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**DRAGONFLIES AND DAMSELFLIES (INSECTA: ODONATA) AS INDICATORS FOR
RIPARIAN CONDITION IN OZARK SPRING STREAMS**

A Master's Thesis

Presented to

The Graduate College of
Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science, Biology

By

Cameron Riley Cheri

May 2020

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DRAGONFLIES AND DAMSELFLIES (INSECTA: ODONATA) AS INDICATORS FOR RIPARIAN CONDITION IN OZARK SPRING STREAMS

Biology

Missouri State University, May 2020

Master of Science

Cameron Riley Cheri

ABSTRACT

The Odonata (dragonflies and damselflies) are often ineffectively sampled during standard stream bioassessments in North America. Subsequently, odonates are not frequently regarded as informative taxa for stream assessment, particularly when monitoring the ecological impacts of organic pollution. I hypothesized that stream-dwelling odonates should be more useful bioindicators for the assessment of riparian conditions surrounding streams because vegetation associated with streams is used for oviposition, roosting and to establish breeding territories. I selected twelve Ozark spring streams that satisfied a broad array of riparian conditions for study. I sampled each stream's odonate and total benthic community along with both instream and vegetation-specific environmental variables. Odonate and total benthic communities were compared across study sites to identify differences in community structure and identify sensitivity to different environmental variables. Odonate community structure alone was highly correlated with riparian-specific vegetation variables. Meanwhile, standard water-quality assessment metrics used by the Missouri Department of Natural Resources were not useful to indicate riparian habitat condition, based on the total benthic community. I developed tolerance values for use in an odonate-oriented biotic index as a more appropriate metric for assessment of Ozark spring stream riparian conditions. I additionally examined two abundant damselfly species using occupancy modeling associated with riparian habitat. The two species showed different occupancy patterns in relation with the level of riparian impactedness around study sites. Overall, odonates showed greater sensitivity to riparian conditions than did total benthic communities, supporting the idea that this taxon alone is useful for biomonitoring associated with riparian structure around Ozark spring streams. The sensitivity of odonates to riparian conditions in stream ecosystems found in other Nearctic regions should be further studied to identify regional and species differences. Future studies can help land managers make informed decisions concerning riparian conservation efforts around streams by employing biomonitoring practices that incorporate this apparently riparian-sensitive taxon.

KEYWORDS: Odonata, springs, bioindicators, riparian zones, Ozark Highlands, stream bioassessment

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In the interest of academic freedom and the principle of free speech, approval of this thesis indicates the format is acceptable and meets the academic criteria for the discipline as determined by the faculty that constitute the thesis committee. The content and views expressed in this thesis are those of the student-scholar and are not endorsed by Missouri State University, its Graduate College, or its employees.

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TABLE OF CONTENTS

Overview	Page 1
Introduction	Page 3
Methods	Page 9
Study Sites	Page 9
Habitat Variables	Page 11
Collecting Community Data	Page 12
Standard Stream Metrics	Page 15
Statistical Analyses	Page 16
Results	Page 20
Physical Habitat	Page 20
Spring Communities	Page 21
Standard Metrics and Tolerance Values	Page 24
Discussion	Page 26
Community Responses to Riparian Condition	Page 27
Odonate Responses to Riparian Condition	Page 28
Bioassessment of the Benthos	Page 33
Bioassessment with Odonata	Page 36
References	Page 38
Occupancy Modeling with Odonata	Page 57
Introduction	Page 57
Objective	Page 58
Methods	Page 60
Study Sites	Page 60
Vegetation Covariates	Page 61
Odonate Data	Page 61
Occupancy Models	Page 62
Results	Page 66
Discussion	Page 68
References	Page 71
Summary	Page 74
Appendix	Page 82

LIST OF TABLES

Table 1. General location and characteristics of study springs	Page 44
Table 2. Physicochemical environmental variables	Page 45
Table 3. Vegetation-associated environmental variables	Page 46
Table 4. Odonate community data	Page 47
Table 5. Correlation coefficients and P-values for fitted vectors plotted in NMS ordinations	Page 48
Table 6. Results from Indicator Species Analysis	Page 49
Table 7. Estimates for standard stream bioassessment metrics applied by MDNR	Page 50
Table 8. Calculated tolerance values for resident odonates	Page 51
Table 9. Detections for <i>Calopteryx maculata</i> and <i>Argia plana</i>	Page 76
Table 10. Model selection results for each study species	Page 77
Table 11. Parameter estimates for predictive occupancy models	Page 78

LIST OF FIGURES

Figure 1. Study Map	Page 52
Figure 2. Odonate Community Dissimilarity Dendrogram	Page 53
Figure 3. NMS ordinations for odonate, general benthic, and insect-only general benthic communities	Page 54
Figure 4. Pearson correlation matrix comparing standard metric estimates and vegetation-specific environmental variables	Page 55
Figure 5. Boxplots for one-way ANOVA testing for differences between site classes with standard stream bioassessment metrics	Page 56
Figure 6. Predicted occupancy of <i>Calopteryx maculata</i> across a local-scale riparian gradient	Page 79
Figure 7. Predicted occupancy of <i>Argia plana</i> across a local-scale riparian gradient	Page 80
Figure 8. Predicted occupancy of <i>Argia plana</i> across a landscape-scale riparian gradient	Page 81

OVERVIEW

Bioassessment is commonly applied by conservationists and researchers to evaluate environmental quality and ecological integrity for a variety of ecosystem types, especially in freshwater systems like streams. Stream bioassessment techniques usually involve sampling a very small portion of a total stream ecosystem for their macroinvertebrate communities and then drawing conclusions on environmental integrity based on community composition of the samples. Several metrics are routinely used to describe stream quality, especially biotic indices based on tolerance values (e.g. Hilsenhoff 1977), and taxonomic diversity indices. Other routine approaches to ecological bioassessment include indicator species analysis and occupancy modeling, both of which consider species detections from field observations or collections to make inferences about species-habitat affinities and population dynamics.

Theoretically, by maximizing the number of organisms that are collected from an environment for use in a bioassessment survey, we should improve our understanding of the corresponding environment's conditions. Nevertheless, there are often organisms which can tolerate a wider range of environmental conditions than others, and consequently the presence of hardy organisms is less informative of certain environmental conditions than sensitive ones. For this reason, identifying taxonomic groups with the greatest sensitivity to the ecosystem component of interest can facilitate quick and reliable bioassessment.

It has been long suspected that the diurnal adults of the Odonata navigate their world principally by their vision (Corbet 1962). Many odonates select breeding sites based on vegetation structure in and around their preferred aquatic habitats. Based on the known associations held between adult odonates and vegetation, I suspected that among the various

aquatic invertebrate taxa collected during stream bioassessments, the Odonata would likely be the most sensitive taxon to the physical structure of the riparian zone.

In the first chapter of my thesis, I selected 12 study sites with riparian zones that I categorized into three levels of impactedness based on their physical structure. Then I compared the sensitivity of thoroughly sampled odonate communities to the overall benthic macroinvertebrate communities commonly used in bioassessment at each site to identify which community type was most informative of riparian condition. I subsequently discuss whether odonates would be ideal subjects of riparian bioassessment based on my results. In chapter two I applied occupancy modeling techniques for two damselfly species frequently encountered at spring streams to identify the efficacy of this common monitoring technique for odonate-riparian bioassessment.

INTRODUCTION

Riparian zones are important habitat for many riverine organisms and serve as the primary ecotone between streams and terrestrial ecosystems (Thomas et al. 1979; Gregory et al. 1991). Degradation to the riparian zone of a stream system can greatly impact that stream's biological community. Lack of shading over streams following disappearance of canopy species can increase water temperatures and increase algal growth. At the local scale, removal of riparian vegetation can increase fine sediment inputs to streams, which may reduce heterogeneity of substrata habitat for macroinvertebrate organisms or alter channel morphology (Henley et al. 2000). Riparian degradation can also reduce allochthonous inputs to aquatic ecosystems. A lack of allochthonous material can affect the base of stream food webs and alter biological community composition (Vannote et al. 1980; Cummins et al. 1989). The importance of riparian zones around streams merits action to preserve them in an effort to conserve aquatic resources, so there is a need for rapid assessment methods that indicate the biological state of riparian habitats.

Biological monitoring is a common endeavor for assessing environmental conditions, particularly in fresh waters. Both managers and researchers worldwide use macroinvertebrates as bioindicators for rapid assessment of freshwater ecosystem impairment (Barbour et al. 1999). In streams, macroinvertebrates primarily occupy the benthos or substratum and are often sensitive to changes in their environment (Hynes 1970). Biotic indices used for streams often characterize benthic macroinvertebrate groups by their sensitivity to water quality (i.e., chemical parameters associated with organic pollution). Tolerance values are numeric values that reflect the sensitivity of specific benthic taxa to changes in environmental variables of interest. Tolerance

values are proportionally combined across full communities to calculate a single biotic index score for an individual sample site. Most of the commonly applied biotic indices in streams follow the general approach of Chutter (1972) and Hilsenhoff (1977), applying tolerance values associated with responses to organic pollution.

Chutter (1972), Hilsenhoff (1977) and other authors (e.g., Lenat and Resh 2001) also stressed the importance of refining identifications to the lowest taxonomic level (species) and using species-specific tolerance values to achieve the most accurate biological index calculations, because closely related taxa can sometimes have substantially different responses. Finer levels of taxonomic resolution can be difficult to accomplish for many to most macroinvertebrate taxa, a problem that is exacerbated by an individual's identification skills and access to reliable, easy-to-use taxonomic keys. Conversely, there are multiple keys that work well for species level identifications of most larval odonates in North America (Daigle 1991; Westfall and May 1996; Landwer and Sites 2010; Tennessen 2019). Larval odonates are also easier to refine to lower taxonomic levels due to their relatively large size and elaborate morphological characters that can be more readily identified under dissecting microscopes than taxonomic groups with smaller size and less distinctive characters. Public databases such as BugGuide.net (2019) and OdonataCentral.org (Abbott 2019) provide additional species distribution and adult identification information for most Nearctic odonates. Due to their popularity as charismatic insects, adult odonates are also well described by naturalists in several regional field guides accessible to the general public.

However, despite their appeal, odonates are infrequently used as focal taxa during biological assessments, due in large part to their presence in rarely sampled habitat components of streams and to their general tolerance to organic pollution. Time and funding constraints can

limit the thoroughness of stream bioassessment fieldwork, which can result in non-representative collection of odonates when using only the most common standard stream assessment collection methods (e.g., Surber sampling) or restricting sampling to select habitats (e.g. riffle units). Restricting sampling to one habitat type is an attractive bioassessment approach aimed at maximizing cost and time efficiency. Some studies have shown that invertebrates occupying different stream habitats have similar sensitivities to human stressors (e.g. pollution) (Ostermiller and Hawkins 2004; Gerth and Herlihy 2006), while others have identified greater sensitivity to stressors by invertebrates in one specific habitat (Roy et al. 2003; Chessman et al. 2007; Carlson et al. 2013). For example, Carlson et al. (2013) found that riffle communities better discriminated against agricultural land use effects in Sweden than did pool communities. Stream benthic samples are sometimes taken solely from one habitat (e.g. riffles) (Bowles et al. 2008) where taxa most sensitive to a focal stressor like organic pollution tend to be abundant. As such, only a few odonate taxa adapted to that microhabitat are collected while a greater diversity of odonates may occupy other instream habitats. Accordingly, the largely pollution-tolerant odonates should be sampled using multihabitat methodologies at the reach scale for proper stream bioassessment application.

The Odonata of North America are comprised of two suborders, Anisoptera (dragonflies) and Zygoptera (damselflies). Members of both suborders require aquatic ecosystems to complete their life cycle and serve as important predators in both their immature and adult stages. The nymphs are sometimes the top predators in small lentic systems while adults can be voracious predators of many flying insects. Many odonate nymphs occupy specific aquatic microhabitats, exhibit specific behaviors, or have variable developmental phenologies that can reduce interspecific competition and lead to greater diversity of this predatory taxon within a single

ecosystem (e.g., stream or pond) (Johannsson 1978; Pierce et al. 1985; Suhling 1996; Khelifa et al. 2013). Odonates target their prey by sight, and adults have large compound eyes with a wide field of vision that they use to identify predators, prey, mates, rivals and suitable habitat (Corbet 1999; Bybee et al. 2016).

Odonates can be especially sensitive to physical habitat structure, like riparian conditions (Smith et al. 2007). Because odonates often have little relevance in standard biotic indices compared to more abundant and pollution-sensitive insect orders usually present in the benthos of streams, vegetation structure would likely be a more appropriate environmental character for odonate-related bioassessment. The adults of many odonates, particularly anisopterous species, are strong fliers that can travel long distances to find suitable breeding sites that they select primarily according to local environmental conditions. Sensitive environmental conditions for many species include shading around water bodies, specific vegetation structure for breeding and oviposition (Corbet 1962), or larval habitat (e.g. Rantala et al. 2004). Changes in habitat structure driven by invasive tree species (Remsburg, Olson, and Samways 2008), agricultural practices (Hornung and Rice 2003; García-García et al. 2017), urbanization (Córdoba-Aguilar and Rocha-Ortega 2019) and deforestation within riparian zones (Subramanian, Ali, and Ramachandra 2008; Miguel et al. 2017) can affect odonate species residency and abundance in streams and ponds, usually in the absence of altered water chemistry.

The Ozark Highlands is a karst-dominated ecoregion supplying a plethora of groundwater-fed spring systems that provide unique habitat for a diverse array of freshwater macroinvertebrates. The region is home to 258 odonate species (George Sims, Central Wyoming College 2015, unpublished data) of which several are known to use Ozark springs and spring-fed systems (Trial 2005; Zeller 2010). Specific habitat data for odonates in the Ozark Highlands is

limited for many species, and it is not well understood which specific habitat variables odonate species in this region primarily respond to when choosing a breeding site. Odonate abundance data are likely to be useful to land managers to monitor large-scale changes to environmental conditions, especially in riparian zones, surrounding Ozark springs. Furthermore, managers or researchers should be able to identify important components of habitat suitability by monitoring odonates at multiple life stages (Oertli 2008).

In this study, I evaluated macroinvertebrate community structure in Ozark spring streams along a steep gradient of riparian structure. Throughout this paper I will refer to two principle communities of interest: 1) the odonate community, which I define as all odonate taxa collected by qualitatively sampling all habitats at an Ozark spring stream, and 2) the general benthic community, defined by the macroinvertebrates collected from standard quantitative sampling of riffle habitat. My overarching hypothesis was that physical characteristics of vegetation that can be visually observed in and around Ozark springs by adult insects is an important predictor of macroinvertebrate communities in those systems. Subsequently I made two specific predictions about which habitat variables predominantly drive total odonate versus general benthic community structure. Prediction 1: In comparison to odonate communities, general benthic communities respond more strongly to environmental variables associated with water chemistry (e.g., pH or dissolved oxygen) and benthic substrate characteristics. Prediction 2: In comparison to general benthic communities, odonate communities respond more strongly to the condition of riparian zones around spring habitats than water chemistry and other abiotic instream variables. I further explored whether characteristics of odonate communities can better indicate the quality of local riparian conditions around spring streams than existing bioassessment metrics that require extensive taxonomic expertise across numerous orders. I used general benthic community data to

calculate four standard metrics used to assess water quality in Missouri streams and evaluated their predictability along the riparian gradient. Finally, I propose new tolerance values calculated from abundance and habitat data that could be applicable for later use in an odonate-specific biotic index specifically for assessing riparian conditions.

METHODS

Study Sites

I located several potential spring study sites using the ‘Springs’ data layer in the Geosciences Technical Resource Assessment Tool (GeoSTRAT) provided by the Missouri Department of Natural Resources (MDNR). Further discovery of potential study sites was supplemented from information provided by Vineyard et al. (1974), and by David Bowles (National Park Service Heartland Network, pers. comm), followed by communicating with private land owners. I scouted sites in the spring of 2018 and selected twelve springs based on two primary criteria: 1) consistent year-round flow magnitude (spring size), and 2) riparian zone conditions immediately adjacent to each spring fell along a gradient of structure. Aquatic habitat permanence is integral for odonates during their immature stage, so I only used springs known to have consistent flow. Spring size is categorized by classes of magnitude ranging from ‘first magnitude’ springs (highest discharge) to ‘eighth magnitude’ (lowest discharge) (Meinzer 1927). Because stream size influences macroinvertebrate community structure (Barbour et al. 1999), and springs located on the Ozark Plateau vary greatly in magnitude, I primarily selected third magnitude (28-280 L/s) springs (N=10) and larger fourth magnitude (6.3-28 L/s) springs (N=2) for this study (Meinzer 1927). I confirmed the magnitude of some springs with historic flow records from the MDNR springs data layer or flow data published by Vineyard et al. (1974). For springs without flow records, I estimated their magnitudes using a flow meter during scouting and calculated discharge. I identified twelve springs that met my criteria and were accessible to sample (Table 1). Each spring feeds a larger stream that is a tributary within the White or Sac river watersheds of the Ozark Plateau. Those twelve sites were subsequently categorized into

three classes of riparian impactedness based on observed land use data for each spring: minimally impacted (N=4), moderately impacted (N=5), and highly impacted (N=3) (Figure 1). Minimally impacted field sites included springs with riparian zones that were undisturbed by human use and were located in mostly forested settings. Moderately impacted sites were usually in rural areas with varying degrees of agricultural use but at least partially intact riparian zones. Sometimes these sites were modified for public recreational use or partly deforested by landowners. Highly impacted sites had riparian zones that were reduced to manicured lawn or inorganic rubble, lacked natural forest settings and may be concrete-lined adjacent the spring streams.

At each of the 12 springs, I defined the length of the sampling reach according to United States Geological Survey (USGS) protocols as twenty times the wetted width of the sampling point (Fitzpatrick et al. 1998). For this study, the sampling point was wherever benthic samples were taken at either a riffle or shallow run along the channel. In cases where a stream segment's total length was less than twenty times its wetted width (Silver and Chiles springs), I sampled its entire length. At most springs I selected sampling reaches that were in close proximity to their spring's source (within 50m). East Ritter, Doling, Brown and Patterson springs had sampling reaches more distant (72 – 260m) from the source (Table 1) either due to greater ease of access to the spring stream or the presence of favorable habitat (i.e., shallow riffles or runs) to take benthic samples. I defined the riparian zone adjacent to each sampling reach similarly to Environmental Protection Agency (EPA) protocols as four times the wetted width of the stream (Barbour et al. 1999) on each side of the stream.

Habitat Variables

I measured fifteen environmental variables within each sampling reach during daylight hours in June and July 2018. The springs in this study are fed by aquifers that produce year-round flows, so their water chemistry parameters were not expected to vary seasonally to the same degree as streams primarily sourced by run-off from precipitation. Ozark springs in particular exhibit great thermal and physicochemical stability (Carroll and Thorp 2014). The environmental data collected represent in-stream or riparian conditions during warmer months when most odonates and many other benthic insects reach developmental maturity, breed, and oviposit.

Physicochemical variables including pH, specific conductivity (Cond), total dissolved solids (TDS), oxidation-reduction potential (ORP), dissolved oxygen (DO) and temperature (Temp) were measured with a Hanna Instruments HI98194 Multiparameter Meter (Hanna Instruments, Woonsocket, Rhode Island). I used Hach Model PO-19 and Model NI-12 test kits (Hach Instruments, Loveland, Colorado) to measure orthophosphate (PO_4^{3-}), nitrate (NO_3^-) and nitrite (NO_2^-) concentrations, respectively. Nitrite concentrations were below detection level at all springs and were omitted from later analyses. I used a Hach FH950 portable velocity meter (Hach Instruments, Loveland, Colorado) fitted to a top-setting wading rod to measure velocity for estimation of discharge (Q) in fast-flowing, shallow areas of each sampling reach. To assess dominant substrate size (Substrate), I conducted pebble counts in riffles where benthic samples were taken and assigned size classes for twenty particles following Bowles et al. (2008) to calculate median size (D50). I implemented modified EPA protocols for visual estimation (Barbour et al. 1999) to categorize the embeddedness (Embed) of each site into quinaries of roughly 0, 25, 50, 75 and 100 percent after physically disturbing the substrate with a

garden claw cultivator during Surber sampling. I similarly categorized canopy cover (CC) into quaternaries for visual estimation from following modification of the EPA protocols (Barbour et al. 1999).

I visually estimated percent cover of instream emergent vegetation (EmVC) at springs during the summer sampling period when many species of aquatic vegetation display emergent forms. I evaluated riparian conditions for each study site at both local (RipC) and landscape (LSRC) scales. To estimate RipC, I visually approximated the proportion of the defined riparian zone that was primarily undisturbed and contained mostly natural vegetation (i.e. trees and shrubs). My RipC assessment was based on a modification of the Barbour (1999) visual-assessment protocols. For estimation of LSRC, I used ArcMap (ver. 10.5.1; ESRI 2012) to approximate the percent of undisturbed riparian conditions around each site. The USGS National Land Cover Database (NLCD) provides a file with a US map overlay with land use classification data for years 1992, 2001, 2006 and 2011. I used the 2011 land-use data provided in this file. Using the draw toolbar, I placed a circle 1-km in diameter over the center of each spring's sampling reach to create a 500-m buffer around each sample site. I visually estimated what percentage of each buffer area was 'disturbed' or 'undisturbed' habitat based on the NLCD land use classes. Areas I considered undisturbed habitat were classified as follows: deciduous forest, evergreen forest, mixed forest, woody wetlands, emerging herbaceous wetlands, and shrub/scrub.

Collecting Community Data

I conducted sampling of general benthic communities during June and early July 2018. Three replicate samples were collected at each site using a Slack Surber sampler with a 500 μm mesh net fitted with a 0.25 m^2 sampling frame. Collection of replicate samples (henceforth

‘samples’) involved disturbing the substrate within the sampling frame with a long-handled garden claw cultivator for two minutes (Bowles et al. 2008). Each sample was collected from riffles or shallow runs with predominantly large pebble substrates (32-64 mm in diameter). Benthic samples were stored in 95% ethanol and the invertebrates collected were later identified to the lowest practical taxonomic level, primarily using the keys of Holsinger (1972), Williams (1972), Moulton and Stewart (1996), Merritt et al. (2008) and Morse et al. (2017). For most taxa, the lowest taxonomic level recorded was genus with the exception of Chironomidae, which were only identified to the family level. Amphipods were abundant in many samples, often numbering in the thousands. Individuals of *Gammarus* (Fabricius) and *Crangonyx* (Bate) were present in several samples, but a large proportion were tiny and difficult to confidently distinguish so these two genera were grouped together for statistical analysis. Large samples with >2000 specimens were sub-sampled according to methods described by Bowles et al. (2008), using a round 500- μ m USGS sieve marked into quadrants. All subsamples were sorted at 25% and contained >500 specimens.

To characterize the odonate communities at each spring, I sampled once during each of three seasons, including summer (June/July 2018), fall (October 2018) and spring (March 2019). The rationale for sampling sites over three seasons was to aid identification of any species existing as early instar nymphs in one season and account for differences in developmental phenologies among resident species. Here, ‘resident’ refers to odonate species using the springs to complete their life cycles. This is opposed to an ‘occupant’, which is a species present at a spring for foraging or exploration for potential breeding habitat (Chovanec and Waringer 2001). Nymphs and exuviae were the focus of collection because their presence indicates habitat suitability for completing the life cycle. I recorded the presence of every species of adult

odonate, but only individuals breeding and ovipositing were used to indicate species residency. I collected nymphs using the qualitative multihabitat methods for sampling wadeable and nonwadeable streams described by Moulton et al. (2002) by sampling all microhabitats found in each sampling reach with a 500 μ m mesh D-frame kick net. Sites were qualitatively sampled for fifteen minutes during summer, following collection of benthic and environmental data. However, time spent sampling was increased to sixty minutes during fall and spring collecting to ensure thorough investigation of all microhabitats in each sampling reach for detection of rare or cryptic species. All odonates collected during Surber sampling were included in qualitative specimen counts. Exuviae were collected anywhere encountered while collecting nymphs. Collected nymphs and exuviae were stored in 75% ethanol. Whenever possible I collected at least one adult for each odonate species observed at a spring, including tandem pairs, to verify habitat use regardless of the presence or absence of nymphs of the same species. Adults were captured by aerial net and temporarily stored in glassine envelopes. Most adult odonates present at a spring were identified on the wing but some ambiguous specimens were collected for confident identification with adult keys (Abbott 2005). Collected adults were placed in acetone and bathed over a ~16 hour period following the end of fieldwork on the day they were collected. Dried specimens were subsequently stored in clear polypropylene envelopes with a note card containing date, locale, collector, and species information. Final species counts recorded for each spring included combined specimen counts for all collected odonate materials. The odonate materials used for each species total included all nymphs collected from three Surber samples, nymphs collected per hour sample effort using kick-nets, any exuviae collected along a streambank during kick-net sampling, and breeding or ovipositing adults recorded during fieldwork, for all three sampling seasons.

Standard Stream Metrics

I calculated four standard stream assessment metrics applied by Missouri Department of Natural Resources (MDNR) stream bioassessment procedures (MDNR 2014), including the Ephemeroptera, Plecoptera and Trichoptera Taxa index (EPTT), the total taxonomic richness index (TTR), a biotic index (BI) and the Shannon Diversity index (SDI). Each metric was calculated using general benthic community data. These four metrics are combined by MDNR to create a macroinvertebrate stream condition index (MSCI) score used to compare reference streams with non-reference streams in the state. Higher values of EPTT, TTR and SDI are considered healthier, but for BI, lower values are healthier. The EPTT is calculated by summing the total number of distinct taxa representing the EPT orders at each site. The TTR is similar but sums the total of all distinct taxa. The BI assigns tolerance values reported in MDNR (2016) and is calculated with a weighted average equation equivalent to that used by Chutter (1972) and Hilsenhoff (1977),

$$\text{Equation 1: } BI = \frac{\sum X_i T_i}{n}$$

where X_i is the number of individuals of taxon i , T_i is the tolerance value assigned to that taxon, and n is the total sample size for all taxa in the sample that have tolerance values. Shannon diversity integrates richness and evenness of all taxonomic groups at a site and follows the equation,

$$\text{Equation 2: } SDI = - \sum_{i=1}^S (p_i)(\ln p_i)$$

where S is the total number of taxa in the sample, and p_i is the proportion for each taxon i .

Statistical Analyses

I used the statistical software package R version 3.5.2 (R Core Team 2018) to conduct all statistical analyses. I performed MANOVA (with $\alpha = 0.05$) to look for differences in non-vegetation related environmental variables among site classes and investigated relationships between vegetation-specific environmental variables (RipC, LSRC, CC, EmVC) and non-vegetation specific variables using Pearson correlation analysis. I adjusted α for multiple comparisons using the Holm-Bonferroni method.

I used the ‘vegan’ package (Oksanen et al. 2019) to run multivariate analyses aimed at uncovering differences in the structure of communities defined in three different ways: odonates, general benthic community (all taxa), and the insect component of the general benthic community. I examined the insect-only component of the general benthic community in case it responded differently compared to the two communities of focal interest. To examine the variability of all three communities across study sites, I plotted cluster dendrograms from pairwise Bray-Curtis dissimilarity matrices using the *vegdist* function.

I used the *metaMDS* function in the vegan package to perform nonmetric multidimensional scaling (NMS) analyses to further assess variation in community structure for the three community types across the three riparian-associated site classes. Input data for NMS were square-root transformed total abundance of each taxon standardized by Wisconsin double standardization with Bray-Curtis dissimilarities. The best configuration for each NMS ordination was determined by selecting the analysis with the lowest stress value after twenty random starts. Bi-plots to visualize NMS results were half-change scaled, rotated so that the first axis explained the most variance, and scaled so the origin of ordination was the average of both axes. The closeness of site scores in ordination space reflects how similar their communities are relative to

one another. I performed multiple response permutation procedure (MRPP) analyses using Bray-Curtis distance with 9999 permutations to identify whether there were consistent community differences among the three site classes for each type of community analyzed by NMS (general benthic community, insect-only benthic community, and odonate community). Then I plotted 95% confidence ellipses in each of the three NMS bi-plots around each class of sites in ordination space.

I combined all raw environmental variable data (Disch, pH, ORP, DO, Cond, TDS, Temp, NO₃, PO₄, RipC, CC, EmVC, LSRC, Embed, and Substrate) into an additional matrix and then fitted these data as vectors to community ordinations based on correlation coefficients between these data and each ordination. Before plotting the vectors, they were scaled by their correlation so that the length of each arrow represents the predictive strength of its corresponding variable (longer arrows are “stronger” predictors). The direction of a vector represents the gradient along which its corresponding environmental variable experiences the most rapid change. Significance of the vectors was assessed following 9999 random permutations of the data (Oksanen 2015). I plotted environmental vectors on the NMS bi-plots for any variable with significance at $\alpha < 0.1$.

To determine whether any odonate species was strongly associated with springs of a particular riparian class I used the ‘indicspecies’ package (De Cáceres and Legendre 2009) to run an indicator species analysis (ISA). I used untransformed abundance data for odonate species that had multiple detections among sites and across sampling events ($n=13$) in ISA. Using the *multipatt* function, I calculated point biserial correlation coefficients (r_{pb}) for each species in the ISA, which are Pearson correlations that compare the abundance of a species within a site class

or grouping of site classes to its abundance within every other site class (De Cáceres and Legendre 2009).

In order to evaluate differences among riparian site classes for each of the four standard stream metrics, I performed one-way ANOVAs with a Tukey post-hoc test. I then examined Pearson correlation matrices to identify relationships between the four stream metrics and individual, vegetation-specific variables. I created the correlation matrices with the ‘PerformanceAnalytics’ package (Peterson and Carl 2019), which plots Pearson’s product moment correlation coefficients above the matrix diagonal and bivariate scatterplots with a fitted line below.

Contingent upon the findings of the aforementioned multivariate analyses and standard biomonitoring metrics, I wanted to develop a more practical metric for bioassessment of stream riparian zones in case the standard stream metrics were not useful. Indeed, no clear relationships were discovered between any of the four standard metrics and riparian variables. Because odonate communities showed the closest relationship to riparian variables among the three types of communities assessed, I took a first step towards establishing tolerance values for an odonate-specific biotic index that assesses local riparian conditions along Ozark spring streams. Several approaches have been used to calculate tolerance values from biological survey data. I chose a simple but effective method, weighted averaging, to create tolerance values that should accurately reflect species-level sensitivity to an environmental stressor. I created tolerance values by weighted averaging using local riparian condition (RipC) estimates and the total number of individuals of each odonate species collected. RipC varied greatly along the gradient of my study sites, so weighted averaging using this variable was an informative approach for calculating species-specific tolerance values. The weighted averaging equation I used to calculate tolerance

values is modified from Yuan (2006) as follows,

$$\text{Equation 3: } WA_j = \frac{\sum_{i=1}^N (Y_{ij} X_{in})}{\sum_{i=1}^N (Y_{ij}) M}$$

where N is the total number of sites, Y_{ij} is the absolute abundance for a species j at a site i , X_{in} is the value for an environmental variable n (here, RipC) at a site i , M is the total number of environmental variables evaluated (just one here, see Discussion) and WA is the weighted average value for species j . I divided the WA for each species by ten and subtracted from ten to produce tolerance values ranging from 0 to 10, where 0 represents the lowest tolerance value (intolerant species) and 10 represents the highest tolerance value (tolerant species). This approach was used to keep the calculated tolerance values consistent with those reported in familiar biotic indices (Chutter 1972; Hilsenhoff 1977) where lower values typically represent higher sensitivity of a taxon to the variable of interest.

RESULTS

Physical Habitat

Many instream physicochemical habitat variables spanned a wide range across field sites, with exception of pH and ORP (Table 2). Temperature was similar (16.88 ± 3.31 , mean \pm standard deviation) across all but one site, Doling spring, where the sampling reach was located well downstream of the source allowing this shallow stream to warm considerably as it flowed through a concrete channel. Dissolved oxygen was variable across springs, ranging from 52.9% saturation at East Ritter spring to 107.1% saturation at Valley Water Mill spring. Specific conductivity ranged from 316 to 616 $\mu\text{S}/\text{cm}$ and TDS from 158 to 308 mg/L, where Patterson and Gilbert springs had the lowest measurements and East Ritter spring had the highest. Substrate size across springs was similar (Table 2), falling within the small to large pebble range on the Wentworth scale. Most springs had high to moderate embeddedness ($70.83 \pm 23.43\%$, where 100% equates to fully embedded substrate), except for Patterson spring which had the least embedded substrate. Nutrient concentrations, nitrate and orthophosphate, varied considerably across sites (12.83 ± 5.12 , 0.03 ± 0.04 , mg/L concentrations respectively). In spite of this substantial among-site variability in many physicochemical variables, MANOVA analysis did not identify any differences among the three riparian-associated site classes.

Vegetation-specific habitat variables including local (RipC) and landscape (LSRC) scale riparian conditions, canopy cover and emergent vegetation cover were correlated with one another. Higher RipC estimates were usually associated with higher LSRC estimates ($r = 0.62$, $P = 0.031$) and greater canopy cover ($r = 0.80$, $P = 0.0019$). Emergent vegetation cover generally decreased with increasing canopy cover ($r = -0.59$, $P = 0.042$) and LSRC ($r = -0.63$, $P = 0.029$).

Excluding RipC, which site classes were directly based on, only canopy cover ($F = 4.765$, $Df = 2,9$, $P = 0.038$) was significantly different among site classes. The four minimally impacted sample sites had primarily intact local riparian zones and likewise, mostly intact riparian vegetation at the broader spatial scale (LSRC) and 50% or greater canopy cover over their sampling reaches (Table 3). Their channels were occupied by $\leq 50\%$ emergent vegetation. Moderately impacted sites had RipC estimates between 40 and 65% and LSRC estimates higher than their corresponding RipC estimates, with the exception of Double spring (LSRC = 10%). Moderately impacted sites also had $\leq 50\%$ canopy cover and a wide range of emergent vegetation cover (Table 3). Silver, Doling and Brown springs were heavily impacted with RipC ranging from 0 to 20%. Silver and Doling springs also had an LRSC of 0 and 10%, respectively, whereas Brown spring had a LSRC of 80%. Doling spring had a more developed canopy (50%) around the spring than Silver and Brown springs (0%). Emergent vegetation peaked at Silver spring (90%), while Doling and Brown springs had emergent vegetation occupying approximately one third of their channels.

Spring Communities

Eighty-five unique taxa were identified from general benthic community Surber samples collected across the 12 springs. Dominant orders were Diptera (24 taxa), Coleoptera (14), Ephemeroptera (13) and Trichoptera (13). Other orders with just two or three taxa each were Plecoptera, Megaloptera, Odonata, and Hemiptera. Additionally, nine non-insect taxa were present (Appendix). Several of the non-insect taxa were infrequently encountered in benthic samples, but amphipods and isopods were occasionally the overwhelmingly dominant representatives in a sample.

After three seasons of kick net sampling the odonate communities from all instream microhabitats at each site I identified twenty-two resident odonate taxa represented by eleven zygopterous and eleven anisopterous species (Table 4). Ten resident odonate taxa occupied the minimally impacted sites, fourteen occupied moderately impacted sites, and six occupied highly impacted sites. A single odonate nymph identification was left at the generic level. It was an early instar nymph in the genus *Gomphurus* (Needham) which belonged to either *G. ozarkensis* (Westfall) or *G. vastus* (Walsh). The nymph was found in a Surber sample taken at Mitch Hill spring and was the only nymph in the family Gomphidae found during the study.

Cluster analysis of the 12 odonate communities showed three broad clusters (Figure 2). All minimally impacted sites fell within the largest cluster, along with two moderately impacted sites. All sites within this cluster were represented by a high abundance of *Calopteryx maculata* (Palisot de Beauvois). The smallest cluster includes Brown (highly impacted) and Chiles (moderately impacted) springs, which together had the highest densities of *Ischnura posita* (Hagen). The final cluster contained the remaining two highly impacted springs and two faunistically similar moderately impacted sites. Silver and Doling springs had a high abundance of *Argia plana* (Calvert) while Double and Crane Creek springs had a moderate abundance of this species. All four of these springs with moderate to high abundances of *Argia plana* had dense patches of *Nasturtium officinale* (W.T. Aiton). Results from cluster analysis of the general benthic communities were best explained by relative abundances of peracaridan crustaceans and proximity between sites rather than riparian condition. Cluster analysis of the insect-only component of the benthic communities was difficult to interpret with no obvious taxonomic patterns between the most closely related sites.

Stress decreased with each NMS ordination and clusters of sites according to the three categories of riparian impactedness became increasingly differentiated (i.e., general benthic communities > total insects > odonates) (Figure 3). Multi-response permutation procedure revealed a significant difference among site classes only in the odonate community ordination (MRPP, $A = 0.1187$, $P = 0.04$). In the odonate community ordination, the minimally impacted sites were significantly different from moderately impacted (MRPP, $A = 0.1111$, $P = 0.04$) and highly impacted sites (MRPP, $A = 0.2137$, $P = 0.01$). MRPP showed no significant differences among site classes in the general benthic and insect-only benthic communities (MRPP, $A = 0.03671$, $P = 0.187$ and $A = 0.01609$, $P = 0.307$, respectively). Plotted 95% confidence ellipses in Fig. 3 help visualize these MRPP results.

Environmental variables significantly associated with multivariate community structure varied according to community type. Variation among general benthic communities was best described by temperature ($r = 0.77$, $P = 0.0066$) and to a lesser extent landscape riparian conditions ($r = 0.64$, $P = 0.0759$). When assessing only the insect taxa within benthic samples, local ($r = 0.75$, $P = 0.0239$) and landscape ($r = 0.74$, $P = 0.0274$) riparian conditions were more strongly correlated. The odonate community was strongly correlated with local riparian conditions ($r = 0.90$, $P = 0.0003$), followed by canopy cover ($r = 0.76$, $P = 0.0193$), landscape riparian condition ($r = 0.73$, $P = 0.0246$) and emergent vegetation ($r = 0.63$, $P = 0.0985$). Most vegetation-related fitted vectors correlated more strongly with finer community examination (Table 5), suggesting that these variables better explain variation among odonate communities than general benthic communities.

Among the 22 collected species of odonates, only two were significant indicators of riparian class in the indicator species analysis. The dragonfly *Cordulegaster obliqua* (Say) was

detected at each minimally impacted site on at least two sampling occasions and it was a significant indicator species ($r_{pb} = 0.84$, $P = 0.0071$) for springs with minimally impacted local riparian conditions. The damselfly *Argia plana* was also a significant indicator species ($r_{pb} = 0.61$, $P = 0.048$), but for springs with highly impacted local riparian conditions, despite its apparent absence from the highly impacted Brown spring. None of the other odonate species included in the indicator species analysis were determined significant indicator species for site classes (Table 6).

Standard Metrics and Tolerance Values

Biotic index scores fell within a narrow range (7.16 ± 0.64) (Table 7) across all 12 springs. Silver spring had the most highly impacted riparian conditions of any study site and had the highest BI at 8.14 while a moderately impacted site, Double spring, had the lowest BI at 6.12. Double spring also had the highest Shannon diversity, EPTT, and TTR (2.77, 20, and 47, respectively). Valley Water Mill spring had the lowest TTR with 13 taxa and the lowest EPTT along with Silver spring having just one EPT taxon (*Stenonema femoratum* (Say) and *Baetis flavistriga* (McDunnough), respectively). Shannon diversity was lowest at the minimally impacted West Ritter spring (SDI = 0.59) and second lowest at the moderately impacted Patterson spring (SDI = 0.69). Amphipods were the overwhelmingly dominant taxon at both of these sites, but Patterson spring also had high abundance of the mayfly *Acentrella turbida* (McDunnough) and Chironomidae. None of the standard metric scores correlated significantly with any of the vegetation-specific variables (Figure 4). Additionally, one-way ANOVA did not identify any significant differences between site classes for any of these metrics (Figure 5).

Odonate species tolerance values associated with riparian structure ranged from 0.65 to 8.51 (Table 8). *Argia plana* received the highest tolerance value because this species was most abundant in springs with highly impacted riparian zones (as demonstrated by the indicator species analysis). *Argia translata* (Hagen in Selys) also had a high tolerance value, but this species was only present at Doling spring. *Cordulegaster obliqua* had the lowest tolerance value because it was only found in springs with fully intact local riparian zones. Four other species, *Somatochlora tenebrosa* (Say), *Aeshna umbrosa* (Walker), *Basiaeschna janata* (Say) and *Boyeria vinosa* (Say) also had low tolerance values. The latter three species only occurred as residents at Mitch Hill spring. *Somatochlora tenebrosa* was a resident at the minimally impacted East Ritter and Mitch Hill springs, but one larval specimen occurred in samples from the moderately impacted Valley Water Mill spring. Forktail species (*Ischnura* spp.) occurred at moderately to highly impacted springs. The bluets (*Enallagma* spp.) only occurred in the same moderately impacted springs where *Ischnura* (Charpentier) was found. *Erythemis simplicicollis* (Say) was resident at Brown and Chiles springs. *Calopteryx maculata* occurred as a resident species at all but Silver and Chiles springs and was overall most abundant in springs with less impacted local riparian conditions so it received a relatively low tolerance value.

DISCUSSION

The twelve spring streams in this study presented a catholic array of both instream and riparian conditions, in addition to odonate communities. Despite high variability among abiotic instream conditions across sites, I did not find evidence that the general benthic communities responded to water chemistry and substrate size beyond a significant response to temperature. Additionally, odonate communities showed no response to abiotic variables and the standard stream metrics that I applied to general benthic community data were not useful for characterizing the state of the riparian zone. Aquatic macroinvertebrates usually occupy specific types of microhabitats in streams (Downing 1991) and routine benthic sampling in North American streams is often targeted at a narrow range of microhabitats best represented by specific taxa (i.e., EPT), so it can often miss much of the odonate community. However, the odonate communities that I sampled across multiple habitat types at each site were more strongly associated with riparian conditions than general benthic communities sampled using a typical stream bioassessment method. Odonates were even more sensitive to riparian conditions than the insect-only component of general benthic communities, supporting my prediction that odonates respond more strongly to the riparian structure around streams than to other physical or chemical instream variables. While odonates only make up a small component of the biotic community in a stream, they appear to be a reliable indicator for riparian condition and because they are much easier to identify to species than other benthic macroinvertebrates, odonates may be the most appropriate indicators for riparian bioassessment. The behavior of odonates by which they use vegetation not only for cover, like other aquatic insect taxa, but for perching when hunting and establishing breeding territories (Corbet, 1999) may contribute to my observations. Sampling a

broad range of microhabitats available at a site was necessary in order to characterize entire odonate communities. Only two odonate species were detected from benthic samples taken across all twelve sites, but twenty-one species were identified from kick-net samples from the various microhabitats throughout each sampling reach. Because many odonate taxa are infrequently collected in standard benthic collections for bioassessment, these results have strong implications for future management and bioassessment of riparian ecosystems.

Community Responses to Riparian Condition

Benthic communities characterized at the general and insect-specific levels could not be well differentiated according to their respective riparian site classes from ordination analysis, however, some vegetation-specific variables were related to community structure at both of these levels. General benthic community structure appeared to be most closely associated with water temperature. Sornom et al. (2010) found that temperature and salinity had significant effects on mortality and respiratory ventilation in *Gammarus roeseli* amphipods. Temperature may be the most sensitive measured variable for the Crustacea that often comprised a major proportion of spring communities in this study. NMS analysis of only the insects within the general benthic community revealed a significant relationship with local and landscape riparian conditions but these communities could not be separated by site classification using MRPP. Surprisingly, general and insect-only benthic community NMS analyses did not show significant responses to other in-stream environmental variables. However, all measured vegetation-related variables were significantly associated with odonate community structure, hence the distinct separation of odonate communities among site classes across the riparian gradient. The odonate community

also correlated most strongly with local riparian conditions and was the only community that showed a significant response to canopy cover.

Odonate Responses to Riparian Conditions

Riparian condition is likely important to odonate communities both directly (as visual cues for adults) but also indirectly, in that the heterogeneity of instream habitat important for the nymph stage is also strongly influenced by riparian condition. Local riparian conditions in particular (especially within ~2m of the streambank) will directly affect the abundance of many of the instream habitats at most springs. For example, springs with minimally impacted riparian zones often had more trees along the streambank and as a result, more root-wad and overhanging bank habitat. This is likely a key factor dictating species-specific sensitivities to local riparian conditions because riparian vegetation structure can greatly influence instream conditions that are optimal for larval development (Gregory et al. 1991). *Argia* spp. were usually within the benthos or thick bundles of emergent-vegetation, *Calopteryx maculata* were more abundant in root-wads than other microhabitats, *C. obliqua* were frequently buried in fine sediments, *Enallagma* and *Ischnura* spp. were typically clinging to in-stream vegetation, aeshnids were most common along wetted-edges characterized by tall grasses, and emeralds (*Somatochlora* and *Epitheca*) were found beneath undercut banks and in root-wads. Fourteen odonate species were identified in moderately impacted sites whereas six and ten species were identified from highly and minimally impacted sites, respectively. Higher odonate species richness observed at the moderately impacted sites of this study may also be attributable to a wider array of suitable in-stream microhabitat diversity. Local riparian conditions at these sites

may be deemed acceptable by adult odonates that usually prefer conditions nearer one end of the riparian gradient.

Odonate communities varied considerably across the 12 sample springs, but their multivariate dissimilarities were likely driven largely by the two most abundant species, *A. plana* and *C. maculata*. These species were often either mostly absent from springs or the dominant taxon at springs. In springs where *A. plana* was abundant, there were dense patches of *N. officinale* which may serve as a preferred substrate for oviposition of *A. plana* (Bick and Bick 1972), but *N. officinale* was present at every spring and had dense patches in some springs where *A. plana* was rare, like Patterson spring. All anisopterous species, with exception of *E. simplicicollis* were restricted to the springs falling into the largest cluster. The springs belonging to this cluster have more shading than springs in the other clusters, save Doling spring, with 50% or more canopy cover. This observation conflicts with the findings from Miguel et al. (2017) who studied odonate communities in streams with variously impacted riparian zones in Brazil. There, anisopterous species were more common at streams with more exposure to sunlight resulting from environmental degradation, and zygopterous species tended to prefer more shaded streams with natural vegetation. In my study there were several zygopterous species like *A. plana* that were most abundant in less shaded springs, while Miguel et al (2017) observed most *Argia* spp. seeming to prefer or largely tolerate well shaded streams in Brazil. However, there was one particularly exceptional zygopterous species observed in my study, *Calopteryx maculata*, that mostly preferred minimally impacted riparian conditions with ample shade. Biogeographical differences between ecoregions (ie. temperate and tropical zones) observed here and in Brazil, may explain why members of each suborder had opposite environmental responses between studies.

Compared to dragonflies, damselflies tend to have small bodies and spend most of their time as adults perched on vegetation, so it is presumed that they rely largely on climactic conditions and habitat structure for thermoregulation (Miguel et al. 2017). The larger dragonflies include many species that spend most daylight hours on the wing and those that do are believed to achieve thermoregulation chiefly by heat generation from flight muscle activity (Corbet and May 2008). Most of the resident anisopterous species reported here are fliers, excluding *E. simplicicollis* and *Sympetrum corruptum* (Hagen), which spend much of their time perched when active, so perhaps these exceptions can thermoregulate sufficiently in sunnier environments. At least three anisopterous species observed during this study, including *A. umbrosa*, *B. vinosa* and *S. tenebrosa*, have been shown to prefer shaded habitats (Abbott 2005). Immediately following emergence, odonates typically disperse from their natal habitat to forage and mature before returning to a suitable breeding habitat (Corbet 1980). I have observed many of these anisopterous species foraging in open habitats that are nearby shaded streams they breed in, as opposed to streams with more open canopies, so shading may be an important factor influencing their residence in spring streams.

The Aeshnidae species observed at Mitch Hill spring were not observed as resident species at the other minimally impacted springs, but I suspect that *B. janata* and *A. umbrosa* may be occasional residents of either West or East Ritter springs. In spring, I found emerging *B. janata* about two kilometers downstream of the spring branch where the Ritter springs merge while scouting potential field sites. I also observed multiple *A. umbrosa* flying alongside both Ritter springs during fall sampling. Gilbert spring (also minimally impacted) probably lacks all three aeshnid species resident at Mitch Hill spring because they are endophytic ovipositors and Gilbert spring lacked emergent vegetation except for a couple sprigs of *N. officinale*.

The two springs in the smallest cluster from the cluster analysis (Figure 2) had high counts of *Ischnura* spp., which is likely a result of both springs containing calm stretches along their channels with similar types of emergent vegetation, including floating algae and duckweed as substrates for adult oviposition (Paulson 2011). These hydrologic and vegetation characters were not exclusive to these springs. *Ischnura* spp. occurred at most springs that had quiet stretches within their sampling reach with floating or emergent vegetation. The middle cluster in figure 2 was particularly characterized by springs dominated by *Argia plana* and contained little or no canopy cover, typically lots of emergent vegetation, and mostly shallow sampling reaches. The degree to which all of these environmental conditions might influence residency for *A. plana* is befuddling in large part because breeding adults were observed at the minimally impacted Mitch Hill and Patterson springs, which also had some sunny, shallow and well-vegetated portions of their sampling reaches. Despite breeding activity from adults around otherwise ideal-looking habitat, the nymphs were never detected at these springs. I also observed one female *A. plana* ovipositing into moss on an exposed rock in the first riffle habitat of the minimally impacted West Ritter spring during summer. But again, in spite of rigorous efforts to locate nymphs within this stream over the course of three seasons, none were collected.

One explanation for the apparent absence of *A. plana* nymphs at West Ritter spring is possible predation from the incredibly abundant amphipods, of which mature specimens appeared to be *Gammarus*. These amphipods were less abundant in other springs that had *A. plana* and while often regarded as shredders/collectors, Kelly et al. (2002) ran experiments that show these crustaceans to be facultative predators and they showed a preference for *Baetis* (Leach) mayflies over leaf litter when offered both as a food source under laboratory conditions. Gammarid amphipods can be found by the dozens in clumps of submersed mosses in several of

the springs I sampled, and I would expect them to be capable of feeding on eggs or very early instars of *A. plana*. Incidentally, the amphipods may also be capable of dislodging eggs while scavenging for food, leaving eggs vulnerable to currents. *Argia plana* was determined an indicator species for highly impacted riparian zones in this study but did not occur at the highly impacted Brown spring which mostly lacked shallow reaches and had the highest abundance of amphipods from any spring. A shortage of shallow habitat within this spring's channel or amphipod abundance were perhaps responsible for its absence as a resident during sampling, but adults were observed around the spring's margins. Breeding adults were observed at Patterson and Mitch Hill springs, but I never witnessed oviposition, nor detected larvae. Patterson spring had plenty of shallow habitat with dense patches of *N. officinale*, but also high densities of amphipods. Mitch Hill had sparse patches of *N. officinale* near shallow margins and lower densities of amphipods. Some species are well adept at colonizing suboptimal habitats, but are ill-adapted for handling interspecific interactions that are more likely to be encountered in more optimal locations (Connell 1961). Some aquatic insect taxa have been identified that can tolerate a wide range of habitat conditions but are poor competitors, restricting their distributions to harsh extremes of environmental conditions (eg. Flory and Milner 2000; Madsen et al. 2015). In the case of *Argia plana*, nymphs may fail to thrive in springs where prime nymphal habitats are overcrowded by high abundances of Gammarid amphipods (ie. interspecific interactions).

Cordulegaster obliqua was an indicator for minimally impacted springs and the nymphs in this genus are burrowers in fine sediments (Tennessen 2019). All springs in this study had either sand, silt or clay substrates dispersed throughout their sampling reaches, and nymphs for this species were discovered in all three types of fine sediments. The fine sediments occupied by *C. obliqua* nymphs are typically overlooked during most standard benthic sampling practices

where there is usually preference for larger substrates that provide more interstitial space for other benthic taxa (particularly EPT). Regardless of quantity or type (clay, silt, sand) of nymphal habitat, this species was a resident at every minimally impacted site, and I failed to detect any in highly to moderately impacted sites even as non-breeding adults. There are two past records of a rarer Ozark species within this genus, *Cordulegaster maculata* (Selys), that had been recovered as mature nymphs from Mitch Hill spring (personal data, 2017), but it was never observed in the current study. I suspect that both species of *Cordulegaster* within the Ozarks are among the most sensitive dragonflies with respect to riparian integrity because they seek primarily pristine habitats for reproduction. It appears as though favorable environmental conditions for adult *Cordulegaster* are more important dictators of species distribution than other factors like presence of nymphal habitat. Adults may depend on undisturbed, forested riparian zones as foraging or breeding habitat, protection from predators, or other reasons not yet understood.

Bioassessment with the General Benthic Community

Because standard bioassessment approaches may possibly be related to riparian structure, an additional component of my research included running four standard stream metrics. I achieved this by using the common approach for wadeable streams in the state of Missouri and the four standard metrics I used to characterize benthic macroinvertebrate stream conditions at my field sites were not useful for distinguishing the three riparian site classes. Additionally, no vegetation-specific environmental variables were significantly related to metric estimates after analyzing multiple Pearson correlations, which can be explained by a wide range of estimates for each metric across the 12 study sites. A few springs had particularly high or low metric estimates for which there are no obvious explanations. For example, Double spring was a moderately

impacted site that had the lowest BI score and the highest SDI, EPTT and TTR scores, but there were no particularly outstanding environmental measurements or other noteworthy differences distinguishing this spring from the others. Valley Water Mill and West Ritter springs had the lowest TTR scores (13 and 14, respectively) with few insect taxa, a pattern that might be attributable to their rather high conductivity. The vast majority of insect specimens collected from Valley Water Mill spring were chironomid larvae, while the West Ritter spring insect count was dominated by elmids larvae. Clements et al. (2016) conducted experiments using representatives from both of these taxa that occur in montane streams and found that both taxa were moderately tolerant to elevated conductivity levels. Valley Water Mill spring also had a much higher DO value than any other spring, but with no other outstanding environmental parameters, it is uncertain why this spring lacks many non-odonate insects.

Generally, instream variables were wide ranging across field sites, independent of riparian conditions. Consequently, general benthic community patterns were not expected to, nor did they vary strongly with respect to the riparian gradient. There was some disparity among water chemistry including parameters related to dissolved ions or dissolved oxygen but these parameters were not skewed by the condition of a site's riparian zone. Jones et al. (1981) calculated BI and SDI for nearly pristine to grossly enriched surface-fed Ozark streams within and around the same study region. The BI estimates they reported highly correlated with nutrient levels in their study streams. In my study, nutrient data was measured with test kits that use a color-wheel indicator to provide a rough estimation of nutrient concentration. The estimates I recorded were larger than are likely realistic but because all testing was performed uniformly across field sites, they should be comparable. Some Ozark streams are known to receive high fluxes of nutrients in the summer months, including Brown Spring in this study (OWW, 2019),

so while the nutrient concentrations reported here are high, they should scale down to their realistic concentrations evenly and should have no dubious effect on comparative analysis. Additionally, there were no significant correlations made between nutrient variables and community compositions.

Most benthic samples were taken near the source of each spring, but as noted previously, some springs had samples taken further from the source due to either ease of access or more favorable sampling habitat. This may have produced confounding variables for benthic community characterizations from each spring. Carroll and Thorp (2014) observed ecotonal shifts in the diversity of benthic communities at three Ozark springs within the same study region. Changes included shifts from community dominance by peracaridan crustaceans (amphipods and isopods) near spring sources to dominance by insects further downstream from the source (up to 145 m), even though physicochemical variability throughout each spring's channel was negligible. Of note here is that Brown spring had the highest abundance of both amphipods and isopods (see Appendix) but was sampled further from its source than any other spring in this study with exception of East Ritter and Doling springs. East Ritter spring was sampled furthest from its source (240 m) of any of the sample springs and had the fourth highest abundance of amphipods, more so than springs sampled very near their source like Mitch Hill and Chiles springs, therefore it is difficult to attribute community differences I observed to distance of samples from spring sources. Additionally, water chemistry did not appear to vary with distance sampled from a spring's source, just as Carroll and Thorp (2014) observed in their study. Based on this observation and similar findings from other spring stream studies, Carroll and Thorp (2014) concluded that physicochemical variables had little effect on macroinvertebrate community composition in the spring streams they studied, further supporting

my own results that show almost no response to physicochemical variables by general benthic communities.

Bioassessment with Odonata

While Odonata have not been considered useful for standard bioassessment associated with organic pollution, the results I present here suggest that odonates may be especially useful for bioassessment of riparian conditions. I initiated the first steps in that pursuit by developing preliminary tolerance values based solely on local riparian condition estimates which was the environmental variable that odonate communities responded most strongly to. Future studies should analyze odonate community responses to riparian variables at several spatial scales and then combine whichever riparian variables are deemed essential to create more accurate tolerance values using equation 3. Additionally, tolerance values should be attained from a larger dataset produced over a longer time span and incorporates as many sample sites as possible. Another approach could mirror a biotic index proposed by Huggins and Moffett (1988) that incorporates six independent tolerance values calculated from six specific types of stream pollution. Their reasoning behind the creation of several tolerance values was that different aquatic taxa can be more or less sensitive to specific forms of stream pollution (i.e., not necessarily ‘organic pollution’). Perhaps additional riparian variables accounted from intensive evaluation of riparian conditions around sample sites can tease out fine-scale riparian characteristics that odonates respond to strongly.

The tolerance values that I created were specific to odonates using Ozark spring streams, so their effectiveness in other locations will need to be tested before they could be regularly applied elsewhere. Future sampling of other Ozark streams will also help fine tune the tolerance

values I created for accurate bioassessment throughout the region just as Hilsenhoff (1987) improved tolerance values for Wisconsin taxa after collecting data from many additional streams to bolster his original set of tolerance values (Hilsenhoff 1977). Ecoregion differences may greatly influence the effectiveness of odonates as riparian bioindicators. For example, Lenat (1993) developed tolerance values for benthic macroinvertebrates of the southeastern United States that sometimes contrasted to the ones created by Hilsenhoff, even among the same species. The Odonata are less species rich at higher elevations (Stevens and Bailowitz 2009) and probably would not be reliable bioindicators in most montane situations. Another extreme may include arid environments with streams that lack wide, well-vegetated riparian structure. These streams can sometimes become intermittent during dry seasons, but there exist odonate species adapted to living in these environments, including the desert southwestern US. It stands to reason that odonate species living in these extremes may have greater sensitivity to the thin riparian buffers that exist around their streams compared to streams found in temperate settings. As a result, the Odonata could still be useful bioindicators even in areas with extreme environmental constraints where natural riparian conditions take on an unusual configuration.

Odonates are shown here to be the most useful group in Ozark spring streams for riparian bioassessment. This new understanding should allow land managers to effectively monitor the quality of stream riparian zones without spending long hours processing and identifying general benthic taxa for use in standard stream metrics that are insufficient for thorough riparian bioassessment. In addition, tolerance values, like the ones I have created here, can be prepared for additional odonate species and improved upon with continued application of odonate bioassessment using a biotic index for riparian condition.

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Table 1: Location and general characteristics of study springs. Includes numeric values in meters (m) for the distance between the source of each spring to the beginning of the sampling reach (Source to Reach), the total length of each spring's sampling reach (Reach Length) and the estimated width of the local-scale riparian zone from the left and right streambanks (RipC Width).

Spring	Spring code	Spring Magnitude	State	County	Source to Reach (m)	Reach Length (m)	RipC Width (m)	Channel Morphology	Class of Riparian Impactedness
West Ritter	WR	3 rd	MO	Greene	16.5	68	13.6	riffle/run/pool	Minimal
Valley Water Mill	VWM	3 rd	MO	Greene	43.0	124	24.8	riffle/run/pool	Moderate
Silver	S	3 rd	MO	Greene	0.0	41	17.2	riffle/run	High
Patterson	P	3 rd	MO	Christian	72.0	68	13.6	riffle/run	Moderate
Chiles	CH	3 rd	MO	Greene	0.0	33	10.6	riffle/run/pool	Moderate
East Ritter	ER	3 rd	MO	Greene	260.0	62	12.4	riffle/run/pool	Minimal
Doling	D	4 th	MO	Greene	180.0	34	6.8	riffle/run/pool	High
Mitch Hill	MH	3 rd	AR	Newton	0.0	82	16.4	riffle/run/pool	Minimal
Gilbert	G	3 rd	AR	Searcy	11.0	74	14.8	riffle/run	Minimal
Double	DO	3 rd	MO	Christian	0.0	56	11.2	riffle/run	Moderate
Brown	B	3 rd	MO	Christian	140.0	54	10.8	riffle/run/pool	High
Crane Creek	CR	4 th	MO	Stone	0.0	24	4.8	riffle/pool	Moderate

Table 2: Physicochemical environmental variables used in ordination analyses. Variables listed here are discharge (Q), pH, oxidation-reduction potential (ORP), dissolved oxygen percent saturation (DO), specific conductivity (Cond), total dissolved solids (TDS), temperature (Temp), nitrates (NO_3^-), orthophosphates (PO_4^{3-}), embeddedness (Embed), and substrate D50 scores (Substrate). All measurements are mid-day readings during summer sampling.

Spring	Q	pH	ORP	DO	Cond	TDS	Temp	NO_3^-	PO_4^{3-}	Embed	Substrate
	(m^3/s)		(mV)	(%)	($\mu\text{S}/\text{cm}$)	(mg/L)	($^{\circ}\text{C}$)	(mg/L)	(mg/L)	(%)	(D50)
West Ritter	0.107	6.93	261.7	73.2	586	293	14.99	13.2	0.08	75	14.0
Valley Water Mill	0.082	6.88	277.7	107.1	596	298	16.50	8.8	0.00	50	14.5
Silver	0.030	6.93	308.3	70.1	499	250	17.37	8.8	0.04	100	13.0
Patterson	0.170	7.16	246.2	73.2	316	158	14.07	4.4	0.00	25	15.0
Chiles	0.029	7.03	313.9	72.9	434	217	14.54	17.6	0.14	75	15.0
East Ritter	0.039	7.05	204.4	52.9	616	308	16.24	13.2	0.04	75	14.0
Doling	0.006	7.59	231.7	56.8	594	297	26.63	8.8	0.00	100	16.0
Mitch Hill	0.100	6.77	328.7	62.1	457	229	15.73	13.2	0.00	75	15.0
Gilbert	0.033	7.47	264.5	84.3	317	159	18.27	8.8	0.06	100	14.0
Double	0.060	7.16	275.2	70.6	357	178	17.29	17.6	0.04	50	15.0
Brown	0.053	7.17	265.8	79.0	448	224	16.00	22.0	0.02	50	14.5
Crane Creek	0.009	6.86	321.1	58.9	405	203	14.96	17.6	0.04	75	14.0

Table 3: Vegetation-associated variables used in ordination analyses. Variables listed here are local riparian conditions (RipC), landscape riparian conditions (LSRC), canopy cover (CC) and emergent vegetation cover (EmVC). All measurements are estimations made during summer sampling. See Table 1 for spring code designations.

Spring	RipC	LSRC	CC	EmVC
	(% undisturbed)	(% undisturbed)	(% cover)	(% cover)
West Ritter	95	78	75	20
Valley Water Mill	65	65	50	70
Silver	0	0	0	90
Patterson	45	60	50	50
Chiles	40	50	25	20
East Ritter	95	80	50	50
Doling	20	15	50	35
Mitch Hill	85	100	50	10
Gilbert	100	75	100	0
Double	50	10	25	90
Brown	10	80	0	35
Crane Creek	40	75	0	60

Table 4: Odonate community data from resident larval detections made from multi-habitat and Slack Surber collections and breeding adult sightings at each spring. Counts represent larval abundance for one-hour-effort kick-net sampling from all different microhabitats within a sampling reach combined with any breeding/ovipositing adult detections and Surber data across 3 sampling seasons. See text (Methods) for more detail and Table 1 for spring code designations.

Species	WR	VWM	S	P	CH	ER	D	MH	G	DO	B	CR
<i>Aeshna umbrosa</i>	0	0	0	0	0	0	0	16	0	0	0	0
<i>Anax junius</i>	0	1	0	0	0	0	0	0	0	0	0	0
<i>Argia plana</i>	1	0	244	2	0	0	85	2	0	51	0	48
<i>Argia translata</i>	0	0	0	0	0	0	6	0	0	0	0	0
<i>Basiaeschna janata</i>	0	0	0	0	0	0	0	7	0	0	0	0
<i>Boyeria vinosa</i>	0	0	0	0	0	0	0	4	0	0	0	0
<i>Calopteryx maculata</i>	19	33	0	24	0	42	18	18	28	10	2	2
<i>Cordulegaster obliqua</i>	14	0	0	0	0	2	0	9	8	0	0	0
<i>Enallagma basidens</i>	0	25	0	0	0	0	0	0	0	0	0	0
<i>Enallagma divagans</i>	0	0	0	0	0	0	0	1	0	0	0	0
<i>Enallagma geminatum</i>	0	6	0	0	8	0	0	0	0	0	0	0
<i>Enallagma signatum</i>	0	3	0	0	16	0	0	0	0	0	0	0
<i>Enallagma vesperum</i>	0	1	0	0	0	0	0	0	0	0	0	0
<i>Epitheca cyanosura</i>	0	0	0	0	0	0	0	1	0	0	0	0
<i>Erythemis simplicicollis</i>	0	0	0	0	3	0	0	0	0	0	1	0
<i>Gomphurus</i> sp.	0	0	0	0	0	0	0	1	0	0	0	0
<i>Ischnura hastata</i>	0	0	0	0	4	0	0	0	0	0	0	0
<i>Ischnura posita</i>	0	13	0	0	22	0	5	0	0	0	43	0
<i>Ischnura verticalis</i>	0	2	0	0	18	0	0	0	0	0	2	0
<i>Libellula incesta</i>	0	1	0	0	0	0	0	0	0	0	0	0
<i>Somatochlora tenebrosa</i>	0	1	0	0	0	11	0	2	0	0	0	0
<i>Sympetrum corruptum</i>	0	1	0	0	0	0	0	0	0	0	0	0

Table 5: Correlation coefficients (*r*) and *P*-values for fitted vectors in NMS bi-plots produced from permutation procedure (*n* = 9999 permutations). See Tables 1 and 2 for explanation of variable abbreviations. Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1.

Fitted Vector	NMS A	NMS A	NMS B	NMS B	NMS C	NMS C
	<i>r</i>	<i>P</i> Value	<i>r</i>	<i>P</i> Value	<i>r</i>	<i>P</i> Value
RipC	0.57	0.158	0.75	0.0239*	0.90	0.0003***
LSRC	0.64	0.0759	0.74	0.0274*	0.73	0.0246*
CC	0.48	0.291	0.49	0.278	0.76	0.0193*
EmVC	0.49	0.287	0.67	0.0648	0.63	0.0985
Temp	0.77	0.0066**	0.58	0.151	0.17	0.908

Table 6: Results from Indicator Species Analysis for resident odonate taxa with multiple detections across sampling seasons. Point-biserial correlation coefficients are designated as (r_{pb}) and P-values were calculated using 9999 permutations. Class of impactedness refers to the site class each odonate species is an indicator for.

Odonate Taxa	r_{pb}	P value	Class of Impactedness
<i>Cordulegaster obliqua</i>	0.84	0.0071	Minimally impacted
<i>Argia plana</i>	0.61	0.048	Highly impacted
<i>Calopteryx maculata</i>	0.58	0.14	Minimally impacted
<i>Enallagma geminatum</i>	0.54	0.28	Moderately impacted
<i>Argia translata</i>	0.50	0.24	Highly impacted
<i>Somatochlora tenebrosa</i>	0.49	0.15	Minimally impacted
<i>Enallagma signatum</i>	0.44	0.48	Moderately impacted
<i>Ischnura posita</i>	0.42	0.32	Highly impacted
<i>Aeshna umbrosa</i>	0.42	0.57	Minimally impacted
<i>Boyeria vinosa</i>	0.42	0.57	Minimally impacted
<i>Ischnura verticalis</i>	0.38	0.73	Moderately impacted
<i>Enallagma basidens</i>	0.37	1.0	Moderately impacted
<i>Erythemis simplicicollis</i>	0.28	1.0	Moderate and Highly impacted

Table 7: Calculated standard metrics used by Missouri’s Department of Natural Resources when conducting water quality surveys. Metrics are abbreviated here for the biotic index (BI), the Shannon diversity index (SDI), the Ephemeroptera, Plecoptera and Trichoptera taxonomic richness index (EPTT) and the total taxonomic richness index (TTR).

Spring code	BI	SDI	EPTT	TTR
WR	7.40	0.59	3	14
VWM	8.00	1.41	1	13
S	8.14	1.14	1	24
P	7.64	0.69	4	28
CH	6.82	1.69	8	26
ER	6.93	1.06	3	21
D	6.61	1.95	5	20
MH	7.14	1.24	6	24
G	6.23	1.94	8	22
DO	6.12	2.77	20	47
B	7.53	1.31	16	41
CR	7.46	1.70	11	35

Table 8: Calculated tolerance values for resident odonate taxa with multiple detections in the present study. The tolerance value for each species is based on the local riparian condition estimates for all springs where that species was observed. Tolerance values are scaled 0-10 where 0 represents complete intolerance to riparian degradation and 10 represents complete tolerance to riparian degradation.

Odonate Taxa	Tolerance Value
<i>Aeshna umbrosa</i>	1.50
<i>Argia plana</i>	8.51
<i>Argia translata</i>	8.00
<i>Basiaeschna janata</i>	1.50
<i>Boyeria vinosa</i>	1.50
<i>Calopteryx maculata</i>	2.69
<i>Cordulegaster obliqua</i>	0.65
<i>Enallagma basidens</i>	3.50
<i>Enallagma geminatum</i>	4.92
<i>Enallagma signatum</i>	5.60
<i>Erythemis simplicicollis</i>	6.75
<i>Ischnura hastata</i>	6.00
<i>Ischnura posita</i>	7.28
<i>Ischnura verticalis</i>	6.04
<i>Somatochlora tenebrosa</i>	0.85

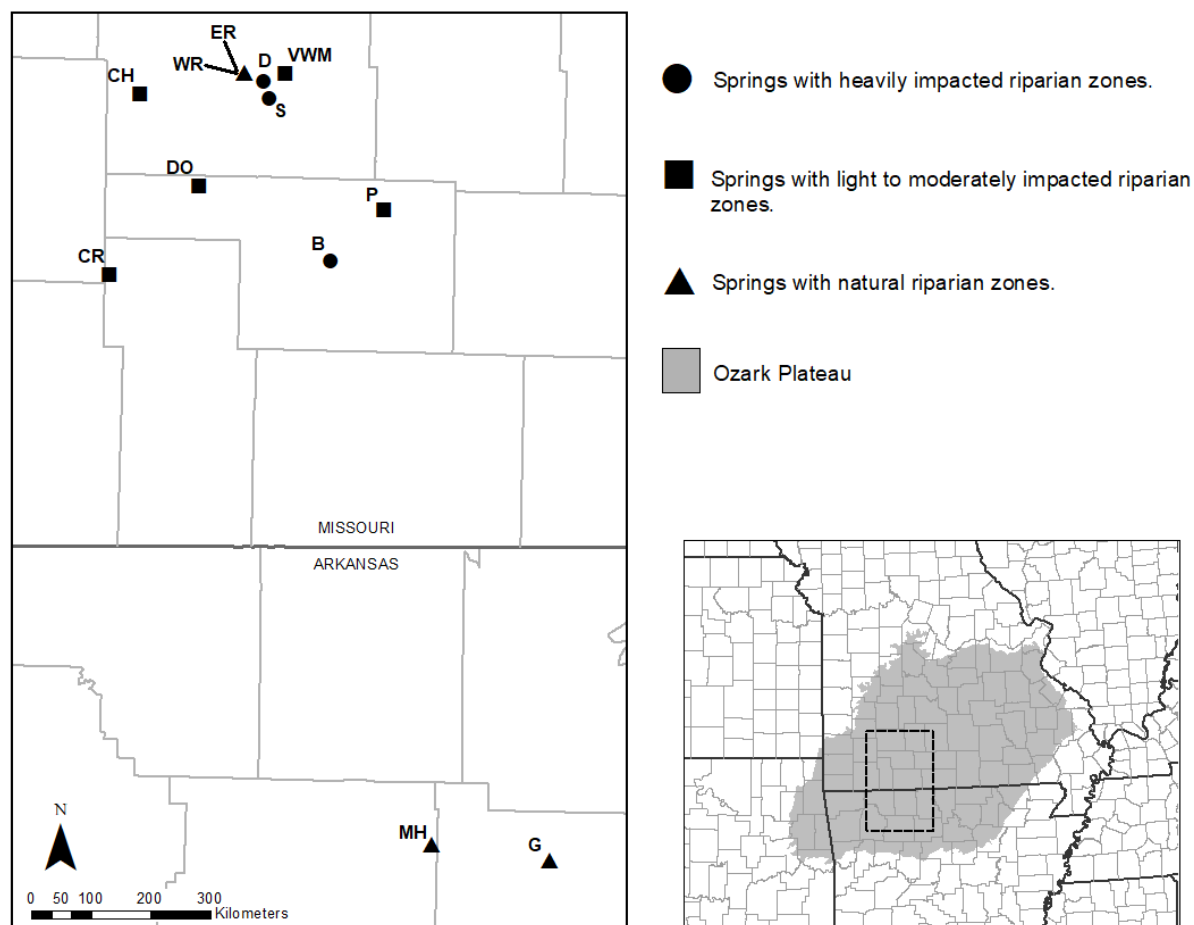


Figure 1: Map of the spring locations in the Ozark Plateau region of Arkansas and Missouri. This map was created with ArcMap 10.5.1 GIS software (ESRI 2012). Site abbreviations can be found in Table 1.

Odonata Dissimilarity Dendrogram

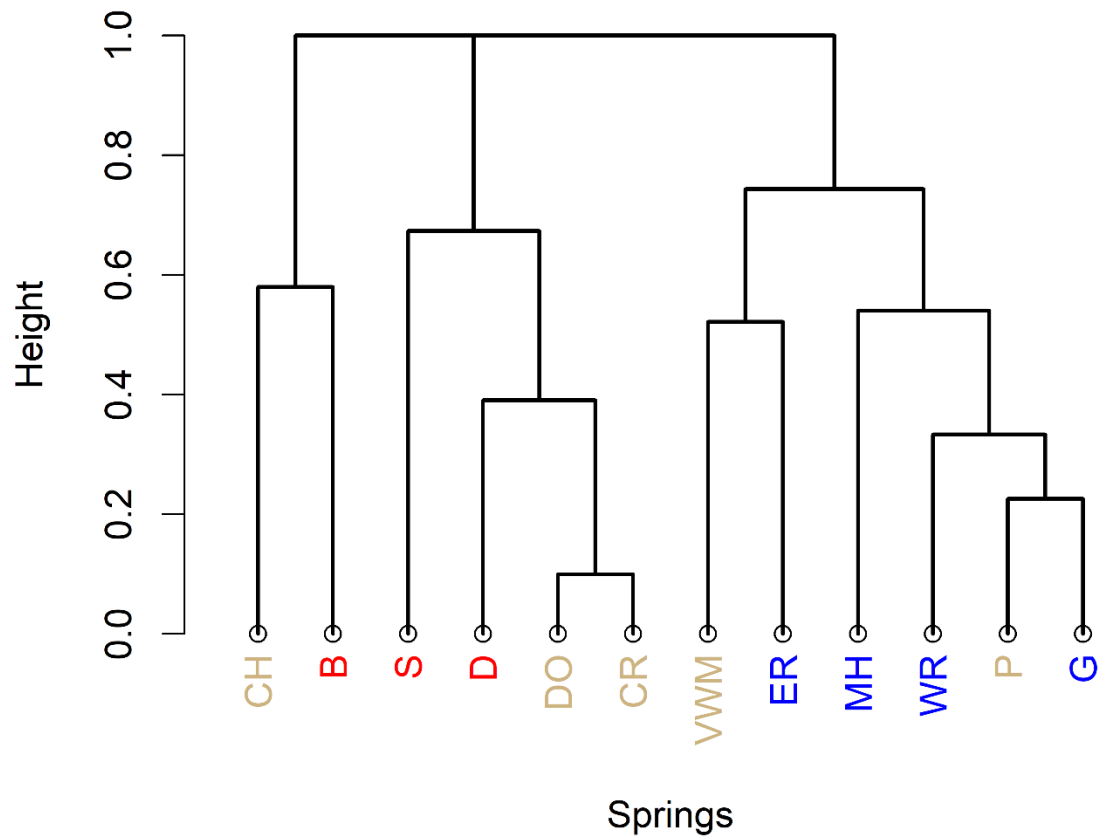


Figure 2: A Bray-Curtis dissimilarity dendrogram of the resident odonate communities at 12 investigated springs. Colors indicate the three classes of riparian impactedness: Red = highly impacted, Tan = moderately impacted, Blue = minimally impacted. Site codes can be found in Table 1. The ‘height’ scale on the vertical axis of cluster dendrograms gives Bray-Curtis distance measure values between clusters of communities.

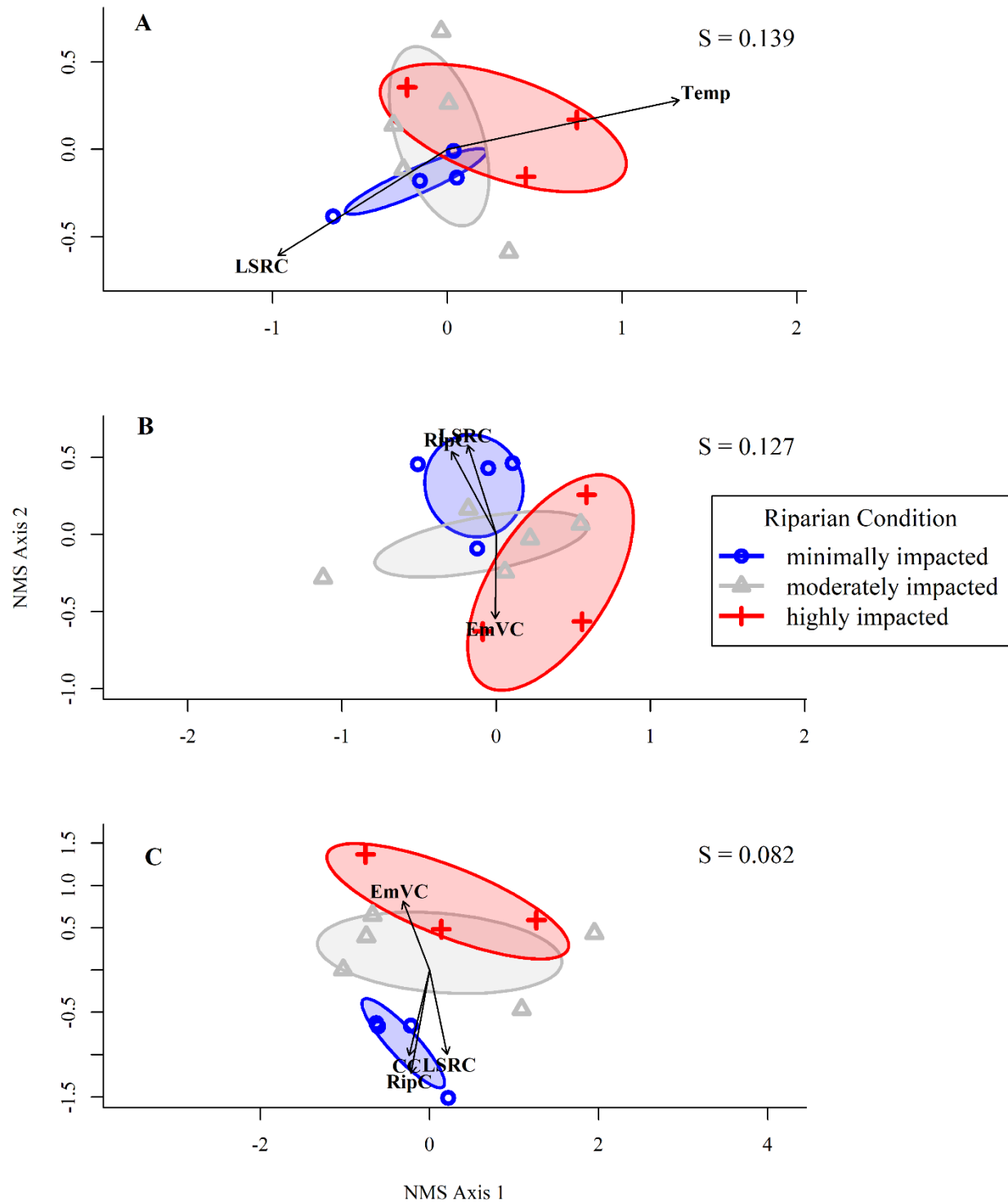


Figure 3: NMS ordination bi-plots plotting sites according to their: (A) general benthic community, (B) insect-only benthic community and (C) odonate community. 95% confidence ellipses are plotted for minimally, moderately and highly impacted sites. Vectors represent environmental variables that significantly ($P < 0.1$) correlated with each ordination after running permutation tests ($n = 9999$ permutations). Vector length is proportional to the correlation between the environmental variable and the ordination. Stress (S) is reported for each model.

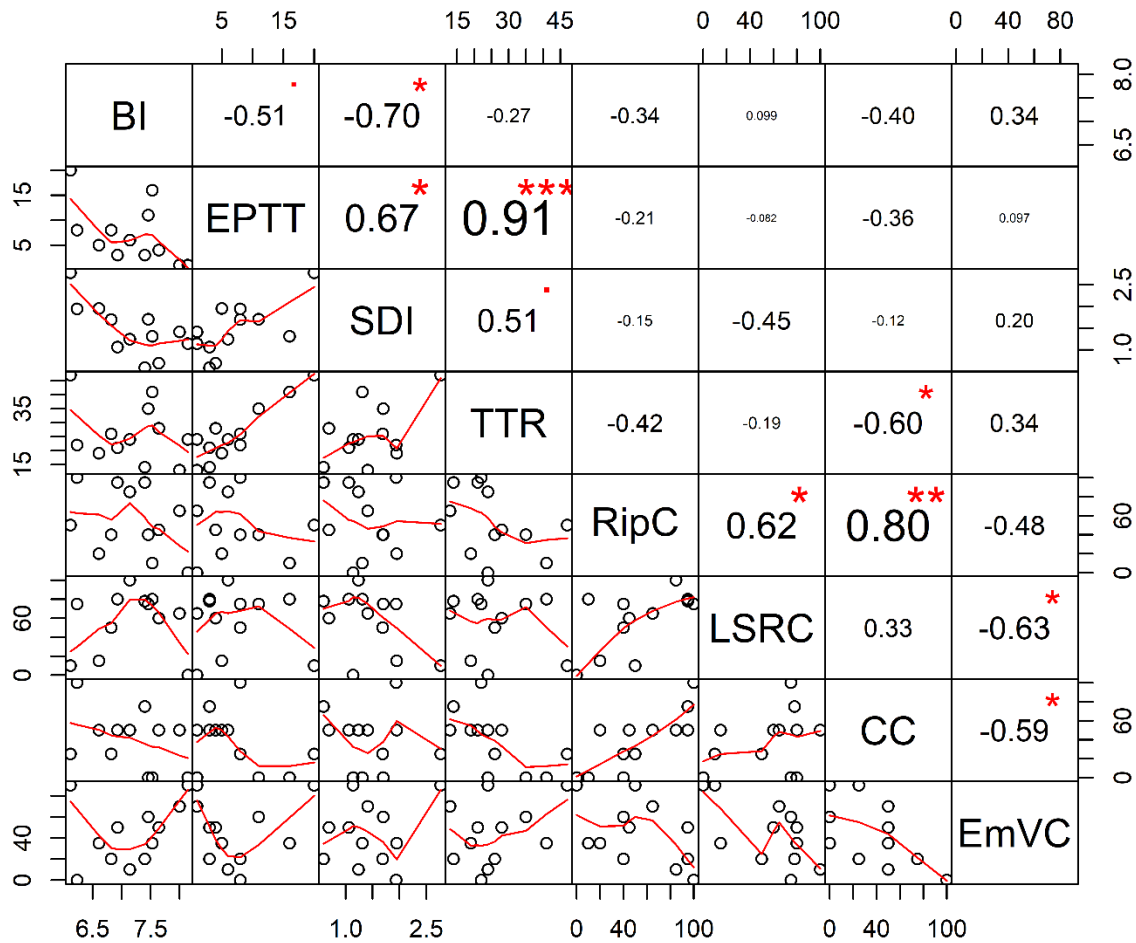


Figure 4: A correlation matrix plotting bivariate scatterplots in the bottom diagonal and corresponding Pearson's product moment correlation coefficients are in the top diagonal. Standard metric scores (BI = Biotic Index, EPTT = Ephemeroptera Plecoptera Trichoptera Taxonomic Index, SDI = Shannon Diversity Index, TTR = Total Taxonomic Richness) are correlated against estimates for local riparian conditions (RipC), landscape riparian conditions (LSRC), canopy cover (CC) and emergent vegetation cover (EmVC) from 12 spring sites. Scales indicating the value of each matrix item at each spring are listed on a horizontal and vertical axis. P-value significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

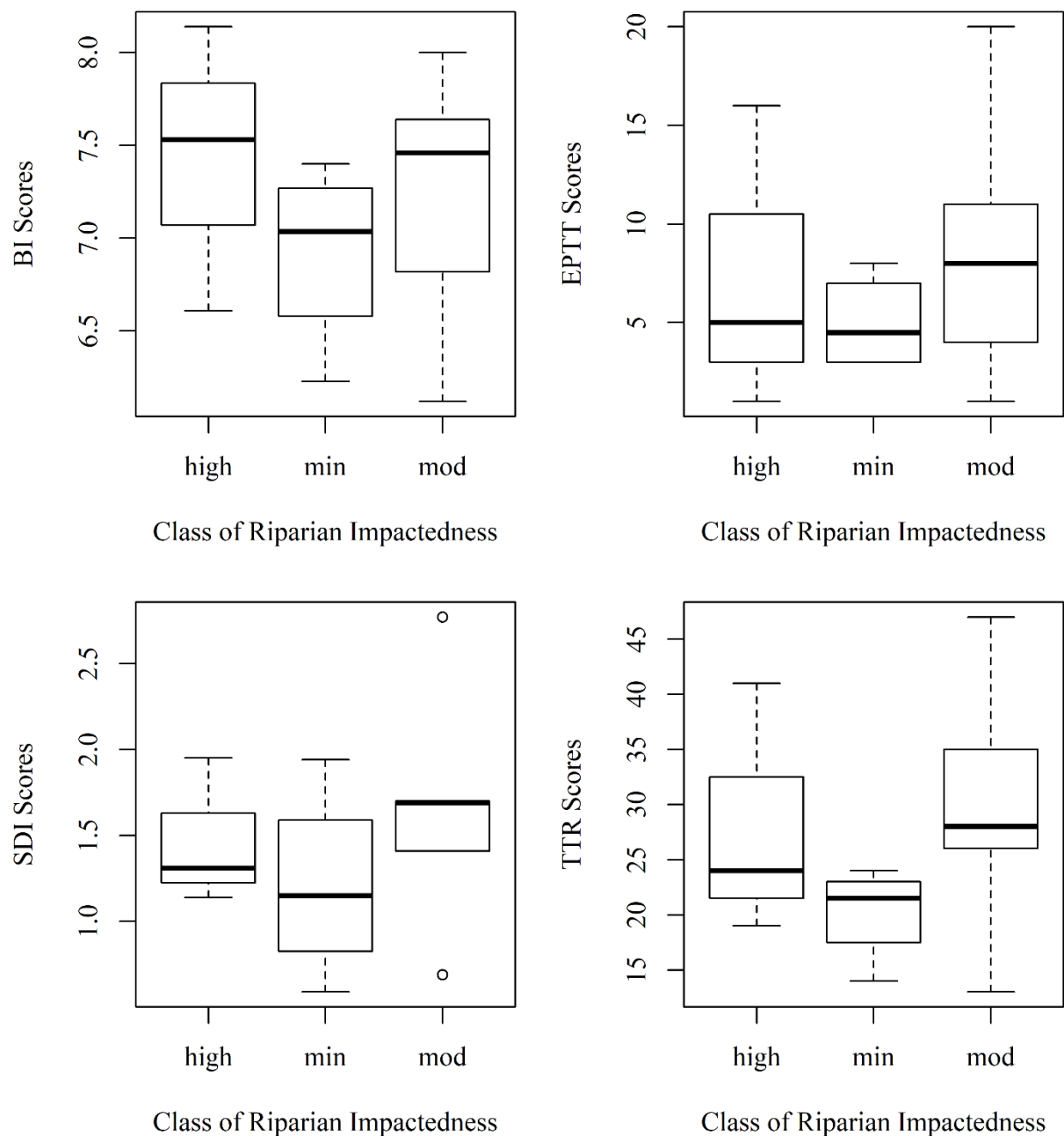


Figure 5: Boxplots illustrating differences between the standard metric scores (BI = Biotic Index, EPTT = Ephemeroptera Plecoptera Trichoptera Taxonomic Index, SDI = Shannon Diversity Index, TTR = Total Taxonomic Richness) and site classes. Site classes are designated as “high” = highly impacted, “min” = minimally impacted, “mod” = moderately impacted. One-way ANOVA testing ($\alpha = 0.05$) found no differences among site classes and standard bioassessment metrics. Horizontal bars represent means, boxes represent standard error, and vertical dashed lines represent ranges.

OCCUPANCY MODELING TO IDENTIFY SUITABLE RIPARIAN HABITAT FOR TWO DAMSELFLY SPECIES

Introduction

The Odonata are a widespread, charismatic insect group known for their sensitivity to physical habitat alteration (Corbet, 1999). In the past decade, odonates have become prevalent in ecological studies focused on identifying habitat suitability because they are useful indicators for habitat alteration (e.g., Kéry et al. 2010; van Strien et al. 2010; Harms et al. 2014; Rapacciuolo et al. 2017). Odonate adults have so far been used almost exclusively for these studies. Insufficient research has been applied to the nymph stage, but nymphs should be a more useful indicator for habitat suitability than their adult counterparts, because they spend the majority of their life cycle in the aquatic, immature stage (Tennessen 2019). The nymphs designate an odonate species' residence at a site whereas adults may temporarily occupy sites unsuitable for reproduction (Chovanec and Waringer 2001; Tennessen 2019). The idea of species 'residency', whereby a species is using its present habitat to complete its life cycle, is distinguishable from species 'occupancy', where a species may be temporarily occupying a habitat unsuitable for reproduction. Residency status should be an important factor for ecologists and odonatologists to consider when analyzing qualitative (presence/absence) odonate data to assess effects of habitat alteration.

Adult odonates must use visual cues to choose a suitable habitat for oviposition so that their offspring can mature successfully, therefore riparian and aquatic vegetation structure likely plays an important role in this process. The adults are diurnal predators that navigate the environment strictly by flight with the help of their large, well-developed eyes (Corbet 1999;

Bybee et al. 2016). Many odonate species also exhibit obligate endophytic oviposition where the eggs are inserted into plant tissues. Many adult odonates also use riparian vegetation for thermoregulation (Corbet and May 2008), while the nymph stage for some species may rely on aquatic vegetation as suitable habitat (Corbet 1999). Given the structural vegetation requirements many species rely on to complete their life histories and our understanding of how odonates traverse their environments to find suitable nymph habitat, it is likely that vegetation-specific habitat variables influence residency of the Odonata in various aquatic environments.

The Ozark Highlands are a karst geographic region with an abundance of spring stream habitats. The springs in this ecoregion are rich in dissolved minerals and support an abundance of aquatic macrophytes that likely serve as excellent oviposition sites for adult odonates. The region is home to 258 species of odonates (George Sims, Central Wyoming College 2015, unpublished data), several of which are known to use spring habitats (Trial 2005; Zeller 2010). Despite thin soils and rocky conditions, much of the land throughout the region has been modified for agricultural and forestry purposes that have resulted in extensive clear cutting, impoundment and other modifications, and there has been substantial urban development in some areas as well. Accordingly, numerous Ozark spring habitats have varying degrees of impact within their riparian zones.

Objective

My objective in the present study was to explore how the degree of human impact on Ozark spring riparian zones may affect native odonate residency. To address my objective, I applied an occupancy modeling approach (MacKenzie et al. 2006) that incorporated nymphs as the primary method of detection. I focused on the effect that four independent variables related to

aquatic and riparian vegetation structure at nested spatial scales had on residency of abundant odonate taxa over three sampling occasions. Furthermore, I examined the effect that season, time spent sampling, and precipitation had on probability of detection of resident odonates.

METHODS

Study Sites

I selected several (N=12) Ozark spring stream study sites as described in chapter 1. Study sites had adjacent riparian conditions that represented a steep gradient of impact (Table 1). Additionally, these springs had a variable amount of instream vegetation and canopy cover that should be a consequence of their riparian condition. I chose springs that had permanent and stable flows and were similar in size, because aquatic permanence is integral to nymph survival and some odonates may prefer larger or smaller size streams for reproduction (Abbott 2005). Spring size is categorized by classes of magnitude ranging from ‘first magnitude’ springs (highest discharge) to ‘eighth magnitude’ (lowest discharge, Meinzer (1927)). I selected third magnitude (28-280 L/s) springs (N=10) and larger fourth magnitude (6.3-28 L/s) springs (N=2) for the current study (Meinzer 1927).

At most of the 12 springs, I selected focal sampling reaches that were located within 50m of the spring’s source. East Ritter, Doling, Brown and Patterson springs had sampling reaches more distant (72 – 260m) from the source (Table 1) where there was greater ease of access to the spring stream. I defined the length of the sampling reach at each spring according to United States Geological Survey (USGS) protocols as twenty times the wetted width of the access point (Fitzpatrick et al. 1998). In some cases, a stream’s total length was less than twenty times its wetted width, in which case I sampled its entire length (Table 1). I defined the riparian zone adjacent to each sampling reach similarly to Environmental Protection Agency (EPA) protocols (Barbour et al. 1999) as four times the wetted width of the stream on each side of the stream.

Vegetation-specific Independent Variables

For each study site, I assessed four vegetation-specific environmental variables that I expected to influence the residence of odonates, including riparian conditions at the local (RipC) and landscape (LSRC) scales, canopy cover (CC) and emergent vegetation cover (EmVC). To estimate RipC, I visually approximated the proportion of a site's defined riparian zone that was primarily undisturbed and contained mostly natural vegetation (i.e., trees and shrubs). For estimation of LSRC, I used ArcMap (ver. 10.5.1; ESRI 2012) to approximate the percent of undisturbed riparian conditions around each site. To evaluate CC, I implemented modified EPA protocols for visual estimation (Barbour et al. 1999) to categorize CC into quinarys of roughly 0, 25, 50, 75 and 100 percent at each site. I visually estimated percent cover of EmVC at springs during the summer sampling period when many species of aquatic vegetation display emergent forms. More detail on the methods used to define each variable listed here can be found in Chapter 1.

Odonate Data

I sampled odonates once during each of three seasons, including summer (June/July 2018), fall (October 2018) and spring (March 2019). Nymphs and exuviae were the focus of collection because their presence indicates habitat suitability for completing the odonate life cycle (Tennessen 2019) hence, 'residency'. I also recorded the presence of adult odonates; however, I only recorded the presence of breeding and ovipositing individuals as resident species detections. I collected nymphs using the qualitative multihabitat methods for sampling wadeable and nonwadeable streams described by Moulton et al. (2002) by sampling all microhabitats found in each sampling reach with a 500 μm mesh D-frame kick net within a limited time span.

Sites were qualitatively sampled for fifteen minutes during summer, but time spent sampling was increased to sixty minutes during fall and spring collecting to ensure thorough investigation of all microhabitats in each sampling reach for detection of rare or cryptic species. Exuviae were collected anywhere encountered while collecting nymphs. Collected nymphs and exuviae were stored in 75% ethanol. Whenever practical, I collected at least one adult of each odonate species observed at each spring, including tandem pairs, to verify resident or occupant status regardless of the presence or absence of nymphs of the same species. Adults were captured by aerial net and temporarily stored in glassine envelopes. Most adult odonates present at a spring were identified on the wing, but some ambiguous specimens were collected for confident identification with adult keys (Abbott 2005). Collected adults were bathed in acetone for about ~16 hours following the end of fieldwork on the day they were collected. Once removed from the acetone, dried specimens were stored in clear polypropylene envelopes with a note card containing collection date and identification.

Occupancy Models

I applied an occupancy modeling approach to assess the importance of riparian habitat conditions on odonate presence/absence across the 12 sample springs. Occupancy modeling is a method for estimating the probability of an organism's presence or absence in an environment using field detections, on a species-by-species basis. Single season occupancy models estimate probability of detection (p) and probability of occupancy (ψ) (MacKenzie et al. 2006). There can be many independent or confounding variables that influence response variables (i.e. presence/absence data of the focal species) and these variables are referred to as covariates in occupancy modeling. In the present study, the four vegetation-specific variables under

investigation are henceforth referred to as vegetation covariates and were used to calculate ψ for species of interest. I regarded covariates that could influence my ability to make perfect detections as sampling covariates and modeled their effects on p .

I expected three sampling covariates to influence odonate nymph detection probability, including the sampling season, the amount of time spent sampling (Time) and the amount of precipitation recorded prior to sampling (Precip). Some species that I detected may only exist as nymphs for part of the year, so I recorded seasons as qualitative factors. Time spent sampling was increased after the summer sampling season, which could influence the detection of rare species residing in a study site. I collected precipitation estimates within a 6.43 kilometer radius of each study site 48 hours prior to sampling from the National Weather Service's quantitative precipitation estimates database, which provided rainfall estimates around each study site. I recorded combined precipitation estimates (mm) for the two days prior to sampling a site.

I constructed occupancy models with the statistical software program R version 3.5.2 (R Core Team 2018) using the 'unmarked' package (Fiske and Chandler 2011). I used the *unmarkedFrameOccu* function to combine resident species presence data with sampling and vegetation covariates and the *occu* function to fit the combined data to a MacKenzie et al. (2002), single season occupancy model (Fiske and Chandler 2011). I modeled vegetation covariate effects on the two most abundant resident species detected during sampling, *Calopteryx maculata* (Palisot) and *Argia plana* (Say). Both of these species are reported to prefer well shaded stream habitats (Abbott 2005) and I frequently encountered both at a majority of the study sites. Additionally, *C. maculata* is known to readily disperse to more suitable habitats in response to environmental changes (Jonsen and Taylor 2000). *Argia plana* is a common resident of springs and is known to have a close association with typical spring type vegetation,

especially *Nasturtium officinale* (W.T. Aiton) (watercress), which it frequently uses for oviposition (Bick and Bick 1972).

I evaluated occupancy models by using Akaike's Information Criterion (AIC) corrected for small sample sizes (AIC_c) using a similar approach to Harms et al. (2014). I generated AIC_c values using the 'AICcmodavg' package (Mazerolle 2019). Initially, I examined sampling covariate effects on detection probability both independently and in combination while holding probability of occupancy constant. Subsequently, I selected the best models with effects on detection probability to create explanatory models that examined the effect of vegetation covariates on probability of occupancy. Finally, I selected the best models (lowest AIC_c value) as well as competitive ($\Delta\text{AIC}_c \leq 2$) models with effects on both detection and occupancy parameters to interpret species habitat selection. My objective in this study was particularly aimed at identifying which covariates had the strongest effects on p and ψ , so models without effects on ψ were ignored, although their AIC_c values were the lowest among models for both species. Henceforth the 'best' models that I report here are the most parsimonious where covariates had effects on both detection and occupancy probabilities. In order to interpret the strength of the relationship between covariates and their effect on p and ψ (effect size) and determine whether the results of each model were significant, I calculated beta coefficients (β) and 95% confidence intervals (CI) for the covariates used in each model. I calculated beta coefficients which are a direct measure of effect size in each model using the *coef* function in R. To produce 95% confidence intervals, I used the *confint* function.

I averaged the best models and all competitive models for *C. maculata* and *A. plana* to predict site occupancy under fixed environmental conditions during a summer season, while evenly varying precipitation and sampling time covariates across observed minimum and

maximum values. I evenly varied the vegetation covariate specific to site occupancy from the best model for each species similarly to the quantitative sampling covariates. All other vegetation covariates in each model were assigned mean values from observed data. Then I plotted the predictive models for both species. To compare the effect of local riparian conditions (RipC) between species, I also plotted a predictive model where RipC had an effect on site occupancy for *A. plana*. Additionally, I recorded point estimates for detection and occupancy probabilities in each predicted model.

RESULTS

I observed the adults of *C. maculata* and *A. plana* occupying the riparian zones at all 12 sample sites during at least one field season, with the exception of Valley Water Mill spring where *A. plana* was never spotted. *Calopteryx maculata* was a resident species at 10 study sites and *A. plana* was a resident at seven (Table 9). *Calopteryx maculata* nymphs were present at every site where it was resident except for Brown spring where residency was based on observations of breeding adults. *Argia plana* nymphs were detected at Silver, Doling, Double and Crane Creek springs, while only breeding or ovipositing adults were observed at West Ritter, Patterson and Mitch Hill springs.

The best model for *C. maculata* showed time spent sampling as a weak negative effect on p ($\beta = -0.03$, CI from -0.08 to 0.02) and RipC as a weak positive effect on ψ ($\beta = 0.05$, CI from -0.02 to 0.13), but these effects were not significant because their 95% confidence intervals included zero. In the most competitive model ($\Delta AIC_c = 0.36$), precipitation prior to sampling had a weak negative relationship with p ($\beta = -0.03$, CI from -0.08 to 0.01). Other competitive models ($\Delta AIC_c \leq 2$) for *C. maculata* also included LSRC and CC as effects on ψ (Table 10), but the effect sizes in every model were non-significant. Emergent vegetation cover was not included as an effect in any competitive models for *C. maculata*.

In the best model for *A. plana*, time spent sampling had a weak negative effect on p ($\beta = -0.02$, CI from -0.07 to 0.02) and LSRC showed a weak negative effect on ψ ($\beta = -0.02$, CI from -0.07 to 0.02). Similar to *C. maculata*, the best model and all competitive models for *A. plana* were non-significant. Time spent sampling had an effect on p in the top three *A. plana* models and precipitation was an effect in other competitive models (Table 10). The most competitive

model ($\Delta AIC_c = 1.03$) that included precipitation as an effect on p also presented a very weak negative relationship ($\beta = -0.01$, CI from -0.05 to 0.04). Competitive models included all other vegetation covariates as effects on ψ and both LSRC and RipC showed negative effects ($\beta = -0.02$, CI from -0.07 to 0.02 and $\beta = -0.01$, CI from -0.05 to 0.02, respectively).

The predictive models for *C. maculata* and *A. plana* showed opposite responses to RipC as an effect on site occupancy. The plotted *C. maculata* model suggests that RipC positively influenced ψ (Figure 6), while the plotted *A. plana* model where RipC was varied for ψ suggests a negative relationship (Figure 7). The *A. plana* model where LSRC was varied for ψ also showed a negative relationship between the covariate and ψ (Figure 8). Overall, the predictive models illustrate how *C. maculata* appears more likely to occupy spring streams with less impacted riparian conditions, while *A. plana* is more likely to occupy streams with highly impacted riparian conditions (Table 11).

DISCUSSION

The general trends observed in the occupancy models that I presented closely match general predictions based on natural history observations of the two focal species. However, the effects of model parameters were small and not significant in this study, which is likely due to a relatively small sample size either spatially, temporally, or a combination of both. As a result, inferences from the models should be interpreted with caution. The types of occupancy models I created here are the first occurrence of anyone exploring the p and ψ parameters exclusively using resident detections instead of occupant detections. Because the ‘detections’ used to create these models are specific to continuation of the odonate life cycle, they perhaps should be more appropriately termed ‘residency models’ that calculate ‘probability of residence’, a modified ψ parameter. Residency modeling can be further developed for more ecologically meaningful application with other organisms that have niche habitat preferences or requirements for completing their life-cycle.

All vegetation covariates showed an effect on the residency of both species in their competitive models with the exception of EmVC in *C. maculata* models. My model results suggested that RipC, LSRC, and CC have opposite effects on each species, where ψ for *C. maculata* was positively influenced by these covariates and negatively influenced for *A. plana*. In my study, landscape scale riparian structure was assessed within a 500-meter buffer around sample sites and was included as an effect on ψ for competitive models of both species. In an Iowa study, landscape configuration covariates at a 600 meter spatial scale were included in all best and competitive occupancy models for four odonate species (Harms et al. 2014). Harms et al. (2014) also evaluated site occupancy for *C. maculata*, and they found that although wetland

patches within a 600-meter buffer around sample sites were included in their best models as an effect on ψ for this species, it was not deemed significant. *Calopteryx maculata* is a large, darkly pigmented damselfly that uses riparian vegetation as a perch when mating or hunting, and shade for thermoregulation (Kirkton and Schultz 2001). The positive effect that vegetation covariates had on *C. maculata* residency reported here is likely a reflection of the true biological importance that resource has on this species. The opposite effect that vegetation covariates had on *A. plana* occupancy models may also reflect their relative importance on *A. plana* residency. Adult *A. plana* are small and lightly pigmented damselflies that probably tolerate sunlight exposure to a better degree than *C. maculata*. They were also abundant where *C. maculata* was rare so I suspect interspecific competition may have a direct influence on *A. plana* adults trying to occupy those streams, which is supported by the nymphs of both species typically occupying separate microhabitats (pers. obv.).

Prior precipitation and the amount of time spent sampling were the most influential sampling covariates for detection of *A. plana* and *C. maculata*. Nymphs for both of these species were usually detected within the first few minutes of sampling when they were resident at a site, so it makes sense that time spent sampling showed a negative effect on detection probabilities. The negative influence that precipitation events prior to sampling had on detection is not as easily understood from collected data. It stands to reason that spurts of increased flow at springs may trigger a sheltering response in odonate nymphs, where those that frequently inhabit the substrate, like *A. plana*, may take shelter in the hyporheic zone (Palmer et al. 1992, Dole-Olivier 2011). Nymphs of other *Argia* spp. have been recovered from hyporheic samples after floods from a run-off stream within the same region as this study (Dorff 2019). A sheltering response to unknown variables (e.g. predators, recent flooding), or perhaps sampling inaccessibility to

hyporheic habitat may explain why *A. plana* nymphs were not detected at sites where adults were observed breeding (Mitch Hill and Patterson springs) or even ovipositing (West Ritter spring). *Calopteryx maculata* nymphs were sometimes undetected at sites where they were previously or later detected. Precipitation might influence presence of breeding and ovipositing adults, because adults remain perched during rainfall (pers. obv.) and the quantity of food consumed daily by female damselflies probably influences their fecundity as reported for *Ischnura verticalis* (Say) (Richardson and Baker 1997).

Occupancy modeling is a recent innovation for understanding odonate species distributions and dispersal capabilities for species of interest (e.g. Harms et al. 2014; Andersen et al. 2016; Rapacciuolo et al. 2017), as well as habitat conditions favored for reproduction (this paper). To my knowledge, this is the one of very few studies (but see Andersen et al. 2016) that attempt modeling odonate occupancy with nymph detections. It is an introductory attempt to specifically model the residency status of odonates, which should be a more informative method of monitoring the habitat preferences for many species when compared to monitoring individual adults on the wing. Odonates can respond quickly to environmental changes and should serve as useful bioindicators for the state of lateral connectivity between aquatic-terrestrial ecotones (Foote and Rice Hornung 2005). Odonate residency modeling endeavors should be an effective way to gauge which environmental variables are most important for species life history preferences. The results from such studies can inform future land management decisions that can influence odonate species distributions.

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SUMMARY

The community composition of odonates was compared to that of the general benthic community in Ozark spring streams with variably-impacted riparian zones. Odonate communities were significantly associated with riparian categories, while general benthic communities were not. Similar to the findings of Miguel et al. (2017), odonate communities belonging to streams with mostly unimpacted conditions varied from those with moderate to high levels of impact. The sensitivity of odonates in other US ecoregions to riparian conditions should be further studied to better our understanding of the environmental stressors that impact species dynamics for this taxonomic group. Discovery of community-level and species-specific environmental sensitivities will no doubt be useful for developing this common and charismatic order for increased use in bioassessment by researchers and land managers alike. An odonate biotic index for riparian condition assessment, such as the one that I developed in Chapter 1, has strong promise for application with tolerance values developed from larger datasets gathered over multiple seasons as a reliable, time and cost-effective solution to monitoring riparian degradation.

Habitat preference and distributional data are still lacking among many North American odonate fauna. Efforts to preserve the habitats critical for reproduction of rarer species and identify land-management practices that minimize impairment are necessary for their survival. In Chapter 2, I illustrated an approach based on modeling the residency of two common spring-dwelling species in the Ozark Highlands. Although the dataset from my thesis did not have the statistical power required to make strong inference, I have highlighted the ability for occupancy modeling to describe species presence trends against multiple habitat gradients. Additionally, I

suggest that using nymph detections should better inform which habitats a species is using for reproduction and how fragmented species populations are across a given landscape.

Table 9. Resident detections for *Calopteryx maculata* and *Argia plana* over three sampling seasons.

Spring Stream	<i>Calopteryx maculata</i>			<i>Argia plana</i>		
	Summer	Fall	Spring	Summer	Fall	Spring
West Ritter	1	0	1	1	0	0
Valley Water Mill	1	1	1	0	0	0
Silver	0	0	0	1	1	1
Patterson	1	0	1	1	0	0
Chiles	0	0	0	0	0	0
East Ritter	1	1	1	0	0	0
Doling	1	1	1	1	1	1
Mitch Hill	1	0	1	0	1	0
Gilbert	1	1	1	0	0	0
Double	1	1	0	1	1	1
Brown	1	0	0	0	0	0
Crane Creek	0	0	1	1	1	1

Table 10. Competitive models ($\Delta AIC_c \leq 2$) including both detection (p) and site occupancy (ψ) probabilities of *Calopteryx maculata* and *Argia plana*. The best model for each species has a $\Delta AIC_c = 0.00$. K denotes the number of parameters in each model, w denotes the AIC_c weight and LL denotes Log likelihood. RipC, LSRC, CC and EmVC denote the local riparian conditions, landscape riparian conditions, canopy cover, and emergent vegetation cover respectively. Time refers to time spent sampling and Precip refers to the amount of precipitation 48 hours prior to sampling.

Model	AIC_c	ΔAIC_c	w	K	LL
<i>Calopteryx maculata</i>					
$p(\text{Time})\psi(\text{RipC})$	53.80	0.00	0.19	4	-20.04
$p(\text{Precip})\psi(\text{RipC})$	54.17	0.36	0.16	4	-20.23
$p(\text{Time})\psi(\text{LSRC})$	54.22	0.41	0.16	4	-20.25
$p(\text{Time})\psi(\text{CC})$	54.39	0.58	0.14	4	-20.34
$p(\text{Precip})\psi(\text{LSRC})$	54.58	0.77	0.13	4	-20.43
$p(\text{Precip})\psi(\text{CC})$	54.75	0.95	0.12	4	-20.52
<i>Argia plana</i>					
$p(\text{Time})\psi(\text{LSRC})$	52.19	0.00	0.22	4	-19.24
$p(\text{Time})\psi(\text{EmVC})$	52.81	0.61	0.16	4	-19.55
$p(\text{Time})\psi(\text{RipC})$	53.17	0.97	0.13	4	-19.73
$p(\text{Precip})\psi(\text{LSRC})$	53.23	1.03	0.13	4	-19.76
$p(\text{Time})\psi(\text{CC})$	53.43	1.24	0.12	4	-19.86
$p(\text{Precip})\psi(\text{EmVC})$	53.83	1.64	0.10	4	-20.06
$p(\text{Precip})\psi(\text{RipC})$	54.19	2.00	0.08	4	-20.24

Table 11. Parameter estimates for both detection (p) and site occupancy (ψ) probabilities resulting from predicted models by model averaging of competitive models within 2 AICc of the best model for two species. Vegetation covariates include local scale riparian conditions (RipC) and landscape scale riparian conditions (LSRC).

Models	Predicted ψ (SE)	Predicted p (SE)
<i>Calopteryx maculata</i> ψ (RipC)	0.88 (0.14)	0.71 (0.15)
<i>Argia plana</i> ψ (LSRC)	0.62 (0.18)	0.72 (0.14)
<i>Argia plana</i> ψ (RipC)	0.61 (0.17)	0.72 (0.14)

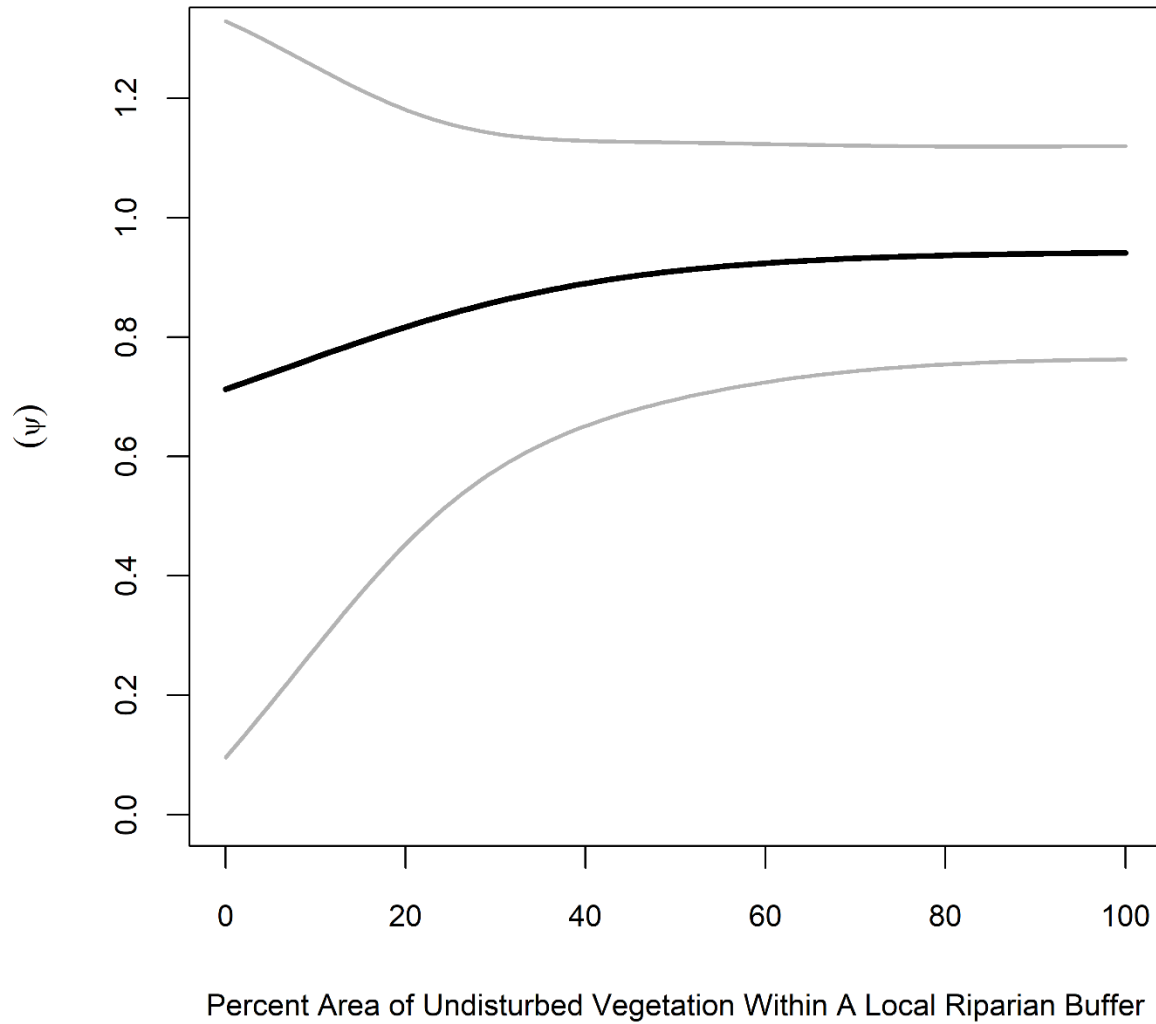


Figure 6. Predicted probability of site occupancy (ψ) for *Calopteryx maculata* in response to local riparian conditions (RipC) across 12 study sites during summer. Gray lines represent 95% confidence intervals.

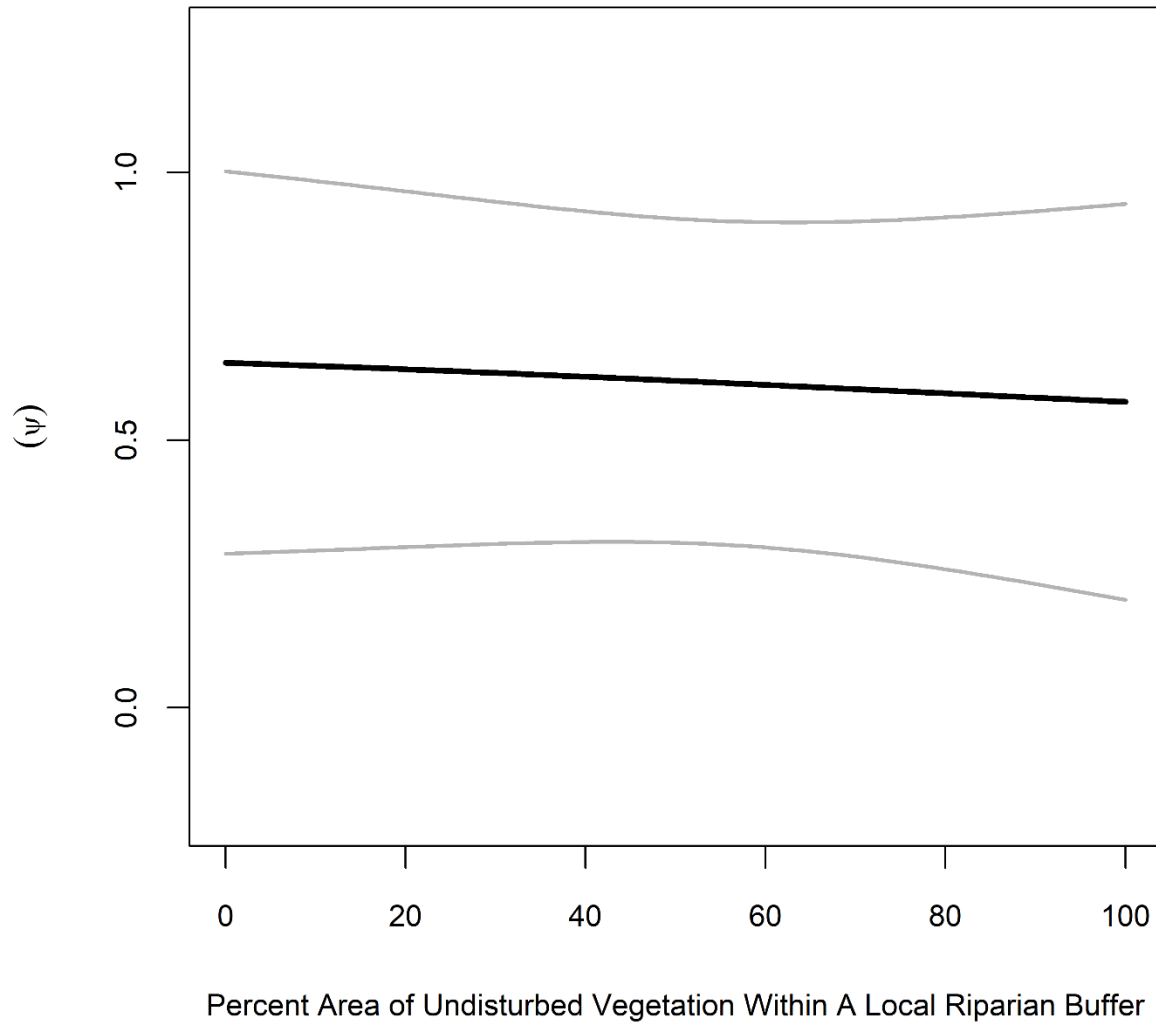


Figure 7. Predicted probability of site occupancy (ψ) for *Argia plana* in response to local riparian conditions (RipC) across 12 study sites during summer. Gray lines represent 95% confidence intervals.

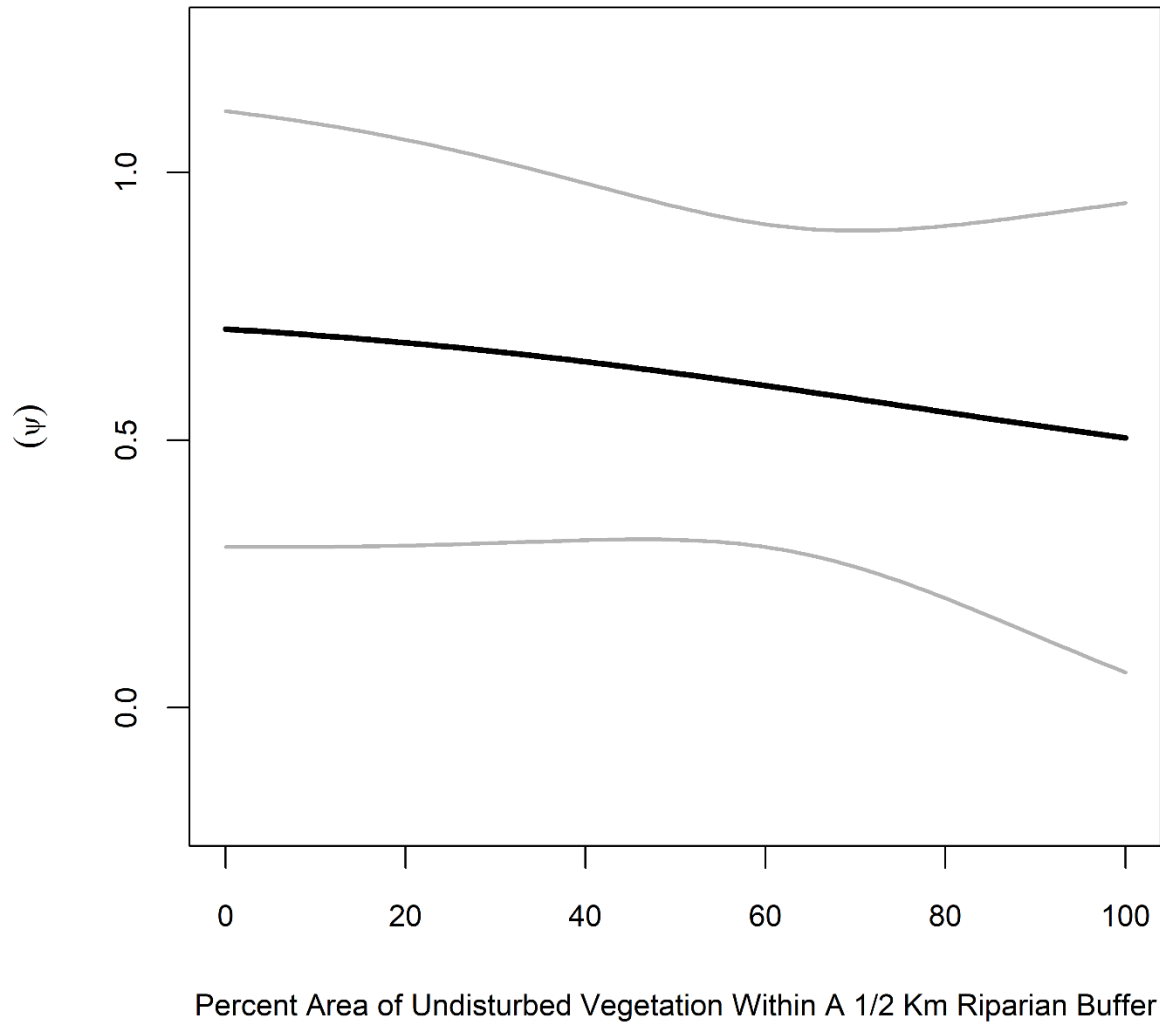


Figure 8. Predicted probability of site occupancy (ψ) for *Argia plana* in response to landscape riparian conditions (LSRC) across 12 study sites during summer. Gray lines represent 95% confidence intervals.

APPENDIX

Appendix: Benthic macroinvertebrate communities recorded from Slack Surber samples taken at each spring site. Counts are per 0.25m² sampling area. See Table 1 for spring code designations.

Phylum													
Class													
Order													
Family													
<i>Specific Taxon</i>	WR	VWM	S	P	CH	ER	D	MH	G	DO	B	CR	
Annelida													
Hirudinea	0	10	0	0	0	0	0	0	0	0	0	0	0
Oligocheata	6	120	192	65	29	94	44	14	94	76	125	94	
Mollusca													
Bivalvia	0	3	9	0	1	9	1	1	7	1	8	5	
Gastropoda	0	3	2	8	21	13	0	28	12	11	58	19	
Nematoda													
	2	0	4	0	4	3	3	1	2	8	87	1	
Platyhelminthes													
Rhabditophora													
Tricladida	118	42	12	53	70	70	1	29	9	50	624	53	
Arthropoda													
Arachnida													
Trombidiformes	1	0	0	47	71	14	0	87	7	46	111	21	

Entognatha												
Collembola	10	4	17	96	8	1	2	3	4	4	1	4
Insecta												
Coleoptera												
Elmidae												
<i>Dubiraphia</i> sp.	0	0	0	0	0	0	0	0	0	0	1	0
<i>Microcylleopus pusillus</i>	0	0	0	0	0	0	0	0	0	0	1	0
<i>Optioservus sandersoni</i>	347	2	0	0	624	724	0	11	170	56	643	85
<i>Stenelmis</i> sp.	0	0	1	0	0	0	30	0	0	2	0	0
Dytiscidae												
<i>Hygrotus</i> sp.	0	0	0	0	0	0	0	0	0	0	1	0
(other)	0	0	1	0	0	0	0	0	0	0	0	0
Hydrophilidae												
<i>Hydrobius</i> sp.	0	0	0	4	0	0	0	1	0	0	0	1
(other)	0	0	0	0	0	0	0	0	0	0	31	0
Psephenidae												
<i>Ectopria nervosa</i>	0	0	0	0	0	0	0	0	3	1	0	0
<i>Psephenus herricki</i>	0	0	0	0	0	0	0	0	0	0	0	1
Staphylinidae												
<i>Stenus</i> sp.	1	0	0	4	0	0	0	0	0	0	0	6
(other)	1	0	0	0	2	0	0	0	0	0	0	0
unknown	0	0	0	0	0	0	0	0	0	1	0	0

Diptera

Canacidae

<i>Canace</i> sp.	0	0	1	1	1	1	0	0	0	0	0	0
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Ceratopogonidae

<i>Atrichopogon</i> sp.	0	0	0	0	0	0	0	0	0	0	4	0
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<i>Bezzia/Palpomyia</i> sp.	0	0	0	1	0	0	0	0	0	2	2	0
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<i>Ceratopogon</i> sp.	0	1	0	0	0	0	0	0	0	0	0	0
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<i>Culicoides</i> sp.	0	0	0	0	0	1	0	0	0	1	0	0
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Chironomidae	16	425	991	320	203	147	29	387	8	118	34	116
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Dixidae

<i>Dixa</i> sp.	3	0	0	0	1	0	0	1	0	7	0	4
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Empididae

<i>Hemerodromia</i> sp.	0	0	0	0	0	0	0	0	0	1	0	0
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<i>Neoplasta</i> sp.	0	0	1	4	13	13	0	2	3	1	8	2
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<i>Trichoclinocera ozarkensis</i>	0	0	0	0	0	0	0	0	0	0	0	5
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Ephydriidae

<i>Coenia curvicauda</i>	0	0	3	0	0	0	0	0	0	0	0	0
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<i>Hydrellia</i> sp.	0	0	0	0	0	0	0	1	0	0	0	1
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(other)	0	0	4	2	1	0	0	0	0	0	0	1
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Limoniidae

<i>Antocha</i> sp.	0	0	0	1	0	0	0	0	0	0	2	0
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<i>Hexatoma</i> sp.	0	0	0	15	0	0	0	0	0	3	0	0
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Muscidae												
<i>Limnophora</i> sp.	0	0	2	7	0	0	0	0	0	0	1	0
Psychodidae												
<i>Pericoma/Clogmia</i> sp.	0	0	3	4	0	0	1	0	0	0	0	0
<i>Psychoda</i> sp.	0	0	30	0	0	0	0	0	0	0	0	0
Simuliidae												
<i>Prosimulium</i> sp.	0	0	0	0	0	0	0	0	0	61	4	0
<i>Simulium</i> sp.	0	0	0	3	0	2	0	0	0	4	1	0
Stratomyiidae												
<i>Allognosta</i> sp.	0	0	0	1	0	0	0	0	0	0	0	0
<i>Caloparyphus</i> sp.	0	0	0	0	0	0	0	0	0	0	0	1
Tabanidae												
<i>Tabanus fairchildi</i>	0	0	0	0	0	0	0	0	0	0	0	1
Tipulidae												
<i>Tipula</i> sp.	0	0	0	1	1	0	2	0	0	0	0	0
Ephemeroptera												
Baetidae												
<i>Acentrella turbida</i>	15	0	0	507	38	67	0	0	1	6	1	1
<i>Acerpenna pygmaea</i>	0	0	0	0	0	0	0	0	0	4	0	0
<i>Baetis flavistriga</i>	0	0	3	0	0	0	2	0	6	27	0	1
<i>Baetis intercalaris</i>	0	0	0	0	0	0	0	0	0	5	0	0
<i>Baetis tricaudatus</i>	0	0	0	0	278	1	0	57	9	0	0	0

<i>Dipheter hageni</i>	0	0	0	34	0	15	2	1	7	56	51	12
<i>Labiobaetis propinquus</i>	0	0	0	0	0	0	0	0	0	9	0	0
Leptohyphidae												
<i>Tricorythodes</i> sp.	0	0	0	0	0	0	0	0	0	30	4	0
Leptophlebiidae												
<i>Habrophlebiodes americana</i>	0	0	0	0	0	0	0	0	0	1	0	0
Heptageniidae												
<i>Heptagenia</i> sp.	0	0	0	0	0	0	0	0	0	0	1	0
<i>Stenacron</i> sp.	0	0	0	0	0	0	21	0	0	0	28	0
<i>Stenonema femoratum</i>	0	1	0	0	0	0	0	0	0	0	0	0
unknown	0	0	0	0	0	0	0	0	0	0	1	0
Hemiptera												
Gerridae	0	0	0	0	0	3	1	0	0	1	0	1
Hebridae	0	0	1	0	0	0	0	0	0	0	0	0
Veliidae												
<i>Microvelia</i> sp.	0	0	4	1	0	1	3	0	0	2	0	8
Megaloptera												
Corydalidae												
<i>Nigronia serricornis</i>	0	0	0	0	0	0	0	0	0	0	2	0
<i>Sialis</i> sp.	0	0	0	0	0	0	0	0	0	1	1	0
Odonata												
Coenagrionidae	0	0	15	0	0	0	1	0	0	4	0	7

<i>Argia plana</i>												
Gomphidae												
<i>Gomphurus</i> sp.	0	0	0	0	0	0	0	1	0	0	0	0
Plecoptera												
Leuctridae												
<i>Leuctra tenuis</i>	0	0	0	0	0	0	0	4	3	74	10	7
Perlidae												
<i>Agnetina</i> sp.	0	0	0	0	2	0	0	0	0	13	3	2
Trichoptera												
Glossostomatidae												
<i>Agapetus illini</i>	4	0	0	0	74	0	0	209	35	5	0	2
Helicopsychidae												
<i>Helicopsyche borealis</i>	0	0	0	0	5	0	0	0	3	0	1	0
Hydropsychidae												
<i>Ceratopsyche slossonae</i>	0	0	0	8	0	0	0	0	0	0	132	0
<i>Cheumatopsyche</i> sp.	0	0	0	0	0	0	143	14	0	14	443	3
<i>Diplectrona modesta</i>	0	0	0	4	59	0	0	0	6	10	1	16
<i>Hydropsyche</i> sp.	0	0	0	0	0	0	0	0	0	1	0	1
Hydroptilidae												
<i>Hydroptila</i> sp.	2	0	0	0	8	0	0	0	0	23	96	0
<i>Oxyethira</i> sp.	0	0	0	0	0	0	0	0	0	2	58	10
Leptoceridae	0	0	0	0	0	0	0	0	0	1	0	0

<i>Nectopsyche</i> sp.												
Limnephilidae												
<i>Ironoquia punctatissima</i>	0	0	0	0	0	0	0	0	0	1	0	0
Philopotamidae												
<i>Chimarra</i> sp.	0	0	0	0	1	0	26	2	0	4	17	1
Polycentropodidae												
<i>Polycentropus</i> sp.	0	0	0	0	0	0	0	0	0	0	11	0
Psychomyiidae												
<i>Lype diversa</i>	0	0	0	0	0	0	0	0	0	2	0	0
Malacostraca												
Amphipoda												
Crangonyctidae/												
Gammaridae												
<i>Crangonyx</i> sp./												
<i>Gammarus</i> sp.	2977	783	101	7556	1814	2660	8	1602	257	55	9728	833
Decapoda												
Cambaridae												
<i>Orconectes</i> sp.	0	9	5	0	0	0	14	1	2	2	15	0
Isopoda												
Asellidae												
<i>Asellus</i> sp.	0	0	1	28	0	0	0	2	0	0	0	0
<i>Lirceus</i> sp.	0	357	3	55	1531	5	0	2	49	287	5346	107
