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Hydrology-Biology Response Relationships in the Ozark Highlands

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Biological Sciences

by

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December 2015
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Abstract

I examined flow-ecology relationships among stream communities in the Ozark Highlands, USA. I sampled fish, crayfish, and benthic macroinvertebrates during two consecutive summers, including a drought year (2012) and a flood year (2013). Biological response variables related to community structure were assessed via two different statistical methods: an Information Theoretic approach relating response variables to *a priori* selected predictor variables incorporating hydrology, habitat, geomorphology, and water quality, and canonical ordination using forward selection to relate these same response variables to a large assortment of hydrologic metrics. In addition to assessing metrics related to predicted natural flow, flow alteration at gaged sites was also quantified and community metrics were assessed with respect to flow alteration. Additionally, I conducted a manipulative laboratory greenhouse experiment to examine the effects of stream drying, one of the major components of the natural hydrologic disturbance regime in the region, on stream fishes as well as benthic community structure. Hydrologic variation was often less important than other environmental variables and substantial temporal variation existed in flow-ecology relationships. Stream flow magnitude was the most important category of hydrologic metric overall, but there were key differences in which metrics were important for each assemblage and how those assemblages responded to those metrics. Flow alteration has a strong effect on Ozark riverine communities, and the most important categories of flow alteration affecting these communities are magnitude of average flows, and frequency, magnitude, and duration of high flows. The large number of important high flow metrics suggests that flood events may play a particularly crucial role in structuring aquatic assemblages in the region. I found that seasonal stream drying had strong species-specific effects on organisms in pool refuges, and that type of drying specifically affected

periphyton growth. Overall, I found that the elucidation of flow-ecology relationships and management decisions that are based on those relationships face a variety of challenges: the complex interaction of hydrology with other kinds of environmental variables, temporal variation in the aquatic community, and the differential effects of flow metrics on different assemblages.

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Table of Contents

Introduction.....	1
Literature Cited	7
Stream Macroinvertebrate Flow-Ecology Relationships Vary Temporally: Importance of Geomorphology, water Quality, and Disturbance	11
Abstract	12
Introduction	13
Methods	16
Results	27
Discussion	30
Acknowledgements	37
Literature Cited	39
Tables and Figures	46
Relative Influence of Hydrology, Geomorphology, Water Quality, and Disturbance on Fish and Crayfish Assemblages in Ozark Highland Streams	55
Abstract	56
Introduction	57
Methods	59
Results	67
Discussion	69
Acknowledgements	75
Literature Cited	77
Tables, Figures, and Appendices.....	83
Alteration of Stream Flow in the Ozark Highlands: Consequences for Fish, Crayfish, and Macroinvertebrate Communities	95
Abstract	96
Introduction	97
Methods	100
Results	108
Discussion	112
Acknowledgements	118
Literature Cited	120

Tables, Figures, and Appendices.....	126
Effects of Pulse and Press Drying Disturbance on Benthic Stream Communities	136
Abstract	137
Introduction	138
Methods	142
Results	148
Discussion	150
Acknowledgements	157
Literature Cited	159
Tables and Figures	164
Conclusion	174
Literature Cited	178

Introduction

Anthropogenic alteration of freshwater ecosystems is among the most severe current threats to biodiversity, particularly in developed countries such as the U.S., which possess some of the most threatened aquatic ecosystems in the world (Benke 1990, Jelks 2008). In North America, extinction rates for freshwater organisms may be five times greater than species losses in terrestrial systems (Dudgeon et al. 2006, Vaughn 2010). Human influence on watershed hydrology is a pervasive phenomenon that may be the chief cause of ecological impairment in stream ecosystems, and altered flow regimes have contributed significantly to the loss of species (Postel and Richter 2003, Carlisle et al. 2010). The cumulative effects of hydrologic alteration have global-scale environmental consequences, yet the study of these effects are relatively recent compared to human development of dams, reservoirs, and other technologies that have allowed us to alter rivers for human needs (Rosenberg et al. 2000).

Streamflow plays a crucial role in determining habitat and biotic composition in lotic ecosystems (Vannote et al. 1980, Poff and Ward 1990), and hydrologic variation may be among the most critical environmental variables for stream biota (Poff et al. 1997). The concept of the natural flow regime (Poff et al. 1997) posits that the ecological integrity of rivers depends on their natural dynamic character rather than just minimum low flows that have historically been the focus of stream management (Bunn and Arthington 2002, Poff et al. 2010). The natural flow regime includes magnitude, frequency, duration, timing, and rate of change of streamflows (Poff et al. 1997, Poff et al. 2010) and dictates not only the structure and function of stream ecosystems, but also the evolutionary adaptations of stream organisms (Bunn and Arthington 2002, Carlisle et al. 2010).

Hydrologic alteration negatively effects ecosystem function as well as biodiversity (Bunn and Arthington 2002, Harris and Heathwaite 2011, Warfe et al. 2014). Water managers face the growing crisis of balancing the water needs of growing human populations with the conservation of stream ecosystems (Poff et al. 2010, Olden et al. 2014). In the U.S., natural flow regimes are threatened by an assortment of factors, including construction of dams and diversion structures, groundwater withdrawals from aquifers, and inter-basin transfers (Carlisle et al. 2010). Furthermore, extreme climate events are expected to increase as a result of global climatic change, including many events that directly impact lotic ecosystems, such as an increase in the frequency, duration, and intensity of drought (Beniston et al. 2007, Beche et al. 2009). These factors may interact with one another in ways that amplify the impact that individual stressors may have alone. Water withdrawals during drought years can further reduce habitat connectivity and result in critical flow reductions (Beche et al., 2009). Altered flow regimes can also facilitate species invasions, another pervasive phenomenon in rivers (Closs and Lake 1996, Bunn and Arthington 2002, Poff et al. 2010). The maintenance of naturally variable hydrologic regimes may provide a safeguard against many of these impacts, and is a crucial challenge to the protection of rivers, their biota, and people who depend on them (Carlisle et al., 2010).

It is important to consider that natural disturbance is a critical component of rivers and streams, as it is in most ecosystems (Sousa 1984, Pickett and White 1985, Lytle and Poff 2004). In lotic ecosystems, the natural disturbance regime typically consists of cycles of seasonal flooding and drought (Lytle and Poff 2004). These events may play a major role in the structuring of aquatic communities (Resh et al. 1988, Poff and Allan 1995). Although seasonal drought is a part of the natural disturbance regime in many streams, there is increasing evidence that human activities may strongly exacerbate its effects (Bond et al. 2008). Anthropogenic

alteration of natural disturbance regimes can have major consequences for biodiversity and ecosystem function (Benke 1990, Ward 1998, Bunn and Arthington 2002), both in cases where the magnitude, extent, and frequency of disturbance are increased or when they are diminished (Carlisle et al. 2010).

The environmental flow paradigm is an approach to the management and conservation of freshwater ecosystems that incorporates the complexity of the natural flow regime and its effect on stream biota. Environmental flows have been defined in a variety of ways. The ICUN defines them as "the water regime provided within a river, wetland, or coastal zone to maintain ecosystems and their benefits where there are competing water uses and where flows are regulated" (Dyson et al. 2003). The Brisbane Declaration (2007) defines environmental flows as "the quantity, timing, and quality of water flows required to sustain freshwater and estuarine ecosystems and the human livelihood and well-being that depend on these ecosystems". Poff et al.'s (2010) Ecological Limits of Hydrologic Alteration (ELOHA) approach emphasizes that environmental flows consist of the quantity, timing, and quality of stream flows required to maintain the function of particular organisms or overall ecosystem structure and function.

The imperative to incorporate freshwater ecosystem needs and region-wide water resources planning has been increasingly recognized at national and international scales (Petts 1996, Poff et al. 2010, Kendy et al. 2012, Olden et al. 2014). Understanding the role of flow alteration in modifying the ecological processes of rivers has become a key element in the development of regional flow-standards Olden et al. 2014). Implementation of the environmental flows-based approach to stream management faces several unique challenges. A critical step in the process is classification of streams into distinct natural flow regimes; different

flow regimes within the same region may be more or less susceptible to particular forms of flow alteration (Leasure et al. 2014). Furthermore, the hydrologic regime is highly interrelated with a complex suite of other important variables including water quality, land-use, habitat structure, and stream geomorphology (Poff et al. 2010, McManamay and Frimpong 2015). The interaction of all of these variables shapes the structure and function of streams (Dudgeon et al. 2006), but the relative importance of each, and the potential interactions between them, have remained challenges for stream ecologists attempting to elucidate relationships between flow variables and biota, as well as biological responses to flow alteration (Olden et al. 2014). Another challenge is temporal variation in biological communities. While ideally, natural temporal variation, including infrequent disturbance events such as severe floods or suprasedasonal droughts, should be incorporated into such studies (Sousa 1984, Stoddard 2006), this may be challenging due to the logistical considerations of monitoring many sites over extended periods (MacDonald and Cote 2014). Strong temporal variation in communities can confound the formulation of predictable flow-ecology relationships (Rolls et al. 2012, Katz and Freeman 2015).

The goal of the research presented in this dissertation was to examine relationships between hydrology and stream communities in the Ozark Highlands, USA. My objectives included: elucidating flow-ecology relationships between biota and the natural flow regime, assessing the relative importance of hydrology among an assortment of other kinds of environmental variables, quantifying the effects of hydrologic alteration on stream communities, and examining the effects of a particular component of the disturbance regime, i.e. seasonal stream drying, on aquatic community structure and function. The Ozark Highlands is a Level III Ecoregion (Omernik and Griffith 2014) that stretches across portions of four states in the Interior

Highlands of the central U.S. This region contains a diversity of freshwater habitats, including fens, sinkholes, springs, and the headwaters of clear, free-flowing streams (TNC-OEAT 2003). It is home to a unique assemblage of fish species, including 10 endemic species, has remarkably rich crayfish and mussel faunas with a number of endemic species, and a highly diverse aquatic herpetofauna, many of which are near-endemics found otherwise only in the adjacent Ouachita Highlands (TNC-OEAT 2003). The remarkable freshwater biodiversity in this region is potentially threatened by a host of anthropogenic threats, including rapidly growing urban areas and agricultural development that affect water quality (Petersen et al. 2005, Haggard 2010, Scott et al. 2011), expansion of natural gas extraction (Johnson et al. 2015), displacement of native fauna due to the spread of invasive species (Magoulick and DiStefano 2007, Larson et al. 2009), and direct hydrologic alteration of streams via construction of reservoirs, dams, and watershed development (TNC-OEAT 2003).

To achieve these objectives, I carried out a two-year field study of environmental flow-biological response relationships in the Ozark Highlands, focusing on several aquatic assemblages: fish, crayfish, and benthic macroinvertebrates. This study was carried out in conjunction with a classification of Ozark-Ouachita Interior Highland streams into seven distinct hydrologic flow regimes (Leasure et al. 2014). I focused on one of the predominant flow regimes in the region, Groundwater Flashy Streams, in order to facilitate biological comparisons. I approached these objectives in a variety of ways, incorporating both multimetric and multivariate analyses and assessing biological responses both in the context of a large assortment of hydrologic metrics and a smaller set of a priori selected predictor variables incorporating habitat, geomorphology, water quality, and watershed-scale disturbance. In addition to examining relationships between aquatic assemblages and predicted natural hydrology, I also

assessed the effects of hydrologic alteration on the aquatic community. Finally, I performed a manipulative lab experiment designed to explore the effects of stream drying, a critical component of the regional disturbance regime that may be exacerbated by a variety of anthropogenic causes, on benthic community structure.

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**Stream Macroinvertebrate Flow-ecology Relationships Vary Temporally: Importance of
Geomorphology, Water Quality and Disturbance**

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Abstract

Uncertainty is inherent in the establishment of flow-ecology relationships because they may vary temporally and they are affected by the complex interaction with other environmental variables, including geomorphology and water quality. We examined flow-ecology relationships in benthic macroinvertebrate communities in Ozark Highland streams, USA, over two years with contrasting environmental conditions, a drought year (2012) and a flood year (2013). We used a quantitative richest-targeted habitat method and a qualitative multi-habitat method to collect macroinvertebrates at 20 sites. Metrics of community structure were assessed via two different statistical methods: an Information Theoretic approach relating response variables to *a priori* selected predictor variables incorporating hydrology, habitat, geomorphology, and water quality, and canonical ordination using forward selection to relate these same response variables to a large assortment of hydrologic metrics. We found that hydrology was less important than other environmental variables and that there was substantial temporal variation in environment-ecology relationships, with fewer significant relationships during the drought year. Canonical ordination showed that stream flow magnitude was the most important category of hydrologic metric overall, but that there was a shift in relative importance from magnitude of low flow metrics in 2012 to magnitude of average flow metrics in 2013, and that specific metrics of importance varied markedly between sampling type and year. We suggest that further examination of the temporal variation in flow-ecology relationships is warranted, and that the effects of flow, while potentially important, are best considered within a wider framework of environmental variables, including geomorphology, water quality, and disturbance.

Introduction

Environmental flows are defined as the quantity, timing, and quality of stream flows required to maintain the function of particular organisms or overall ecosystem structure and function (Poff et al. 2010). The historic approach to water management has often involved advocating minimum low flows necessary to sustain lotic habitats and their communities. However, it has become increasingly clear in recent decades that a naturally variable flow regime, rather than a minimum low flow, is vital to sustaining freshwater ecosystems (Poff et al. 1997, Bunn and Arthington 2002). Hydrologic variation in streams may potentially be the most important environmental variable for biota (Poff et al. 1997, Larson et al. 2009), overriding even predation and competition as the main determinant of community structure and use of resources in stream ecosystems (Grossman et al. 1998, Magoulick and Kobza 2003). Following this recent paradigm shift, relationships between hydrologic variables and stream community structure have become the focus of many regional environmental flow studies that have begun to inform the management of freshwater ecosystems, often with uneven results (Gillespie et al. 2014, Olden et al. 2014).

One potential difficulty in developing quantifiable flow-ecology relationships is temporal variation in biological communities. Bioassessment techniques are generally based on comparing ecological conditions in disturbed areas to those in unimpacted or reference-condition streams, which are thought to show less natural variation than variation due to anthropogenic impact (Reynoldson et al. 1997, Stoddard et al. 2006). Ideally, natural temporal variation, including infrequent disturbance events, e.g. severe droughts and floods, should be incorporated into the reference conditions in such studies (Sousa 1984, Stoddard 2006) but this is often

challenging due to the time and expense required to monitor many sites over extended periods (MacDonald and Cote 2014). Methods such as the Ecological Limits of Hydrologic Alteration (ELOHA) framework depend to a large extent on predictable relationships between flow variables and metrics related to stream ecosystems and organisms (Poff et al. 2010), but this could potentially be confounded by strong temporal variation in biological communities.

Uncertainty is also inherent in the establishment of flow-ecology relationships due to the complex interaction of many other important variables such as habitat structure, geomorphology, and water quality (Poff et al. 2010). The important role of all of these factors in shaping the structure and function of riverine ecosystems has been well established (Poff et al. 1997, Dudgeon et al. 2006), but the relative importance of each and potential interactions between them has remained a challenge in the establishment of regional flow standards (Poff et al. 2010, Olden et al. 2014). In this study, we used an approach that incorporated all of these components in addition to hydrologic data to facilitate comparison of the relative importance of these factors or combinations of factors. Furthermore, Reynoldson et al. (1997), in a comparison of multimetric and multivariate analyses, suggested that both approaches had strengths and weaknesses and that they were best used in a complementary fashion in studies relating water-quality impairment to benthic macroinvertebrate communities; we have taken the same approach with respect to hydrology metrics in this study.

Human influence on watershed hydrology is a pervasive world-wide phenomenon that may be the chief cause of ecological impairment in stream ecosystems (Sparks 1995, Bunn and Arthington 2002, Carlisle et al. 2010) and one of the most severe current threats to biodiversity (Benke 1990). Water managers are increasingly challenged to provide reliable and affordable

water supplies to growing human populations, while at the same time mediating the degradation of freshwater ecosystems (Poff et al. 2010, Olden et al. 2014). In the U.S., natural stream-flow regimes are influenced by anthropogenic factors such as construction of dams and diversion structures, land uses that alter runoff to stream channels, groundwater withdrawals from aquifers, and inter-basin water transfer (Carlisle et al. 2010). Because natural timing, magnitude, and frequency of stream-flows dictate the evolutionary adaptations of many river biota (Bunn and Arthington 2002, Carlisle et al. 2010), and control physical and chemical processes (Carlisle et al. 2010, Poff et al. 2010), anthropogenic alterations of stream-flows may have profound effects on ecosystem structure and function.

The Ozark Highlands is a Level III Ecoregion (Omernik and Griffith 2014) that stretches across portions of four states in the Interior Highlands of the central U.S (Fig. 1). It is home to an assortment of freshwater habitats with diverse assemblages of many taxonomic groups, including amphibians, fish, crayfish, mussels, and aquatic insects, and is a crucial center of biodiversity and endemism for many of these groups (TNC-OEAT 2003). This study focuses on flow-ecology relationships among the benthic macroinvertebrate community in the Ozark Highlands. Macroinvertebrates have long been used as water quality indicators in lotic systems (Karr 1991); they are a critical part of stream food webs, show a wide variety of tolerances to pollution and other anthropogenic stressors, are relatively easy to sample, and can show the effects of both long- and short-term environmental effects on streams (USEPA 2007). While there is a long history of use of macroinvertebrates as indicators of water quality (Armitage et al. 1983, Reynoldson et al. 1997, Hawkins et al. 2000), specific relationships between macroinvertebrate taxa and hydrologic variables is a newer area of study (Extence et al. 1999, Carlisle et al. 2010).

Our objectives were to develop environment-biology relationships for benthic macroinvertebrate communities in the Ozark Highlands and to test hypotheses relating biology to hydrology, water quality, stream geomorphology and disturbance. We approached these objectives in two ways: multiple regression analysis incorporating a small set of environmental variables from several categories including hydrology, and multivariate analysis incorporating a larger set of only hydrologic variables.

Methods

Site Selection

Aquatic community sampling was conducted at 20 sites over two summer field seasons (May-July) during 2012 and 2013 in northwest Arkansas, southwest Missouri, and northeast Oklahoma (Fig. 1). The two years in which this study was conducted contrasted strongly in flow conditions. During the summer of 2012, there was a severe to extreme drought throughout the study area as measured on the Palmer Drought Severity Index (PDSI), while 2013 saw higher than normal precipitation and flows, including summer flooding at many of the sites (NOAA 2015).

To facilitate biological comparisons, all sites were selected within a single ecoregion, the Ozark Highlands; a single physiographic region, the Springfield Plateau; and within a single flow regime, Groundwater Flashy streams, where flow regime was based on a classification of Ozark streams into seven natural flow regimes (Leasure et al. 2014). Streams selected ranged from 22 to 542 km² total drainage area. Sites encompassed a wide gradient of conditions, ranging from reference quality (6) to highly disturbed (29) on a Hydrologic Disturbance Index (HDI) developed by Falcone et al. (2010). This HDI consists of seven watershed scale metrics of

disturbance, including presence of major dams; change in reservoir storage from 1950-2009; percentage of streamlines coded as canals, ditches or pipelines; road density; distance to nearest major pollutant discharge site; freshwater withdrawal estimates; and fragmentation of undeveloped land in the watershed (Falcone et al. 2010).

The majority of sites (18) were located at USGS stream gages where hydrologic data could be obtained, but un-gaged sites (2) were also included in the study (Fig. 1). All available gaged sites within the flow class and physiographic province that were suitable to our sampling methods were selected. Un-gaged site selection was based on Leasure et al.'s (2014) flow regime map of the Ozark Highlands; sites were randomly selected from stream segments classified within the Groundwater Flashy flow regime. Natural flow conditions were predicted for both gaged and un-gaged sites, based on 171 flow metrics relating to magnitude (M), frequency (F), duration (D), timing (T), and rate of change (R) of flow events (Leasure et al. 2014).

Benthic macroinvertebrate community sampling

At each site, we sampled a reach consisting of three riffles, three pools, and three runs. All habitat units were located a minimum of 100 m from road crossings to avoid the hydrologic influence of bridge abutments, culverts, or any other man-made structures that could influence physical stream habitat characteristics or create artificial habitat. Benthic macroinvertebrates were sampled using modified versions of two different methodologies devised for the National Water Quality Assessment (NAWQA) program by Moulton et al. (2002), consisting of a semi-quantitative richest-targeted habitat (RTH) method and a qualitative multiple habitat (QMH) method.

In the RTH method, a quantitative sample of invertebrates was taken from riffles only, the habitat type determined to support the richest invertebrate community in high-gradient wadeable streams (Moulton et al. 2002). A 0.25 m² pvc quadrat frame was used at 3 randomly selected locations within each riffle, in conjunction with a Slack sampler consisting of a wooden handle attached to a rectangular net frame (50 cm × 30 cm) fitted with a tapered, 500-μm NitexTM collection net. The slack sampler was positioned immediately downstream of the quadrat and perpendicular to the direction of flow. Large cobble and debris were removed by hand from the sampling area and inspected for attached organisms, which were then removed from the surface in front of the slack sampler. The sampling area was disturbed by digging into the substrate and agitating it to stir up invertebrates in the benthos, which were then collected by moving the slack sampler in a forward motion and retrieving it. The nine discrete subsamples were then combined and placed into a 19-L plastic bucket for processing. Processing consisted of rinsing and removal of large debris, followed by elutriation and sieving (with a 500-μm sieve) of the samples to separate invertebrates and organic debris from inorganic debris.

The QMH method was used to document invertebrate taxa present in all habitat types throughout our sampling reaches (Moulton et al. 2002). Before QMH sampling began, crew members assessed the entire reach to determine number of different instream habitat types present and to estimate proportions of each type present. Then, QMH collections were taken from each of the different habitats present in the reach and combined into a single composited sample. A D-frame kicknet with 500-μm mesh was used to collect invertebrates from each habitat type present in relative proportion to habitat area for a total standard time of one hour per reach. Samples were processed in the field as described for the RTH method mentioned above.

In the laboratory, invertebrate samples were sorted on a square gridded subsampling frame of 25, 5×5 cm squares using a fixed-count approach targeting a minimum of 300 organisms (Barbour et al. 1999, Moulton et al. 2000). After pouring the sample into the frame and allowing it to settle evenly, an initial inspection was performed to remove large and rare organisms likely to be missed during subsampling. A grid square was randomly selected and all of the organisms present were counted. Subsampling proceeded in this fashion until a minimum of 300 organisms were counted, with the square in which the 300th organism was counted also being fully counted. All macroinvertebrates were identified to the lowest practical taxonomic level, generally family or genus. To estimate total numbers of organisms in samples, a laboratory subsampling correction factor was used (Moulton et al. 2000) in which the total number of grids was divided by the number of grids sorted during subsampling, and multiplied by the number of organisms subsampled. Large and rare organisms taken from the sample as a whole were added to these numbers without a correction factor. Invertebrate community response variables were then calculated based on these numbers.

Habitat, geomorphology, and water quality measurements

Measured habitat variables included wetted width, current velocity, depth, substrate composition, and canopy cover. Length and wetted width of each habitat unit were measured with a tape measure; length at the middle of the stream and width at a minimum of 5 transects along each habitat unit. For habitat units exceeding 10 m in length, an additional transect was added for each additional 5 m. At five evenly spaced points along each transect, depth and current velocity were measured with a Model 2000 Portable Flowmeter (Marsh McBirney, Inc.) and substrate size was recorded on a modified Wentworth scale ranging from 1 (silt) to 7

(bedrock). Canopy angle was measured from the midpoint of each transect using a clinometer. For RTH samples, habitat predictor variables were calculated based only on measurements in riffle units; for QMH samples, measurements taken over the entire reach were used.

Stream geomorphology was assessed at each reach using a protocol specifically designed for high-gradient streams (Willard et al. 2004), including measurements of bankfull width, bankfull depth and low bank height, counts of debris jams and sediment storage bars, visual estimation of vegetative buffer widths, and assessment of near bank vegetation type and other categorical variables. Finally, a qualitative Rapid Habitat Assessment (RHA) was given to each site with a maximum possible score of 200. RHA consisted of 10 different habitat parameters targeted specifically at the assessment of high-gradient streams: epifaunal substrate and available cover, embeddedness, velocity/depth patterns, sediment deposition, channel flow status, channel alteration, frequency of riffles, bank stability, bank vegetative protection, and riparian vegetative buffer width. Each parameter was ranked from 1 (low quality) – 20 (high quality) and tallied, for a maximum total score of 200 (Willard et al. 2004).

Water samples were taken from each site for analysis at the Arkansas Water Resources Center (AWRC) Water Quality Lab (WQL) three times during each sampling year: spring, summer, and winter. Samples were collected at the thalweg of the stream in a 500 mL NalgeneTM sampling bottle which was first rinsed by filling and emptying with stream water three times, then stored on ice in a cooler for transport to the lab (USEPA 2009). Water quality parameters measured at the AWRC included chloride, conductivity, fluoride, nitrate, soluble reactive phosphorus (P), sulfate, total nitrogen (N), total P, total suspended solids, and turbidity. Additionally, at the time of biological sampling, physical-chemical data, including temperature,

pH, dissolved oxygen, conductivity and salinity were collected using a Model MS5 multi-parameter sonde (Hydrolab, Inc.).

Hydrologic variable estimation

USGS daily flow data were obtained for 208 gages within the Interior Highlands region, including the South Central Plains of Arkansas, using the R package *dataRetrieval* (Hirsch and De Cicco 2015). Water years include the period from October 1 to September 30, and they are named for the year they end. Every water year in each daily flow record was assessed to identify the number of days of data, number of days missing data, and the largest contiguous block of days with missing data. Years were removed from daily flow records if they had more than 30 days of missing data, or if they had a contiguous block of missing data greater than 7 days. These criteria are similar to those used by the Hydrologic Index Tool (HIT, Henriksen et al. 2006).

Sixty-four reference gages were previously identified in the Interior Highlands region for the river classification study (Leasure et al. 2014). Flow metrics were calculated for reference gages for their entire periods of record using the R package *EflowStats*. All gages had more than 15 years of data to minimize measurement uncertainty that may affect some flow metrics when using short periods of record (Kennard et al. 2010). The 187 flow metrics calculated by the R package *EflowStats* included the 171 metrics calculated by HIT.

A set of 187 random forest models was developed to predict the 187 flow metrics. Flow metrics from 64 streams in least-disturbed reference condition were used as response variables. Full models were built initially that included 144 predictor variables describing climate and

landscape characteristics within reference watersheds. Importance of each variable was assessed using the default method of the *randomForest* R package (Liaw and Wiener 2002) which is based on increase of mean squared error resulting from random permutations of the variable. A reduced model was built for each flow metric that included only the 30 most important predictor variables.

Comparing predicted values from the random forest models to observed values from the gage data, overall prediction error for each model was assessed as:

$$error = \frac{median(|predicted - observed|)}{IQR(observed)}$$

where IQR is the interquartile range.

Bias was measured as:

$$bias = \frac{median(predicted - observed)}{IQR(observed)}$$

Precision was measured as:

$$precision = \frac{IQR(predicted - observed)}{IQR(observed)}$$

Data were collected at all 208 USGS gages in the Interior Highlands for any predictor variable selected for at least one of the reduced random forest models. The reduced random forest models were used to predict values of each flow metric expected under natural conditions, as well as the distribution of expected values. The spread of these predicted distributions included both natural variation and model error. The expected value for each flow metric under natural conditions was taken as the median of the predicted distribution. The mean of the predicted distributions was also recorded.

Response Variable Selection

We calculated five biological response metrics for use in macroinvertebrate community data analysis: total number of individuals per sample (density was not used due to the qualitative nature of the QMH method); taxa richness; Simpson's diversity; percentage of individuals in the total sample belonging to Orders Ephemeroptera, Plecoptera, and Tricoptera (EPT), considered to be taxa associated with good water quality and less disturbed habitat (Karr 1991); and percentage of total individuals belonging to the Family Chironmidae, generally considered a more tolerant taxon that is predicted to increase in abundance with increasing stream perturbation (Barbour et al. 1999) (Table 1). Response variables calculated from RTH and QMH samples were analyzed separately. Mean values for biological response variables are summarized in Table 2.

Data Analysis

We examined environment-ecology relationships among macroinvertebrate communities with two approaches: 1) a multiple regression analysis incorporating a small number of predictor variables related to habitat, water quality, and geomorphology, as well as hydrology, and 2) a canonical ordination procedure using only hydrologic variables in which we used forward selection to select predictors that were most related to our response variables.

Comprehensive Multiple Regression Analysis

For the comprehensive analysis we used an information theoretic (IT) approach (Burnham and Anderson 2002). The multiple regression analyses were designed to assess importance of hydrology in relation to other categories of predictors, including local habitat,

stream geomorphology, watershed-scale disturbance and water quality. We selected predictor variables from each of these categories that we hypothesized to have greatest biological significance.

We selected substrate size for the local habitat variable. Macroinvertebrate community structure in streams has long been known to be heavily influenced by the average size of particles in the substrate (Rabeni and Minshall 1977, Erman and Erman 1984, Culp et al. 2011). Substrate size was selected over other variables such as depth, current velocity, or temperature because it varies less based on conditions at the time of sampling than these other variables. We selected Total P for the water quality variable. Recent studies have shown increases in P concentrations can cause reductions in macroinvertebrate diversity and increase in abundance, biomass, and secondary production of P-rich consumers (Cross et al. 2006, Davis et al. 2010, Prater et al. 2015). Karst landscapes such as the Springfield Plateau are thought to be particularly vulnerable to P-enrichment from agricultural sources (Jarvie et al. 2014), and the western Ozark Highlands have become widely known in recent years for excess P enrichment due to poultry production throughout the region (Haggard et al. 2010, Scott et al. 2014). We selected RHA for the geomorphology variable and HDI for watershed-scale disturbance variable due to the inclusive nature of these indices, which both encompass a wide variety of ecologically relevant parameters. Mean values for environmental predictor variables are summarized in Table 2.

To select a single hydrologic variable to use in our multiple regression analysis, we first ran a PCA on the 171 log transformed flow metrics from all categories (M,F,D,T,R) based on predicted natural flows for our sites (Leasure and Magoulick, unpublished data). We dropped

flow metrics that were outside the threshold criteria for bias, precision and accuracy (Leasure et al. 2014). Then we selected the flow metrics with the top 10 loadings on PC1 and examined their distributions, R^2 , and measurement uncertainty and selected the best metric. We ultimately selected MA32, a measurement of flow variability based on the coefficient of variation in September flows (Olden and Poff 2003) as the hydrologic variable for analyses, because it had the best combination of top loading on PC1, distribution, high R^2 , and low measurement uncertainty. MA32 was highly correlated (0.89) with MA4, the coefficient of variation in daily flows, but was better with respect to all of these characteristics, making it a good measure of flow variability. Flow variability has previously been shown to be related to macroinvertebrate community structure (Monk et al. 2006).

We examined bivariate correlations among predictor variables and among response variables and dropped variables that were highly correlated. We graphically examined variables via box-plots and histograms to check for normality of distributions. Variables were transformed as needed to improve normality and to meet the assumptions of analyses. This was done separately for the 2012 and 2013 datasets.

We developed a priori hypotheses resulting in 12 models relating our response variables to our predictors (Table 1). Models consisted of single-variable models for each of the five above-mentioned variables, the global model with all five predictors, and combination models that we developed based on combinations of variables we felt had biological significance, including an "anthropogenic impact" model of variables most likely to be impacted by human alteration (RHA, HDI, Total P, and MA32), a "habitat only" model (substrate and RHA), a "hydrology and water quality" model (MA32 and Total P), and models individually combining

HDI with Total P, MA32, and RHA (Table 1). Multiple regression analyses were performed in SYSTAT 13 and models were ranked using Akaike Information Criteria corrected for small sample size (AICc). We used 95% confidence intervals to determine whether parameter estimates differed from 0. Delta AICc values were calculated, and in cases where these values were within 2 points of the top models, both models were considered equally valid and are reported in the results. Residual plots were visually inspected for all regressions.

Multivariate Hydrologic Analysis

We used Redundancy Analysis (RDA) to determine response variable-hydrology relationships for both sampling years and methods separately. As in the multiple regression analysis, we used predicted natural hydrology rather than observed hydrology to incorporate both our gaged and ungaged sites, and we used the same selection criteria for bias, precision and accuracy to eliminate variables from the analysis. After eliminating variables that did not meet our criteria, the hydrologic variable set was reduced from the initial 171 to 154 variables. RDA was appropriate because preliminary Detrended Correspondence Analyses (DCA) indicated that species gradient lengths were less than 1 standard deviation (ter Braak 1995). We used forward selection in CANOCO 4.5 to select hydrologic variables that were related to response variables. We limited the hydrologic variables to those with $\lambda \geq 0.07$ after entry into the model.

We centered and standardized response variables before running the RDA's because response variables were measured in different units. Because we were interested in relationships among response variables, scaling of ordination scores was focused on inter-response variable correlation rather than inter-sample distance and the response variable scores were standardized to prevent response variables with large variances from unduly influencing ordination diagrams

(ter Braak and Smilauer 1998). Monte Carlo permutations testing the significance of canonical axes together were then performed for each RDA to determine the overall importance of remaining hydrologic variables in influencing response variables. Values and definitions for all significant hydrologic variables in RDA analysis are presented in Table 3.

Results

2012 (drought year)

For RTH macroinvertebrate assemblages, the top model predicting percent Chironomidae was the Substrate model, with percent Chironomidae positively related to Substrate (Table 4, Fig. 2). None of the models predicting total number of individuals, richness, diversity, or percent EPT were significant. For QMH macroinvertebrate assemblages, the top model predicting total number of individuals was the Total P model, with number of individuals positively related to Total P (Table 4, Fig. 2). The top model predicting taxa richness was the Substrate+RHA model, with taxa richness positively related to Substrate and RHA, but the Substrate model was also supported (Table 4, Fig. 2). The top model predicting percent EPT was the RHA model, with percent EPT positively related to RHA. None of the models predicting diversity or percent Chironomidae were significant (Table 4, Fig. 2).

RDA analysis showed RTH macroinvertebrate assemblages were significantly related to magnitude of average flow, magnitude of low flow, magnitude of high flow, and frequency of high flow (Table 3, Fig. 3). QMH assemblages were significantly related to magnitude of average flow, magnitude of low flow, magnitude of high flow, and duration of high flow (Table 3, Fig. 3). Magnitude was the most important category of hydrologic metric overall, with 11 of

13 significant predictors between the two sampling methods belonging to this category. Particularly important were metrics related to magnitude of low flows, which made up more than half of those significant magnitude metrics. Two metrics, ML14 and ML17, were important in both RTH and QMH assemblages (Table 3, Fig. 3).

In both RTH and QMH assemblages, response variables fell into two groups - richness, diversity, and percent EPT were related to one another, and total number and percent Chironomidae were related to each other (Fig. 3). Associations between richness, diversity and percent EPT were stronger in RTH assemblages than QMH. In RTH assemblages, richness, diversity and percent EPT were positively associated with higher flow magnitudes and negatively associated with flow variability. In QMH assemblages, these three response variables were negatively associated with mean number of flood free days (Fig 3). Total number and percent Chironomidae in QMH assemblages were positively related to range and variability in flow magnitudes and negatively related to higher low flow magnitudes (Fig 3).

2013 (flood year)

For RTH macroinvertebrate assemblages, the top model predicting percent EPT was the RHA model, with percent EPT positively related to RHA (Table 4, Fig. 4). The top model predicting percent Chironomidae was the RHA model, with percent Chironomidae negatively related to RHA, but the Substrate+RHA model was also supported, with percent Chironomidae positively related to Substrate and negatively related to RHA (Table 4, Fig. 4). None of the models predicting total number of individuals, taxa richness, or diversity were significant (Table 4, Fig. 4). For QMH assemblages, the top model predicting richness was the HDI model, with richness negatively related to HDI, but the MA32+HDI model was also supported, with HDI

negatively related to richness but MA32 was not significant (Table 4, Fig. 4). The top model predicting percent EPT was RHA, with percent EPT positively related to RHA (Table 4, Fig. 4). The top model predicting percent Chironomidae was the Substrate model, with percent Chironomidae positively related to Substrate (Table 4, Fig. 4). None of the models predicting total number of individuals or diversity were significant (Table 4, Fig. 4).

RDA analysis showed RTH macroinvertebrate assemblages were significantly related to magnitude of average flow, magnitude of high flow, duration of low flow, and timing of average flow (Table 3, Fig. 3). QMH assemblages were significantly related to magnitude of average flow, magnitude of high flow, and timing of low (Table 3, Fig. 3). Magnitude was again the most important category of hydrologic metric overall, with 6 of 9 significant predictors. There was, however, a shift in importance from metrics related to magnitude of low flows to magnitude of average flows. Metrics related to magnitude of average flows comprised 4 of the 6 significant magnitude metrics in 2013 (Table 3).

Richness and diversity remained strongly associated, but in the RTH assemblages in 2013, percent EPT was more closely associated with total number (Fig 3). Richness and diversity in RTH assemblages were positively associated with variability in high flows and negatively associated with low flow duration, while total number was positively associated with skewness in daily flow and annual runoff (Fig 3). Richness, diversity, and percent EPT in QMH assemblages were positively associated with predictability of low flow and negatively associated with percent Chironomidae, while total number was negatively associated with magnitude of high flow (Fig 3).

With respect to average, low, and high flow conditions, metrics related to low flow were relatively more important during the drought year, with 50% of all metrics being low flow-related in 2012 while only 22.2% were low-flow related in 2013 (Table 3). Average flow conditions were relatively more important in the flood year, with 55.6% of important metrics being average-flow related in 2013, while only 25% were average flow-related in 2012. Metrics relating to high flow were the least important of the three and remained most consistent between the two years (25% and 22.2% of all important metrics, respectively, in 2012 and 2013)

Discussion

Comprehensive Analysis

Considering both years and sampling methods, RHA was the single most important predictor variable, showing up in 6 significant top models, while flow variability (MA32) was the least important, showing up as part of a single top combination model, but not as a significant parameter of that model. Monk et al. (2006) showed that magnitude metrics were the most strongly related to macroinvertebrate assemblage structure of any flow metric category. Thus, it is somewhat surprising that flow, at least in relation to the measure of flow variability that we selected (MA32), was not an important predictor in any of our biological response variables. It is possible that other flow metric categories may be more important to stream communities in Groundwater Flashy streams of the Ozark Highlands, or that magnitude metrics relating to low or high flows rather than average flow conditions may be more important. The much greater prominence of both RHA and substrate in our top models, however, suggests that local habitat and geomorphology may be the most important determinants of macroinvertebrate assemblage structure, overriding the influence of hydrologic variation in these systems. Total P and HDI fell

in between these variables in importance, appearing as significant top models once each, in 2012 QMH total number and 2013 QMH taxa richness, respectively.

Our results are typical of what others have found regarding chironomids increasing and EPT taxa decreasing with increased perturbation (Karr 1991, Barbour et al. 1999). Both HDI and RHA are indices that measure various aspects of ecological disturbance on two different spatial scales; our results demonstrate the usefulness of macroinvertebrate community response variables as indicators of ecological condition at differing spatial scales within these systems. RHA and substrate size were most important in the drought year, whereas RHA and HDI were most important in the flood year. The shift in importance towards a smaller-scale variable such as substrate size versus a broad-scale variable such as HDI during drought may be related to the role that refuges play in these systems. During drought, biota are packed at higher densities into smaller suitable habitats, experiencing harsher biotic and abiotic stressors than those to which they are typically exposed (Magoulick and Kobza 2003). Under such conditions, microhabitat and water quality could become relatively more important than watershed-level variables; we also saw that Total P followed the same pattern of being more important during the drought year than the flood year, which supports this notion. RHA was shown to be a consistently good predictor of response variables generally related to environmental quality, both taxa richness and percent EPT in the QMH assemblages.

Both QMH richness and percent EPT averaged higher in 2013 than 2012, trends that support the findings of previous studies of the effects of drought vs. high flows on freshwater communities (Lake 2000, Lake 2003, Suren and Jowett 2006). The relationship between percent EPT and RHA remained consistent between the two years, in addition to being consistent with

percent EPT in 2013 RTH assemblages. This may indicate that reach-scale habitat structure and quality, which does not tend to vary as much as hydrology or water quality, could play a more consistently important role with respect to these taxa. In the case of taxa richness, we saw a complete shift in which metrics were important between the drought year (substrate and RHA) and the flood year (HDI). It is possible that larger-scale disturbance typically has a strong effect on richness in these communities, but that drought in 2012 negated its importance in favor of reach and microhabitat scale variables.

The positive relationship between taxa richness and substrate size in the 2012 QMH assemblages could reflect more diverse microhabitats suitable for a wider variety of taxa at sites that had larger proportions of boulder and cobble. While all sites had large amounts of pebble and gravel substrate (percent pebble ranged from 11.97% to 47.19%, gravel from 22.59% to 78.29%), many sites had little or no cobble (0.01% to 29.56%) or boulder (0% to 11.96%). Sites with a higher mean substrate size had a large amount of cobble or boulder substrate in addition to pebble and gravel, tended to be more variable, and were therefore probably more suitable to a larger range of taxa. Surprisingly, substrate size and percent Chironomidae in the 2013 QMH assemblages were positively related, despite the fact that chironomids are often associated with finer sediments (Barnes et al. 2013). It should be noted, however, that these sites included a relatively low overall percentage of sandy or silty microhabitat. The site with the lowest mean substrate size still fell between gravel and pebble on the modified Wentworth scale used in this study, and mean percentages of sand and silt across all sites were generally so low (2.68% and 3.34%, respectively), that this is unlikely to have played a large role in the overall percentage of chironomids in the assemblages.

The positive relationship between Total P and total number of individuals in 2012 QMH assemblages could be due to bottom-up trophic effects (Rosemond et al. 2001, Cross et al. 2005). Previous studies have directly linked increased phosphorus concentration to increased invertebrate biomass in various types of stream ecosystems (Peterson et al. 1993, Cross et al. 2006). Phosphorus concentrations in streams of the Ozark Highlands increase with the fraction of human-altered land use within the catchment (Haggard et al. 2007, Giovannetti et al. 2013). Anthropogenic sources such as wastewater discharges can have profound influence on phosphorus concentrations in streams (Haggard et al. 2005, Ekka et al. 2006, Migliaccio et al. 2007, Haggard 2010, Scott et al. 2011), which can increase concentrations for tens of river kilometers downstream.

With respect to the two methods of invertebrate sampling used, the QMH method detected more biological relationships than the RTH method in 2012; we found significant relationships for three of the five QMH variables tested but only one of the RTH variables. A possible explanation for this could be that riffles are the habitat most heavily affected by drought (Dekar and Magoulick 2007, Chester and Robson 2011) which could potentially push the biota below some threshold where many of the relationships are no longer apparent. The effects of the drought may have been somewhat ameliorated by inclusion of pool and run habitats in the QMH assemblages, which may act as refuges for macroinvertebrates during summer drying (Chester and Robson 2011). Stubbington et al. (2015) found that diversity of benthic macroinvertebrates decreased during a major drought, and that assemblages at individual streams became more similar, reflecting lower diversity and abundance overall; this increased homogeneity across sites would have the effect of making relationships more difficult to detect.

Magnitude was the most important category of flow metric in terms of influence on macroinvertebrate communities in Groundwater Flashy streams in the Ozark Highlands. Although there are a greater percentage of magnitude metrics than any other category (54.55%), this category comprised a much higher percentage (77.27%) of the metrics that we found to be important overall between the two years and sampling methods. Other metric categories (timing, frequency, and duration) did have some influence, but to a much lesser extent, appearing only once each in RDA result. While few studies have directly compared the relative importance of flow metric categories, regional environmental flow studies in recent years have suggested that magnitude of flow is an important influence on aquatic communities (Monk et al. 2006, Kendy et al. 2012). In a statewide study of streams in Massachusetts, Armstrong et al. (2011) found that metrics related to flow magnitude were good predictors of biological integrity in aquatic communities compared to other chemical and physical covariates.

While we found some consistent trends in these analyses (e.g. the overall importance of magnitude metrics), the important flow variables changed almost completely between sampling types and years. It is important to note that these predicted natural flow metrics are based on a consistent period of record and do not actually change between the two years; it is the invertebrate communities themselves that strongly differ between one year and the next and between one sampling method and the other. These shifts are enough to change the relationships between response variable and flow metrics. While it has been a traditional tenet of long-term biomonitoring programs that reference-quality streams show relatively little inter-annual variation in macroinvertebrate communities (Robinson et al. 2000), recent studies have shown

that this is not always the case. MacDonald and Cote (2014) compared year-to-year variation in benthic macroinvertebrate stream communities at reference and urbanized sites over a six year period and found significant temporal change in communities in both stream types, although there was a much a greater degree of variation at urbanized sites. Our study encompassed a gradient of sites ranging from highly disturbed to reference condition. Carter and Fend (2001) found that strong inter-annual differences in discharge regimes, similar to those seen in our study, resulted in strong inter-annual variation in percentages of both taxonomic groups and functional feeding groups among macroinvertebrate communities.

The dramatic shift in biological response variables between two years with different environmental conditions suggests that in order to form a strong picture of relationships between biota and hydrologic variables, long term biological monitoring over multiple years encompassing a variety of environmental extremes may be crucial. Had our study taken place during two consecutive drought summers, for example, we likely would have found more consistent results between the two years. However, flow-ecology relationships underpinned by that dataset would be missing the strong temporal variation in these communities. Ideally, bioassessment protocols should incorporate spatial and temporal variation in a system, including more infrequent and extreme disturbances (Sousa 1984, Stoddard et al. 2006).

In 2012 RTH assemblages, richness, diversity, and percent EPT were strongly positively related to average and low flow magnitude metrics, and negatively related to flow variability. This is unsurprising given the relatively stable nature of groundwater streams (Hughes and Hannart 2003, Leasure et al. 2014). The closer association between these three response variables in RTH assemblages compared to QMH assemblages could be explained by the fact

that EPT taxa are often associated with riffles and would account for more of the taxa found in that habitat (Gregory 2007).

In 2012 QMH assemblages, there was a negative relationship between richness, diversity, and percent EPT and mean flood-free days per year. The relationship between flooding and macroinvertebrate community response variables is complex and can strongly differ between stream type and ecoregion (Agerich et al. 2004, Fritz and Dodds 2004, Bae et al. 2012), but this result suggests that floods in Groundwater Flashy streams in the Ozark Highlands may be positively associated with richness, diversity, and EPT taxa. This may have been due to the inclusion of refuge habitats in the QMH assemblages, which are utilized by macroinvertebrates during flood events while a higher number of animals are typically washed out of riffles (Palmer et al. 1995, Szczerkowska-Majchrzak and Lik 2014).

Total number and percent Chironomidae were positively associated with increased range and variability in average and low flow conditions, metrics that may be associated with more disturbed hydrologic regimes (Walsh et al. 2005, Bond et al. 2008). Although Groundwater Flashy streams do possess a somewhat high level of natural flow variability compared to Groundwater or Groundwater Stable streams, all groundwater-fed streams in the region tend to be relatively stable when compared to runoff or intermittent streams, typically experiencing few, if any, days of zero flow annually, for example (Leasure et al. 2014). Previous studies have shown that in reliably flowing waters, biota are more sensitive to fluctuations in flow (Hughes and Hannart 2003). This has important conservation, management, and restoration ramifications, as both hydrologic alteration and land-use practices generally increase flashiness and instability in streams (Walsh et al. 2005, Bond et al. 2008).

Conclusions

An implicit assumption of environmental flows theory is that flow-ecology relationships will be somewhat temporally invariant and allow us to make predictions about how biota will respond to changes in their environment (Poff et al. 2010, Carlisle et al. 2010, Olden et al. 2014); this notion is crucial to implementing environmental flows-based frameworks such as ELOHA with regard to management decisions (Gillespie et al. 2014, Olden et al. 2014). Development of these models and any policies informed by them must be implemented in an active framework supported by biological data (Poff et al. 2010, King et al. 2015), yet there is a great deal of variation inherent in the collection of biological data (Hurlbert 1984). While long-term studies sampling the same sites year after year to encompass all of the natural temporal variation in a system would be the ideal way to elucidate environmental flow relationships (MacDonald and Cote 2015), limitations of time and funding mean this is not always a practical option. Most studies examining flow-ecology relationships are carried out in a shorter time frame, and some studies are based on a single sample from a given time period to categorize a site (Olden et al. 2014). We found substantial differences in strength and patterns of relationships over a two year period. We suggest that further examination of the temporal variation in flow-ecology relationships is warranted. Furthermore, we found that other categories of environmental variables, including geomorphology, water quality, and watershed-scale disturbance, were more strongly related to macroinvertebrate assemblages than hydrology. This suggests that hydrologic metrics are best considered within the more inclusive context of a complex framework that includes other types of environmental variables.

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Table 1. Response and predictor variables and models used in comprehensive multiple regression analyses. RTH = Richest Targeted Habitat; QMH = Qualitative Multi-Habitat.

Response Variables	Predictor Variables	Models
RTH Total Number	Substrate (modified Wentworth Scale)	Substrate
RTH Taxa Richness	RHA (Rapid Habitat Assessment)	RHA
RTH Simpson's Diversity	HDI (Hydrologic Disturbance Index)	HDI
RTH % EPT	Total P (mg/L)	Total P
RTH % Chironomidae	MA32 (Coefficient of variation in September flows)	MA32
QMH Total Number		Substrate+RHA
QMH Taxa Richness		Total P+MA32
QMH Simpson's Diversity		HDI+Total P
QMH % EPT		HDI+MA32
QMH % Chironomidae		HDI+RHA
		Total P+MA32+HDI+RHA
		Substrate+Total
		P+MA32+HDI+RHA

Table 2. Mean (\pm SE) values for biological response and predictor variables used in comprehensive multiple regression analysis in 2012 and 2013. Substrate size based on modified Wentworth scale; RTH substrate size measured in riffles only, QMH in all habitats. RHA = Rapid Habitat Assessment, maximum possible score of 200. HDI = Hydrologic Disturbance Index, maximum possible score of 42. HDI and MA32 do not vary between years.

Variable	2012	2013
RTH Total Number	2382 (\pm 625.13)	4014 (\pm 659.32)
RTH Taxa Richness	22 (\pm 1.25)	19 (\pm 1.02)
RTH Simpson's Diversity	0.808 (\pm 0.02)	0.776 (\pm 0.02)
RTH % EPT Taxa	0.496 (\pm 0.04)	0.599 (\pm 0.042)
RTH % Chironomidae	0.084 (\pm 0.03)	0.148 (\pm 0.047)
QMH Total Number	2306 (\pm 662.40)	3067 (\pm 355.42)
QMH Taxa Richness	24 (\pm 1.98)	27 (\pm 1.19)
QMH Simpson's Diversity	0.722 (\pm 0.05)	0.834 (\pm 0.02)
QMH % EPT Taxa	0.234 (\pm 0.05)	0.352 (\pm 0.04)
QMH % Chironomidae	0.069 (\pm 0.03)	0.189 (\pm 0.04)
RTH Substrate Size	3.63 (\pm 0.08)	3.67 (\pm 0.08)
QMH Substrate Size	3.55 (\pm 0.09)	3.66 (\pm 0.09)
RHA	165.2 (\pm 3.61)	158.2 (\pm 3.69)
HDI	14.45 (\pm 1.46)	14.45 (\pm 1.46)
Total P (mg/L)	0.07 (\pm 0.02)	0.06 (\pm 0.01)
MA32 (Coefficient of Variation in September Flows)	106.12 (\pm 8.77)	106.12 (\pm 8.77)

Table 3. Hydrologic metrics (Olden and Poff 2003) used in multivariate hydrologic analysis for 2012 and 2013 with mean (\pm SE) values.

Code	Definition	Category	Mean (\pm SE)
2012 RTH			
FH3	High flood pulse count (number of annual occurrences with 3x mean daily flow)	Frequency of High Flows	79.88 (\pm 2.23)
MA23	Mean monthly December flows	Magnitude of Average Flows	67.25 (\pm 13.93)
MA25	Coefficient in variation of February flows	Magnitude of Average Flows	99.82 (\pm 4.25)
MH22	High flow volume (mean of area between hydrograph and upper threshold of 3x median annual flow)	Magnitude of High Flows	81.74 (\pm 8.27)
ML10	Mean minimum October flows	Magnitude of Low Flows	5.98 (\pm 1.66)
ML14	Mean of annual minimum flows	Magnitude of Low Flows	0.19 (\pm 0.01)
ML17	Baseflow index (Seven-day minimum flow divided by mean annual daily flow averaged across all years)	Magnitude of Low Flows	0.09 (\pm 0.01)
2012 QMH			
DH24	Mean annual number of flood free days	Duration of High Flows	156.57 (\pm 0.98)
MA7	Range in daily flow (ratio of 20th/80th percentile in daily flow over all years)	Magnitude of Average Flows	9.96 (\pm 0.74)
MH6	Mean maximum June flows	Magnitude of High Flows	274.41 (\pm 68.5)
ML17	Baseflow index (Seven-day minimum flow divided by mean annual daily flow averaged across all years)	Magnitude of Low Flows	0.09 (\pm 0.01)
ML18	Variability in baseflow index (Coefficient in variation in ML17)	Magnitude of Low Flows	87.19 (\pm 11.13)
ML14	Mean of annual minimum flows	Magnitude of Low Flows	0.19 (\pm 0.01)
2013 RTH			
MA38	Variability in monthly flows divided by median monthly flows	Magnitude of Average Flows	5.66 (\pm 0.25)
MA41	Mean annual runoff (Mean annual flow divided by catchment area)	Magnitude of Average Flows	0.88 (\pm 0.02)
MA5	Skewness in daily flows (Mean daily flows divided by median daily flows)	Magnitude of Average Flows	4.06 (\pm 0.33)

Table 3 (cont.). Hydrologic metrics (Olden and Poff 2003) used in multivariate hydrologic analysis for 2012 and 2013 with mean (\pm SE) values.

Code	Definition	Category	Mean (\pm SE)
MH18	Variability across annual maximum flows	Magnitude of High Flows	11.03 (\pm 0.21)
TA2	Predictability of flow composed of two additive components: constancy and contingency (Olden and Poff 2003)	Timing of Average Flows	49.23 (\pm 0.04)
DL16	Low flow pulse duration (mean duration of all occurrences during which magnitude of flow remains below the 25th percentile)	Duration of Low Flows	13.51 (\pm 0.28)
2013 QMH			
MA24	Coefficient of variation in January flows	Magnitude of Average Flows	106.3 (\pm 2.89)
MH14	Mean of annual maximum flows	Magnitude of High Flows	115.48 (\pm 11.09)
TL3	Seasonal predictability of low flows (proportion of low-flow events \geq 5-year magnitude falling in a 60-day seasonal window)	Timing of Low Flows	0.18 (\pm <0.01)

Table 4. Best models for 2012 and 2013 response variables. Significant relationships in bold.

Response Variable	Best Model	Std. Coefficient	R ²	C.I.
2012 (Drought Year)				
RTH Total Number	Total P	0.373	0.139	-0.205 - 2.939
RTH Taxa Richness	RHA	0.362	0.131	-0.024 - 0.266
RTH Simpson's Diversity	Total P	-0.275	0.076	-47.147 - 11.101
RTH % EPT	Total P	-0.300	0.090	-1.236 - 0.234
RTH % Chironomidae	Substrate	0.433	0.188	0.009 - 0.437
QMH Total Number	Total P	0.514	0.265	1.827 - 14.089
QMH Taxa Richness	Substrate+RHA	0.671, 0.369	0.393	6.134 - 24.480, -1.87 - 0.422
	Substrate	0.527	0.278	3.0738 - 20.989
QMH Simpson's Diversity	RHA	0.372	0.139	-0.049 - 4.069
QMH % EPT	RHA	0.517	0.267	0.006 - 0.013
QMH % Chironomidae	Substrate	0.152	0.152	-0.016 - 0.384
2013 (Flood Year)				
Response Variable	Best Model	Std. Coefficient	R ²	C.I.
RTH Total Number	Total P	0.291	0.085	-1.099 - 5.357
RTH Taxa Richness	HDI	-0.296	0.088	-13.732 - 3.427
RTH Simpson's Diversity	Total P	-0.339	0.115	-59.216 - 7.272
RTH % EPT	RHA	0.476	0.227	0.002 - 0.938
RTH % Chironomidae	RHA	-0.531	0.282	-0.009 - -0.001
	Substrate+RHA	0.231, -0.487	0.333	-0.074 - 0.282, -0.009 - -0.001
QMH Total Number	MA32	-0.310	0.096	-1365.529 - 5675.696
QMH Taxa Richness	HDI	-0.537	0.288	-25.742 - -4.076
	MA32+HDI	-0.294, -0.482	0.372	-24.174 - 3.180, -24.062 - -2.752
QMH Simpson's Diversity	RHA	0.370	0.137	-0.024 - 0.320
QMH % EPT	RHA	0.540	0.291	0.001 - 0.008
QMH % Chironomidae	Substrate	0.462	0.214	0.002 - 0.271

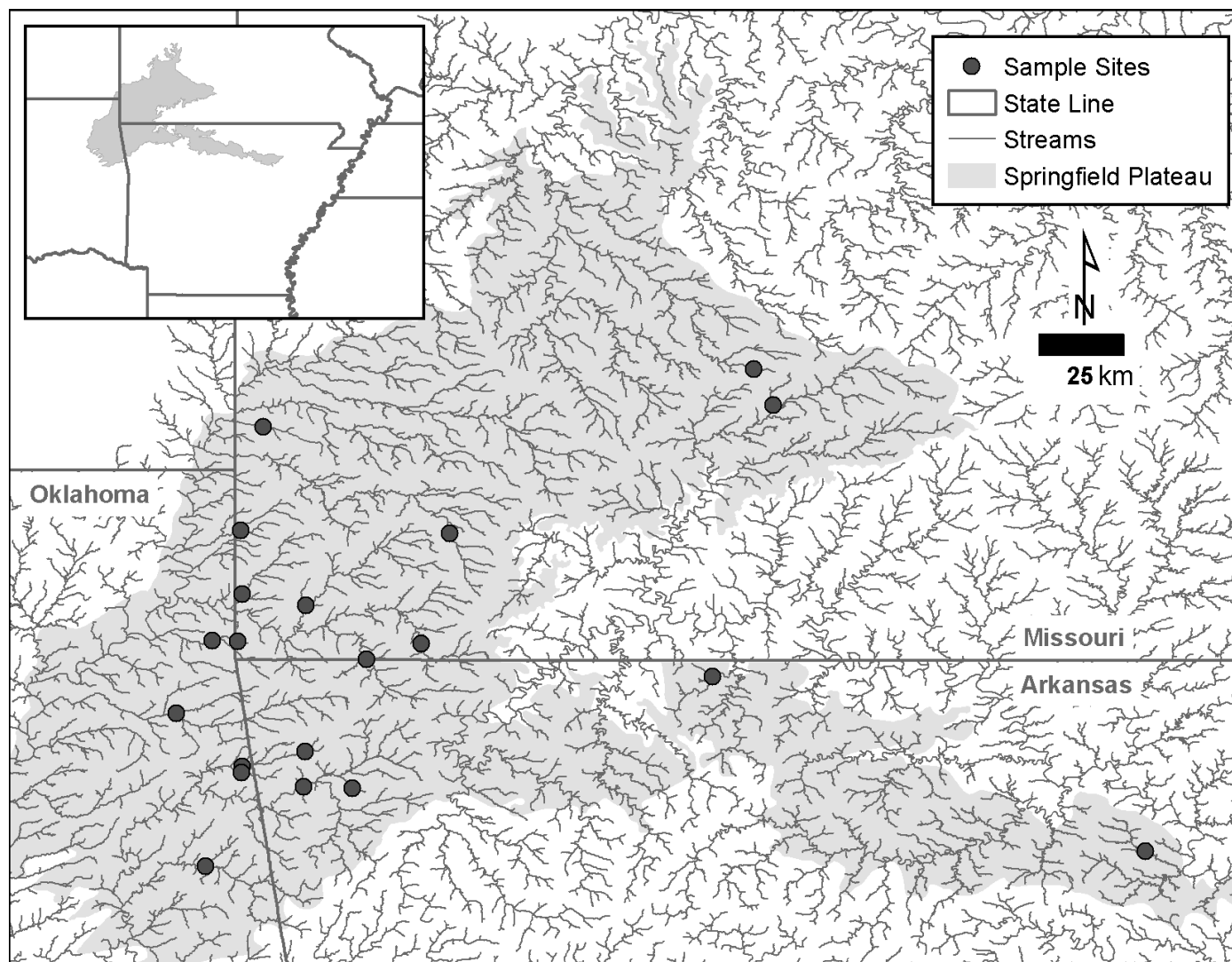


Figure 1. Map of study area showing sample sites, stream network, and Springfield Plateau.

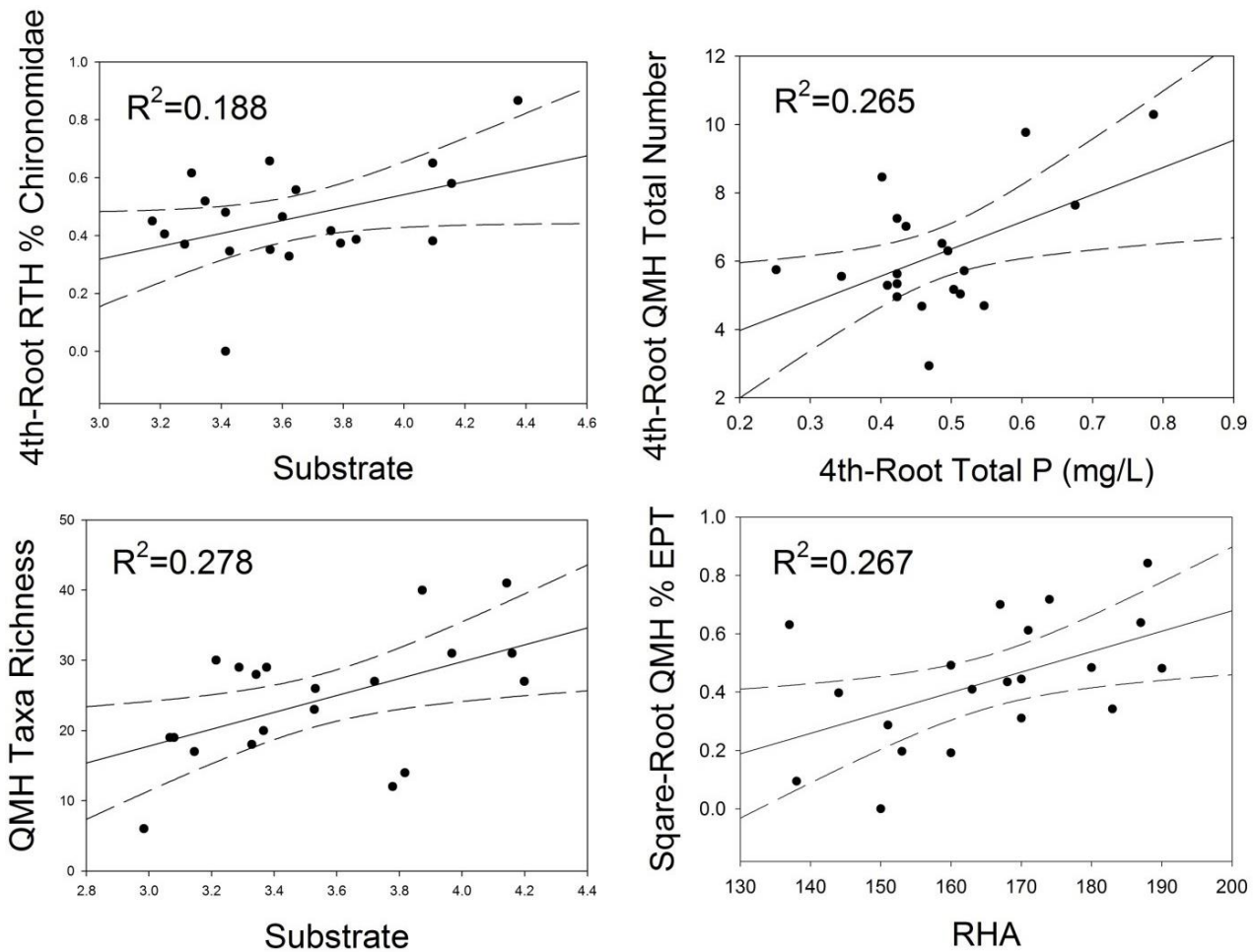


Figure 2. Important RTH and QMH macroinvertebrate-environment relationships in 2012. Only significant relationships in the best models are shown. Dashed lines indicate 95% confidence intervals.

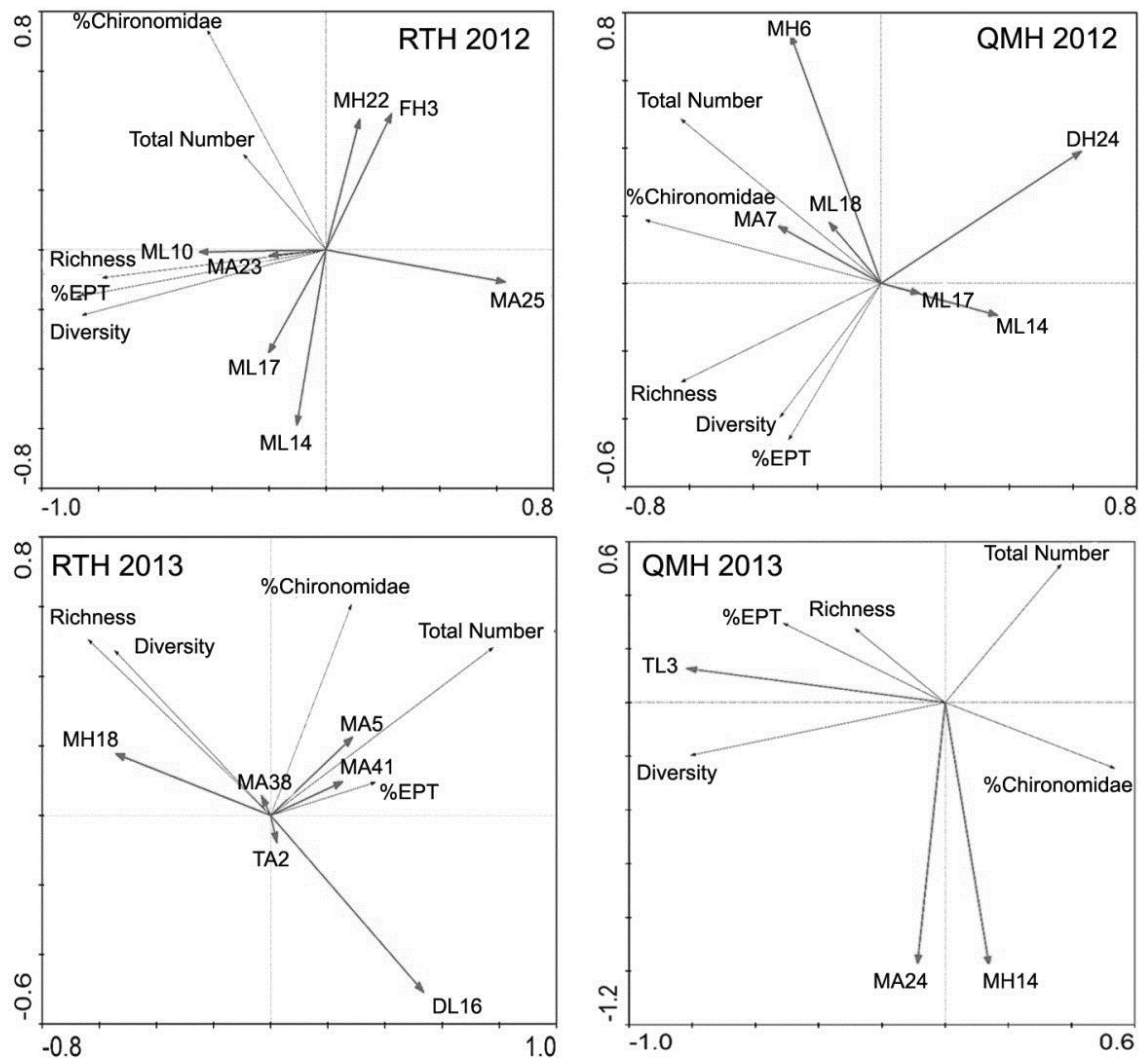


Figure 3. Redundancy analysis ordination plots relating RTH and QMH macroinvertebrate and selected hydrologic metrics in 2012 and 2013. Angles of arrows indicate associations and length of arrows indicate strength of the relationship.

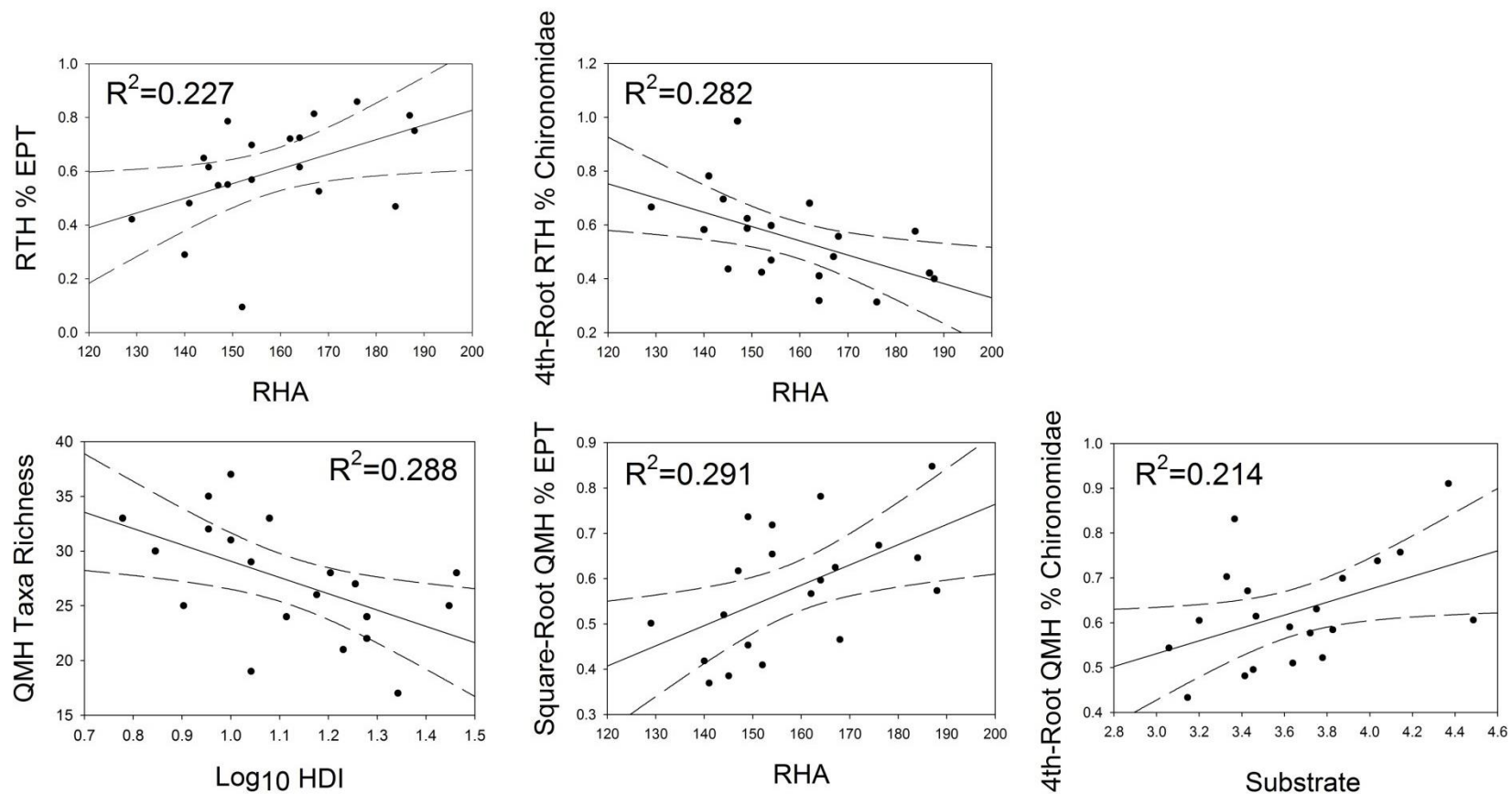


Figure 4. Important RTH and QMH macroinvertebrate-environment relationships in 2013. Only significant relationships in the best models are shown. Dashed lines indicate 95% confidence intervals

**Relative Influence of Hydrology, Geomorphology, Water Quality, and Disturbance on Fish
and Crayfish Assemblages in Ozark Highland Streams**

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Abstract

We examined flow-ecology relationships in fish and crayfish assemblages in Ozark Highland streams, USA, using three-pass backpack electrofishing to sample at 21 sites in 2012 and 18 sites in 2013. We measured in-stream habitat, water quality, and stream geomorphology, and calculated watershed-scale hydrologic disturbance index. Additionally we calculated hydrologic metrics based on predicted natural predicted flows in the region. Biological metrics were examined via two different statistical methods: an information theoretic approach relating response variables to *a priori* selected predictor variables incorporating hydrology, habitat, geomorphology, and water quality, and canonical ordination using forward selection to relate these same response variables to a large assortment of hydrologic metrics. Substrate was the most important environmental variable overall and that flow magnitude was the most important category of hydrologic metric. Increasing disturbance was associated with decreasing richness and diversity and flood frequency was positively related to diversity. Our findings suggest that hydrologic metrics are best considered within a framework that includes other types of environmental data such as water quality, stream geomorphology, and local habitat.

Introduction

Flow is a major determinant of physical habitat and biotic composition in streams at multiple spatial and temporal scales (Vannote et al. 1980, Poff and Ward 1990, Bunn and Arthington 2002). Hydrologic variation may be one of the single most important environmental variables for stream biota (Poff et al. 1997), overriding even predation and competition as the main determinant of community structure and use of resources in stream ecosystems (Grossman et al. 1998). It is not hydrology alone, however, but the complex interaction between flow regime, stream geomorphology, and local habitat that largely determines the distribution, abundance, and diversity of stream organisms (Schlosser 1982, Poff and Allan 1995, Ward et al. 1999, Bunn and Arthington 2002). Fish assemblage structure is highly dependent on habitat structure (Pusey et al. 1993, Bunn and Arthington 2002) and richness and diversity of fish fauna typically increase as habitat complexity increases (Gorman and Karr 1978, Schlosser 1982). Water quality is another crucial influence on the composition and structure of stream communities. Declining water quality has long been linked to dramatic changes in assemblages of both aquatic macroinvertebrates (Armitage et al. 1983, Karr 1991, Reynoldson 1997) and fish (Katz and Gauvin 1953, Reash and Berra 1987).

The concept of environmental flows has gained increasing momentum in recent years in the management of stream ecosystems (Gillespie 2014, Olden et al. 2014). The International Union for Conservation of Nature (IUCN) defines environmental flow as "the water regime provided within a river, wetland, or coastal zone to maintain ecosystems and their benefits where there are competing water uses and where flows are regulated" (Dyson et al. 2003) while the Brisbane Declaration (2007) more specifically defines environmental flow as "the quantity,

timing, and quality of water flows required to sustain freshwater and estuarine ecosystems and the human livelihood and well-being that depend on these ecosystems". Given the complex interaction of hydrology with many other important variables such as water quality, habitat structure, and stream geomorphology (Poff et al. 2010), it is a challenge to elucidate precise flow-ecology relationships. In the present study, we used an approach to modeling that included all of these components.

Poff et al. (2010) developed the Ecological Limits of Hydrologic Alteration (ELOHA) framework, an approach consisting of both scientific and social processes working in tandem to address the needs of ecosystems and stakeholders, a framework that has since been implemented in six states and three inter-state river basins (Kendy et al. 2012). The scientific portion of this framework consists of a thorough examination of both the hydrology and the biology of these systems to determine predictable relationships between flow variability and stream organisms and ecosystems. Critical to the goal of establishing biology-hydrology response relationships is comparing streams within the same flow regime (Poff et al. 2010), as rivers with differing flow regimes within the same region can support distinctive ecologies (Warfe et al. 2014), making the recognition of hydrologic variation at multiple scales one of the most crucial first steps in setting environmental flow management strategies (Kennard et al. 2010, Belmar et al. 2011).

The Ozark Highlands ecoregion extends across southern Missouri, northern Arkansas, and northeast Oklahoma, USA (Omernik and Griffith 2014). This ecoregion contains a diversity of freshwater habitats and is home to a unique assemblage of fish, including 10 endemic species, as well as disjunct, relict populations of more northern-distributed and Appalachian species (TNC-OEA 2003). The ecoregion also has remarkably rich crayfish and mussel faunas, with a

number of endemic species, and a very diverse aquatic herpetofauna of some 40 species, many of which are near-endemics found otherwise only in the adjacent Ouachita Highlands (TNC-OEAT 2003). The freshwater biodiversity in this region is potentially threatened by a host of anthropogenic impacts, including rapidly growing urban areas and extensive agricultural development that affect water quality (Petersen et al. 2005, Haggard 2010, Scott et al. 2011), an expansion in natural gas extraction in the region (Johnson et al. 2015), displacement of native crayfish due to the spread of invasive species (Magoulick and DiStefano 2007, Larson et al. 2009), and direct hydrologic alteration of streams via construction of dams and reservoirs and watershed development (TNC-OEAT 2003).

The primary goal of this study was to develop regional-level environment-biology response relationships for fish and crayfish communities in the Ozark Highlands. We approached this objective in two ways: a multiple regression analysis incorporating environmental variables from several categories in addition to hydrology, and multivariate analysis incorporating a larger set of only hydrologic variables.

Methods

Site Selection

Aquatic community sampling was conducted at 26 sites over two summer field seasons (May-July) during 2012 and 2013 in northwest Arkansas, southwest Missouri, and northeast Oklahoma (Fig. 1). All sites were selected within a single flow regime, Groundwater Flashy streams, based on a classification of Ozark streams into seven natural flow regimes (Leasure et al. 2014) to facilitate biological comparison. Additionally, all sites were confined to a single

ecoregion, the Ozark Highlands, and a single physiographic region, the Springfield Plateau. Streams selected ranged from 16 to 542 km² total drainage area. Sampling was conducted at sites along a gradient of conditions, ranging from reference quality (6) to highly disturbed (29) on a Hydrologic Disturbance Index (HDI) developed by Falcone et al. (2010). This HDI is comprised of seven watershed-scale metrics of disturbance, including presence of major dams; change in reservoir storage from 1950-2009; percentage of streamlines coded as canals, ditches or pipelines; road density; distance to nearest major pollutant discharge site; freshwater withdrawal estimates, and fragmentation of undeveloped land in the watershed (Falcone et al. 2010). The two years presented a strong contrast in flow conditions throughout the study area; an extreme drought as measured on the Palmer Drought Severity Index (PDSI) was present for most of summer 2012, whereas there was sustained higher than normal precipitation leading to much higher than normal flows and summer flooding at many of the sites throughout summer 2013 (NOAA 2015).

The majority of sites (20) were located at USGS stream gages but un-gaged sites (6) were also included in the study. All available gaged sites within the flow class, ecoregion, and physiographic province were selected. Selection of un-gaged sites was random and based on Leasure et al.'s (2014) map of flow regimes in the Ozark Highlands. Natural flow conditions were predicted for both gaged and un-gaged sites, based on 171 flow metrics relating to magnitude (M), frequency (F), duration (D), timing (T), and rate of change (R) of flow events (Leasure et al. 2014). Due to extreme differences in sampling conditions between the two years (drought in 2012 versus extensive flooding in 2013), we were unable to resample 8 of the largest sites from the first field season for fish and crayfish during the second season, but did add five

additional sites following the same site selection criteria. Twenty-one sites were sampled for fish and crayfish in 2012, 18 in 2013, with 13 overlapping sites between the two years.

Fish and crayfish community sampling

Sampling was stratified by habitat to include three units each of riffles, pools, and runs, for a total of nine habitat units per reach. Total area sampled at sites ranged from 140 – 957 m². All habitat units were located at least 100 m away from road crossings to minimize the influence of any man-made structures that could influence hydrology, physical stream habitat characteristics, or result in the creation of artificial habitats (Barbour et al. 1999).

Fish were collected using backpack electrofishing, a method shown to be effective for fish community sampling in Ozark streams (Dauwalter and Pert 2003). Prior to sampling, 1.6 cm² mesh block-nets were placed at the end of each habitat unit to prevent fish from escaping or biasing sampling data by moving from one unit to another. Three upstream sampling passes were conducted per habitat unit consisting of one individual operating a backpack electroshocker (Smith-Root Model LR-24) and three individuals collecting fish with dip-nets. Fish from each pass were kept in separate buckets until all passes were completed. Each pass was processed separately and all fish were identified to species level and released live back into the stream. Crayfish were sampled at the same time and using the same methods as fish, as backpack electroshocking has been shown to be an effective way to sample crayfish (Rabeni et al. 1997). Crayfish were kept in separate buckets for each pass and processed separately from fish. All crayfish were identified to species and released live back into the stream.

Habitat, geomorphology, and water quality measurements

Length and wetted width of each habitat unit were measured with a tape measure; length at the middle of the stream and width at a minimum of 5 transects along each habitat unit. For habitat units exceeding 10 m in length, an additional transect was added for each additional 5 m. At five evenly spaced points along each transect, depth and current velocity were measured with a Model 2000 Portable Flowmeter (Marsh McBirney, Inc.) and substrate size was recorded on a modified Wentworth scale ranging from 1 (silt) to 7 (bedrock).

Stream geomorphology was assessed at the reach scale using a protocol specifically designed for high-gradient streams (Willard et al. 2004), which included a qualitative Rapid Habitat Assessment (RHA) with a maximum possible score of 200. RHA consisted of 10 different habitat parameters targeted specifically at the assessment of high-gradient streams: epifaunal substrate and available cover, embeddedness, velocity/depth patterns, sediment deposition, channel flow status, channel alteration, frequency of riffles, bank stability, bank vegetative protection, and riparian vegetative buffer width. Each parameter was ranked from 1 (low quality) – 20 (high quality) and tallied, for a maximum total score of 200 (Willard et al. 2004).

Water samples were taken for analysis at the Arkansas Water Resources Center (AWRC) three times during each sampling year: spring, summer, and winter. Samples were collected at the thalweg of the stream in a 500 mL NalgeneTM sampling bottle which was first rinsed by filling and emptying with stream water three times, then stored on ice in a cooler for transport to the lab (USEPA 2009). Water quality parameters measured included chloride, conductivity, fluoride, nitrate, soluble reactive phosphorus (P), sulfate, total nitrogen (N), total P, total suspended solids, and turbidity.

Hydrologic variable estimation

Sixty-four reference gages were previously identified in the Interior Highlands region for the river classification study (Leasure et al. 2014). Flow metrics were calculated for reference gages for their entire periods of record using the R package *EflowStats* (Archfield et al. 2013). A set of 187 random forest models was developed to predict the 187 flow metrics. Flow metrics from 64 streams in least-disturbed reference condition were used as response variables. A reduced model was built for each flow metric that included only the 30 most important predictor variables. The reduced random forest models were used to predict values of each flow metric expected under natural conditions, as well as the distribution of expected values. The expected value for each flow metric under natural conditions was taken as the median of the predicted distribution.

Response Variable Selection

We calculated five biological response metrics for use in fish community data analysis and three for use in crayfish community analysis (Table 1). For fish, our five response variables were: species richness, Simpson's diversity, total fish density (per volume sampled), percentage of total individuals belonging to Family Centrarchidae, and percentage of total individuals belonging to species categorized as intolerant in an index of biotic integrity specifically developed for fish communities of the Ozark Highlands (Dauwalter et al. 2003). For crayfish, our three response variables were Simpson's diversity, total crayfish density per volume sampled, and percentage of total individuals belonging to species designated as extraregional invaders in Larson and Olden's (2010) assessment of invasion risks of crayfish in the eastern U.S. These are large, highly fecund, generalist crayfish represented in our dataset by two species, *Orconectes*

neglectus neglectus and *Orconectes virilis*. It is important to note that these two species are native within the study area, but are extraregional invaders in other regions (Larson and Olden 2010). This was chosen as a community response metric due to the variety of life history parameters that comprised the designation. Species richness was not used as a response variable for crayfish due to the generally low and relatively uniform richness across sites. For both fish and crayfish, estimates based on three-pass removal were calculated in R-package Unmarked (Fiske and Chandler 2011), but ultimately raw abundances were used instead for all metrics due to the poor fit of the removal models. Mean values for biological response variables are summarized in Table 2.

Data Analysis

We examined environment-ecology relationships among fish and crayfish communities using two approaches: 1) a multiple regression analysis incorporating a small number of predictor variables related to habitat, water quality, and geomorphology, as well as hydrology, and 2) a canonical ordination procedure using only hydrologic variables in which we used forward selection to select predictors that were most related to our response variables.

Comprehensive Multiple Regression Analysis

We used an information theoretic (IT) approach (Burnham and Anderson 2002) to assess the relative importance of hydrology in relation to other categories of predictors, including local habitat, stream geomorphology, watershed-scale disturbance and water quality. We selected predictor variables from each of these categories that we hypothesized to have greatest biological significance. We selected substrate size for the local habitat variable. Substrate size has been

shown to be an important influence on species composition of fishes at the reach scale in Ozark Highland streams (Magoulick 2000, Dauwalter et al. 2008) and varies less due to conditions at the time of sampling than other variables such as depth, current velocity, or temperature. We selected total P for the water quality variable. Levels of total P are a significant current environmental and political issue in the Ozark Highlands; the 2005 lawsuit filed by the Oklahoma attorney general against several poultry companies in Northwest Arkansas focused on enrichment of total P, among other elements, in the Illinois watershed (Scott et al. 2011). In addition to agriculture-related direct nutrient enrichment such as application of poultry waste to pastureland (Haggard 2010), P concentration in streams is associated with land use characteristics such as the amount of forested land in catchments, and with anthropogenic sources such as wastewater treatment, runoff, and erosion from construction sites (Scott et al. 2011). We selected RHA for the geomorphology variable and HDI for the watershed-scale disturbance variable; both are multi-metric indices that encompass a variety of ecologically relevant parameters but at two different spatial scales (Falcone et al. 2010, Willard et al. 2004). Mean values for environmental predictor variables are summarized in Table 2.

To select a single hydrologic variable, we ran a PCA on the 171 log transformed flow metrics based on predicted natural flows for our sites (Leasure and Magoulick, unpublished data). We dropped flow metrics that were outside our threshold criteria for bias, precision and accuracy (Leasure et al. 2014), then selected metrics with the top 10 loadings on PC1 and examined their distributions, R^2 , and measurement uncertainty. We selected MA32, a measurement of flow variability based on the coefficient of variation in September flows (Olden and Poff 2003), because it had the best combination of top loading on PC1, distribution, high R^2 , and low measurement uncertainty. MA32 was highly correlated (0.89) with MA4, coefficient of

variation in daily flows, but better with respect to all of these characteristics, making it a good overall measure of flow variability.

We examined bivariate correlations among predictors and among response variables and dropped highly correlated variables. All variables were examined via box-plots and histograms to check for normality of distributions. Variables were transformed as needed to improve normality and to meet the assumptions of analyses. This was done separately for the 2012 and 2013 datasets.

We developed a priori hypotheses resulting in 12 models relating biological response variables to predictors, including single-variable models for each of the five predictor variables, the global model, and combination models developed based on variables we felt had biological significance when combined (Table 1). We performed multiple regression analyses in SYSTAT 13 (Systat Software Inc., San Jose, CA) and ranked models by Akaike Information Criteria corrected for small sample size (AICc). We used 95% confidence intervals to determine whether parameter estimates differed from 0. In cases where delta AICc values were within 2 points of the top model, both models were considered equally valid and are reported in the results. We visually inspected residual plots for all regressions.

Multivariate Hydrologic Analysis

We used Redundancy Analysis (RDA) to determine biological response-hydrology relationships during both years. As in the multiple regression analysis, we used predicted natural hydrology in order to incorporate both gaged and ungaged sites. We used the same selection criteria for bias, precision and accuracy to eliminate variables, reducing the hydrologic variable

set from the initial 171 to 154 variables. RDA was appropriate because preliminary Detrended Correspondence Analyses (DCA) indicated that species gradient lengths were less than 1 standard deviation (ter Braak 1995). We used forward selection in CANOCO 4.5 to select hydrologic variables that were related to response variables and limited hydrologic variables to those with $\lambda \geq 0.07$ after entry into the model. Response variables were centered and standardized before running the RDA's because response variables were measured in different units. Because we were interested in relationships among response variables, scaling of ordination scores was focused on inter-response variable correlation rather than inter-sample distance. Response variable scores were standardized to prevent response variables with large variances from unduly influencing ordination diagrams (ter Braak and Smilauer 1998). We performed Monte Carlo permutations to test the significance of canonical axes together for each RDA in order to determine the overall importance of remaining hydrologic variables in influencing response variables. Values and definitions for all significant hydrologic variables in RDA analysis are presented in Table 3.

Results

2012 (drought year)

For fish, the top model predicting species richness was the RHA model, with richness positively related to RHA (Table 4, Fig. 2). The top model predicting species diversity was the HDI model, with diversity negatively related to HDI. The top model predicting percent intolerant species was the total P model, with percent intolerant species negatively related to total P. The top model predicting percent Centrarchidae was the substrate model, with percent

Centrarchidae positively related to substrate size. The top model predicting fish density was also the substrate model, with density negatively related to substrate size (Table 4, Fig. 2).

For crayfish, the top model predicting species diversity was the HDI+total P model, with diversity negatively related to HDI and positively related to total P (Table 4, Fig. 3). The top model predicting crayfish density was the substrate model, with crayfish density negatively related to substrate size. The top model predicting percent extraregional crayfish was the HDI model, with percent extraregional crayfish positively related to HDI (Table 4, Fig. 3).

RDA analysis showed fish assemblages were significantly related to magnitude of average flow, duration of high and low flow, and rate of change metrics (Table 3, Fig. 4). Crayfish assemblages were significantly related to duration and magnitude of low flow and timing of high flow metrics. Duration and magnitude were the most important categories of hydrologic metric overall, with 7 of 10 significant predictors belonging to one of these two categories. Metrics relating to low, high, and average flows were of almost equal importance, with four, three, and three of each of the significant metrics belonging to those categories, respectively. No single metric was important to both fish and crayfish assemblages (Table 3, Fig. 4).

2013 (flood year)

For fish, the top model predicting species richness was the RHA model, with richness positively related to RHA (Table 4, Fig. 5). The top model predicting percent Centrarchidae was the substrate model, with percent Centrarchidae positively related to substrate size. The top model predicting fish density was the MA32 model, with density negatively related to flow

variability. None of the models predicting diversity or percent intolerant species were significant (Table 4, Fig. 5).

For crayfish, the top model predicting density was the substrate model, with density negatively related to substrate size (Table 4, Fig. 6). The top model predicting percent extraregional crayfish was the RHA model with percent extraregional crayfish negatively related to RHA. None of the models predicting diversity were significant (Table 4, Fig. 6).

RDA analysis showed fish assemblages were significantly related to magnitude of average and high flow, timing of low flow, and frequency of high flow metrics (Table 3, Fig. 4). Crayfish assemblages were significantly related to magnitude of average, high, and low flow, timing of low flow, and frequency of high flow metrics. Magnitude was the most important category of hydrologic metric overall, with 7 of 11 significant predictors belonging to this category. Metrics relating to low, high, and average flows were of almost equal importance, with three, four, and four of each of the significant metrics belonging to those categories, respectively (Table 3, Fig. 4).

Discussion

Comprehensive Analysis

In 2012 we found important relationships between biological response variables and environmental predictor variables for all 8 response variables, while in 2013 we found significant relationships for 5 of the 8 response variables. Differences between the two years included: 1) a lack of significant top models for diversity in either fish or crayfish and for percent intolerant fish in 2013, 2) RHA, rather than HDI, being the top model for percent extraregional crayfish in

2013, and 3) MA32, rather than substrate, being the top model for total density in fish in 2013. It is important to note that the lack of overlap in sites limits the strength of conclusions that can be made about temporal variation in fish and crayfish communities in the region between years.

Substrate was the single most important predictor variable, appearing in five top models between the two years and also showing the most consistent relationship between both years of any predictor variable. Substrate size was strongly negatively correlated with densities of fish and crayfish; it was the best model for both in 2012 and for crayfish in 2013. While these relationships are likely related to habitat preference by many of the species that comprise the biota of these streams (Dauwalter et al. 2008), they may also be related to sampling efficiency, given the fact that larger substrate size can lead to lower capture probability due to fish hiding in more complex habitat (Peterson et al. 2004) or immobilized individuals becoming lodged there (Meyer and High 2011). The positive relationship with percent Centrarchidae and substrate size in both years is unsurprising given the association of most Centrarchid species in the region with larger substrate and more complex microhabitat (Pflieger 1975, Robison and Buchanan 1988).

In 2012, HDI was a good predictor of species diversity in both fish and crayfish, showing a negative relationship between disturbance and diversity in both cases. In an overview of the principles relating altered flow regimes to aquatic biodiversity, Bunn and Arthington (2002) showed that streams with more disturbed hydrologic regimes are often associated with a decrease in diversity of aquatic organisms; this has been demonstrated in fish (Stanford and Ward 1986, Copp 1990) as well as macroinvertebrates (Munn and Brusven 1991). Falcone et al.'s (2010) HDI heavily incorporates land-use metrics in addition to direct hydrologic impacts at the watershed scale; the relationship between aquatic biodiversity and land-use has also been

demonstrated in a number of studies among different taxonomic groups (Walser and Bart 1999, Wang et al. 2001, Allan 2004). The positive association between HDI and percent extraregional crayfish in 2012 may be a result of the specific life history parameters that comprise that designation. In addition to high fecundity that may offset more disturbed ecological conditions (Carlisle et al. 2010), these species are also generalists with regard to substrate (Larson and Olden 2010) and are likely better equipped to deal with forms of disturbance such as increased siltation. Alteration of natural flow regimes and other forms of disturbance have been shown to facilitate invasion and success of exotic species (Moyle and Light 1996, Bunn and Arthington 2002). Both of the crayfish species designated as “extraregional invaders” in this study are actually native to the study area but have been highly successful invaders in other regions (Larson and Olden 2010), and it is logical that more disturbed streams would help facilitate the relative success of such species within their natural ranges as well.

Total P was negatively related to percent intolerant fish species in 2012. Whereas the effects of P enrichment in the Ozark Highlands due to agricultural and other land-use practices has been a major environmental issue in recent years, the general recent trend in watersheds such as the Illinois River has been a decrease in concentrations and transport of P, due to watershed management changes in both wastewater treatment and land management over the last two decades (Haggard 2010, Scott et al. 2011). The results of this study support the role of such practices in the conservation of less tolerant species in this region. One surprising relationship was the positive association between total P and crayfish diversity as part of the top combination model with HDI in 2012. Whereas the HDI component of this relationship follows the expected pattern, the positive relationship between increased levels of total P and crayfish diversity are contrary to what would be expected based on studies among other taxonomic groups in similar

streams (Evans-White et al. 2009). Both HDI and total P played a significant role in top models in 2012 but neither appeared in any top models in 2013.

RHA was positively associated with fish species richness in both years. Several of the parameters that comprise the RHA score are related to heterogeneity of habitat (Willard et al. 2004); this may account for a greater number of species being able to utilize those sites with a higher RHA score. Many studies (Gorman and Karr 1978, Schlosser 1982, Heithaus and Grame 1997) have shown that metrics relating to fish diversity are positively associated with increasing habitat complexity while other studies (Closs and Lake 1996, Herbert and Gelwick 2003) have shown that local hydrological disturbances such as those reflected at sites with lower RHA scores can strongly impact fish community dynamics. The negative relationship between percent extraregional crayfish and RHA in 2013 suggests again that these species tend to proliferate in more disturbed habitats; this was demonstrated at the reach scale via RHA in 2013 as opposed to the watershed scale via HDI in 2012.

The least important predictor variable overall was the hydrologic variable MA32, which appeared only once in any top model in the two years. This suggests that hydrologic variability, or at least the metric that we chose for this analysis, may not be as important as other environmental variables. The one case in which MA32 was a significant top model was total fish density in 2013, where it was negatively related to density. Craven et al. (2010) showed that fish density can be negatively related to flow variability, due largely to density of young of year fish being negatively impacted by flow variability during the rearing period.

Multivariate Hydrologic Analysis

Magnitude was the most important category of flow metric in terms of influence on fish and crayfish communities in Groundwater Flashy streams in the Ozark Highlands. Of the 21 important flow metrics between both taxonomic groups and years, 10 belonged to this category. Duration and timing were also important categories, each comprising 4 of the 21 important flow metrics overall, while frequency and rate of change were less important, comprising 2 and 1 of the 21 important flow metrics respectively. The relative importance of each of these categories was fairly consistent between the two taxonomic groups. Numbers of important magnitude, frequency, and duration metrics were identical in fish and crayfish assemblages. While few studies have directly compared the relative importance of flow metric categories, regional environmental flow studies in recent years have suggested that magnitude of flow may be one of the most important influences on aquatic communities (Monk et al. 2006, Armstrong et al. 2011, Kendy et al. 2012).

In 2012, fish species richness and percent intolerant species were closely associated, and both variables were negatively related to measurements of flow variability and duration of low flows. Poff and Allan (1995) showed that stable streams are characterized by the presence of intolerant and specialized species which are often not found in streams with more hydrologic variability. In contrast, percent Centrarchidae was positively related to flow variability. A possible explanation is that these species tend to prefer pools, which may be more stable habitats during both low and high flow events compared to riffles and runs (Magoulick and Kobza 2003); this could somewhat ameliorate the effects of increased flow variability on these species compared to other groups (Poff et al. 2010). In 2012, crayfish diversity was positively associated with duration of low flows, while extraregional crayfish were positively associated with magnitude of low flows and Julian date of high flows, and negatively associated with

predictability of high flows and duration of low flows. In 2012, timing of flows appears to be more important to crayfish than fish assemblages. This is most apparent in the percent extraregional crayfish metric; less predictable timing of floods and floods later in the year seem to favor these species, perhaps because of life history traits (Pflieger 1996, Carlisle et al. 2010, Larson and Olden 2010). No single flow variable in 2012 was important to both fish and crayfish communities, which illustrates that flow can differentially affect different taxonomic assemblages in the same region.

In 2013 there was more similarity in metrics important to both fish and crayfish assemblages compared to 2012. Two metrics, MA39 and FH5, were important to both groups and showed similar relationships. MA39 was strongly negatively related to total density in both fish and crayfish, while FH5 was closely positively related to diversity in both groups. The negative relationship between flow variability and density follows the same pattern that we found in the comprehensive multiple regression analysis as well as previous studies, at least with regard to fish density (Craven et al. 2010). With regard to the positive relationship between FH5 and diversity, fishes and other aquatic fauna in Ozark streams are typically well adapted to cope with non-catastrophic flooding (Dodds et al. 2004, Matthews et al. 2014). Flooding may generally have less of a detrimental effect than drought in riverine ecosystems (Lake 2000, Matthews et al., 2013). In long-term studies, Matthews et al. (2013) found that frequency, rather than magnitude, of flooding events may be more important in the community structure of stream fishes. It is possible that more frequent flooding may help facilitate greater diversity in aquatic communities in these streams.

Conclusions

In our comprehensive analysis, we found that other categories of environmental variables, including geomorphology, water quality, and watershed-scale disturbance, were more strongly related to fish and crayfish assemblages than the measure of hydrologic variation used in this study. In our multivariate hydrologic analysis, we found that flow magnitude was most related to fish and crayfish assemblage structure, followed by duration and timing. We found strong similarities between fish and crayfish community responses to some hydrologic variables, e.g. flood frequency and average flow variability, while others differed considerably between the two groups, e.g. the importance of low versus average flows in crayfish assemblages compared to fish.

Increased hydrologic disturbance at both the reach and watershed scale was associated with a loss of species richness and diversity as well as an increase in the success of generalist species. Flood frequency was positively related to diversity and flow alteration that diminishes this aspect of the natural flow regime could have a detrimental effect on diversity in the region. Our findings suggest that hydrologic metrics are best considered within a complex framework that includes other types of environmental data such as water quality, stream geomorphology, and local habitat.

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Table 1. Response and predictor variables and models used in comprehensive multiple regression analyses.

Response Variables	Predictor Variables	Models
Fish Species Richness	Substrate (modified Wentworth Scale)	Substrate
Fish Simpson's Diversity	RHA (Rapid Habitat Assessment)	RHA
Fish Total Density	HDI (Hydrologic Disturbance Index)	HDI
Fish % Intolerant	Total P (mg/L)	Total P
Fish % Centrarchidae	MA32 (Coefficient of variation in September flows)	MA32
Crayfish Simpson's Diversity		Substrate+RHA
Crayfish Total Density		Total P+MA32
Crayfish % Extraregional		HDI+Total P
		HDI+MA32
		HDI+RHA
		Total P+MA32+HDI+RHA
		Substrate+Total P+MA32+HDI+RHA

Table 2. Mean (\pm SE) values for biological response and predictor variables used in comprehensive multiple regression analysis in 2012 and 2013. Substrate size based on modified Wentworth scale. RHA = Rapid Habitat Assessment, maximum possible score of 200. HDI = Hydrologic Disturbance Index, maximum possible score of 42. HDI and MA32 do not vary between years.

Variable	2012	2013
Fish Species Richness	16 (\pm 0.17)	15 (\pm 0.19)
Fish Simpson's Diversity	0.73 (\pm 0.01)	0.75 (\pm 0.01)
Fish Total Density	11.00 (\pm 0.25)	15.54 (\pm 0.69)
Fish % Intolerant	0.70 (\pm 0.01)	0.68 (\pm 0.01)
Fish % Centrarchidae	0.03 (\pm 0.01)	0.05 (\pm 0.01)
Crayfish Simpson's Diversity	0.21 (\pm 0.01)	0.30 (\pm 0.01)
Crayfish Total Density	3.68 (\pm 0.19)	7.30 (\pm 0.38)
Crayfish % Extraregional	0.75 (\pm 0.02)	0.44 (\pm 0.02)
Substrate Size	3.55 (\pm 0.02)	3.67 (\pm 0.02)
RHA	165.33 (\pm 0.75)	153.61 (\pm 1.30)
HDI	14.71 (\pm 0.31)	13.5 (\pm 0.34)
Total P (mg/L)	0.07 (\pm 0.01)	0.06 (\pm 0.01)
MA32 (Coefficient of Variation in September Flows)	111.09 (\pm 2.07)	120.27 (\pm 2.33)

Table 3. Hydrologic metrics (Olden and Poff, 2003) used in multivariate hydrologic analysis for 2012 and 2013 with mean (\pm SE) values.

Code	Definition	Category	Mean (\pm SE)
2012 Fish			
DH17	High Flow Duration where upper threshold is defined as 7 times median flow	Duration of High Flows	27.42 (\pm 0.34)
DL16	Low flow pulse duration	Duration of Low Flows	13.60 (\pm 0.30)
MA4	Coefficient in variation of logs in daily flows	Magnitude of Average Flows	126.81 (\pm 2.78)
MA5	Skewness in daily flows	Magnitude of Average Flows	4.20 (\pm 0.36)
RA40	Variability in fall rate	Rate of Change in Flows	626.34 (\pm 11.00)
2012 Crayfish			
DL13	Mean of 30-day minima of daily discharge	Duration of Low Flows	0.24 (\pm 0.02)
DL14	Low exceedence flows (magnitude of flows exceeded 75% of the time divided by median daily flow over all years)	Duration of Low Flows	0.36 (\pm 0.02)
ML6	Mean minimum June flows	Magnitude of Low Flows	9.11 (\pm 2.18)
TH1	Julian date of annual maximum	Timing of High Flows	94.87 (\pm 2.34)
TH3	Seasonal predictability of non-flooding (maximum proportion of year during which no floods have occurred during period of record)	Timing of High Flows	0.21 (\pm 0.01)
2013 Fish			
FH5	Flood frequency (mean number of high flow events per year)	Frequency of High Flows	7.96 (\pm 0.10)
MA36	Variability across monthly flows 1 (variability in daily flows divided by median monthly flows where variability is calculated as range)	Magnitude of Average Flows	21.79 (\pm 1.11)
MA39	Variability across monthly flows 2 (coefficient of variation in mean monthly flows)	Magnitude of Average Flows	132.53 (\pm 1.55)
MH6	Mean maximum June flows	Magnitude of High Flows	143.09 (\pm 12.67)
TL3	Seasonal predictability of low flow	Timing of Low Flows	0.18 (\pm <0.01)

Table 3 (cont.). Hydrologic metrics (Olden and Poff, 2003) used in multivariate hydrologic analysis for 2012 and 2013 with mean (\pm SE) values.

Code	Definition	Category	Mean (\pm SE)
TL3	Seasonal predictability of low flow	Timing of Low Flows	0.18 ($\pm <0.01$)
2013 Crayfish			
FH5	Flood frequency (mean number of high flow events per year)	Frequency of High Flows	7.96 (± 0.10)
MA39	Variability across monthly flows 2 (coefficient of variation in mean monthly flows)	Magnitude of Average Flows	132.53 (± 1.55)
MA41	Mean annual runoff	Magnitude of Average Flows	0.88 (± 0.02)
MH2	Mean maximum February flows	Magnitude of High Flows	269.26 \pm 35.20)
ML2	Mean minimum February flows	Magnitude of Low Flows	12.67 (± 1.32)
TL3	Seasonal predictability of low flows (proportion of low-flow events \geq 5-year magnitude falling in a 60-day seasonal window)	Timing of Low Flows	0.18 ($\pm <0.01$)

Table 4. Best models for 2012 and 2013 response metrics (fish and crayfish). Significant relationships in bold.

Response Variable	Best Model	Std. Coefficient	R ²	C.I.
2012				
Fish Species Richness	RHA	0.432	0.187	0.006 – 0.186
Fish Simpson's Diversity	HDI	-0.487	0.237	-28.772 – -3.092
Fish % Intolerant	Total P	-0.550	0.302	-83.823 – -15.767
Fish % Centrarchidae	Substrate	0.604	0.365	0.019 – 0.073
Fish Total Density	Substrate	-0.835	0.668	-0.719 – -0.3954
Crayfish Simpson's Diversity	HDI+Total P	-0.601, 0.775	0.505	-80.145 – -13.945, 59.619 – 127.658
Crayfish % Extraregional	HDI	0.436	0.190	1.026 – 109.266
Crayfish Total Density	Substrate	-0.477	0.227	-0.771 – -0.049
2013				
Fish Species Richness	RHA	0.460	0.211	-0.003 – -0.125
Fish Simpson's Diversity	Substrate	0.337	0.113	-2.735 – 17.493
Fish % Intolerant	MA32	-0.191	0.036	-0.739 – 0.319
Fish % Centrarchidae	Substrate	0.652	0.425	0.044 – 0.162
Fish Density	MA32	-0.517	0.267	-2.391 – -0.247
Crayfish Simpson's Diversity	Total P	0.232	0.054	-0.428 – 1.238
Crayfish % Extraregional	RHA	-0.440	0.194	-0.012 – -0.001
Crayfish Total Density	Substrate	-0.562	0.316	-1.922 – -0.310

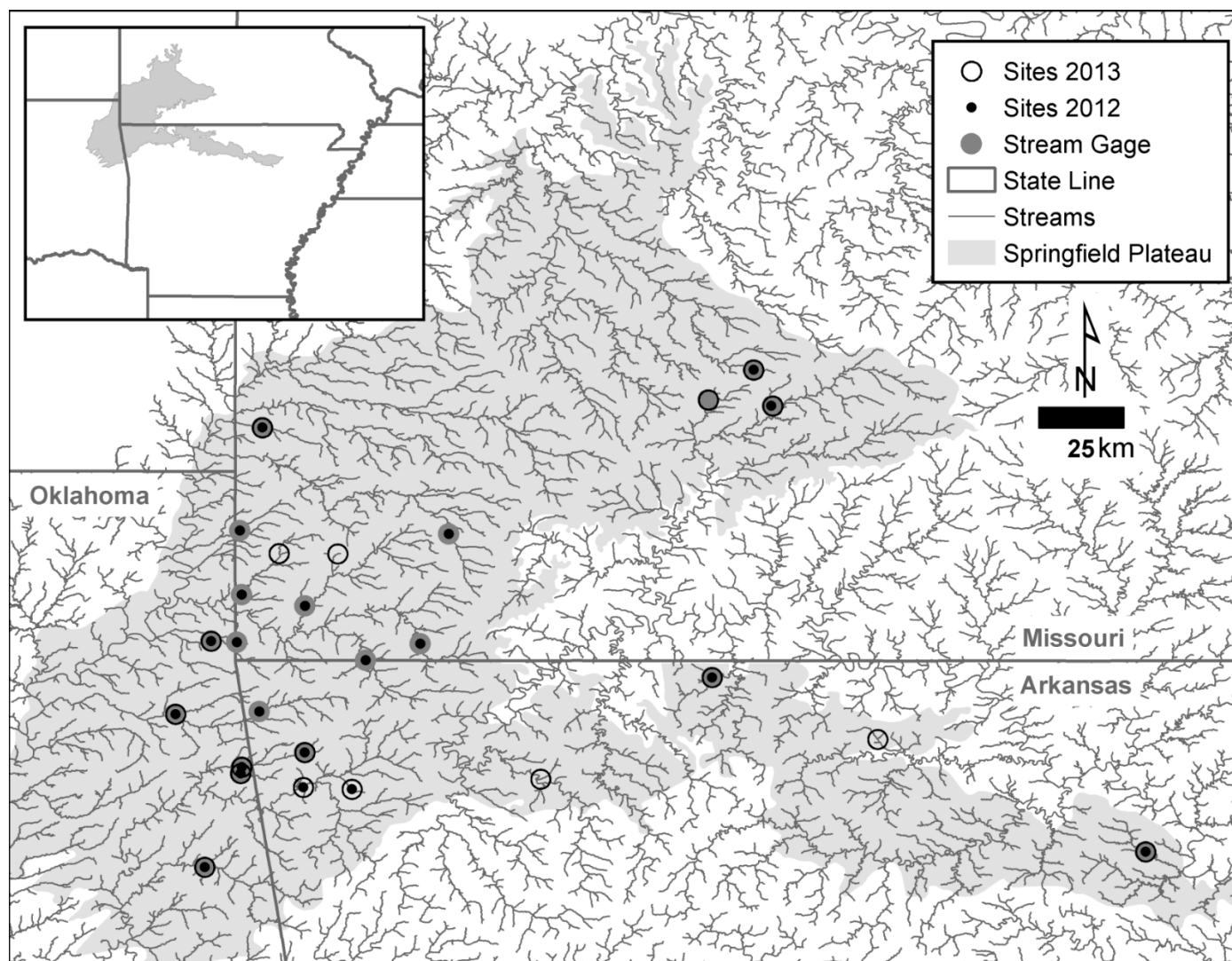


Figure 1. Map of study area showing sample sites, stream network, and Springfield Plateau.

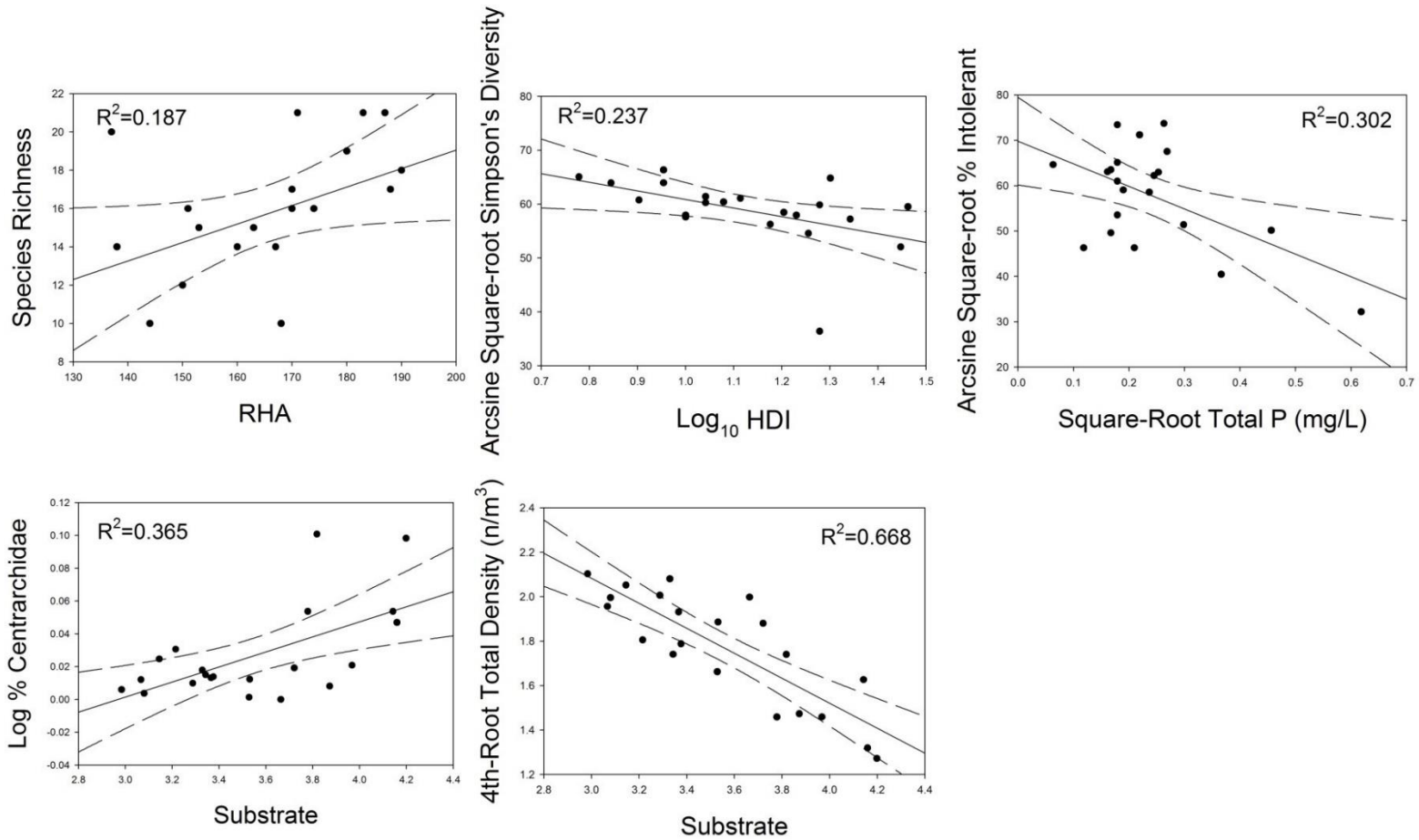


Figure 2. Important fish-environment relationships in 2012. Only significant relationships in the best models are shown. Dashed lines indicate 95% confidence intervals.

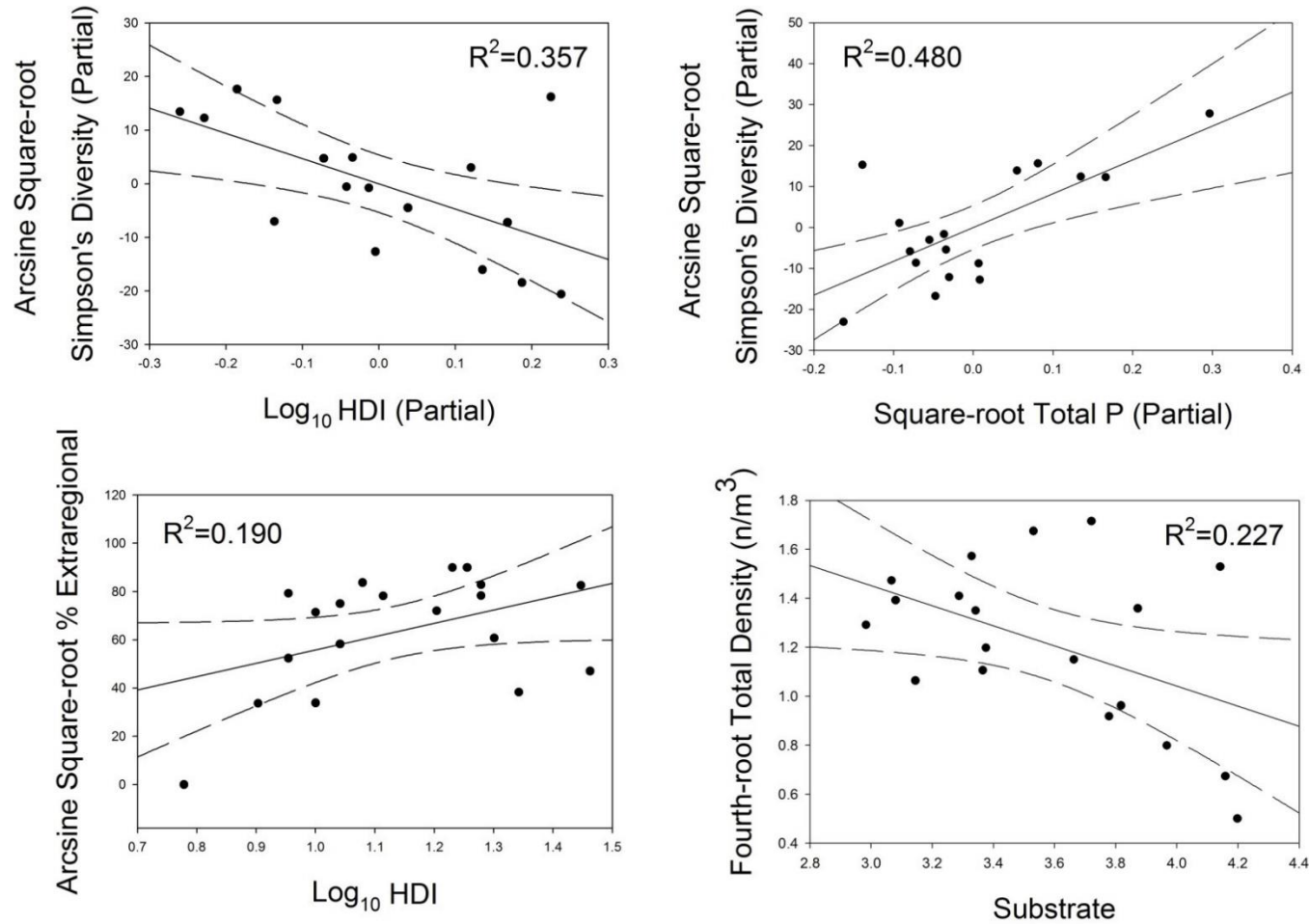


Figure 3. Important crayfish-environment relationships in 2012. Only significant relationships in the best models are shown. Partial regression values used for combination models. Dashed lines indicate 95% confidence intervals.

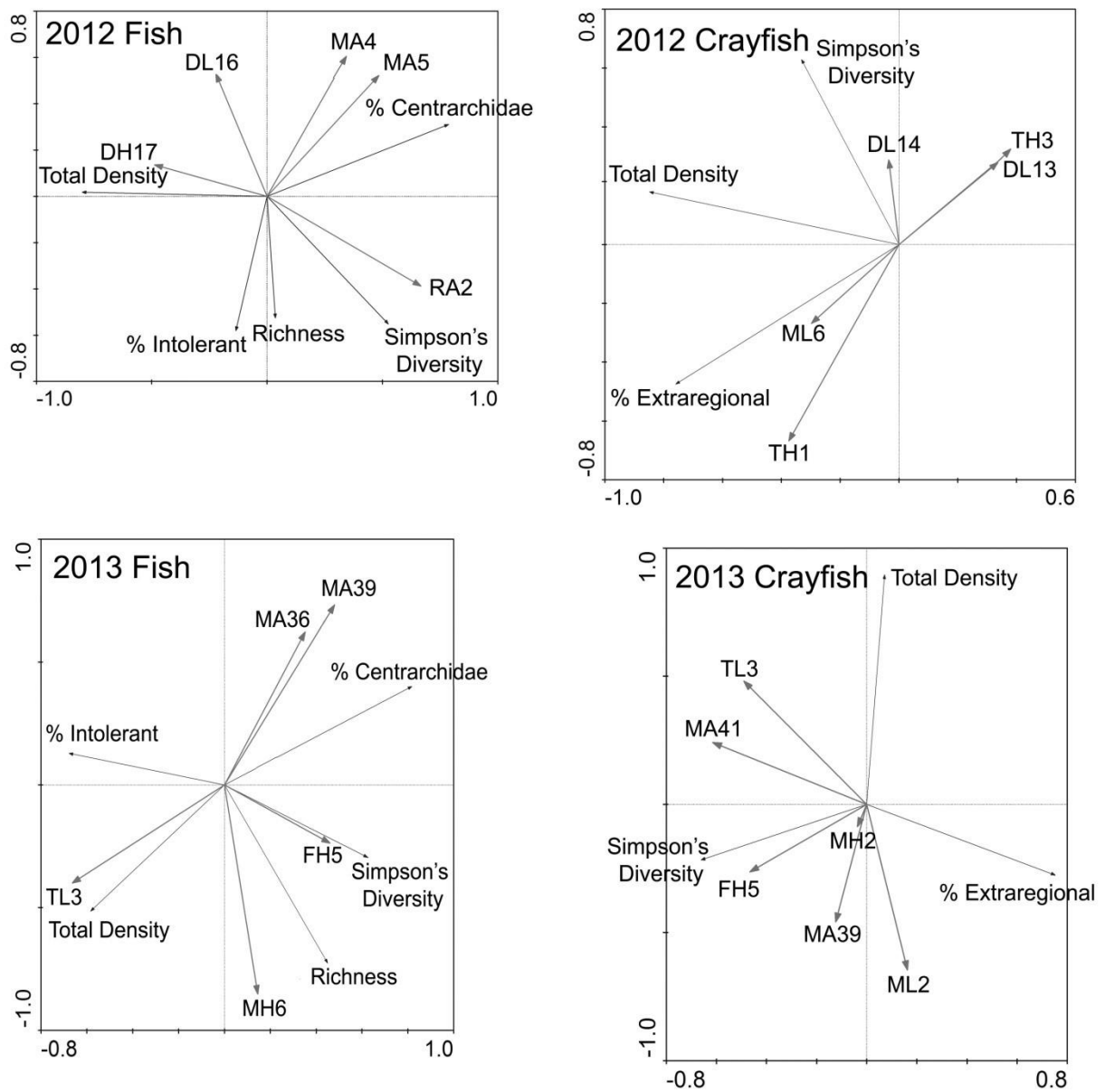


Figure 4. Redundancy analysis ordination plots relating fish and crayfish response variables and selected hydrologic metrics in 2012 and 2013. Angles of arrows indicate associations and length of arrows indicate strength of the relationship.

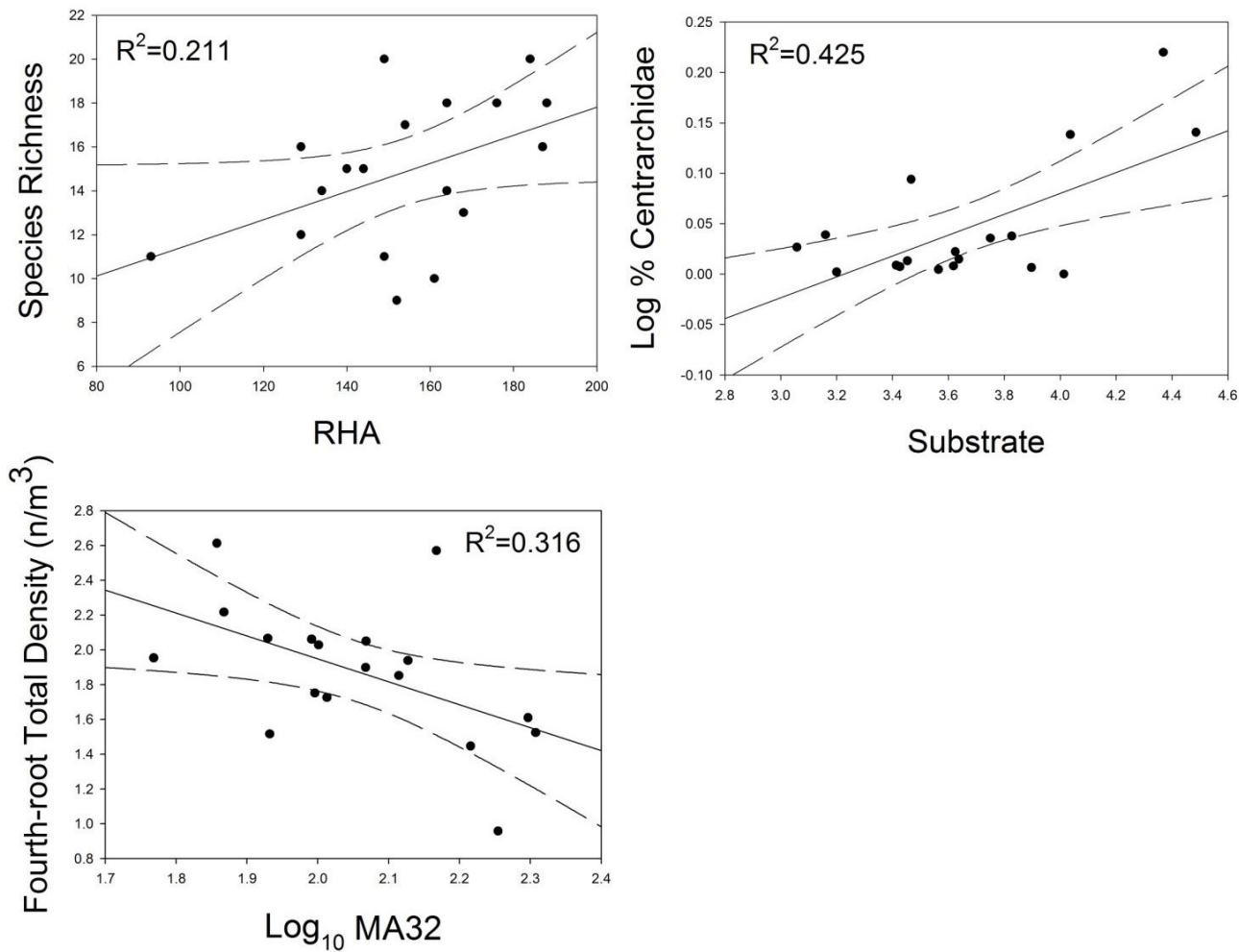
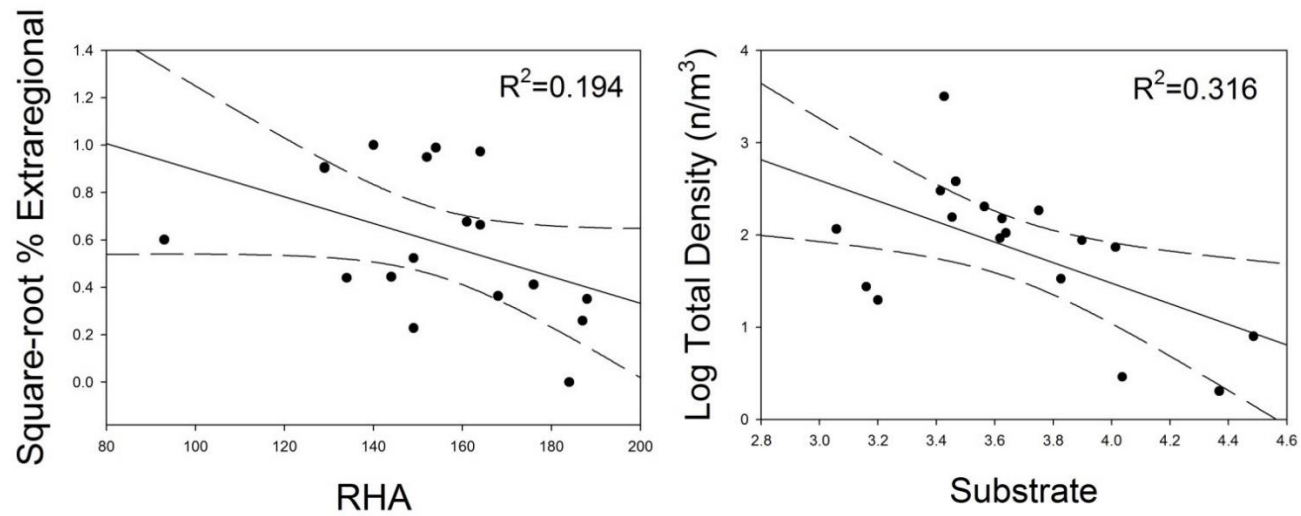


Figure 5. Important fish-environment relationships in 2013. Only significant relationships in the best models are shown. Dashed lines indicate 95% confidence intervals.



96

Figure 6. Important crayfish-environment relationships in 2013. Only significant relationships in the best models are shown. Dashed lines indicate 95% confidence intervals.



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MEMORANDUM

TO: Dan Magoulick
FROM: Craig N. Coon, Chairman
Institutional Animal Care
And Use Committee
DATE: February 4, 2011
SUBJECT: **IACUC PROTOCOL APPROVAL**
Expiration date : **January 31, 2013**

The Institutional Animal Care and Use Committee (IACUC) has **APPROVED** Protocol #11018-
"CLASSIFICATION OF ARKANSAS FLOW REGIMES, REGIONAL ECOLOGICAL-FLOW
RESPONSE RELATIONSHIPS AND ENVIRONMENTAL FLOWS ASSESSMENT FOR THE
OZARK REGION ". You may begin this study immediately.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any changes in the protocol during the research, please notify the IACUC in writing **prior** to initiating the changes. If the study period is expected to extend beyond **01-31-2013**, you may request an extension (up to 02-04-2014, which is 3 years from original approval date) via a Modification Request form. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

cnc/car

cc: Animal Welfare Veterinarian

The University of Arkansas is an equal opportunity/affirmative action institution.

Appendix 1. IACUC approval letter for protocol #11018.

**Stream Flow Alteration in the Ozark Highlands: Consequences for Fish, Crayfish, and
Macroinvertebrate Communities**

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Abstract

We examined flow alteration-ecology relationships in fish, crayfish, and benthic macroinvertebrate communities in Ozark Highland streams, USA, over two years with contrasting environmental conditions, a drought year (2012) and a flood year (2013). We used three-pass backpack electrofishing to sample fish and crayfish at 17 USGS-gaged sites in 2012 and 11 sites in 2013. We also used a quantitative richest-targeted habitat (RTH) method and a qualitative multi-habitat (QMH) method to collect macroinvertebrates at 16 gaged sites during both years. We used redundancy analysis to relate biological response variables, including richness, diversity, density, and community-based metrics to metrics of flow alteration. We found that flow alteration had a strong influence on Ozark Highland stream communities. Magnitude of average flow, frequency of high flow, magnitude of high flow, and duration of high flow were the most important categories of flow alteration metrics across taxa. Alteration of high and average flows were more important than alteration of low flows. Of the 32 important flow alteration metrics across years and assemblages, 19 were significantly altered relative to expected values. Fish, crayfish, and QMH macroinvertebrate assemblages showed similar importance of magnitude alteration metrics, while high flow frequency alteration metrics were also important in fish and QMH macroinvertebrates but not crayfish. No particular category of alteration metrics was most important in RTH macroinvertebrate assemblages. We found strong temporal variation among flow alteration-ecology relationships in macroinvertebrate assemblages. Differences in flow alteration-ecology relationships among taxonomic groups and temporal variation in relationships illustrate that a complex suite of variables should be considered for effective conservation of stream communities related to flow alteration.

Introduction

The natural flow regime paradigm posits that the ecological integrity of rivers depends on their natural dynamic character (Poff et al. 1997), and that traditional approaches to managing streams by simply focusing on minimum low flows may be inadequate to protect these ecosystems and their biota (Bunn and Arthington 2002, Poff et al. 2010). One of the great challenges in the implementation of the environmental flows approach to management and restoration is accounting for natural variability and complexity among different types of streams, even those within the same geographic region (Arthington et al. 2006, Kennard et al. 2010, Poff et al. 2010). The Ecological Limits of Hydrologic Alteration (ELOHA) framework (Poff et al. 2010) consists of scientific and social processes working in tandem to address the needs of both ecosystems and stakeholders. In addition to quantifying hydrologic and biological data, the ELOHA approach stresses the importance of regional stream natural flow regime classification as a critical initial step (Poff *et al.* 2010). Although lotic ecologists possess a solid general knowledge of how ecological processes and ecosystem structure and function depend on hydrologic variation, only recently have studies been published in which specific ecological metrics have been quantified in response to various degrees of flow alteration (Kendy et al. 2012, McManamay and Frimpong 2015). Quantifying flow alteration, the degree of variation away from the natural flow regime, is a crucial step in environmental-flows based management approaches such as the ELOHA framework (Poff et al. 2010).

While there is strong evidence that flow alteration generally negatively effects biodiversity as well as ecosystem function (Bunn and Arthington 2002, Harris and Heathwaite 2011, Warfe et al. 2014), there are challenges to establishing transferable relationships between

flow alteration and ecological response (Poff and Zimmerman 2010). Crucial steps in the ELOHA process include regional flow regime classification and the quantification of flow alteration; these steps are often made difficult by lack of hydrological data due to the somewhat sparse nature of stream gages, which are often placed only on larger order stream segments. Determining quantifiable relationships between hydrologic alteration and biological data is not only of great interest in informing management decisions relating to issues of water conservation and restoration (McManamay et al. 2014), but could potentially also be a critical tool in the assessment of the possible impacts of climate change on stream ecosystems and organisms (Xenopolous et al. 2005).

Maintenance of naturally variable hydrological regimes is critical in the face of pervasive human influence on watershed hydrology (Carlisle et al. 2010) as water managers are increasingly challenged to balance the water supply needs of growing human populations with the conservation of stream ecosystems and their biota (Poff et al. 2010, Olden et al. 2014). Freshwater biodiversity has declined faster than either terrestrial or marine diversity over the past 30 years, with altered flow rates contributing significantly to the loss of species (Jenkins 2003, Postel and Richter 2003, Xenopolous et al. 2005). North America in particular possesses some of the most threatened aquatic ecosystems in the world (Jelks et al. 2008).

In the U.S., natural streamflow regimes are threatened by a host of anthropogenic factors including construction of dams and diversion structures and groundwater withdrawals from aquifers, (Carlisle et al. 2010). Additionally, extreme climate events are expected to increase as a result of global climatic change, including many events that directly impact lotic ecosystems, such as increases in drought frequency, duration, and intensity in many regions of the world (Beniston et al. 2007, Beche et al. 2009). The potential interactive effects of natural and

anthropogenic stressors such as drought, climate change, and human water use on ecosystems highlight the need for increased understanding of each stressor (Christensen et al. 2006, Beche et al. 2009). For example, water withdrawals during dry years can reduce habitat connectivity and result in critical flow reductions (Beche et al. 2009). The maintenance of natural hydrological regimes can also provide resistance to species invasion (Closs and Lake 1996, Caiola et al. 2014), another pervasive world-wide phenomenon in freshwater habitats, often facilitated by anthropogenic alteration of flow regimes (Bunn and Arthington 2002). For example, naturally flashy streams or rivers typified by frequent or rapid onset of high flows can prevent the establishment of non-native fish species that lack behavioral adaptations to rapid onset of flows (Meffe 1984, Poff et al. 2010) or have a vulnerable juvenile stage present during periods of peak flows (Fausch et al. 2001, Poff et al. 2010).

The Ozark Highlands ecoregion of southern Missouri, northern Arkansas, and northeast Oklahoma, USA (Omernik and Griffith 2014), is heavily affected by a suite of anthropogenic impacts, including rapidly growing urban areas and agricultural development that affect water quality (Petersen et al. 2005, Haggard 2010, Scott et al. 2011), expansion of natural gas extraction (Johnson et al. 2015), displacement of native fauna due to the spread of invasive species (Magoulick and DiStefano 2007, Larson et al. 2009), and direct hydrologic alteration of streams via construction of reservoirs, dams, and watershed development (TNC-OEAT 2003). This region is home to a diverse assortment of freshwater habitats and aquatic species, including endemic fish, crayfish, mussels, macroinvertebrates, and herpetofauna (TNC-OEAT 2003). Understanding the impacts of hydrologic alteration could be a crucial step in the formulation of guidelines for protection and restoration of stream ecosystems in the region.

The objective of this study was to examine flow alteration-biological response relationships for fish, crayfish, and benthic macroinvertebrate assemblages in the Ozark Highlands. We conducted aquatic community sampling at 18 sites in Groundwater Flashy streams in the Ozark Highlands over two years and used redundancy analysis (RDA) to relate biological response variables to metrics of flow alteration, including magnitude, frequency, duration, timing, and rate of change.

Methods

Site Selection

Aquatic community sampling was conducted at 18 sites with USGS stream gages over two summer field seasons (May-July) during 2012 and 2013 in northwest Arkansas, southwest Missouri, and northeast Oklahoma (Figure 1). To facilitate biological comparisons, all sites were selected within a single ecoregion, the Ozark Highlands; a single physiographic region, the Springfield Plateau; and within a single flow regime, Groundwater Flashy streams, where flow regime was based on a classification of Ozark-Ouachita Interior Highland streams into 7 different hydrologic flow regimes (Leasure et al. 2014). Streams selected ranged from 16 to 542 km² total drainage area. Sites encompassed a wide gradient of conditions, ranging from reference quality (6) to highly disturbed (29) on a Hydrologic Disturbance Index (HDI) developed by Falcone et al. (2010).

Natural flow conditions were predicted for all sites based on 171 flow metrics relating to magnitude (M), frequency (F), duration (D), timing (T), and rate of change (R) of flow events (Leasure et al. 2014). Due to extreme differences in sampling conditions between the two years (drought in 2012 versus extensive flooding in 2013), we were unable to resample 7 of the largest

sites from the first field season for fish and crayfish during the second season, but did add one additional site. Seventeen sites were sampled for fish and crayfish in 2012, 11 in 2013, with 10 overlapping sites between the two years. Macroinvertebrate collections were taken at 16 sites which were the same in both years (Figure 1).

Aquatic community sampling

At each site, we sampled for fish, crayfish, and benthic macroinvertebrates. All sampling was stratified by habitat to include three units each of riffles, runs and pools, for a total of nine habitat units per reach. Total area sampled at sites ranged from 140 – 957 m². All habitat units were located at least 100 m from road crossings to avoid the hydrologic influence of bridge abutments, culverts, or any other man-made structures that could influence physical stream habitat characteristics or create artificial habitat (Barbour et al. 1999).

Fish were collected using backpack electrofishing, a method shown to be effective for fish community sampling in Ozark streams (Dauwalter and Pert 2003). Prior to sampling, 1.6 cm² mesh block-nets were placed at the end of each habitat unit to prevent fish from escaping or biasing sampling data by moving from one unit to another. Three upstream sampling passes were conducted per habitat unit consisting of one individual operating a backpack electroshocker (Smith-Root Model LR-24) and three individuals collecting fish with dip-nets. Fish from each pass were kept in separate buckets until all passes were completed. Each pass was processed separately and all fish were identified to species level and released live back into the stream. Crayfish were sampled at the same time and using the same methods as fish, as backpack electroshocking has been shown to be an effective way to sample crayfish (Rabeni et al. 1997). Crayfish were kept in separate buckets for each pass and processed separately from fish. All

crayfish were identified to species and released live back into the stream. Total time spent shocking for fish and crayfish was recorded for each pass.

Benthic macroinvertebrates were sampled using two different methodologies devised for the National Water Quality Assessment (NAWQA) program by Moulton et al. (2002), consisting of a semi-quantitative richest-targeted habitat (RTH) method and a qualitative multiple habitat (QMH) method. Invertebrate collections were sorted and identified in the laboratory during the year following the second field season (2013-2014).

In the RTH method, a quantitative sample of invertebrates was taken from riffles only, the habitat type determined to support the richest invertebrate community in high-gradient wadeable streams (Moulton et al. 2002). A 0.25 m² pvc quadrat frame was used at 3 randomly selected locations within each riffle, in conjunction with a slack sampler consisting of a wooden handle attached to a rectangular net frame (50 cm × 30 cm) fitted with a tapered, 500-μm mesh NitexTM collection net. The slack sampler was positioned immediately downstream of the quadrat and perpendicular to the direction of flow. Large cobble and debris were removed by hand from the sampling area and inspected for attached organisms, which were then removed from the surface in front of the slack sampler. The sampling area was disturbed by digging into the substrate and agitating it to stir up invertebrates in the benthos, which were then collected by moving the slack sampler in a forward motion and retrieving it. The nine discrete subsamples were then combined and placed into a 19-L plastic bucket for processing. Processing consisted of rinsing and removal of large debris, followed by elutriation and sieving (with a 500-μm sieve) of the samples to separate invertebrates and organic debris from inorganic debris.

The QMH method was used to document invertebrate taxa present in all habitat types throughout our sampling reaches (Moulton et al. 2002). Before QMH sampling began, crewmembers assessed the entire reach to determine number of different instream habitat types present and to estimate proportions of each type present. Then, QMH collections were taken from each of the different instream habitats (riffle, run, pool) present in the reach and combined into a single composited sample. A D-frame kicknet with 500- μm mesh was used to collect invertebrates from each habitat type present in relative proportion for a total standard time of one hour per reach. Samples were processed in the field as described for the RTH method mentioned above.

In the laboratory, invertebrate samples were sorted on a square gridded subsampling frame of $25 \times 5 \times 5$ cm squares using a fixed-count approach targeting a minimum of 300 organisms (Barbour et al. 1999, Moulton et al. 2000). After pouring the sample into the frame and allowing it to settle evenly, an initial inspection was performed to remove large and rare organisms likely to be missed during subsampling. A grid square was randomly selected and all of the organisms present were counted. Subsampling proceeded in this fashion until a minimum of 300 organisms were counted, with the square in which the 300th organism was counted also being fully counted. All macroinvertebrates were identified to the lowest practical taxonomic level, generally family or genus. To estimate total numbers of organisms in samples, a laboratory subsampling correction factor was used (Moulton et al. 2000) in which the total number of grids was divided by the number of grids sorted during subsampling, and multiplied by the number of organisms subsampled. Large and rare organisms taken from the sample as a whole were added to these numbers without a correction factor. Invertebrate community metrics were then calculated based on these numbers.

Hydrologic variable and flow alteration estimation

USGS daily flow data were obtained for 208 gages within the Interior Highlands region, including the South Central Plains of Arkansas, using the R package *dataRetrieval* (Hirsch and De Cicco 2015). Water years include the period from October 1 to September 30, and they are named for the year they end. Every water year in each daily flow record was assessed to identify the number of days of data, number of days missing data, and the largest contiguous block of days with missing data. Years were removed from daily flow records if they had more than 30 days of missing data, or if they had a contiguous block of missing data greater than 7 days. These criteria are similar to those used by the Hydrologic Index Tool (HIT; Henrickson et al. 2006).

Sixty-four reference gages were previously identified in the Interior Highlands region for the river classification study (Leasure et al. 2015). Flow metrics were calculated for reference gages for their entire periods of record using the R package *EflowStats*. All gages had more than 15 years of data to minimize measurement uncertainty that may affect some flow metrics when using short periods of record (Kennard et al. 2010). The 187 flow metrics calculated by the R package *EflowStats* included the 171 metrics calculated by HIT (Olden and Poff 2003).

A set of 187 random forest models was developed to predict the 187 flow metrics. Flow metrics from 64 streams in least-disturbed reference condition were used as response variables. Full models were built initially that included 144 predictor variables describing climate and landscape characteristics within reference watersheds. Importance of each variable was assessed using the default method of the *randomForest* R package, which is based on increase of mean-

squared error resulting from random permutations of the variable. A reduced model was built for each flow metric that included only the 30 most important predictor variables.

Data were collected at all 208 USGS gages in the Interior Highlands for any predictor variable selected for at least one of the reduced random forest models. The reduced random forest models were used to predict values of each flow metric expected under natural conditions, as well as the distribution of expected values. The spread of these predicted distributions included both natural variation and model error. The expected value for each flow metric under natural conditions was taken as the median of the predicted distribution.

Flow metrics were calculated for every complete 15 year period within the daily flow records of 208 gages within the Interior Highlands region. Flow alteration was calculated as:

$$flow\ alteration = \frac{observed - median(predicted)}{std.dev(predicted)}$$

where *observed* is the value of the flow metric from a specific period with a gage's record, and *predicted* is the distribution of values expected under natural conditions predicted by the random forest models. The standard deviation (*std. dev*) of predicted natural values was used for standardization rather than the interquartile range because the interquartile range may be zero for random forest models with high accuracy. We decided not to assess flow alteration as *observed / expected* as recommended by Carlisle et al. (2010) because of issues arising when expected values are zero. Flow alteration was measured for each 15 year period in the flow records of 208 gage sites. We dropped flow metrics that were outside our threshold criteria for bias, precision and accuracy (Leasure *et al.* 2014), reducing our initial set of 171 metrics to 154.

Data Analysis

Biological response variables were calculated for fish, crayfish, and macroinvertebrate datasets (Table 1). For fish, our five response variables were: species richness, Simpson's diversity, total fish density (per volume sampled), percentage of total individuals belonging to Family Centrarchidae, and percentage of total individuals belonging to species categorized as intolerant in an index of biotic integrity specifically developed for fish communities of the Ozark Highlands (Dauwalter et al. 2003). Percent Centrarchidae was chosen as a community metric because these species are the predominant larger-bodied, higher trophic level piscivores in the region, but a stricter “top carnivore” trophic designation would have included too few individuals in our samples (Dauwalter et al. 2003). For crayfish, our three response variables were Simpson's diversity, total crayfish density (per volume sampled), and percentage of total individuals belonging to species designated as extraregional invaders in Larson and Olden's (2010) assessment of invasion risks of crayfish in the eastern U.S. These are large, highly fecund, generalist crayfish represented in our dataset by two species, *Orconectes neglectus* and *Orconectes virilis*. It is important to note that these two species are native, i.e. not actually extraregional invaders in our study area, but have been shown capable of invading across major drainage barriers in other parts of their range (Larson and Olden 2010). This was chosen as a community response metric due to the variety of life history parameters that comprised the designation. Species richness was not used as a response variable for crayfish due to the generally low and relatively uniform richness across sites. For both fish and crayfish, raw abundances were used for all metrics rather than estimates based on three-pass removal as calculated in R-package Unmarked (Fiske and Chandler 2011) due to the poor fit of the removal models.

For benthic macroinvertebrates, our five response variables were: total number of individuals per sample (density was not used due to the qualitative nature of the QMH method); taxa richness; Simpson's diversity; percentage of individuals in the total sample belonging to Orders Ephemeroptera, Plecoptera, and Tricoptera (EPT), considered to be taxa associated with high water quality (Karr 1991); and percentage of total individuals belonging to the Family Chironomidae, generally considered a more tolerant taxon that is predicted to increase in abundance with increasing stream perturbation (Barbour et al. 1999). Response variables calculated from RTH and QMH samples were analyzed separately.

We examined relationships between response variables and flow alteration variables using redundancy analysis (RDA). Linear model RDA's were appropriate because preliminary Detrended Correspondence Analyses (DCA) indicated that species gradient lengths were less than 1 standard deviation (ter Braak 1995). We used forward selection in CANOCO 4.5 to select flow alteration variables that were related to response variables. We limited the flow alteration variables to those with $\lambda \geq 0.7$ after entry into the model.

We centered and standardized response variables before running the RDA because response variables were measured in different units. Scaling of ordination scores was focused on inter-response variable correlations rather than inter-sample distances and the response variable scores were standardized to prevent response variables with large variances from unduly influencing ordination diagrams because we were interested in relationships among response variables, (ter Braak and Smilauer 1998). Monte Carlo permutations testing the significance of canonical axes together were then performed for each RDA to determine the overall importance of remaining environmental variables in influencing response variables. Analyses of response

variable-flow alteration relationships were performed separately for each taxon and sampling type. All significant hydrologic alteration metrics are listed and defined in Table 2.

Results

Fish

In 2012, fish response variables were significantly related to alteration of MA22, MA36, FH1, FH2, FH8, and DH7 (RDA $p < 0.001$, Table 2, Figure 2). MA22, MA36, FH1, and FH2 were significantly reduced relative to expected values (Figure 2). Diversity and richness were positively related to alteration of MA22, FH2, and MA36 and negatively related to alteration of DH7 (Figure 2). Percent intolerant fish was negatively related to alteration of FH1 and FH8 (Figure 2).

In 2013, fish response variables were significantly related to alteration of MH13, MH18, FH11, DH7, and RA3 (RDA $p < 0.001$, Table 2, Figure 2). MH18 was significantly increased and RA3 significantly reduced relative to expected values (Figure 2). Percent intolerant fish was positively related to alteration of MH18, total density negatively related to alteration of RA3, and richness and diversity negatively related to alteration of MH18 and DH7 (Figure 2).

In fish assemblages, magnitude and frequency were the most important categories of flow alteration metrics; eight of the 11 important alteration metrics between years were in these two categories (Table 2, Figure 2). Of the remaining three important metrics, two belonged to the duration category and one to the rate of change category. Eight of the 11 metrics were high flow metrics and the remaining three were in the average flow category. No metrics belonging to the low flow category were important. No metrics were important in fish assemblages in both years.

MA22 and RA3 were also important metrics in RTH macroinvertebrate assemblages and FH11 in QMH macroinvertebrate assemblages (Table 2).

Crayfish

In 2012, crayfish response variables were significantly related to alteration of MA3, MA32, MA33, DL18 and RA2 (RDA $p < 0.001$, Table 2, Figure 3). DL18, MA32, and MA33 were significantly reduced and RA2 significantly increased relative to expected values (Figure 3). Diversity was negatively related to alteration of DL18, while total density and percent extraregional crayfish were positively related to alteration of RA2 (Figure 3).

In 2013, crayfish response variables were significantly related to alteration of MA3, MA21, DH1, and TH1 (RDA $p < 0.001$, Table 2, Figure 3). DH1 was significantly reduced and TH1 significantly increased relative to expected values (Figure 3). Total Density was positively related to alteration of DH1 while diversity was negatively related to alteration of MA21 (Figure 3).

In crayfish assemblages, magnitude was the most important category of alteration metric; five of the nine important alteration metrics between years belonged to this category (Table 2, Figure 3). Of the remaining four important metrics, two belonged to the duration category, one to the timing category, and one to the rate of change category. Six of the nine metrics were average flow metrics, with two high flows, and one low flow. One metric, MA3, was an important metric in crayfish assemblages in both years. No specific metrics important to crayfish assemblages were important in other taxonomic groups (Table 2).

RTH Macroinvertebrates

In 2012, RTH macroinvertebrate response variables were significantly related to alteration of DH18, TA3, and RA3 (RDA $p < 0.001$, Table 2, Figure 4). RA3 was significantly reduced relative to expected values (Figure 4). Diversity, richness, and percent EPT were all negatively related to alteration of TA3, while total number and percent Chironomidae were positively related to alteration of RA3 (Figure 4).

In 2013, RTH macroinvertebrate assemblages were significantly related to alteration of MA22, FH3, DH23, and TH2 (RDA $p < 0.001$, Table 2, Figure 4). MA22 and FH3 were significantly reduced relative to expected values (Figure 4). Diversity was positively related to alteration of MA22, while percent Chironomidae was negatively related to alteration of MA22 (Figure 4). Percent EPT was positively related to alteration of TH2 (Figure 4).

In RTH macroinvertebrate assemblages, no category of alteration metric stood out as most important. Of the seven important flow alteration metrics, two were duration, two were timing, one was magnitude, one was frequency, and one was rate of change (Table 2, Figure 3). Four of seven metrics were high flow metrics, and the remaining three were average flow metrics. No metrics belonging to the low flow category were important. No metrics were important in RTH macroinvertebrate assemblages in both years. One metric, DH23, was also an important metric in QMH macroinvertebrate assemblages, and two others, MA22 and RA3, were also important metrics in fish assemblages (Table 2).

QMH Macroinvertebrates

In 2012, QMH macroinvertebrate response variables were significantly related to alteration of MA12, MH3, MH20, ML12, and FH11 (RDA $p < 0.001$, Table 2, Figure 5). MA12

and MH3 were significantly reduced and ML12 significantly increased relative to expected values (Figure 5). Richness, diversity, and percent EPT were all negatively related to alteration of FH11, while percent Chironomidae was positively related to alteration of MH3 (Figure 5).

In 2013, QMH macroinvertebrate response variables were significantly related to alteration of MA29, MH17, FH4, FH5, and DH23 (RDA $p < 0.001$, Table 2, Figure 5), and MA29, MH17, and FH4 were significantly reduced relative to expected values (Figure 5). Percent EPT was negatively related to alteration of FH5 and DH23, while alteration of both of these metrics were positively related to percent Chironomidae (Figure 5).

In QMH macroinvertebrate assemblages, magnitude was the most important category of alteration metric; six of the ten important alteration metrics belonged to this category (Table 2, Figure 5). Frequency was the second most important category, with three of the ten. One metric belonged to the duration category. Seven of the ten metrics were high flow metrics, with three average flow and one low flow. No metrics were important in QMH assemblages in both years. One metric, DH23, was also important in RTH macroinvertebrate assemblages, and another, FH11, in fish assemblages (Table 2).

Aquatic Community

Considering all four assemblages over both years, 32 different metrics of hydrologic alteration were significantly related to biological response variables (Table 2). In order of importance, the five categories were ranked: magnitude (14), frequency (7), duration (6), timing (3) and rate of change (2). In terms of average, low, and high flows, metrics relating to alteration of high flows were the most important (19), followed by average flows (11), with a much lower

number of important alteration metrics relating to low flows (2). All seven frequency metrics and all but one of the duration metrics were related to high flows, while the majority of important magnitude metrics were related to average flows. The four most important specific categories were MA (8), FH (7), MH (5), and DH (5). Four specific alteration metrics were important in multiple assemblages: MA22, FH11, DH23, and RA3 (Table 2).

Discussion

The overall importance of magnitude and frequency alteration metrics is of particular interest given that regional environmental flow studies have suggested that magnitude of flow is an important influence on aquatic communities (Monk et al. 2006, Armstrong et al. 2011, Kendy et al. 2012), while other studies (Dodds et al. 2004, Matthews et al. 2013, Matthews et al. 2014) have found that frequency of floods may be one of the most important determinants of community structure in streams. Anthropogenic alteration of streamflow magnitudes is a widespread phenomenon; in an assessment of 2,888 streamflow monitoring sites throughout the conterminous U.S., Carlisle et al. (2010) found that streamflow magnitude was altered at 86% of assessed streams, and that diminished magnitudes were better predictors of biological integrity in both fish and macroinvertebrate communities than other physical and chemical covariates. Reduction in high flow frequencies has also been linked to a decrease in the ecological integrity of river systems (Ward and Stanford 1995). The general trend in our study area in both magnitude and frequency metrics was towards reduction relative to expected values.

Compared to alteration of high and average flows, alteration of low flows appears to be a considerably less important influence on stream biota in the region; only two of 32 important metrics across years and assemblages were low flow related. Although both floods and droughts

act as major hydrologic disturbances in stream ecosystems and can exert significant influence on biota (Lake 2000), the alteration of low-flow hydrology has been relatively less studied than that of high flows (Rolls *et al.* 2012). In this study, we focused on Groundwater Flashy streams, one of the most common flow regimes in the Ozark Highlands (Leasure *et al.* 2014), but it is possible that in other flow regimes, alteration of low flows may be more important. Different natural flow regimes within the same region may be more or less susceptible to particular forms of flow alteration (Leasure *et al.* 2014), which is the reason that flow regime classification is a crucial step in the assessment of hydrologic alteration (Poff *et al.* 2010). The seven distinct flow regimes in the Ozark Highlands can be divided into three broad categories – groundwater, runoff, and intermittent streams (Leasure *et al.* 2014). Runoff and intermittent flow regimes are categorized by more frequent low flow spells and lower base flows than groundwater streams; it may be that low flow metrics play a greater role in the life history of biota in these streams and therefore alteration of those metrics would have greater impact. Poff (1992) suggested that perennial runoff and intermittent streams may be more strongly affected by alteration of low flows and groundwater streams more affected by alteration of high flows; the latter at least appears to be reflected in the present study.

In fish assemblages, the association between richness and diversity and alteration of variability in both average flow magnitude (MA22 and MA36) and high flow frequency, (FH2) is supported by studies relating hydrologic variation to North American stream fishes (Ward 1998, Niu *et al.* 2012, but see McGarvey 2014). The trend towards reduction of these metrics in our study area could be associated with an overall decline in richness and diversity of stream fishes in the region. While previous studies have suggested that aquatic biodiversity is often lower in modified or disturbed streams than in those with relatively intact natural flow regimes

(Ward and Stanford 1995, Gehrke et al. 1999), it has been an ongoing challenge for stream ecologists to unravel the direct effects of flow alteration from multiple associated stressors that often accompany development in watersheds, e.g. land-use factors or declining water quality (Bunn and Arthington 2002). Our study provides evidence that alteration of specific flow metrics can influence richness and diversity in stream biota.

Magnitude alteration was also the most important category in crayfish assemblages. Fish and crayfish assemblages strongly differ in one important way, however – the lack of any important frequency alteration metrics in crayfish assemblages. The ability of crayfish species in the region to more fully utilize the hyporheic zone during dry periods (DiStefano et al. 2009, Larson et al., 2009) may make them less dependent on frequent high flow events than fish assemblages; this may lessen the impact that alteration of flood frequency has on them. MA3, variability in daily flows, was a consistently important metric in crayfish assemblages in the region, as it was selected in both 2012 and 2013 despite a lack of overlap between sites. The relationship between alteration of flow variability and density was similar to that observed in fish assemblages in this study, *i.e.* the relationship between fish density and MA36 in 2012, and is also supported by previous studies of flow variability and fish density (Craven et al. 2010).

The relationship between percent extraregional crayfish species and MA3 is interesting in that it appears quite different between sampling years, being more positively associated in 2012 and negatively in 2013. This could be evidence of temporal variation in environmental flow-biological response relationships (Katz and Freeman 2015), although it is difficult to draw such conclusions due to the lack of total overlap between sites for crayfish sampling during the two years. In general, it is thought that more altered hydrologic regimes may facilitate invasion and

success of invasive species (Moyle and Light 1996, Bunn and Arthington 2002). While the two crayfish species designated “extraregional invaders” in this study are actually native to the study area, both are highly successful invaders in other regions (Larson and Olden 2010), so it is logical that more altered flow regimes would facilitate their success within their own natural ranges as well.

RTH macroinvertebrate assemblages differed in key ways from the others in this study. This was the only group in which magnitude was not the most important category of flow alteration metric or that no category was more prominent than the others. Furthermore, the complete overlap of sites for invertebrate collections makes it possible to draw temporal comparisons in flow alteration-ecological response relationships between the two years for macroinvertebrate assemblages. It is somewhat surprising then to see no consistently important metrics between the two years, and in some cases quite different relationships between variables, e.g. the association between percent EPT and diversity and richness in 2012 versus the association between percent EPT and total number in 2013. It is important to note the drastically different sampling conditions during the two summers – severe drought in 2012 versus flooding in 2013. Temporal variation can complicate our ability to formulate predictable flow-ecology relationships (Poff et al. 2010, Rolls et al. 2012, Katz and Freeman 2015). RTH samples were collected only from riffles, and riffles were the habitat most heavily affected by drought (Dekar and Magoulick 2007, Chester and Robison 2011). In the case of the relationship between percent EPT and these other response variables, it may be that during a wet summer, EPT taxa make up a larger portion of the total number of invertebrates occupying riffles than during an extreme drought where conditions may be less suitable for them (Karr 1991, Barbour et al. 1999). Conversely, percent Chironomidae was more closely associated with total number during

2012, perhaps because of their higher tolerance for ecological perturbations such as drought compared to other invertebrate taxa (Karr 1991, Barbour et al. 1999).

Predictability of flooding (TA3) appears critically important to RTH assemblages, at least during drought years; alteration of this metric was negatively related to all response metrics in 2012. Fritz and Dodds (2005) found that streams with low flow predictability had consistently lower macroinvertebrate taxa richness than those with greater predictability. Alteration of the variability in high flow timing (TH2) was also related to both percent EPT taxa and total number in 2013. Predictable timing of floods may be very important in aquatic macroinvertebrates that rely on life-history adaptations to avoid disturbances rather than escaping on a per-event basis, particularly taxa that require gill respiration as juveniles but have an aerial adult stage, e.g. EPT taxa (Lytle 2008). When floods are timed earlier or later than they typically occur, it can dramatically effect these organisms (Lytle 2003).

Unlike RTH assemblages, QMH assemblages showed a pattern consistent with fish and crayfish assemblages with respect to the prominence of magnitude alteration metrics. Interestingly, QMH assemblages show more of an affinity with fish than crayfish assemblages in terms of the importance of the high flow frequency (FH) category. As with RTH assemblages, the total overlap of sites makes it possible to draw temporal comparisons in relationships. Relationships between response variables in the two years are more consistent in QMH than RTH samples. It is possible that the inclusion of pool and run habitats, which act as refuges for macroinvertebrates during summer drying (Chester and Robson 2011), may have somewhat ameliorated the effects of drought in 2012 in QMH compared to RTH samples. Temporal variation in relationships was also apparent in QMH assemblages, however, as no individual

metrics were significant in both years. General trends among QMH macroinvertebrate assemblages in the region include reduction of important metrics relating to magnitude and variability in average and high flows, as well as frequency and duration of high flows. These may have a variety of effects on QMH macroinvertebrate assemblages in the region; in a few cases, some trends may actually offset one another. For example, decreasing magnitude of average flows (MA12) may lead to a decrease in richness which could be somewhat ameliorated by the trend toward decreasing flood frequency (FH11).

Conclusions

Flow alteration is an important influence on community structure in Ozark Highland streams. The most important categories of alteration influencing stream biota in the region were MA, FH, MH, and DH. The fact that three of these categories were high flow-related suggests the overall importance of floods as a determinant of community structure and composition in Groundwater Flashy Ozark Highland streams, which has been supported by previous studies (Matthews et al. 2013, Matthews et al. 2014). Of the 32 important metrics across years and assemblages, 19 were significantly altered relative to expected values. The general trend in the region was towards reduction of flow metrics; 15 of the 19 significantly altered metrics were reduced relative to expected values. General patterns were apparent across assemblages that may be useful to managers and stakeholders attempting to conserve and manage freshwater ecosystems in the region, but key differences between taxonomic groups and temporal variation in relationships suggest that a complex suite of flow metrics should be considered for effective conservation of stream communities related to flow alteration. Furthermore, while hydrology plays a major role in structuring aquatic assemblages, it is heavily interrelated to many other

factors, including geomorphology, land-use, and water quality; the ecological effects of hydrologic alteration are best examined within the context of this suite of factors (McManamay and Frimpong 2015). Finally, while we examined flow alteration-ecology relationships in a predominant flow regime (Groundwater Flashy streams) in the Ozark Highlands, these relationships may strongly differ in other flow regimes (Poff 1992, Poff *et al.* 2010, Leasure *et al.* 2014). Future studies of flow alteration-ecology relationships focused on other flow regimes would help to form a more complete picture of the impact of hydrologic alteration on stream communities.

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#11018 (Appendix 1). Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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Table 1. Mean (\pm SE) values for biological response variables.

Variable	2012	2013
Fish Species Richness	16 (\pm 0.94)	15 (\pm 1.15)
Fish Simpson's Diversity	0.73 (\pm 0.03)	0.73 (\pm 0.04)
Fish % Intolerant	70.35 (\pm 4.21)	71.56 (\pm 4.03)
Fish % Centrarchidae	2.68 (\pm 0.77)	5.08 (\pm 1.67)
Fish Total Density	11.66 (\pm 0.77)	11.73 (\pm 1.51)
Crayfish Simpson's Diversity	0.20 (\pm 0.05)	0.32 (\pm 0.06)
Crayfish % Extraregional	80.10 (\pm 8.34)	40.46 (\pm 11.07)
Crayfish Total Density	3.54 (\pm 1.05)	8.00 (\pm 2.66)
RTH Macroinvertebrate Taxa Richness	22 (\pm 1.48)	19 (\pm 1.09)
RTH Macroinvertebrate Simpson's Diversity	0.81 (\pm 0.03)	0.77 (\pm 0.02)
RTH Macroinvertebrate % EPT	49.65 (\pm 4.93)	59.93 (\pm 4.65)
RTH Macroinvertebrate % Chironomidae	9.31 (\pm 3.48)	11.54 (\pm 2.50)
RTH Macroinvertebrate Total Number	2568 (\pm 757.14)	4064 (\pm 809.97)
QMH Macroinvertebrate Taxa Richness	25 (\pm 2.28)	27 (\pm 1.29)
QMH Macroinvertebrate Simpson's Diversity	0.73 (\pm 0.05)	0.85 (\pm 0.02)
QMH Macroinvertebrate % EPT	22.67 (\pm 0.04)	33.94 (\pm 0.04)
QMH Macroinvertebrate % Chironomidae	6.33 (\pm 0.03)	17.46 (\pm 2.94)
QMH Macroinvertebrate Total Number	2710 (\pm 799.82)	3292 (\pm 398.98)

Table 2. Important hydrologic alteration metrics (Olden and Poff, 2003) used in RDA analysis for 2012 and 2013 with mean (\pm SE) values.

Code	Definition	Category	Mean (\pm SE)
2012 Fish			
MA22	Mean November flows	Magnitude of Average Flows	-0.29 (\pm 0.05)
MA36	Variability across monthly flows	Magnitude of Average Flows	-0.82 (\pm 0.14)
FH1	High flood pulse count (pulse defined as 75th percentile)	Frequency of High Flows	-0.74 (\pm 0.31)
FH2	Variability in high flood pulse count	Frequency of High Flows	-0.75 (\pm 0.30)
FH8	Flood frequency (25th percentile upper threshold)	Frequency of High Flows	-0.73 (\pm 0.32)
DH7	Variability in annual maxima of 3 day mean daily discharge	Duration of High Flows	-0.04 (\pm 0.12)
2013 Fish			
MH13	Variability across maximum monthly flows	Magnitude of High Flows	0.26 (\pm 0.38)
MH18	Variability across annual maximum flows	Magnitude of High Flows	1.60 (\pm 1.00)
FH11	Flood frequency (mean number of discrete flood events per year)	Frequency of High Flows	-0.18 (\pm 0.41)
DH17	High flow duration (upper threshold 1 times median flows)	Duration of High Flows	-0.54 (\pm 0.69)
RA3	Fall rate	Rate of Change of Average Flows	-0.11 (\pm 0.09)
2012 Crayfish			
MA3	Variability in daily flows	Magnitude of Average Flows	-0.42 (\pm 0.26)
MA32	Variability in September flows	Magnitude of Average Flows	-0.51 (\pm 0.2)
MA33	Variability in October flows	Magnitude of Average Flows	-0.41 (\pm 0.19)
DL18	Number of zero-flow days	Duration of Low Flows	-0.18 (\pm 0.13)
RA2	Variability in rise rate	Rate of Change of Average Flows	2.31 (\pm 0.38)
2013 Crayfish			
MA3	Variability in daily flows	Magnitude of Average Flows	-0.85 (\pm 0.32)
MA21	Mean October flows	Magnitude of Average Flows	-0.01 (\pm 0.06)
DH1	Annual maxima of daily mean discharge	Duration of High Flows	-0.24 (\pm 0.08)
TH1	Julian date of annual maximum	Timing of High Flows	0.55 (\pm 0.22)

Table 2 (cont.). Important hydrologic alteration metrics (Olden and Poff, 2003) used in RDA analysis for 2012 and 2013 with mean (\pm SE) values.

Code	Definition	Category	Mean (\pm SE)
2012 RTH Macroinvertebrates			
DH18	High flow duration (upper threshold 3 times median flows)	Duration of High Flows	-0.41 (\pm 0.22)
TA3	Seasonal predictability of flooding	Timing of Average Flows	1.20 (\pm 0.59)
RA3	Fall rate	Rate of Change of Average Flows	-0.13 (\pm 0.05)
2013 RTH Macroinvertebrates			
MA22	Mean November flows	Magnitude of Average Flows	-0.27 (\pm 0.05)
FH3	High flood pulse count (upper threshold 3 times median daily flow)	Frequency of High Flows	-0.95 (\pm 0.24)
DH23	Flood duration (mean annual number of days that flow remains above threshold averaged over all years)	Duration of High Flows	-0.27 (\pm 0.16)
TH2	Variability in Julian date of annual maximum	Timing of High Flows	-1.06 (\pm 0.57)
2012 QMH Macroinvertebrates			
MA12	Mean January flows	Magnitude of Average Flows	-0.16 (\pm 0.06)
ML12	Mean minimum December flows	Magnitude of Low Flows	0.13 (\pm 0.04)
MH3	Mean maximum March flows	Magnitude of High Flows	-0.14 (\pm 0.06)
MH20	Specific mean annual maximum flows (maximum flows divided by catchmen area)	Magnitude of High Flows	0.01 (\pm 0.27)
FH11	Flood frequency (mean number of discrete flood events per year)	Frequency of High Flows	-0.56 (\pm 0.32)
2013 QMH Macroinvertebrates			
MA29	Variability in June flows	Magnitude of Average Flows	-0.74 (\pm 0.21)
MH17	High flow discharge	Magnitude of High Flows	-0.58 (\pm 0.15)
FH4	High flood pulse count (upper threshold 7 times median daily flow)	Frequency of High Flows	-0.72 (\pm 0.20)
FH5	Flood frequency (upper threshold times median flow over all years)	Frequency of High Flows	1.08 (\pm 0.68)

Table 2 (cont.). Important hydrologic alteration metrics (Olden and Poff, 2003) used in RDA analysis for 2012 and 2013 with mean (\pm SE) values.

Code	Definition	Category	Mean (\pm SE)
DH23	Flood duration (mean annual number of days that flow remains above threshold averaged over all years)	Duration of High Flows	-0.27 (\pm 0.16)

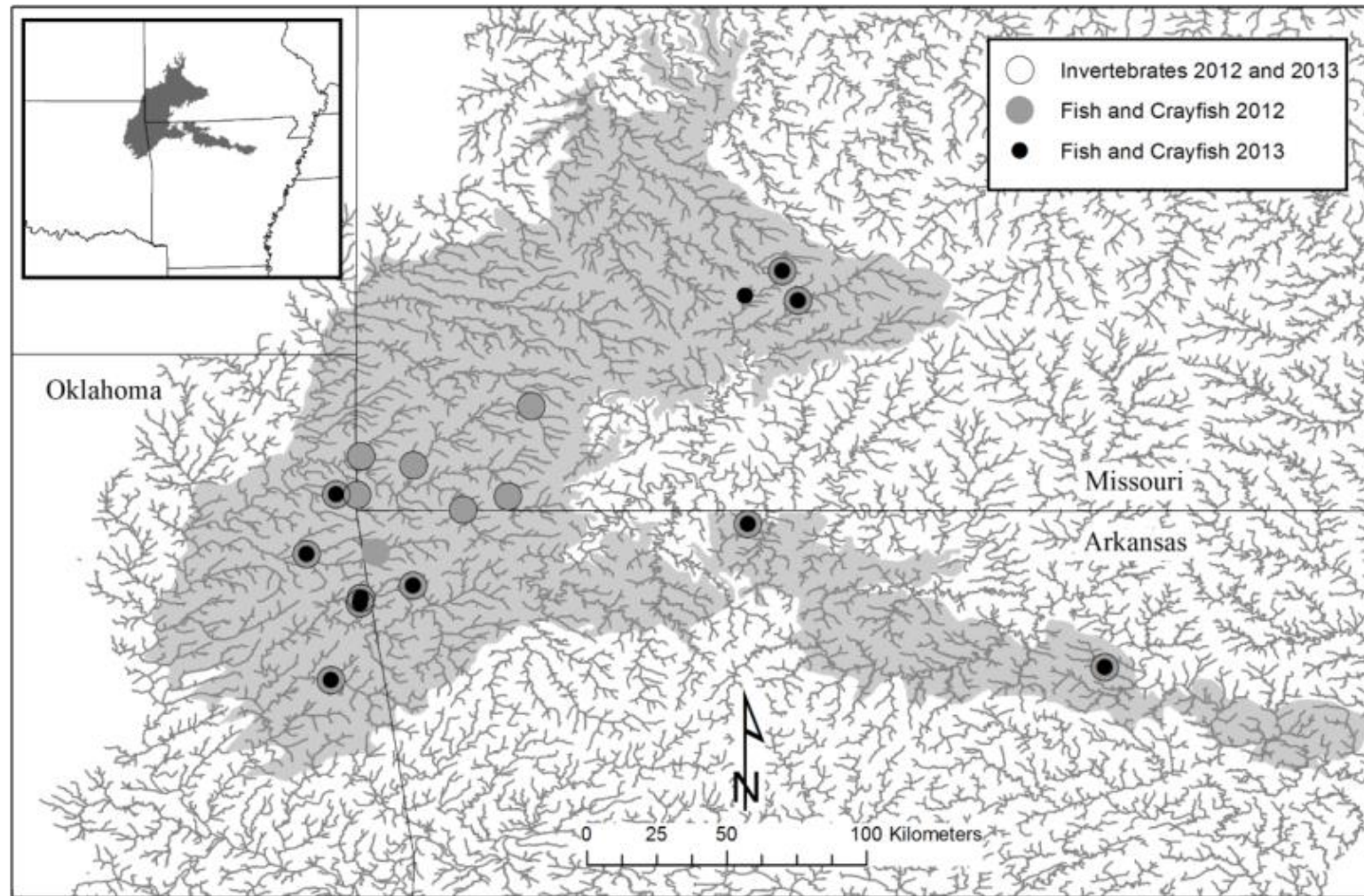


Figure 1. Map of study area showing sample sites, stream network, and Springfield Plateau.

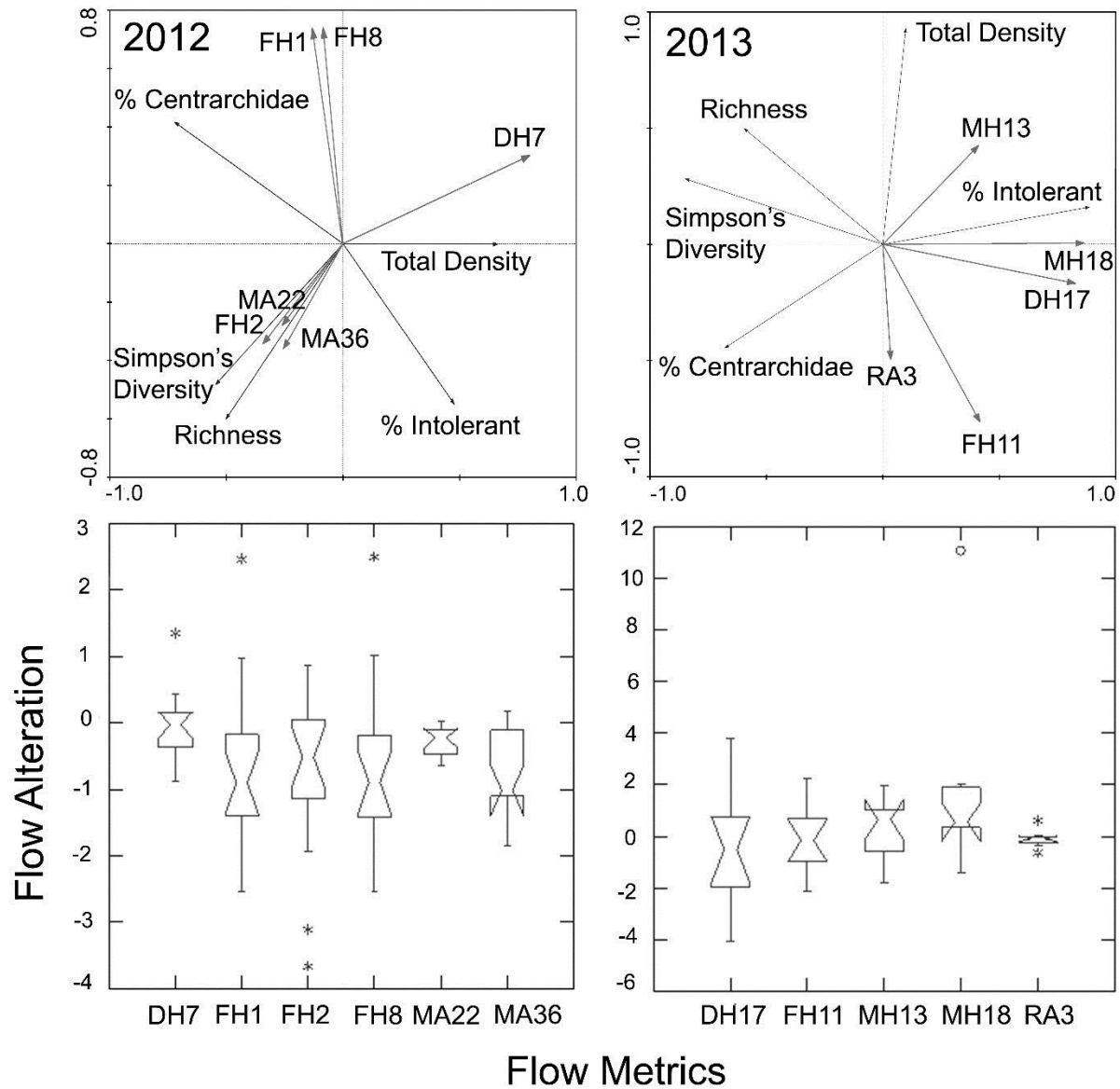


Figure 2. Redundancy analysis ordination plot relating fish assemblages and selected flow alteration variables in 2012 and 2013. Boxplots show flow alteration variables used. Angles of arrows indicate associations and length of arrows indicate strength of the relationship. Environmental variable abbreviations and descriptions are given in Table 2.

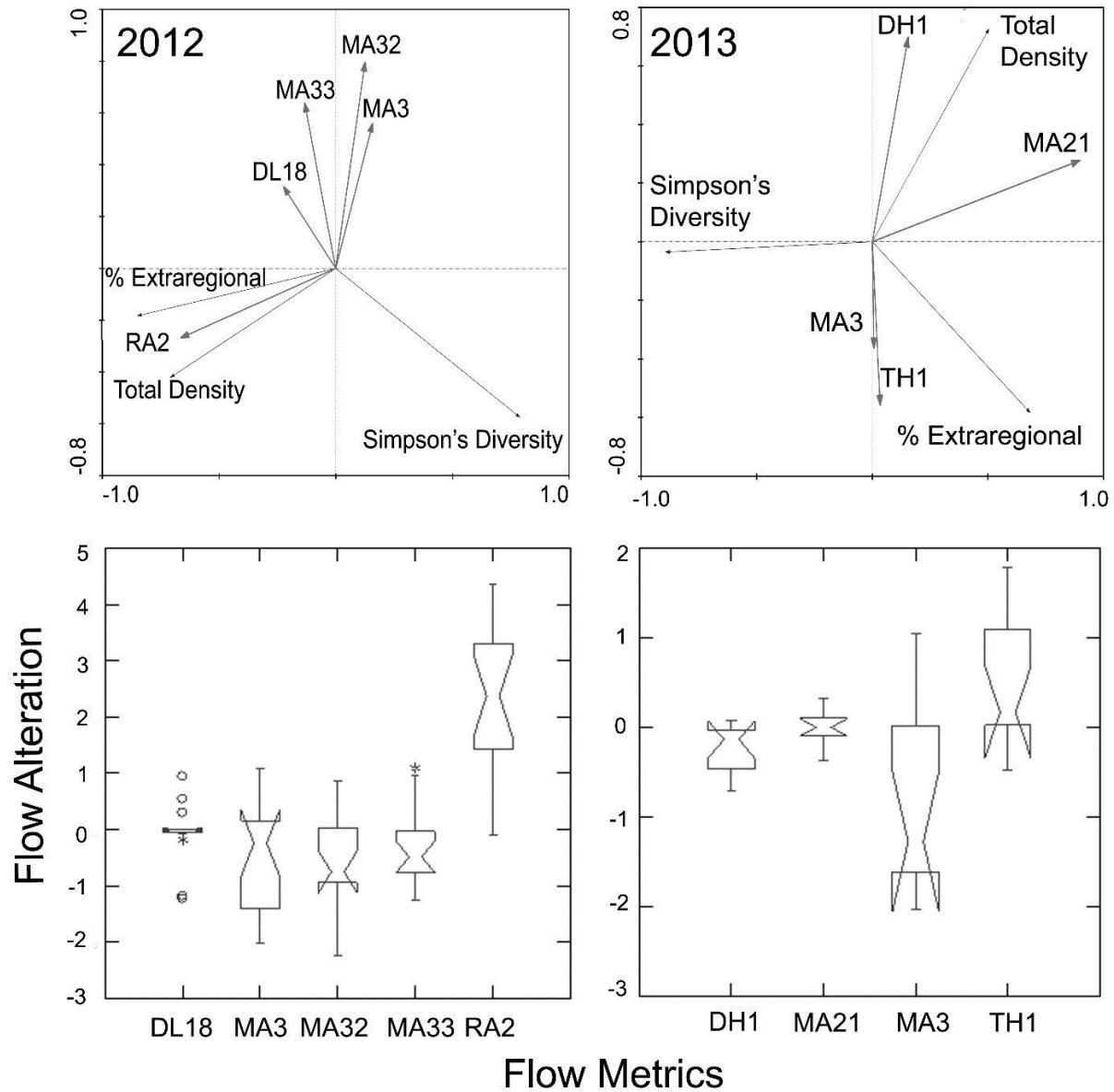


Figure 3. Redundancy analysis ordination plot relating crayfish assemblages and selected flow alteration variables in 2012 and 2013. Boxplots show flow alteration variables used. Angles of arrows indicate associations and length of arrows indicate strength of the relationship. Environmental variable abbreviations and descriptions are given in Table 2.

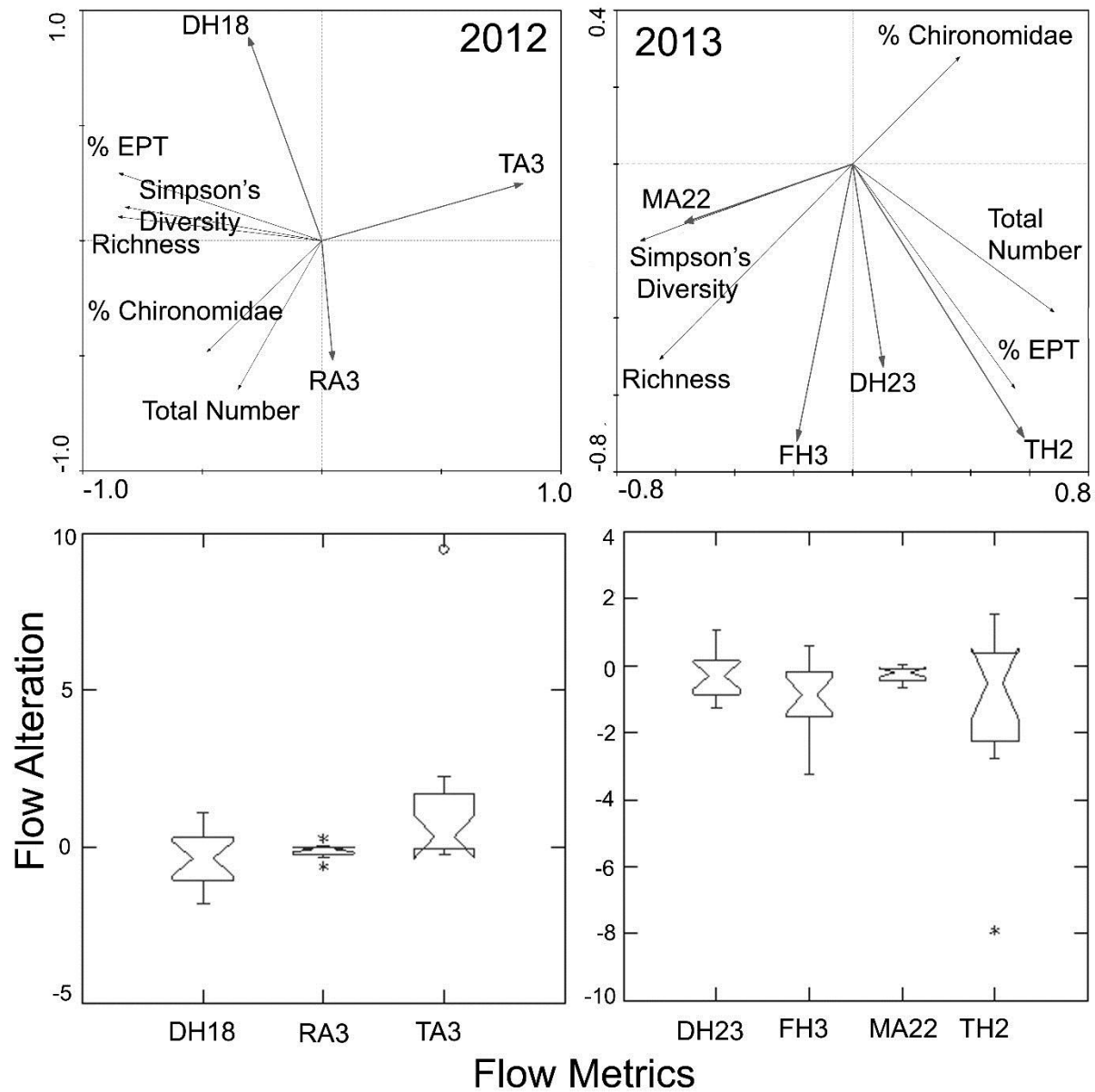


Figure 4. Redundancy analysis ordination plot relating RTH (Richest Targeted Habitat) macroinvertebrate assemblages and selected flow alteration variables in 2012 and 2013. Boxplots show flow alteration variables used. Angles of arrows indicate associations and length of arrows indicate strength of the relationship. Environmental variable abbreviations and descriptions are given in Table 2.

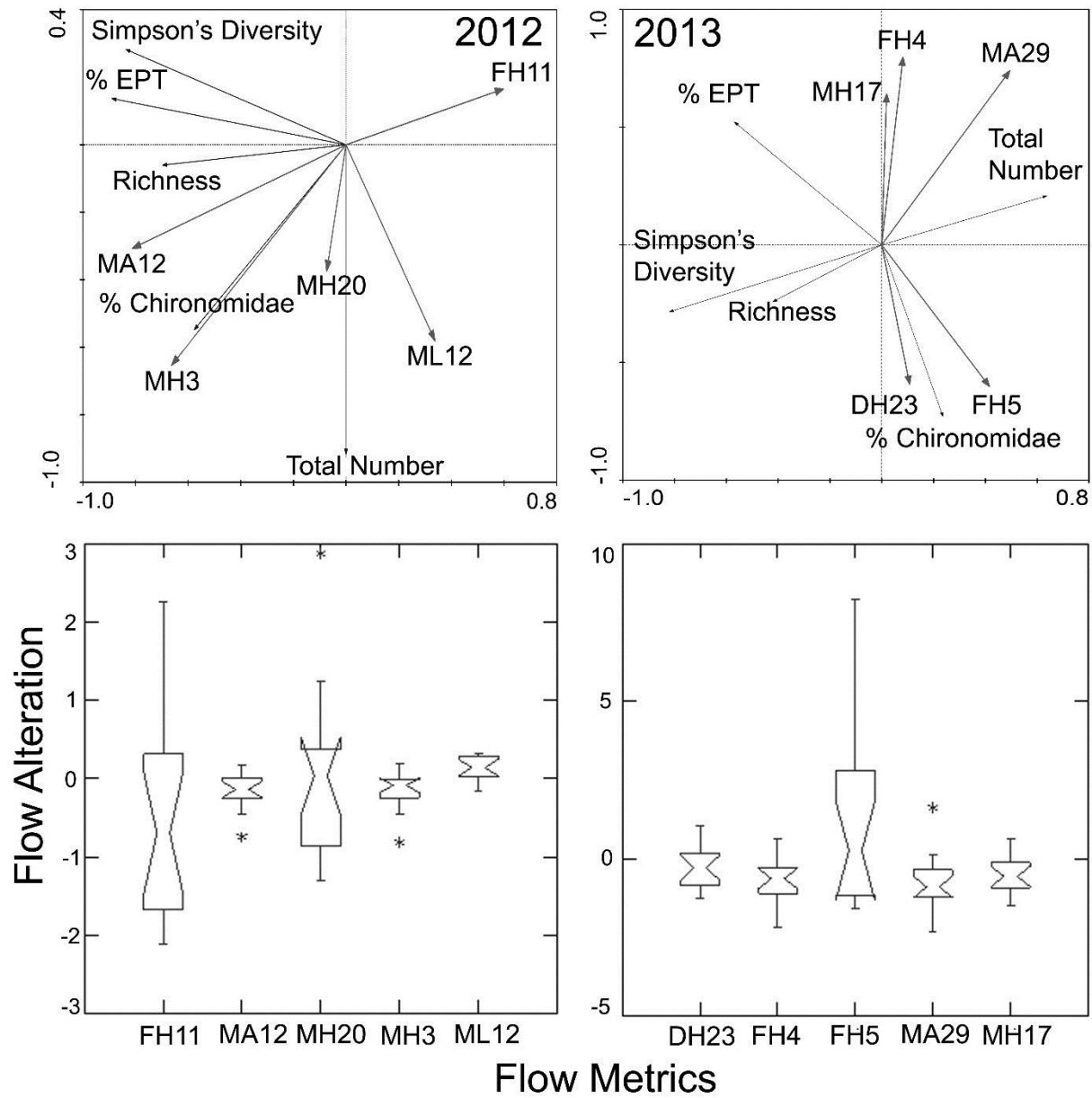


Figure 5. Redundancy analysis ordination plot relating QMH (Qualitative Multi-Habitat) macroinvertebrate assemblages and selected flow alteration variables in 2012 and 2013. Boxplots show flow alteration variables used. Angles of arrows indicate associations and length of arrows indicate strength of the relationship. Environmental variable abbreviations and descriptions are given in Table 2.



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MEMORANDUM

TO: Dan Magoulick
FROM: Craig N. Coon, Chairman
Institutional Animal Care
And Use Committee
DATE: February 4, 2011
SUBJECT: **IACUC PROTOCOL APPROVAL**
Expiration date : **January 31, 2013**

The Institutional Animal Care and Use Committee (IACUC) has **APPROVED** Protocol #11018-
“**CLASSIFICATION OF ARKANSAS FLOW REGIMES, REGIONAL ECOLOGICAL-FLOW
RESPONSE RELATIONSHIPS AND ENVIRONMENTAL FLOWS ASSESSMENT FOR THE
OZARK REGION**”. You may begin this study immediately.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any changes in the protocol during the research, please notify the IACUC in writing **prior** to initiating the changes. If the study period is expected to extend beyond **01-31-2013**, you may request an extension (up to 02-04-2014, which is 3 years from original approval date) via a Modification Request form. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

cnc/car

cc: Animal Welfare Veterinarian

The University of Arkansas is an equal opportunity/affirmative action institution.

Effects of Pulse and Press Drying Disturbance on Benthic Stream Communities

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Abstract

Natural disturbance is an integral component of most ecosystems that occurs in three different forms, pulse, press, and ramp. In lotic ecosystems, seasonal drought acts as a major form of disturbance, particularly in intermittent headwater streams, which are often reduced to pools that serve as refuges for biota. We used simulated intermittent stream pools to compare the effects of control, pulse, and press drying on growth and survival in three fish species: *Lepomis megalotis*, *Camptostoma anomalum*, and *Etheostoma spectabile*, commonly found together in drought-prone streams in the Ozark Highlands, USA. We also compared effects on benthic community structure, including periphyton and chironomid density and sediment in deep (permanently watered) and shallow (intermittently dewatered) habitat. Only one species, *L. megalotis*, showed a significant reduction in length and mass growth in press drying compared to control. There was no effect of drying or type of drying on survival of any fish species. Drying and type of drying had strong overall effects on periphyton growth in shallow habitats, driven by reduction in ash-free dry-mass (AFDM) and increase in autotrophic index (AI) in drying versus control and press versus pulse treatments. Drying also negatively affected sediment accumulation in shallow habitat and chironomid density in deep habitat. Drying in intermittent streams has species-dependent effects on fish growth and benthic structure, and pulse and press drying differ in their effects on periphyton in these systems. These effects may have important consequences in seasonally-drying streams as anthropogenic influence on stream drying increases.

Introduction

Natural disturbance is an integral component of most ecosystems that operates at many spatial and temporal scales and at multiple levels of ecological and evolutionary organization (Sousa 1984, Pickett and White 1985, Lytle and Poff 2004). Disturbance is one of the single most important influences on spatial and temporal heterogeneity, species diversity, population size, and community composition (Sousa 1984, Ward 1998, Lytle and Poff 2004).

Anthropogenic alteration of natural disturbance regimes can have major consequences on both biodiversity and ecosystem function (Benke 1990, Ward 1998, Bunn and Arthington 2002), both in cases where the magnitude, extent, and frequency of natural disturbance are increased or when they are diminished (Carlisle et al. 2010).

Ecological perturbation consists of two sequential events: a disturbance, or some change in environmental conditions, followed by the response of the affected biota (Rykiel 1985, Lake 2000, Lake 2003). It is now generally accepted that there are three classes of ecological perturbations - pulse, press, and ramp (Bender et al. 1984, Glasby and Underwood 1996, Lake 2003). In a pulse perturbation, the disturbance occurs and then conditions return to former levels; pulses are short-term and typically intense events (Bender et al. 1984). In a press, the disturbance continues to occur at some steady rate that is maintained (Bender et al. 1984). The more recently described ramp has been described as a perturbation in which disturbance increases in strength and sometimes spatial extent over time (Lake 2000).

In rivers and streams, the natural disturbance regime is represented by cycles of flooding and drought (Lytle and Poff 2004) that are major factors in the structuring of aquatic communities (Resh et al. 1988, Poff and Allan 1995). While both floods and droughts are

common in lotic ecosystems, the role of floods has historically received more attention than that of droughts (Lake 2000, Lake 2003, Magoulick and Kobza 2003) despite the fact that drought can have important effects on stream ecosystem structure and function (Magoulick and Kobza 2003, Bond 2008, Beche et al. 2009, Lake 2011). While seasonal drought is a part of the natural disturbance regime in many lotic ecosystems, there is increasing evidence that human activities have the potential to amplify and exacerbate its effects (Bond et al. 2008). In order to better mitigate the potential impacts of water abstraction, climate change, and other anthropogenic stressors, we must first understand the effects of drought on organisms and ecosystems (Payne et al. 2004).

Refuges are crucial in allowing populations and communities of organisms to persist in disturbed environments such as streams undergoing seasonal drought. Not only can refuges function to reduce population loss in the face of disturbance, conferring increased resistance on populations, but they can also serve as sources of recolonization after disturbance, conferring increased resilience as well (Poff and Ward 1990, Townsend and Hildrew 1994, Magoulick and Kobza 2003). In regions such as the Ozark Highlands and Boston Mountains of northern Arkansas, USA, seasonal drying typically reduces small streams to intermittent pools that often persist and provide refuge to biota throughout the summer (Dekar and Magoulick 2007), and during this period of isolation, aquatic organisms can be exposed to harsher abiotic and biotic factors than those experienced during the rest of the year (Magoulick and Kobza 2003, Dekar and Magoulick 2007). Anthropogenic alterations to the hydrologic regime, such as dewatering due to the construction of dams and water withdrawals, can also mimic drought conditions (Pringle et al. 2000), and responses of stream organisms in those situations should be similar to

those found during drought, with refuges serving in a similar capacity (Magoulick and Kobza 2003).

In relation to drying in stream ecosystems, a pulse represents the type of disturbance experienced during an intense but relatively brief drought event, a press represents the type of disturbance experienced during predictable and periodic seasonal droughts, and a ramp represents longer supra-seasonal drought events (Gasith and Resh 1999). Most systems are characterized by a mixture of these different disturbance types, which may interact to affect biota in different ways (Parkyn and Collier 2004). Responses of stream biota are thought to differ in terms of resistance and resilience to different types of perturbations, although this has been relatively understudied (Lake 2003, but see Parkyn and Collier 2004). Understanding responses of stream biota to drought could be critical, given predictions that global climate change could lead to increased intensity, duration, and extent of drought events (Xenopoulous et al. 2005). Specific types of drought disturbance, the crucial role of refuge habitats, and the varied responses of aquatic communities are all concepts that should inform management and conservation decisions that affect stream ecosystems.

Our objective was to examine effects of drought disturbance on stream communities in mesocosms representing pool refuges in intermittent Ozark Highland streams over the course of a three-month experiment intended to simulate the typical drying season in the region. We examined a pulse treatment consisting of two intense brief water withdrawals, a press treatment consisting of gradual water withdrawal, and a no drying control. We used mesocosms designed to replicate a range of depths, including deep continuously wetted habitats and shallow habitats that experienced complete drying during portions of the experiment. We examined growth (both

mass and length) and survival in three species of fish, longear sunfish (*Lepomis megalotis*), central stoneroller (*Camptostoma anomalum*), and orangethroat darter (*Etheostoma spectabile*), commonly found together in these ecosystems (Magoulick 2000, Ludlam and Magoulick 2009). Additionally, we examined several metrics potentially impacted by both the direct abiotic and indirect biotic effects of drought, including periphyton growth, accumulation of inorganic sediment, and chironomid density. These experiments trade some of the realism of field studies for the ability to precisely quantify not only a disturbance itself, but also the response of biota to that disturbance; this allows for greater insight into mechanisms that would be difficult to address in in-situ studies in which there are many more variables at play and where disturbance is beyond direct experimenter control (Pickett and White 1985, Gelwick and Matthews 1993).

We hypothesized that growth and survival of all species of fish would be lowest in press, intermediate in pulse, and highest in control treatments due to more chronic effects of drought as a stressor in the press treatment. Diminished water volume forces fish to persist at higher densities at which growth rates and survival are typically decreased, often due to increased competition for space and food (Holm et al. 1990, Anderson et al. 2002). We hypothesized that in shallow habitat exposed to complete drying for portions of the experiment in the drying treatments, periphyton and chironomid densities would be highest in press, intermediate in pulse, and lowest in control treatments due to the shortest period for growth and colonization being in the press treatment. We hypothesized that in deeper, continuously wetted habitat, periphyton and chironomid densities would be lowest in press, intermediate in pulse, and highest in control treatments due to the concentration effect of predators for the most prolonged period being in the press treatment. We hypothesized that inorganic sediment in shallow habitat would be highest in control, intermediate in pulse, and lowest in press treatments due to greater time for the

accumulation of sediments from the water column in the control treatment. We hypothesized that we would see a reversal of this pattern in deep habitats due to concentration of fish in the deep habitats for more prolonged periods in the press treatment where they would be expected to entrain more sediment into the water column while moving and foraging.

Methods

Experimental design

We evaluated the effects of different drought treatments on growth and survival of three species of fish, as well as on periphyton growth, accumulation of inorganic sediment (hereafter sediment), and chironomid density, in a three-month mesocosm experiment during the summer of 2012 (May 27 - Aug 8). There were three treatments: 1) a pulse treatment consisting of two rapid water withdrawals, meant to simulate intense short-term drought events with normal conditions before, between, and after the drying, 2) a press treatment consisting of steady, gradual water withdrawal, a long period of low conditions, and then gradual rewetting, meant to simulate seasonal stream drying, and 3) a no-drying control (Fig. 1). We chose fish species commonly associated with Ozark streams that undergo annual seasonal drought. We also used ceramic tiles to evaluate periphyton growth, accumulation of sediment, and chironomid colonization, and built the substrate in the tanks on a sloping gradient that allowed us to compare these effects in deep versus shallow habitats.

Mesocosms were housed in a climate-controlled greenhouse under natural light on a 3 x 10 grid. Each mesocosm consisted of a 416 L oval polyethylene tank (1.26 m long × 0.84 m wide × 0.49 m deep) with a mixed substratum of gravel (< 0.03 m diameter) and pebbles (0.03 -

0.06 m diameter). Substrate was placed along a sloping gradient ranging from 0.10 m to 0.32 m from the bottom of the tank, so that approximately one third of the tank habitat consisted of a level shallow end, one third a slope, and one third a level deep end. We did this for several reasons: 1) to simulate the variety of depths and slopes naturally found in pool refuges (Magoulick 2000, Ludlam and Magoulick 2009), 2) to add the component of diminishing pool surface area along with diminishing volume to more accurately reflect the effects of drought-related stress (Magoulick and Kobza 2003), and 3) to allow us to evaluate drought effects on periphyton and chironomids separately for deep (continuously underwater) versus shallow (exposed for portions of the experiment) habitats.

Water was circulated and filtered by canister aquarium filters (Fluval 205, Hagen, Quebec, Canada), which provided only slight flow, as is often found in natural pools in the region during summer drying (Magoulick 2000, Magoulick and Kobza 2003, Ludlam and Magoulick 2010). Although abiotic effects of drying were potentially lessened by filtering the water, we chose to do this in order to avoid catastrophic mortality in the relatively confined space of our mesocosms, as well as to better mimic natural conditions in this system. Even during seasonal stream drying, most of these streams have some degree of surface or subsurface flow-through and are not merely stagnant pools (Magoulick 2000). We constructed lids for the tanks from 0.6 cm mesh plastic hardware cloth lined with Velcro strips to prevent escapes while still allowing for natural lighting.

Two weeks before the beginning of the experiment, we filled the tanks with water to a level of 0.4 m above the bottom, initiated the pumps, and treated the water with nitrifying bacteria (Proline Freshwater Nitrifying Bacteria, Pentair Aquatic Eco-Systems, Sanford, North

Carolina) at a dose of 3 g/L. We placed four 11 × 11 cm unglazed ceramic tiles in each mesocosm for later measurement of periphyton and chironomid densities. We placed two tiles into the deep end of each mesocosm and two in the shallow end. Chironomids and periphyton were not seeded from local stream populations, but allowed to colonize naturally. It had been determined that Chironomidae in the tribe Tanytarsini readily colonized the mesocosms in previous experiments (Ludlam and Magoulick 2010). We allowed natural colonization rather than seeding with local macroinvertebrates in order to control the inherent complexity of the invertebrate community that would be present in a stream slurry; this allowed us to use Chironomid density alone as a simpler response variable that was comparable across all mesocosms. We performed all water withdrawals throughout the experiment by removing the pump outputs and pumping water out to the desired level before returning outputs to the tank. Water levels were monitored daily in each tank and during all withdrawals with a meter-stick pressed to the bottom of the tank in the shallow end of the mesocosm.

We collected three species of fish that are locally abundant in the region and commonly co-inhabit small, drought-prone headwater tributaries in the Ozark Highlands and Boston Mountains ecoregions of northwest Arkansas. Species collected were: central stoneroller, longear sunfish, and orangethroat darter. These three species represent an array of taxonomic groups (Families Cyprinidae, Centrarchidae, and Percidae, respectively), as well as a wide assortment of life history traits and habits (Robison and Buchanan 1988). Central stonerollers are largely herbivorous cyprinids with a specialized ridge on the lower jaw for scraping algae off rocks, and are often the most abundant species in small Ozark streams (Robison and Buchanan 1988). They act as a keystone species in these environments; their feeding habits and high densities in these systems can have an important impact on the distribution of algae in small

streams (Matthews et al. 1986). Adults are primarily associated with raceways and pools, but juveniles, such as those used in this experiment, often prefer shallow margins and backwaters (Robison and Buchanan 1988). Longear sunfish are primarily invertivorous centrarchids that are most frequently associated with pools in small upland streams but found in a variety of other habitats (Pflieger 1975), and are the predominant species of sunfish in highland streams in the Ozark Highlands and Boston Mountains. Orangethroat darters are benthic-dwelling, strict invertivores that prefer shallow riffles and pool margins with moderate current (Robison and Buchanan 1988). Individuals of all three species were collected from populations in three local Illinois River tributaries: Chamber Springs (36.164° N, -94.437° W), Mud Creek (36.119° N, -94.149° W), and Scull Creek (36.087° N, -94.168° W). In addition, we collected one species of crayfish common in headwater streams in the region, Meek's crayfish. This species is commonly found in small clear creeks having stable substrate of bedrock, cobble, pebble, and gravel in the Ozark Highlands and Boston Mountains (Pflieger 1996). Crayfish were collected from Little Mulberry Creek (35.768° N, -93.589° W), a tributary of the Mulberry River in the Boston Mountains ecoregion on the southern edge of the Ozark Highlands. Fish and crayfish were collected via a combination of kickseining and backpack electrofishing.

We stocked each mesocosm with 13 fish (5 stonerollers, 5 darters, and 3 sunfish) and 3 crayfish. These densities fall within the observed ranges for the species in the region (Dekar and Magoulick 2007, Ludlam and Magoulick 2009). At the time of stocking, we recorded length and mass of each individual (to the nearest 0.1 cm and 0.1 g, respectively) and obtained mean length and mass for each species for each tank (Table 1). Total length was used for fish and carapace length for crayfish. Initial mean length and mass for all species was kept as close as possible for all mesocosms. Because tanks were not seeded with macroinvertebrates and Chironomid

colonization alone was insufficient to sustain them, we fed fish and crayfish daily a 5 mL total combination of commercially available flake and pellet food in order to avoid catastrophic mortality.

The three different drying treatments were systematically interspersed throughout the 3×10 grid of mesocosms for a total of 10 replicates of each treatment. The experiment began on 27-May-2012 and ended on 8-Aug-2012, a period of 74 days. All mesocosms underwent an initial two-week acclimation period in which the full 0.4 m water levels were maintained before the different drying treatments began. In the no-drying control, water was maintained at this 0.4 m level for the duration of the experiment. Water in the control mesocosms was only added as needed to offset the effects of evaporation in order to maintain full levels (Fig. 1).

In the pulse treatment, after the initial 2-week acclimation period, tanks were subjected to a rapid withdrawal of 0.125 m per day for two days until the water was 0.15 m above the bottom. During this period, the shallow habitat was exposed. Water was maintained at this low level for one week and then rapidly returned to the full 0.4 m level at the same rate of 0.125 m per day for two days. Water was maintained at the full level for 27 days, then the process of rapid 2-day withdrawal, maintenance at low level for a week, and rapid 2-day refilling was repeated. Pulse mesocosms remained at the full level for the final 11 days of the experiment (Fig. 1).

In the press treatment, after the initial 2-week acclimation period, tanks underwent a gradual water withdrawal at a rate of 0.015 m per day for 17 days, until the water was 0.15 m above the bottom. As in the pulse treatment, the shallow habitat was exposed during this period. Water was maintained at this low level for three weeks and then gradually returned to the full 0.4

m level at the same rate of 0.015 m per day for 17 days. Press mesocosms remained at the full level for the final 6 days of the experiment (Fig. 1).

Laboratory methods

At the end of the experiment, we tallied surviving fish and crayfish to calculate survival (proportion of individuals remaining) and re-measured all surviving individuals in order to calculate mean mass growth and mean length growth for each mesocosm. Recovery of crayfish from mesocosms was low. Despite efforts to create escape-proof lids primarily to keep crayfish in, we found individuals outside the mesocosms in the greenhouse facility twice during the experiment, indicating that escapes were occurring, likely contributing to this low recovery rate. This makes it difficult to draw conclusions about possible drought effects on crayfish based on the outcome of this experiment, so crayfish were excluded from further analyses.

We collected each tile and scraped the periphyton from it into a pan using a brush and distilled water. We poured 10 ml of the periphyton slurry onto a pre-ashed (550° C for 2 h) 24 cm filter (Pall GF/F), which we stored at -20° C until Chl a analysis. We then searched the rest of the periphyton slurry for chironomids and calculated chironomid no. cm⁻² for each tile. We extracted Chl a by placing filters in tubes with 10 mL of 95% ethanol, incubated in a water bath of 78°C for 5 minutes (Sartory and Grobbelaar 1984) and storing them in the dark at 4 °C for 24 h. After extraction absorbance was measured on a Genesys 10 VIS spectrophotometer (Thermo Fischer Scientific inc., Waltham, MA) as described in APHA (2005). In addition to calculating Chl a in µg cm⁻², we also measured ash-free dry mass (AFDM) and sediment for each tile (both in mg cm²) and calculated autotrophic index (AI) as Chl a/AFDM. For each tank, we averaged

the two shallow tile replicates and two deep tile replicates to obtain mean deep and shallow habitat values for chironomid density, Chl a, sediment and AI.

Statistical analyses

We performed one-way analysis of variance (ANOVA) to test treatment effects on mean length growth (cm), mean mass growth (g), and survival in each fish species, and on sediment (mg cm^{-2}) and chironomid density (no. cm^{-2}) separately for deep and shallow tiles. We used multivariate analysis of variance (MANOVA) to analyze periphyton data separately for deep and shallow tiles using Chl a, AFDM and AI as response variables. Prior to all analyses, we used visual inspection of box plots and scatterplot matrices to check that data met the assumptions of homoscedasticity and normal distribution and transformed data as needed to meet these assumptions. We performed square root transformation on AFDM, sediment, and chironomid density. We then used Levene's Test to check for homogeneity of variances and Kolmogorov-Smirnov Test to check for normality in all ANOVAs. We examined all pairwise-comparisons between control, pulse and press using Tukey's HSD for ANOVAs and multivariate post-hoc comparisons for MANOVAs. MANOVA tests consisted of univariate F-tests for specific effects as well as Pillai Trace Test for overall multivariate effects. An alpha level of 0.05 was used to indicate significance for all ANOVA results. To control for type I error, the alpha level for MANOVA results on specific effects was adjusted using false discovery rate control (Verhoeven et al. 2005). Residual plots were examined for all tests performed. We performed all statistical analyses in SYSTAT 13 (Systat Software, Inc., San Jose, California).

Results

Fish growth and survival

We found significant reductions in both length growth and mass growth in control vs. press in longear sunfish, but no effect on survival (Table 2, Fig. 2). We found no significant effects on length growth, mass growth, or survival in longear sunfish in either control vs. pulse or pulse vs. press (Table 2, Fig. 2). We found no significant effects on length growth, mass growth, or survival in any comparisons in either central stonerollers or orangethroat darters (Table 2, Fig. 2, Fig. 3).

Periphyton, sediment, and chironomids

In shallow habitat, we found significant overall effects of control vs. pulse, control vs. press, and press vs. pulse on periphyton, which was driven in each case by a significant decrease in AFDM and a significant increase in AI, but no significant effect on Chl a (Table 3, Fig. 5). In shallow habitat, AFDM was highest in the control, intermediate in the pulse treatment, and lowest in the press treatment, while the reverse pattern was true for AI; no such pattern was apparent in Chl a, which did not significantly differ between treatments (Table 3, Fig. 5). In deep habitat, we found no significant overall or individual effects in any comparisons (Table 3).

In shallow habitat, sediment was significantly reduced in both press vs. control and pulse vs. control, but not in press vs. pulse (Table 3, Fig. 6). In deep habitat, there were no significant effects on sediment in any comparisons (Table 3, Fig. 6).

Chironomid density in shallow habitat did not differ significantly in any comparisons (Table 3, Fig. 6). In deep habitat, both press and pulse significantly reduced chironomid density

compared to control, but there was no significant difference between press and pulse (Table 3, Fig. 6).

Discussion

Fish growth and survival

Drying in general appears to have relatively little effect on fish growth and no effect on fish survival in fish occupying refuges in these systems. Only length and mass growth in longear sunfish in the control vs. press comparison were affected. There were no significant effects of type of drying on fish growth or survival in any comparisons.

Longear sunfish may have been most affected by drying because this species is more associated with pools and deeper water habitats than the other two fish species (Pflieger 1975, Robison and Buchanan 1988). The shift from typical conditions experienced during most of the year to those experienced in dwindling drought refuges may be more extreme for sunfish, hence a greater overall effect on that species. Sammons and Maceina (2009) demonstrated that growth increments in redbreast sunfish (*Lepomis auritus*), a related species that occupies similar habitats, were much greater in a wet year versus a dry year and predicted a reduction of growth with increased water withdrawals.

The lack of effects of drying type on fish growth indicates that stream drying may be important in some cases, e.g. growth of pool-dwelling species such as longear sunfish, but the specific type of drying experienced was not as important as we hypothesized. A potentially interesting avenue for further exploration of this question would be to compare the effects of seasonal drought (press treatment) to those of supra-seasonal drought (ramp treatment).

Physiological and behavioral responses to drought have been documented in many fish communities. Hodges and Magoulick (2011) found that extent of movement to pool refuges and subsequent rates of survival and abundance in those refuges varied greatly between co-occurring species of minnows in small Ozark streams. Mesocosm experiments conducted under differing types of water withdrawal regimes, including rate and extent of withdrawal, have revealed widely differing responses of freshwater fish taxa. Fischer and Ohl (2005) found that under baseline water levels, burbot (*Lota lota*) form a hierarchy of competition for shelter, with larger individuals able to outcompete smaller ones; however, this hierarchy ceases to exist when water levels are reduced to a certain point and the larger individuals abandon the use of shelters while smaller ones retain it. In the same experiment, the authors found that there was no hierarchical order in shelter use among a commonly co-occurring species, the stone loach (*Barbatula barbatula*). Davey et al. (2006) found that two species of fish living in New Zealand streams, Canterbury galaxias (*Galaxias vulgaris*) and upland bullies (*Gobiomorphus breviceps*), differed greatly in their response to drought, with upland bullies being more likely than galaxias to become stranded on coarse substrata during rapid flow reductions, while they were less likely to become stranded on impermeable substrata during gradual flow reductions. Experiments such as these show that responses to drought events differ among species, and also interact with factors such as rate of water withdrawal and substrate type. The precise effects of drought at the level of fish communities depends on the absolute and relative survival rates of the component species in those communities (Davey et al. 2006), as well as physical aspects of refuge habitat (Fisher and Ohl 2005).

Drying had no effect on fish survival in this experiment. Overall fish survival was generally high, particularly for sunfish and stonerollers but considerably lower in darters. We

chose to keep our stocking densities within the natural range (Dekar and Magoulick 2007, Ludlam and Magoulick 2009) for these species in the region observed during previous field studies, although drying treatments increased densities to higher than normal levels typical of what would be experienced in dwindling pool refuges in the Ozark Highlands during seasonal drought (Magoulick and Kobza 2003). Previous studies have shown that stonerollers (Hodges et al. 2011) and various darters (Weston et al. 2006, Wine et al. 2008) in the region have adaptations allowing them to deal with strong seasonal drying and prolonged confinement to pool refuges through increased resistance and/or resilience.

Benthic community structure

The effects of drying and drying type on periphyton in shallow habitats were driven by significant negative effects on AFDM and positive effects on AI, but no effects on Chl a, which indicates that other components of the biomass, e.g. detritus or heterotrophic organisms, were more heavily affected than viable photosynthesizing algae in the periphyton. AFDM was highest in control, intermediate in pulse, and lowest in press, while AI showed the reverse pattern. Longer periods of exposure left less time for the overall accumulation of biomass on the tiles in the two drought treatments, but viable algae (as indicated by the Chl a measurements) were apparently able to recolonize quickly after periods of exposure and remained at a fairly high level of the overall biomass in all three treatments. Dried algal biofilms on substrata in intermittent streams are a critical source of algal propagules for recolonization once substrata are rewetted following drought (Robson 2000, Cowell et al. 2006), although pools are an even more important refuge for benthic algal regrowth (Robson and Matthews 2004). As the intensity, extent, and duration of drought increases due to anthropogenic causes, one potential effect could

be the loss of some pool refuges that may dry entirely (Magoulick and Kobza 2003). While this would have an obvious effect on fish survival in these systems, it could also have an effect on algal densities even after rewetting occurs, making dried algal biofilm the only source of propagules for recolonization. Robson and Matthews (2004) showed that drying streams that retained permanent pools reached higher algal density after flow resumed and suggested that loss of pool refuges due to water abstraction or drought may reduce algal productivity and limit the supply of autochthonous carbon in these systems. Furthermore, intermittent streams relying only on dried biofilm for recolonization would be more susceptible to terrestrial disturbances, including wildfires (Cowell et al. 2006).

Given the difference we found in the effects of drying type on periphyton growth in shallow habitats, it is logical to conclude that the effects would be even more pronounced in supra-seasonal drought events such as those forecasted by many climate-change models (Xenopoulos et al. 2005), although long-term experiments that can simulate such events and compare them to seasonal drought are an important avenue for further study. Seasonal droughts are predictable events; this allows for the adaptation of life history traits among stream biota to survive them. The lack of predictability in timing and duration of supra-seasonal drought events makes them more difficult for organisms to cope with (Lake 2003). Differences in the severity of effects between pulse, press, and ramp disturbances, as well as potential interactions between all three types, may have particularly critical implications in refuge habitats, given the importance of such refuges in both minimizing mortality and in providing sources of individuals for recolonization following disturbance.

In deep habitat, we found no effects of drying or drying type on periphyton. Although we hypothesized that overall periphyton biomass in deep habitat would be lower in the drought treatments due to the effect of more concentrated grazing by stonerollers (Power et al. 1988, Gelwick and Matthews 1997), this was not shown to be the case. As with fish survival, this effect may have been more apparent if we had used higher stocking densities at which the rate of periphyton growth may not have been able to compensate the rate of foraging by stonerollers in the tanks. At the natural range of fish and crayfish densities we used, any adverse effect on periphyton growth by stoneroller grazing may have been offset by reduction in density of herbivorous chironomids by foraging of fish and crayfish (Charlebois and Lamberti 1996, Ludlam and Magoulick 2010). Additional proposed mechanisms by which consumers can benefit algal abundance include nutrient excretion (Flecker et al. 2002) or altered algal composition (Abe et al. 2007). While Magoulick (2014) found that drought significantly reduced AI in permanently watered habitats in a somewhat similar mesocosm experiment but stocked with only crayfish, we did not observe such an effect, potentially due to these consumer-driven positive effects on algae resulting from the addition of fish.

With respect to sediment in shallow habitat, there was a significant effect of both types of drying vs. control, which, as in biomass, is likely due to increased time for accumulation of particulate matter from the water column on the tiles that were continuously underwater compared to those that were exposed during portions of the experiment. Drying type does not appear to be as important in accumulation of sediment in shallow habitat as it is for periphyton biomass. Although previous studies (Flecker et al. 2002) have shown that herbivorous fish can limit sediment deposition, we saw no effects of drying or drying type on sediment in deep

habitat, which would be expected due to increased concentration of fish in that habitat during water withdrawals.

We found what appears to be a biotically-driven concentration effect of foraging on chironomid density. This effect is suggested by the lack of significant effects of drying on chironomid density in shallow habitat, but a significant effect in deep habitat. Unlike algal mats consumed by stonerollers, chironomids make up part of the diet of all fish and crayfish species used in this experiment (Pflieger 1975, Robison and Buchanan 1988). During portions of the experiment in which shallow habitats were inaccessible to the fish in the drying treatments, deep habitats seem to have experienced increased amounts of foraging on chironomids. Magoulick (2014) found no effect of drought on chironomid density in a similar mesocosm experiment with crayfish, again suggesting that increased concentration of fish in the deep habitat played an important role in our results. Dramatic changes in biomass or density of benthic invertebrates due to increased densities of consumers have been well-documented in some experiments (Forrester 1994, Baxter et al. 2004) although results on this topic have been inconsistent (Allan 1982, Miyasaka et al. 2003). Winkelmann et al. (2011) found that at moderate fish stocking densities, total benthic invertebrate biomass and density at the reach scale were not affected, but that effects differed between pools and riffles at the habitat unit scale. As with algal communities, permanent pools serve as crucial sources for benthic macroinvertebrate recolonization following drought in intermittent streams (Miller and Golladay 1996, Dodds et al. 2004), and loss or reduction of these refuges could have important ecological ramifications.

In a review of case studies of recovery following disturbances, Detenbeck et al. (1992) found that temperate stream fish communities were generally less resilient to press disturbances,

while recovery from pulse disturbances varied depending on many factors, including taxonomic differences. While the results we found in one species, longear sunfish, seem to support these ideas with respect to resistance, it must also be considered that ecosystems rarely experience discrete disturbance regimes, but rather mixtures of disturbance types (Underwood 1994, Parkyn and Collier 2004). Interaction between different disturbances could potentially have a significant effect on stream communities in ways that one discrete disturbance event of any type may not (Underwood 1994). For example, a mixture of direct and indirect anthropogenic influences on lotic ecosystems could increase the frequency of pulse disturbances, the severity of press disturbances, and initiate ramp disturbances on an overlapping time-scale (Poff et al. 1997, Lake 2003). A pulse disturbance due to a one-time water withdrawal that depletes pool refuges during an ongoing press or ramp drought exacerbated by land-use practices or climate change could have a more dramatic impact than either would alone (Bond et al. 2015). Furthermore, the effects on different aspects of the benthic community due to any type of drying could interact with each other in significant ways during the post-disturbance recovery period (Detenbeck et al. 1992). During post-drought recolonization of streams, the lack of available periphyton to consume could affect growth, survival, and ultimately the ability of stonerollers to fully recolonize some portions of a system. Likewise, reduced colonization by chironomids due to depleted populations in deeper refuge habitats could mean less food for recolonizing darters, potentially reducing their growth, survival, or recolonization ability.

Conclusions

Our results suggest that some fish species in the Ozark Highlands are better adapted to resist intense drying than others. Differential effects on fish species as a result of

anthropogenically induced or exacerbated drying events could play a role in restructuring the composition of fish communities in drought prone regions. Additionally, the effects of drying on sediment in shallow habitats and chironomids in deep habitats demonstrates that drying is an important influence on aspects of benthic community structure in a range of microhabitats within these systems. While the specific type of drying was not important in terms of impact on sediment, chironomids, or fish growth and survival, it did differ in its effects on periphyton growth. Effects of drying disturbance are likely to become more important with anthropogenically-influenced increase in the frequency, intensity, and duration of drought, the potential loss or depletion of critical refuge habitats, and increasingly complex, unpredictable interactions between pulse, press, and ramp disturbances in stream ecosystems.

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trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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Table 1. Initial mean length and mass of fish and crayfish species (standard deviation in parentheses).

	Length (cm)	Mass (g)
Longear Sunfish (<i>Lepomis megalotis</i>)	6.50 (0.93)	5.05 (2.46)
Central Stoneroller (<i>Campostoma anomalum</i>)	6.85 (1.14)	3.23 (1.63)
Orangethroat Darter (<i>Etheostoma spectabile</i>)	4.27 (0.47)	0.81 (0.3)

Table 2. Results of ANOVAs for length growth, mass growth, and survival in longear sunfish, central stoneroller and orangethroat darter. Significant values ($p < 0.05$) in bold.

		Length Growth	Mass Growth	Survival
Longear Sunfish	Control vs. Press	<0.001	0.028	0.870
	Control vs. Pulse	0.087	0.412	0.870
	Press vs. Pulse	0.062	0.333	1.000
Central Stoneroller	Control vs. Press	0.082	0.063	0.905
	Control vs. Pulse	0.313	0.149	1.000
	Press vs. Pulse	0.734	0.900	0.905
Orangethroat Darter	Control vs. Press	0.054	0.246	0.930
	Control vs. Pulse	0.222	0.215	0.640
	Press vs. Pulse	0.715	0.999	0.850

Table 3. Results of MANOVA for periphyton (Chl a , AFDM, AI), and ANOVA for sediment and chironomid density. Significant values in bold ($p < 0.05$ for ANOVAs; False Discovery Rate control used for MANOVAs).

		Overall Effect (Periphyton)	Chl a	AFDM	AI	Sediment	Chironomids
Shallow	Control vs. Press	<0.000	0.478	<0.001	<0.001	<0.001	0.191
	Control vs. Pulse	0.021	0.536	0.015	0.009	0.003	0.536
	Press vs. Pulse	0.028	0.190	0.003	0.028	0.315	0.757
Deep	Control vs. Press	0.180	0.343	0.353	0.048	0.172	0.020
	Control vs. Pulse	0.764	0.348	0.868	0.333	0.623	0.048
	Press vs. Pulse	0.368	0.992	0.444	0.287	0.634	0.921

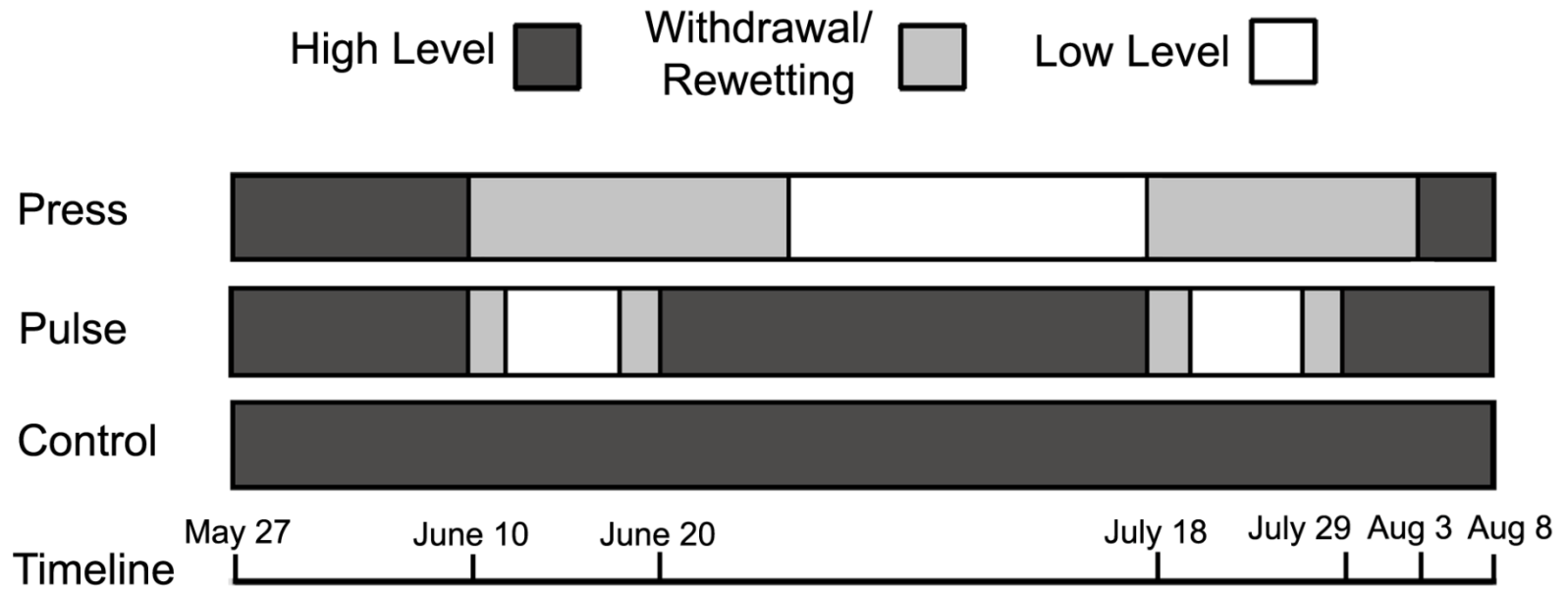


Figure 1. Experimental timeline. High water level was 0.40 m above the bottom of the tank, low level was 0.15 m. Withdrawal/rewetting rate was 0.125 m/day in the pulse treatment and 0.015 m/day in the press treatment. Key dates on timeline: start of experiment, beginning and end of each pulse, beginning and end of press, and end of experiment.

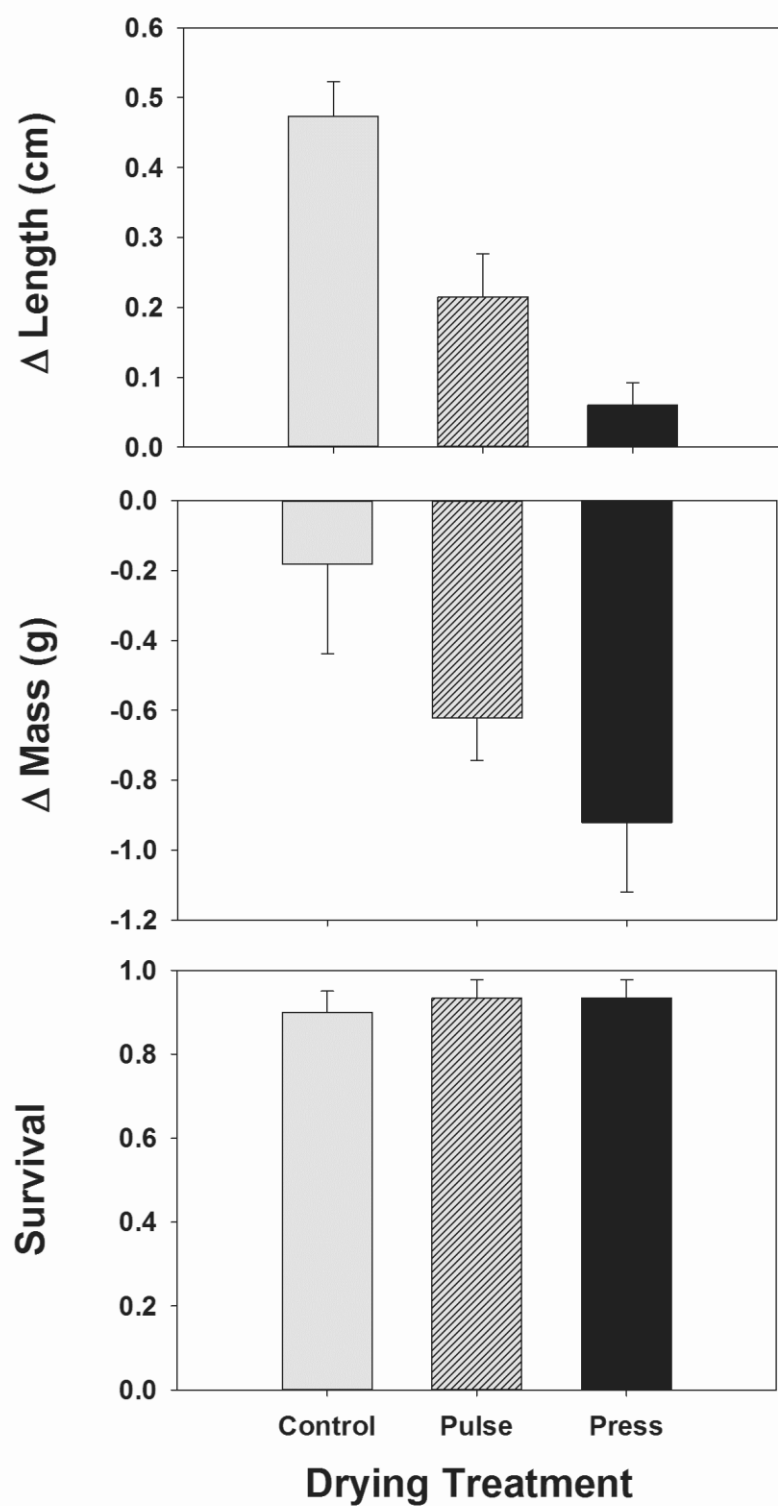


Figure 2. Length growth, mass growth, and survival in longear sunfish (*Lepomis megalotis*).

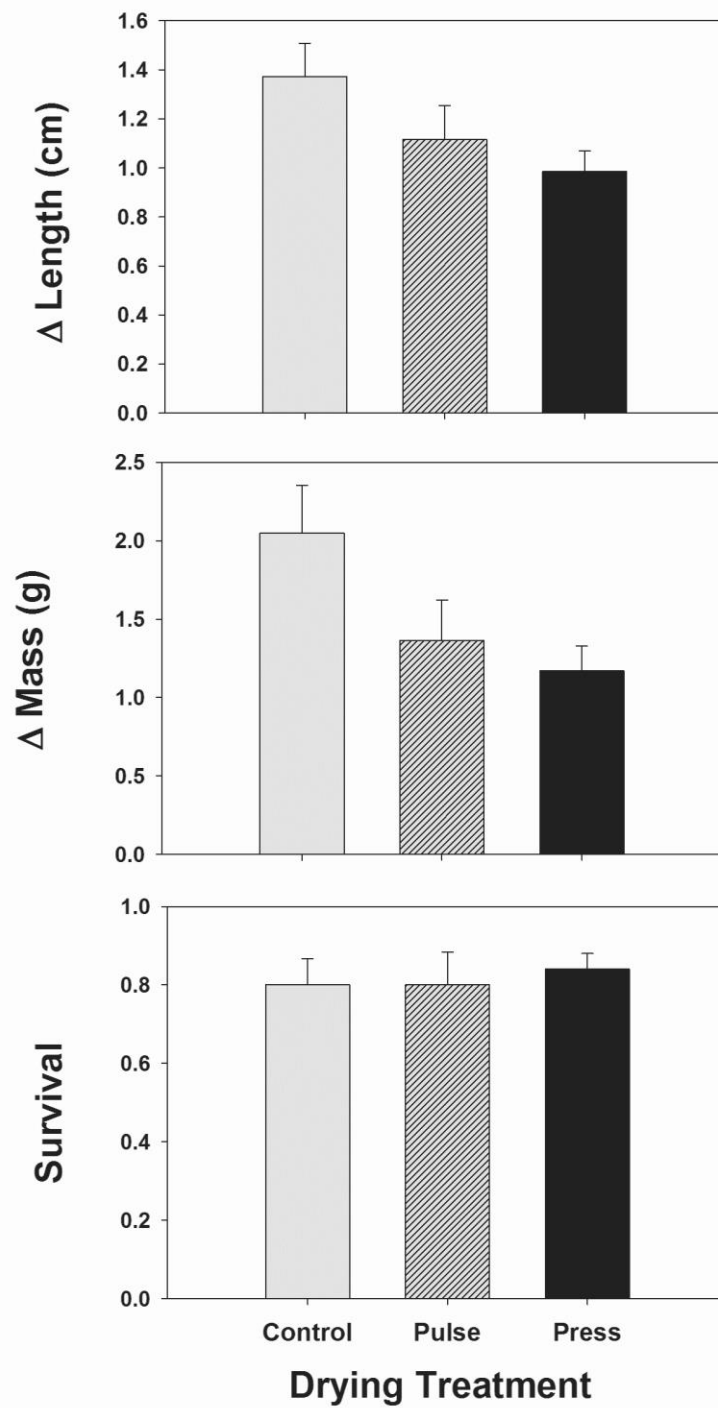


Figure 3. Length growth, mass growth, and survival in central stoneroller (*Campostoma anomalum*).

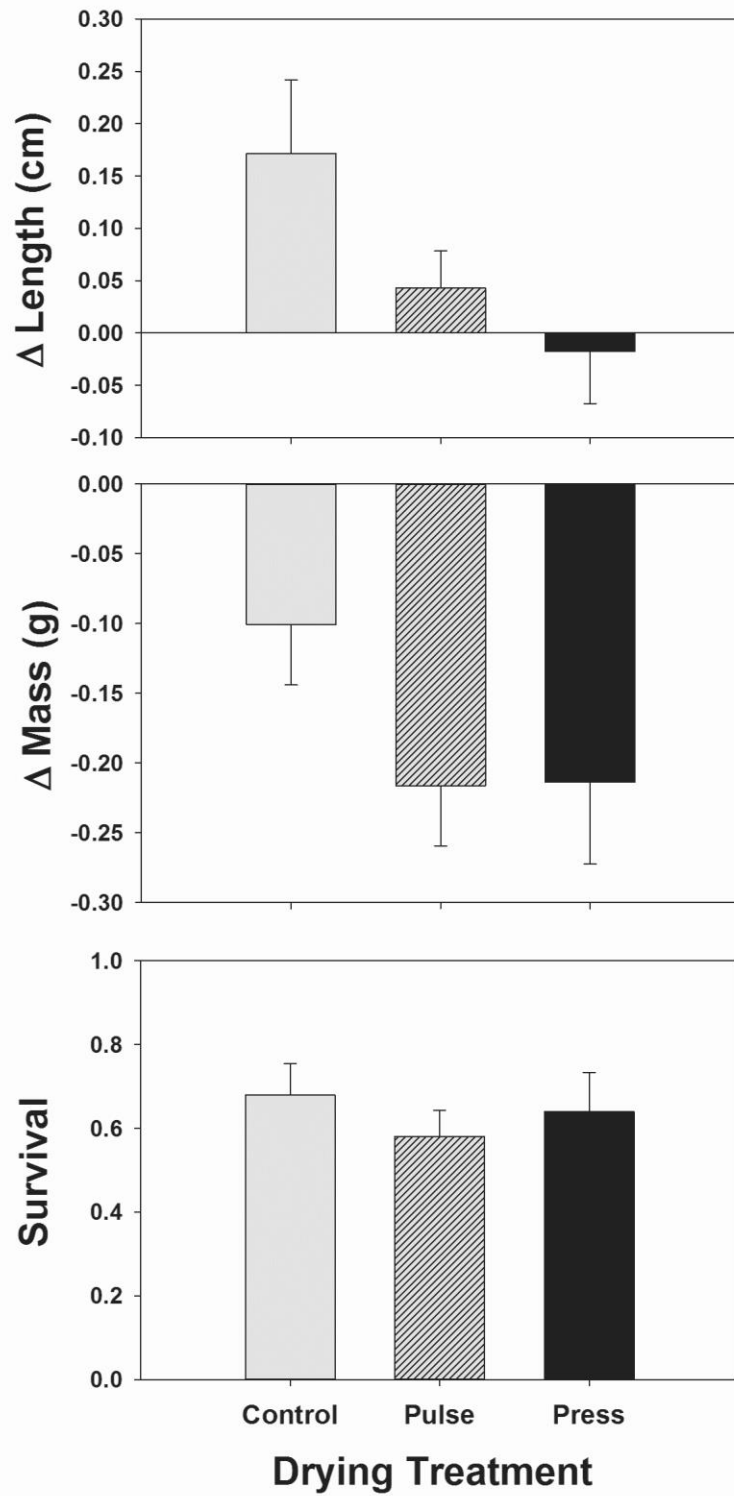


Figure 4. Length growth, mass growth, and survival in orangethroat darter (*Etheostoma spectabile*).

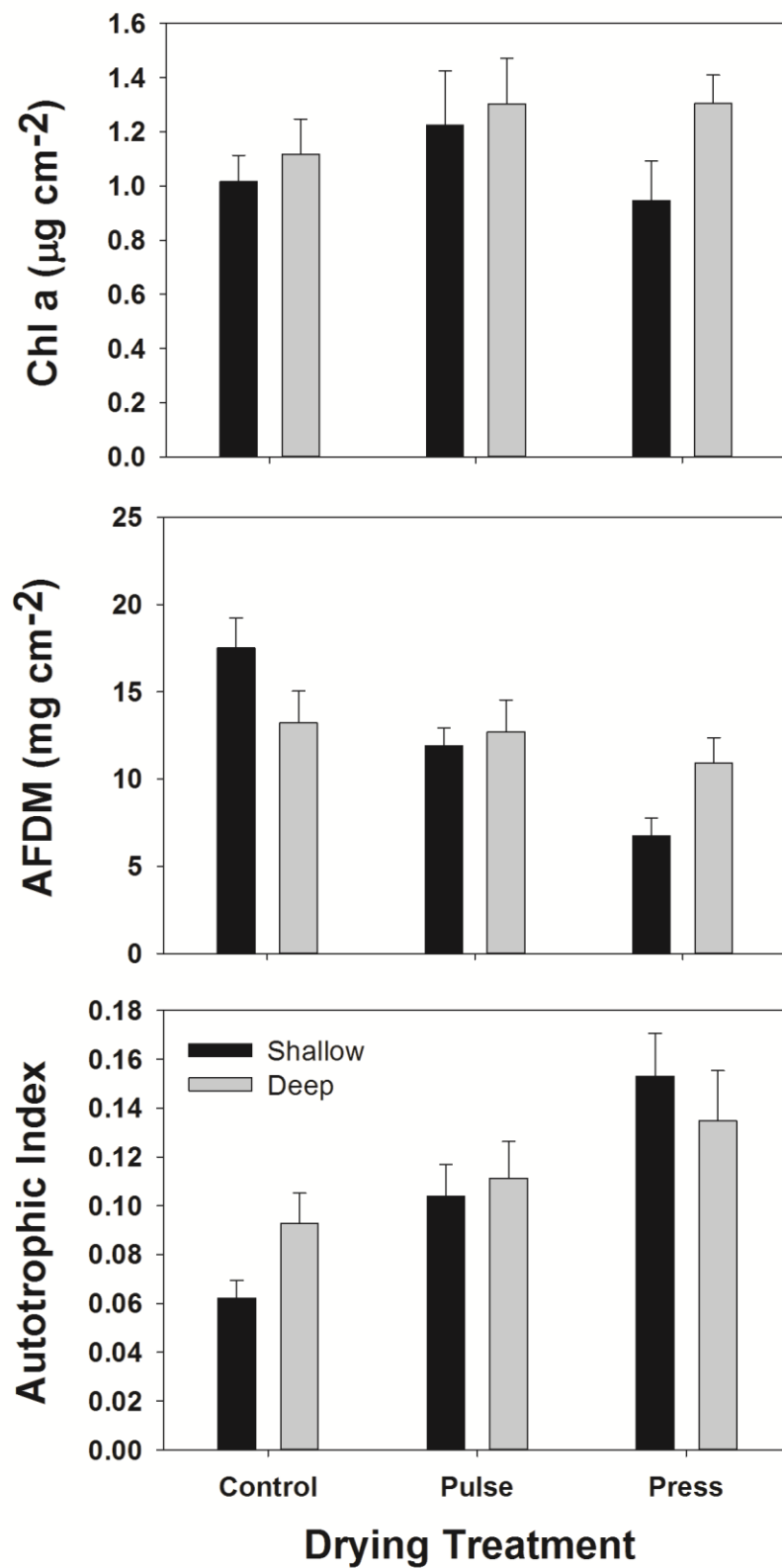


Figure 5. Periphyton (Chl a, AFDM, and AI) on shallow and deep tiles.

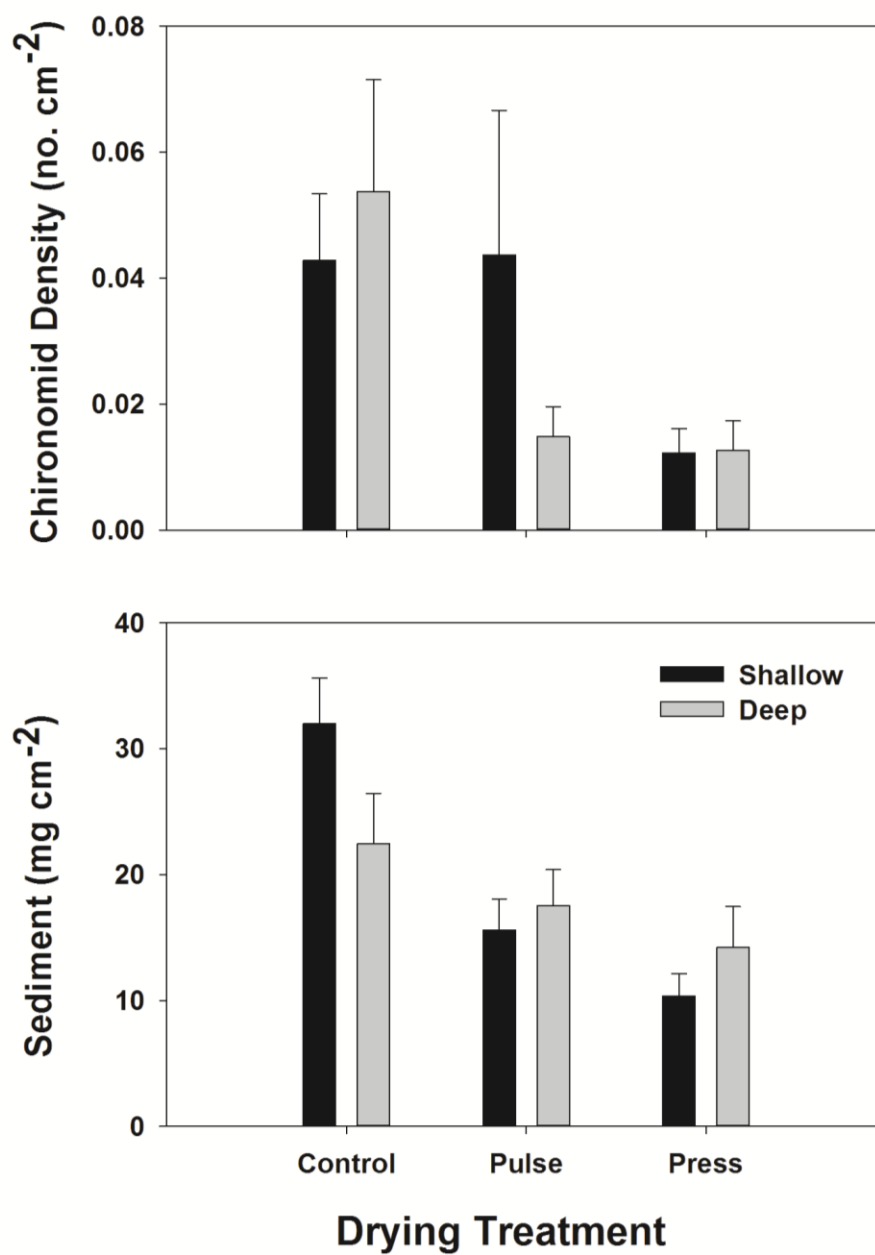


Figure 6. Chironomid density and sediment on shallow and deep tiles.

MEMORANDUM

TO: Daniel Magoullick
Dustin Lynch

FROM: Craig N. Coon, Chairman
Institutional Animal Care
And Use Committee

DATE: March 29, 2012

SUBJECT: IACUC PROTOCOL APPROVAL
Expiration date : **June 30, 2012**

The Institutional Animal Care and Use Committee (IACUC) has **APPROVED** Protocol #12036-
"OZARK HIGHLANDS FISH COMMUNITY RESPONSES TO SIMULATED DROUGHT AND
PULSE DROUGHT PERTURBATIONS IN STREAM MESOCOSMS ". You may begin this study
immediately.

The IACUC encourages you to make sure that you are also in compliance with other UAF committees
such as Biosafety, Toxic Substances and/or Radiation Safety if your project has components that fall
under their purview.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any
changes in the protocol during the research, please notify the IACUC in writing [Modification Request
form] **prior** to initiating the changes. If the study period is expected to extend beyond **06-30-2012**, you
may request an extension [via the Modification Request form] up to 03-26-2015 (3 years from original
approval date). By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for
research involving animal subjects.

cnc/car

cc: Animal Welfare Veterinarian

Conclusion

In this study, I examined hydrology-biology relationships among aquatic communities in the Ozark Highlands. When assessing the importance of hydrologic variation relative to other environmental variables, I found that it was often less important to fish, crayfish, and benthic macroinvertebrate assemblages than local habitat, stream geomorphology, and water quality. Of course the nature of these analyses necessitated greatly reducing the hydrologic data a priori to a single measure of variation. It is possible other hydrologic variables would be more important relative to other kinds of environmental data. Nevertheless, the results of this study suggest that hydrologic variation is best considered within a wider framework of other environmental variables which can strongly affect stream organisms. The idea that it is not hydrology alone, but the complex interaction between stream-flow, geomorphology, land-use, and other factors that largely determines the distribution, abundance, and diversity of stream organisms is not new (Schlosser 1982, Poff and Allan 1995, Ward et al. 1999, Bunn and Arthington 2002). Only in recent years have studies such as this one attempted to quantify the relationship between specific hydrologic metrics and stream communities (Poff et al. 2010, Olden et al. 2014) and isolate the influence of hydrology from these other factors. This is inherently challenging; the degree to which hydrologic variation is interrelated to other factors makes it difficult to isolate. McManamay and Frimpong (2015) found that models that incorporated only hydrologic variables performed poorly compared to models constructed with both hydrology and landscape variables. Further studies attempting to quantify biological responses to specific hydrologic metrics, particularly metrics of hydrologic alteration, are certainly warranted, but from a management perspective it is important to consider this broader context of many environmental

factors given that ecological response to hydrologic alteration is likely to be highly heterogeneous (McManamay and Frimpong 2015).

Another issue that can confound attempts to elucidate flow-ecology relationships is temporal variation. An implicit assumption of environmental flows theory is that flow-ecology relationships will be at least somewhat temporally invariant and allow us to make predictions about how biota will respond to changes in their environment (Poff et al. 2010, Carlisle et al. 2010, Olden et al. 2014). Temporal variation in aquatic communities can make this difficult. I encountered that issue in this study due to the extremely different flow conditions between the two sampling seasons. While I cannot draw strong conclusions about variation in the fish and crayfish assemblages due to lack of total overlap between the sites, I did find evidence of temporal variation in benthic macroinvertebrate assemblages, which differed strongly from year to year. It is important to note that flow metrics in this study were based on a consistent period of record and did not change between the two years; it was the invertebrate communities themselves that strongly varied. This variation was enough to considerably shift relationships between response variable and flow metrics. Other studies have suggested that macroinvertebrate communities can significantly vary from year to year even in reference streams (MacDonald and Cote 2014, Darter and Fend 2001). A single sampling is a snapshot which may vary greatly depending on the conditions under which it is taken. This study highlights the importance of long-term biomonitoring to establish quantifiable relationships between hydrology, along with other kinds of environmental variables, and stream biota in order to form a bigger picture of how streamflow affects aquatic communities. Ideally, monitoring would occur over a long enough period to encompass extreme ends of the disturbance spectrum such as the drought and flooding encountered in this study.

From a management perspective, another challenge is that different taxa within the same streams may be affected in very different ways by streamflow and flow alteration. While we saw some general patterns that were similar between groups, we also saw important differences. For example, while metrics related to high flow frequencies may be among the most important to fish assemblages, they appear to be less important to crayfish, whereas metrics related to timing of flows may be much more crucial to riffle-dwelling macroinvertebrates than they are to other groups. These differences are likely due to the very different life history strategies of stream organisms and how they cope with disturbance (Bunn and Arthington 2002, Lytle 2008, Carlisle et al. 2010). A complicated suite of metrics must be considered to best manage stream ecosystems for the benefit of the entire aquatic community. All of these challenges – the relationship between hydrology and other environmental variables, temporal variation in aquatic communities, and the differential effects of hydrology on different taxonomic groups – are related to the inherent complexity of streams and suggest that a holistic approach is needed in the conservation and management of lotic ecosystems.

I found that flow alteration is an important influence on community structure in Ozark Highland streams. The most important categories of alteration influencing stream biota in the region were magnitude of average flows, and frequency, duration, and magnitude of high flows. The fact that three of these categories were high flow-related suggests the overall importance of floods as a determinant of community structure in these streams, and that altered flood frequency, which is typically reduced compared to expected values in the region, may have serious consequences for aquatic communities. Although I saw generally less impact of alteration of low flow metrics, this may be due to flow regime that was the focus of this study,

Groundwater Flashy streams; other regimes in the region may be impacted very differently by different forms of alteration (Leasure et al. 2014).

Finally, I saw that different forms of stream drying can have differential effects on stream communities. While seasonal stream drying is an integral part of the natural disturbance regime in the region, the fact that organisms can be impacted differently by different forms of drought may have conservation implications. Supraseasonal drought events are expected to become more common due to global climatic change (Lake 2000, Xenopolous 2005), and anthropogenic impacts on streams can exacerbate all forms of drought (Beche et al., 2009, Magoulick and Kobza 2003). The effects of drying and type of drying on periphyton growth in particular could have important ecological consequences, particularly in refuge habitats that are critical sources of organisms and propagules for recolonization following seasonal droughts.

In conclusion, Ozark Highland streams are complex and fascinating ecosystems in which streamflow plays a critical role shaping and influencing aquatic communities. While it is challenging for a variety of reasons to disentangle the effects of specific hydrologic metrics on biota, it is clear that anthropogenic alteration of the natural flow regime has major consequences for these systems.

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