PRAIRIE STREAM ECOLOGY: TEMPORAL AND SPATIAL
PATTERNS OF MACROINVERTEBRATES

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" 'Have you also learned that secret from the river; that there is no such thing as time?' That the river is everywhere at the same time, at the source and at the mouth, at the waterfall, at the ferry, at the current, in the ocean and in the mountains, everywhere and that the present only exists for it, not the shadow of the past nor the shadow of the future. "

– Hermann Hesse, Siddharta

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ABSTRACT

Tallgrass prairie streams and their fauna are understudied, due in part to their rarity. The composition of prairie stream communities differs geographically due to regionally and locally determined environmental characteristics and varies over time due to disturbance events. This research focused on expanding the current knowledge of temporal and spatial variation in the macroinvertebrate communities of Missouri prairie streams.

Intra-seasonal variation in the macroinvertebrate community of one prairie stream was correlated with changes in the stream environment and resulted in three relatively short-lived but significant community groupings. These intra-seasonal community shifts also affected functional feeding group structure and led to significant differences in biomonitoring measures over the course of weeks. For situations in which small, dynamic stream systems need to be monitored, sampling should be conducted at frequent intervals to capture changes in communities that may occur within seasons.

The regional diversity and community structure of prairie headwater stream communities is in part driven by both the intermittent nature and the relative scarcity of prairie stream habitat. The macroinvertebrate communities of the headwater networks from five prairies in Missouri were unique. Differences in their taxonomic composition led to differences in biomonitoring metric values. The distinct community compositions of the headwater prairie streams studied and the high regional diversity documented are likely driven by geographic isolation rather than local environmental factors. Each of these prairie stream systems is an important contributor to species diversity at a regional

level. Conservation and restoration efforts must work to preserve the biotic diversity in prairie streams to support existing macroinvertebrate communities that remain.

For lotic insects to maintain stable populations across fragmented prairie patches and their intermittent streams in Missouri, dispersal must occur between patches to enable recolonization of streams after disturbance. *Leptophlebia konza* Burian was thought to be restricted to its type locality of Konza Prairie, Kansas; however, a population was discovered at Hi Lonesome Prairie in Missouri during an extensive search of 54 prairies. DNA sequence data from cytochrome oxidase subunit 1, CO1, show that both populations of *L. konza* lost genetic diversity during a recent bottleneck, either because of habitat fragmentation or competition from other species, including *Leptophlebia johnsoni* McDunnough. The rarity of *L. konza* populations across Missouri when taken into consideration with DNA sequence data suggest that *L. konza* populations are unstable and unable to disperse between remaining prairie patches.

Water quality monitoring and research activities taking place in prairie streams must take into account spatial and temporal variation in both community structure and genetic diversity across these landscapes.

CHAPTER 1

INTRODUCTION

Before the introduction of agriculture, 162 million hectares of grassland covered the heartland of the United States extending from Canada to Mexico and from Indiana to the foothills of the Rocky Mountains (Samson and Knopf 1994). The tallgrass prairie, the easternmost portion of the Great Plains, extended through the Upper Midwest between the eastern deciduous forest and the central mixed grass prairie, encompassing an area that included most of Iowa and significant portions of Illinois, Kansas, Minnesota, Missouri, Nebraska, North Dakota, and South Dakota (Smith et al. 2010).

Tallgrass prairies are unique ecosystems characterized by a wide variety of highly drought-tolerant grasses, sedges, and forbs; a healthy prairie may harbor nearly 300 species of native plants (Smith et al. 2010), 330 species of birds (Samson and Knopf 1994), and many species of insects (Whiles and Charlton 2006). Many of these prairie species are of conservation concern; large, charismatic species, such as the plains wolf and plains grizzly bear, have already succumbed to extinction (Samson and Knopf 1994). Undoubtedly, many smaller species have become extinct as well.

Prairie streams support an important portion of the biodiversity present in prairie systems (Dodds et al. 2004). Headwater prairie streams provide aquatic macroinvertebrate populations with refuges from downstream predators and competitors; with spawning, nursery, and feeding grounds; and with colonists to

downstream areas (Meyer et al. 2007). The insectivore fauna of the terrestrial prairie also relies on headwater streams to provide important food resources through the export of adult insects into the surrounding areas (Meyer et al. 2007).

Since the introduction of agriculture, the tallgrass prairie has lost more area than has any other ecosystem in North America, declining to approximately 97 percent of its original land cover (Smith et al. 2010). In Missouri alone, tallgrass prairie covered 6.5 million ha but now coverage has declined 99.5 percent to an estimated 30,000 ha of remnant prairie (Samson and Knopf 1994). Thus, this once pervasive, widespread ecosystem and its streams are now endangered. Once damaged, restoration of prairies can require decades because of the multitude of biotic interactions present in a healthy ecosystem (Schramm 1990).

Most areas of remaining tall grass prairie exist as small acreage remnants (Toney 1982, U.S. Fish and Wildlife Service 2004) that do not comprise entire watersheds; thus, prairie streams are more threatened than is the tall grass prairie itself (Dodds et al. 2004). What once were prairie watersheds are now mostly agricultural or urban land with fragmented prairie remnants and their streams scattered throughout.

The condition of the remaining prairie streams is threatened by widespread pollution, hydrologic disturbance, and physical modification (Dodds et al. 2004). Agriculture, logging, urbanization, and mining practices from the surrounding watersheds introduce pollution into prairie streams (Dodds et al. 2004). Agricultural irrigation practices remove water from the prairie streams, thus reducing the habitat quality for a variety of stream organisms. Streams running

through cropland often are channelized, leading to increased erosion rates and subsequent inputs of nutrients and sediment than would naturally occur (Dodds et al. 2004). These anthropogenically modified waterways are no longer able to support the biodiversity they once did.

Most of the research published on prairie stream systems has come from the Konza Prairie Biological Station (Dodds et al. 2004, Larson et al. 2013) on the western edge of the historic range of tallgrass prairie in east-central Kansas. Konza provides a unique opportunity for researchers because it is one of the largest areas of tallgrass prairie preserved in the United States; thus, it is one of the only prairies that encompasses complete watersheds (Dodds et al. 2004, Larson et al. 2013). Much less research has been conducted on tallgrass prairies in other areas of the historic range. One study compared the structure and function of headwater streams in a tallgrass prairie preserve in Missouri with a Konza Prairie stream and found that they were comparable in physiochemical characters but differed in macroinvertebrate abundance and taxonomic composition (Larson et al. 2013).

Knowledge of the adaptive fauna and the hydrologic dynamics of prairie streams will become increasingly important with global climate change. Global climate change is likely to result in increased fluctuation in the amount of precipitation and flow in many stream systems (Dodds et al. 2004). Prairie headwater streams are typically intermittent in flow due to the low levels of run-off that are characteristic of grasslands, and are particularly susceptible to flash flooding (Dodds et al. 2004). Thus, prairie streams represent an excellent model system in which to study hydrologic variability (Dodds et al. 2004). Additionally, as

precipitation patterns continue to change, it will be increasingly important to understand the biology of the fauna that is adapted to intermittent habitats, like that of prairie streams, so monitoring and management strategies can be appropriately modified for the increased hydrologic fluctuation predicted to occur in other stream systems.

Without an understanding of the community structure of the fauna inhabiting prairie headwater streams, it is impossible to gauge the current health of these stream environments for application in prairie stream conservation and management decision-making and for use as a potential model system.

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CHAPTER 2

LITERATURE REVIEW

Little is known about the aquatic biodiversity of prairies, due in part to the relative scarcity of the prairie ecosystem. Prairies were once the most widespread ecosystem in North America (Samson and Knopf 1994), but now have declined to approximately three percent of their original land cover (Smith et al. 2010). In Missouri, coverage has declined more than 99 percent (Samson and Knopf 1994). Since healthy prairie streams rely on healthy prairie watersheds, this range loss means that prairie streams and the organisms that inhabit them are even more threatened than are the prairies themselves.

Historically, prairie watersheds were composed of large networks of low order streams (Dodds et al. 2004) in which in-stream habitat varied longitudinally. Most typical prairie streams have open reaches in headwaters (Smith 1986, Harris et al. 1999, Vandermyde and Whiles 2015) that receive a great amount of sunlight and little terrestrial input of organic material, which is mostly in the form of grass litter. Extensive algal growth in these open-canopied reaches provides the basal resource for this reach of the stream. Farther downstream, trees and large shrubs become more common, organic matter input in the form of leaves and woody detritus increases, and less light reaches the streambed. Therefore, the primary basal resource in these areas is leaves and woody debris, known as coarse particulate organic matter (CPOM) and is terrestrial in origin. In these reaches, the decomposition of CPOM, specifically leaf litter, plays important roles as a source of nutrition (Hax and Golladay 1993) and as shelter (Smith 1986) for

macroinvertebrates. Overall, riparian vegetation influences the composition of the macroinvertebrate community due to a longitudinal pattern of organic matter and light input into the stream, which influences the basal resources available to primary consumers.

Regionally, prairie streams vary widely in riparian vegetation and substrate composition, both of which influence in-stream habitat and the macroinvertebrate community. The riparian characteristics of several prairie regions across South Dakota varied widely when compared by width and vegetation composition (Rasmussen et al. 2008). Specifically, the banks of prairie streams in one region were characterized by the complete absence of trees, while those of a nearby region were characterized by shrubs and trees as are forested streams (Rasmussen et al. 2008). The basal resources of these streams differ and likely affect the identity of primary consumers, as can the composition of the streambed. Streams in the northern Great Plains are marked by more consistent flow and cobble substrates, whereas streams in the southern Great Plains have more irregular flow and finer substrates (Harris et al. 1999). Since in-stream substrate composition affects macroinvertebrates abundance and identity (Merritt et al. 2008), this also can affect the composition of the macroinvertebrate community. In contrast, headwater streams in a Missouri prairie and the Konza Prairie are comparable in physical characters (Larson et al. 2013), but differ in macroinvertebrate community composition.

Healthy prairie streams support a diversity of aquatic life (Dodds et al. 2004, Whiles and Charlton 2006). Insects are the most common resident macroinvertebrates in prairie streams (Rust and Troelstrup 2006). Published faunal lists from prairie streams contain insects from the orders Coleoptera, Diptera, Ephemeroptera, Odonata, Plecoptera,

and Trichoptera as well as other invertebrates (i.e., Amphipoda, Gastropoda, Hirudinea, Isopoda, Oligochaeta, and Pelecypoda) (Bryant and Wilhm 1990, Bass 1994, Fritz and Dodds 2002, Hall et al. 2003, Phillips et al. 2008, Alamayehu 2016).

Collector-gatherers dominate the macroinvertebrate community throughout both gallery-forest and grassland reaches of prairie streams (Menzel et al. 1984, Duehr et al. 2006, Rust and Troelstrup 2006, Phillips et al. 2008, Vander Vorste et al. 2008, Larson et al. 2013, Jackson et al. 2015), sometimes composing approximately 60% of the insect community (Harris et al. 1999). Collector-gatherers consume most available food resources (Whiting et al. 2011, Stagliano and Whiles 2002) in the form of fine organic particulate matter (FPOM) resulting from the decomposition, physical abrasion, and excretion of leaf litter. Chironomidae (Diptera) is the most common collector-gatherer taxon in these systems (Harris et al. 1999, Vander Vorste et al. 2008).

In most prairie streams, other functional feeding groups are present at lower percent abundances than collector-gatherers. Predators were more abundant in one headwater prairie stream than in similar sized streams in forest and desert (Gray and Johnson 1988), and their abundances are limited by food (Huryn 1998, Stagliano and Whiles 2002, Whiting et al. 2011, Vandermyde and Whiles 2015). Shredders play an important role in breaking down CPOM and have been documented in varying abundances. One study found a nearly complete lack of shredders throughout an entire branch of a stream running through Konza Prairie (Larson et al. 2013), whereas other studies at Konza have shown a high abundance of shredders (Gray and Johnson 1988, Stagliano and Whiles 2002). High shredder abundance (~20 percent) in prairie streams have been attributed to the existence of constant sources of water, so that longer-lived

shredder taxa were able to complete their life cycle (Gray and Johnson 1988) or to the presence of large amounts of CPOM in perennial gallery-forested reaches of streams which support higher populations (Bryant and Wilhm 1990). Shredders, in general, are not resource limited (Whiting et al. 2011, Vandermyde and Whiles 2015). Scraper abundance increases with removal of riparian vegetation, which is linked to increased light levels and therefore increased primary production (Vandermyde and Whiles 2015). Scrapers dominate the invertebrate community in some prairie streams (Bryant and Wilhm 1990, Phillips et al., 2008, Vandermyde and Whiles 2015) but are rare in others (Jackson et al. 2015).

Prairie stream macroinvertebrate communities are made unique by regional and local physical characteristics of streams, but they also can be shaped by sporadic disturbances such as fire and grazing. Fire and grazing animals have been shown to be components of the disturbance cycle of the prairie, especially for terrestrial vegetation (Matthews 1988, Dodds et al. 2004), but few studies have shown that there is an effect on the macroinvertebrate communities of the streams. Studies at Konza Prairie on the effect of fire on the macroinvertebrate community have found both no effect (Larson et al. 2013) and a reduction in sensitive insect taxa (Jackson et al. 2015). Bison increase stream bank erosion which adds fine sediment to the water column, in turn providing more resources for taxa that feed on fine particulate organic matter (Fritz et al. 1999). However, riparian fencing can prevent changes to stream communities under grazing pressure, according to a study at Osage Prairie (Jackson et al. 2015).

The most important disturbances influencing the community composition of aquatic macroinvertebrates are hydrologic (Fritz and Dodds 2002); specifically in prairie

streams, the hydrologic disturbances are periodic flooding and seasonal drying (Jewett 1927, Matthews 1988). For the prairie stream fauna in general, communities recover better after flooding than drying (Fritz and Dodds 2004), and rapidly return to pre-disturbance density and biomass (Bertrand et al. 2013). Several factors influence the ability of communities to recover better after floods. Large substrates can act as flow refuges, aiding resistance to floods (Hax and Golladay 1998). Available colonists and growth rates also play a role; recovery is slower after fall floods than spring floods due to the reduced availability of colonists and lower temperatures (Miller and Golladay 1996). In contrast to flood response, only specialized drought adapted macroinvertebrates exhibit resistance to droughts (Miller and Golladay 1996), and resilience to droughts is by far the most important factor for post-disturbance recovery and assemblage structuring (Fritz and Dodds 2004). Drying can determine the distribution of stream invertebrates in intermittent streams because riffle-inhabiting taxa are pushed into pools as the streams become dry (Miller and Golladay 1996).

Prairie streams are increasingly affected by human activities, especially agriculture; thus, they no longer support the same communities as do healthy prairie streams. Irrigation practices remove water from these streams, thereby reducing habitat quality for a variety of stream organisms by reducing the wetted area of the stream (Dodds et al. 2004). In addition to water loss, when streams run through cropland they are often channelized, which leads to increased erosion and a much higher input of nutrients and sediment than would naturally occur in a grassland stream (Dodds et al. 2004). These impacted waterways are no longer able to provide the same level of support for the diversity and ecosystem function they did previously.

Since the Clean Water Act of 1972, stream health has been assessed through biological monitoring using aquatic macroinvertebrates, now primarily through rapid assessment techniques (Barbour et al. 1999, Sarver et al. 2002). Aquatic invertebrates are sampled and identified, and various community attributes such as abundance, species richness, evenness and dominance are used to calculate numerical values that are treated as indicators of water or habitat quality. Biomonitoring activities utilize the results of these measures, either taken individually or integrated with other indicators into an overall score or index, as the basis for assigning a rating that reflects relative quality, biotic condition or stream health (Barbour et al. 1999).

Two common metrics used to compare the health of streams are the Ephemeroptera, Plecoptera, and Trichoptera richness index (EPT) (Lenat 1988) and the Biotic Index (BI) (Hilsenhoff 1977). Higher EPT values indicate healthier streams because EPT taxa are sensitive to environmental perturbation and pollution. The Biotic Index is calculated based on the pollution tolerance values and relative abundances of all macroinvertebrates in the sample. In Missouri, additional macroinvertebrate metrics that are calculated include overall richness and Shannon diversity (Sarver et al. 2002). Shannon diversity values consider the number of unique taxa present in a community but also the evenness, or proportionality of representation, of those taxa (Sarver et al. 2002). More diverse and presumably healthy communities have many species that are evenly represented and are not dominated by one or a few species in particular. Values for these community-level metrics can also be used to make spatial and temporal comparisons between sites, streams or entire watersheds.

Many of these community measures have been shown to vary among prairie streams. Both relatively high and low levels of EPT taxa have been documented from prairie streams (Harris et al. 1999, Rust and Troelstrup 2006, Alemayehu 2016). Low levels of EPT taxa can be attributed to past system pollution (Harris et al. 1999). Levels of EPT taxa can be similar in both small prairie streams and large, non-wadeable prairie streams (Rust and Troelstrup 2006). The overall diversity measures of prairie streams have been shown to vary from low (Bryant and Wilhm 1990, Harris et al. 1999, Alemayehu 2016) to high (Gray and Johnson 1988, Bass 1994, Vander Vorste et al. 2008). The differences in metrics between sampled streams can be due to environmental pollution (Wilhm and Dorris 1966, Bryant and Wilhm 1990, Phillips et al. 2008, Alemayehu 2016), physical stream characteristics (Bass 1994, Vander Vorste et al. 2008, Vandermyde and Whiles 2015) or to disturbance regime (Wilhm and Dorris 1966, Bass 1994, Fritz and Dodds 2002, Hall et al. 2003, Lunde et al. 2013).

Prairie stream invertebrate communities vary regionally and locally when compared by community metrics as well as functional group composition; however, much published research on prairie streams is from a single prairie at the Konza Prairie Biological Station (Smith 1986; Tate and Gurtz 1986; Gray and Johnson 1988; Dodds et al. 1996, 2004; Miller and Golladay 1996; Fritz et al. 1999; Fritz and Dodds 2002, 2004; Stagliano and Whiles 2002; Whiting et al. 2011; Riley and Dodds 2012; Betrand et al. 2013; Larson et al. 2013; Vandermyde and Whiles 2015), a long term ecological research facility in east-central Kansas. Some research has been conducted on prairies spanning other areas of the historic range including Iowa (Duehr et al. 2006), Minnesota (Harris et al. 1999), Missouri (Hoel 1998, Hall et al. 2003, Meade 2004, Larson et al. 2013, Jackson

et al. 2015), Nebraska (Harris et al. 1999), Oklahoma (Bryant and Wilhm 1990, Bass 1994, Alemayehu 2016), South Dakota (Rust and Troelstrup 2006, Rasmussen et al. 2008, Vander Vorste et al. 2008) and Texas (Hax and Golladay 1998).

In Missouri, prairie stream invertebrate research has focused on the responses of communities to disturbances. After a chemical spill in Muddy Creek, a stream in central Missouri, the invertebrate community had not stabilized after 50 weeks (Meade 2004). In a comparison of a regulated prairie river with an unregulated river, community composition differed along with invertebrate biomass and density (Hoel 1998). Adjacent land use affected stream community composition in the prairie region (Hall et al. 2003). Patch burn grazing affected the physical and chemical characteristics of Osage Prairie streams, which shifted invertebrate communities (Jackson et al. 2015). Although Missouri prairie stream research has focused on community responses to chemical or physical disturbances, our knowledge of the natural communities of Missouri is lacking.

Since prairie stream communities vary widely over space due to regionally and locally determined physical characteristics, as well as over time due to disturbance events, my dissertation research focuses on expanding the current knowledge of temporal and spatial variation of the macroinvertebrate communities of Missouri prairie streams. Specifically, I explore 1) the intra-seasonal variation in the macroinvertebrate community of one prairie stream, 2) the spatial variation in the macroinvertebrate community among several prairie streams, and 3) the genetic variation in a rare prairie stream insect.

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CHAPTER 3

COMPOSITIONAL DYNAMICS OF THE MACROINVERTEBRATE COMMUNITY IN A MISSOURI PRAIRIE STREAM

Abstract

Intra-seasonal shifts in community structure can affect biomonitoring metrics. Prairie streams likely undergo intra-seasonal shifts in community structure because they fluctuate widely in hydrologic condition annually as well as seasonally. Intra-seasonal variation in the macroinvertebrate community of one prairie stream was correlated with changes in the stream environment and resulted in three relatively short-lived but significant community groupings. These shifts in community structure led to significant differences in total taxa richness and EPT richness over the course of weeks. For situations in which small, dynamic stream systems need to be monitored, sampling should be conducted at frequent intervals to capture changes in communities that may occur within seasons.

Introduction

The distribution of lotic aquatic invertebrates is determined by the complex interaction between a species life history and its environment. Species distributions vary on a season to season basis due to species-specific life history traits as well as environmental conditions such as water temperature, water chemistry, photoperiod, food availability, and biotic interactions (Hynes 1970, Sweeney 1984, Thorp and Covich 2001). Variation in any of these conditions can affect the distribution of individual

invertebrate species and at a larger scale the entire community of invertebrates present during any season.

Seasonal shifts in the invertebrate communities affect the results of biomonitoring measures used to establish water quality ratings. Biological assessment, or bioassessment, protocols are often adapted to account for seasonal variation in aquatic communities. Values for diversity (Robinson and Minshall 1986), richness (Linke et al. 1999, Johnson et al. 2012), and biotic indices (Linke et al. 1999) are known to change on a seasonal basis (Barbour et al. 1999). Whereas functional feeding groups (Cummins 1974) are not used to establish water quality ratings directly, they are often used as a component in some measures of stream quality and to gain additional insight into the structure of aquatic communities (Stagliano and Whiles 2002, Whiting et al. 2011, Larson et al. 2013, Jackson et al. 2015) and are also known to shift according to season (Larson et al. 2013).

When values of individual biomonitoring metrics are integrated to provide an overall score or rating that is used to compare relative biotic condition or impairment among and between sites, they are referred to as multi-metric indices. These indices are also known to differ seasonally (Johnson et al. 201). The Missouri stream condition index (MSCI) is calculated using a combination of four metrics and their observed values in relation to that of reference streams: total richness, EPT (Ephemeroptera, Plecoptera, and Trichoptera) richness, Shannon Weiner diversity, and Biotic Index (Sarver et al. 2002). Changes in any of the four metrics that compose the MSCI could potentially change the impairment rating of a stream and affect management decisions. Because of seasonal variation in stream communities, many water quality sampling protocols at state

and federal levels suggest seasonal or biannual sampling of invertebrate communities to account for this variation during biological monitoring activities (Barbour et al. 1999, Sarver et al. 2002).

Less well understood is variability in stream communities that exists within a season, on the scale of days or weeks, and its potential effect on the results of these macroinvertebrate bioassessments. During the wet season in monsoonal streams, variation in invertebrate community composition exists on the scale of days due to the occurrence of spates (Leung and Dudgeon 2011, Leung et al. 2012). After the spring rewetting of an intermittent prairie stream, invertebrate structure shifted over the course of weeks (Fritz and Dodds 2002). Headwater and intermittent stream systems are likely especially prone to shifts in community structure because of their increased hydrologic variability (Feminella 1996, Williams 1996, Fritz and Dodds 2004, Wood et al. 2005, Lunde et al 2013).

Prairie streams can serve as model high disturbance systems in which intra-seasonal shifts in community structure can be investigated. Intermittent streams are characteristic of prairies because of the low level of runoff associated with grasses that thrive in the hot, dry environment of prairies (Dodds et al. 2004) and prairie streams in general have high flood frequency and low predictability (Dodds et al. 2004), leading to wide variation in hydrologic conditions over the course of a season.

This study aims to increase the knowledge of intra-seasonal changes in macroinvertebrate community structure by examining the macroinvertebrate community of an intermittent prairie stream. Specific objectives are to:

- 1) Identify intra-seasonal variation in macroinvertebrate community composition and link variation to environmental changes
- 2) Determine how values of commonly-used biomonitoring indicators change over the course of the season
- 3) Examine how functional feeding group composition changes over the course of the season

Materials and Methods

Study Site

Hi Lonesome Prairie is a 627 acre native prairie remnant located in Benton County approximately 1.1 miles northwest of Cole Camp, Missouri. It is one of the largest remaining native tracts of prairie in Missouri and lies on the western edge of the historic extension of the tallgrass prairie into Missouri in the Western Missouri Prairie Region near the transition to deciduous forest (Toney 1982, CARES 2009). Gravelly silt loam derived from cherty limestone covers the rolling hills of the prairie (CARES 2009). Land cover around Hi Lonesome Prairie consists mainly of pasture and cropland at an elevation of 1000–1100 ft. above sea level (CARES 2009). A headwater tributary of Cole Camp Creek flows through Hi Lonesome Prairie and was the study site for this project.

Sampling methods

Macroinvertebrate samples were collected approximately weekly from March 7 to May 15, 2016 on 11 sampling dates. In total, 40 potential sampling sites were identified along 750 meters of the stream. Each week, samples were collected from three sites randomly chosen from among the 40 potential sites, which averaged 268 meters apart. At each site, two random samples were taken, one each from riffle and marginal vegetation

(Fig. 1), for a total of 6 samples per sample date. In early season (i.e., March), marginal vegetation was absent; thus, these samples were taken from depositional areas at the edge of the stream where marginal vegetation was likely to grow. Samples were collected using a stovepipe sampler with an area of 792 cm² (Fig. 1). After the sampler was rapidly pushed into the substrate, the substrate within the perimeter of the sampler was disturbed for a period of two minutes and organisms and particulate matter in the water column were removed using an aquarium style fish net. The contents of the net were rinsed into a plastic storage container, preserved with 80% ethanol, labeled, and brought to the laboratory. Large stream bed material within the sampler, including cobble and woody debris, was examined for any clinging invertebrates, which were added to the samples.

At each site the following measures were taken: dissolved oxygen (mg L⁻¹), pH, water temperature (°C), turbidity (NTU), canopy cover (%), velocity (m/s), stream depth (m), width (m), and sample distance from source (m). For each sample, substrate composition was estimated visually using a modification of the Wentworth scale that includes the percentage of substrate in each of seven groups: cobble, gravel, sand, silt, silt, bedrock, and leaf pack and woody debris (Ohio EPA 2012). The number of substrate types present in each sample was used as a measure of habitat heterogeneity. Substrate types were scored and totaled as in the Ohio EPA (2012) protocol for headwater streams. Visual estimations of the percentage cover of macrophytes, periphyton, filamentous algae, organic matter, and embeddedness were recorded for each sample. Additionally, each sample was scored for an overall algal coverage index (Feminella and Hawkins 2000). Precipitation data were taken from the NOAA weather station at Cole Camp and were used to calculate four-day, weekly, and two-week rainfall totals for each collection

date. GPS coordinates were used to determine the distance of each site from the stream confluence.

In the laboratory, the samples were sorted and specimens identified to the lowest possible taxonomic level. Samples were processed by fixed count subsampling until a minimum of 200 organisms were picked (Barbour and Gerritsen 1996, Hughes et al. 2009). All organisms from one randomly selected subsample were removed. If the minimum count was not reached, another random subsample was taken and all organisms were counted from that subsample. Counts for each subsample were extrapolated to achieve estimates of whole sample relative abundance.

Statistical Analyses

Ordination is a statistical technique that is commonly used to visualize patterns of similarity and difference in community datasets by creating coordinate frames in which samples are represented as points within the frame. Similar samples will cluster together in the coordinate frame and dissimilar communities will be located farther apart.

In this case, ordination, specifically non-metric multidimensional scaling (NMS), was used to identify if temporal patterns exist within the macroinvertebrate community over the course of the spring season by looking at clustering of samples within the coordinate frame. Since each sample represents the macroinvertebrate community present during a given week, samples located closer together in the ordination space represent similar communities over time.

All multivariate analyses were performed in PC-ORD v. 6.2 (McCune and Mefford 2011). For each habitat (riffle and marginal vegetation), two matrices were constructed using Sørensen dissimilarity values: a matrix of species abundance data and a

matrix of environmental variables. Species that appeared only once in the dataset were removed because they may be adventitious, and they do not contribute to the detection of patterns. Species abundance data were relativized by maximum abundance for each species to reduce the influence of highly abundant species. This relativization transforms every abundance value for a particular species to a proportion of that species maximum abundance. Additionally, a third matrix was created by combining data from both habitats.

For each set of matrices, dimensionality of the coordinate frame was determined through three NMS runs on autopilot settings. The number of dimensions chosen for the final autopilot run was based on the stress value and the significance of a randomization test (McCune et al. 2002). After determination of dimensionality, final NMS plots were calculated based on 1000 runs and a Monte Carlo test was based on 250 runs of randomized data. The final NMS plots were then examined to identify any clustering patterns that emerged, if so, whether those clusters related to the time of season. Time-related clusters represent the different macroinvertebrate community groups present in this stream over the spring season.

Multi-response permutation procedure (MRPP) was used to test for significant differences between the clusters of samples apparent on each final NMS plot, with each cluster representing a seasonal grouping. MRPP first calculates an effect size, A , and then performs a randomization test to yield a p-value for overall significance. Pairwise comparisons of seasonal groupings were performed after verifying overall significance (McCune et al 2002).

After seasonal groupings were confirmed by MRPP, indicator species analysis was performed to reveal significant associations between species and seasonal groupings (McCune et al. 2002).

To determine if changes in community composition were related to any environmental variables, coefficients of determination between the ordination distances and the distances in the original dataset were calculated for each axis (McCune et al. 2002). Pearson correlation coefficients were calculated for each environmental variable and each axis.

For each sample, a variety of metrics were calculated. Four common biomonitoring metrics were used: total taxa richness, EPT richness, Shannon-Weiner diversity, and Biotic Index. Species-specific tolerance values used in the calculation of the Biotic Index were taken from SOP 209 of MDNR (2016). Macroinvertebrates were classified into five functional feeding groups (FFG) according to Merritt et al. (2008) and Viera et al. (2006): collector-gatherers, collector-filterers, predators, scrapers, and shredders. For each sample, FFG richness and relative abundance were calculated.

The impacts of seasonal grouping and habitat on these four metrics were tested using separate two-way analyses of variance (Proc Mixed, SAS 9.3., SAS Institute, Cary, NC). EPT richness was log transformed and Shannon-Weiner diversity was reflected and square root transformed to meet assumptions of normality. Functional feeding group relative abundances were logit transformed to meet assumptions of normality. Pairwise comparisons of least squared means were conducted with sequential Bonferroni adjustments.

Results

Community Composition

Over the course of this study, 80 unique taxa and 59,320 individuals were collected from the stream. Of these, 64 taxa were found in riffles and 61 in the marginal vegetation habitat (Table 1). The insect community was dominated by non-tanypodine Chironomidae, which were the most common macroinvertebrates overall in the riffles. Cyclopoid copepods were the most common macroinvertebrates in the marginal vegetation habitat.

For the riffle dataset, I interpreted a significant two-dimensional NMS solution with a final stress of 9.92 (Monte Carlo randomization test, 250 runs, $p=0.0040$) after verifying consistency of interpretation among several NMS solutions (Fig. 2). The proportion of variation explained by axes one and two was 28.9 and 55.2%, respectively, for a total of 84.0% of variation captured from the original dataset. Ordinations are shown with an overlay of convex hulls to define the ordination space of seasonal groupings (Fig. 2). Invertebrate communities separated into three distinct groupings according to time of season (Table 2, MRPP, $A=0.07$, $p<0.0001$). The early season grouping included the first four sampling dates, the mid-season grouping included the middle three sampling dates, and the final grouping included the last four sampling dates. The composition of all three groups was significantly different from each other group (Table 2).

Indicator species analysis for the riffle dataset identified the taxa that were significantly more frequent and abundant in one seasonal group than the others (Table 3). Two taxa (*Rhyacophila lobifera* Betten and *Hexatoma*) were indicators of the early

season group, one genus (*Simulium*) of the mid-season group, and one species (*Perlesta fusca* Poulton & Stewart) of the late season group.

A joint biplot of the community seasonal groupings and environmental variables revealed associations between the three riffle seasonal groups and their environment (Fig. 3). Axis one was correlated with percent filamentous algae, algal coverage index, number of substrate types, canopy cover, substrate score, percentage of gravel, percent leaf pack woody debris, and two-week precipitation amount (Table 4). Axis two was correlated with percent periphyton, percent embeddedness, and temperature (Table 4).

For the marginal vegetation dataset, no NMS solution was significant at the $p < 0.05$.

For the combined riffle and marginal vegetation dataset, I interpreted a significant three-dimensional NMS solution with a final stress of 13.19 (Monte Carlo randomization test, 250 runs, $p = 0.004$). The proportion of variation in the dataset explained by axes 1–3 was 38.5, 29.4, and 10.0%, respectively, for a total of 79.9% of variation captured from the original dataset.

The riffle and marginal vegetation samples were not significantly different in ordination space when all sampling dates were considered (MRPP, $A = 0.0068$, $p = 0.0911$; Fig. 4); however, when the three riffle seasonal groupings were considered as separate groups, the early and mid-season riffle groups are significantly different from the marginal vegetation (Fig. 5, Table 5). Late season riffles were not significantly different from marginal vegetation.

A joint biplot of the environmental variables revealed the same associations between environmental variables and seasonal groupings as were present in the riffle

dataset alone (Fig. 6). Axis one was moderately correlated with percentages of periphyton, filamentous algae, organic material, embeddedness, and temperature, algal coverage index, number of substrates, and silt (Table 6). Axis two was moderately correlated with percentage gravel and leaf pack/woody debris (Table 6).

Biomonitoring Metrics

Interaction in the effects of habitat and seasonal group identity on total richness was detected ($F=8.05$, $p<0.0008$; Fig. 7). Total richness was highest in the riffle in the early season and highest in the marginal vegetation in late season. EPT richness was low overall with an average overall value of 2.45 ± 0.20 . Habitat and seasonal group interacted to affect EPT richness ($F=3.70$, $p=0.0314$; Fig. 8). EPT richness dropped in the riffle over time and peaked in the marginal vegetation at mid-season. The Biotic Index of the marginal vegetation community was significantly lower than that of the riffle community ($F=6.95$, $p=0.0106$; Fig. 9), and was not affected by seasonal group. Shannon-Wiener diversity indices did not differ according to habitat ($F=0.43$, $p=0.5121$) or seasonal grouping ($F=0.90$, $p=0.4108$). The overall average diversity was 1.78 ± 0.05 .

Functional Feeding Groups

Functional feeding group compositions also varied over the season. Seasonal group and habitat interacted to affect the richness values of collector-gatherers ($F=3.98$, $p=0.0239$; Fig. 10) and predators ($F=5.91$, $p=0.045$; Fig. 11). Collector-gatherer richness and predator richness decreased sharply from early to mid-season in the riffle, while increasing over the season in the marginal vegetation. Collector-filterer ($F=0.03$, $p=0.8604$), scraper ($F=0.93$, $p=0.3383$), and shredder ($F=0.95$, $p=0.3341$) richness did not differ between habitats. Collector-filterer ($F=1.46$, $p=0.2393$) and shredder ($F=1.46$,

$p=0.2393$) richness did not differ among seasonal groups, although scraper richness was highest in late season ($F=10.83$, $p<0.0001$; Fig. 12). Scrapers, shredders, and collector filterers had low richness values overall.

Relative abundance of functional feeding groups varied by seasonal grouping and habitat (Fig. 13). Collector-gatherers dominated both riffle and marginal vegetation communities throughout all samples (Fig. 13). There was no overall effect of either habitat ($F=1.53$, $p=0.220$) or seasonal group ($F=0.43$, $p=0.650$) on collector-gatherer abundance, but there was a significant interaction between habitat and seasonal group ($F=3.39$, $p=0.040$). The relative abundance of collector gatherers increased over time in the riffle, while relative abundances in the marginal vegetation peaked in the mid-season group.

Predators were on average second most abundant functional feeding group. Habitat and seasonal group identity interacted to affect the relative abundance of predators ($F=3.52$, $p=0.035$). Predators averaged 22% of the community in the riffle and 13% of the community in the marginal vegetation, primarily because of the higher abundance of *Rhyacophila*, *Isoperla* and *Perlesta*. In both habitats, the lowest abundances of predators occurred during the late season when the predaceous limoniid flies, *Hexatoma*, *Erioptera*, *Pilaria* and *Limnophila* were absent from late season samples, and *Rhyacophila* and *Isoperla* were less abundant.

The relative abundances of the collector-filterers, scrapers, and shredders could not be analyzed with parametric tests because they did not meet assumptions of normality after transformation; however, several trends were evident. Collector-filterers appeared to have higher relative abundances in the marginal vegetation. Scraper and shredder

relative abundances appeared highest in the late season group as compared to early and mid season. This was likely due to the abundance of the scrapers *Physa* and *Stenelmis* and the shredders *Lirceus*, *Hyaella*, and *Synurella* in the late season community.

Discussion

Biomonitoring protocols incorporate seasonal community changes (Barbour et al. 1999, Hughes et al. 2009, Sarver et al. 2002), although changes in community composition may also occur intra-seasonally (Fritz and Dodds 2002, Leung and Dudgeon 2011, Leung et al. 2012). I found that intra-seasonal changes in the stream community were correlated with changes in the stream environment, which underscores the relationship between the invertebrate community and stream ontogeny.

Community Composition

Overall, the community of this prairie stream is similar to those documented from other prairie streams. Many species found in these communities are documented from prairie streams in other regions of the prairie range (Gray and Johnson 1988, Bass 1994, Fritz and Dodds 2002, Stagliano and Whiles 2002, Dueher et al. 2006, Jackson et al. 2015). The average diversity value of 1.78 recorded in this study was lower than the average diversity value of 2.1 from a study of Osage Prairie streams (Larson et al. 2013), but higher than the average diversity across several intermittent streams on Konza Prairie, which ranged from 0.340 to 0.845 (Fritz and Dodds 2002). Collector-gatherers were the most prevalent functional feeding group across all seasonal groups, corroborating findings documented in other studies (Stagliano and Whiles 2002, Rust and Troelstrup 2006, Larson et al. 2013, Jackson et al. 2015).

The community composition of the three seasonal groupings in this study are distinct from the successional assemblages in a prairie stream at Konza Prairie, Kansas noted by Fritz and Dodds (2002) with a few similarities. *Hexatoma* and *Siphonurus* appeared in the late season Fritz and Dodds (2002) assemblage; however, they occurred in early season in this study. *Simulium* was present in their study at 3–4 weeks and in this study at mid-season.

The early season riffle group in this study was correlated with lower temperatures and less periphyton, as well as higher amounts of leaf pack and woody debris and more substrate types. One indicator species associated with the early season riffle group, *Rhyacophila lobifera* is known to mature in early spring in the Midwest and favors small rapid clear streams (Ross 1944). Since *Rhyacophila* is an active predator that inhabits the stream bed (Ross 1944), it follows that leaf pack and woody debris would provide additional surface area within which it can search for prey.

The mid-season riffle group was correlated with a higher percentage of gravel and lower amount of leaf pack and woody debris and with increasing total precipitation values. It follows that with an increase in precipitation, lighter debris remaining from the winter season was swept away from the stream bed, leading to an increase in the amount of gravel on the stream bed. This likely created higher quality habitat for the indicator species for this group, *Simulium*, which attaches to coarse stream-bed substrates in order to feed (Crosskey 1990).

The late season group was correlated with an increase in periphyton, embeddedness, and temperature. As the spring season progressed, algae and plants had more time to become established on the stream bed, while water temperatures continued

to increase. The indicator species for this group, *Perlesta fusca*, has been collected from small streams in the plains region of Missouri, and has emergence dates from April to June (Poulton and Stewart 1991), which coincide with the collection of late instars in May during this study.

No significant pattern was found in the marginal vegetation community data, likely because in early and mid-spring the marginal vegetation was unestablished except for remnant vegetation from the previous year. When the marginal vegetation and riffle communities were analyzed together, the late season riffle group and the marginal vegetation community were not statistically different. Higher stream temperatures in late season likely decreased the quality of the riffle habitat (Hynes 1970, Sweeney 1984), led to the loss of some unique species from the riffle, and shifted community composition toward that of the marginal vegetation.

Biomonitoring Metrics

The changes in these community groups over the season were echoed by changes in two of the biomonitoring metrics. Total richness and EPT richness decreased from early season to mid-season in the riffle along with increasing water temperatures, as several predators (*Sialis*, *Chauliodes*, *Hexatoma*, *Erioptera*, *Pilaria*, and *Limophila*) were found in only the early season riffles. As the marginal vegetation became more established over time, total richness of invertebrates in the marginal vegetation increased. Richness values are known to differ among seasons in prairie streams (Larson et al. 2013) and after major hydrologic disturbances (Fritz and Dodds 2004, Bertrand et al. 2013), but have not been previously recorded to vary significantly within season in prairie streams.

In Missouri, stream condition is summarized using a multi-metric stream condition index, the MSCI (Sarver et al. 2002). MSCI is calculated based on a summation of diversity, Biotic Index, total richness, and EPT richness values, and a subsequent comparison to values from reference streams. In theory, a change in any or all these metrics could affect the MSCI. Hence, the changes I identified in total richness and EPT richness values among seasonal groupings could lead to changes in overall MSCI and could potentially affect the management of the stream.

While not used in the calculation of multi-metric indices (e.g., MSCI), functional feeding groups can provide insight into stream ecosystem functioning and are increasingly suggested for use in the monitoring of water quality along with other trait-based metrics (Doledec et al. 2006, Nichols et al. 2016). In this study, the richness and relative abundance of several function feeding group varied according to time of sampling and align with changes in environmental parameters.

These results confirm that significant changes in community composition and functional feeding group structure can occur intra-seasonally in intermittent streams. The mid-season community was present over a sampling period of only less than two weeks, from April 6 to April 18, whereas the late season community was present in the riffle for less than four weeks. These seasonal groupings may have been entirely missed with a monthly or seasonal sampling regime because they both lasted for less than a month. This rapid shift in community structure reflects the unique hydrologic characteristics of intermittent streams and their highly adapted fauna (Fritz and Dodds 2004, Meyer et al. 2007).

The intra-seasonal shifts in functional feeding group structure and community groupings in this small dynamic system led to significant differences in biomonitoring metrics over the course of weeks, not seasons. Since these metrics are used to calculate stream condition indices (Sarver et al. 2002) used by state and federal (Barbour et al. 1999) conservation agencies, differences in their values could impact the impairment ratings of streams and the resultant management decisions and strategies. Admittedly, most reference streams used to monitor overall water quality are larger, perennial systems, but in some cases, the health of smaller water systems might be required for use when larger reference streams are unavailable. In the case where smaller systems are to be monitored, sampling should be conducted at more frequent intervals to capture changes in communities that may occur over shorter time frames.

Adapting sampling regimes to account for intra-seasonal variation may be vital for the monitoring of prairie stream water quality. Whereas they were once major ecosystems in North America (Samson and Knopf 1994), now most prairies exist only in small fragments (Toney 1982, Smith et. al 2010) and, therefore, are not sufficiently expansive to harbor a watershed. Headwater and intermittent streams are generally the only stream systems that can be fully contained within these prairie fragments. In these streams, unique sampling regimes will be necessary to make appropriate resource conservation and water quality management decisions.

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Table 1. Species presence by seasonal and habitat groupings.

		Habitat			
		Riffle			Marginal Vegetation
		Early	Mid	Late	
Insecta					
Coleoptera					
Dytiscidae	<i>Agabus</i>				x
	<i>Heterosternuta</i>	x			x
Dryopidae	<i>Helichus fastigatus</i>	x			x
Elimidae	<i>Dubiraphia vittata</i>				x
	<i>Stenelmis crenata</i>	x	x	x	x
Haliplidae	<i>Peltodytes muticus</i>				x
Helophoridae	<i>Helophorus</i>		x		
Hydrophilidae	<i>Helocombus</i>			x	x
Psephenidae	<i>Ectopria nervosa</i>	x			
Ptilodactylidae	<i>Paralichus</i>				x
Odonata					
Anisoptera		x			x
Coenagrionidae	<i>Ischnura posita</i>				x
	<i>Argia bipunctulata</i>	x			
Ephemeroptera					
Baetidae				x	x
Caenidae	<i>Caenis punctata</i>	x	x	x	x
Ephemeridae	Hexagenia				x
Heptageniidae		x		x	
Leptophlebiidae	<i>Leptophlebia konza</i>	x			x
Siphonuridae	<i>Siphonurus</i>	x			x
Diptera					
Chironomidae	Tanypodinae	x	x	x	x
	Non-Tanypodinae	x	x	x	x
Ceratopogonidae	<i>Bezzia/Palpomyia</i>	x	x	x	x
	<i>Ceratopogon</i>	x	x	x	x
	<i>Probezzia</i>	x	x	x	x
	<i>Mallochohelea</i>			x	x
	<i>Serromyia</i>				x
Culicidae	<i>Culex</i>				x
Simuliidae	<i>Prosimulium</i>	x	x		
	<i>Simulium</i>	x	x	x	x
	<i>Stegopterna</i>	x	x		
Tabanidae		x	x	x	x

Tipulidae	<i>Tipula</i> spp.	x		x	x
Limoniidae	<i>Erioptera</i>	x			x
	<i>Hexatoma</i>	x			x
	<i>Limnophila</i>	x			
	<i>Pilaria</i>	x	x		x
	<i>Pseudolimnophila</i>				x
Dolichopodidae		x			
Muscidae				x	x
Sciomyzidae					x
Stratomyidae					x
Empididae	<i>Hemerodromia</i>	x			
Hemiptera					
Corixidae	<i>Trichocorixa</i>				x
Veliidae				x	
Plecoptera					
Capniidae	<i>Allocapnia</i>	x			x
Perlodidae	<i>Isoperla mohri</i>	x	x	x	x
Perlidae	<i>Perlesta fusca</i>	x	x	x	x
Megaloptera					
Corydalidae	<i>Chauliodes rastricornis</i>	x			
Sialidae	<i>Sialis</i>	x			
Lepidoptera					
		x			x
Trichoptera					
Rhyacophilidae	<i>Rhyacophila lobifera</i>	x	x		x
	<i>Rhyacophila fenestra/ledra</i>	x	x	x	
Limnephilidae	<i>Ironoquia punctatissima</i>	x	x	x	x
Polycentropidae	<i>Polycentropus</i>				
Leptoceridae	<i>Triaenodes</i>	x			x
Philopotamidae	<i>Chimarra</i>	x			
Phryganeidae	<i>Ptilostomis</i>	x	x		x
	<i>Pycnopysche</i>	x			
Non-Insecta					
Collembola					
					x
Oligochaeta					
Tubificidae		x			
Naididae		x	x	x	x
Tricladida					
		x			x
Nematoda					
		x	x	x	x
Cladocera					
				x	x
Copepoda					
Calanoid			x		

Cyclopoida		X	X	X	X
Harpacticoida		X	X	X	X
Amphipoda					
Hyaellidae	<i>Hyaella azteca</i>	X	X	X	X
Gammaridae	<i>Synurella</i>	X		X	X
Isopoda					
Asellidae	<i>Lirceus</i>	X	X	X	X
Gastropoda					
Ancylidae				X	X
Hydrobiidae				X	X
Planorbidae		X		X	X
Physidae	<i>Physa</i>			X	X
Lymnaeidae	<i>Lymnaea</i>				X
Ostracoda		X	X	X	X
Decapoda		X		X	X
Hirudinea		X	X	X	X
Bivalvia					
Sphaeriidae		X	X	X	X

Table 2. Multi-response permutation procedure for riffle seasonal groupings.

		A	<i>p</i>
Overall MRPP		0.0704	0.0001
Pairwise comparisons	Early vs. Mid	0.0618	0.0172
	Early vs. Late	0.0531	0.0046
	Mid vs. Late	0.0542	0.0192

Table 3. Indicator species analysis for riffle seasonal groups.

Taxon	Group Association	Indicator Value	<i>p</i>
<i>Rhyacophila lobifera</i>	Early	74.6	0.0302
<i>Hexatoma</i>	Early	100.0	0.0072
<i>Simulium</i>	Mid	79.0	0.0234
<i>Perlesta fusca</i>	Late	100.0	0.0070

Table 4. Pearson correlation coefficients for riffle ordination environmental variables and ordination axes.

		Axis 1		Axis 2	
Environmental Variable	Abbreviation	r	r ²	r	r ²
Macrophyte (%)	Macrophyte	-0.002	0.000	-0.317	0.100
Periphyton (%)	Periphyton	-0.273	0.075	0.859	0.738
Filamentous Algae (%)	Filamentous Algae	-0.608	0.370	0.533	0.284
Organics (%)	Organics	-0.437	0.191	0.079	0.006
Embeddedness (%)	Embeddedness	-0.230	0.053	0.715	0.512
Temperature	Temperature	0.062	0.004	0.825	0.680
Dissolved Oxygen	DO	0.098	0.010	-0.438	0.192
pH	pH	-0.445	0.198	-0.25	0.063
Depth	Depth	0.434	0.188	0.413	0.170
Width	Width	0.116	0.013	-0.138	0.019
Velocity (m/s)	Velocity	0.329	0.108	0.017	0.000
Algal Coverage Index	ACI	-0.674	0.454	0.262	0.069
Turbidity	Turbidity	-0.104	0.011	-0.544	0.296
Canopy Cover	Canopy Cover	-0.597	0.357	0.368	0.135
Number of Substrate Types	Number of Substrates	-0.691	0.477	-0.009	0.000
Substrate Score	Substrate Score	0.757	0.573	0.329	0.108
Cobble (%)	Cobble	-0.231	0.053	-0.183	0.034
Gravel (%)	Gravel	0.831	0.690	0.090	0.008
Sand (%)	Sand	-0.200	0.040	0.298	0.089
Silt	Silt	-0.495	0.245	0.328	0.108
Leaf Pack/ Woody Debris	Leaf Pack/Woody Debris	-0.598	0.358	-0.302	0.091
Distance from confluence	Distance	-0.212	0.045	0.476	0.227
Four day precipitation	4 day precipitation	0.287	0.082	-0.167	0.028
One week precipitation	1 week precipitation	0.406	0.165	-0.017	0.000
Two week precipitation	2 day precipitation	0.628	0.000	-0.023	0.001

Table 5. Multiple response permutation procedure for composite dataset ordination.

		A	<i>p</i>
Overall MRPP		0.04420	0.00009
Pairwise comparisons	Marginal Vegetation vs. Early Season Riffle	0.03333	0.00106
	Marginal Vegetation vs. Mid-Season Riffle	0.02506	0.01411
	Marginal Vegetation vs. Late Season Riffle	0.00534	0.20440

Table 6. Pearson correlation coefficients for composite dataset ordination axes and environmental variables.

	Axis 1		Axis 2		Axis 3	
Environmental Variable	r	r ²	r	r ²	r	r ²
Macrophyte (%)	0.320	0.102	0.093	0.009	0.519	0.270
Periphyton (%)	0.763	0.582	-0.144	0.021	-0.281	0.079
Filamentous Algae (%)	0.745	0.555	0.204	0.042	-0.321	0.103
Organics (%)	0.509	0.259	0.312	0.097	0.150	0.022
Embeddedness (%)	0.725	0.526	-0.016	0.000	-0.206	0.042
Temperature	0.619	0.383	-0.450	0.203	-0.261	0.068
Dissolved Oxygen	-0.413	0.170	0.271	0.073	-0.016	0.000
pH	0.091	0.008	0.382	0.146	-0.424	0.180
Depth	0.292	0.085	-0.418	0.175	-0.098	0.010
Width	0.055	0.003	0.035	0.001	-0.062	0.004
Velocity (m/s)	-0.276	0.076	-0.273	0.074	-0.185	0.034
Algal Coverage Index	0.657	0.431	0.445	0.198	-0.258	0.067
Turbidity	-0.428	0.183	0.277	0.077	-0.171	0.029
Canopy Cover	0.156	0.024	0.180	0.032	-0.287	0.083
Number of Substrate Types	0.569	0.323	0.142	0.020	0.073	0.005
Substrate Score	-0.457	0.209	-0.278	0.077	-0.447	0.199
Cobble (%)	-0.163	0.027	0.286	0.082	-0.345	0.119
Gravel (%)	-0.437	0.191	-0.670	0.449	-0.190	0.036
Sand (%)	0.256	0.066	-0.131	0.017	0.106	0.011
Silt	0.598	0.358	0.301	0.091	0.265	0.070
Leaf Pack/ Woody Debris	0.252	0.064	0.565	0.319	0.190	0.036
Distance from confluence	0.362	0.131	-0.053	0.003	0.076	0.006
Four day precipitation	-0.191	0.036	-0.204	0.042	0.311	0.097
One week precipitation	-0.129	0.017	-0.325	0.106	0.292	0.085
Two week precipitation	-0.158	0.025	-0.537	0.288	0.346	0.120

Figure 1. (A) Modified stovepipe sampler used for sampling benthic invertebrates. (B) Habitats sampled for benthic macroinvertebrates. Marginal vegetation circled in red. Riffle circled in blue.

Figure 2. Non-metric multidimensional scaling plot for invertebrate communities of riffle habitat ($p=0.0040$).

Figure 3. Non-metric multidimensional scaling plot for riffle invertebrate communities with joint biplot of environmental variables overlaid ($p=0.0040$). See Table 4 for abbreviations.

Figure 4. Three-dimensional non-metric multidimensional scaling plot of combined riffle and marginal vegetation invertebrate communities ($p=0.0040$). (A) axis 1 versus 2, (B) axis 1 versus 3.

Figure 5. Three-dimensional non-metric multidimensional scaling plot of riffle and marginal vegetation invertebrate communities with riffle seasonal groupings outlined ($p=0.0040$). (A) axis 1 versus 2, (B) axis 1 versus 3.

Figure 6. Non-metric multidimensional scaling plot of riffle and marginal vegetation invertebrate communities with joint biplot of environmental variables. Only two dimensions shown ($p=0.0040$). See Table 4 for abbreviations.

Figure 7. Interactive effect of habitat and seasonal group on macroinvertebrate total richness. LS means ± 1 SEM shown.

Figure 8. Interactive effect of habitat and seasonal group on EPT richness. LS means ± 1 SEM shown.

Figure 9. Effect of habitat on Missouri Biotic Index. LS means ± 1 SEM. Different letters represent significant differences at the $p < 0.05$ level.

Figure 10. Interactive effects of seasonal group and habitat on collector-gatherer richness. LS means ± 1 SEM shown.

Figure 11. Interactive effects of seasonal group and habitat on predator richness. LS means ± 1 SEM shown.

Figure 12. Effect of seasonal group on scaper richness. LS means ± 1 SEM. Different letters represent significant differences at the $p < 0.05$ level.

Figure 13. Invertebrate functional feeding group relative abundances by habitat and seasonal grouping.

CHAPTER 4

MACROINVERTEBRATES OF PRAIRIE HEADWATER STREAMS: SPATIAL PATTERNS OF COMMUNITY STRUCTURE

Abstract

The regional diversity and community structure of prairie headwater stream communities is driven by both the intermittent nature and the relative scarcity of prairie stream habitat. My objective was to compare the macroinvertebrate communities of the headwater networks from several disjunct Missouri prairies and to determine what factors influence the structuring of these assemblages. The macroinvertebrate communities of the headwater networks from five prairies in Missouri were unique and each prairie was associated with at least one indicator species. Differences in their taxonomic composition led to differences in diversity and richness biomonitoring metric values. Whittaker's beta diversity was 3.9. The distinct community compositions of the headwater prairie streams studied and the high regional diversity documented are likely driven by geographic isolation rather than local environmental factors. Each of these prairie stream systems is an important contributor to species diversity at a regional level. Prairie conservation and restoration efforts must work to preserve the extant faunal diversity in prairie streams to maintain support for the macroinvertebrate communities that remain.

Introduction

The study of headwater streams and the diversity of their biota is vital to the understanding of riverine systems. Headwater streams are first order streams that mark the origin of perennial water flow in a river network. These streams are abundant throughout stream networks and can contribute 70–80% of the catchment area (Santos

and Stevenson 2011) and three-quarters of channel length to a watershed (Clarke et al. 2008). Further, interactions of headwater streams with the riparian zone provide nutrients, organic matter, and sediment to higher order streams and their fauna (Gomi et al. 2002, Clarke et al. 2008). Healthy headwaters contribute to the biotic diversity of higher order streams by providing refuge for macroinvertebrates from downstream predators and competitors; spawning, nursery, and feeding grounds; and colonists to downstream reaches (Labbe and Fausch 2000, Meyer et al. 2007).

Headwater biodiversity has been understudied due in part to the river continuum concept, which emphasizes a linear approach to the study of streams and predicts that the highest levels of diversity occur in mid-order streams (Clarke et al. 2008, Finn et al. 2011). Historically overlooked, studies from headwater streams consistently yield a wide array of new, uniquely adapted species-, genus-, and family-level invertebrate descriptions (Meyer et al. 2007). Because headwater streams span broad catchment areas, they encompass a wide range of local environmental conditions and therefore stream conditions (Meyer et al. 2007, Clarke et al. 2008, Finn et al. 2011). Variation in the stream environment influences species distributions, which leads to differences in measures of taxonomic richness and species diversity among individual streams (Clarke et al. 2008). When the individual richness values of all the headwater streams in a network are considered together, headwater streams can contribute most of the taxonomic richness in a catchment because of their high beta diversity, or species turnover (Clarke et al. 2008, Finn et al. 2011). Headwater streams are important contributors to regional diversity and their loss will affect regional diversity levels.

The differences in richness, diversity, and community compositions among individual headwater streams in a region is well documented (Dieterich and Anderson 2000, Fritz and Dodds 2002, Heino 2005, Chaves et al. 2008) and can be driven by geographical factors as well as disturbance patterns. The local physical environment varies within headwater stream networks, so headwaters streams closer to each other within a drainage should harbor more similar fauna than streams farther apart (Heino et al. 2003). Significant differences in functional richness, evenness, and diversity were recorded from a wide region encompassing over one hundred headwater streams in Finland (Heino 2005). However, patterns of diversity unrelated to variation in local environmental condition, but related instead to habitat stability have also been recorded in studies of headwater stream networks (Mykra et al. 2007). For example, in a study of five alpine headwater streams, diversity and richness variation was attributed to disturbance-driven population dynamics despite their extreme spatial proximity (Chaves et al. 2008).

Stream permanence is an important driver of the regional diversity of headwater streams because it determines habitat availability and stability (Heino et al. 2003). In the study of alpine headwaters, community variability over space was related to flow intermittency (Chaves et al. 2008). Even though habitat availability fluctuates in many headwaters streams, in a study of seven intermittent streams within 12 km of one another 202 aquatic insect species were identified and two of these streams had a greater diversity of insect species than did a perennial stream in the same environment (Dieterich and Anderson 2000). Thus, intermittent streams can be important in harboring regional diversity even though stream habitat is not available continuously.

Historically, the headwater networks that covered the Midwest drained vast areas of prairie, once covering 162 million hectares (Samson and Knopf 1994). These networks were characterized by high prevalence of intermittent streams with high flood frequencies and low predictabilities (Dodds et al. 2004). In Missouri alone, tallgrass prairie covered once 6.5 million hectares; coverage has declined 99.5 percent to an estimated 30,000 ha of remnant prairie (Samson and Knopf 1994). The fragmentation of Missouri prairie habitat has increased the geographic isolation of these headwater networks from one another and, therefore, has decreased connectivity between regionally dispersing populations of stream macroinvertebrates. Stream rarity and isolation likely compounds with habitat instability caused by stream intermittency to lead to high levels of regional diversity and community differentiation in these systems.

Few studies have examined the regional diversity and community structure of prairie streams. One study compared the invertebrate communities across headwater streams at Konza Prairie and found that taxonomic composition was more correlated with disturbance patterns than with local environmental conditions (Fritz and Dodds 2002). The only Midwestern study of regional community structure outside of Konza compared the structure and function of one Missouri prairie headwater stream to a stream in Konza Prairie; the streams were comparable in physiochemical characters but differed in macroinvertebrate abundance and taxonomic composition (Larson et al. 2013b), suggesting that regional variation in these systems may be driven by factors other than the local environment.

Knowledge of regional macroinvertebrate community structure in headwater streams will be a critical component in understanding the effects of anthropogenic

disturbances to these environments and in preventing further disruption of these ecosystems. Because of the close interface between aquatic and terrestrial resources in headwater streams, these streams are increasingly threatened by a loss of species diversity from disturbance caused by anthropogenic activities (Clarke et al. 2008). Headwater streams are increasingly known to vary regionally, thereby making appropriate comparisons between reference and impacted sites difficult when performing assessments across regions (Mykra et al. 2007).

Because the regional diversity and community structure of prairie headwater stream communities is driven by the both intermittent nature and the relative scarcity of prairie streams, my objective was to compare the macroinvertebrate communities of the headwater networks from several disjunct prairies within the Osage Plains region of Missouri and to determine what factors influence the structuring of these assemblages. Specifically, my aims were to:

- 1) Compare the macroinvertebrate community structure of headwater streams of several prairies
- 2) Identify local environmental factors that are important in explaining macroinvertebrate community composition
- 3) Determine whether differences in community structure lead to differences in biomonitoring metrics values among prairies
- 4) Examine regional macroinvertebrate diversity in prairie headwater streams

Methods

Study Sites

Several tallgrass prairie stream systems in Missouri were sampled: two headwater streams at Golden Prairie and three at Osage Prairie Conservation Area, Prairie State Park, Taberville Prairie Conservation Area, and Schwartz Prairie (Fig. 1, Table 1). These streams systems are encompassed within approximately 2,300 square kilometers in southwestern Missouri (Fig. 1). These sites were chosen because they each contain a series of headwaters of the same higher order stream within the confines of the prairie. The prairies are managed by three different conservation agencies (Missouri Prairie Foundation, Missouri Department of Conservation, Missouri Department of Natural Resources).

Because this study required multiple streams in each prairie, prairies with different management strategies and schedules were used. All five prairies undergo scheduled burns, although only Golden Prairie was burned in the winter prior to sampling. Schwartz Prairie supports a small herd of cattle and Prairie State Park is home to a herd of bison.

Samples were collected from March 5–7, 2015 from Golden, Osage, Prairie State, and Taberville prairies. Due to a rain event on March 8, Schwartz Prairie was sampled on March 15.

Sampling methods

Macroinvertebrate samples were collected from riffle and marginal vegetation at three random sites in each of the headwater streams. Samples were collected using a stovepipe sampler with an area of 792 cm². After the sampler was rapidly pushed into the substrate, the substrate within the perimeter of the sampler was disturbed for a period of two minutes and organisms in the water column removed using an aquarium style fish

net. The contents of the net were rinsed into a plastic storage container, preserved with 80% ethanol, labeled, and brought to the laboratory. Large particulate matter contained within the sampler was examined for any clinging invertebrates that had not been swept into the water column, which were added to the samples.

At each site the following measures were taken: dissolved oxygen (mg L^{-1}), pH, water temperature ($^{\circ}\text{C}$), canopy cover (%), velocity (m/s), average stream depth (m), and width (m). For each sample, percentages of substrate types were estimated visually using a modification of the Wentworth scale that includes seven groups: cobble, gravel, sand, silt, silt, bedrock, and leaf pack and woody debris (Ohio EPA 2012). The number of substrate types present in each sample was used as a measure of habitat diversity. Substrate types were scored and totaled as in the Ohio EPA (2012) protocol for headwater streams. Visual estimations of the percentage cover of macrophytes, periphyton, filamentous algae, organic matter, and embeddedness were recorded for each sample. Additionally, each sample was scored for an overall algal coverage index (Feminella and Hawkins 2000).

In the laboratory, the samples were sorted and specimens identified to the lowest possible taxonomic level. Samples were processed by fixed count subsampling until a minimum of 200 organisms were identified (Barbour and Gerritsen 1996, Hughes et al. 2009). All organisms from one randomly selected subsample were removed. If the minimum count was not reached, another randomly selected subsample was taken and all organisms were counted from that subsample. Counts for each subsample were extrapolated to achieve estimates of whole sample abundance. Macroinvertebrate data from riffle and marginal vegetation samples were pooled for each site.

Statistical Analyses

Non-metric multidimensional scaling (NMS) was used to assess differences in macroinvertebrate community composition across prairies and to associate the variation in community composition with differences in the environment. All multivariate analyses were performed in PC_ORD v. 6.2 (McCune and Mefford 2011). Two sets of multivariate analyses were conducted: one with the entire dataset and one with a subset including only Chironomidae. Two matrices were constructed for the NMS analyses: a matrix containing species abundance data and a matrix containing the environmental data using Sørensen measures of dissimilarity.

For each set of matrices, dimensionality was determined through three NMS runs on autopilot settings. The number of dimensions chosen for the final autopilot run was based on the stress value and the significance of a randomization test (McCune et al. 2002). After determination of dimensionality, final NMS plots were calculated based on 1000 runs, and a Monte Carlo test was based on 250 runs of randomized data. Coefficients of determination between the ordination distances and the distances in the original dataset were calculated for each axis (McCune et al. 2002). Pearson correlation coefficients were calculated for each environmental variable and each axis.

Multi-response permutation procedures (MRPP) were used to verify significant differences between the aquatic invertebrate communities. Pairwise comparisons were performed after verifying overall significance. Indicator species analysis was run using the Dufrene and Legendre method (Dufrene and Legendre 1997).

Total richness and Shannon-Wiener diversity were calculated for each sample. Macroinvertebrates were classified into five functional feeding groups (FFG) according

to Merritt et al. (2008) and Viera et al. (2006): collector-gatherers, collector-filterers, predators, scrapers, and shredders. For each sample, FFG relative abundances were calculated. The effect of prairie identity on richness and diversity metrics was tested using separate analyses of variance (Proc Mixed, SAS 9.3., SAS Institute, Cary, NC). The effect of prairie identity on FFG relative abundance was tested using analysis of variance (Proc Mixed, SAS 9.3., SAS Institute, Cary, NC) after logit transformation to meet assumptions of normality.

For calculations of species turnover (beta diversity), alpha diversity was calculated as the average number of species present at each prairie. Gamma diversity was the total number of unique species recorded across all five prairies. Whittaker's beta diversity (Whittaker 1960) was calculated as gamma diversity divided by the average of the alpha diversities of all five prairies. This multiplicative metric represents how many times more diverse a region is than its component units. In addition, beta diversity can be described in an additive fashion as gamma minus the average alpha diversities, and represents the regional increase in diversity as compared to the average of a single within-region unit (Anderson et al. 2011). Because estimates of regional richness did not exist before this study, it is also important to note that these measures of beta diversity are inherently correlated.

Because of the conservative sampling of these environments, with only three samples taken per stream, estimated species richness was calculated using the first order jackknife estimator in PC_ORD v. 6.2. This measure predicts the richness that was underestimated by the sampling regime.

Results

Community Composition

In total, 79 invertebrate taxa, including 64 insect taxa, were collected from the five prairies (Table 2). Diptera dominated the insect fauna at all prairies in terms of richness by contributing 40 taxa, with 26 of those taxa from the midge family Chironomidae. Insect community abundance was dominated by Chironomidae and Ceratopogonidae. No insect taxa other than Diptera were recorded from Golden Prairie. *Polypedilum* (Chironomidae), *Corynoneura* (Chironomidae), *Cricotopus/Orthocladius* (Chironomidae), *Bezzia/Palpomyia* (Ceratopogonidae), *Culicoides* (Ceratopogonidae), *Probezzia* (Ceratopogonidae), tabanid flies, dolichopodid flies, *Tipula* (Tipulidae), and *Hexatoma* (Limoniidae) were the only insects found at every prairie. Seventeen species of insects were unique to only one prairie. *Hyalella azteca* Saussure (Hyalellidae), an amphipod, was found only at Golden Prairie.

I interpreted a significant three-dimensional NMS solution with a final stress of 14.3 (Monte Carlo randomization test, 1000 runs, $p=0.0004$) after verifying consistency of interpretation among several NMS solutions. 79.8 percent of the variation in the original species abundance dataset was captured by the ordination with 29.1, 15.2, and 35.5 percent of the variation captured by axes 1–3, respectively. Ordinations are shown with an overlay of convex hulls to define the ordination space of each prairie community (Fig. 2). Invertebrate communities were largely separated in ordination space according to prairie (Fig. 2). The community of Golden Prairie was entirely separated in ordination space from those of the other prairies, which overlapped in the ordination space. The community of Schwartz Prairie was the most variable and occupied a large portion of the

NMS plot. Osage and Taberville Prairies were distinct from each other in ordination space.

Invertebrate community composition differed significantly according to prairie (MRPP, $A=0.1224$, $p<0.0001$; Table 3). All pairwise comparisons revealed significant differences between prairie communities with the exception of Osage Prairie and Prairie State Park (Table 3).

Distinctness of these communities was also supported by an indicator species analysis, which identified 18 taxa, including at least two taxa from each prairie, that were significant indicator species ($p<0.05$, Table 4). Of these taxa, 12 were genera of Chironomidae. Other indicator taxa were two orders of copepods, one species of amphipod, and one genus of Ceratopogonidae, Odonata, and Plecoptera. Prairie State Park, Osage, Taberville, and Schwartz prairies each had at least one genus of Chironomidae as an indicator taxon; Taberville Prairie had six genera of Chironomidae as indicator taxa (Table 4).

Environmental Factors

A joint biplot of the environmental variables revealed only minor correlations of environmental variables with axes (Fig. 3, Table 5). Axis 1 was correlated with percentage cover of filamentous algae ($r^2 = 0.355$) and the algal coverage index ($r^2=0.318$) (Table 5).

Chironomidae Communities

Because many genera of Chironomidae were indicator species and almost half of all insect taxa were Chironomidae, an additional NMS ordination was performed to determine if a Chironomidae-only dataset revealed the same patterns as in the original

dataset. I interpreted a significant three-dimensional NMS solution with a final stress of 12.3 (Monte Carlo randomization test, 1000 runs, $p=0.0120$) after verifying consistency of interpretation among several NMS solutions (Fig. 4). 74.5 percent of the variation in the Chironomidae-only dataset was captured by the ordination with 35.7, 15.6, and 23.2 percent of the variation in axes 1–3, respectively.

The Chironomidae communities were separated in ordination space in a similar fashion as the overall community ordination, with Golden Prairie distinct from the other prairies (Fig. 4). Overlap existed among the remaining communities again (Fig. 4).

This result was supported by a significant MRPP, which indicated that Chironomidae community composition differed significantly among prairies (Table 6). Pairwise comparisons revealed significant differences between all prairie chironomid communities from one another at the $p=0.05$ level (Table 6), unlike the community wide results in which Osage Prairie and Prairie State Park were not significantly different from each other.

Biomonitoring Metrics

Diversity ($F=11.15$, $p<0.0001$; Fig. 5) and richness ($F=14.44$, $p<0.0001$; Fig. 6) were both affected by prairie (Figs. 5, 6). Diversity was lowest at Golden Prairie and highest at Osage and Taberville prairies (Fig. 5). Richness was lowest at Golden Prairie and highest at Osage, Taberville and Schwartz prairies (Fig. 6).

To better understand which species might be influencing these trends in diversity, I examined relative abundances of functional feeding groups at each prairie (Fig. 7). Collector-gatherers did not differ significantly among prairies ($F=1.47$, $p=0.232$) and relative abundance averaged 67% across all prairies. The other functional feeding groups

could not be analyzed with parametric tests because none met the assumptions of normality after transformation.

Although not statistically significant, the second most abundant functional feeding groups changed according to prairie; shredders at Golden Prairie, predators at Schwartz, collector-filterers at Prairie State, and scrapers at Osage. High relative abundances of shredders were due to one amphipod, *Synurella bifurca* (Amphipoda: Gammaridae). Ostracods were at high abundance at Prairie State. *Hydrobaenus* (Chironomidae) was abundant at Osage Prairie where Dryopidae were also found. Predators were highest in relative abundance at Schwartz Prairie due to high abundances of ceratopogonids and *Natarsia* (Chironomidae) and to the presence of *Isoperla mohri* Frison (Perlodidae) and *Rhyacophila lobifera* Betten (Rhyacophilidae).

Regional diversity

Gamma diversity was determined to be 79, as the total number of unique species recorded across all prairies. Alpha diversities ranged from 11.00 to 24.78 (Table 7). Whittaker's beta diversity was 3.9, meaning that regional richness was 3.9 times the richness of any one prairie. Whittaker's beta diversity indicates that if these prairies were populated to average diversity with only unique species, nearly four prairies would be full. Additive beta diversity was 58.8, meaning that the average number of species in any one prairie was exceeded by 58.8 in the region.

Observed richness levels were within 78.0 ± 1.3 percent of expected values (Table 8), meaning that the sampling regime sufficiently captured the species richness present at each prairie.

Discussion

The distinct community compositions of the headwater prairie streams studied and the high regional diversity documented are likely driven by geographic isolation rather than local environmental factors. The macroinvertebrate communities of five headwater prairies were unique contributors to regional diversity. Many of the organisms identified in this study (Table 2) have been documented in other studies of Midwestern prairies (Gray and Johnson 1988, Bass 1994, Fritz and Dodds 2002, Stagliano and Whiles 2002, Dueher et al. 2006, Jackson et al. 2015).

Community Composition

The community of Schwartz Prairie occupied a large area of ordination space, which might be because the samples from Schwartz Prairie were collected one week later than the other samples because of a rain event. As such, stream community recovery following the disturbance might require more time, and I already documented the transient nature of prairie headwater stream communities (see Chapter 3). Spates are known to shift the communities of prairie streams by washing away non-resistant invertebrates from the streambed (Hax and Golladay 1998, Fritz and Dodds 2004), so the high variation among samples from Schwartz prairie may reflect communities at different stages of recovery depending on the local effects of rainfall on each stream. In addition, since the most important source of recovery from flooding is in the return of colonists (Miller and Golladay 1996, Fritz and Dodds 2004), the source locations of colonists could result in different recovery times for each stream based on colonist proximity.

Each prairie has several indicator species associated with it, suggesting that even the communities of Osage Prairie and Prairie State Park support slightly different sets of

species, although the communities are not statistically significantly different. When we consider rare species, in this case meaning those unique to a single prairie, all but three were found in only one sample from each respective prairie. This could mean either they were adventitious or that these species may have been rare, but not necessarily unique to individual prairies.

Environmental Factors

Few environmental variables were correlated with the ordination axes. High percentages of filamentous algae were correlated with Golden Prairie. *Hyalella azteca*, found only at Golden Prairie, is known to feed on filamentous algae (Hargrave 1970). The high abundance of filamentous algae could have resulted from the winter burn, which reduced the amount of riparian vegetation, increasing amount of light reaching the stream bed. Increased light facilitates the growth of in-stream filamentous algae, an effect which has been documented in other studies (Riley and Dodds 2012, Vandermyde and Whiles 2015).

However, the macroinvertebrate communities could be responding to water chemistry measures such as phosphorus, nitrogen, suspended solids, or conductivity, which were not among the environmental variables measured here. These factors are known to affect the distribution of some stream biota (Thorp and Rogers 2015) and could have played a role in determining the distribution of the macroinvertebrates. Concentrations of nitrogen can influence community structure, as dissolved inorganic nitrogen concentrations were correlated with macroinvertebrate community structure after a flood in a prairie stream (Bertrand et al. 2013).

Biomonitoring Metrics

Golden Prairie was distinct from the other prairies in two commonly used biomonitoring metrics: Shannon-Wiener diversity and total richness. Shannon-Wiener diversity values for all prairies, except Golden Prairie, were similar to the 2.1 recorded from Osage Prairie streams (Larson et al. 2013b). The same study from Larson et al. (2013b) recorded an average of 31 taxa from Osage Prairie, which was higher than the average of each of the prairies in this study, and almost three times higher than the average richness of Golden Prairie.

Golden Prairie may have been lowest in these metrics because of several reasons. Disturbance can lead to greater richness and diversity than undisturbed communities (Connell 1978, Death and Winterbourn 1995). Because Golden Prairie is not grazed, the stream community was less disturbed; therefore, richness and diversity remained low. This stability also was evident in the ordination plot in which Golden Prairie occupied the least space of all the prairie communities, and therefore represents the least variable community.

Since two other prairies were also free from grazing animals, their low diversity and richness values were likely due to the recent burns. Fire has been documented to affect the water chemistry of streams in various ways (Bixby et al. 2015). While not increasing the amount of black carbon in streams (Ding et al. 2013), total nitrogen and phosphorus levels decrease in prairies after fire (Larson et al. 2013a), reducing the amount of these vital nutrients available for growth of stream biota. Additionally, burning negatively affects the abundance of the EPT taxa, the most sensitive members of the prairie stream communities (Jackson et al. 2015). Because no insects other than

dipterans, which are generally one of the most tolerant groups of insects (Merritt et al. 2008), were recorded from Golden Prairie, the recent burn explains the absence of other groups of insects and, therefore, the low diversity and richness of Golden Prairie.

When functional traits are considered, prairies were broadly similar in functional structure with collector-gatherers dominating as has been documented in other prairie streams (Menzel et al. 1984, Duehr et al. 2006, Rust and Troelstrup 2006, Phillips et al. 2008, Vander Vorste et al. 2008, Larson et al. 2013b, Jackson et al. 2015), although the identity of the second most dense functional feeding group differed among prairies. As in other studies, predators, shredders, and scrapers were found at high abundances (Gray and Johnson 1988, Bryant and Wilhm 1990, Stagliano and Whiles 2002, Phillips et al. 2008, Vandermyde and Whiles 2015). The high relative abundance of shredding organisms in Golden Prairie could be attributed to the recent burn, which increased the amount of coarse organic particulate matter in streams, an association documented in other recently burned prairie streams (Bryant and Wilhm 1990).

Chironomidae Community

Chironomids composed almost half of the insect richness found in these prairie streams, and more than half of the indicator species. Many studies of stream communities do not identify Chironomidae to the genus level and the identification of Chironomidae was shown to not significantly influence the results of water quality monitoring studies in Missouri (Rabeni and Wang 2001). In these prairie headwater streams, chironomid genera represent nearly a third of the total richness across the region. Because of the highly variable nature of prairie headwater streams where the short lived, rapid life cycles of most Chironomidae are advantageous, as well as in other similarly

unpredictable environments, Chironomidae should be identified to finer taxonomic levels to accurately characterize the composition of the community. In this study, the identification of Chironomidae to the genus level led to the discovery that one rarely encountered genus, *Heterotrissocladius*, is locally abundant in prairie streams.

Regional Diversity

Each prairie community is unique as documented in the ordination and indicator species analyses, and these differences in taxonomic composition led to measurable differences in biomonitoring metrics. Alone, these results suggest region-wide variation in prairie stream communities and are further reinforced by beta diversity metrics. Whittaker's beta diversity was almost 4, suggesting that nearly four prairies could be entirely populated with unique species at the overall average level of alpha diversity of 22. Even Golden Prairie, where alpha-diversity was lowest, harbored a unique species. Additive beta diversity was almost 60, meaning that regional diversity was sixty species greater than the average diversity of a single prairie. Taken together, these measures support that each prairie is an important contributor to species diversity at a regional level, a result that has been echoed by diversity studies of other headwater stream systems (Clarke et al. 2008). Regional diversity estimates would be likely to increase with sampling during the summer, fall, and winter, as the fauna of prairie streams changes seasonally (Larson et al. 2013b) as well as within season (see Chapter 3).

Prairie headwater streams are isolated from one another due to high levels of habitat fragmentation, whereas prairies and the streams that drained them once were continuous across much of central North America. Within this continuum of prairie, stream communities are undoubtedly structured by constant cycles of extinction and

recolonization brought about by their intermittent and rapidly changing nature. However, with the ever-increasing geographic isolation of prairies, the metapopulation dynamics that once connected these prairie communities are no longer the same, and recolonization is less likely. The cycle of flooding and drying typical of prairie streams make recolonization a vital part of the prevention of local extinctions (Miller and Golladay 1996, Fritz and Dodds 2004, Bertrand et al. 2013). Dispersal capabilities of some of these taxa may be too low, either through short life cycles or poor flight ability, making recolonization increasingly unlikely. Differences in community structure among the prairie streams of this study is likely a reflection of their geographic isolation.

It is unfortunate that no historical record exists that describes the communities of “ideal” undisturbed and unmodified prairie streams. Because current prairie stream communities cannot be compared to those that existed before the introduction of agriculture, the goal must be to preserve the remaining diversity of these systems to maintain support for the macroinvertebrates and stream function that remain.

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Table 1. Locations and characteristics of streams in five Missouri prairies.

Prairie	County	Stream System	Soil type	Size (acres)	Management Strategy
Golden Prairie	Barton	Coon Creek- Sac River	Silt loam (sandstone)	320	Burn
Schwartz Prairie	St. Clair	Allen Branch- Sac River	Sandy loam (sandstone)	240	Burn, graze (cattle)
Prairie State Park	Barton	Drywood Creek	Sandy/silt loam (sandstone and shale)	3,942	Burn, graze (bison)
Osage Prairie	Vernon	Landon Branch- Osage River	Silt loam (sandstone and shale)	1,547	Burn
Taberville Prairie	St. Clair	Baker Branch- Osage River	Silt loam (sandstone and shale)	1,360	Burn

Table 2. Macroinvertebrate taxa collected in headwater streams in five Missouri prairies.

		Prairie				
		Golden	Osage	Prairie State	Taberville	Schwartz
Insecta						
Coleoptera						
Dytiscidae	<i>Agabus</i>		x		x	x
	<i>Ilybius</i>				x	
	<i>Lioporeus</i>		x			x
	<i>Neoporus undulatus</i>		x			
	<i>Hydroporus</i>					x
	<i>Neobidessus</i>				x	
Dryopidae			x			
Haliplidae	<i>Peltodytes</i>			x		x
Hydrochidae	<i>Hydrochus</i>			x		
Hydrophilidae	<i>Helocombus</i>			x		
	<i>Tropisternus lateralis</i>			x	x	
Odonata						
Libellulidae	<i>Erythemis simplicicollis</i>		x			
Cordulegastridae	<i>Cordulegaster</i>		x		x	
Ephemeroptera						
Baetidae			x	x		x
Leptophlebiidae	<i>Leptophlebia</i>			x		
Siphonuridae	<i>Siphonurus</i>		x	x		
Diptera						
Chironomidae	<i>Hydrobaenus</i>	x	x	x	x	
	<i>Polypedilum</i>	x	x	x	x	x
	<i>Cricotopus/Orthocladius</i>	x	x	x	x	x
	<i>Mesosmittia</i>	x		x	x	x
	<i>Corynoneura</i>	x	x	x	x	x
	<i>Tvetnia</i>		x	x	x	x
	<i>Diplocladius</i>		x	x	x	x
	<i>Eukiefferiella</i>				x	
	<i>Larsia</i>		x	x	x	x
	<i>Orthocladius</i>		x		x	x
	<i>Parametriocnemus</i>				x	
	<i>Tanytarsus</i>		x		x	x

	<i>Heterotrissocladius</i>		x	x	x	x
	<i>Paraphaenocladius</i>		x		x	x
	<i>Psectrocladius</i>		x	x	x	x
	<i>Stictochironomus</i>		x		x	x
	<i>Gymnomectrionemus</i>			x	x	x
	<i>Smittia</i> group		x	x	x	
	<i>Mesocricotopus</i>		x	x	x	x
	<i>Rheocricotopus</i>				x	
	<i>Thienemanniella</i>				x	x
	<i>Pseudorthocladius</i>			x		x
	<i>Limnophyes</i>		x			
	<i>Natarsia</i>				x	x
	<i>Zavreliomyia</i>		x	x	x	x
Ceratopogonidae	<i>Bezzia/Palpomyia</i>	x	x	x	x	x
	<i>Culicoides</i>	x	x	x	x	x
	<i>Probezzia</i>	x	x	x	x	x
	<i>Dasyhelea</i>		x	x		x
Simuliidae	<i>Simulium</i>		x		x	x
	<i>Stegopterna</i>		x	x	x	x
Tabanidae		x	x	x	x	x
Tipulidae	<i>Tipula</i> spp.	x	x	x	x	x
Limoniidae	<i>Hexatoma</i>	x	x	x	x	x
Dolichopodidae		x	x	x	x	x
Sciomyzidae			x			
Stratomyidae			x	x	x	x
Athericidae	<i>Atherix</i>		x	x		
Empididae			x	x		
Hemiptera						
Corixidae	<i>Trichocorixa</i>					x
Plecoptera						
Capniidae			x	x	x	x
Leuctridae	<i>Zealeuctra</i>			x		x
Perlodidae			x			x
Perlidae			x	x		
Trichoptera						
Rhyacophilidae	<i>Rhyacophila lobifera</i>					x
Limnephilidae	<i>Ironoquia punctatissima</i>		x	x	x	x
Polycentropidae	<i>Polycentropus</i>		x	x	x	
Non-Insecta						
Tubificidae		x	x	x	x	x
Naididae		x	x		x	x

Tricladida	x	x	x	x	x
Cladocera	x	x	x	x	x
Copepoda					
Cyclopoida	x	x	x	x	x
Harpacticoida	x	x	x	x	x
Amphipoda					
Hyaellidae <i>Hyaella azteca</i>	x				x
Gammaridae <i>Synurella</i>	x	x		x	
Isopoda					
Asellidae	x	x	x	x	x
Planorbidae		x			x
Physidae <i>Physa</i>	x	x	x	x	x
Ostracodae	x	x	x	x	x
Decapoda	x		x	x	x
Hirudinea	x			x	x
Bivalvia					
Sphaeriidae		x			

Table 3. Multi-response permutation procedure by prairie for all taxa prairie stream invertebrate community analysis.

		A	<i>p</i>
Overall MRPP		0.1224	0.0000
Pairwise comparisons	Golden vs. Osage	0.1637	0.0000
	Golden vs. Prairie	0.1424	0.0001
	Golden vs. Taberville	0.1986	0.0000
	Golden vs. Schwartz	0.1341	0.0001
	Osage vs. Prairie	0.0134	0.1411
	Osage vs. Taberville	0.0571	0.0006
	Osage vs. Schwartz	0.0631	0.0003
	Prairie vs. Taberville	0.0588	0.0004
	Prairie vs. Schwartz	0.0448	0.0017
	Taberville vs. Schwartz	0.0374	0.0025

Table 4. Indicator species analysis of prairie stream invertebrate communities according to prairie.

Taxon	Group Association	Indicator Value	<i>p</i>
<i>Synurella bifurca</i>	Golden	98.9	0.0002
Harpacticoida	Golden	53.3	0.0060
<i>Hydrobaenus</i>	Osage	41.2	0.0392
<i>Mesocricotopus</i>	Osage	60.3	0.0012
<i>Probezzia</i>	Osage	52.0	0.0046
<i>Heterotrissocladius</i>	Prairie	40.7	0.0278
Cyclopoida	Prairie	54.0	0.0176
<i>Cordulegaster</i>	Taberville	38.0	0.0202
<i>Corynoneura</i>	Taberville	45.3	0.0128
<i>Diplocladius</i>	Taberville	65.1	0.0006
<i>Eukiefferiella</i>	Taberville	33.3	0.0316
<i>Orthocladius</i>	Taberville	35.4	0.0284
<i>Paramecetrionemus</i>	Taberville	44.4	0.0086
<i>Tanytarsus</i>	Taberville	50.6	0.0224
<i>Isoperla mohri</i>	Schwartz	39.5	0.0164
<i>Paraphaenocladius</i>	Schwartz	74.2	0.0004
<i>Pseudorthocladius</i>	Schwartz	32.1	0.0296
<i>Natarsia</i>	Schwartz	51.5	0.0026

Table 5. Pearson correlation coefficients of environmental variables for non-metric multidimensional scaling axes of all taxa prairie stream invertebrate community analysis.

	Axis 1		Axis 2		Axis 3	
Environmental Variable	r	r ²	r	r ²	r	r ²
Macrophyte (%)	-0.082	0.007	0.167	0.028	-0.408	0.167
Periphyton (%)	0.175	0.031	-0.007	0.000	-0.217	-0.098
Filamentous Algae (%)	0.596	0.355	0.209	0.044	-0.251	0.063
Organics (%)	-0.095	0.009	-0.211	0.044	-0.158	0.025
Embeddedness (%)	-0.067	0.004	-0.024	0.001	-0.241	0.058
Temperature	0.087	0.007	-0.137	0.019	-0.191	0.036
Dissolved Oxygen	0.213	0.045	-0.226	0.051	-0.054	0.003
pH	0.314	0.098	-0.420	0.177	-0.210	-0.004
Depth	0.186	0.035	0.002	0.000	-0.287	0.082
Width	-0.163	0.026	0.127	0.016	-0.191	0.036
Canopy Cover	0.055	0.003	-0.192	0.037	0.249	0.062
Algal Coverage Index	0.564	0.318	0.243	0.059	-0.229	0.053
Velocity (m/s)	0.268	0.072	0.069	0.005	-0.115	0.013
Number of Substrate Types	-0.241	0.058	0.217	0.047	-0.065	0.004
Substrate Score	-0.208	0.043	0.052	0.003	0.107	0.011

Table 6. Multi-response permutation procedure by prairie for chironomid-only community analysis.

		A	<i>p</i>
Overall MRPP		0.1437	0.0000
Pairwise comparisons	Golden vs. Osage	0.1069	0.0004
	Golden vs. Prairie	0.1791	0.0000
	Golden vs. Taberville	0.1600	0.0001
	Golden vs. Schwartz	0.1332	0.0001
	Osage vs. Prairie	0.0428	0.0109
	Osage vs. Taberville	0.0542	0.0032
	Osage vs. Schwartz	0.0958	0.0000
	Prairie vs. Taberville	0.0855	0.0004
	Prairie vs. Schwartz	0.0978	0.0001
	Taberville vs. Schwartz	0.0608	0.0033

Table 7. Alpha diversities of stream invertebrate communities of five Missouri prairies.

	α -diversity
Golden Prairie	11.00
Osage Prairie	24.78
Prairie State Park	19.89
Taberville Prairie	24.22
Schwartz Prairie	21.33
Average α-diversity	22.24$\pm$2.48

Table 8. Expected species richness of prairie stream invertebrate communities using first order jack-knife estimator.

	Observed Species Richness	Expected Species Richness	Observed/ Expected
Golden Prairie	28	37	0.76
Osage Prairie	72	95	0.76
Prairie State Park	63	83	0.76
Taberville Prairie	69	86	0.80
Schwartz Prairie	69	88	0.78
Regional	107	128	0.84

Figure 1. Locations of prairies in southwestern Missouri used for sampling of headwater stream macroinvertebrate communities.

Figure 2. Three-dimensional non-metric multidimensional scaling plot of prairie stream invertebrate communities ($p=0.0040$). (A) axis 1 versus 3, (B) axis 2 versus 3.

Figure 3. Non-metric multidimensional scaling plot of prairie stream invertebrate communities with joint biplot of environmental variables overlaid ($p=0.0040$). ACI, algal coverage index; Filamentous algae, percentage of filamentous algae.

Figure 4. Non-metric multidimensional scaling plot of prairie stream chironomid communities ($p=0.0120$). (A) axis 1 versus 2, (B) depicts axis 1 versus 3.

Figure 5. Shannon-Weiner diversity index values for prairie stream invertebrate communities. LS means ± 1 SEM. Different letters represent significant differences ($p < 0.05$).

Figure 6. Taxonomic richness of prairie stream invertebrate communities. LS means ± 1 SEM. Different letters represent significant differences ($p < 0.05$).

Figure 7. Functional feeding group percentages of total abundance of prairie stream invertebrate communities.

CHAPTER 5

GENE FLOW IN TWO SPECIES OF *LEPTOPHLEBIA* (EPHEMEROPTERA: *LEPTOPHLEBIIDAE*) ACROSS A TALLGRASS PRAIRIE STREAM LANDSCAPE

Abstract

For lotic insects to maintain stable populations across fragmented prairie patches and their intermittent streams in Missouri, dispersal must occur between patches to enable recolonization of streams after disturbance. *Leptophlebia konza* Burian was thought to be restricted to its type locality, Konza Prairie, Kansas; however, a population was discovered at Hi Lonesome Prairie in Missouri during an extensive search of 54 prairies. A congeneric species, *Leptophlebia johnsoni* McDunnough, is distributed widely through the northeastern United States and Canada from Nova Scotia to Ontario with records south to South Carolina and has also been found at Konza Prairie. My hypothesis was that *L. konza* would exhibit isolation by distance due to the fragmentation of its habitat, whereas *L. johnsoni* would exhibit evidence of gene flow. DNA sequence data from cytochrome oxidase subunit 1, COI, showed no genetic differentiation between the Kansas and Missouri populations of *L. konza* and an extremely reduced haplotype network with an apparent bottleneck. The population of *L. johnsoni* had high haplotypic diversity and a simple haplotype network, indicating recent expansion into the area. Evidence suggests that *L. konza* was once widely distributed from Kansas to Missouri, and recently lost genetic diversity during a bottleneck, likely because of habitat fragmentation. The rarity of *L. konza* populations across Missouri when taken in consideration with DNA sequence data suggests that *L. konza* populations are unstable and unable to disperse between remaining prairie patches.

Introduction

Lowland areas are barriers to dispersal of upland species of insects (Finn et al. 2007, Baggiano et al. 2011), but how landscape affects the distribution of lowland aquatic insect species is poorly understood (Murria et al. 2013). Habitat fragmentation, habitat loss, and habitat instability may pose barriers (Zwick 1992, Watanabe et al. 2010, Alexander et al. 2011, Chester et al. 2015) that affect the dispersal patterns of lowland aquatic insects, including those inhabiting intermittent prairie streams. Historically, prairies were areas of high headwater density (Nelson 1987) where intermittent streams were common (Fritz and Dodds 2004). However, what was once a broad expanse of prairie is now a divided landscape consisting of agricultural and urban lands with scattered remnant prairies.

For stable insect populations to exist in prairie streams, insects must either exhibit resistance, the ability to withstand disturbance, or resilience, the ability to recolonize after disturbances such as floods or droughts. Resistance in the form of adaptive life histories or behaviors is often less important in prairie streams than is resilience (Fritz and Dodds 2004). One important form of resilience is recolonization through dispersal either overland or in-stream. Many aquatic insects disperse terrestrially as adults; overland dispersal is an integral part of the maintenance of genetic diversity in several populations of stream insects (Miller et al. 2002, Schultheis and Hughes 2005, Zickovich and Bohonak 2007, Hughes et al. 2008, Sproul et al. 2014). Aquatic insects also are known to disperse actively and passively via drifting within stream channels; they also can recolonize following disturbances by drift (Sheldon 1984).

Adult flight is the major dispersal mechanism for stream insects (Hughes et al. 2008, Young et al. 2013). The ability of aquatic insects to disperse successfully as adults depends on a suite of life history features. As adults, many aquatic insects including mayflies and some caddisflies are non-feeding; thus, they are limited to the energy gained as immatures for all reproductive and dispersal activities. Additionally, mayfly dispersal is constrained by short adult lifespans (Peckarsky et al. 1999). Dispersal ability differs among mayfly taxa, even within the same genus (Hughes et al. 2008) and has been linked to wing length (Malmqvist 2000). Although mayflies can fly as adults, it is generally accepted that they are not able to consistently disperse over long distances (Malmqvist 2000, Chester et al. 2015) because of these constraints.

For stream insects to maintain stable populations across the fragmented prairie patches and their intermittent streams in Missouri, dispersal must occur between patches to enable recolonization of streams after disturbance. Because dispersal correlates with genetic variability (Miller et al. 2002), the dispersal of stream insects among patches can be examined using DNA sequence data. Many recent studies have examined the population genetic structure of aquatic insects to explore their dispersal abilities and distribution patterns (Miller et al. 2002, Schultheis and Hughes 2005, Finn et al. 2007, Zickovich and Bohonak 2007, Downes and Reich 2008, Watanabe et al. 2010, Baggiano et al. 2011, Sproul et al. 2014, Wickson et al. 2014).

The population genetic structure of the aquatic insects inhabiting intermittent streams of this landscape likely reflects the patchy nature of their habitat. *Leptophlebia konza* Burian is a mayfly recently described from Kings Creek in Konza Prairie, Kansas (Burian 2001). Initially, *L. konza* was thought to be restricted to its type locality;

however, a population was recently discovered at Hi Lonesome Prairie in Missouri (Fig. 1). A congeneric species, *Leptophlebia johnsoni* McDunnough, is distributed widely through the northeastern United States and Canada from Nova Scotia to Ontario with records south to South Carolina (Burian 2001) and has also been found at Konza Prairie (Fig. 2). The widespread distribution of *L. johnsoni* suggests a stronger dispersal ability and less restrictive habitat requirement than those of *L. konza* (Burian 2001). Because strong dispersers are known to be more resilient to habitat fragmentation and habitat instability (Watanabe et al. 2010, Baggiano et al 2011, Wickson et al. 2014, Chester et al. 2015), I predict that the genetic structure of prairie stream populations of these species will differ. Specifically, my hypotheses are that:

1) Populations of *L. konza* will exhibit evidence of isolation by distance, due to a lack of dispersal between habitat patches.

2) Populations of *L. johnsoni* will be structured by gene flow because it is a strong disperser with a wide distribution.

Methods

Study area

In total, 54 Missouri prairies were surveyed for *Leptophlebia konza* and *Leptophlebia johnsoni*. These prairies are located throughout nine counties and over an area of approximately 7000 square kilometers in the Central Irregular Plains ecoregion of Missouri (Chapman et al. 2002). The Central Plains ecoregion was not glaciated during the Pleistocene and is characterized by rolling hills gradually transitioning to flat plains in the south and was naturally covered by a mixture of grasslands and oak-hickory

woodlands (Chapman et al. 2002). Most extant prairies are remnants consisting of less than 200 acres with only one intermittent drainage occurring within the boundaries. Several of the larger prairies include multiple headwaters of the same stream system within the confines of the prairie (Osage Prairie, Prairies State Park, Schwartz Prairie, Taberville Prairie).

Konza Prairie is a long-term ecological research facility located in the Flint Hills of Kansas southwest of Manhattan, Kansas and is managed by Kansas State University. The Flint Hills ecoregion of Kansas is characterized by flat plains to moderate limestone hills eroded by streams (Oviatt 1998). Konza Prairie is located approximately 260 kilometers from the center of the Missouri sampling locations.

Data Collection

In the early spring of 2016, late instars of *Leptophlebia* were collected from leaf-packs in pools at three prairies: Taberville Prairie and Hi Lonesome Prairie, Missouri, and Konza Prairie, Kansas. At the remaining 51 prairies surveyed, no immatures of *Leptophlebia* were found. Larvae were immediately preserved in 95% EtOH and transferred to the laboratory. In the laboratory, specimens were identified and stored at -80°C before DNA extraction. Species identifications were verified by Luke Jacobus (Assistant Professor, Indiana University).

Leptophlebia konza was found at only two prairies, Hi Lonesome Prairie (Benton County, Missouri) and two locations in Konza Prairie (Fig. 1). The two collections in Konza Prairie were taken from the same stream but were separated by approximately 500 meters. Despite intensive sampling, only one individual of *L. konza* was collected at its

type locality (location 5, Fig. 2), and no individuals were collected from any other locations across Konza Prairie.

Leptophlebia johnsoni was not found in any prairies in Missouri, but was found in streams throughout Konza Prairie (Fig. 2).

Two other species of *Leptophlebia* were collected from Missouri prairies. Specifically, *L. intermedia* (Traver) was identified from Taberville Prairie and *L. cupida* Say co-occurred with *L. konza* at Hi Lonesome Prairie. Specimens of *L. cupida* were earlier instars than those of *L. konza*. For the present study, *L. johnsoni* and *L. konza* were selected for use because of their co-occurrence at Konza Prairie and dramatically different distributions.

Mayflies are widely considered to be weak fliers not capable of consistent long-distance dispersal, although many have been shown to disperse consistently across distances of 15 to 64 km (Zickovich and Bohonak 2007, Hughes et al. 2011, Young et al. 2013, Spitzer 2014). Since the sampling locations at Konza Prairie were separated by a maximum distance of only 5 km, all samples taken from Konza Prairie were considered to represent a single population. Thus, samples from one population of *L. johnsoni* and two populations of *L. konza* were collected.

DNA extraction, PCR amplification, and DNA sequencing

Genomic DNA was isolated from metathoracic coxal muscle tissue of 31 individuals of *L. konza* and 54 individuals of *L. johnsoni* (Table 1) with the DNeasy Blood and Tissue Kit (Qiagen). The mitochondrial gene cytochrome oxidase subunit 1 (COI) was targeted for sequencing using primers C_LepFolF and C_LepFolR (Hendrich et al. 2015) using the polymerase chain reaction (PCR). Reactions were performed in

25µL volumes consisting of approximately 15ng of DNA, 1X AmpliTaq Gold PCR buffer (Applied Biosystems), 200µM dNTPs, 2mM MgCl₂, 0.8mM BSA, 0.4µL of each primer, and 1 unit of AmpliTaq Gold (Applied Biosystems). Amplifications were performed on Eppendorf thermocyclers using a profile consisting of an initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 1 min, annealing at 43°C for 1 min, and primer extension for 1 min at 72°C with a final extension at 72°C for 10 min. PCR products were verified via gel electrophoresis before purification using the QIAQuick PCR Purification Kit (Qiagen). Purified products were sequenced directly on a 3730xl DNA Analyzer with Applied Biosystems Big Dye Terminator cycling chemistry at the University of Missouri DNA Core Facility.

Genetic Analyses

For each species, all nucleotide sequences were edited and aligned using Sequencher v5.1 (Gene Codes) resulting in a total length of 658 base pairs of sequence from CO1. Sequence identification was verified through BLAST on the NCBI GenBank.

DnaSP 6.0 (Rozas et al. 2017) was used to calculate haplotype diversity (Hd) and nucleotide diversity (π) for each population. Haplotype diversity was defined as the probability that any two randomly chosen haplotypes from the population are different. Nucleotide diversity is the average number of nucleotide differences per site between any two sequences. Pairwise genetic differentiation (F_{ST}) between the two populations of *L. konza* was calculated in DnaSP 6.0 (Rozas et al. 2017).

Since COI is a coding gene, it is subject to natural selection; thus, the null hypothesis of neutrality was tested with Tajima's D in DnaSP 6.0 (Rozas et al. 2017) for

each population. Significant negative values can indicate recent population expansion, whereas significant positive values can indicate recent population contraction.

Haplotype networks were constructed in PopArt (Leigh and Bryant 2015) using statistical parsimony methods (Clement et al. 2000).

Results

Genetic diversity

Four haplotypes of *L. konza* and three haplotypes of *L. johnsoni* were identified (Tables 2, 3). Two haplotypes of *L. johnsoni*, were found at every sampling location at Konza Prairie with the third haplotype present at three locations (Fig. 1). All four haplotypes of *L. konza* were present in the Konza prairie population, whereas only two were present at Hi Lonesome Prairie in Missouri (Fig. 2).

Haplotypic diversity was much higher in *L. johnsoni* than in *L. konza*, whereas nucleotide diversity was higher in *L. konza* (Table 4), meaning that the haplotypes of *L. konza* are highly differentiated from each other.

Genetic structure

Genetic differentiation between the two populations of *L. konza* was effectively zero ($F_{ST} = -0.050$) meaning that there is no genetic variation between the two populations in Kansas and Missouri. The value of Tajima's D was significantly negative for the Missouri population (Table 5) indicating fewer haplotypes than the number of segregating sites and, therefore, a possible recent population expansion.

The haplotype network for *L. konza* was highly reduced (Fig. 3). Most individuals sampled were from a single haplotype, K1, while the other three haplotypes were represented by either one or two individuals. Haplotypes K3 and K4, clade 2, were

separated from haplotypes K1 and K2, clade 1, by nine steps. No obvious geographic pattern was present in the haplotype network, as samples from Missouri and Kansas were nearly equally represented in each clade, supporting the conclusion of low genetic differentiation as determined by the F_{ST} calculation.

The value of Tajima's D for *L. johnsoni* was significantly positive, indicating an excess of haplotypes as compared to segregating sites, and, therefore a recent population contraction (Table 5). The haplotype network for *L. johnsoni* is simple and linear with two dominant haplotypes (Fig. 4), with no obvious geographic pattern.

Discussion

Genetic structure in *Leptophlebia konza*

The pattern evident on the haplotype network of *L. konza* conflicts with the indication of population expansion based on Tajima's D . Expanding populations are typically represented by star-shaped patterns of less frequent haplotypes radiating from dominant, presumably ancestral haplotypes. In the case of *L. konza*, no star shaped pattern is evident. The separation of clade 1 and clade 2 by a nine bp difference suggests a bottleneck that resulted in the loss of the intermediate haplotypes that linked the two clades.

This widespread loss of haplotype diversity corresponds to high levels of mortality (Poff et al. 2018), which may relate to recent habitat loss. Although mayflies are generally capable of consistent dispersal on the scale of only tens of kilometers (Zickovich and Bohonak 2007, Hughes et al. 2011, Young et al. 2013, Spitzer 2014), they are capable of widespread gene flow by small increments given a continuous habitat and a large time scale (Lester et al. 2007, Monaghan and Sartori 2009). The habitat of *L. konza* was once a

continuous network of tallgrass prairie and headwater streams (Matthews 1988). Before the introduction of agriculture this habitat existed for thousands of years (Axelrod 1985), giving *L. konza* ample time to distribute across the lowlands from Kansas to Missouri. The relatively recent loss of habitat with the onset of agriculture corresponds with a loss of continuous habitat and the subsequent loss of all but the most common haplotypes from the network.

No geographic pattern is present in the haplotype network because only the most common haplotypes are remaining. Haplotypes K3 and K4 were identified in only two and one individuals, respectively, and are the only haplotypes remaining from clade 2. The haplotype network probably was once a star-like radiant pattern with many intermediate haplotypes, similar to those found in other widespread mayfly species (Baggiano et al. 2011, Chester et al. 2015).

A negative Tajima's D indicates a case of recent population expansion in *L. konza*. It is unlikely that eggs could have been transferred from Konza to Hi Lonesome Prairie either naturally or anthropogenically. Although the oviposition habits of *L. konza* are unknown, three species of leptophlebiid mayflies are known to oviposit directly into the water rather than onto a substrate (Takemon 2000, Encalada and Peckarsky 2006, Gaino et al. 2008), making it unlikely that eggs would be transported by any mechanism other than female dispersal. Additionally, no materials have ever been transported from Konza Prairie to Hi Lonesome Prairie for the purposes of restoration according to the current prairie manager (J. Coy, pers. comm.). Mayflies rarely disperse over 146 km (Hughes et al. 2011) and generally only when appropriate habitat is unavailable (Smith and Collier 2005, Young et al. 2013). For *L. konza*, the negative value of Tajima's D is

due to the 9 bp difference between the two clades, which increased the relative number of segregating sites to haplotypes and is likely not linked to a recent population expansion.

An F_{ST} value of zero indicates that there is no genetic variation between the Missouri and Kansas populations, which ordinarily would suggest that consistent dispersal occurs between the two populations and a high level of gene flow. F_{ST} is calculated by comparing the variation in haplotype frequencies between the populations of Kansas and Missouri. In this case, allele frequencies between the two populations probably are similar not because of widespread gene flow, but because of a recent bottleneck event which resulted in the loss of all but the most common haplotypes from the two populations.

These results could be the result of insufficient sampling, although this is unlikely. Streams at 54 prairies across Missouri were sampled and *L. konza* was found at only one of the prairies. Other members of the genus are known to inhabit similar stream environments (Burian 2001) and were rarely encountered, which suggests that suitable habitat is rare in Missouri. The Kansas City metropolitan area separates the mayfly populations of Missouri and Kansas with a wide swath of uninhabitable environment. Even though Konza Prairie is pristine, unfragmented tallgrass habitat, individuals were collected from only one stream on the prairie. An additional population of *L. konza* was recorded from a stream draining to Geary Lake south of Konza Prairie (Burian 2001). Genetic material from this population would provide additional insight into dispersal patterns.

The initial hypothesis of isolation by distance in *L. konza* was not supported. Tests for isolation by distance require three or more sampled populations; thus, such tests

were not performed, although results from other analyses suggest widespread loss of genetic diversity after a bottleneck.

Genetic structure in *Leptophlebia johnsoni*

A simple linear haplotype network indicates that only a few haplotypes of *L. johnsoni* have colonized this area. *L. johnsoni* is likely on the edge of its range at Konza Prairie because the species has never been recorded this far west; the westernmost record for the species was Ohio (Randolph 2002). Additionally, *L. johnsoni* was included in a key to the mayfly species of Wisconsin as an informal record (Hilsenhoff 1929). The presence of *L. johnsoni* at Konza Prairie is not unexpected, as its range has been thought to extend farther west than has been recorded (Burian 2001). The expansion of the range of *L. johnsoni* is recent. Konza Prairie is a well-studied prairie and *L. johnsoni* was not included in any faunal records or from the study in which *L. konza* was identified (Fritz and Dodds 2002). The relatively even abundance of individuals across the three haplotypes indicates either the success of individuals after colonization or of a constant colonization of Konza Prairie from other more central populations of *L. johnsoni*.

A positive value of Tajima's D indicates a lack of rare alleles and a possible range contraction. The lack of rare alleles in the *L. johnsoni* population is consistent with the concept that the species is on the edge of its range and only a few haplotypes have been able to colonize. The population of *L. johnsoni* is likely not going through a range contraction in this area because it is a new colonist. This population may, however, be a sink population for source populations deeper within the center of the range, leading to a lack of rare alleles and the positive value of Tajima's D.

The haplotype network is suggestive of *L. johnsoni* newly colonizing Konza Prairie and existing at the edge of its range, even though it was not discovered in Missouri. Insect species with expanding ranges can jump ahead of the leading edge and back-fill the distribution voids over time (Lester et al. 2007). The range of *L. johnsoni* extends from Nova Scotia west to Ontario and south to Ohio, with disjunct populations in Virginia and South Carolina (Burian 2001). It is more probable that *L. johnsoni* may have expanded its range south from Ontario rather than west from Ohio because of the large urban areas in Indiana and Illinois, which could have impeded dispersal. Dispersal from Ontario south into Minnesota and Kansas would have encountered fewer urban centers.

Implication for species interactions

Leptophlebia konza was found only at the southernmost sampled stream in Konza Prairie, which is the type locality of *L. konza*. The species also has been recorded from location 5 (Fig. 2) (Fritz and Dodds 2002). I collected only one individual of *L. konza* from location 5; however, *L. johnsoni* was in abundance there. *Leptophlebia konza* was abundant at site 6 (Fig. 2), which is farther upstream from site 5 and where *L. johnsoni* was not collected. *Leptophlebia konza* never co-occurred with *L. johnsoni* in high numbers. This single individual of *L. konza* at site 5 was of a unique haplotype, K4 (Fig. 2), which suggests that this individual did not drift downstream from site 6.

The presence of *L. johnsoni* may be shifting the distribution of *L. konza* at Konza Prairie. *L. konza* was previously abundant at site 5, but is now locally rare. If *L. johnsoni* had been locally abundant at site 5 as was the case in this study, Fritz and Dodds (2002) would have collected *L. johnsoni* at location 5 along with *L. konza*. *Leptophlebia konza*

remains abundant at the southernmost sampled site (6), which is consistent with a range expansion of *L. johnsoni* into Konza Prairie from the north.

In Missouri, habitat changes may have harmed *L. konza* while benefiting the congeners *L. intermedia* and *L. cupida*. *Leptophlebia cupida* was found syntopically with *L. konza* at Hi Lonesome Prairie; however, the individuals of *L. cupida* were earlier instars than individuals of *L. konza* collected from the same habitat at the same time. *Leptophlebia konza* may be able to co-exist with *L. cupida* because of ontogenetic partitioning. At Taberville Prairie in Missouri, *L. intermedia* was found in pools in leaf-packs very similar to those inhabited by *L. konza* at Konza Prairie. *L. intermedia* is commonly found with several other species of *Leptophlebia* (Burian 2001) in other areas of its range. The individuals of *L. intermedia* collected were of similar size to those of *L. konza* that were collected at Konza Prairie, which suggests similar ontogenies between the two species.

One mayfly species, *Baetis tricaudatus*, has been documented to expand its range into areas previously occupied by other species after an extreme disturbance event caused widespread mortality (Poff et al. 2018). Since prairie streams are relatively unpredictable hydrologic environments (Fritz and Dodds 2004), extreme drought or a major flooding event could have led to the mortality of other populations of *L. konza* in Missouri. Without source populations nearby, *L. intermedia* and *L. cupida* could have supplanted *L. konza* in its absence.

Dispersal and Habitat Fragmentation

Based on the lack of any geographic pattern evident in the haplotype network, lowlands did not previously prevent the dispersal of *L. konza*, a historical pattern

documented in a few other species of mayflies (Schultheis and Hughes 2005, Phillipsen et al. 2015). The most common haplotype was found with even frequency at the two remnant populations sampled, suggesting that *L. konza* was once distributed across the lowlands between Kansas and Missouri.

These results suggest that *L. konza* has recently lost genetic diversity during a bottleneck, likely because of habitat fragmentation of its habitat. Only four haplotypes of *L. konza* were identified, three of which were extremely rare. Despite extensive sampling, *L. konza* was found at only two locations. This suggests that useable prairie habitats may now be too far apart for the regular dispersal needed to sustain populations. Additionally, other congeners are using the same habitat as *L. konza*. Although members of the genus are often found syntopically in other areas of North America (Burian 2001), *L. konza* may not be able to co-exist with congeners.

The current range of *L. konza* is extremely restricted and the remaining populations of *L. konza* have lost genetic diversity. The rarity of *L. konza* populations when taken in consideration with DNA sequence data suggest that *L. konza* populations are unstable and unable to disperse between remaining prairie patches. As such, *L. konza* is a rare mayfly species that appears to be in decline and should be considered for protected status.

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Table 1. Locality and abundance of two species of *Leptophlebia* collected in seven streams in Kansas and Missouri.

	Stream	Latitude	Longitude	Individuals	
				<i>L. johnsoni</i>	<i>L. konza</i>
Kansas	1	39.11263	-96.54419	7	0
	2	39.10866	-96.60873	8	0
	3	39.10085	-96.57526	9	0
	4	39.08537	-96.58277	17	0
	5	39.08529	-96.58311	10	1
	6	39.08129	-96.58211	3	16
Missouri	7	39.07924	-95.266952	0	14

Stream numbers correspond to sampling locations of Figures 1 and 2.

Table 2. Nucleotide polymorphisms across 658 bp of COI mtDNA sequences in four haplotypes of *Leptophlebia konza*.

Nucleotide Position												
Haplotype	40	79	85	88	160	181	232	271	356	388	502	550
K1	G	G	G	A	A	G	C	G	A	A	A	A
K2	G	G	G	A	A	G	C	G	G	C	A	A
K3	T	A	T	G	G	A	T	A	G	C	C	A
K4	T	A	T	G	G	A	T	A	G	C	C	G

Table 3. Nucleotide polymorphisms across 658 bp of COI mtDNA sequence in three haplotypes of *Leptophlebia johnsoni*.

Nucleotide Position		
Haplotype	223	544
J1	T	G
J2	T	A
J3	C	A

Table 4. Genetic diversity of two species of *Leptophlebia* based on COI mitochondrial sequence data.

	S	H	Hd	π
<i>L. johnsoni</i>	2	3	0.582	0.00158
<i>L. konza</i>	12	4	0.243	0.00338
Kansas	12	4	0.331	0.00423
Missouri	11	2	0.143	0.00246

(S) number of nucleotide polymorphisms, (H) number of haplotypes, (Hd) haplotypic diversity, (π) nucleotide diversity

Table 5. Test of selective neutrality for populations of two species of *Leptophlebia*.

	Tajima's D	p value
<i>L. johnsoni</i>	2.21132	<0.05
<i>L. konza</i>	-0.91581	>0.10
Kansas	-0.87642	>0.10
Missouri	-2.1508	<0.01

Figure 1. Distribution of *Leptophlebia johnsoni* haplotypes across Konza Prairie, Kansas according to sampling location (see stream numbers in Table 1).

Figure 2. Distribution of *Leptophlebia konza* haplotypes across Kansas and Missouri according to sample location (see stream numbers in Table 1).

Figure 3. Mitochondrial haplotype network for *Leptophlebia konza* populations from Kansas and Missouri. Circles represent haplotypes and each dash represents a single nucleotide substitution.

Figure 4. Mitochondrial haplotype network for *Leptophlebia johnsoni* population at Konza Prairie, Kansas. Circles represent haplotypes and each dash represents a single nucleotide substitution.

VITA

Jessica Warwick was born to Thomas and Cathleen Warwick on July 23, 1989 in Baraboo, Wisconsin. She spent her youth in Baraboo with her parents and her two younger siblings. Jessica graduated from Baraboo High School in June 2007 before attending the University of Wisconsin- Madison beginning in August 2007. In May 2012, Jessica graduated from the University of Wisconsin- Madison with a Bachelor of Science degree in Natural Resources with majors in Entomology and Botany along with a certificate in Environmental Sciences. While at the University of Wisconsin- Madison, Jessica worked in the Departments of Botany, Entomology, and Dairy Sciences where she worked in a variety of laboratory and field settings. In the spring of 2013, Jessica was accepted into the Division of Plant Sciences at the University of Missouri and was awarded a Life Sciences Fellowship to investigate the macroinvertebrate communities of Missouri prairie streams. She graduated with her PhD in 2018.