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Aquatic Invertebrate Community Structure, Biological Condition, Habitat, and Water Quality at Ozark National Scenic Riverways, Missouri, 2005-2014

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Running title: Aquatic Invertebrate Community Structure

Abstract

Ozark National Scenic Riverways (OZAR) was established to protect the corridor of the Current River and its major tributary, the Jacks Fork. The Current River is one of the few remaining free-flowing rivers in the U.S., with much of its base flow coming from several large springs. To assess the biological condition of these rivers, aquatic invertebrate community structure was monitored from 2005 to 2014. Benthic invertebrate samples and associated habitat and water quality data were collected from each of nine sampling sites using a Slack-Surber sampler. The Stream Condition Index (SCI), a multimetric index that incorporates taxa richness, EPT (Ephemeroptera, Plecoptera, Trichoptera) richness, Shannon's diversity index, and Hilsenhoff Biotic Index (HBI), was calculated. The benthic invertebrate fauna was diverse with 155 distinct taxa identified from all sites. Mean taxa richness was high, ranging from 22 to 30 among sites. The invertebrate taxa of the Current River and Jacks Fork are largely intolerant across all taxa represented (mean tolerance value= ~4.25). Mean HBI did not exceed 3.9 in the Current River or 4.4 for the Jacks Fork. Mean SCI scores across sampling sites generally were well above 16, indicating they are not impaired. Habitat and water quality data were summarized, but they were poorly correlated with individual invertebrate metrics. Sørensen's similarity index was used to assess community similarity among sites, and similarity scores were then analyzed using ascendant hierarchical cluster analysis. Similarity among sites was 72% or greater. Cluster analysis showed that Current River and Jacks Fork sites clustered separately and in a downstream progression. The uppermost collection site on the Current River was most unlike the other sites, which probably relates to the distinct physical features of that site compared to the others. Nonmetric Multidimensional Scaling (NMDS) was used to evaluate the relationship of invertebrate metrics to habitat and water quality. The NMDS model

was found to be a good fit (stress=0.04) and specific conductance, temperature, discharge, filamentous algae and aquatic vegetation were among the most important habitat variables in defining the relationship among sampling sites. The three lower Current River and Jacks Fork sites each were closely grouped in ordination space, but the three upper Current River sites were farther apart from each other. The influence of several large volume springs near those sites is suspected of producing such disparity through press type disturbances. Although the invertebrate communities and water quality in the Current River and Jacks Fork are largely sound and have high biological condition, ongoing and projected threats to these resources remain, and those threats largely originate outside park jurisdictional boundaries. Inherent variability of invertebrate community diversity across sites and years highlights the importance of using multi-metric assessments and multiyear monitoring to support management decisions.

Introduction

Aquatic invertebrates are useful for understanding and detecting changes in biological condition because they reflect cumulative impacts not typically detected through traditional water quality monitoring (Barbour *et al.* 1999; Moulton *et al.* 2000, 2002). The occurrence of pollution sensitive taxa, dominance by a particular taxon combined with low overall taxa richness, or appreciable shifts in community composition relative to a reference condition are all ways that invertebrates are useful for assessing stream biological condition (Lazorchak *et al.* 1998; Barbour *et al.* 1999; Bonada *et al.* 2006).

Short-term, single event invertebrate monitoring is a strategy commonly used by resource and regulatory agencies for assessing stream stressors such as habitat disturbance, and chemical and biological pollution (Bonada *et al.* 2006). While short-term invertebrate monitoring serves a valuable purpose, evaluation of long-term variability helps researchers and managers

better understand and gauge chronic alterations in stream condition relative to climatic variability and change, as well as other anthropogenic disturbances (Bruce 2002; Jackson and Füreder 2006; Mazor *et al.* 2009; Vaughan and Ormerod 2012; Bowles *et al.* 2013a, 2013b).

Study area

Ozark National Scenic Riverways (OZAR), located in southeastern Missouri, was established in 1964 to protect the corridor of the Current River, its tributaries (including the Jacks Fork), and springs. The Current River is one of the few remaining large, free-flowing streams in the U.S. The extensive karst topography of the region results in formation of springs, of which there are more than 425 in the Current River basin (Bowles and Dodd 2015). Several of these springs are 1st and 2nd magnitude (Meinzer 1927; Bowles and Dodd 2015) and they provide the bulk of the baseflow for these rivers. The boundary of OZAR encompasses only 4% of the watershed, leaving much of it unprotected from human activities (e.g., agriculture, urbanization, and logging), which could result in alteration of water quantity and quality. Protecting and maintaining the integrity of the natural resources at OZAR is a high priority because it also serves as a major economic contributor to the region (Cui *et al.* 2013; Cullinane *et al.* 2014; NPS 2014).

Past disturbances and current threats

Although wadeable streams in the Ozark region, including those at OZAR, are generally considered to be in good condition, multiple stressors threaten their integrity (Davis and Richards 2002; Petersen and Femmer 2002; Huggins *et al.* 2005; USEPA 2006; Heth *et al.* 2016). Due to the karst topography, interbasin groundwater connections make these streams vulnerable to contamination that may originate from adjacent watersheds (Adamski *et al.* 1995; Mugel *et al.* 2009). Stressors such as deforestation and other land management practices in the watershed are particularly problematic because they tend to overwhelm localized protection of stream corridors at the watershed level (Roth *et al.* 1996; Heino *et al.* 2003; Zumberge *et al.* 2003). For example, increases in bank erosion rates and changes in channel morphology through time have been correlated with increased land clearing of steep uplands within a stream basin, as well as historical riparian land clearing (Jacobson and Primm 1997, Panfil and Jacobson 2001).

Previous aquatic invertebrate studies

Several previous studies have been conducted on

stream invertebrate communities at OZAR to assess water quality impacts and biological condition. They include Clifford (1966), Duchrow (1977), Doisy *et al.* (1997, 2002), Rabeni *et al.* (1997), Doisy and Rabeni (1999, 2001), Sarver *et al.* (2002), Heth (2015), and Heth *et al.* (2016). With the exception of Doisy *et al.* (1997), Doisy and Rabeni (2001) and Heth *et al.* (2016), all of these works exist as gray literature and have not been published. Additionally, these studies were based on either single season events, or multiple season events within the same year. We do not attempt to summarize those studies here.

Other aquatic invertebrate studies at OZAR have attempted to take a more comprehensive and long-term approach to assessing invertebrate community dynamics and stream biological condition. For example, the National Park Service's Heartland Inventory and Monitoring Network (HTLN) began monitoring invertebrates, habitat and water quality at OZAR in 2005. Bowles *et al.* (2016) presented a summary of the first few years of this monitoring program for mainstem river sampling locations.

The purpose of this paper is three fold. First, we describe patterns in selected characteristics of invertebrate community structure, habitat, and water quality at OZAR. Second, we assess the biological condition of those invertebrate communities relative to regional reference sites. Third, we determine the strength of relationships between invertebrate community metrics and environmental variables (habitat and water quality).

Methods and Materials

Site Selection

Sampling was conducted at six permanent mainstem river sites on the Current River and three sites on the Jacks Fork annually from 2005 to 2009, and again in 2012 and 2014 (Fig. 1). All samples were collected from riffles during November through early January.

Invertebrate Sampling

Three benthic invertebrate samples were collected from each of three successive riffles at each sampling site using a Slack-Surber sampler (500 μ m mesh, 0.25 m², n=9; Moulton *et al.* 2002). The sample area was agitated for 2 minutes with a garden cultivation tool. Large pieces of substrate were scrubbed with a brush as necessary to remove attached invertebrates. Samples were placed in plastic jars and preserved with 99% isopropyl or 95% ethyl alcohol. Samples were sorted in the laboratory following a subsampling routine

Figure 1. Location of water quality, habitat, and benthic invertebrate sampling sites at Ozark National Scenic Riverways, Missouri.

described in Bowles *et al.* (2007), and taxa were identified to the lowest practical taxonomic level (usually genus) and counted. We recognize that raw taxa richness estimates based on our subsampling routine (≥ 200 organisms, plus large and rare search) possibly may result in biased estimates of that metric, but as noted by Vinson and Hawkins (1996), taxa richness increases rapidly in samples up to 200 individuals but it increases at a much slower rate thereafter. So, we contend our data reasonably reflect richness in our samples without using rarefaction procedures.

Habitat and Water Quality

Qualitative habitat variables (percent substrate embeddedness, periphyton, filamentous green algae, and aquatic vegetation) were estimated within the sampling net frame as percentage categories (0, <10, 10-40, 40-75, >75). Habitat data were analyzed as midpoints of each category across years for each site to estimate the general condition of those resources.

Dominant substrate size was visually estimated within the sampling net frame using the Wentworth scale (Wentworth 1922). Depth (cm) and current velocity (m/sec) were measured immediately in front of the sampling net frame using a top-setting wading rod fitted with a calibrated Marsh-McBirney Flow-Mate 2000 flow meter. Discharge was taken from appropriate USGS gages or measured by hand using the method of Carter and Davidian (1969). Discrete readings of water quality parameters (temperature, dissolved oxygen, specific conductance, and pH) were recorded at each riffle sampled with calibrated, hand-held instruments (YSI models 55, 63, ProPlus). In addition, hourly readings of water quality parameters (temperature, dissolved oxygen, specific conductance, pH, and turbidity) were recorded continuously at least 1 week prior to sampling using calibrated data loggers (YSI models 6600, 6920) at two fixed sites on the Current River and one site on the Jacks Fork (Fig. 1). Water quality data were summarized as means across years for

each site to estimate the general condition of those resources. The water quality data collected for this study only describe the prevailing conditions that may influence the structure of invertebrate communities, and they represent only a small snapshot of the broader range of possible conditions over longer periods.

Statistical Analysis

On the recommendation of Reynoldson *et al.* (1997) we used both a multimetric index and multivariate statistical analyses to analyze our data to maximize their interpretive value.

Multimetric analysis

The Stream Condition Index (SCI), a multimetric index developed by Rabeni *et al.* (1997) for the state of Missouri, was used to assess biological condition of invertebrate community data. The SCI is founded on data collected from 26 reference streams in the Ozarks region (Rabeni *et al.* 1997). It is calculated using four metrics as measures of community structure and balance, including taxa richness, EPT (Ephemeroptera, Plecoptera, Trichoptera) richness, Shannon's diversity index, and Hilsenhoff Biotic Index (HBI; Hilsenhoff 1982, 1987, 1988). Procedures for calculating and scoring these four metrics and the SCI can be found in Bowles *et al.* (2007). For this study, we used only that portion of the index as it relates to single habitat, coarse substrates (i.e., riffles) during a fall index period (Rabeni *et al.* 1997).

High values are preferred for all metrics used in the index, except for HBI, where smaller values are the desired response. An increase in HBI values over time is undesired, because that would reflect the community's increasing tolerance to disturbance. See Bowles *et al.* (2007) for sources of assigned invertebrate tolerance values. The chosen metrics are sound measures of community structure and balance and are generally considered sufficiently sensitive to detect a variety of potential pollution problems in Ozark streams (Rabeni *et al.* 1997) (Table 1). The lower or upper quartile of the distribution for each metric is used as the minimum value representative of reference conditions (Table 1). Mean metric values were established by averaging the values for each of three samples per riffle and then averaging the means for the three riffles to establish a site mean. The SCI produces three possible levels of stream condition: 1) fully biologically supporting (unimpaired), 2) partially biologically supporting (impaired), and 3) non-biologically supporting (very impaired). Unimpaired or reference

sites score ≥ 16 and have the capability of supporting and maintaining a balanced, integrated, adaptive community of organisms having a taxa composition, diversity, and functional organization comparable to that of the natural habitat of the region. Both partially biologically supporting (SCI 10-14) and non-biologically supporting (SCI 4-8) categories indicate impaired streams that do not fully meet the beneficial use of protection of aquatic life.

Multivariate analyses

Pairwise correlation coefficients for each pair of invertebrate metrics and habitat and water quality variables were calculated using nonparametric Kendall's tau (Daniel 1990), because examination of histograms revealed lack of normality for many of the habitat variables. SPSS version 20.0 was used to calculate correlation coefficients (IBM Corp. 2011).

This analysis evaluated correlations between the four biological metrics calculated from aquatic invertebrate samples and 11 habitat variables. Data were grouped separately and analyzed by year and by site. When grouped by year, all riffles from all sites were included in the same analysis, and the analysis was repeated for each year ($N = 7$ years; $n = 18$ observations for each correlation: 3 riffles $\times$ 9 sites) (4 metrics $\times$ 11 habitat variables $\times$ 7 years = 308 total correlations). This approach provided the strongest level of independence among observations. When grouped by site, all years of data for all riffles of each site were included, and the analysis was repeated for each site ($N = 9$ sites; $n = 21$ observations for each correlation: 3 riffles $\times$ 7 years) (4 metrics $\times$ 11 habitat variables $\times$ 9 sites = 396 total correlations). Because these analyses produced many correlation coefficients and P-values, with an unknown actual type I error rate, a meta-analytic approach was applied to these data, and the number of "significant" ($\alpha = 0.05$) correlations was summarized for each pair of metrics and habitat variables. The percentages of "significant" correlations for each pair of metrics and habitat variables were summarized over all metrics. Habitat variables with a greater percentage of "significant" correlations are likely to have, in general, greater potential to explain variability in these metrics.

Because we anticipated there would be differences in the invertebrate community structure along the river continuum, we used Sørensen's similarity index (presence/absence) to analyze similarity of taxa occurrences across years among the different sampling sites (Vannote *et al.* 1980; Southwood and Henderson 2000; Hammer *et al.* 2001). Similarity index scores

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Table 1. Descriptive statistics, quartiles and scores for aquatic invertebrate metrics calculated using single habitat coarse substrate (riffle) data during a fall index period (from Rabeni *et al.* 1997). Summary statistics are from riffle habitat of reference streams (n=18) in the Ozark ecoregion during the fall index period.

Metric	Statistics				Quartiles			Scores		
	Mean	Standard Error	Minimum	Maximum	25%	50%	75%	5	3	1
Taxa Richness	28.3	3.3	23.5	41.0	21	26	29	≥21	20-11	<11
EPT Richness	13.1	0.7	11.5	15.0	9	11	12	≥9	8-5	<5
HBI	4.3	0.3	3.3	5.0	3.6	4.9	5.3	≤5.3	5.4-7.7	>7.7
Shannon's Diversity Index	2.4	0.1	2.1	2.7	2.29	2.44	2.61	≥2.29	2.28-1.15	<1.15

SCI Scoring: ≥16 not impaired, 10-14 impaired, 4-8 very impaired.

among sites were subsequently analyzed using ascendant hierarchical cluster analysis (Ward 1963) following the recommendation of Magurran (2004). Sørensen's similarity index and cluster analysis were conducted using PAST statistical software (Hammer *et al.* 2001).

Nonmetric multidimensional scaling (NMDS) with a Bray-Curtis distance measure was used to evaluate the relationship of invertebrate metrics (taxa and EPT richness, Shannon diversity index, HBI) and associated environmental variables among collection sites (PAST statistical software, Hammer *et al.* 2001). Variables were transformed prior to analysis using Log₁₀ for water quality data and ArcSin Square Root for proportional data to reduce skew and increase interpretability. Data were averaged over all years for each site. Depth and current velocity were not included in this analysis due to their relative uniformity among samples.

Results and Discussion

Aquatic invertebrates

The aquatic invertebrate faunas of the Current River and Jacks Fork are diverse and many taxa are shared across sampling sites. Among all sites, 155 distinct taxa were identified with similarities ranging from 72% to 86% (Table 2). We identified Chironomidae (Diptera) only to the family level because doing so does not appreciably change the metrics used in this paper (Rabeni and Wang 2001). However, we recognize that by making this grouping the number of distinct taxa is likely much higher. A complete list of invertebrate taxa at each site, their abundances and associated environmental data are too voluminous to present here, but can be obtained from the authors.

The invertebrate metric values recorded among sites exceeded the minimum reference stream values (maximum for HBI) across years (Table 1, Figs. 2A-D).

Table 2. Sørensen similarity index for aquatic invertebrate taxa among river collecting sites on the Current River (C1-C6) and Jacks Fork (J1-J3), Missouri. Taxa compositions were accumulated over 7 years (2005-2009, 2012 and 2014).

	C2	C3	C4	C5	C6	J1	J2	J3
C1	0.76	0.76	0.73	0.75	0.72	0.72	0.73	0.73
C2		0.82	0.78	0.79	0.76	0.73	0.73	0.76
C3			0.85	0.81	0.80	0.81	0.77	0.79
C4				0.86	0.86	0.78	0.78	0.82
C5					0.83	0.77	0.79	0.81
C6						0.78	0.76	0.79
J1							0.79	0.80
J2								0.85

Individual metrics were highly variable among years and sites, although such variability is expected (Mazor *et al.* 2009). Mean taxa richness ranged from 22.0 to 30.4 among sites with the lowest richness values occurring at sites C1 and C2 (22.0 and 23.1, respectively) (Fig. 2A). It is particularly noteworthy that representatives of intolerant EPT taxa were abundant at all sampling sites with mean EPT richness values ranging from 10.9 to 16.1 among sites. Site C1 also had the lowest EPT richness among all sites (Fig. 2B). In contrast, taxa and EPT richness were highest at Current River sites 3 and 4. Taxa and EPT richness values for all three Jacks Fork sites were generally lower than those observed for the Current River. Mean Shannon's diversity index values ranged from 1.9 to 2.5 among sites, with the two upper Current River sites (C1,

Figures 2A-D. Aquatic invertebrate community metrics for 9 sites on the Current River and Jacks Fork, Missouri. Values are means averaged over 7 years (2005-2009, 2012 and 2014) and vertical bars are standard errors. The horizontal line conforms to the minimum reported value for Ozark reference streams, except for HBI, which is the maximum reported value (from Rabeni *et al.* 1997).

C2) consistently having values below 2 (Fig. 2C). For biological data, Shannon's diversity index ranges generally from 1.5 (low taxa richness and evenness) to 3.5 (high taxa evenness and richness) (McDonald 2003), but the actual value is contingent on the number of taxa in the community.

Mean HBI values were low at all sites and well below that for Ozark reference streams (Fig. 2D) and other regional streams (Rabeni *et al.* 1997; Bowles *et al.* 2016). The invertebrate taxa of the Current River and Jacks Fork are largely intolerant (mean tolerance value=4.2, and HBI values generally were below 4.5 at all sites. Mean HBI across years for all sites ranged from 3.1 to 4.4, which reflects good conditions (Hilsenhoff 1982, 1988).

In general, SCI scores showed that the invertebrate communities in this study are indistinguishable from those of reference streams. All SCI scores indicated that our sampling sites are not impaired and are fully biologically-supporting (Fig. 3). Lower scores observed in some years are likely due to interannual variability of invertebrate communities coupled with instream flow dynamics (flood, drought) that occur at those sites rather than anthropogenic disturbances. These data also show the importance of collecting data during multiple years and at multiple sites so that low scores in any given year or location do not overly influence management decisions for corrective actions (Mazor *et al.* 2009). The data further illustrate the importance of using a multimetric index for stream assessment so that too much weight is not placed on the value of a single metric. Environmental stressors, such as extended drought and flooding, may impact invertebrate communities and influence assessment results in any given year.

Habitat and Water Quality

Only summary habitat data are presented here to generally characterize the conditions in which samples were collected. Exclusive of discharge, habitat conditions were generally consistent among sites and years (Figs. 4-7). Mean depth and current velocities where samples were collected were typical for Ozark stream riffles (depth range=25 to 33 cm, current velocities range=0.6 to 0.9 m/sec). Discharge predictably increased in a downstream progression for both the Current River and Jacks Fork (Fig. 4). Smallest mean substrate size for the Current River was at sites C1 and C6 (32.8 mm and 37.9 mm, respectively) (Fig. 5). Site C2 had the largest average substrate size (55.08 mm), while the remaining sites had smaller and more similar sized substrates (42-48 mm). Substrate size for

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Figure 3. Mean SCI values averaged over 7 years (2005-2009, 2012 and 2014) and standard errors for 9 sites on the Current River and Jacks Fork. The horizontal line represents an SCI of 16, the lower limit for rating a site unimpaired.

the Jacks Fork was largest at site J1 (50.2 mm) and became increasingly smaller at the downstream sites (44.6 mm and 41.1 mm, respectively). Embeddedness was generally similar at all sites on both rivers (~26-29%), except site C6 on the Current River, which was about 38% (Fig. 5). Aquatic vegetation (mosses and various angiosperms) and filamentous green algae were poorly represented at all sampling sites (<11%) (Fig. 6). Periphyton densities growing on the rock substrates were generally consistent among sites, ranging from 26 to 34%.

Water quality met Missouri standards in all instances (Missouri Department of Natural Resources, 2014) (Fig. 7A-D). Temperature was variable among (means=8.7-11.9 °C) sampling sites and years, which is expected due to climatic variations among years sampled as well as location of sampling sites along the length of the river. Dissolved oxygen levels were high in all instances and were at or above saturation across years and sites (means=10.21-12.27 mg/liter). Specific conductance was generally consistent among sites, but slightly higher for the Jacks Fork as measured using the hand-held instruments. Overall values were highest for the three sites where dataloggers were deployed, which suggests differences in instrument sensitivities. In all instances, specific conductance ranged from 248 to 328 $\mu\text{m}/\text{cm}$. pH was consistent and similar among all sampling sites and years sampled (means=7.7-8.2). Turbidity, not shown here, was nearly always below 10 NTU. The water quality values we report are consistent with those summarized by Huggins *et al.* (2005), with the exception of temperature because their data were recorded during different seasons.

Figure 4. Mean discharge for the Current River and Jacks Fork, Missouri averaged over 7 years ((2005-2009, 2012 and 2014) with standard errors. See methods for site details.

Figure 5. Mean substrate size (mm) and percent substrate embeddedness associated with benthic invertebrate samples from the Current River and Jacks Fork, Missouri. Values are means averaged over 7 years (2005-2009, 2012 and 2014) with standard errors. See methods for site details.

Figure 6. Percent vegetation, filamentous algae and periphyton occurring in samples from the Current River and Jacks Fork, Missouri. Values are means averaged over 7 years (2005-2009, 2012 and 2014) with standard errors. See methods for site details.

Figures 7A-D. Water physical-chemical data for sampling sites on the Current River and Jacks Fork, Missouri. Values are means averaged over 7 years (2005-2009, 2012 and 2014) with standard errors. Data were collected as discrete readings using hand-held meters at sampling sites 1-6, while data were collected continuously using dataloggers at fixed locations. See methods for site details and Fig. 1 for datalogger locations.

Overall, no habitat variables exhibited persistently strong correlations with any of the metrics, and the percentage of “significant” correlations was relatively low (<30%) in all cases (Table 3). In addition, a certain

number of spurious correlations are expected (1 in 20 for $\alpha = 0.05$) in analyses such as those conducted here. The number of expected spurious correlations ranged from 32 to 37% of the observed “significant” correlations (Table 3). Specific conductance, temperature, dissolved oxygen, substrate size, depth, periphyton, and filamentous algae usually had a greater percentage of “significant” correlations than the other variables, across all analyses, but some of these variables are autocorrelated, hence their biological significance may not be relevant. The low number of significant correlations for some habitat variables is likely due to the categorical scale used to assess some habitat data (see Methods), and the low variability among observed values. This analysis shows that the habitat data collected in relation to benthic invertebrate samples presently has limited value for correlating with community and diversity metrics, but that finding does not rule out further analyses with individual invertebrate taxa or groups of taxa (e.g., EPT), or assessing the collective relationships among habitat variables on the benthic communities.

Cluster analysis of Sørensen’s similarity values showed that Current River and Jacks Fork sites clustered separately and in a downstream progression, with those sites closest to one another in linear distance generally being the most closely related (Fig. 8). The uppermost collection site on the Current River was most unlike the other sites, which probably relates to the distinct physical features of that site compared to the others. Our observations and collected data show the physical

Figure 8. Dendrogram showing results for ascendant hierarchical cluster analysis and relative distance of Sørensen’s similarity index scores of the aquatic invertebrate communities at sampling sites along the Current River (C1-C6) and Jacks Fork (J1-J3), Missouri. Taxa compositions were accumulated over 7 years (2005-2009, 2012 and 2014).

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Table 3. Summary of OZAR pairwise correlations organized by site (i.e., correlations conducted among all years at each site, n=396) and by year (i.e., correlations conducted among all sites in each year, n=308). Values are number of significant correlations/percentage of significant correlations of total.

Variables	HBI	Taxa Richness	EPT Richness	Shannon Diversity Index	Total
By Site					
Depth	3/0.33	2/0.22	5/0.55	0/0	10/0.28
Specific conductance	1/0.11	2/0.22	3/0.333	2/0.22	8/0.22
Current Velocity	2/0.22	1/0.11	1/0.11	3/0.33	7/0.19
Periphyton	3/0.33	2/0.22	1/0.11	1/0.11	7/0.19
Substrate size	1/0.11	2/0.22	2/0.22	2/0.22	7/0.19
Dissolved oxygen	1/0.11	2/0.22	0/0	1/0.11	4/0.11
Filamentous algae	2/0.22	1/0.11	1/0.11	0/0	4/0.11
Vegetation	0/0	1/0.11	0/0	2/0.22	3/0.08
pH	2/0.22	0/0	0/0	0/0	2/0.06
Temperature	1/0.11	1/0.11	0/0	0/0	2/0.06
Substrate embeddedness	0/0	0/0	0/0	0/0	0/0
Total / %	16/0.16	14/0.14	13/0.13	11/0.11	54/0.14
Expected number of spurious correlations =20					
By Year					
Temperature	2/0.29	1/0.14	4/0.57	1/0.14	8/0.29
pH	1/0.14	3/0.43	0/0	4/0.57	8/0.29
Specific conductance	3/0.43	2/0.29	1/0.14	2/0.29	8/0.29
Filamentous Algae	1/0.14	2/0.29	2/0.29	2/0.29	7/0.25
Dissolved oxygen	3/0.43	1/0.14	2/0.29	0/0	6/0.21
Vegetation	0/0	2/0.29	3/0.43	0/0	5/0.18
Periphyton	2/0.29	0/0	1/0.14	0/0	3/0.11
Current Velocity	0/0	0/0	0/0	1/0.14	1/0.04
Substrate size	1/0.14	0/0	0/0	0/0	1/0.04
Substrate Embeddedness	0/0	0/0	0/0	0/0	0/0
Depth	0/0	0/0	0/0	0/0	0/0
Total / %	13/0.17	11/0.14	13/0.17	10/0.13	47/0.15
Expected number of spurious correlations =15					

condition at the three upper Current River sites is more variable both within and among the sites. Site C1 had higher dissolved oxygen concentrations, lower specific conductance, and smaller substrate size compared to all other sites. In contrast, site C2 had the largest substrate, lowest pH, and greatest abundance of filamentous algae and aquatic vegetation among all sites.

The results of the cluster analysis were corroborated by a NMDS analysis (Fig. 9). The NMDS model for the diversity and environmental data was found to be a good fit (Shepard plot stress value =0.04; Axis 1=0.61, Axis 2=0.22). The three Jacks Fork sites grouped closely to

one another as did the three lower Current River sites. In contrast, the three upper Current River sites were more widely separated in ordination space. Correlations of the habitat variables with the ordination axes indicate associations of the Jacks Fork sites with higher specific conductance and pH, and to a lesser extent higher temperature and periphyton density (Fig. 9, Table 4). In contrast, Current River sites 4 through 6 were associated with higher embeddedness and discharge (Fig. 9, Table 4). Current River sites 2 and 3 were associated with higher dissolved oxygen and greater abundance of filamentous algae and aquatic plants (Table 4, Fig. 9).

The relatively wider spacing of sites C1 through C3 may be due, in part, to the influences of two first magnitude springs (Montauk and Welch, $\geq 2,800$ liter/sec) and three second magnitude springs (Cave, Pulltite and Round, ≥ 280 liter/sec) located in the upper river basin where those sites are located. The Current River is formed by Montauk Spring approximately 14 km upstream of site C1. Welch Spring, Cave Spring and Pulltite Spring are located approximately 17 km, 8 km, and 3.5 km, respectively, upstream of site C2. Round Spring is located approximately 0.5 km upstream of site C3.

Because these springs produce cold, thermally consistent flows and are environmentally stable and uniform, they exhibit strong localized influences on the structure and functioning of the three upper sampling sites, thus giving them their unique character. Inflows

Table 4. NMDS correlation coefficients for habitat variables. See methods for details.

Variable	Axis 1	Axis 2
Discharge	0.77	-0.20
Temperature	-0.46	0.14
Dissolved oxygen	0.21	-0.49
Specific conductance	-0.004	0.68
pH	-0.14	0.41
Filamentous algae	0.10	-0.72
Vegetation	0.30	-0.79
Periphyton	-0.29	0.11
Substrate size	0.29	-0.02
Substrate embeddedness	0.33	0.19

Figure 9. NMDS biplot with convex hulls for invertebrate diversity metrics by sampling sites and associated environment variables at Ozark National Scenic Riverways, Missouri. Triangles represent Jacks Fork sites (J1-J3), and circles (C1-C3) and squares (C4-C6) represent upper and lower Current River sites, respectively.

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from these large springs influence surface stream character through thermal consistency (warmer in winter, colder in summer), higher dissolved calcium and specific conductance levels, lower dissolved oxygen concentrations, and potentially higher nutrient concentration (Smartt *et al.* 2013; Westhoff and Paukert 2014). Spring dominated streams also typically have lower faunal diversity and higher floral diversity in comparison to streams that receive most of their flow from surface sources because they generally have greater physical and chemical uniformity (Williams and Hogg 1988; Danks and Williams 1991; Varza and Covich 1995; Bowles and Dodd 2015). However, increased occurrence of aquatic vegetation and spring adapted aquatic invertebrates may occur in the mixing zone of springs and streams (Reiser *et al.* 2004; Barquín and Death 2011; Westhoff and Paukert 2014; Heth 2015).

Punctuated inflows of multiple large volume springs into the upper Current River effectively serve as predictable press type disturbances (Poff 1992; Lake 2000). Moreover, large spring inflows constantly reset or alter the predicted river continuum model (Vannote *et al.* 1980), and they mitigate patchiness associated with many surface fed streams (Resh *et al.* 1988; Lake 2000; Dornelas 2010). The uniformity and stability of the spring flows may also serve as a refugium for aquatic life from other disturbances (Lake 2000; Westhoff and Paukert 2014; Heth 2015), including floods, drought, and anthropogenic impacts. In contrast to the upper river, sampling sites on the lower Current River (C4-C6) have most of their baseflows originating from high magnitude springs (>90%, Mugel *et al.* 2009) so the punctuated disturbances from spring inflows observed upstream are not as pronounced. In addition, Blue Spring (first magnitude) is located approximately 8 km upstream of site C4 and Bass Rock Spring (second magnitude) and Big Spring (first magnitude) are located approximately 18 km and 10 km, respectively, upstream of site C6. The Jacks Fork is the major tributary of the Current River and most of its flows originate from surface flows. An additional first magnitude spring feeds the Jacks Fork downstream of our sampling sites with its confluence approximately 10 km upstream of Site C4. Finally, Current River basin tributaries located downstream of the confluence with Blue Spring have smaller drainage basins than those upstream, which may further increase the influence of springs in the lower river.

Conclusions

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River and Jacks Fork is diverse and reflects above average water quality. These two rivers are fully biologically supporting and meet Ozark reference stream criteria at all sites sampled. Inherent variability of invertebrate community diversity across sites and years highlights the importance of multiyear assessment and monitoring to support management decisions. Large volume springs likely serve as sustained and predictable sources of disturbance for the Current River, but this unique type of disturbance remains incompletely quantified. Although the condition of invertebrate communities and water quality at OZAR are largely sound and have high integrity, numerous ongoing and projected threats to these resources remain, and those threats largely originate outside of the park's jurisdictional boundaries.

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