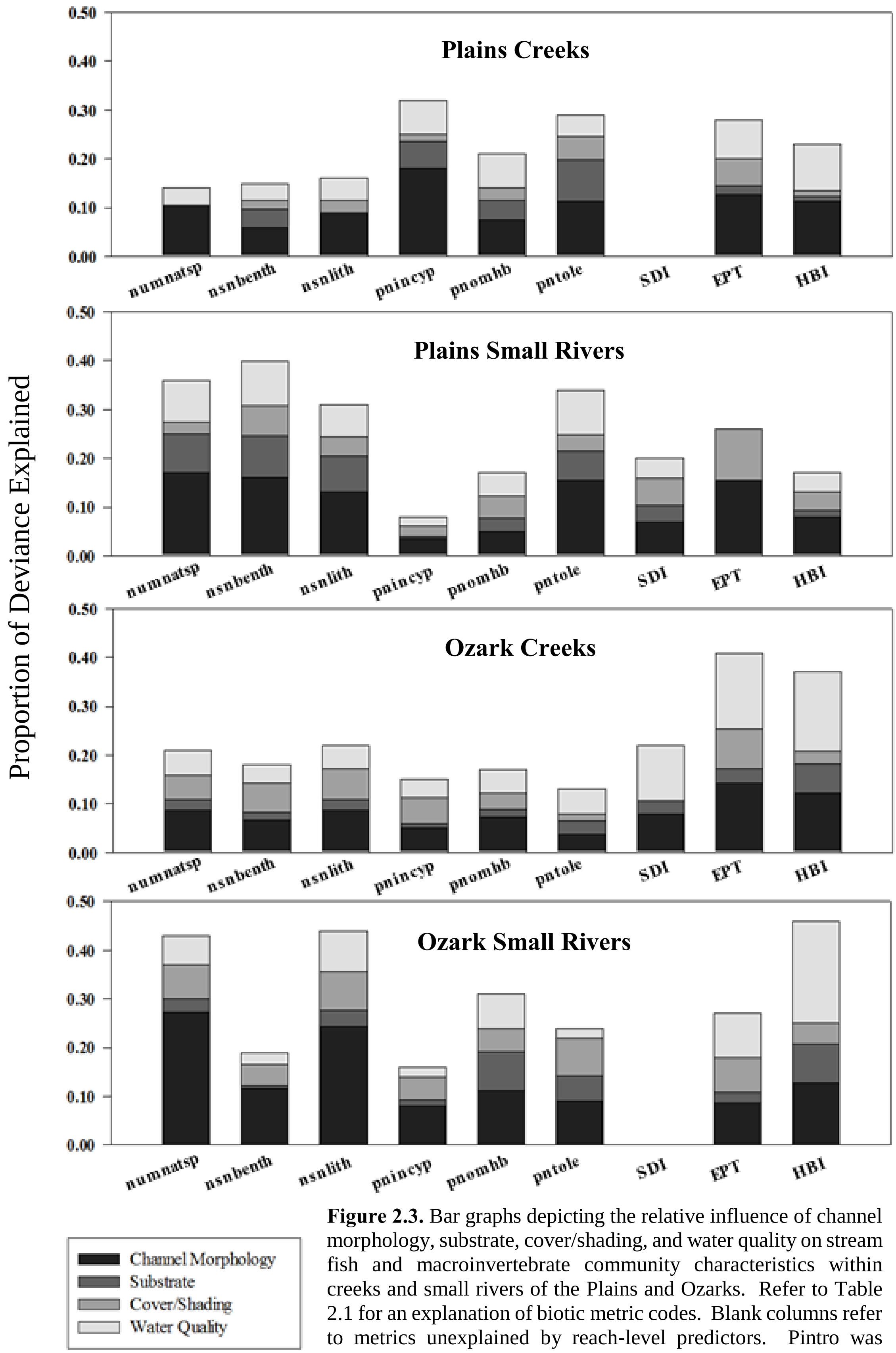

Figure 2.3. Bar graphs depicting the relative influence of channel morphology, substrate, cover/shading, and water quality on stream fish and macroinvertebrate community characteristics within creeks and small rivers of the Plains and Ozarks. Refer to Table 2.1 for an explanation of biotic metric codes. Blank columns refer to metrics unexplained by reach-level predictors. Pintro was unexplained at every spatial scale.

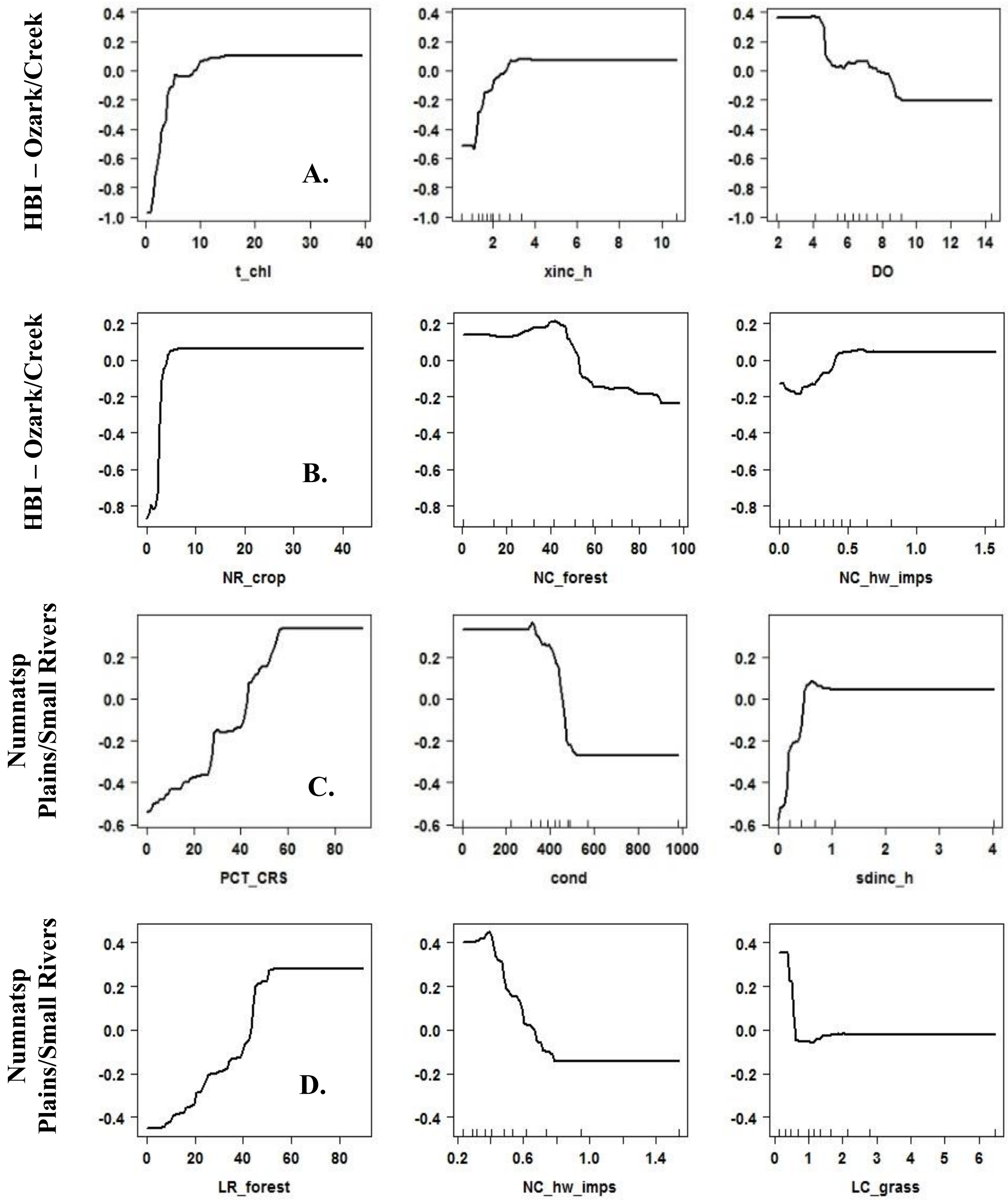

Figure 2.4. Example partial dependence plots of top influential environmental variables for **A.** – Hilsenhoff Biotic Index (HBI) – Ozark creeks, reach-level model, **B.** – Hilsenhoff Biotic Index (HBI) – Ozark creeks, watershed-level model, **C.** – Number of native species (Numnatsp) – Plains small rivers, reach-level model, and **D.** – Number of native species (Numnatsp) – Plains small rivers, watershed-level model. The y axes are presented as dimensionless transformations of each response. Rug plots at inside bottom of plots show the distribution of sites across the given predictor variable.

Invertebrate metrics (SDI, EPT, and HBI), in general, were more strongly related to water quality parameters than were fish metrics, with total chlorophyll, dissolved oxygen, and conductivity routinely accounting for 30-40% of the explained variation in each metric (Figure 2.3). In the Plains region, SDI values increased with coarse substrate, woody debris, and riffle percentage. Surprisingly, SDI values were also negatively related to dissolved oxygen, peaking at ~ 7 mg/L and then declining. In the Ozark region, SDI values were also highest in riffle-dominated streams and were shown to decrease sharply with increased total chlorophyll levels (Table 2.4, Appendix 3). The EPT richness was routinely most strongly related to reach-level environmental conditions and responded to metrics consistently across stream size and aquatic subregion, with values increasing with bank-full width and bank-full width/depth ratios, dissolved oxygen, and woody debris volume, and decreasing with increased total chlorophyll, conductivity, and mid-channel canopy cover (Table 2.4; Appendix 3). Conversely, HBI values decreased with increased dissolved oxygen, bank-full width, and percent riffle, and increased with total chlorophyll and channel embeddedness (Figure 2.4; Table 2.4; Appendix 3).

WATERSHED-LEVEL ENVIRONMENTAL INFLUENCE ON BIOTIC METRICS

We reduced our initial set of 62 watershed-level predictors to 28 after highly correlated variables were removed, and successfully constructed models for nine of ten biotic metrics for at least one stream size classification. We were unable to reduce model deviance within the first 1000 tree iterations within both subregions and stream size classes for the proportion of non-native individuals, as well as for Plains creek Shannon Diversity Index (SDI) values, Ozark small river SDI values, Plains Hilsenhoff Biotic Index (HBI) values in small rivers, and Ozark small river values of the proportion of native insectivorous cyprinids (Table 2.4). On average, watershed-level models explained ~ 18% of the variation in fish and invertebrate metrics in the Plains region, with the number of native lithophilic species in creeks having the lowest explained variation (4%), and the number of native lithophilic species in small rivers having the highest (31%). In the Ozark region, watershed-level models on average explained ~ 20% of the variation in biotic metrics, with the SDI value in creeks being the lowest (7%), and HBI values in small rivers being the highest (51%).

Percent forest, together with disturbance metrics related to fragmentation and flow modification, were consistently among the top predictors of fish and invertebrate community characteristics and frequently accounted for 50-75% of the explained variation in each metric. Conversely, metrics representing point-source pollution consistently showed the weakest relationship with biotic metrics within both subregions, on average accounting for less than 10% of the explained variation in fish and invertebrate metrics (Figure 2.4). Agricultural and urban impairments typically accounted for moderate amounts of explained variation (~ 20-30%), but their influence varied significantly among biotic response and between aquatic subregion (Figure 2.4). Within the Plains region, the density of headwater impoundments, road crossings, and pasture landcover within the local and network catchments consistently had the strongest negative influence on the number of native fish species, native benthic species, and native lithophilic species (Table 2.4; Appendix 4).

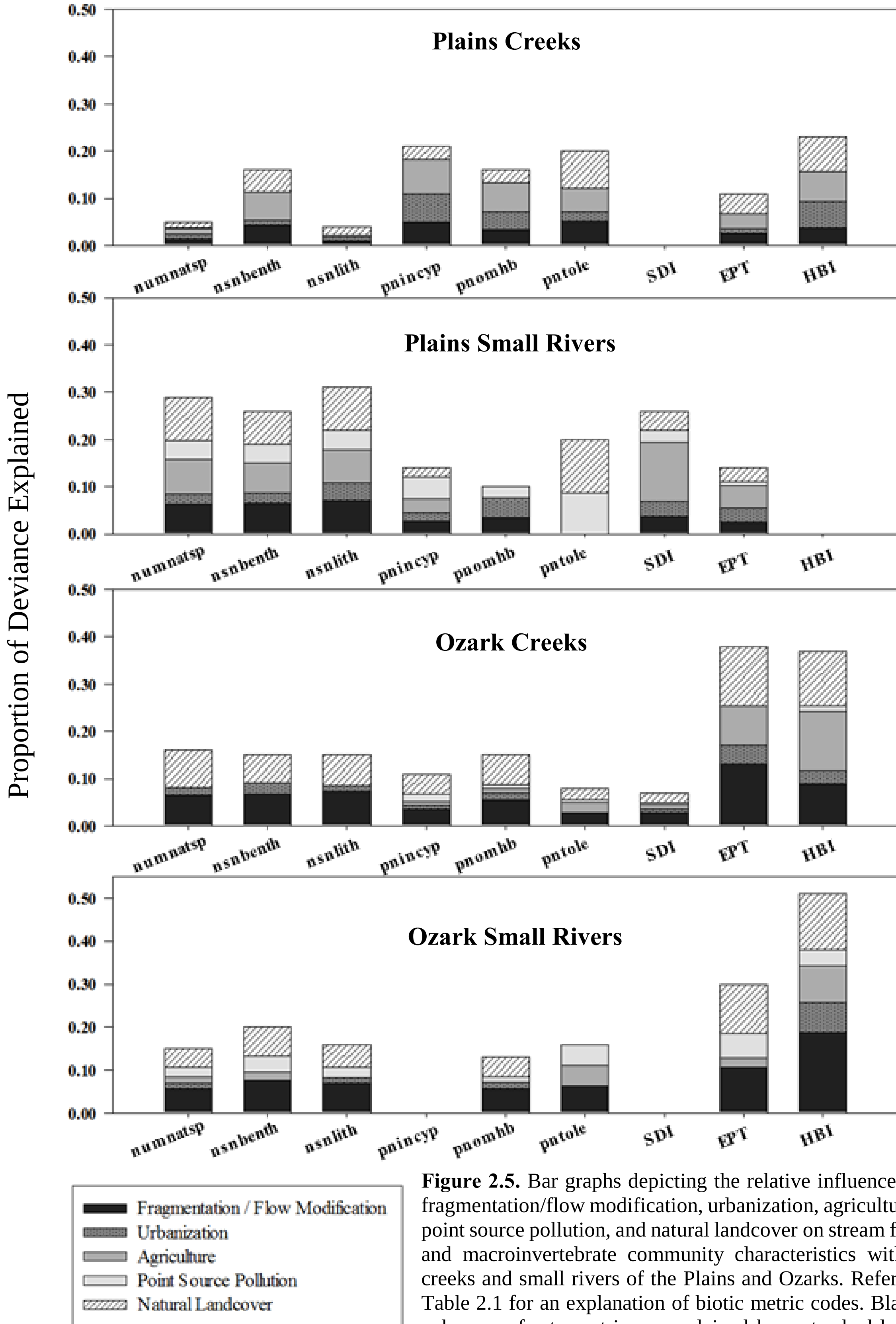

Figure 2.5. Bar graphs depicting the relative influence of fragmentation/flow modification, urbanization, agriculture, point source pollution, and natural landcover on stream fish and macroinvertebrate community characteristics within creeks and small rivers of the Plains and Ozarks. Refer to Table 2.1 for an explanation of biotic metric codes. Blank columns refer to metrics unexplained by watershed-level predictors. Pintro was unexplained at every spatial scale.

Within the Ozark region, fish richness measures also increased with higher percentages of forested landcover, and showed slightly higher sensitivity to urban impairment. Consistent with the Plains region, the number of native fish species, native benthic species, and native lithophilic species in the Ozarks were negatively influenced by increased road crossing density. In contrast with the Plains, fish richness measures in the Ozark region were positively related with low-level headwater impoundment density.

In general, fish metrics related to trophic ecology (proportion of native insectivorous cyprinids, proportion of native omnivorous/herbivorous individuals) showed stronger relationships to urban impairment than did species richness metrics, though responded to point-source pollution sources counter to what was predicted (Table 2.4; see Table 2.1 for predicted responses). The proportion of native insectivorous cyprinids increased with decreased local catchment population density, and with decreased developed, low-intensity and pasture landcover. Surprisingly, the proportion of native insectivorous cyprinids increased with increased confined animal feeding operations in the Plains region, and with lead-mining density in the Ozark region (Table 2.4; Appendix 4), although average densities of both stressors were still relatively low, at 0.013/km^2 and 0.038/km^2, respectively. In contrast to insectivorous cyprinids, the proportion of native omnivorous/herbivorous individuals increased with local catchment population density and local riparian imperviousness in the Plains region, and decreased with increased forest cover in the network catchments of Ozark creeks and small rivers (Table 2.4; Appendix 4).

Within both aquatic subregions, invertebrate metrics (SDI, EPT, HBI) typically showed the strongest relationship with agricultural disturbance (Figure 2.5). Within the Plains region, SDI values decreased with increased crop and pasture landcover, and increased with forested area. Similarly, EPT values decreased with increased pasture land in the network riparian zone, and local riparian crop at the small river level, although they had a slight positive relationship with local riparian crop at the creek level (Table 2.4; Appendix 4). Invertebrate community tolerance, as measured by HBI, was negatively related to local catchments with higher percentages of both forest and grassland, and was positively associated with local population density. In general, invertebrate metrics were more sensitive to agricultural disturbances in the Ozark subregion, with SDI values and EPT richness decreasing with increased network riparian row-cropping, and HBI values increasing (Figure 2.4; Table 2.4; Appendix 4).

LINKING REACH AND WATERSHED-LEVEL ENVIRONMENTAL VARIABLES

Each of the top 15 reach-level variables were significantly correlated ($P<0.05$) to at least 5 of the top 14 watershed level predictors (Table 2.5). The density of headwater impoundments within the network catchment, along with local and network riparian agriculture showed the most measurable response in the in-stream habitat. Increased headwater impoundment density was associated with narrower, incised stream channels with lower percentages of coarse substrate, and lower dissolved oxygen levels (Table 2.5). Similarly, increased row crop agriculture was linked with deeper, more heavily incised streams, fewer aquatic macrophytes and lower dissolved oxygen, and much higher levels of fine sediment and total chlorophyll. In contrast, the percentage of low intensity development, though showed to be detrimental to both fish and invertebrates, manifested in the fewest measurable habitat and water quality metrics, with higher conductivity levels being the most significant indicator of that source of impairment (Table 2.5)

Table 2.5. Spearman's rank correlation relating drainage area (Drainage_Ar), reach gradient, and top influential watershed –level landuse/landcover metrics to top influential reach-level environmental characteristics. Bold values indicate correlations significant at P=0.05.

	xdepth	xbkf_w	bfwd_rat	rpmx_dep	xinc_h	pct_pool	pct_crs	pct_fn	xfc_brs	xfc_aqm	xcdenmid	DO	t_chl	cond	pH
NC_hw_imps	**-0.08**	**-0.22**	**-0.23**	**0.09**	**0.15**	**0.09**	**-0.16**	**0.29**	**0.14**	**-0.14**	**0.34**	**-0.13**	**0.32**	**0.10**	-0.07
NC_rd_crs	0.06	-0.04	**-0.13**	0.01	0.00	**0.14**	0.02	**0.08**	-0.03	**0.08**	**0.12**	-0.01	**0.12**	0.05	0.07
LC_dev_low	0.04	-0.04	-0.05	-0.04	0.03	0.06	-0.04	**0.12**	-0.03	-0.04	0.02	-0.01	**0.21**	**0.20**	**0.10**
NC_dev_low	0.03	-0.02	-0.04	-0.03	0.02	0.04	-0.03	**0.12**	-0.04	-0.06	0.02	0.02	**0.19**	**0.20**	**0.11**
NR_crop	0.03	**-0.24**	**-0.28**	**-0.20**	**0.50**	0.02	**-0.53**	**0.51**	-0.03	**-0.31**	0.02	**-0.17**	**0.62**	0.00	**-0.11**
LR_crop	-0.03	**-0.20**	**-0.21**	**-0.25**	**0.45**	-0.02	**-0.50**	**0.36**	**-0.07**	**-0.28**	**-0.12**	**-0.10**	**0.50**	0.07	**-0.07**
LC_pasture	**-0.08**	-0.06	-0.01	**0.10**	**0.16**	0.03	-0.07	**0.18**	**0.16**	**-0.09**	**0.19**	**-0.13**	**0.17**	**-0.03**	**-0.05**
NR_pasture	-0.04	-0.02	0.03	**0.14**	**0.10**	0.05	0.03	**0.11**	**0.14**	-0.01	**0.15**	**-0.11**	**0.08**	**-0.15**	-0.03
LC_forest	-0.01	**0.31**	**0.34**	**0.19**	**-0.49**	**-0.09**	**0.50**	**-0.53**	-0.03	**0.25**	**-0.08**	**0.18**	**-0.59**	0.06	**0.11**
NC_forest	0.00	**0.30**	**0.33**	**0.15**	**-0.48**	**-0.09**	**0.47**	**-0.52**	-0.04	**0.26**	**-0.12**	**0.19**	**-0.58**	**0.07**	**0.12**
LR_forest	-0.05	0.07	**0.10**	**0.24**	**-0.40**	0.04	**0.36**	**-0.31**	**0.07**	**0.11**	**0.28**	**0.09**	**-0.34**	0.03	0.01
LC_grass	**-0.07**	**0.12**	**0.21**	**0.10**	**-0.18**	**-0.10**	**0.07**	**-0.21**	0.05	-0.01	-0.03	0.03	**-0.18**	**0.19**	**0.10**
NC_lndfl	**0.16**	**0.17**	0.06	**0.08**	**0.08**	-0.01	-0.02	**0.07**	0.02	0.06	**-0.10**	0.04	**0.10**	0.05	0.01
NC_npdes	**0.25**	**0.20**	**0.08**	0.05	0.01	0.06	**0.09**	0.04	**-0.10**	**0.07**	**-0.08**	0.04	0.06	**0.08**	**0.11**

PREDICTING STATEWIDE BIOLOGICAL INTEGRITY

After modeling the effect of watershed-level environmental conditions on fish and invertebrate community metrics, we used our boosted regression trees to predict estimated biotic metric values at over 19,700 creek and 8,900 small river reaches across the Plains and Ozark aquatic subregions. Rescaling each predicted value to a continuous 0-10 scale and summing to create a cumulative biological integrity score resulted in scores ranging from 0-80 within both Plains stream size classes, 0-90 for Ozark creeks, and 0-70 for Ozark small rivers. Maximum values were based on the number of biotic metrics related to watershed-level anthropogenic disturbance variables, and were thus able to be modeled, as described in Table 2.4 (e.g., 7 of the biotic metrics within the Ozarks small rivers spatial scale). Streams scoring at the high end of the continuum reflect least-disturbed watershed conditions relative to other reaches within the same aquatic subregion and stream size class, and are estimated to exhibit water quality and physical habitat characteristics suitable for a wider range of fish and invertebrate species. Conversely, streams scoring on the lower end of the spectrum reflect heightened impairment, and thus are estimated to exhibit degraded conditions (Figure 2.6). No stream reaches received maximum scores for all 7-9 metrics. The median score for Plains creeks was 38.6/80, with the maximum score of 63.4/80 (Table 2.5). Plains small river scores were on average slightly lower, with median and maximum scores of 34.2/80 and 61.8/80, respectively. Ozark creeks exhibited the highest concentration of least-impaired stream reaches, as indicated by median and maximum biological integrity scores of 52/90 and 77.4/90, respectively. Similar to the Plains region, high quality small river reaches in the Ozarks were fewer, with median biological integrity scores of 37.7/70, and with a maximum score of 51.8/70 (Table 2.5).

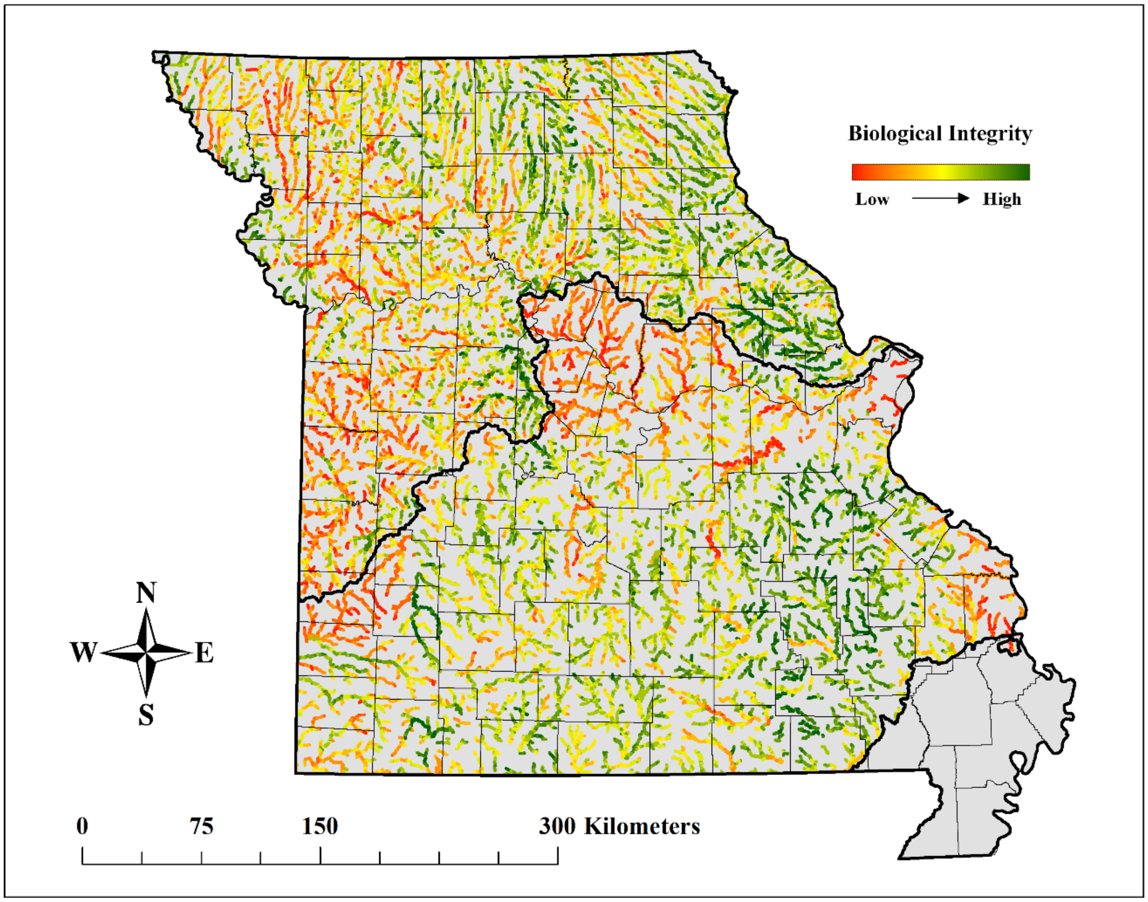

Figure 2.6. Map depicting the predicted biological integrity of creek and small river segments in the Plains and Ozark aquatic subregions of Missouri.

Table 2.6. Summary of cumulative biological integrity scores for creeks and small rivers of the Plains and Ozark aquatic subregions of Missouri.

Scale	Max Score	25th Percentile	Median	75th Percentile	95th Percentile
Plains - Creeks	63.4 / 80	32.6	38.6	45.2	57.3
Plains - Small Rivers	61.8 / 80	28.1	34.2	40.6	55.5
Ozark - Creeks	77.4 / 90	43.9	52.0	58.3	69.1
Ozark - Small Rivers	51.8 / 70	32.7	37.7	41.2	48.4

After calculating overall biological integrity scores, we retained those sites scoring in the top 95th percentile within each aquatic subregion and stream size classification to serve as candidate reference reaches. Within the Plains region, we retained 448 creek and 236 small river segments. In the Ozark region, 532 creek segments and 208 small river segments were selected (Figure 2.7). Streams varied considerably between and within aquatic ecoregion in terms of landcover/landuse within their watersheds, although several patterns were evident. Within the Plains region, candidate creek reference reaches exhibiting above average forested landcover (31.3%), below average pasture cover (29.8%), slightly below average cultivated crop (27.1%), and near average imperviousness (2.26% ; Table 2.6). Small river candidates in the Plains region showed a similar pattern with forest (18.3%), pasture (38.9%), and imperviousness (1.5%), though exhibited slightly above average levels of cultivated crop (38.9%). Candidate reference reaches within the Ozark region exhibited 88.9% forested local catchments, with low levels of pasture (5.2%) and imperviousness (0.8%), and extremely low levels of cultivated crop (0.08%). On average, Ozark small rivers had local catchments that were 54.5% forested, 36.9% pasture land, 1.5% impervious, and 0.4% cultivated crop.

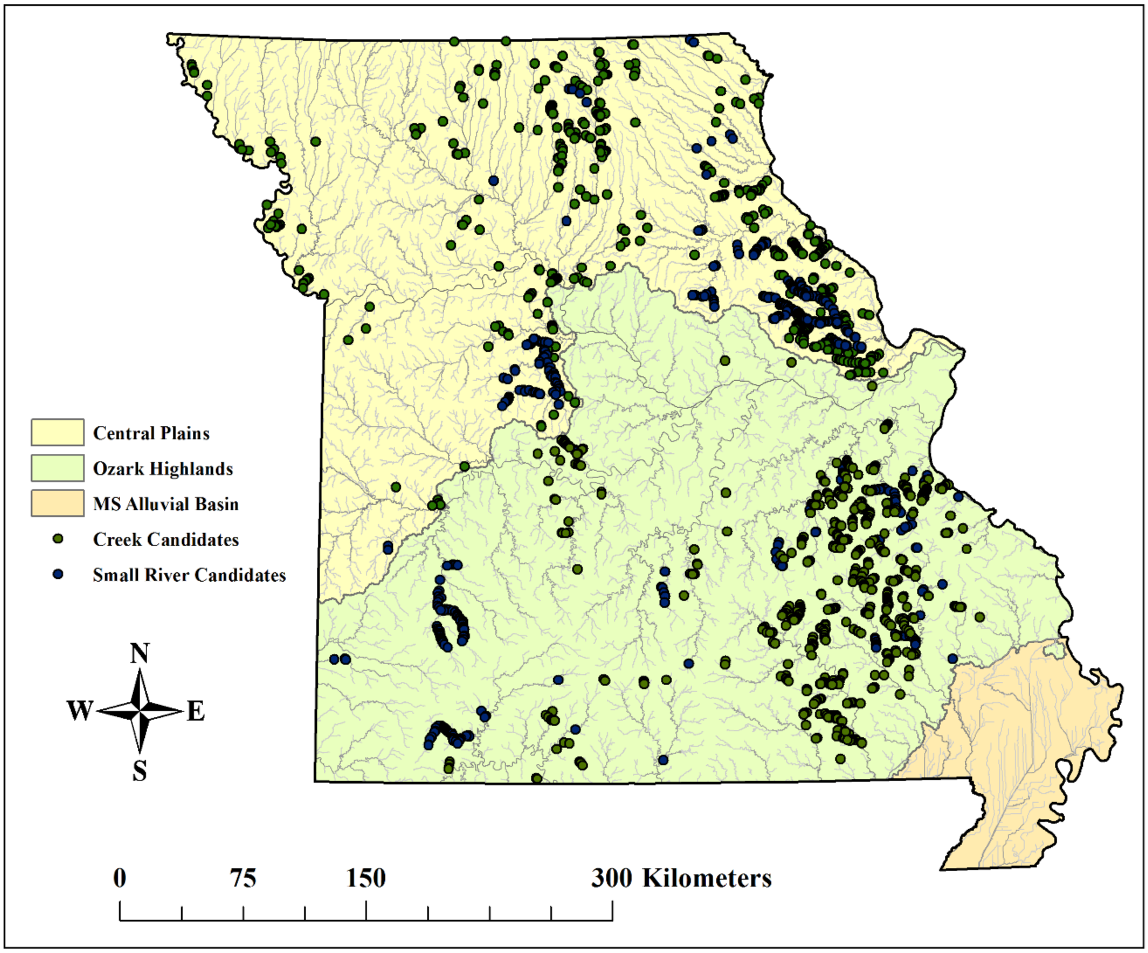

Figure 2.7. Map depicting candidate creek and small river reference reaches in the Central Plains and Ozark Highlands aquatic subregions of Missouri. (Candidates consisted of streams scoring in the top 95th percentile of predicted biological integrity.

Table 2.7. Mean and (standard deviation) values of general landcover/landuse metrics within the local watersheds of candidate creek and small river reference reaches within the Central Plains and Ozark Highlands aquatic subregions of Missouri.

	Central Plains		Ozark Highlands	
Landcover/Landuse	Creeks	Small Rivers	Creeks	Small Rivers
Forest	31.3 (15.9)	18.3 (9.44)	88.9 (6.0)	54.5 (26.4)
Cultivated Crop	27.1 (19.6)	38.9 (16.6)	0.08 (0.28)	0.4 (0.6)
Pasture	29.8 (18.6)	27.6 (9.8)	5.2 (4.5)	36.9 (25.4)
Impervious Surface	2.26 (3.1)	1.5 (0.6)	0.8 (0.5)	1.5 (0.7)
Localities	**448**	**236**	**532**	**208**

DISCUSSION

Our study is one of the first efforts to model the estimated biological integrity of Missouri's wadeable streams using predicted values of fish and invertebrate community characteristics from watershed-level human disturbance models. Additionally, our study represents a novel framework for relating watershed-level anthropogenic disturbances to in-stream physical habitat and biotic condition, and for identifying candidate least-disturbed stream reaches.

Reach-level physical habitat and water quality variables consistently explained greater proportions of variation in biotic metrics, on average accounting for approximately 26% of total variation, as opposed to 19% for watershed-level variables. This finding corroborates previous work showing the importance of reach-level environmental characteristics on fish and invertebrate communities (Richards et al. 1987; Wang et al. 2003), but does contradict other studies suggesting that watershed characteristics were greater determinants of stream biota than reach-level habitat and water quality (Roth et al. 1996; Allan et al. 1997). This discrepancy is scale dependent (Lammert and Allan 1999), and by modeling environmental influences within individual aquatic subregions, we likely reduced the influence of large-scale landuse/landcover patterns previously shown to be important (Infante et al. 2009).

In general, channel morphological characteristics were the most influential predictors of fish richness measures at the reach-level, and routinely accounted for ~ 40 to 50% of the explained variation in the total number of native species, the number of native benthic species, and the number of native lithophilic species. Even after removing the effect of drainage area, these richness measures still showed strong positive associations with increasing bank-full width, bank-full width/depth ratio, and standard deviation of channel incision, highlighting the importance of wider, and more variable channel conditions in maintaining habitat heterogeneity and species richness (Gorman and Karr 1978). In contrast, invertebrate metrics and proportional fish metrics (proportion of native insectivorous cyprinids, proportion of native omnivore/herbivore, and proportion of tolerant individuals) typically exhibited stronger relationships with water quality parameters (i.e. DO, total chlorophyll, conductivity), and to a lesser extent, substrate characteristics (i.e. channel embeddedness). These results support previous conclusions regarding the non-concordance of fish and invertebrate responses to disturbance (Infante et al. 2009), suggesting fish as good determinants of habitat degradation, while invertebrates serve as better indicators of water quality impairment (Rabeni 1997; Wang et al. 1997; Bramblett et al. 2005), further indicating the necessity to incorporate both into an overall biological integrity model. Our results indicate that increased percentages of fine sediment and substrate embeddedness, linked to riparian agricultural and urban development, result in increased invertebrate community tolerance (HBI), greater proportions of tolerant fish species, and lower fish species richness. This finding is similar to others that have documented the loss of interstitial benthic habitat and spawning substrate as a result of excess sedimentation, resulting in degraded fish and invertebrate communities (Berkman and Rabeni 1987; Berry et al. 2003; Kemp et al. 2011). However, substrate characteristics in general accounted for the least amount of explained variation in biotic metrics, although this may be attributable to the subjective determination of particle size and embededness in relation to more precisely measured features, such as stream widths and depths (Kauffman 1999).

The relationships between biotic metrics and watershed-level environmental variables differed slightly between stream size class and aquatic subregion, though several patterns were evident. Forested landcover, together with measures of fragmentation and flow modification were consistently among the top watershed predictors, frequently accounting for between 50 and 75% of the total explained variation in biotic metrics. In the Plains region, this largely consisted of decreasing fish richness values as road crossing and headwater impoundment densities increased, and increased fish richness and invertebrate metric values (SDI, EPT) with increased forested land cover, particularly at the local catchment and local riparian scale. The strength of these relationships corresponds with previous suggestions that fish species of the Plains are strongly influenced by flow conditions and water availability (Matthews 1988; Dodds et al. 2004). The amount of pastureland, used as a surrogate for cattle production, in the local catchment and riparian zone also appears to be a significant source of fish community impairment, even more so than cultivated crops within the Plains region. This finding corroborates various studies documenting the negative influence of riparian cattle grazing, including dramatically increased phosphorous contributions and destabilized stream bank conditions following riparian grazing (Quinn et al. 1992; James et al. 2007). Row-crop agriculture, specifically in the local and network riparian zones, however, more strongly influenced invertebrate metrics than fish richness metrics, likely due to increased sedimentation and nutrient contributions, as measured by channel embeddedness and total chlorophyll. Numerous studies highlight invertebrate sensitivity to row-crop agriculture and the resultant channel and water quality degradation (Lenat and Crawford 1994; Lammert and Allan 1999; Allan 2004). Even within the agriculturally dominated Plains region, invertebrate community metrics were able to differentiate streams along a continuum of agricultural impairment. Similarly, invertebrate metrics of the Ozark region were negatively influenced by agricultural landcover, and demonstrated much higher sensitivity, suggesting that values as low as 5-10% of cultivated crop within the network riparian zone can lead to degraded stream conditions, much lower values than previously found in the Midwest using fish community data (Wang et al. 1997).

Urban sources of impairment (i.e. population density, imperviousness, etc.) showed little influence on fish richness measures, however, had a greater impact on invertebrate metrics and stream fish trophic ecology. The proportion of native insectivorous cyprinids decreased with increased population density and low intensity development, while the proportion of omnivorous/herbivorous individuals increased with population density and local riparian imperviousness. Relatively few studies have documented the specific effects of urbanization on fish community structure (Paul and Meyer 2001), but our results do coincide with the general finding of decreased fish community integrity with increased urbanization (Wang et al. 2001; Morgan and Cushman 2005). In the Ozark region, HBI values begin to increase once development exceeds ~ 6-8%, while EPT richness appears to decline rapidly after ~10%, resembling previous estimates of urban impairment thresholds (Yoder et al. 1999; Paul and Meyer 2001).

Despite its history as a major source of stream impairment (Cairns and Pratt 1993), point-source pollution consistently proved to be a weak predictor of fish and invertebrate characteristics, and when present, tended to influence biotic metrics contrary to what was expected. For instance, the proportion of native insectivorous cyprinids in the Plains region showed a positive relationship with the density of confined animal feeding operations

(CAFO), and increased with lead mine density in the Ozark region. Still, average densities of both stressors were relatively low within our study area, at 0.013/km^2 and 0.038/km^2, respectively. Additionally, both of these stressors were concentrated in remote, largely forested areas within each subregions, perhaps indicating false detection of point-source influence, with the surrounding natural landcover responsible for the resultant biotic metric values.

By correlating influential variables from both our reach and watershed-level predictor sets, we can begin developing a mechanistic view of the specific ways human landscape alterations impact the physical and chemical condition of receiving waters (Rabeni 2000; Infante and Allan 2010). The density of headwater impoundments within the network catchment, along with local and network riparian agriculture showed the most measurable response in the in-stream habitat. Increased headwater impoundment density was associated with narrower, more incised stream channels with lower percentages of coarse substrate, and lower dissolved oxygen levels. Similarly, increased row crop agriculture was linked with deeper, more heavily incised streams, fewer aquatic macrophytes and lower dissolved oxygen, and much higher levels of fine sediment and total chlorophyll. In contrast, the percentage of low intensity development, though showed to be detrimental to both fish and invertebrates, manifested in the fewest measurable habitat and water quality metrics, with higher conductivity levels being the most significant indicator of that source of impairment. This result coincides with other studies finding urbanization to be more weakly tied to physical habitat integrity than to biotic integrity (Wang et al. 1997), suggesting we are currently limited in our ability to identify urban impairment using common habitat and water quality metrics.

Using the results from our watershed-level models, we were able to predict biotic metric values at over 19,700 creek and 8,900 small river reaches across the Plains and Ozark aquatic subregions using existing landscape-level environmental variables. By rescaling and summing predicted fish and invertebrate metric values, we were able to generate cumulative scores representing an estimate of overall biological integrity. Rather than simply assigning impaired and unimpaired statuses to stream reaches, our scoring system reflects a continuum of degraded conditions (Davies and Jackson 2006). By incorporating multiple ecological indicators into our estimate, we were able to identify a wider range of disturbances than detectable using single indicators, a noted advantage (Dale and Beyeler 2001).

We identified streams scoring in the top 95th percentile within each stream size class and aquatic subregion as candidate least-disturbed reference reaches. By focusing on these predicted scores rather than designating set landscape criteria for candidate determination, we retained the full complexity of watershed conditions, and did not sacrifice any ability to describe biotic conditions (Leathwick et al. 2006; Elith et al. 2008). Additionally, by replacing previous best-professional judgment techniques (Hughes et al. 1986; Rabeni et al. 1997) for determining reference reaches with a stepwise, data-based approach, we were able to increase repeatability and lower bias in identifying high and low quality stream reaches (Doisy et al. 2008).

Our model's ability to predict biological integrity and identify candidate reference reaches can be supplemented and improved in several ways. Because our watershed-based models used coarse surrogates for in-stream environmental conditions, our predictors explained at best ~ 50% of the variation in a given response metric, meaning subsequent model refining and field verification will be necessary prior to designating streams as reference (Wang et al. 2008). Additionally, a major assumption of our modeling approach is that the full range of stream conditions within a given size class and aquatic subregion is represented in our data. Because stream monitoring programs often focus efforts in more pristine regions with enhanced conservation opportunities, this assumption may not have been fully met. By targeting streams on both ends of our scoring spectrum for sampling, we can better hone our expectations for biotic metric values under both least and most-disturbed conditions (Sarver 2002). Furthermore, by sampling reaches exhibiting specific human threats (e.g. landfills, mining operations, etc.), we can better quantify their effect on biotic communities, and they may then carry more significance in our predictive model. Additionally, because biotic metrics exhibited variation in both their predictability, and sensitivity to anthropogenic disturbance, assigning weights to individual metrics may improve our estimates of overall stream health (Wang et al. 2008).

Our study represents a critical first step toward refining existing biological criteria and developing a companion physical habitat index in wadeable streams of Missouri. Moreover, our results can be used to designate areas of high conservation value, and restoration need.

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CHAPTER THREE

TOWARDS A BETTER UNDERSTANDING OF HEADWATERS: DEVELOPING A PROVISIONAL REGIONAL THREAT INDEX

Ethan R. Kleekamp

ABSTRACT

Headwater streams are varied and diverse members of steam networks responsible for maintaining natural discharge regimes, sediment transport, nutrient export and retention, often serving as critical spawning habitat and diverse refugia for downstream aquatic organisms. Despite their importance, headwaters are largely neglected in many states' stream sampling protocols, representing both a knowledge gap and barrier to their conservation. Close terrestrial linkages make these streams highly susceptible to anthropogenic disturbances, thus, managers need the ability to predict areas of potentially high and low biological integrity to inform management decisions and meet conservation needs. We summarized existing landscape-level anthropogenic threat data for 92,500 headwater stream segments in Missouri and developed a regionally-specific, multimetric threat index to estimate in-stream habitat and biotic condition based on land-use and point source disturbances (e.g. agriculture, urban areas, mining operations, landfills). Catchments varied considerably in disturbance type and intensity within and across major physiographic boundaries, resulting in varied estimates of relative impairment between aquatic subregions. Using our indexing approach, we identified a suite of 984 sites as potential candidate headwater reference reaches. These consisted of 95 Central Plains sites, 558 Ozark Highlands sites, and 331 sites within the Ozark Border transitional region. Candidate reaches averaged less than 1% impervious surface, fewer than 0.2 stream crossings/km^2, and on average ranged from 66% forested in the Central Plains to 94%

forested in the Ozark Highlands. The Ozark Border region contained higher average pasture land (20%), though varied considerably in watershed condition. After subsequent field verification and biological sampling, data collected from these sites will assist in recalibrating existing biotic indices and help inform a companion physical habitat index for Missouri headwater streams.

KEYWORDS: *Headwater streams, Threat index, Watershed condition, Conservation*

INTRODUCTION

Headwater streams are unique and varied members of stream networks, crucial in maintaining the structure, function, and biological integrity of downstream river reaches (Lowe and Likens 2005; Meyer et al. 2007). These upper branches maintain natural discharge regimes, sediment transport, and nutrient export and retention (Likens and Bormann 1974) and often serve as critical spawning habitat and diverse refugia for downstream aquatic organisms (Meyer et al. 2007). Their close terrestrial linkages make headwaters both highly sensitive to landscape modifications (Meyer and Wallace 2001), and ideal targets for ecosystem restoration and designation in protected-area networks (Saunders et al. 2002; Lowe et al. 2006).

Given their importance, surprisingly little sampling and management effort has been directed toward headwaters. Despite approximating 75 percent of the United States' stream channel length (Leopold et al. 1964), headwaters are largely neglected in many states' stream sampling protocols, likely due to their characteristic intermittency and unpredictable nature (Lowe and Likens 2005). In Missouri, headwaters account for less than 15 percent of the state's sampled stream reaches, representing a significant knowledge gap and effective barrier to their conservation. Moreover, current assessments of the state's headwaters likely fail to describe their true biological condition due to unrealistic criteria developed from larger streams (Doisy et al. 2008).

There has been growing concern over the condition of Missouri's flowing waters over the last several decades. Widespread deforestation and increasing agricultural and urban expansion have highlighted the need to conserve remaining high quality stream reaches and restore those that are now degraded. However, because Missouri, like much of the United States, is lacking sufficient biological data from headwater streams to guide such management efforts, resource agencies must instead use coarser conservation planning and prioritization tools developed at larger spatial scales (Fore et al. 2014). Often these efforts make use of available natural and anthropogenic landscape data to forecast in-stream physical habitat and biotic conditions.

Given the importance of headwater streams, we need the ability to predict areas of high and low biological integrity to inform management decisions and meet conservation needs. Thus, the objectives of this study were to: 1) summarize existing human threat data for each headwater stream segment in Missouri, 2) develop a provisional, multi-metric headwater threat index, and 3) identify a suite of least-disturbed candidate headwater reference reaches to serve as benchmarks of physical habitat, water quality, and biotic potential in headwater streams. This work will assist in recalibrating existing biotic indices and help inform a companion physical habitat index for Missouri headwater streams.

METHODS

STUDY REGIONS AND SPATIAL FRAMEWORK

Our study focused exclusively on headwater streams in Missouri, as identified in the state's aquatic community classification system (Pflieger 1989). These streams range from Strahler orders 1-3, and typically exhibit drainage areas less than 10km^2, though may exceed 200km^2 in areas of lower drainage density. We used stream reaches from a modified version of the 1:100,000-scale National Hydrography Dataset (NHDPlus V1, 2008; Annis et al. 2010) and attributed each reach's upstream catchment with landscape-level environmental variables using ArcGIS 10.2. (ESRI, Redlands, CA, USA), and the stream network topology tool, RivEX (Hornby, 2013).

Missouri is a physiographically diverse state exhibiting three primary aquatic subregions, each containing distinct geology, soils, landform, vegetative cover, groundwater influence, and aquatic fauna (Sowa et al. 2007). The dissected till plains (hereafter, Plains), comprise much of the northern half of Missouri, and contain low, rolling hills, broad river valleys, and generally low gradient streams with fine substrate (Pflieger 1971; Figure 3.1). The Ozark highlands (hereafter, Ozarks), encompasses much of southern Missouri and is characterized by high local relief, deep and narrow river valleys, and much higher stream gradients than commonly seen in the plains region (Figure 3.1). Ozark streams often have substantial groundwater influence, and commonly exhibit low turbidity, high dissolved oxygen levels, and coarse gravel substrates (Sowa et al. 2007). The Mississippi Alluvial Basin (hereafter, MS Alluvial Basin), is an agriculturally dominated region, characterized by streams that are often highly vegetated, exhibit relatively low dissolved oxygen levels, and consist primarily of silty and fine gravel substrates (Pflieger 1971; Figure 3.1).

Though these subregions can account for much of the variation in stream habitat and fish community structure, intraregional differences do exist. To control for patterns of species endemism and other potential drainage-specific characteristics, a suite of ecological drainage units (EDU) have been delineated along major basin boundaries within each subregion (Sowa et al. 2007; Figure 3.1). These 17 distinct EDUs account for taxonomic differences within aquatic subregions and allow for more informative stream comparisons, and served as our base spatial layer for index development.

DATA SUMMARIZATION

We used landscape-level stressor data primarily compiled by the Missouri Resource Assessment Partnership (MORAP) as the basis for our threat index. These metrics represent known environmental stressors, and were selected by an EPA work group for inclusion in MORAP's Synoptic Human Threat Index (Annis et al. 2010; Table 3.1).

We calculated land-use metrics from the 2006 National Land Use/Land Cover dataset (Fry et al. 2011) (e.g. agricultural cover, urban land cover, etc.) as percentages of each headwater segment's upstream catchment, and converted point-stressors (e.g. stream crossings, mining operations, landfills, etc.) to upstream densities (number/km^2) (Table 3.1).

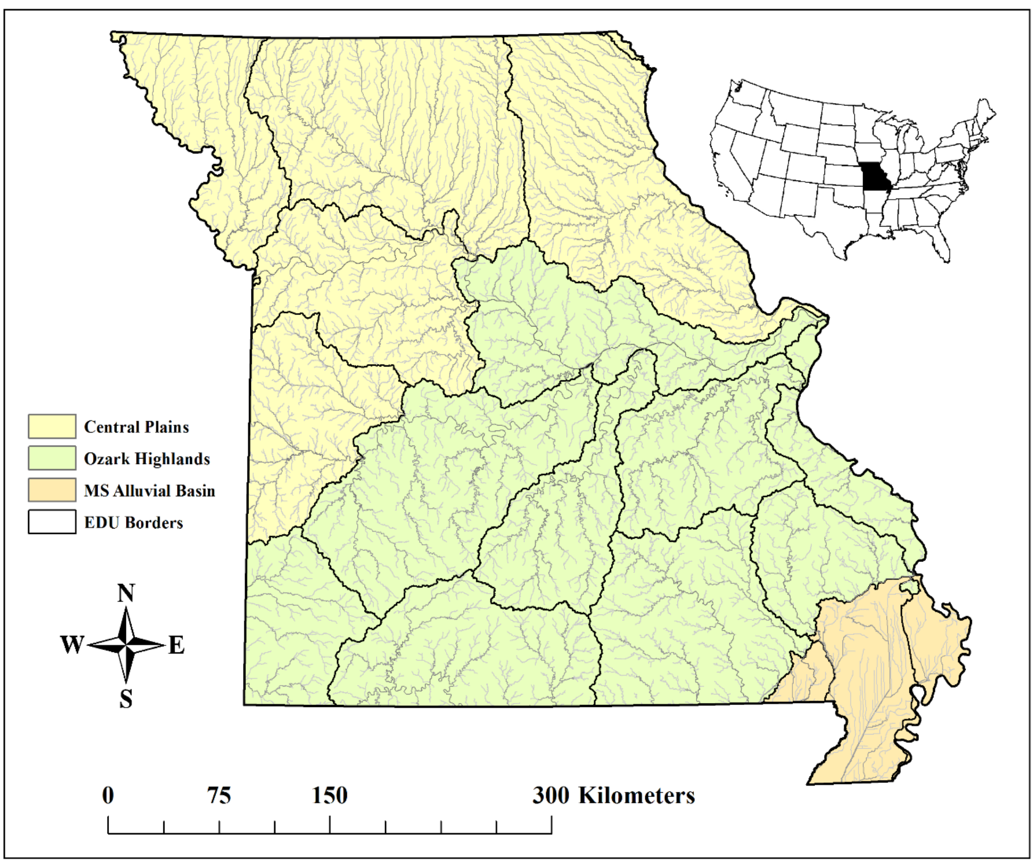

Figure 3.1. Map depicting Missouri's major aquatic subregions and ecological drainage units.

THREAT INDEX DEVELOPMENT

Just as biological indices are often regionally-specific to account for natural differences in biotic potential, threat indices too must be developed regionally to best reflect stream conditions. Because threats are rarely ubiquitous across a state (i.e. agricultural production, lead mining, etc.), and because certain stressors may have differential effects on species whose life history traits and physiological tolerances evolved under different environmental conditions (Matthews 1998), index structure and scoring systems require regionally-specific calibration.

Table 3.1. Landscape-level threat metrics summarized for each headwater catchment. CAFO* - Confined Animal Feeding Operation, NPDES† - National Pollution Discharge Elimination System.

Metric	Date Published	Source
CAFO* Sites (no./km^2)	2003	Environmental Protection Agency
NPDES† Sites (no./km^2)	2007	Environmental Protection Agency
Landfills (no./km^2)	2001	Environmental Protection Agency
Registered Hazardous Waste Sites (no./km^2)	2007	Environmental Protection Agency
Superfund Sites (no./km^2)	2007	Environmental Protection Agency
Dams (no./km)	2010	Missouri Department of Natural Resources
Road/Stream Crossings (no./km)	2007	Missouri Resource Assessment Partnership
Coal Mines (no./km^2)	2001	Environmental Protection Agency
Lead Mines (no./km^2)	2001	Environmental Protection Agency
Mines (Other) (no./km^2)	2001	Environmental Protection Agency
Sand/Gravel Mines (no./km)	2002	National Atlas
Cultivated Crop (% watershed area)	2006	Multi-Resolution Land Characteristics Consortium
Pasture/Hay (% watershed area)	2006	Multi-Resolution Land Characteristics Consortium
Imperviousness (% watershed area)	2006	Multi-Resolution Land Characteristics Consortium

To meet these requirements, we first calculated density quartiles for each of the 14 threat metrics at the EDU level to assess each metric's range and variability. Next, we generated individual threat-severity rankings based either on known literature thresholds, (impervious surface <5%, 5%-10%, 10%-15%, >15% ;Yoder et al. 1999; Paul and Meyer 2001) or on density quartiles when no empirical threshold evidence could be obtained (Table 3.2). Scoring exceptions were made, however, for those EDUs spanning the transitional zone between the plains and Ozark regions. Because inherent environmental differences (e.g. soil type, vegetative cover, groundwater influence) within these "border" regions could potentially misconstrue index results, we calibrated our stream scores nested by Aquatic Ecological Classification Unit (AES). These units were delineated to account for finer scale natural environmental differences, thus allowing for more realistic forecasting and assessment of streams' true physical and biological condition (Sowa et al. 2007; Figure 3.2).

Table 3.2. Example index scoring criteria for the White River Drainage Unit, Ozark Region.

	Relative Ranks				
Metric	0	1	2	3	4
CAFO Site (no./km^2)	0	0.01 - 0.10	0.11 - 0.23	0.24 - 0.31	0.32 ≤
NPDES Site (no./km^2)	0	0.01 - 0.14	0.15 - 0.25	0.26 - 0.49	0.5 ≤
Landfills (no./km^2)	0	0.01 - 0.08	0.09 - 0.17	0.18 - 0.29	0.30 ≤
Registered Hazardous Waste Sites (no./km^2)	0	0.01 - 0.12	0.13 - 0.26	0.27 - 0.50	0.51 ≤
Superfund Sites (no./km^2)	0	0.01 - 0.06	0.07 - 0.11	0.12 - 0.28	0.29 ≤
Dams (no./km)	0	0.01 - 0.11	0.12 - 0.19	0.20 - 0.31	0.32 ≤
Road/Stream Crossings (no./km)	0	0.01 - 0.40	0.41 - 0.59	0.60 - 0.98	0.99 ≤
Coal Mines (no./km^2)	-	-	-	-	-
Lead Mines (no./km^2)	0	0.01 - 0.12	0.13 - 0.20	0.21 - 0.41	0.42 ≤
Mines (Other) (no./km^2)	0	0.01 - 0.10	0.11 - 0.16	0.17 - 0.30	0.31 ≤
Sand/Gravel Mines (no./km)	0	0.01 - 0.07	0.08 - 0.32	0.33 - 0.52	0.53 ≤
Cultivated Crop (% watershed area)	0	0.1 - 0.16	0.17 - 0.50	0.51 - 1.29	1.3 ≤
Pasture/Hay (% watershed area)	0	0.1 - 12.2	12.3 - 28.6	28.7 - 51	51.1 ≤
Imperviousness (% watershed area)	0	0.01 - 5	5.1 - 10	10.1 - 15	15.1 ≤

Individual threat metric scores ranged from 0-4, and once all 14 metrics were assigned relative severity rankings, they were summed to create an overall, cumulative threat score (Table 3.2). This overall score could range from 0-56, with low numbers indicating relatively low estimated anthropogenic impact, and either varied or intense threats occurring for higher scoring streams.

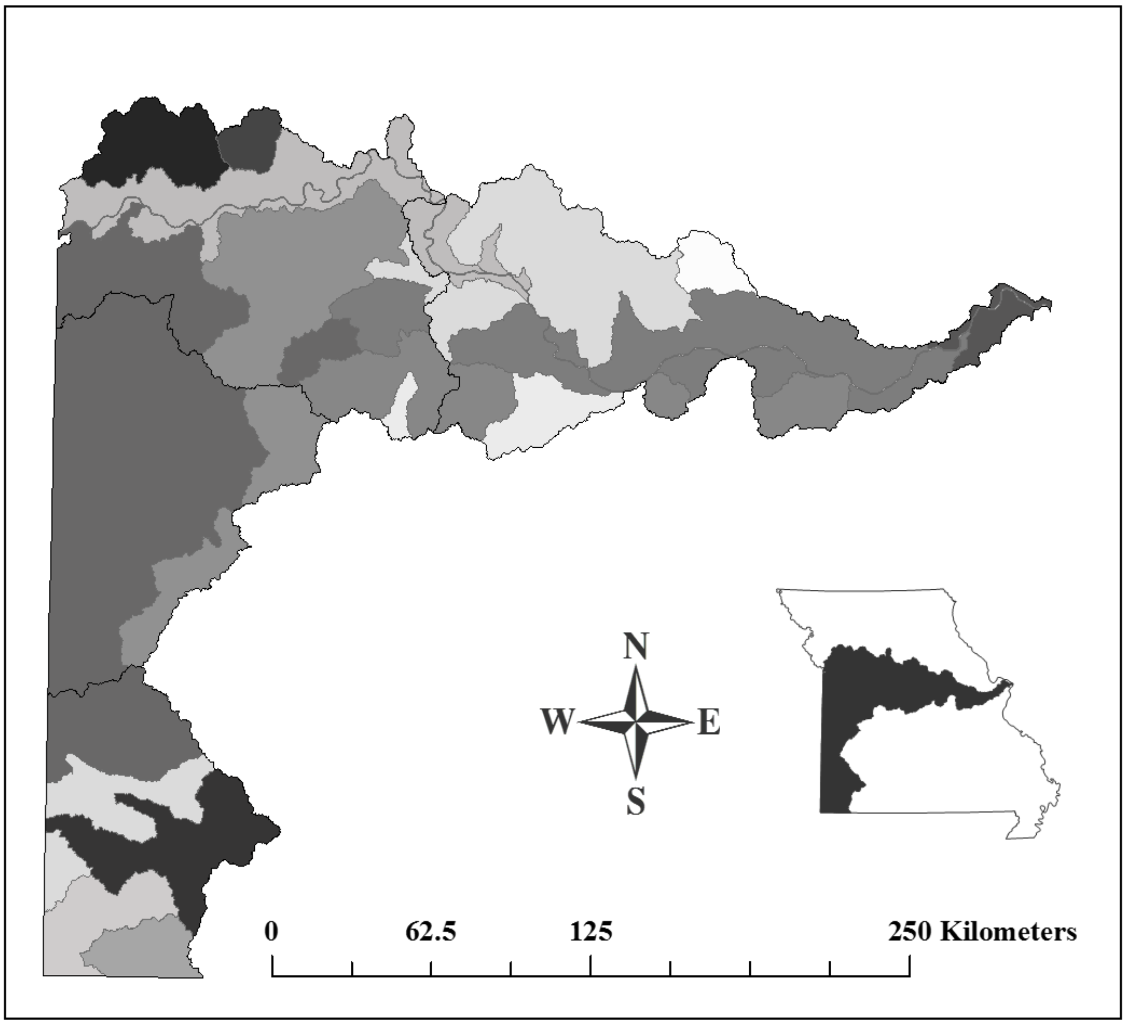

Figure 3.2. Map depicting the Aquatic Ecological System units used as the base spatial scale for threat index development for the four EDUs occurring within the Ozark Border region.

CANDIDATE REFERENCE REACH DETERMINATION

We used threat index results to identify a suite of "least-disturbed" candidate headwater reference stream reaches (hereafter, candidates) within each EDU, or AES unit in the Border Region. We employed a stepwise filtering process to locate reaches exhibiting potentially high biotic integrity. First, we retained for each region only those streams with threat index scores in the top one percentile (lowest overall scores). We then recalculated individual threat metric quartiles for these top tier reaches, adjusted their scores based on previously described methods, and generated a new ranking for each. To account for those smaller catchments (e.g. < 0.5 km^2) exhibiting homogenous agricultural or urban land use, and thus low overall threat scores, any catchments scoring a 3 or 4 for any single threat metric were excluded from consideration as candidates. To help direct field verification efforts, we generated lists of the top scoring streams (n~30) in each EDU/AES. Project personnel from both the Missouri Department of Conservation and Missouri Department of Natural Resources then proceeded to visit selected sites between March and September 2014, identify potential disturbances undetected at the landscape-level, and make recommendations concerning their inclusion, or exclusion from the final candidate list (See Appendix 5 for field reconnaissance form).

RESULTS

THREAT PREVALENCE

We summarized landscape-level anthropogenic stressor data for a total of 92,500 headwater stream segments in Missouri. Catchments showed considerable variation in disturbance type and intensity within and across major physiographic boundaries (Table 3.3). Major land-use patterns were evident at regional scales, but point stressors showed slightly less predictability in their spatial distributions. Agricultural land-use was highest in the southeast lowlands (~70%), followed by the Plains region (~37%) and Ozarks (~3.86%). Despite registering fewer agriculturally dominated watersheds and slightly fewer pastured watersheds, the Ozark region contains much of the state's lead mining activity, and several regions within exhibited watersheds with heavy (~2.5/km^2) CAFO concentrations. Mean catchment imperviousness varied little, and was commonly found to be below estimated impairment thresholds (~ 5%) (Paul and Meyer 2001).

Table 3.3. Landscape-level headwater threat metric summary for each aquatic subregion.

	Central Plains	**Ozark Highlands**	**MS Alluvial Basin**
Metric	Mean (Stdev)	Mean (Stdev)	Mean (Stdev)
CAFO Sites (no./km^2)	0.0051 (0.07)	0.003 (0.05)	0.0006 (0.011)
NPDES (no./km^2)	0.0117 (0.107)	0.0247 (0.155)	0.0072 (0.091)
Landfills (no./km^2)	0.001 (0.026)	0.0015 (0.03)	0.0005 (0.014)
Registered Hazardous Waste (no./km^2)	0.0064 (0.068)	0.0097 (0.099)	0.0019 (0.028)
Superfund Sites (no./km^2)	0.0014 (0.029)	0.0035 (0.045)	0.0005 (0.017)
Dams (no./km)	0.0711 (0.294)	0.0353 (0.276)	0.0099 (0.107)
Road/Stream Crossings (no./km)	0.6582 (0.659)	0.5785 (0.731)	0.6278 (1.556)
Coal Mines (no./km^2)	0.0117 (0.101)	0.0031 (0.084)	0 (0)
Lead Mines (no./km^2)	0.0003 (0.016)	0.0148 (0.229)	0 (0)
Mines (Other) (no./km^2)	0.0004 (0.014)	0.001 (0.027)	0.0001 (0.005)
Sand/Gravel Mines (no./km)	0.0001 (0.004)	0.0002 (0.016)	0.001 (0.03)
Cultivated Crop (% watershed area)	36.82 (28.883)	3.86 (12.33)	69.94 (34.32)
Pasture/Hay (% watershed area)	36.52 (24.101)	29.04 (24.897)	5.82 (14.308)
Imperviousness (% watershed area)	2.27 (5.254)	2.06 (5.234)	1.63 (3.036)

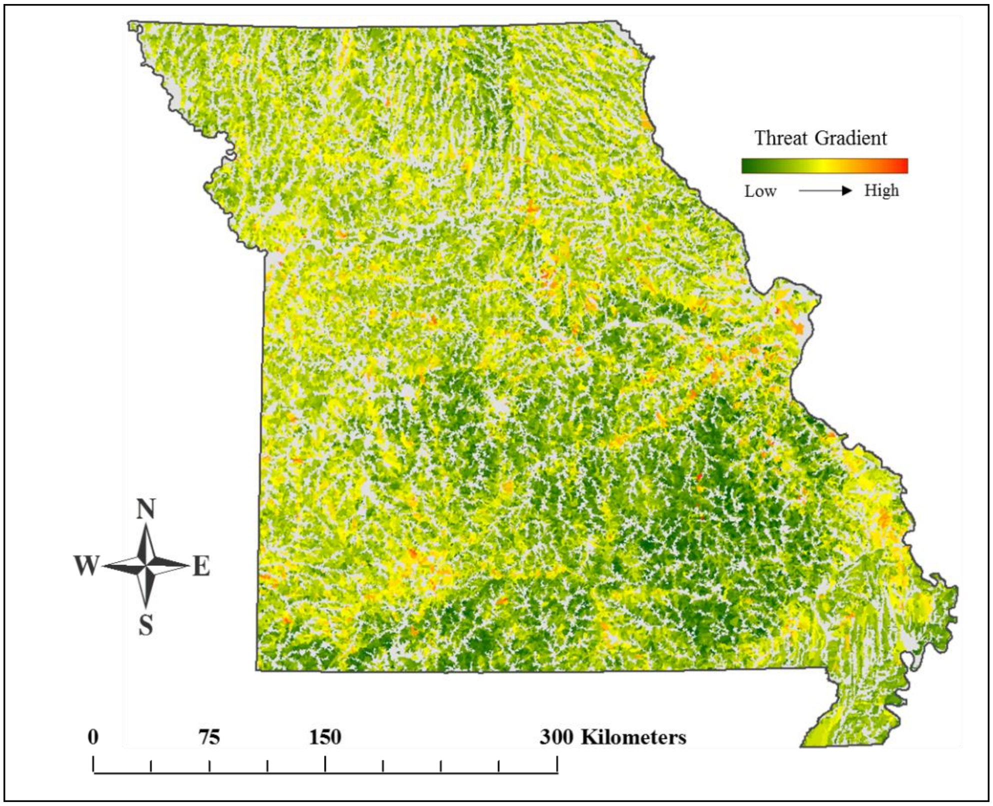

Figure 3.3. Results of headwater threat index mapped at the catchment scale. Colors reflect threat level relative to other sites within their respective EDUs, or AESs for border region streams.

STREAM SCORING AND CANDIDATE DETERMINATION

Though the maximum cumulative threat index score could reach 56, no catchments exceeded 28. The Current River drainage unit (Ozark region) had the highest concentration of streams without any upstream disturbances (all metrics ranked 0; n=276), while the Platte River drainage unit (plains region) had the fewest (n=2). By limiting our search for candidate headwaters to the top one percentile in each assessment unit, we eliminated over 90,900 stream reaches from consideration as reference sites. After rescoring and generating new rankings, our final candidate list consisted of 984 total sites, including 95 Plains streams (mean index value=3.14), 558 Ozark streams (mean index value=1.06) and

331 Ozark Border Streams (mean index value=3.15) (Figure 3.4). Due to the nearly homogenous agricultural makeup of watersheds in the MS Alluvial Basin, along with insufficient biological samples for site validation, the decision was made to presently forego candidate screening in the region.

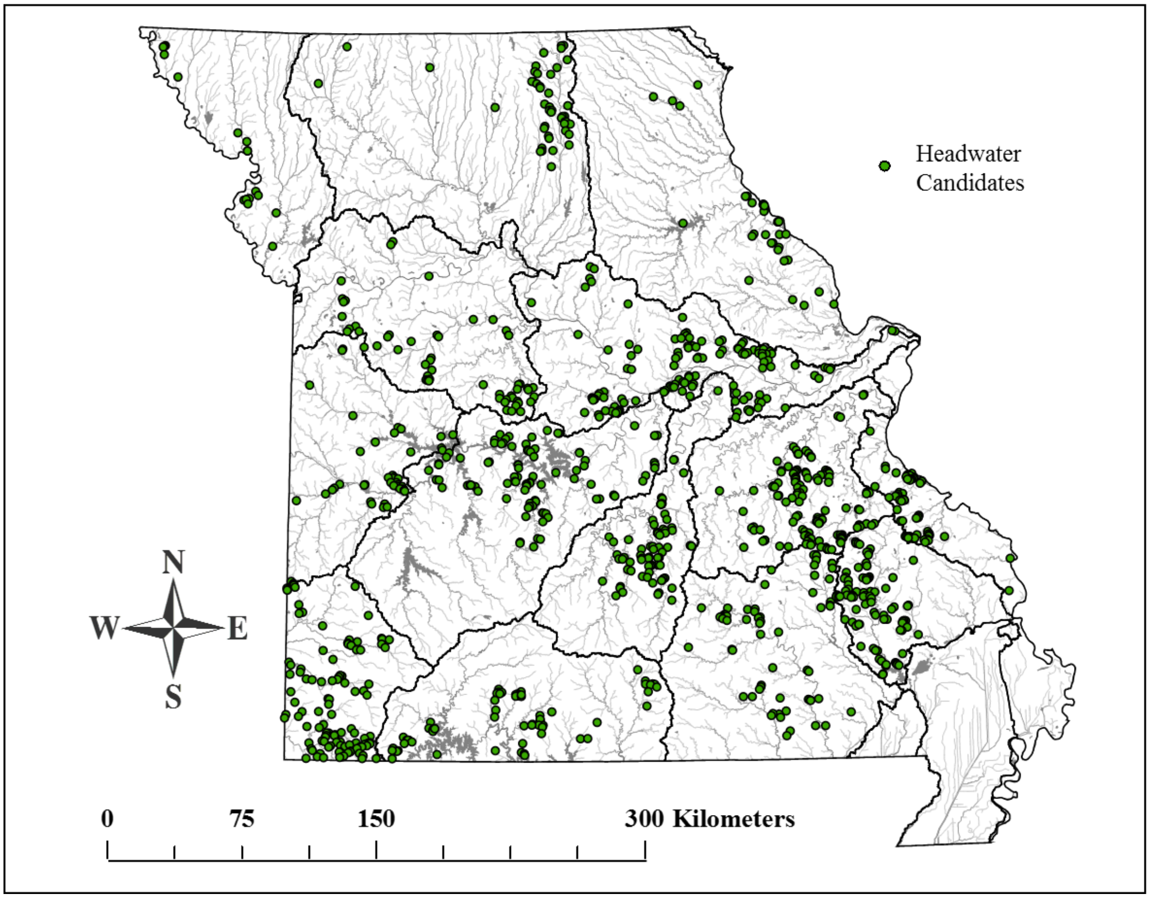

Figure 3.4. Map depicting candidate headwater reference reach locations (n=984).

Table 3.4. Mean and (standard deviation) values of landcover/landuse metrics summarized for headwater candidates in the Plains, Ozark, and Ozark Border regions.

Landcover/Landuse	**Plains**	**Ozark**	**Border**
Forest	66.2 (19.1)	94.0 (8.6)	64.6 (23.8)
Cultivated Crop	4.3 (7.1)	0.02 (0.38)	4.1 (9.8)
Pasture	9.9 (10.7)	1.88 (4.5)	20.7 (15.0)
Impervious Surface	0.94 (0.65)	0.38 (0.42)	0.9 (0.75)
Road Crossings	0.15 (0.34)	0.02 (0.07)	0.17 (0.23)
Localities	**95**	**558**	**331**

Candidates exhibited a range of conditions across the Plains, Ozark, and Ozark Border regions (Table 3.4). Despite occurring in an agriculturally dominated region, headwaters with relatively unaltered watersheds did exist in the Plains, with average forest cover exceeding 66%, and total imperviousness less than 1%. In the Ozark region, candidates averaged nearly 95% forested, with less than 0.4% imperviousness and less than 2% pasture land. The high number of relatively unaltered reaches in the region resulted in a greater number of sites meeting the requirements for inclusion on the candidate list. Because scoring exceptions were made for the Ozark Border region, sites there exhibited less forest cover (~ 65%) and higher percentages of pasture land (~ 21%) on average than either the Plains or Ozark regions, though watershed conditions were highly variable, reflecting the transitional nature of the region (Table 3.4).

To date, 171 candidate sites have been field verified, with 65 being recommended for candidate consideration and further biological sampling. 84 sites were eliminated due to low or intermittent flow incapable of supporting permanent fish populations, while the remaining 22 sites were removed from consideration due to impairments unaccounted for using our landscape-level screening process (Appendix 5). Additionally, 118 of the original 984 candidates were removed from consideration following GIS screening of land-use practices either unaccounted for in the original threat index or that have changed since publication of the 2006 NLCD data. The remaining 695 candidates will be field verified and assessed by Missouri Department of Conservation and Missouri Department of Natural Resources personnel over the course of the next several field seasons.

DISCUSSION

Our multi-metric threat index represents a cumulative, regionally-specific estimate of anthropogenic disturbance on Missouri's headwater streams. By adjusting scoring criteria based on EDUs, and in certain instances by AES type, our index allows researchers and managers to estimate stream disturbance levels relative to other locations within their overall watershed. Because our index values describe relative threat levels, our mapped results do not reflect land-use patterns as closely as previous works have (Sowa et al. 2007; Fore et al. 2014). Instead, our mapped index lowers expectations for streams occurring in highly impacted areas (i.e. MS Alluvial Basin), and enforces stricter criteria in relatively pristine areas, such as the south-flowing Ozark drainages (Figures 3.1, 3.3). In doing so, our candidate reference list depicts estimated "least-disturbed" conditions as determined by EDU/AES-specific threat prevalence (Stoddard et al. 2006).

Our index was developed using landscape-level surrogates for in-stream habitat and water quality characteristics. Because stream habitat and biotic composition ultimately result from a series of complex, hierarchical interactions between broad climatic and geologic conditions, anthropogenic disturbances, and finer scale physical and ecological processes (Frissell et al. 1986; Montgomery and Buffington 1997), our simplified index results can be expected to show moderate correlations with in-stream biotic data, at best (Annis et al. 2010; Fore et al. 2014). Therefore, field verification will be needed to finalize the candidate set of reference stream reaches. These field visits will determine whether or not streams are suffering from localized disturbances unaccounted for using landscape-level metrics (i.e. unmapped discharge pipes, creek crossings, gravel mining operations, etc.)

Additional shortcomings of our candidate list stem from our general lack of knowledge regarding seasonal fluctuations in headwater flow stability and biotic composition. Of our 984 initial candidates, over 65% of sites had drainage areas less than 5 km^2, many of which were either intermittent during spring field visits or determined to be ephemeral upon later verification. Similar scenarios were seen throughout the Ozark Border region, where sites with drainage areas > 30 km^2 exhibited intermittency due to the local prevalence of karst geology and losing streams. Though potentially ecologically meaningful, these sites represent unique stream conditions unsuitable for comparison with larger, or more permanently flowing waters.

We recommend using our index primarily as a sampling guide to start acquiring a more thorough, and comprehensive knowledge of the state's headwater streams and their biota. Also, there exists a need to develop a more specific stream size/flow classification for Missouri's headwaters, as the current single size classification fails to recognize the extreme variability in flow conditions and biotic community stability, greatly reducing our ability to predict stream conditions. For instance, a basic tiered approach incorporating flow permanence and thermal regime would likely enhance our biological expectations for these varied systems. Lastly, we recommend prioritizing sampling at both ends of our scoring spectrum to better represent the full range of stream conditions in Missouri. This added information will eventually allow methodology described in chapters 1 and 2 to be applied to headwaters, providing a more defensible, biologically relevant approach to identifying candidate reference reaches.

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CONCLUSIONS AND CONSERVATION IMPLICATIONS

Maintaining and/or restoring the integrity of flowing waters will continue to be a tremendous natural resources challenge. Expanding urbanization and agricultural production, along with the pervasive threats of global climate change, invasive species, and the increasing demand for water and other valuable ecosystem services has, and will continue to render streams vulnerable and impaired (Dudgeon et al. 2006). Nearly a third of fishes and over half of all mussel species in Missouri are listed as either imperiled or of conservation concern (Missouri Natural Heritage Program 2013). Thus, managers need the ability to predict areas of high and low biological integrity and to identify the effects and sources of impairment to be able to conserve remaining high quality stream reaches and mitigate and restore those already impaired. Our study offers a stepwise, inductive approach to characterizing the influence of natural environmental gradients and anthropogenic disturbance on stream fish and macroinvertebrate communities. Our models allow us to predict up to nine fish and aquatic invertebrate metrics at unsampled creek and small river reaches throughout Missouri, and by overlaying predicted metric values, estimate overall biotic integrity at each site. By linking reach and watershed-level environmental conditions, we can assemble a better mechanistic understanding of the many adverse ways in which human landscape-alterations influence the physical, chemical, and biological condition of flowing waters.

Because stream habitat and biotic composition ultimately result from a series of complex, hierarchical interactions between broad climatic and geologic conditions, anthropogenic disturbances, and finer scale physical and ecological processes (Frissell et al. 1986; Montgomery and Buffington 1997), understanding and accounting for the influence of natural environmental gradients is a key first step in evaluating stream health. In our study, we determined that measures of stream size and gradient, surficial geology, spring density, and network positioning all may exert significant influence on fish and invertebrate community characteristics, and must be taken into consideration when modeling the effects of anthropogenic disturbance and ultimately when designating biological criteria. We accounted for the influence of these natural gradients by retaining the residual biotic metric values from our natural models for further analysis, allowing us to model biotic condition relative to other streams exhibiting similar natural characteristics. Following the final determination of reference stream reaches, we recommend quantifying the specific relationship between these natural environmental gradients and biotic metrics under least-disturbed conditions to develop correction factors for applying biological criteria to streams with a diversity of natural landscape characteristics.

We related multiple stream fish and invertebrate metrics to both reach and watershed-level environmental variables, and provided a detailed account of the many ways anthropogenic disturbances influence both biotic and abiotic stream characteristics. Using existing landscape-level data, we were able to generate predicted biological metric values for every creek and small river reach in Missouri. By predicting fish and invertebrate metrics related to various aspects of stream health (i.e. richness/diversity, habitat preference, trophic and reproductive ecology, sensitivity to disturbance), we were able to generate an overall estimate of biological integrity for each reach. Lastly, we selected reaches at the top of our biological integrity gradient within each stream size classification and aquatic subregion to serve as candidate reference reaches. These 980 creek segments (Plains – 448, Ozark – 532) and 444 small river segments (Plains – 236, Ozark – 208) are predicted to contain the least-impaired stream fish and macroinvertebrate communities within their respective size classes and subregion and may serve as benchmarks of high quality physical habitat, water quality, and biotic integrity.

Because our watershed-based models used coarse surrogates for in-stream environmental conditions, our predictors explained, at best, ~ 50% of the variation in a given response metric, meaning subsequent model refining and field verification will be necessary prior to designating streams as reference. A major assumption of our modeling approach is that the full range of stream conditions within a given size class and aquatic subregion is represented in our data. By targeting streams on both ends of our scoring spectrum for sampling, we can better hone our expectations for biotic metric values under least and most-disturbed conditions. Furthermore, by sampling reaches exhibiting specific human threats (e.g. landfills, mining operations, etc.), we can better quantify their effect on biotic communities, and they may then carry more significance in our predictive model. Because our watershed-level predictors do not entirely overlap with in-stream physical habitat and water quality metrics, field verification will be necessary to screen for stressors unaccounted for in our coarse-filter estimates. Additionally, professional judgment may be necessary to determine whether or not candidate reference streams are anomalous or are truly representative of their respective region.

We still know relatively little about the effects of natural environmental gradients and anthropogenic disturbances on the fish and invertebrate communities of Missouri's headwater streams. Due to sparse biological sampling, we were unable to apply the same methodology for identifying candidate reference headwater reaches as we used for creeks and small rivers. Instead, we developed a provisional threat headwater threat index to assign impairment levels and guide future biological sampling. Additional research will be required to quantify the influence of drainage area, network position, movement barriers, and other anthropogenic disturbances on the physical and biotic characteristics of headwaters. Developing a flow-based classification system will likely increase our ability to predict biotic assemblage characteristics at headwater sites and will help ensure that realistic expectations are developed for these unique and varied communities.

Successful identification and restoration of impaired streams is reliant on an intimate understanding of the many ways stream habitat, water quality, and biotic communities respond to both natural environmental gradients and anthropogenic disturbance. For this, no single stream-health index is sufficient, rather, managers must rely on every tool available, including fish and invertebrate indices, physical habitat indices, available landscape-level data, and some level of professional judgment. This study represents a strong first step toward refining existing biological indices, developing a companion physical habitat index, and ultimately conserving the integrity, and diversity of Missouri’s flowing waters.

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APPENDICES

Appendix 1. Species occurrences expressed as percentage of occupied headwater (Hw), creek (Crk), and small river (Riv) stream reaches within each aquatic subregion in Missouri. MAB – Mississippi Alluvial Basin.

		Plains			**Ozarks**			**MAB**		
Common Name	**Scientific Name**	Hw	Crk	Riv	Hw	Crk	Riv	Hw	Crk	Riv
Bowfin	*Amia calva*	-	-	-	-	-	-	-	17.5	10.0
Pirate Perch	*Aphredoderus sayanus*	-	0.8	-	-	2.5	1.8	-	35.0	-
Brook Silverside	*Labidesthes sicculus*	-	9.7	20.8	2.0	21.3	45.6	-	37.5	80.0
Highfin Carpsucker	*Carpiodes velifer*	-	-	0.9	-	-	-	-	-	10.0
Quillback	*Carpiodes cyprinus*	1.4	0.4	21.7	-	0.3	6.1	-	12.5	30.0
Northern Hog Sucker	*Hypentelium nigricans*	-	2.5	12.3	6.1	50.7	87.7	-	-	-
Bigmouth Buffalo	*Ictiobus cyprinellus*	1.4	1.7	4.7	-	-	0.9	-	17.5	40.0
Black Buffalo	*Ictiobus niger*	-	-	-	-	-	1.8	-	10.0	10.0
Smallmouth Buffalo	*Ictiobus bubalus*	1.4	0.4	7.5	-	0.3	4.4	-	27.5	60.0
Spotted Sucker	*Minytrema melanops*	-	0.4	-	2.0	6.1	7.0	-	12.5	-
Black Redhorse	*Moxostoma duquesnei*	-	2.5	12.3	4.1	17.2	66.7	-	-	-
Golden Redhorse	*Moxostoma erythrurum*	-	5.5	29.2	4.1	18.6	56.1	-	-	-
River Redhorse	*Moxostoma carinatum*	-	-	2.8	-	0.3	3.5	-	-	-
Shorthead Redhorse	*Moxostoma macrolepidotum*	-	0.4	16.0	-	3.9	15.8	-	7.5	20.0
Silver Redhorse	*Moxostoma anisurum*	-	0.4	7.5	-	-	3.5	-	-	-
Ozark Bass	*Ambloplites constellatus*	-	-	-	2.0	8.9	12.3	-	-	-
Rock Bass	*Ambloplites rupestris*	-	0.8	-	-	15.5	45.6	-	-	-
Shadow Bass	*Ambloplites ariommus*	-	-	-	-	6.4	7.9	-	5.0	-
Flier	*Centrarchus macropterus*	-	-	-	-	-	-	-	2.5	-
Bluegill	*Lepomis macrochirus*	56.9	79.8	89.6	28.6	72.0	84.2	100.0	92.5	100.0
Green Sunfish	*Lepomis cyanellus*	76.4	93.7	97.2	51.0	84.2	86.0	100.0	97.5	90.0
Longear Sunfish	*Lepomis megalotis*	-	9.7	13.2	20.4	69.5	95.6	-	95.0	100.0
Orangespotted Sunfish	*Lepomis humilis*	13.9	13.9	39.6	2.0	5.3	7.9	-	55.0	80.0
Redear Sunfish	*Lepomis microlophus*	-	1.3	8.5	2.0	5.3	14.0	-	17.5	20.0
Redspotted Sunfish	*Lepomis miniatus*	-	-	-	2.0	1.9	6.1	-	40.0	20.0
Warmouth	*Lepomis gulosus*	1.4	8.0	3.8	2.0	6.9	9.6	-	40.0	50.0
Largemouth Bass	*Micropterus salmoides*	33.3	64.7	76.4	14.3	45.7	61.4	-	50.0	50.0
Smallmouth Bass	*Micropterus dolomieu*	-	0.4	10.4	4.1	33.0	73.7	-	-	-
Spotted Bass	*Micropterus punctulatus*	9.7	2.1	11.3	2.0	10.0	23.7	-	35.0	40.0
Black Crappie	*Pomoxis nigromaculatus*	-	0.4	1.9	2.0	1.9	1.8	-	5.0	-
White Crappie	*Pomoxis annularis*	1.4	9.7	27.4	-	0.8	6.1	-	12.5	30.0
Gizzard Shad	*Dorosoma cepedianum*	1.4	7.1	28.3	-	1.7	17.5	-	52.5	90.0

Appendix 1. *Species occurrences continued.*

Common Name	Scientific Name	Plains			Ozarks			MAB		
		Hw	Crk	Riv	Hw	Crk	Riv	Hw	Crk	Riv
Banded Sculpin	*Cottus carolinae*	-	3.8	-	20.4	33.2	41.2	-	-	-
Mottled Sculpin	*Cottus bairdii*	-	-	-	10.2	18.8	17.5	-	-	-
Ozark Sculpin	*Cottus hypselurus*	-	-	-	22.4	16.9	20.2	-	-	-
Central Stoneroller	*Campostoma a. pullum*	63.9	72.7	63.2	65.3	82.8	80.7	-	-	10.0
Largescale Stoneroller	*Campostoma oligolepis*	-	1.3	5.7	34.7	54.6	46.5	-	-	-
Goldfish	*Carassius auratus*	-	0.8	-	-	-	0.9	-	-	-
Grass Carp	*Ctenopharyngodon idella*	-	0.4	3.8	-	1.1	1.8	-	-	-
Blacktail Shiner	*Cyprinella venusta*	-	-	-	-	0.3	1.8	-	67.5	70.0
Red Shiner	*Cyprinella lutrensis*	31.9	68.9	94.3	2.0	12.7	22.8	-	5.0	-
Spotfin Shiner	*Cyprinella spiloptera*	-	0.4	-	4.1	2.5	11.4	-	-	-
Steelcolor Shiner	*Cyprinella whipplei*	-	7.1	11.3	2.0	4.7	9.6	-	-	-
Whitetail Shiner	*Cyprinella galactura*	-	-	-	-	1.7	4.4	-	-	-
Common Carp	*Cyprinus carpio*	1.4	10.9	26.4	-	1.9	9.6	-	42.5	50.0
Gravel Chub	*Erimystax x-punctatus*	-	-	1.9	-	-	7.9	-	-	-
Ozark Chub	*Erimystax harryi*	-	-	-	-	1.1	7.0	-	-	-
Brassy Minnow	*Hybognathus hankinsoni*	4.2	5.9	7.5	-	-	-	-	-	-
MS Silvery Minnow	*Hybognathus nuchalis*	-	0.8	-	-	-	-	-	-	-
Plains Minnow	*Hybognathus placitus*	-	-	2.8	-	-	-	-	-	-
Wstrn. Silvery Minnow	*Hybognathus argyritis*	-	0.8	0.9	-	-	-	-	-	-
Bigeye Chub	*Hybopsis amblops*	-	-	-	2.0	11.1	26.3	-	-	-
Bighead Carp	*Hypophthalmichthys nobilis*	-	-	-	-	0.3	-	-	-	-
Silver Carp	*Hypophthalmichthys molitrix*	-	0.4	1.9	-	-	1.8	-	2.5	10.0
Bleeding Shiner	*Luxilus zonatus*	-	-	-	16.3	49.6	57.9	-	-	-
Cardinal Shiner	*Luxilus cardinalis*	-	-	0.9	4.1	12.5	17.5	-	-	-
Common Shiner	*Luxilus cornutus*	4.2	13.9	16.0	-	3.0	4.4	-	-	-
Duskystripe Shiner	*Luxilus pilsbryi*	-	-	-	12.2	15.2	12.3	-	-	-
Striped Shiner	*Luxilus chrysocephalus*	-	14.3	11.3	10.2	36.0	57.9	-	2.5	-
Redfin Shiner	*Lythrurus umbratilis*	16.7	49.6	65.1	8.2	19.7	21.9	-	37.5	10.0
Ribbon Shiner	*Lythrurus fumeus*	-	-	-	-	-	-	-	10.0	-
Shoal Chub	*Macrhybopsis hyostoma*	-	-	4.7	-	-	-	-	5.0	-
Silver Chub	*Macrhybopsis storeriana*	-	0.4	2.8	-	-	-	-	-	-
Hornyhead Chub	*Nocomis biguttatus*	-	0.8	5.7	6.1	34.6	47.4	-	-	-
Redspot Chub	*Nocomis asper*	-	-	-	2.0	8.0	16.7	-	-	-
Golden Shiner	*Notemigonus crysoleucas*	13.9	26.1	9.4	4.1	5.8	2.6	-	27.5	40.0
Bigeye Shiner	*Notropis boops*	-	10.5	17.9	10.2	18.3	37.7	-	-	-
Bigmouth Shiner	*Notropis dorsalis*	20.8	41.6	54.7	-	1.4	0.9	-	-	-

Appendix 1. *Species occurrences continued.*

Common Name	Scientific Name	Plains			Ozarks			MAB		
		Hw	Crk	Riv	Hw	Crk	Riv	Hw	Crk	Riv
Blacknose Shiner	*Notropis heterolepis*	-	-	-	4.1	0.8	2.6	-	-	-
Carmine Shiner	*Notropis percobromus*	-	1.3	5.7	-	13.3	50.0	-	-	-
Emerald Shiner	*Notropis atherinoides*	1.4	2.1	7.5	-	0.6	1.8	-	52.5	80.0
Ghost Shiner	*Notropis buchanani*	-	0.4	2.8	-	-	-	-	-	-
Ironcolor Shiner	*Notropis chalybaeus*	-	-	-	-	-	-	-	2.5	20.0
Mimic Shiner	*Notropis volucellus*	-	0.4	7.5	-	0.6	8.8	-	55.0	30.0
Ozark Minnow	*Notropis nubilus*	-	-	-	14.3	55.4	75.4	-	-	-
Ozark Shiner	*Notropis ozarcanus*	-	-	-	-	1.7	0.9	-	-	-
River Shiner	*Notropis blennius*	-	0.4	-	-	-	-	-	-	-
Sand Shiner	*Notropis stramineus*	19.4	45.4	84.9	-	10.5	21.9	-	-	-
Silverjaw Minnow	*Notropis buccatus*	-	-	-	-	2.5	2.6	-	-	-
Telescope Shiner	*Notropis telescopus*	-	-	-	-	8.9	10.5	-	-	-
Wedgespot Shiner	*Notropis greenei*	-	-	-	-	3.9	19.3	-	-	-
Weed Shiner	*Notropis texanus*	-	-	-	-	-	-	-	50.0	50.0
Pugnose Minnow	*Opsopoeodus emiliae*	-	-	-	-	-	-	-	22.5	10.0
Suckermouth Minnow	*Phenacobius mirabilis*	6.9	35.3	61.3	-	3.9	7.9	-	10.0	20.0
So. Redbelly Dace	*Phoxinus erythrogaster*	11.1	4.6	2.8	57.1	47.9	7.9	-	-	-
Bluntnose Minnow	*Pimephales notatus*	43.1	75.2	93.4	14.3	44.6	60.5	-	65.0	50.0
Bullhead Minnow	*Pimephales vigilax*	-	1.7	11.3	-	0.8	-	-	37.5	60.0
Fathead Minnow	*Pimephales promelas*	19.4	30.3	44.3	2.0	3.9	0.9	100.0	5.0	10.0
Creek Chub	*Semotilus atromaculatus*	87.5	89.1	69.8	67.3	73.7	36.8	-	7.5	10.0
Blackspotted Topminnow	*Fundulus olivaceus*	2.8	2.9	1.9	24.5	50.1	52.6	-	67.5	60.0
Blackstripe Topminnow	*Fundulus notatus*	-	16.0	17.9	8.2	25.5	31.6	-	72.5	50.0
Golden Topminnow	*Fundulus chrysotus*	-	-	-	-	0.3	-	-	2.5	-
Northern Plains Killifish	*Fundulus kansae*	-	-	0.9	-	-	-	-	-	-
Northern Studfish	*Fundulus catenatus*	-	8.0	3.8	18.4	47.4	65.8	-	2.5	-
Plains Topminnow	*Fundulus sciadicus*	1.4	1.3	-	6.1	4.7	-	-	-	-
Starhead Topminnow	*Fundulus dispar*	-	-	-	-	-	-	-	5.0	-
Banded Pigmy Sunfish	*Elassoma zonatum*	-	-	-	-	-	-	-	7.5	-
Chain Pickerel	*Esox niger*	-	-	-	2.0	1.7	7.0	-	12.5	-
Grass Pickerel	*Esox americanus vermiculatus*	-	-	-	6.1	3.0	7.0	-	2.5	-
Goldeye	*Hiodon alosoides*	-	-	-	-	-	0.9	-	-	-
Black Bullhead	*Ameiurus melas*	13.9	29.4	16.0	10.2	9.7	8.8	100.0	30.0	30.0
Brown Bullhead	*Ameiurus nebulosus*	-	-	-	-	-	-	-	2.5	-
Yellow Bullhead	*Ameiurus natalis*	9.7	58.0	53.8	12.2	44.0	52.6	-	47.5	60.0
Blue Catfish	*Ictalurus furcatus*	-	-	0.9	-	-	-	-	-	-

Appendix 1. *Species occurrences continued.*

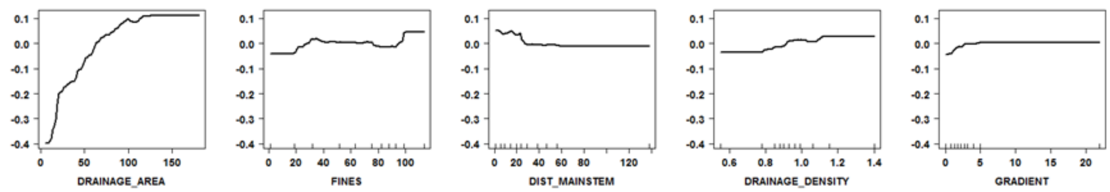
		Plains			Ozarks			MAB		
Common Name	**Scientific Name**	Hw	Crk	Riv	Hw	Crk	Riv	Hw	Crk	Riv
Channel Catfish	*Ictalurus punctatus*	1.4	9.2	64.2	2.0	1.4	17.5	-	60.0	50.0
Brindled Madtom	*Noturus miurus*	-	-	-	-	0.8	1.8	-	-	-
Checkered Madtom	*Noturus flavater*	-	-	-	2.0	0.3	1.8	-	-	-
Freckled Madtom	*Noturus nocturnus*	-	-	1.9	-	-	-	-	7.5	-
Mountain Madtom	*Noturus eleutherus*	-	-	-	-	0.3	-	-	-	-
Ozark Madtom	*Noturus albater*	-	-	-	2.0	5.0	11.4	-	-	-
Slender Madtom	*Noturus exilis*	-	16.4	23.6	26.5	65.9	61.4	-	-	-
Stonecat	*Noturus flavus*	-	1.7	14.2	-	0.6	1.8	-	-	-
Tadpole Madtom	*Noturus gyrinus*	-	2.5	10.4	-	-	-	-	52.5	20.0
Flathead Catfish	*Pylodictis olivaris*	-	1.7	21.7	-	0.6	8.8	-	2.5	30.0
Longnose Gar	*Lepisosteus osseus*	-	0.4	17.0	-	2.2	18.4	-	27.5	30.0
Shortnose Gar	*Lepisosteus platostomus*	-	2.9	24.5	-	1.1	7.9	-	25.0	10.0
Spotted Gar	*Lepisosteus oculatus*	-	-	-	2.0	-	1.8	-	65.0	40.0
Striped Bass	*Morone saxatilis*	-	-	-	-	-	-	-	2.5	-
White Bass	*Morone chrysops*	-	-	2.8	-	-	1.8	-	-	-
Western Sand Darter	*Ammocrypta clara*	-	-	0.9	-	-	-	-	-	-
Arkansas Darter	*Etheostoma cragini*	-	-	-	4.1	5.3	1.8	-	-	-
Arkansas Saddled Darter	*Etheostoma euzonum*	-	-	-	-	0.3	0.9	-	-	-
Banded Darter	*Etheostoma zonale*	-	0.8	-	-	5.0	36.0	-	-	-
Bluntnose Darter	*Etheostoma chlorosoma*	-	-	0.9	-	-	-	-	5.0	10.0
Brook Darter	*Etheostoma burri*	-	-	-	-	1.1	4.4	-	-	-
Current Darter	*Etheostoma uniporum*	-	-	-	-	1.4	1.8	-	-	-
Cypress Darter	*Etheostoma proeliare*	-	-	-	-	-	-	-	25.0	10.0
Fantail Darter	*Etheostoma flabellare*	8.3	21.4	26.4	38.8	64.3	54.4	-	-	-
Greenside Darter	*Etheostoma blennioides*	-	2.1	2.8	4.1	42.7	84.2	-	-	-
Harlequin Darter	*Etheostoma histrio*	-	-	-	-	-	-	-	2.5	-
Johnny Darter	*Etheostoma nigrum*	27.8	48.3	60.4	6.1	18.0	25.4	-	2.5	20.0
Least Darter	*Etheostoma microperca*	-	0.4	-	-	0.8	-	-	-	-
Missouri Saddled Darter	*Etheostoma tetrazonum*	-	-	-	-	1.9	12.3	-	-	-
Mud Darter	*Etheostoma asprigene*	-	-	-	-	-	-	-	2.5	-
Orangethroat Darter	*Etheostoma spectabile*	37.5	55.9	41.5	69.4	90.9	83.3	-	-	-
Rainbow Darter	*Etheostoma caeruleum*	-	0.8	0.9	22.4	51.0	63.2	-	-	-
Slough Darter	*Etheostoma gracile*	1.4	1.7	-	-	0.3	-	-	7.5	-
Speckled Darter	*Etheostoma stigmaeum*	-	-	-	-	0.8	0.9	-	-	-
Stippled Darter	*Etheostoma punctulatum*	-	-	-	14.3	40.4	36.0	-	-	-
Yoke Darter	*Etheostoma juliae*	-	-	-	-	-	6.1	-	-	-

Appendix 1. *Species occurrences continued.*

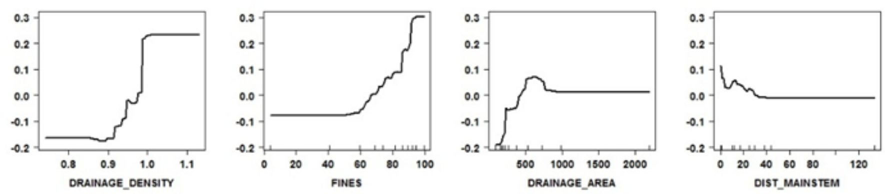
		Plains			Ozarks			MAB		
Common Name	**Scientific Name**	Hw	Crk	Riv	Hw	Crk	Riv	Hw	Crk	Riv
River Carpsucker	*Carpiodes carpio*	-	3.4	37.7	-	0.3	3.5	-	15.0	20.0
White Sucker	*Catostomus commersonii*	18.1	49.6	32.1	14.3	29.9	13.2	-	-	-
Creek Chubsucker	*Erimyzon oblongus*	-	-	-	6.1	7.5	14.9	-	32.5	-
Lake Chubsucker	*Erimyzon sucetta*	-	-	-	-	0.6	-	-	2.5	-
Yellow Perch	*Perca flavescens*	-	-	-	-	0.3	-	-	-	-
Blackside Darter	*Percina maculata*	2.8	3.8	23.6	-	0.3	0.9	-	2.5	20.0
Bluestripe Darter	*Percina cymatotaenia*	-	-	-	-	0.3	0.9	-	-	-
Channel Darter	*Percina copelandi*	-	-	-	-	-	2.6	-	-	-
Dusky Darter	*Percina sciera*	-	-	-	-	-	-	-	5.0	20.0
Gilt Darter	*Percina evides*	-	-	-	-	0.3	4.4	-	-	-
Logperch	*Percina caprodes*	1.4	12.6	32.1	2.0	20.8	51.8	-	7.5	-
Saddleback Darter	*Percina vigil*	-	-	-	-	-	-	-	2.5	-
Slenderhead Darter	*Percina phoxocephala*	1.4	0.8	23.6	-	-	7.0	-	-	-
Walleye	*Sander vitreus*	-	0.4	1.9	-	-	1.8	-	-	-
Trout-Perch	*Percopsis omiscomaycus*	-	0.4	3.8	-	-	-	-	-	-
Chestnut Lamprey	*Ichthyomyzon castaneus*	-	-	0.9	-	-	1.8	-	-	-
Northern Brook Lamprey	*Ichthyomyzon fossor*	-	-	-	-	0.3	0.9	-	-	-
Southern Brook Lamprey	*Ichthyomyzon gagei*	-	-	-	-	-	2.6	-	-	-
Least Brook Lamprey	*Lampetra aepyptera*	-	-	-	-	1.1	0.9	-	-	-
Western Mosquitofish	*Gambusia affinis*	8.3	19.7	38.7	22.4	28.0	37.7	100.0	95.0	70.0
Rainbow Trout	*Oncorhynchus mykiss*	-	-	-	6.1	5.8	0.9	-	-	-
Brown Trout	*Salmo trutta*	-	-	-	-	0.6	0.9	-	-	-
Freshwater Drum	*Aplodinotus grunniens*	-	0.4	23.6	-	-	10.5	-	20.0	50.0
	Sampling Localities	**72**	**238**	**106**	**49**	**361**	**114**	**1**	**40**	**10**

Appendix 2. Boosted regression tree partial dependence plots of influential natural environmental variables for fish and invertebrate community characteristics (presented as a dimensionless transformation of each response, centered to zero mean). Rug plots at inside bottom of plots show the distribution of sites across the given predictor variable.

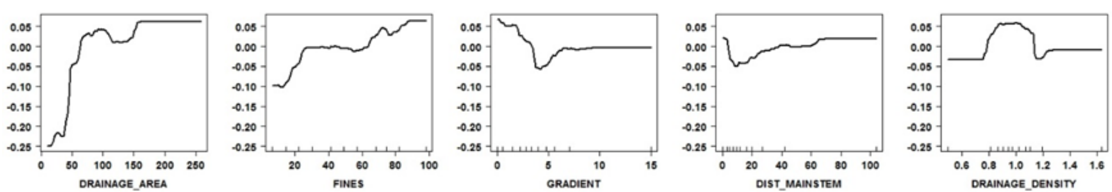
Number of Native Fish Species – Central Plains Creeks



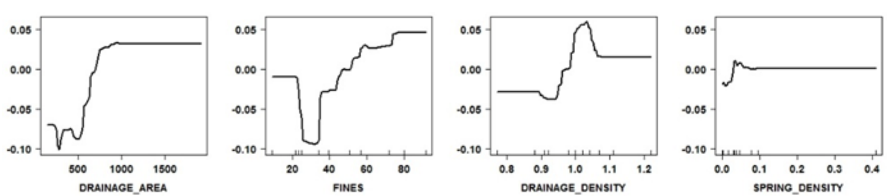
Number of Native Fish Species – Central Plains Small Rivers



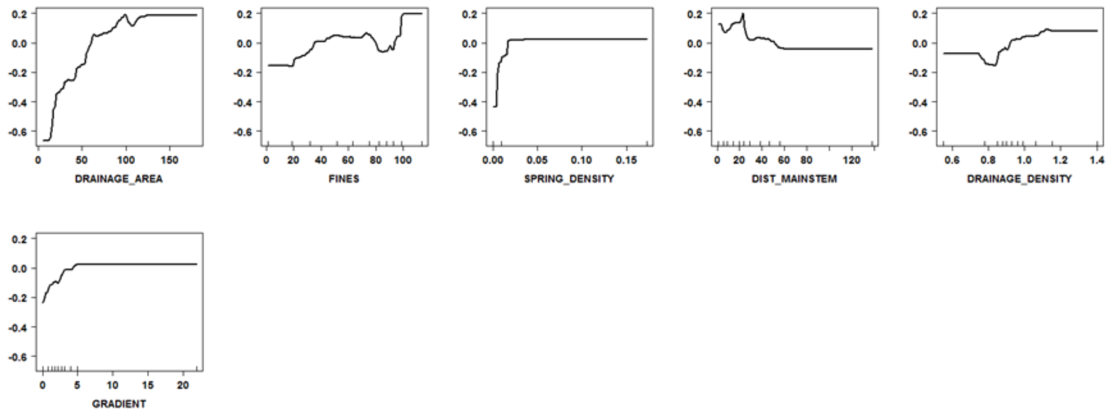
Number of Native Fish Species – Ozark Highlands Creeks



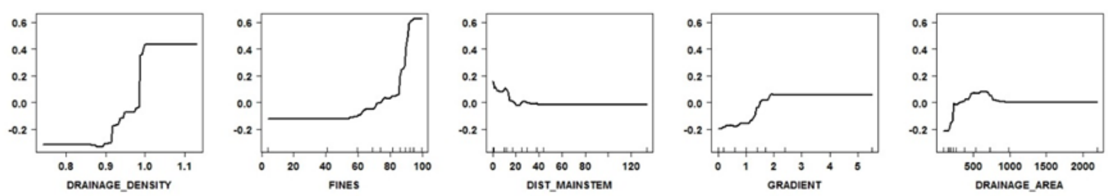
Number of Native Fish Species – Ozark Highlands Small Rivers



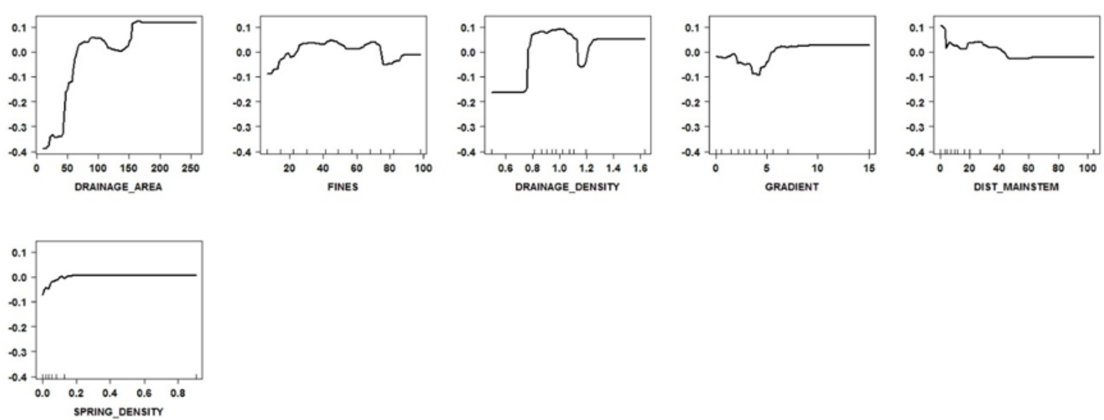
Number of Native Benthic Fish Species – Central Plains Creeks



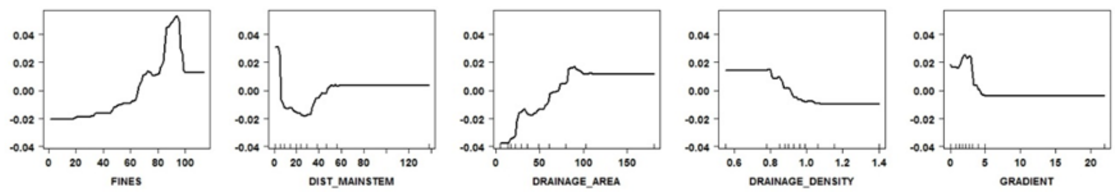
Number of Native Benthic Fish Species – Central Plains Small Rivers



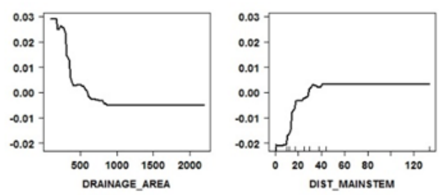
Number of Native Benthic Fish Species – Ozark Highlands Creeks



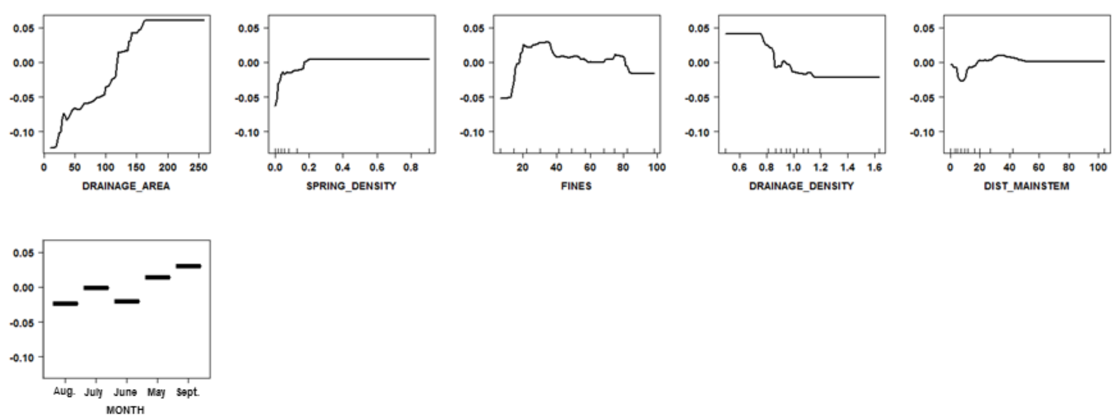
Proportion of Native Insectivorous Cyprinid Individuals – Central Plains Creeks



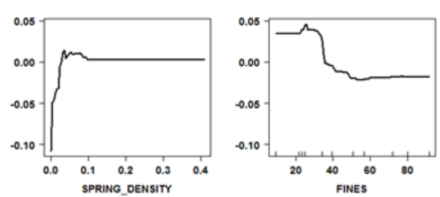
Proportion of Native Insectivorous Cyprinid Individuals – Central Plains Small Rivers



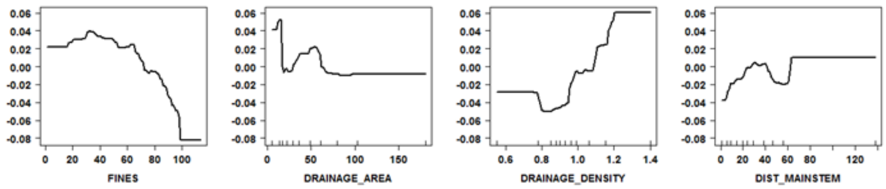
Proportion of Native Insectivorous Cyprinid Individuals – Ozark Highlands Creeks



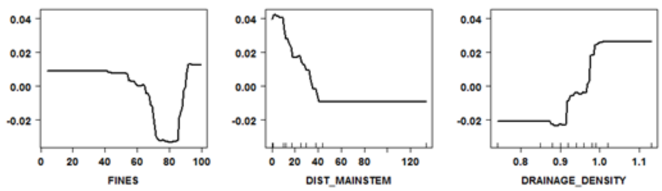
Proportion of Native Insectivorous Cyprinid Individuals – Ozark Highlands Small Rivers



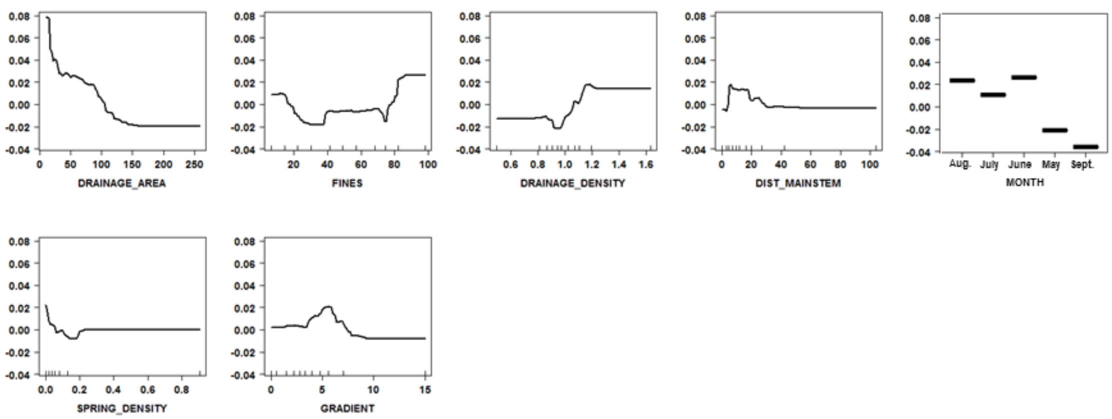
Proportion of Native Omnivorous/Herbivorous Individuals – Central Plains Creeks



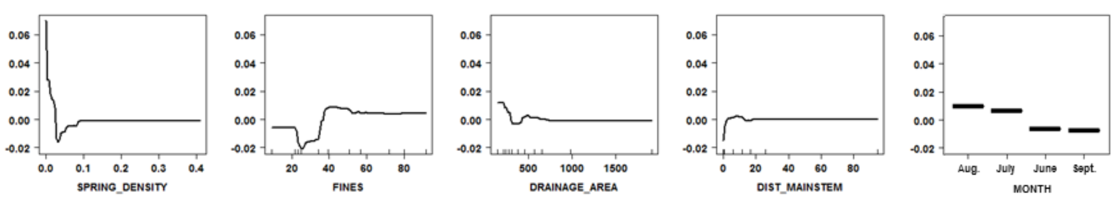
Proportion of Native Omnivorous/Herbivorous – Central Plains Small Rivers



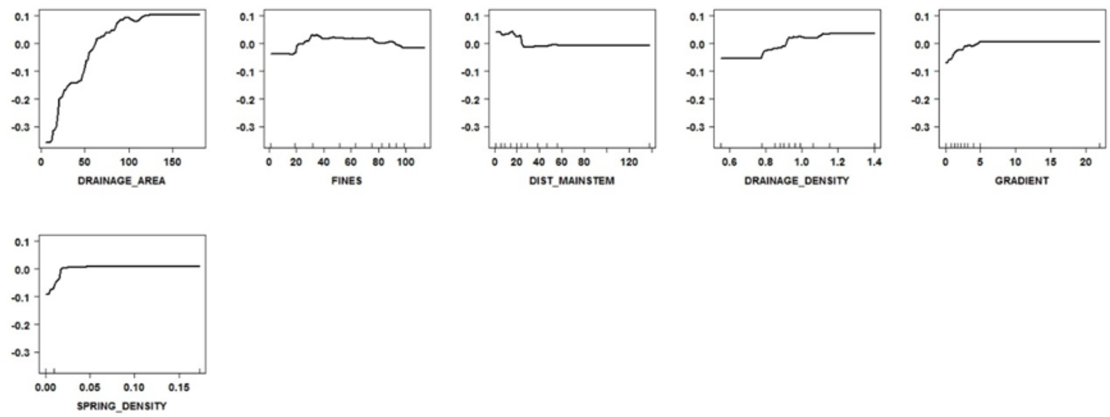
Proportion of Native Omnivorous/Herbivorous – Ozark Highlands Creeks



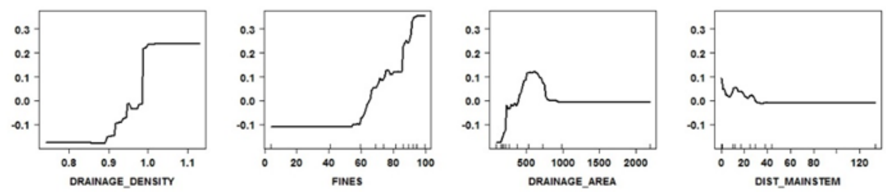
Proportion of Native Omnivorous/Herbivorous – Ozark Highlands Small Rivers



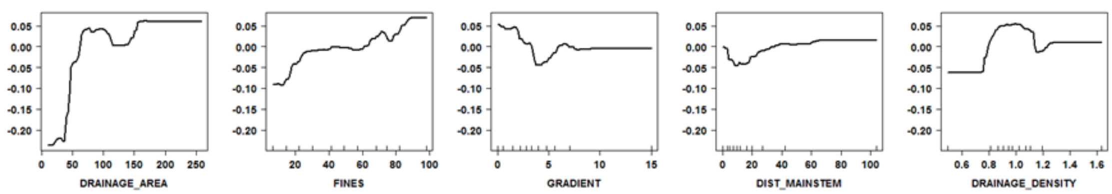
Number of Native Lithophilic Fish Species – Central Plains Creeks



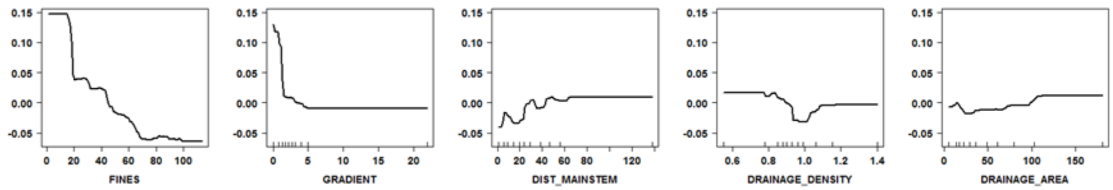
Number of Native Lithophilic Fish Species – Central Plains Small Rivers



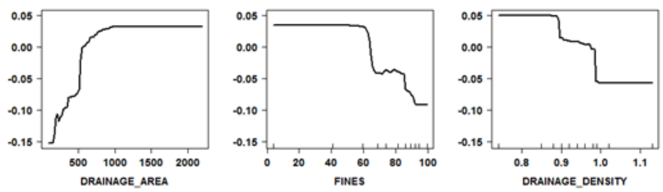
Number of Native Lithophilic Fish Species – Ozark Highlands Creeks



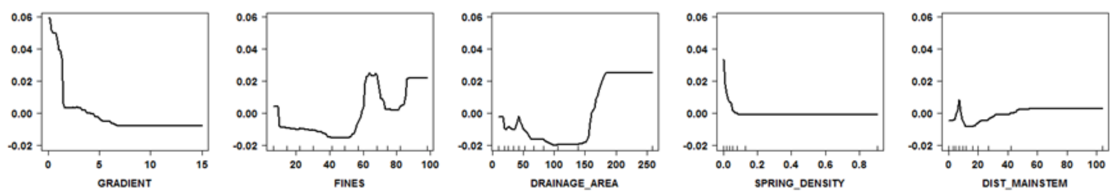
Proportion of Native Tolerant Individuals – Central Plains Creeks



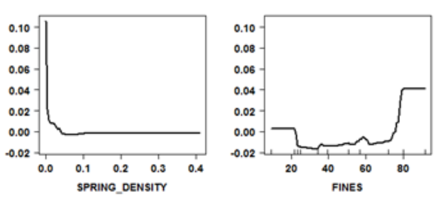
Proportion of Native Tolerant Individuals – Central Plains Small Rivers



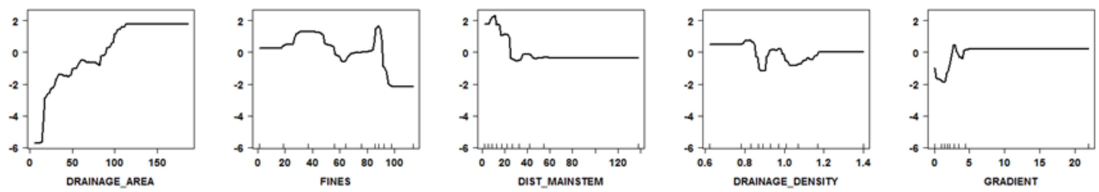
Proportion of Native Tolerant Individuals – Ozark Highlands Creeks



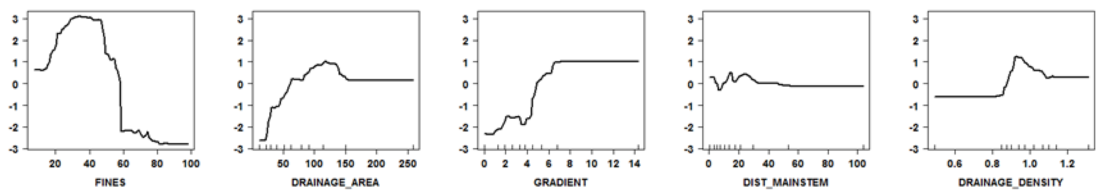
Proportion of Native Tolerant Individuals – Ozark Highlands Small Rivers



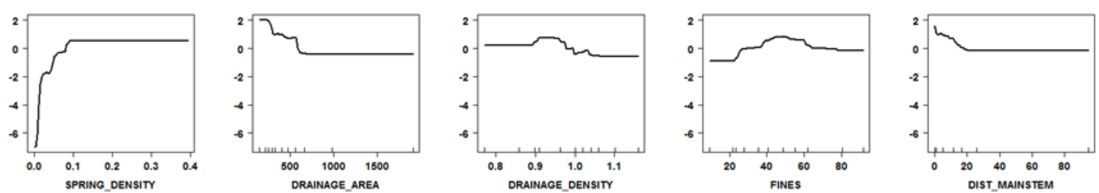
EPT Richness (Invertebrate) – Central Plains Creeks



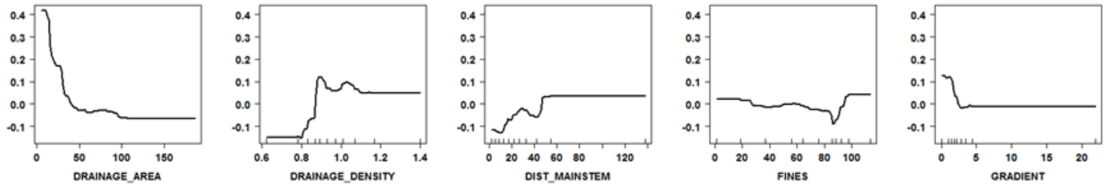
EPT Richness (Invertebrate) – Ozark Highlands Creeks



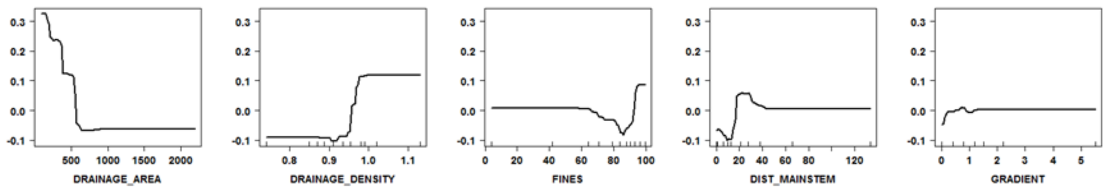
EPT Richness (Invertebrate) – Ozark Highlands Small Rivers



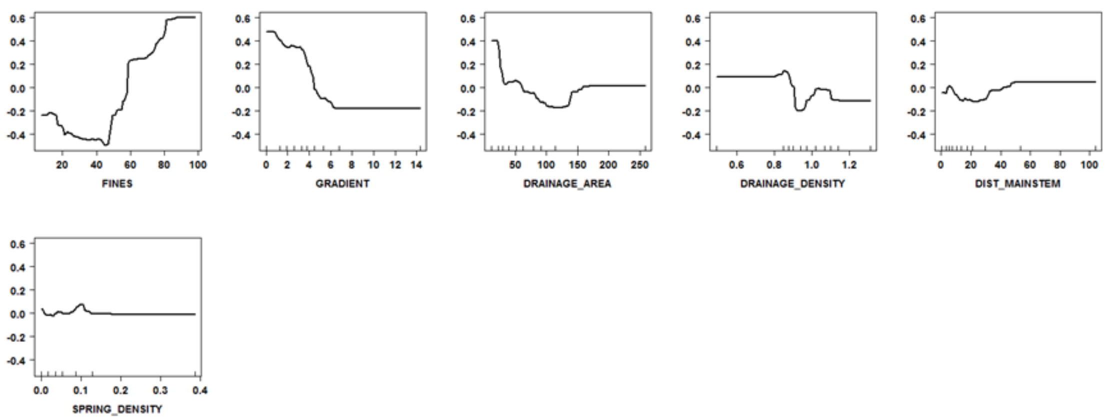
Hilsenhoff Biotic Index (Invertebrate) – Central Plains Creeks



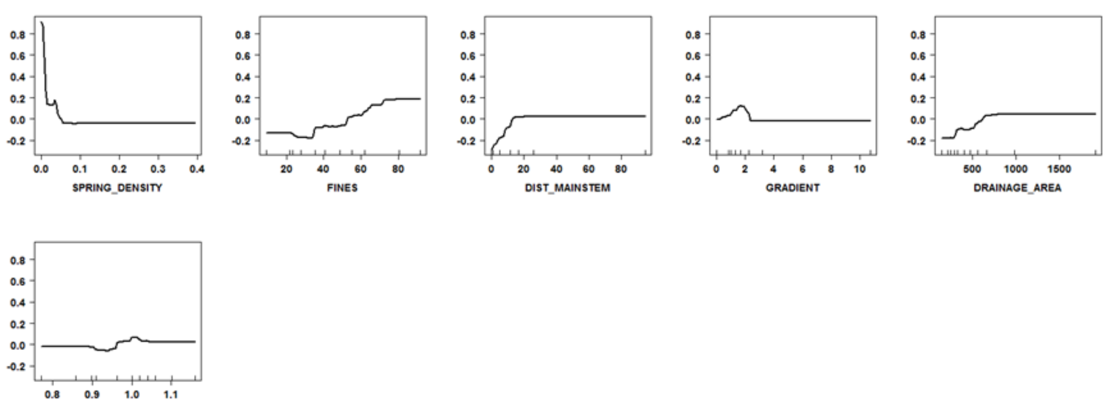
Hilsenhoff Biotic Index (Invertebrate) – Central Plains Small Rivers



Hilsenhoff Biotic Index (Invertebrate) – Ozark Highlands Creeks

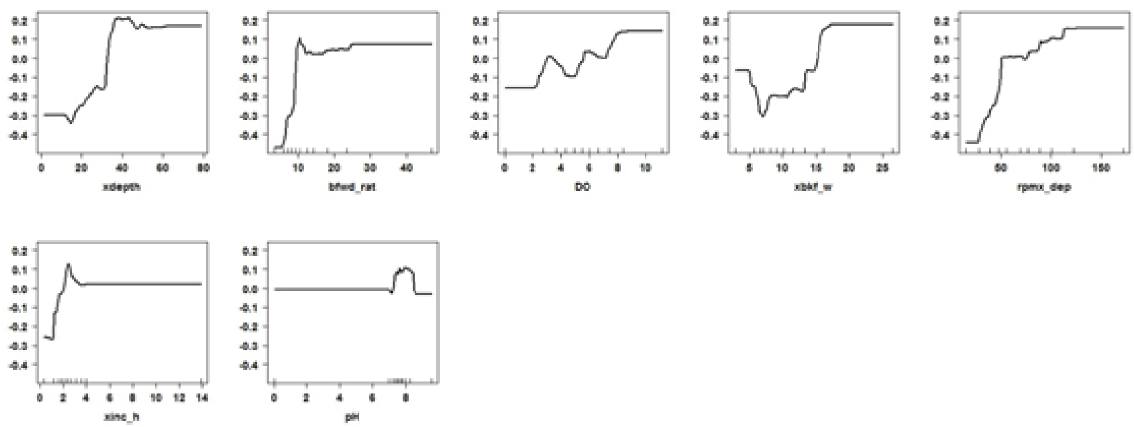


Hilsenhoff Biotic Index (Invertebrate) – Ozark Highlands Small Rivers

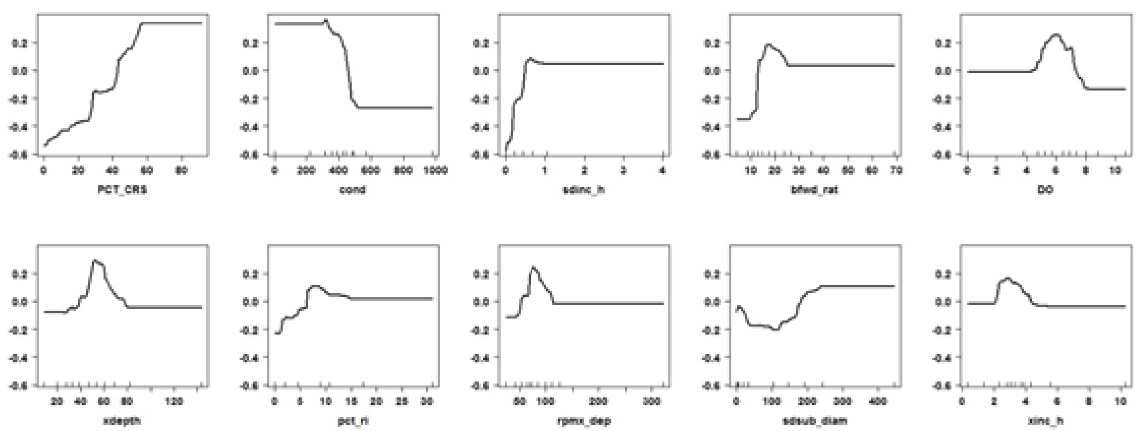


Appendix 3. Boosted regression tree partial dependence plots of influential reach-level environmental variables for fish and invertebrate community characteristics (presented as a dimensionless transformation of each response, centered to zero mean). Rug plots at inside bottom of plots show the distribution of sites across the given predictor variable.

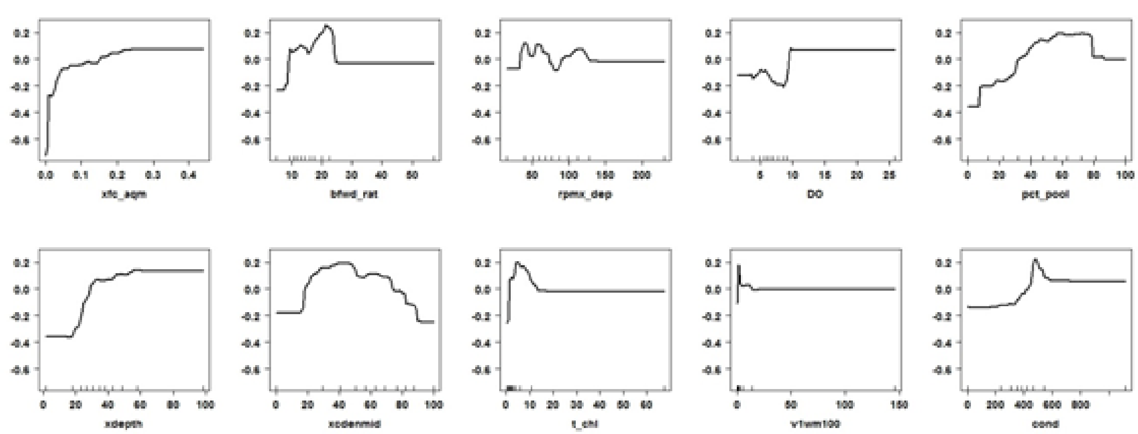
Number of Native Fish Species (numnatsp) – Central Plains Creeks



Number of Native Fish Species (numnatsp) – Central Plains Small Rivers

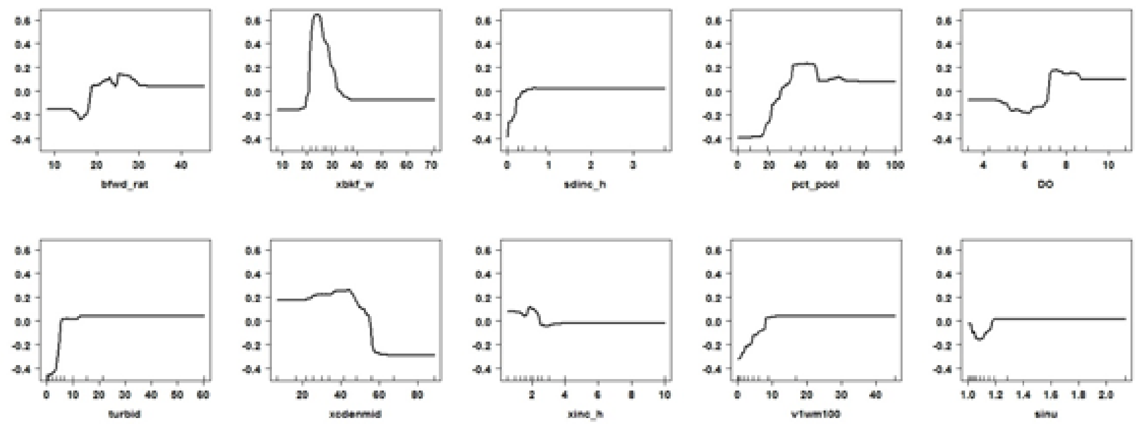


Number of Native Fish Species (numnatsp) – Ozark Highlands Creeks

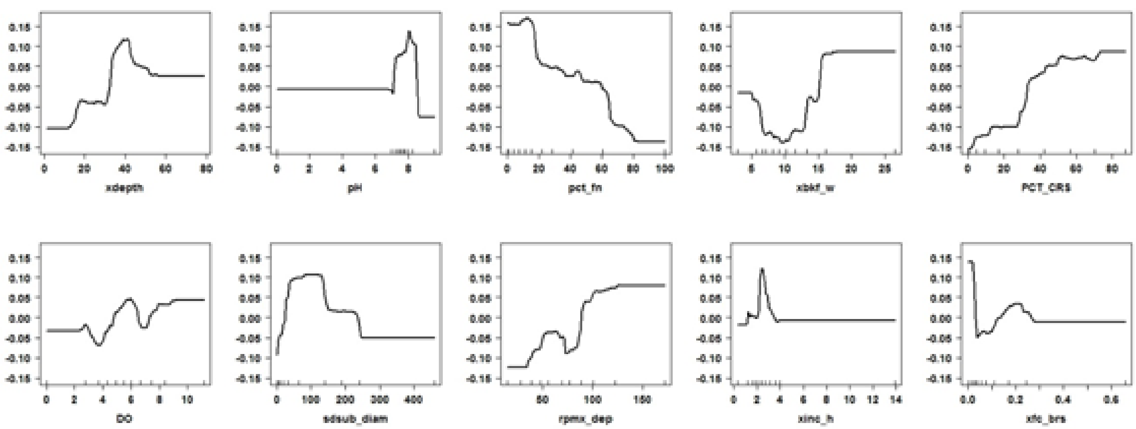


Appendix 3. *Continued*

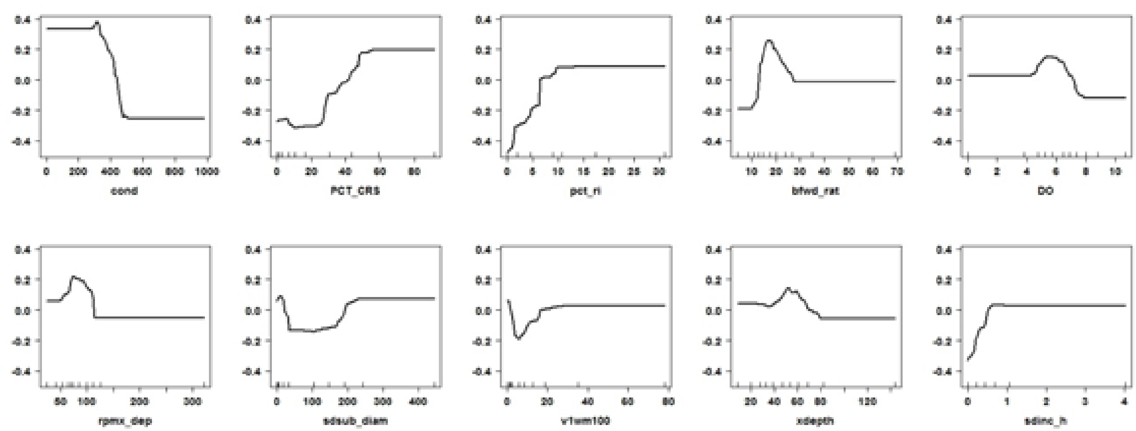
Number of Native Fish Species (numnatsp) – Ozark Highlands Small Rivers



Number of Native Benthic Species (nsnbenth) – Central Plains Creeks

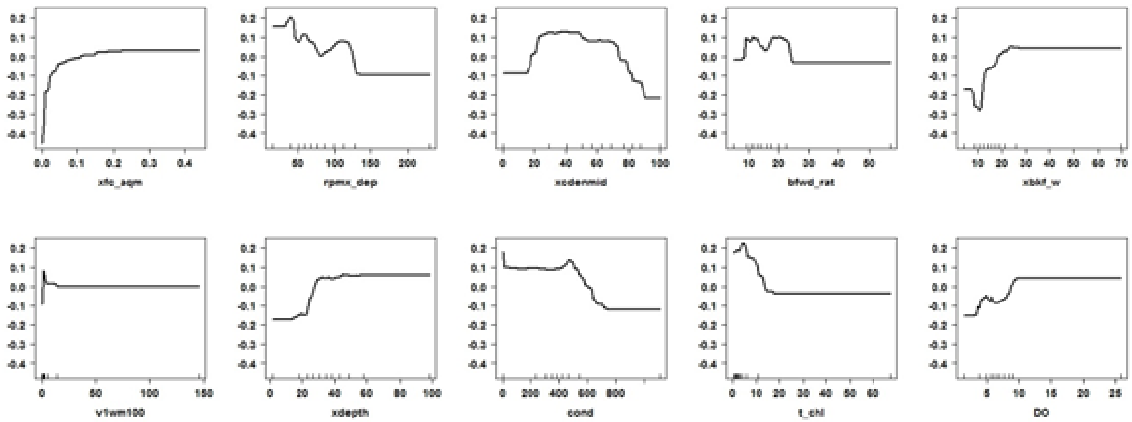


Number of Native Benthic Species (nsnbenth) – Central Plains Small Rivers

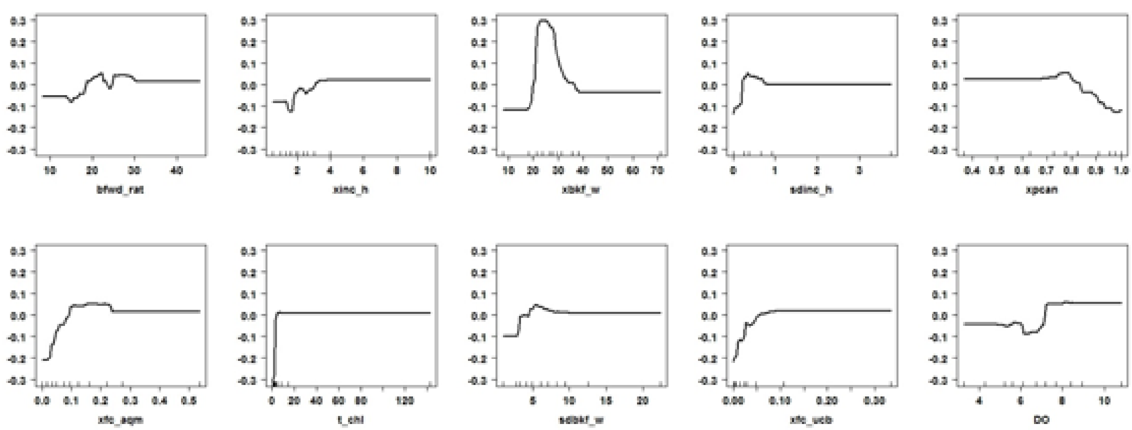


Appendix 3. *Continued*

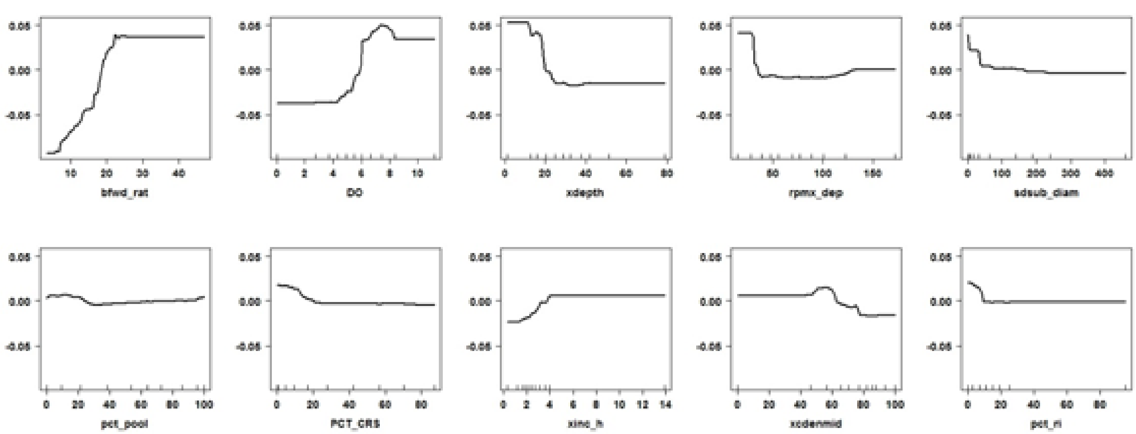
Number of Native Benthic Species (nsnbenth) – Ozark Highlands Creeks



Number of Native Benthic Species (nsnbenth) – Ozark Highlands Small Rivers

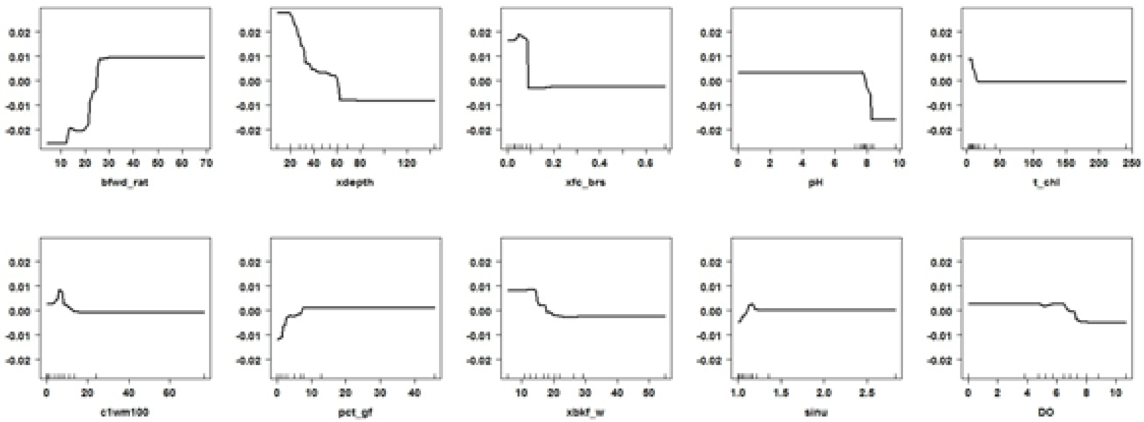


Proportion of Native Insectivorous Cyprinid Individuals (pnincyp) – Central Plains Creeks

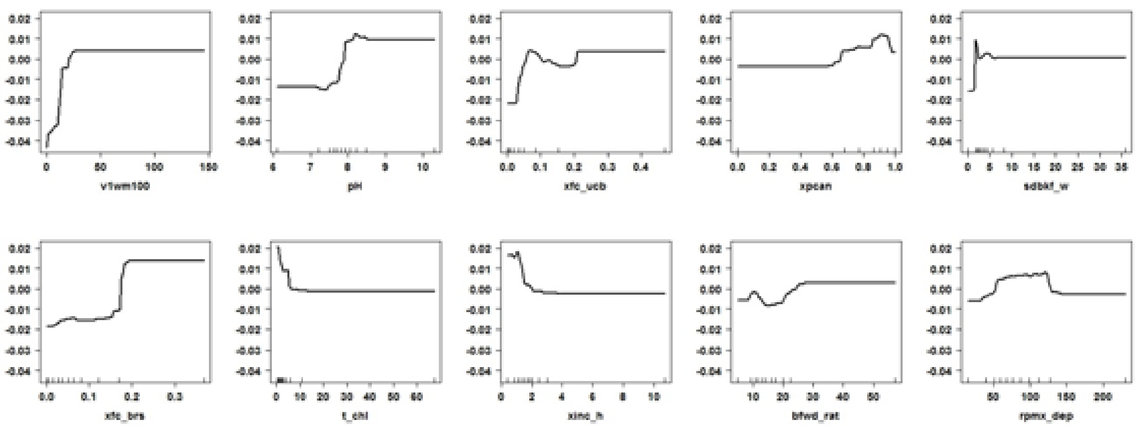


Appendix 3. *Continued*

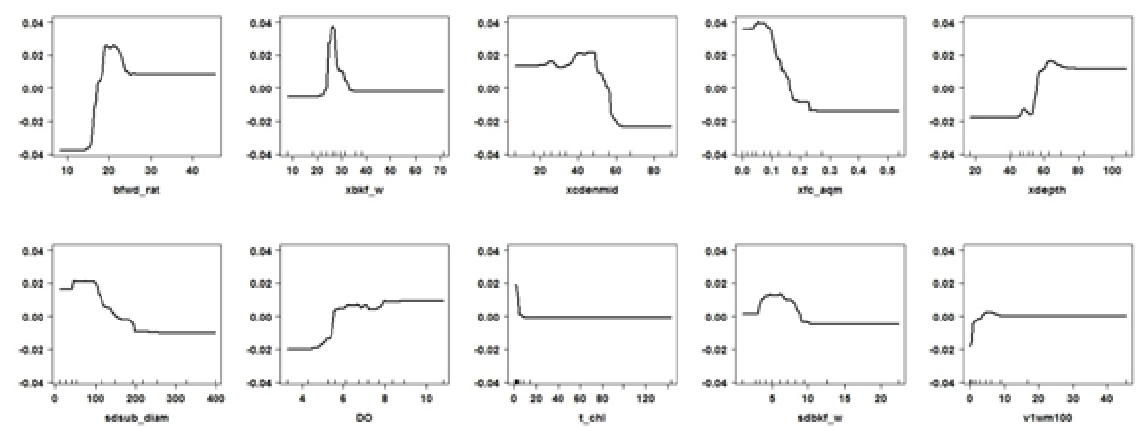
Proportion of Native Insectivorous Cyprinid Individuals (pnincyp) – Central Plains Small Rivers



Proportion of Native Insectivorous Cyprinid Individuals (pnincyp) – Ozark Highlands Creeks

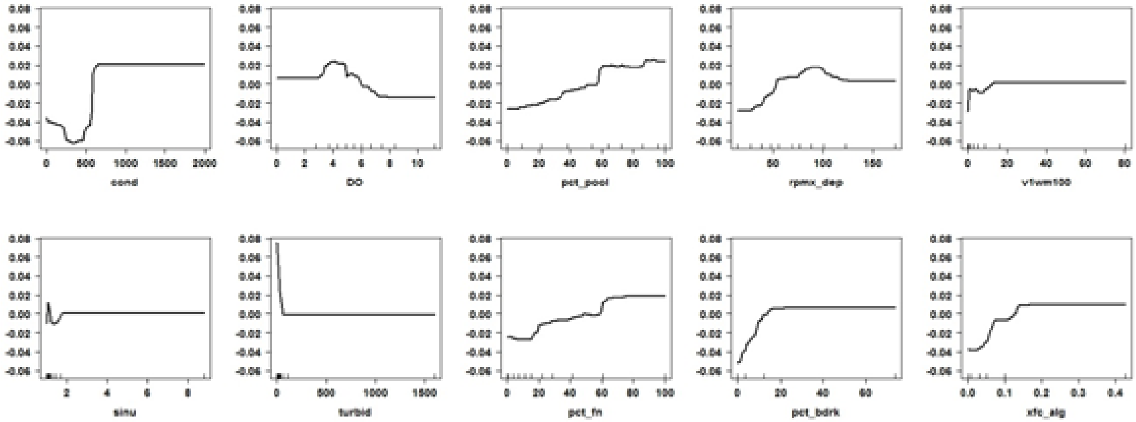


Proportion of Native Insectivorous Cyprinid Individuals (pnincyp) – Ozark Highlands Small Rivers

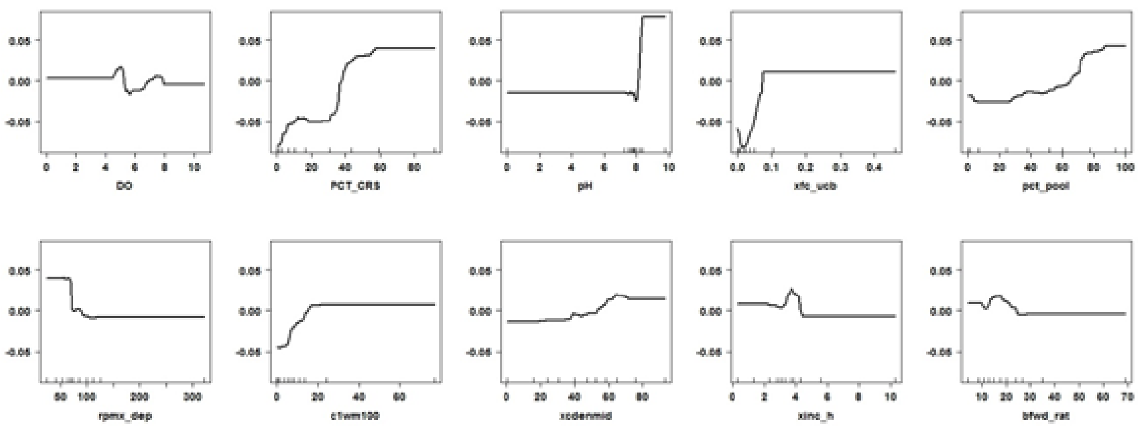


Appendix 3. *Continued*

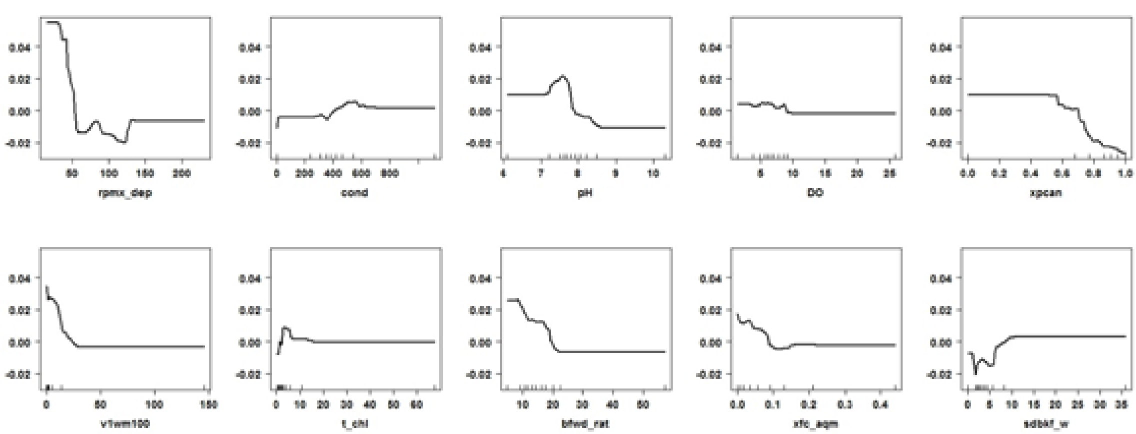
Proportion of Native Omnivorous/Herbivorous Individuals (pnomhb) – Central Plains Creeks



Proportion of Native Omnivorous/Herbivorous Individuals (pnomhb) – Central Plains Small Rivers

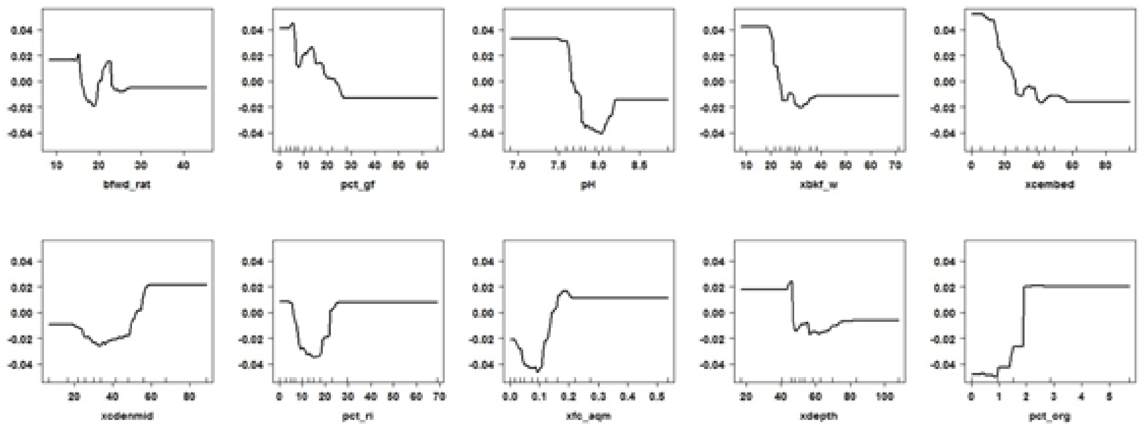


Proportion of Native Omnivorous/Herbivorous Individuals (pnomhb) – Ozark Highlands Creeks

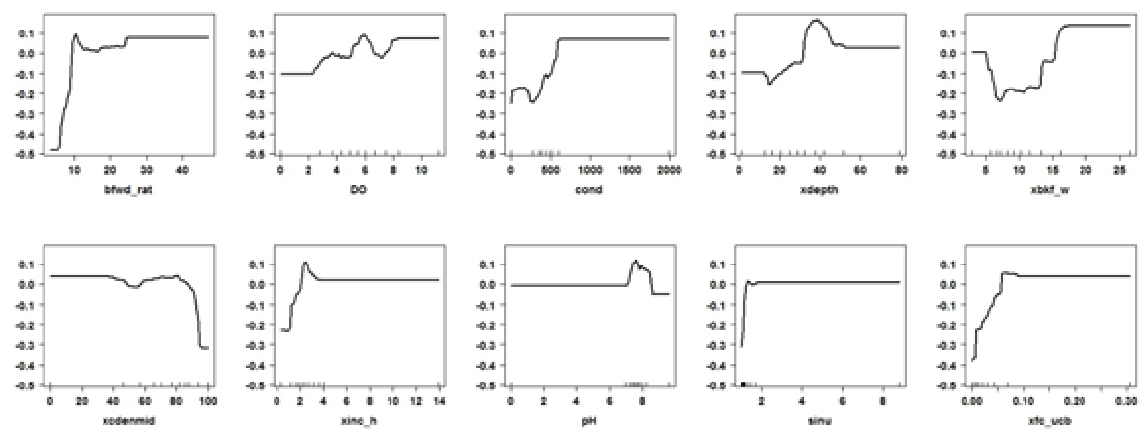


Appendix 3. *Continued*

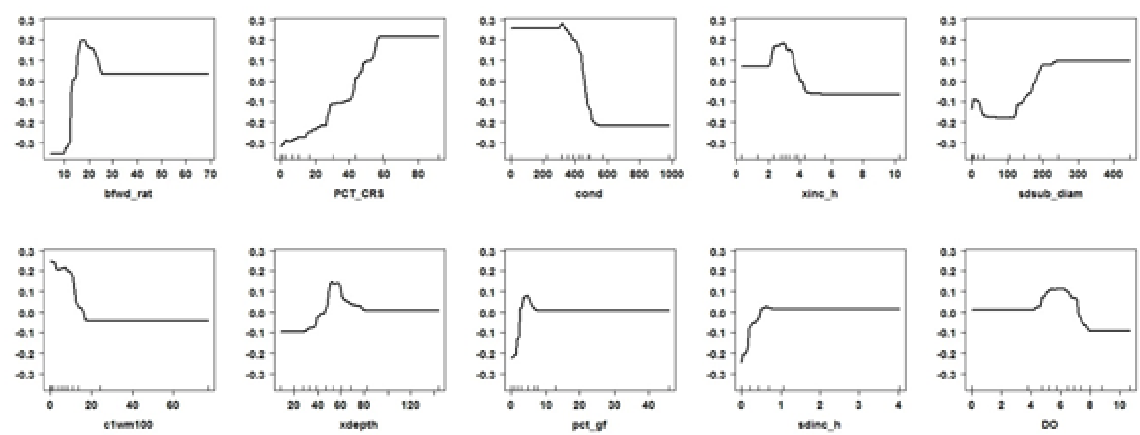
Proportion of Native Omnivorous/Herbivorous Individuals (pnomhb) – Ozark Highlands Small Rivers



Number of Native Lithophilic Species (nsnlith) – Central Plains Creeks

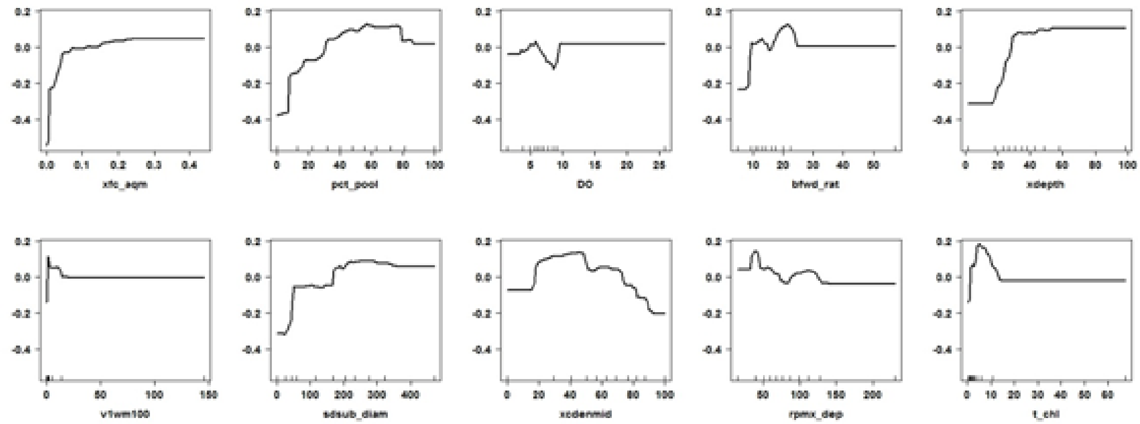


Number of Native Lithophilic Species (nsnlith) – Central Plains Small Rivers

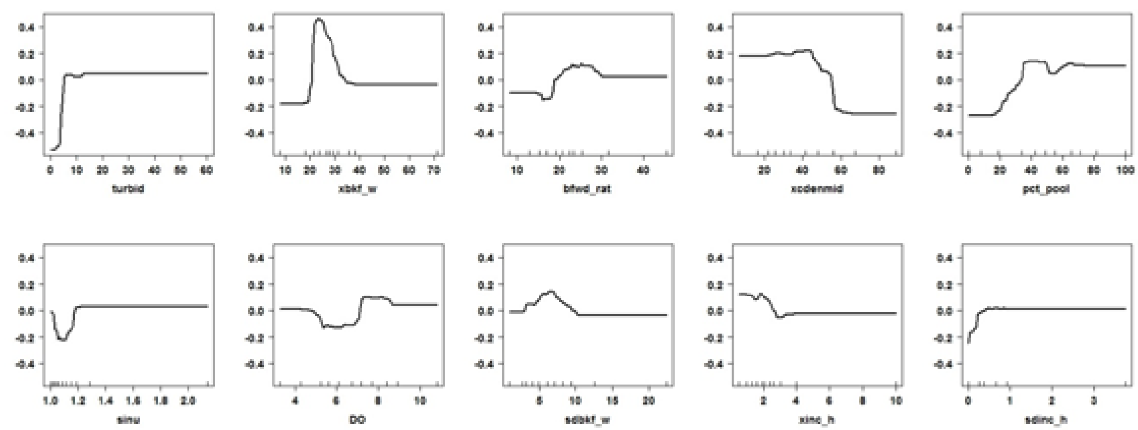


Appendix 3. *Continued*

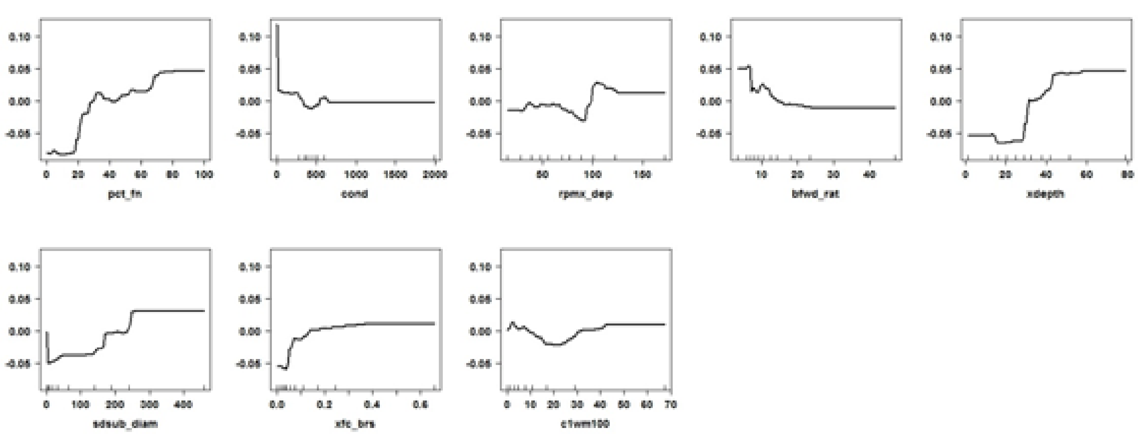
Number of Native Lithophilic Species (nsnlith) – Ozark Highlands Creeks



Number of Native Lithophilic Species (nsnlith) – Ozark Highlands Small Rivers

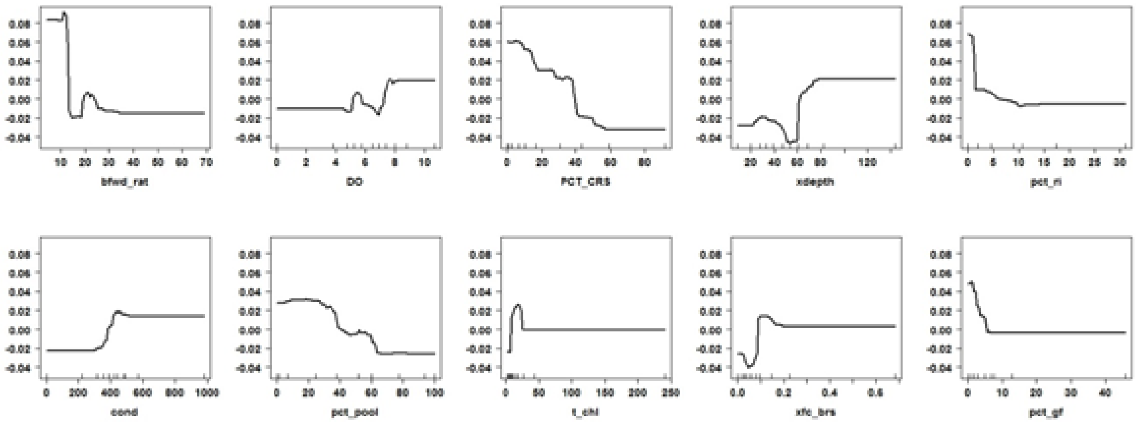


Proportion of Native Tolerant Individuals (pntole) – Central Plains Creeks

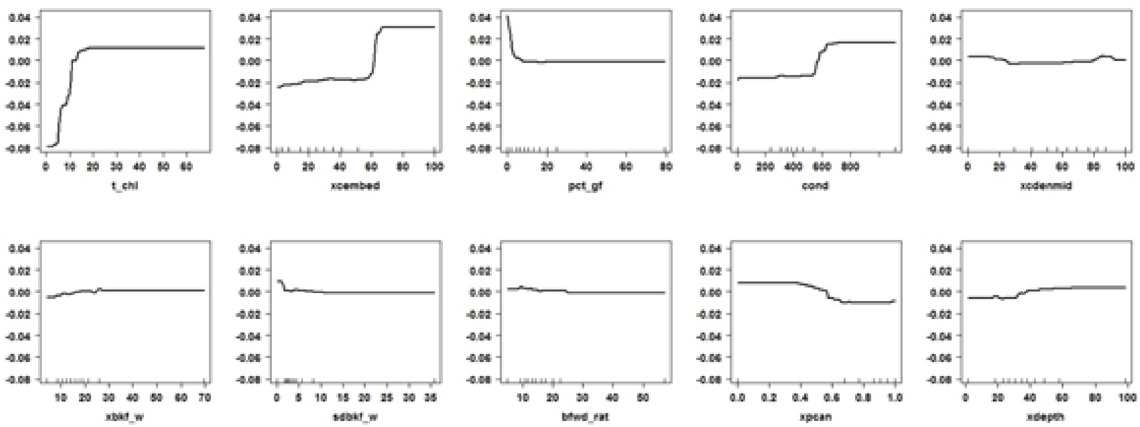


Appendix 3. *Continued*

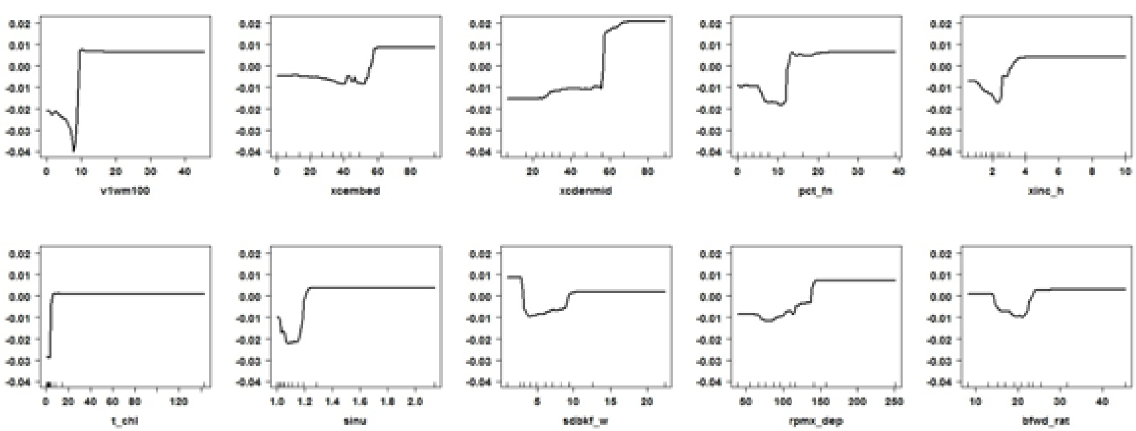
Proportion of Native Tolerant Individuals (pntole) – Central Plains Small Rivers



Proportion of Native Tolerant Individuals (pntole) – Ozark Highlands Creeks



Proportion of Native Tolerant Individuals (pntole) – Ozark Highlands Small Rivers

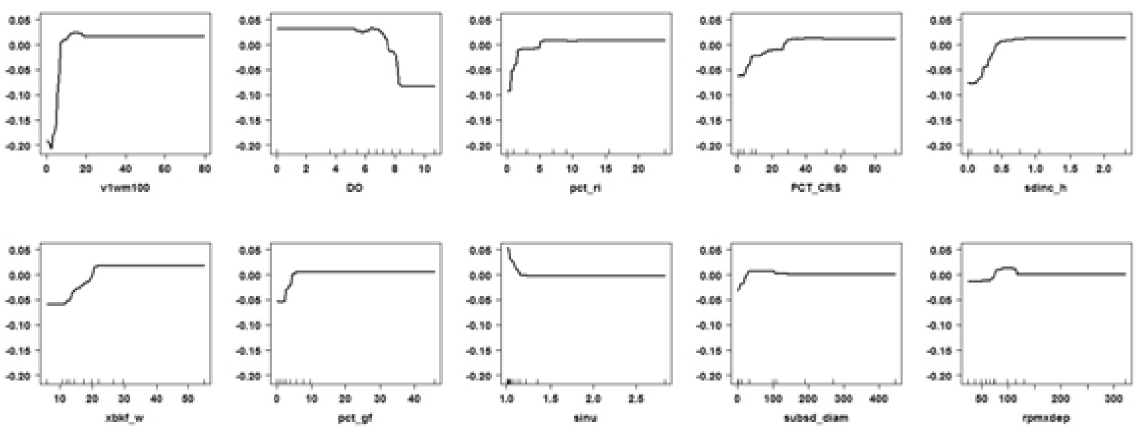


Appendix 3. *Continued*

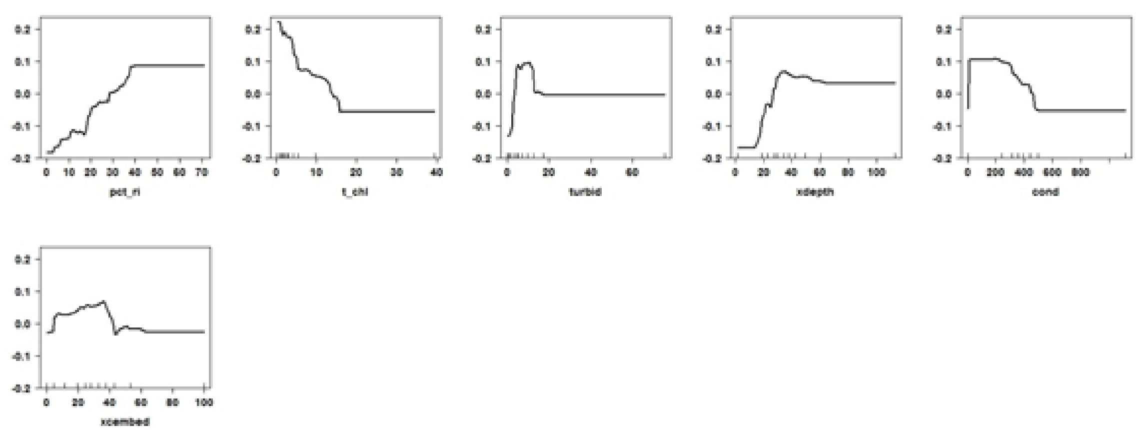
Invertebrate Shannon's Diversity Index (SDI) – Central Plains Creeks

-- No Influential Predictors --

Invertebrate Shannon's Diversity Index (SDI) – Central Plains Small Rivers



Invertebrate Shannon's Diversity Index (SDI) – Ozark Highlands Creeks

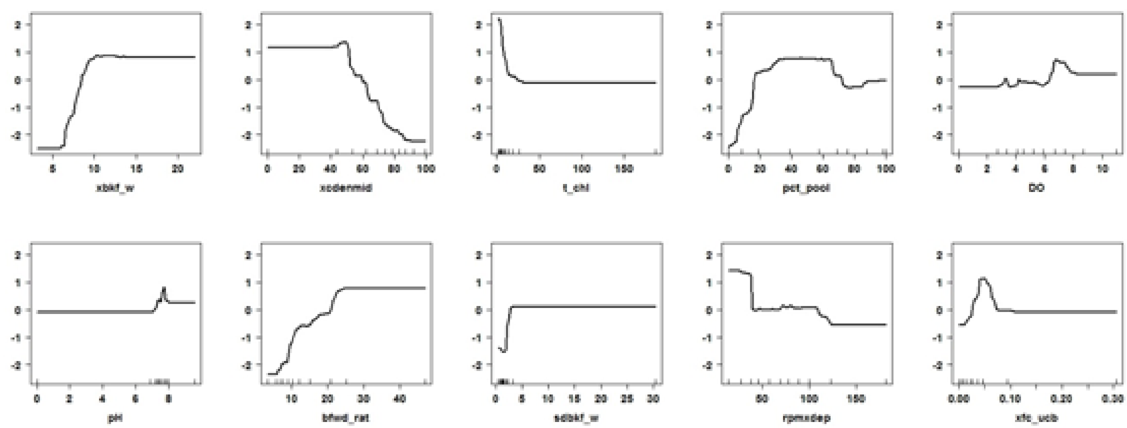


Appendix 3. *Continued*

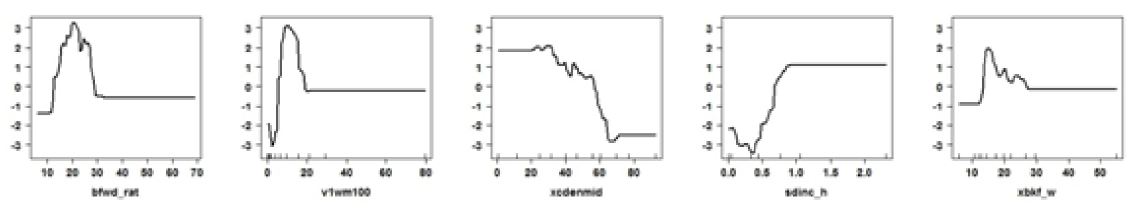
Invertebrate Shannon's Diversity Index (SDI) – Ozark Highlands Small Rivers

-- No Influential Predictors --

Ephemeroptera, Plecoptera, Trichoptera Richness (EPT) – Central Plains Creeks

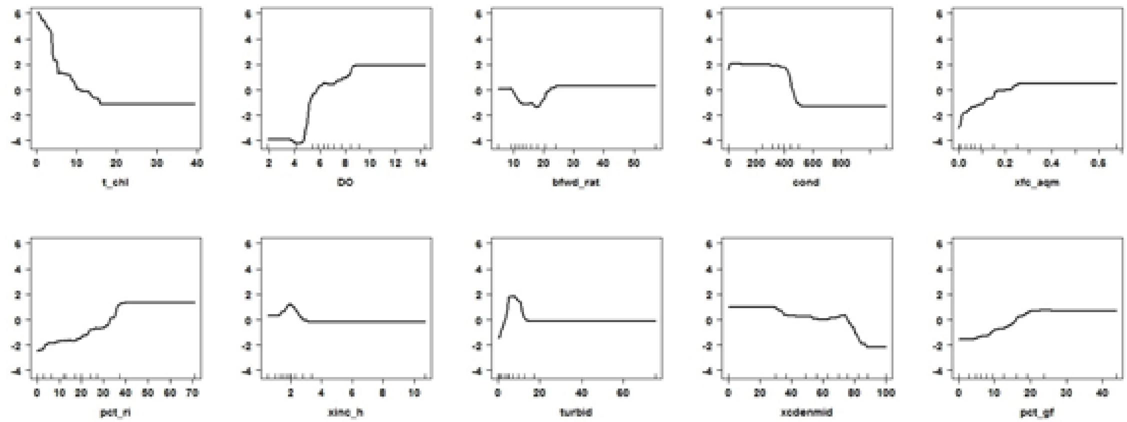


Ephemeroptera, Plecoptera, Trichoptera Richness (EPT) – Central Plains Small Rivers

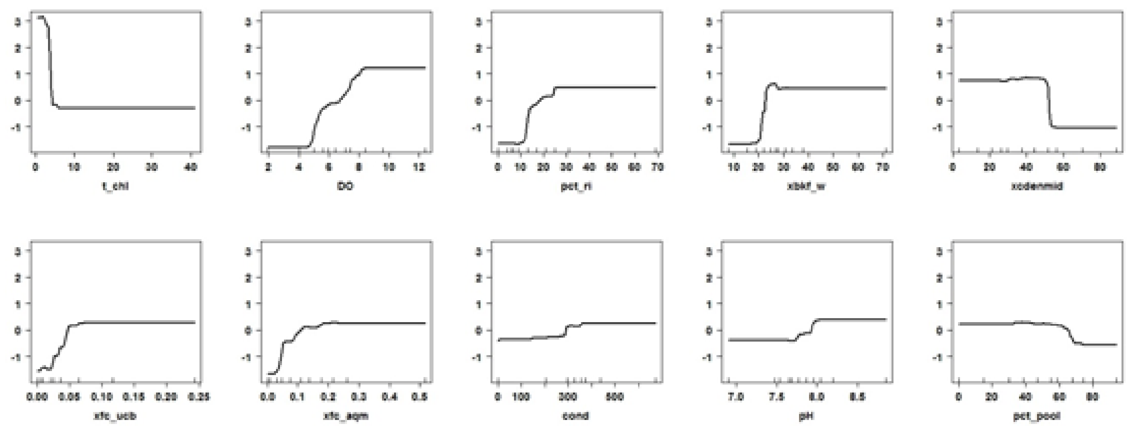


Appendix 3. *Continued*

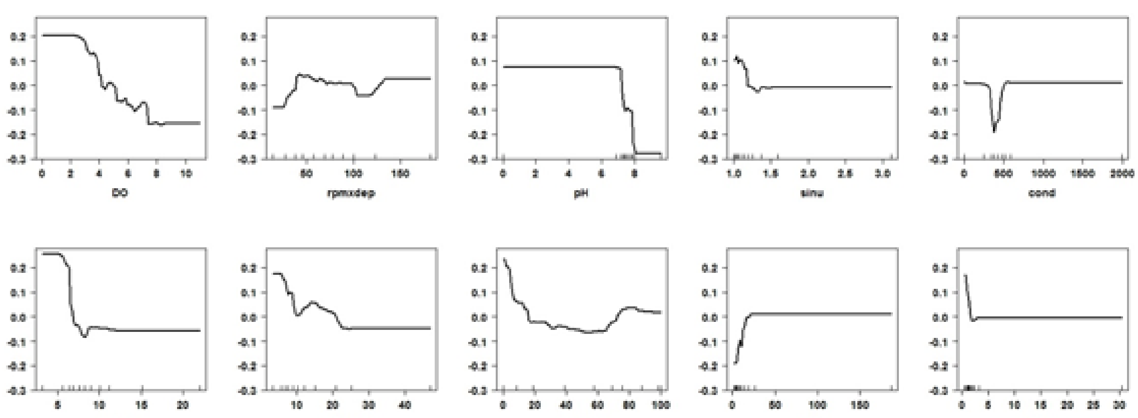
Ephemeroptera, Plecoptera, Trichoptera Richness (EPT) – Ozark Highlands Creeks



Ephemeroptera, Plecoptera, Trichoptera Richness (EPT) – Ozark Highlands Small Rivers

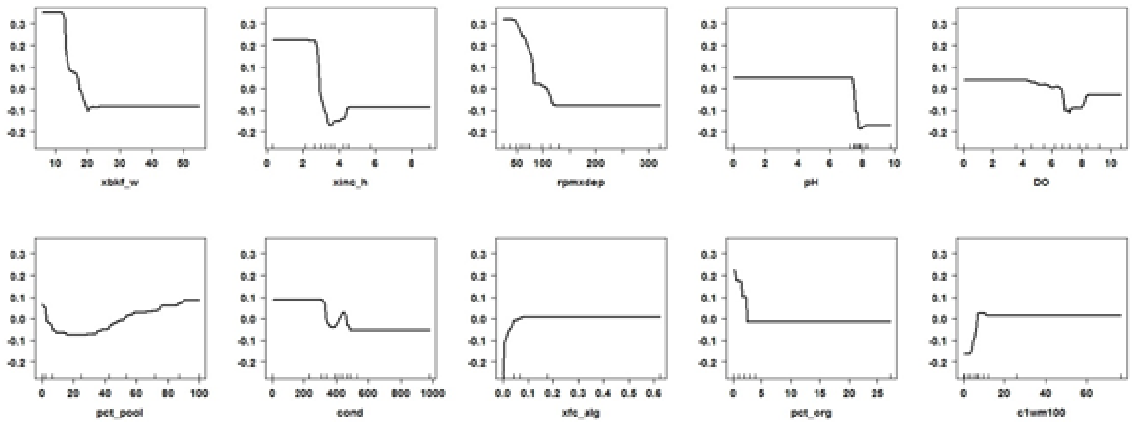


Invertebrate Hilsenhoff Biotic Index (HBI) – Central Plains Creeks

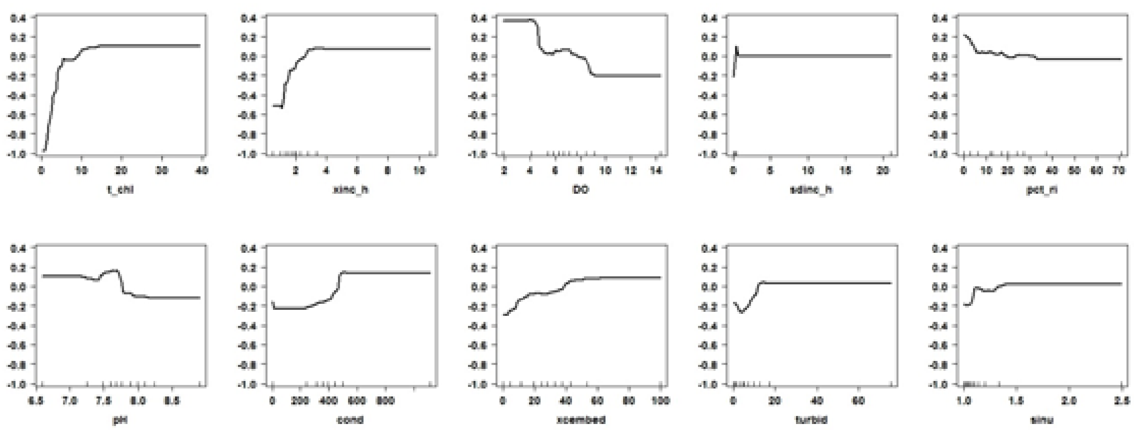


Appendix 3. *Continued*

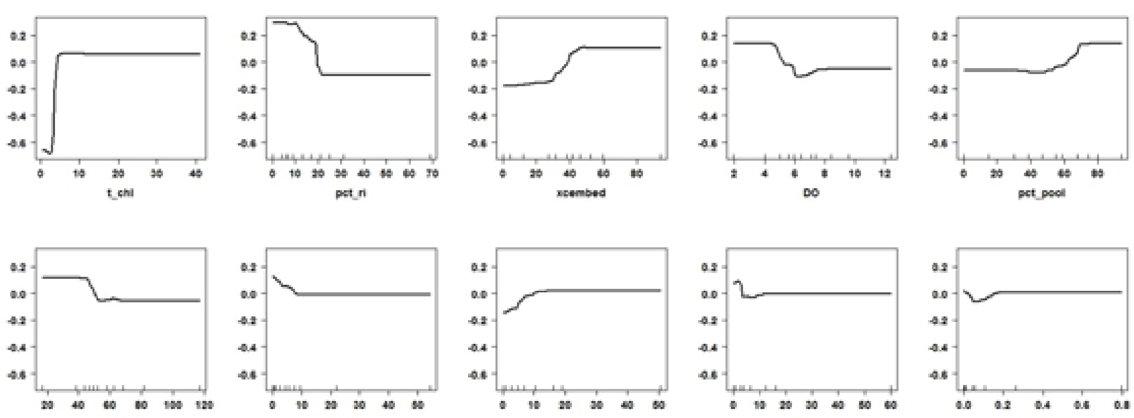
Invertebrate Hilsenhoff Biotic Index (HBI) – Central Plains Small Rivers



Invertebrate Hilsenhoff Biotic Index (HBI) – Ozark Highlands Creeks

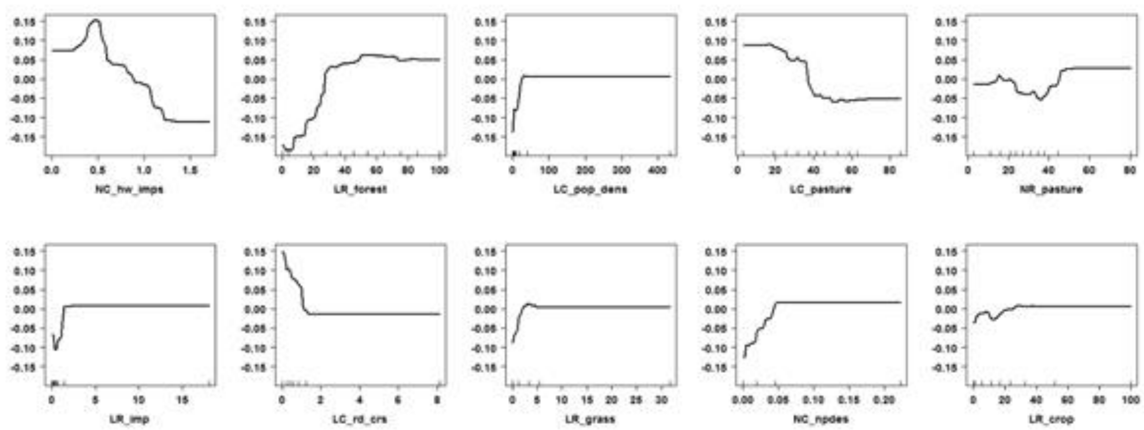


Invertebrate Hilsenhoff Biotic Index (HBI) – Ozark Highlands Small Rivers

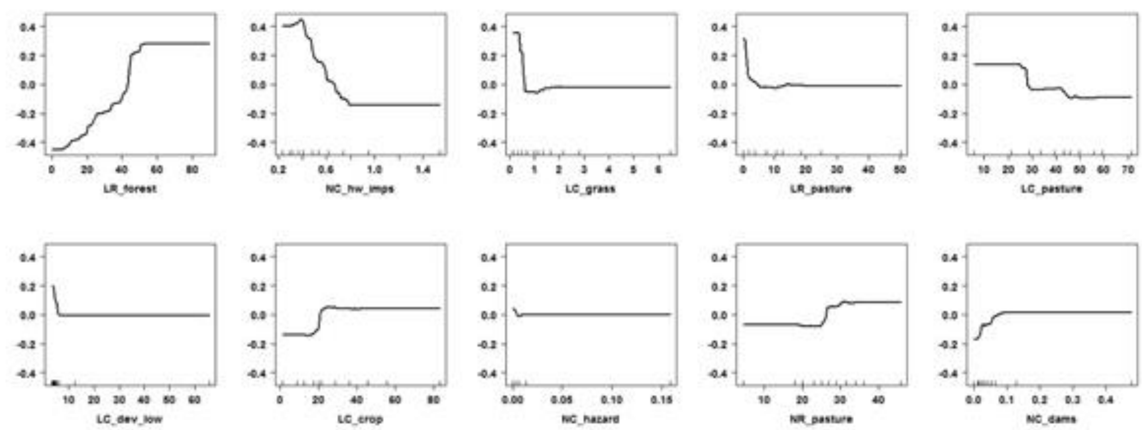


Appendix 4. Boosted regression tree partial dependence plots of influential watershed-level environmental variables for fish and invertebrate community characteristics (presented as a dimensionless transformation of each response, centered to zero mean). Rug plots at inside bottom of plots show the distribution of sites across the given predictor variable.

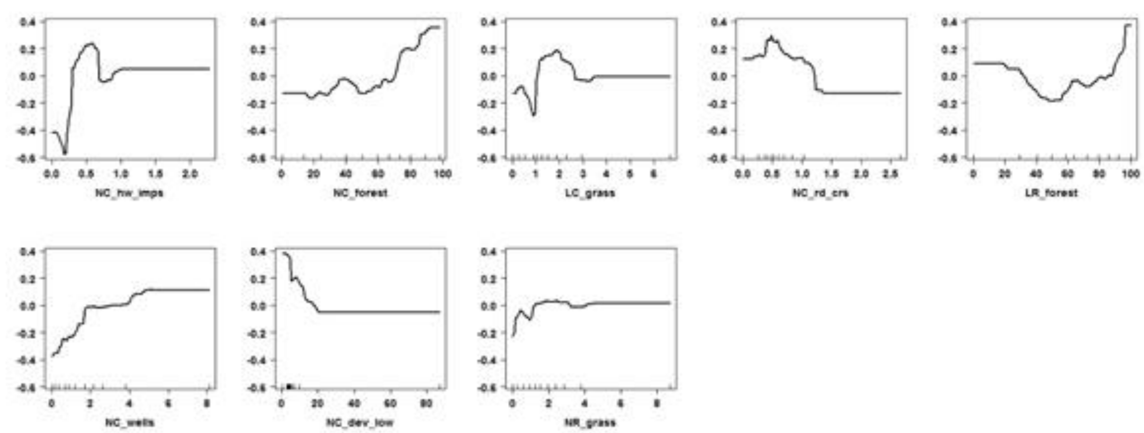
Number of Native Fish Species (numnatsp) – Central Plains Creeks



Number of Native Fish Species (numnatsp) – Central Plains Small Rivers

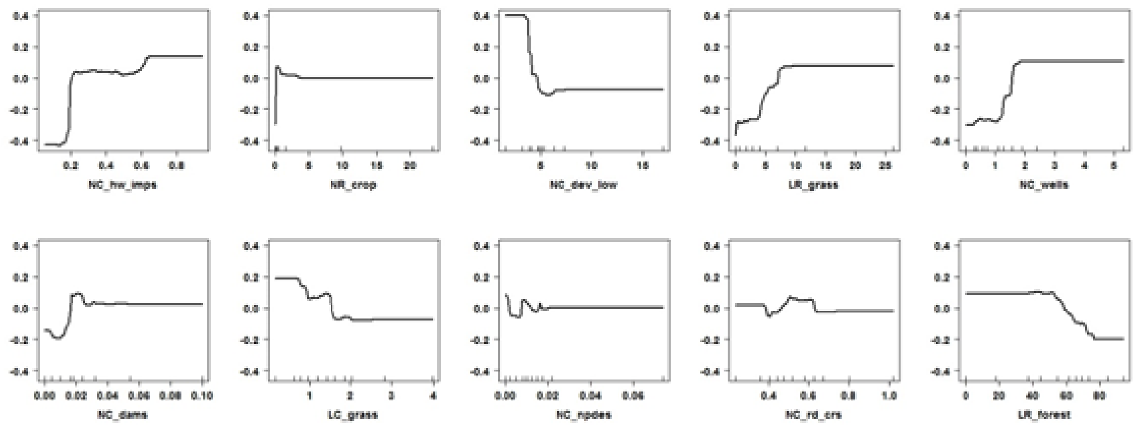


Number of Native Fish Species (numnatsp) – Ozark Highlands Creeks

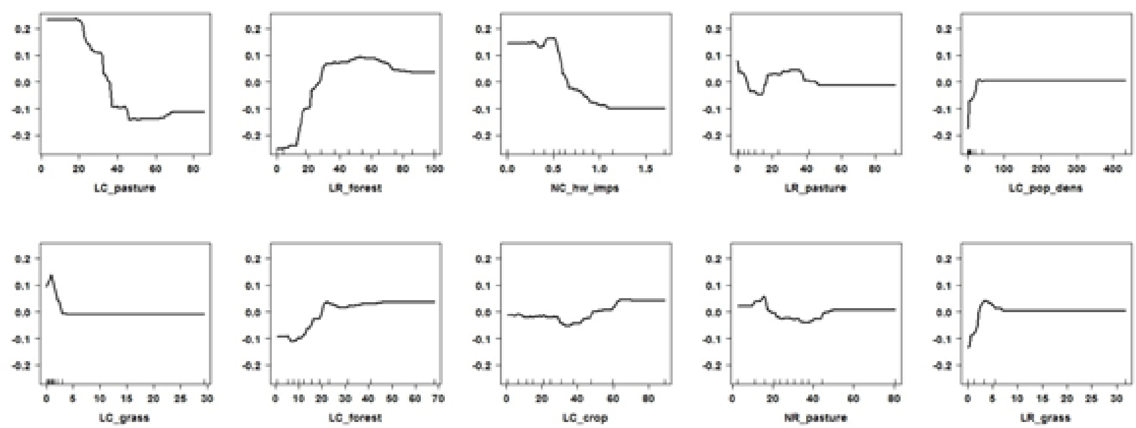


Appendix 4. *Continued*

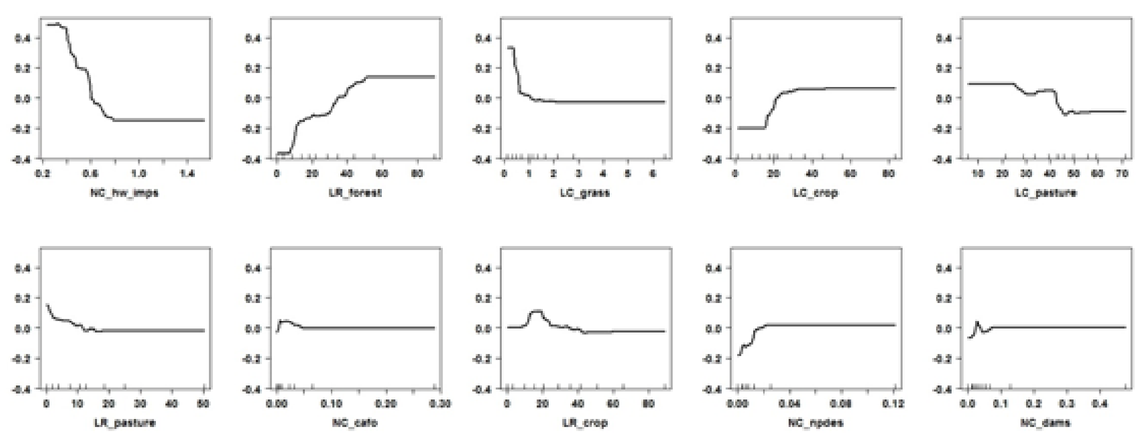
Number of Native Fish Species (numnatsp) – Ozark Highlands Small Rivers



Number of Native Benthic Species (nsnbenth) – Central Plains Creeks

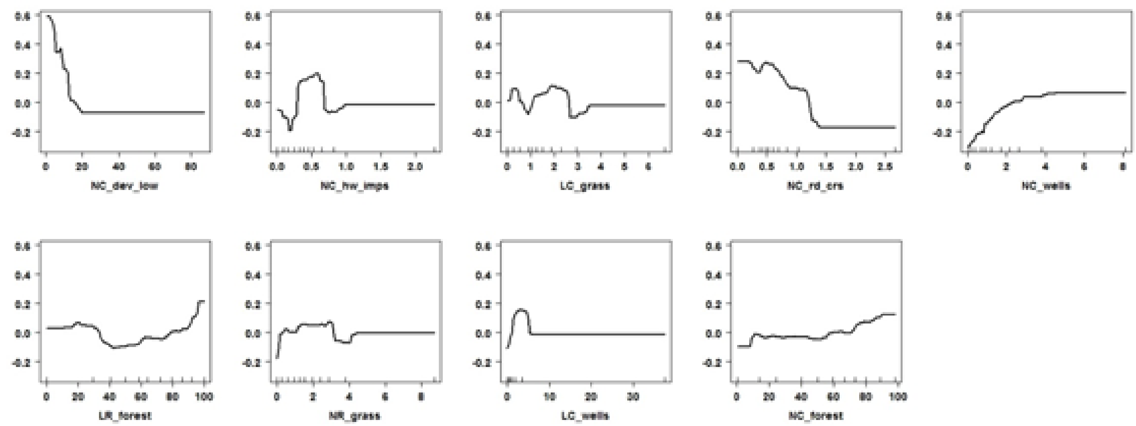


Number of Native Benthic Species (nsnbenth) – Central Plains Small Rivers

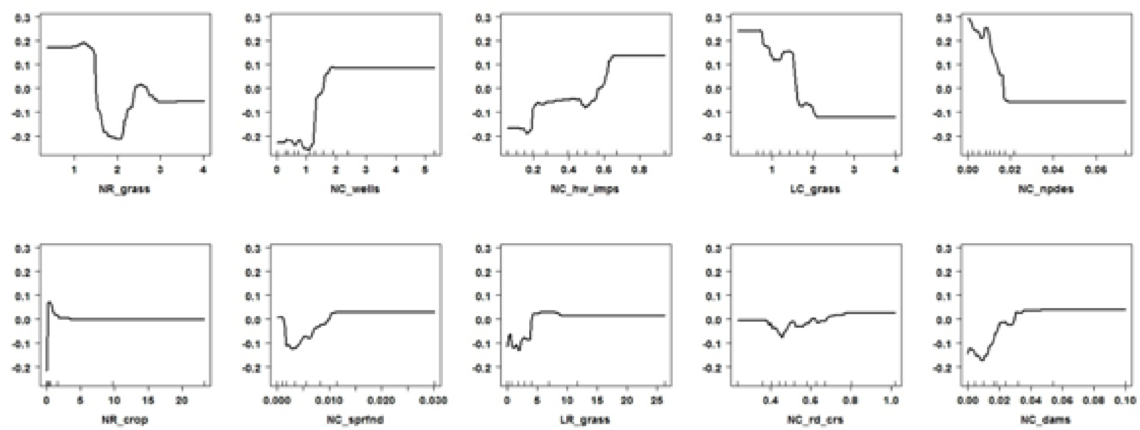


Appendix 4. *Continued*

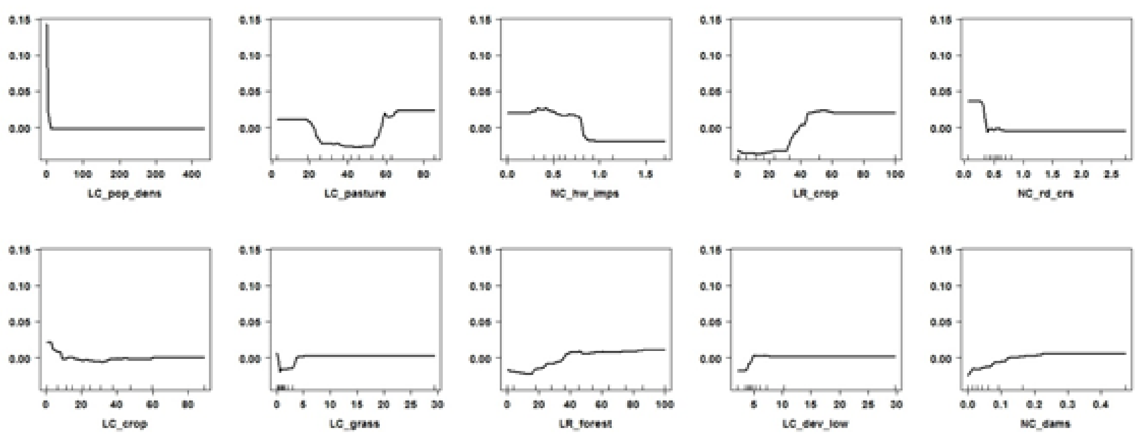
Number of Native Benthic Species (nsnbenth) – Ozark Highlands Creeks



Number of Native Benthic Species (nsnbenth) – Ozark Highlands Small Rivers

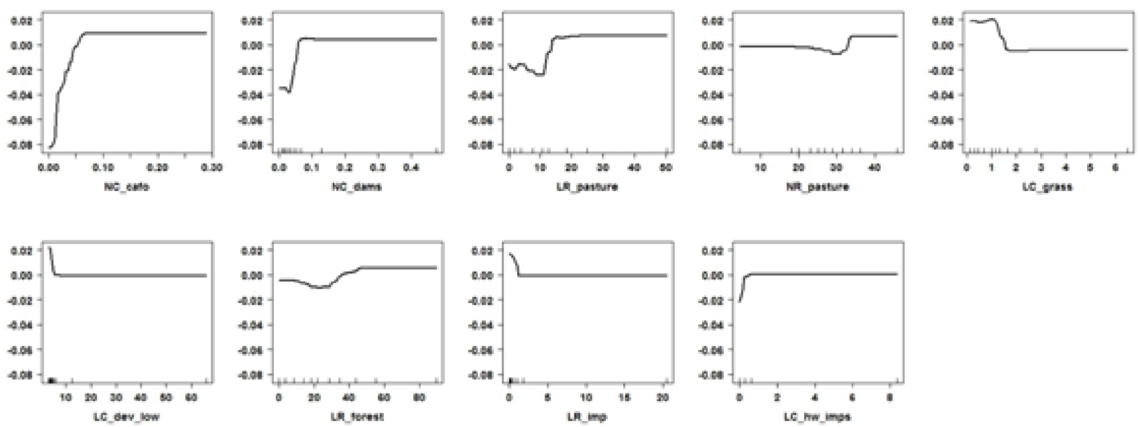


Proportion of Native Insectivorous Cyprinid Individuals (pnincyp) – Central Plains Creeks

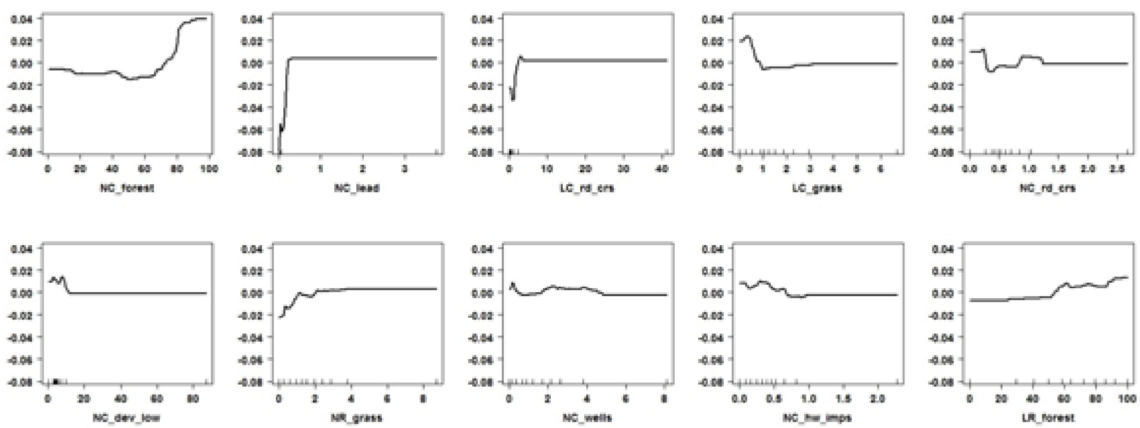


Appendix 4. *Continued*

Proportion of Native Insectivorous Cyprinid Individuals (pnincyp) – Central Plains Small Rivers



Proportion of Native Insectivorous Cyprinid Individuals (pnincyp) – Ozark Highlands Creeks

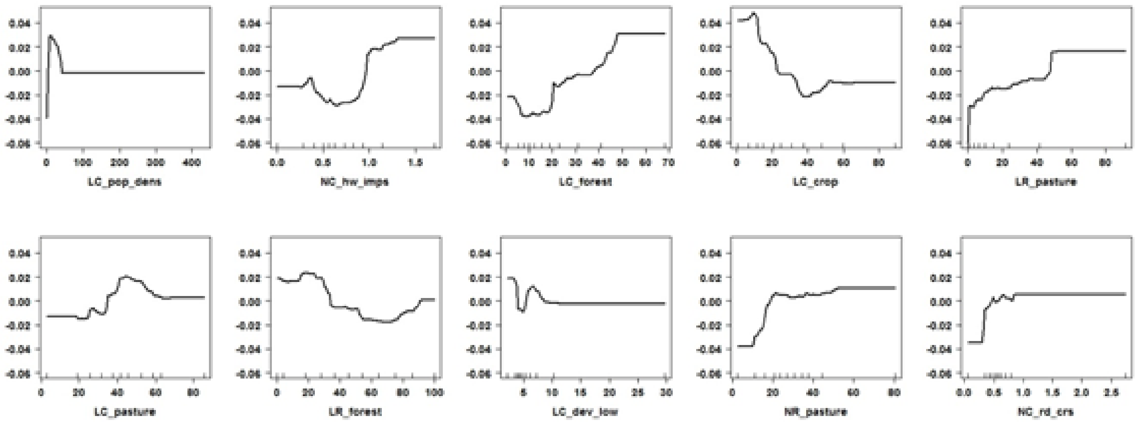


Proportion of Native Insectivorous Cyprinid Individuals (pnincyp) – Ozark Highlands Small Rivers

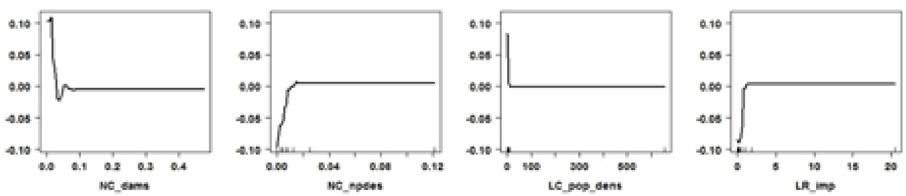
-- No Influential Predictors --

Appendix 4. *Continued*

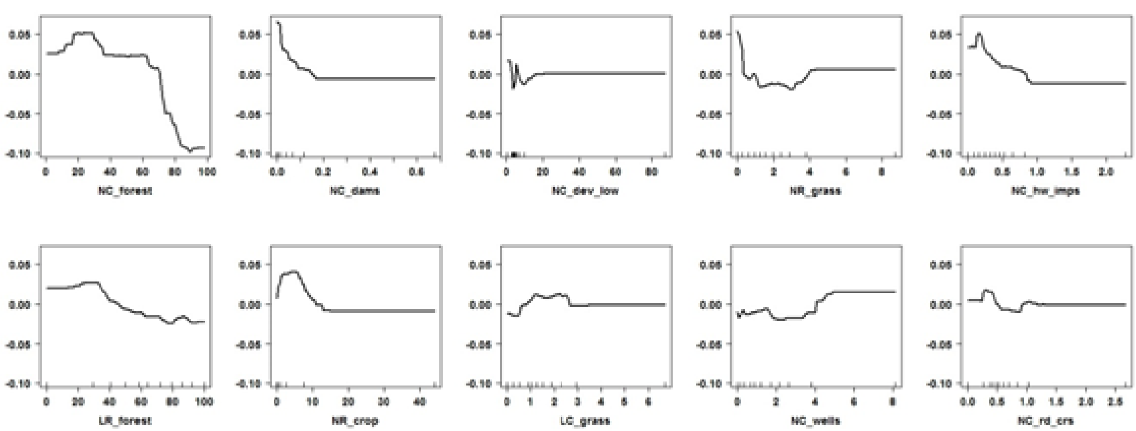
Proportion of Native Omnivorous/Herbivorous Individuals (pnomhb) – Central Plains Creeks



Proportion of Native Omnivorous/Herbivorous Individuals (pnomhb) – Central Plains Small Rivers

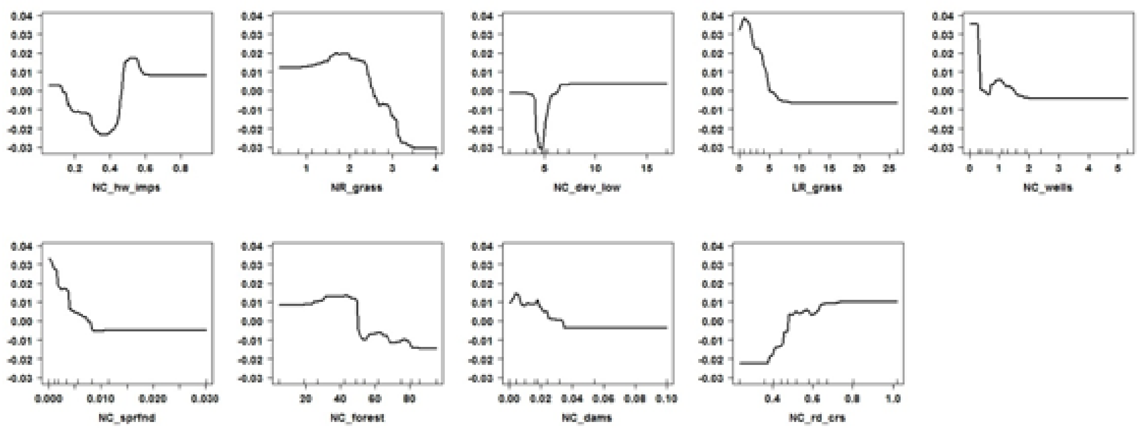


Proportion of Native Omnivorous/Herbivorous Individuals (pnomhb) – Ozark Highlands Creeks

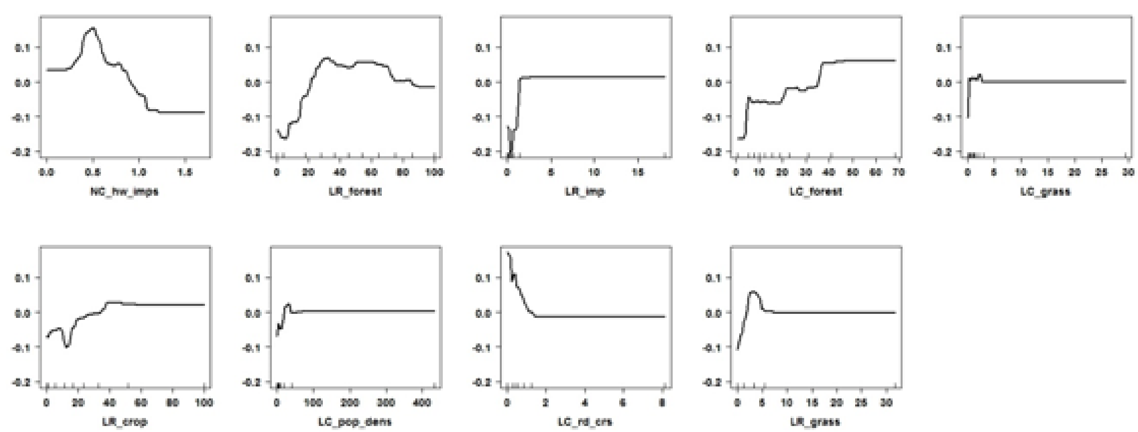


Appendix 4. *Continued*

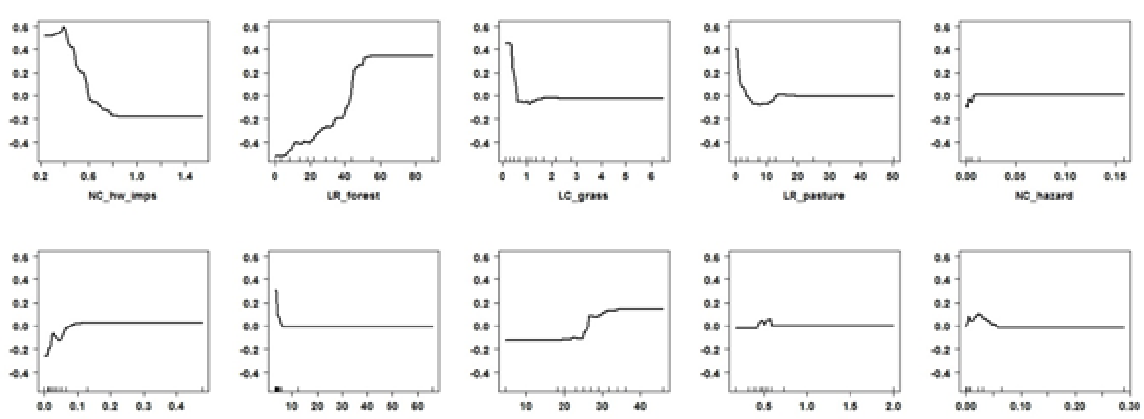
Proportion of Native Omnivorous/Herbivorous Individuals (pnomhb) – Ozark Highlands Small Rivers



Number of Native Lithophilic Species (nsnlith) – Central Plains Creeks

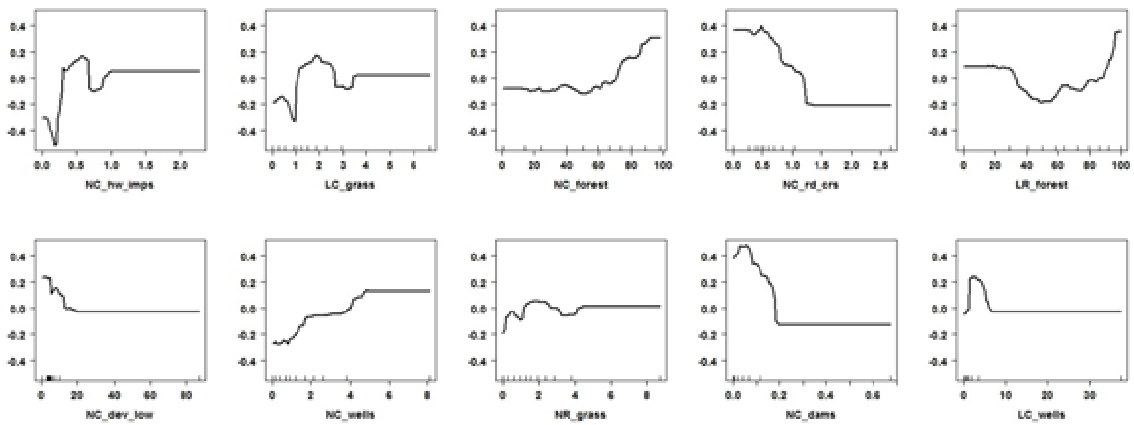


Number of Native Lithophilic Species (nsnlith) – Central Plains Small Rivers

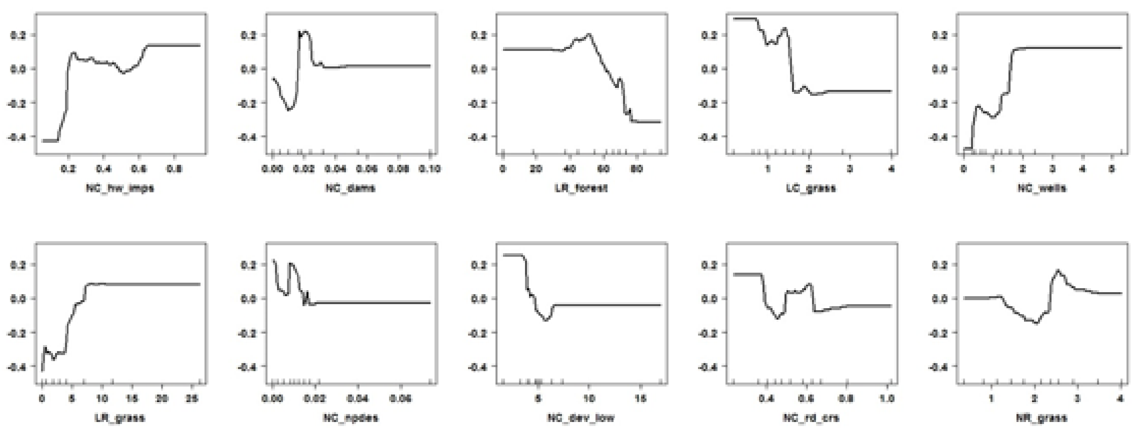


Appendix 4. *Continued*

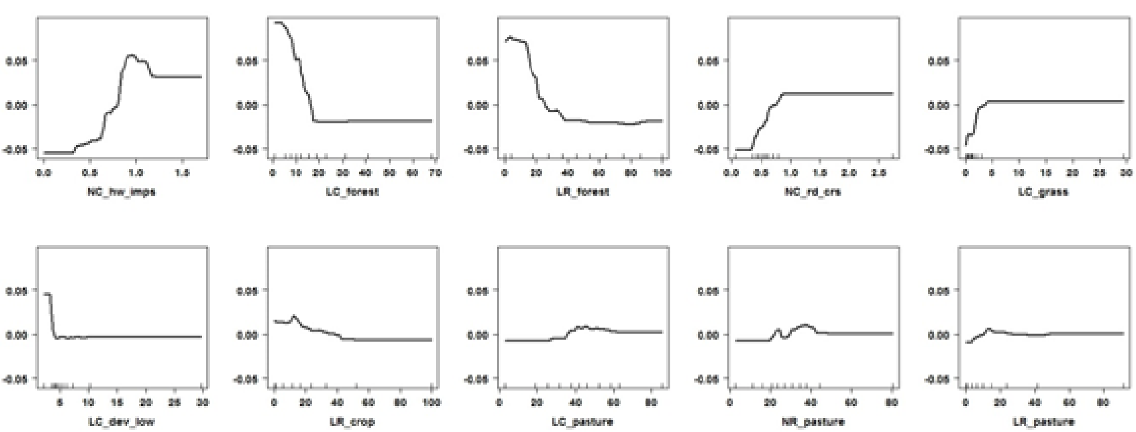
Number of Native Lithophilic Species (nsnlith) – Ozark Highlands Creeks



Number of Native Lithophilic Species (nsnlith) – Ozark Highlands Small Rivers

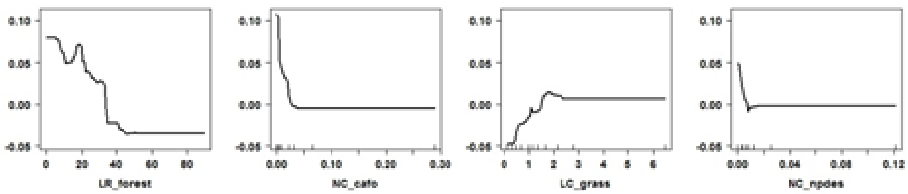


Proportion of Native Tolerant Individuals (pntole) – Central Plains Creeks

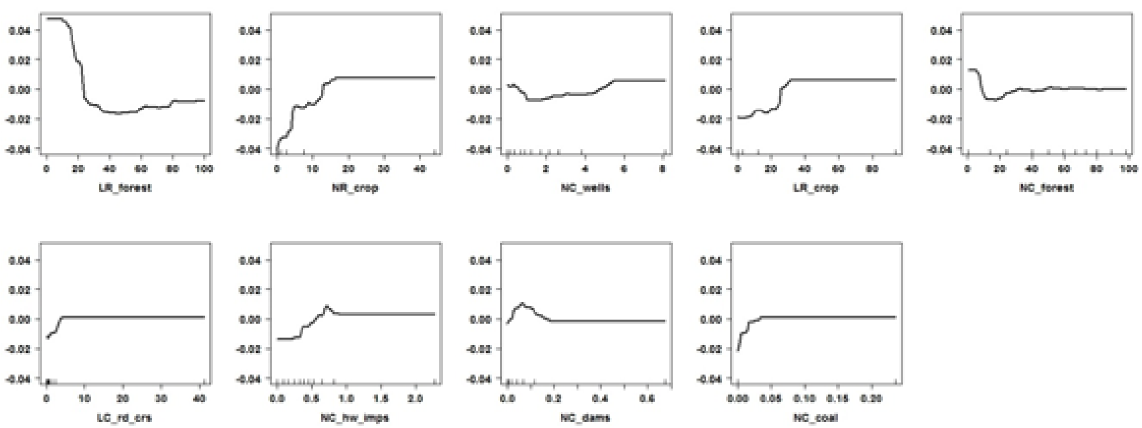


Appendix 4. *Continued*

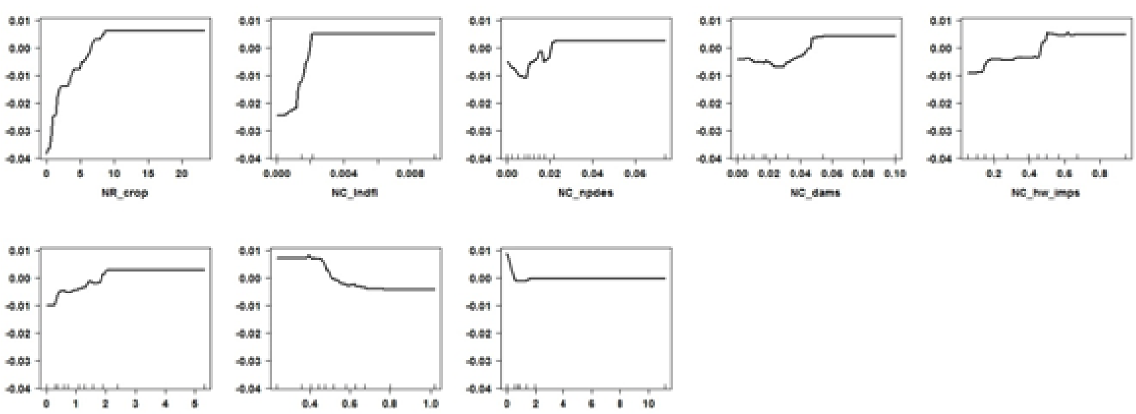
Proportion of Native Tolerant Individuals (pntole) – Central Plains Small Rivers



Proportion of Native Tolerant Individuals (pntole) – Ozark Highlands Creeks



Proportion of Native Tolerant Individuals (pntole) – Ozark Highlands Small Rivers

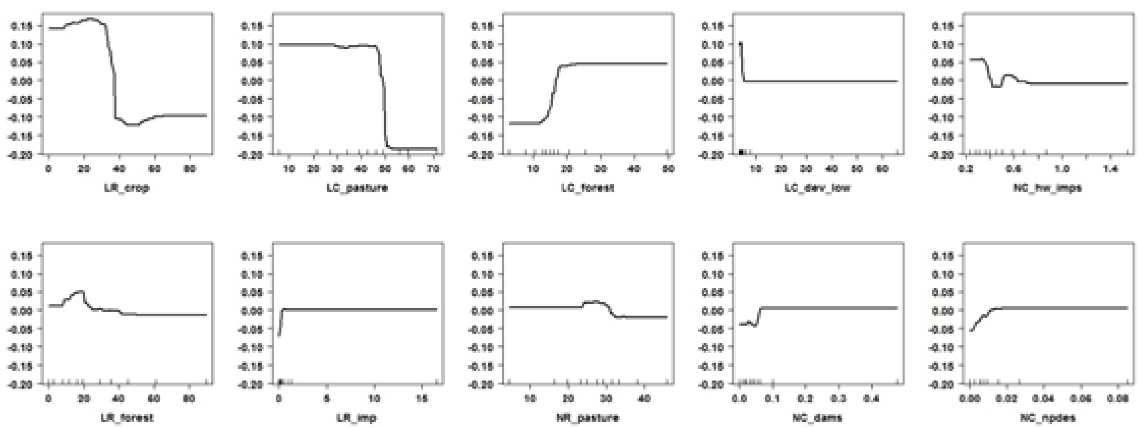


Appendix 4. *Continued*

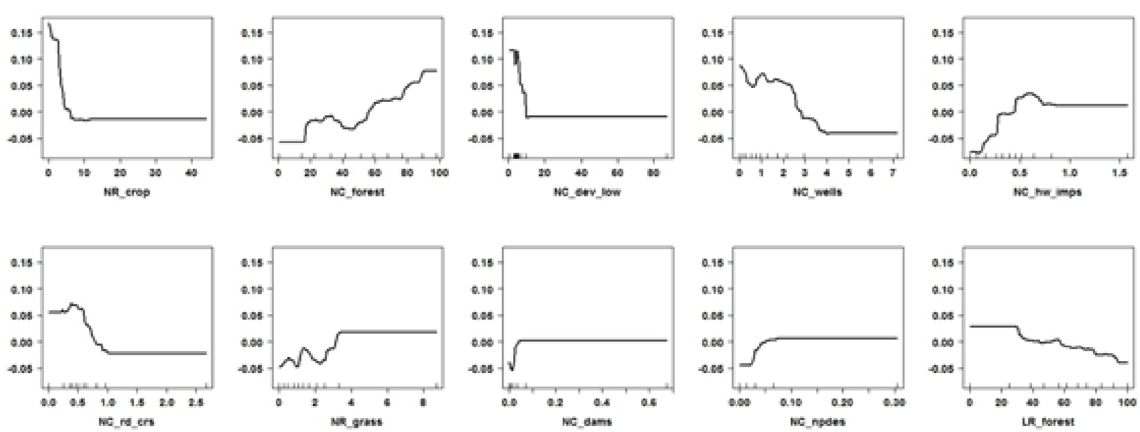
Invertebrate Shannon's Diversity Index (SDI) – Central Plains Creeks

-- No Influential Predictors --

Invertebrate Shannon's Diversity Index (SDI) – Central Plains Small Rivers



Invertebrate Shannon's Diversity Index (SDI) – Ozark Highlands Creeks

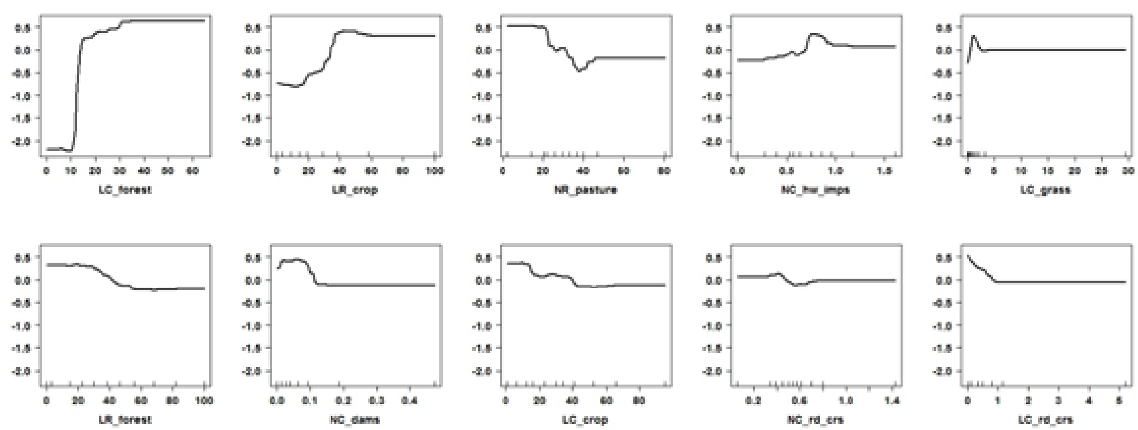


Appendix 4. *Continued*

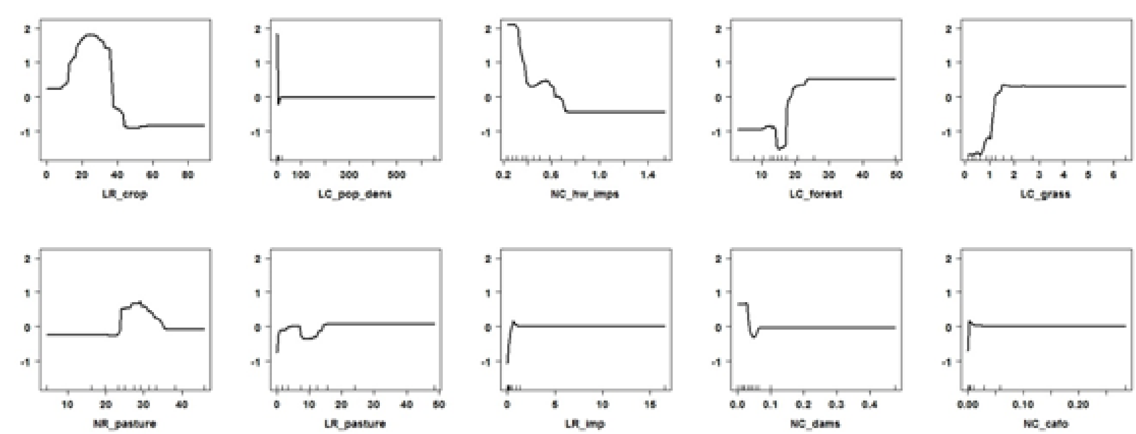
Invertebrate Shannon's Diversity Index (SDI) – Ozark Highlands Small Rivers

-- No Influential Predictors --

Ephemeroptera, Plecoptera, Trichoptera Richness (EPT) – Central Plains Creeks

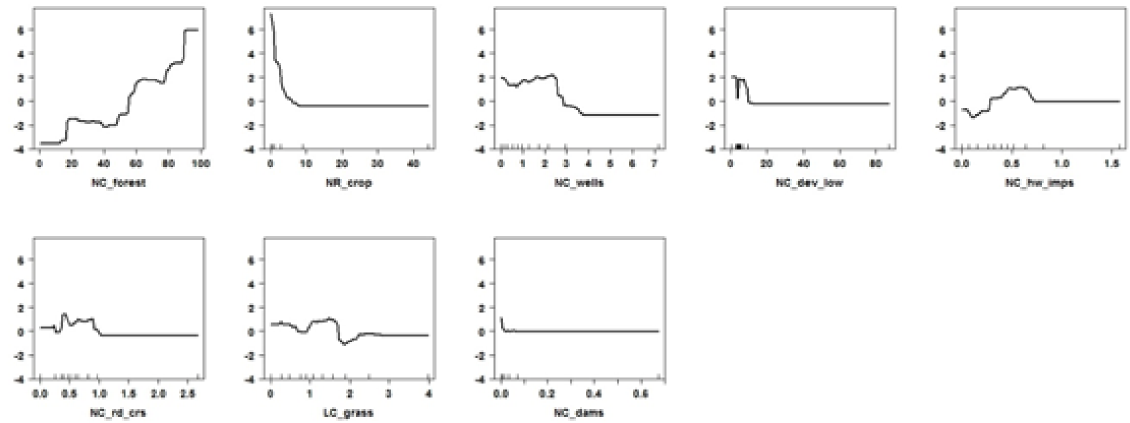


Ephemeroptera, Plecoptera, Trichoptera Richness (EPT) – Central Plains Small Rivers

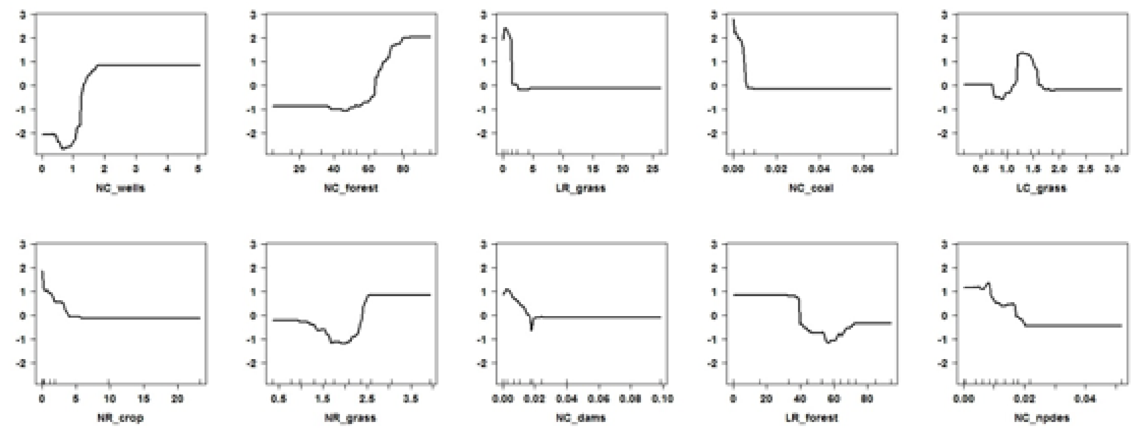


Appendix 4. *Continued*

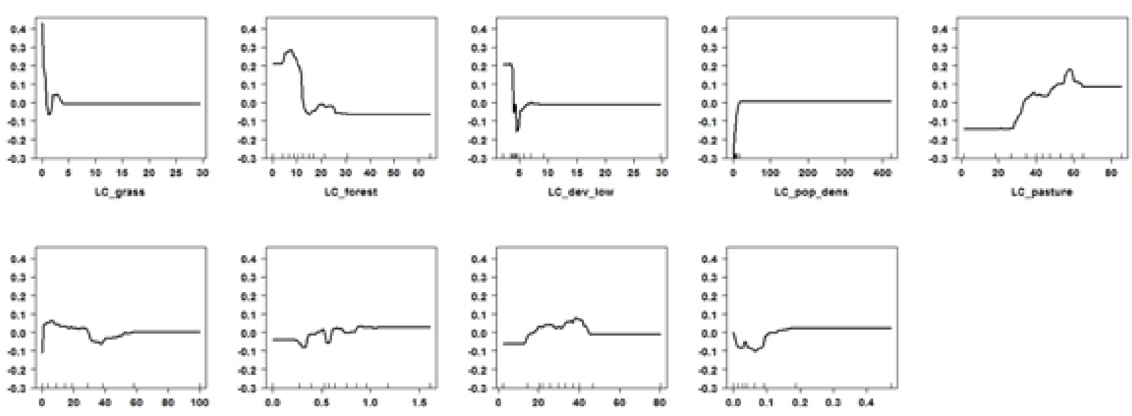
Ephemeroptera, Plecoptera, Trichoptera Richness (EPT) – Ozark Highlands Creeks



Ephemeroptera, Plecoptera, Trichoptera Richness (EPT) – Ozark Highlands Small Rivers



Invertebrate Hilsenhoff Biotic Index (HBI) – Central Plains Creeks

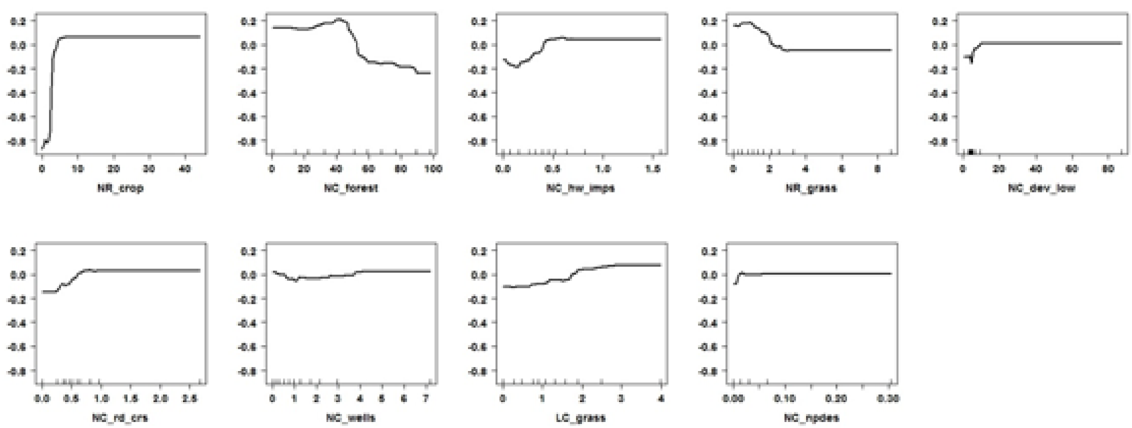


Appendix 4. *Continued*

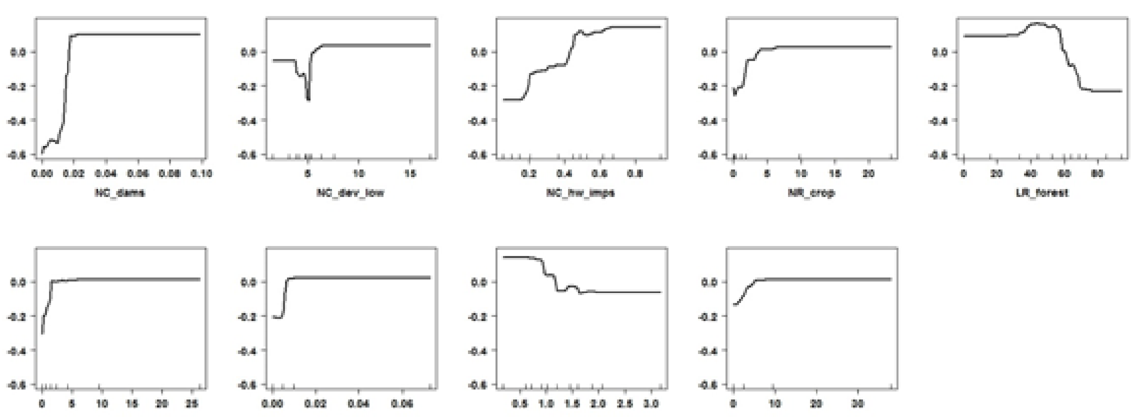
Invertebrate Hilsenhoff Biotic Index (HBI) – Central Plains Small Rivers

-- No Influential Predictors --

Invertebrate Hilsenhoff Biotic Index (HBI) – Ozark Highlands Creeks



Invertebrate Hilsenhoff Biotic Index (HBI) – Ozark Highlands Small Rivers



Appendix 5. Field reconnaissance form for screening and recommending candidate headwater reference reaches.

Missouri Candidate Reference Streams Project -Field Reconnaissance Form

Date:____________________________ Investigator:______________________ County:______________

AES Type:________________________ Stream Reach Code:____

EDU: ____________________________ UTM Coordinates:________________(E)_________________(N)

Directions to Site:____

Land Owner Name, Address, Phone:____

Water Permanence (circling either of the first two bullet points eliminates the candidate)

- No Water
- Potential Losing Stream
- Evidence of Spring Influence

Instream Reach (circling any one bullet point eliminates the candidate)

- Cattle
- Graveling
- Low Water Bridges
- Impoundments
- Extensive Bedrock
- Fish Barriers
- Channel Alterations
- Discharge Pipe

Bank and Riparian

Bank stability - (Score each bank) Left Bank Right Bank	Bank stable; evidence of erosion or bank failure absent or minimal; little potential for future problems; <5% of bank affected. 10-9 ________ 10-9 ________	Moderately stable; infrequent, small areas of erosion, mostly healed over; 5-29% of bank in reach has areas of erosion. 8-6 ________ 8-6 ________	Moderate unstable; 30-59% of bank in reach has areas of erosion; high erosion potential during floods. 5-3 ________ 5-3 ________	Unstable; many eroded areas; "Raw" areas frequent along straight sections and bends; obvious bank sloughing; 60- 100% of bank has erosion scars. 2-0 ________ 2-0 ________
Riparian vegetative zone width (Score each bank) Left Bank Right Bank	Width of riparian zones > 18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone. 10-9 ________ 10-9 ________	Width of riparian zones 17-12 meters; human activities have impacted zone minimally. 8-6 ________ 8-6 ________	Width of riparian zones 11-6 meters; human activities have impacted zone a great deal. 5-3 ________ 5-3 ________	Width of riparian zones <6 meters; little or no riparian vegetation due to human activities. 2-0 ________ 2-0 ________

Appendix 5. *Field reconnaissance form continued.*

Vegetative protection– (Score each bank) Left Bank Right Bank	More than 90% of the stream bank surfaces and immediate riparian zone covered by native vegetation, including trees, understory, or herbaceous growth; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally. 10-9 ________ 10-9 ________	90-70% of the stream bank surface covered by native vegetation; but one class of plants is not well represented; disruption evident but not affecting full plant growth potential To any great extent; more than one-half of the potential plant stubble height remaining. 8-6 ________ 8-6 ________	69-50% of the stream bank surface covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining. 5-3 ________ 5-3 ________	Less than 50% of the stream bank surface covered by vegetation; disruption of stream bank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height. 2-0 ________ 2-0 ________

Flood Plain and Watershed Issues

- CAFO
- Land Use
- Roads
- Upstream or Downstream Impoundments
- Downstream Fish Barriers

Overall Ranking for EDU: 1 2 3 4 5 6 7 8 9

Notes:____

____

____

____

____

Recommendation:____

____

____

Photo Log (Description & Number):____

____