# Biological Criteria for Stream Fish Communities of Missouri


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## Acknowledgments

This research is a contribution of the Missouri Cooperative Fish and Wildlife Research Unit (U.S. Geological Survey, Missouri Department of Conservation, University of Missouri, and Wildlife Management Institute cooperating).

## Executive Summary

The objective of this project was to develop a fish Index of Biological Integrity for wadeable streams in Missouri. Because the development of any biological "index" is fairly subjective we followed a stringent protocol documented here, ensuring that the final system was sensitive to human-induced changes, reproducible, with good sensitivity and low variability.

**Step 1. Data collection, validation and usability**
Fish collections (>450) used in this project were made using identical protocols (EPA – REMAP) between 1994 and 2005. Verified data were processed and analyzed by the Missouri Department of Conservation's Resource Assessment and Monitoring (RAM) coordinator using SAS programs developed by Environmental Monitoring and Assessment Program (EMAP) personnel (Kaufmann et al. 1999).

**Step 2. Selection of reference sites**
Data were reviewed by personnel from Missouri Department of Natural Resources, Missouri Department of Conservation and the University of Missouri to determine all preexisting MDNR reference sites and any others that would meet reference standards. Seventy-two candidate reference sites were retained. Additional GIS analysis of possible anthropogenic stressors resulted in a final list of 43 reference sites.

**Step 3. Defining reference conditions for two major ecoregions**
The two major ecoregions of Missouri (Plain and Ozark) are generally considered to harbor different fish communities and habitat conditions. We quantitatively evaluated these differences in several ways. First, ordination analysis of fish communities showed distinct separation of communities based on ecoregion. Statistical analyses indicated significant differences between the ecoregions in the overwhelming majority of watershed landscape and channel morphology variables. Fish communities (ordination site scores) of these reference streams from each ecoregion were not significantly associated with any human disturbance or land cover variables. Additionally, multiple habitat variables were determined to be statistically different between the two ecoregions. Finally a suite of fish metrics (n = 39) was compared between the 2 ecoregions and 62% were significantly different between ecoregions. The conclusion was to develop a unique IBI for each ecoregion.

**Step 4. Metric evaluation and selection using reference sites**
Forty-two candidate fish metrics were evaluated (Ozark n = 26, Plain n =17) following the process outlined by Hughes et al. (1998):

1) Criteria relating to the **range, normality and variability** with reference conditions resulted in elimination of 3 Plain metrics and 1 Ozark metric.
2) Responsiveness to **anthropogenic disturbance** was evaluated by statistically testing metric scores between reference and impaired sites. Thirteen metrics from the Ozark and 8 from the Plain ecoregions were deemed sufficiently sensitive. Sensitivity was scored on retained metrics using box plots on a score

from 0 - 3. Nine metrics using Ozark data showed good sensitivity. While 8 metrics showed good sensitivity in the Plain ecoregion, 4 metrics responded in the opposite direction of the prediction. Analysis of physical habitat for both ecoregions showed 23 variables that were significantly different between reference and impaired sites in the Ozark ecoregion, while only 9 variables were significantly different within the Plain ecoregion. The data support the conclusion that, for the Plain ecoregion, reference site conditions are scarcely better than those for the impaired sites. We concluded that the development of a useful IBI for the Plain ecoregion is not possible at this time. The following work focused exclusively on development of an IBI for the Ozark ecoregion.

3) **Metric precision** was analyzed by examining the ratio of among site variance (or signal) to within site variance from replicated sites (noise).
4) **Metric redundancy** was examined using correlation analysis. Based upon box plot sensitivity and precision, one of the pair was dropped.

**Step 5. Final metric selection** consisted of five metrics from the richness category: number of native darter species, number of native benthic species, number of native water column species, number of native minnow species, number of all native lithophilic species, two metrics from the balance/diversity/composition category: proportion of native sunfishes, and proportion of the 3 dominant species; one metric from the trophic and reproductive category: proportion of native insectivore cyprinid species, and one metric from the abundance category: number of native individuals.

**Step 6. IBI Development**

Metric values were converted into unitless scores of 1, 3, or 5 (poor to good). Out of a total possible score of 45, the mean for reference and impaired sites was 40.5 and 32.3 respectively (significantly different at $p = 0.0001$). Eight of the 9 metrics were positively related to the IBI score. Modeling indicated all metrics had roughly the same influence on the final IBI score.

A validation data set (n = 19) containing a wide range of habitat scores was assembled with sites different than those used in the calibration tests. Several statistical tests indicated a good ability for the IBI to distinguish the “good” from “poor” sites.

The calibration and verification data sets were then combined to develop quartiles for the final IBI scores. We suggest criteria for a three-level classification of stream condition of *no impairment*, *impaired* and *highly impaired*.

## Conclusions and Recommendations

The development of a fish IBI for the Ozark ecoregion was successful and should serve as a useful indicator of the biological condition of wadeable streams in this area. Failure to achieve biocriteria for the Plain ecoregion may be due to fewer environmental differences in reference versus impaired streams, the lack of true reference streams, or the more tolerant nature of the current assemblage of fishes.

We suggest that future work on IBI development in Missouri, either for refinement of this IBI for the Ozark ecoregion or towards efforts to produce a viable IBI for the Plain ecoregion, be directed towards a more data-based approach to the screening process of the reference and impaired sites. Site selection could be improved and variance reduced if inclusion of sites was not based exclusively on Best Professional Judgment, but also included elimination criteria whereupon known “reference” or “impaired” sites could be dropped from use based on the associated physical habitat data or additional water quality data.

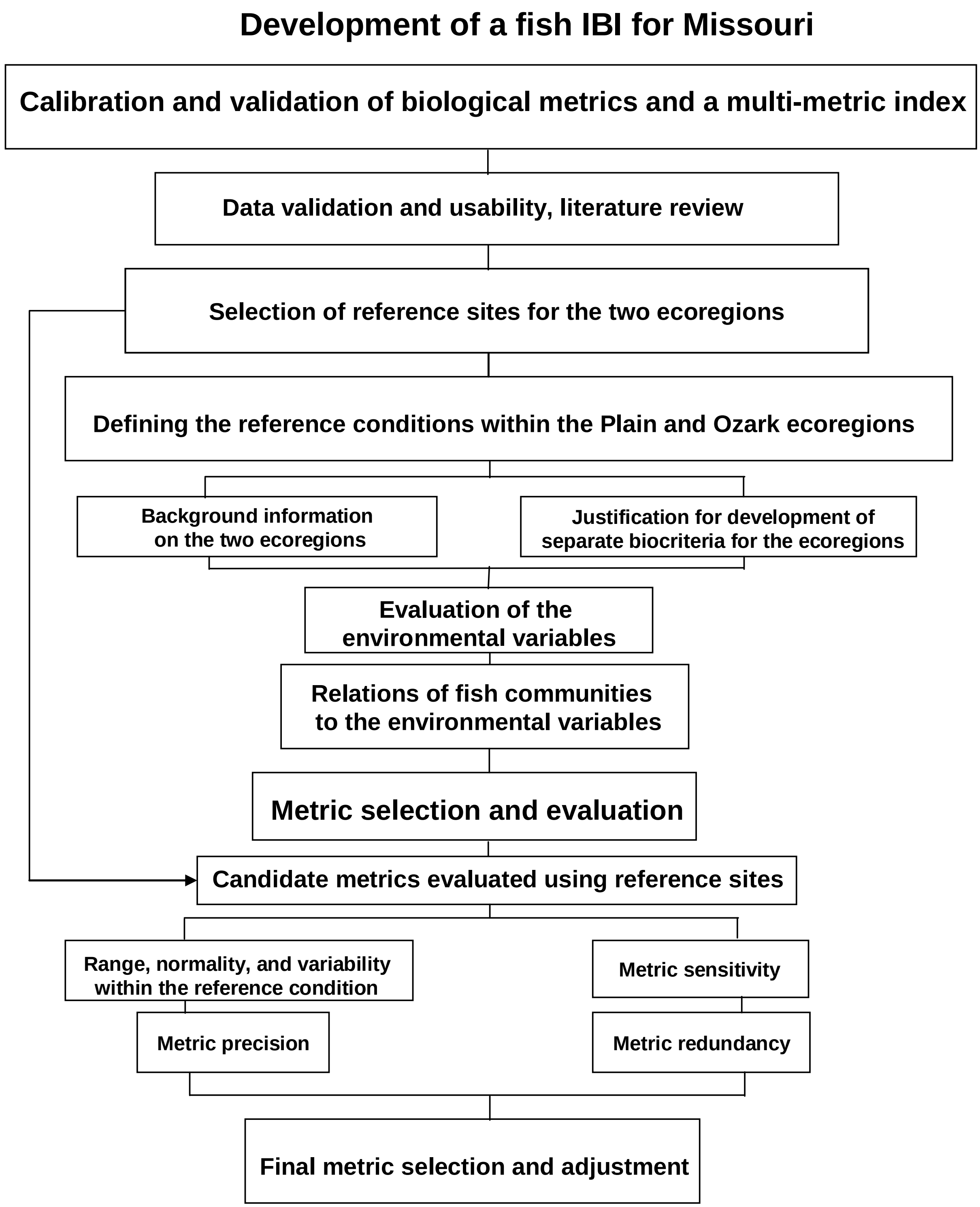
# Introduction

The concept of biological integrity was codified by the federal Clean Water Act Amendments of 1972, which mandated that the condition of the aquatic life existing in streams and rivers be an endpoint that could be measured. This has proven to be difficult because biological integrity is not definable in absolute terms. That is to say that while most people agree on exactly what represents a temperature of 20 degrees Celsius many people would disagree on a number representing biological integrity. The difference is that while temperature has the underpinnings of a physical law – motion of molecules – biological integrity is merely an idea. Nevertheless biologists have given the concept a definition – the most accepted one being the ability to support and maintain *"a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitat of the region"* (Karr and Dudley 1981). Biological integrity is equated with pristine conditions or those conditions with no or minimal disturbance, and it is used as the baseline for the IBI. While at the University of Illinois, Jim Karr (1981) produced the milestone system of using fish communities to evaluate stream health – The Index of Biological Integrity (IBI). This index has been widely used with considerable modification ever since. Karr's initial IBI used 12 metrics representing fish species composition and richness, and ecological factors most closely representing the concept of biological integrity.

The concept has been shown to be useful in a variety of aquatic systems and geographic areas. Along with usable indices there have been publications advocating particular protocols in the development of an IBI to insure scientifically sound results. This is particularly important, as the IBI is an index, one which could be developed in a number of different ways.

An Index of Biological Integrity should embody a number of attributes. Desirable attributes include the use of biological measures that can be interpreted at several trophic levels and are sensitive to the environmental conditions being monitored within a suitable range. These measures must be reproducible, with good precision and low variability (Simon 1999).

The objective of this project was to develop a fish Index of Biological Integrity for wadeable streams in Missouri. Because the development of any biological "index" is fairly subjective we followed a stringent protocol documented here.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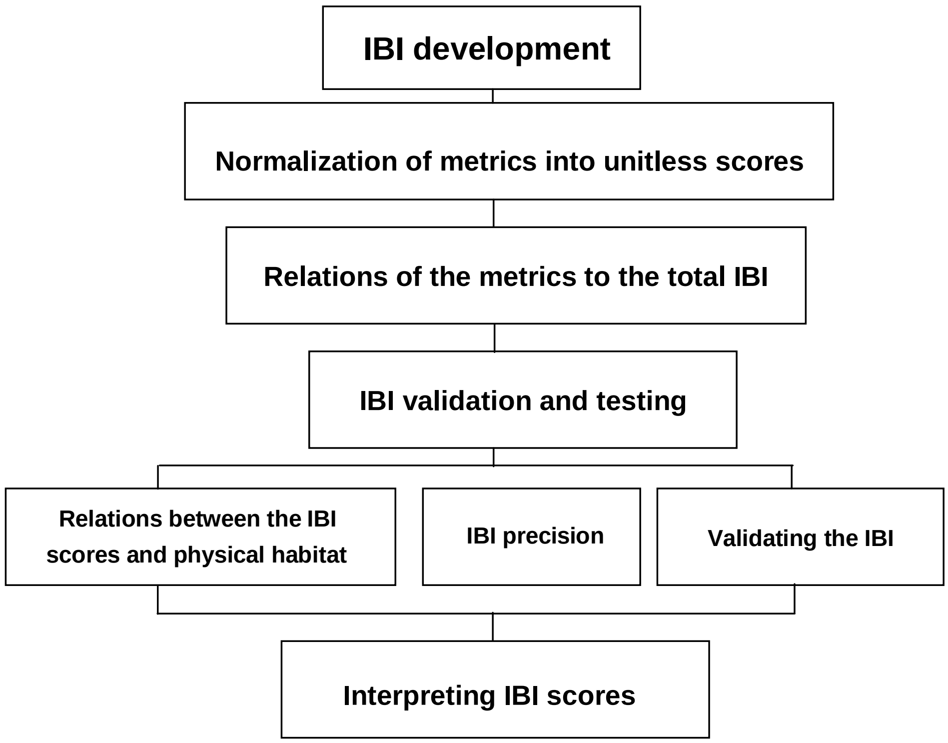
## Development of a fish IBI for Missouri continued



## Background

A wealth of fish community and associated water quality and physical habitat data have been collected by the Missouri Department of Conservation (MDC) Resources Assessment and Monitoring (RAM) program and the Department of Fisheries and Wildlife at the University of Missouri (UMC). All of these data have been accumulated using standardized procedures (developed by EMAP) for collecting community level data in a range of wadeable streams and rivers throughout Missouri (Strahler orders 2 – 5). Compatible data collections within both the Central Plain and Ozark ecoregions[1] (Figure 1) began in 1994 and 1995, and continued in 2000, 2001, 2002, 2004, and 2005. The sample sites were randomly selected using EMAP protocols, chosen due to their status as Missouri Department of Natural Resources (MDNR) reference sites, or handpicked to meet other research needs of MDC. Additional data for each sample site such as Strahler order, watershed size, and basic land use were determined using the perennial stream layers developed by the Missouri Resource Assessment Partnership (MoRAP).

Prior to this project, the RAM program could only provide a ranking of the fish community by stream size. Through informal discussions among Missouri members of the EPA Region 7 Biocriteria Workgroup [Dr. Charles Rabeni (UMC), Randy Sarver (MDNR) and Matt Combes (MDC)] it was agreed that in order to improve both MDNR and MDC abilities' to assess the biological integrity and/or impairment of Missouri's rivers and streams, the data must be developed into biological criteria for each of the major ecoregions of the state for use in monitoring sites of concern such as the growth areas of Branson and the Lake of the Ozarks thereby meeting the EPA's subobjective 2.2.1 of Goal 2, Improving water quality via watersheds, with the target activity of developing effective water quality standards (WQS) that protect existing high quality waters and achieve fishable and swimmable uses.

Although the development of a stream classification system for Missouri by the Missouri Resource Assessment Partnership (MoRAP) has greatly improved the ecological framework for biological criteria, there are still a number of steps that need to be taken to create biological criteria for fish communities. These basic steps include: 1) Better quantitative criteria for defining reference stream reaches, 2) Calibration and validation of biological metrics and a multi-metric index and 3) Development of criteria that establish the status of the community. The objective of this project was to accomplish steps 2 and 3.

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[1] Biocriteria development for streams of the Mississippi Alluvial Basin was not addressed due to inadequate reference sites.

## Calibration of biological metrics

### *Data validation and usability*

An extensive literature review of criteria development for fish communities was performed prior to the start to ensure the incorporation of the latest techniques and results in the process. A compilation of pertinent publications and citations for this document can be found in Appendix A.

Verified data collected using the EMAP protocol were processed and analyzed by the RAM coordinator (Matt Combes, MDC) using SAS programs developed by EMAP personnel specifically for that protocol EPA/620/R-99/003 "Quantifying Physical Habitat in Wadeable Streams" by Kaufmann, Levine, Robison, Seeliger, and Peck (1999). The EMAP protocol and computer programs provide six general procedures for data verification. These include: data file structure, missing values, allowable ranges, unusual values, plausible channel morphology, and other evaluations of internal logic and consistency. These programs provide data summaries that are spatially representative estimates of the habitat characteristics measured.


Figure 1. Missouri Aquatic Subregions (MoRAP 2004).

### *Selection of reference sites for the two ecoregions*

The next step in biocriteria development was to identify a group of reference sites for each of the ecoregions. There are several methods of determining reference conditions. Reference sites may be selected from a group of streams that are minimally disturbed, or by using known disturbance gradients, historical and paleoecological information, and/or best professional judgment (Hughes and Oberdorff 1999). The committee agreed to use those reference sites previously determined by MDNR and the University of Missouri (Rabeni et al. 1997) to meet reference quality standards based on best professional judgment (BPJ) of the associated water quality, physical habitat, and the benthic invertebrate communities. The available RAM data (collections from over 450 streams) were reviewed to determine all the pre-existing MDNR reference sites that were assessed as a part of the RAM program and to determine other sites which fell within MDNR reference stream reaches. All data were again reviewed for obvious problems such as missing data, prior to entry into the candidate reference data set. Once entered, additional exploratory statistics were conducted using SAS to validate and verify the data prior to further analyses and to identify any outlying data that might indicate data entry errors. The remaining data were inspected so as to retain only those with complete fish, water quality, and physical habitat databases for use in biometric development.

As a result of these procedures 72 candidate reference sites were retained for possible use in either the calibration or validation data sets. After a review by the committee, nine of the candidate reference sites presented in Quarterly Report #1 (September 2006) were dropped for various reasons. An ArcView analysis of possible impacts was developed to aid in the evaluation of the remaining candidate reference sites, and to supplement those numbers. Each of the candidate sites was evaluated for mining, hazardous waste, NPDES, landfills, dams, 303d waters, losing stream sections, large springs, and connectivity to mainstem rivers, using GIS data layers that were downloaded from the University of Missouri's Missouri Spatial Data Information Service website. These layers included:

Ecological Sections and Subsections of State of Missouri — Projection Units: UTM

Geo-dataset delineating the ecological sections and subsections of Missouri.

Stream Valley Segment Classification of State of Missouri — Projection Units: UTM

This data was created as part of the Missouri Aquatic Gap Project. This coverage contains selected arcs from the 1:100,000 National Hydrography Dataset (NHD) that was developed by the USGS and EPA. The selected arcs represent the centerlines of wide streams.

Biological Reference Stream Segments of State of Missouri — Projection Units: UTM

Biological reference stream segments are segments of streams that represent the best stream conditions for support of aquatic life for a given area. The spatial framework for these areas is the Ecological Drainage Unit (EDU).

Missouri Dams of State of Missouri — Projection Units: UTM

This data set contains the locations of regulated and non-regulated dams in Missouri.

Inventory of Mines, Occurrences, and Prospects in Missouri of State of Missouri — Projection Units: UTM

| | |
|---|---|
| This data set contains a partial inventory of mines, occurrences, and prospects for the State of Missouri. | |
| Missouri Department of Natural Resources Hazardous Waste Program - Permits of State of Missouri | Projection Units: UTM |
| This data set contains sites permitted to treat, store or dispose of hazardous waste and facilities that are certified for resource recovery. Some of the permitted sites have known or suspected hazardous contamination. | |
| Landfills of State of Missouri | Projection Units: UTM |
| This data set contains locations for all permitted active landfills in Missouri. | |
| National Pollutant Discharge Elimination System (NPDES) Outfalls of State of Missouri | Projection Units: UTM |
| This is a point data set depicting outfall locations of wastewater facilities with Missouri NPDES Operating Permits. The permittee through permit application provided attribute information. Locational data was obtained using a variety of methods. | |
| Missouri 2002 303(d) Listed Waters of State of Missouri | Projection Units: UTM |
| Line work representing streams, lakes and reservoirs were selected from the USGS 1:100,000 NHD files using the Missouri 2002 303(d) list. Only those features on the 303(d) list appear in this shapefile. | |
| Department of Natural Resources - State Losing Streams - 2006 of State of Missouri | Projection Units: UTM |
| This data set contains stream segments classified by the Missouri Department of Natural Resources, Division of Geology and Land Survey (DGLS). Stream segments are classified as either losing or gaining. | |
| Department of Natural Resources - State Known Spring Locations - 2006 of State of Missouri | Projection Units: UTM |
| Known spring locations. | |

As a result of these efforts, 43 reference sites were selected by the committee for use in the calibration data set (n = 26 from the Ozark ecoregion, n = 17 from the Plain ecoregion) to define the natural variation of the fish communities within the state (Appendix B, Figure 2).

## *Defining the reference conditions within the Plain and Ozark ecoregions*

### Background information on the two ecoregions

The Ozark ecoregion is characterized by limestone and dolomite bedrocks with upland elevations commonly above 1000 feet and local relief along major streams greater than 300 feet. Streams within this ecoregion usually occur in narrow, sinuous, entrenched valleys and may have high bluffs. Gradients are high and the channels follow a pattern of well-defined riffles and pools. Substrates are coarse and water clarity is high (Pflieger 1989; Nigh and Schroeder 2002).

The Plain ecoregion is characterized by shale and thin sandstone bedrock with limestone outcroppings along the big river areas. Loess and glacial till blanket this area varying from near absence to over 300 feet deep. Elevations away from large rivers exceed 1000 feet but local relief is typically less than 200 feet. Prior to settlement, streams in this area were meandering. Today, channels are straighter with high alluvial banks. Pools are longer than in the Ozarks, and riffles are generally lacking. Stream sediments are fine with silt and sand being the most common substrates (Pflieger 1989; Nigh and Schroeder 2002).

Justification for development of separate biocriteria for the ecoregions

Selection of the final reference sites for use in biocriteria development (the calibration data set) was finalized by the committee after consideration of the available GIS data, physical habitat data, and field knowledge of the proposed sites for which there was available fish community data. The committee agreed upon 17 reference sites from the Plain ecoregion and 26 sites from the Ozark ecoregion. Although Strahler order ranges from 2 – 4 for the Plain ecoregion, and 3 – 5 for the Ozark ecoregion, neither the mean watershed size or channel length sampled were significantly different between the reference sites from each ecoregion (172 km$^2$/268 m for the Plain and 173 km$^2$/276 m for the Ozark). Mean fish species richness was significantly higher in the Ozark ecoregion (22.0 v. 17.5, $p$ = 0.002).

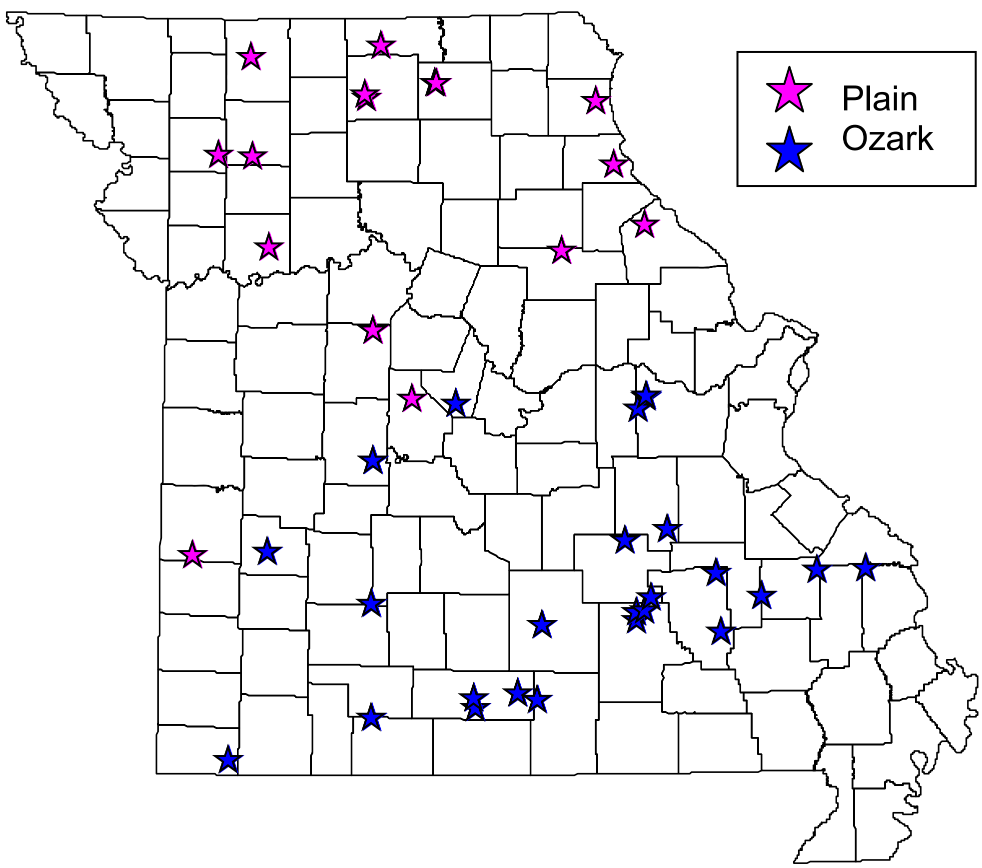



Figure 2. Final reference sites used in the calibration data set for the RAM biocriteria project.

The fish collection data for the reference sites from both ecoregions were evaluated using Detrended Correspondence Analyses (DCA) to confirm the need for separate biocriteria for the two ecoregions (Ozark and Plain). DCA was performed using PC-ORD (version 5.0, MjM software, Gleneden Beach, Oregon) with rare species downweighted. In DCA the sites are distributed along the axes based on their similarity in species composition, with equal distances in the ordination corresponding to equal differences in species composition, and with axis 2 derived independently of axis 1. DCA showed two distinct sites (Figure 3) with a slight mixing in the middle due to sites from EDU 26 (the Ozark border). This area has been shown with invertebrate data to be a transitional area between the two ecoregions (Rabeni and Doisy 2000) and is treated as part of the Ozark ecoregion in these analyses.

The fish community of the reference sites in the Plain ecoregion was represented by 52 species. The 17 sites were dominated by seven species: the red shiner, *Cyprinella lutrensis* (18%); the central stoneroller, *Campostoma anomalum* (16%); the bigmouth shiner, *Notropis dorsalis* (13%); the bluntnose minnow, *Pimephales notatus* (12%); the creek chub, *Semotilus atromaculatus* (6%); the sand shiner, *Notropis stramineus* (6%), and the green sunfish, *Lepomis cyanellus* (6%). The fish community of the reference sites in the Ozark ecoregion was represented by 75 species. The 26 sites were dominated by six species: the central stoneroller, *Campostoma anomalum* (18%); the bleeding shiner, *Luxilus zonatus* (11%); the largescale stoneroller, *Campostoma oligolepis* (9%); the Ozark minnow, *Notropis nubilus* (8%); the orangethroat darter, *Etheostoma specatabile* (5%); and the longear sunfish, *Lepomis megalotis* (5%).

Wilcoxon rank sum tests of the water quality and GIS data showed significant differences in the soil, water quality, and land use of two ecoregions (Table 1). These results, along with significantly higher species richness in the Ozark ecoregion and dominance by different species, support our development of separate biocriteria for the two ecoregions. This regionalization should help account for as much natural variation in the metrics as possible, enhancing the ability of the index to detect the effects of anthropogenic influences on the fish communities.

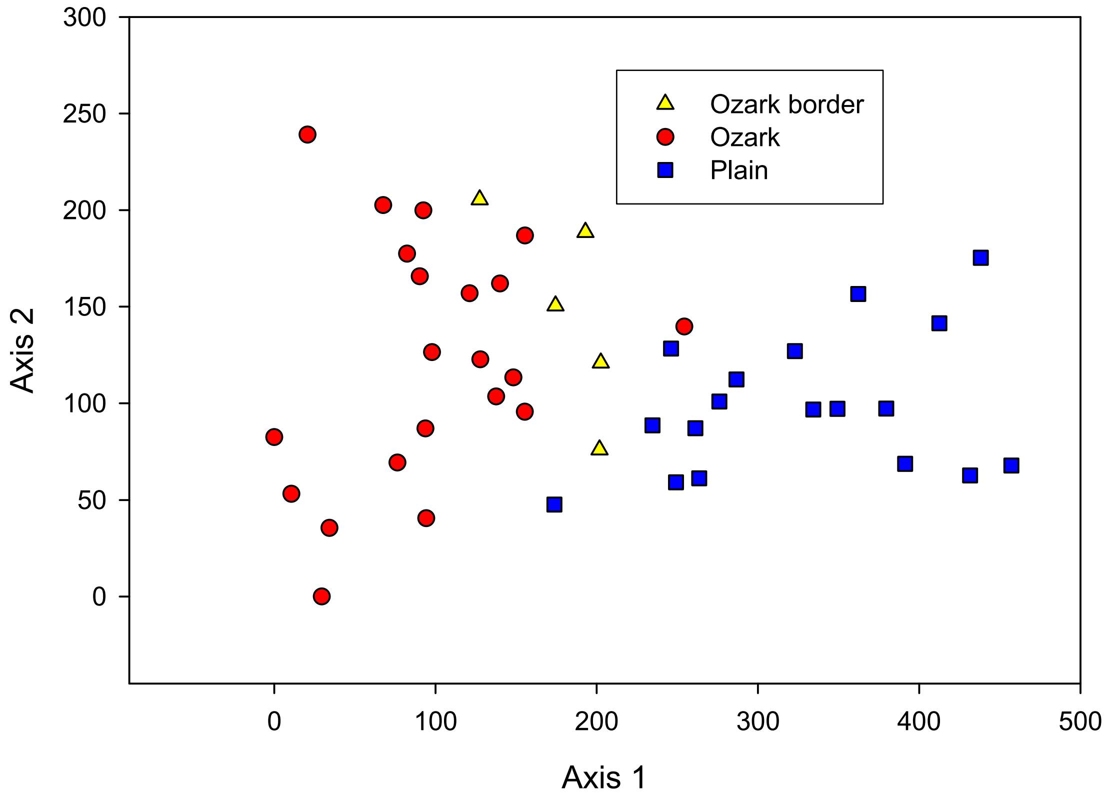



Figure 3. Detrended Correspondence Analysis of the Plain and Ozark (including the Ozark border) ecoregion reference sites.

### *Evaluation of the environmental variables*

In addition to sampling the fish communities of all the selected streams, the EMAP protocol for quantifying the associated physical habitat was followed, allowing for detailed characterization of each stream with over 400 variables related to measures of the bank angle, bankfull, canopy, embeddedness, fish cover, habitat type, residual pools, sinuosity, slope, substrate, thalweg, woody debris, and human disturbance (Kaufmann et al. 1999). We retained 130 of these variables that were relevant to conditions found in Missouri. These variables, along with basic GIS landcover and water quality data, were analyzed with Spearman rank correlations to test for redundancy ($R_s \geq 0.80$). If two or more variables were redundant, the most ecologically relevant variable was retained. After removal of redundant variables, there were 73 remaining variables (Appendix C). Differences in the environmental variables of the reference conditions for the two ecoregions were determined using Wilcoxon rank sum tests (Table 1). Analyses of basic GIS data showed that mean slope of the local watershed, along with hydrologic soil group B and coarse soils are significantly higher in the Ozark ecoregion. Land cover analyses indicated that row crop cover is significantly higher in the watersheds of the reference sites of the Plain ecoregion, while forest cover is significantly higher in those within the Ozark ecoregion. However, the slope and sinuosity of the sampled reaches are not significantly different for the two ecoregions. Basic water quality[1] appears to vary only in conductivity and turbidity (turbidity was very low in both ecoregions).

The majority of the significant differences exist within the substrate data. The Ozark ecoregion has significantly higher percentages of rough bedrock and coarse and fine gravel, while the Plain ecoregion has higher sand, fine sediment and embeddedness. There are no significant differences in bank angle or thalweg between the two ecoregions. Bankfull width is greater in the Ozark ecoregion, while channel incision height is greater in the Plain ecoregion. Significantly higher percentages of reaches within the Ozark ecoregion were fast water habitat, while the Plain ecoregion contained higher percentages of pool habitat. However, residual pool measurements indicate no significant differences in number or volume between the two ecoregions. Measures of fish cover indicate no significant differences except for higher macrophyte cover in the Ozark ecoregion, while the Plain ecoregion has higher levels of canopy cover. There are no differences in available woody debris.

### *Relations of the fish communities of each ecoregion to the environmental variables*

Detrended Correspondence Analyses (DCA) of the fish communities within the reference sites of the Plain and Ozark ecoregions were performed separately. Ordinations were followed with an independent assessment of the importance of the environmental variables by relating them to the coordinates (or scores) of the sites along the 2 dominant axes. Because data for many of the environmental variables could not be normalized, correlations between all variables including the ordination site scores were determined using Spearman's two-tailed rank correlation method. Alphas (0.05) for all correlations were adjusted using the Bonferroni multiple test procedure to minimize type I errors that might occur because of the high number of comparisons. There were no significant relations for the fish communities of the reference sites from the Plain or Ozark ecoregions with any of the land cover or human disturbance variables. The fish communities of the Ozark ecoregion were significantly related to the mean slope of the local watershed and the percent of the pool head length with fine sediment. The fish communities of the Plain ecoregion were significantly related to the percent of substrate < 2 mm in diameter and the number, volume and length of residual pools in the reach.

[1] These data are based on only one sample taken during collection of the phyhab data.

| Table 1. Mean values and significance of Wilcoxon rank sum tests of the phyhab and other variables for the two ecoregions in the RAM biocriteria project. | PLAIN (n=17) mean | OZARK (n=26) mean | *p* - value |
|---|---|---|---|
| LANDCOV1 - percentage of urban land cover in watershed | 0.13 | 0.08 | NS |
| LANDCOV2 - percentage of row crop cover in watershed | 32.1 | 2.7 | <0.0001 |
| LANDCOV3 - percentage of grassland in watershed | 45.2 | 33.2 | NS |
| LANDCOV4 - percentage of forest cover in watershed | 17.0 | 62.6 | <0.0001 |
| LANDCOV6 - percentage of water cover in watershed | 0.27 | 0.10 | 0.048 |
| Water temperature – degrees centigrade | 23.4 | 24.2 | NS |
| Dissolved oxygen – mg/l | 6.9 | 6.2 | NS |
| Conductivity – umhoms/cm | 430 | 338 | 0.002 |
| pH – standard units | 7.7 | 7.8 | NS |
| Turbidity - NTU | 16.0 | 0.8 | 0.0001 |
| MNSLOPE - mean slope of local watershed | 5.7 | 13.3 | <0.0001 |
| HGB_IP - percentage of local segmentshed in hydrologic soil group B | 22.4 | 59.1 | 0.003 |
| HGD_IP - percentage of local segmentshed in hydrologic soil group D | 15.8 | 0.7 | NS |
| COARS_SL - percentage of watershed in coarse soils | 1.2 | 63.3 | <0.0001 |
| Sinuosity | | | |
| SINU = 'Channel Sinuosity (m/m)' | 1.21 | 1.12 | NS |
| Slope | | | |
| XSLOPE = 'Channel Slope -- reach mean (%)' | 0.47 | 0.57 | NS |
| Embeddedness export | | | |
| XCEMBED = 'Mean Embeddedness--Channel (%)' | 67.6 | 24.4 | 0.0001 |
| Substrate | | | |
| SUB_X = 'Substrate--Mean Size Class (1-6)' | 2.7 | 3.3 | 0.036 |
| PCT_RR = 'Substrate Rough Bedrock (%)' | 0 | 3.6 | 0.014 |
| PCT_RS = 'Substrate Smooth Bedrock (%)' | 4.0 | 1.2 | 0.030 |
| PCT_CB = 'Substrate Cobbles -- 64-250 mm (%)' | 11.2 | 18.7 | 0.015 |
| PCT_GC = 'Substrate Coarse Gravel -- 16-64 mm (%)' | 16.8 | 41.4 | 0.0009 |
| PCT_GF = 'Substrate Fine Gravel -- 2-16 mm (%)' | 4.7 | 16.0 | 0.0005 |
| PCT_SA = 'Substrate Sand -- .06-2 mm (%)' | 35.8 | 4.4 | 0.0008 |
| PCT_FN = 'Substrate Fines -- Silt/Clay/Muck (%)' | 18.1 | 8.7 | 0.021 |
| PCT_HP = 'Substrate Hardpan -- (%)' | 4.1 | 0.6 | 0.026 |
| PCT_SAFN = 'Substrate Sand & Fines -- <2 mm (%)' | 53.9 | 13.1 | <0.0001 |
| PCT_BDRK = 'Substrate Bedrock (%)' | 4.0 | 4.8 | NS |
| Bank angle export | | | |
| XBKA = 'Bank Angle--mean (degrees)' | 30.9 | 30.4 | NS |
| XUN = 'Undercut Distance--Mean (m)' | 0.01 | 0.02 | NS |
| Bankfull export | | | |
| XINC_H = 'Channel Incision Ht.-Mean (m)' | 3.2 | 1.9 | <0.0001 |
| XBKF_W = 'Bankfull Width--Mean (m)' | 16.5 | 26.2 | 0.0002 |
| XBKF_H = 'Bankfull Height-Mean (m)' | 0.77 | 0.86 | NS |

Table 1 continued.

| | | | |
|---|---|---|---|
| Thalweg | | | |
| WD_RAT = 'Mean Width/Depth Ratio (m/m)' | 43.0 | 51.4 | NS |
| Habitat type export | | | |
| PCT_PB = 'Backwater Pool (% of reach length)' | 1.8 | 0.04 | 0.0019 |
| PCT_FAST = 'Fast Wtr Hab (% riffle & faster)' | 9.1 | 24.0 | 0.0004 |
| PCT_POOL = 'Pools -- All Types (% of reach)' | 56.3 | 30.9 | 0.014 |
| Residual pool labels | | | |
| NRP = 'Number of residual pools in reach' | 10.2 | 8.8 | NS |
| PCTRCHRP = 'Resid. pool length percentage (%of rch)' | 84.4 | 84.3 | NS |
| RPGT50 = 'Resid Pools >50cm deep (number/reach)' | 1.71 | 1.92 | NS |
| RPGT75 = 'Resid Pools >75cm deep (number/reach)' | 0.94 | 1.0 | NS |
| RPMDEP = 'Maximum residual depth in reach (cm)' | 97.9 | 98.3 | NS |
| RPMLEN = 'Max. resid pool length in reach (m/pool)' | 78.0 | 90.1 | NS |
| RPMWID = 'Max resid width of any pool in reach (m)' | 13.1 | 15.9 | 0.01 |
| RPMVOL = 'Max volume of any pool in reach (m^3)' | 169.9 | 195.9 | NS |
| PCTUSED = '% of pool head length with sediment' | 87.4 | 65.9 | 0.008 |
| Fish cover export | | | |
| XFC_ALG = 'Fish Cvr-Filamentous Algae (Areal Prop)' | 0.03 | 0.02 | NS |
| XFC_AQM = 'Fish Cvr-Aq. Macrophytes (Areal Prop)' | 0.02 | 0.11 | 0.0001 |
| XFC_BRS = 'Fish Cvr-Brush&Small Debris (Areal Prop)' | 0.07 | 0.07 | NS |
| XFC_HUM = 'Fish Cvr-Artif. Structs. (Areal Prop)' | 0.002 | 0.002 | NS |
| XFC_LWD = 'Fish Cvr-Large Woody Debris (Areal Prop)' | 0.05 | 0.07 | NS |
| XFC_NAT = 'Fish Cvr-Natural Types (Sum Areal Prop)' | 0.28 | 0.31 | NS |
| XFC_OHV = 'Fish Cvr-Overhang Veg (Areal Prop)' | 0.03 | 0.07 | NS |
| XFC_RCK = 'Fish Cvr-Boulders (Areal Prop)' | 0.08 | 0.07 | NS |
| XFC_UCB = 'Fish Cvr-Undercut Banks (Areal Prop)' | 0.04 | 0.02 | NS |
| Canopy | | | |
| XPCAN = 'Rip Canopy Present (Fraction of reach)' | 0.91 | 0.76 | 0.004 |
| XPMID = 'Rip MidLayer Present (Fraction of reach)' | 0.96 | 0.87 | 0.007 |
| XPGVEG = 'Rip Ground Layer Present (Fract. reach)' | 0.99 | 0.94 | 0.027 |
| Canopy export | | | |
| XCDENBK = 'Mean Bank Canopy Density (%)' | 85.0 | 76.0 | NS |
| XCDENMID = 'Mean Mid-channel Canopy Density (%)' | 63.6 | 46.0 | 0.007 |
| Woody debris | | | |
| C1Wm100 = 'LWD in Bkf chnl (#/100m-all sizes)' | 10.7 | 7.3 | NS |
| V1Wm100 = 'LWD Vol in Bkf chnl (m3/100m-all sizes)' | 11.4 | 6.1 | NS |

## Metric selection and evaluation

### *Candidate metrics*

The candidate metrics that were selected focused on native species because the wadeable streams of Missouri that were sampled contained less than 1% non-native species (Matt Combes, personal communication). Autecology of fish species was determined by a panel of regional experts put together by MDC. Candidate metrics calculated by the EMAP SAS programs (some of which have been modified by MDC to apply to MO fish communities) and their predicted responses to impairment are listed in Table 2. In addition to the 34 candidate metrics calculated by the EMAP program, eight additional metrics were calculated that represent variants of lithophilic species composition in an effort to include a measure of reproductive condition, and percentages of dominant species for a total of 42 candidate metrics. Each of these 42 metrics was calculated for each of the calibration reference sites for both ecoregions (n = 26 for the Ozark ecoregion, and n = 17 for the Plain ecoregion).

The candidate metrics were tested for significant differences using Wilcoxon rank sum tests of the reference sites for the two ecoregions to further justify the development of different biocriteria for the two ecoregions (Table 3). The Ozark ecoregion had significantly higher numbers of species, darter species, small benthic species, round bodies sucker species, benthic species, water column species, insectivore cyprinid species, long-lived species, insectivore and invertivores species, all types of lithophilic species, non-guarding lithophilic species, and water column specialist feeder species. The Ozark ecoregion also had higher percentages of darter individuals, small benthic individuals, round bodies sucker individuals, benthic individuals, omnivore and herbivore individuals, insectivore cyprinid individuals, insectivore and invertivores individuals, and carnivore individuals, while the Plain ecoregion had a higher number and percentage of simple lithophilic species, and percentages of tolerant and carnivore individuals.

Table 2. Candidate metrics for development of biocriteria for the fish communities of the Plain and Ozark ecoregions in Missouri and their predicted response to impairment.

| Metric | | Predicted response |
|---|---|---|
| numnativ | Number of native individuals | negative |
| numspec | Number of native species | negative |
| numnatfm | Number of native families | negative |
| nsnsen | Number of native sensitive species | negative |
| pnsen | Percentage of native sensitive individuals | negative |
| nsntole | Number of native tolerant species | positive |
| pntole | Percentage of native tolerant individuals | positive |
| nsndart | Number of native darter species | negative |
| pndart | Percentage of native darter individuals | negative |
| nsnsmben | Number of native small benthic species | negative |
| pnsmben | Percentage of native small benthic individuals | negative |
| nsnrbs | Number of native round bodies sucker species | negative |
| pnrbs | Percentage of native round bodies sucker individuals | negative |
| nsnbenth | Number of native benthic species | negative |
| pnbenth | Percentage of native benthic individuals | negative |
| nsnwcol | Number of native water column species | negative |
| pnwcol | Percentage of native water column individuals | negative |
| nsnlunk | Number of native long lived species | negative |
| pnlunk | Percentage of native long lived individuals | negative |
| nsnincyp | Number of native insectivore cyprinid species | negative |
| pnincyp | Percentage of native insectivore cyprinid individuals | negative |
| nsintro | Number of introduced species | positive |
| numintro | Number of introduced individuals | positive |
| pintro | Percentage of introduced individuals | positive |
| pnativ | Percentage of native individuals | negative |
| nsnsnfsh | Number of native sunfish species | negative |
| pnsnfsh | Percentage of native sunfish individuals | negative |
| nsnminn | Number of native minnow species | negative |
| pnminn | Percentage of native minnow individuals | negative |
| nsnomhb | Number of native omnivore and herbivore species | positive |
| pnomhb | Percentage of native omnivore and herbivore individuals | positive |
| nsnisiv | Number of native insectivore and invertivore species | negative |
| pninsiv | Percentage of native insectivore and invertivore individuals | negative |
| nsncarn | Number of native carnivore species | negative |
| pncarn | Percentage of native carnivore individuals | negative |
| persimp | Percentage of native simple lithophilous individuals | negative |
| pernong | Percentage of native non-guarding lithophilous individuals | negative |
| simprich | Number of native simple lithophilous species | negative |
| nongrich | Number of native non-guarding lithophilic species | negative |
| perall | Percentage of all native lithophilic individuals | negative |
| allrich | Number of all native lithophilic species | negative |
| nsnwcsp | Number of species of native water column specialists feeders | negative |
| pdom | Percentage of the top dominant species | positive |
| pdom3 | Percentage of the three dominant species | positive |

| Table 3. Significant differences in the fish community metrics between the two ecoregions using Wilcoxon rank sum tests ($p < 0.05$ for significance). | Ozark<br>n = 26 | Plain<br>n = 17 | *p* value |
|---|---|---|---|
| Number of native individuals | 669.4 | 641.6 | NS |
| Number of native species | 22.0 | 17.5 | 0.003 |
| Number of native families | 7.1 | 6.2 | NS |
| Percentage of native tolerant individuals | 0.04 | 0.32 | <0.0001 |
| Number of native tolerant species | 1.80 | 3.90 | <0.0001 |
| Number of native sensitive species | 0.23 | 0.00 | NS |
| Percentage of native sensitive individuals | 0.002 | 0.00 | NS |
| Percentage of native darter individuals | 0.11 | 0.04 | 0.0003 |
| Number of native small benthic species | 5.5 | 3.7 | 0.002 |
| Percentage of native small benthic individuals | 0.16 | 0.10 | 0.025 |
| Number of native round bodies sucker species | 1.80 | 0.60 | 0.001 |
| Percentage of native round bodies sucker individuals | 0.02 | 0.01 | 0.001 |
| Number of native benthic species | 7.3 | 4.2 | <0.0001 |
| Percentage of native benthic individuals | 0.18 | 0.11 | 0.001 |
| Number of native water column species | 9.0 | 6.5 | 0.001 |
| Percentage of native water column individuals | 0.37 | 0.43 | NS |
| Number of native long-lived species | 12.2 | 9.3 | 0.004 |
| Percentage of native long-lived individuals | 0.30 | 0.38 | NS |
| Number of native insectivore cyprinid species | 2.8 | 1.9 | 0.026 |
| Percentage of native insectivore cyprinid individuals | 0.25 | 0.16 | 0.03 |
| Number of native sunfish species | 2.7 | 2.4 | NS |
| Percentage of native sunfish individuals | 0.1 | 0.1 | NS |
| Number of native minnow species | 7.7 | 8.1 | NS |
| Percentage of native minnow individuals | 0.68 | 0.70 | NS |
| Number of native omnivore and herbivore species | 5.4 | 5.5 | NS |
| Percentage of native omnivore and herbivore individuals | 0.44 | 0.35 | NS |
| Number of native insectivore and invertivore species | 7.1 | 4.2 | 0.0001 |
| Percentage of native insectivore and invertivore individuals | 0.18 | 0.11 | 0.003 |
| Number of native carnivore species | 2.7 | 2.1 | 0.012 |
| Percentage of native carnivore individuals | 0.03 | 0.12 | 0009 |
| Percentage of native simple lithophilous individuals | 0.08 | 0.17 | 0.022 |
| Number of native simple lithophilous species | 2.8 | 4.1 | 0.022 |
| Percentage of native non-guarding lithophilous individuals | 0.18 | 0.19 | NS |
| Number of native non-guarding lithophilic species | 6.3 | 4.9 | 0.04 |
| Percentage of all native lithophilic individuals | 0.66 | 0.79 | 0.0209 |
| Number of all native lithophilic species | 16.8 | 14.3 | 0.019 |
| Number of native water column specialist feeders | 7.1 | 5.5 | 0.022 |
| Percentage of the top dominant species | 0.30 | 0.34 | NS |
| Percentage of the three dominant species | 0.60 | 0.62 | NS |

### *Evaluating the candidate metrics using the reference sites*

Each potential metric was evaluated based on the 4-step process developed by Hughes et al. (1998). First the candidate metrics are evaluated for range, normality, and variability within the reference condition. Retained metrics need to vary adequately across reference sites with a symmetrical distribution and no extreme outliers. In the second step metrics are evaluated for their responsiveness to anthropogenic disturbance. Metrics that respond the most strongly to human influence such as significantly different t-tests or box plots between reference and impaired sites, or significant relations with at least 3 measures—and no significant relations to other natural variables such as watershed size, gradient, sinuosity, or soil types—should be retained. The third step is to evaluate the precision of each metric. Metrics should discriminate among reaches but remain relatively constant at the same site. Finally, metrics need to be evaluated for redundancy. If two responsive metrics have a Spearman ($R_s$) greater than 0.75 the metric with the most significant correlations to human influence variables should be retained. For these data, candidate metrics were eliminated if they did not meet the assumptions of step 1. However, after those initial eliminations, no metrics were eliminated until steps 2, 3, and 4 were performed to allow for a comprehensive evaluation of the remaining metrics.

Range, normality, and variability within the reference condition

First, metrics were evaluated for their range within the reference condition. Candidate metrics with extreme outliers (> or < 2 standard deviations from the mean) and those composed of three or less species were eliminated. This resulted in the elimination of the number and percentage of native sensitive species for the Plain ecoregion, and the number and percentage of native or introduced species and individuals from both ecoregions. The candidate metrics were then evaluated for normality (Shapiro-Wilk test) and low variability [Coefficient of variation (CV) <100] using the reference site data for each ecoregion. The metrics that met these conditions were retained for that ecoregion, while those that were eliminated are indicated by hyphens in the columns under the *p* value and CV (Table 4). Following this process the candidate metrics for the reference site data were correlated with watershed size within each ecoregion separately using Pearson correlations to see if they would require adjustment as described by Emery et al. (2003). For the Ozark ecoregion (n = 26) two metrics were positively correlated with watershed size indicating they would need adjustment if used in the final IBI: number of non-guarding lithophilic species and number of native insectivore and invertivore species. For the Plain ecoregion (n = 17) the percentage of native tolerant individuals and percentage of native water column individuals were positively related to watershed size, while percentage of native omnivore and herbivore individuals and percentage of all lithophilic individuals were negatively correlated with watershed size.

| Table 4. Normality (Shapiro – Wilk test) of each metric for the reference sites within each ecoregion (*p* values >0.05 indicate normally distributed data) followed by the Coefficient of Variation. Metrics that were close to normality were retained. | Ozark (n=26) | | Plain (n=17) | |
|---|---|---|---|---|
| | normal | CV | normal | CV |
| Number of native individuals | 0.07 | 55 | 0.33 | 59 |
| Number of native species | 0.63 | 19 | 0.82 | 23 |
| Number of native families | - | - | 0.68 | 32 |
| Number of native sensitive species | - | - | - | - |
| Percentage of native sensitive individuals | - | - | - | - |
| Number of native tolerant species | - | - | - | - |
| Percentage of native tolerant individuals | - | - | 0.86 | 56 |
| Percentage of native darter individuals | 0.71 | 54 | 0.07** | 48 |
| Number of native darter species | (0.04) | 35 | - | - |
| Number of native small benthic species | 0.55 | 30 | 0.11 | 53 |
| Percentage of native small benthic individuals | 0.12 | 54 | 0.11 | 74 |
| Number of native round bodies sucker species | - | - | - | - |
| Percentage of native round bodies sucker individuals | 0.17** | 67 | - | - |
| Number of native benthic species | 0.07 | 30 | 0.37* | 34 |
| Percentage of native benthic individuals | 0.053 | 39 | 0.16 | 64 |
| Number of native water column species | (0.037) | 24 | 0.16* | 16 |
| Percentage of native water column individuals | 0.31** | 28 | 0.73 | 43 |
| Number of native long-lived species | 0.14 | 21 | - | - |
| Percentage of native long-lived individuals | 0.40 | 35 | 0.14** | 41 |
| Number of native insectivore cyprinid species | - | - | - | - |
| Percentage of native insectivore cyprinid individuals | 0.12 | 59 | 0.38** | 55 |
| Number of introduced species | - | - | - | - |
| Number of introduced individuals | - | - | - | - |
| Percentage of introduced individuals | - | - | - | - |
| Number of native sunfish species | (0.03) | 40 | - | - |
| Percentage of native sunfish individuals | 0.35** | 54 | 0.40** | 61 |
| Number of native minnow species | 0.09 | 24 | 0.10 | 24 |
| Percentage of native minnow individuals | 0.21 | 15 | 0.36 | 29 |
| Number of native omnivore and herbivore species | 0.053 | 24 | - | - |
| Percentage of native omnivore and herbivore individuals | - | - | 0.63 | 69 |
| Number of native insectivore and invertivore species | 0.15 | 30 | 0.37* | 34 |
| Percentage of native insectivore and invertivore individuals | 0.05 | 42 | 0.16 | 64 |
| Number of native carnivore species | - | - | - | - |
| Percentage of native carnivore individuals | 0.52** | 44 | 0.27** | 60 |
| Percentage of native simple lithophilous individuals | - | - | 0.10** | 60 |
| Number of native simple lithophilous species | - | - | 0.18* | 33 |
| Percentage of non-guarding lithophilous individuals | - | - | 0.32** | 49 |
| Number of native non-guarding lithophilic species | 0.14* | 20 | - | - |
| Percentage of all native lithophilic individuals | 0.69 | 20 | 0.75** | 20 |
| Number of all native lithophilic species | 0.30 | 19 | 0.41 | 24 |
| Number of native water column specialists feeders | 0.068 | 29 | 0.18* | 22 |
| Percentage of the top dominant species | - | - | 0.34 | 38 |
| Percentage of the three dominant species | 0.05** | 13 | 0.52 | 20 |

* Significant with a natural log transformation.
** Significant with an ARCSIN Square root transformation.

### Responsiveness of metrics to anthropogenic disturbance

Unpaired t-tests.—The second step of metric evaluation is testing for responsiveness (or sensitivity) to anthropogenic disturbances for those metrics that were normally distributed and had low variation. First, unpaired t-tests were run using the calibration reference sites against groups of impaired sites that were suggested by MDNR and MDC personnel using best professional judgment. There were 18 impaired sites for the Ozark ecoregion and 11 impaired sites for the Plain ecoregion for which a full complement of physical habitat variables, land cover, and metrics were available. A portion of these sites was retained for each ecoregion for later use in validation testing of the IBI, leaving 12 impaired sites for calibration testing in the Ozark ecoregion, and 8 impaired sites for calibration testing in the Plain ecoregion. Results (Table 5) showed 13 metrics that were significantly different within the reference and impaired sites from the Ozark ecoregion, and 8 for the Plain ecoregion when the *p* value was increased to 0.10.

| Table 5. Unpaired t-tests of the reference and impaired stream metrics that were retained for each ecoregion (*p*<0.10 for significance). | Ozark Ref = 26 | Ozark Imp = 12 | Ozark *p* value | Plain Ref = 17 | Plain Imp = 8 | Plain *p* value |
|---|---|---|---|---|---|---|
| Number of native individuals | 669.4 | 506.8 | NS | 641.6 | 346.3 | 0.06 |
| Number of native species | 22.0 | 18.0 | 0.010 | 17.5 | 14.8 | NS |
| Number of native families | - | - | - | 6.2 | 6.0 | NS |
| Percentage of native tolerant individuals | - | - | - | 0.32 | 0.35 | NS |
| Percentage of native darter individuals | 0.11 | 0.11 | NS | 0.04 | 0.06 | NS |
| Number of native darter species | 3.6 | 2.2 | 0.003 | - | - | - |
| Number of native small benthic species | 5.5 | 3.9 | 0.006 | 3.7 | 3.3 | NS |
| Percentage of native small benthic individuals | 0.16 | 0.15 | NS | 0.10 | 0.20 | 0.005 |
| Percentage of native round bodied sucker individuals | 0.02 | 0.02 | NS | - | - | - |
| Number of native benthic species | 7.3 | 4.5 | 0.006 | 4.2 | 3.4 | NS |
| Percentage of native benthic individuals | 0.18 | 0.17 | NS | 0.10 | 0.20 | 0.005 |
| Number of native water column species | 9.0 | 6.7 | 0.005 | 6.5 | 5.6 | NS |
| Percentage of native water column individuals | 0.37 | 0.34 | NS | 0.43 | 0.43 | NS |
| Number of native long-lived species | 12.2 | 9.9 | 0.020 | - | - | - |
| Percentage of native long-lived individuals | 0.30 | 0.37 | NS | 0.38 | 0.34 | NS |
| Percentage of native insectivore cyprinid individuals | 0.25 | 0.15 | 0.059 | 0.16 | 0.07 | 0.06 |
| Number of native sunfish species | 2.7 | 2.3 | NS | - | - | - |
| Percentage of native sunfish individuals | 0.08 | 0.09 | NS | 0.14 | 0.10 | NS |
| Number of native minnow species | 7.7 | 6.4 | 0.032 | 8.1 | 5.9 | 0.018 |
| Percentage of native minnow individuals | 0.68 | 0.68 | NS | 0.70 | 0.64 | NS |
| Number of native omnivore & herbivore species | 5.4 | 4.8 | NS | 5.5 | 4.0 | 0.031 |
| Percentage of native omnivore & herbivore individuals | - | - | - | 0.35 | 0.30 | NS |
| Number of native insectivore & invertivore species | 7.1 | 5.0 | 0.009 | 4.3 | 3.7 | NS |
| Percentage of insectivore & invertivore individuals | 0.18 | 0.17 | NS | 0.11 | 0.20 | 0.005 |
| Percentage of native carnivore individuals | 0.03 | 0.05 | NS | 0.12 | 0.08 | NS |
| Percentage of simple lithophilous individuals | - | - | - | 0.18 | 0.17 | NS |
| Number of native simple lithophilous species | - | - | - | 4.0 | 3.4 | NS |

| Table 5 continued. | Ozark<br>Ref = 26 | <br>Imp = 12 | <br>$p$ value | Plain<br>Ref = 17 | <br>Imp = 8 | <br>$p$ value |
|---|---|---|---|---|---|---|
| Percentage non-guarding lithophilous individuals | - | - | - | 0.20 | 0.28 | NS |
| Number of native non-guarding lithophilic species | 6.3 | 4.5 | 0.011 | - | - | - |
| Percentage of all native lithophilic individuals | 0.66 | 0.74 | NS | 0.79 | 0.68 | NS |
| Number of all native lithophilic species | 16.8 | 13.3 | 0.003 | 14.3 | 11.6 | 0.051 |
| Number of native water column specialist feeder | 7.1 | 5.6 | 0.054 | 5.5 | 4.6 | NS |
| Percentage of the top dominant species | - | - | - | 0.34 | 0.32 | NS |
| Percentage of the three dominant species | 0.60 | 0.68 | 0.014 | 0.62 | 0.62 | NS |

Box plots.—After completion of the unpaired t-tests, box plots were made for the retained metrics from each ecoregion (Appendix D). The “box” in the box plots represents the interquartile range from the 25$^{th}$ percentile to the 75$^{th}$ percentile. The horizontal line within the box represents the median or 50$^{th}$ percentile, while the end points of the vertical lines that extend off either end of the box represent the 10$^{th}$ and 90$^{th}$ percentiles. Any points beyond the 10$^{th}$ and 90$^{th}$ percentile are outliers. The sensitivity of each metric was determined from the box plots based on the scoring system developed by Barbour et al. (1996). Metrics were determined to have a sensitivity of 3 if no overlap existed in the interquartile range (strong discriminatory power), a sensitivity of 2 if there was some overlap that did not extend to the medians, a sensitivity of 1 if there was a moderate overlap of interquartile ranges but at least one median was outside the range, and a sensitivity of 0 if interquartile overlap was considerable with weak discriminatory power between reference and impaired sites.

Results for the Ozark ecoregion showed that none of the metrics were highly sensitive (scoring a 3). The number of native water column species, percentage of native insectivore cyprinid individuals, and number of native lithophilic species scored a value of 2 for sensitivity; the number of native fishes, number of native species, number of native benthic species, number of native darter species, number of native minnow species, and number of insectivore and invertivores species scored a value of 1 for sensitivity; and the number and percentage of native small benthic individuals, percentage of native benthic individuals, number of native sunfish species, percentage of native minnow individuals, number of omnivore and herbivore species, number of long-lived species, percentage of long-lived individuals, percentage of native darter individuals, percentage of insectivore and invertivores individuals, percentage of all native lithophilic individuals, and number of water column specialist individuals all scored a value of 0, meaning weak discriminatory power. None of the metrics for the Plain ecoregion scored a 3; but the number of native fish, percentage of small benthic individuals, percentage of benthic individuals, number of native minnow species, and percentage of insectivore and invertivore individuals scored a 2; and the number of native omnivore and herbivore species, number of all lithophilic species, and percentage of insectivore cyprinid individuals all scored a value of 1.

Problems with the calibration data set for the Plain ecoregion.—However, inspection of the unpaired t-tests average values and the box plots indicate a serious problem regarding the development of biocriteria for the Plain ecoregion data. Of the 8 significant metrics, 4 reflect a response that is the opposite of the prediction (Table 2). The percentage of small benthic individuals, percentage of benthic individuals, and percentage of insectivore and invertivore individuals increased with impairment, while the number of omnivore and herbivore species decreased. One possible explanation was put forth by Matt Combes (MDC Resource Science Division, Resource Scientist),

> "Almost all metrics giving a correct response were based on "number of", but all metrics with incorrect responses were "percentage of". In short, the benthic species found at the impaired sites are the core species in the prairie region so are found at both reference and impaired sites in about equal amounts. However, communities at reference locations have the benthic species plus other species, so the percentage of benthic species is less than at impaired sites where the benthic species are all that's left. Sand shiner, bigmouth shiner, and johnny darters are found in almost all small prairie streams, and are small benthic species. If an impaired site has 10 species and a reference has 20, then the percentage of these common species is .30 and .15 respectively. All this just supports the idea that our prairie reference sites are barely better than our prairie impaired sites."

To further investigate this condition Wilcoxon rank sum tests were performed on all of the retained EMAP variables for the reference and impaired sites in both ecoregions (Table 6). Although the overall measures of physical habitat (the final habitat indices—QCPH, QPH, and QTPH) were significant (or close to significant <0.10) for both the Plain and Ozark ecoregions, the results of the individual environmental variables underscore the inadequate range of physical habitat conditions within the Plain ecoregion. For the Ozark ecoregion 23 variables were significantly different ($p$<0.05) between the reference and impaired streams (excluding the final habitat indices), but only 9 variables were significantly different between the reference and impaired sites for the Plain ecoregion: conductivity, % of backwater pools, mean slope, % cobble substrate, landfills on the bank, number of pieces of large woody debris in the bankfull channel, the % of residual pools in the reach, the amount of sediment in the head of the pools, and the % of hardpan substrate. However in contrast to expectations, the % of backwater pools was higher in the impaired streams of the Plain ecoregion, while the amount of fine sediment in the head of pools and the mean number of landfills on the bank were higher in the reference streams of the Plain ecoregion.

Table 6. Results of Wilcoxon rank sum tests for the reference and impaired streams of the Ozark and Plain ecoregions ($p$<0.05 for significance). See Appendix C for variable definitions.

| | OZARK | | | PLAIN | | |
|---|---|---|---|---|---|---|
| | REF = 26 | IMP = 12 | p value | REF = 17 | IMP = 8 | p value |
| MNSLOPE | 13.3 | 7.2 | 0.0013 | 5.6 | 5.4 | NS |
| HGB_IP | 59.1 | 36.0 | NS | 21.2 | 16.6 | NS |
| HGD_IP | 0.7 | 9.3 | 0.016 | 20.4 | 30.5 | NS |
| LANDCOV1 | 0.1 | 11.9 | 0.002 | 0.14 | 0.35 | NS |
| LANDCOV2 | 2.7 | 9.0 | 0.011 | 33.2 | 24.8 | NS |
| LANDCOV3 | 33.2 | 54.5 | 0.005 | 44.6 | 41.2 | NS |
| LANDCOV4 | 62.6 | 24.1 | <0.0001 | 16.8 | 15.5 | NS |
| LANDCOV6 | 0.1 | 0.40 | <0.0001 | 26.8 | 55.4 | NS |
| TEMP | 23.4 | 22.7 | NS | 24.2 | 23.7 | NS |
| DO | 6.9 | 6.8 | NS | 6.1 | 5.9 | NS |
| CONDUCT | 338.4 | 632.8 | <0.0001 | 429.5 | 676.9 | 0.022 |
| PH | 7.7 | 7.7 | NS | 7.8 | 7.7 | NS |
| NTU | 0.8 | 7.9 | 0.009 | 15.3 | 24.3 | NS |
| XBKA | 30.4 | 38.6 | 0.044 | 31.5 | 36.0 | NS |
| XUN | 0.016 | 0.004 | NS | 0.01 | 0.002 | NS |
| XBKF_W | 26.2 | 20.0 | 0.023 | 16.8 | 18.0 | NS |
| XBKF_H | 0.86 | 0.88 | NS | 0.8 | 1.1 | NS |
| XINC_H | 1.9 | 2.8 | 0.022 | 3.2 | 3.6 | NS |
| XCDENBK | 76.3 | 80.9 | NS | 84.6 | 80.3 | NS |
| XCDENMID | 46.0 | 60.6 | 0.034 | 62.2 | 52.3 | NS |
| XCEMBED | 24.4 | 33.0 | NS | 67.0 | 76.9 | NS |
| XFC_ALG | 0.02 | 0.16 | 0.023 | 0.03 | 0.10 | NS |
| XFC_AQM | 0.11 | 0.04 | NS | 0.02 | 0.04 | NS |
| XFC_LWD | 0.07 | 0.02 | NS | 0.05 | 0.04 | NS |
| XFC_BRS | 0.07 | 0.04 | NS | 0.08 | 0.09 | NS |
| XFC_OHV | 0.07 | 0.03 | NS | 0.03 | 0.02 | NS |
| XFC_UCB | 0.02 | 0.04 | NS | 0.04 | 0.02 | NS |
| XFC_RCK | 0.07 | 0.06 | NS | 0.07 | 0.01 | NS |
| XFC_HUM | 0.0 | 0.03 | NS | 0.0 | 0.0 | -- |
| XFC_NAT | 0.31 | 0.20 | NS | 0.27 | 0.18 | NS |
| PCT_PB | 0.04 | 0.42 | NS | 1.8 | 0.0 | 0.04 |
| PCT_FAST | 24.0 | 12.3 | 0.020 | 9.6 | 8.3 | NS |
| PCT_POOL | 30.9 | 27.3 | NS | 54.0 | 36.8 | NS |
| NRP | 8.8 | 8.3 | NS | 10.2 | 8.8 | NS |
| RPGT50 | 1.9 | 1.4 | NS | 1.8 | 1.6 | NS |
| RPGT75 | 1.0 | 0.8 | NS | 0.94 | 0.44 | NS |
| RPMLEN | 90.1 | 98.7 | NS | 80.7 | 94.7 | NS |
| RPMDEP | 98.3 | 73.9 | NS | 97.9 | 70.0 | NS |
| RPMWID | 15.9 | 11.4 | 0.032 | 13.2 | 11.2 | NS |

| Table 6 continued. | | | | | | |
|---|---|---|---|---|---|---|
| | OZARK | | | PLAIN | | |
| | REF = 26 | IMP = 12 | p value | REF = 17 | IMP = 8 | p value |
| RPMVOL | 195.9 | 120.2 | NS | 185.1 | 182.6 | NS |
| PCTRCHRP | 84.3 | 62.0 | 0.010 | 84.3 | 85.3 | 0.014 |
| PCTUSED | 65.9 | 88.6 | 0.034 | 87.6 | 85.0 | 0.012 |
| SINU | 1.12 | 1.05 | NS | 1.2 | 1.1 | NS |
| XSLOPE | 0.57 | 0.23 | 0.034 | 0.47 | 0.14 | 0.017 |
| SUB_X | 3.3 | 3.2 | NS | 2.7 | 2.2 | NS |
| PCT_CB | 18.7 | 20.2 | NS | 11.2 | 5.3 | 0.019 |
| PCT_FN | 8.7 | 16.7 | NS (0.053) | 18.1 | 32.3 | NS |
| PCT_GC | 41.4 | 32.2 | NS | 17.8 | 16.0 | NS |
| PCT_GF | 16.0 | 10.3 | NS | 4.7 | 13.6 | NS |
| PCT_SA | 4.4 | 5.8 | NS | 33.8 | 28.4 | NS |
| PCT_RS | 1.9 | 6.2 | NS | 3.8 | 1.1 | NS |
| PCT_RR | 3.6 | 0.6 | NS | 0.0 | 0.4 | NS |
| PCT_SAFN | 13.1 | 22.6 | NS | 53.6 | 62.4 | NS |
| PCT_BDRK | 4.8 | 6.8 | NS | 3.8 | 1.6 | NS |
| PCT_HP | 0.63 | 0.0 | NS | 4.1 | 0.0 | 0.016 |
| XWD_RAT | 51.4 | 40.4 | NS | 42.9 | 54.6 | NS |
| XPCAN | 0.76 | 0.88 | 0.049 | 0.91 | 0.84 | NS |
| XPMID | 0.87 | 0.89 | NS | 0.96 | 0.94 | NS |
| XPGVEG | 0.94 | 0.97 | NS | 0.99 | 1.0 | NS |
| C1WM100 | 7.3 | 1.7 | 0.043 | 10.7 | 1.9 | 0.016 |
| V1WM100 | 6.1 | 2.9 | NS | 10.8 | 3.0 | NS |
| BXPLDFL | 0.05 | 0.00 | NS | 0.05 | 0.0 | 0.04 |
| BXPPARK | 0.0 | 0.0 | NS | 0.003 | 0.0 | NS |
| BXPPSTR | 0.01 | 0.0 | NS | 0.003 | 0.0 | NS |
| BXPROAD | 0.01 | 0.0 | NS | 0.005 | 0.0 | NS |
| BXPMINE | 0.02 | 0.0 | NS | 0.0 | 0.0 | NS |
| CXPCROP | 0.0 | 0.15 | 0.034 | 0.02 | 0.16 | NS |
| CXPPSTR | 0.06 | 0.14 | NS | 0.04 | 0.04 | NS |
| CXPROAD | 0.01 | 0.03 | NS | 0.005 | 0.02 | NS |
| XB_HALL | 0.11 | 0.02 | NS | 0.06 | 0.03 | NS |
| XC_HALL | 0.08 | 0.36 | 0.001 | 0.08 | 0.25 | NS |
| XCB_HALL | 0.19 | 0.39 | NS | 0.14 | 0.28 | NS |
| X_HALL | 0.58 | 0.81 | NS | 1.03 | 0.97 | NS |
| QCPH1 | 0.73 | 0.65 | 0.040 | 0.63 | 0.52 | NS (0.07) |
| QPH1 | 0.71 | 0.65 | NS (0.06) | 0.64 | 0.53 | 0.045 |
| QTPH1 | 0.71 | 0.64 | 0.048 | 0.63 | 0.53 | 0.034 |
| QCPH2 | 0.69 | 0.60 | 0.042 | 0.59 | 0.47 | NS (0.06) |
| QPH2 | 0.68 | 0.61 | NS (0.06) | 0.61 | 0.49 | 0.033 |
| QTPH2 | 0.68 | 0.61 | NS (0.05) | 0.61 | 0.50 | 0.018 |

The results for the Plain ecoregion of both the metric and environmental variable analyses indicate problems with either the available data or the methodology being used for development of biocriteria for that portion of the state. Several of the reference and impaired sites in both the Plain and Ozark ecoregion have final habitat indices that are much lower or higher than might be expected in reference or impaired sites. Further confirmation of possible problems with site selection are seen in the results of preliminary Spearman rank correlations run using only the reference and impaired sites for the Plain ecoregion. All the metrics had at least 3 significant correlations to physical habitat variables and 9 of the metrics were significantly correlated to at least 10 variables and/or significantly related to the final habitat index. To find such strong correlations between the metrics and physical habitat variables despite few findings of significant difference between the reference and impaired sites indicates that some of the selected sites may not be representative of the group to which they were attributed (based on BPJ). It was decided at this point to defer development of biocriteria for the Plain ecoregion until additional data and funding were available.

<u>Spearman rank correlations for the Ozark ecoregion</u>.—Spearman rank correlations between all the metrics (which met assumptions of normality) and the environmental variables were performed for the Ozark ecoregion using the calibration reference sites (n = 26), impaired sites (n = 12), and 13 randomly selected sites (Table 7). All the metrics were significantly correlated to at least 3 of the environmental parameters except for the number of water column specialist feeders, and 5 were significantly related to at least one of the measures of the final habitat quality: the percentage of darter individuals, the percentage of small benthic individuals, the percentage of benthic individuals, the percentage of insectivore cyprinid individuals, and the percentage of insectivore and invertivore individuals. Correlations between the metrics and the water quality indicators of turbidity (NTU) or ammonia could not be performed due to a lack of data for many of the sites. A summary of overall metric responsiveness is presented in Table 7. Metrics in italics were not found to be significantly different in the unpaired t-tests of the reference and impaired sites (Table 5).

After correlation analyses, all the retained metrics were evaluated using methodology proposed by Karr and Chu (1999) where scatter plots of each metric to the final habitat index were visually inspected for response patterns and thresholds (Appendix E). As indicated in Table 7, five of the metrics were significantly related to the final habitat index therefore indicating a broad response. No threshold responses were observed for any of the metrics with non-significant relations to the final habitat index. Those metrics were rated as having an uncertain response.

| Table 7. Results of Spearman rank correlations between metrics and environmental variables using reference, impaired and random sites (*n* = 51) and box plot sensitivity rating. | Ozark ecoregion | | |
|---|---|---|---|
| | Box plot sensitivity rating | Number of significant correlations | Significantly correlated to the final habitat index |
| *Number of native individuals* | 1 | 6 | |
| Number of native species | 1 | 7 | |
| *Percentage of native darter individuals* | 0 | 4 | Yes |
| Number of native darter species | 1 | 5 | |
| Number of native small benthic species | 0 | 9 | |
| *Percentage of native small benthic individuals* | 0 | 9 | Yes |
| *Percentage of native round bodied sucker individuals* | 0 | 7 | |
| Number of native benthic species | 1 | 8 | |
| *Percentage of native benthic individuals* | 0 | 7 | Yes |
| Number of native water column species | 2 | 8 | |
| *Percentage of native water column individuals* | 0 | 3 | |
| Number of native long-lived species | 0 | 7 | |
| Percentage of native long-lived individuals | 0 | 6 | |
| Percentage of native insectivore cyprinid individuals | 2 | 13 | Yes |
| *Number of native sunfish species* | 0 | 3 | |
| *Percentage of native sunfish individuals* | 1 | 8 | |
| Number of native minnow species | 1 | 7 | |
| *Percentage of native minnow individuals* | 0 | 3 | |
| Number of native omnivore and herbivore species | 0 | 3 | |
| Number of native insectivore and invertivore species | 1 | 7 | |
| *Percentage of insectivore and invertivore individuals* | 0 | 7 | Yes |
| *Percentage of native carnivore individuals* | 0 | 6 | |
| Number of native non-guarding lithophilic species | 1 | 12 | |
| *Percentage of all native lithophilic individuals* | 0 | 4 | |
| Number of all native lithophilic species | 2 | 11 | |
| Number of native water column specialist feeder | 0 | 2 | |
| Percentage of the three dominant species | 1 | 6 | |

## Metric precision

Metrics were evaluated for their precision because metrics that discriminate among reaches but remain relatively constant at the same site are desirable. Metric precision was calculated based on the methodology of Kaufmann et al. (1999) where they described the precision of stream habitat measurements by the ratio of among-site variance (signal) to within-site variance from replicated sites (noise). Nine of the sites from the calibration data set (representing reference, impaired and random conditions) had duplicate samples from the same season and year. These data were used to evaluate metric precision within a site in a single year, to compare to variance among the sites. The signal to noise variance ratio was calculated as the among-site variance divided by the within-site variance. A high signal to noise ratio is desirable. Precision results are shown in Table 8.

### Metric redundancy

Metric redundancy was calculated using Pearson correlation analyses. Metrics that had correlation coefficients equal to or greater than 0.80 were considered redundant. Metrics that were redundant with one another are shown in Table 8.

### ***Final metric selection and adjustment***

A final summary of all the metric analyses is presented in Table 8. Box plot sensitivity ratings were the first selection criteria. All metrics with a box plot sensitivity rating of zero were dropped from further consideration. Other metrics that were dropped due to redundancy with a superior metric included the number of native species and the number of native non-guarding lithophilic species (compare with number of all native lithophilic species) and the number of native insectivore and invertivore species (compare with number of native benthic species). The remaining metrics with a high S/N ratio and a high number of significant correlations are highlighted in bold font. They include six metrics from the richness category: number of native darter species, number of native benthic species, number of native water column species, number of native minnow species, number of native insectivore and invertivore species, and number of all native lithophilic species; two metrics from the balance/diversity/composition category: percentage of native sunfish individuals, and percentage of the three dominant species; one metric from the trophic and reproductive category: percentage of native insectivore cyprinid individuals, and one metric from the fish abundance category: number of native individuals. Although some of these metrics did not achieve a significant t-test between the reference and impaired sites they were retained for IBI development due to a need for metrics in that category.

Spearman rank correlations of these metrics with the phyhab data showed several highly significant ($p$<0.005) relations with features of the environment associated with or impacted by anthropogenic activities. The number of native darter species and the number of native benthic species were negatively related to the percentage of urban land cover in the watershed; the number of native water column species was negatively related to the bank angle; the number of all native lithophilic species was positively related to the maximum residual pool width of the reach and the mean bankfull width; the percentage of native sunfish individuals was positively related to the percentage of water land cover in the watershed, and negatively related to the areal percentage of overhanging vegetation; the percentage of the three dominant species was negatively related to the percentage of rough bedrock; the percentage of native insectivore cyprinid individuals was positively related to the maximum volume of any pool in the reach and the areal percentage of brush and small debris, and negatively related to the percentage of water land cover in the watershed; while the number of native individuals was highly related to the percentage of pools in the reach.

Table 8. Summary of results of coefficient of variation, t-tests, box plot sensitivity, Spearman rank correlations, signal-noise variance ratios, and metric redundancy for final determination of metrics for the Ozark ecoregion. See Table 2 for definitions of abbreviations used in final column.

| | CV | t-test *p* value | Box plot sensitivity rating | Number of significant correlations | Signal/ noise ratio | Metric redundancy |
|---|---|---|---|---|---|---|
| **Number of native individuals** | 55 | NS | 1 | 6 | 0.4 | none |
| Number of native species | 19 | 0.010 | 1 | 7 | 12.7 | nsnlunk, nongrich, allrich |
| Percentage of darter individuals | 54 | NS | 0 | 4 | 1.1 | none |
| **Number of native darter species** | 35 | 0.003 | 1 | 5 | 4.6 | none |
| Number of native small benthic species | 30 | 0.006 | 0 | 9 | 4.1 | nsnisiv, nsnbenth |
| Percentage of native small benthic individuals | 54 | NS | 0 | 9 | 1.2 | pninsiv, pnbenth |
| Percentage of native round bodied sucker individuals | 67 | NS | 0 | 7 | 2.4 | none |
| **Number of native benthic species** | 30 | 0.006 | 1 | 8 | 8.5 | nsnisiv, nongrich, nsnsmben |
| Percentage of native benthic individuals | 39 | NS | 0 | 7 | 1.2 | pninsiv, pnsmben |
| **Number of native water column species** | 24 | 0.005 | 2 | 8 | 7.7 | nsnwcsp |
| Percentage of native water column individuals | 28 | NS | 0 | 3 | 1.1 | pnincyp |
| Number of native long-lived species | 21 | 0.020 | 0 | 7 | 10.6 | numspec |
| Percentage of native long-lived individuals | 35 | NS | 0 | 6 | 0.5 | pnminn |
| **Percentage of native insectivore cyprinid individuals** | 59 | 0.059 | 2 | 13 | 1.3 | pnwcol |
| Number of native sunfish species | 40 | NS | 0 | 3 | 4.8 | none |
| **Percentage of native sunfish individuals** | 54 | NS | 1 | 8 | 5.5 | none |
| **Number of native minnow species** | 24 | 0.032 | 1 | 7 | 0.8 | none |
| Percentage of minnow individuals | 15 | NS | 0 | 3 | 2.0 | pnlunk |
| Number of native omnivore and herbivore species | 24 | NS | 0 | 3 | 1.1 | none |
| **Number of native insectivore and invertivore individuals** | 30 | 0.009 | 1 | 7 | 8.1 | nsnbenth, nsnsmben, nongrich |
| Percentage of native insectivore and invertivore individuals | 42 | NS | 0 | 7 | 1.2 | pnbenth, pnsmben |
| Percentage of native carnivore individuals | 44 | NS | 0 | 6 | 4.2 | none |
| Number of native non-guarding lithophilic species | 20 | 0.011 | 1 | 12 | 0.3 | allrich, nsnbenth, numspec, nsnisiv |
| Percentage of all native lithophilic individuals | 20 | NS | 0 | 4 | 0.8 | none |
| **Number of all native lithophilic** | 19 | 0.003 | 2 | 11 | 6.2 | numspec, |

| | | | | | | |
|---|---|---|---|---|---|---|
| **individuals** | | | | | | nongrich |
| Number of native water column specialist feeder | 29 | 0.054 | 0 | 2 | 13.5 | nsnwcol |
| **Percentage of the three dominant species** | 13 | 0.014 | 1 | 6 | 4.3 | none |

In their study of IBI development in different regions of Virginia, Smogor and Angermeier (1999) found that the relations between taxonomic metrics and stream size varied from region to region (e.g. darters were related to stream size in one region, not in another) and were actually reversed in some regions (fewer species in larger streams). They also found that functional metrics were related to stream size in some regions. They reported that

> "Contrary to prior IBI emphases, our results showed that only a few taxonomic but several functional metrics varied with stream size and that most of these relations differed among as well as within IBI regions. Despite conceptual arguments and some prior evidence to the contrary, we found few generally applicable patterns in the way taxonomic or functional metrics varied with stream size...First IBI regions and relevant environmental gradients in each region should be explicitly defined and justified. Then for each region, metric criteria and their adjustments should be determined by examining empirical relations between each metric and each environmental gradient. Adjustments…should not be universally applied."

Adjustment based on stream size for each of the retained metric was evaluated as suggested above. Watershed size was correlated to the metrics using Pearson's correlation since the data for the retained metrics and watershed size were normally distributed. This analysis revealed that of the retained metrics only the number of native insectivore and invertivore species was significantly related to watershed size. Due to redundancy between this metric and the number of native benthic species, which performed comparably but was not related to watershed size, the latter was retained for IBI development. The remaining metrics were then correlated to the natural variables using Spearman rank correlations with the Bonferroni multiple test correction factor. Only one of the metrics was significantly related to any of the following natural variables: channel sinuosity, percentage of local segmentshed in hydrologic soil group B or hydrologic soil group D, percentage of the watershed in coarse soils, or channel slope of the reach. The number of all native lithophilic species was significantly correlated to the mean slope of the local watershed (calculated with GIS), but not significantly related to the mean slope of the reach (calculated with EMAP).

The following metrics were retained for use in IBI development. They include five metrics from the richness category: number of native darter species, number of native benthic species, number of native water column species, number of native minnow species, and number of all native lithophilic species; two metrics from the balance/diversity/composition category: percentage of native sunfish individuals, and percentage of the three dominant species; one metric from the trophic and reproductive category: percentage of native insectivore cyprinid species, and one metric from the fish abundance category: number of native individuals.

## IBI development

### *Normalization of metrics into unitless scores*

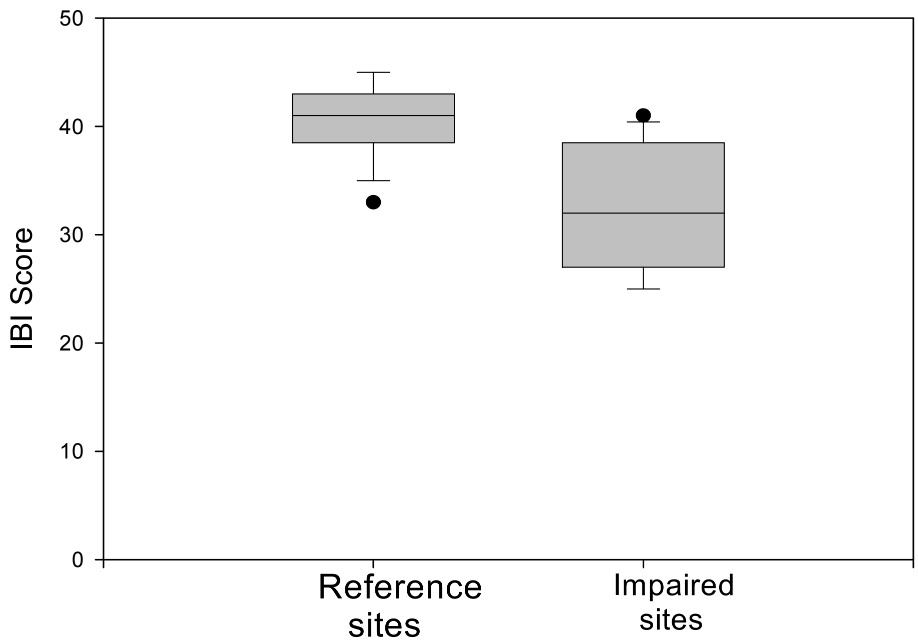
To convert the metrics into unitless scores, data values for each metric were scored with a 5, 3, or 1 following methods similar to those of Barbour et al. (1996) and Angermeier et al. (2000). For metrics that were positively related to stream quality, the lowest quartile (25$^{th}$ percentile) of the distribution of the metric values from the reference quality sites was used as the minimum value for scoring a 5. The minimum metric value for scoring a 3 was set as ½ the value of the 25$^{th}$ percentile and everything below that value was scored a 1. For the metric that was negatively related to stream quality (percentage of the three dominant species) the reverse procedure was followed using the 75$^{th}$ percentile.

Table 9. Metric scoring criteria for calculating the index of biotic integrity (IBI) for the Ozark ecoregion.

| | Metric score | | |
|---|---|---|---|
| | 1 | 3 | 5 |
| Number of native individuals | < 216 | 216 - 431 | ≥ 432 |
| Number of native darter species | < 2 | 2 | ≥ 3 |
| Number of native benthic species | < 3 | 3 - 5 | ≥ 6 |
| Number of native water column species | < 4 | 4 - 7 | ≥ 8 |
| Number of native minnow species | < 3 | 3 - 5 | ≥ 6 |
| Number of all native lithophilic species | < 7 | 7- 13 | ≥ 14 |
| Percentage of native insectivore cyprinid individuals | < 0.067 | 0.067 – 0.134 | ≥ 0.135 |
| Percentage of native sunfish individuals | < 0.013 | 0.013 – 0.026 | ≥ 0.027 |
| Percentage of the three dominant species | > 0.815 | 0.64 – 0.815 | ≤ 0.63 |

All the metrics for the reference (n = 26) and impaired (n = 12) sites from the calibration data set were normalized into unitless scores. Out of a total possible score of 45, the mean (standard deviation) of the IBI scores for the reference and impaired sites were 40.5 (3.5) and 32.3 (5.8), respectively. An unpaired t-test of the IBI scores showed that the IBI scores for the reference sites were significantly higher ($p$ <0.0001). A one-sided nonparametric median test was performed between the two types of sites (Mundahl and Simon 1999). This test calculated the median value of the IBI for the ranked reference and impaired sites combined. It then tested the predicted number of sites above this median for each type against the actual number of sites above the median for each type. For these data PR < Z = 0.0008 indicating that the IBI was able to distinguish between the reference and impaired sites. A box and whisker plot of the reference and impaired sites (Figure 4) revealed that the interquartile ranges of the reference and impaired sites did not overlap (1st quartile of reference sites = 39, 3rd quartile of the impaired sites = 38.5).

Figure 4. Box and whisker plot of IBI scores for the reference and impaired sites of the calibration data set -- Ozark ecoregion.




### *Relations of the metrics to the total IBI*

Each metric was correlated with the total IBI scores for the reference and impaired sites (calibration data set) using Spearman rank correlations. Eight of the nine selected metrics were significantly related to the total IBI score (Appendix F). Only the percentage of native sunfishes was not significantly correlated to the total IBI scores ($R_s = 0.20$, $p = 0.24$). To assess whether any of the metrics had an excessive influence on the IBI, the IBI scores for the reference, impaired and random sites from the calibration data set were recalculated by dropping one metric at a time from the total IBI score and then correlating these new scores with the total IBI score. The results showed highly significant relations ($p < 0.0001$) for each recalculation indicating that each of the metrics had roughly the same influence on the total IBI score.

Scatter plots of raw metric values and the IBI scores (Appendix F) were made to allow visual assessment of the metrics to the total IBI scores with the range of total IBI scores across the metric values that changed most rapidly determined to be the range of primary sensitivity for that metric (Angermeier and Karr 1986). All appeared to have a broad range of sensitivity.

### *Additional IBI validation and testing*

Relations between the IBI scores and the physical habitat

Spearman rank correlations (with a Bonferroni multiple test correction factor) were run with the IBI score for each of the reference, impaired and random sites against associated data for all the environmental variables and watershed area. There was only one significant relation ($p = 0.0002$), a negative correlation between the IBI scores and the percentage of urban land cover in the watershed. The IBI scores were not significantly related to any of the individual physical habitat variables or the syntheses of those variables, the final habitat indices (channel phyhab quality, channel plus riparian phyhab quality, channel plus riparian phyhab quality including human disturbance). This indicates that factors other than physical habitat may also be influencing these communities. This wasn't surprising since selection of the reference and impaired sites was based on best professional judgment rather than the physical habitat data collected through the EMAP protocol. Further analysis showed that while percentage of urban land cover was not related to the final habitat indices, it was positively related to the percentage of row crop land cover ($p = 0.0008$) for these sites. In contrast to urban land cover, row crop land cover was related (negatively) to two of the final habitat indices (channel phyhab quality, $p < 0.0001$, channel plus riparian phyhab quality, $p < 0.0001$).

IBI precision

Precision of the IBI was calculated in the same fashion as for the individual metrics based on the methodology of Kaufmann et al. (1999) where they described the precision of stream habitat measurements by the ratio of among-site variance (signal) to within-site variance from replicated sites (noise). Duplicate samples from the same season and year for nine reference, impaired and random sites of the calibration data set were used to evaluate metric precision within a site in a single year, to compare to variance among the sites. The signal to noise variance ratio was calculated as the among-site variance divided by the within-site variance. A high signal to noise ratio is desirable. Precision for the IBI scores was good at 5.9. It should be noted that variance within a year at the same site was higher among the impaired sites than the reference sites.

Validating the IBI

A validation data set was developed for use in testing the ability of the IBI to discern stream conditions. Nineteen additional sites were chosen from the RAM data set that had high and low ranges of the final habitat indices. None of these sites had been used in the calibration portion of this process. Seven "good" sites were chosen that had final habitat indices >0.8 (out of 1.0) and 5 "poor" sites were chosen that had final habitat indices <0.4. The rest of the 19 sites were chosen based on their occurrence within targeted watersheds listed by the EPA rather than physical habitat condition (see Appendix G). These random sites covered EPA targeted watersheds that were not all ready represented in earlier data sets presented here. All the metrics were calculated for each of these sites, normalized to unitless scores, and the final IBI for each was calculated for use in the validation data set. IBI scores for these sites were normally distributed.

The mean IBI scores for the sites ranked as "good" and "poor" were 40.4, and 24.6, respectively, and an unpaired t-test of these data was significant (*p* = 0.0006). Results of a one-sided nonparametric median test performed between the good and poor sites also indicated that the IBI was able to distinguish between the sites with good and poor habitat (PR < Z = 0.0025). A box and whisker plot of these data showed no overlap in the interquartile range (Figure 5).

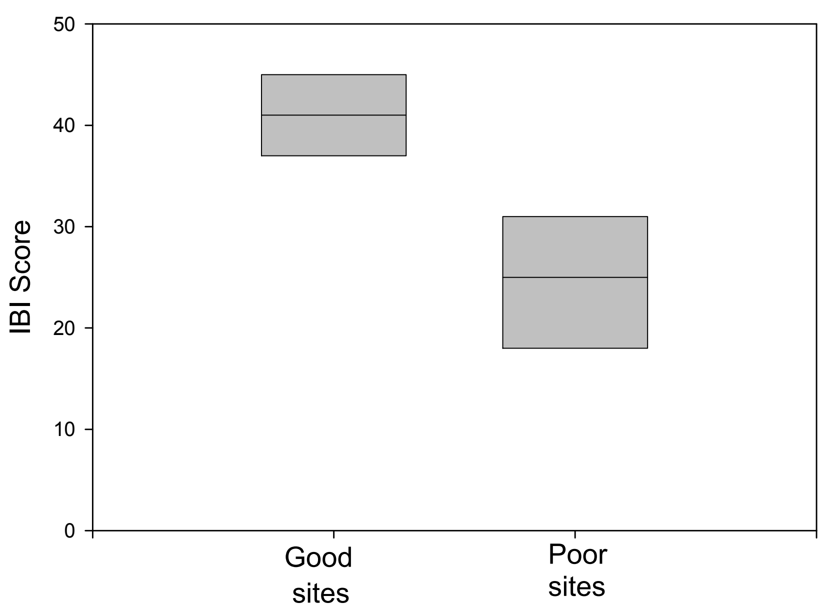



Figure 5. Box and whisker plot of the IBI validation data set – Ozark ecoregion.

Spearman rank correlations were run between the IBI scores for the validation sites with the land cover data and final habitat indices. Land cover data were only available for 17 of the 19 sites. Results of these analyses showed that rather than being negatively related to the percentage of urban land use like the original calibration data set, these validation sites (that were chosen based on EMAP final habitat indices instead of BPJ) were instead negatively related to the percentage of row crop cover ($p$ <0.0001). The IBI scores for these validation data ($n$ = 19) were also significantly positively related to the final habitat indices (channel plus riparian phyhab quality, $p$ = 0.0016; channel phyhab quality, $p$ = 0.0062; channel plus riparian phyhab quality including human disturbance impacts, $p$ = 0.010). Final habitat indices for these data were also significantly negatively related to the percentage of row crop cover (channel plus riparian phyhab quality, $p$ = 0.0059; channel phyhab quality, $p$ = 0.0082; channel plus riparian phyhab quality including human impacts, $p$ = 0.0125). As we saw with the original calibration data set, relations to the final habitat index that includes human disturbance impacts were not as strong as for the other habitat indices.

### *Interpreting the IBI scores*

Due to the highly significant differences in IBI scores between the good and poor sites selected for the IBI validation data, IBI scores from those sites were added to the reference and impaired sites of the calibration data for calculations made to determine level classifications of the IBI scores. This resulted in a total of 33 reference sites and 17 impaired sites. Percentiles for these two groups were determined (Table 10).

Table 10. Quartiles for the IBI scores of the reference and impaired sites for the Ozark ecoregion.

| | Impaired | Reference |
|---|---|---|
| 25$^{th}$ percentile | 25 | 37 |
| 50$^{th}$ percentile | 29 | 41 |
| 75$^{th}$ percentile | 35 | 43 |

We suggest a three-level classification of stream condition of *no impairment, impaired*, and *highly impaired* based on the following criteria. Any streams with an IBI score greater than or equal to the 25$^{th}$ percentile of the reference sites (37) would indicate *no impairment*. Streams with an IBI score higher than the median of the impaired streams (29) up to 36 would be deemed *impaired*, and anything less than 29 would be considered *highly impaired*. This establishes an IBI score of 36 as the threshold of impairment for any stream for which the IBI score is determined through this process.

## Discussion and recommendations

Anyone who has frequented the state of Missouri inherently knows that development of biocriteria for the benthic invertebrates or fish requires the separation of the state into at least three different ecoregions: the Central Plain, Ozark, and Mississippi Alluvial Basin. Analyses of the fish community and physical habitat presented in this document clearly support the separation of the Central Plain and Ozark ecoregions (Figure 3, Tables 1 and 3).

The development of biocriteria for these two major ecoregions of the state was only successful for the Ozark ecoregion. Failure to achieve biocriteria for the Plain ecoregion may be the result of any or all of the following reasons. First, the current natural state of the streams in the Plain ecoregion in many ways—such as fine sediment deposition and water clarity—embodies qualities that science equates with water pollution and stream degradation. In addition, fishes of prairie streams are more tolerant of these conditions. Those facts, along with a history of extensive stream channel modification in the region, has led to the identification (and probably the existence) of an inadequate number of reference sites within the Plain ecoregion of Missouri. An additional source of variance may have been introduced in these data by the use of reference and impaired sites that were selected based on best professional judgment. Analyses of these BPJ sites for the Plain ecoregion (Table 6) highlight the lack of any basis for these stream condition assignments based on the land use, physical habitat or water quality. Still another source of critical information that needs improvement for both the Plain and Ozark ecoregions are water quality data. If funding is available, potential ways to address these problems might be the addition of data from a similar study in Iowa using reference sites from the Central Irregular Plains. Another possible approach might be the selection of both reference and impaired sites based solely on the physical habitat variables instead of including water quality concerns that are a part of selecting sites when using BPJ.

In contrast, conditions are still good within a large portion of the streams in the Ozark region. Current natural conditions of some of the streams in this ecoregion are comparable to the best found anywhere in the country. This allowed for a relatively wide selection of reference sites for biocriteria development. After metric screening that included tests of range, normality, variance, redundancy, responsiveness to human influences, and the ability to discern between reference and impaired conditions, nine metrics remained for IBI development. Data for these metrics from the calibration data set were developed into an IBI to which they all contributed approximately equally. The IBI was then assessed for variance, responsiveness to human influences and the overall ability to discern between reference and impaired conditions. Sites within the calibration data set were highly related to the percentage of urban land cover within the watershed, and although they were not related to the final habitat indices, the IBI was able to differentiate between reference and impaired conditions.

A final test of the IBI was performed using a validation data set that was developed based on the physical habitat (final habitat index scores) rather than best profession judgment[3]. These validation data showed that the IBI developed from the calibration data set was able to discriminate between reference and impaired conditions of other sites. These data also revealed that the primary relation to the physical habitat of Ozark streams was with the percentage of row crop land cover in the watershed, and the sites were (as expected) significantly related to the final habitat indices.

[3] Reasons for this were two-fold—an inadequate number of impaired sites (based on BPJ) for both calibration and validation data sets, and earlier analyses of the calibration data set indicating no relation to the final habitat indices.

Although this index is able to easily discern between reference and impaired conditions, improvement of this index and development of an index for the Plain ecoregion, might be achieved in the future by a more careful data-based approach to the screening process of the reference and impaired sites. Several of the impaired sites (chosen by BPJ) that were used in the calibration data set had final habitat index scores that did not fit the expectation (e.g. impaired sites with very good physical habitat). Discussion of these sites with committee members who suggested them indicated that often sites were considered impaired based on repeated fish kills or suspected influences of urban development. These sites were retained for the process since development of biocriteria was meant to aid these agencies with assessing all types of stream perturbations. However, as the analyses progressed it became obvious to the analyst that many of these impaired sites with good physical habitat were introducing excessive variance into the process. The negative effect of this site selection method was corroborated when analyses of the validation sites (selected solely on the physical habitat data) revealed a stronger ability to discern between reference and impaired conditions (Figures 4 and 5).

We suggest the possibility of a two-pronged approach to stream monitoring in the state. Biocriteria development for the invertebrate communities of the state (Rabeni et al. 1997) showed that invertebrates were quite good at detecting water quality issues such as organic pollution but failed at the detection of habitat quality impairment. In contrast, studies of the development of biocriteria using fish communities have reported that physical habitat rather than water quality is the major influence (Wang et al. 1997, Bramblett et al. 2005). In the future, it might make sense for agencies to assess streams of concern within Missouri using invertebrate biocriteria for water quality issues and fish communities for physical habitat issues.

## Appendix A. Literature review on development and use of biocriteria for fish communities of wadeable streams.


Allan, J. D. 2004. Landscapes and riverscapes: The influence of land use on stream ecosystems [review]. Annual Review of Ecology Evolution & Systematics 35:257-284.

Angermeier, P. L., and J. R. Karr. 1986. Applying an index of biotic integrity based on stream-fish communities: Considerations in sampling and interpretation. North American Journal of Fisheries Management 6:418-429.

Angermeier, P. L., and I. J. Schlosser. 1987. Assessing biotic integrity of the fish community in a small Illinois stream. North American Journal of Fisheries Management 7(3):331-338.

Angermeier, P. L., and I. J. Schlosser. 1989. Species-area relationships for stream fishes. Ecology 70:1450-1462.

Angermeier, P. L., and J. R. Karr. 1994. Biological integrity versus biological diversity as policy directives: Protecting biotic resources. BioScience 44(10):690-697.

Angermeier, P. L., R. A. Smogor, and J. R. Stauffer. 2000. Regional frameworks and candidate metrics for assessing biotic integrity in mid-Atlantic highland streams. Transactions of the American Fisheries Society 129(4):962-981.

Barbour, M. T., J. B. Stribling, and J. R. Karr. 1995. Multimetric approach for establishing biocriteria and measuring biological condition. Pages 63-77 *in* W. S. Davis, and T. P. Simon, editors. Biological assessment and criteria: Tools for water resource planning and decision making. Lewis Publishers, Boca Raton.

Barbour, M. T., J. Gerritsen, G. E. Griffith, R. Frydenborg, E. McCarron, J. S. White, and M. L. Bastian. 1996. A framework for biological criteria for Florida streams using benthic macroinvertebrates. Journal of the North American Benthological Society 15:185-211.

Bramblett, R. G., and K. D. Fausch. 1991. Variable fish communities and the index of biotic integrity in a western Great Plains river. Transactions of the American Fisheries Society 120:752-769.

Bramblett, R. G., T. R. Johnson, A. V. Zale, and D. G. Heggem. 2005. Development and evaluation of a fish assemblage index of biotic integrity for northwestern Great Plains streams. Transactions of the American Fisheries Society 134(3):624-640.

Bressler, D. W., J. B. Stribling, M. J. Paul, and M. B. Hicks. 2006. Stressor tolerance values for benthic macroinvertebrates in Mississippi. Hydrobiologia 573:155-172.

Dauwalter, D. C., and E. J. Pert. 2003. Effect of electrofishing effort on an index of biotic integrity. North American Journal of Fisheries Management 23(4):1247-1252.

Dauwalter, D. C., and J. R. Jackson. 2004. A provisional fish index of biotic integrity for assessing Ouachita Mountains streams in Arkansas, USA. Environmental Monitoring & Assessment 91(1-3):27-57.

Emery, E. B., T. P. Simon, F. H. McCormick, P. L. Angermeier, J. E. Deshon, C. O. Yoder, R. E. Sanders, W. D. Pearson, G. D. Hickman, R. J. Reash, and J. A. Thomas. 2003. Development of a multimetric index for assessing the biological condition of the Ohio River. Transactions of the American Fisheries Society 132(4):791-808.

Fausch, K. D., J. R. Karr, and P. R. Yant. 1984. Regional application of an index of biotic integrity based on stream fish communities. Transactions of the American Fisheries Society 113:39-55.

Fausch, K. D., J. Lyons, J. R. Karr, and P. L. Angermeier. 1990. Fish communities as indicators of environmental degradation. Pages 123-144 *in* Adams, S. M., editor. Biological indicators of stress in fish. American Fisheries Society. Bethesda, Maryland.

Fitzpatrick, F. A., B. C. Scudder, B. N. Lenz, and D. J. Sullivan. 2001. Effects of multi-scale environmental characteristics on agricultural stream biota in eastern Wisconsin. Journal of the American Water Resources Association 37(6):1489-1507.

Fore, L. S., J. R. Karr, and L. L. Conquest. 1994. Statistical properties of an index of biological integrity used to evaluate water resources. Canadian Journal of Fisheries & Aquatic Sciences 51(5):1077-1087.

Freeman, M. C., Z. H. Bowen, and J. H. Crance. 1997. Transferability of habitat suitability criteria for fishes in warmwater streams. North American Journal of Fisheries Management 17(1):20-31.

Hall, L., M. Scott, W. Killen, and R. Anderson. 1996. The effects of land-use characteristics and acid sensitivity on the ecological status of Maryland Coastal Plain streams. Environmental Toxicology & Chemistry 15:384-394.

Hued, A., and M. Bistoni. 2005. Development and validation of a biotic index for evaluation of environmental quality in the central region of Argentina. Hydrobiologia 543:279-298.

Hughes, R. M. and T. Oberdorff. 1999. Applications of IBI concepts and metrics to waters outside the United States and Canada. Pages 79 - 81 *in* T. Simon, editor. Assessing the sustainability and biological integrity of water resources using fish communities. CRC Press, Washington, D. C.

Hughes, R. M., P. R. Kaufmann, A. T. Herlihy, T. M. Kincaid, L. Reynolds, and D. P. Larsen. 1998. A process for developing and evaluating indices of fish assemblage integrity. Canadian Journal of Fisheries & Aquatic Sciences 55(7):1618-1631.

Hughes, R. M., S. Howlin, and P. R. Kaufmann. 2004. A biointegrity index (IBI) for coldwater streams of western Oregon and Washington. Transactions of the American Fisheries Society 133(6):1497-1515.

Kaufmann, P. R., P. Levine, E. G. Robison, C. Seeliger, and D. V. Peck. 1999. Quantifying physical habitat in wadeable streams. US Environmental Protection Agency, EPA/620/R-99/003, Washington, D.C.

Karr, J. R. 1981. Assessment of biotic integrity using fish communities. Fisheries 6:21-27.

Karr, J. R. 1991. Biological integrity: A long-neglected aspect of water resource management. Ecological Applications 1(1):66-84.
Karr, J. R. and D. R. Dudley. 1981. Ecological perspective on water quality goals. Environmental Management 5:55-68.
Karr, J. R., and E. W. Chu. 1997. Biological monitoring - Essential foundation for ecological risk assessment. Human & Ecological Risk Assessment 3(6):993-1004.
Karr, J. R., and E. W. Chu. 1999. Restoring life in running waters: Better biological monitoring. Island Press, Washington, D.C.
Karr, J. R., K. D. Fausch, P. L. Angermeier, P. R. Yant, and I. J. Schlosser. 1986. Assessing biological integrity in running waters a method and its rationale. Illinois Natural History Survey.
Karr, J. R., P. R. Yant, K. D. Fausch, and I. J. Schlosser. 1987. Spatial and temporal variability of the index of biotic integrity in three Midwestern streams. Transactions of the American Fisheries Society 116:1-11.
Kerans, B. L., and J. R. Karr. 1994. A benthic index of biotic integrity (B-IBI) for rivers of the Tennessee valley. Ecological Applications 4(4):768-785.
Klauda, R., P. Kazyak, S. Stranko, M. Southerland, N. Roth, and J. Chaillou. 1998. Maryland biological stream survey: A state agency program to assess the impact of anthropogenic stresses on stream habitat quality and biota. Environmental Monitoring & Assessment 51:299-316.
Langdon, R. 2001. A preliminary index of biological integrity for fish assemblages of small coldwater streams in Vermont. Northeastern Naturalist 8(2):219-232.
Leonard, P. M., and D. J. Orth. 1986. Application and testing of an index of biotic integrity in small, coolwater streams. Transactions of the American Fisheries Society 115:401-415.
Liang, S.-H., and B. W. Menzel. 1996. The functional characteristics of headwater fish communities in Iowa. Acta Zoologica Taiwanica 7(2):61-71.
Lydy, M. J., A. J. Strong, and T. P. Simon. 2000. Development of an index of biotic integrity for the little Arkansas River basin, Kansas. Archives of Environmental Contamination & Toxicology 39(4):523-530.
Lyons, J. 1992. Using the index of biotic integrity (IBI) to measure environmental quality in warmwater streams of Wisconsin, U.S. Forest Service General Technical Report NC-149.
Lyons, J., L. Wang, and T. D. Simonson. 1996. Development and validation of an index of biotic integrity for coldwater streams in Wisconsin. North American Journal of Fisheries Management 16:241-256.
Lyons, J., R. R. Piette, and K. W. Niermeyer. 2001. Development, validation, and application of a fish-based index of biotic integrity for Wisconsin's large warmwater rivers. Transactions of the American Fisheries Society 130(6):1077-1094.
McCormick, F. H., R. M. Hughes, P. R. Kaufmann, D. V. Peck, J. L. Stoddard, and A. T. Herlihy. 2001. Development of an index of biotic integrity for the Mid-Atlantic Highlands region. Transactions of the American Fisheries Society 130(5):857-877.

Mebane, C., T. Maret, and R. Hughes. 2003. An index of biological integrity (IBI) for Pacific northwest rivers. Transactions of the American Fisheries Society 132:239-261.

Miller, D., P. Leonard, R. Hughes, J. Karr, P. Moyle, L. Schrader, B. Thompson, R. Daniels, K. Fausch, G. Fitzhugh, J. Gammon, D. Halliwell, P. Angermeier, and D. Orth. 1988. Regional applications of the index of biotic integrity for use in water resource management. Fisheries 13(5):12-20.

Morris, C. C., P. M. Stewart, and T. P. Simon. 2007. Development of an index of biotic integrity for a Southeastern Coastal Plain Watershed, USA. Journal of the American Water Resources Association 43(2):295-307.

Mundahl, N., and T. Simon. 1999. Development and application of an index of biotic integrity for coldwater streams of the upper Midwestern United States. Pages 383-415 *in* T. Simon, editor. Assessing the sustainability and biological integrity of water resources using fish communities. CRC Press, Washington, D. C.

Nigh, T., and W. Schroeder. 2002. Atlas of Missouri ecoregions. Missouri Department of Conservation, Jefferson City, Missouri.

Oberdorff, T., D. Pont, B. Hugueny, and J. P. Porcher. 2002. Development and validation of a fish-based index for the assessment of 'river health' in France. Freshwater Biology 47(9):1720-1734.

Paller, M. H., M. J. M. Reichert, and J. M. Dean. 1996. Use of fish communities to assess environmental impacts in South Carolina Coastal Plain streams. Transactions of the American Fisheries Society 125(5):633-644.

Pirhalla, D. E. 2004. Evaluating fish-habitat relationships for refining regional indexes of biotic integrity: Development of a tolerance index of habitat degradation for Maryland stream fishes. Transactions of the American Fisheries Society 133(1):144-159.

Pflieger, W. 1989. Aquatic community classification system for Missouri. Aquatic Series no. 19. Missouri Department of Conservation, Jefferson City, Missouri.

Rabeni, C. F., R. J. Sarver, N. Wang, G. S. Wallace, M. Weiland, and J. T. Peterson. 1997. Development of regionally based biological criteria for Missouri. Final Report to the Missouri Department of Natural Resources, P.O. Box 176, Jefferson City, Missouri 65102.

Rabeni, C. F., and K. E. Doisy. 2000. Correspondence of stream benthic invertebrate assemblages to regional classification schemes in Missouri. Journal of the North American Benthological Society 19(3):419-428.

Rankin, E. and C. Yoder. 1999. Methods for deriving maximum species richness lines and other threshold relationships. Pages 611-624 *in* T. Simon, editor. Assessing the sustainability and biological integrity of water resources using fish communities. CRC Press, Washington, D.C.

Schleiger, S. L. 2000. Use of an index of biotic integrity to detect effects of land uses on stream fish communities in west-central Georgia. Transactions of the American Fisheries Society 129(5):1118-1133.

Scott, M. C., and L. W. Hall. 1997. Fish assemblages as indicators of environmental degradation in Maryland Coastal Plain streams. Transactions of the American Fisheries Society 126(3):349-360.

Shearer, J. S., and C. R. Berry. 2002. Index of biotic integrity utility for the fishery of the James River of the Dakotas. Journal of Freshwater Ecology 17(4):575-588.

Simon, T. P. 1999. Assessing the sustainability and biological integrity of water resources using fish communities. CRC Press, WAshington, D.C.

Smogor, R. A, and P. L. Angermeier. 1999. Effects of drainage basin and anthropogenic disturbance on relations between stream size and IBI metrics in Virginia. Pages 249-272 *in* T. Simon, editor. Assessing the sustainability and biological integrity of water resources using fish communities. CRC Press, Washington, D. C.

Smogor, R. A., and P. L. Angermeier. 2001. Determining a regional framework for assessing biotic integrity of Virginia streams. Transactions of the American Fisheries Society 130(1):18-35.

Snyder, C. D., J. A. Young, R. Villella, and D. P. Lemarie. 2003. Influences of upland and riparian land use patterns on stream biotic integrity. Landscape Ecology 18(7):647-664.

Stewart, J. S., L. Z. Wang, J. Lyons, J. A. Horwatich, and R. Bannerman. 2001. Influences of watershed, riparian-corridor, and reach-scale characteristics on aquatic biota in agricultural watersheds. Journal of the American Water Resources Association 37(6):1475-1487.

Stranko, S. A., M. K. Hurd, and R. J. Klauda. 2005. Applying a large, statewide database to the assessment, stressor diagnosis, and restoration of stream fish communities. Environmental Monitoring & Assessment 108(1-3):99-121.

USEPA. 1996. Biological Criteria: Technical guide for streams and small rivers. Revised Edition. Office of Science and Technology, United States Environmental Protection Agency. Washington, D.C. EPA-822-B-96-001.

USEPA. 2000. Stressor Identification guidance document. Office of Water, Office of Research and Development, United States Environmental Protection Agency. Washington, D.C. EPA-882-B-00-025.

VDEC. 2004. Biocriteria for fish and macroinvertebrate assemblages in Vermont wadeable streams and rivers. Water Quality Division, Biomonitoring and Aquatic Studies Section, Vermont Department of Environmental Conservation. Waterbury, Vermont.

Wang, L., J. Lyons, P. Kanehl, and R. Gatti. 1997. Influences of watershed land use on habitat quality and biotic integrity of Wisconsin streams. Fisheries 22(6):6–12.

Wang, L. Z., J. Lyons, and P. Kanehl. 2001. Impacts of urbanization on stream habitat and fish across multiple spatial scales. Environmental Management 28(2):255-266.

Wilton, T. 2004. Biological assessment of Iowa's wadeable streams. TMDL and Water Quality Assessment Section, Environmental Services Division, Iowa Department of Natural Resources. Des Moines, Iowa.

Yoder, C., and M. Smith. 1999. Using fish assemblages in a state biological assessment and criteria program: Essential concepts and considerations. Pages 17-56 *in* T. Simon, editor. Assessing the sustainability and biological integrity of water resources using fish communities. CRC Press, Washington, D.C.

## Appendix B. List of sites used for IBI development in Missouri.

| Stream name | Storet | Quality[1] | Ecoregion | Unique ID[2] | Segment ID[3] | EDU[4] | Watershed area ($km^2$) | rf3 order | UTM_X | UTM_Y | county |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Gravois Creek | 0001 | Impaired | OZARK | 00012-00 | 7140101 699 | 24 | 31 | 2 | 731699 | 4268438 | 29189 |
| Hinkson Creek | 0005 | Impaired | OZARK | 00051-00 | 10300102 1881 | 26 | 198 | 4 | 552192 | 4307272 | 29009 |
| Flat River | 0010 | Impaired | OZARK | 00101-00 | 7140104 6649 | 25 | 74 | 3 | 719587 | 4194107 | 29187 |
| North Moreau Creek | 0012 | Impaired | OZARK | 00121-00 | 10300102 4377 | 26 | 415 | 5 | 539395 | 4270434 | 29135 |
| Buffalo Creek | 0014 | Impaired | OZARK | 00141-00 | 11070208 4252 | 22 | 67 | 3 | 366714 | 4074038 | 29145 |
| Indian Creek | 0017 | Impaired | OZARK | 00171-00 | 11070208 4270 | 22 | 267 | 5 | 389174 | 4072773 | 29145 |
| Bear Creek | 0101 | Impaired | OZARK | 01011-01 | 10300102 1361 | 26 | 38 | 2 | 556096 | 4315467 | 29009 |
| Wilson Creek | 0103 | Impaired | OZARK | 01031-01 | 11010002 521 | 29 | 134 | 3 | 466303 | 4110171 | 29077 |
| Hubble Creek | 0113 | Impaired | OZARK | 01131-01 | 7140107 1078 | 28 | 50 | 3 | 793408 | 4138704 | 29031 |
| Hinkson Creek | 2080 | Impaired | OZARK | 20801-04 | 10300102 1747 | 26 | 134 | 3 | 558965 | 4309451 | 19 |
| Straight Fork | 2081 | Impaired | OZARK | 20811-04 | 10300102 4494 | 26 | 125 | 3 | 520839 | 4268711 | 135 |
| Little Sac River | 9674 | Impaired | OZARK | 96741-02 | 10290106 4949 | 27 | 488 | 4 | 458845 | 4144911 | 29167 |
| Big Sugar Creek | 2013 | Reference | OZARK | 20131-02 | 11070208 4872 | 22 | 223 | 4 | 397806 | 4049562 | 29119 |
| Burris Fork | 2014 | Reference | OZARK | 20141-03 | 10300102 4799 | 26 | 163 | 4 | 534719 | 4263052 | 29135 |
| Boeuf Creek | 2015 | Reference | OZARK | 20151-02 | 10300200 7515 | 26 | 174 | 4 | 647745 | 4266740 | 29071 |
| Apple Creek | 2016 | Reference | OZARK | 20161-02 | 7140105 3655 | 24 | 122 | 4 | 779940 | 4164309 | 29031 |
| Meramac River | 2017 | Reference | OZARK | 20171-02 | 7140102 4283 | 25 | 419 | 5 | 635969 | 4180819 | 29065 |
| Cedar Creek | 2018 | Reference | OZARK | 20181-02 | 10290106 4087 | 27 | 287 | 4 | 421239 | 4173472 | 29039 |
| Deer Creek | 2020 | Reference | OZARK | 20201-01 | 10290109 7203 | 27 | 161 | 4 | 484388 | 4228010 | 29015 |
| Deer Creek | 2020 | Reference | OZARK | 20201-02 | 10290109 7203 | 27 | 161 | 4 | 484388 | 4228010 | 29015 |
| North Fork White River | 2021 | Reference | OZARK | 20211-02 | 11010006 643 | 29 | 191 | 5 | 570948 | 4089057 | 29067 |
| Sinking Creek | 2022 | Reference | OZARK | 20221-02 | 11010007 5118 | 21 | 170 | 4 | 692861 | 4125785 | 29179 |
| Sinking Creek | 2023 | Reference | OZARK | 20231-02 | 11010008 718 | 21 | 302 | 4 | 642456 | 4132827 | 29203 |
| Marble Creek | 2024 | Reference | OZARK | 20241-02 | 8020202 1044 | 28 | 109 | 3 | 717796 | 4147674 | 29123 |
| Boeuf Creek | 2077 | Reference | OZARK | 20771-04 | 10300200 7675 | 26 | 78 | 3 | 643429 | 4260055 | 71 |
| Boeuf Creek | 2092 | Reference | OZARK | 20921-04 | 10300200 7408 | 26 | 255 | 5 | 648514 | 4267252 | 71 |
| Barren Fork | 561 | Reference | OZARK | 21071-04 | 11010008 561 | 21 | 124 | 4 | 642295 | 4137666 | 203 |
| Shut in Creek | 4139 | Reference | OZARK | 21181-04 | 110100074139 | 21 | 56 | 3 | 689721 | 4161159 | 179 |
| Sinking Creek | 529 | Reference | OZARK | 21271-04 | 11010008 529 | 21 | 117 | 4 | 647215 | 4139054 | 203 |
| Spring Creek | 845 | Reference | OZARK | 22201-05 | 11010006 845 | 29 | 42 | 3 | 582492 | 4085550 | 91 |
| Rippee Creek | 940 | Reference | OZARK | 22241-05 | 11010006 940 | 29 | 45 | 3 | 545895 | 4080107 | 67 |
| Bull Creek | 9548 | Reference | OZARK | 95481-01 | 11010003 6215 | 29 | 272 | 4 | 483897 | 4074766 | 29043 |

**Appendix B continued.**

| Stream name | Storet | Quality[1] | Ecoregion | Unique ID[2] | Segment ID[3] | EDU[4] | Watershed area ($km^2$) | rf3 order | UTM_X | UTM_Y | county |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Pomme De Terre River | 9673 | Reference | OZARK | 96731-02 | 10290107 15553 | 27 | 237 | 4 | 483863 | 4143121 | 29167 |
| Sinking Creek | 9675 | Reference | OZARK | 96751-02 | 11010008 276 | 21 | 55 | 3 | 651273 | 4146797 | 29065 |
| Castor River | 9690 | Reference | OZARK | 96901-02 | 7140107 196 | 28 | 89 | 3 | 750450 | 4162752 | 29123 |
| Huzzah Creek | 9807 | Reference | OZARK | 98071-02 | 7140102 4048 | 25 | 290 | 3 | 660877 | 4187202 | 29055 |
| West Piney River (creek) | 05 | Reference | OZARK | RES051-05 | 10290202 4819 | 23 | 219 | 4 | 585830 | 4129784 | 215 |
| Hunter Creek | 05 | Reference | OZARK | RES131-05 | 11010006 749 | 29 | 137 | 4 | 544892 | 4085962 | 67 |
| Big Berger Creek | 2094 | Random | OZARK | 20941-04 | 10300200 7126 | 26 | 58 | 3 | 643130 | 4273837 | 71 |
| Hillers Creek | 2095 | Random | OZARK | 20951-04 | 10300102 3716 | 26 | 81 | 3 | 584545 | 4281782 | 27 |
| Hungry Mother Creek | 2098 | Random | OZARK | 20981-04 | 10300102 374 | 26 | 41 | 3 | 539057 | 4335240 | 89 |
| Middle Fork Black River | 4058 | Random | OZARK | 21081-04 | 11010007 4058 | 21 | 66 | 4 | 680343 | 4163800 | 93 |
| Pine Creek | 2145 | Random | OZARK | 21121-04 | 11010008 2145 | 21 | 52 | 3 | 598989 | 4101378 | 215 |
| South Fork Buffalo Creek | 3263 | Random | OZARK | 21141-04 | 11010008 3263 | 21 | 65 | 3 | 679297 | 4064627 | 181 |
| Panther Creek | 302 | Random | OZARK | 22151-05 | 11010002 302 | 29 | 106 | 3 | 497736 | 4116927 | 225 |
| Indian Creek | 658 | Random | OZARK | 22181-05 | 11010006 658 | 29 | 127 | 4 | 576568 | 4091933 | 67 |
| Noblett Creek | 733 | Random | OZARK | 22191-05 | 11010006 733 | 29 | 49 | 3 | 580881 | 4086421 | 91 |
| Fox Creek | 1098 | Random | OZARK | 22251-05 | 11010006 1098 | 29 | 64 | 4 | 554800 | 4076014 | 67 |
| Pine Creek | 1655 | Random | OZARK | 22291-05 | 11010006 1655 | 29 | 53 | 3 | 560820 | 4057824 | 153 |
| S. Fk. Bratten Spring Creek | 7611 | Random | OZARK | 22501-05 | 11010003 7611 | 29 | 43 | 3 | 537615 | 4045763 | 153 |
| Pearson Creek | 0107 | Random | OZARK | 01071-01 | 11010002 219 | 29 | 59 | 2 | 482645 | 4114093 | 29077 |
| Deer Creek | 2020 | Duplicate | OZARK | 20202-01 | 10290109 7203 | 27 | 161 | 4 | 484388 | 4228010 | 29015 |
| Deer Creek | 2020 | Duplicate | OZARK | 20202-02 | 10290109 7203 | 27 | 161 | 4 | 484388 | 4228010 | 29015 |
| Bull Creek | 9548 | Duplicate | OZARK | 95482-01 | 11010003 6215 | 29 | 272 | 4 | 483897 | 4074766 | 29043 |
| Castor River | 9690 | Duplicate | OZARK | 96902-02 | 7140107 196 | 28 | 89 | 3 | 750450 | 4162752 | 29123 |
| Gravois Creek | 0001 | Duplicate | OZARK | 00011-00 | 7140101 699 | 24 | 30.78 | 2 | 731699 | 4268438 | 29189 |
| Hinkson Creek | 0005 | Duplicate | OZARK | 00052-00 | 10300102 1881 | 26 | 198.23 | 4 | 552192 | 4307272 | 29009 |
| North Moreau Creek | 0012 | Duplicate | OZARK | 00122-00 | 10300102 4377 | 26 | 415.36 | 5 | 539395 | 4270434 | 29135 |
| Bear Creek | 0101 | Duplicate | OZARK | 01012-01 | 10300102 1361 | 26 | 38.46 | 2 | 556096 | 4315467 | 29009 |
| Pine Creek | 2145 | Duplicate | OZARK | 21122-04 | 11010008 2145 | 21 | 51.52 | 3 | 598989 | 4101378 | 215 |
| Mill Creek | 0006 | Impaired | PLAIN | 00061-00 | 7110008 669 | 16 | 43.32 | 3 | 668340 | 4332376 | 29113 |
| West Fork Tebo Creek | 0013 | Impaired | PLAIN | 00131-00 | 10290108 17557 | 15 | 46.2 | 2 | 443544 | 4252182 | 29083 |
| Big Creek | 0016 | Impaired | PLAIN | 00161-00 | 10290108 16114 | 15 | 316.7 | 4 | 398233 | 4286778 | 29037 |
| Middle Fork Salt River | 0102 | Impaired | PLAIN | 01021-01 | 7110006 14808 | 16 | 312.04 | 4 | 554611 | 4389916 | 29121 |

**Appendix B continued.**

| Stream name | Storet | Quality[1] | Ecoregion | Unique ID[2] | Segment ID[3] | EDU[4] | Watershed area ($km^2$) | rf3 order | UTM_X | UTM_Y | county |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Blue River | 0111 | Impaired | PLAIN | 01111-01 | 10300101 7984 | 11 | 229.6 | 4 | 362246 | 4304048 | 29095 |
| Spring Creek | 0114 | Impaired | PLAIN | 01141-01 | 10280202 2018 | 12 | 206.89 | 4 | 520700 | 4455711 | 29001 |
| Little Medicine Creek | 0115 | Impaired | PLAIN | 01151-01 | 10280103 3897 | 12 | 174.42 | 3 | 467622 | 4463311 | 29129 |
| Middle Fork Grand River | 0117 | Impaired | PLAIN | 01171-01 | 10280101 3046 | 12 | 199.5 | 4 | 382526 | 4482746 | 29227 |
| Middle Richland Creek | 0110 | Reference | PLAIN | 01101-01 | 10300103 4130 | 11 | 55.8 | 3 | 507853 | 4265148 | 29141 |
| Dog Creek | 0116 | Reference | PLAIN | 01161-01 | 10280101 8074 | 12 | 55.98 | 3 | 412535 | 4410710 | 29061 |
| Grindstone Creek | 2006 | Reference | PLAIN | 20061-02 | 10280101 8030 | 12 | 153.4 | 4 | 392102 | 4411932 | 29063 |
| Spring Creek | 2008 | Reference | PLAIN | 20081-02 | 10280202 2032 | 12 | 216.2 | 4 | 521367 | 4454264 | 29001 |
| Heaths Creek | 2011 | Reference | PLAIN | 20111-02 | 10300103 2623 | 11 | 190.9 | 3 | 484140 | 4306586 | 29159 |
| Heaths Creek | 2011 | Reference | PLAIN | 20111-05 | 10300103 2623 | 11 | 190.9 | 3 | 484140 | 4306586 | 159 |
| Little Dry Wood Creek | 2012 | Reference | PLAIN | 20121-02 | 10290104 1701 | 15 | 159.5 | 4 | 376236 | 4172223 | 29217 |
| East Fork Crooked River | 5330 | Reference | PLAIN | 21631-05 | 10300101 5330 | 11 | 217.29 | 4 | 422334 | 4356113 | 177 |
| West Fork of Big Creek | KKK3 | Reference | PLAIN | 21721-05 | 10280101 3788 | 12 | 332.38 | 4 | 411517 | 4470325 | 81 |
| Spring Creek | KKK19 | Reference | PLAIN | 21831-05 | 10280202 2036 | 12 | 222.69 | 4 | 522810 | 4454091 | 1 |
| Locust Creek | KKK34 | Reference | PLAIN | 21911-05 | 10280103 3444 | 12 | 234.88 | 4 | 489065 | 4476748 | 171 |
| West Locust Creek | RWL6 | Reference | PLAIN | 22051-05 | 10280103 5521 | 12 | 198.01 | 3 | 480924 | 4445789 | 211 |
| West Locust Creek | RWL5 | Reference | PLAIN | 22141-05 | 10280103 5349 | 12 | 188.28 | 3 | 480082 | 4447621 | 211 |
| Sugar Creek | 9532 | Reference | PLAIN | 95321-02 | 7110001 11866 | 16 | 117.2 | 3 | 617658 | 4443306 | 29111 |
| South River | 9663 | Reference | PLAIN | 96631-02 | 7110004 7335 | 16 | 99.1 | 2 | 628763 | 4405824 | 29127 |
| Peno Creek | 9801 | Reference | PLAIN | 98011-02 | 7110007 13132 | 16 | 86.7 | 4 | 647032 | 4369279 | 29163 |
| Youngs Creek | 9803 | Reference | PLAIN | 98031-00 | 7110006 15614 | 16 | 197.2 | 4 | 597771 | 4354618 | 29007 |

[1] Assigned condition of site. "Duplication" indicates additional reference sites used for validation purposes.
[2] Assigned by MDC--final two digits indicate the year of sampling 2000-2005.
[3] Stream segment identification number.
[4] Ecological drainage unit.

## Appendix C. Definitions of the variables retained for use in the development of biocriteria for the fish communities in the wadeable streams of Missouri. The majority of these variables were derived from the EMAP protocol.

MNSLOPE – mean slope of local watershed
HGB_IP – percentage of local segmentshed in hydrologic soil group B
HGD_IP – percentage of local segmentshed in hydrologic soil group D

<u>Bank angle export</u>
XBKA = 'Bank Angle--mean (degrees)'
XUN = 'Undercut Distance--Mean (m)'

<u>Bankfull export</u>
XINC_H = 'Channel Incision Ht.-Mean (m)'
XBKF_W = 'Bankfull Width--Mean (m)'
XBKF_H = 'Bankfull Height-Mean (m)'

<u>Canopy export</u>
XCDENBK = 'Mean Bank Canopy Density (%)'
XCDENMID = 'Mean Mid-channel Canopy Density (%)'

<u>Embeddedness export</u>
XCEMBED = 'Mean Embeddedness--Channel only (%)'

<u>Fish cover export</u>
XFC_ALG = 'Fish Cvr-Filamentous Algae (Areal Prop)'
XFC_AQM = 'Fish Cvr-Aq. Macrophytes (Areal Prop)'
XFC_BRS = 'Fish Cvr-Brush&Small Debris (Areal Prop)'
XFC_HUM = 'Fish Cvr-Artif. Structs. (Areal Prop)'
XFC_LWD = 'Fish Cvr-Large Woody Debris (Areal Prop)'
XFC_NAT = 'Fish Cvr-Natural Types (Sum Areal Prop)'
XFC_OHV = 'Fish Cvr-Overhang Veg (Areal Prop)'
XFC_RCK = 'Fish Cvr-Boulders (Areal Prop)'
XFC_UCB = 'Fish Cvr-Undercut Banks (Areal Prop)'

<u>Habitat type export</u>
PCT_PB = 'Backwater Pool (% of reach length)'
PCT_FAST = 'Fast Wtr Hab (% riffle & faster)'
PCT_POOL = 'Pools -- All Types (% of reach)'

<u>Residual pool labels</u>
NRP = 'Number of residual pools in reach'
PCTRCHRP = 'Resid. pool length proportion (%of rch)'
RPGT50 = 'Resid Pools >50cm deep (number/reach)'
RPGT75 = 'Resid Pools >75cm deep (number/reach)'
RPMDEP = 'Maximum residual depth in reach (cm)'
RPMLEN = 'Max. resid pool length in reach (m/pool)'
RPMWID = 'Max resid width of any pool in reach (m)'
RPMVOL = 'Max volume of any pool in reach (m^3)'
PCTUSED = '% of pool head length with sediment'

<u>Sinuosity</u>
SINU = 'Channel Sinuosity (m/m)'

<u>Slope</u>
XSLOPE = 'Channel Slope -- reach mean (%)'

Substrate
SUB_X = 'Substrate--Mean Size Class (1-6)'
PCT_RR = 'Substrate Rough Bedrock (%)'
PCT_RS = 'Substrate Smooth Bedrock (%)'
PCT_CB = 'Substrate Cobbles -- 64-250 mm (%)'
PCT_GC = 'Substrate Coarse Gravel -- 16-64 mm (%)'
PCT_GF = 'Substrate Fine Gravel -- 2-16 mm (%)'
PCT_SA = 'Substrate Sand -- .06-2 mm (%)'
PCT_FN = 'Substrate Fines -- Silt/Clay/Muck (%)'
PCT_HP = 'Substrate Hardpan -- (%)'
PCT_SAFN = 'Substrate Sand & Fines -- <2 mm (%)'
PCT_BDRK = 'Substrate Bedrock (%)'

Thalweg
WD_RAT = 'Mean Width/Depth Ratio (m/m)'

Canopy
XPCAN = 'Rip Canopy Present (Fraction of reach)'
XPMID = 'Rip MidLayer Present (Fraction of reach)'
XPGVEG = 'Rip Ground Layer Present (Fract. reach)'

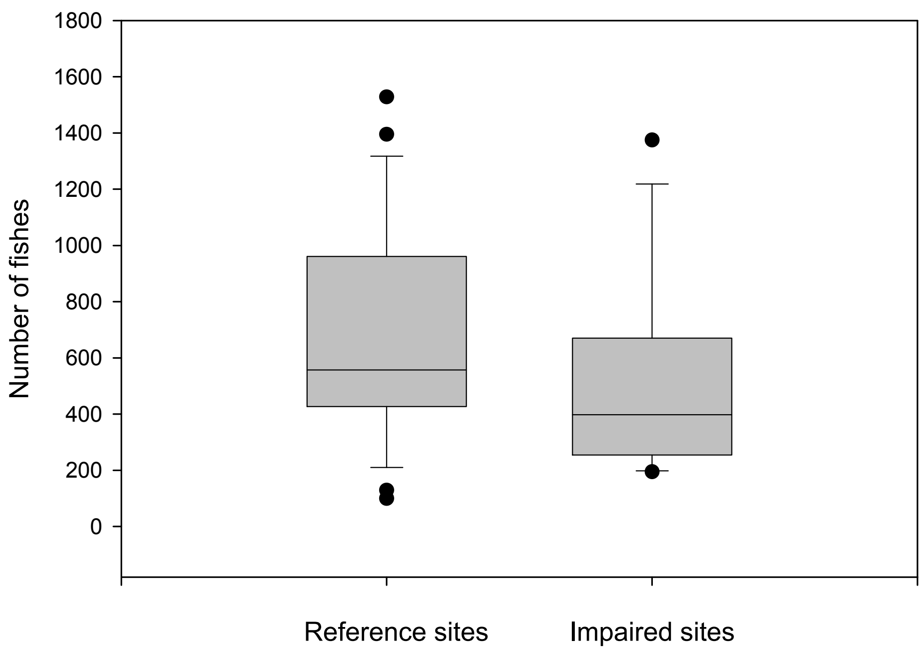
Woody debris
C1Wm100 = 'LWD in Bkf chnl (#/100m-all sizes)'
V1Wm100 = 'LWD Vol in Bkf chnl (m3/100m-all sizes)'

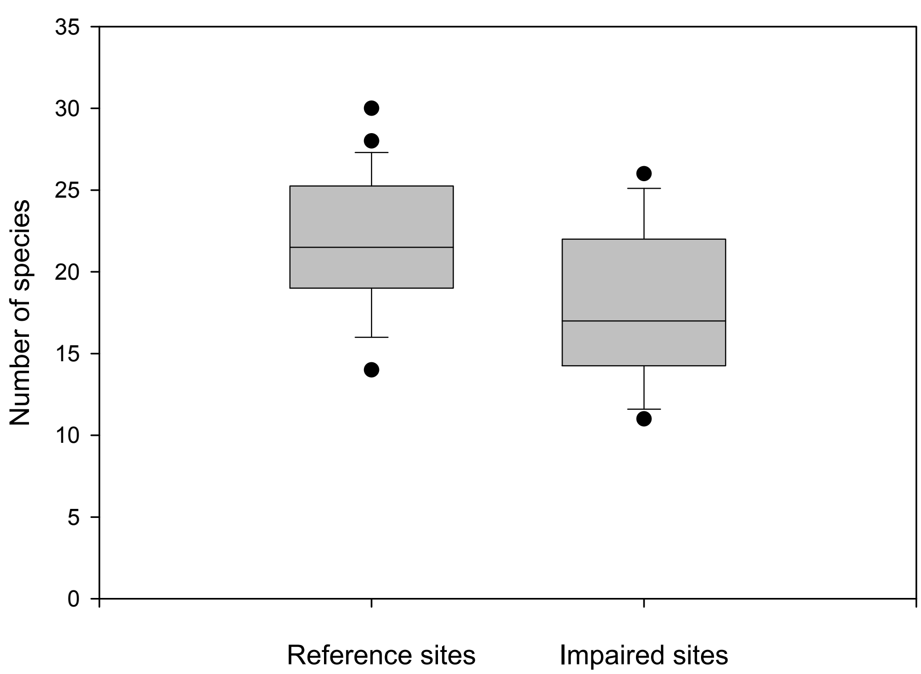
Human disturbance
LAND COVER 1 - percentage of urban land cover in watershed
LAND COVER 2 – percentage of row crop cover in watershed
LAND COVER 3 – percentage of grassland cover in the watershed
LAND COVER 4 – percentage of forest cover in the watershed
LAND COVER 6 – percentage of water cover in the watershed
Water temperature – (degrees centigrade) based on one measurement at time of field collections
Dissolved oxygen – (mg/l) based on one measurement at the time of field collections
Conductivity – (umhos/cm) based on one measurement at time of field collections
pH – (standard units) based on one measurement at time of field collections
Turbidity (NTU) - based on one measurement at time of field collections
BXPBLDG = 'The mean, BLDG, on Bank'
BXPMINE = 'The mean, MINE, on Bank'
BXPPARK = 'The mean, PARK/LAWN, on Bank'
BXPPSTR = 'The mean, PASTURE, on Bank'
BXPROAD = 'The mean, ROAD, on Bank'
BXPWALL = 'The mean, WALL, on Bank'
CXPCROP = 'The mean, CROP, in Riparian Plot'
CXPPSTR = 'The mean, PSTR, in ripar Plot'
CXPROAD = 'The mean, ROAD, in Ripar Plot'
XB_HALL = 'Rip Dist--Sum All Types instrm & on bank'
XCB_HALL = 'Rip Dist--Sum All Types instrm & in plot'
XC_HALL = 'Rip Dist--Sum All Types in Ripar Plots'
X_HALL = 'Rip Dist--Sum All Types str plt & beyond'

Final habitat indices
QCPH = Channel phyhab quality
QPH = Channel plus Riparian phyhab quality
QTPH = Channel plus Riparian phyhab quality including Riparian Human Disturbance
If followed by a ‘1’ it indicates data not adjusted to watershed size, adjusted to watershed size if followed by a ‘2’.

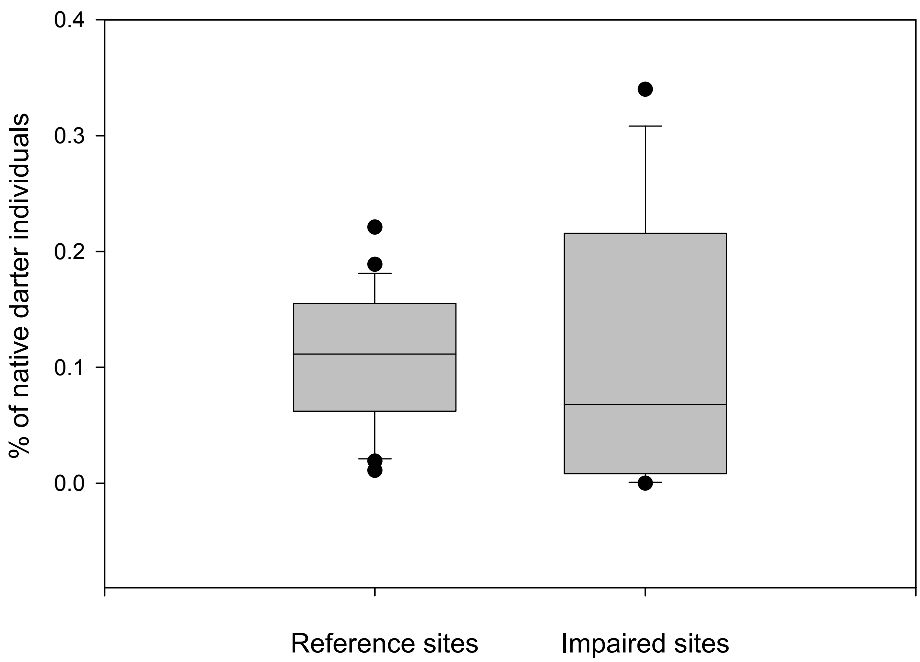
**Appendix D. Metric comparisons for the Ozark and Plain ecoregions – reference versus impaired sites.**

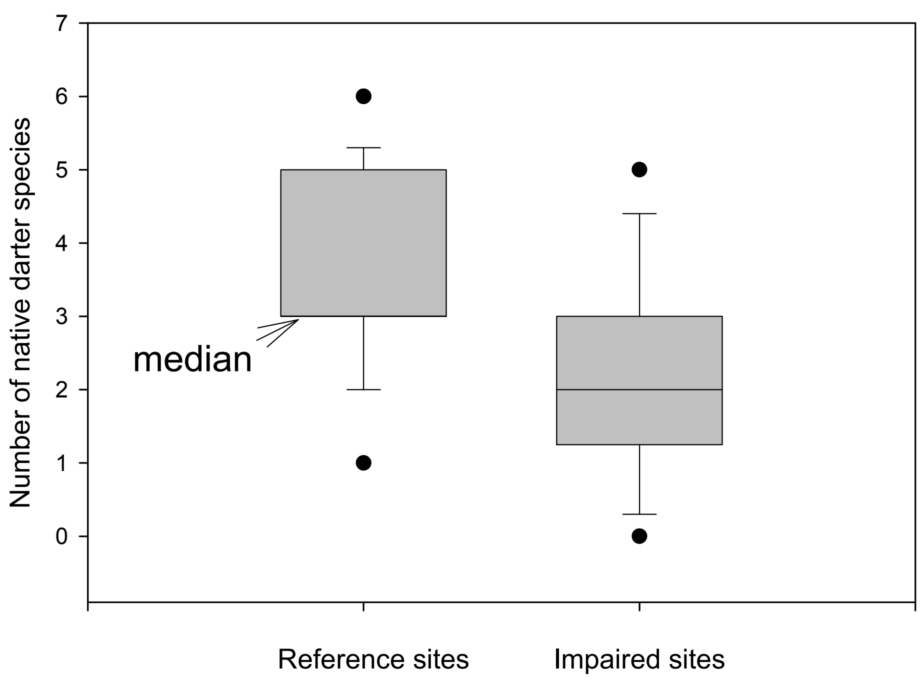
Ozark ecoregion metrics



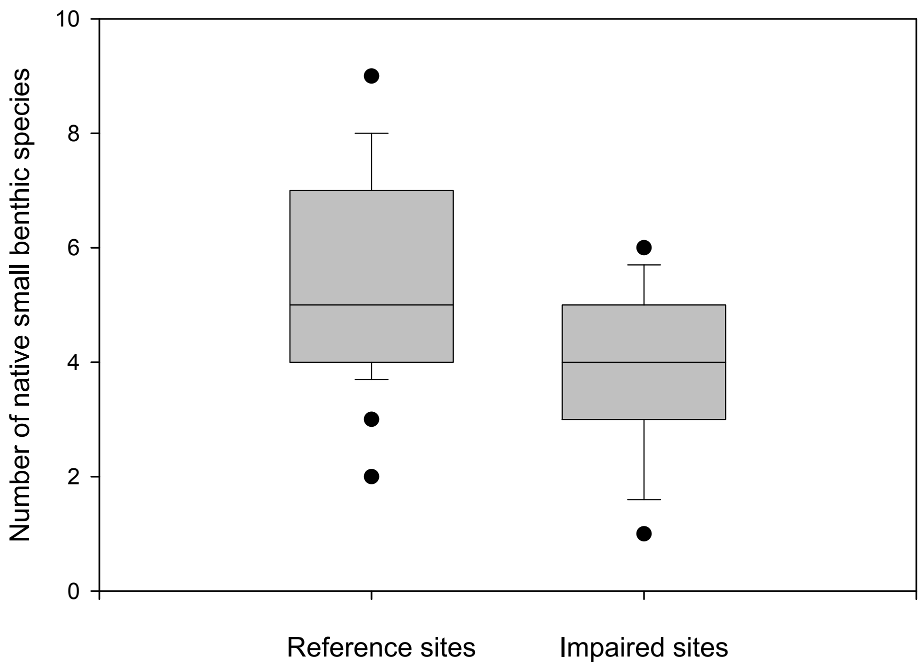



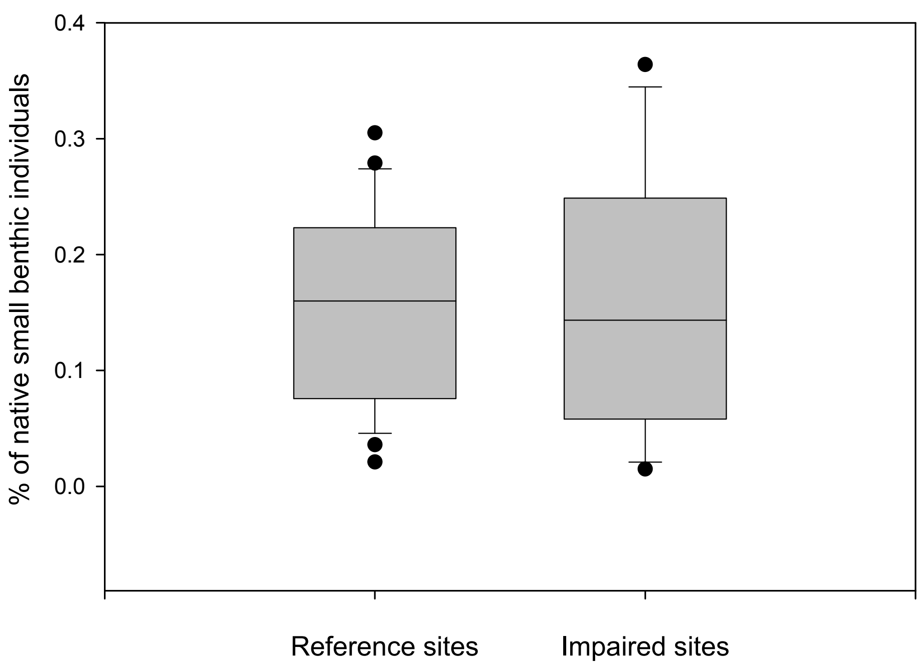
Ozark ecoregion metrics



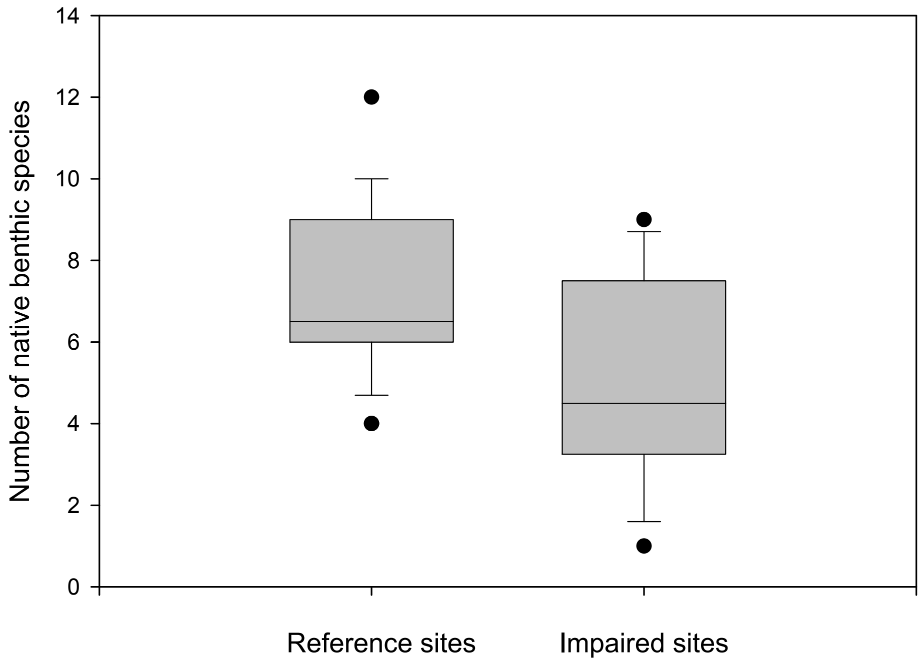



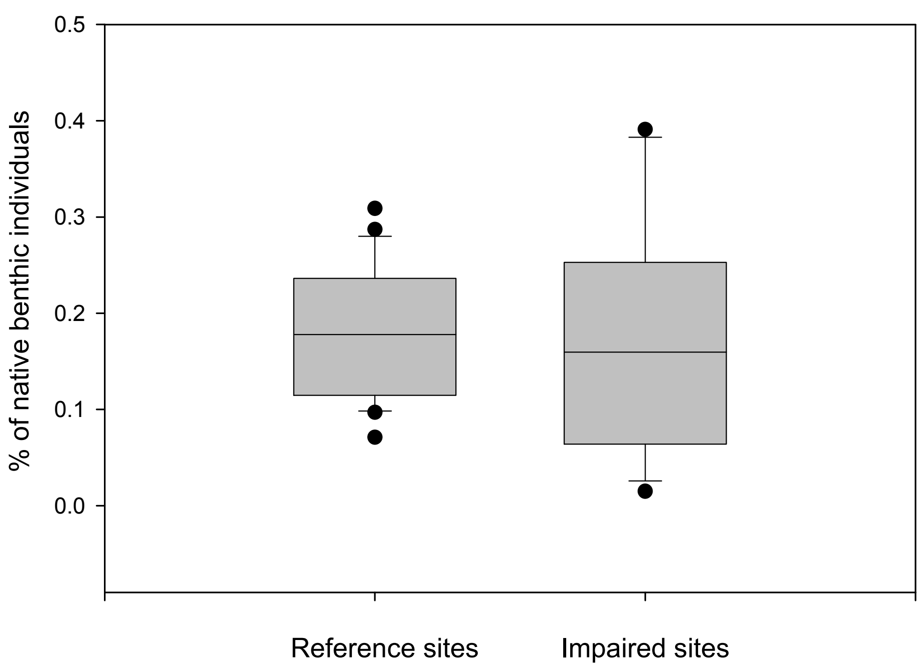
Ozark ecoregion metrics



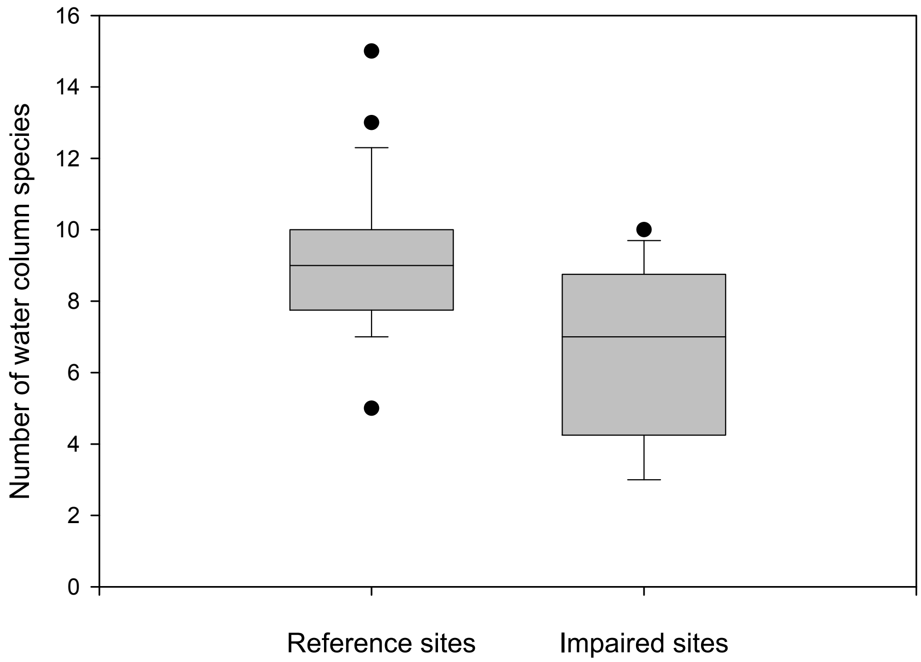



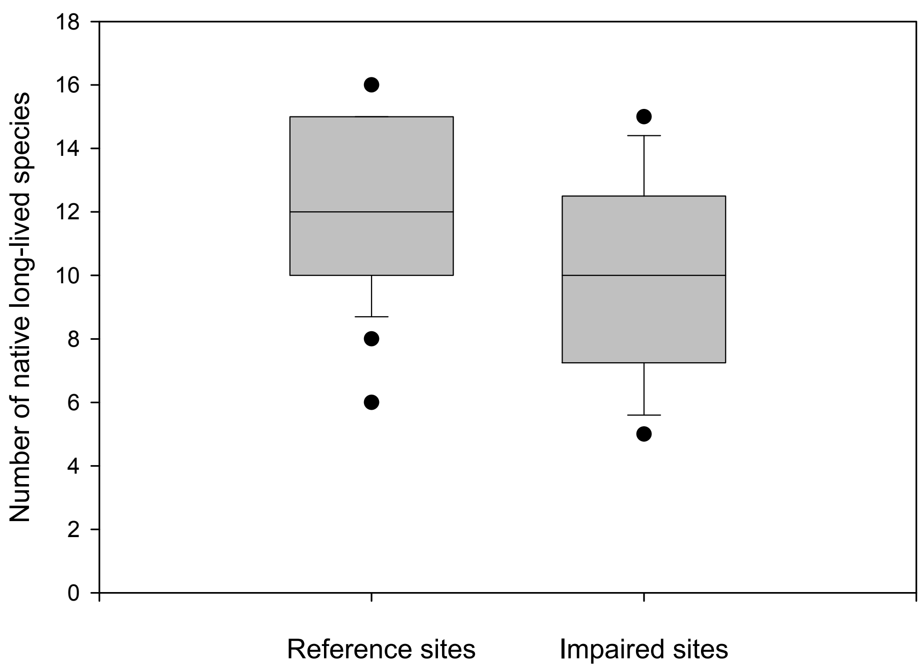
Ozark ecoregion metrics



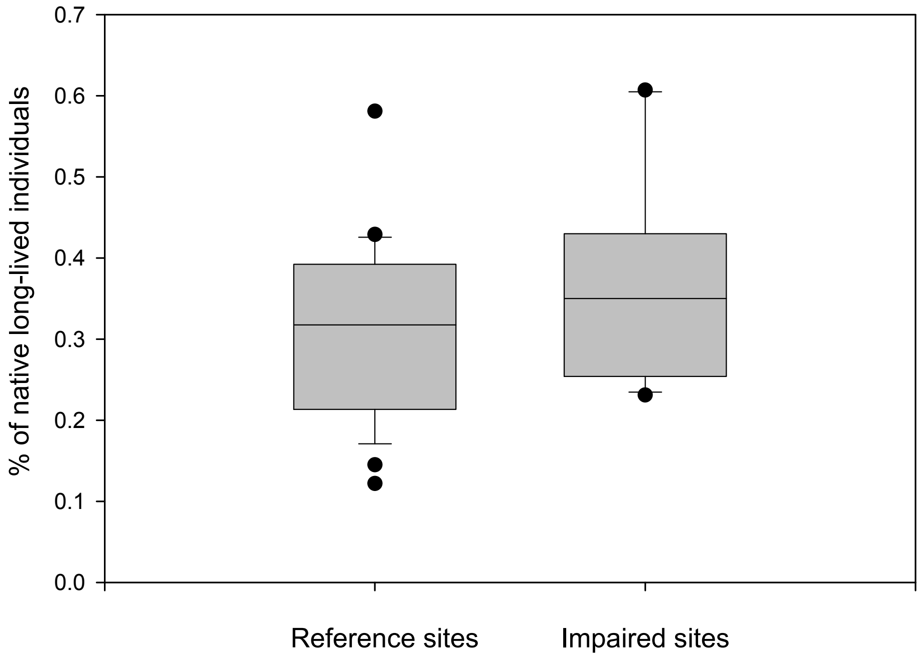



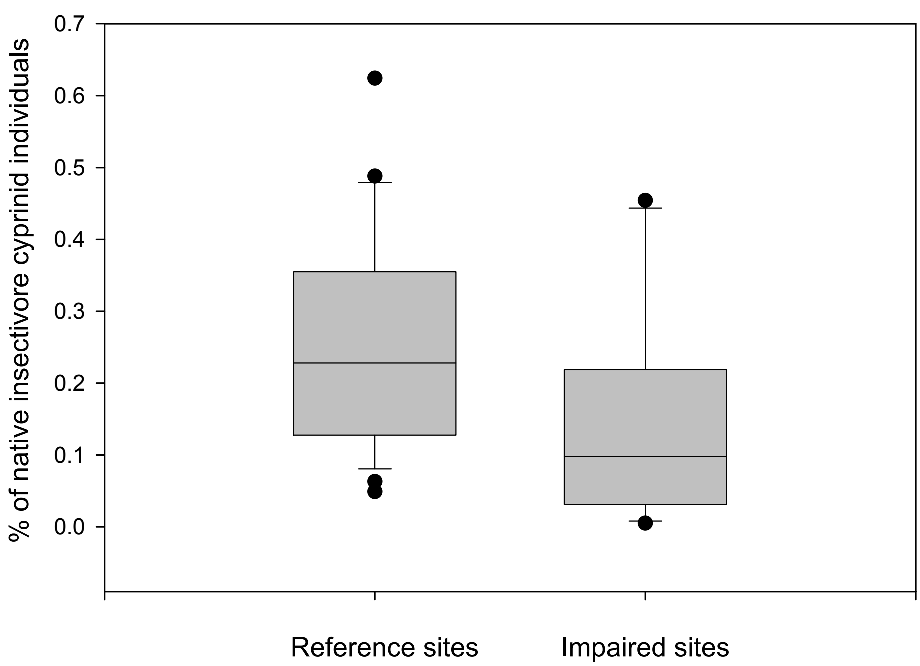
Ozark ecoregion metrics



Ozark ecoregion metrics



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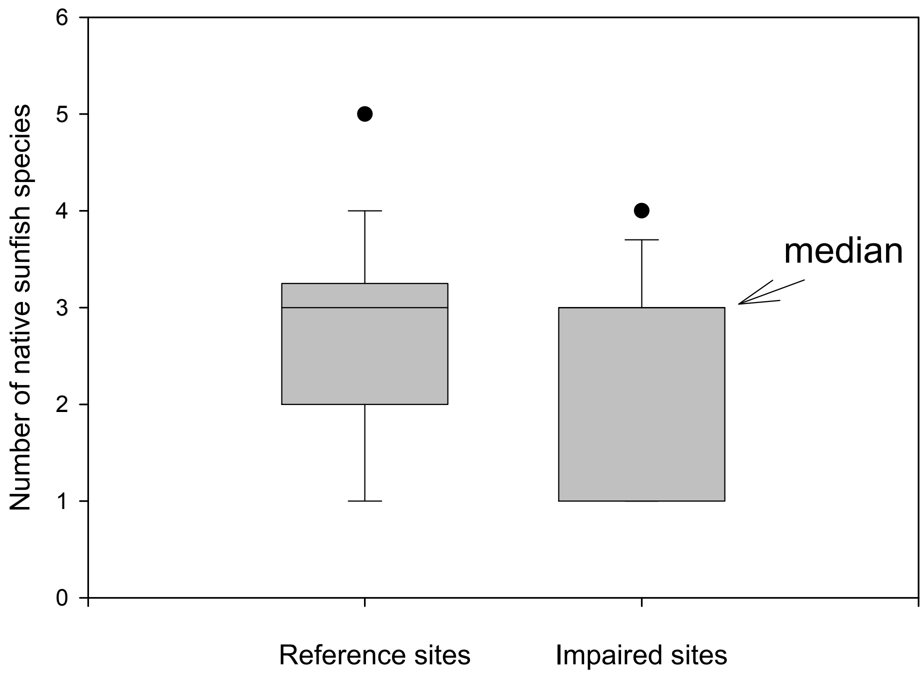
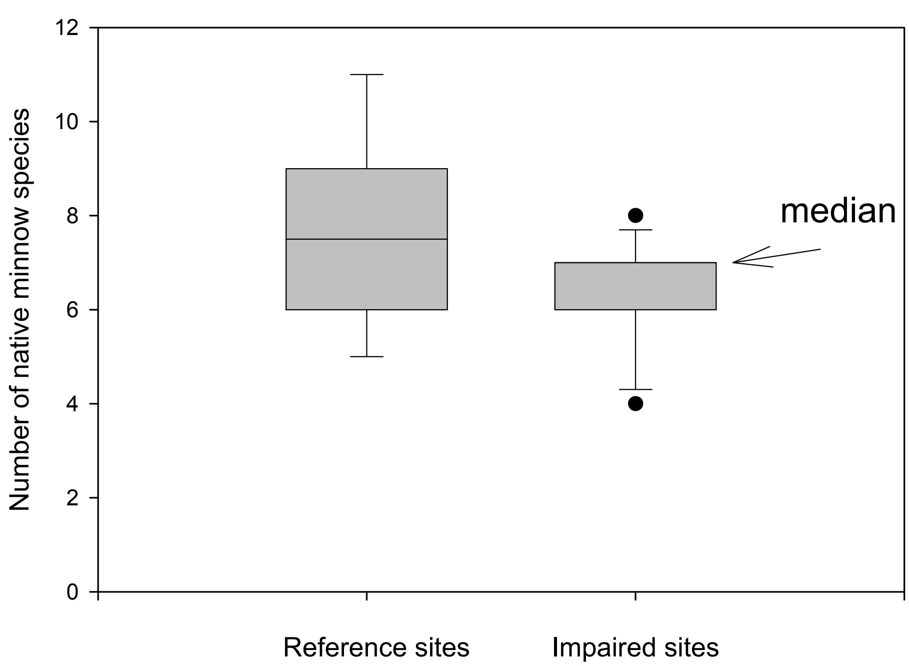
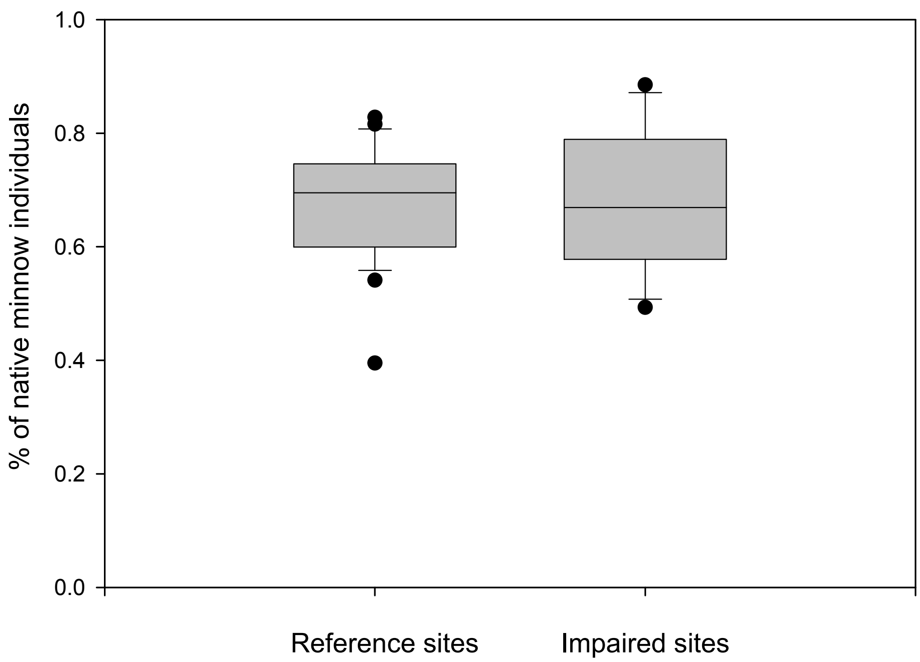
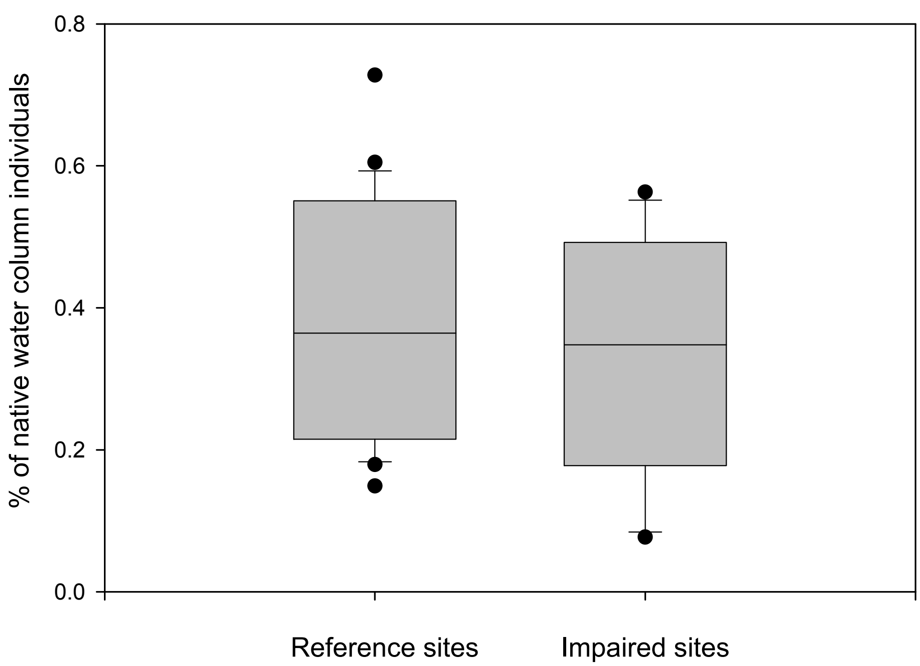


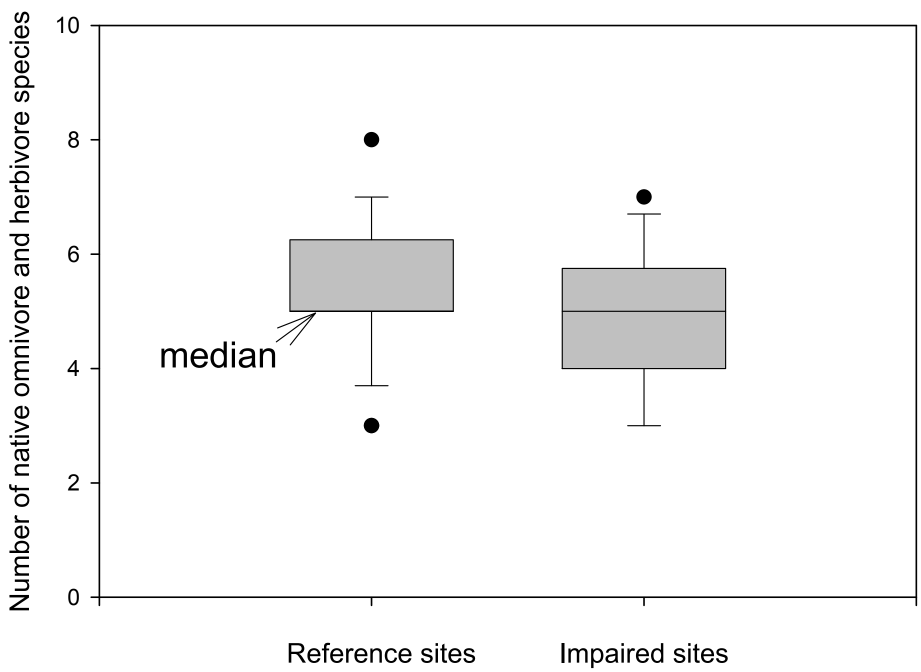



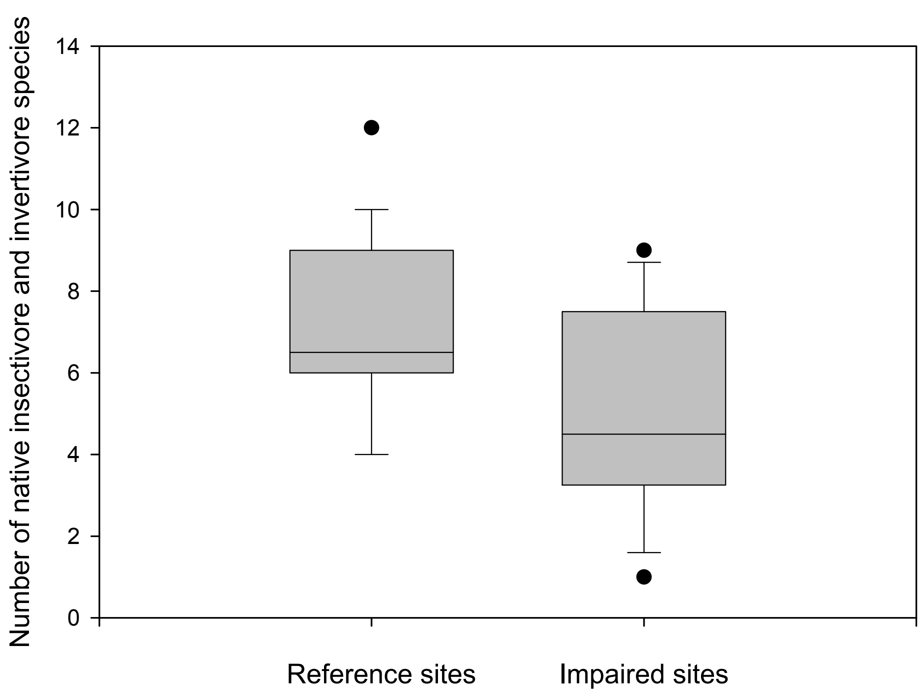
Ozark ecoregion metrics



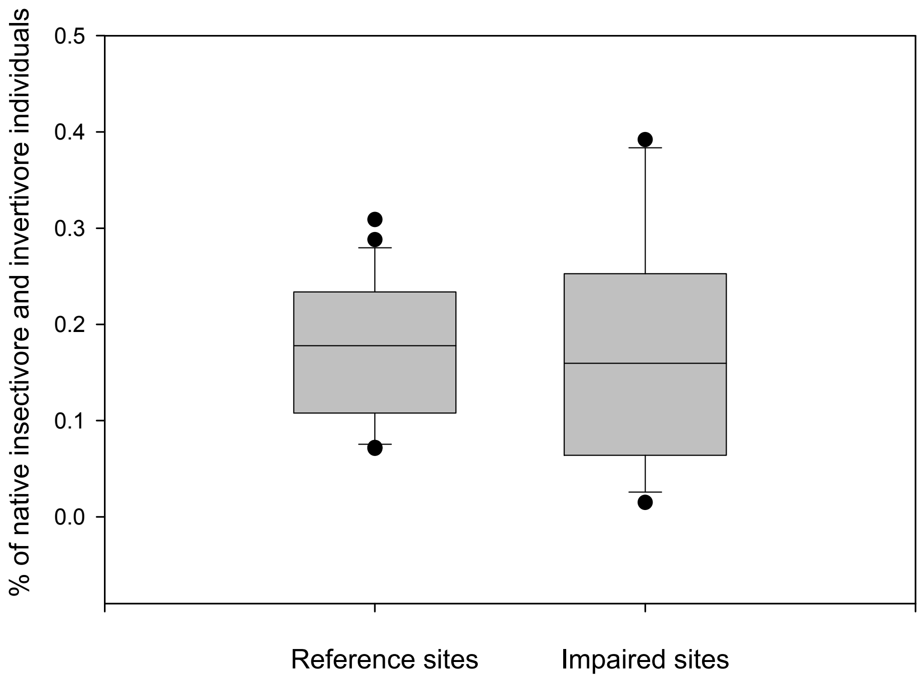



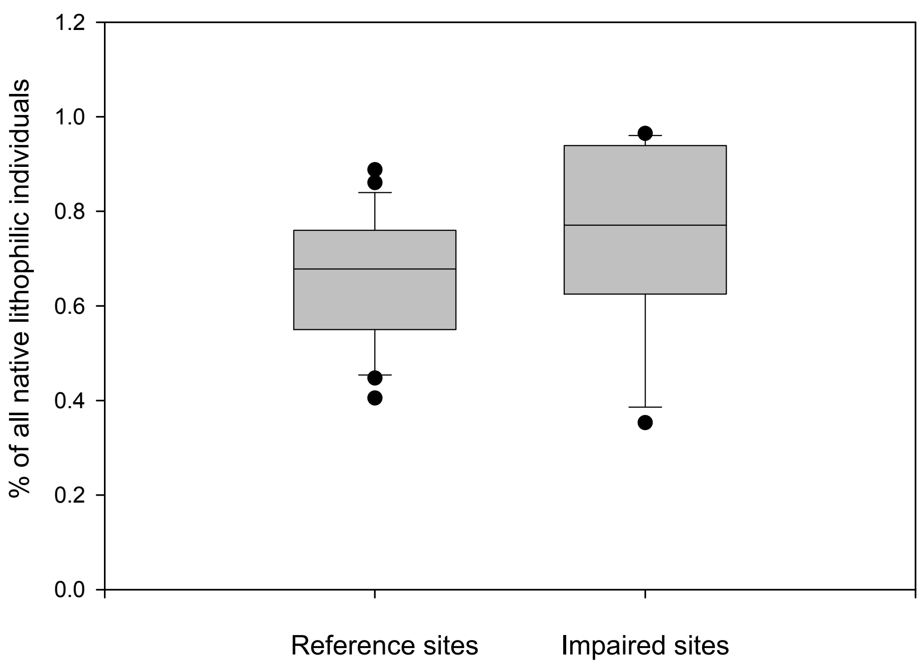
Ozark ecoregion metrics



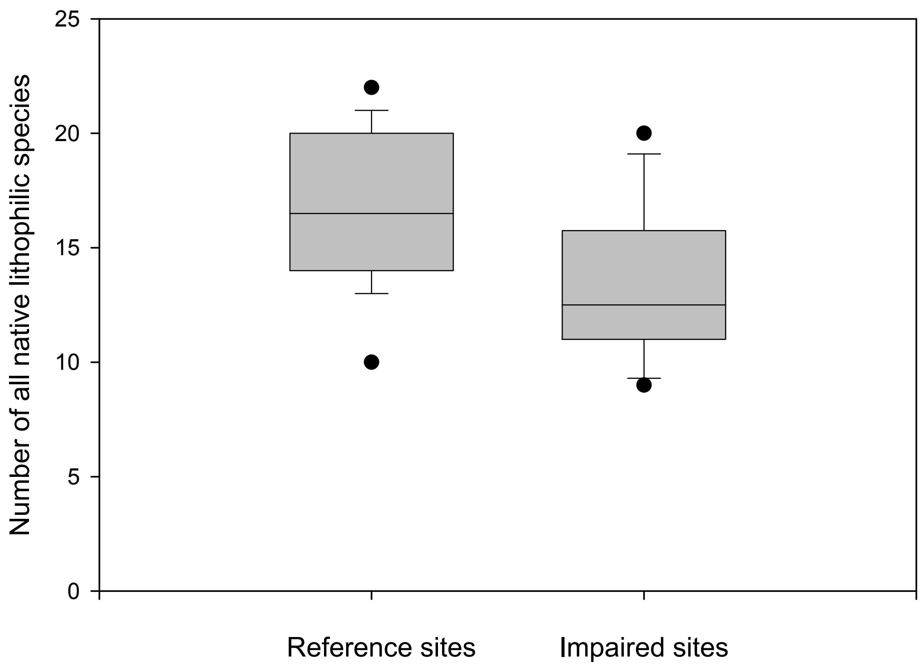



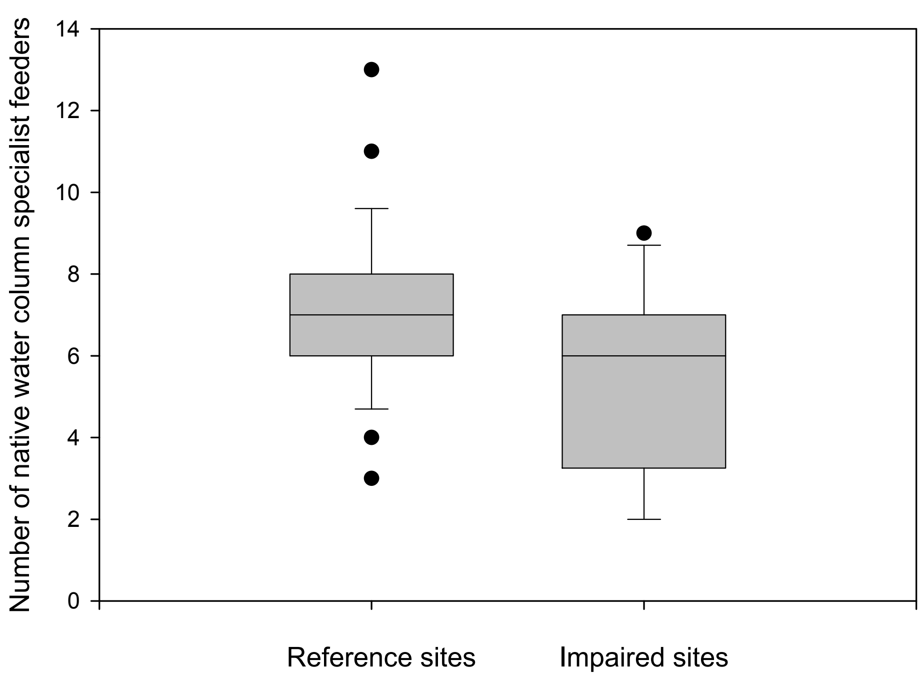
Ozark ecoregion metrics



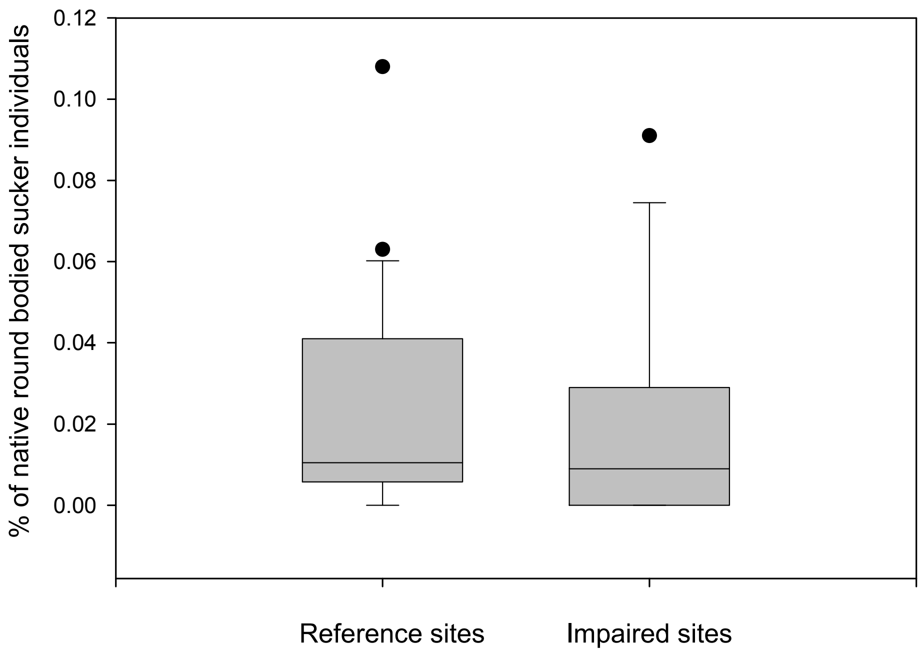



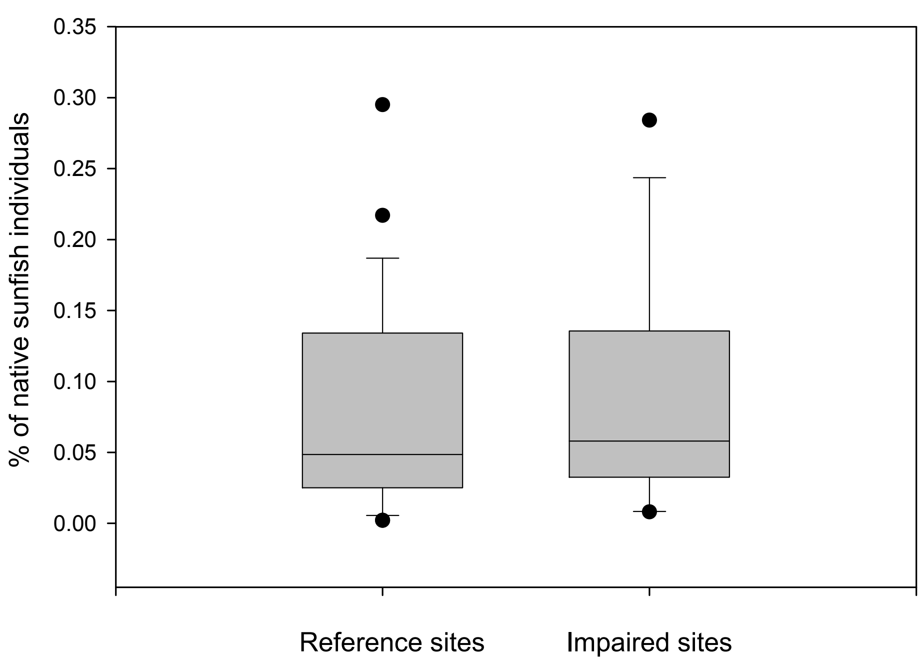
Ozark ecoregion metrics



Ozark ecoregion metrics



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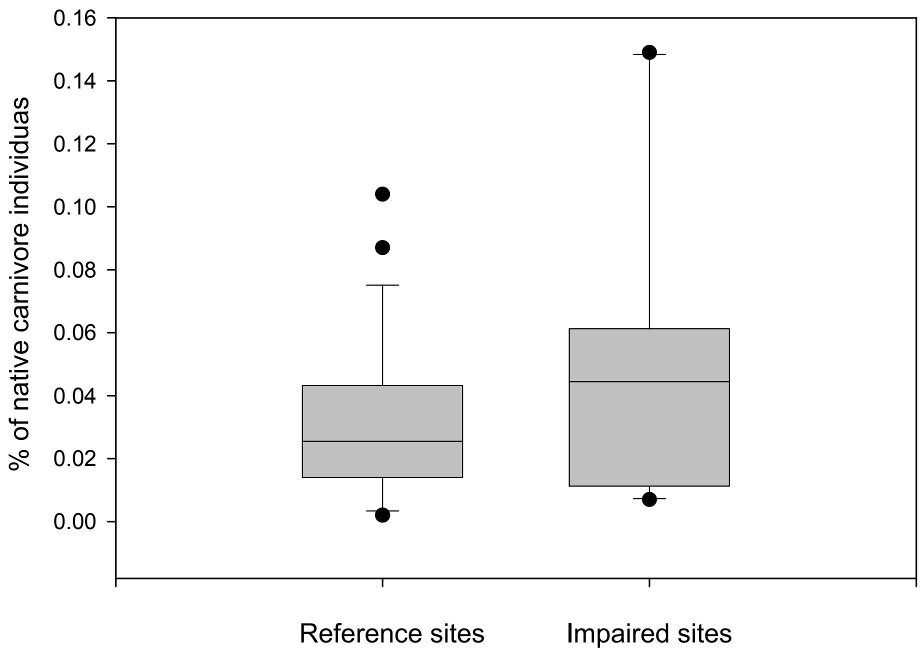
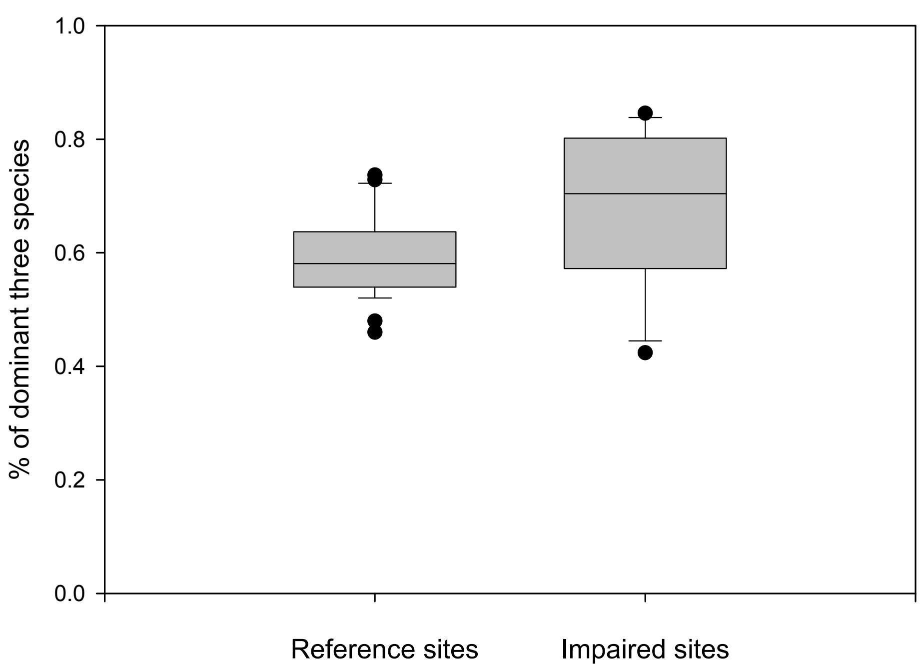
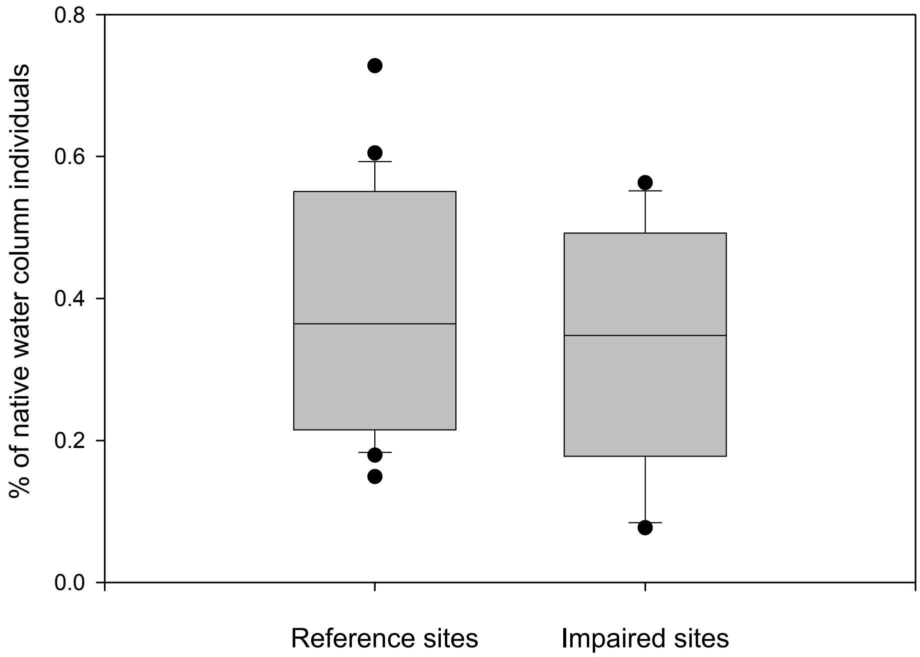
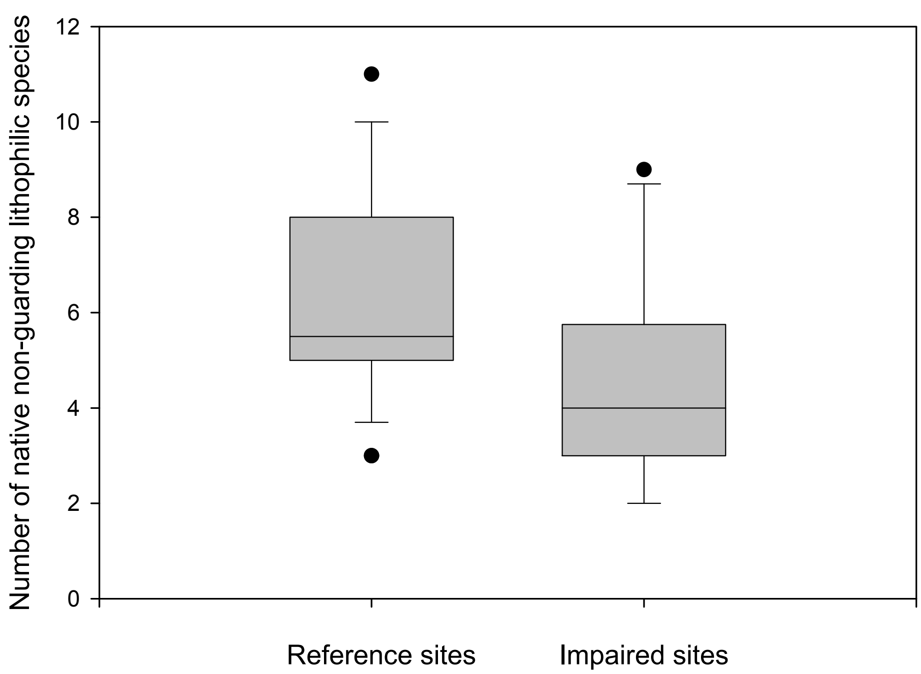


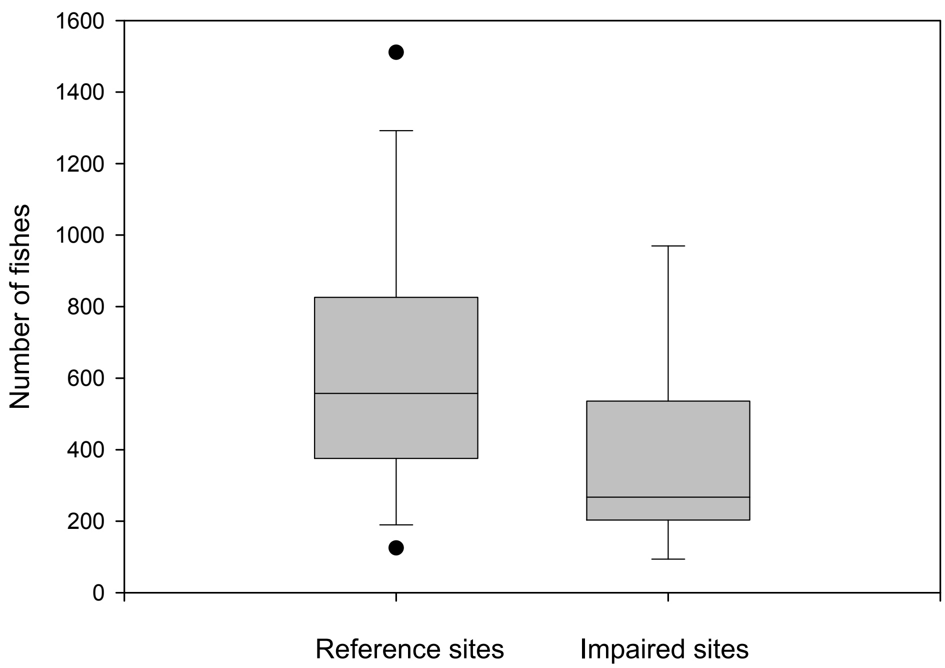
Ozark ecoregion metrics

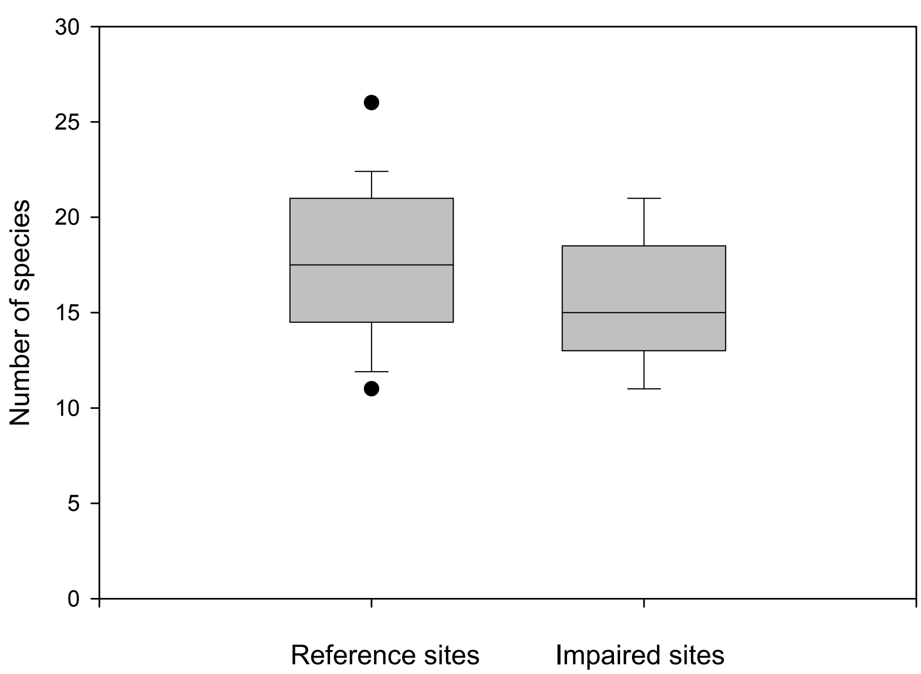


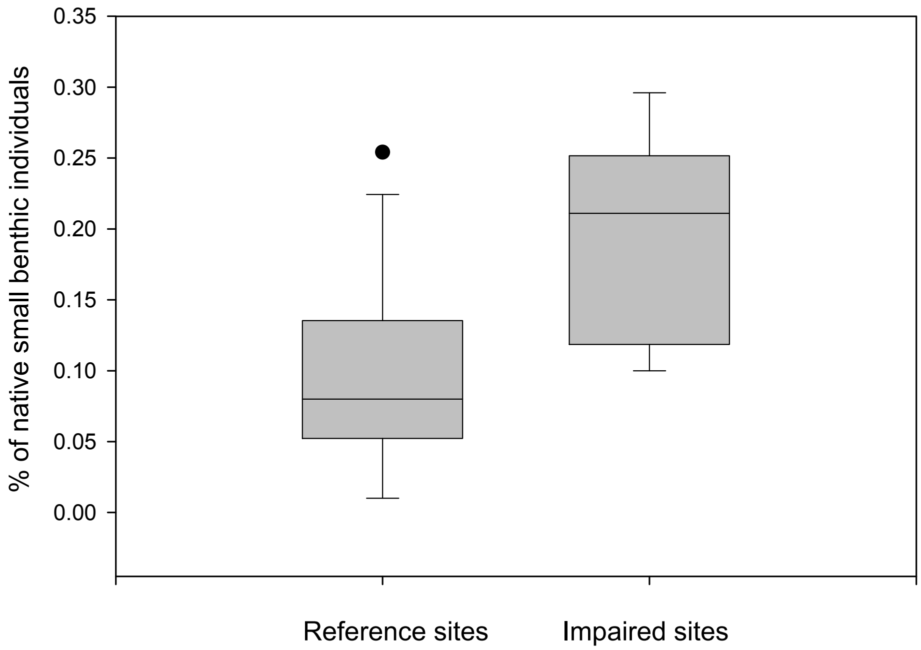
## Metric comparisons for the Plain ecoregion – reference versus impaired sites

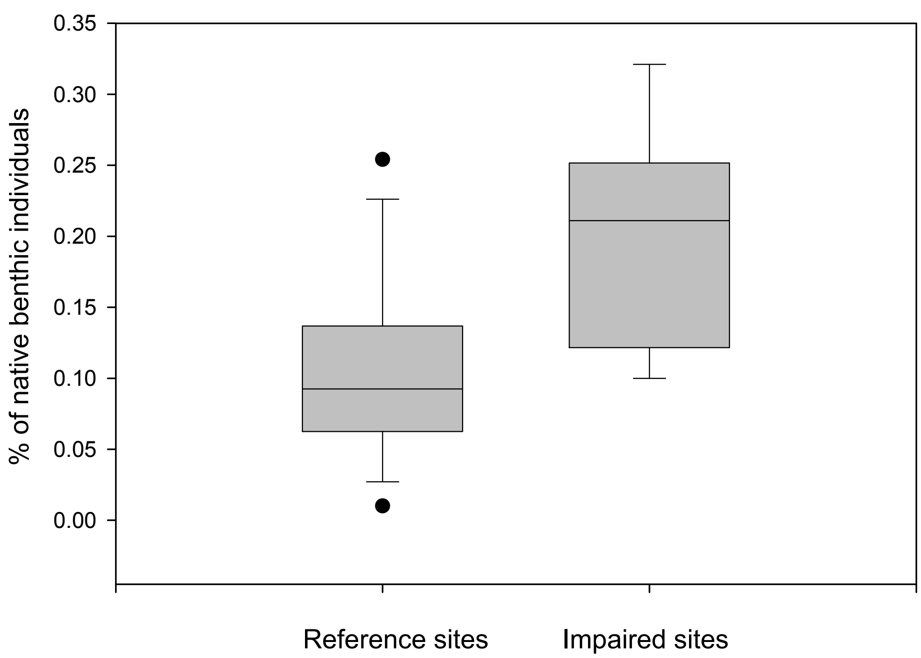
Plain ecoregion metrics



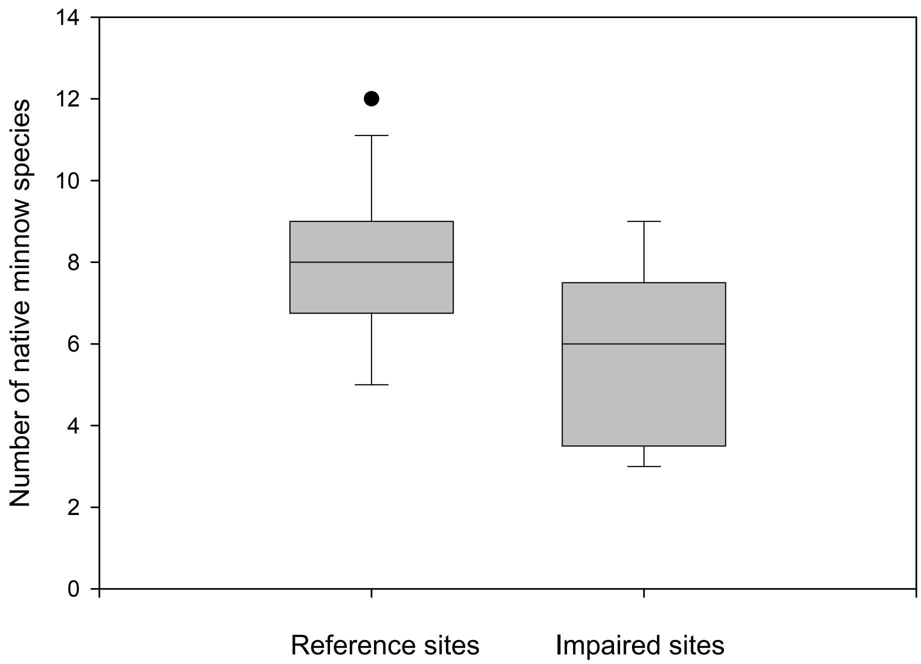



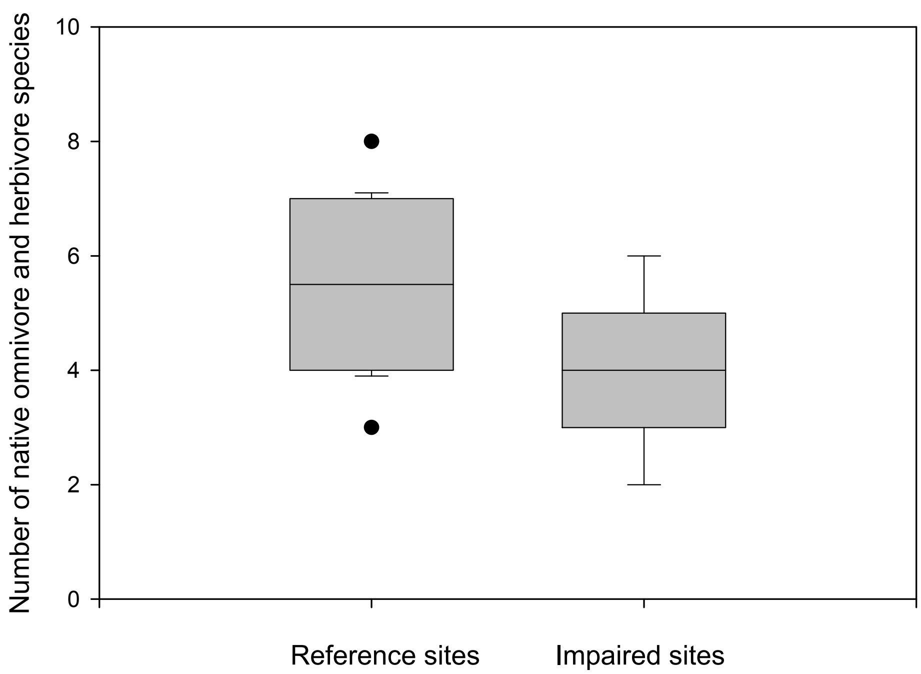
Plain ecoregion metrics



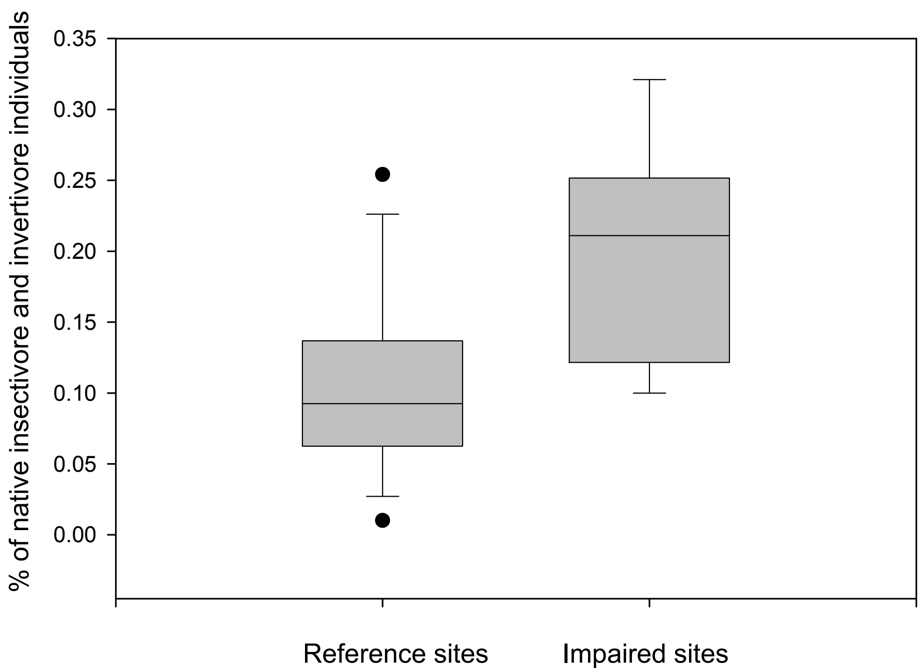



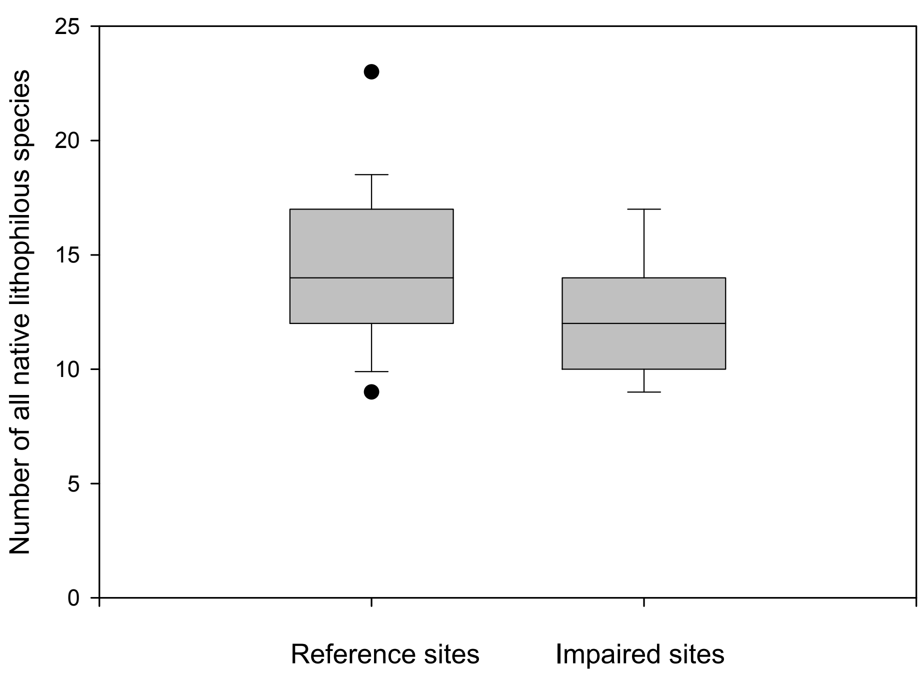
Plain ecoregion metrics

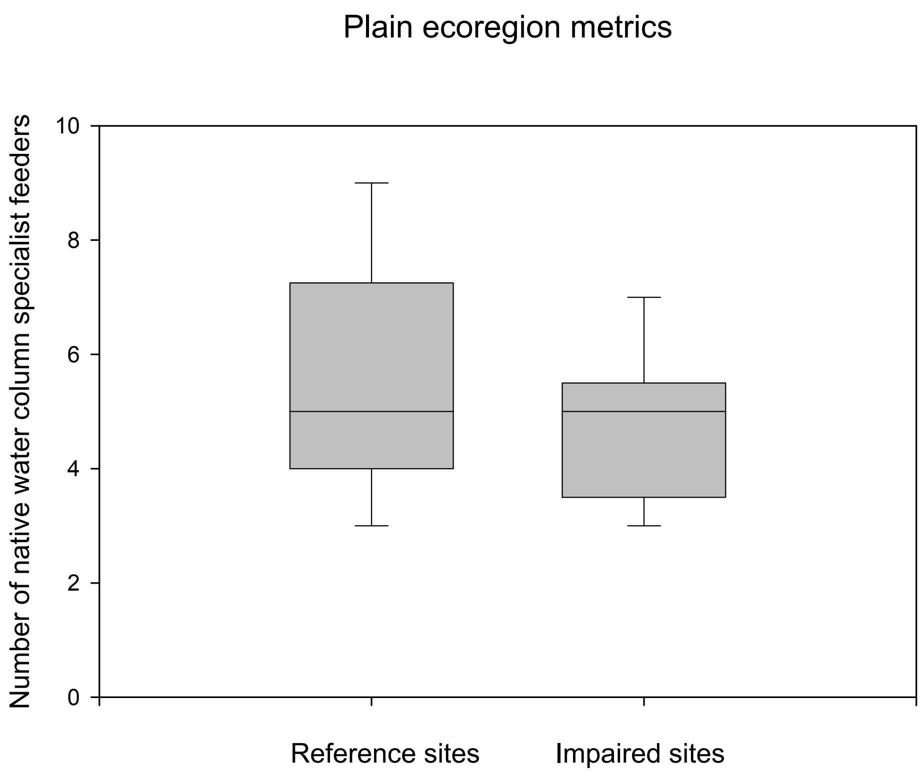


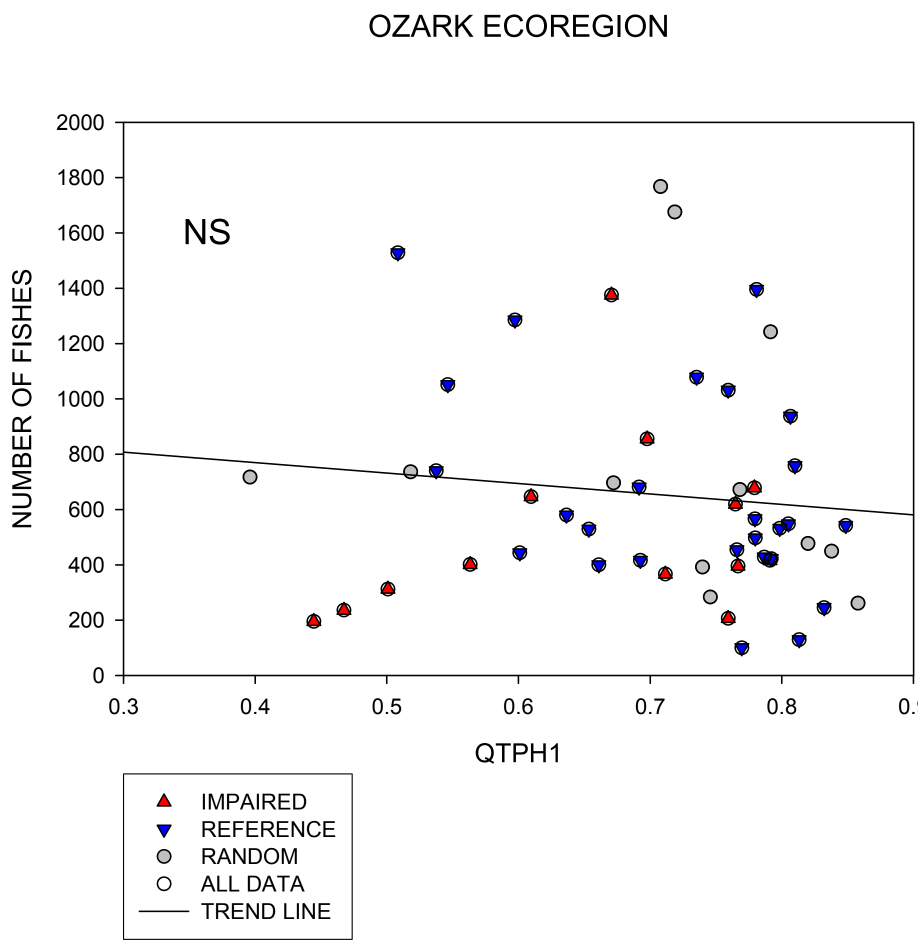
Plain ecoregion metrics

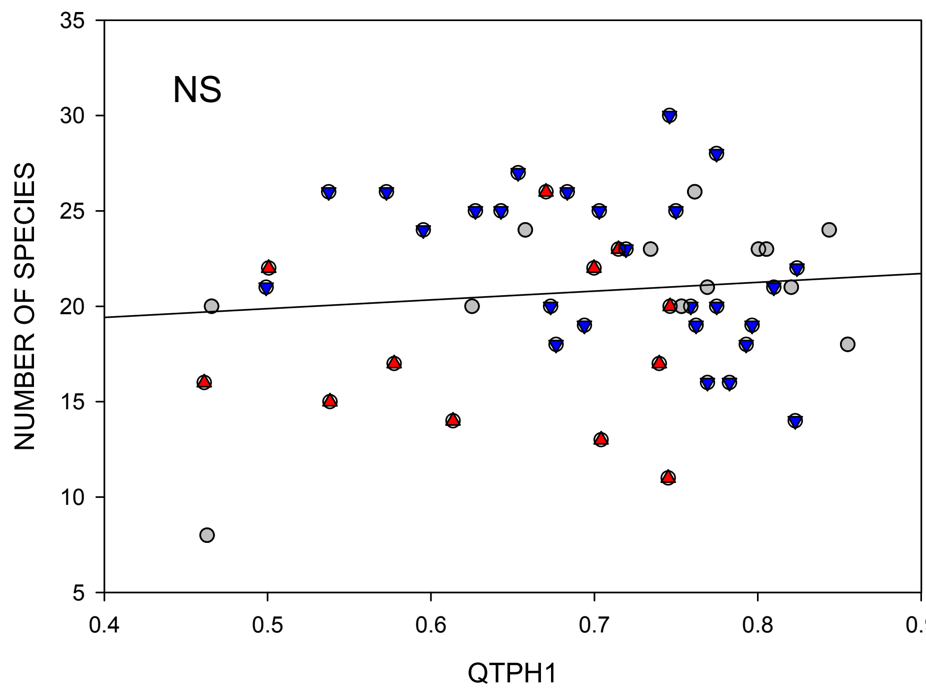


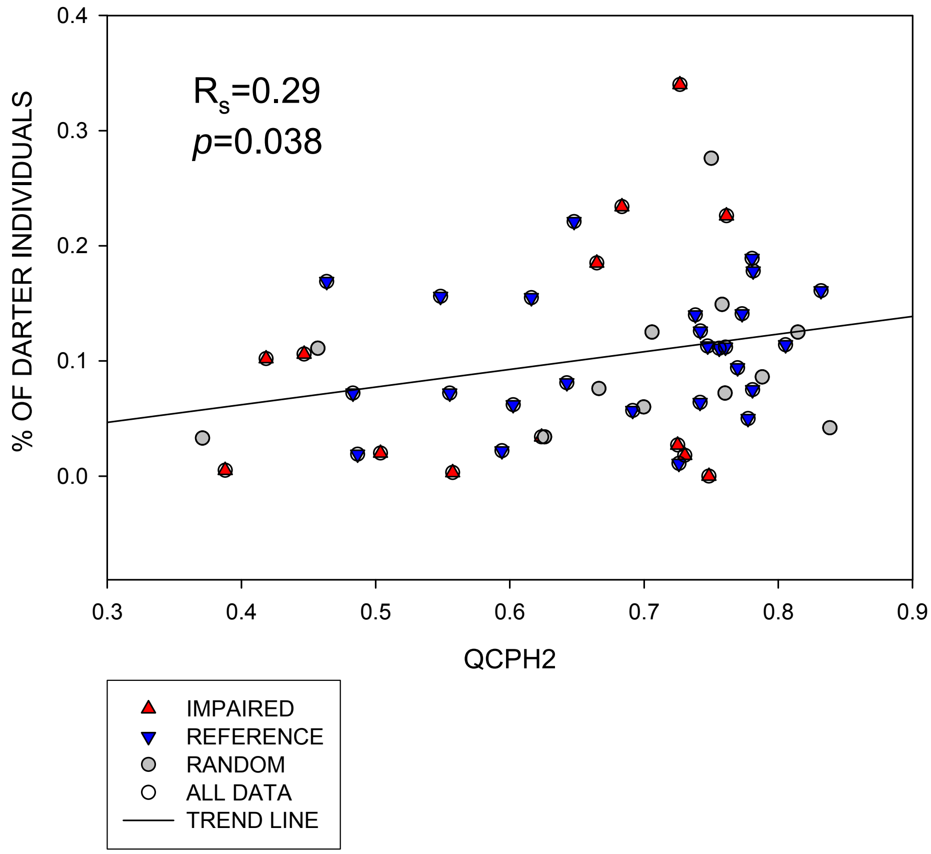

Plain ecoregion metrics
Number of native water column specialist feeders
10
8
6
4
2
0
Reference sites
Impaired sites

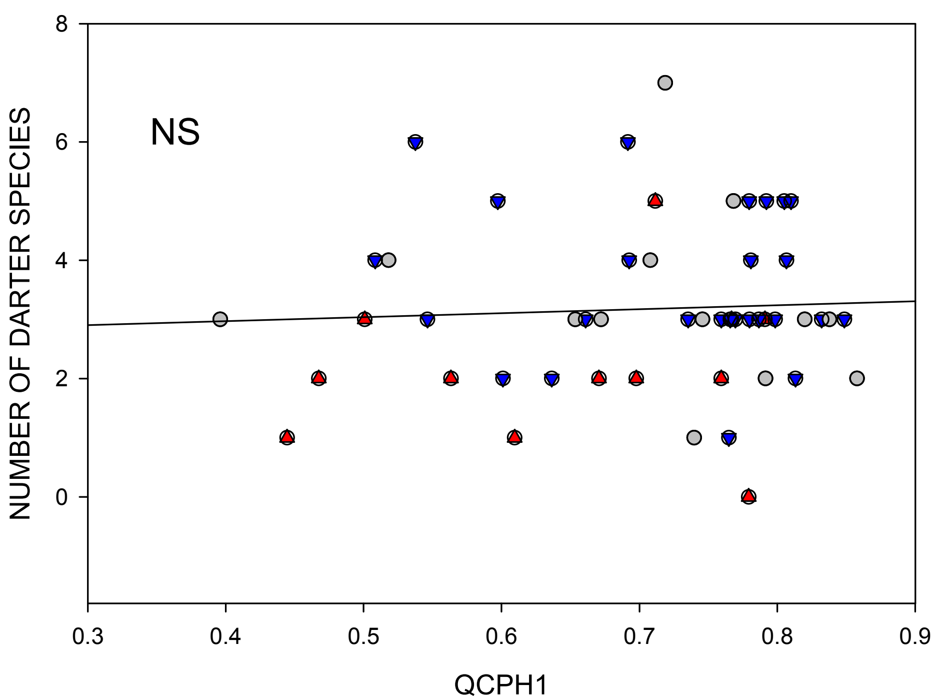
**Appendix E. Spearman rank correlations of Ozark metrics with the final habitat index scores.**



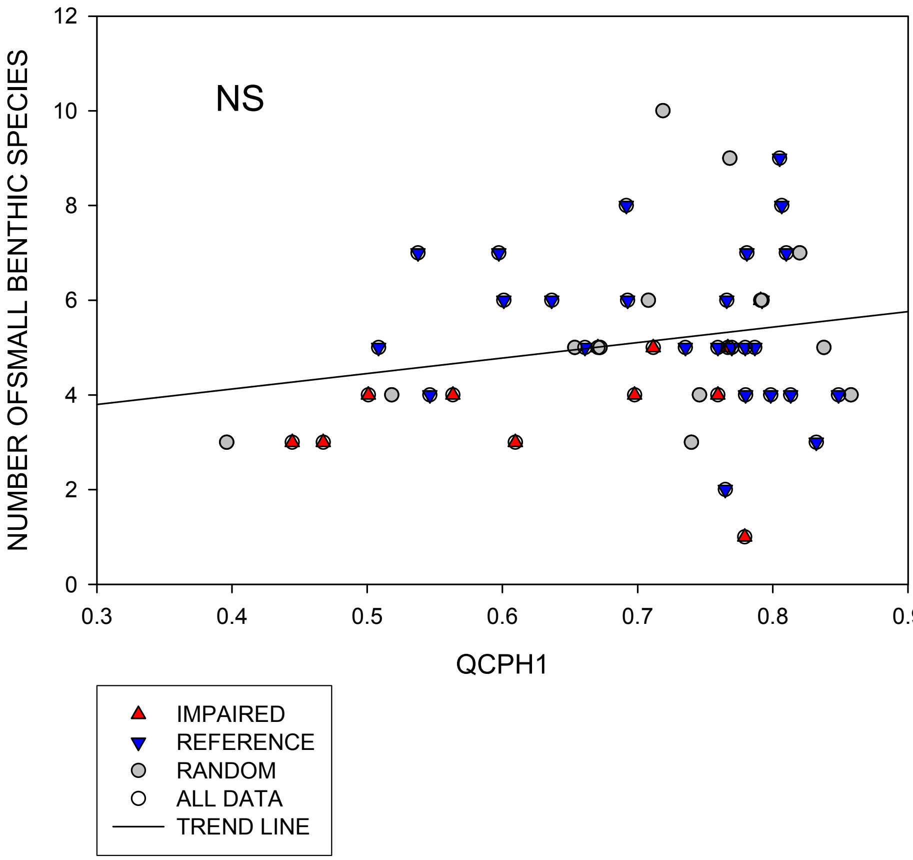



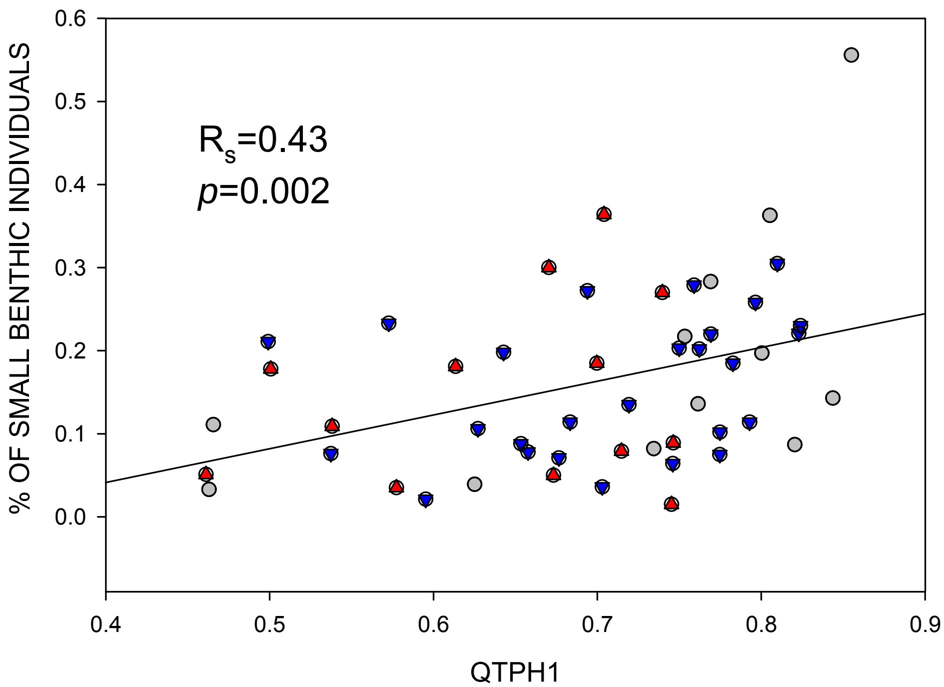
## OZARK ECOREGION



OZARK ECOREGION



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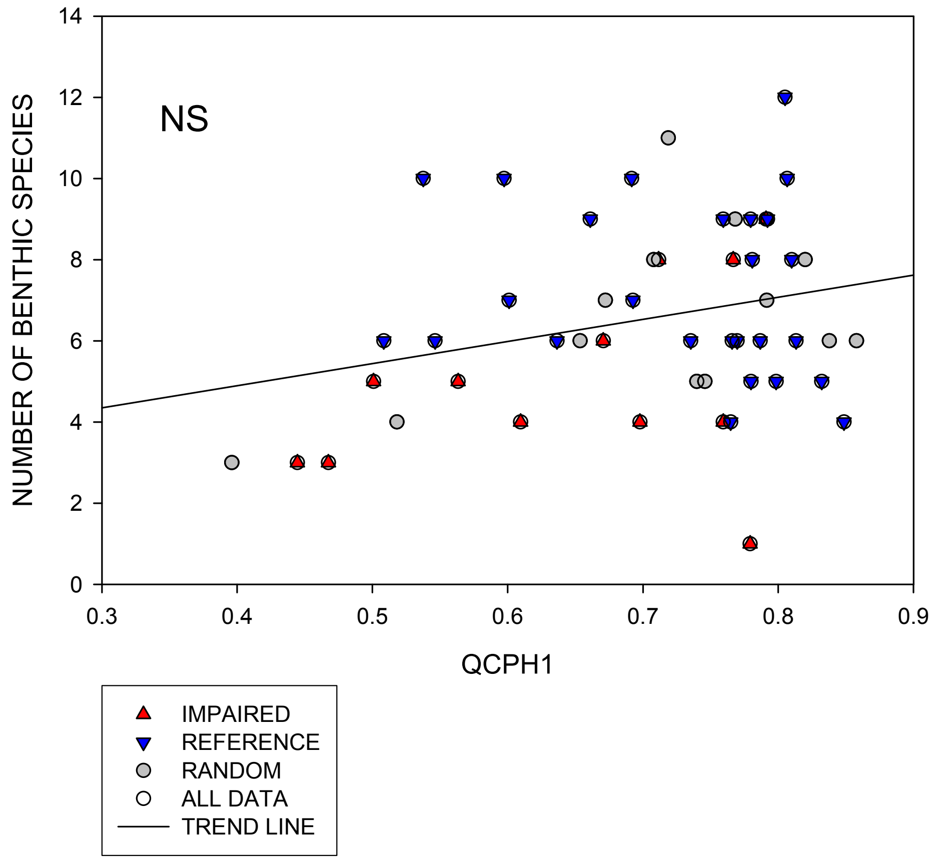
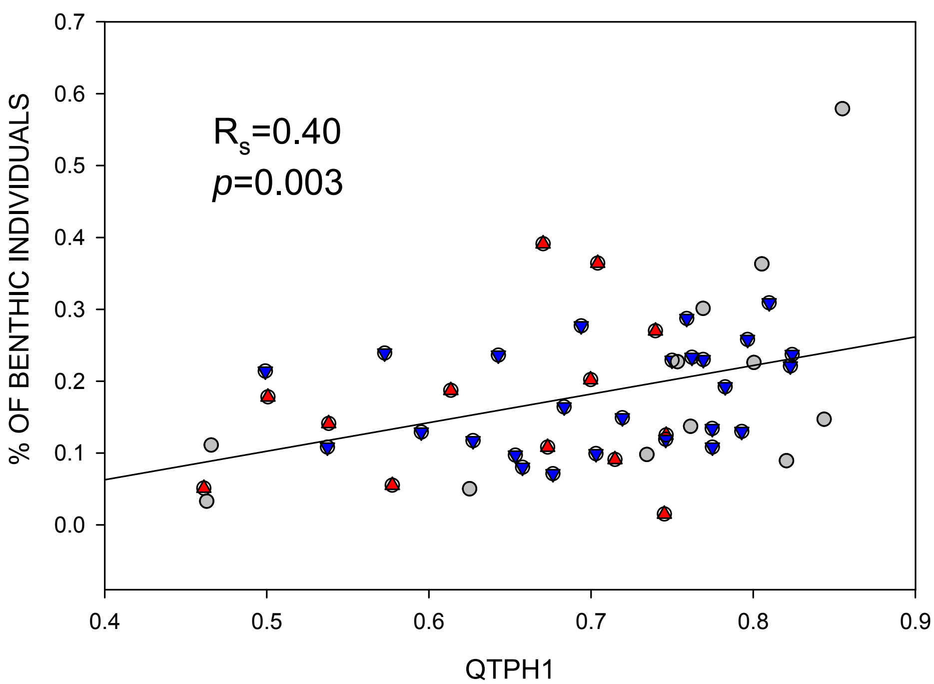
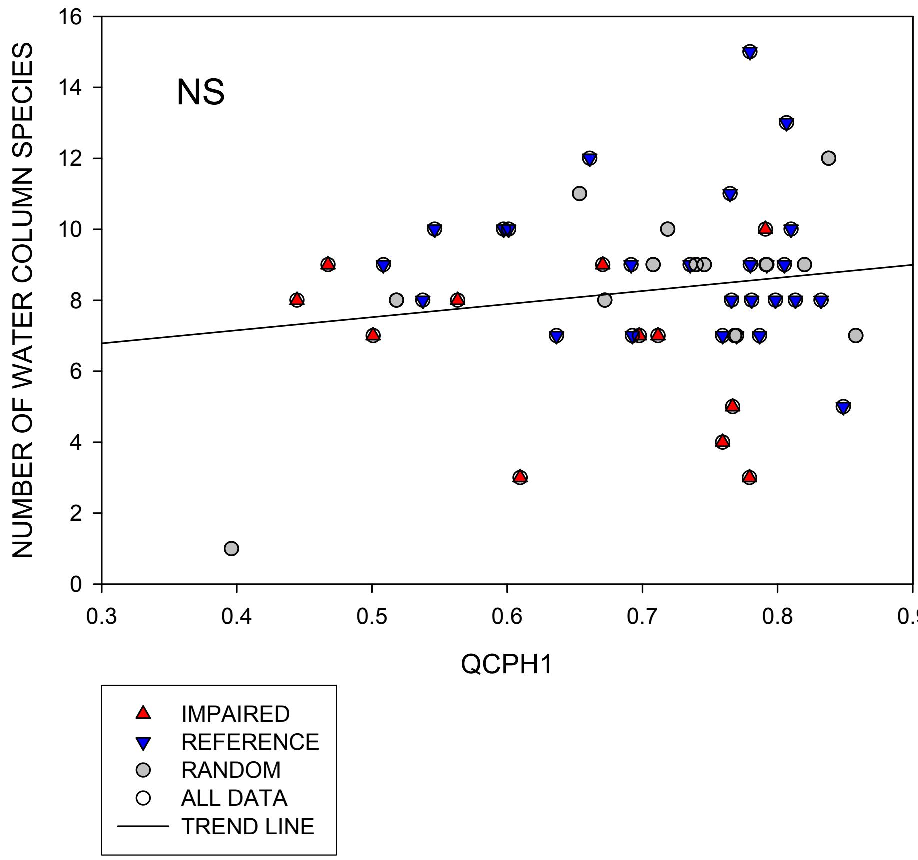
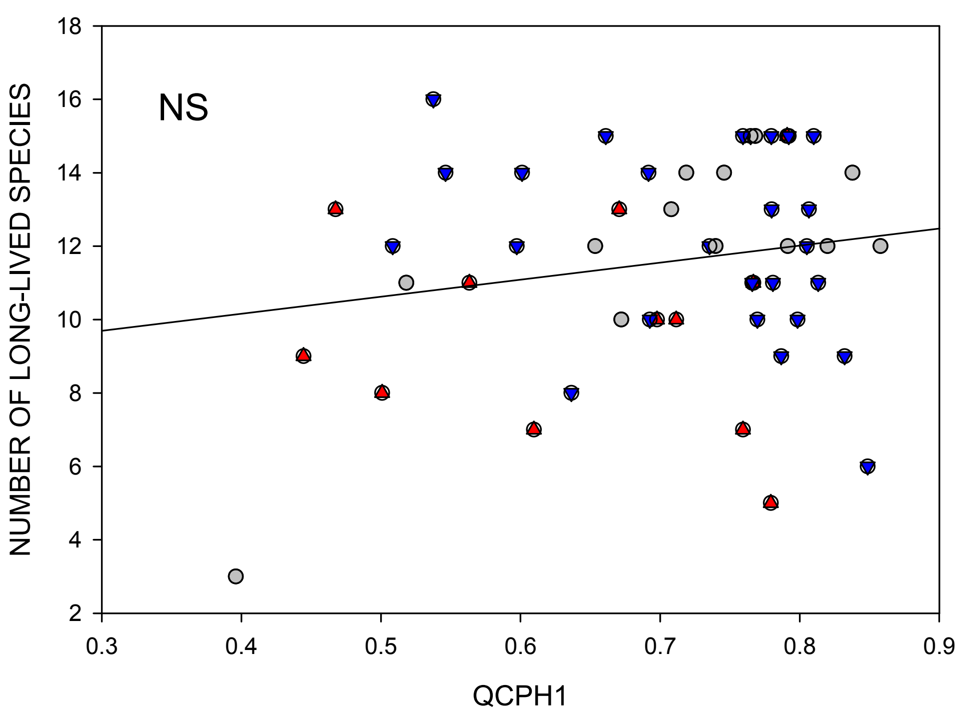


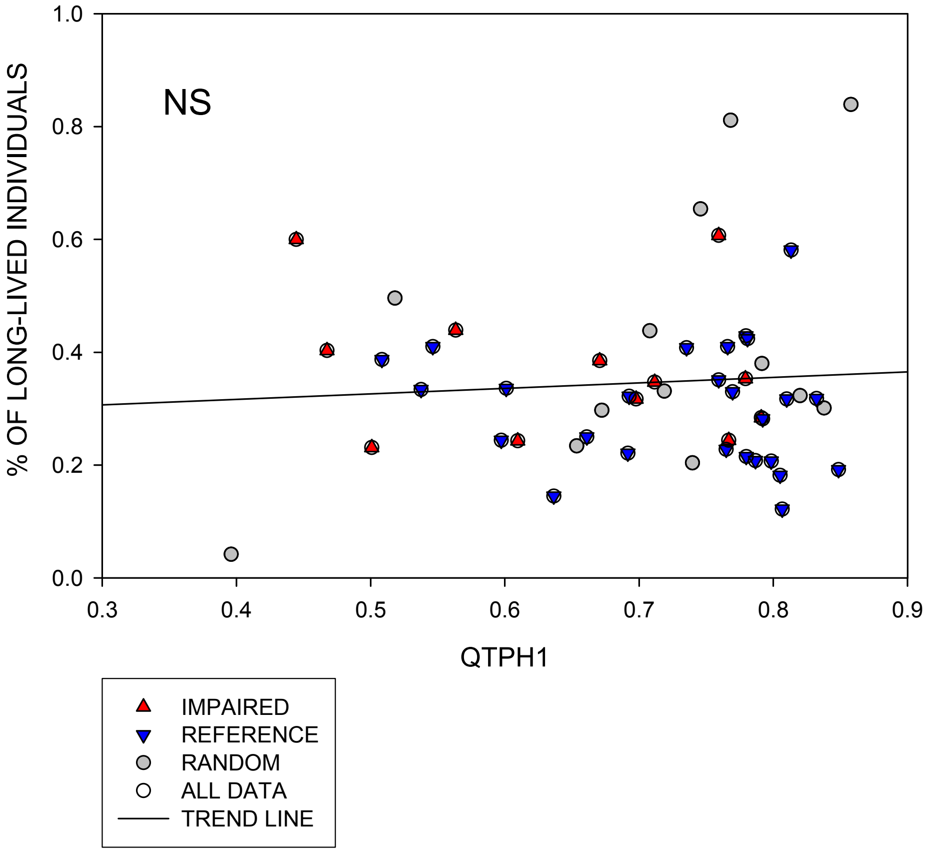



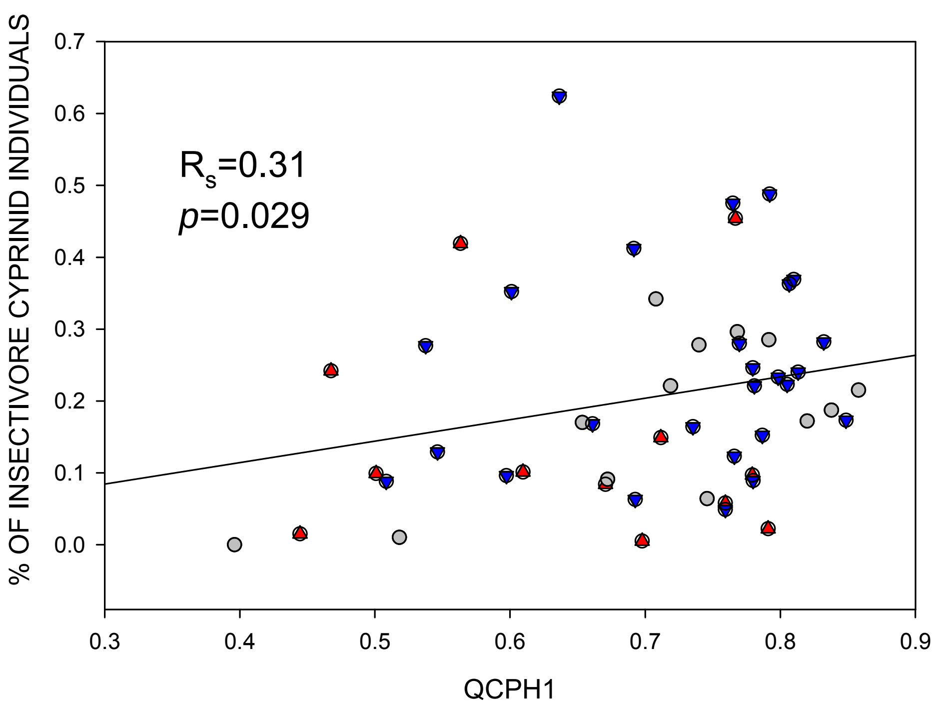
OZARK ECOREGION



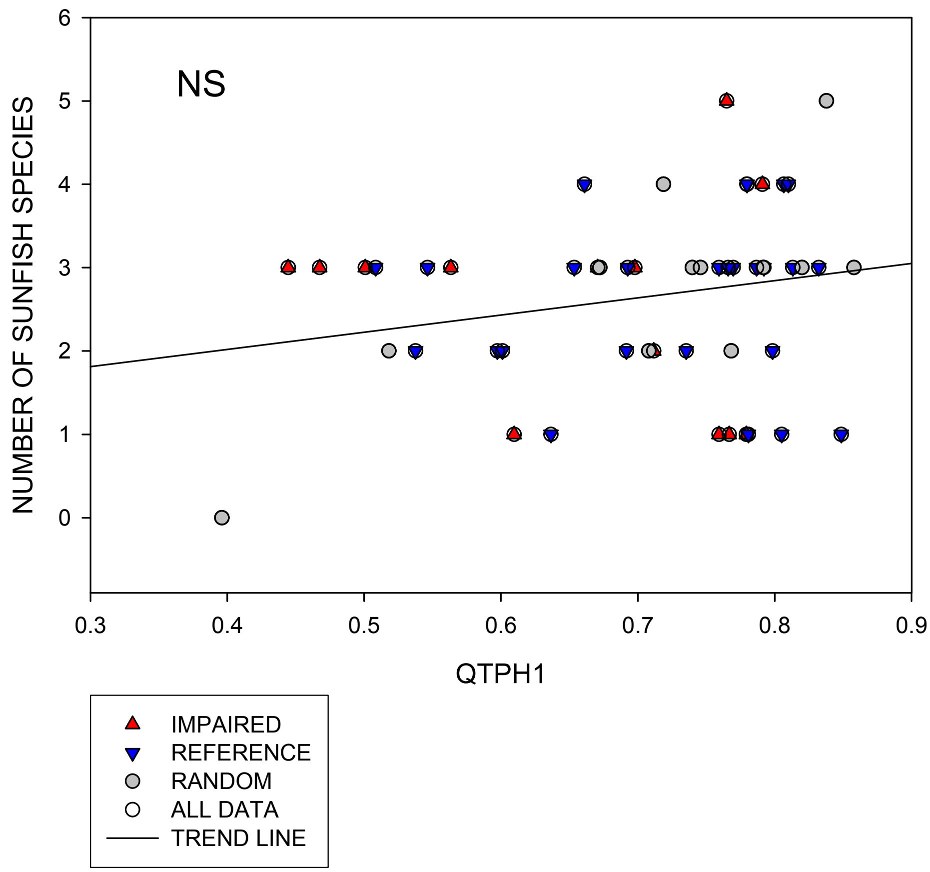



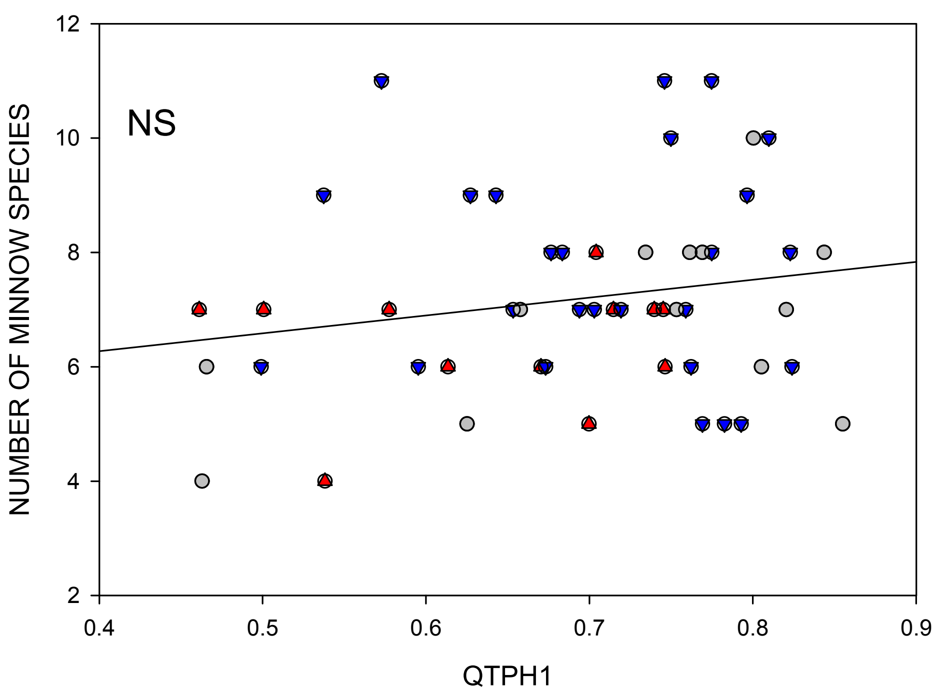
## OZARK ECOREGION



OZARK ECOREGION



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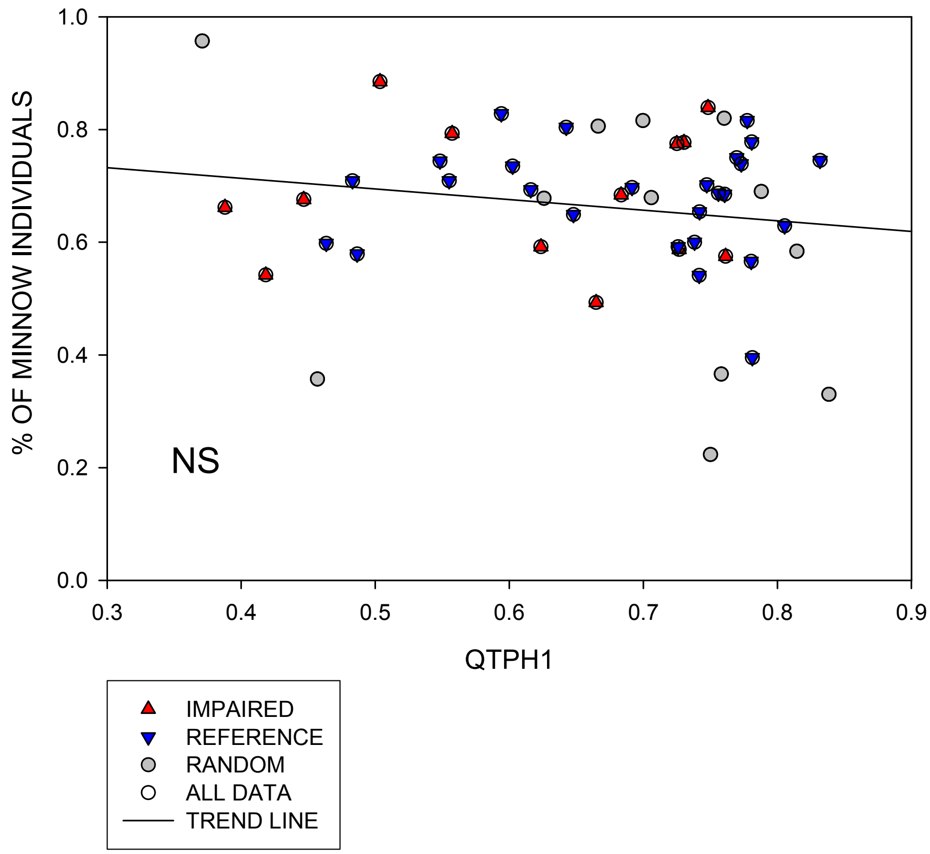


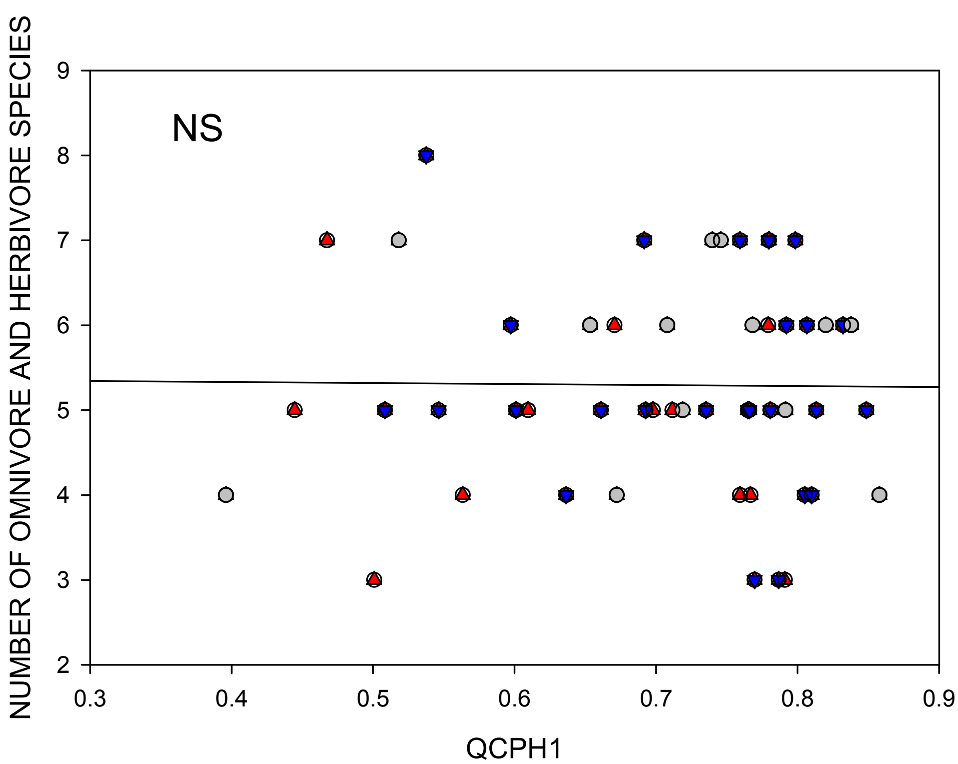

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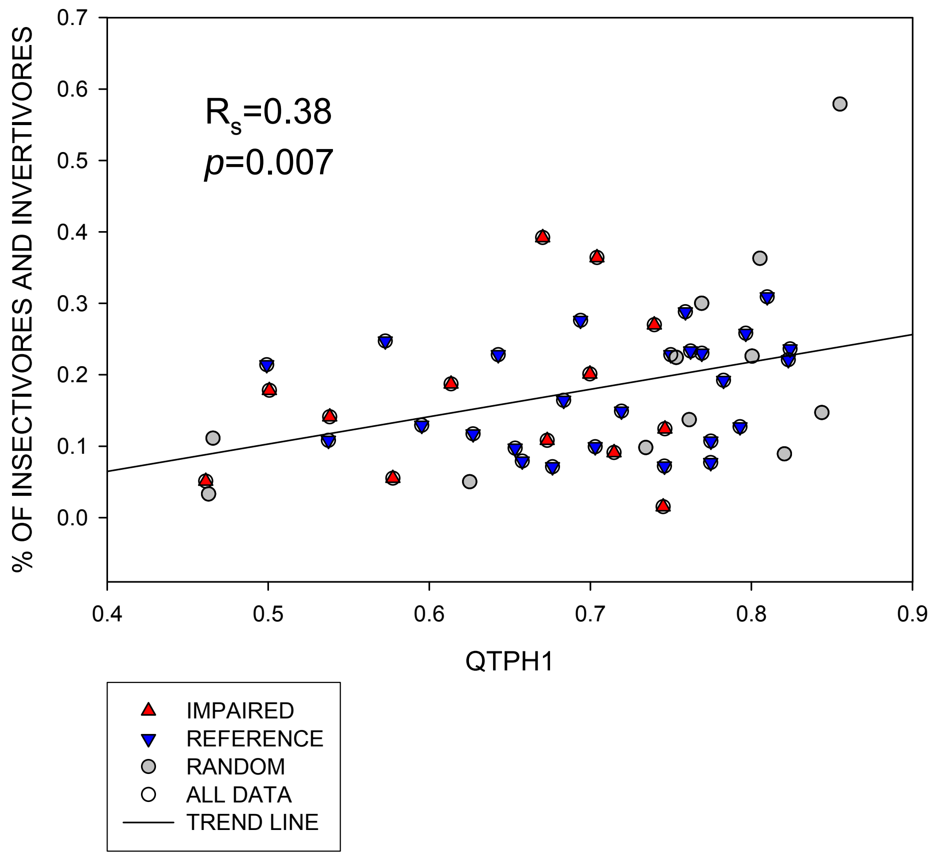
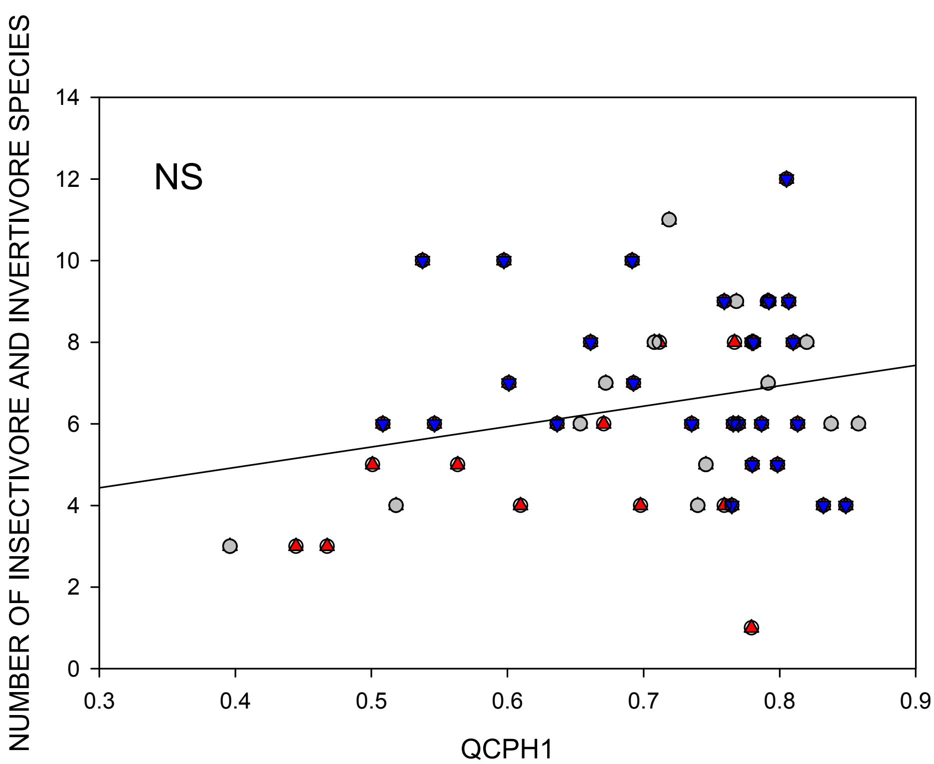
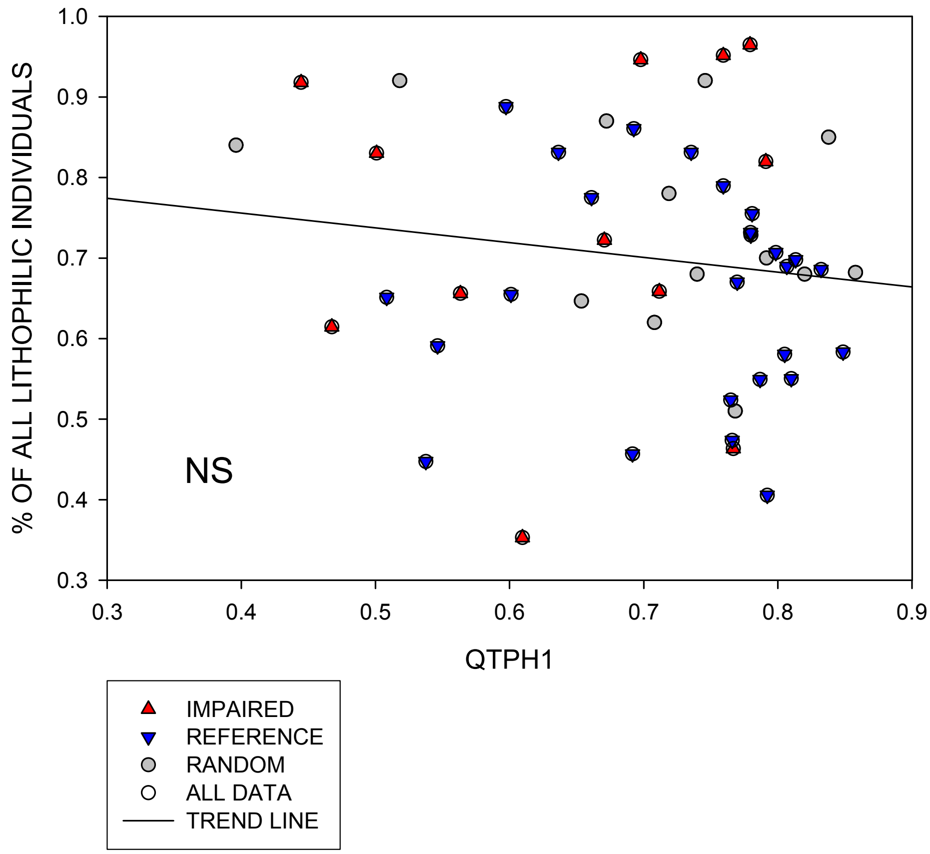
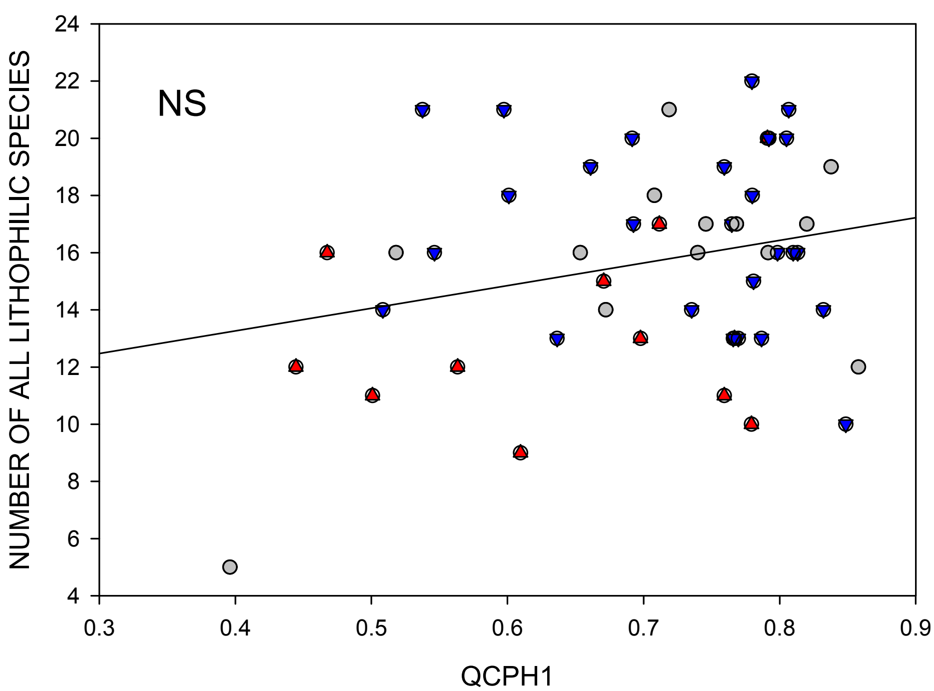
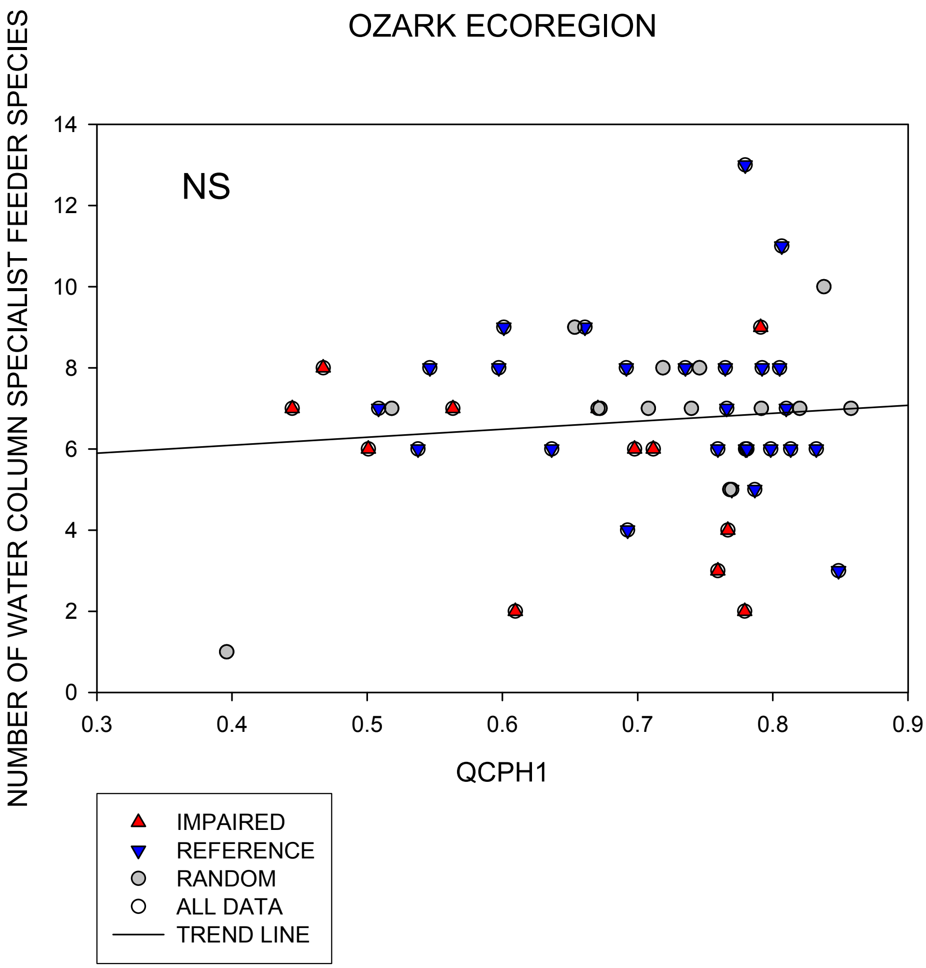


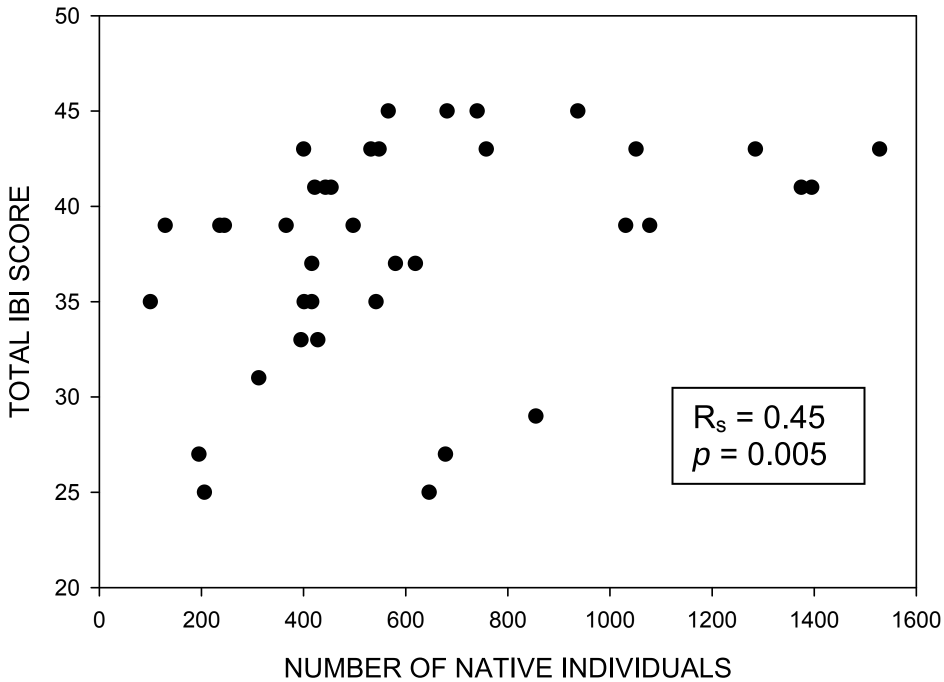
## OZARK ECOREGION

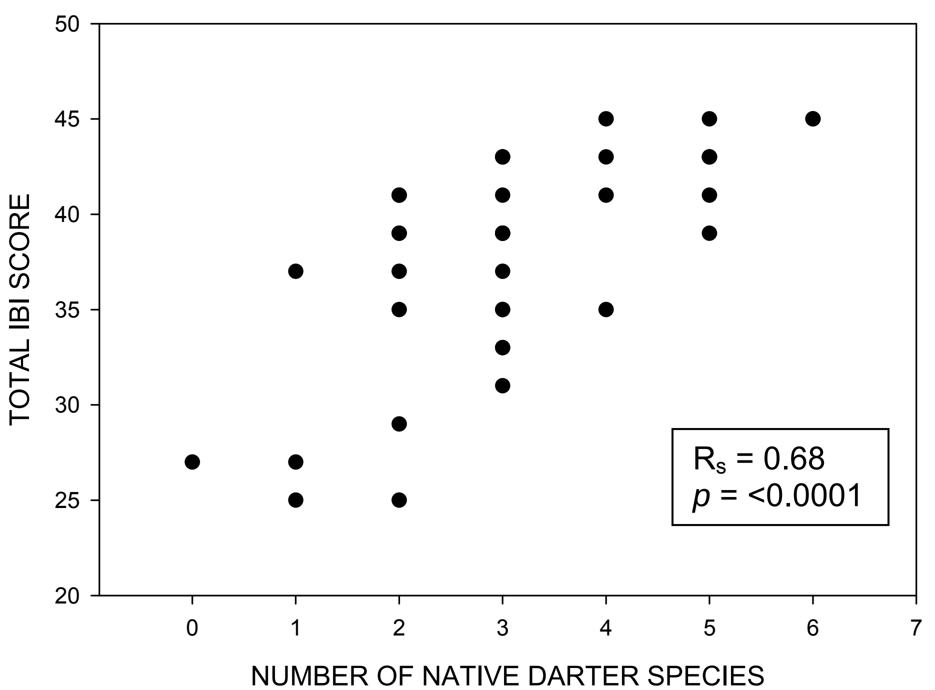


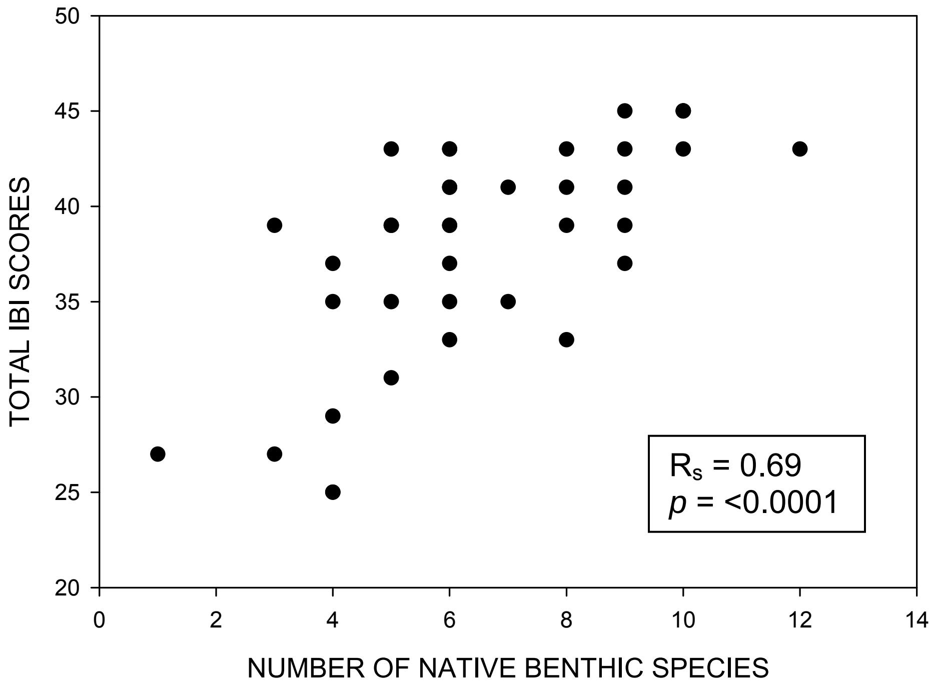



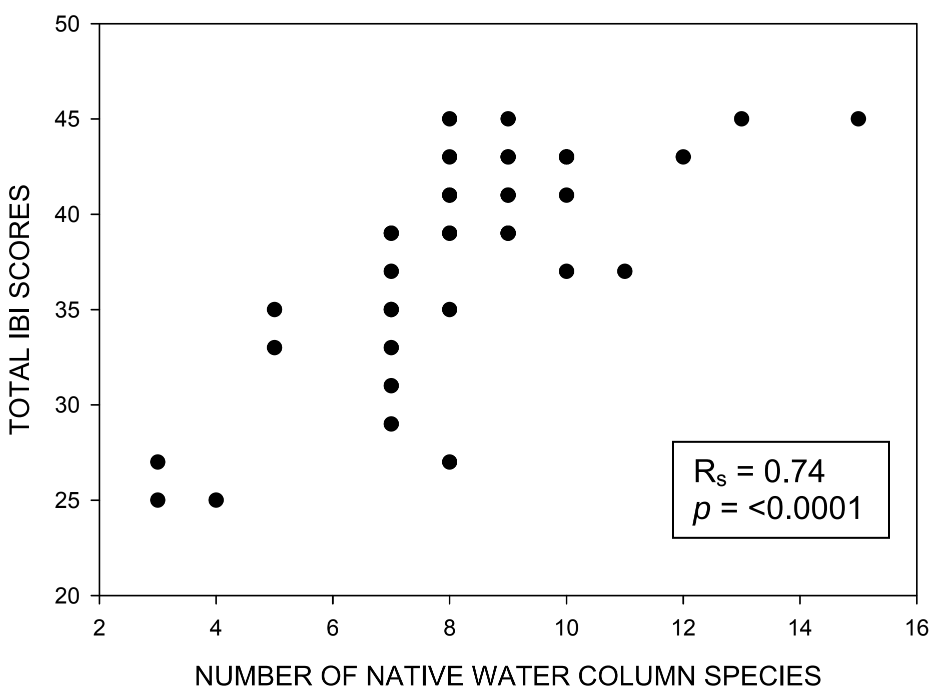

OZARK ECOREGION
NS
NUMBER OF WATER COLUMN SPECIALIST FEEDER SPECIES
QCPH1
0
2
4
6
8
10
12
14
0.3
0.4
0.5
0.6
0.7
0.8
0.9
IMPAIRED
REFERENCE
RANDOM
ALL DATA
TREND LINE

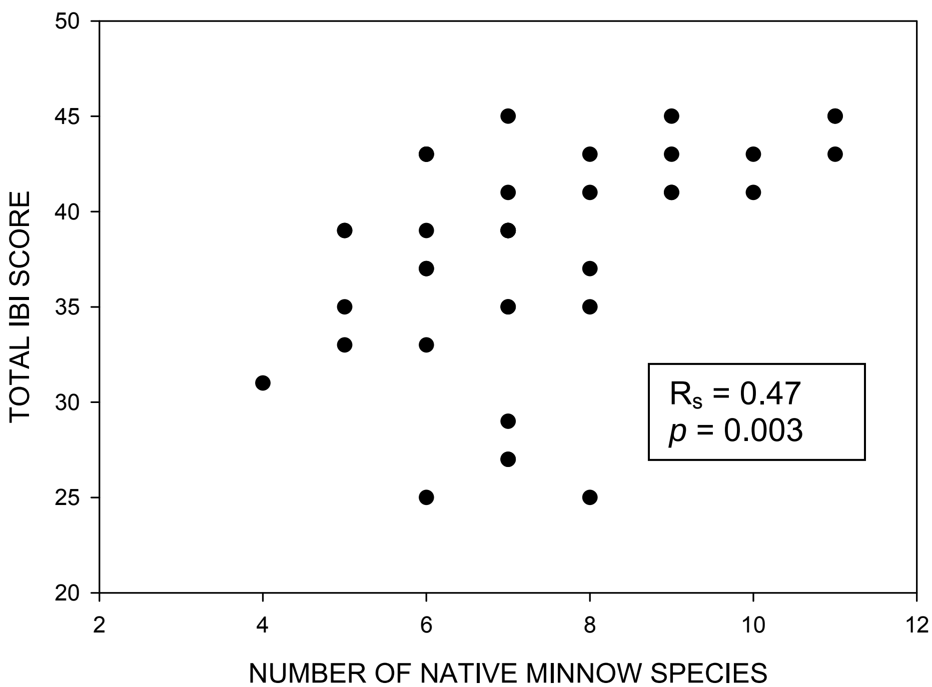
**Appendix F. Scatter plots of the raw metric data to the total IBI scores for the Ozark ecoregion and the result of a Spearman rank correlation between the variables.**



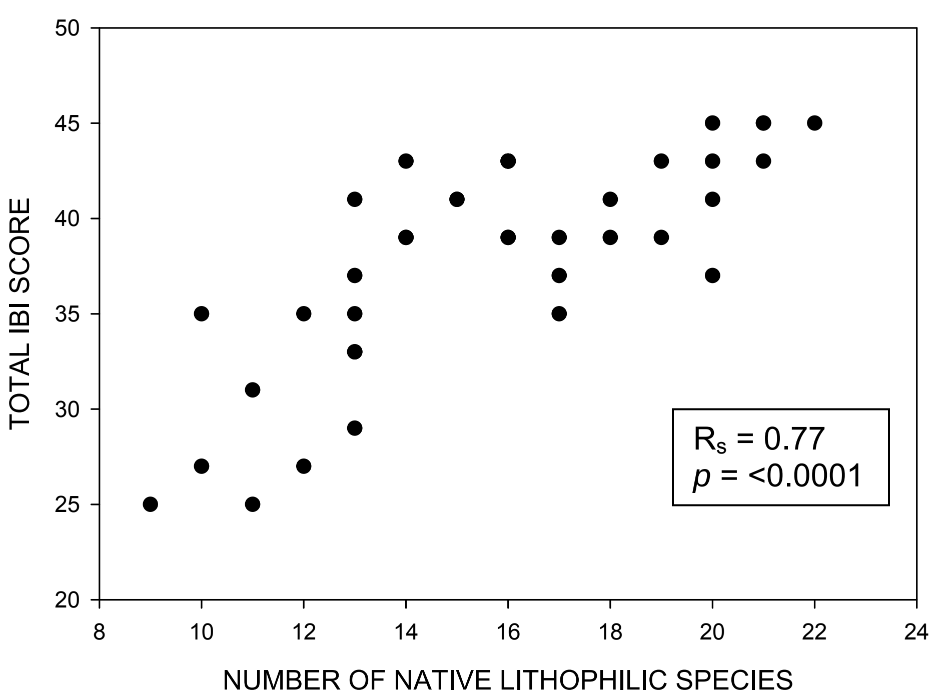




TOTAL IBI SCORES
50
45
40
35
30
25
20
0
2
4
6
8
10
12
14
NUMBER OF NATIVE BENTHIC SPECIES
$R_s$ = 0.69
*p* = <0.0001

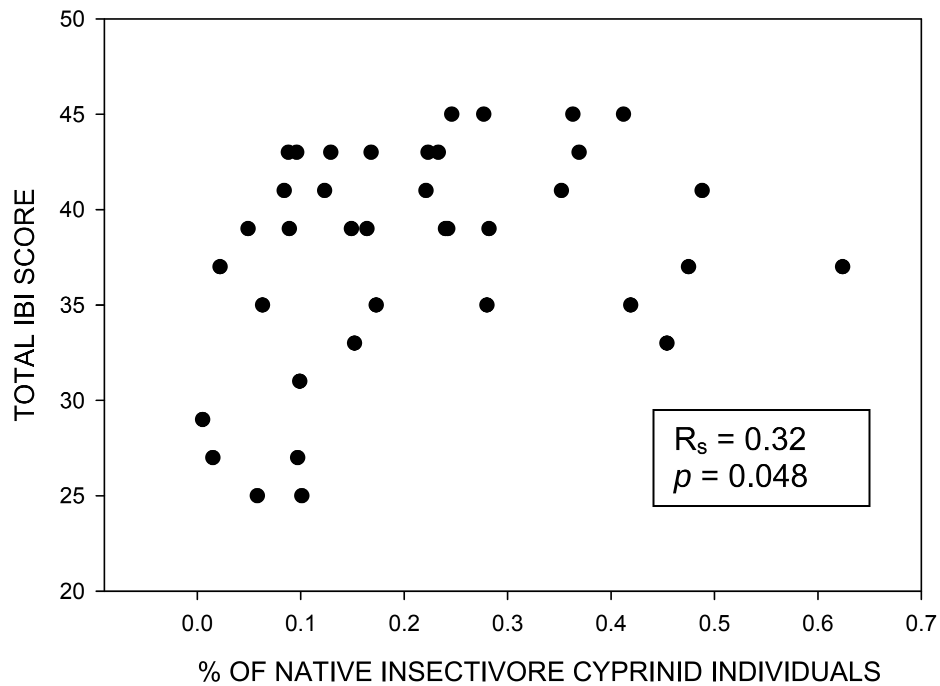

TOTAL IBI SCORES
50
45
40
35
30
25
20
2
4
6
8
10
12
14
16
NUMBER OF NATIVE WATER COLUMN SPECIES
$R_s$ = 0.74
*p* = <0.0001



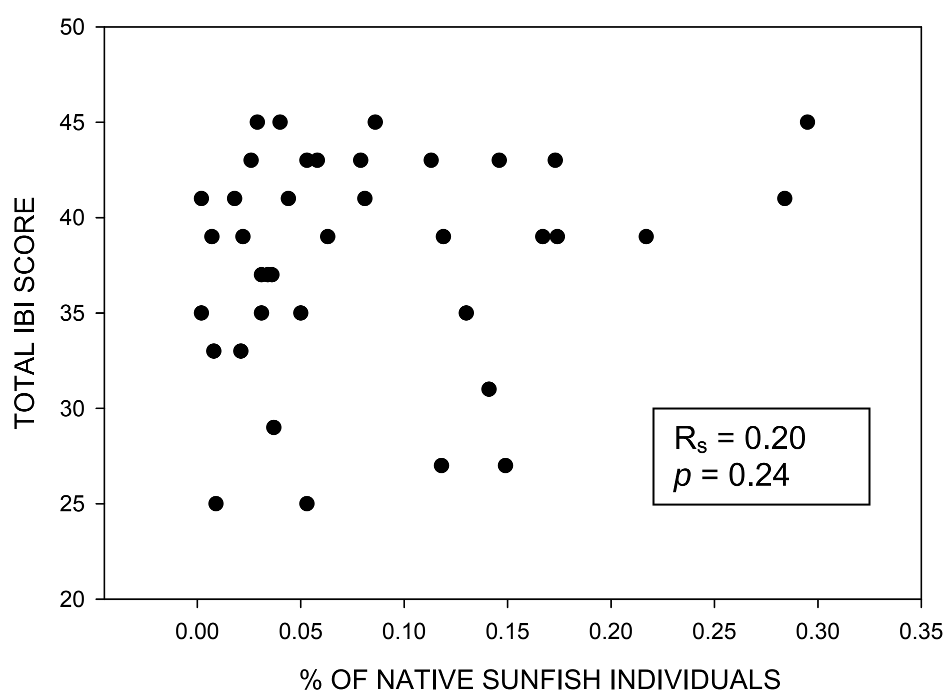
50
45
40
35
30
25
20
TOTAL IBI SCORE
2
4
6
8
10
12
NUMBER OF NATIVE MINNOW SPECIES
$R_s$ = 0.47
$p$ = 0.003

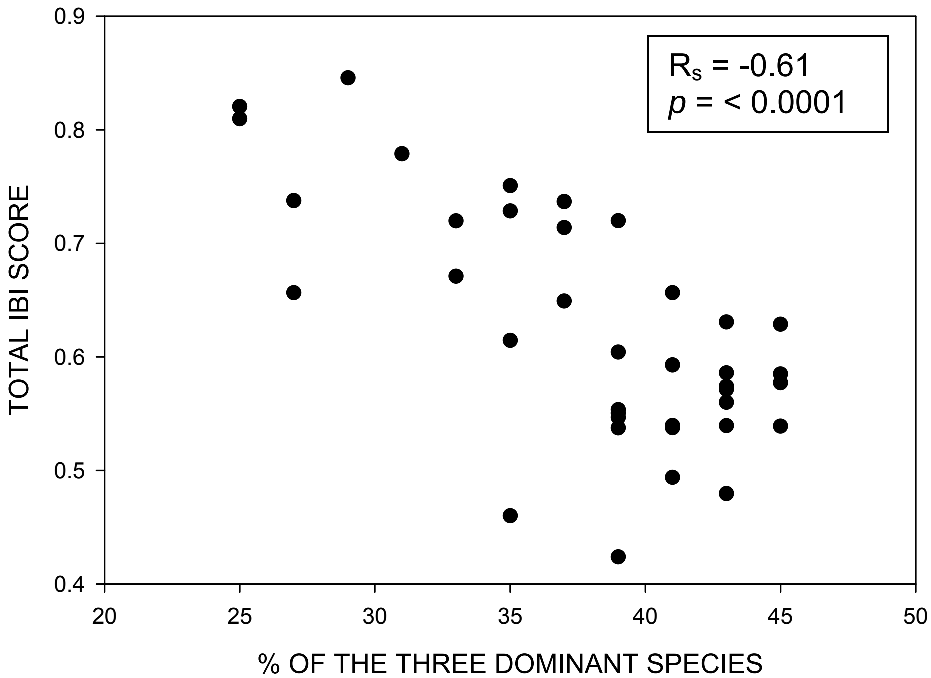
50
45
40
35
30
25
20
TOTAL IBI SCORE
8
10
12
14
16
18
20
22
24
NUMBER OF NATIVE LITHOPHILIC SPECIES
$R_s$ = 0.77
$p$ = <0.0001



TOTAL IBI SCORE
50
45
40
35
30
25
20
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
% OF NATIVE INSECTIVORE CYPRINID INDIVIDUALS
$R_s$ = 0.32
*p* = 0.048

TOTAL IBI SCORE
50
45
40
35
30
25
20
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
% OF NATIVE SUNFISH INDIVIDUALS
$R_s$ = 0.20
*p* = 0.24



$R_s = -0.61$
$p = < 0.0001$
0.9
0.8
0.7
0.6
0.5
0.4
TOTAL IBI SCORE
20
25
30
35
40
45
50
% OF THE THREE DOMINANT SPECIES

## Appendix G. Streams and associated IBI scores for targeted watersheds (EPA-R7WWPD-05-005) taken from the RAM data sets.

| Unique ID | Storet | Stream name | HUC | Watershed | IBI score[1] |
|---|---|---|---|---|---|
| 95221-02 | 9522 | Gallinipper Creek | 10290105 | Harry S Truman Reservoir | 31 |
| 96741-02 | 9674 | Little Sac River | 10290106 | Sac | 39 |
| 20181-02 | 2018 | Cedar Creek | 10290106 | Sac | 45 |
| 96731-02 | 9673 | Pomme De Terre R. | 10290107 | Pomme de Terre | 45 |
| 20201-01 | 2020 | Deer Creek | 10290109 | Lake of the Ozarks | 39 |
| 20201-02 | 2020 | Deer Creek | 10290109 | Lake of the Ozarks | 35 |
| RES061-05 | 030231-05 | Roubidoux Creek | 10290201 | Upper Gasconade | 41 |
| 95211-02 | 9521 | Big Piney River | 10290202 | Big Piney | 45 |
| RES051-05 | 020221-05 | West Piney River | 10290202 | Big Piney | 43 |
| 96941-02 | 9694 | Gourd Creek | 10290203 | Lower Gasconade | 39 |
| 00051-00 | 0005 | Hinkson Creek | 10300102 | Lower Missouri- Moreau | 39 |
| 00121-00 | 0012 | North Moreau Creek | 10300102 | Lower Missouri- Moreau | 37 |
| 01011-01 | 0101 | Bear Creek | 10300102 | Lower Missouri- Moreau | 25 |
| 20141-03 | 2014 | Burris Fork | 10300102 | Lower Missouri- Moreau | 39 |
| 20801-04 | 2080 | Hinkson Creek | 10300102 | Lower Missouri- Moreau | 29 |
| 20811-04 | 2081 | Straight Fork | 10300102 | Lower Missouri- Moreau | 41 |
| 20951-04 | 2095 | Hillers Creek | 10300102 | Lower Missouri- Moreau | 39 |
| 20981-04 | 2098 | Hungry Mother Creek | 10300102 | Lower Missouri- Moreau | 21 |
| 20991-04 | 2099 | Koch Creek | 10300200 | Lower Missouri | 19 |
| 20151-02 | 2015 | Boeuf Creek | 10300200 | Lower Missouri | 41 |
| 20771-04 | 2077 | Boeuf Creek | 10300200 | Lower Missouri | 43 |
| 20921-04 | 2092 | Boeuf Creek | 10300200 | Lower Missouri | 43 |
| 20941-04 | 2094 | Big Berger Creek | 10300200 | Lower Missouri | 45 |
| 01031-01 | 0103 | Wilson Creek | 11010002 | James | 35 |
| 22151-05 | 302 | Panther Creek | 11010002 | James | 37 |
| 01071-01 | 0107 | Pearson Creek | 11010002 | James | 33 |
| 22411-05 | 5538 | Beaver Creek | 11010003 | Bull Shoals Lake | 37 |
| 22501-05 | 7611 | S. Fk. Bratten Spring Ck. | 11010003 | Bull Shoals Lake | 39 |
| 95481-01 | 9548 | Bull Creek | 11010003 | Bull Shoals Lake | 41 |
| 20211-02 | 2021 | North Fork White River | 11010006 | North Fork of the White | 43 |
| 22181-05 | 658 | Indian Creek | 11010006 | North Fork of the White | 41 |
| 22191-05 | 733 | Noblett Creek | 11010006 | North Fork of the White | 43 |
| 22201-05 | 845 | Spring Creek | 11010006 | North Fork of the White | 41 |
| 22241-05 | 940 | Rippee Creek | 11010006 | North Fork of the White | 41 |
| 22251-05 | 1098 | Fox Creek | 11010006 | North Fork of the White | 43 |
| 22291-05 | 1655 | Pine Creek | 11010006 | North Fork of the White | 43 |
| RES131-05 | 040041-05 | Hunter Creek | 11010006 | North Fork of the White | 39 |

## Appendix G continued.

| Unique ID | Storet | Stream name | HUC | Watershed | IBI score[1] |
|---|---|---|---|---|---|
| 20221-02 | 2022 | Sinking Creek | 11010007 | Upper Black | 45 |
| 21181-04 | 4139 | Shut In Creek | 11010007 | Upper Black | 43 |
| 21081-04 | 4058 | Middle Fork Black River | 11010007 | Upper Black | 37 |
| 20231-02 | 2023 | Sinking Creek | 11010008 | Current | 35 |
| 21071-04 | 561 | Barren Fork | 11010008 | Current | 35 |
| 21121-04 | 2145 | Pine Creek | 11010008 | Current | 45 |
| 21122-04 | 2145 | Pine Creek | 11010008 | Current | 45 |
| 21141-04 | 3263 | South Fork Buffalo Creek | 11010008 | Current | 45 |
| 21271-04 | 529 | Sinking Creek | 11010008 | Current | 37 |
| 21301-04 | 3200 | Buffalo Creek | 11010008 | Current | 45 |
| 96751-02 | 9675 | Sinking Creek | 11010008 | Current | 33 |
| 21201-04 | 7691 | Hurricane Creek | 11010011 | Eleven Point | 37 |
| 21251-04 | 8408 | Mill Creek | 11010011 | Eleven Point | 45 |
| 01081-01 | 0108 | North Fork Spring River | 11070207 | Spring | 33 |
| 00141-00 | 0014 | Buffalo Creek | 11070208 | Elk | 25 |
| 00171-00 | 0017 | Indian Creek | 11070208 | Elk | 33 |
| 20131-02 | 2013 | Big Sugar Creek | 11070208 | Elk | 37 |

[1] scores > 37 indicate *no impairment*, scores from 29 - 36 indicate *impaired* conditions, and scores < 29 indicate *highly impaired* conditions.