Flow Alteration-Ecology Relationships in Ozark Highland Streams: Consequences for Fish, Crayfish and Macroinvertebrate Assemblages

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Abstract

We examined flow alteration-ecology relationships in benthic macroinvertebrate, fish, and crayfish assemblages in Ozark Highland streams, USA, over two years with contrasting environmental conditions, a drought year (2012) and a flood year (2013). We hypothesized that:

1) there would be temporal variation in flow alteration-ecology relationships between the two years, 2) flow alteration-ecology relationships would be stronger during the drought year vs the flood year, and 3) fish assemblages would show the strongest relationships with flow alteration. We used a quantitative richest-targeted habitat (RTH) method and a qualitative multi-habitat (QMH) method to collect macroinvertebrates at 16 USGS gaged sites during both years. We used backpack electrofishing to sample fish and crayfish at 17 sites in 2012 and 11 sites in 2013. We used redundancy analysis to relate biological response metrics, including richness, diversity, density, and community-based metrics, to flow alteration. We found temporal variation in flow alteration-ecology relationships for all taxa, and that relationships differed greatly between assemblages. We found that relationships were stronger for macroinvertebrates during the drought year but not for other assemblages, and that fish assemblage relationships were not stronger than the invertebrate taxa. 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 was more important than alteration of low flows. Of 32 important flow alteration metrics across years and assemblages, 19 were significantly altered relative to expected values. Ecological responses differed substantially between drought and flood years, and this is likely to be exacerbated with predicted climate change scenarios. 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). A concept related to the natural flow regime is the environmental flow regime; the key difference is that environmental flow regimes allow for some degree of hydrologic alteration in an attempt to balance the needs of humans as well as stream ecosystems, while still resulting in the same patterns and ecological outcomes as the natural flow regime (Poff *et al.* 1997, Bunn and Arthington 2002, Poff *et al.* 2010). One of the great challenges in the implementation of the environmental flows (e-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).

Natural streamflow regimes are threatened worldwide by a host of anthropogenic factors, including construction of dams and diversion structures, groundwater withdrawals from aquifers, and other hydromorphological alterations (Sondergaard and Jeppesen 2007, 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), including in the focal region of this study, where these phenomena have already had consequences for rare and imperiled aquatic species (Magoulick and Lynch 2015). 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 hydrologic 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).

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; Kendy et al., 2012; Gillespie *et al*., 2014, McManamay & Frimpong, 2015; King *et al*., 2015, Sengupta *et al*., 2018). While there is strong evidence that flow alteration generally negatively affects 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 and may not represent all stream types in an area (Zimmerman *et al*., 2018). 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; Farjad *et al*., 2015).

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 hypothesized that: 1) there would be temporal variation in flow alteration-ecology relationships between the two years (drought year versus flood year), 2) flow alteration-ecology relationships would be stronger during the drought year vs the flood year, and 3) fish assemblages would show the strongest relationships with flow alteration.

We hypothesized that the potential interactive effects between the dual stressors of drought and flow alteration would lead to stronger relationships during the drought year (Acuna et al. 2005, Beche *et al*., 2009, Bunn and Arthington 2002, Dodds et al. 2004, Poff and Allan 1995, Lynch et al. 2018). We hypothesized that fish would be more strongly impacted by drought than the other groups due to the ability of benthic macroinvertebrates and crayfish to utilize the hyporheic zone as a refuge (DiStefano *et al.*, 2009; Wood, *et al*. 2010; Stubbington 2012) or to utilize drought-coping life history strategies ranging from aestivation (Wickson *et al*., 2012) and desiccation-resistant eggs (Pallares *et al*., 2016) to overland escape (Chester *et al*., 2014) or shifts in timing of emergence (Stenroth *et al*., 2010). To address our objectives, 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.

Study Area

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 rapid development of urban areas and agricultural practices 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 and dams (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.

Methods

Site Selection

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). Precipitation and flow conditions contrasted strongly between the two years. In summer 2012 the study area experienced an extreme drought, as measured on the Palmer Drought Severity Index (Palmer, 1965), while sustained higher than normal precipitation resulted in summer flooding at most sites during 2013 (NOAA, 2015). To facilitate biological comparisons, site selection was limited to a single ecoregion, the Ozark Highlands, a single physiographic region, the Springfield Plateau, and a single flow regime, Groundwater Flashy streams, based on a classification of Ozark-Ouachita Interior Highland streams into seven different hydrologic flow regimes (Leasure *et al.*, 2016). Streams selected ranged in drainage area from 16 to 542 km^2.

Macroinvertebrate collections were taken at 16 sites that were the same in both years (Figure 1). Due to extreme differences in sampling conditions between the two years (drought in 2012 versus extensive flooding in 2013), we were unable to resample seven 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 and 11 in 2013, with 10 overlapping sites between the two years (Figure 1).

Hydrologic variable and flow alteration estimation

We identified 64 USGS gaged streams in our study area in least-disturbed reference condition based on a composite hydrologic disturbance index (Falcone et al. 2010) using water withdrawals, density of major dams, change in dam storage between 1950 and 2009), percent canals in the watershed, water discharge locations, road density, and land cover fragmentation (Leasure *et al.* 2016). All streams selected in least-disturbed condition had an index less than the median of all gaged streams in the study area (Leasure et al. 2016). Using these streams, we developed a set of random forest models to predict 171 flow metrics (Olden and Poff 2003). Full models were built initially that included 282 predictor variables describing climate, geology, soils, topography, groundwater and landscape characteristics within reference watersheds (Appendix A). Importance of each variable was assessed using the default method of the *randomForest* R package (Liaw and Wiener 2002) that is based on how much prediction error increases when each variable is permuted while others are left the same. A benefit of using random forest models for this approach is that they are not sensitive to the number of variables at each node or the number of trees (Liaw and Wiener 2002). 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 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 18 gages used in this study. 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. We dropped flow metrics that were outside our threshold criteria for bias, precision and accuracy, reducing our initial set of 171 metrics to 154 (Appendix B).

Aquatic community assessment

Benthic macroinvertebrates, fish, and crayfish were sampled at each site. Reaches were defined by the presence of at least three discrete units of riffles, runs, and pools, and a qualitative attempt was made to ensure that sampling reaches were as comparable as possible between sites. Sampling was stratified by habitat to include three units each of riffles, runs and pools for a total of nine units per reach. Total area of reaches ranged from 140 – 957 m^2 and units averaged 8.3 m (SD = 3.2) in length across all habitat types. Habitat units were located at least 100 m from road crossings to avoid the hydrologic influence of man-made structures that could affect stream habitat (Barbour *et al.*, 1999).

Benthic macroinvertebrate samples were typically collected at sites a few hours prior to fish and crayfish sampling. Macroinvertebrates were sampled using two different, complementary methods at each site, a semi-quantitative richest-targeted habitat (RTH) method and a qualitative multiple habitat (QMH) method, both developed for the U.S. Geological Survey National Water Quality Assessment (NAWQA) program (Moulton *et al*., 2002). Quantitative RTH collections were taken in riffles using a 500-µm mesh Slack sampler (50 cm × 30 cm) equipped with a 0.25 m^2 area PVC frame attached to the upstream end of the sampler (Moulton *et al*., 2002). The Slack sampler was positioned immediately downstream of the chosen quadrat perpendicular to the direction of flow. Large cobble and woody debris were lightly brushed, inspected for any remaining invertebrates, and then removed from the sampling area. The sampling area was then agitated by disturbing the substrate upstream of the mouth of the sampler, allowing the dislodged invertebrates to flow into the trailing net. The nine discrete subsamples were composited into a 19-L plastic bucket for processing, which consisted of rinsing and removing large debris from the samples, followed by elutriation and sieving (with a 500 µm sieve) in order to separate invertebrates and organic debris from inorganic material.

The QMH method was used to document invertebrate taxa present in all habitat types throughout the reach (Moulton *et al.*, 2002). Crew members assessed the entire reach to determine the number of different instream habitat types present and to estimate the proportion of each type. QMH samples were collected using a D-frame kicknet with 500-µm mesh. Each habitat type was sampled in proportion to total habitat area for a standardized time of one hour per reach. Samples were processed in the field as described for the RTH method.

In the laboratory, RTH and QMH 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 organisms present were removed from the grid and processed. Subsampling proceeded in this fashion until a minimum of 300 organisms were counted, with the square in which the 300th organism was counted also fully processed. Macroinvertebrates were identified to the lowest practical taxonomic level, generally family or genus. To estimate total numbers of organisms, 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. These numbers were then used to calculate invertebrate community response metrics.

Fish and crayfish were collected using a Smith-Root Model LR-24 backpack-electrofishing unit which has been shown effective for fish and crayfish sampling in Ozark streams (Rabenin *et al*., 1997; Dauwalter and Pert, 2003). Standard LR-24 settings for power output based on ambient stream conductivity were used. Prior to sampling, 1.6 cm^2 mesh block-nets were placed at the end of each habitat unit to prevent animals from escaping or biasing sampling data by moving from one unit to another. A four-person team conducted three upstream passes per habitat unit. Collections from all passes were kept in separate buckets until all passes were completed. Each pass was processed separately and all specimens were identified to species and released live back into the stream.

Biological Response Metric Selection

Biological response metrics were calculated for macroinvertebrate, fish, and crayfish assemblages (Table 1). A subset of metrics considered most ecologically relevant was chosen based on published relationships and best professional judgment. The five macroinvertebrate response metrics chosen were: abundance; taxa richness; Simpson's diversity; percent contribution of individuals belonging to Orders Ephemeroptera, Plecoptera, and Trichoptera (EPT), taxa that are associated with undisturbed habitat and high water quality (Karr, 1991); and percent contribution of the family Chironomidae, considered a generally tolerant taxon predicted to increase in abundance with increasing levels of perturbation (Barbour *et al.*, 1999). Response metrics calculated from RTH and QMH samples were analyzed separately.

For crayfish, the three response metrics chosen were: Simpson's diversity, total crayfish density (per volume sampled), and percent contribution of species designated as habitat generalists in an assessment of invasion risk of crayfish in the eastern United States (Larson and Olden 2010). These are represented in our dataset by two species, *Faxonius neglectus neglectus* and *Faxonius virilis*. Species richness was not used as a community response metric for crayfish due to the generally low and relatively uniform richness across sites.

For fish, the five biological response metrics chosen were: species richness, Simpson's diversity, total fish density (per volume sampled), percent of total individuals belonging to Family Centrarchidae, and percent of total individuals belonging to species categorized as intolerant, i.e. sensitive to various environmental perturbation, in an Index of Biotic Integrity specifically developed for fish assemblages of the Ozark Highlands (Dauwalter *et al.*, 2003). Percent Centrarchidae was chosen as a response metric because most Ozark centrarchids are ecologically tolerant habitat generalists (Dauwalter *et al.*, 2003; Robison & Buchanan, 1988).

Data Analysis

Redundancy analysis (RDA) was used to evaluate flow alteration-ecology relationships separately for assemblages and sampling years. RDA is a canonical ordination procedure that examines relationships among response variables and predictor variables in multivariate space (ter Braak, 1995). 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 (ter Braak and Smilauer, 2002) to select flow alteration variables that were related to response metrics. We limited the flow alteration variables to those with lambda ≥ 0.7 after entry into the model.

Prior to RDA analysis, response variables were centered and standardized. 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 variables with large variances from disproportionately influencing ordination diagrams (ter Braak and Smilauer, 2002). Monte Carlo permutations were performed for each RDA to test the significance of the canonical axes together and 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 year, taxonomic assemblage and sampling type (for macroinvertebrates). All significant hydrologic alteration metrics are listed and defined in Table 2. Percent variance explained in assemblage-environment relationships was examined by comparing eigenvalues from RDA analysis in order to test our second and third hypotheses.

Results

RTH Macroinvertebrates

In 2012, RTH macroinvertebrate response metrics were significantly related to alteration of DH18, TA3, and RA3 (RDA $p<0.001$, Table 2, Figure 2). RA3 was significantly reduced relative to expected values (Figure 2). Diversity, richness, and percent EPT were all negatively related to alteration of TA3, while abundance and percent Chironomidae were positively related to alteration of RA3 (Figure 2). Cumulative percent variance explained by flow alteration was 88.6% for Axis 1 and 2 (Table 3).

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

(Figure 2). Percent EPT was positively related to alteration of TH2 (Figure 2). Cumulative percent variance explained by flow alteration was 80.2% for Axis 1 and 2 (Table 3).

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 2). 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 metrics were significantly related to alteration of MA12, MH3, MH20, ML12, and FH11 (RDA $p<0.001$, Table 2, Figure 3). MA12 and MH3 were significantly reduced and ML12 significantly increased relative to expected values (Figure 3). Richness, diversity, and percent EPT were all negatively related to alteration of FH11, while percent Chironomidae was positively related to alteration of MH3 (Figure 3). Cumulative percent variance explained by flow alteration was 93.6% for Axis 1 and 2 (Table 3).

In 2013, QMH macroinvertebrate response metrics were significantly related to alteration of MA29, MH17, FH4, FH5, and DH23 (RDA $p<0.001$, Table 2, Figure 3), and MA29, MH17, and FH4 were significantly reduced relative to expected values (Figure 3). 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 3). Cumulative percent variance explained by flow alteration was 69.6% for Axis 1 and 2 (Table 3).

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 3). 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 macroinvetebrate assemblages, and another, FH11, in fish assemblages (Table 2).

Crayfish

In 2012, crayfish response metrics were significantly related to alteration of MA3, MA32, MA33, DL18 and RA2 (RDA $p<0.001$, Table 2, Figure 4). DL18, MA32, and MA33 were significantly reduced and RA2 significantly increased relative to expected values (Figure 4). Diversity was negatively related to alteration of DL18, while total density and percent generalist crayfish were positively related to alteration of RA2 (Figure 4). Cumulative percent variance explained by flow alteration was 88.6% for Axis 1 and 2 (Table 3).

In 2013, crayfish response metrics were significantly related to alteration of MA3, MA21, DH1, and TH1 (RDA $p<0.001$, Table 2, Figure 4). DH1 was significantly reduced and TH1 significantly increased relative to expected values (Figure 4). Total Density was positively related to alteration of DH1 while diversity was negatively related to alteration of MA21 (Figure

4). Cumulative percent variance explained by flow alteration was 90.7% for Axis 1 and 2 (Table 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 4). 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).

Fish

In 2012, fish response metrics were significantly related to alteration of MA22, MA36, FH1, FH2, FH8, and DH7 (RDA $p<0.001$, Table 2, Figure 5). MA22, MA36, FH1, and FH2 were significantly reduced relative to expected values (Figure 5). Diversity and richness were positively related to alteration of MA22, FH2, and MA36 and negatively related to alteration of DH7 (Figure 4). Percent intolerant fish was negatively related to alteration of FH1 and FH8 (Figure 5). Cumulative percent variance explained by flow alteration was 72.4% for Axis 1 and 2 (Table 3).

In 2013, fish response metrics were significantly related to alteration of MH13, MH18, FH11, DH7, and RA3 (RDA $p<0.001$, Table 2, Figure 5). MH18 was significantly increased and RA3 significantly reduced relative to expected values (Figure 5). 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 5). Cumulative percent variance explained by flow alteration was 86.7% for Axis 1 and 2 (Table 3).

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 5). 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).

Aquatic Community

Considering all four assemblages over both years, 32 different metrics of hydrologic alteration were significantly related to biological response metrics (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 numerous (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

Our hypothesis that there would be temporal variation in flow alteration-ecology relationships was supported. The complete overlap of sites for macroinvertebrate collections makes it possible to draw temporal comparisons in flow alteration-ecological response relationships between the two years for macroinvertebrate assemblages. The contrast between years was most pronounced in the RTH samples, which may be because they are collected only from riffles, the stream habitat most heavily affected by drought (Dekar and Magoulick, 2007; Chester and Robison, 2011). While we expected some differences between the years, it was still somewhat surprising to see no consistently important metrics between the two years in either RTH or QMH collections. In some cases, there were seemingly different relationships between response metrics. For example, RTH percent EPT was negatively related to alteration of TA3 (seasonal predictability of flooding) in 2012, whereas percent EPT was positively related to alteration of TH2 (variability in Julian date of annual maximum) in 2013. However, in both cases percent EPT was reduced with altered flow timing (discussed further below). It appears that alteration of flow timing is important for percent EPT, but specific relationships vary temporally.

Non-stationarity in environmental conditions can complicate our ability to formulate predictable flow-ecology relationships and pose challenges for the implementation of e-flows science (Poff *et al.*, 2010; Rolls *et al.*, 2012; Katz and Freeman, 2015; Poff 2017; Lynch *et al.* 2018). As the hydrologic cycle is further altered by global climate change and the severity, duration and frequency of droughts increases (Masson-Delmotte et al., 2013, Farjad *et al.*, 2015) it is crucial to incorporate strategies that realistically account for these phenomena when implementing e-flows science into management decisions (Poff 2017). One such strategy is to focus on resilience, the maintenance of processes and relationships that are robust and able to maintain integrity despite anticipated changes in environmental conditions (Poff 2017; Mazor *et al.*, 2018).

Our hypotheses that flow alteration-ecology relationships would be stronger during the drought year and that fish assemblages would show the strongest flow-alteration relationships, had much less support, and showed year- and taxa-dependent caveats. Relationship strength, as indicated by cumulative percent variance explained in Axes 1 and 2 of the RDA's, was greater during the drought year for benthic macroinvertebrate assemblages, but weaker for fish and crayfish, with the pattern reversed during the flood year. Fish assemblage relationships were stronger than macroinvertebrates and lower than crayfish during the flood year, but weaker than all other groups during the drought year. Overall, relationships were slightly stronger during the drought year than the flood year, and relationships were actually weaker in fish than in the macroinvertebrate assemblages.

With respect to which categories of alteration metrics were most important, the prominence of magnitude and frequency is of particular interest given that regional e-flows 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 others have found that frequency of floods may be one of the most important determinants of community structure in streams (Dodds *et al.*, 2004; Matthews *et al.*, 2013; Matthews *et al.*, 2014). 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 assemblages 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 appeared to be a considerably less important influence on biota in Groundwater Flashy streams in the Ozark Highlands; 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 the present study, we focused on Groundwater Flashy streams, one of the most common flow regimes in the Ozark Highlands (Leasure *et al.*, 2016), but it is likely 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, 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.*, 2016). 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.

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. It was also the only assemblage in which no flow alteration category was clearly more prominent than the others. Predictability of flooding is thought to be critically important to macroinvertebrate assemblages. 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 abundance 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). In the present study, predictability of flooding (TA3) was altered towards higher predictability and was negatively related to all response variables in 2012. Likewise, alteration towards increased variability in high flow timing (TH2) was positively related to increased percent EPT taxa and abundance in 2013, but it is important to note that TH2 alteration ranges from strongly negative to slightly positive so percent EPT and abundance increased with less altered (i.e., more normal) TH2. Therefore, in both cases percent EPT was reduced with altered flow timing. It appears that alteration of flow timing, regardless of direction, may negatively influence RTH macroinvertebrate assemblages in these systems.

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 high flow frequency (FH). Relationships between response variables in the two years were 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 flow alteration 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 each other. 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) (Fig. 3). Examining synergies and indirect effects of flow alteration on ecosystem structure and function could be a fruitful avenue for future research.

It should be noted that care should be taken in interpreting the flow alteration-ecology relationships because flow can be negatively or positively altered or unaltered. Therefore, negative alteration can be positively correlated with a response variable. For example, in Fig. 2 the alteration of FH3 is positively related to Taxa Richness and negatively related to %Chironomidae, but FH3 is negatively altered and high FH3 values are near normal (i.e., FH3=0). Therefore, Taxa Richness is greater and %Chironomidae is reduced when FH3 is near normal.

Magnitude alteration was the most important category in both crayfish and fish assemblages. The two 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).

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.

Overall, our results show the importance of magnitude and high flow alteration to stream assemblages in these systems. However, patterns related to alteration of specific flow metrics between years or between taxa are less obvious. It is possible that redundancy between tested flow metrics used to explain similar ecological processes may be responsible for lack of patterns with specific flow metrics. Future research that builds on established flow alteration-biological response relationships in a way that specifically elucidates functional links would be worthwhile.

Another caveat to consider is that, while relationships appeared to vary between years, our ability to detect these relationships may have varied as well based on the very different sampling conditions between drought and flood years. Detection probability of freshwater fish can vary strongly between samples taken at different flow magnitudes, and this may influence inferences based on fish community-flow relationships (Pregler *et al*., 2015, Gwinn *et al*., 2016). Similar factors may affect detection probability in benthic macroinvertebrate assemblages (Meador *et al*., 2011; Wisniewski *et al*., 2013). 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 (Poff *et al*., 2006; McManamay and Frimpong, 2015, Lynch, *et. al* 2018). 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 even within the same ecoregion (Poff, 1992; Poff *et al.*, 2010; Leasure *et al.*, 2016). 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.

Conclusions

Flow alteration appears to be an important influence on community structure in Groundwater Flashy streams in the Ozark Highlands. The most important categories of alteration influencing stream biota were MA, FH, MH, and DH. The fact that three of these categories were high flow-related suggests the overall importance of high flows as a determinant of community structure and composition in these systems. Of the 32 important metrics across years and assemblages, 19 were significantly altered relative to expected values. General patterns, such as the importance of magnitude and high flow alteration, were apparent across assemblages and may be useful to managers and stakeholders attempting to conserve freshwater ecosystems in the region. However, key differences between taxonomic groups, as well as 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. Environmental flows concepts are increasingly finding traction in regions across the world (Belmar et al., 2011; Buchanan et al., 2013; Rolls and Arthington, 2014; O'Brien, et al., 2017; Zhang et al., 2017), but could be enhanced by a better understanding of complexity with respect to interactions between temporal variation, disturbance, and taxa-dependent response differences.

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Table 1. Mean (±SE) values for biological response metrics.

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

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

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

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

Code	Definition	Category	Mean ((±SE)
DH23	Flood duration (mean annual number of days that flow remains above threshold averaged over all years)	Duration of High Flows	-0.27 (± 0.16)
	2012 Fish		
MA22	Mean November flows	Magnitude of Average Flows	-0.29 (± 0.05)
MA36	Variability across monthly flows	Magnitude of Average Flows	-0.82 (± 0.14)
FH1	High flood pulse count (pulse defined as 75th percentile)	Frequency of High Flows	-0.74 (± 0.31)
FH2	Variability in high flood pulse count	Frequency of High Flows	-0.75 (± 0.30)
FH8	Flood frequency (25th percentile upper threshold)	Frequency of High Flows	-0.73 (± 0.32)
DH7	Variability in annual maxima of 3 day mean daily discharge	Duration of High Flows	-0.04 (± 0.12)
	2013 Fish		
MH13	Variability across maximum monthly flows	Magnitude of High Flows	0.26 (± 0.38)
MH18	Variability across annual maximum flows	Magnitude of High Flows	1.60 (± 1.00)
FH11	Flood frequency (mean number of discrete flood events per year)	Frequency of High Flows	-0.18 (± 0.41)
DH17	High flow duration (upper threshold 1 times median flows)	Duration of High Flows	-0.54 (± 0.69)
RA3	Fall rate	Rate of Change of Average Flows	-0.11 (± 0.09)
	2012 Crayfish		
MA3	Variability in daily flows	Magnitude of Average Flows	-0.42 (± 0.26)
MA32	Variability in September flows	Magnitude of Average Flows	-0.51 (± 0.2)
MA33	Variability in October flows	Magnitude of Average Flows	-0.41 (± 0.19)
DL18	Number of zero-flow days	Duration of Low Flows	-0.18 (± 0.13)
RA2	Variability in rise rate	Rate of Change of Average Flows	2.31 (± 0.38)
	2013 Crayfish		
MA3	Variability in daily flows	Magnitude of Average Flows	-0.85 (± 0.32)
MA21	Mean October flows	Magnitude of Average Flows	-0.01 (± 0.06)

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

Code	Definition	Category	Mean ((±SE)
DH1	Annual maxima of daily mean discharge	Duration of High Flows	-0.24 (± 0.08)
TH1	Julian date of annual maximum	Timing of High Flows	0.55 (± 0.22)

Table 3. Cumulative % Variance in RDA axes.

Assemblage	Cumulative % Variance Explained	
2012 (drought year)	Axis 1	Axis 2
RTH Macroinvertebrates	68.5	88.6
QMH Macroinvertebrates	60.9	93.6
Fish	44.8	72.4
Crayfish	68.5	88.6
2013 (flood year)		
RTH Macroinvertebrates	54.9	80.2
QMH Macroinvertebrates	43.4	69.6
Fish	54.0	86.7
Crayfish	57.7	90.7

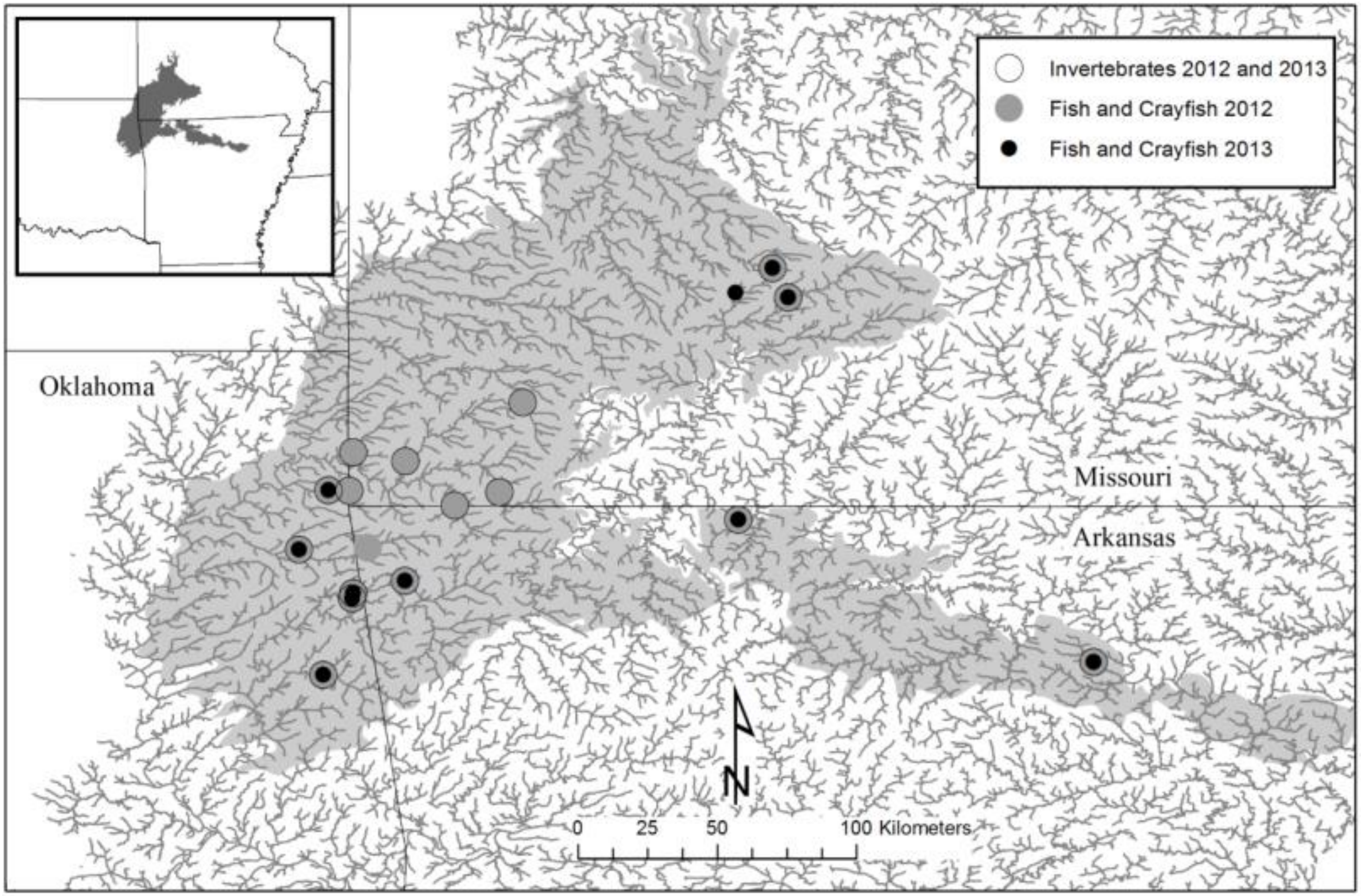

Figure 1. Map of study area showing sample sites, stream network, and Springfield Plateau.

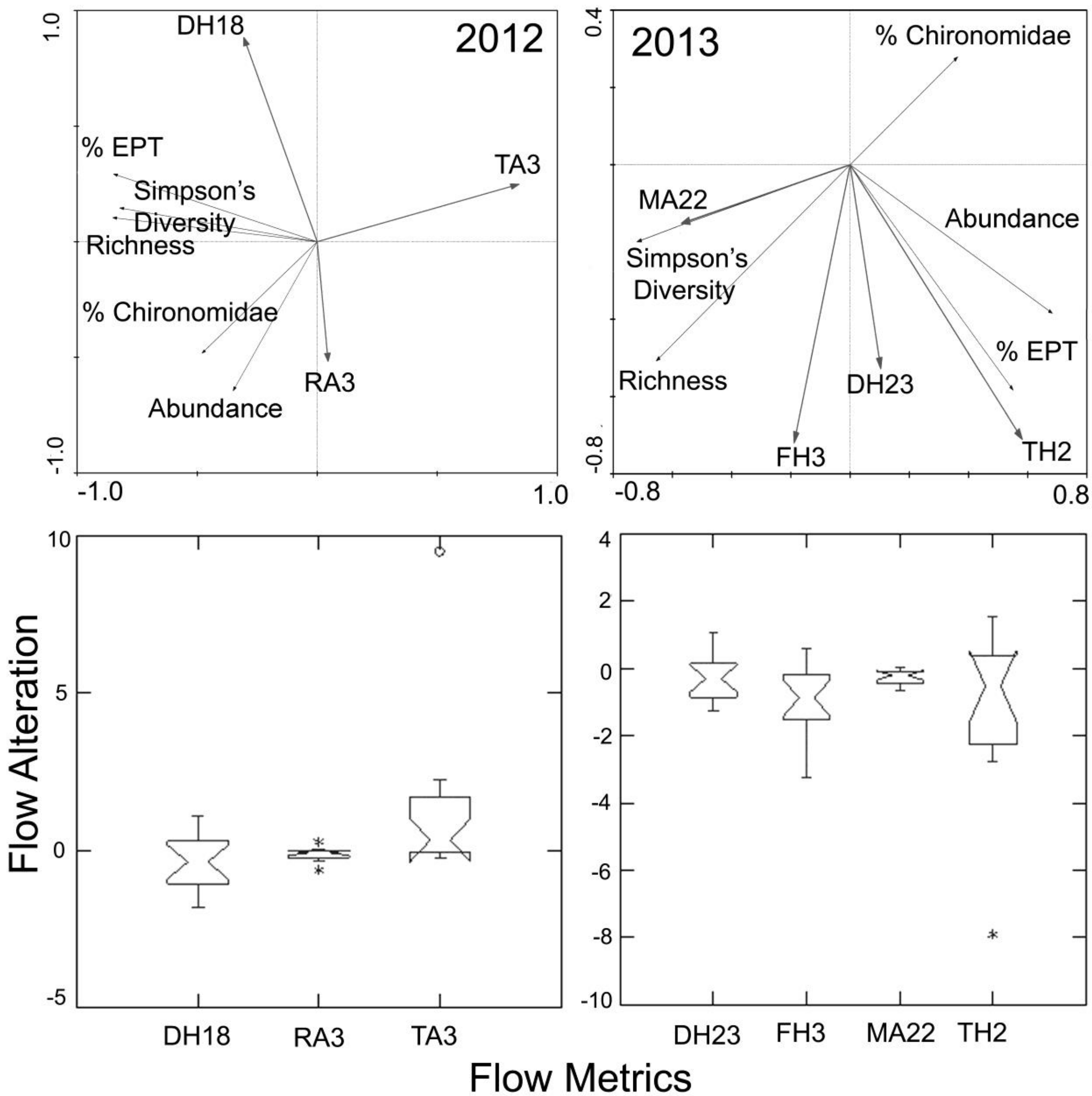

Figure 2. Redundancy analysis ordination plot relating RTH (Richest Targeted Habitat) macroinvertebrate assemblages and selected flow alteration variables in 2012 and 2013. Boxplots show flow alteration variables used with notches indicating 95% CI. Angles of arrows indicate associations and length of arrows indicate strength of the relationship. Flow alteration variable abbreviations and descriptions are given in Table 2.

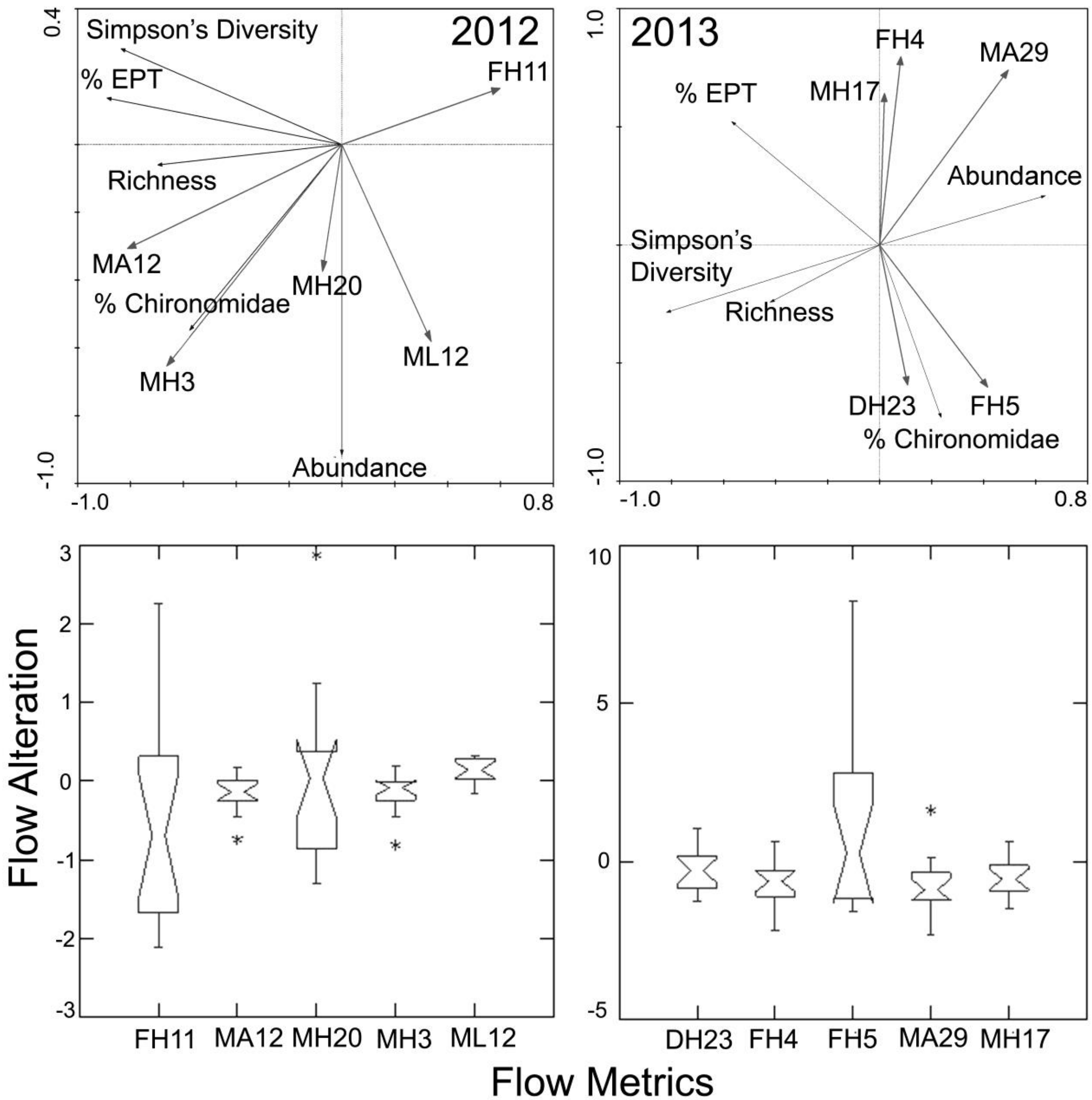

Figure 3. Redundancy analysis ordination plot relating QMH (Qualitative Multi-Habitat) macroinvertebrate assemblages and selected flow alteration variables in 2012 and 2013. Boxplots show flow alteration variables used with notches indicating 95% CI. Angles of arrows indicate associations and length of arrows indicate strength of the relationship. Flow alteration variable abbreviations and descriptions are given in Table 2.

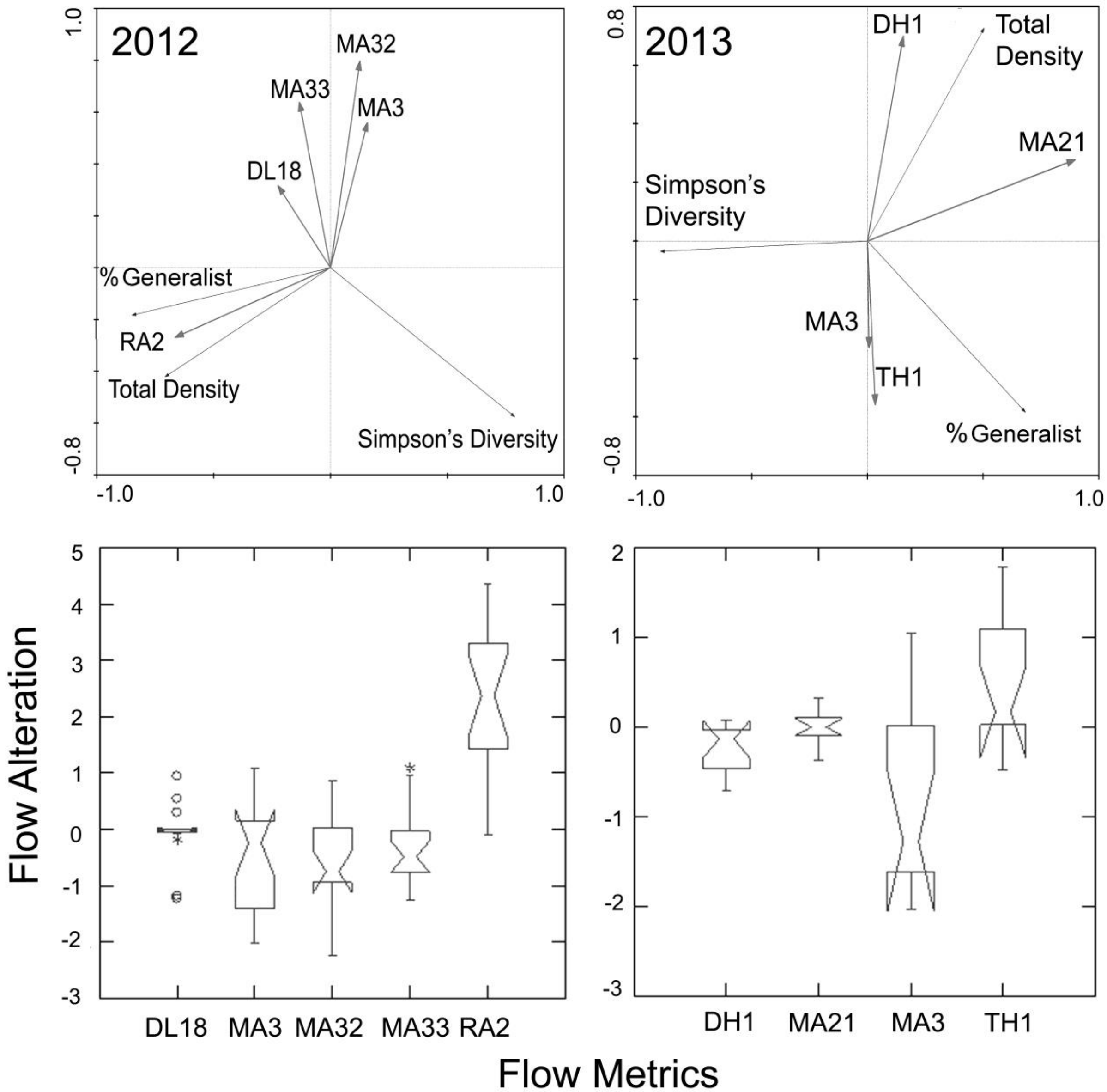

Figure 4. Redundancy analysis ordination plot relating crayfish assemblages and selected flow alteration variables in 2012 and 2013. Boxplots show flow alteration variables used with notches indicating 95% CI. Angles of arrows indicate associations and length of arrows indicate strength of the relationship. Flow alteration variable abbreviations and descriptions are given in Table 2.

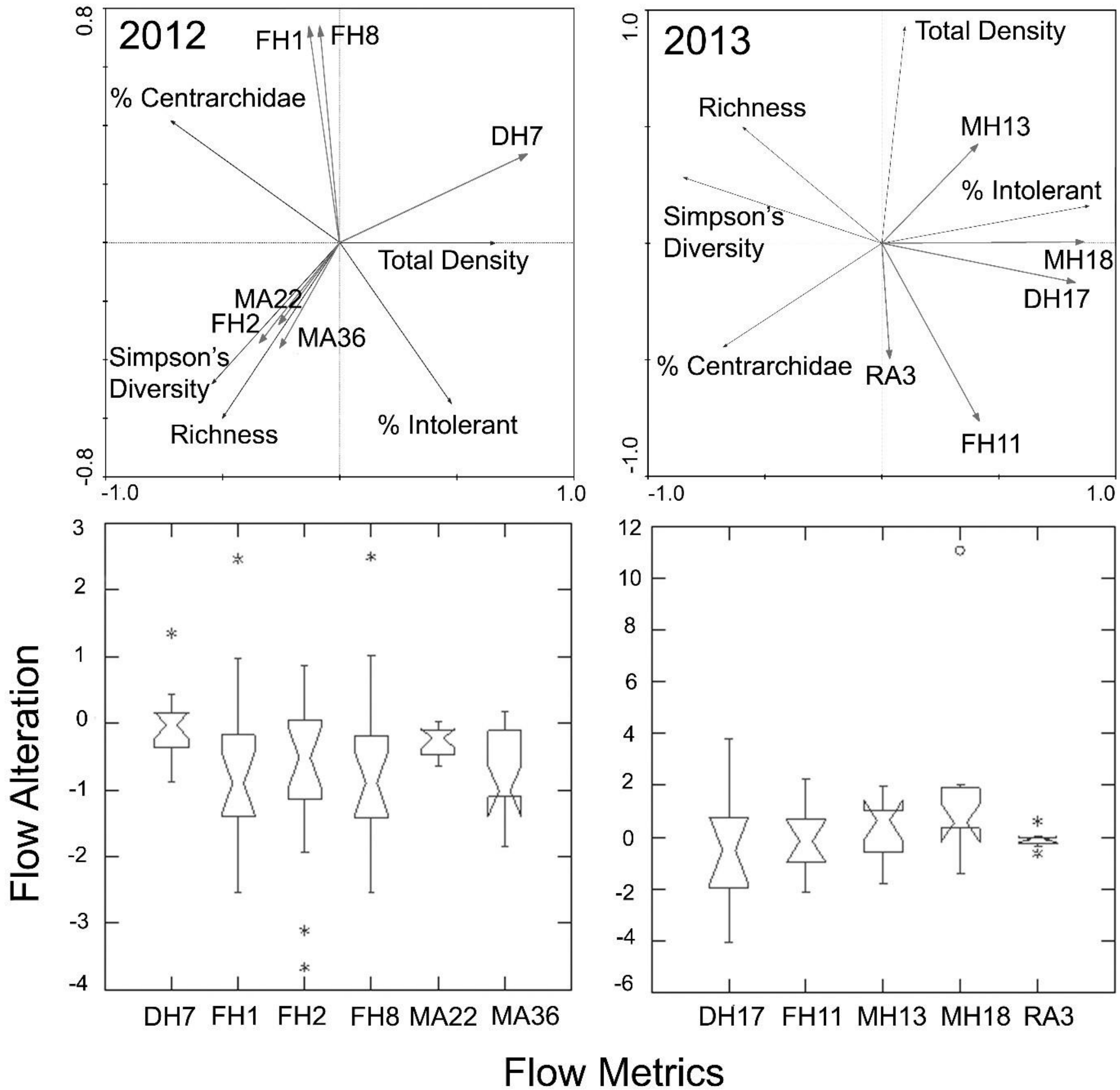

Figure 5. Redundancy analysis ordination plot relating fish assemblages and selected flow alteration variables in 2012 and 2013. Boxplots show flow alteration variables used with notches indicating 95% CI. Angles of arrows indicate associations and length of arrows indicate strength of the relationship. Flow alteration variable abbreviations and descriptions are given in Table 2.