

# Diatoms in Indiana Streams as Indicators of Biological Condition

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## Executive Summary

### ***Background and Purpose***

The Indiana Department of Environmental Management's (IDEM) mission statement includes an objective to implement federal and state regulations to protect human health and the environment while allowing the environmentally sound operations of industrial, agricultural, commercial, and governmental activities vital to a prosperous economy. Protection of the environment begins with monitoring and assessment of environmental resources, such as surface waters of the state. In accordance with the Clean Water Act (CWA) requirements to restore and maintain the chemical, physical, and biological integrity of the nation's waters, the Indiana Administrative Code has narrative biological criteria that states "all waters, except those designated as limited use, will be capable of supporting a well-balanced, warm water aquatic community." The water quality standard definition of a "well-balanced aquatic community" is "an aquatic community which is diverse in species composition, contains several different trophic levels, and is not composed mainly of strictly pollution tolerant species".

To assess whether or not the aquatic biological community is well-balanced, IDEM currently use Indices of Biotic Integrity (IBI) for fish and macroinvertebrates. IDEM has recently collected data on benthic diatoms along with other data (chemical parameters, nutrients, and habitat) to monitor the health of this third assemblage in streams and rivers in Indiana. The purpose of this project is to develop a diatom IBI for assessment of biological conditions using the IDEM data.

### ***Approach and Methods***

This project examined the classification of diatom communities to recognize natural variation across the different types of streams in Indiana (e.g., ecoregions, temperature, stream size, and geology). Human disturbances in sites were also evaluated using criteria related to landscape activities and land cover. In this way, a diatom reference condition could be described from the samples relative to changes in natural expectations. The reference condition was described by the biological metrics calculated from samples and taxa traits. The metrics that differed between reference and stressed sites within site classes were tested as candidates for inclusion in the multimetric index. All combinations of responsive and non-redundant metrics were used in creating and evaluating possible assessment indices. The best index alternatives were selected after applying explicit selection criteria.

### ***Reference conditions***

Criteria for determining site disturbance categories were the same as those used in other IDEM biological assessments (macroinvertebrate and fish index development). Of the 409 sites with diatom samples, 61 were least disturbed reference and 92 were most disturbed, or stressed. The reference sites were used in site classification analyses and to characterize the reference condition. Stressed sites were used to test for metric differences across the disturbance gradient.

### ***Site Classification***

Natural variation in the diatom community was investigated through ordination of taxa and metrics within reference sites. Classification exercises followed preliminary index developments in an iterative process: 1) attempted distinct classification, 2) index formulation without classes, 3) application of metric adjustments to the preliminary index, 4) assessment of the preliminary indices and metrics relative to classification variables, and 5) repeated index formulation to recognize variability in the preliminary indices and metrics. The iterative classification process resulted in the recognition of two distinct site classes and adjustment of a limited number of metrics to natural gradients. The two classes were related to geologic nitrogen composition. Diatom samples from sites with low geologic nitrogen composition (< 0.089 % nitrogen) were distinct from those with high geologic nitrogen composition. Metric adjustments within the classes only applied to metrics that varied appreciably compared to natural factors with the distinct site classes. For example, the proportion of sensitive taxa varied with the base-flow index in the high nitrogen site class.

### ***Responsive metrics***

The metrics were screened for responsiveness so that the most sensitive metrics could be included in the index. While 308 metrics were calculated, 88 metrics in the high nitrogen class and 181 in the low nitrogen class had responses that were sensitive to disturbance and had a non-contradicting response trend among the classes. Of the responsive metrics, 20 were selected in each site class as candidates for inclusion in the site-class-specific indices. These were selected not only to be responsive to disturbance, but also to represent diverse response mechanisms to diverse stressors.

### ***Index Composition***

Index composition included scoring metrics and combining them in a numeric index that consistently distinguishes reference conditions from non-reference and severely stressed conditions. Metrics were scored on a linear 100-point scale so that each metric had equal weight in the indices and the 100 points spanned the most effective metric value range. The scores were averaged to arrive at a single index value for each sample and index alternative. More than 100,000 index alternatives were calculated and evaluated for sensitivity to stress. The final indices were selected to be responsive to the disturbance gradient, precise within reference sites, and representing several metric types and response mechanisms.

### ***Conclusions***

The final indices selected for the low nitrogen and high nitrogen site classes had five metrics each (Table ES-1). In the high nitrogen site class (HiN), the index had a calibration discrimination efficiency (DE) of 71.7%. That means the 71.7% of stressed sites had index values less than the 25<sup>th</sup> percentile of reference sites. In the low nitrogen site class (LoN), the DE was 100%. The indices were validated using independent data withheld from the calibration analyses and through comparison to individual stressors. The indices were effective at discriminating reference and stressed sites. Overlap in reference and stressed index values indicates that there will be some error in assessments, but that it is slight (Figure ES-1). Biological condition thresholds are preliminary until approved by IDEM.

*Table ES-1. Index metrics for the high nitrogen and low nitrogen sites classes, showing metric codes, categories, discrimination efficiency (DE), trend with increasing stress, and metric scoring formulae. DEC = decreasing trend and INC = increasing trend.*

High Nitrogen Index Metrics	Metric Code	Metric Category	Metric DE	Metric Scoring Formula
Number of low nitrogen taxa	nt_LOW_N	Nutrients	43.5 (DEC)	$100*(\text{metric} - 1) / 7$
Proportion of pollution tolerant taxa	pt_PT_12	Pollution Tolerance	50 (INC)	$100*(0.20 - \text{metric}) / 0.13$
Proportion of tolerant valves	pi_Tol_13	Tolerance Analysis	52.2 (INC)	$100*(49.6 - \text{metric}) / 48$
Proportion of taxa that are <i>Achnanthidium</i> or <i>Navicula</i>	pt_Achnan_Navic	Taxa Groups	45.7 (DEC)	$100*(\text{metric} - 0.14) / 0.18$
Proportion of sensitive taxa (adjusted to the base flow index)	pt_BC_12_adj	Biological Conditions	54.3 (DEC)	$100*(\text{metric} + 0.09) / 0.16$
$\text{pt\_BC\_12\_adj} = \begin{cases} \text{If } (\text{BFIcat} < 30, \\ \text{then pt\_BC\_12} - 0.105, \\ \text{else pt\_BC\_12} - 0.151) \end{cases}$				
Low Nitrogen Index Metrics	Metric Code	Metric Category	Metric DE	Metric Scoring Formula
Proportion of taxa that are <i>Achnanthidium</i> or <i>Navicula</i>	pt_Achnan_Navic	Taxa Groups	81.8 (DEC)	$100*(\text{metric} - 0.14) / 0.18$
Number of low phosphorus taxa	nt_LOW_P	Nutrients	81.8 (DEC)	$100*(\text{metric} - 1) / 6$
Proportion of taxa tolerant of salts	pt_SALINITY_34	Salts	90.9 (INC)	$100*(0.25 - \text{metric}) / 0.18$
Proportion of taxa associated with low dissolved oxygen	pt_O_345	Dissolved Oxygen	72.7 (INC)	$100*(0.47 - \text{metric}) / 0.23$
Proportion of sensitive taxa	pt_Sens_810	Pollution Tolerance	81.8 (DEC)	$100*(\text{metric} - 0.10) / 0.30$

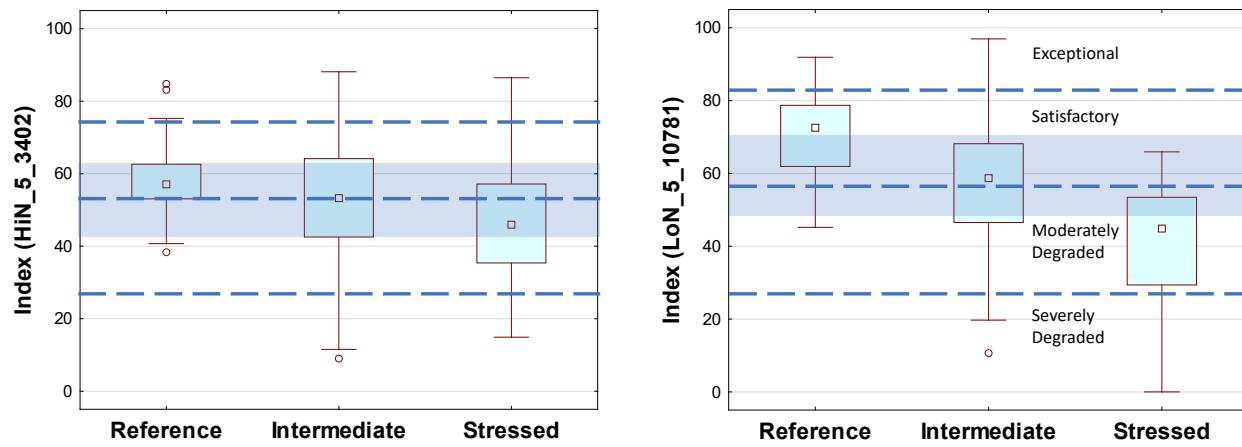


Figure ES-1. Indiana diatom index value distributions in disturbance categories for the HiN (left) and LoN (right) site classes, showing suggested condition thresholds and the range of possible values for the general condition threshold.

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The Indiana Department of Environmental Management staff provided the diatom data for analysis and participated in all interim analytical decisions. The staff included Stacey Sobat, Kristen Arnold, Joanna Wood, and Kassia Groszewski. Their technical reviews resulted in an assessment tool that will be applicable and useful to fulfill IDEM's mission to implement federal and state regulations to protect human health and the environment.

The principal authors and analysts for the project included Ben Jessup, Ben Block, Jen Stamp, and Erik Leppo, all from Tetra Tech. Their interpretations of the IDEM data resulted in development of the Indiana diatom IBI presented in this report.

The cover and closing photos show periphyton being scraped from a stream cobble and being composited, as two steps in the process of sampling diatoms. The photos were provided by IDEM.

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## 1. Introduction

### 1.1 Background

The Indiana Department of Environmental Management (IDEM) monitors the conditions of streams and rivers in Indiana to inform management decisions regarding protection, mitigation, and restoration of aquatic life and other designated uses. The Indiana water quality standards (Administrative Code Article 2<sup>1</sup>) describe minimum acceptable conditions in terms of narrative biological criteria as follows:

“all waters, [except those designated as limited use,] will be capable of supporting a well-balanced, warm water aquatic community” [327 IAC 2-1-3], where a well-balanced aquatic community is “an aquatic community which is diverse in species composition, contains several different trophic levels, and is not composed mainly of pollution tolerant species” [327 IAC 2-1-9].

The U.S. Environmental Protection Agency (U.S. EPA) evaluated IDEM’s biological assessment program in January 2014. The review provided information on the strengths and limitations of the bioassessment program, resource allocation and prioritization for improving the bioassessment program, and integration of biological assessments to more precisely describe aquatic life uses and develop numeric biological criteria. Following recommendations of that review, IDEM developed new numeric Indices of Biological Integrity (IBI) for fish and macroinvertebrates based on regional reference conditions. An IBI for diatoms would provide a third biological assemblage for assessing attainment of biological criteria.

The IBIs calculate measures of the aquatic community (metrics) that, when combined, indicate the similarity of a biological sample to expected conditions of a well-balanced community. The process by which the metrics are selected and combined in an index follows established and innovative analytical methods of the reference condition approach (Hughes et al. 1986, Bailey et al. 2004). In this approach, biological conditions that are sampled from relatively undisturbed sites and that account for natural variability are set as a standard (or reference) to which other samples are compared (Stoddard et al. 2006). Using metrics to establish the numeric index scale, IBI values that resemble those found in reference sites are determined to meet the expectations for a well-balanced aquatic community. IBI values that are unlike the reference values indicate departure from the acceptable biological conditions and probable impairment of aquatic life uses.

The algal assemblage is an important indicator of biological integrity (McCormick et al. 1994, Pan et al. 1996, Hill et al. 2000, Wang et al. 2005, Cao et al. 2007, Stevenson et al. 2008a, Stevenson et al. 2010, Fetscher et al. 2013, 2014, Hausmann et al. 2016, Paul et al. 2020). Algae were part of the early saprobic indicator system for assessing the biological state of waters in relation to organic pollution (Kolkwitz and Marrson 1908). Algae were one of the first assemblages used in biological assessment in the United States (Stevenson 2014; Stevenson et al. 2010; Stevenson and Smol 2003). Algae exhibit a wide variety of sensitivity

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<sup>1</sup> [http://www.in.gov/legislative/iac/iac\\_title?iact=327](http://www.in.gov/legislative/iac/iac_title?iact=327)

among taxa and algal physiologies are conducive to investigating biological responses across a range of stressors. The diatom assemblage, metrics, and indices can indicate relative condition among samples and in relation to stressors, such as nutrients (Porter et al. 2008, Potapova and Charles 2007). Diatom responses to disturbance differ from the responses of other organism groups (benthic macroinvertebrates, fish, and macrophytes) and might be differently suited for detecting certain types of disturbance (Johnson et al. 2006a, Hering et al. 2006, Justus et al. 2010) and over different time periods (Lavoie et al. 2008, Smucker and Vis 2011, Johnson et al. 2006b, McCormick and Scinto 1999). In addition, algal measurements are readily interpreted and understood by scientists, policy makers, and the public (U.S. EPA 2000). Therefore, diatom samples and metrics of diatom community traits were used to explore biological responses to stressor conditions.

## 1.2 Purpose

The purpose of this report is to describe the use of diatom community data and other data collected by IDEM (chemical parameters, nutrients, and habitat) to develop a diatom Index of Biotic Integrity. The general approach to IBI development included identification of a disturbance gradient of the sampled sites throughout Indiana. Samples from sites with least disturbance were used to identify sources of natural variability and to establish the biological reference condition. Samples from sites with most disturbance were used to find metrics that responded consistently to stressors and that could be used in the index. Index compilation and evaluation followed standard scoring techniques to calculate metric combinations that accounted for natural variability and that distinguished acceptable conditions from degraded conditions.

A diatom IBI will enhance the state of Indiana's monitoring and assessment strategy by adding a numeric indicator of diatom community structure that could be used to assess aquatic life use in:

- IDEM's Integrated Report, thus satisfying 305(b) and 303(d) reporting requirements to U.S. EPA.
- Watershed characterization projects which identify critical areas and chemical/physical stressors to the biological communities.
- Identifying improvements in the biological communities following watershed restoration efforts.

In addition, a diatom IBI will provide an accurate assessment of ecological effects on a third assemblage in Indiana, which might respond to different stressors than those indicated by the existing fish and macroinvertebrate assemblages, thus improving IDEM's diagnostic ability to identify causes of degradation in water quality. Diatoms are typically associated with nutrient availability (Charles et al. 2019) and the diatom IBI might be useful to evaluate direct biological responses to excessive nutrients.

## 2. Data Description

### 2.1 Study area

Streams sampled in the monitoring program were wadeable, perennial, and have watersheds draining less than 1,000 square miles. Most sites were selected as part of IDEM probabilistic sampling surveys with a rotating basin design. Sites were not targeted to represent least disturbed or most disturbed environmental settings. Over the eight years of sampling, the entire state of Indiana was represented, with fairly even spatial coverage. In two smaller river basins (Miami River and Patoka River), sample sites were more densely spaced compared to the other basins (Figure 1). The diatom data used for the index development analysis included a total of 497 samples from 409 sites. Repeated samples at a site were collected as field duplicates on the same day as the primary sample, lab replicates of subsamples, or site revisits over years.

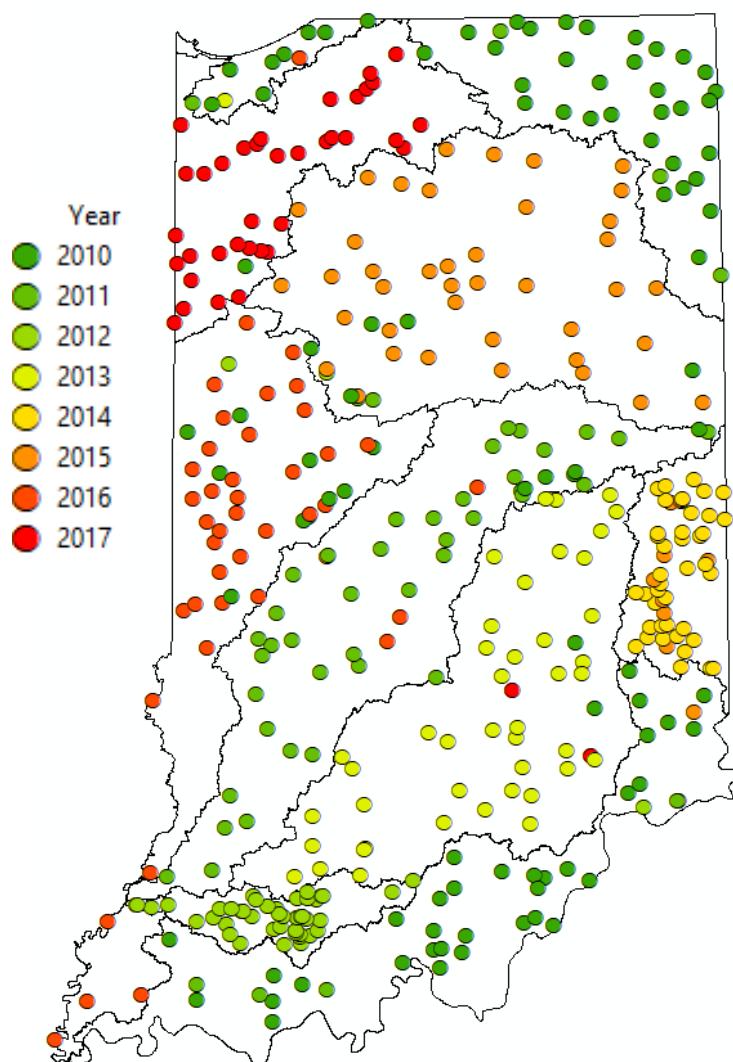


Figure 1. Diatom sample sites throughout Indiana, showing year sampled and outlines of major river basins.

## 2.2 Sample methods

All diatom samples used in the analyses were collected by IDEM field staff according to the Technical Standard Operating Procedures for periphyton sampling (IDEM 2018). Periphyton samples are collected during low flow, from late August through October. Periphyton samples were collected from one of three possible habitats in the following priority: 1) epilithic habitat in shallow stream riffles with coarse-grained substrates, 2) epidendric habitat from woody snags in streams with fine-grained substrates, and 3) episammic or epipellic habitat from sandy depositional areas along stream margins.

For an epilithic sample, the field crew collects ten algae covered rocks from a transect near the middle of the sampling reach. Five of the ten rocks are retained for sampling and five are returned without scraping. A modified syringe is placed on the algae covered surface of a rock. The outline of the syringe area is scribed on the rock and algal material is scraped, brushed, and collected from within the scribed circle. A second area on the same rock is scraped and then two areas on the four other rocks are scraped. The scrapings from the 10 areas are composited into a single periphyton sample. The sample is then diluted and split into one sample for diatom identification and enumeration and another sample for periphyton chlorophyll *a* and pheophytin *a* analysis.

If the sample is epidendric (with predominant woody substrate at the site), five submerged sticks with visible algae covering are collected near the center of the sampling reach. The sticks are brushed clean of algae and the composite sample is diluted and split as described for the epilithic sample. To determine the area of the brushed substrate, the length of the selected sticks is limited to 7-20 cm. After brushing, the length and circumference of the sticks are recorded and surface area is calculated.

For episammic or epipellic samples (from sand and finer substrates), field crews select five locations in the transect that have a depositional zone consisting of sand or silt substrates with visible algae growth. The sample is collected by slowly pressing the top half of a 47 mm petri dish into the sand or silt substrate. The sample is contained by sliding a spatula under the petri dish, then lifting it out of the water and into a collection pan. This is repeated in four other areas and the composite sample is diluted and split as described for the epilithic sample.

The diatom sample is preserved with formalin, using 2 mL of 100% formalin for every 50 mL of sample. Diatoms were extracted from the periphyton sample and processed and identified in the IDEM laboratory (IDEM 2015). The processing and identification procedure includes decanting excess water overlying the settled diatom sample, centrifuging, rinsing, oxidizing, mounting, and fixing the sample onto microscope slides. IDEM biologists count and identify six hundred diatom valves for each sample. Identifications were mostly to the species taxonomic level, though varieties and genera were also identified.

The periphyton chlorophyll *a* and pheophytin *a* samples were chilled until processed on-site or in the lab. Processing included vacuum-assisted filtration through glass fiber filters and preservation of the filters on dry ice. Chlorophyll *a* and pheophytin *a* were analyzed by the U.S. Geological Survey (USGS).

Other sampling at each diatom sampling site included general site and sample observations, field water quality, analytical chemistry, and habitat evaluations. These data were collected by IDEM using standard procedures. The Data Summary (Appendix A) describes data completeness (numbers of records for each variable) and data quality (potential outliers or

questionable values). Outliers were flagged but were not removed from analysis unless directed by IDEM after their review.

### 2.3 GIS Analysis

The sampled sites were analyzed in a Geographical Information System (GIS) to identify landscape features and characteristics that determine disturbance intensity or natural factors that could affect diatom sample results. Much of the GIS analysis was to replicate previous analyses for identifying the disturbance status of each site. Several sites ( $N = 224$ ) had been analyzed for development of fish and macroinvertebrate indicators. IDEM decided to continue using the information initially derived for these sites and to analyze another 185 sites to derive similar information. Therefore, the landscape variables used previously were used again, using updated information when available and refined delineations. This included information from the National Hydrography Dataset Plus version 2.1 (NHDPlusV2.1). Watersheds and buffers around the stream channels were delineated using tools available in StreamStats (<https://streamstats.usgs.gov/>). For the classification analysis, additional variables were associated with site watershed and local catchments using the StreamCat database (Hill et al. 2016) and GIS analysis. Additional details on the GIS analysis are described in the Data Summary (Appendix A). After QC checks of watershed delineations, six sites had uncertain delineations and therefore unreliable GIS statistics. These six were not used in index calibration analyses.

### 2.4 Traits and metric calculations

Diatom data were transferred from IDEM as a collection of tables in a relational Microsoft Access database. The 497 samples included 25,225 records of unique diatom taxa identified within the samples. The 709 diatom taxa identified by IDEM were reconciled with standardized taxa names in the U.S. Geological Survey BioData database<sup>2</sup>. The taxa reconciliation process included the IDEM data set, BioData, and two taxa traits databases. Once the taxa were associated using the most current authority for the final standardized name, the list was reviewed by biologists from IDEM and USGS. After review, there were 33 taxa that were unresolved; they were not matched to BioData or taxa traits. Most of these taxa were uncommon in the IDEM data set.

Diatom taxa were associated with traits based on two sources. One source was provided by the Diatoms of North America (DONA) workgroup, as described in Tyree et al. (2020). The second source was a database of trait values compiled by Tetra Tech and based on literature and regional studies (Potopova et al. 2004, Stevenson and Wang 2001, Stevenson et al. 2008a, Porter et al. 2008, Bahls 1993, Teply and Bahls 2005, Kelly and Whitton 1995, van Dam et al. 1994). In some cases, the literature sources from Tetra Tech were the same sources used to inform the DONA trait assignments.

Diatom metrics that have typically shown relationships in stream assessment studies were calculated in an Access database and using R statistical software (Attachment 1). For each of the DONA traits, taxa richness, relative richness, and relative abundance metrics were calculated for each sample using R code. Metrics based on the Tetra Tech traits were calculated in an Access database. These metrics included taxa richness, relative richness, and relative abundance of taxa groups and traits as well as weighted averages of trait values,

<sup>2</sup> <https://my.usgs.gov/confluence/display/biodata/About+BioData> (diatom taxa list retrieved on February 26, 2020).

indicator species metrics, and metrics based on regional tolerance values (Appendix B). All metric calculations were based on unique standardized identifications within the samples. The original record set was reduced to 24,797 records because some taxa appeared twice in a sample after standardization. The R code for DONA traits automatically excludes taxa that are not matched, resulting in a total record set of 24,585 records. There was no exclusion of taxa or individuals due to lack of standardization or ambiguous redundancy.

### 3. Analytical Methods

#### 3.1 Approach

Development of an IBI for diatoms in Indiana streams was approached primarily as an application of the reference condition framework to calibrate a multimetric index (MMI). In this approach, there are sequential and iterative steps for characterizing the reference condition, identifying natural variability in the reference condition (site classification), finding community metrics that are sensitive to human disturbance, and combining the metrics so that the resulting index consistently identifies biological conditions that corresponds with the environmental setting for each sample.

Biological criteria are based on biological integrity, defined as “the capability of supporting and maintaining a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitats of the region” (Karr and Dudley 1981 after Frey 1977), and has been widely adopted by U.S. EPA to be consistent with the purpose of the U.S. Clean Water Act (CWA). The definition relies on the natural condition, and bioassessment is based on a comparison of conditions in assessable waterbodies to relatively natural reference conditions. Reference conditions are derived from observations from relatively undisturbed waterbodies, and account for natural biological types that can be classified with environmental predictors.

States typically have reference criteria based on land use/land cover, activities in the watershed, and possibly habitat or water quality (Buss et al. 2014).

Common categories of land use that are summarized and used as criteria include forests, all-natural cover, agriculture, and urban (including residential, commercial, industrial, and transportation). Habitat and water quality measures can be incomplete or variable if obtained in one-time grab samples. They are often used as secondary screening variables for identifying disturbance, considered in addition to the land cover variables.

#### ***Why Designate Disturbance Conditions?***

- To determine a gradient of stream settings ranging from least disturbed to most disturbed
- The least disturbed streams will represent reference streams, from which the reference condition will be described
- The reference condition will represent the natural condition, or, in areas with ubiquitous stressors, the most natural observable condition
- The most disturbed streams will be used in calibration of the bioassessment index to test responsiveness of metrics and indices to stressors

Site classification is the process by which natural effects on assemblage characteristics are identified so that reference expectations are appropriate for the environmental setting and so that metric responses can be correctly associated with human disturbance. In developing a multimetric IBI, natural variability among biological community types is accounted for through explorations of sample characteristics in the least disturbed reference sites. One way

to account for the natural variability in biological metrics is to create distinct classes of streams that have similar biological expectations within each class. By examining the structure and composition of samples in the least disturbed reference sites, a reasonable number of biological types can be recognized so that variability is reduced relative to the variability in all sites. Distinct classes that reflect the biological types are assigned by identifying the environmental conditions that determine the biological variation among sites.

Distinct classes are not always apparent. Classification can also be approached through adjustment to continuous variables or through adjustment of expectations one metric at a time. Correlation analysis, classification and regression trees (CART), and random forest models can account for natural variability for individual metrics.

Diatom community metrics were calculated based on sample composition and taxonomic traits. The diatom community is effective for assessing waterbodies because species have different environmental tolerances, optima, requirements, and adaptations for environmental conditions. Community metrics based on these species traits primarily quantify taxa richness, relative richness, and relative abundance for each of the traits. Given the variety of metric types and trait types, numerous metrics can be calculated.

Metric responses were evaluated along the disturbance gradient and relative to the site classes. Metrics that show disparate value distributions between the least disturbed and most disturbed sites were candidates for inclusion in the multimetric index. Combinations of the best candidate metrics were tested to find IBI alternatives that were responsive to the stressor gradient, contained metrics from various trait and calculation types, did not contain redundant metrics, and that had plausible response mechanisms. The best IBI alternatives were selected and validated for application as biological indicators in Indiana streams.

### 3.2 Site Disturbance Designations

Sites were classified as either reference, intermediate, or stressed depending on the criteria previously established for reference designations (Jessup and Stamp 2017; Table 1). Criteria were set based on distributions of values in the entire upstream catchments and partial catchments 1 and 5 km upstream from the sampling sites as derived from the GIS analysis. Disturbance categories were assigned to sites based on the numbers of primary reference criteria that indicated either reference or stressed conditions (Table 2). For a site to pass a criterion for a reference designation, it was required to pass at all three spatial scales. Failure of a stressed criterion was based on the failure of one or more of the spatial scales. For a site to be considered in any of the reference categories, it could not have any indications of stress. In contrast, the levels of stress were assigned regardless of the number of reference criteria that were passed.

As a quality control process, new site designations (i.e., Reference or Stressed) were compared to previously reported site designations for those sites that were previously analyzed. Any differences in site designation are likely due to updated source data for land cover (i.e., NLCD 2016 vs NLCD 2011) and refined watershed delineations.

The disturbance designations resulting from the disturbance criteria were reviewed by the IDEM staff biologists who were familiar with the sites and had additional information to inform the disturbance designations. Analytical designations that matched previous designations were accepted as the final designation. For those that differed between the

previous analysis and the current analysis were mostly designated according to the current analysis under the assumption that the recent data coverages and analyses are more detailed and accurate than the earlier ones. IDEM biologists re-designated some sites that were on their approved list of reference sites or that could be associated with stressors and sources that were not detected with the disturbance criteria.

*Table 1. Reference (Ref) and Stressed (Strs) criteria for 13 disturbance variables, by catchment delineation: whole catchment (W), within 5 km (5K), and within 1 km (1K).*

	Ref Criterion	Strs Criterion	Units
POP_DENSITY	<10	>200	People/km2
W_%_URBAN	<5	>40	% in catchment
5K_%_URBAN	<5	>40	% within 5km
1K % URBAN	<5	>40	% within 1km
W_%_AGRIC	<40	>90	% in catchment
5K_%_AGRIC	<40	>90	% within 5km
1K % AGRIC	<40	>90	% within 1km
W_%_IMPERV	<2	>12	% in catchment
5K_%_IMPERV	<2	>12	% within 5km
1K % IMPERV	<2	>12	% within 1km
Wd_RD_CROSS	<50	>100	#/100km2
5K_RD CROSS	<5	>25	# within 5km
1K RD CROSS	<1	>5	# within 1km
W_RD_DENSE	<1	>5	km road/km2
5K_RD_DENSE	<1	>5	km/km2 within 5km
1K RD DENSE	<1	>5	km/km2 within 1km
W_%_CANAL/PIPE	<20	>80	% in catchment
5K_%_CANAL/PIPE	<20	>80	% within 5km
1K % CANAL/PIPE	0	>80	% within 1km
Wd_MINES	<0.1	>0.5	#/100km2
5K_MINES	<1	>2	# within 5km
1K_MINES	0	>1	# within 1km
Wd_NPDES	<1	>15	#/100km2
5K_NPDES	0	>10	# within 5km
1K_NPDES	0	>2	# within 1km
Wd_CERCLIS	<0.5	>2	#/100km2
5K_CERCLIS	0	>2	# within 5km
1K_CERCLIS	0	>1	# within 1km
Wd_CAF0	<2	>10	#/100km2
5K_CAF0	<1	>4	# within 5km
1K_CAF0	0	>1	# within 1km
Wd_TRI	<1	>10	#/100km2
5K_TRI	<1	>10	# within 5km
1K_TRI	0	>2	# within 1km
Wd_dDAMS	<0.1	>1	#/100km2
5K_DAMS	<1	>2	# within 5km
1K DAMS	0	>1	# within 1km

*Table 2. Preliminary disturbance category assignments based on reference and stressed criteria.*

Category	Rule or description
Reference	No criteria indicate stress for BestRef, Ref, or SubRef
BestRef	12 -13 of 13 criteria indicate reference
Ref	10 - 11 of 13 criteria indicate reference
SubRef	8 - 9 of 13 criteria indicate reference
Other	<8 criteria indicate reference or <2 criteria indicate stress
Stressed	Stressed designations do not depend on reference indications
Strs	2 – 3 of 13 criteria indicate stress
HiStrs	4 or more variables indicate stress

### 3.3 Site Classification

The classification analysis for Indiana diatoms evolved from familiar and simple categorical classification, to complex clustering and discriminant function analysis, to correction and regression of individual metrics, and finally to classification and regression trees (CART) on metrics and a preliminary diatom index. The initial analyses were informative, leading to refinement of the subsequent analyses, but were ultimately inconclusive. These methods were largely exploratory and depended on a weight of evidence and discussions with the IDEM staff to ascertain the logic behind recognizing naturally distinct stream types. Preliminary analyses are shown in Appendix E.

In CART analysis, metric or index values are predicted based on multiple possible classification variables. Of the multiple predictors, a subset of variables was selected based on correlation analyses. For each candidate index metric and for a preliminary index, the values were bifurcated to optimize precision on either side of the split. Splits can be categorical or thresholds of continuous variables and each node at the end of a branch has a predicted metric or index value.

Classification for individual metrics was accomplished by applying the split criteria in the sampled site, identifying the expected value based on the predicted node, and calculating a residual from the expected value (Cao et al. 2007). Classification for the index was accomplished by recognizing common classification variables among metrics and with a preliminary index. The preliminary multimetric index was established based on responsive and intelligible metrics that discriminated reference and stressed sites statewide. The split identified for the index was used to establish categorical classes based on the split.

The adjustment process applied CART analysis in the rpart (recursive partitioning) package of the R programming language (R Core Team 2021). The classification trees were pruned to the complexity parameter (CP) associated with the minimum cross-validation error (xerror). With this limitation, there is less chance of over-fitting the model with too many splits. From the CART results, the mean values at the terminal nodes were used as the metric expectations in natural settings. Adjusted metric values and scores were then calculated based on residuals from those means. An example of the CART analysis results is shown in Figure 2.

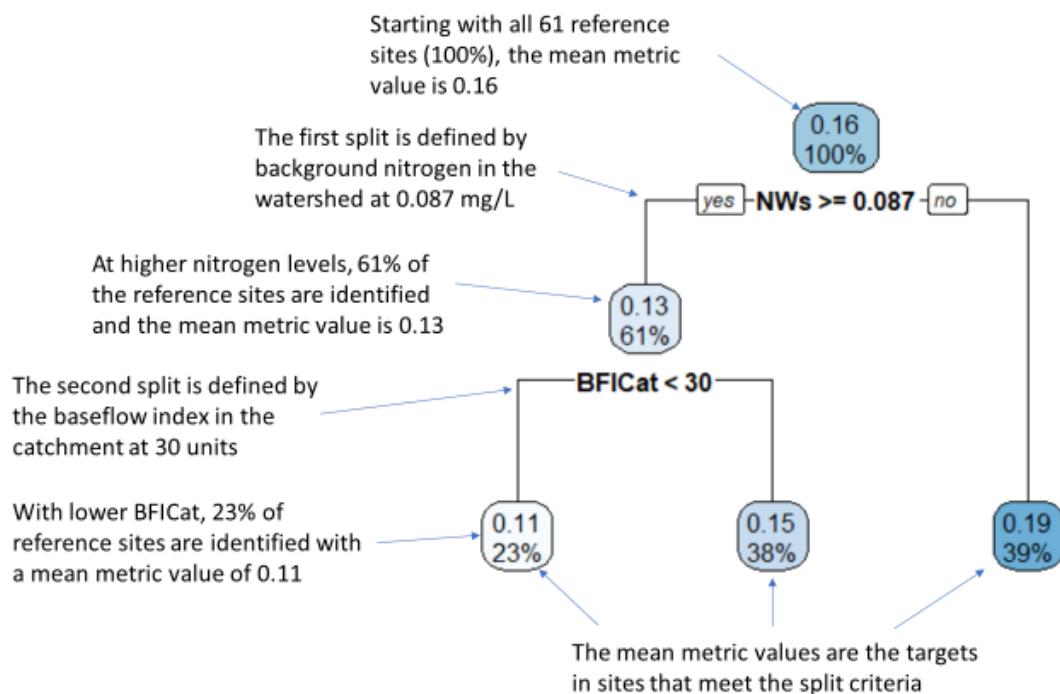


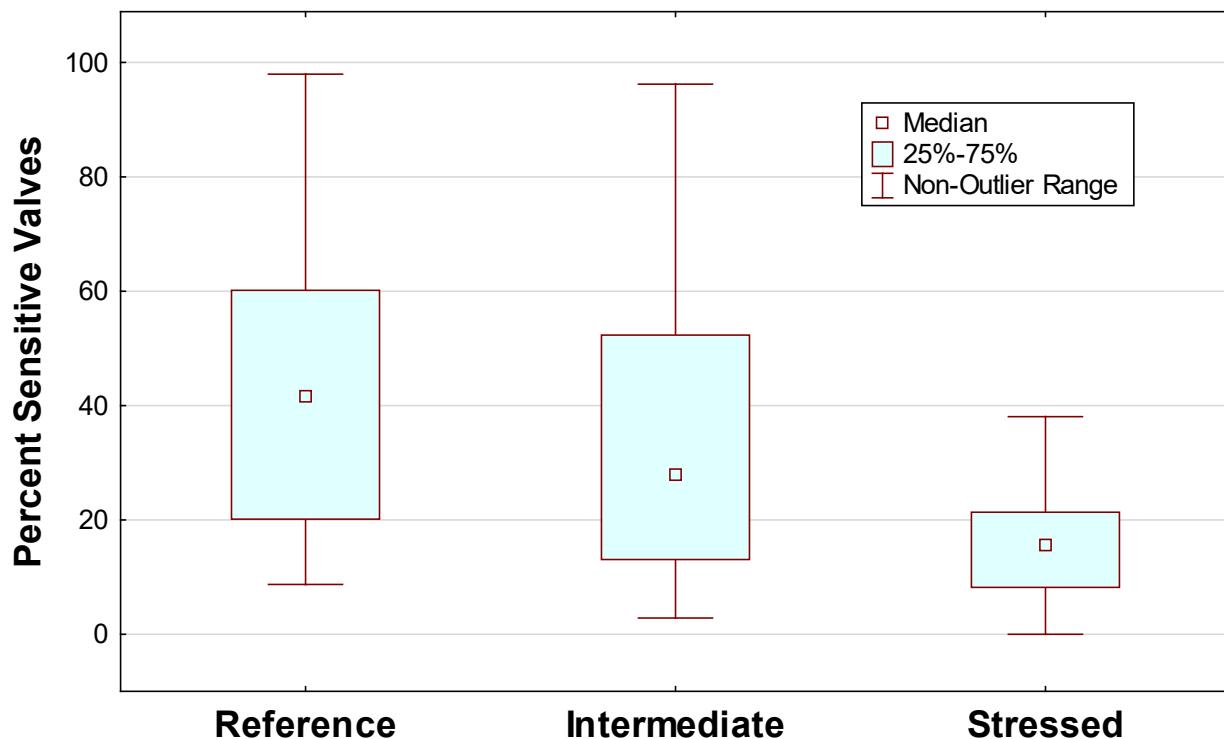
Figure 2. Example of CART results for the pt\_BC\_12 metric (percent sensitive taxa).

### 3.4 Metric Responses

The ability of each metric to distinguish between reference and stressed sites within a site class was measured as discrimination efficiency (DE) (Flotemersch et al. 2006, Maxted et al. 2000). The distinction between reference and stressed site values is illustrated with box plots that show the median, upper and lower quartiles, minimum, maximum, and outliers (Barbour et al. 1999). The DE is a quantification of the visually apparent distinctions. DE was calculated as the percentage of metric scores in stressed sites that were worse than the worst quartile of those in the reference sites. For metrics with a pattern of decreasing value with increasing environmental stress, DE is the percentage of stressed values below the 25<sup>th</sup> percentile of reference site values. For metrics that increase with increasing stress, DE is the percentage of stressed sites that have values higher than the 75<sup>th</sup> percentile of reference values. DE can be visualized on box plots of reference and stressed metric or index values with the inter-quartile range plotted as the box (Figure 3). Higher DE denotes more frequent correct association of metric values with site conditions. DE values  $\leq 25\%$  show no discriminatory ability in one direction. Metrics with DE values  $\geq 50\%$  were generally considered for inclusion in the index. However, metric selection was usually dependent on relative DE values within a metric category.

The Z-score was a second measure of metric sensitivity to stress. It was calculated as the difference between mean reference and stressed metric or index values divided by the standard deviation of reference values. The Z-score is similar to Cohen's D (Cohen 1992) and gives a combined measure of index sensitivity and precision. There is no absolute Z-score value that indicates adequate metric performance, but among metrics or indices, higher Z-scores suggest better separation of reference and stressed values. Cohen proposed that Z values  $\geq 0.80$  indicated a "large" effect.

The DE and Z-scores summarize the difference in distributions at critical potential threshold levels and incorporate the precision of the reference distribution. They were used in favor of a t-test or signal to noise (S:N) ratio. The DE is an estimate of the percentage of correct impaired assessments and can be interpreted for management applications. While the *t*-test has been used elsewhere (Stoddard et al. 2008), we are not testing a hypothesis about the difference between reference and stressed sites. The Z-score and S:N ratio are similar measures of responsiveness as a function of variability.



*Figure 3. Box and whisker plot illustrating the percent sensitive valves metric distributions among disturbance categories in the low nitrogen site class. The metric decreases with increasing stress and has a DE slightly less than 75% (estimating from the lower quartile of reference values compared to the distribution of stressed values).*

Another component of metric performance is precision of repeated measures. Precision was analyzed as the metric coefficient of variation (CV) of sample sets that were collected at the same site on the same day. This characterizes the metric precision attributable to sampling protocol. A low CV ( $\sim < 30\%$ ) would indicate a precise metric. Metrics with CVs near and greater than 100 are imprecise and might be avoided when a precise assessment is needed.

From an ANOVA using a replicate set identifier as the grouping variable and metrics as dependent variables, the Root Mean Squared Error (RMSE) was derived as an estimate of the standard deviation of each metric or index. The RMSE was standardized to the replicate sample mean to give the coefficient of variability (CV), which is comparable among metrics. Low CVs (e.g.,  $< 30\%$ ) would indicate high precision for a metric and if included in an index might contribute to a precise index. Conversely, high CVs (e.g.,  $> 75\%$ ) could contribute to more variability in an index. The index 90% confidence interval was calculated for each site class as  $1.645 * \text{RMSE}$ . Metric precision statistics were calculated for all replicate samples in

the data set (not separately by site class). Index precision was calculated using the same methods, but within site classes.

### 3.4 Index Composition

#### **Metric Scoring**

Metric values vary in scale depending on the units in the measurement and the ranges in the data sets. To give each metric equal weight in the index, metric values were converted to metric scores on a 100-point scale, using the effective range of each metric. The 5<sup>th</sup> and 95 percentile metric values from all sites were used as the effective ranges of metric variation in the Indiana samples. This recognized the possible range of metric values throughout the state while discounting values that were unusually extreme and possibly outliers (Blocksom 2003). For metrics that decreased with increasing stress, a metric score was calculated as follows:

$$\text{Decreaser metric score} = 100 * \frac{\text{Metric value} - 5\text{th percentile}}{95\text{th percentile} - 5\text{th percentile}}$$

For metrics that increase with increasing stress, the calculation was:

$$\text{Increaser metric score} = 100 * \frac{95\text{th percentile} - \text{metric value}}{95\text{th percentile} - 5\text{th percentile}}$$

#### **Metric Selection**

Metrics were selected as candidates for inclusion in the index based on several factors, including the following.

- Sensitivity
  - How well does the metric distinguish between reference and stressed sites?
  - What is the relationship between the metric and the disturbance variables?
    - Direction of response
    - Strength/significance
    - Consistency of response among site classes
- Redundancy
- Representation across metric categories (richness, composition, tolerance to stressors, functional characteristics, etc.)
- Precision

Metric responses that were consistently effective across site classes were preferred to those that were responsive in only one class. The confirmation of response patterns is an important indication of robust response mechanisms, especially when sample sizes are small. The consistent response guards against overfitting the model that might occur when selecting metrics that are responsive only in one site class.

#### **Index Calculations**

Index compositions were formulated from the best performing metrics in each metric category. The metrics were combined by scoring each on the 0 to 100 scale and then averaging the scores. Each index alternative was then evaluated for discrimination efficiency and other measures of representativeness and sensitivity. Index formulations were created and

evaluated in two ways: manual metric substitutions and automatic all-subsets modeling. Initial combinations using manual metric substitutions were used for exploratory analysis. Metrics with high sensitivity in multiple metric categories were scored, combined into an index, and evaluated for sensitivity using DE and Z-scores.

The all-subsets analysis allowed consideration of diverse index compositions that are too numerous to be computed by hand. Twenty candidate metrics were selected for inclusion in index trials based on DE, Z-score, and professional opinion of the working group. An “all subsets” routine in R software (R Core Team 2020) was used to combine up to 8 metrics in multiple index trials. Each index alternative was evaluated for performance using DE, Z-score, number of metric categories, and redundancy of component metrics. Those models including two or more correlated metrics (Spearman  $|r| \geq 0.80$ ) were excluded from consideration. As many metric categories as practical were represented in the index alternatives so that signals of various stressor-response relationships would be integrated into the index. While several metrics should be included to represent biological integrity, redundant metrics can bias an index to show responses specific to certain stressors or taxonomic responses.

### ***Index Selection***

The multiple possible indices were evaluated by applying a series of criteria for retaining or eliminating indices based on criteria related to sensitivity, redundancy, representation of multiple metric categories, adequate ranges of values, and intelligible response mechanisms. After reducing the possibilities to less than 20 indices, the team of IDEM biologists reviewed and selected the index combination that suited programmatic needs.

## **3.5 Index Validation**

A portion of the data were set aside before testing metric responses and index composition, so that the index could be tested with an independent data set. These were randomly selected with the intention of using sufficient sample sizes to represent reference and stressed conditions in all site classes in both calibration and validation analyses. For calibration, at least 10 samples were required so that a reliable reference condition could be characterized and so that stable stressed performance statistics could be calculated. For validation, a minimum of 5 sites were targeted to estimate index performance in sites that were not used in calibration.

The validation process included comparison of the reference and stressed validation index values to the 25<sup>th</sup> percentile of calibration reference index values. In a perfect validation, 75% of validation reference sites would be greater than the calibration reference 25<sup>th</sup> percentile and the percentage of stressed sites below that threshold would be as much or more than the DE in each site class. This is a comparison of calibration and validation Type I and Type II errors. Validation error might increase compared to calibration, but it is expected to be within 10% of the calibration error. Greater validation error would indicate the index was too specific to the calibration data, or overfit.

Validation can also compare index values to stressor gradients that were not used in defining the stressor gradient. If the stressors are independent and the index is responsive, then the stressor will likely be detected using the biological index.

### 3.6 Condition Thresholds

Biological indices are intended to describe biological conditions relative to human disturbances. When an index is accurate and precise, it can be used to indicate biological conditions that warrant special protection, are adequate for maintaining aquatic life uses, or are impaired and in need of pollution mitigation. Impairment thresholds are not defined in this report. Rather, approaches and analyses are presented and could be used to justify the selection of thresholds through policy discussions within IDEM.

Once site classes were established and indices were calibrated, some condition thresholds were associated with the 100-point index for assessment of biological condition and possibly for establishing numeric biological criteria. Multiple analyses were used to identify possible thresholds associating ranges of index values with biological condition categories. These included reference distribution statistics, balanced error types, and proportional odds logistic regression.

#### ***Reference Distribution Statistics***

The reference condition (RC) approach is the most commonly used method to derive biological thresholds (e.g., Yoder and Rankin 1995, DeShon 1995, Barbour et al. 1996, Roth et al. 1997). With the RC approach, IBI scores are calculated from a reference site dataset, and then a percentile of the IBI scores, such as the 25<sup>th</sup> or 10<sup>th</sup>, is chosen to represent the RC. If a reference condition is not defined, the 75<sup>th</sup> percentile of all site values could be considered (U.S. EPA 2000).

The Indiana diatom IBI was developed using reference condition concepts to identify sites with relative degrees of disturbance due to human activities. The reference and highly stressed conditions for sampled sites were defined using quantitative criteria of stressors and stressor sources. The absolute degree of disturbance is undefined, though there are relatively fewer stressors in the reference sites compared to intermediate and high-stress sites.

Distribution statistics in reference sites and all sites can inform possible thresholds, allowing assessment of sites that are similar to reference. These reference sites have few stressors and a biological condition representing a somewhat natural standard. Any index value above the minimum of reference index values might be a reference site. However, it is likely that the minimum value is not representative of acceptable reference conditions because a) the reference sites were defined with relative, not absolute, stressor criteria, b) there is variability in biological conditions, and c) there might be undetected stressors due to limited data availability. Rather, the minimum reference index value probably should not be recognized as an acceptable natural standard. In contrast, a threshold set at the median of index values would discount half of the reference sites, which would suggest that the reference sites were poorly defined, and the reference condition has substantial errors.

Thresholds based on a lower percentile of reference IBI scores describe points on the index scale above which conditions represent predominantly natural community types and below which biological conditions are departing from the core natural standard and might be impacted, erroneously designated reference sites, or simple errors due to biological and site

variability. The 10<sup>th</sup> - 25<sup>th</sup> percentiles of reference index values are common thresholds used in bioassessments. One of these percentiles could be selected as a threshold for assessing low gradient biological conditions using the IBI.

### ***Balanced Errors***

One strategy for selecting a threshold is to balance errors in assessing reference and highly stressed sites: there should be as many reference sites identified as impacted as there are highly stressed sites identified as unimpacted. This is based on the premise that each data set and condition was identified with equal degrees of certainty and therefore error should be the same. Type I and Type II errors are associated with reference sites erroneously identified as impacted and highly stressed sites identified as unimpacted, respectively.

### ***Secondary Thresholds***

Secondary thresholds can be identified within the generally unimpacted and generally impacted index ranges. This would allow for refined emphasis in biological condition when prioritizing or justifying management decisions. Within the generally unimpacted index range, refined conditions could be described as Exceptional or Satisfactory based on a secondary threshold derived from a simple bisection of the unimpacted index range, half-way between the general threshold and the maximum of the index scale. Similarly, the impacted range of the index scale could be bisected to describe a threshold between Moderately Degraded and Severely Degraded.

A more complex determination of secondary thresholds can be explored using proportional odds logistic regression (polr). This technique estimates the probabilities of membership in the reference, moderately stressed, and highly stressed groups based on index values within those categories. The points at which there is an equal probability between groups can describe a potential threshold that would evenly divide the Exceptional and Satisfactory index values and also the Moderately Degraded and Severely Degraded index values. These thresholds recognize the observed range of index values within disturbance groups, as opposed to the simple bisection, which uses the entire range of index values, regardless of the observed range. Recognition of the observed range of values is a more empirical method that is recommended.

## 4. Results

### 4.1 Site Disturbance

Of the 409 sites, 61 were designated as reference sites, in the sub-categories of BestRef, Ref, and SubRef (Table 3). Eighty-two (82) sites were designated as stressed (Strs and HighStrs) and 266 as intermediate stress sites. Relatively undisturbed and stressed sites were found throughout Indiana, though some regions (such as the Central Corn Belt Plains in the northwest) were not represented by reference sites. A high percentage of sites in Interior Plateau ecoregion (#71) were relatively undisturbed and few sites in this ecoregion were stressed (Figure 4). The greatest number of sites were in the Eastern Corn Belt Plains ecoregion (#55), which had the greatest number of reference and stressed sites compared to other ecoregions. Reference sites also occurred in the Southern Michigan / Northern Indiana Drift Plains (#56) and the Interior River Lowlands (#72). Figure 5 shows the range of stressor values represented across the disturbance gradient, as measured by percent urban land use, percent agricultural land use, total phosphorus concentration, qualitative habitat evaluation index (QHEI), chloride concentration, and total copper concentration. Appendix C contains additional box plots with disturbance variables as well as natural variables (such as drainage area, slope, and elevation). Appendix D contains the site list with final disturbance category assignments.

Though the criteria for designating sites in disturbance categories included agricultural land use, it was possible to attain reference status if intensive land use was the only one or one of few stressors. Several of the criteria were related to urban activities, such as urban land use coverage, percent imperviousness, population density, and road crossings. Habitat quality was not an explicit criterion and the QHEI did not vary appreciably among disturbance categories. Several chemical pollutant concentrations, including nutrients, metals and salts, increased as disturbance categories represented increasing stress.

*Table 3. Tabulation of diatom sampling sites by disturbance category and ecoregion in Indiana.*

Ecoregion:		54	55	56	57	71	72	Totals
Reference	BestRef					1		1
	Ref		6	4		9	1	20
	SubRef		17	5		10	8	40
Intermediate	Intermediate	30	110	25	1	48	52	266
Stressed	Strs	9	27	8	2	4	10	60
	HighStrs	4	16	2				22
Totals	Totals	43	176	44	3	72	71	409

Ecoregions include the Central Corn Belt Plains (54), the Eastern Corn Belt Plains (55), the Southern Michigan / Northern Indiana Drift Plains (56), the Huron/Erie Lake Plains (57), the Interior Plateau (71), and the Interior River Lowlands (72).

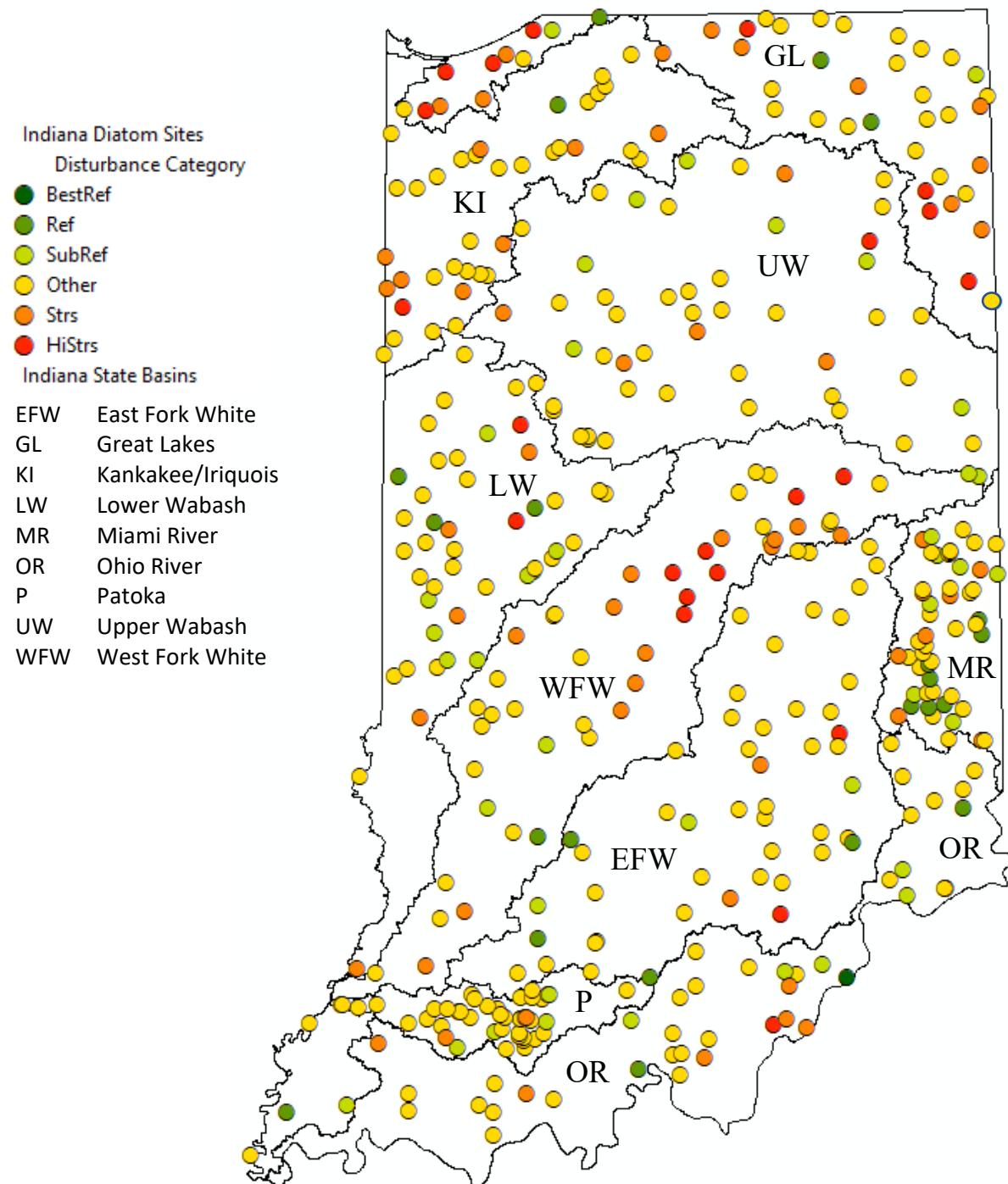


Figure 4. Diatom sampling sites in river basins of Indiana color-coded by disturbance category.

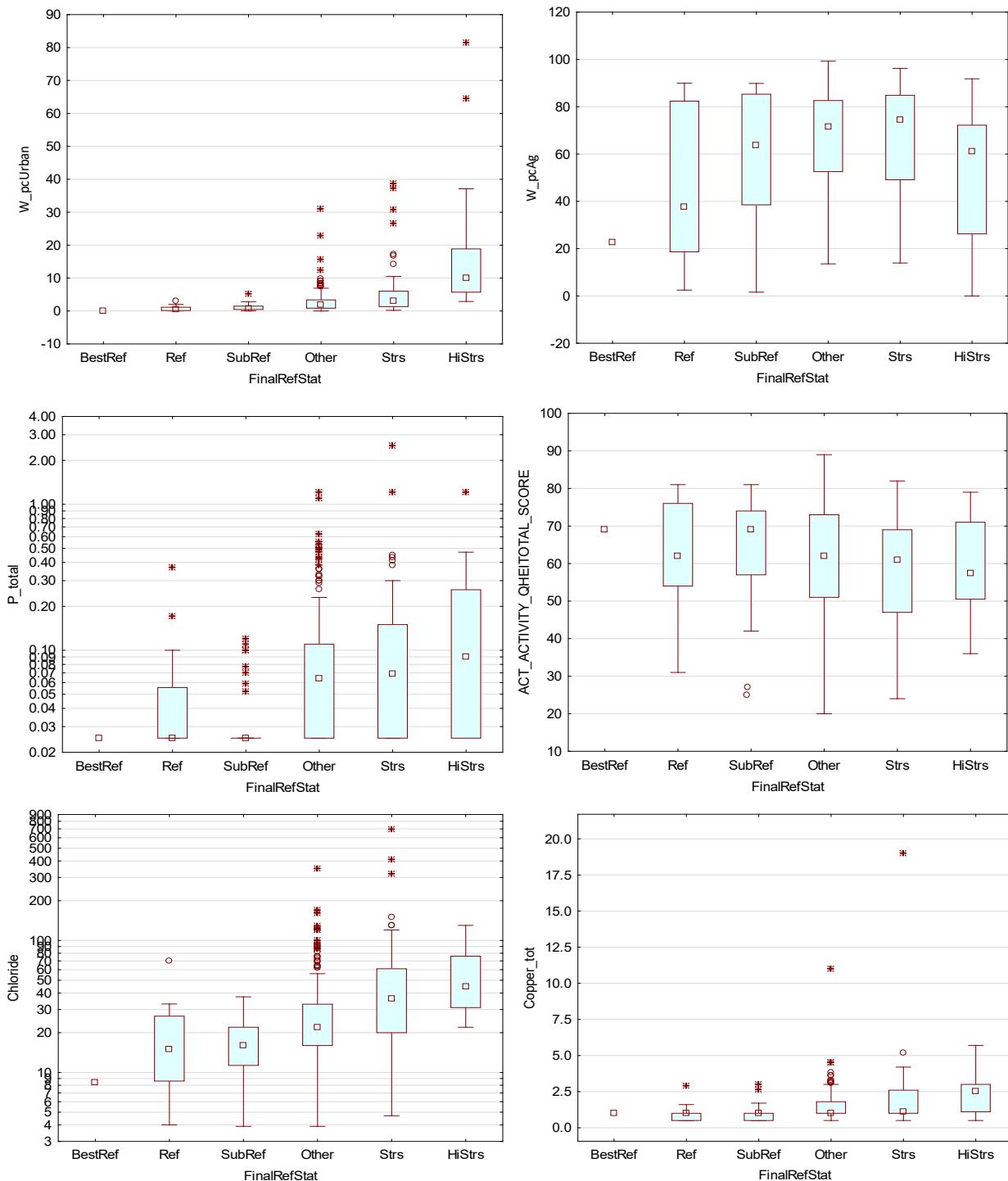
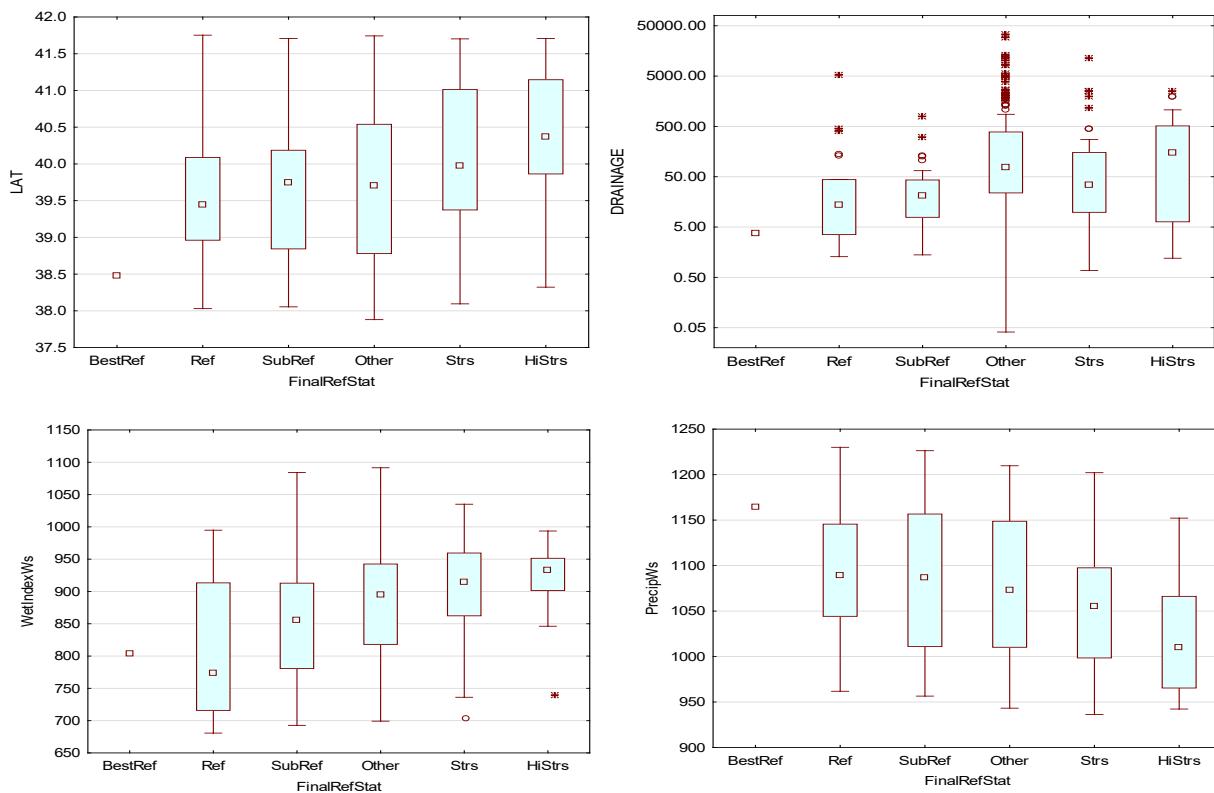


Figure 5. Distributions of selected stressors among disturbance categories, showing percent urban land use, percent agricultural land use, total phosphorus concentration, qualitative habitat evaluation index (QHEI), chloride concentration, and total copper concentration.

The disturbance categories were established based on the process outlined in Section 3.2. Though it was not intended, there were some natural variables showing patterns across the disturbance gradient, such as latitude, drainage area, the wetness index (related to topography), and precipitation (Figure 6). Other variables did not vary with disturbance

category, such as elevation, base flow index (BFI), and K-factor (related to soil erodibility) (Appendix C).



*Figure 6. Distributions of selected natural characteristics among disturbance categories, showing latitude, drainage area, the wetness index (related to topography), and precipitation.*

## 4.2 Site Classification

### Natural Background Conditions

Biological samples from 65 reference sites (BestRef, Ref, and SubRef) were used in the classification analysis, with only one sample per site included. The reference sites were fairly well distributed across the state, though there were spatial gaps in central and northwest Indiana (Figure 7). Reference sites were found in all ecoregions except the Central Corn Belt Plains (54) and the Huron/Erie Lake Plains (57). River basins, ecoregions, and benthic macroinvertebrate classification regions were tested as potential regional classification schemes for diatoms (Figures 7 and 8). Several other environmental variables were tested for correlations with ordination axes, including the base flow index (BFI) and site elevation (Figure 9).

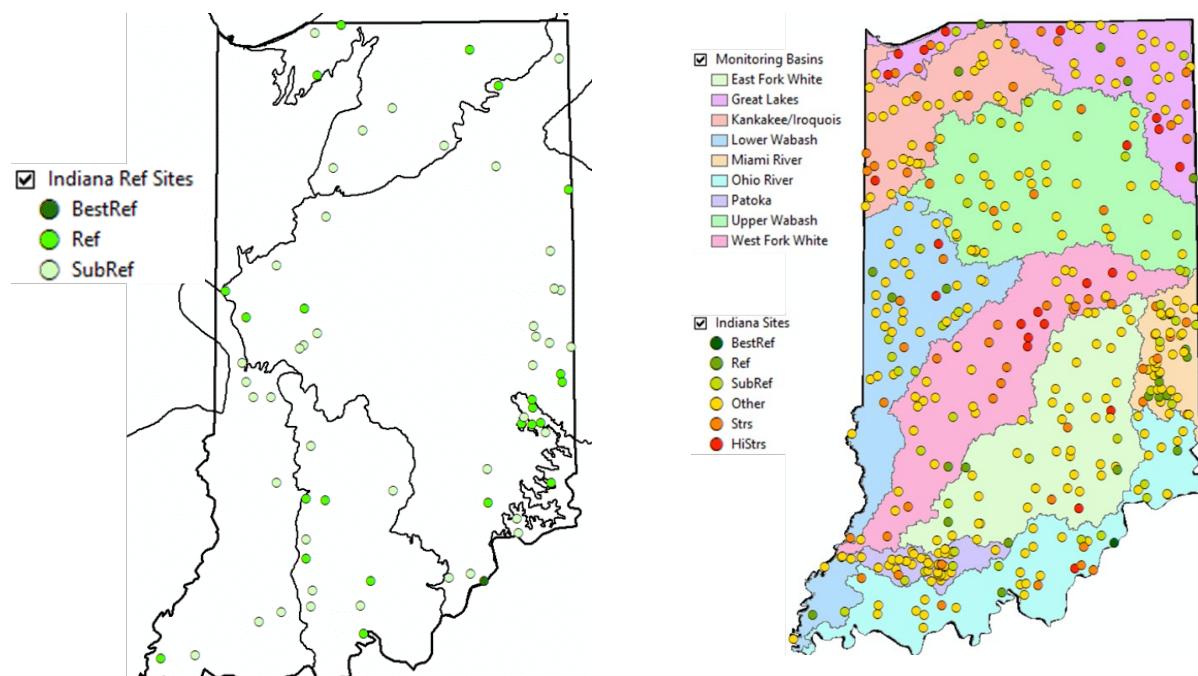


Figure 7. Reference sites in Indiana ecoregions (left, see ecoregion map in Figure 8) and all sites by disturbance category in river basins (right)

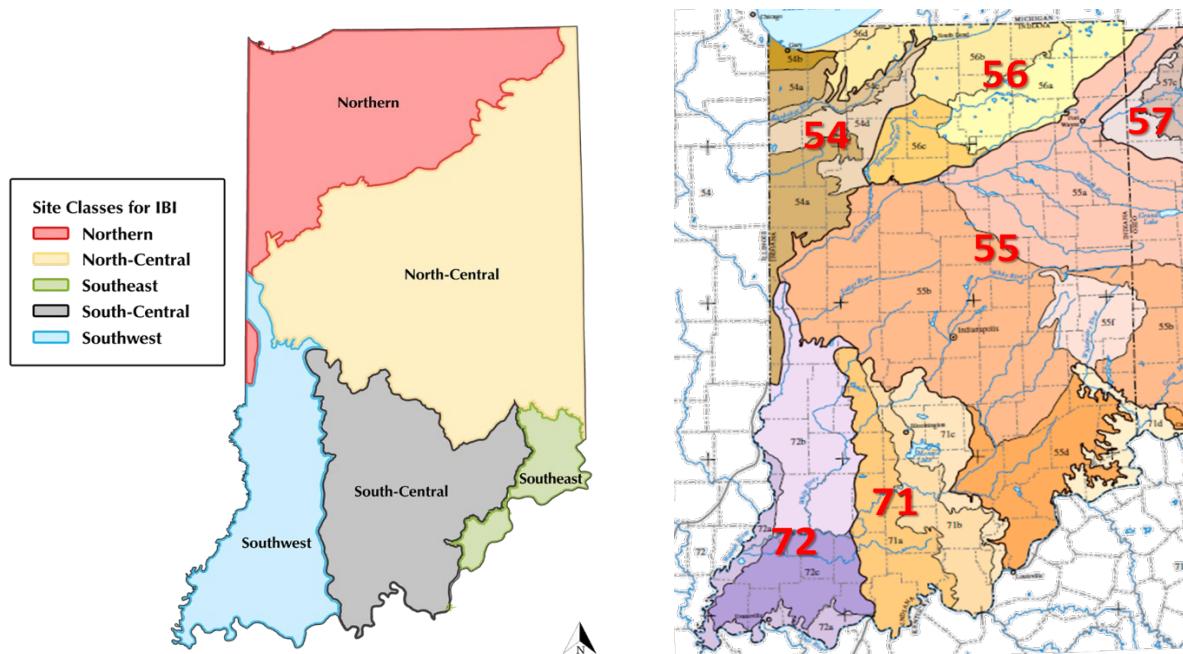


Figure 8. Macroinvertebrate assessment regions in Indiana (left) and Indiana ecoregions (right). Ecoregions include the Central Corn Belt Plains (54), the Eastern Corn Belt Plains (55), the So. Michigan / No. Indiana Drift Plains (56), the Huron/Erie Lake Plains (57), the Interior Plateau (71), and the Interior River Lowlands (72).

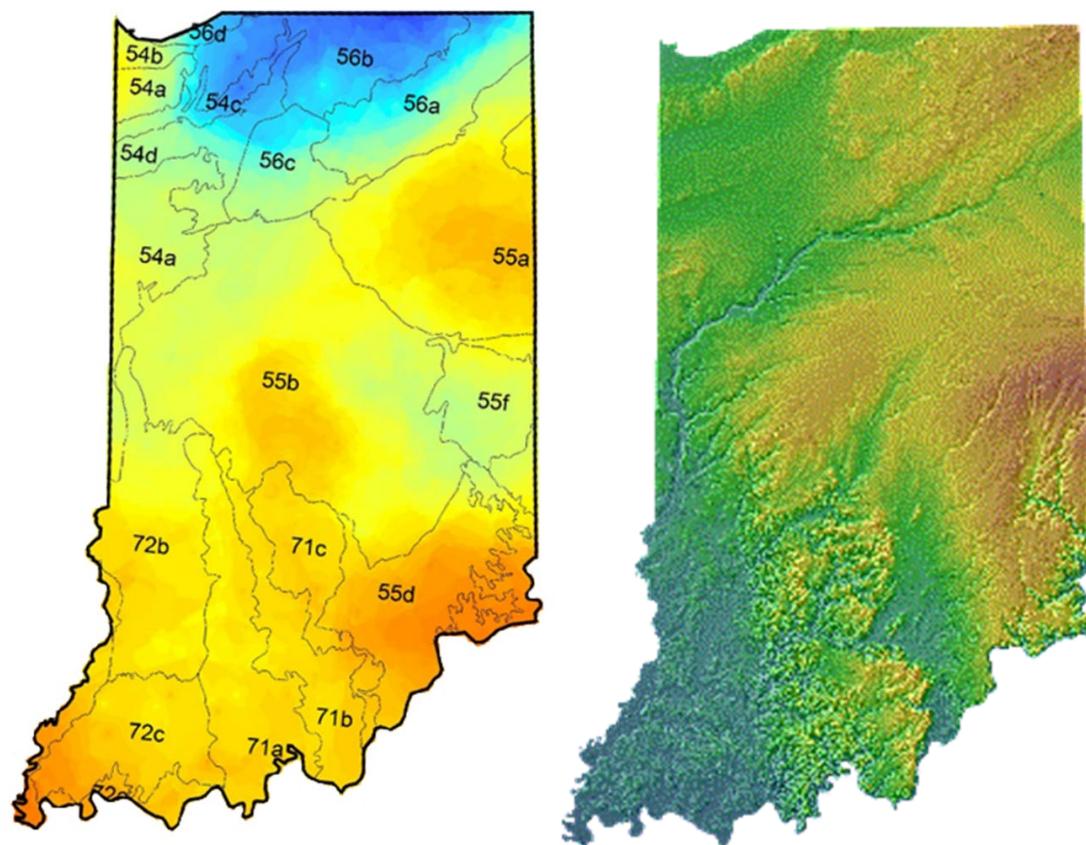
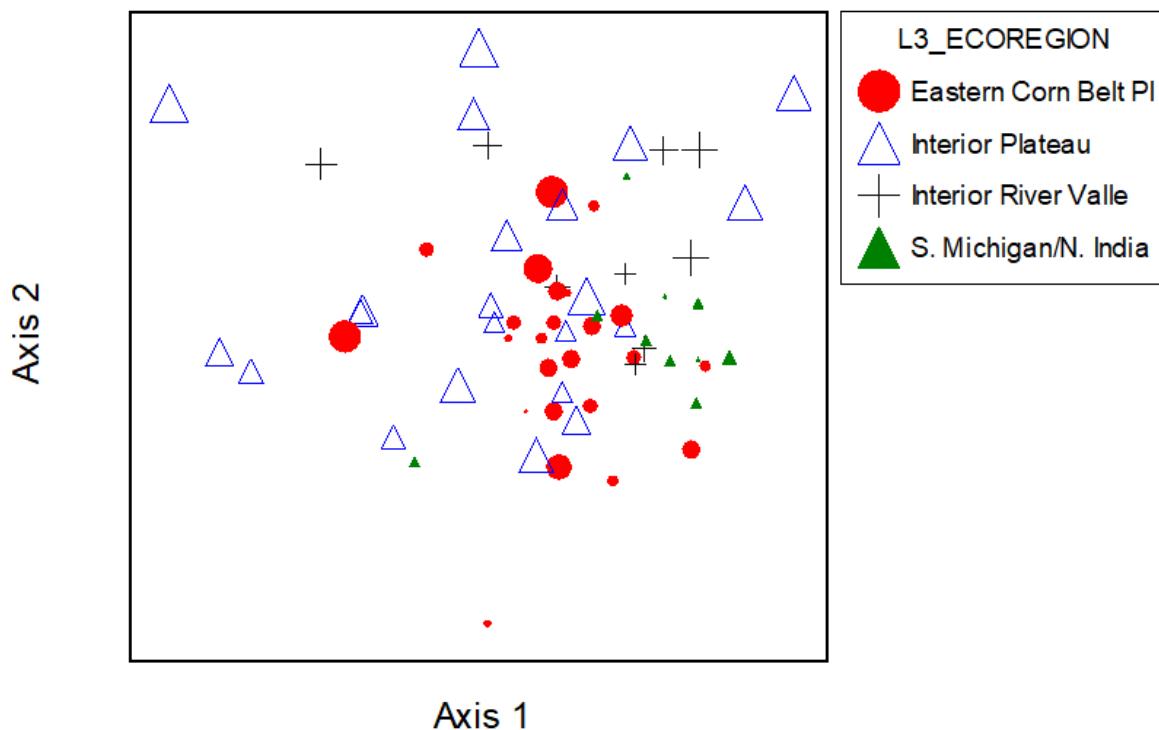


Figure 9. Base flow index (BFI) in Indiana ecoregions (left) and topographic elevation (right). For the BFI, blue tones indicate greater groundwater influence on stream flow and orange indicates less influence of groundwater. For elevation, brown tones are higher and blue tones are lower.

### Preliminary Classification Analysis

One hundred eighty-four (184) taxa, 29 metrics, and 59 environmental variables were included in the exploratory site classification analyses using NMS ordination of taxa, PCA ordination of metrics, and clustering of taxa with CART analysis. The analyses were informative, but ultimately inconclusive because they did not result in recognizable and distinct categories of site types. The ordination diagrams with taxa similarities, metric similarities, and clusters resulted in plots of overlapping sites when plotted by multiple categorical variables, such as ecoregion and precipitation (Figure 10). Though the categorical site classes had precedent in Indiana biological assessment (Jessup and Stamp 2017), they were not apparent as diatom site classes (Appendix E).



*Figure 10. Non-metric multidimensional scaling (NMS) diagram showing reference sites arranged by taxonomic similarity and marked by ecoregion and precipitation (size of the marker).*

### ***Correlation Analysis***

The lack of a clear site classification in the preliminary analyses lead to a correlation analysis with metrics and environmental variables so that individual metrics might be adjusted as needed. The correlation analysis was used to select the variables and metrics used for individual metric adjustments. Acidity (pH and alkalinity) was suspected of having some effects on metrics and of having primarily natural origins. The range of pH in reference and non-reference sites was similar, supporting the idea that pH was not a stressor variable. Pearson correlation coefficients had magnitudes  $> 0.25$  for pH in 27 of 270 metrics tested. The highest correlations were with oligotrophic taxa richness; high pH was associated with few oligotrophic taxa in reference sites and in all sites. High pH was also associated with fewer eutrophic individuals. The correlation coefficients and mixed signals suggest that pH has minor effects on some metrics.

Other correlations with water quality were apparent but were possibly related to stressor effects. For example, richness metrics were correlated with water temperature. Chloride was correlated with reference metrics, but is likely a stressor indicator and not an appropriate classification variable. Turbidity was correlated with metrics, also associated with chloride and nutrients, and not an appropriate classification variable. The variables that had high correlation coefficients with several metrics included slope, wet index, BFI, precipitation, temperature, and predicted lithologic chemistry (Ca, Na, Fe, and N) (Table 4). There were also some weak signals with elevation and catchment size.

*Table 4. Correlation coefficients for selected metrics and environmental variables with relatively high correlation coefficients (Spearman  $|rho| > 0.30$ ). Metric and variable descriptions are in Attachment 1 and Appendix F, respectively.*

Variable	Wet								
	SLOPE	BFI Cat	Index Cat	Precip Ws	Tmean Ws	Na2O Cat	CaO Ws	Fe2O3 Ws	N Ws
MMI_NRSA	0.36	-0.44	-0.56	0.53	0.51	-0.42	0.51	-0.48	-0.46
nt_HIGH_N		0.33	0.45	-0.49	-0.47				
pi_Diatas_TN_2						-0.30	0.44		-0.39
pi_Diatas_TP_2						-0.32	0.41	-0.30	-0.38
pi_HIGH_N				-0.46	-0.35				
pt_N_FIXER	0.40								
pt_NON_N_FIXER	-0.40								
pi_Diat_CA_1				-0.43	-0.39				
nt_O_3		0.33	0.34						
pi_O_2	0.47								
pt_O_2	0.44								
nt_TROPHIC_3		-0.31	-0.39	0.49	0.43				
pi_TROPHIC_4		0.45	0.39			0.43	-0.33	0.35	
pt_TROPHIC_3		-0.40	-0.52	0.57	0.50	-0.37	0.41	-0.44	-0.39
pt_SAP_2	0.53								
pt_BC_3	0.40								
pi_BC_4		0.34	0.37	-0.53	-0.50				
nt_PT_4		0.40		-0.56	-0.61				
pt_PT_4	0.41								
pt_Bahls_3	0.45								
pt_SALINITY_2	0.46								
nt_BIG		0.55	0.31	-0.5	-0.54	0.39	-0.30	0.47	0.37
pi_BIG		0.44							
pi_SMALL							-0.39	0.34	0.37
pt_BIG	0.51			-0.43	-0.48	0.43		0.49	0.39
pt VERY_SMALL						-0.31	0.47	-0.33	-0.35
WA_Size_USGS	0.40								
wa_Moisture	-0.47	-0.47	0.32	0.44	-0.50	0.39	-0.45	-0.39	
pi_NAVICULA	0.41	0.48	-0.47	-0.5	0.31	-0.38	0.41	0.35	

### Metric Adjustment

Variables were selected for metric adjustment based on the correlation analysis and judgement on their appropriateness for classification (e.g., not subject to human disturbance, reliable measurements, etc.). Variables were selected if they were consistently and strongly correlated to metrics at a Spearman  $|rho| > 0.40$  in reference sites. Variables were also selected if they were not the strongest correlations, but that were common over several metrics. For example, BFICat. BFICat was selected for metric adjustments over BFIWs because they consistently had similar correlation coefficients with environmental variables, but BFICat was correlated at  $|rho| > 0.40$  for 10 metrics, while BFIWs was correlated to 8 metrics. The selected variables for adjustment included BFICat (base flow), TminCat

(temperature), PrecipCat (precipitation), WetIndexCat (topography), CaOWs (background calcium oxide), NWs (background nitrogen), and SLOPE (stream gradient). Metrics from the preliminary index were adjusted by one or two variables identified in CART analysis (Table 5). The purpose of the metric adjustment was to refine the discrimination performance of the metrics to recognize natural background expectations. However, after adjustment, the preliminary index had a lower DE than the unadjusted version (65.6% vs. 75.4 %), and the adjustments were not applied as planned.

*Table 5. Metrics in the preliminary index and the classification variables used for adjustments.*

Index Metric	Adjustment Variables
pt_BC_12	NWs and BFICat
nt_LOW_P	PrecipCat
pt_O_345	NWs
pt_HIGHLY_MOTILE	WetIndexCat and BFICat
pt_SESTONIC_HABIT	SLOPE and TminCat
pt_RefIndicators	NWs and PrecipCat

### ***Final Classification Analysis***

The final classification analysis included CART analysis of the preliminary index. At first, background nitrogen (NWs<sup>3</sup>) and precipitation (PrecipCat) were the first and second splits of the reference index values. Results showed that precipitation explained relatively little variability after accounting for background nitrogen. Ultimately, the best classification scheme used a background nitrogen threshold of 0.089 % to describe high nitrogen (HiN) and low nitrogen (LoN) conditions (Figure 11). The breakpoint is supported by similar breakpoints derived from CART analysis of individual metrics.

Within the two site classes, metric adjustments were again evaluated. For most metrics, CART analysis did not identify different expectations. The nitrogen classes accounted for most variability. This was evident in values of the environmental variables, which were distinguishable in the two nitrogen classes (Figure 12). Sites in both site classes were distributed throughout the state, though most low nitrogen sites were in the southern half of the state (Figure 13).

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<sup>3</sup> NWs = Mean % of lithological nitrogen (N) content in surface or near surface geology within the watershed, derived from StreamCat (Hill et al. 2016). See derivation details in Appendix A.

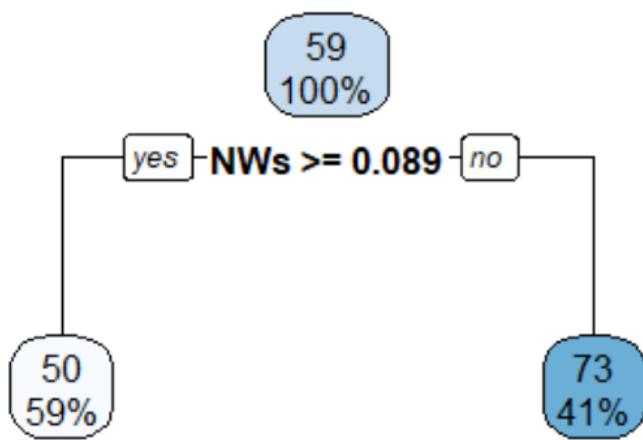


Figure 11. CART diagram showing that reference preliminary index values were split into high nitrogen and low nitrogen categories based on a geological nitrogen (NWs) threshold of 0.089 %.

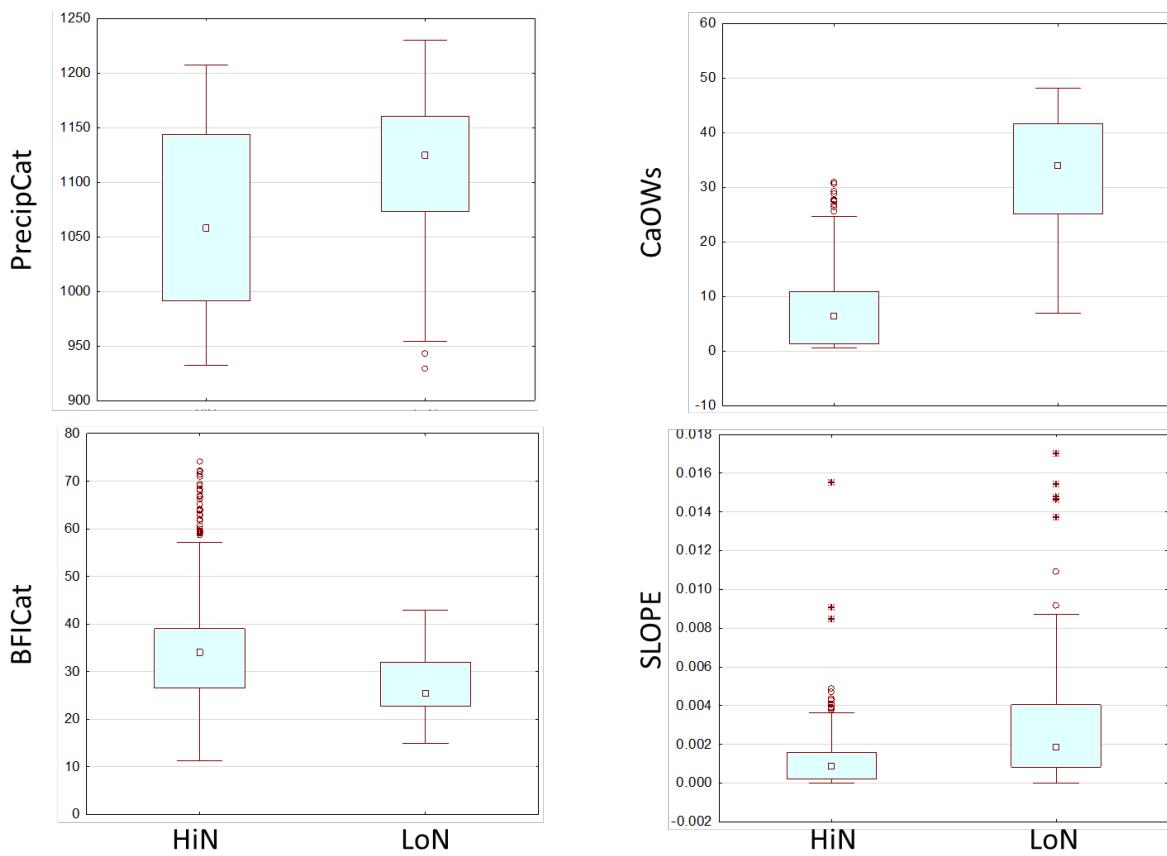


Figure 12. Classification variable distributions in high nitrogen (HiN) and low nitrogen (LoN) site classes.

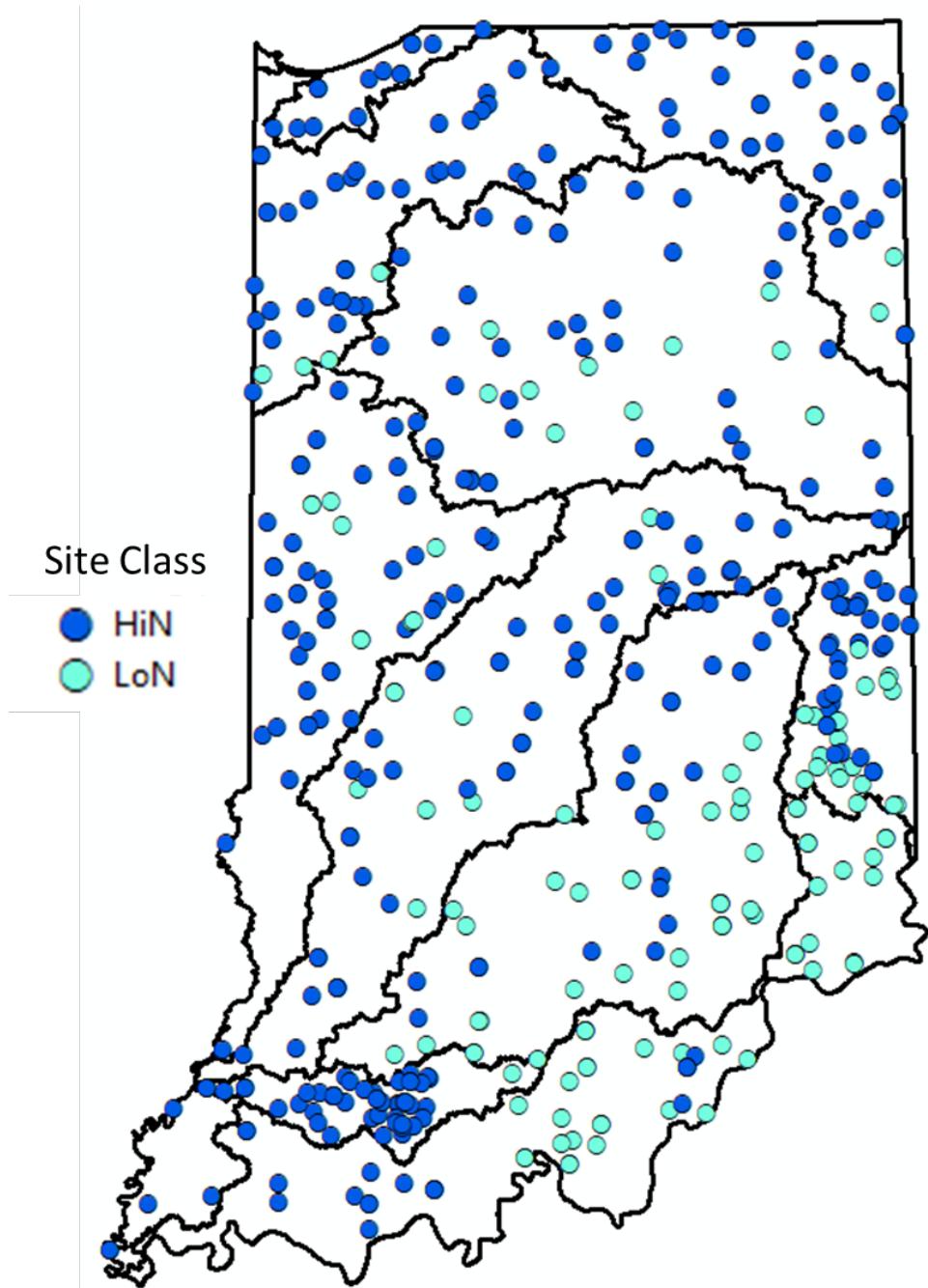


Figure 13. Diatom sampling site locations marked by site class; high nitrogen (HiN) or low nitrogen (LoN).

#### 4.3 Traits and Tolerance Analysis

To help inform tolerance value assignments, we ran taxa tolerance analyses on the Indiana diatom dataset to explore the distribution of taxa across four generalized disturbance measures: the Index of Watershed Integrity (IWI, Thornbrugh et al. 2018, Johnson et al. 2018), Qualitative Habitat Evaluation Index (QHEI), stream conductivity (Cond) and watershed imperviousness (Imperv\_W). Taxa that occurred at fewer than 10 sites were excluded from the analysis because low numbers of occurrences gave unreliable results. Tolerance analyses allow for visualization of the shape of the taxon-stressor relationship across a continuous numerical scale and can be used to identify optima (the point at which the taxon has the highest probability of occurrence) as well as tolerance limits (the range of conditions in which the taxon can persist) (Yuan 2006).

Taxa traits and tolerance values were used to calculate 308 metrics. The traits and tolerance values are in Attachment 2. Tolerance value graphic output for diatom genera are in Appendix G. Metric descriptions are in Attachment 1.

#### 4.4 Metric Responses

The metrics were screened for responsiveness so that the most sensitive metrics could be included in the index. While 308 metrics were calculated, 88 metrics in the HiN class and 181 in the LoN class had responses that were sensitive ( $DE > 40$ ) and had a non-contradicting response trend among the classes (Appendix H). Consistently responsive metrics were identified in most of the metric categories. Of the 15 metric categories in the metric list, all were represented in the consistently responsive metrics in the LoN class and all but two were represented in the HiN class: total taxa richness and affinity for moisture.

For the all-subsets modeling, the metrics were limited to about 20 metrics, which is a practical limit for running the iterative code. The selection criteria included metric performance (DE), representation within a metric category, independence from other selected metrics (non-redundant), and adequate range of values. The resulting selections are as in Tables 6 and 7 for the LoN and HiN site classes, respectively.

In the LoN site class, no metrics were selected from the following categories because the metric DEs were relatively low: acidity, habit, moisture, motility, richness, saprobity, and size. In the HiN site class, no metrics were selected from the following categories: acidity, moisture, motility, richness, saprobity, size, and trophic.

Table 6. Candidate metrics for the LoN All-subsets model analysis

Metric	Metric Category	DE (trend)	Z-score
nt_BC_12	Biological	90.9 (DEC)	1.9
pt_BC_12	Condition	81.8 (DEC)	1.4
pt_O_345	Oxygen	72.7 (INC)	-1.2
pt_RefIndicators	Indicators	100 (DEC)	1.4
nt_LOW_P		81.8 (DEC)	1.7
nt_LOW_N		72.7 (DEC)	1.3
pt_LOW_P	Nutrients	72.7 (DEC)	0.9
pt_HIGH_N		90.9 (INC)	-2.0
pi_LOW_P		72.7 (DEC)	0.7
pi_LOW_N		72.7 (DEC)	0.7
pt_PT_12		63.6 (INC)	-0.5
pt_Bahls_1	Pollution	54.5 (INC)	-0.6
wa_Poll_Tol	Tolerance	72.7 (DEC)	0.7
pt_Sens_810		81.8 (DEC)	1.7
nt_SALINITY_12	Salts	54.5 (DEC)	0.7
pt_SALINITY_34		90.9 (INC)	-2.0
nt_Achnan_Navic	Taxa Group	81.8 (DEC)	1.1
pt_Achnan_Navic		81.8 (DEC)	1.5
pi_Achnan_Navic		72.7 (DEC)	0.9
pt_TROPHIC_456	Trophic	63.6 (INC)	-0.5

Table 7. Candidate metrics for the HiN all-subsets model analysis

Metric	Metric Category	DE (trend)	Z-score
pt_BC_12		50 (DEC)	0.6
pt_BC_12_adj	BC	54.3 (DEC)	0.6
pt_BC_45		50 (INC)	-0.1
pt_O_345_adj	DO	45.7 (INC)	-0.3
pt_SESTONIC_HABIT	Habit	54.3 (INC)	-0.6
pt_RefIndicators_adj	Indicators	52.2 (DEC)	0.5
pi_RefIndicators		50 (DEC)	0.1
nt_LOW_P		52.2 (DEC)	0.3
nt_LOW_N		43.5 (DEC)	0.3
pt_LOW_P	Nutrients	43.5 (DEC)	0.3
pi_LOW_P		43.5 (DEC)	0.0
pi_HIGH_P		50 (INC)	-0.6
pt_PT_12		50 (INC)	-0.2
pt_Bahls_1		52.2 (INC)	-0.6
pi_PT_45	PollToler	41.3 (DEC)	0.0
wa_Poll_Tol		41.3 (DEC)	0.0
pi_Tol_13		52.2 (INC)	-1.1
pi_SALINITY_4	Salts	47.8 (INC)	-1.3
pi_Diat_CL_1_ASSR		52.2 (INC)	-1.2
pt_Achnan_Navic	TaxaGroup	45.7 (DEC)	0.5

In the HiN site class, three of the selected metrics for the all-subsets modeling were adjusted using CART analysis to account for natural variability. These included pt\_BC\_12\_adj, pt\_O\_345\_adj, and pt\_RefIndicators\_adj (Table 8). Also in the HiN site class, the pi\_Diat\_CL\_1\_ASSR metric used the *arcsine (square root)* transformation to expand the range of low percentage values. None of the selected metrics were adjusted or transformed in the LoN site class.

Table 8. Adjustment formulae for candidate HiN index metrics.

Metric	Adjustment Variable and Threshold	Adjustment Formula
pt_BC_12_adj	BFIcat at 30 units	pt_BC_12_adj = If BFIcat < 30, then pt_BC_12 - 0.105, else pt_BC_12 - 0.151
pt_O_345_adj	NWs at 0.158 %	pt_O_345_adj = If NWs < 0.158, then pt_O_345 - 0.312, else pt_O_345 - 0.387
pt_RefIndicators_adj	PrecipCat at 1006 mm/yr	pt_RefIndicators_adj = If PrecipCat < 1006, then pt_RefIndicators - 6.29, else pt_RefIndicators - 10.9

Metric scoring proceeded using the 5<sup>th</sup> and 95<sup>th</sup> percentile of all data (both site classes and all disturbance categories) except when substantial differences were noted in statistics between site classes (Table 9). The reliance on the whole data set assured that the entire range of possible values would be addressed, but when the metric values were limited in one site class, the limited range was used to optimize the metric response within the effective range.

Table 9. Scoring formulae for candidate metrics in the LoN and HiN site classes. In the scoring formulae, replace 'metric' with the sample metric value to calculate the metric score.

Metric	Metric Category	LoN Formula	HiN Formula
nt_BC_12		$100*(\text{metric} - 2)/(13 - 2)$	
pt_BC_12	BC	$100*(\text{metric} - 0.05)/(0.23 - 0.05)$	$100*(\text{metric} - 0.05)/(0.23 - 0.05)$
pt_BC_12_adj			$100*(\text{metric} + 0.09)/(0.07 + 0.09)$
pt_BC_45			$100*(0.75-\text{metric})/(0.75-0.43)$
pt_O_345	Dissolved Oxygen	$100*(0.47-\text{metric})/(0.47-0.24)$	
pt_O_345_adj	Oxygen		$100*(0.14-\text{metric})/(0.14+0.12)$
pt_SESTONIC_HABIT	Habit		$100*(0.20-\text{metric})/(0.20)$
pi_RefIndicators <sup>†</sup>			$100*(\text{metric} - 0.7)/(27.6 - 0.7)$
pt_RefIndicators <sup>†</sup>	Indicators	$100*(\text{metric} - 4.5)/(26.2 - 4.5)$	
pt_RefIndicators_adj			$100*(\text{metric} + 10.9)/(7.5 + 10.9)$
nt_LOW_N		$100*(\text{metric} - 1)/(8 - 1)$	$100*(\text{metric} - 1)/(8 - 1)$
nt_LOW_P		$100*(\text{metric} - 1)/(7 - 1)$	$100*(\text{metric} - 1)/(7 - 1)$
pi_HIGH_P			$100*(0.75-\text{metric})/(0.75-0.11)$
pi_LOW_N <sup>†</sup>	Nutrients	$100*(\text{metric} - 0.01)/(0.70 - 0.01)$	
pi_LOW_P <sup>†</sup>		$100*(\text{metric} - 0.01)/(0.70 - 0.01)$	$100*(\text{metric})/(0.26)$
pt_HIGH_N		$100*(0.55-\text{metric})/(0.55-0.33)$	
pt_LOW_P		$100*(\text{metric} - 0.05)/(0.15 - 0.05)$	$100*(\text{metric} - 0.05)/(0.15 - 0.05)$
pi_PT_45			$100*(\text{metric} - 0.03)/(0.59 - 0.03)$
pi_Tol_13			$100*(49.6-\text{metric})/(49.6-1.6)$
pt_Bahls_1	Pollution	$100*(0.18-\text{metric})/(0.18-0.03)$	$100*(0.18-\text{metric})/(0.18-0.03)$
pt_PT_12	Tolerance	$100*(0.20 - \text{metric})/(0.20 - 0.07)$	$100*(0.20 - \text{metric})/(0.20 - 0.07)$
pt_Sens_810		$100*(\text{metric} - 0.10)/(0.40 - 0.10)$	
wa_Poll_Tol		$100*(\text{metric} - 1.9)/(3.9 - 1.9)$	$100*(\text{metric} - 1.9)/(3.9 - 1.9)$
nt_SALINITY_12		$100*(\text{metric} - 17)/(47.2 - 17)$	
pi_Diat_CL_1_ASSR	Salts		$100*(0.20-\text{metric})/(0.20)$
pi_SALINITY_4			$100*(0.40-\text{metric})/(0.40)$
pt_SALINITY_34		$100*(0.25-\text{metric})/(0.25-0.07)$	
nt_Achnan_Navic	Taxa Groups	$100*(\text{metric} - 4.8)/(18 - 14.8)$	
pi_Achnan_Navic		$100*(\text{metric} - 0.04)/(0.49 - 0.04)$	
pt_Achnan_Navic		$100*(\text{metric} - 0.14)/(0.32 - 0.14)$	$100*(\text{metric} - 0.14)/(0.32 - 0.14)$
pt_TROPHIC_456	Trophic Status	$100*(0.66-\text{metric})/(0.66-0.43)$	

<sup>†</sup> Metric scoring was based on the 5<sup>th</sup> and 95<sup>th</sup> percentiles of all data within the site class (all other metrics used the 5<sup>th</sup> and 95<sup>th</sup> percentile across both site classes).

#### 4.5 Index Composition

The all-subsets model calculation and screening resulted in thousands of valid index combinations. Initially, the all-subsets analysis resulted in more than 110,000 different index combinations of non-redundant metrics in both the HiN and LoN site classes. To identify the most sensitive, comprehensive, and practical index alternatives, the characteristics of the alternatives were screened for favorable characteristics such as high index DEs and

representation of multiple metric categories. Metrics with conceptual redundancy were identified and index alternatives with more than one similar metric were removed from consideration. Index alternatives were also discounted if they used metrics that had relatively low DE in the metric category, if the metric response mechanism was poorly understood, if the range of metric values was limited, or if metric calculation was complex relative to alternative metrics. Application of index removal criteria was done with approval of the IDEM workgroup and a final index was selected and justified.

In the HiN site class, 137,678 index models were systematically reduced to two alternatives using exclusion criteria (Table 10). The final index was selected because it had more metric calculation types, which is assumed to allow a broader diversity of responses and an index that will be more effective at detecting disturbance. In the LoN site class, 113,618 index models were systematically reduced to 37 alternatives using similar exclusion criteria (Table 11). The final index selection for the LoN site class was based on preference for some metrics and exclusion of others using nuanced criteria that were not applied as quantitative or uniform rules (Table 12). Of the 37 alternative that remained after application of the criteria in Table 11, one alternative was selected and approved by IDEM.

The final indices for the HiN and LoN site classes each had five component metrics (Table 13). The HiN index had a DE of 71.7% and a Z-score of 1.1. The LoN index had a DE of 100% and a Z-score of 3.2. The index scores reflected the relative degrees of stress in the sites. The HiN index performs well in both site classes, though it was customized for only the HiN class (Figure 14). The LoN index performs best in the LoN class (Figure 15). In both indices, the sites with intermediate disturbance status showed a span of values that was broader than either the reference or stressed values. The intermediate disturbance sites had median values and interquartile ranges that were intermediate to the reference and stressed statistics.

Table 10. Conditions for exclusion of the HiN index alternatives from consideration as final indices

Index alternative exclusion criteria	Initial Number of Models	Remaining Models
	137,678	
<b>Number of metrics &lt; 5:</b> A limited number of metrics will limit the index sensitivity to few stressors and response mechanisms	132,316	
<b>Number of metric categories &lt; 4:</b> A limited number of metric categories will limit the index sensitivity to few stressors and response mechanisms	129,802	
<b>Number of nutrient metrics &gt; 2:</b> If the index is overweighted in one metric category, sensitivity will be biased towards the targeted stressor (nutrients)	113,926	
<b>DE &lt; 60:</b> A low DE indicates poor sensitivity compared to other models	21,290	
<b>Conceptually redundant low P metrics (nt_LOW_P, pt_LOW_P and pi_LOW_P):</b> Though not statistically correlated at $ r  > 0.80$ , multiple low P metrics would bias the index towards sensitivity to phosphorus	15,456	
<b>Conceptually redundant regionally calibrated tolerance metrics (pi_RefIndicators and pi_Tol_13):</b> Multiple regionally calibrated tolerance metrics would bias the index and might be overfit to this data set	10,761	
<b>Conceptually redundant salts metrics (pi_SALINITY_4 and pi_Diat_CL_1_ASSR):</b> Multiple metrics related to dissolved salts would bias the index towards sensitivity to ion content	9,118	
<b>Conceptually redundant biological condition (BC) metrics (pt_BC_12, pt_BC_12_adj and pt_BC_45):</b> Multiple BC metrics would bias the index towards sensitivity to this tolerance characteristic	6,679	
<b>DE &lt; 69:</b> A lower DE indicates poor sensitivity compared to other models	273	
<b>Index alternatives with pi_LOW_P:</b> This metric was included in the sensitive index alternatives less often than other nutrient metrics	257	
<b>Index alternatives with pi_PT_45 or wa_Poll_Tol:</b> These metrics had lower DE than the remaining pollution tolerance metric and were included in fewer of the sensitive index alternatives	247	
<b>Index alternatives with pi_RefIndicators:</b> This metric had a lower DE and was included in fewer sensitive index alternatives compared to the other tolerance metrics	238	
<b>Index alternatives with pi_Diat_CL_1_ASSR:</b> This metric had a limited range of values	141	
<b>Index alternatives with pt_SESTONIC_HABIT:</b> Though responsive to stress, this metric was also responsive to catchment size, which is not a disturbance	75	
<b>Index alternatives with pt_Bahls_1:</b> This trait was calibrated for Montana conditions that might be unlike Indiana conditions	61	
<b>Index alternatives with pt_RefIndicators_adj:</b> This metric relies on reference sites and it requires adjustment, which is a more complex calculation than simpler and effective alternatives	9	
<b>Included an alternative with pt_RefIndicators_adj:</b> This alternative illustrates that the DE could be improved in comparisons to other alternatives without it	10	
<b>Index alternatives with pi_SALINITY_4:</b> This metric had a limited range of values	4	
<b>DE &lt; 71:</b> A lower DE indicates poor sensitivity compared to other models	2	
<b>Select preferred index</b> - with a variety of metric types (including pt, nt, and pi calculation types)	5_3402	

Table 11. Conditions for removal of the LoN index alternatives from consideration as final indices

Index alternative removal criteria	Initial Number of Models	Remaining Models
	113,618	
<b>Number of metrics &lt; 5:</b> A limited number of metrics will limit the index sensitivity to few stressors and response mechanisms	108,526	
<b>Number of metric categories &lt; 4:</b> A limited number of metric categories will limit the index sensitivity to few stressors and response mechanisms	106,519	
<b>Number of nutrient metrics &gt; 2:</b> If the index is overweighted in one metric category, sensitivity will be biased towards the targeted stressor (nutrients)	98,619	
<b>DE &lt; 80:</b> A low DE indicates poor sensitivity compared to other models	94,972	
<b>DE &lt; 90:</b> A low DE indicates poor sensitivity compared to other models	84,846	
<b>Conceptually redundant taxa group metrics (nt_Achnan_Navic, pt_Achnan_Navic, and pi_Achnan_Navic):</b> Though not statistically correlated at $ r  > 0.80$ , many measures of the same taxa would bias the index towards this indicator	55,032	
<b>Conceptually redundant nutrient metrics (nt_LOW_N and pi_LOW_N or nt_LOW_P, pi_LOW_P, and pi_LOW_P):</b> Multiple low P or low N metrics would bias the index towards sensitivity to phosphorus or nitrogen	44,885	
<b>Conceptually redundant salts metrics (nt_SALINITY_12 and pt_SALINITY_34):</b> Multiple metrics related to dissolved salts would bias the index towards sensitivity to ions	37,396	
<b>Conceptually redundant biological condition (BC) metrics (nt_BC_12 and pt_BC_12):</b> Multiple BC metrics would bias the index towards sensitivity to this tolerance characteristic	30,995	
<b>Conceptually redundant pollution tolerance (PT) metrics (pt_PT_12 and wa_Poll_Tol):</b> Multiple metrics based on pollution tolerance traits would bias the index towards a single characteristic	25,448	
<b>Conceptually redundant regionally calibrated tolerance metrics (pt_RefIndicators and pt_Sens_810):</b> Multiple regionally calibrated tolerance metrics would bias the index and might be overfit to this data set	18,815	
<b>DE &lt; 99:</b> A lower DE indicates poor sensitivity compared to other models – there were several models with a DE of 100%	11,897	
<b>Index alternatives with pi_Achnan_Navic:</b> This metric had a lower DE and was included in fewer sensitive index alternatives compared to the other taxa group metrics	10,503	
<b>Index alternatives with pt_RefIndicators:</b> The other regionally calibrated metric was based on analysis of a broader data set and specific stressors	6,138	
<b>Index alternatives with nt_SALINITY_12:</b> This metric had a lower DE compared to pt_SALINITY_34	4,402	
<b>Index alternatives with pt_Bahls_1:</b> This trait was calibrated for Montana conditions that might be unlike Indiana conditions	2,575	
<b>Index alternatives with nt_Achnan_Navic:</b> The pt_Achnan_Navic metric was favored because it was also used in the HiN index and consistency among site classes shows robust metric response	1,444	
<b>Conceptually redundant N metrics (pt_HIGH_N and other N metrics):</b> Multiple N metrics would bias the index towards sensitivity to nitrogen	1,249	
<b>Index alternatives with pt_TROPHIC_456 metric:</b> this metric had a relatively low DE	565	
<b>Z-score &lt; 3:</b> The discrimination indicated by the Z-score differentiates among the several models with a DE of 100%	77	
<b>Index alternatives with wa_Poll_Tol:</b> This metric is somewhat complex to calculate	37	

Table 12. Selection rationale for index metrics in the LoN site class.

<b>Selected Metrics with Preferred Characteristics</b>
The nt_LOW_N and nt_LOW_P metrics were considered as index components because they have a different calculation format than many others - number of taxa instead of percent of taxa. This diversity of metric types might allow for broader sensitivity of the index. nt_LOW_P had a higher DE than nt_LOW_N. Other nutrient metrics were also viable candidates.
The pt_SALINITY_34 metric was considered as an index component because it had a high DE (90.9%) and an adequate range of values
The pt_O_345 metric was considered because it might indicate low oxygen conditions
The pt_Achnan_Navic metric was considered because it was a common metric in many of the high performance indices
The pt_Sens_810 metric was considered because it was regionally calibrated to specific stressors using all samples in the analysis. The BC and PT metrics were also based on tolerance to stressors, but they were not calibrated in Indiana

Table 13. Component metrics and scoring formulae for the HiN and LoN diatom indices.

HiN ModelID: 5_3402	Metric DE	Metric Scoring Formula	Metric Category
nt_LOW_N	43.5 (DEC)	100*(metric - 1) / 7	Nutrients
pt_PT_12	50 (INC)	100*(0.20 - metric) / 0.13	Pollution Tolerance
pi_Tol_13	52.2 (INC)	100*(49.6 - metric) / 48	Tolerance Analysis
pt_Achnan_Navic	45.7 DEC	100*(metric - 0.14) / 0.18	Taxa Groups
pt_BC_12_adj	54.3 (DEC)	100*(metric + 0.09) / 0.16  pt_BC_12_adj = If (BFIcat < 30, then pt_BC_12 - 0.105, else pt_BC_12 - 0.151)	Biological Conditions
LoN ModelID: 5_10781	Metric DE	Metric Scoring Formula	Metric Category
pt_Achnan_Navic	81.8 (DEC)	100*(metric - 0.14) / 0.18	Taxa Groups
nt_LOW_P	81.8 (DEC)	100*(metric - 1) / 6	Nutrients
pt_SALINITY_34	90.9 (INC)	100*(0.25-metric) / 0.18	Salts
pt_O_345	72.7 (INC)	100*(0.47-metric) / 0.23	Dissolved Oxygen
pt_Sens_810	81.8 (DEC)	100*(metric - 0.10) / 0.30	Pollution Tolerance

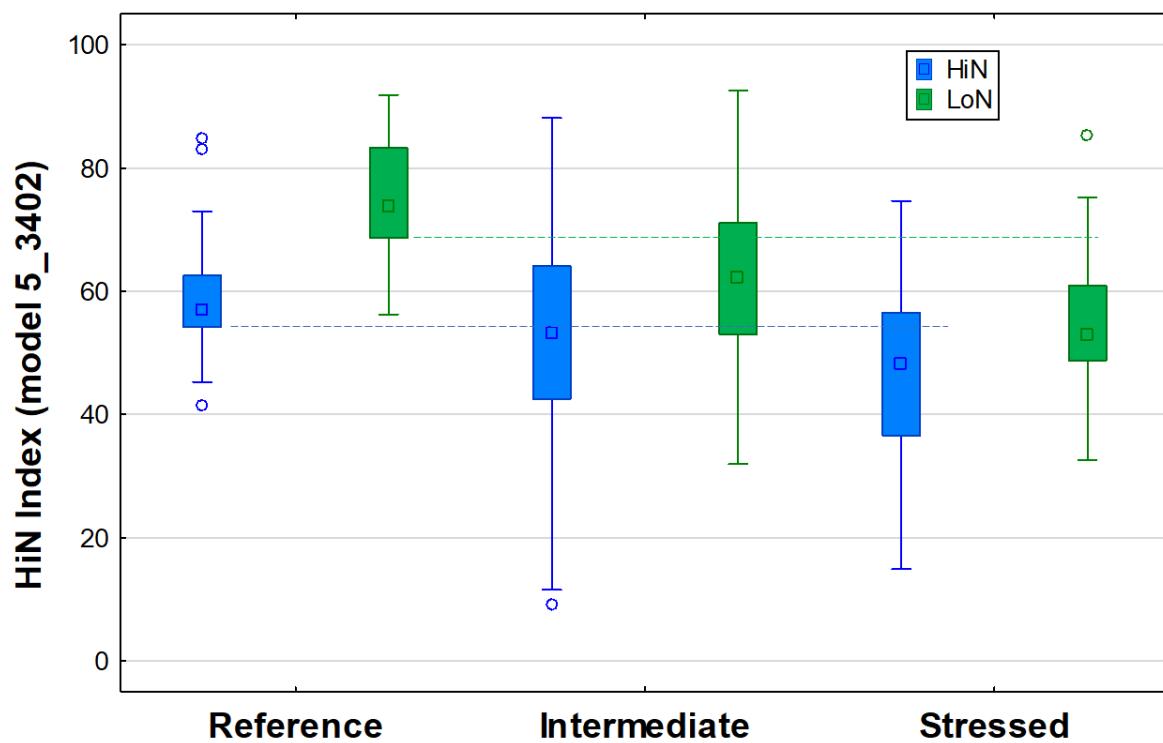


Figure 14. HiN calibration index value distributions by disturbance category and site class. This index was calibrated to the HiN site class (blue). LoN values are shown for comparison and interpretation in case of a mis-identified site class. The dotted horizontal lines are the 25<sup>th</sup> percentiles of reference sites in each site class, which was used in calculating the discrimination efficiency.

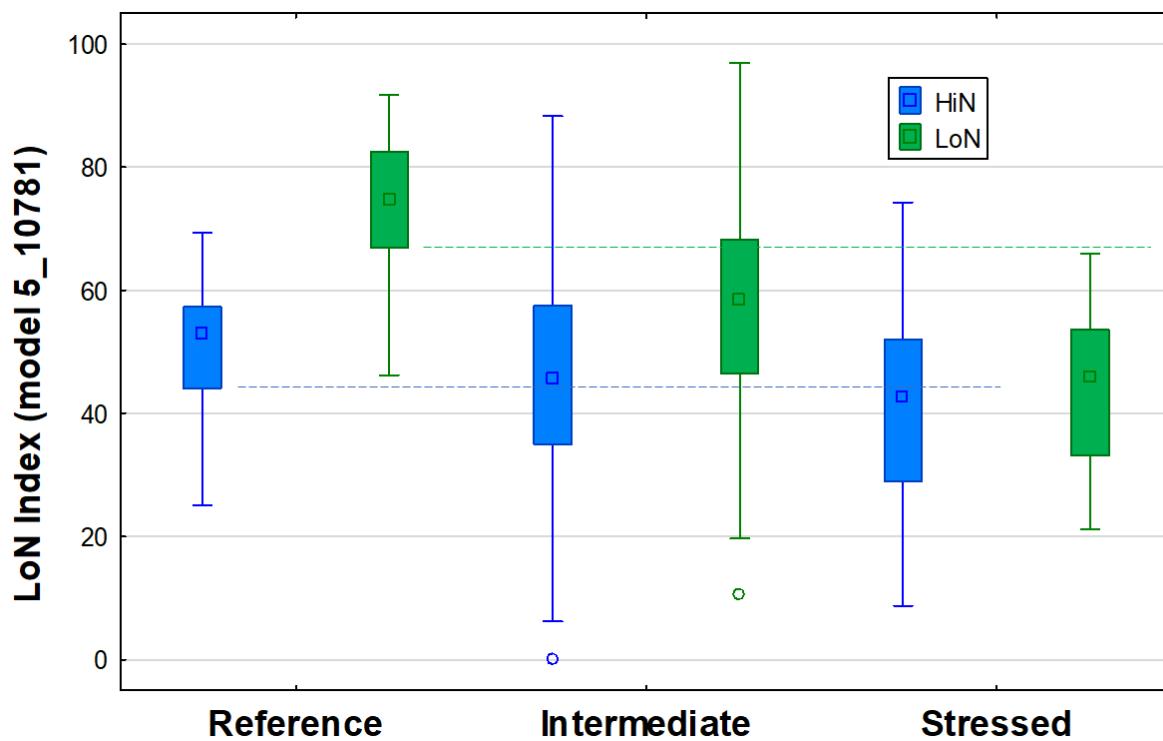


Figure 15. LoN calibration index value distributions by disturbance category and site class. This index was calibrated to the LoN site class (green). HiN values are shown for comparison and interpretation in case of a mis-identified site class. The dotted horizontal lines are the 25<sup>th</sup> percentiles of reference sites in each site class, which was used in calculating the discrimination efficiency.

#### 4.6 Validation

Index scores were calculated for the validation samples that were reserved and not used in calibration. Their performance statistics were compared to calibration results. Validation data were expected to perform nearly as well as calibration data, with DEs not more than 10% less than the calibration data. This was evaluated by comparing reference and stressed validation data to the reference calibration 25<sup>th</sup> percentiles. Validation samples were reserved based in the HiN and LoN site classes. In one validation category, the number of validation samples was < 10, which can result in unreliable validation percentages.

In the HiN site class, 68.2% of 22 stressed validation samples were below the calibration reference 25<sup>th</sup> percentile (Table 14). Of the 10 HiN reference validation samples, 6 were greater than the 25<sup>th</sup> percentile of reference calibration samples. This represents a good validation of the HiN index in identifying stressed sites and only a fair validation of the index in identifying reference sites, because the expectation was for at least 65% of validation reference sites to have index values greater than the 25<sup>th</sup> percentile of calibration reference sites.

In the LoN site class, validation results were similar to results in the HiN site class. All stressed validation index scores were below the 25<sup>th</sup> percentile of calibration reference values (100% correct designation). However, only 5 of the 10 HiN reference validation samples were greater than the 25<sup>th</sup> percentile of reference calibration samples (50% correct designation).

*Table 14. Index performance statistics (N, DE, and percent of reference validation samples greater than the 25<sup>th</sup> percentile of reference calibration samples) for validation samples in comparison to calibration DE.*

Site Class	Calibration DE	Validation Strs N	Validation DE	Validation Ref N	% Validation Ref > 25 <sup>th</sup>
HiN	71.7%	22	68.2%	10	60%
LoN	100%	5	100%	10	50%

As an additional validation, Spearman rank correlations were calculated to evaluate the responsiveness of the indices to stressors. The indices had significant and relatively high correlation coefficients with conductivity, chloride, total phosphorus, percent urban and percent impervious cover (Table 15). In the HiN site class, correlations were also relatively strong with turbidity, TKN, and the riffle/run habitat score. In the LoN class, relatively strong correlations were also with benthic chlorophyll a, channel and bank habitat scores, and percent agriculture. Indices were not correlated with drainage area, but they were correlated with air temperature. Scatter plots illustrating these relationships are in Appendix I).

*Table 15. Spearman rank correlation coefficients relating the diatom indices to stressors and natural variables within site classes. Values marked with an asterisk (\*) were significant at  $p < 0.05$ . Values marked with two asterisks (\*\*) were significant at  $p < 0.01$ .*

	HiN Index (5_3402)	LoN Index (5_10781)
DisOxy	-0.02	-0.04
pH	-0.08	-0.10
Chloride	<b>-0.34**</b>	<b>-0.62**</b>
Conductivity	<b>-0.30**</b>	<b>-0.48**</b>
Turbidity	<b>-0.25**</b>	-0.14
N_TKN	<b>-0.39**</b>	-0.24
P_total	<b>-0.48**</b>	<b>-0.55**</b>
CHLa_benthic	0.11	<b>-0.29**</b>
CHLa_water	-0.15*	0.06
Act_Activity_QHEltotal_Score	0.09	0.14
Substr_Score_Val_Txt	0.06	0.13
Instr_Cover_Score_Val_Txt	0.12*	-0.05
Chan_Score_Val_Txt	0.04	<b>0.28**</b>
Bank_Score_Val_Txt	0.11	<b>0.30**</b>
Pool_Score_Val_Txt	0.02	-0.07
Riffle_Rif_Run_Score_Val_Txt	<b>0.15**</b>	0.18
Gradient_Score_Val_Text	-0.03	0.09
W_pcUrban	<b>-0.19**</b>	<b>-0.50**</b>
W_pcAg	0.07	<b>-0.39**</b>
W_pcImp	<b>-0.23**</b>	<b>-0.47**</b>
Drainage	-0.13*	-0.04
TmeanWs	<b>-0.21**</b>	<b>0.33**</b>

#### 4.7 Index Precision

Index precision was compared among replicate samples collected at the same sites on the same days. The replicates included 69 replicate sets, including 144 total replicates. Analyses were separated by site class (HiN and LoN). We calculated the variability of index scores for the replicated samples as an estimate of index precision related to sampling error and variability (Table 16). Index RMSE values (approximating within site standard deviation) were 9.6 index points in both site classes. The RMSEs were used to calculate the CI90s of 15.8 index points in both site classes.

The 90% CI of 15.8 index points indicates that this amount of difference between index scores would represent a meaningful change in index values when measured in the same year, with 90% confidence. On a 100-point index scale, this precision suggests that 3 – 5 condition levels could be detected in the index when accounting for sampling error. The confidence interval does not apply to any comparisons to condition thresholds, only to comparisons among individual samples.

*Table 16. Index precision statistics for same-day same-site replicates. Mean squared error (MSE) and root mean squared error (RMSE) values are derived from one-way ANOVA. RMSE is an approximation of standard deviation. Coefficient of variability (CV) was calculated as the RMSE standardized by the Index Mean. The 90% confidence interval (CI90) was calculated as 1.645 \* RMSE.*

Site Class	N Stations	N Replicates	Index MSE	Index Mean	RMSE	CV	CI
HiN	52	108	92.7	48.3	9.6	20.0	15.8
LoN	17	36	92.3	57.7	9.6	16.6	15.8

#### 4.8 Condition Thresholds

The distribution of index values in the reference condition can be used in setting thresholds of impairment: How much deviation from the reference condition is the State willing to tolerate under the CWA? How many species is the State willing to lose from a stream assemblage before triggering a management action? Application of thresholds based on the reference distribution of index values must account for the expectations of reference conditions relative to the reference criteria and whether the reference sites represent a minimally disturbed condition or only the least disturbed, or best observed, condition.

The indices were shown to distinguish between reference, intermediate, and stressed sites. Reference index distribution percentiles and associations with Type I and Type II error are shown in Table 17. These statistics were derived from the combined calibration and validation data sets.

Impairment thresholds based on the 10<sup>th</sup> through 25<sup>th</sup> percentiles of the reference data sets correspond to 43.2 and 53.1 index points in the HiN site class and 46.7 and 61.9 in the LoN site class. In the HiN site class, The Type I and Type II errors are balanced at the 30<sup>th</sup> reference percentile; an index score of 54.3. In the LoN site class, the balance of errors occurs at the 20<sup>th</sup> reference percentile, or 56.9 index points. The difference in index value statistics between the two site classes can be attributed to different index formulations. The difference in index percentiles at the point of balanced error can be attributed to a more sensitive index in the LoN site class.

The standard deviation of the reference HiN index distribution was 10.6 index points. The mean reference index score (58.2) minus 1 standard deviation is 47.6 index points. The standard deviation of the reference LoN index distribution was 13.4 index points. The mean reference index score (70.2) minus 1 standard deviation is 56.8 index points.

*Table 17. Diatom index distribution statistics and error associated with reference percentiles.*

Statistic	All sites index score	Reference index score	Type I error (%)	DE (%)	Type II error (%)	All Below (%)
<b>HiN</b>						
Valid N	323	37		68		323
Minimum	8.9	38.3	0	35.3	64.7	19.8

5 <sup>th</sup> Percentile	24.3	40.7	2.7	39.7	60.3	23.8
10 <sup>th</sup> Percentile	32.7	43.2	8.1	45.6	54.4	28.8
15 <sup>th</sup> Percentile	35.5	50.3	13.5	57.4	42.6	44.0
20 <sup>th</sup> Percentile	38.3	51.7	18.9	63.2	36.8	47.7
Lower Quartile	41.4	53.1	24.3	67.6	32.4	51.1
30 <sup>th</sup> Percentile	43.5	54.3	29.7	70.6	29.4	55.7
Mean	51.9	58.2	62.2	76.5	23.5	66.9
Median	53.0	56.8	48.6	73.5	26.5	62.8
Upper Quartile	61.8	62.6	73.0	85.3	14.7	75.5
Maximum	88.1	84.6	97.3	98.5	1.5	98.8
<b>LoN</b>						
Valid N	112	24		16		112
Minimum	0	45.2	0	50.0	50	23.2
5 <sup>th</sup> Percentile	23.0	46.2	4.2	56.3	43.7	24.1
10 <sup>th</sup> Percentile	37.3	46.7	8.3	56.3	43.7	25.9
15 <sup>th</sup> Percentile	41.1	52.8	12.5	68.8	31.2	37.5
20 <sup>th</sup> Percentile	44.3	56.9	16.7	81.3	18.7	45.5
Lower Quartile	46.3	61.9	25.0	93.8	6.2	55.4
Mean	57.5	70.2	41.7	100	0	75.0
Median	58.5	72.5	50.0	100	0	79.5
Upper Quartile	70.2	78.7	75.0	100	0	90.2
Maximum	96.7	91.9	95.8	100	0	98.2

A synopsis of the possible index thresholds to describe a general distinction between acceptable and degraded biological conditions is shown in Table 18. In both site classes, the potential threshold derived from a balancing of Type I and Type II errors is corroborated by one other analytical result. In the HiN site class, the 25<sup>th</sup> percentile of reference site index values is a strong candidate for a threshold because the use of this statistic for setting condition thresholds has precedent in the literature and the errors are nearly balanced. The reference 25<sup>th</sup> percentile (53.1 index points) is the median of the thresholds derived in the analyses. The threshold derived from the balanced errors (54.3 index points) is also a potential threshold, though it is the 30<sup>th</sup> percentile of reference.

In the LoN site class, the balanced errors and the 20<sup>th</sup> percentile of reference sites (56.9 index points) coincide with the mean of reference minus one standard deviation. The mean minus one standard deviation is a descriptive threshold that allows statistical conceptualization of the core reference values and the departure from them.

The 75<sup>th</sup> percentile of all sites is a high outlier of the potential thresholds identified in both site classes. This threshold is based on an assumption that approximately 75% of the general population of sites have a degraded biological condition. This assumption is not supported by the other threshold-setting methods that have a basis in the reference condition concept. The 10<sup>th</sup> percentile of reference sites is the low end of the range of possible thresholds in both site classes.

*Table 18. Diatom index thresholds resulting from reference distributions and balancing Type I and Type II errors.*

Threshold Rationale	Corresponding HiN Index Value	Corresponding LoN Index Value
Reference 10 <sup>th</sup> percentile	43.2	46.7
Reference 25 <sup>th</sup> percentile	53.1	61.9
Reference mean minus one standard deviation	47.6	56.8
Balanced Type I and Type II errors	54.3	56.9
75 <sup>th</sup> percentile of all sites	61.8	70.2

Within the generally acceptable index ranges (above the suggested general threshold), Exceptional and Satisfactory conditions could be distinguished based on a bisection of the unimpacted index range, half-way between the general threshold and the maximum of the index scale. For the preliminary index thresholds of 53.1 in the HiN and 56.9 in the LoN, these secondary thresholds would be at 76.5 and 78.5, respectively. Similarly, the impacted range of the index scale could be bisected into Moderately Degraded and Severely Degraded conditions at secondary thresholds of 26.6 and 28.5 for the HiN and LoN indices, respectively.

Based on proportional odds logistic regression, a threshold between Exceptional and Satisfactory conditions was identified at 82 index points for the LoN diatom index. The threshold between Moderately Degraded and Severely Degraded conditions was identified at 26 LoN index points (Figure 16). The crossover for stressed and reference membership probabilities is at 54 LoN index points. We have less confidence in this as a potential general threshold because of the influence of the mid-stress distribution. Because of the broad distribution of index values in the HiN intermediate and stressed disturbance categories, the proportional odds logistic regression results were unintelligible; the cross-over values with the intermediate distribution were outside of the 100-point scale.

These indications from reference distributions, standard deviations, and balanced errors suggest that a general condition threshold dividing satisfactory conditions from moderately degraded conditions should be in the range of 53 - 57 index points, depending on site class (Table 19 and Figure 17). Secondary thresholds are 76 – 82 index points to describe Exceptional and Satisfactory conditions within the acceptable index range and 26 - 27 to describe Moderately Degraded and Severely Degraded conditions.

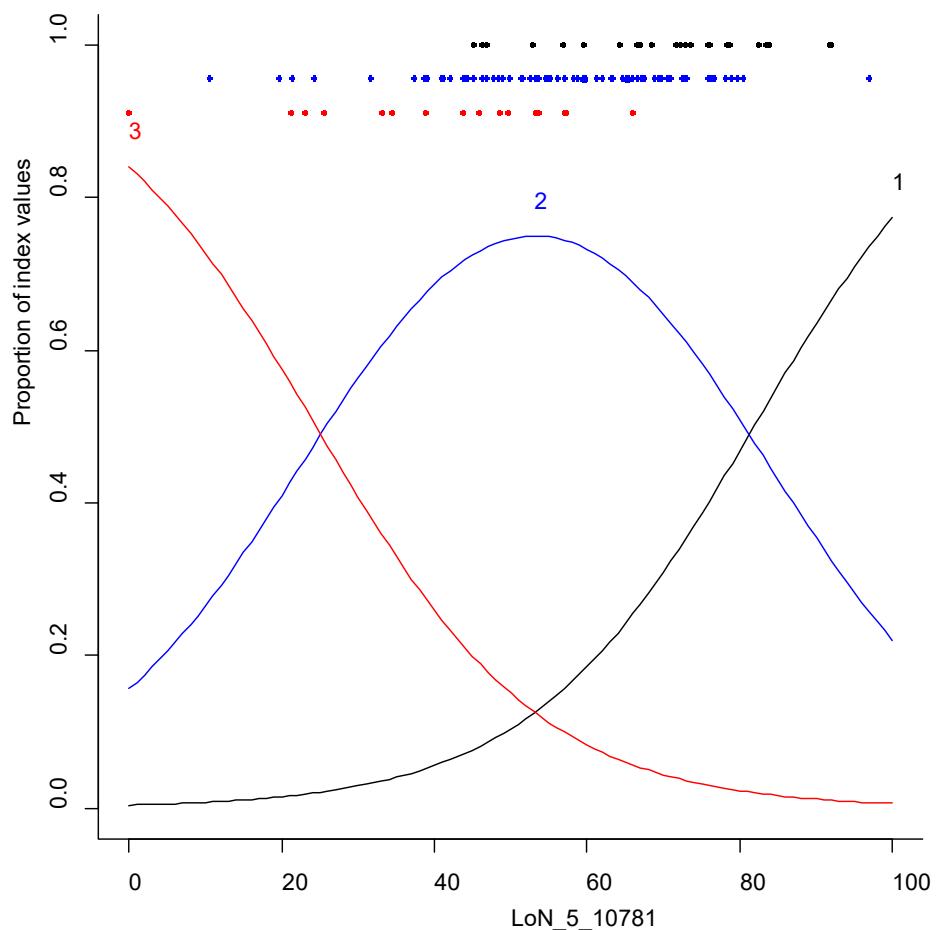


Figure 16. Proportional odds logistic regression graph for the LoN index, showing probability of membership in the reference (1), moderately stressed (2), and highly stressed (3) disturbance categories. Actual data points for the revised index are plotted at the top of the graph.

Table 19. Threshold ranges and recommended index values for indication of biological conditions in Indiana streams.

		General unimpacted conditions		General impacted conditions	
		Exceptional Conditions	Satisfactory Condition	Moderately Degraded Condition	Severely Degraded Condition
HiN	Index threshold range		76.5	43.2 – 61.8	26.6
HiN	Suggested index threshold		76.5	53.1	26.6
LoN	Index threshold range		78.5 - 82	49.7 – 70.2	26 - 28.5
LoN	Suggested index threshold		82	56.9	26

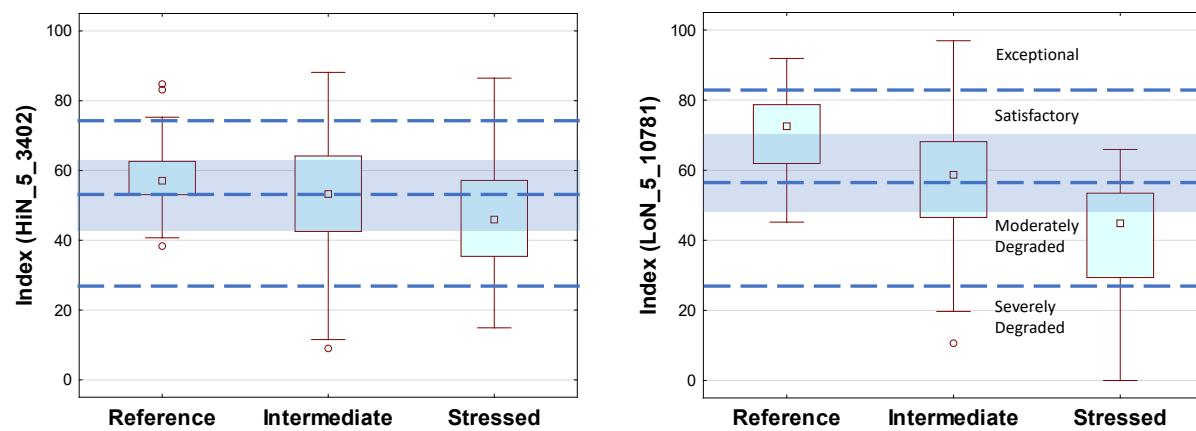


Figure 17. Indiana diatom index value distributions in disturbance categories for the HiN (left) and LoN (right) site classes, showing suggested condition thresholds and the range of possible values for the general condition threshold.

The map in Figure 18 shows the spatial distribution of sites in the four biological condition categories based on the recommended thresholds. These thresholds are preliminary and are subject to further review, refinement, and approval by IDEM before they are applicable in biological assessment programs.

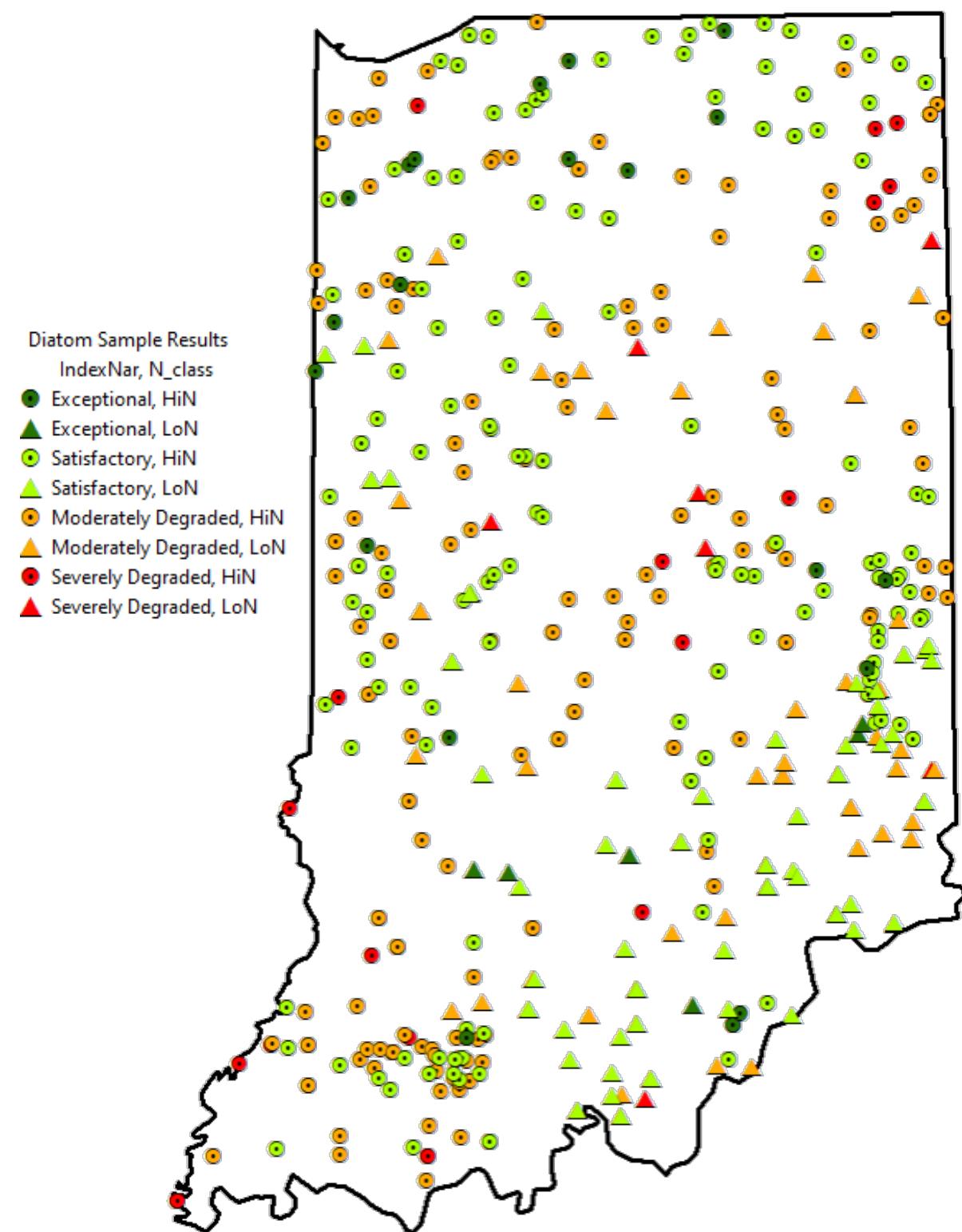


Figure 18. Indiana diatom sites color-coded by biological condition category based on the recommended IBI thresholds in Table 19, also showing site classes.

## 5. Conclusions, Discussion, and Recommendations

### 5.1 Conclusions

The multimetric diatom index developed for Indiana streams is calibrated to the natural background settings and is efficient at indicating diatom conditions relative to stressors. The main influence on the diatom assemblage was identified as geologic sources of nitrogen. This was used to define site classes HiN and LoN. In general, the metrics that were responsive to the stressor gradient had optimal values when background nitrogen was low. When background nitrogen was high, the diatom metrics were suboptimal. This natural factor should be considered if using the diatom indices in relation to nutrients either as stressors or as natural conditions.

The indices (in both low and high background nitrogen) were responsive to the general disturbance gradient that was identified largely from land use cover and activities. The index as calibrated to this gradient was successfully validated with independent data. The validation was especially successful at correctly identifying stressed sites. The error associated with identifying least-disturbed reference sites was higher in the validation data than it was in the calibration data. When compared to some of the stressors that were not used in defining the disturbance categories, the indices showed responses to salts (conductivity and chloride) and to nutrients (TKN and total phosphorus). Depending on the site class, the indices were also responsive to turbidity and habitat features. The index was also responsive to long term air temperature; negatively correlated in the HiN site class and positively correlated in the LoN.

The index was more responsive to the stressor gradient in low nitrogen background settings (the LoN site class) than it was with higher background nitrogen (HiN). This might be attributed to the natural effect of nutrients, which are typically understood as stressors in the context of algal assemblages. With high nitrogen even in reference sites, the metrics showed sub-optimal values over the entire stressor gradient and less of a difference between the least disturbed and most disturbed sites. Differences in index value statistics between the two site classes can also be attributed to different index formulations.

The diatom index will enhance the state of Indiana's monitoring strategy by adding another core indicator (diatom community structure) used to assess aquatic life use in:

- IDEM's Integrated Report, thus satisfying CWA 305(b) and 303(d) reporting requirements to EPA.
- Watershed characterization projects which identify critical areas and chemical/physical stressors to the biological communities.
- Identifying improvements in the biological communities following watershed restoration efforts.

## 5.2 Specific metric responses

The metrics included in the indices showed consistent responses to the stressor gradient across the site classes. They were selected based on empirical evidence of the response within the calibration data set, diversity of metric types and traits, and plausible rationale for the response mechanism.

### **Nutrient Indicators**

Nutrient concentrations are associated with nutrient based diatom metrics in both site classes of the Indiana data set as well as in other settings (Porter et al. 2008, Pillsbury et al. 2019). Diatom responses to phosphorus include productivity and growth rates and shifts in taxonomic composition from oligotrophic species to those capable of faster growth under P-enriched conditions. For example, the low phosphorus diatom metric has been shown to decrease substantially at phosphorus concentrations above 75 to 150 µg/L (Smucker et al. 2020). Periphyton responses to phosphorus enrichment precede those of other biota (e.g., emergent macrophytes) and, thus, provide a valuable early indicator of eutrophication, as seen in the Florida Everglades and other wetlands (McCormick and Scinto 1999). Because diatoms have specific tolerance and preference for nutrient conditions, nutrient inference models have been calibrated using nutrient-based taxa traits (Ponader et al. 2007, Kelly and Whitton 1995).

### ***Achnanthidium* and *Navicula***

The *Achnanthidium* and *Navicula* genera were defined in the strict sense for current taxa identifications and calculation of metrics. In other words, the most current genus designations for the taxa were used to identify these genera, even though the taxon name in the database might differ. As an example, *Navicula minima* is identified in the taxa list from IDEM, but is now considered *Eolimna minima*, not in the *Navicula* genus. These designations were derived from the USGS/DONA traits table.

*Achnanthidium* and *Navicula* were responsive to the stressor gradient in the Indiana data set and their use as indicators have precedence as indicators, though often at the species level. Low-nutrient streams can be dominated by a few small species of *Achnanthidium* (Kawcka 1993; Stevenson et al. 2008b) and increases in nutrient concentrations may increase dominance of other taxa more than the small *Achnanthidium* (Manoylov and Stevenson 2006).

While the genera were responsive as taxa groups it is evident that there is variable sensitivity among species (Ponader and Potapova 2007, Paul et al. 2020, Tang et al. 2016). *Achnanthidium* and *Navicula* are taxa-rich genera with diverse species characteristics. The most responsive *Achnanthidium* and *Navicula* metric forms were related to richness and relative richness. Although some taxa might not be sensitive, as a group, taxa richness of these groups would indicate greater complexity with more sensitive taxa.

### **Biological Condition Attributes**

The Biological Condition rating (diatoms.org) takes many factors into account (alkalinity, salinity, organic nutrients, etc.) based on a number of reports (Lange-Bertalot 1979, Van Dam et al. 1994, Bahls 1993, Porter et al. 2008) merged with professional experience following the Biological Condition Gradient (BCG) approach (Davies and Jackson 2006, Paul et al. 2020). The BC traits were established by staff at the EPA and USGS working with diatom taxa traits for the Diatoms of North America website (diatoms.org).

For the index metrics, the proportional richness of BC 1 and BC 2 taxa was responsive along the stressor gradient. BC\_1 represents the most sensitive diatoms and BC\_2 represents moderately sensitive taxa. The scale is up to BC\_5, the most tolerant taxa. In the BCG framework, the most sensitive and moderately sensitive taxa are proportionally diverse when environmental conditions are as naturally occurs. As stressors increase, the sensitive diatom taxa perish or emigrate, resulting in proportionally fewer BC 1 and BC 2 taxa (Hausmann et al. 2016, Paul et al. 2020, Charles et al. 2019).

The BC metric was adjusted to the BFI in the HiN site class and was recognized as having a unique reference distribution in the LoN site class (Figure 19). The metric adjustment for the index in the HiN site class was the residual of the metric value minus 0.15 metric units if the BFI was > 30 BFI units. If the BFI was < 30, the residual was calculated as the metric value minus 0.10 metric units.

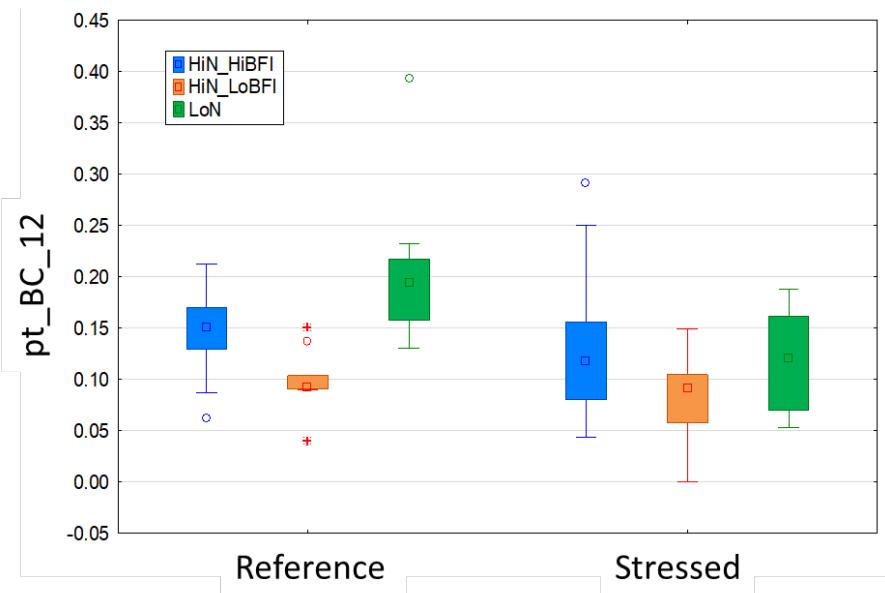


Figure 19. Distributions of the proportion of BC 1 or 2 taxa metric (pt\_BC\_12) by disturbance category, site class, and base-flow index (BFI) category.

### **Pollution Tolerance**

The pollution tolerance system of Lange-Bertalot (1979) was based on a four-year study of the Rhine-Main river system in Germany. At the time, the river was the most polluted river in Central Europe. Water was not treated until 1975 and included effluent of the paper industry. This trait includes some overlap with the saprobian system, which is largely based on biological oxygen demand. PT\_1 represents taxa that are very tolerant of extremely degraded conditions and PT\_5 represents taxa that are found in water with low organic enrichment. The metric format used in the HiN site class calculates the proportion of taxa that are tolerant and very tolerant of extremely degraded conditions.

### **Sensitivity Metrics**

The sensitivity values for common taxa were derived from the tolerance analysis that compared taxa occurrence and abundance in relation to four stressors, including the QHEI habitat score, percent watershed imperviousness, the index of watershed integrity, and measured stream conductivity (Appendix B). The analysis was specific to the Indiana data set. This has the advantage of calibrating taxa sensitivity to the stressors of concern and the taxa commonly collected. There might be disadvantages to defining sensitivity specifically within Indiana (excluding regional conditions and distributions) and to focusing on common taxa. However, the sensitivity metrics were responsive to the disturbance gradient and were included in the indices for both site classes. In the HiN, the proportion of valves that were tolerant (pi\_Tol\_13) were indicators of the disturbance gradient. In the LoN, the proportion sensitive taxa (pt\_Sens\_810) were indicators.

### **Sestonic Habit Attributes**

Metrics related to traits for general habitat, nitrogen fixation, motility, and cell size are documented in the autecology section of Diatoms of North America (diatoms.org). Sestonic species are primarily suspended in the plankton and include species that are found in lentic habitats of lakes, reservoirs, or slow moving rivers. Benthic species are primarily bottom dwellers, or species that are attached to surfaces.

In this analysis, taxa with sestonic habits were more common in samples from stressed sites than they were in samples from reference sites. Although it was responsive in smaller sites (<500 sq mi), it was not used because it was consistently high in larger sites, for which there were no comparable reference sites. In addition, this metric does not have obvious precedent in the literature as an indicator of general disturbance or as a component of a multi-metric index.

### **Chloride Indicator**

The chloride tolerance metric had a very limited range of values, being especially low in reference samples. The tolerant taxa included 16 species, 10 of which were in the genera *Nitzchia* and *Navicula* (Potapova and Charles 2003). These chloride tolerant species were almost entirely absent in reference sites with low background nitrogen. The metric had greater percentages in stressed sites in the LoN site class, so it was more responsive in that class. Diatom community changes have been noted in association with increases in chlorides due to urbanization and road salting (Porter-Goff et al. 2013, Newall and Walsh 2005). Salt tolerant

diatoms have been found to increase in catchments influenced by land use, including agriculture and urbanization (Leland & Porter, 2000, Rott et al., 1998; Munn et al., 2002, Sonneman et al. 2001, Potapova and Charles 2003). The metric was not selected for the final index because of the low range of values.

### 5.3 Index response to nutrients

The diatom index will provide a more accurate assessment of ecological effects, thus improving IDEM's diagnostic ability to identify causes of degradation in water quality. Diatoms are commonly associated with nutrient availability (Charles et al. 2019, Haussman et al. 2016, Justus 2010, Ponader et al. 2007, Porter et al. 2008, Stevenson et al. 2008b). The IDEM diatom IBI might become useful to evaluate direct biological responses to excessive nutrients. The relationship of the diatom IBI and nutrients was explored to begin understanding whether this relationship might be strong enough to be used in establishing nutrient criteria for streams throughout Indiana. The proof-of-concept analysis is preliminary and should not yet be interpreted as recommendations for nutrient thresholds. A complete and varied analysis might be warranted to confirm and refine these preliminary findings.

The analysis included a simple correlation analysis followed by a change-point analysis (CPA, Paul and McDonald 2005). The data included all samples (regardless of reference status and replication) in the LoN (N=126) and HiN (N=370) site classes. Spearman rank correlation *rho* values were calculated between the diatom index and predictor variables related to both stressors and natural variables, including nutrients (TP, TN, NO<sub>3</sub>NO<sub>2</sub>, and TKN). The CPA identifies the point on the x-axis (nutrients) at which response variables (index values) are the most disparate (homogenous within each side of the point and different on either side of the point). The change-point (CP) was evaluated using precision of the confidence interval (derived through bootstrap iterations) and confirmation of the LOESS slope with the 95<sup>th</sup> quantile slope. A draft index (Carlisle et al. [in review]) developed from National Water Quality Assessment (NAWQA) and National River and Stream Assessment (NRSA) data was included in the analysis in addition to the index developed for IDEM.

Results are presented by site class, starting with the LoN. Based on the Spearman *rho* correlation coefficients, the LoN diatom index is sensitive to stressors, especially chloride, conductivity, solids, TP, some metals, and land uses (especially urban development) (Table 20). The LoN index is not very sensitive to nitrogen. The index is also related to natural variables, with somewhat higher index values in warmer, wetter watersheds. Compared to the IDEM-calibrated index, the NAWQA/NRSA MMI is more sensitive to nitrogen, site characteristics, climate factors, and agriculture. Plots of the LoN diatom index against nutrients show that the relationship with total phosphorus (TP) is stronger than with total nitrogen (TN) (Figure 20).

*Table 20. Spearman rank correlation coefficients (*rho*) to show relationships between the IDEM LoN diatom index (LoN\_5\_10781), the NAWQA/NRSA index (NRSA\_MMII), and selected site and water quality variables (see variable code descriptions in Appendix F). Coefficients are shown in bold type to emphasize the stronger relationships. Variables with correlation coefficients  $|\rho| < 0.30$  for both indices are not shown.*

Variable	LoN_5_10781	MMI_NRSA	Variable	LoN_5_10781	MMI_NRSA
MMI_NRSA	<b>0.56</b>	<b>1.00</b>	Solids_TDS	-0.43	-0.36
LoN_5_10781 (Index)	<b>1.00</b>	<b>0.56</b>	Solids_tot	-0.49	-0.44
WsAreaSqKm	-0.03	-0.02	N_NO3NO2	-0.11	-0.32
Elev_m	-0.26	<b>-0.45</b>	P_total	<b>-0.55</b>	-0.45
SLOPE	0.09	0.19	Cadmium_diss	0.04	<b>0.31</b>
WetIndexCat	<b>-0.41</b>	<b>-0.57</b>	Cadmium_tot	0.10	<b>0.39</b>
WetIndexWs	<b>-0.39</b>	<b>-0.53</b>	Chrom_diss	0.05	<b>0.30</b>
BFIICat	-0.16	<b>-0.36</b>	Chrom_tot	0.13	<b>0.43</b>
BFIWs	-0.15	<b>-0.38</b>	COD	<b>-0.37</b>	-0.22
MgOCat	-0.20	<b>-0.43</b>	Copper_diss	-0.26	<b>-0.35</b>
MgOWs	<b>-0.30</b>	<b>-0.48</b>	Copper_tot	<b>-0.36</b>	-0.34
NCat	-0.20	<b>-0.41</b>	Lead_diss	0.04	<b>0.33</b>
NWs	-0.23	<b>-0.44</b>	Lead_tot	0.12	<b>0.38</b>
PrecipCat	<b>0.38</b>	<b>0.55</b>	Magnesium	-0.23	<b>-0.37</b>
TmaxCat	<b>0.32</b>	<b>0.62</b>	Nickel_diss	<b>-0.42</b>	-0.42
TmeanCat	<b>0.31</b>	<b>0.61</b>	Nickel_tot	<b>-0.31</b>	-0.20
TminCat	<b>0.34</b>	<b>0.59</b>	Selenium_tot	0.10	<b>0.39</b>
PrecipWs	<b>0.39</b>	<b>0.55</b>	Silver_diss	-0.04	<b>-0.31</b>
TmaxWs	<b>0.32</b>	<b>0.62</b>	Silver_tot	-0.07	<b>-0.33</b>
TmeanWs	<b>0.30</b>	<b>0.60</b>	Zinc_diss	<b>-0.35</b>	-0.23
TminWs	<b>0.32</b>	<b>0.60</b>	Zinc_tot	<b>-0.33</b>	0.01
Alkalinity	-0.19	<b>-0.33</b>	W_pcUrban	<b>-0.50</b>	-0.29
Chloride	<b>-0.62</b>	<b>-0.34</b>	W_pcAg	<b>-0.37</b>	<b>-0.42</b>
Conductivity	<b>-0.48</b>	<b>-0.40</b>	W_pcImp	<b>-0.47</b>	-0.24

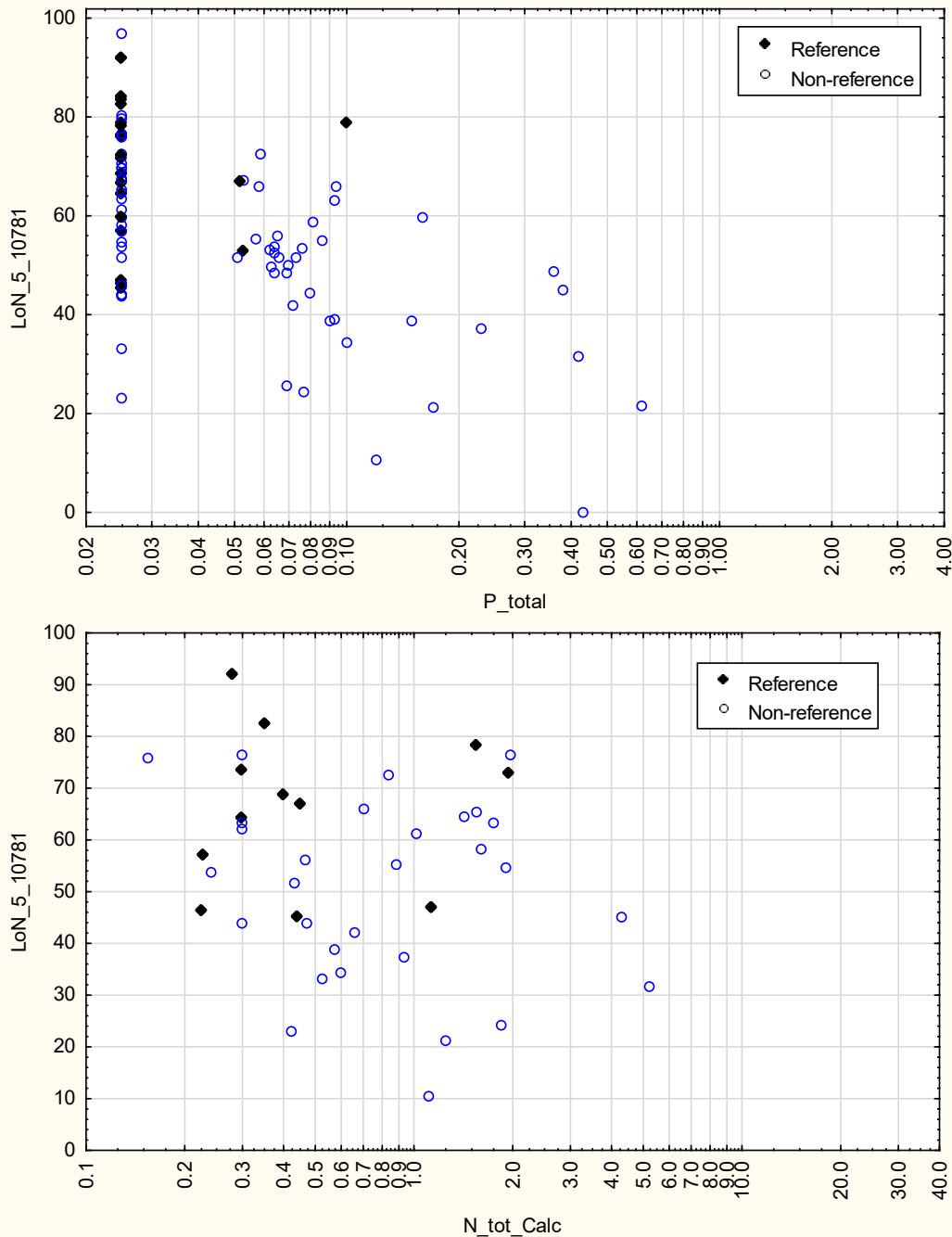
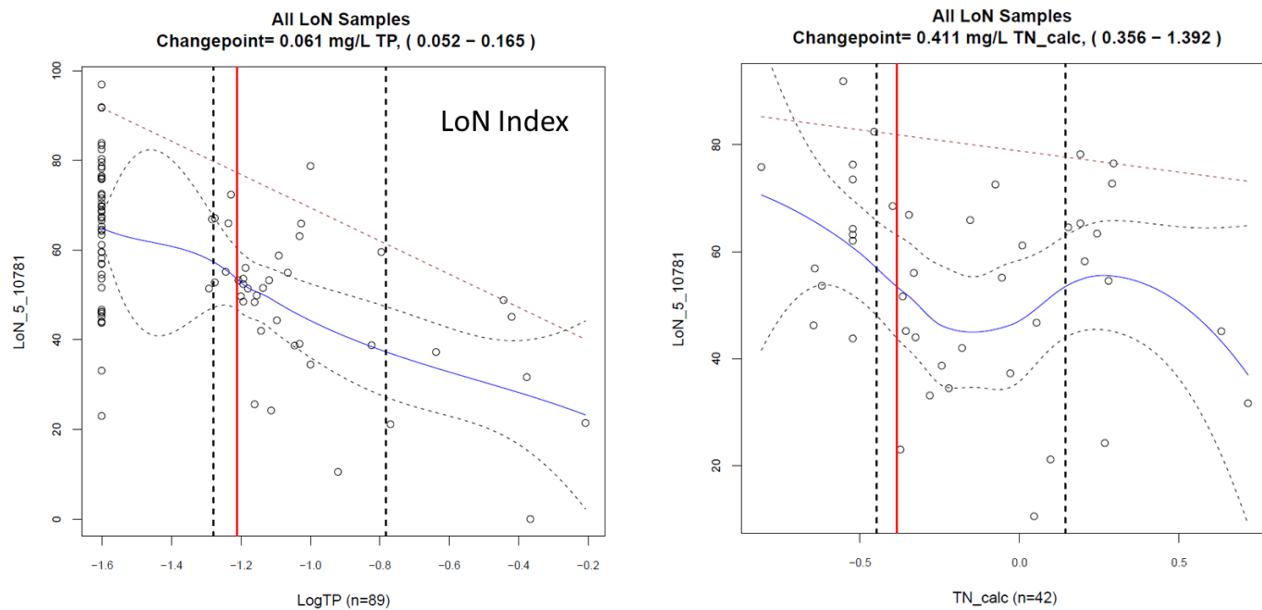


Figure 20. LoN diatom index values in comparison to TP (top) and TN (bottom), with reference sites marked.

The CPA indicates that there is an important shift to lower LoN index values when TP exceeds 0.061 mg/L (Figure 21). The LOWESS regression line at the CP is negative and is confirmed with a similar slope of the 95<sup>th</sup> quantile regression line. The CP for TP derived from the NAWQA/NRSA index was similar, at 0.074 mg/L. A log-linear regression interpolation of the index value from TP suggests that the TP CP is associated with a LoN index value of 53.3 mg/L ( $\text{LoN\_5\_10781} = 16.361 - 30.372 * \log(0.061)$ ). This interpolated index value compares to the

index threshold of 56.9 index points, as suggested through multiple analysis in Section 4.8. Through back-calculation with the same regression equation, the 56.9 index value is associated with 0.046 mg/L TP.



*Figure 21. Change-point analysis (CPA) plots to illustrate the relationships between the LoN diatom index and TP (left) and TN (right). Vertical lines and the change-points (CP) and 90% confidence intervals. Blue curves are the LOWESS regression line with dashed confidence envelopes. The dashed slope is the 95<sup>th</sup> quantile regression line.*

Therefore, when LoN diatom index values are below 56.9, biological impairment was suggested based on the recommended diatom index threshold. When LoN index values are below 53.3, biological impairment is commonly associated with high TP ( $> 0.061 \text{ mg/L TP}$ ). When TP is greater than 0.046 mg/L, biological impairment is suggested (on average), but might not be due to excess nutrients until TP is greater than 0.061 mg/L.

The same analysis was used to relate the LoN diatom index to TN values, though the correlation coefficient suggested a relatively weak relationship ( $\rho > -0.30$ ). In addition, the LOWESS regression slope varied over the nutrient gradient and the NAWQA/NRSA index was even less convincing (contradictory 95<sup>th</sup> quantile value). Nevertheless, the CP of the two indices were fairly close, ranging from 0.411 to 0.431 mg/L TN. The regression analysis showed agreement between the index threshold (56.9 index units) derived in Section 4.8 and as interpolated ( $57.3 = 52.794 - 11.731 * \log(0.411)$ ). Through back-calculation with the same regression equation, the 57.3 index value is associated with 0.45 mg/L TN.

When LoN index values are below 56.9, biological impairment was suggested based on the recommended diatom index threshold. When LoN index values are below 57.3, biological impairment is commonly associated with high TN ( $> 0.41 \text{ mg/L TN}$ ). When TN is greater than 0.45 mg/L, biological impairment is suggested (on average), and might be due to high TN.

However, TN was not highly correlated with the LoN diatom index nor the NRSA MMI. Also, CPs are always identifiable regardless of ecological significance

The analysis was repeated in the HiN site class. The HiN diatom index was correlated to stressors, including TP, TKN, chloride, conductivity, solids, some metals, and imperviousness (though weakly) (Table 21). Relative to the HiN index, the NAWQA/NRSA MMI was more sensitive to imperviousness, watershed area, slope, and carbon. The HiN diatom index values were related to natural conditions, with somewhat higher values in watersheds with high groundwater input (high BFI) and low modeled summer stream temperature (MSST). Neither index was sensitive to agricultural land cover. Plots of the HiN diatom index against nutrients show that the relationship with total phosphorus (TP) is stronger than with total nitrogen (TN) (Figure 22).

*Table 21. Spearman rank correlation coefficients (*rho*) to show relationships between the IDEM LoN diatom index (HiN\_5\_3402), the NAWQA/NRSA index (NRSA\_MM), and selected site and water quality variables (see variable code descriptions in Appendix F). Coefficients are shown in bold type to emphasize the stronger relationships. Variables with correlation coefficients |*rho*| < 0.30 for both indices are not shown.*

	HiN_5 3402	MMI NRSA
MMI_NRSA	0.55	1.00
HiN_5_3402	1.00	0.55
WsAreaSqKm	-0.11	<b>-0.35</b>
Elev_m	0.19	0.05
SLOPE	0.13	<b>0.36</b>
BFICat	<b>0.35</b>	0.14
BFIWs	<b>0.31</b>	0.08
Avg_MSST	<b>-0.30</b>	<b>-0.32</b>
Chloride	<b>-0.35</b>	<b>-0.32</b>
Conductivity	<b>-0.30</b>	-0.22
N_TKN	<b>-0.39</b>	<b>-0.39</b>
P_total	<b>-0.48</b>	<b>-0.41</b>
C_tot_inorg	<b>-0.36</b>	<b>-0.47</b>
C_tot_partic	<b>-0.36</b>	<b>-0.47</b>
Chrom_diss	<b>-0.30</b>	-0.21
Copper_diss	<b>-0.31</b>	-0.28
Copper_tot	<b>-0.32</b>	-0.28
Nickel_diss	<b>-0.40</b>	<b>-0.36</b>
Nickel_tot	-0.37	<b>-0.36</b>
W_pcImp	-0.24	<b>-0.32</b>

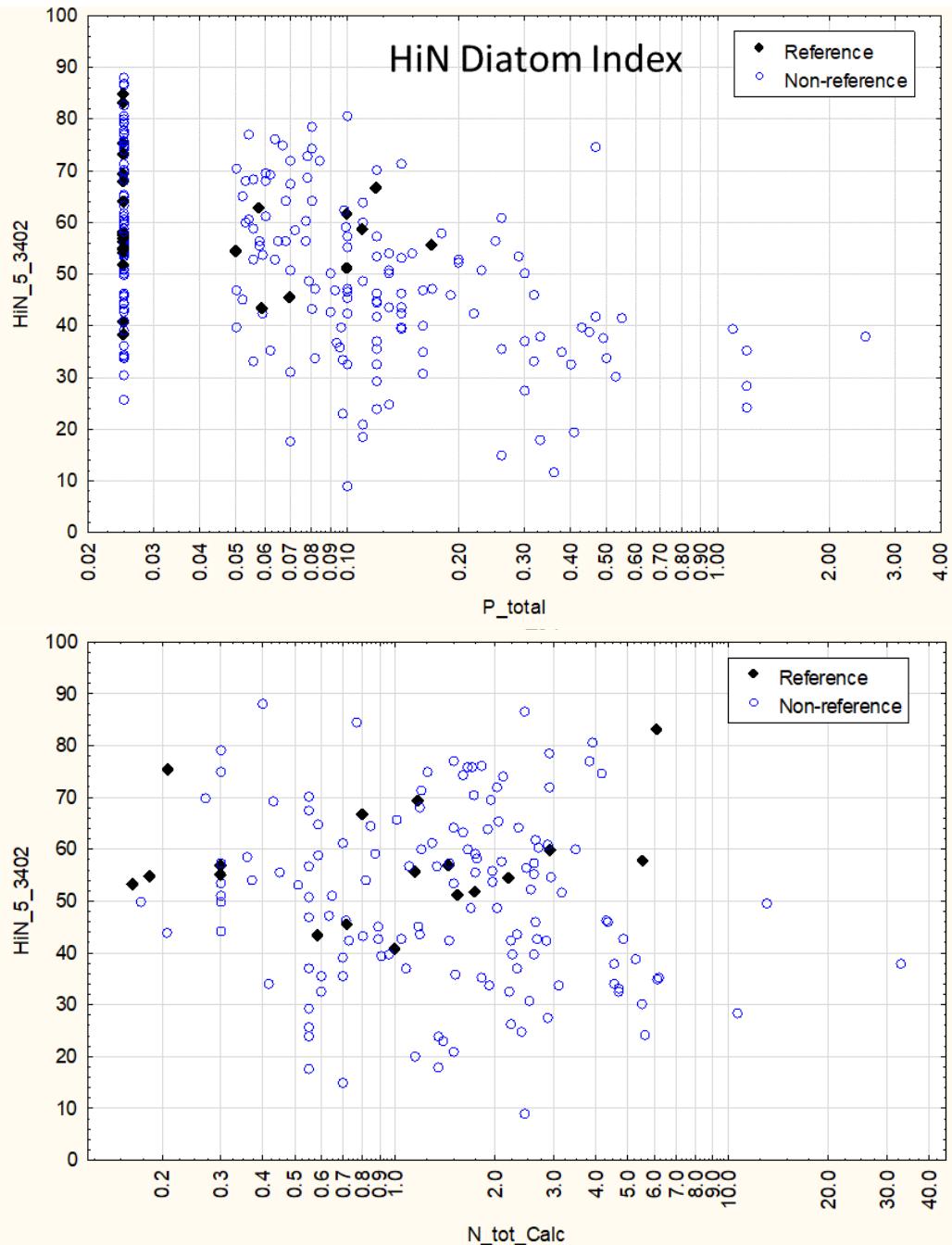


Figure 22. HiN diatom index values in comparison to TP (top) and TN (bottom), with reference sites marked.

The CPA indicates that there is an important shift to lower HiN index values when TP exceeds 0.083 mg/L (Figure 23). The LOWESS regression line at the CP is negative and is confirmed with a similar slope of the 95<sup>th</sup> quantile regression line. In addition, the confidence interval around the CP is relatively narrow. The CP for TP derived from the NAWQA/NRSA index was similar, at 0.069 mg/L. A log-linear regression interpolation of the index value from TP suggests that the TP CP is associated with a HiN index value of 50.9 mg/L (HiN\_5\_3402 = 31.6193 -

$17.823 * \log(0.083)$ ). This interpolated index value compares to the index threshold of 53.1 index points, as suggested through multiple analysis in Section 4.8. Through back-calculation with the same regression equation, the 50.9 index value is associated with 0.062 mg/L TP.

Therefore, when HiN diatom index values are below 53.1, biological impairment was suggested based on the recommended diatom index threshold. When HiN index values are below 50.9, biological impairment is commonly associated with high TP ( $> 0.083$  mg/L TP). When TP is greater than 0.062 mg/L, biological impairment is suggested (on average), but might not be due to excess nutrients until TP is greater than 0.083 mg/L.

The HiN diatom index was not strongly related to TN values ( $\rho = -0.16$ ) but there was a stronger correlation with TKN. TKN is not typically used in nutrient criteria, but it is a major component of TN, with nitrate and nitrite. The CPA analysis with the HiN diatom index and TKN showed that there was an important shift in HiN index values when TKN exceeded 0.42 mg/L. A CPA with the NAWQA/NRSA index indicated a similar CP of 0.39 mg/L TKN. The regression analysis showed agreement between the index threshold (53.1 index units) derived in Section 4.8 and as interpolated ( $52.3 = 44.329 - 20.956 * \log(0.415)$ ). Through back-calculation with the same regression equation, the 52.3 index value is associated with 0.42 mg/L TKN.

When HiN index values are below 53.1, biological impairment was suggested based on the recommended diatom index threshold derived in Section 4.8. When HiN index values are below 52.3, biological impairment is commonly associated with high TKN ( $> 0.42$  mg/L). If TKN would be used in nutrient criteria instead of TN, it could be supported with a CPA. However, the lack of a strong relationship with TN would need further exploration.

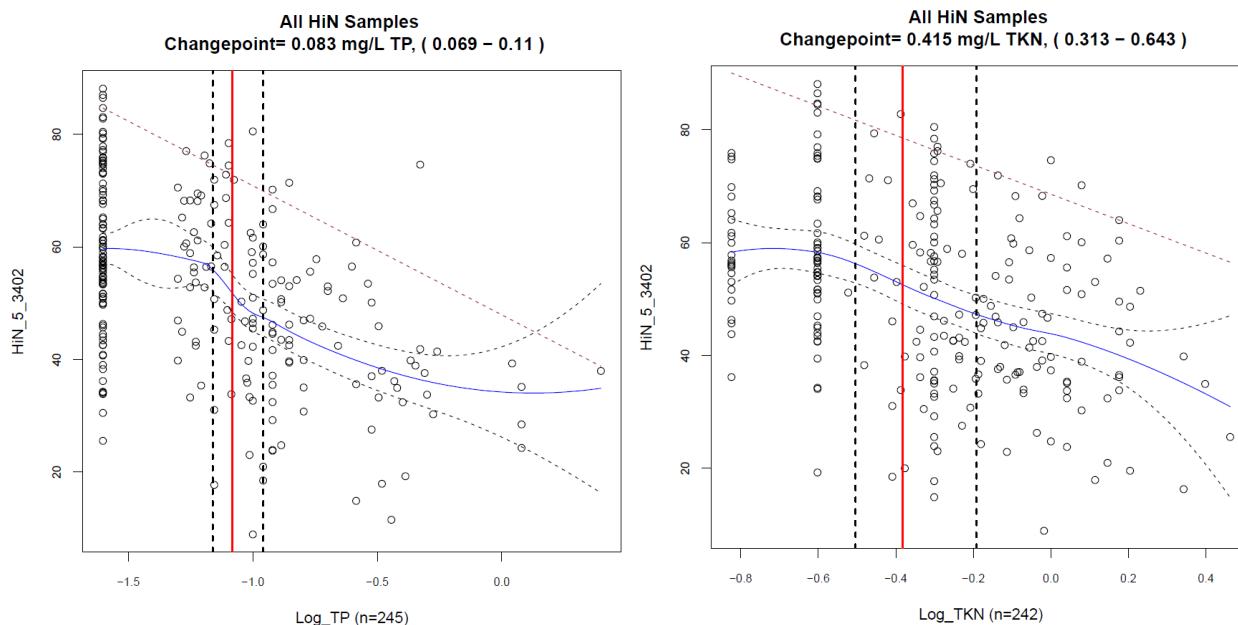


Figure 23. Change-point analysis (CPA) plots to illustrate the relationships between the LoN diatom index and TP (left) and TN (right). Vertical lines and the change-points (CP) and 90% confidence intervals. Blue curves are the LOWESS regression line with dashed confidence envelopes. The dashed slope is the 95<sup>th</sup> quantile regression line.

The nutrient CP at which the diatom indices show the greatest differences in values are closely associated with the diatom index thresholds of impairment derived through other methods in Section 4.8. Below identifiable diatom index values, TP concentrations are higher and might contribute to or signal biological impairment. Diatom index associations with TN are not as robust, or not evident, compared to associations with TP. The LoN diatom index was more responsive to stressors than the NRSA MMI, with the exception of some metals and NO<sub>3</sub>NO<sub>2</sub>. The HiN diatom index was more responsive to stressors than the NRSA MMI, with the exception of carbon and % imperviousness. These analyses show potential for using the diatom index as one line of evidence in evaluating direct biological responses to excessive nutrients.

The nutrient benchmarks identified in this preliminary analysis are comparable to nutrient benchmarks identified through diatom analyses in other states, such as New Jersey (Charles et al. 2019, Hausmann et al. 2016) and Ohio (Smucker et al. 2020). The New Jersey nutrient benchmarks were derived by comparison to Level 4 of the Biological Condition Gradient (Davies and Jackson 2006). For the Ohio study in the Little Miami River, benchmarks were derived using three nonparametric statistical analyses to characterize change-points in the diatom community relative to nutrients concentrations. Benchmarks for TN as suggested in Ohio was a broad range that included the range suggested in the LoN site in the current study (a TN benchmark was not suggested in the HiN site class) (Table 22). In New Jersey, the TN benchmark was more than double the concentration suggested for the LoN site class. For TP, the other studies suggested benchmarks that spanned the range of (Ohio) and were lower than (New Jersey) the range of benchmarks suggested in the current analysis (Table 22).

*Table 22. Comparison of preliminary nutrient benchmarks in the LoN and HiN site classes with benchmarks suggested for Ohio and New Jersey.*

Nutrient	LoN	HiN	Ohio <sup>1</sup>	New Jersey <sup>2</sup>
Nitrogen (mg TN/L)	0.41 – 0.43	(0.42 TKN <sup>3</sup> )	0.150 – 0.850	1.00
Phosphorus (mg TP/L)	0.061 – 0.074	0.069 – 0.083	0.020 - 0.150	0.050

<sup>1</sup> Smucker et al. 2020

<sup>2</sup> Charles et al. 2019

<sup>3</sup> TKN benchmarks were explored in the HiN site class instead of TN

#### 5.4 Index Application

Index application for biological assessments depends on consistent collection of samples from valid site types, appropriate calculation and scoring of metrics, combination of metric scores, comparison to approved condition thresholds, and awareness of potential error.

Sample collection and processing must follow protocols according to the Technical Standard Operating Procedures for periphyton sampling (IDEM 2018). Periphyton samples are collected during low flow, from late August through October. The stream sampling frame includes flowing wadeable perennial streams. Except for a few outliers, site watershed ranged from 0.5 – 5,000 sq mi. However, index results might be more reliable in the range of 2 – 500 sq mi, which is the range of most reference site watershed sizes.

Site classes are based on geologic nitrogen composition (NWs<sup>4</sup>). Low nitrogen sites have NWs < 0.089 %.

Taxonomic standards and traits must be as described in the electronic Attachments 1 and 2. Metric calculations should follow the conventions for taxa richness, proportion or percent of taxa, and proportion of valves (individuals). Metric scoring is according to the formulae in Table 13. Metric calculation, scoring, and combination in the indices can be accomplished using the R-Shiny app<sup>5</sup>. This index calculation tool requires input of sample taxa lists with specific formats that are detailed in an instruction page in the R-Shiny app. As the metrics were developed in the R-Shiny app, it became apparent that some traits and some metric calculations needed slight revisions. These revisions were necessary and valid. They were not substantial enough to warrant revisions in analysis (Appendix J).

Index values can be used in assessment for characterizing waterbody biological conditions and for prioritizing management actions. The thresholds for condition categories presented in Table 17 are preliminary until approved by IDEM. Thresholds could be refined in accordance with concepts of the Biological Condition Gradient (BCG; U.S. EPA 2016). Once a threshold is decided, conditions resulting from comparisons of index values to the threshold should be qualified by stating the Type I and Type II errors associated with the thresholds. For simplicity, a general rule of thumb could be applied to associate conditions with indices: Index values > 80 indicate exceptional conditions, 55 – 80 are satisfactory, 25 – 55 are moderately degraded, and < 25 are severely degraded.

When comparing one sample to another, the CI90 in Table 16 can be used to determine whether the index values are significantly different. There is some evidence that least disturbed index values are more precise than scores for non-reference sites (Smucker and Vis 2011), but this was not confirmed with the Indiana data due to low numbers of replicate sets in each category. The CI90 should not be used in comparison to the threshold because the error associated with the threshold is already described using the Type I and Type II errors.

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<sup>4</sup> NWs = Mean % of lithological nitrogen (N) content in surface or near surface geology within the watershed, derived from StreamCat (Hill et al. 2016).

<sup>5</sup> IDEM Diatom IBI Calculator R Shiny App: <https://tetratech-wtr-wne.shinyapps.io/IDEMtools/>

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*Streamside diatom sample processing in Indiana.*

# Appendix A

## IDEM GIS Analyses and Data Summaries

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September 4, 2020

### Summary Statement

This report contains GIS analysis methodologies used to analyze 409 IDEM sites for the designation of site disturbance classification. Succeeding the GIS Analysis Plan are data summaries for water chemistry, diatom, and habitat data provided to Tetra Tech, Inc. by Indiana Department of Environmental Management (IDEM). Any questions related to the GIS Analysis Plan should be directed to Ben Block ([Ben.Block@trectech.com](mailto:Ben.Block@trectech.com)).

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### IN diatom sites GIS analysis plan

The following is a detailed description of the GIS analysis done on 224 existing sites and 185 new sites in Indiana. Twenty existing sites were initially analyzed and used as a quality control measure to assure current and previous GIS methods gave similar results. The following GIS analysis will allow the designation of site disturbance status and natural classification of the 185 new IDEM sites and the updated classification of 224 previous IDEM sites (hereafter collectively referred to as Diatom sites). We begin with the quality control analysis of 20 previously analyzed sites. The second section details the exact sources, steps in ArcGIS, and data transformations for each of the disturbance variables (e.g., % Urban Land Cover or Population Density). Thirteen previously chosen disturbance variables were calculated for each site and were used for disturbance classification.

#### *Quality control analyses*

For quality control, I randomly selected 20 sites that were previously analyzed for disturbance status by Chris Wharton (Tetra Tech, Inc.) in 2015. The following steps were conducted to obtain updated watershed delineations and to compare disturbance variable values to previously reported values:

- 1.) Randomly selected 20 sites from DiatomSites sheet that were previously analyzed by Tetra Tech in 2015.
- 2.) Site names, latitude, and longitude were imported into ArcMap as a point shapefile.
- 3.) The sites point shapefile was projected into a coordinate system that matched the flowline raster file produce by StreamStats (<https://streamstats.usgs.gov/>).
- 4.) Sites need to intersect the StreamStats flowline raster prior to watershed delineation. Sites were manually arranged to intersect the nearest Streamstats flowline using the Editor tool. Note, the NHD flowline shapefile ([NHDPlus V2.1](#)) and satellite imagery were used to ensure that sites were associated with the correct stream.
- 5.) Watersheds were delineated using the [StreamStats Batch Processor](#). The site point shapefile, which was intersected with the flowline raster, was uploaded to the batch processor and results were later emailed to the recipient.
- 6.) Upon completion, watershed delineations were imported back into ArcMap.
- 7.) The exact drainage area of each watershed was calculated using the Calculate Geometry tool in the attribute table of the watershed delineation shapefile.
- 8.) The attribute table of the watershed delineation shapefile was exported to MS Excel.
- 9.) Disturbance variables were calculated, as described in the next section, using the source data collected by Chris Wharton (Tetra Tech) and compared to previously reported values (taken from Indiana\_GIS\_Data\_20150220.xlsx).
- 10.) QC analyses completed using the previous source data confirmed that the updated GIS methods provided nearly identical results to previous scores.

In a second QC check compared catchment size as calculated in the current GIS analysis to catchment size provided in the IDEM database. There were five sites for which the catchment sizes were unequal (Table 1). These sites included three that appeared to have reference characteristics when analyzed using the Tetra Tech delineations, which were ultimately incorrect or questionable. They were removed from the data set for index calibration.

Another site (LES030-0002) was removed from the reference set because it was close to the state border, much of the watershed was in Ohio, and the watershed statistics were incomplete.

**Table 1.** Sites with contradicting drainage areas based on IDEM data set (Drainage SqMi) and Tt GIS analysis (WsArea SqKm).

SITE	LAT	LONG	Disturbance Designation	Drainage SqMi	WsArea SqKm	Resolution
WEL-14-0001	38.62088	-86.8426	Ref	5130	14	Removed from reference data set
WWL-08-0004	38.81238	-87.2428	Other	4976	68	
WTI-06-0008	41.13368	-86.3959	SubRef	772	5	Removed from reference data set
LMJ190-0031	41.50085	-85.7798	Other	377	115	
GMW-06-0003	39.40026	-85.0681	Ref	138	8	Removed from reference data set

## ***Disturbance variables and GIS analyses***

**Table 2.** Data sources for GIS analyses

Data sources	Variable
National Hydrography Dataset Plus version 2.1 (NHDPlusV2.1) <a href="https://nhdplus.com/NHDPlus/index.php">https://nhdplus.com/NHDPlus/index.php</a>	Canal and Pipes, point sources (NPDES, CERCLIS, CAFO, TRI)
National Land Cover Database 2016 (NLCD2016) <a href="https://www.mrlc.gov/data/nlcd-2016-land-cover-conus">https://www.mrlc.gov/data/nlcd-2016-land-cover-conus</a>	Land use categories (% Urban and % Ag), road density and crossings
National Land Cover Database 2016 Percent Developed Imperviousness <a href="https://www.mrlc.gov/data/nlcd-2016-percent-developed-imperviousness-conus">https://www.mrlc.gov/data/nlcd-2016-percent-developed-imperviousness-conus</a>	Percent developed imperviousness
USGS Active Mines and Mineral Plants in the US <a href="http://mrdata.usgs.gov/mineplant/">http://mrdata.usgs.gov/mineplant/</a>	Mine locations
National Inventory of Dams <a href="https://nid.sec.usace.army.mil/">https://nid.sec.usace.army.mil/</a>	Dam locations
Population density at the site (individuals / km <sup>2</sup> ) <a href="https://hub.arcgis.com/datasets/9fdfa3bdfb134e9cb458a84e5d28a6d3_0?selectedAttribute=POPDENS">https://hub.arcgis.com/datasets/9fdfa3bdfb134e9cb458a84e5d28a6d3_0?selectedAttribute=POPDENS</a>	2000 Census data

## *Watershed delineation 1km and 5km buffers*

1. Watershed delineations were subset into 1 km and 5 km upstream buffers from each Diatom site.
2. Upstream buffers were created as an indirect result of the RMN Land Cover tool (see *Land Use* section; Gibbs and Bierwagen, 2017). Note, each site/watershed has two buffer shapefiles, one for the 1 km buffer and another for the 5 km buffer.
3. Buffers for each watershed were merged using the Merge tool in ArcGIS. The subsequent shapefile contains buffer attributes for all watersheds. Note, there is one shapefile for the 1 km buffers and one shapefile for the 5 km buffers.
4. The following disturbance variables were calculated for the whole watershed, 5 km buffer, and 1 km buffer scales. Note, Population Density was estimated at the site itself rather than estimated across the whole watershed or buffer areas.

## *Land use (% Urban and % Agriculture)*

1. Land use statistics (% Urban and % Agriculture) for each Diatom site were calculated using the Regional Monitoring Network (RMN) toolbox for ArcGIS developed by U.S. EPA (Gibbs and Bierwagen, 2017).
2. The RMN land-use composition tool calculates the percentage of each land use at all three spatial scales (whole watershed and both buffer distances).
3. Simply, the required data to use the tool includes the sampling stations, watershed delineations, and the NLCD land use raster (Table 2).
4. The output of the tool produces a watershed delineation shapefile with one field added for each land use-area extent combination (whole watershed, 1,000 m, and 5,000 m). Note, the tool also produces the 1 km and 5 km buffers needed for the remaining analyses.
5. The attribute table of the land use shapefile was exported to MS Access.
6. Percent urban land cover was calculated as the sum of different levels of “developed” land use (i.e., land use codes (LUC) 22, 23, 24). Percent agricultural land cover was calculated as the sum of agricultural land uses (i.e., LUC 81 and 82). At each site, we obtained estimates of % Urban and % Ag for the whole watershed and both upstream buffers.

#### *Population density*

1. The Population density (PopDens) shapefile (Table 2) was imported into ArcGIS.
2. PopDens shapefile was intersected (Intersect tool) with Diatom sites.
3. The attribute table of the new shapefile was exported to MS Excel.
4. The Population Density variable, from the original PopDens shapefile, was used to estimate the population density at the site (individuals / km<sup>2</sup>).

#### *Impervious land cover*

1. Percent impervious land cover was estimated at all three spatial scales (whole watershed and both buffer distances) using NLCD data (Table 2).
2. Percent impervious was calculated for each watershed using the Tabulate Area tool. The tool was iterated over each watershed and an output table was produced that included the area (m<sup>2</sup>) within each percent imperviousness category (i.e., 0 – 100 %). The output tables for all watersheds were merged (Merge tool) to create one summary table for all watersheds. The table was exported to MS Excel.
3. The percent impervious land cover for each watershed was estimated using a weighted average calculation – area was weighted by its percent imperviousness category and then divided by the total area of the watershed.
4. The process was repeated with the 1 km and 5 km buffers.

#### *Density of road crossings and road densities*

1. The density of road crossings and road densities were estimated at all three spatial scales (whole watershed and both buffer distances) using Indiana road data (Table 2).
2. To calculate the density of road crossings, a spatial join (Spatial Join tool) was done between the road crossings point shapefile and the watershed delineations shapefile. This

step produces the number of road crossings in each watershed. Note, see [Technical Article](#) as a guide.

3. The attribute table of the output shapefile was exported to MS Excel and density was estimated.
4. To calculate the road density, the roads shapefile was spatially joined (Spatial Join tool) with the watershed delineations shapefile. The watershed shapefile was the target feature and the roads shapefile was the join feature. The shape length field was aggregated (summed). See [Article](#) as an example.
5. The attribute table of the output shapefile was exported to MS Excel and density was estimated.
6. These processes were repeated with the 1 km and 5 km buffers.

#### *Percent canals or pipelines*

1. The percentage of flowlines found in watersheds that are classified as either canals or pipelines was estimated at all three spatial scales (whole watershed and both buffer distances) using NHD flowline data (Table 2).
2. NHD flowlines were intersected (Intersect tool) with the watershed delineation shapefile and the output shapefile was exported to MS Access.
3. A crosstab query was used to obtain the count of each type of flowline (FTYPE) in each watershed. The percentage of flowlines classified as either canals or pipelines was calculated for each watershed.
4. These processes were repeated with the 1 km and 5 km buffers.

#### *Mines, dams, NPDES, CERCLIS, CAFO, and TRI sites*

1. The number of mines, dams, NPDES, CERCLIS, CAFO, and TRI sites were estimated at all three spatial scales (whole watershed and both buffer distances) using the respective site data (Table 2).
2. To calculate the number of each site type (i.e., CAFO, mines, etc.), a spatial join (Spatial Join tool) was done between the respective point shapefile and the watershed delineations shapefile. This step produces the number of sites in each watershed. Note, see [Technical Article](#) as a guide.
3. The attribute table of each resulting shapefile was exported to MS Excel and the densities of each site type were estimated.
4. These processes were repeated with the 1 km and 5 km buffers.

#### *Reference site designation*

Following the GIS analysis, sites were classified as either stressed, reference, or other depending on the criteria previously established for reference designation (Jessup and Stamp, 2017; Table 3). Criteria were previously set based on distributions of values in the entire upstream catchments and partial catchments 1 and 5 km upstream from the sampling sites. The preliminary disturbance categories were assigned to sites based on the numbers of primary reference criteria that indicated either reference or stressed conditions (Table 4). For a site to pass a criterion for a reference designation, it was required to pass at all 3 spatial scales. Failure of a stressed criterion

was based on the failure of one or more of the spatial scales. For a site to be considered in any of the reference categories, it could not have any indications of stress. In contrast, the levels of stress were assigned regardless of the number of reference criteria that were passed.

As a quality control process, new site designations (i.e., Reference or Stressed) were compared to previously reported site designations. Differences in site designation are likely due to updated source data (i.e., NLCD 2016 vs NLCD 2011).

**Table 3.** Reference (Ref) and Stressed (Strs) criteria for 13 disturbance variables, by catchment delineation: whole catchment (W), within 5 km (5K), and within 1 km (1K).

	Ref Criterion	Strs Criterion	Units
POP_DENSITY	<10	>200	People/km2
W_%_URBAN	<5	>40	% in catchment
5K_%_URBAN	<5	>40	% within 5km
1K % URBAN	<5	>40	% within 1km
W_%_AGRIC	<40	>90	% in catchment
5K_%_AGRIC	<40	>90	% within 5km
1K % AGRIC	<40	>90	% within 1km
W_%_IMPERV	<2	>12	% in catchment
5K_%_IMPERV	<2	>12	% within 5km
1K % IMPERV	<2	>12	% within 1km
Wd_RD_CROSS	<50	>100	#/100km2
5K_RD_CROSS	<5	>25	# within 5km
1K RD CROSS	<1	>5	# within 1km
W_RD_DENSE	<1	>5	km road/km2
5K_RD_DENSE	<1	>5	km/km2 within 5km
1K RD DENSE	<1	>5	km/km2 within 1km
W_%_CANAL/PIPE	<20	>80	% in catchment
5K_%_CANAL/PIPE	<20	>80	% within 5km
1K % CANAL/PIPE	0	>80	% within 1km
Wd_MINES	<0.1	>0.5	#/100km2
5K_MINES	<1	>2	# within 5km
1K MINES	0	>1	# within 1km
Wd_NPDES	<1	>15	#/100km2
5K_NPDES	0	>10	# within 5km
1K NPDES	0	>2	# within 1km
Wd_CERCLIS	<0.5	>2	#/100km2
5K_CERCLIS	0	>2	# within 5km
1K CERCLIS	0	>1	# within 1km
Wd_CAFO	<2	>10	#/100km2
5K_CAFO	<1	>4	# within 5km
1K CAFO	0	>1	# within 1km
Wd_TRI	<1	>10	#/100km2
5K_TRI	<1	>10	# within 5km
1K TRI	0	>2	# within 1km
Wd_dDAMS	<0.1	>1	#/100km2
5K_DAMS	<1	>2	# within 5km
1K DAMS	0	>1	# within 1km

**Table 4.** Preliminary disturbance category assignments based on reference and stressed criteria.

Category	Rule or description
Reference	No criteria indicate stress for BestRef, Ref, or SubRef
BestRef	12 -13 of 13 criteria indicate reference
Ref	10 - 11 of 13 criteria indicate reference
SubRef	8 - 9 of 13 criteria indicate reference
Other	<8 criteria indicate reference or <2 criteria indicate stress
Stressed	Stressed designations do not depend on reference indications
Strs	2 – 3 of 13 criteria indicate stress
HiStrs	4 or more variables indicate stress

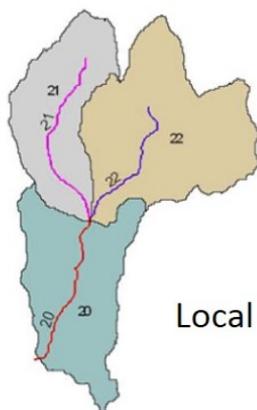
### ***StreamCat Variables for Site Classification***

Landscape-scale metrics were obtained for site classification using the USEPA Stream-Catchment (StreamCat) Dataset (Hill et al. 2016), which covers the contiguous US. StreamCat is an extensive database of natural and anthropogenic landscape metrics that are associated with the National Hydrography Dataset (NHD) Plus Version 2 (NHDPlusV2) stream segments (McKay et al. 2012). StreamCat data are available at two spatial scales: local catchment and full upstream watershed (Figure 1). Some variables address site disturbance characterization (e.g., overall watershed condition (ICI and IWI), percent agricultural cover, percent urban cover, road density, and specific discharges or activities (National Pollutant Discharge Elimination System discharges, Confined Animal Feeding Operations, mining activity, etc.). Natural (classification) variables include geologic types, elevation, stream slope, catchment size, ecoregion, mean annual temperature, and precipitation, among others. In addition, NHDPlusV2 attribute data for flowline type (stream/river, canals/ditches, coastline, and artificial pathway) and slope were associated with biological sampling sites, as were EPA level III and IV ecoregions.

To associate the biological sampling sites with the StreamCat dataset, an intersect procedure was performed with Geographic Information System software (ArcGIS 10.7.1), which created an attribute table with a list of the biological sampling stations and unique identifiers for the NHDPlusV2 catchments (COMID/FEATUREID). The COMID was then used to link the biological sampling sites with the StreamCat data tables, which were downloaded from the StreamCat website<sup>1</sup>. The data were uploaded to MS Access and queries were created to generate tables with the desired StreamCat metrics.

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<sup>1</sup> <https://www.epa.gov/national-aquatic-resource-surveys/streamcat-dataset-0>



A. Local Catchments for Reaches 20, 21, and 22

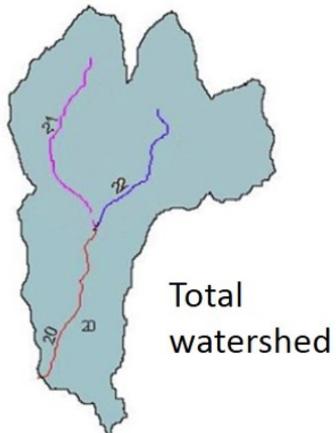
### Local catchment

Definition: the landscape area draining to a single stream segment, excluding upstream contributions.

In this example, there are three local catchments (associated with unique flowline segments) –

- # 20 (green)
- # 21 (gray)
- # 22 (brown)

Each local catchment has a unique identifier (COMID or FEATUREID).



B. Total Upstream Watershed for Reach 20

### Watershed-level

Definition: the local catchment plus the accumulated area of all upstream catchments

In this example there is one total watershed, comprised of the three local catchments (#20 + #21 + #22).

Figure 1. USEPA's StreamCat metrics (Hill et al. 2016) cover two spatial scales: local catchment and total watershed.

## References

- Gibbs, D.A., and B. Bierwagen. 2017. Procedures for delineating and characterizing watersheds for stream and river monitoring programs. (EPA/600/R-17/448F). Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development.
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- Jessup, B.K., and J. Stamp 2017. Development of multimetric indices of biotic integrity for assessing macroinvertebrate and fish assemblages in Indiana streams. Prepared for US EPA Region 5 and the Indiana Department of Environmental Management.

McKay, L., T. Bondelid, T. Dewald, J. Johnston, R. Moore, and A. Reah. 2012. NHDPlus Version 2: User Guide. Washington, DC: U.S. Environmental Protection Agency, Office of Water.

## Water chemistry data summary

Water chemistry data, provided by IDEM, have been summarized for overall completeness (Table 4). Possible outliers occur in 35 of 63 analytes – see Notes in Table 4. The outliers were reviewed by IDEM staff and for the most part, verified as valid values. Also, 6 of 63 analytes had low sample counts and should not be considered for further analyses – see Notes in Table 4. Note, a possible exception can be made for CBOD (n = 84).

**Table 4.** Summary statistics for Indiana water chemistry data. Notes indicate whether an analyte has samples that are considered outliers or whether the analyte summary statistics are represented by a low sample count.

Analyte	Min	Mean	Median	q95	Max	Count	Notes
AFDM	5.83	69.91	60.22	175.70	475.76	256	two outliers >400
Al_diss	0.01	0.06	0.03	0.18	2.60	922	seven outliers >1
Al_tot	0.00	0.67	0.17	2.86	31.00	1190	three outliers >15
Alkalinity	5.00	201.46	207.00	302.05	636.00	1280	two outliers >600
Antim_diss	0.50	0.52	0.50	0.50	4.20	977	
Antim_tot	0.50	0.53	0.50	0.50	7.70	1217	
Arsen_Diss	0.50	1.89	2.50	2.50	12.00	977	
Arsen_Tot	0.50	2.10	2.50	3.10	13.00	1217	
Barium	81.50	93.25	93.25	103.83	105.00	2	Low count
Beryllium	0.10	0.10	0.10	0.10	0.10	2	Low count
C_organic	0.50	5.07	4.00	11.91	42.00	1219	two outliers >40
C_part_inorg	0.03	0.06	0.06	0.06	0.80	229	two outliers >0.2
C_tot_inorg	0.12	1.24	0.74	3.26	15.30	229	two outliers >10
C_tot_partic	0.09	1.25	0.74	3.27	15.40	229	two outliers >10
Cadmium_diss	0.03	0.57	1.00	1.00	1.00	990	two outliers >0.50
Cadmium_tot	0.10	0.60	1.00	1.00	1.00	1241	two outliers >0.50
Calcium	7.80	73.39	73.00	103.90	470.00	1223	nine outliers >250
CBOD	1.00	1.21	1.00	2.20	4.00	84	Low count
CHLa_benthic	1.24	74.44	42.72	264.64	795.01	570	two outliers >550
CHLa_water	0.18	17.54	3.03	48.36	1356.52	410	three outliers >450
Chloride	1.50	29.35	20.55	77.96	690.00	1280	four outliers >400
Chrom_diss	0.01	1.30	1.50	1.50	14.00	977	three outliers >5
Chrom_tot	0.01	1.64	1.50	3.40	37.00	1217	two outliers >20
Cobalt	0.67	1.00	1.02	1.29	1.30	4	Low count
COD	2.00	15.51	13.55	35.30	130.00	1246	six outliers >55
Coliform_tot	238.2	2412.8	1209.8	12098	24196	1665	
Conductivity	69.00	636.80	588.00	1130.6	4177	3790	
Copper_diss	0.50	1.62	1.00	4.20	18.00	968	three outliers >12
Copper_tot	0.50	2.22	1.20	6.00	43.00	1200	
Analyte	Min	Mean	Median	q95	Max	Count	Outlier Notes
Cyanide	0.00	0.00	0.00	0.01	0.19	1114	two outliers >0.05

DisOxy	0.22	8.08	8.01	12.13	21.46	3768	
DisOxy_sat	2.70	90.06	88.70	137.26	278.70	3764	
Ecoli	0.50	869.92	261.30	2419.6	24196	1665	
Hardness	36.00	290.05	281.00	413.35	2800	1280	eight outliers >1000
Iron	0.08	0.98	1.30	1.80	1.80	9	Low count
Lead_diss	0.50	0.77	1.00	1.00	2.40	977	
Lead_tot	0.50	1.09	1.00	2.50	30.00	1217	two outliers >15
Magnesium	3.20	25.77	24.00	38.09	380.00	1223	ten outliers >200
Manganese	260	485.00	510.00	651.00	660.00	4	Low count
N_NH4	0.05	0.10	0.05	0.21	12.00	1276	four outliers >5
N_NO3NO2	0.01	4.40	1.40	7.01	1800	1280	three outliers >7
N_TKN	0.15	0.75	0.56	1.80	13.00	1201	two outliers >10
N_tot_part	0.02	0.15	0.10	0.44	1.12	229	
Nickel_diss	0.25	2.36	1.90	5.11	44.00	910	two outliers >30
Nickel_tot	0.25	3.06	2.30	7.20	43.00	1213	five outliers >25
P_Orthoph	0.01	0.09	0.05	0.27	1.04	77	two outliers >0.50
P_total	0.02	0.14	0.08	0.45	2.50	1256	four outliers >1.5
pH	6.02	7.85	7.89	8.39	10.14	3784	
Pheophyt_benthic	1.18	26.87	18.61	78.05	168.37	570	
Pheophyt_water	0.27	7.45	2.95	21.03	361.05	410	five outliers >100
Selenium_diss	0.50	1.29	2.00	2.00	4.30	977	
Selenium_tot	0.50	1.34	2.00	2.00	4.40	1217	
Silver_diss	0.15	0.20	0.15	0.25	0.50	977	
Silver_tot	0.15	0.20	0.15	0.25	0.50	977	
Solids_suspended	0.50	23.29	7.00	86.00	1100	1278	two outliers >700
Solids_TDS	49.00	371.56	330.00	640.00	3900	1277	
Solids_tot	74.00	424.45	380.00	704.60	4100	1275	
Sulfate_diss	1.75	75.83	34.00	159.10	2700	1223	
Sulfate_tot	8.00	39.49	33.00	71.40	239.00	57	three outliers >100
Temp_Water	5.50	20.27	20.63	27.36	170.10	3793	two outliers >50
Turbidity	0.10	25.76	9.30	90.11	1000	3782	three outliers >750
Zinc_diss	1.50	2.89	3.00	6.22	34.00	978	
Zinc_tot	0.00	5.53	3.00	17.00	150.00	1213	two outliers >100

## Diatom data summary

Diatom data, provided by IDEM, have been summarized for overall completeness (Table 5). Many sites are represented by one diatom sample (330/409 sites); however, 70 sites have two samples and nine sites have three samples. The number of taxa and the valve count vary by sample (Table 5). Samples with valve counts 20 percent lower than the target of 600 are flagged with “Low Count”. Only 2 of 497 samples have low counts. Samples AB01153 (n = 51) and AB01172 (n = 59) have extremely low counts and neither are duplicates; therefore, these samples should be removed from further analyses.

**Table 5.** The number of taxa and the valve count per sample in the IDEM diatom dataset. Samples with valve counts lower than 480 (20 % below the target of 600) are flagged “Low Count”.

SampleID	Taxa Count	Valve Count	Valve Target	Target Flag
AB01141	61	600	600	
AB01142	56	600	600	
AB01143	63	600	600	
AB01144	32	600	600	
AB01145	41	599	600	
AB01146	46	600	600	
AB01146(B)	43	600	600	
AB01147	51	600	600	
AB01148	65	600	600	
AB01149	47	600	600	
AB01150	32	600	600	
AB01151	62	600	600	
AB01152	68	600	600	
AB01153	19	51	600	Low Count
AB01156	41	600	600	
AB01157	43	600	600	
AB01158	65	600	600	
AB01159	56	601	600	
AB01160	55	600	600	
AB01161	61	600	600	
AB01162	57	600	600	
AB01163	59	600	600	
AB01164	67	600	600	
AB01164(B)	63	600	600	
AB01165	60	600	600	
AB01166	70	600	600	
AB01167	45	600	600	
AB01170	67	600	600	

AB01171	61	600	600
AB01172	24	59	600
AB01173	78	600	600
AB01173(B)	69	600	600
AB01174	41	600	600
AB01175	67	600	600
AB01176	55	600	600
AB01177	50	600	600
AB01178	61	600	600
AB01178(B)	51	600	600
AB01182	54	596	600
AB01183	54	600	600
AB01184	53	600	600
AB01185	60	600	600
AB01186	39	600	600
AB01187	36	600	600
AB01190	36	600	600
AB01193	58	600	600
AB01193(B)	52	600	600
AB01222	37	600	600
AB01222(B)	38	600	600
AB01223	57	600	600
AB01224	42	600	600
AB01225	32	600	600
AB01226	27	598	600
AB01227	24	600	600
AB01229	32	600	600
AB01230	26	600	600
AB01231	21	600	600
AB01231(B)	25	600	600
AB01234	35	600	600
AB01235	36	600	600
AB01236	52	600	600
AB01237	61	600	600
AB01238	46	600	600
AB01239	52	600	600
AB01240	67	600	600
AB01240(B)	80	600	600
AB01241	97	600	600
AB01245	32	600	600
AB01245(B)	30	600	600
AB01247	27	600	600
AB01248	29	600	600

AB01249	49	600	600
AB01250	51	600	600
AB01253	59	600	600
AB01253(B)	50	600	600
AB01254	82	600	600
AB01255	44	600	600
AB01256	59	600	600
AB01257	29	600	600
AB01258	17	600	600
AB01259	39	600	600
AB01260	73	600	600
AB01263	40	600	600
AB01264	35	600	600
AB01265	90	600	600
AB01266	78	600	600
AB01268	71	600	600
AB01269	49	600	600
AB01271	58	600	600
AB01271(B)	55	600	600
AB01486	6	600	600
AB04675	49	600	600
AB04676	48	600	600
AB04677	47	600	600
AB04679	27	600	600
AB04681	45	600	600
AB04682	36	600	600
AB04684	36	600	600
AB04685	39	600	600
AB04686	53	600	600
AB04687	66	600	600
AB04688	63	600	600
AB04689	52	600	600
AB04690	61	600	600
AB04691	31	600	600
AB04692	40	600	600
AB04693	51	600	600
AB04695	41	600	600
AB04697	36	600	600
AB04698	28	600	600
AB04699	41	600	600
AB04700	53	600	600
AB04701	34	600	600
AB04702	51	600	600

AB04703	25	600	600
AB04705	57	600	600
AB04706	60	600	600
AB04707	42	600	600
AB04708	30	600	600
AB04709	36	600	600
AB04710	64	600	600
AB04711	66	600	600
AB04712	43	600	600
AB04714	33	600	600
AB04715	49	600	600
AB04716	45	600	600
AB04717	28	600	600
AB04718	44	600	600
AB04719	30	600	600
AB04720	41	600	600
AB04721	53	600	600
AB04722	45	600	600
AB04724	48	600	600
AB04725	55	600	600
AB04726	19	600	600
AB04727	35	600	600
AB04728	23	600	600
AB04729	54	600	600
AB04730	65	600	600
AB09763	19	600	600
AB09765	16	600	600
AB09767	47	600	600
AB09769	28	600	600
AB09770	65	600	600
AB09771	33	600	600
AB09772	65	600	600
AB09773	62	600	600
AB09774	65	600	600
AB09775	68	600	600
AB09776	68	600	600
AB09777	62	600	600
AB09778	59	600	600
AB09779	61	600	600
AB09780	55	600	600
AB09781	78	600	600
AB09782	59	600	600
AB09783	32	600	600

AB09784	54	600	600
AB09786	41	600	600
AB09787	42	600	600
AB09788	34	600	600
AB09789	56	600	600
AB09790	52	600	600
AB09791	45	600	600
AB09792	56	600	600
AB09793	68	600	600
AB09794	52	600	600
AB09795	72	600	600
AB09796	53	600	600
AB09798	64	600	600
AB09799	72	600	600
AB09800	71	600	600
AB09801	56	600	600
AB09802	63	600	600
AB09803	61	600	600
AB09804	50	600	600
AB09805	59	600	600
AB09807	72	600	600
AB09808	49	600	600
AB09809	60	600	600
AB09810	57	600	600
AB09811	67	600	600
AB09812	50	600	600
AB09813	46	600	600
AB09814	63	600	600
AB09815	59	600	600
AB09816	50	600	600
AB09817	60	600	600
AB10429	67	600	600
AB14134	87	600	600
AB14135	41	600	600
AB14136	38	600	600
AB14137	43	600	600
AB14138	34	600	600
AB14140	57	600	600
AB14141	49	600	600
AB14252	41	600	600
AB14641	46	600	600
AB14643	36	600	600
AB14644	51	600	600

AB14645	51	600	600
AB14646	36	600	600
AB14647	67	600	600
AB14648	33	600	600
AB14649	56	600	600
AB14650	81	600	600
AB14656	55	600	600
AB14658	52	600	600
AB14659	47	600	600
AB14660	47	600	600
AB14661	48	600	600
AB14662	59	598	600
AB14663	53	600	600
AB14664	31	600	600
AB14669	39	600	600
AB14671	65	599	600
AB14672	33	600	600
AB14673	41	600	600
AB14674	41	600	600
AB14675	44	600	600
AB14676	47	600	600
AB14677	51	600	600
AB14678	58	600	600
AB14679	40	600	600
AB14681	66	600	600
AB14683	40	600	600
AB14684	51	600	600
AB14685	67	600	600
AB14686	45	600	600
AB14687	63	600	600
AB14688	73	600	600
AB14689	53	600	600
AB14934	36	600	600
AB14935	39	600	600
AB14936	49	600	600
AB14937	41	600	600
AB14938	52	600	600
AB14939	46	600	600
AB18800	43	600	600
AB18801	57	600	600
AB18802	41	600	600
AB18803	44	600	600
AB18804	29	600	600

AB18805	40	600	600
AB18806	48	600	600
AB18807	55	600	600
AB18808	66	600	600
AB18809	30	600	600
AB18810	62	600	600
AB18811	51	600	600
AB18812	50	600	600
AB18813	37	600	600
AB18814	59	600	600
AB18815	64	600	600
AB18816	56	600	600
AB18817	41	600	600
AB18818	43	600	600
AB18819	50	600	600
AB18820	50	600	600
AB18821	46	600	600
AB18822	60	600	600
AB18823	39	600	600
AB18826	33	600	600
AB18827	56	600	600
AB18829	37	600	600
AB18830	32	600	600
AB18831	33	600	600
AB18832	67	600	600
AB18833	49	600	600
AB18834	65	600	600
AB18835	49	600	600
AB18836	46	600	600
AB18837	56	600	600
AB18838	46	600	600
AB18839	38	600	600
AB18840	54	600	600
AB18841	26	600	600
AB18842	54	600	600
AB18843	26	600	600
AB18844	49	600	600
AB19126	61	600	600
AB19128	36	600	600
AB19355	71	600	600
AB21959	30	600	600
AB21960	54	600	600
AB21961	62	600	600

AB21962	69	600	600
AB22057	32	602	600
AB22058	71	600	600
AB22086	52	600	600
AB22087	59	600	600
AB22088	65	600	600
AB22089	71	600	600
AB22090	75	600	600
AB22091	70	600	600
AB22092	64	600	600
AB22093	58	600	600
AB22094	36	600	600
AB22095	62	600	600
AB22096	36	600	600
AB22097	61	600	600
AB22098	44	600	600
AB22099	56	600	600
AB22100	37	600	600
AB22515	45	600	600
AB22516	54	600	600
AB22517	37	600	600
AB22518	42	600	600
AB22519	46	600	600
AB22520	37	600	600
AB22521	43	600	600
AB22522	58	600	600
AB22523	63	600	600
AB22524	46	600	600
AB22525	63	600	600
AB22526	37	601	600
AB22528	53	600	600
AB22529	62	600	600
AB22530	48	600	600
AB22531	49	600	600
AB22532	55	600	600
AB22533	42	600	600
AB22534	50	600	600
AB22535	53	599	600
AB22536	59	600	600
AB22537	42	600	600
AB22538	56	600	600
AB22539	55	600	600
AB22540	53	600	600

AB22541	44	600	600
AB22542	41	600	600
AB22543	66	600	600
AB22544	42	600	600
AB22545	74	600	600
AB22547	46	600	600
AB22548	46	600	600
AB22549	59	600	600
AB22551	46	600	600
AB22552	54	600	600
AB22554	30	600	600
AB22556	53	600	600
AB22557	40	600	600
AB22558	44	600	600
AB22559	31	600	600
AB22561	45	600	600
AB22562	65	600	600
AB22563	74	600	600
AB22564	80	600	600
AB22565	52	600	600
AB22566	46	600	600
AB22567	41	600	600
AB22569	56	600	600
AB22570	43	600	600
AB22571	66	600	600
AB22572	78	600	600
AB22575	44	600	600
AB22577	51	600	600
AB22579	42	600	600
AB22580	69	600	600
AB22581	38	600	600
AB22582	70	600	600
AB22583	34	600	600
AB22584	50	600	600
AB22585	46	600	600
AB22586	47	600	600
AB22587	58	600	600
AB22588	70	600	600
AB22589	67	600	600
AB22924	34	601	600
AB23263	45	600	600
AB23268	51	600	600
AB23276	45	600	600

AB23292	41	600	600
AB26562	62	600	600
AB26563	46	600	600
AB26569	58	600	600
AB26573	59	600	600
AB26579	55	600	600
AB26585	55	600	600
AB26597	69	600	600
AB26599	28	600	600
AB26601	68	600	600
AB26602	20	600	600
AB26603	22	600	600
AB26604	48	600	600
AB26610	26	600	600
AB26611	49	600	600
AB26613	72	600	600
AB26615	82	600	600
AB26617	55	600	600
AB26618	67	600	600
AB26621	68	600	600
AB26622	51	600	600
AB26623	64	600	600
AB26624	54	600	600
AB26625	62	600	600
AB26626	45	600	600
AB26627	39	600	600
AB26629	57	600	600
AB26630	38	600	600
AB26631	55	600	600
AB26632	50	600	600
AB26633	77	600	600
AB26634	21	600	600
AB26635	90	600	600
AB26636	73	600	600
AB26637	44	600	600
AB26638	45	600	600
AB26639	36	600	600
AB26640	50	600	600
AB26641	54	600	600
AB26997	72	600	600
AB26998	26	600	600
AB28980	58	600	600
AB28981	42	600	600

AB28982	27	600	600
AB28983	47	600	600
AB28984	41	600	600
AB28985	42	600	600
AB28986	54	600	600
AB28987	22	600	600
AB28988	48	600	600
AB28989	43	600	600
AB28990	59	600	600
AB28991	45	600	600
AB28992	33	600	600
AB28993	52	600	600
AB28994	42	600	600
AB28995	48	600	600
AB28996	41	600	600
AB28997	61	600	600
AB28998	46	600	600
AB28999	42	600	600
AB29000	62	600	600
AB29001	57	600	600
AB29002	37	600	600
AB29003	42	600	600
AB29004	29	600	600
AB29005	47	600	600
AB29006	51	600	600
AB29007	53	600	600
AB29329	33	600	600
AB29331	44	600	600
AB29333	47	600	600
AB30097	57	600	600
AB30098	56	600	600
AB30099	51	600	600
AB30101	55	600	600
AB30103	47	600	600
AB30104	43	600	600
AB30105	64	600	600
AB30106	31	600	600
AB30107	105	600	600
AB30108	64	600	600
AB30110	62	600	600
AB30111	58	600	600
AB30112	58	600	600
AB30113	84	600	600

AB30114	61	600	600
AB30115	78	600	600
AB30116	87	600	600
AB30117	89	600	600
AB30118	66	599	600
AB30119	38	600	600
AB30120	66	600	600
AB30122	49	600	600
AB30123	84	600	600
AB30125	98	600	600
AB30126	70	600	600
AB30127	48	600	600
AB30128	25	600	600
AB30129	48	600	600
AB30130	36	600	600
AB30131	55	600	600
AB30132	50	600	600
AB30135	101	600	600
AB30136	74	600	600
AB30137	97	600	600
AB30138	79	600	600
AB30139	65	600	600
AB30541	71	600	600
AB30546	66	600	600
AB30694	97	600	600
AB30797	68	600	600
AB30893	26	600	600
AB30894	39	600	600
AB30895	48	600	600
AB30896	27	600	600
AB30898	26	600	600
AB30899	15	600	600
AB30900	25	600	600
AB34976	37	600	600
AB34977	46	600	600
AB34978	35	600	600
AB34979	33	600	600
AB34980	54	600	600
AB35723	52	600	600

## Habitat data summary

Habitat data, provided by IDEM, are summarized for completeness (Table 6). Most sites were sampled only once per season (360/390 records); however, 29 site-season combinations were sampled twice, and one site-season combination was sampled three times. Also, 48 of 409 sites did not have any habitat data available (in any season). There were 122 total samples in spring and 299 in summer.

**Table 6.** The number of samples at a given site within a season.

<b>Station Name</b>	<b>Season</b>	<b>Count Samples</b>
GMW-01-0003	Summer	1
GMW-01-0004	Summer	1
GMW010-0044	Spring	1
GMW010-0044	Summer	1
GMW-01-0005	Spring	1
GMW-01-0006	Summer	1
GMW-02-0001	Spring	1
GMW-02-0002	Spring	1
GMW-02-0003	Summer	1
GMW020-0035	Spring	1
GMW-02-0004	Summer	1
GMW-02-0014	Spring	1
GMW-03-0007	Spring	1
GMW-03-0007	Summer	1
GMW-03-0008	Spring	1
GMW-03-0008	Summer	1
GMW-03-0009	Spring	1
GMW-03-0015	Spring	1
GMW-04-0002	Summer	1
GMW-04-0003	Summer	1
GMW-04-0004	Summer	1
GMW040-0040	Summer	1
GMW-04-0005	Spring	1
GMW-04-0008	Spring	1

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GMW-04-0012	Summer	1
GMW-04-0013	Summer	1
GMW-04-0019	Spring	1
GMW-04-0019	Summer	1
GMW-05-0001	Spring	1
GMW-05-0002	Spring	1
GMW-06-0002	Summer	1
GMW060-0021	Spring	1
GMW060-0022	Spring	1
GMW-06-0003	Spring	1
GMW-06-0004	Summer	1
GMW-06-0005	Summer	1
GMW-06-0006	Spring	1
GMW-07-0009	Spring	1
GMW-07-0010	Spring	1
GMW-07-0011	Summer	1
GMW070-0117	Summer	1
GMW-07-0012	Spring	1
GMW-07-0012	Summer	1
GMW-07-0014	Spring	1
GMW-07-0015	Spring	1
GMW-07-0017	Summer	1
GMW-07-0024	Spring	1
GMW-08-0001	Spring	1
GMW-08-0003	Spring	1
GMW-08-0003	Summer	1
GMW-08-0005	Summer	1
GMW-08-0013	Summer	1
LEJ050-0066	Summer	1
LEJ050-0068	Summer	1
LEJ060-0015	Summer	2
LEJ080-0016	Summer	1

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LEJ080-0017	Summer	1
LEJ090-0040	Summer	1
LEJ090-0041	Spring	1
LEJ090-0041	Summer	1
LEJ100-0026	Summer	1
LEM010-0046	Summer	1
LEM010-0050	Summer	1
LEM010-0051	Summer	1
LEM010-0052	Summer	1
LES030-0002	Summer	1
LES040-0104	Summer	1
LMG030-0028	Summer	1
LMG030-0029	Summer	1
LMG040-0012	Summer	1
LMG-04-0039	Spring	1
LMG-05-0015	Summer	1
LMG060-0041	Summer	1
LMG070-0035	Summer	1
LMG100-0009	Spring	1
LMG100-0009	Summer	3
LMJ110-0124	Summer	1
LMJ110-0128	Summer	1
LMJ110-0129	Summer	1
LMJ120-0042	Spring	1
LMJ120-0042	Summer	1
LMJ120-0048	Summer	1
LMJ130-0008	Summer	1
LMJ140-0118	Summer	1
LMJ140-0119	Summer	1
LMJ150-0023	Summer	1
LMJ170-0080	Summer	1
LMJ180-0049	Summer	1

LMJ180-0052	Summer	1
LMJ190-0031	Spring	1
LMJ190-0031	Summer	1
LMJ190-0032	Summer	1
LMJ200-0060	Summer	1
LMJ220-0014	Summer	1
LMJ240-0046	Summer	2
OBS090-0008	Summer	1
OBS090-0009	Summer	1
OBS120-0015	Spring	1
OBS120-0016	Summer	1
OBS140-0010	Summer	1
OBS150-0025	Summer	1
OBS150-0027	Summer	1
OBS180-0022	Spring	1
OBS180-0025	Spring	1
OHP020-0026	Spring	1
OHP020-0026	Summer	1
OHP030-0026	Summer	2
OHP030-0027	Summer	1
OLP070-0064	Spring	1
OLP070-0064	Summer	1
OLP080-0010	Spring	1
OLP110-0001	Summer	1
OLP140-0098	Spring	1
OLP140-0100	Spring	1
OML030-0015	Spring	2
OML040-0008	Summer	1
OML040-0012	Spring	1
OML040-0012	Summer	1
OML040-0014	Spring	1
OML060-0017	Spring	1

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OML060-0019	Summer	2
OML070-0021	Spring	1
OML200-0004	Spring	1
OML200-0018	Summer	1
OSK-02-0016	Spring	1
OSK030-0017	Spring	1
OSK030-0017	Summer	1
OSK030-0019	Spring	2
OSK030-0019	Summer	1
OSK060-0001	Spring	2
OSK070-0018	Summer	1
OSK100-0001	Spring	1
OSK140-0040	Spring	1
OSK140-0040	Summer	1
OSK140-0041	Summer	1
OSK140-0042	Summer	1
OSK140-0043	Summer	1
UMI-02-0014	Spring	2
UMI-02-0015	Spring	2
UMI-02-0016	Spring	1
UMI030-0044	Summer	1
UMI-03-0008	Spring	1
UMI-03-0010	Spring	1
UMI-04-0019	Summer	1
UMI-04-0022	Summer	1
UMI-05-0004	Spring	1
UMI-05-0005	Spring	1
UMI-07-0001	Spring	2
UMI-07-0002	Spring	1
UMI-13-0003	Spring	1
UMK-02-0014	Spring	1
UMK-03-0036	Spring	1

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UMK-04-0009	Summer	1
UMK-04-0010	Summer	1
UMK-04-0011	Summer	1
UMK-04-0012	Spring	1
UMK-05-0016	Spring	1
UMK-05-0017	Spring	1
UMK-05-0018	Spring	1
UMK-05-0019	Spring	1
UMK-05-0022	Spring	1
UMK-08-0001	Summer	1
UMK-10-0007	Summer	1
UMK-10-0009	Spring	1
UMK-10-0014	Spring	1
UMK-11-0001	Summer	1
UMK-11-0002	Summer	1
UMK-12-0001	Summer	1
UMK-13-0013	Spring	1
WAE-02-0002	Summer	1
WAE-04-0001	Summer	1
WAE-06-0003	Summer	1
WAE-07-0002	Summer	1
WAE-07-0003	Summer	1
WAW-01-0001	Summer	1
WAW-03-0036	Summer	1
WAW040-0018	Summer	1
WAW-04-0002	Summer	1
WAW-04-0003	Summer	1
WAW040-0043	Summer	1
WAW040-0065	Summer	1
WAW040-0080	Summer	1
WBU030-0060	Summer	1
WBU-04-0005	Spring	1

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WBU-04-0005	Summer	1
WBU-05-0002	Spring	1
WBU-06-0001	Summer	1
WBU-07-0002	Spring	1
WBU-11-0001	Summer	1
WDE-01-0003	Summer	1
WDE-01-0004	Summer	1
WDE-03-0001	Summer	1
WDE050-0025	Summer	1
WDE050-0031	Summer	2
WDE-05-0007	Summer	2
WED-01-0006	Spring	1
WED-01-0006	Summer	1
WED-01-0008	Spring	1
WED-02-0003	Spring	1
WED-03-0001	Spring	1
WED-03-0001	Summer	1
WED-04-0001	Spring	1
WED-04-0001	Summer	1
WED-05-0001	Spring	1
WED-07-0001	Spring	1
WED-07-0001	Summer	1
WED-07-0003	Spring	1
WEF-01-0001	Spring	1
WEF-02-0001	Spring	1
WEF-02-0001	Summer	1
WEF-06-0001	Spring	1
WEF-06-0001	Summer	2
WEF-06-0002	Spring	1
WEF-06-0002	Summer	1
WEF-06-0003	Summer	1
WEL-01-0003	Summer	1

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WEL-04-0001	Spring	1
WEL-04-0002	Spring	1
WEL-04-0002	Summer	1
WEL-09-0001	Summer	1
WEL-09-0004	Summer	1
WEL-11-0001	Summer	1
WEL-13-0023	Summer	1
WEL-13-0024	Summer	1
WEL-13-0026	Summer	1
WEL-14-0001	Summer	1
WEM-02-0001	Spring	1
WEM-02-0002	Spring	1
WEM-02-0002	Summer	1
WEM020-0036	Summer	1
WEM050-0048	Summer	1
WEM-06-0016	Spring	1
WEM-07-0004	Summer	2
WEM-07-0005	Spring	1
WEM-08-0003	Summer	1
WEM-09-0005	Spring	1
WEU-01-0001	Spring	1
WEU010-0039	Summer	1
WEU010-0040	Summer	1
WEU-02-0001	Summer	1
WEU-03-0034	Spring	1
WEU-03-0035	Spring	1
WEU-04-0002	Spring	1
WEU-05-0001	Summer	1
WEU-06-0001	Summer	1
WLV010-0022	Summer	2
WLV-01-0080	Summer	1
WLV-01-0081	Summer	1

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WLV040-0003	Summer	1
WLV-04-0002	Summer	1
WLV-04-0006	Summer	1
WLV-06-0004	Spring	1
WLV070-0013	Summer	1
WLV-07-0006	Spring	1
WLV-08-0001	Summer	1
WLV080-0015	Summer	2
WLV-09-0001	Spring	1
WLV110-0006	Summer	1
WLV-12-0004	Spring	1
WLV-12-0005	Spring	1
WLV-13-0011	Spring	1
WLV-13-0013	Spring	1
WLV-13-0013	Summer	1
WLV-15-0001	Spring	1
WLV-15-0001	Summer	1
WLV-15-0002	Spring	1
WLV-15-0003	Spring	1
WLV160-0013	Summer	1
WLV160-0019	Summer	1
WLV-16-0002	Summer	1
WLV160-0020	Summer	1
WLV-16-0003	Spring	1
WLV160-0038	Summer	1
WLW-02-0001	Spring	1
WLW-03-0002	Summer	1
WLW-07-0002	Spring	1
WLW-07-0003	Spring	1
WLW-09-0001	Summer	1
WMI-01-0008	Summer	1
WMI-02-0021	Summer	1

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WMI-05-0018	Summer	1
WMI-05-0019	Summer	1
WMI-05-0020	Summer	1
WMI-06-0006	Summer	1
WPA-01-0009	Summer	1
WPA-02-0001	Summer	1
WPA-02-0002	Summer	1
WPA-02-0003	Summer	1
WPA-02-0004	Summer	1
WPA-02-0005	Summer	1
WPA-02-0006	Summer	1
WPA-02-0007	Summer	1
WPA-03-0001	Summer	1
WPA-03-0006	Summer	1
WPA-03-0009	Summer	1
WPA-03-0011	Summer	1
WPA-03-0012	Summer	1
WPA-04-0003	Summer	1
WPA-04-0004	Summer	1
WPA-04-0005	Summer	1
WPA-04-0006	Summer	2
WPA-04-0007	Summer	1
WPA-04-0008	Summer	1
WPA-04-0010	Summer	2
WPA-04-0013	Summer	1
WPA-05-0001	Summer	1
WPA-05-0002	Summer	1
WPA-06-0001	Summer	1
WPA-06-0003	Summer	1
WPA-06-0005	Summer	1
WPA-06-0006	Summer	1
WPA-06-0007	Summer	1

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WPA-07-0001	Summer	2
WPA-07-0002	Summer	1
WPA-07-0003	Summer	1
WPA-07-0004	Summer	1
WPA-08-0007	Summer	1
WPA-08-0008	Summer	1
WPA-08-0009	Summer	1
WPA-08-0010	Summer	2
WPA-08-0011	Summer	1
WSA010-0012	Summer	1
WSA-02-0003	Summer	1
WSU010-0010	Summer	1
WSU-01-0010	Summer	1
WSU040-0020	Summer	1
WSU-04-0003	Summer	1
WSU-05-0001	Spring	1
WSU-05-0001	Summer	1
WSU-06-0010	Spring	1
WSU-06-0014	Spring	1
WTI-03-0014	Summer	1
WTI-05-0015	Summer	1
WTI-06-0008	Summer	1
WTI-06-0010	Summer	1
WTI-08-0004	Summer	2
WTI-10-0012	Summer	1
WTI-12-0004	Summer	1
WTI-13-0002	Summer	1
WUW-07-0014	Summer	2
WUW-10-0001	Summer	1
WUW-10-0002	Summer	1
WUW-11-0004	Summer	2
WUW-14-0002	Summer	1

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WUW-15-0001	Summer	1
WUW-15-0002	Summer	1
WWE-02-0002	Summer	1
WWE-04-0003	Summer	2
WWE-04-0006	Summer	1
WWE-05-0005	Summer	1
WWE-07-0001	Summer	2
WWE-07-0003	Summer	1
WWE-07-0004	Summer	1
WWE-07-0005	Summer	2
WWE-08-0003	Summer	1
WWL-01-0003	Summer	1
WWL-01-0004	Summer	1
WWL-02-0003	Summer	1
WWL-03-0003	Summer	1
WWL-04-0001	Summer	1
WWL-05-0001	Summer	1
WWL-07-0002	Summer	1
WWL-08-0003	Summer	1
WWL-08-0004	Summer	1
WWL-10-0004	Summer	1
WWL-10-0005	Summer	1
WWU010-0039	Summer	1
WWU-01-0006	Summer	1
WWU-03-0001	Summer	1
WWU-03-0002	Summer	1
WWU-05-0001	Summer	1
WWU-07-0001	Summer	1
WWU-07-0002	Summer	1
WWU-08-0002	Summer	1
WWU-08-0004	Summer	1
WWU-09-0002	Summer	1

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WWU-09-0003	Summer	1
WWU-09-0004	Summer	1
WWU-10-0002	Summer	1
WWU100-0040	Summer	1
WWU100-0064	Summer	2
WWU100-0078	Summer	1
WWU100-0098	Summer	1
WWU100-0106	Summer	2
WWU-11-0004	Summer	1
WWU-11-0005	Summer	1
WWU-13-0003	Summer	1
WWU-13-0006	Summer	2
WWU-15-0001	Summer	1
WWU-17-0003	Summer	1

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## *Appendix B*

### *Taxa tolerance analyses*

## Background

Taxon tolerance analyses allow for visualization of the shape of the taxon-stressor relationship across a continuous numerical scale, and can be used to identify optima (the point at which the taxon has the highest probability of occurrence) as well as tolerance limits (the range of conditions in which the taxon can persist) (Yuan 2006). To help inform diatom tolerance value assignments related to sensitivity to stressors in Indiana streams, we ran taxa tolerance analyses on four variables that capture anthropogenic disturbance: stream conductivity, QHEI (habitat) scores, the Index of Watershed Integrity (IWI, Thornbrugh et al. 2018, Johnson et al. 2019), and watershed imperviousness. The tolerance analyses were run on Indiana diatom data as described in Appendix A. The statewide database was consistently developed and will allow derivation of tolerance values specific to the state. Biologists from IDEM reviewed results from the analyses and made revisions based on familiar knowledge when the empirical results were uncertain. In this document, we describe the dataset, methods and results and conclude with recommendations on potential future analyses that could further improve our understanding of taxon-stressor relationships in Indiana streams for diatoms.

## Data compilation

### Diatoms

The statewide dataset was comprised of diatom samples from 2010-2017. Data from 409 sites that spanned six Level 3 ecoregions were included in the analysis (Table 1).

*Table 1. Number of sites in each Level 3 ecoregion.*

Ecoregion Code	Ecoregion Name	# stations
54	Central Corn Belt Plains	43
55	Eastern Corn Belt Plains	176
56	S. Michigan/N. Indiana Drift Plains	44
57	Huron/Erie Lake Plains	3
71	Interior Plateau	72
72	Interior River Valleys and Hills	71

### Disturbance variables

Variables to be included in the tolerance analysis were selected based on a principal components analysis (PCA) of multiple disturbance variables. The variables that were strongly correlated with the major PCA axes were used as indicators of the predominant types of stressors in the data set. The PCA requires complete data for analysis. Variables that were relatively complete were first screened, including those with > 350 of the 409 records complete. Data were transformed by either log10 (for concentrations) or arcsine, square root (for percentages) unless transformation was not necessary to approximate a normal distribution. Average variable values (after transformation) were substituted for

missing data in the included variables. The PCA was conducted in R software (code: PCA\_CorrelationsIN\_Diatoms.R). Three axes were considered based on the proportion of variance included with each axis (Table 2).

*Table 2. PCA of environmental variables, showing statistics for the first five axes.*

Stats	PC1	PC2	PC3	PC4	PC5
StandardDev	3.82	2.93	2.40	2.15	1.74
ProportionOfVariance	0.20	0.12	0.08	0.06	0.04
CumulativeProp	0.20	0.32	0.40	0.46	0.50

On the first axis, the **IWI** was the best representative variable (Table 3). It was opposite % agriculture in the watershed. On axis 2, urban coverage and activities describe the second axis. The % imperviousness in the watershed is a good representative because it incorporates the entire catchment and at that scale is a stronger indicator than % urban. On axis 3, habitat and conductivity were candidates for describing this axis. Total habitat score is a strong indicator on this axis. Hardness and alkalinity might not be true stressors and they can be interpreted like the more familiar stressor: conductivity.

*Table 3. PCA factor scores for the most important variables on the first three axes, showing viable disturbance variables for the tolerance analysis in bold type. See Appendix E for variable descriptions.*

Vars	PC1	Vars	PC2	Vars	PC3
<b>IWI</b>	<b>-0.24</b>	k5_NPDES	0.21	Hardness	-0.27
WTEMP	-0.24	Wd_TRI	0.21	<b>QHEITOTAL_SCORE</b>	<b>-0.27</b>
WHABT	-0.24	k5_RdDens	0.25	Alkalinity	-0.25
WCHEM	-0.23	k1_pcUrban	0.25	Magnesium	-0.24
WHYD	-0.23	k1_pcImp	0.26	SUBSTR_SCORE	-0.23
WCONN	-0.23	<b>W_pcUrban</b>	<b>0.26</b>	CHAN_SCORE	-0.22
ICI	-0.23	<b>W_pcImp</b>	<b>0.27</b>	Solids_TDS	-0.21
CCHEM	-0.23	<b>k5_pcUrban</b>	<b>0.29</b>	pH	-0.21
CTEMP	-0.23	<b>k5_pcImp</b>	<b>0.29</b>	<b>Conductivity</b>	<b>-0.20</b>
CHABT	-0.22			CCONN	-0.20
CHYD	-0.22				
WSED	-0.21				
CSED	-0.20				
<b>W_pcAg</b>	<b>0.21</b>				

We performed the tolerance analysis on four anthropogenic disturbance variables: stream conductivity, QHEI (habitat) scores, the Index of Watershed Integrity (IWI, Thornbrugh et al. 2018, Johnson et al. 2019), and watershed imperviousness (Table 4). The data came from the IDEM database and the USEPA Stream-Catchment (StreamCat) dataset 1 (Hill et al. 2016), and the National Land Cover Database 2016.

<sup>1</sup> <https://www.epa.gov/national-aquatic-resource-surveys/streamcat-dataset-0>

The StreamCat data were joined with the National Hydrography Dataset (NHD) Plus Version 2 (NHDPlusV2) geospatial layer (McKay et al. 2012) via the unique identifiers for the stream segments (COMID) and local catchments (FEATUREID). First we used Geographic Information System software (ArcGIS 10.7.1) to spatially join the biological sampling sites with the NHDPlusV2 dataset. Then we joined the sites with StreamCat data via the NHDPlusV2 identifiers. This was done in a MS Access relational database.

We did several cursory quality control (QC) checks to evaluate whether the biological sampling sites were associated with the correct NHDPlusV2 flowlines. If NHDPlusV2 stream segments had waterbody names (referred to as 'GNIS\_Names'), we checked those against the waterbody names of the sites and flagged mismatches for further evaluation. In the end, all sites were confirmed to be properly associated with flowlines.

StreamCat data are available at two spatial scales: local catchment (Cat) (which is defined as the landscape area draining to a single stream segment, excluding upstream contributions) and total watershed (Ws) (which includes the local catchment plus the accumulated area of all upstream catchments). IWI was calculated at the watershed scale. Because the StreamCat data are not based on exact watershed delineations (except in instances where the site happens to be located at the downstream end of the NHDPlusV2 local catchment), there may be occasional inaccuracies in the attribute data. For example, if a site is located upstream of urban land cover, but the urban land cover is located within the local catchment, the urban land cover data will be (wrongly) associated with the site.

Table 4. Disturbance variables that were included in the taxa tolerance analyses.

Metric (Abbrev)	Description	Source
Qualitative Habitat Evaluation Index (QHEI)	The QHEI as measured and calculated by IDEM field crews	IDEM database table : Data_Environ_WQ
Stream Conductivity (Cond)	Specific conductance as measured by the IDEM field crews at the time of diatom sampling	IDEM database table : Data_Environ_WQ
Index of Watershed Integrity version 2.1 (IWI_21)	Overall watershed condition at the total watershed scale. Scored based on six components: hydrologic regulation, regulation of water chemistry, sediment regulation, hydrologic connectivity, temperature regulation, and habitat provision	EPA StreamCat (Thornbrugh et al. 2018, Johnson et al. 2019)
Watershed Imperviousness (Imperv_W)	Mean imperviousness of anthropogenic surfaces (NLCD 2016) within watershed	National Land Cover Database 2016

Table 5. Summary statistics for the anthropogenic disturbance variables.

Variable	Valid N	Minimum	10th percentile	25th percentile	50th percentile	Mean	75th percentile	90th percentile	Maximum	Std.Dev.
Index of Watershed Integrity version 2.1 (IWI_21)	409	0.38	0.41	0.43	0.47	0.51	0.58	0.69	0.87	0.11
Stream Conductivity (Cond) (Log10 µS/cm)	409	0.00	2.59	2.73	2.80	2.79	2.86	2.98	3.50	0.23
Qualitative Habitat Evaluation Index (QHEI)	409	0.0	0.0	45.0	58.0	53.6	71.0	78.0	89.0	24.0
Percent Watershed Imperviousness (Imperv_W)	409	0.00	0.30	0.50	1.00	2.11	1.94	3.93	49.7	4.43

## **B3 Methods**

### **B3.1 Data preparation**

Data from 409 sites were included in the analysis. To prevent unequal weighting, only the primary sample per site (no duplicates or replicates) was included. For each taxon in each sample, we calculated relative abundance, which was used in the tolerance analysis (vs. straight abundance data).

We generated results for three levels of taxonomic resolution: species, genus, and family. Analyses were limited to taxa that occurred in at least 10 samples. Table 6 shows an example of how data for 12 species of *Achnanthidium* were collapsed to coarser levels of resolution for the genus and family-level analyses. Because all 12 species occurred at 10 or more sites, results were generated for each species. For the genus-level run (*Achnanthidium*), the 12 species were collapsed to genus-level (otherwise their counts would have been excluded from the coarser-level analyses). The species and genus-level identifications were further collapsed for the family-level analyses (and combined with data for other Achnanthidiaceae taxa, as appropriate). Table 7 shows how many taxa within each major taxonomic group were assessed and at what level of taxonomic resolution.

Table 6. Example of how species-level data for *Achnanthidium* were collapsed to coarser levels of resolution for the genus and family-level analyses.

TaxaID	Total # sites	Species	Genus	Family
<i>Achnanthidiaceae</i>	390			<i>Achnanthidiaceae</i>
<i>Achnanthidium</i>	390		<i>Achnanthidium</i>	<i>Achnanthidiaceae</i>
<i>Achnanthidium exilis</i>	10	<i>Achnanthidium exilis</i>	<i>Achnanthidium</i>	<i>Achnanthidiaceae</i>
<i>Achnanthidium altergracillima</i>	15	<i>Achnanthidium altergracillima</i>	<i>Achnanthidium</i>	<i>Achnanthidiaceae</i>
<i>Achnanthidium pyrenaicum</i>	18	<i>Achnanthidium pyrenaicum</i>	<i>Achnanthidium</i>	<i>Achnanthidiaceae</i>
<i>Achnanthidium affine</i>	23	<i>Achnanthidium affine</i>	<i>Achnanthidium</i>	<i>Achnanthidiaceae</i>
<i>Achnanthidium catenatum</i>	14	<i>Achnanthidium catenatum</i>	<i>Achnanthidium</i>	<i>Achnanthidiaceae</i>
<i>Achnanthidium latecephalum</i>	10	<i>Achnanthidium latecephalum</i>	<i>Achnanthidium</i>	<i>Achnanthidiaceae</i>
<i>Achnanthidium saprophilum</i>	37	<i>Achnanthidium saprophilum</i>	<i>Achnanthidium</i>	<i>Achnanthidiaceae</i>
<i>Achnanthidium exiguum</i>	58	<i>Achnanthidium exiguum</i>	<i>Achnanthidium</i>	<i>Achnanthidiaceae</i>
<i>Achnanthidium deflexum</i>	49	<i>Achnanthidium deflexum</i>	<i>Achnanthidium</i>	<i>Achnanthidiaceae</i>
<i>Achnanthidium minutissimum</i>	371	<i>Achnanthidium minutissimum</i>	<i>Achnanthidium</i>	<i>Achnanthidiaceae</i>
<i>Achnanthidium eutrophilum</i>	49	<i>Achnanthidium eutrophilum</i>	<i>Achnanthidium</i>	<i>Achnanthidiaceae</i>
<i>Achnanthidium rivulare</i>	171	<i>Achnanthidium rivulare</i>	<i>Achnanthidium</i>	<i>Achnanthidiaceae</i>

*Table 7. Number of taxa within each diatom family that were assessed with the level of taxonomic resolution.*

Family	# genera	# species
Achnanthaceae	8	20
Achnanthidiaceae	1	12
Amphipleuraceae	2	3
Aulacoseiraceae	1	3
Bacillariaceae	6	51
Berkeleyaceae	1	1
Catenulaceae	2	5
Cymbellaceae	4	9
Diadesmidaceae	2	4
Diploneidaceae	1	5
Eunotiaceae	1	1
Fragilariaceae	8	16
Gomphonemataceae	3	15
Melosiraceae	1	1
Naviculaceae	5	46
Neidiaceae	1	
Pinnulariaceae	2	5
Pleurosigmataceae	1	3
Rhoicospheniaceae	2	3
Rhopalodiaceae	2	1
Sellaphoraceae	2	12
Stauroneidaceae	2	4
Stephanodiscaceae	3	6
Surirellaceae	2	8
Thalassiosiraceae	2	4
Triceratiaceae	1	1

### B3.2 Outputs

We used customized R code to generate weighted average optima (WAopt) and tolerance (WAtol) values for each taxon. The WAopt is a commonly used measure for estimating the central tendency of a taxon along an environmental gradient. The WA is calculated by multiplying taxon relative abundance (=the weighting factor) by the variable of interest (e.g., IWI) for each sample, summing the resulting numbers and dividing that by the sum of all the weights. The width of the bell shape is often called ‘tolerance’ which can also be used to characterize the environmental niche for species along the environmental gradient.

In addition to the WAopt and WAtol values, we generated histograms (Figure B6), relative abundance scatterplots (Figure B7) and cumulative distribution functions (CDFs) (Figure B8) to visualize the

relationship between each taxon's occurrence and the environmental variables. The results provide information on where the taxa occur along stressor gradients and whether they increase or decrease in relative abundance with increasing or decreasing stress. Each output also included taxon distribution maps, with data points sized by relative abundance (such that locations with higher relative abundances had larger dots). Separate sets of output files were generated for each taxonomic group, and disturbance and natural variables were analyzed separately.

The WA optima and tolerance values for each taxon/variable were compiled into a MS Excel worksheet. The worksheet also included sample size. Taxa that occurred in fewer than 25 samples were flagged for low abundance and their outputs were scrutinized. In addition to the numeric WAopt values for each disturbance variable, the worksheet contained columns with categorical, relative rankings for each variable (ten levels, ranging from worst (1) to best (10), based on the criteria in Table 8).

*Table 8. tolerance scores assigned per disturbance variable, showing value thresholds between the 10 tolerance categories*

	Score	QHEI	Cond (log)	IWI	Imp_W
Tolerant	1	41.4	3.35	0.47	9.25
	2	45.0	2.87	0.49	3.15
	3	48.2	2.84	0.50	2.49
	4	50.4	2.82	0.50	2.12
	5	52.1	2.81	0.52	1.93
	6	53.7	2.80	0.53	1.74
	7	55.4	2.79	0.54	1.62
	8	57.6	2.77	0.57	1.42
	9	59.8	2.74	0.60	1.22
	10	72.7	2.70	0.70	0.91
Sensitive					

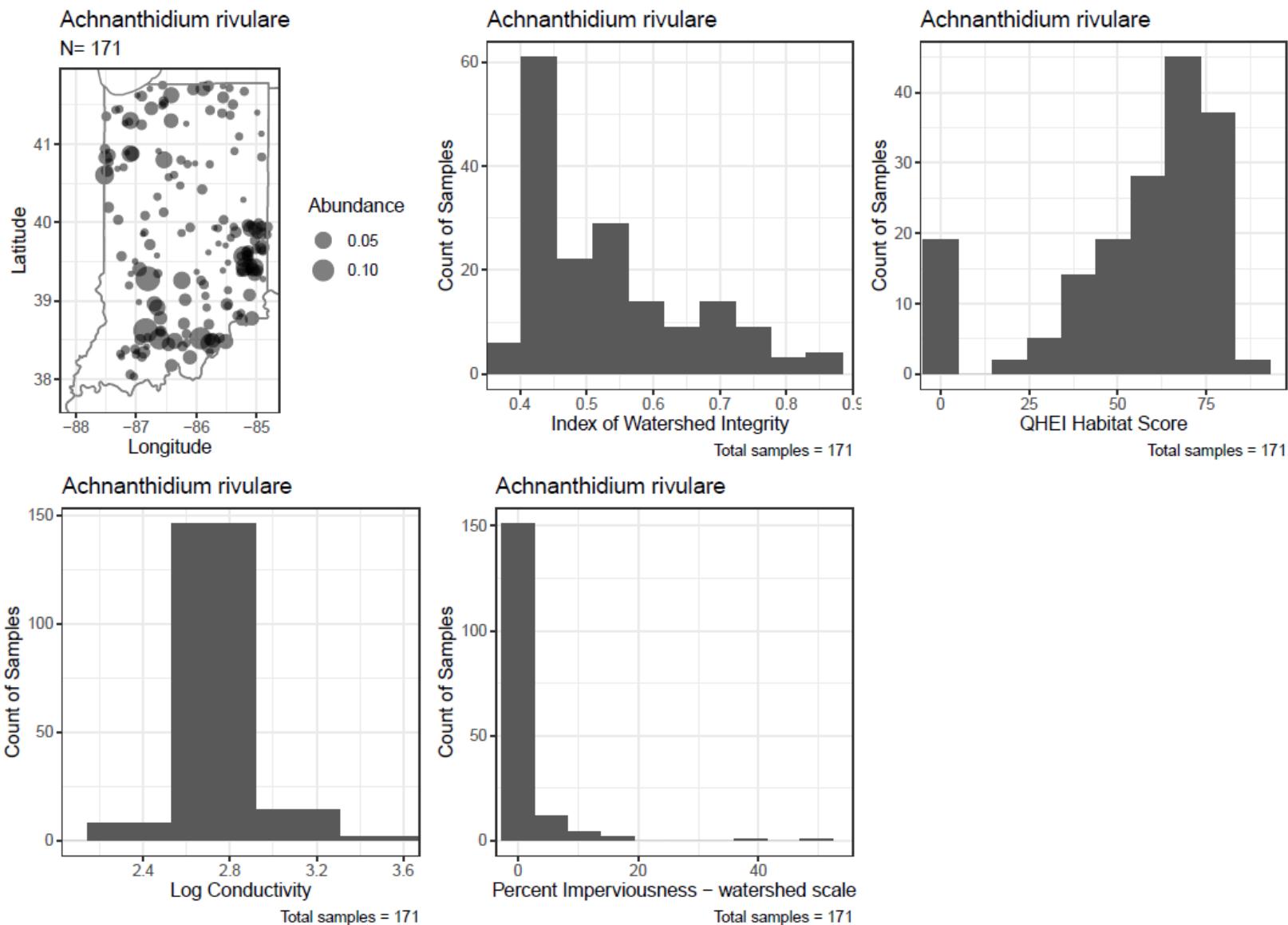


Figure B6. Example of a histogram plot.

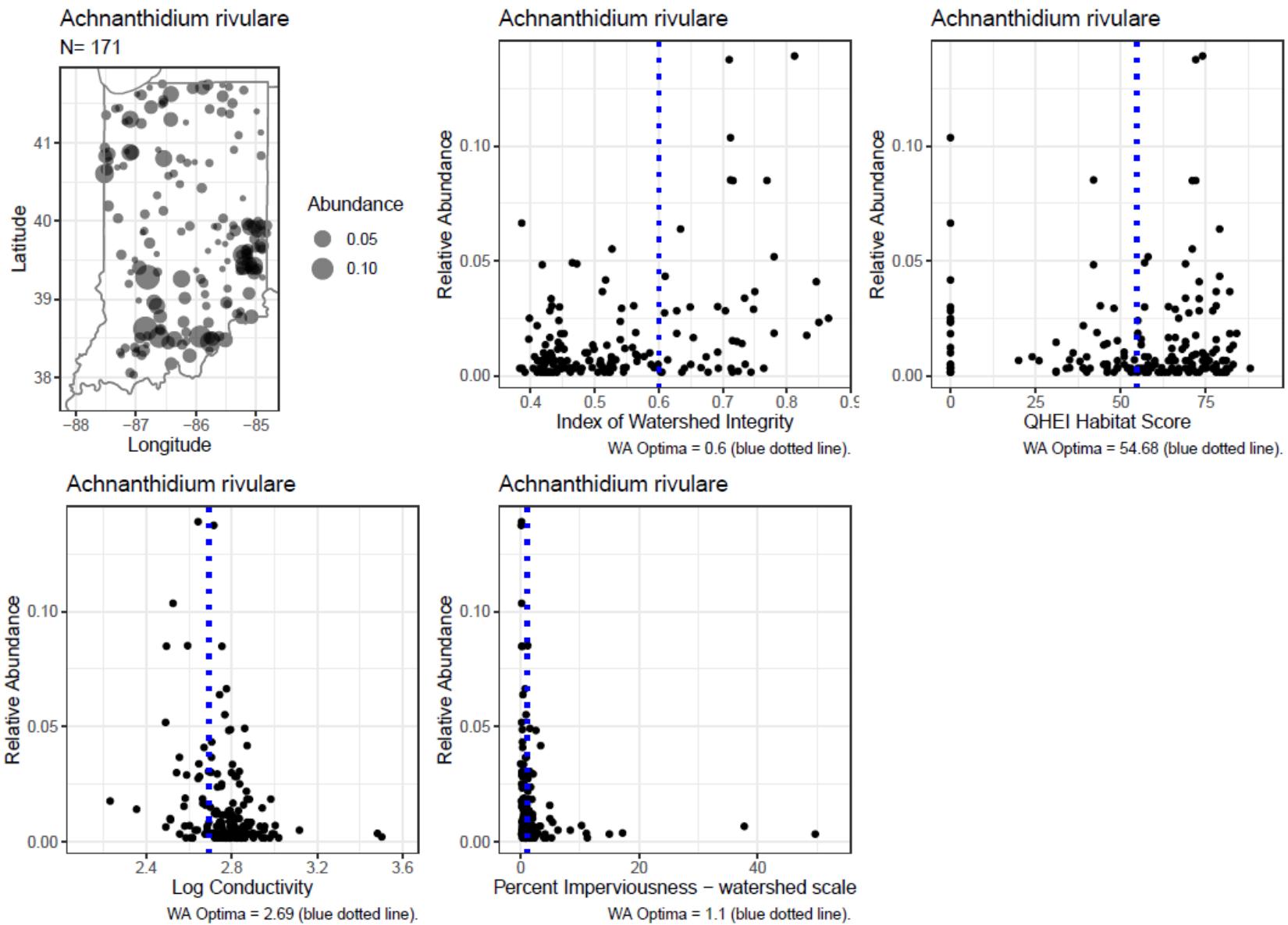


Figure B7. Example of a relative abundance scatterplot. The blue vertical dashed line equals the weighted average optima value.

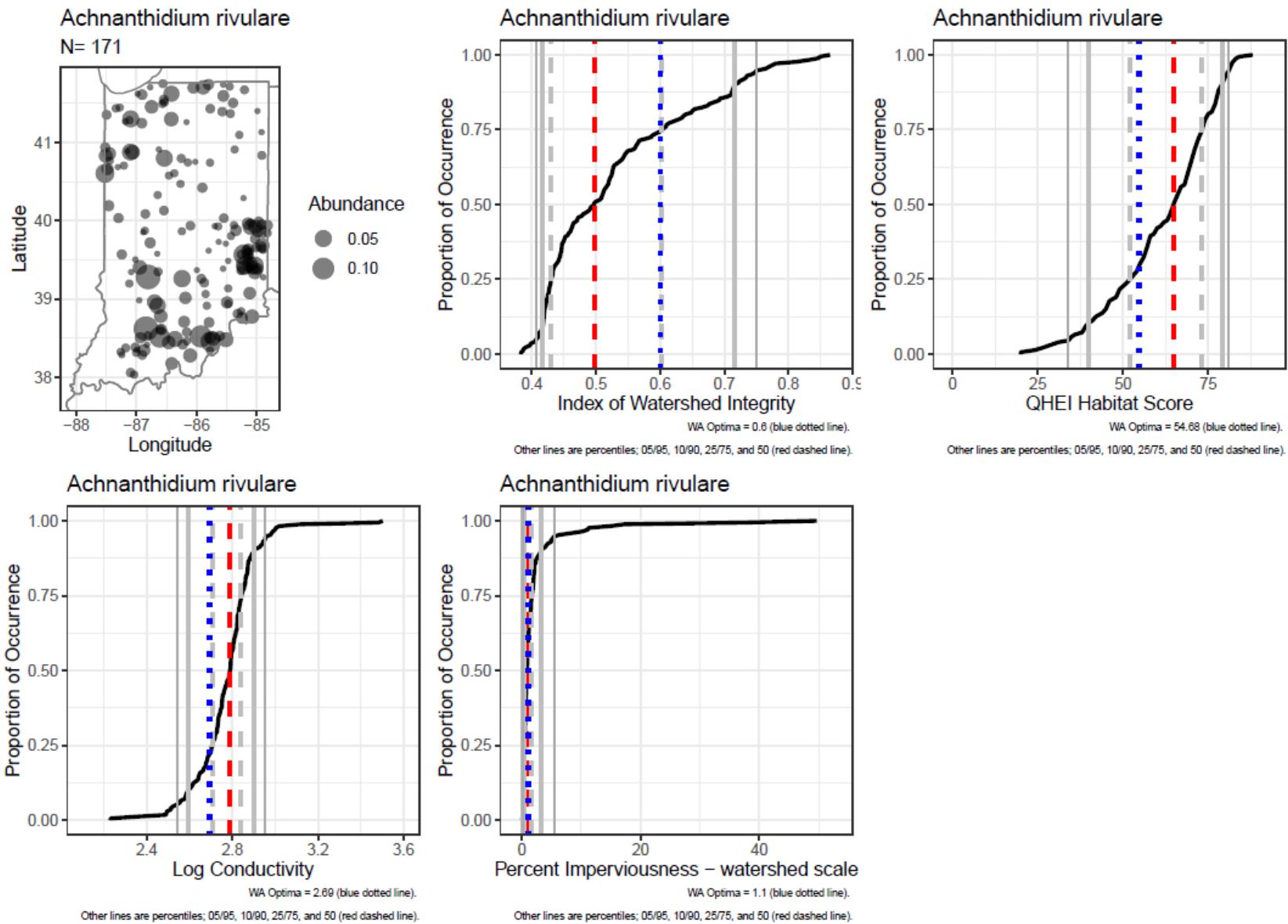


Figure B8. Example of a cumulative distribution function (CDF) plot. The blue vertical dashed line equals the weighted average optima value.

## **Interpretation of results**

The weighted averages of the four disturbance variables per taxon were summarized as averages and products of the tolerance scores. The product was calculated so that low values (tolerant characteristics) would be emphasized, whereas these might be overlooked in an average. From these summarized statistics, a final 10-point sensitivity rating was assigned. The rating was derived from the product score for taxa with sufficient occurrences (>25). For taxa with fewer occurrences, ratings were assigned with some subjectivity. A confidence estimate was included to identify taxa with uncertain (based on low low numbers of occurrences or estimated based on similar taxa (e.g., species tolerance ratings based on the genus-level rating). The confidence scale was from confident (4, with supportive analysis and high precision) to uncertain (1, estimated based on few occurrences, imprecise disturbance variables scores, or associations with genera). Some taxa showed differing sensitivities to the four disturbance variables. In these situations, a high variability was flagged and other factors were considered (e.g., confidence, genus-level results)

Biologists from IDEM reviewed the Excel worksheet and approved the preliminary sensitivity ratings. Sensitive taxa occurred mostly (and in higher relative abundance) at sites with the lowest levels of disturbance (Figure 1). Intermediate taxa were generally ubiquitous and most prevalent in the middle of the disturbance gradient. Tolerant taxa tended to occur throughout the stressor gradient and generally increased in relative abundance as stress levels increased.

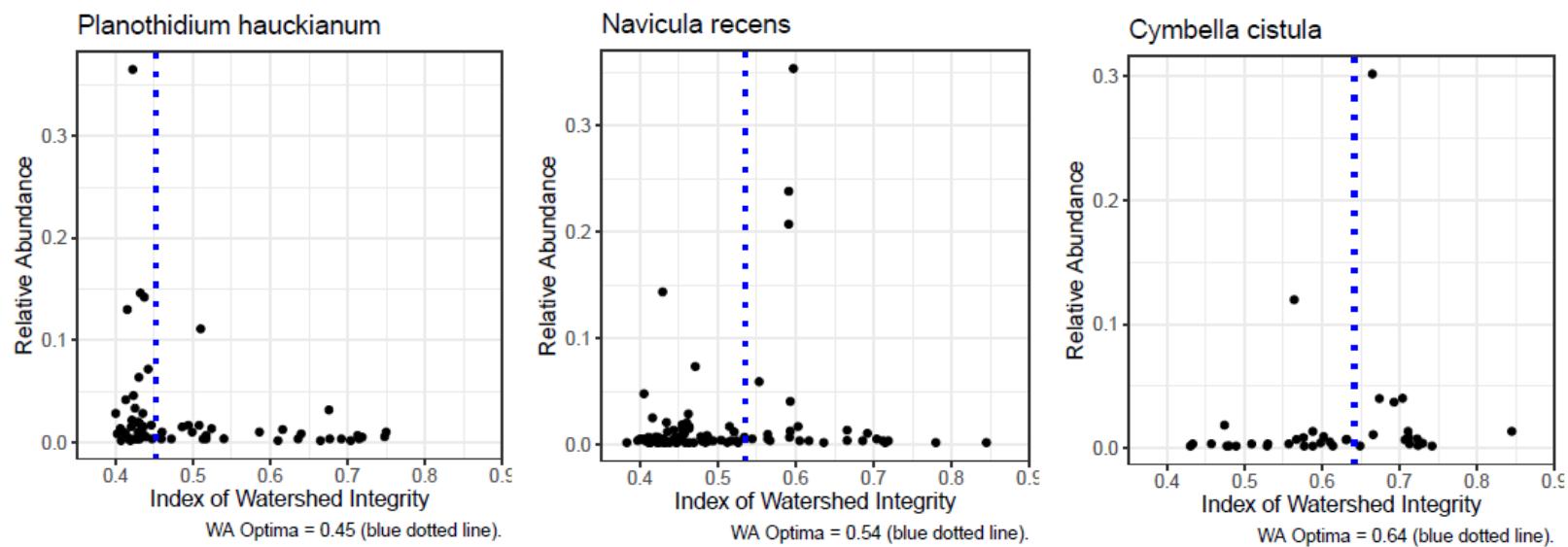


Figure 1. Examples of taxon-response patterns for taxa that were categorized as tolerant, intermediate tolerant and sensitive. The IWI scoring scale ranges from 0 (worst) to 1 (best).

## Results

Table 9 shows the number of taxa in each tolerance category, by family. Most taxa were placed in the intermediate group (257 of the 331 taxa that were assessed). The full set of results (including the plots and worksheet that the reviewers used) are available upon request. The final tolerance values along with other taxa traits are in Attachment [\\_\\_\\_\\_\\_](#).

*Table 9. (Preliminary) Distribution of taxa sensitivity scores by diatom family (1 most tolerant, 10 most sensitive).*

Family	Tolerant								Sensitive	Total	
	1	2	3	4	5	6	7	8	9	10	Taxa
Achnanthaceae	4	2	2	2	3		4	1	5	3	29
Achnanthidiaceae					1			1	2	10	14
Amphipleuraceae				2			1	1	2		6
Aulacoseiraceae	3		1			1					5
Bacillariaceae	3	7	8	22	4	4	3	3		4	58
Berkeleyaceae											3
Catenulaceae			1		2	4		1			8
Cymbellaceae					1	2		6		5	14
Diadesmidaceae	1	2	1		2				1		7
Diplogeiidae	1						1	3		2	7
Eunotiaceae						1	1	1			3
Fragilariaeae	3	3	3	2	5		4	2	1		25
Gomphonemataceae	1		2	1	3	2	3	1	3		19
Melosiraceae		1	1	1							3
Naviculaceae	2	5	6	13	5	2	7	4	6	2	52
Neidiaceae											2
Pinnulariaceae		1			1				6		8
Pleurosigmataceae					1	1	2		1		5
Rhoicospheniaceae				1	1	1				3	6
Rhopalodiaceae											4
Sellaphoraceae	2		1	2		3	3	2		2	15
Stauroneidaceae			1	1		2		3			7
Stephanodiscaceae	5	3					1	1			10
Surirellaceae		1		1		5	3	1			11
Thalassiosiraceae	1			1	4	1					7
Triceratiaceae											3
Totals	27	25	27	50	32	29	33	31	27	34	

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**Table \_\_\_. Taxa tolerance assignments.**

COMMON_NAME	TolNameFinal	Num Samps_TV	TolVal_IN	TV_Notes
Achnanthes amoena	Karayevia amoena			lt10samps
Achnanthes delicatula	Planothidium delicatulum	19	5	Based on Genus
Achnanthes elata	Achnanthes elata			lt10samps
Achnanthes exigua var. constricta	Achnanthidium exiguum	58	8	TV_analysis
Achnanthes exigua var. elliptica	Achnanthidium exiguum	58	8	TV_analysis
Achnanthes exilis	Achnanthidium exilis	10	10	Based on Genus
Achnanthes ploenensis var. gessneri	Karayevia ploenensis	36	1	TV_analysis
Achnanthes ricula	Achnanthes ricula	18	10	Based on Genus
Achnanthes sp.	Achnanthes	57	10	
Achnanthes subhudsonis	Achnanthes subhudsonis	20	9	Based on Genus
Achnanthes subhudsonis var. kraeuselii	Achnanthes subhudsonis	20	9	Based on Genus
Achnanthidium affine	Achnanthidium affine	23	10	Based on Genus
Achnanthidium alpestre	Achnanthidium alpestre			lt10samps
Achnanthidium altergracillima	Achnanthidium altergracillima	15	10	Based on Genus
Achnanthidium caledonicum	Achnanthidium caledonicum			lt10samps
Achnanthidium catenatum	Achnanthidium catenatum	14	10	Based on Genus
Achnanthidium deflexum	Achnanthidium deflexum	49	9	TV_analysis
Achnanthidium druartii	Achnanthidium druartii			lt10samps
Achnanthidium duthiei	Achnanthidium duthiei			lt10samps
Achnanthidium eutrophilum	Achnanthidium eutrophilum	49	10	TV_analysis
Achnanthidium exiguum	Achnanthidium exiguum	58	8	TV_analysis
Achnanthidium exilis	Achnanthidium exilis	10	10	Based on Genus
Achnanthidium latecephalum	Achnanthidium latecephalum	10	10	Based on Genus
Achnanthidium macrocephalum	Achnanthidium macrocephalum			lt10samps
Achnanthidium minutissimum	Achnanthidium minutissimum	371	9	TV_analysis
Achnanthidium pyrenaicum	Achnanthidium pyrenaicum	18	10	Based on Genus

COMMON_NAME	TolNameFinal	Num			
		Samps_TV	TolVal_IN	TV_Notes	
Achnanthidium rivulare	Achnanthidium rivulare	171	10	TV_analysis	
Achnanthidium saprophilum	Achnanthidium saprophilum	37	4	TV_analysis	
Achnanthidium sp.	Achnanthidium			lt10samps	
Adlafia bryophila	Adlafia bryophila			lt10samps	
Adlafia minuscula	Adlafia minuscula			lt10samps	
Adlafia suchlandtii	Adlafia suchlandtii			lt10samps	
Amphipleura pellucida	Amphipleura pellucida	39	9	TV_analysis	
Amphora conspicua	Amphora conspicua			lt10samps	
Amphora copulata	Amphora copulata	10	6	Based on Genus	
Amphora libyca	Amphora ovalis	184	3	TV_analysis	
Amphora ovalis	Amphora ovalis	184	3	TV_analysis	
Amphora pediculus	Amphora pediculus	342	6	TV_analysis	
Amphora sp.	Amphora	382	6		
Asterionella formosa	Asterionella formosa			lt10samps	
Aulacoseira alpigena	Aulacoseira alpigena	45	1	TV_analysis	
Aulacoseira ambigua	Aulacoseira ambigua	40	6	TV_analysis	
Aulacoseira crenulata	Aulacoseira italicica			lt10samps	
Aulacoseira granulata	Aulacoseira granulata	43	3	TV_analysis	
Aulacoseira granulata var. angustissima	Aulacoseira granulata	43	3	TV_analysis	
Aulacoseira italicica	Aulacoseira italicica			lt10samps	
Aulacoseira muzzanensis	Aulacoseira muzzanensis			lt10samps	
Aulacoseira sp.	Aulacoseira	93	1		
Aulacoseira subarctica	Aulacoseira subarctica			lt10samps	
Bacillaria paradoxa	Bacillaria paradoxa	43	4	TV_analysis	
Bacillariophyceae unknown sp.	Bacillariophyceae			lt10samps	
Biremis circumtexta	Biremis circumtexta			lt10samps	
Brachysira microcephala	Brachysira microcephala			lt10samps	
Brachysira vitrea	Brachysira vitrea			lt10samps	
Caloneis amphisbaena	Caloneis amphisbaena	19	2	Basis_ToleranceAnalysis	
Caloneis bacillum	Caloneis bacillum	180	9	TV_analysis	

COMMON_NAME	TolNameFinal	Num		
		Samps_TV	TolVal_IN	TV_Notes
<i>Caloneis schumanniana</i>	<i>Caloneis schumanniana</i>	32	9	TV_analysis
<i>Caloneis silicula</i>	<i>Caloneis silicula</i>	11	5	Basis_ToleranceAnalysis
<i>Caloneis sp.</i>	<i>Caloneis</i>	207	9	
<i>Capartogramma crucicula</i>	<i>Capartogramma crucicula</i>			lt10samps
<i>Cavinula coccineiformis</i>	<i>Cavinula coccineiformis</i>			lt10samps
<i>Cavinula scutiformis</i>	<i>Cavinula scutiformis</i>			lt10samps
<i>Chamaepinnularia mediocris</i>	<i>Chamaepinnularia mediocris</i>			lt10samps
<i>Chamaepinnularia soehrensis</i> var. <i>hassiacum</i>	<i>Chamaepinnularia soehrensis</i>			lt10samps
<i>Chamaepinnularia soehrensis</i> var. <i>muscicola</i>	<i>Chamaepinnularia soehrensis</i>			lt10samps
<i>Cocconeis fluviatilis</i>	<i>Cocconeis fluviatilis</i>			lt10samps
<i>Cocconeis neodiminuta</i>	<i>Cocconeis neodiminuta</i>	14	7	Basis_ToleranceAnalysis
<i>Cocconeis pediculus</i>	<i>Cocconeis pediculus</i>	199	4	TV_analysis
<i>Cocconeis placentula</i>	<i>Cocconeis placentula</i>	360	4	TV_analysis
<i>Cocconeis placentula</i> var. <i>euglypta</i>	<i>Cocconeis placentula</i>	360	4	TV_analysis
<i>Cocconeis placentula</i> var. <i>lineata</i>	<i>Cocconeis placentula</i>	360	4	TV_analysis
<i>Cocconeis pseudolineata</i>	<i>Cocconeis pseudolineata</i>	14	7	Basis_ToleranceAnalysis
<i>Cocconeis sp.</i>	<i>Cocconeis</i>	375	3	
<i>Cocconeis thumensis</i>	<i>Halamphora thumensis</i>			lt10samps
<i>construens</i> fo. var. <i>binodis</i>	<i>Staurosira construens</i>	129	1	TV_analysis
<i>Craticula accomoda</i>	<i>Craticula accomoda</i>			lt10samps
<i>Craticula ambigua</i>	<i>Craticula ambigua</i>			lt10samps
<i>Craticula citrus</i>	<i>Craticula citrus</i>	12	8	Based on Genus
<i>Craticula cuspidata</i>	<i>Craticula cuspidata</i>			lt10samps
<i>Craticula halophila</i>	<i>Craticula halophila</i>	10	8	Based on Genus
<i>Craticula halophiloides</i>	<i>Craticula halophiloides</i>			lt10samps
<i>Craticula minusculoides</i>	<i>Craticula minusculoides</i>			lt10samps
<i>Craticula molestiformis</i>	<i>Craticula molestiformis</i>	38	8	TV_analysis
<i>Craticula sp.</i>	<i>Craticula</i>	78	8	
<i>Craticula subminuscula</i>	<i>Navicula subminuscula</i>	202	2	TV_analysis
<i>Craticula submolesta</i>	<i>Craticula submolesta</i>			lt10samps

COMMON_NAME	TolNameFinal	Num		
		Samps_TV	TolVal_IN	TV_Notes
<i>Ctenophora pulchella</i>	<i>Ctenophora pulchella</i>	14		Uncertain
<i>Cyclostephanos invisitatus</i>	<i>Cyclostephanos invisitatus</i>	60	2	TV_analysis
<i>Cyclostephanos tholiformis</i>	<i>Cyclostephanos tholiformis</i>			lt10samps
<i>Cyclotella aliquantula</i>	<i>Cyclotella aliquantula</i>			lt10samps
<i>Cyclotella atomus</i>	<i>Cyclotella atomus</i>	65	1	TV_analysis
<i>Cyclotella distinguenda</i>	<i>Cyclotella distinguenda</i>			lt10samps
<i>Cyclotella hakanssoniae</i>	<i>Cyclotella hakanssoniae</i>			lt10samps
<i>Cyclotella meneghiniana</i>	<i>Cyclotella meneghiniana</i>	283	2	TV_analysis
<i>Cyclotella pseudostelligera</i>	<i>Cyclotella pseudostelligera</i>			lt10samps
<i>Cyclotella quillensis</i>	<i>Cyclotella quillensis</i>			lt10samps
<i>Cyclotella sp.</i>	<i>Cyclotella</i>	292	1	
<i>Cylindrotheca gracilis</i>	<i>Cylindrotheca gracilis</i>			lt10samps
<i>Cymatopleura elliptica</i>	<i>Cymatopleura elliptica</i>	11	7	Based on Genus
<i>Cymatopleura solea</i> var. <i>apiculata</i>	<i>Cymatopleura solea</i>	73	8	TV_analysis
<i>Cymbella aequalis</i>	<i>Encyonopsis aequalis</i>			lt10samps
<i>Cymbella affinis</i>	<i>Cymbella affinis</i>	84	10	TV_analysis
<i>Cymbella amphioxys</i>	<i>Cymbella amphioxys</i>			lt10samps
<i>Cymbella aspera</i>	<i>Cymbella aspera</i>			lt10samps
<i>Cymbella caespitosa</i>	<i>Encyonema caespitosum</i>			lt10samps
<i>Cymbella cistula</i>	<i>Cymbella cistula</i>	38	10	TV_analysis
<i>Cymbella cymbiformis</i> var. <i>nonpunctata</i>	<i>Cymbella cymbiformis</i>			lt10samps
<i>Cymbella delicatula</i>	<i>Cymbella delicatula</i>			lt10samps
<i>Cymbella hebridica</i>	<i>Encyonema hebridicum</i>			lt10samps
<i>Cymbella helvetica</i>	<i>Cymbella helvetica</i>			lt10samps
<i>Cymbella hustedtii</i>	<i>Cymbella hustedtii</i>			lt10samps
<i>Cymbella incerta</i>	<i>Cymbopleura incerta</i>			lt10samps
<i>Cymbella laevis</i>	<i>Cymbella laevis</i>			lt10samps
<i>Cymbella lapponica</i>	<i>Cymbopleura lapponica</i>			lt10samps
<i>Cymbella lata</i>	<i>Cymbopleura lata</i>			lt10samps
<i>Cymbella leptoceros</i>	<i>Cymbella leptoceros</i>			lt10samps

COMMON_NAME	TolNameFinal	Num		
		Samps_TV	TolVal_IN	TV_Notes
Cymbella mesiana	Cymbella mesiana			lt10samps
Cymbella mexicana	Cymbella mexicana			lt10samps
Cymbella minuta fo. latens	Encyonema latens			lt10samps
Cymbella parva	Cymbella parva			lt10samps
Cymbella pusilla	Cymbella pusilla			lt10samps
Cymbella sp.	Cymbella	168	10	
Cymbella subaequalis	Cymbella subaequalis			lt10samps
Cymbella tumida	Cymbella tumida	56	8	TV_analysis
Cymbella tumidula	Cymbella tumidula			lt10samps
Cymbella turgidula	Cymbella turgidula	13	10	Based on Genus
Cymbella ventricosum	Cymbella ventricosum			lt10samps
Cymbella vulgata	Cymbella vulgata			lt10samps
Cymbopleura amphicephala	Cymbopleura amphicephala			lt10samps
Cymbopleura naviculiformis	Cymbopleura naviculiformis			lt10samps
Cymbopleura subaequalis	Cymbopleura subaequalis			lt10samps
Delicata delicatula	Delicata delicatula			lt10samps
Denticula kuetzingii	Denticula kuetzingii	27	10	TV_analysis
Denticula sp.	Denticula	29	10	
Denticula tenuis	Denticula tenuis			lt10samps
Diadesmis confervacea	Diadesmis confervacea	65	5	TV_analysis
Diadesmis contenta	Diadesmis contenta	28	9	TV_analysis
Diadesmis gallica	Diadesmis gallica			lt10samps
Diadesmis laevissima	Diadesmis laevissima			lt10samps
Diadesmis perpusilla	Diadesmis perpusilla			lt10samps
Diatoma anceps	Diatoma anceps			lt10samps
Diatoma mesodon	Diatoma mesodon			lt10samps
Diatoma moniliformis	Diatoma moniliformis			lt10samps
Diatoma sp.	Diatoma	88	2	
Diatoma tenuis	Diatoma tenuis			lt10samps
Diatoma vulgaris	Diatoma vulgaris	81	3	TV_analysis

COMMON_NAME	TolNameFinal	Num		
		Samps_TV	TolVal_IN	TV_Notes
Didymosphenia geminata	Didymosphenia geminata			lt10samps
Diploneis elliptica	Diploneis elliptica	14	8	Based on Genus
Diploneis marginestriata	Diploneis marginestriata			lt10samps
Diploneis oblongella	Diploneis oblongella			lt10samps
Diploneis ovalis	Diploneis ovalis			lt10samps
Diploneis parma	Diploneis parma	17	1	Basis_ToleranceAnalysis
Diploneis peterseni	Diploneis peterseni	54	10	TV_analysis
Diploneis pseudovalvis	Diploneis pseudovalvis	41	10	TV_analysis
Diploneis puella	Diploneis puella	24	8	Based on Genus
Diploneis smithii	Diploneis smithii			lt10samps
Diploneis sp.	Diploneis	123	8	
Diploneis subovalis	Diploneis subovalis			lt10samps
Discostella asterocostata	Discostella asterocostata			lt10samps
Discostella pseudostelligera	Discostella pseudostelligera			lt10samps
Discostella stelligera	Discostella stelligera	49	5	TV_analysis
Encyonema brehmii	Encyonema brehmii			lt10samps
Encyonema minutum	Encyonema minutum	103	5	TV_analysis
Encyonema perpusillum	Encyonema perpusillum			lt10samps
Encyonema prostratum	Encyonema prostratum	10	6	Based on Genus
Encyonema silesiacum	Encyonema silesiacum	74	8	TV_analysis
Encyonema sp.	Encyonema	151	6	
Encyonema triangulum	Encyonema triangulum			lt10samps
Encyonopsis cesatii	Encyonopsis cesatii			lt10samps
Encyonopsis microcephala	Encyonopsis microcephala	24	8	Based on Genus
Entomoneis alata	Entomoneis alata			lt10samps
Entomoneis sp.	Entomoneis			lt10samps
Eolimna subbadnata	Eolimna subbadnata			lt10samps
Eolimna subminuscula	Navicula subminuscula	202	2	TV_analysis
Epithemia adnata	Epithemia adnata			lt10samps
Epithemia argus	Epithemia argus			lt10samps

COMMON_NAME	TolNameFinal	Num		
		Samps_TV	TolVal_IN	TV_Notes
<i>Epithemia sorex</i>	<i>Epithemia sorex</i>	10		Uncertain
<i>Epithemia sp.</i>	<i>Epithemia</i>	13		Uncertain
<i>Epithemia turgida</i>	<i>Epithemia turgida</i>			lt10samps
<i>Eunotia bilunaris</i>	<i>Eunotia bilunaris</i>	26	8	TV_analysis
<i>Eunotia boomsma</i>	<i>Eunotia boomsma</i>			lt10samps
<i>Eunotia circumborealis</i>	<i>Eunotia circumborealis</i>			lt10samps
<i>Eunotia cristagalli</i>	<i>Eunotia cristagalli</i>			lt10samps
<i>Eunotia diodon</i>	<i>Eunotia diodon</i>			lt10samps
<i>Eunotia exigua</i>	<i>Eunotia exigua</i>			lt10samps
<i>Eunotia formica</i>	<i>Eunotia formica</i>			lt10samps
<i>Eunotia implicata</i>	<i>Eunotia implicata</i>			lt10samps
<i>Eunotia intermedia</i>	<i>Eunotia intermedia</i>			lt10samps
<i>Eunotia monodon</i>	<i>Eunotia monodon</i>			lt10samps
<i>Eunotia naegelii</i>	<i>Eunotia naegelii</i>			lt10samps
<i>Eunotia nymanniana</i>	<i>Eunotia nymanniana</i>			lt10samps
<i>Eunotia paludosa</i>	<i>Eunotia paludosa</i>			lt10samps
<i>Eunotia pectinalis</i> var. <i>undulata</i>	<i>Eunotia pectinalis</i>			lt10samps
<i>Eunotia rabenhorstii</i>	<i>Eunotia rabenhorstii</i>			lt10samps
<i>Eunotia sp.</i>	<i>Eunotia</i>	53	7	
<i>Eunotia subarcuatoides</i>	<i>Eunotia subarcuatoides</i>			lt10samps
<i>Eunotia zygodon</i>	<i>Eunotia zygodon</i>			lt10samps
<i>Eutonia monodon</i>	<i>Eunotia monodon</i>			lt10samps
<i>Fallacia enigmatica</i>	<i>Fallacia enigmatica</i>			lt10samps
<i>Fallacia helensis</i>	<i>Navicula helensis</i>			lt10samps
<i>Fallacia insociabilis</i>	<i>Fallacia insociabilis</i>			lt10samps
<i>Fallacia lenzii</i>	<i>Fallacia lenzii</i>	73	10	TV_analysis
<i>Fallacia monoculata</i>	<i>Fallacia monoculata</i>	40	8	TV_analysis
<i>Fallacia omissa</i>	<i>Fallacia omissa</i>			lt10samps
<i>Fallacia pygmaea</i>	<i>Fallacia pygmaea</i>	15	7	Based on Genus
<i>Fallacia sp.</i>	<i>Fallacia</i>	232	7	

COMMON_NAME	TolNameFinal	Num			
		Samps_TV	TolVal_IN	TV_Notes	
<i>Fallacia subhamulata</i>	<i>Fallacia subhamulata</i>	100	6	TV_analysis	
<i>Fallacia tenera</i>	<i>Fallacia tenera</i>	118	4	TV_analysis	
<i>Fragilaria austriaca</i>	<i>Fragilaria austriaca</i>			lt10samps	
<i>Fragilaria capucina</i>	<i>Fragilaria capucina</i>	79	4	TV_analysis	
<i>Fragilaria capucina</i> var. <i>radians</i>	<i>Fragilaria radians</i>			lt10samps	
<i>Fragilaria capucina</i> var. <i>rumpens</i>	<i>Fragilaria rumpens</i>			lt10samps	
<i>Fragilaria crotonensis</i>	<i>Fragilaria crotonensis</i>			lt10samps	
<i>Fragilaria famelica</i>	<i>Synedra famelica</i>	24	5	Based on Genus	
<i>Fragilaria fasciculata</i>	<i>Tabularia fasciculata</i>			lt10samps	
<i>Fragilaria goulardii</i>	<i>Synedra goulardi</i>	21	5	Based on Genus	
<i>Fragilaria gracilis</i>	<i>Fragilaria gracilis</i>			lt10samps	
<i>Fragilaria mesolepta</i>	<i>Fragilaria capucina</i>	79	4	TV_analysis	
<i>Fragilaria pectinalis</i>	<i>Fragilaria pectinalis</i>			lt10samps	
<i>Fragilaria perminuta</i>	<i>Fragilaria perminuta</i>			lt10samps	
<i>Fragilaria radians</i>	<i>Fragilaria radians</i>			lt10samps	
<i>Fragilaria sepes</i>	<i>Fragilaria sepes</i>	32	7	TV_analysis	
<i>Fragilaria</i> sp.	<i>Fragilaria</i>	158	4		
<i>Fragilaria tenera</i>	<i>Fragilaria tenera</i>	14	7	Basis_ToleranceAnalysis	
<i>Fragilaria vaucheriae</i>	<i>Fragilaria vaucheriae</i>	57	2	TV_analysis	
<i>Fragilaria vaucheriae</i> var. <i>capitellata</i>	<i>Fragilaria vaucheriae</i>	57	2	TV_analysis	
<i>Fragilariforma constricta</i>	<i>Fragilariforma constricta</i>			lt10samps	
<i>Fragilariforma neoproducta</i>	<i>Fragilaria neoproducta</i>			lt10samps	
<i>Fragilariforma</i> sp.	<i>Fragilariforma</i>			lt10samps	
<i>Frustulia amphibleurooides</i>	<i>Frustulia amphibleurooides</i>			lt10samps	
<i>Frustulia crassinervia</i>	<i>Frustulia crassinervia</i>			lt10samps	
<i>Frustulia inculta</i>	<i>Frustulia inculta</i>			lt10samps	
<i>Frustulia latita</i>	<i>Frustulia latita</i>	13	4	Based on Genus	
<i>Frustulia rhomboides</i>	<i>Frustulia rhomboides</i>			lt10samps	
<i>Frustulia rhomboides</i> var. <i>capitata</i>	<i>Frustulia rhomboides</i>			lt10samps	
<i>Frustulia saxonica</i>	<i>Frustulia saxonica</i>			lt10samps	

COMMON_NAME	TolNameFinal	Num		
		Samps_TV	TolVal_IN	TV_Notes
Frustulia sp.	Frustulia	57	4	
Frustulia vulgaris	Frustulia vulgaris	26	7	TV_analysis
Frustulia weinholdii	Frustulia weinholdii			lt10samps
Geissleria acceptata	Geissleria acceptata			lt10samps
Geissleria decussis	Geissleria decussis	140	9	TV_analysis
Geissleria kriegeri	Geissleria kriegeri			lt10samps
Geissleria schoenfeldii	Geissleria schoenfeldii	18	4	Basis_ToleranceAnalysis
Geissleria sp.	Geissleria	156	8	
Gomphoneis herculeana	Gomphoneis herculeana			lt10samps
Gomphoneis herculeanum var. robusta	Gomphoneis herculeanum			lt10samps
Gomphoneis mammilla	Gomphoneis mammilla			lt10samps
Gomphoneis olivaceum	Gomphoneis olivaceum	51	5	TV_analysis
Gomphonema acuminatum	Gomphonema acuminatum			lt10samps
Gomphonema acuminatum var. brebissonii	Gomphonema acuminatum			lt10samps
Gomphonema acuminatum var. coronatum	Gomphonema coronatum			lt10samps
Gomphonema affine	Gomphonema affine	71	6	TV_analysis
Gomphonema americobtusatum	Gomphonema americobtusatum			lt10samps
Gomphonema angustatum	Gomphonema angustatum	82	3	TV_analysis
Gomphonema angustum	Gomphonema angustum			lt10samps
Gomphonema augur	Gomphonema augur			lt10samps
Gomphonema augur var. sphaerophorum	Gomphonema augur			lt10samps
Gomphonema augur var. turris	Gomphonema turris			lt10samps
Gomphonema capitatum	Gomphonema capitatum			lt10samps
Gomphonema cf. minutum	Gomphonema minutum	260	9	TV_analysis
Gomphonema clavatum	Gomphonema clavatum			lt10samps
Gomphonema exilissimum	Gomphonema exilissimum			lt10samps
Gomphonema gracile	Gomphonema gracile	44	6	TV_analysis
Gomphonema insigne	Gomphonema insigne	23	7	Based on Genus
Gomphonema italicum	Gomphonema italicum	10	7	Based on Genus
Gomphonema kobayasi	Gomphonema kobayasi	72	9	TV_analysis

COMMON_NAME	TolNameFinal	Num		
		Samps_TV	TolVal_IN	TV_Notes
Gomphonema lagenula	Gomphonema lagenula			lt10samps
Gomphonema micropus	Gomphonema micropus			lt10samps
Gomphonema minutum	Gomphonema minutum	260	9	TV_analysis
Gomphonema olivaceoides	Gomphonema olivaceoides	18	4	Basis_ToleranceAnalysis
Gomphonema pala	Gomphonema pala			lt10samps
Gomphonema parvulum	Gomphonema parvulum	208	5	TV_analysis
Gomphonema productum	Gomphonema productum			lt10samps
Gomphonema pumilum	Gomphonema pumilum	50	5	TV_analysis
Gomphonema rhombicum	Gomphonema rhombicum			lt10samps
Gomphonema sarcophagus	Gomphonema sarcophagus			lt10samps
Gomphonema sp.	Gomphonema	369	7	
Gomphonema sphaerophorum	Gomphonema sphaerophorum			lt10samps
Gomphonema subclavatum	Gomphonema subclavatum	37	10	TV_analysis
Gomphonema tenellum	Gomphonema tenellum			lt10samps
Gomphonema truncatum	Gomphonema truncatum	14	1	Basis_ToleranceAnalysis
Gomphonema turris	Gomphonema turris			lt10samps
Gomphonitzschia sp.	Gomphonitzschia			lt10samps
Gomphosphenia grovei	Gomphosphenia grovei	24	10	Based on Genus
Gomphosphenia grovei var. lingulata	Gomphosphenia grovei	24	10	Based on Genus
Gomphosphenia lingulatiformis	Gomphosphenia lingulatiformis	18	10	Based on Genus
Gomphosphenia sp.	Gomphosphenia			lt10samps
Gyrosigma acuminatum	Gyrosigma acuminatum	143	9	TV_analysis
Gyrosigma attenuatum	Gyrosigma attenuatum	29	5	TV_analysis
Gyrosigma nodiferum	Gyrosigma nodiferum			lt10samps
Gyrosigma reimeri	Gyrosigma reimeri			lt10samps
Gyrosigma scalproides	Gyrosigma scalproides	102	7	TV_analysis
Gyrosigma sp.	Gyrosigma	198	7	
Gyrosigma spencerii	Gyrosigma acuminatum	143	9	TV_analysis
Halamphora montana	Amphora montana	176	8	TV_analysis
Halamphora sp.	Halamphora	41	6	

COMMON_NAME	TolNameFinal	Num		
		Samps_TV	TolVal_IN	TV_Notes
<i>Halamphora veneta</i>	<i>Halamphora veneta</i>	40	5	TV_analysis
<i>Hannaea arcus</i>	<i>Hannaea arcus</i>			lt10samps
<i>Hantzschia amphioxys</i>	<i>Hantzschia amphioxys</i>	22	5	Based on Genus
<i>Hantzschia distinctepunctata</i>	<i>Hantzschia distinctepunctata</i>			lt10samps
<i>Hantzschia elongata</i>	<i>Hantzschia elongata</i>			lt10samps
<i>Hippodonta capitata</i>	<i>Hippodonta capitata</i>	120	4	TV_analysis
<i>Hippodonta hungarica</i>	<i>Hippodonta hungarica</i>	41	4	TV_analysis
<i>Hippodonta lueneburgensis</i>	<i>Hippodonta lueneburgensis</i>	12	3	Based on Genus
<i>Hippodonta</i> sp.	<i>Hippodonta</i>	139	3	
<i>Karayevia amoena</i>	<i>Karayevia amoena</i>			lt10samps
<i>Karayevia clevei</i>	<i>Karayevia clevei</i>	38	10	TV_analysis
<i>Karayevia laterostrata</i>	<i>Karayevia laterostrata</i>			lt10samps
<i>Karayevia nitidiformis</i>	<i>Karayevia nitidiformis</i>			lt10samps
<i>Karayevia ploenensis</i>	<i>Karayevia ploenensis</i>	36	1	TV_analysis
<i>Karayevia ploenensis</i> var. <i>gessneri</i>	<i>Karayevia ploenensis</i>	36	1	TV_analysis
<i>Karayevia suchlandtii</i>	<i>Karayevia suchlandtii</i>			lt10samps
<i>Kobayasiella subtilissima</i>	<i>Kobayasiella subtilissima</i>			lt10samps
<i>Lemnicola hungarica</i>	<i>Lemnicola hungarica</i>	11		Uncertain
<i>Lindavia ocellata</i>	<i>Cyclotella ocellata</i>	33	7	TV_analysis
<i>Luticola goeppertiana</i>	<i>Luticola goeppertiana</i>	42	2	TV_analysis
<i>Luticola mutica</i>	<i>Luticola mutica</i>	36	1	TV_analysis
<i>Luticola ventricosa</i>	<i>Luticola ventricosa</i>			lt10samps
<i>Martyana atomus</i>	<i>Martyana atomus</i>			lt10samps
<i>Mastogloia elliptica</i>	<i>Mastogloia elliptica</i>			lt10samps
<i>Mayamaea atomus</i>	<i>Mayamaea atomus</i>	47	7	TV_analysis
<i>Mayamaea cahabaensis</i>	<i>Mayamaea cahabaensis</i>			lt10samps
<i>Mayamaea permitis</i>	<i>Mayamaea permitis</i>	149	8	TV_analysis
<i>Mayamaea</i> sp.	<i>Mayamaea</i>	164	9	
<i>Melosira varians</i>	<i>Melosira varians</i>	289	4	TV_analysis
<i>Meridion circulare</i>	<i>Meridion circulare</i>	33	8	TV_analysis

COMMON_NAME	TolNameFinal	Num		
		Samps_TV	TolVal_IN	TV_Notes
Meridion circulare var. constrictum	Meridion circulare	33	8	TV_analysis
Meridion sp.	Meridion	33	7	
Navicula aboensis	Navicula aboensis			lt10samps
Navicula aikenensis	Geissleria aikenensis			lt10samps
Navicula amphiceropsis	Navicula amphiceropsis	155	5	TV_analysis
Navicula angusta	Navicula angusta			lt10samps
Navicula antonii	Navicula antonii	39	9	TV_analysis
Navicula arvensis	Navicula arvensis	13	9	Basis_ToleranceAnalysis
Navicula biconica	Craticula molestiformis	38	8	TV_analysis
Navicula canalis	Navicula canalis	122	7	TV_analysis
Navicula capitata var. lueneburgensis	Hippodonta lueneburgensis	12	3	Based on Genus
Navicula capitoradiata	Navicula capitoradiata	207	5	TV_analysis
Navicula cari	Navicula cari	23	7	Basis_ToleranceAnalysis
Navicula cincta	Navicula cincta	29	3	TV_analysis
Navicula cincta var. minuta	Navicula cincta	29	3	TV_analysis
Navicula concentrica	Navicula concentrica			lt10samps
Navicula constans var. symmetrica	Placoneis symmetrica			lt10samps
Navicula contenta	Humidophila contenta			lt10samps
Navicula cryptocephala	Navicula cryptocephala	89	7	TV_analysis
Navicula cryptotenella	Navicula cryptotenella	351	7	TV_analysis
Navicula cryptotenelloides	Navicula cryptotenelloides	54	10	TV_analysis
Navicula difficillima	Navicula difficillima	72	8	TV_analysis
Navicula difficillimoides	Navicula difficillimoides			lt10samps
Navicula digitulus	Navicula digitulus			lt10samps
Navicula diluviana	Cymbellafalsa diluviana			lt10samps
Navicula duerrenbergiana	Navicula duerrenbergiana			lt10samps
Navicula eidrigiana	Navicula eidrigiana			lt10samps
Navicula erifuga	Navicula erifuga	189	1	TV_analysis
Navicula escambia	Navicula escambia			lt10samps
Navicula exigua	Placoneis exigua			lt10samps

COMMON_NAME	TolNameFinal	Num		
		Samps_TV	TolVal_IN	TV_Notes
<i>Navicula exigua</i> var. <i>signata</i>	<i>Navicula exigua</i>			lt10samps
<i>Navicula exilis</i>	<i>Navicula exilis</i>			lt10samps
<i>Navicula gallica</i>	<i>Diadesmis gallica</i>			lt10samps
<i>Navicula gerloffii</i>	<i>Navicula gerloffii</i>			lt10samps
<i>Navicula germainii</i>	<i>Navicula germainii</i>	313	7	TV_analysis
<i>Navicula gibbosa</i>	<i>Navicula gibbosa</i>			lt10samps
<i>Navicula gregaria</i>	<i>Navicula gregaria</i>	279	2	TV_analysis
<i>Navicula incertata</i>	<i>Navicula incertata</i>			lt10samps
<i>Navicula indifferens</i>	<i>Fallacia indifferens</i>			lt10samps
<i>Navicula ingenua</i>	<i>Navicula ingenua</i>	48	2	TV_analysis
<i>Navicula insociabilis</i>	<i>Fallacia insociabilis</i>			lt10samps
<i>Navicula integra</i>	<i>Prestauroneis integra</i>			lt10samps
<i>Navicula kotschyi</i>	<i>Navicula kotschyi</i>	44	4	TV_analysis
<i>Navicula kriegeri</i>	<i>Geissleria kriegeri</i>			lt10samps
<i>Navicula lacuum</i>	<i>Navicula lacuum</i>			lt10samps
<i>Navicula lanceolata</i>	<i>Navicula lanceolata</i>	38	6	TV_analysis
<i>Navicula latens</i>	<i>Geissleria thingvallae</i>			lt10samps
<i>Navicula lateropunctata</i>	<i>Geissleria lateropunctata</i>			lt10samps
<i>Navicula laterostrata</i>	<i>Navicula laterostrata</i>			lt10samps
<i>Navicula lenzii</i>	<i>Fallacia lenzii</i>	73	10	TV_analysis
<i>Navicula leptostriata</i>	<i>Navicula leptostriata</i>			lt10samps
<i>Navicula libonensis</i>	<i>Navicula libonensis</i>	16	4	Based on Genus
<i>Navicula longicephala</i>	<i>Navicula longicephala</i>			lt10samps
<i>Navicula longicephala</i> var. <i>vilaplanii</i>	<i>Navicula vilaplanii</i>			lt10samps
<i>Navicula lundii</i>	<i>Navicula lundii</i>			lt10samps
<i>Navicula menisculus</i>	<i>Navicula menisculus</i>	179	3	TV_analysis
<i>Navicula menisculus</i> v. <i>upsaliensis</i>	<i>Navicula upsaliensis</i>			lt10samps
<i>Navicula menisculus</i> var. <i>obtusa</i>	<i>Navicula menisculus</i>	179	3	TV_analysis
<i>Navicula meniscus</i>	<i>Navicula meniscus</i>	13	4	Based on Genus
<i>Navicula microcephala</i>	<i>Navicula microcephala</i>			lt10samps

COMMON_NAME	TolNameFinal	Num		
		Samps_TV	TolVal_IN	TV_Notes
Navicula minima	Eolimna minima	351	5	TV_analysis
Navicula minima var. pseudofossilis	Navicula minima	10	4	Based on Genus
Navicula notha	Navicula notha	18	8	Basis_ToleranceAnalysis
Navicula perminuta	Navicula perminuta			lt10samps
Navicula permitis	Mayamaea permitis	149	8	TV_analysis
Navicula perpusilla	Humidophila perpusilla			lt10samps
Navicula phyllepta	Navicula phyllepta	27	10	TV_analysis
Navicula pseudoventralis	Navicula pseudoventralis			lt10samps
Navicula radiosa	Navicula radiosa			lt10samps
Navicula recens	Navicula recens	97	2	TV_analysis
Navicula recta	Navicula recta			lt10samps
Navicula reichardtiana	Navicula reichardtiana	135	5	TV_analysis
Navicula reinhardtii	Navicula reinhardtii			lt10samps
Navicula rhynchocephala	Navicula rhynchocephala			lt10samps
Navicula rostellata	Navicula rostellata	250	3	TV_analysis
Navicula ruttneri var. rostrata	Stauroneis rostrata			lt10samps
Navicula salinarum	Navicula salinarum			lt10samps
Navicula salinicola	Navicula salinicola	14	4	Based on Genus
Navicula sanctaecrucis	Navicula sanctaecrucis			lt10samps
Navicula schadei	Navicula schadei	47	9	TV_analysis
Navicula schroeteri var. escambia	Navicula escambia			lt10samps
Navicula seminulum var. intermedia	Navicula seminulum			lt10samps
Navicula sp.	Navicula	409	4	
Navicula stroemii	Navicula stroemii			lt10samps
Navicula sublucidula	Navicula sublucidula			lt10samps
Navicula submolesta	Craticula submolesta			lt10samps
Navicula submuralis	Navicula submuralis	28	9	TV_analysis
Navicula subplacentula	Placoneis subplacentula			lt10samps
Navicula subrotundata	Navicula subrotundata			lt10samps
Navicula subtilissima	Kobayasiella subtilissima			lt10samps

COMMON_NAME	TolNameFinal	Num		
		Samps_TV	TolVal_IN	TV_Notes
<i>Navicula symmetrica</i>	<i>Navicula symmetrica</i>	211	5	TV_analysis
<i>Navicula tantula</i>	<i>Navicula tantula</i>	146	2	TV_analysis
<i>Navicula tenelloides</i>	<i>Navicula tenelloides</i>	125	3	TV_analysis
<i>Navicula terminata</i>	<i>Luticola terminata</i>			lt10samps
<i>Navicula thienemannii</i>	<i>Navicula thienemannii</i>			lt10samps
<i>Navicula tripunctata</i>	<i>Navicula tripunctata</i>	227	6	TV_analysis
<i>Navicula tripunctata</i> var. <i>schizonemoides</i>	<i>Navicula gracilis</i>			lt10samps
<i>Navicula trivialis</i>	<i>Navicula trivialis</i>	134	4	TV_analysis
<i>Navicula veneta</i>	<i>Navicula veneta</i>	120	1	TV_analysis
<i>Navicula ventralis</i>	<i>Psammothidium ventralis</i>			lt10samps
<i>Navicula vilaplanii</i>	<i>Navicula vilaplanii</i>			lt10samps
<i>Navicula viridula</i>	<i>Navicula viridula</i>	12	4	Based on Genus
<i>Navicula viridulacalcis</i>	<i>Navicula viridulacalcis</i>	67	7	TV_analysis
<i>Navicula vitabunda</i>	<i>Navicula vitabunda</i>			lt10samps
<i>Navicula wildii</i>	<i>Navicula wildii</i>			lt10samps
<i>Neidiomorpha binodiformis</i>	<i>Neidiomorpha binodiformis</i>			lt10samps
<i>Neidium affine</i>	<i>Neidium affine</i>			lt10samps
<i>Neidium binode</i>	<i>Neidium binodis</i>			lt10samps
<i>Neidium binodis</i>	<i>Neidium binodis</i>			lt10samps
<i>Neidium dubium</i>	<i>Neidium dubium</i>			lt10samps
<i>Neidium iridis</i>	<i>Neidium iridis</i>			lt10samps
<i>Neidium</i> sp.	<i>Neidium</i>	20		Uncertain
<i>Nitzschia accomodata</i>	<i>Nitzschia accomodata</i>			lt10samps
<i>Nitzschia acicularis</i>	<i>Nitzschia acicularis</i>	88	7	TV_analysis
<i>Nitzschia acidoclinata</i>	<i>Nitzschia acidoclinata</i>			lt10samps
<i>Nitzschia aequorea</i>	<i>Nitzschia aequorea</i>			lt10samps
<i>Nitzschia agnita</i>	<i>Nitzschia agnita</i>	81	3	TV_analysis
<i>Nitzschia amphibia</i>	<i>Nitzschia amphibia</i>	295	6	TV_analysis
<i>Nitzschia amphibia</i> var. <i>frauenfeldii</i>	<i>Nitzschia amphibia</i>	295	6	TV_analysis
<i>Nitzschia amphibioides</i>	<i>Nitzschia amphibioides</i>			lt10samps

COMMON_NAME	TolNameFinal	Num		
		Samps_TV	TolVal_IN	TV_Notes
<i>Nitzschia angustata</i>	<i>Nitzschia angustata</i>	37	10	TV_analysis
<i>Nitzschia angustatula</i>	<i>Nitzschia angustatula</i>	44	3	TV_analysis
<i>Nitzschia archibaldii</i>	<i>Nitzschia archibaldii</i>			lt10samps
<i>Nitzschia aurariae</i>	<i>Nitzschia aurariae</i>			lt10samps
<i>Nitzschia bacillum</i>	<i>Nitzschia bacillum</i>	12	4	Based on Genus
<i>Nitzschia biacrula</i>	<i>Nitzschia biacrula</i>	16	4	Based on Genus
<i>Nitzschia brevissima</i>	<i>Nitzschia brevissima</i>			lt10samps
<i>Nitzschia capitellata</i>	<i>Nitzschia capitellata</i>	62	8	TV_analysis
<i>Nitzschia clausii</i>	<i>Nitzschia clausii</i>	22	8	Basis_ToleranceAnalysis
<i>Nitzschia closterium</i>	<i>Nitzschia closterium</i>			lt10samps
<i>Nitzschia columbiana</i>	<i>Nitzschia columbiana</i>			lt10samps
<i>Nitzschia communis</i>	<i>Nitzschia communis</i>	14	4	Based on Genus
<i>Nitzschia compressa</i>	<i>Tryblionella compressa</i>			lt10samps
<i>Nitzschia compressa</i> var. <i>balatonis</i>	<i>Tryblionella balatonis</i>	13	7	Basis_ToleranceAnalysis
<i>Nitzschia debilis</i>	<i>Tryblionella debilis</i>	23	5	Basis_ToleranceAnalysis
<i>Nitzschia desertorum</i>	<i>Nitzschia desertorum</i>	20	4	Based on Genus
<i>Nitzschia dissipata</i>	<i>Nitzschia dissipata</i>	283	6	TV_analysis
<i>Nitzschia dissipata</i> var. <i>media</i>	<i>Nitzschia dissipata</i>	283	6	TV_analysis
<i>Nitzschia diversa</i>	<i>Nitzschia diversa</i>			lt10samps
<i>Nitzschia draveillensis</i>	<i>Nitzschia draveillensis</i>			lt10samps
<i>Nitzschia dubia</i>	<i>Nitzschia dubia</i>			lt10samps
<i>Nitzschia elegans</i>	<i>Nitzschia elegans</i>			lt10samps
<i>Nitzschia elegantula</i>	<i>Nitzschia elegantula</i>			lt10samps
<i>Nitzschia filiformis</i>	<i>Nitzschia filiformis</i>	21	4	Based on Genus
<i>Nitzschia filiformis</i> var. <i>conferta</i>	<i>Nitzschia filiformis</i>	21	4	Based on Genus
<i>Nitzschia fonticola</i>	<i>Nitzschia fonticola</i>	112	3	TV_analysis
<i>Nitzschia frustulum</i>	<i>Nitzschia frustulum</i>	233	4	TV_analysis
<i>Nitzschia graciliformis</i>	<i>Nitzschia graciliformis</i>	12	4	Based on Genus
<i>Nitzschia gracilis</i>	<i>Nitzschia gracilis</i>	41	2	TV_analysis
<i>Nitzschia hantzschiana</i>	<i>Nitzschia hantzschiana</i>			lt10samps

COMMON_NAME	TolNameFinal	Num		
		Samps_TV	TolVal_IN	TV_Notes
<i>Nitzschia heufleriana</i>	<i>Nitzschia heufleriana</i>	58	7	TV_analysis
<i>Nitzschia hungarica</i>	<i>Tryblionella hungarica</i>	56	2	TV_analysis
<i>Nitzschia inconspicua</i>	<i>Nitzschia inconspicua</i>	315	4	TV_analysis
<i>Nitzschia innominata</i>	<i>Nitzschia innominata</i>			lt10samps
<i>Nitzschia intermedia</i>	<i>Nitzschia intermedia</i>	27	4	TV_analysis
<i>Nitzschia lacuum</i>	<i>Nitzschia lacuum</i>	43	1	TV_analysis
<i>Nitzschia laevis</i>	<i>Nitzschia laevis</i>			lt10samps
<i>Nitzschia lanceola</i> var. <i>minutula</i>	<i>Nitzschia lanceola</i>			lt10samps
<i>Nitzschia lanceolata</i>	<i>Nitzschia lanceolata</i>	23	4	Based on Genus
<i>Nitzschia lanceolata</i> fo. <i>minima</i>	<i>Nitzschia lanceolata</i>	23	4	Based on Genus
<i>Nitzschia levidensis</i> var. <i>victoriae</i>	<i>Tryblionella victoriae</i>	81	8	TV_analysis
<i>Nitzschia liebetherthii</i>	<i>Nitzschia liebetherthii</i>	92	4	TV_analysis
<i>Nitzschia linearis</i>	<i>Nitzschia linearis</i>	60	3	TV_analysis
<i>Nitzschia linearis</i> var. <i>subtilis</i>	<i>Nitzschia subtilis</i>			lt10samps
<i>Nitzschia littoralis</i>	<i>Tryblionella littoralis</i>			lt10samps
<i>Nitzschia microcephala</i>	<i>Nitzschia microcephala</i>	49	1	TV_analysis
<i>Nitzschia minuta</i>	<i>Nitzschia minuta</i>			lt10samps
<i>Nitzschia nana</i>	<i>Nitzschia nana</i>	12	4	Based on Genus
<i>Nitzschia palea</i>	<i>Nitzschia palea</i>	362	5	TV_analysis
<i>Nitzschia palea</i> var. <i>debilis</i>	<i>Nitzschia palea</i>	362	5	TV_analysis
<i>Nitzschia palea</i> var. <i>tenuirostris</i>	<i>Nitzschia palea</i>	362	5	TV_analysis
<i>Nitzschia paleacea</i>	<i>Nitzschia paleacea</i>	92	3	TV_analysis
<i>Nitzschia paleiformis</i>	<i>Nitzschia paleiformis</i>	20	4	Based on Genus
<i>Nitzschia perminuta</i>	<i>Nitzschia perminuta</i>			lt10samps
<i>Nitzschia perspicua</i>	<i>Nitzschia perspicua</i>			lt10samps
<i>Nitzschia pseudofonticola</i>	<i>Nitzschia pseudofonticola</i>			lt10samps
<i>Nitzschia pumila</i>	<i>Nitzschia pumila</i>			lt10samps
<i>Nitzschia pura</i>	<i>Nitzschia pura</i>			lt10samps
<i>Nitzschia pusilla</i>	<i>Nitzschia pusilla</i>	16	4	Based on Genus
<i>Nitzschia radicula</i>	<i>Nitzschia radicula</i>	40	2	TV_analysis

COMMON_NAME	TolNameFinal	Num			
		Samps_TV	TolVal_IN	TV_Notes	
<i>Nitzschia recta</i>	<i>Nitzschia recta</i>	147	6	TV_analysis	
<i>Nitzschia reversa</i>	<i>Nitzschia reversa</i>	13	4	Based on Genus	
<i>Nitzschia rostellata</i>	<i>Nitzschia rostellata</i>			lt10samps	
<i>Nitzschia scalaris</i>	<i>Tryblionella scalaris</i>			lt10samps	
<i>Nitzschia sigma</i>	<i>Nitzschia sigma</i>	20	4	Based on Genus	
<i>Nitzschia sigmoidea</i>	<i>Nitzschia sigmoidea</i>			lt10samps	
<i>Nitzschia siliqua</i>	<i>Nitzschia siliqua</i>	20	4	Based on Genus	
<i>Nitzschia sinuata var. delognei</i>	<i>Nitzschia sinuata</i>	52	10	TV_analysis	
<i>Nitzschia sinuata var. tabellaria</i>	<i>Nitzschia sinuata</i>	52	10	TV_analysis	
<i>Nitzschia sociabilis</i>	<i>Nitzschia sociabilis</i>	141	6	TV_analysis	
<i>Nitzschia solita</i>	<i>Nitzschia solita</i>			lt10samps	
<i>Nitzschia sp.</i>	<i>Nitzschia</i>	409	4		
<i>Nitzschia subacicularis</i>	<i>Nitzschia subacicularis</i>	14	4	Based on Genus	
<i>Nitzschia subcapitellata</i>	<i>Nitzschia subcapitellata</i>			lt10samps	
<i>Nitzschia suchlandti</i>	<i>Nitzschia suchlandti</i>			lt10samps	
<i>Nitzschia supralitorea</i>	<i>Nitzschia supralitorea</i>			lt10samps	
<i>Nitzschia tropica</i>	<i>Nitzschia tropica</i>			lt10samps	
<i>Nitzschia tubicola</i>	<i>Nitzschia tubicola</i>			lt10samps	
<i>Nitzschia umbonata</i>	<i>Nitzschia umbonata</i>			lt10samps	
<i>Nitzschia valdecostata</i>	<i>Nitzschia valdecostata</i>	15	4	Based on Genus	
<i>Nitzschia vermicularis</i>	<i>Nitzschia vermicularis</i>			lt10samps	
<i>Nitzschia wuellerstorffii</i>	<i>Nitzschia wuellerstorffii</i>			lt10samps	
<i>Nupela neglecta</i>	<i>Nupela neglecta</i>			lt10samps	
<i>Nupela silvahercynia</i>	<i>Nupela silvahercynia</i>			lt10samps	
<i>Nupela sp.</i>	<i>Nupela</i>	11		Uncertain	
<i>Nupela subrostrata</i>	<i>Nupela subrostrata</i>			lt10samps	
<i>Parlibellus crucicula</i>	<i>Parlibellus crucicula</i>	16		Uncertain	
<i>Parlibellus protracta</i>	<i>Parlibellus protracta</i>			lt10samps	
<i>Parlibellus protractoides</i>	<i>Parlibellus protractoides</i>			lt10samps	
<i>Parlibellus sp.</i>	<i>Parlibellus</i>	24		Uncertain	

COMMON_NAME	TolNameFinal	Num		
		Samps_TV	TolVal_IN	TV_Notes
Pinnularia appendiculata	Pinnularia appendiculata			lt10samps
Pinnularia braunii	Pinnularia braunii			lt10samps
Pinnularia gibba	Pinnularia gibba			lt10samps
Pinnularia lundii	Pinnularia lundii			lt10samps
Pinnularia mesogongyla	Pinnularia mesogongyla			lt10samps
Pinnularia microstauron	Pinnularia microstauron			lt10samps
Pinnularia parvulissima	Pinnularia parvulissima			lt10samps
Pinnularia saprophila	Pinnularia saprophila			lt10samps
Pinnularia sp.	Pinnularia	41	9	
Pinnularia subcapitata	Pinnularia subcapitata	15	9	Based on Genus
Pinnularia viridis	Pinnularia viridis			lt10samps
Placoneis clementioides	Placoneis clementioides			lt10samps
Placoneis clementis	Placoneis clementis			lt10samps
Placoneis elginensis	Placoneis elginensis	10	8	Based on Genus
Placoneis exigua	Placoneis exigua			lt10samps
Placoneis explanata	Placoneis explanata			lt10samps
Placoneis gastrum	Placoneis gastrum			lt10samps
Placoneis placentula	Placoneis placentula			lt10samps
Placoneis pseudanglica	Placoneis pseudanglica			lt10samps
Placoneis sp.	Placoneis	38	8	
Plagiotropis lepidoptera	Plagiotropis lepidoptera			lt10samps
Plagiotropis lepidoptera var. proboscidea	Plagiotropis lepidoptera			lt10samps
Planothidium apiculatum	Planothidium apiculatum			lt10samps
Planothidium daui	Planothidium daui	26	7	TV_analysis
Planothidium delicatulum	Planothidium delicatulum	19	5	Based on Genus
Planothidium dubium	Planothidium dubium	57	9	TV_analysis
Planothidium engelbrechtii	Planothidium engelbrechtii			lt10samps
Planothidium frequentissimum	Planothidium frequentissimum	243	8	TV_analysis
Planothidium granum	Planothidium granum			lt10samps
Planothidium hauckianum	Planothidium hauckianum	64	1	TV_analysis

COMMON_NAME	TolNameFinal	Num		
		Samps_TV	TolVal_IN	TV_Notes
Planothidium lanceolatum	Planothidium lanceolatum	181	7	TV_analysis
Planothidium robustum	Planothidium robustum			lt10samps
Planothidium rostratum	Planothidium rostratum	54	3	TV_analysis
Planothidium sp.	Planothidium	336	5	
Platessa bahlsii	Platessa bahlsii	18	2	Based on Genus
Platessa conspicua	Platessa conspicua	43	1	TV_analysis
Pleurosigma angulatum	Pleurosigma angulatum			lt10samps
Pleurosira laevis	Pleurosira laevis	13		Uncertain
Pleurosira sp.	Pleurosira	14		Uncertain
Psammothidium bioretti	Psammothidium bioretii			lt10samps
Psammothidium chlidanos	Psammothidium chlidanos			lt10samps
Psammothidium daonense	Psammothidium daonense			lt10samps
Psammothidium grischunum	Psammothidium grischunum			lt10samps
Psammothidium helveticum	Psammothidium helveticum			lt10samps
Psammothidium lauenburgianum	Psammothidium lauenburgianum	19	9	Based on Genus
Psammothidium sp.	Psammothidium			lt10samps
Psammothidium subatomoides	Psammothidium subatomoides	21	9	Based on Genus
Psammothidium ventralis	Psammothidium ventralis			lt10samps
Pseudostaurosira brevistriata	Pseudostaurosira brevistriata	40	7	TV_analysis
Pseudostaurosira parasitica	Pseudostaurosira parasitica	30	5	TV_analysis
Pseudostaurosira parasitica var. constricta	Pseudostaurosira parasitica	30	5	TV_analysis
Pseudostaurosira parasitica var. subconstricta	Pseudostaurosira parasitica	30	5	TV_analysis
Pseudostaurosira sp.	Pseudostaurosira			lt10samps
Pseudostaurosira trainorii	Pseudostaurosira trainorii			lt10samps
Puncticulata bodanica	Puncticulata bodanica			lt10samps
Reimeria sinuata	Reimeria sinuata	56	10	TV_analysis
Reimeria sp.	Reimeria	164	10	
Reimeria uniseriata	Reimeria uniseriata	116	9	TV_analysis
Rhoicosphenia abbreviata	Rhoicosphenia abbreviata	279	6	TV_analysis
Rhoicosphenia sp.	Rhoicosphenia	279	4	

COMMON_NAME	TolNameFinal	Num			
		Samps_TV	TolVal_IN	TV_Notes	
Rhopalodia acuminata	Rhopalodia acuminata			lt10samps	
Rhopalodia gibba	Rhopalodia gibba			lt10samps	
Rhopalodia gibba var. parallela	Rhopalodia gibba			lt10samps	
Rhopalodia gibberula	Rhopalodia gibberula			lt10samps	
Rhopalodia musculus	Rhopalodia musculus			lt10samps	
Rossithidium duthii	Rossithidium duthii			lt10samps	
Rossithidium linearis	Rossithidium linearis			lt10samps	
Rossithidium nodosum	Rossithidium nodosum			lt10samps	
Rossithidium nodosum 2	Rossithidium nodosum			lt10samps	
Rossithidium petersenii	Rossithidium petersenii			lt10samps	
Rossithidium pusillum	Rossithidium pusillum			lt10samps	
Rossithidium sp.	Rossithidium	23		Basis_ToleranceAnalysis	
Sellaphora atomoides	Sellaphora atomoides	22	1	Basis_ToleranceAnalysis	
Sellaphora bacillum	Sellaphora bacillum			lt10samps	
Sellaphora crassulexigua	Sellaphora crassulexigua			lt10samps	
Sellaphora elliptica	Sellaphora elliptica			lt10samps	
Sellaphora hustedtii	Sellaphora hustedtii	70	10	TV_analysis	
Sellaphora japonica	Sellaphora japonica			lt10samps	
Sellaphora laevissima	Sellaphora laevissima			lt10samps	
Sellaphora nigri	Sellaphora nigri	33	3	TV_analysis	
Sellaphora parapupula	Sellaphora parapupula			lt10samps	
Sellaphora pupula	Sellaphora pupula	210	8	TV_analysis	
Sellaphora pupula var. capitata	Sellaphora pupula	210	8	TV_analysis	
Sellaphora pupula var. mutata	Sellaphora mutata			lt10samps	
Sellaphora pupula var. rectangularis	Sellaphora rectangularis	16	4	Basis_ToleranceAnalysis	
Sellaphora rectangularis	Sellaphora rectangularis	16	4	Basis_ToleranceAnalysis	
Sellaphora rexii	Sellaphora rexii			lt10samps	
Sellaphora saugerresii	Sellaphora saugerresii	46	6	TV_analysis	
Sellaphora schadei	Sellaphora schadei			lt10samps	
Sellaphora seminulum	Sellaphora seminulum	65	1	TV_analysis	

COMMON_NAME	TolNameFinal	Num		
		Samps_TV	TolVal_IN	TV_Notes
<i>Sellaphora</i> sp.	<i>Sellaphora</i>	284	6	
<i>Sellaphora stroemii</i>	<i>Sellaphora stroemii</i>			lt10samps
<i>Sellaphora wallacei</i>	<i>Sellaphora wallacei</i>			lt10samps
<i>Simonsenia delognei</i>	<i>Simonsenia delognei</i>	158	4	TV_analysis
<i>Skeletonema costatum</i>	<i>Skeletonema costatum</i>			lt10samps
<i>Stauroforma exiguiformis</i>	<i>Stauroforma exiguiformis</i>			lt10samps
<i>Stauroneis alpina</i>	<i>Stauroneis alpina</i>			lt10samps
<i>Stauroneis anceps</i>	<i>Stauroneis anceps</i>			lt10samps
<i>Stauroneis kriegeri</i>	<i>Stauroneis kriegeri</i>			lt10samps
<i>Stauroneis phoenicenteron</i>	<i>Stauroneis phoenicenteron</i>			lt10samps
<i>Stauroneis smithii</i>	<i>Stauroneis smithii</i>	20	4	Basis_ToleranceAnalysis
<i>Stauroneis smithii</i> var. <i>incisa</i>	<i>Stauroneis smithii</i>	20	4	Basis_ToleranceAnalysis
<i>Stauroneis</i> sp.	<i>Stauroneis</i>	36	6	
<i>Stauroneis thermicola</i>	<i>Stauroneis thermicola</i>			lt10samps
<i>Staurosira construens</i>	<i>Staurosira construens</i>	129	1	TV_analysis
<i>Staurosira construens</i> var. <i>binodis</i>	<i>Staurosira construens</i>	129	1	TV_analysis
<i>Staurosira construens</i> var. <i>constricta</i>	<i>Staurosira construens</i>	129	1	TV_analysis
<i>Staurosira construens</i> var. <i>venter</i>	<i>Staurosira construens</i>	129	1	TV_analysis
<i>Staurosira elliptica</i>	<i>Pseudostaurosira elliptica</i>			lt10samps
<i>Staurosira trainorii</i>	<i>Pseudostaurosira trainorii</i>			lt10samps
<i>Staurosira zeilleri</i> v. <i>zeilleri</i>	<i>Pseudostaurosira zeilleri</i>			lt10samps
<i>Staurosirella lapponica</i>	<i>Staurosirella lapponica</i>			lt10samps
<i>Staurosirella leptostauron</i>	<i>Staurosirella leptostauron</i>			lt10samps
<i>Staurosirella leptostauron</i> var. <i>rhomboides</i>	<i>Staurosirella rhomboides</i>			lt10samps
<i>Staurosirella martyi</i>	<i>Staurosirella martyi</i>			lt10samps
<i>Staurosirella pinnata</i>	<i>Staurosirella pinnata</i>	71	9	TV_analysis
<i>Staurosirella</i> sp.	<i>Staurosirella</i>	79	8	
<i>Stephanodiscus hantzschii</i>	<i>Stephanodiscus hantzschii</i>	29	8	TV_analysis
<i>Stephanodiscus minutulus</i>	<i>Stephanodiscus minutulus</i>	13	1	Based on Genus
<i>Stephanodiscus minutus</i>	<i>Stephanodiscus minutulus</i>	13	1	Based on Genus

COMMON_NAME	TolNameFinal	Num		
		Samps_TV	TolVal_IN	TV_Notes
<i>Stephanodiscus niagarae</i>	<i>Stephanodiscus niagarae</i>			lt10samps
<i>Stephanodiscus parvus</i>	<i>Stephanodiscus parvus</i>			lt10samps
<i>Stephanodiscus sp.</i>	<i>Stephanodiscus</i>	40	1	
<i>Stephanodiscus tenuis</i>	<i>Stephanodiscus hantzschii</i>	29	8	TV_analysis
<i>Surirella subsalsa</i> var. <i>minuta</i>	<i>Surirella subsalsa</i>			lt10samps
<i>Surirella amphioxys</i>	<i>Surirella amphioxys</i>			lt10samps
<i>Surirella angusta</i>	<i>Surirella angusta</i>	127	6	TV_analysis
<i>Surirella bifrons</i>	<i>Surirella bifrons</i>			lt10samps
<i>Surirella brebissonii</i>	<i>Surirella brebissonii</i>	38	2	TV_analysis
<i>Surirella brebissonii</i> var. <i>kuetzingii</i>	<i>Surirella brebissonii</i>	38	2	TV_analysis
<i>Surirella brebissonii</i> var. <i>punctata</i>	<i>Surirella brebissonii</i>	38	2	TV_analysis
<i>Surirella gracilis</i>	<i>Surirella gracilis</i>			lt10samps
<i>Surirella helvetica</i>	<i>Surirella helvetica</i>	17	6	Based on Genus
<i>Surirella laponica</i>	<i>Surirella laponica</i>			lt10samps
<i>Surirella linearis</i> var. <i>constricta</i>	<i>Surirella linearis</i>			lt10samps
<i>Surirella minuta</i>	<i>Surirella minuta</i>	136	6	TV_analysis
<i>Surirella ovata</i> var. <i>salina</i>	<i>Surirella ovata</i>			lt10samps
<i>Surirella patella</i>	<i>Surirella patella</i>			lt10samps
<i>Surirella robusta</i>	<i>Surirella robusta</i>			lt10samps
<i>Surirella sp.</i>	<i>Surirella</i>	228	6	
<i>Surirella spiralis</i>	<i>Surirella spiralis</i>			lt10samps
<i>Surirella splendida</i>	<i>Surirella splendida</i>	19	6	Based on Genus
<i>Surirella stalagma</i>	<i>Surirella stalagma</i>			lt10samps
<i>Surirella suecica</i>	<i>Surirella suecica</i>	40	7	TV_analysis
<i>Surirella tenera</i>	<i>Surirella tenera</i>			lt10samps
<i>Surirella turgida</i>	<i>Surirella turgida</i>			lt10samps
<i>Synedra rumpens</i>	<i>Synedra rumpens</i>			lt10samps
<i>Tabellaria flocculosa</i>	<i>Tabellaria flocculosa</i>			lt10samps
<i>Tabellaria quadri septata</i>	<i>Tabellaria quadri septata</i>			lt10samps
<i>Tabularia fasciculata</i>	<i>Tabularia fasciculata</i>			lt10samps

COMMON_NAME	TolNameFinal	Num		
		Samps_TV	TolVal_IN	TV_Notes
Tabularia sp.	Tabularia	30	1	
Tabularia tabulata	Tabularia tabulata	25	1	TV_analysis
Thalassiosira lacustris	Thalassiosira lacustris	62	6	TV_analysis
Thalassiosira pseudonana	Thalassiosira pseudonana	18	5	Based on Genus
Thalassiosira sp.	Thalassiosira	136	5	
Thalassiosira visurgis	Thalassiosira visurgis			lt10samps
Thalassiosira weissflogii	Thalassiosira weissflogii	91	1	TV_analysis
Tryblionella acuminata	Tryblionella acuminata			lt10samps
Tryblionella aerophila	Tryblionella aerophila			lt10samps
Tryblionella apiculata	Tryblionella apiculata	195	2	TV_analysis
Tryblionella calida	Tryblionella calida	55	1	TV_analysis
Tryblionella cf. calida	Tryblionella calida	55	1	TV_analysis
Tryblionella coarctata	Tryblionella coarctata	12	3	Based on Genus
Tryblionella debilis	Tryblionella debilis	23	5	Basis_ToleranceAnalysis
Tryblionella hungarica	Tryblionella hungarica	56	2	TV_analysis
Tryblionella levidensis	Tryblionella levidensis	25	2	TV_analysis
Tryblionella littoralis	Tryblionella littoralis			lt10samps
Tryblionella salinarum	Tryblionella salinarum			lt10samps
Tryblionella sp.	Tryblionella	267	3	
Tryblionella victoriae	Tryblionella victoriae	81	8	TV_analysis
Ulnaria acus	Synedra acus	39	3	TV_analysis
Ulnaria ulna	Synedra ulna	166	5	TV_analysis
Unknown centric sp.	Undetermined Centric			lt10samps
Unknown pennate sp.	Undetermined Pennate			lt10samps

# Appendix C

## Site Characteristics by Disturbance Category

Indiana Diatom Sites

# Sample Size

Table 1. Tabulation of diatom sampling sites by disturbance category and ecoregion in Indiana.

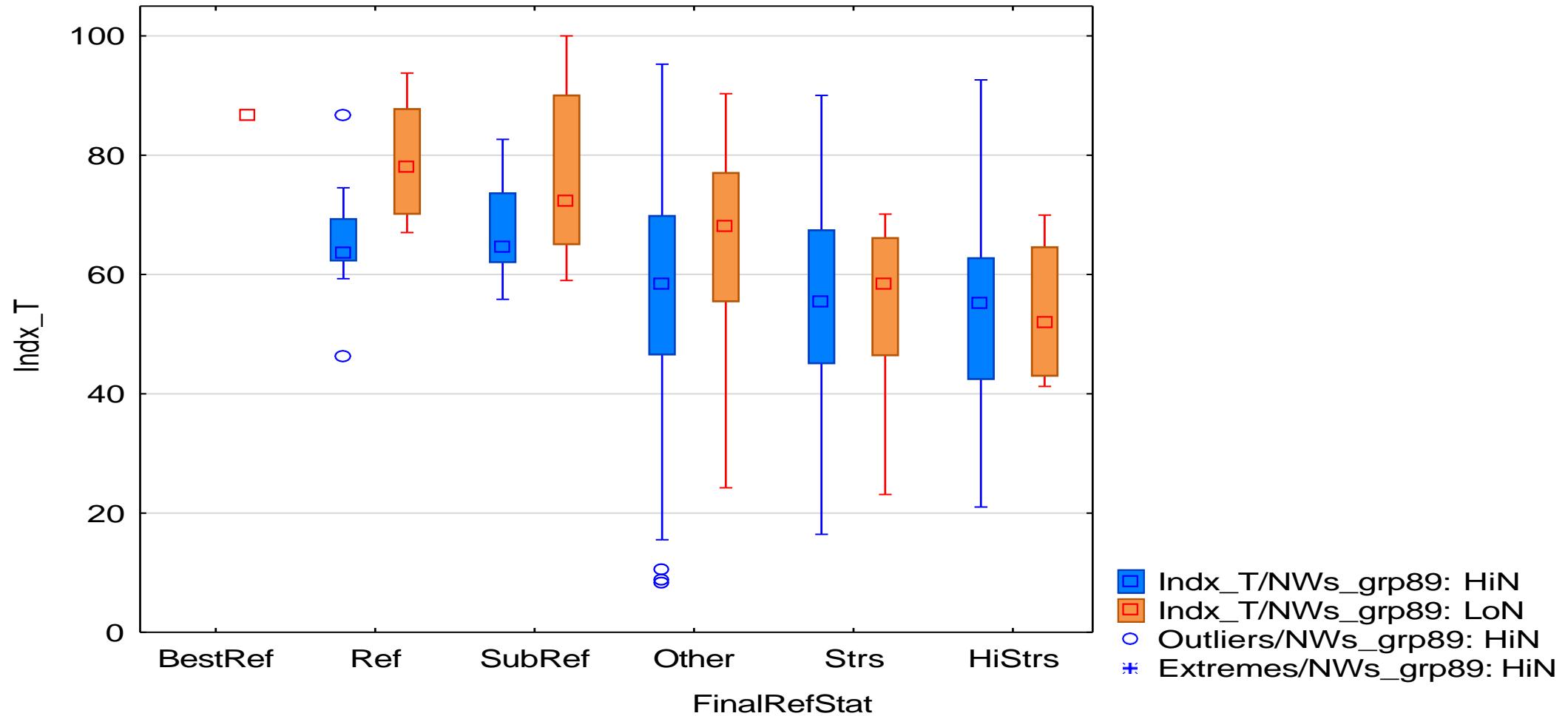
		54	55	56	57	71	72	Totals
Reference	BestRef					1		1
	Ref		7	4		11	1	23
	SubRef		17	6		10	8	41
Intermediate	Intermediate	30	109	24	1	46	52	262
Stressed	Strs	9	27	8	2	4	10	60
	HighStrs	4	16	2				22
Totals	Totals	43	176	44	3	72	71	409

# Index (Preliminary)

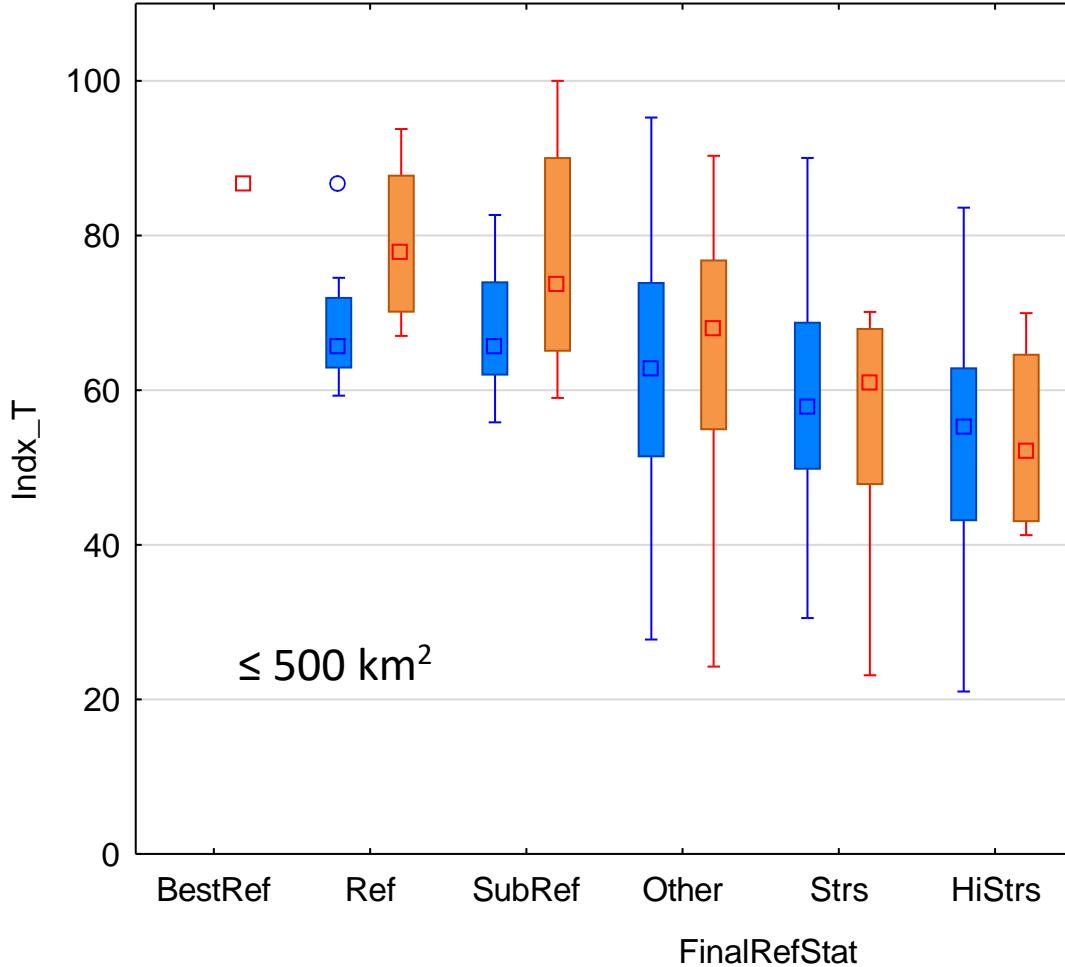
Box Plot of Indx\_T grouped by FinalRefStat; categorized by NWs\_grp89

BIG\_DATA\_IN.sta 458v\*497c

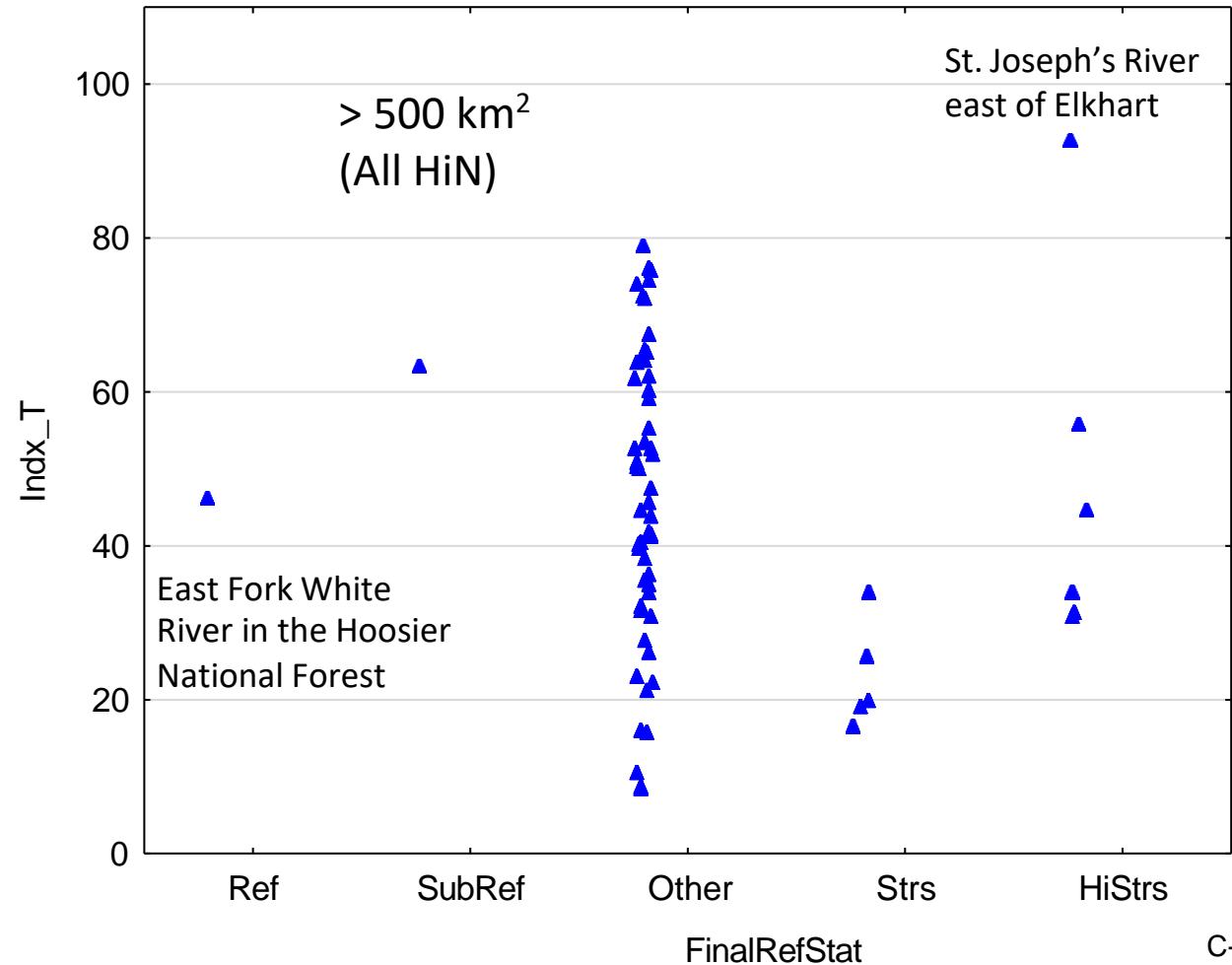
Include condition: RepNum=0



# Index by Drainage Size Category



We discussed that the index might not apply in large sites because there are no comparable reference sites. All large sites (>500 km<sup>2</sup>) are in the HiN class and most are in the intermediate disturbance category. There are outliers, but the index appears to conform to expectations.

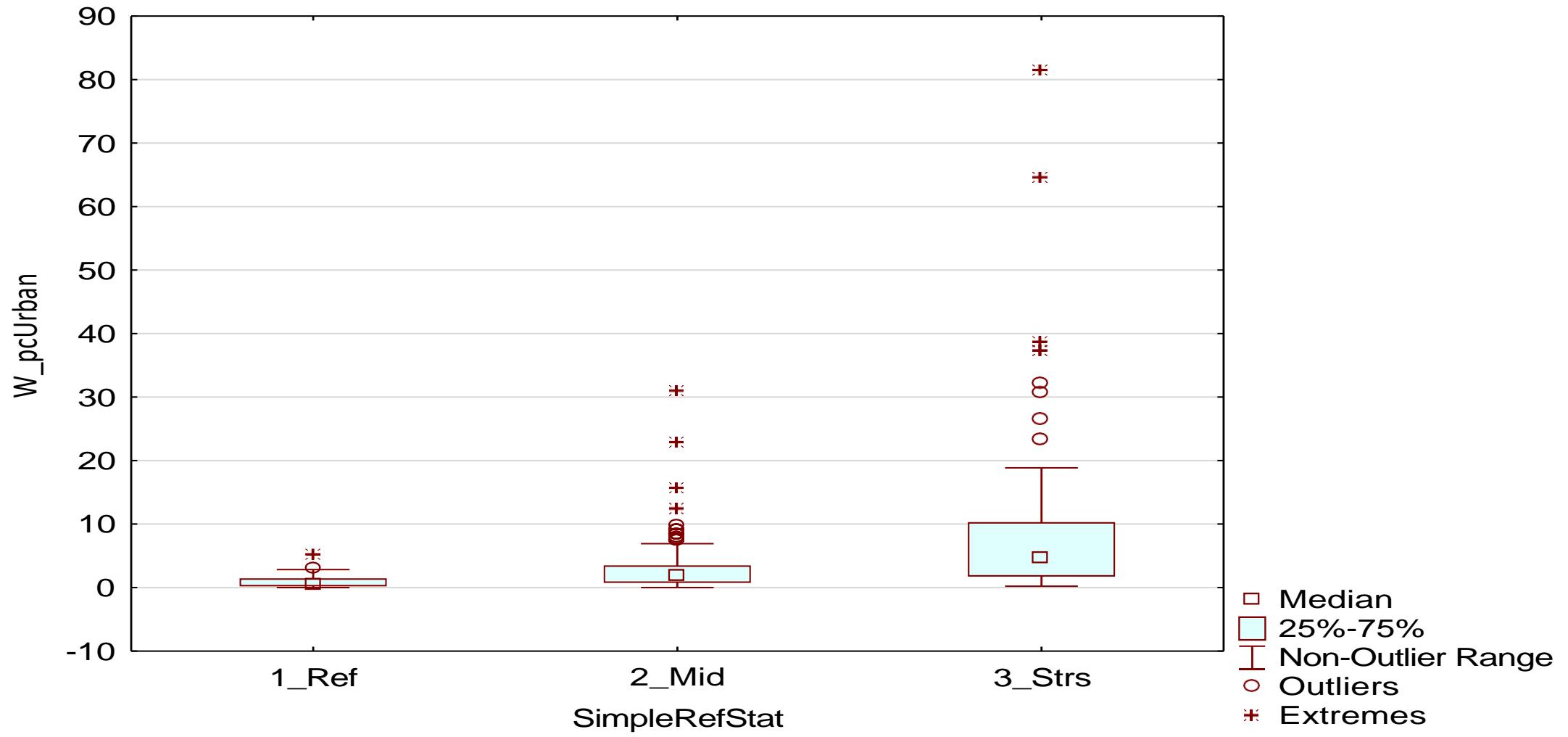


# Land Use

Box Plot of W\_pcUrban grouped by SimpleRefStat

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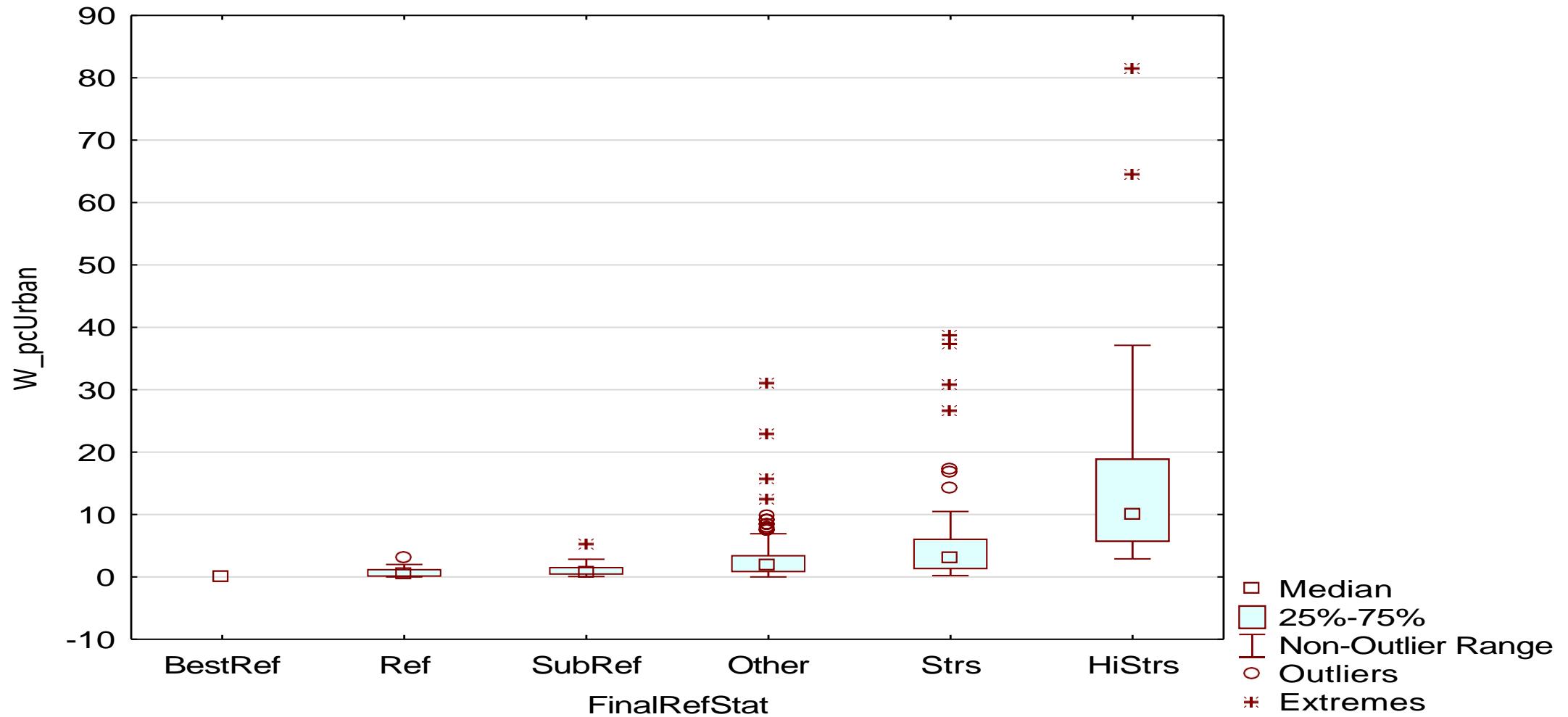
Include condition: RepNum=0



Box Plot of W\_pcUrban grouped by FinalRefStat

BIG\_DATA\_IN.sta 458v\*497c

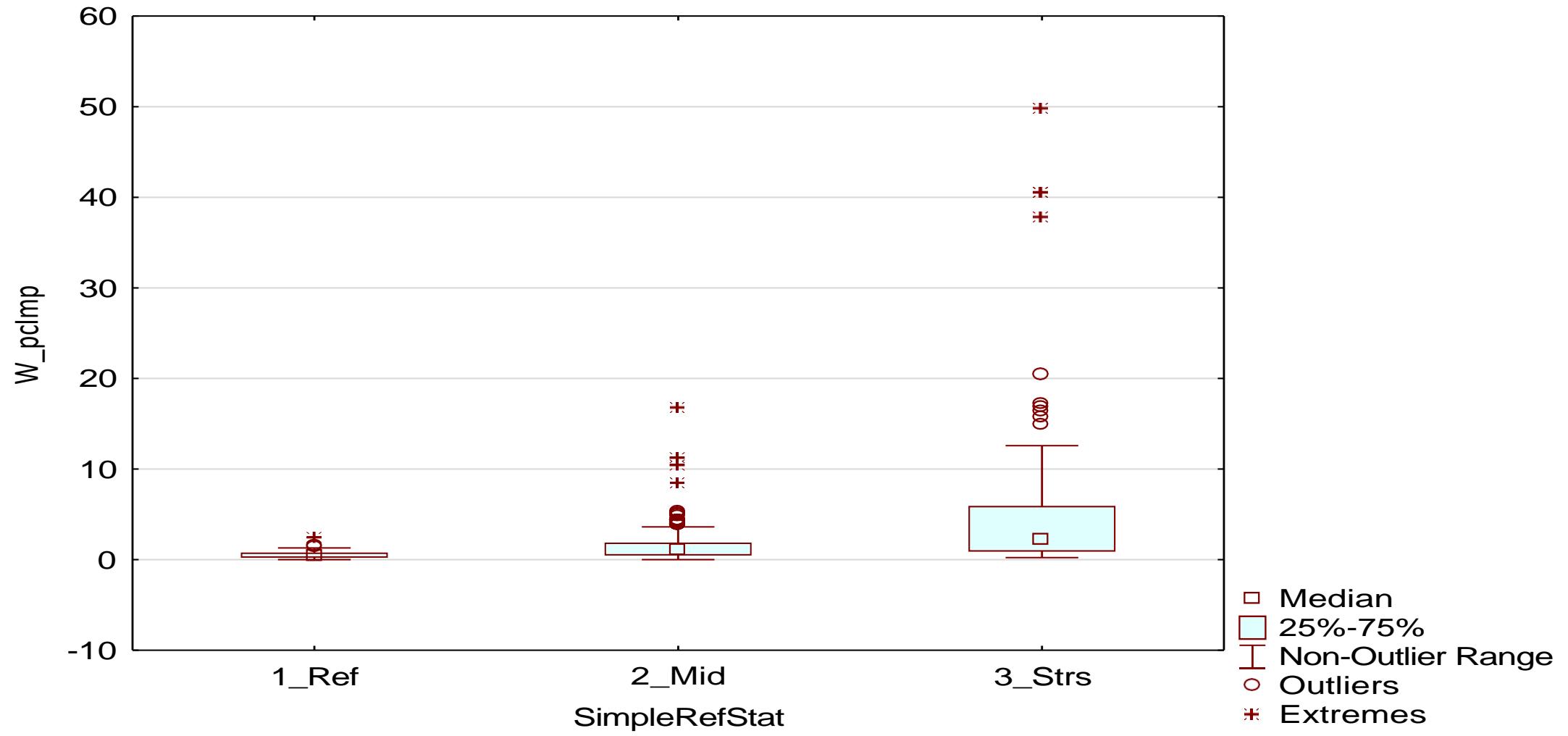
Include condition: RepNum=0



Box Plot of W\_pcImp grouped by SimpleRefStat

BIG\_DATA\_IN.sta 458v\*497c

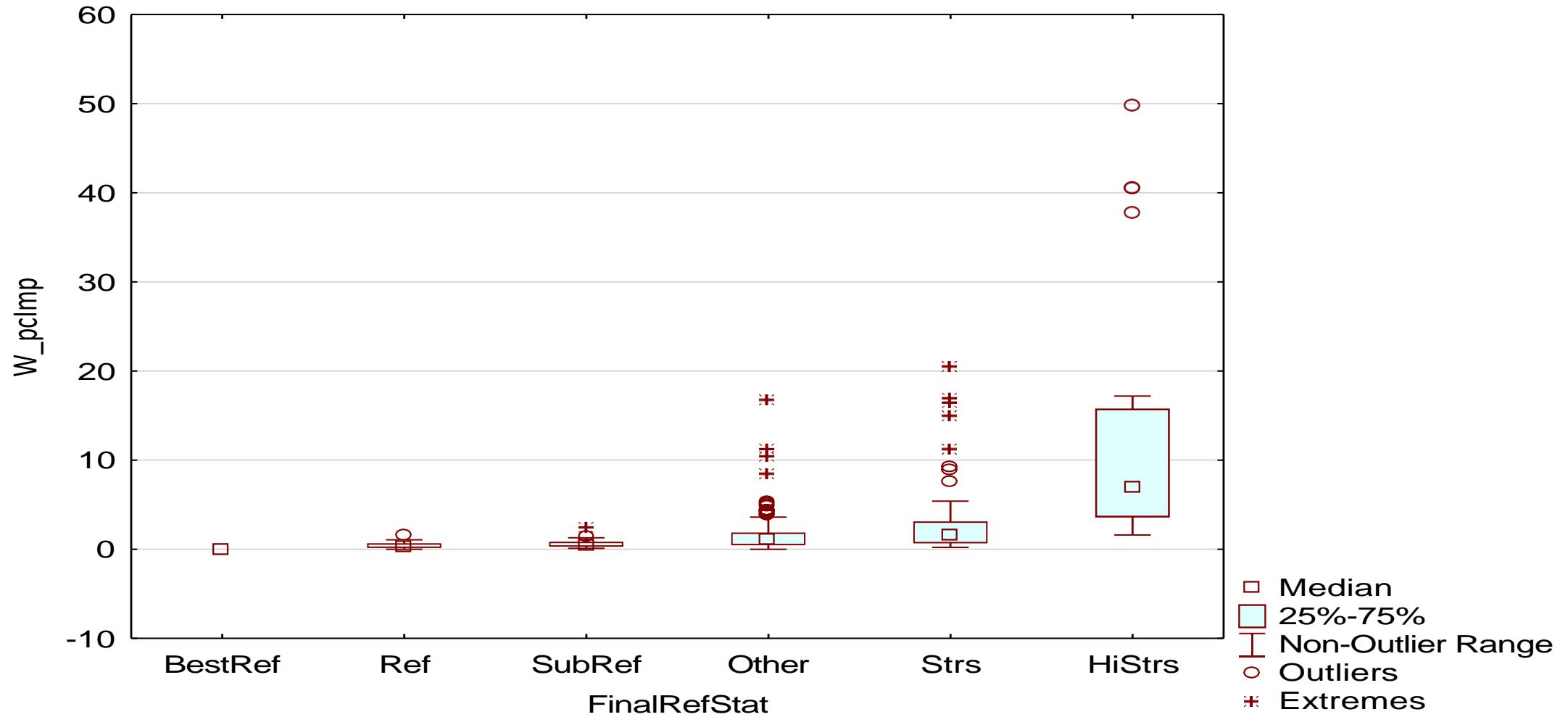
Include condition: RepNum=0



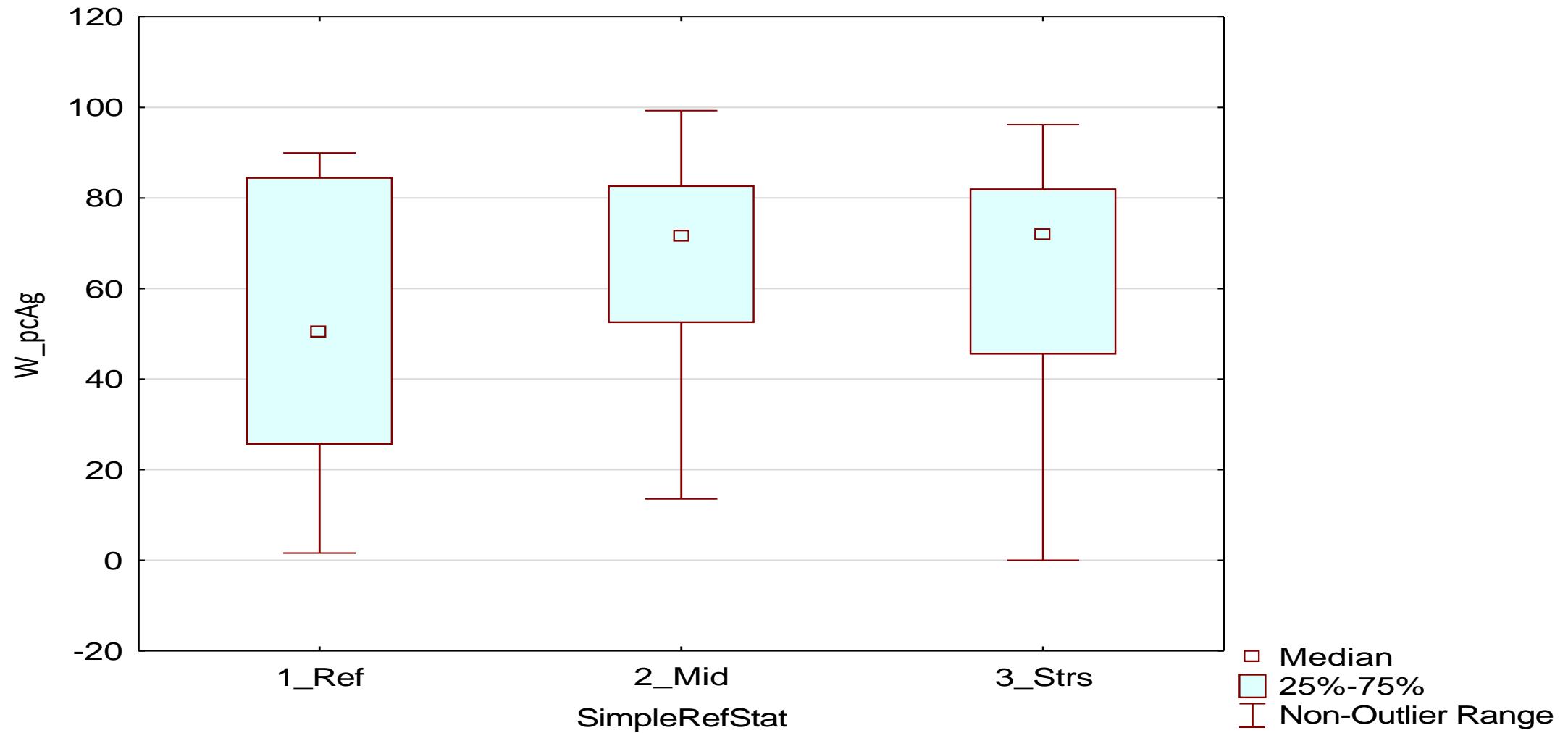
Box Plot of W\_pcImp grouped by FinalRefStat

BIG\_DATA\_IN.sta 458v\*497c

Include condition: RepNum=0



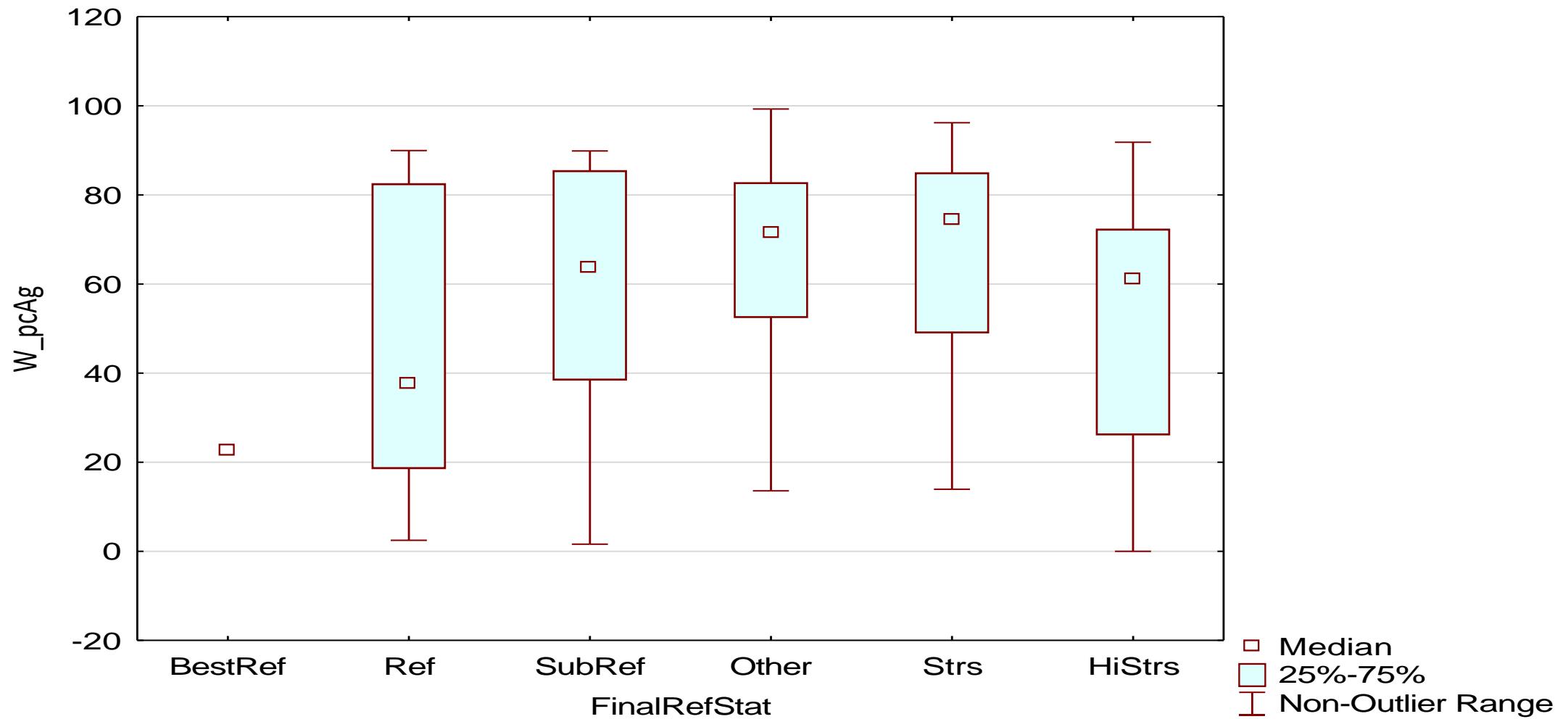
Box Plot of W\_pcAg grouped by SimpleRefStat  
BIG\_DATA\_IN.sta 458v\*497c  
Include condition: RepNum=0



Box Plot of W\_pcAg grouped by FinalRefStat

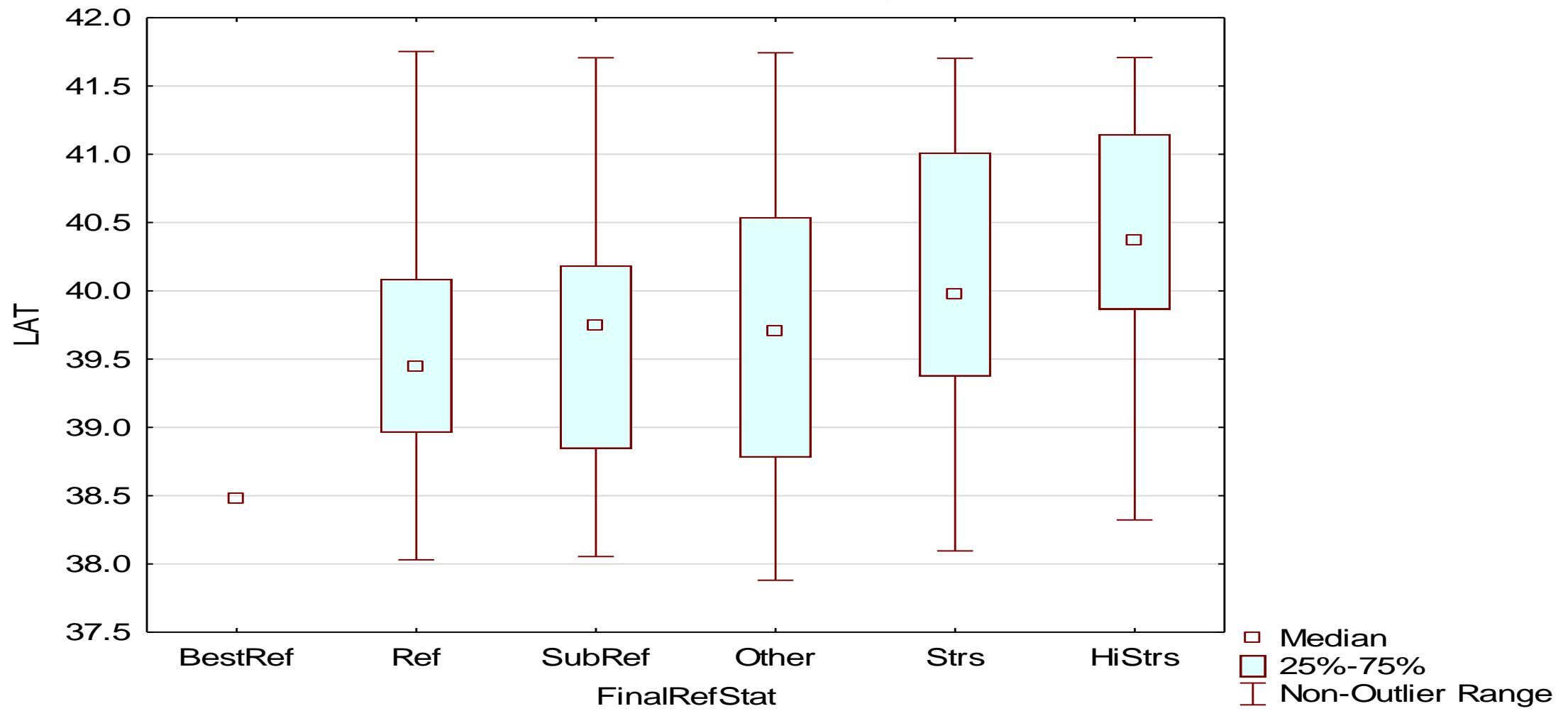
BIG\_DATA\_IN.sta 458v\*497c

Include condition: RepNum=0

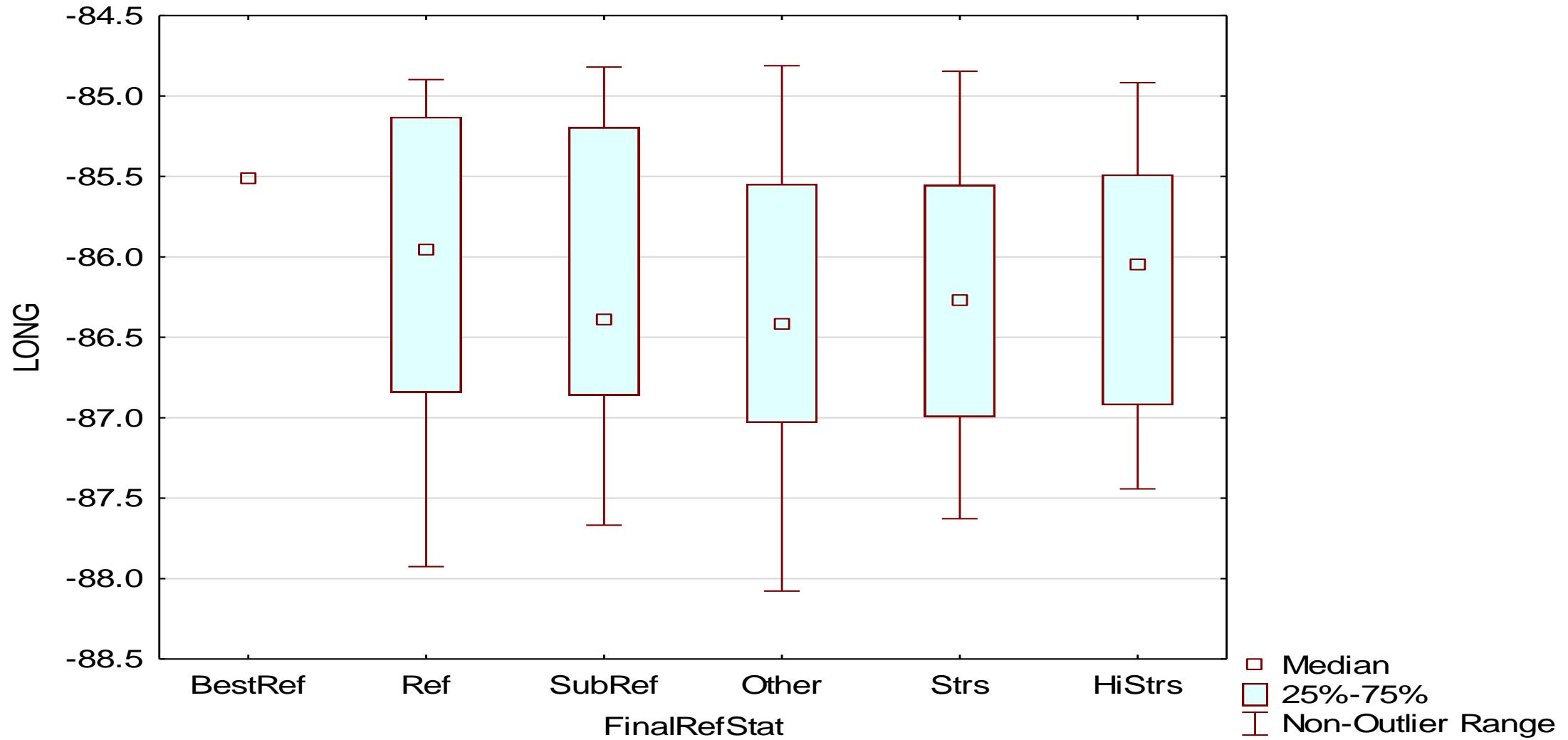


# Natural Characteristics

Box Plot of LAT grouped by FinalRefStat  
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Include condition: RepNum=0



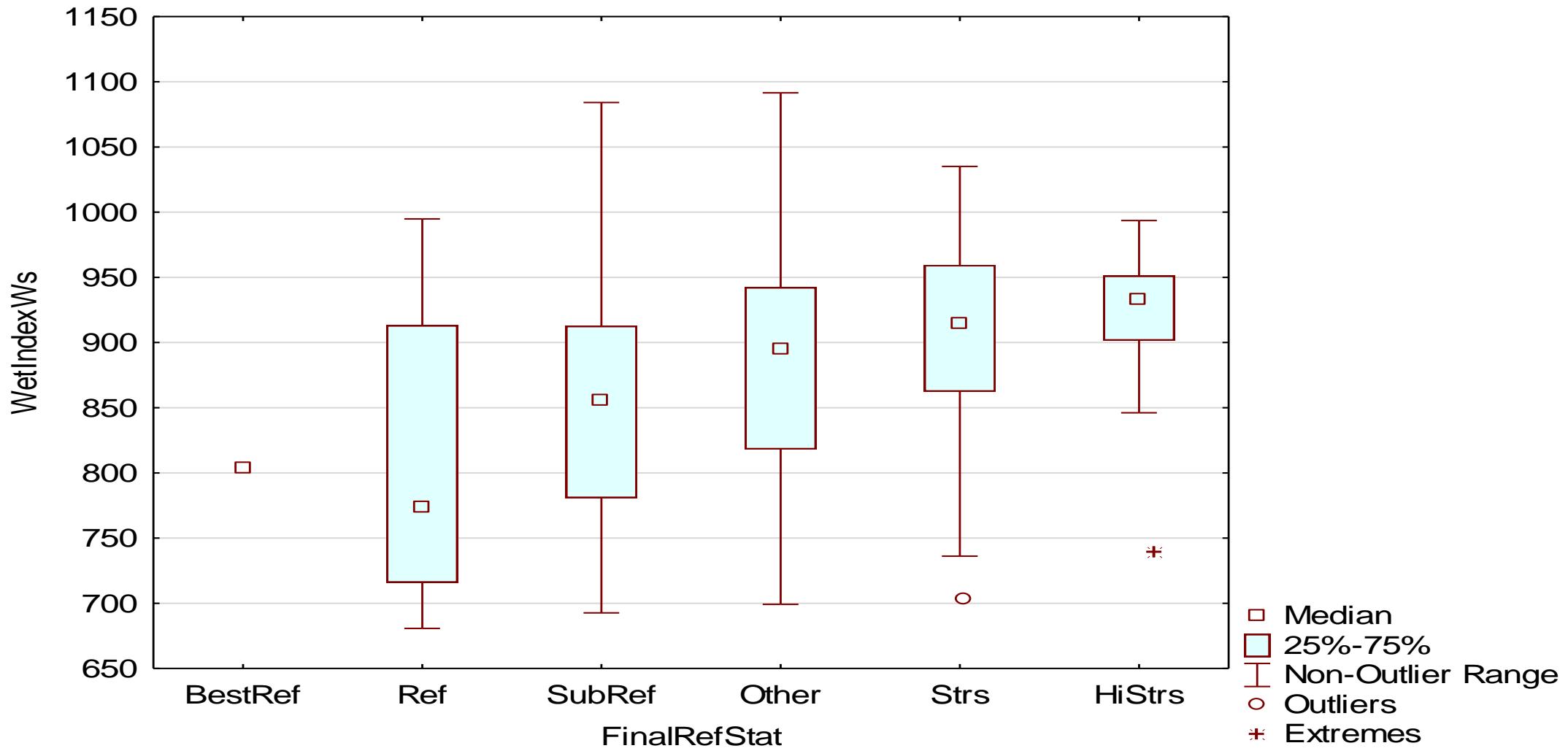
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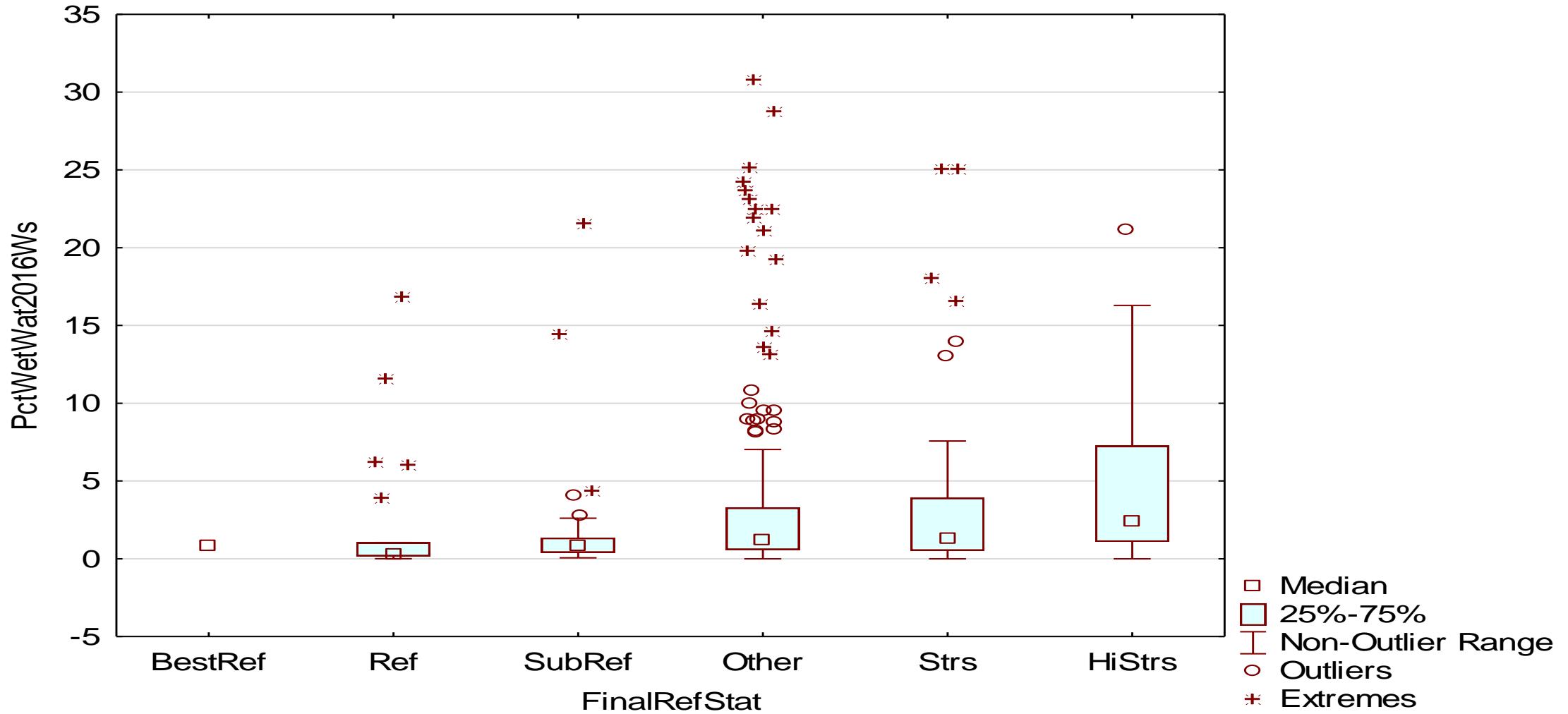
Box Plot of WetIndexWs grouped by FinalRefStat

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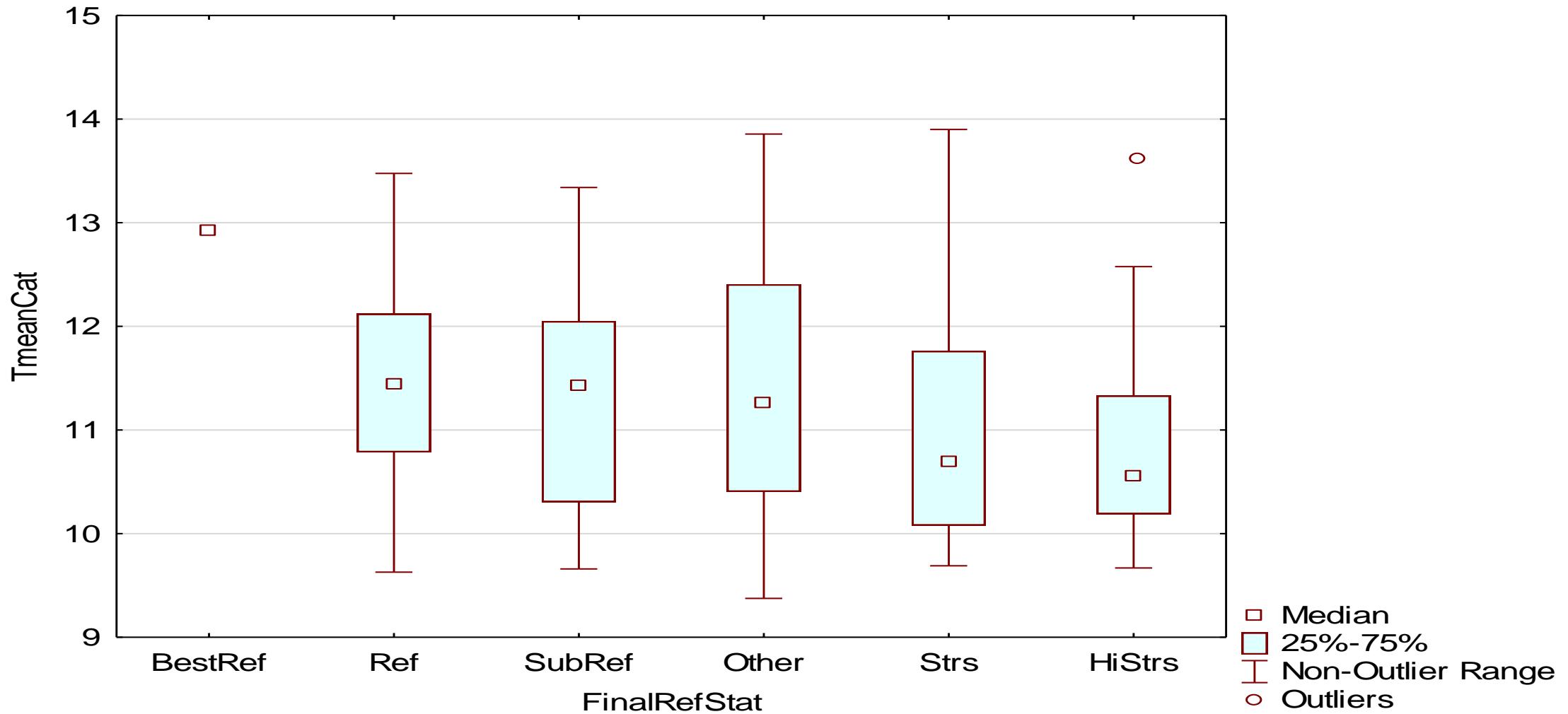
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Box Plot of PctWetWat2016Ws grouped by FinalRefStat  
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Box Plot of TmeanCat grouped by FinalRefStat  
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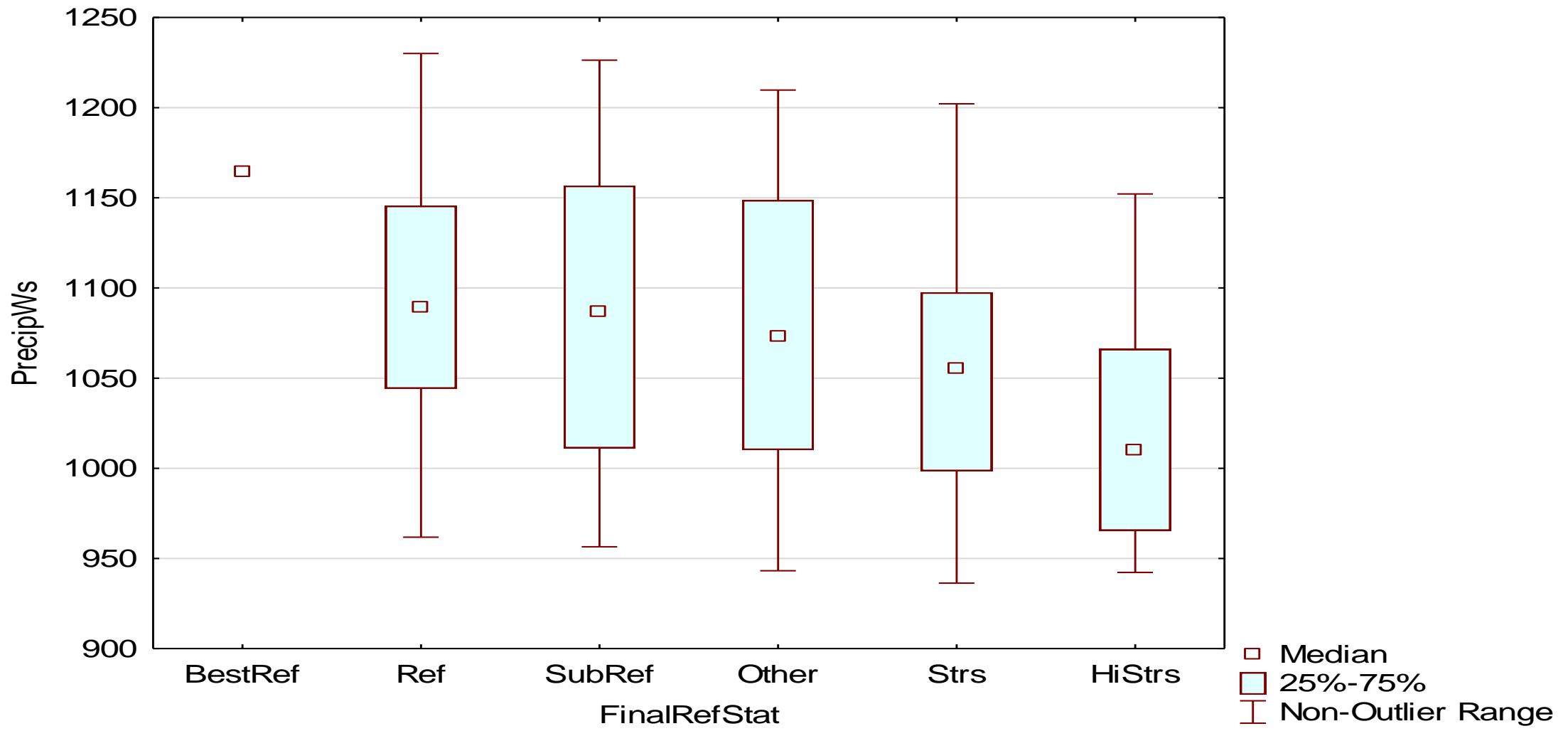




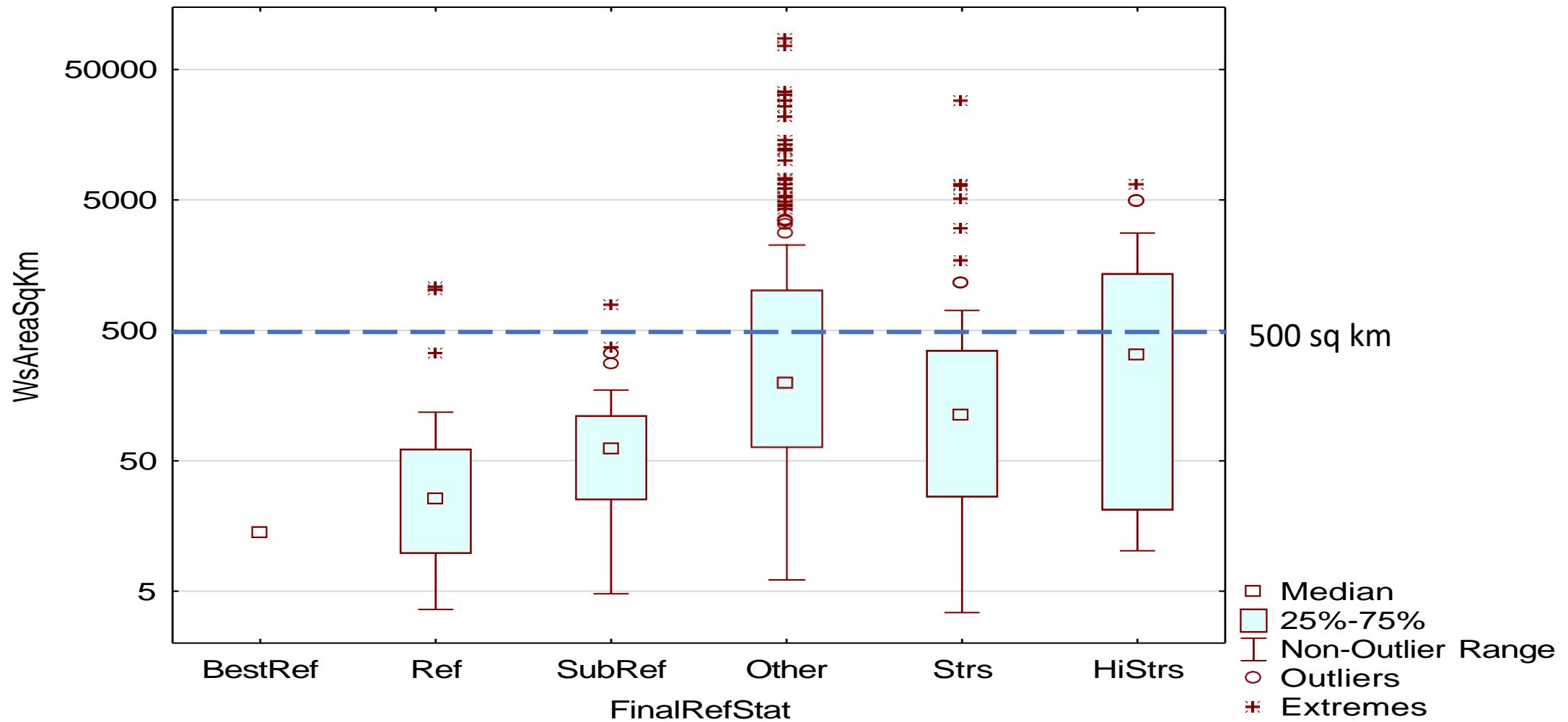
Box Plot of PrecipWs grouped by FinalRefStat

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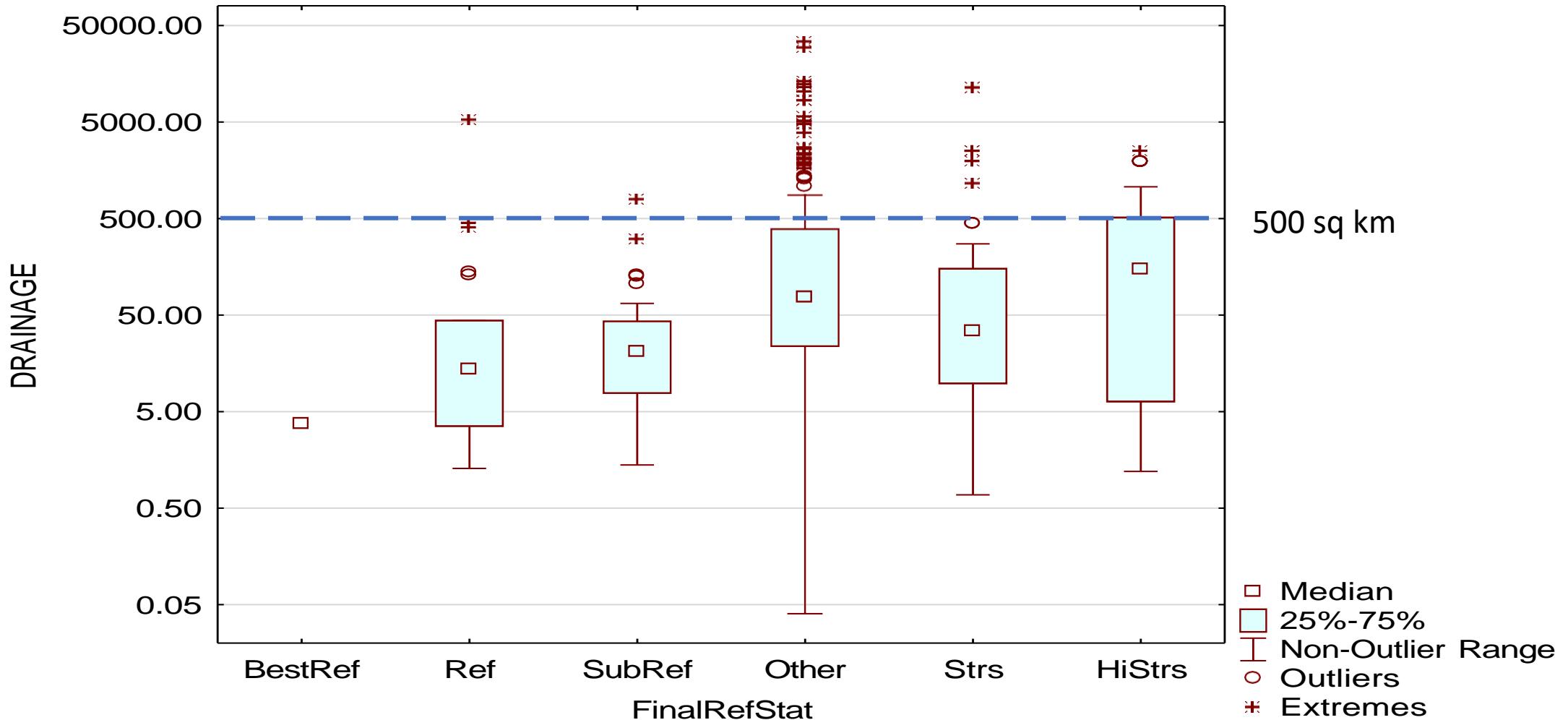
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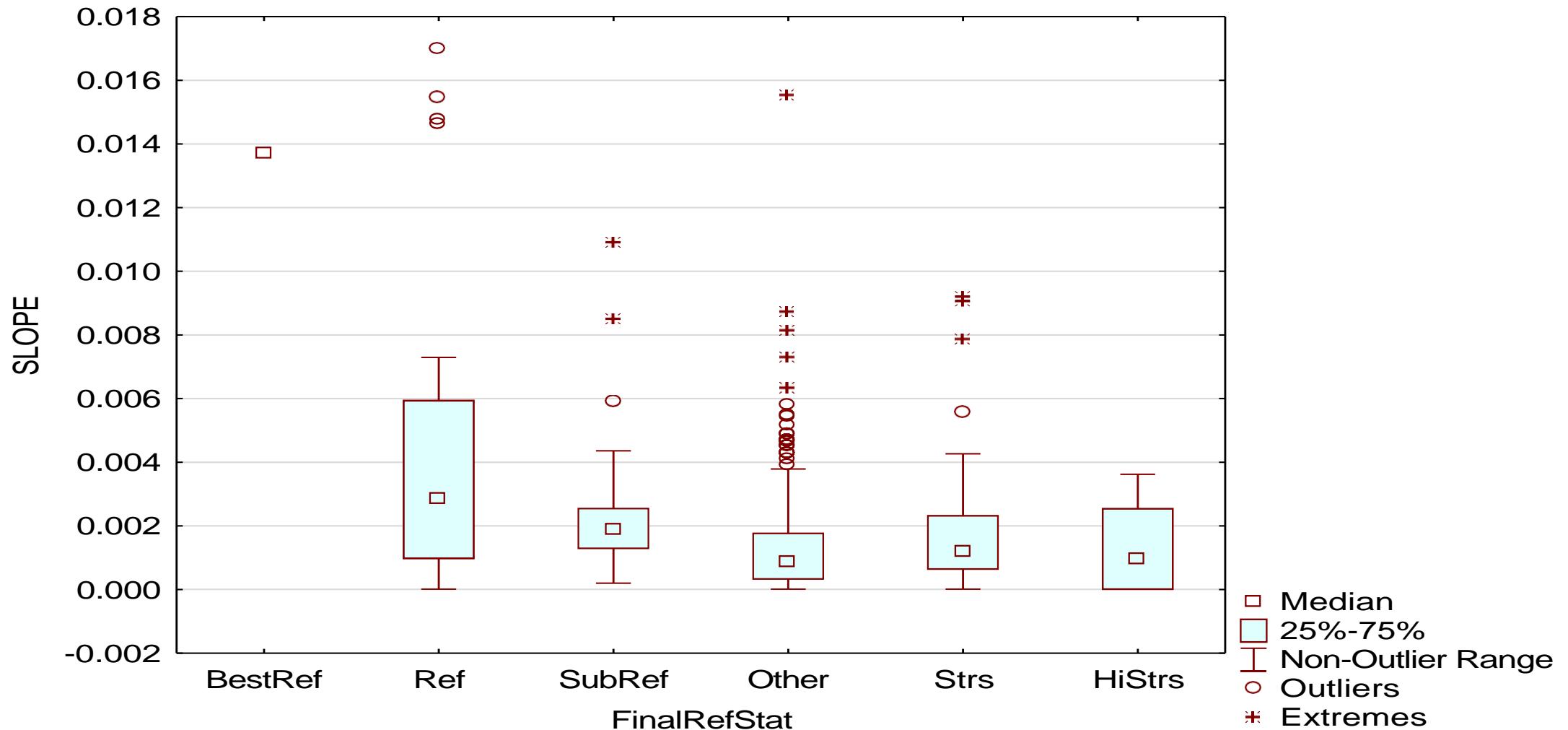
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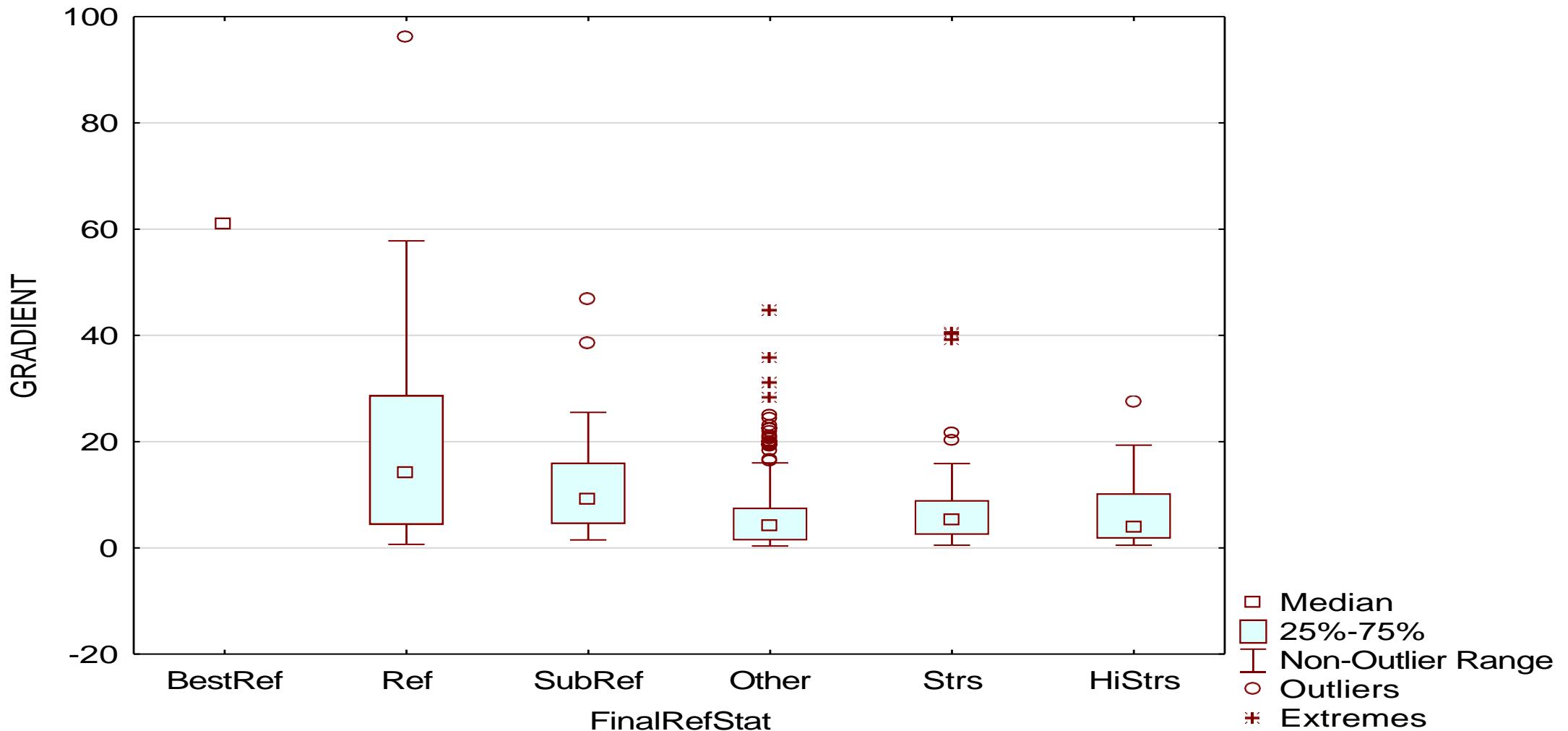
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Box Plot of SLOPE grouped by FinalRefStat  
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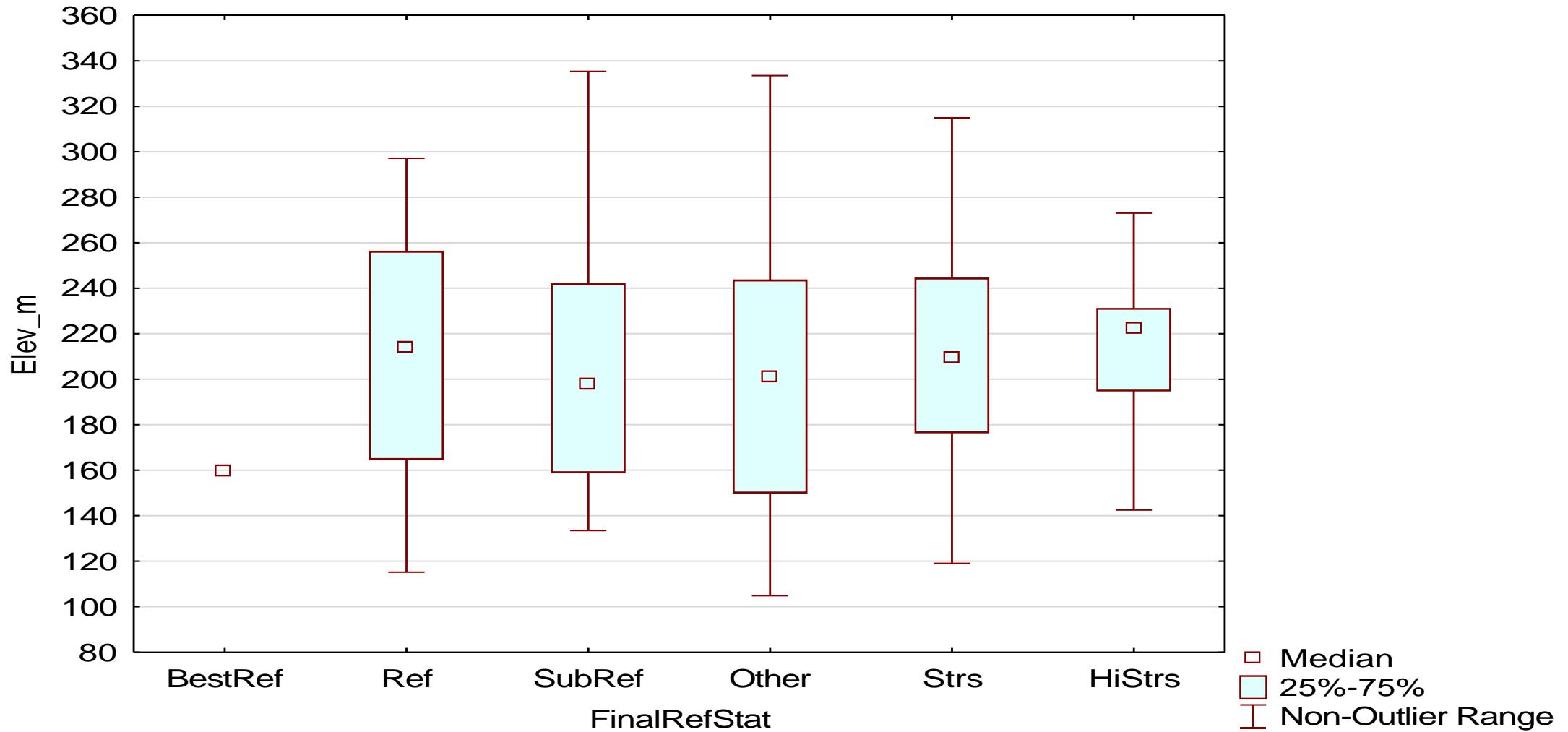
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Box Plot of Elev\_m grouped by FinalRefStat

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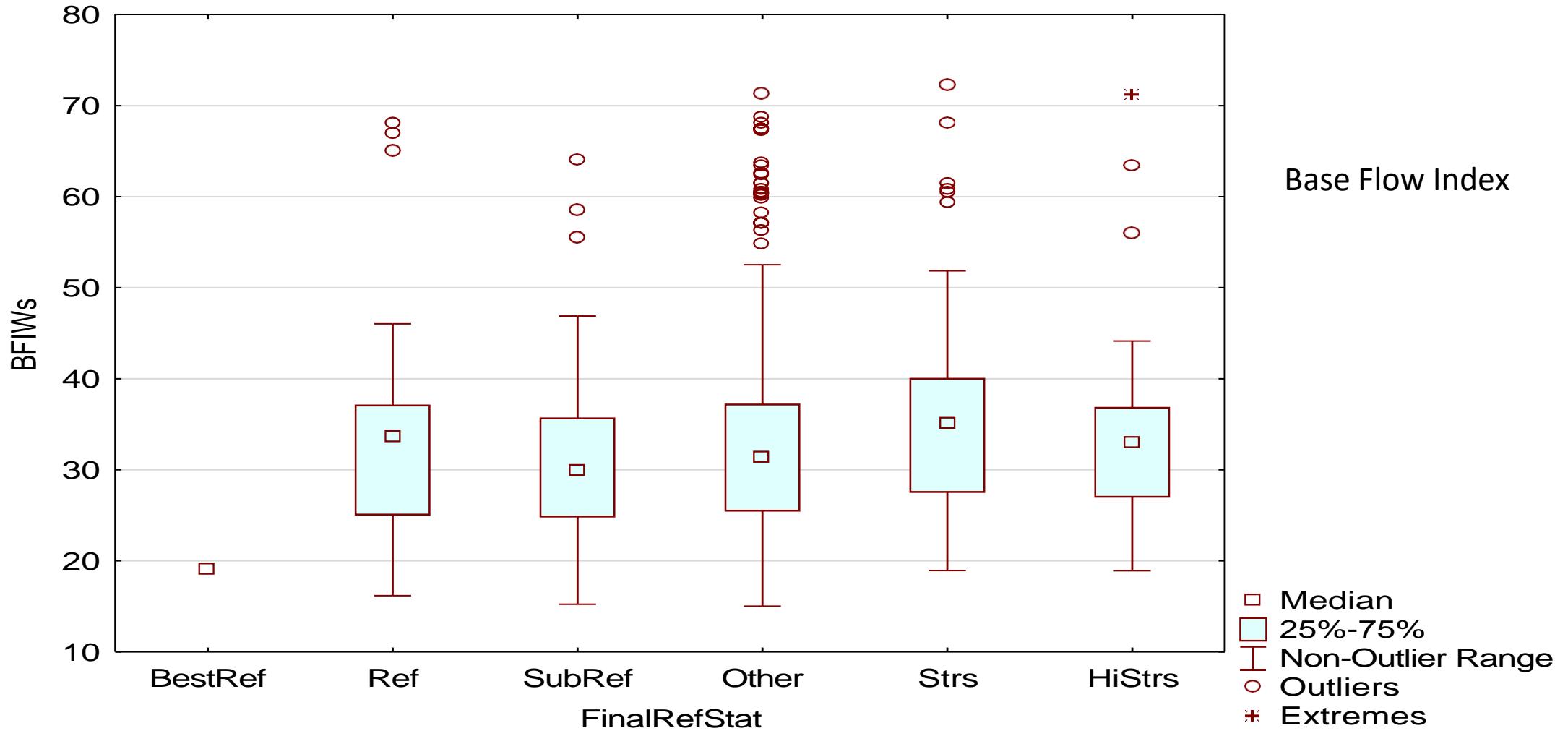
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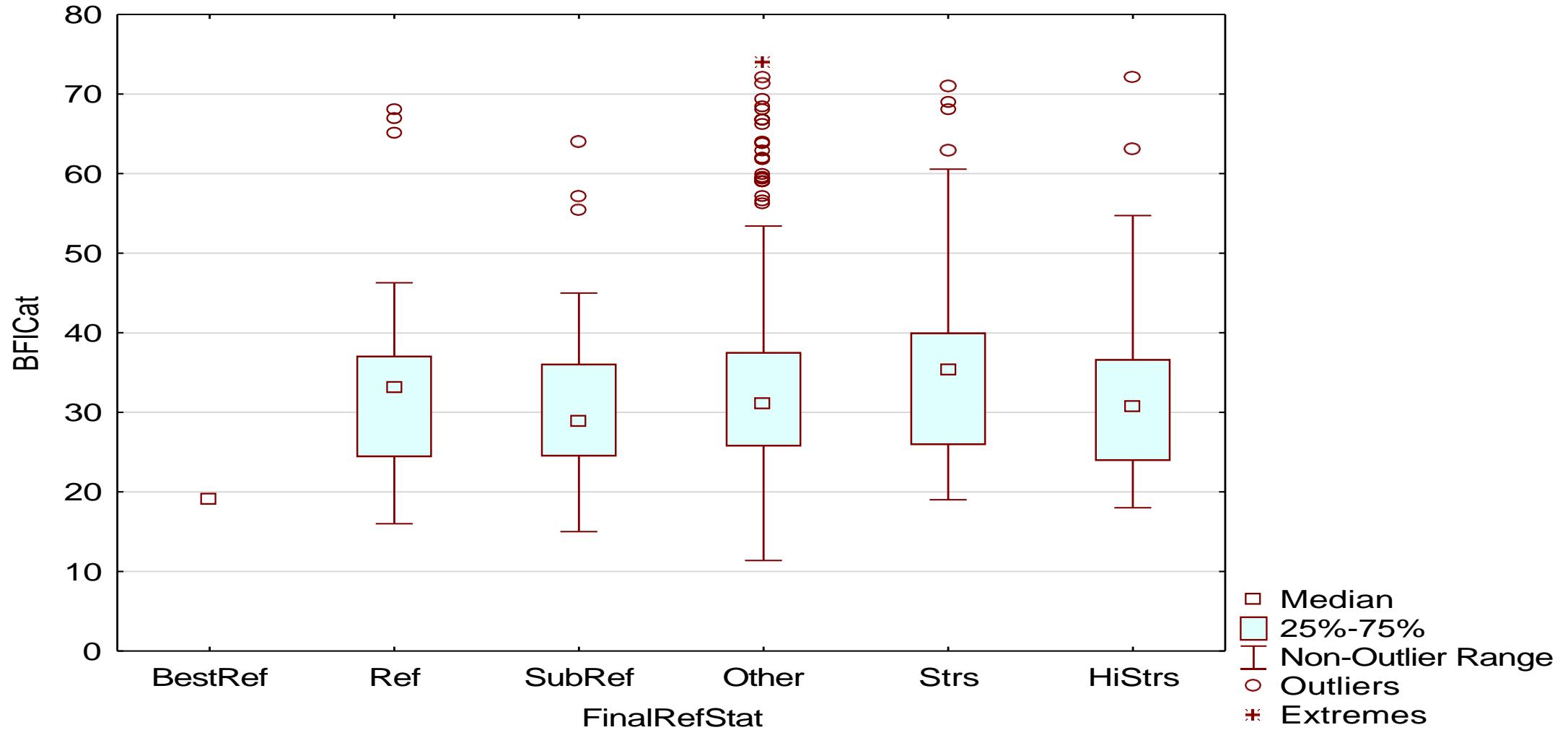
Box Plot of BFIWs grouped by FinalRefStat

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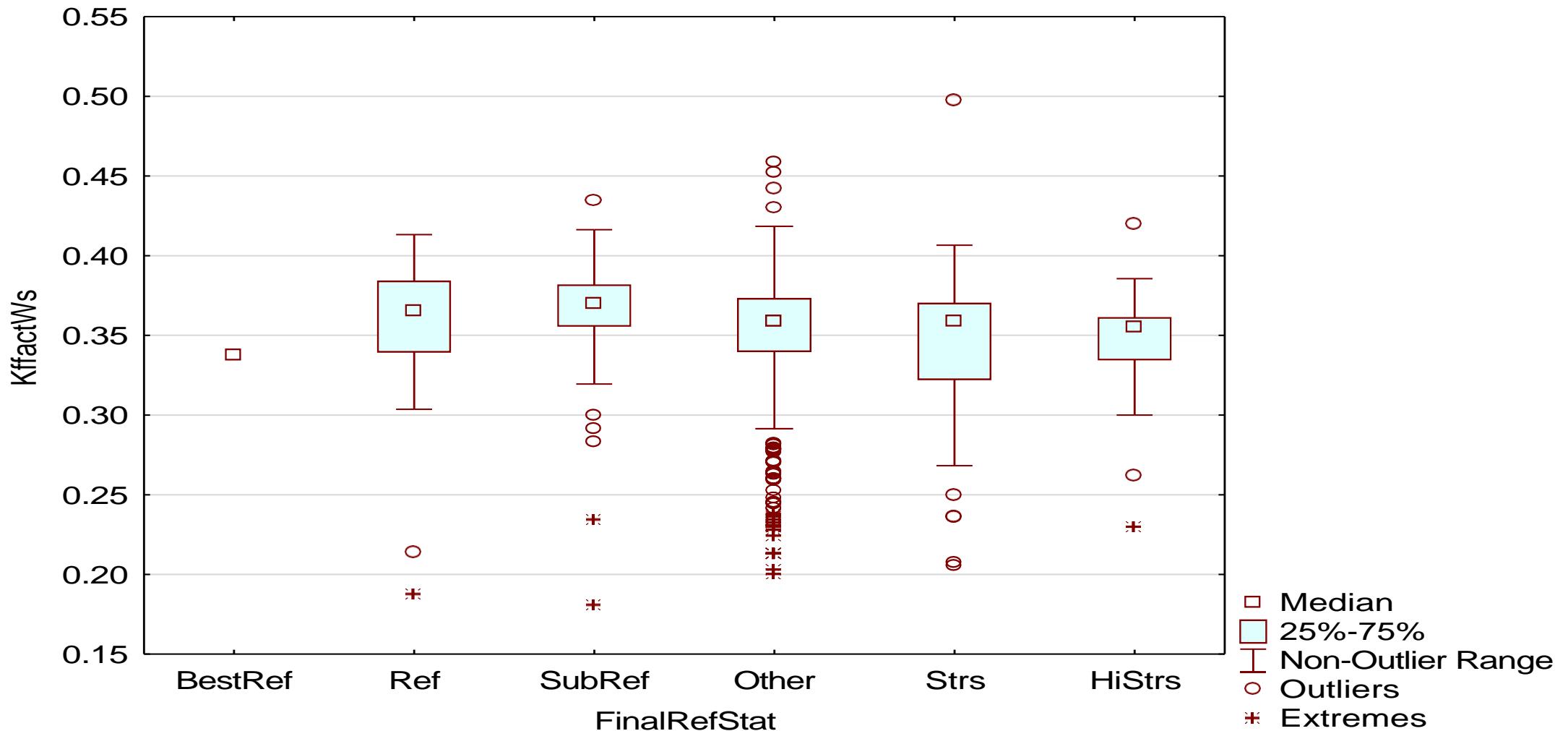
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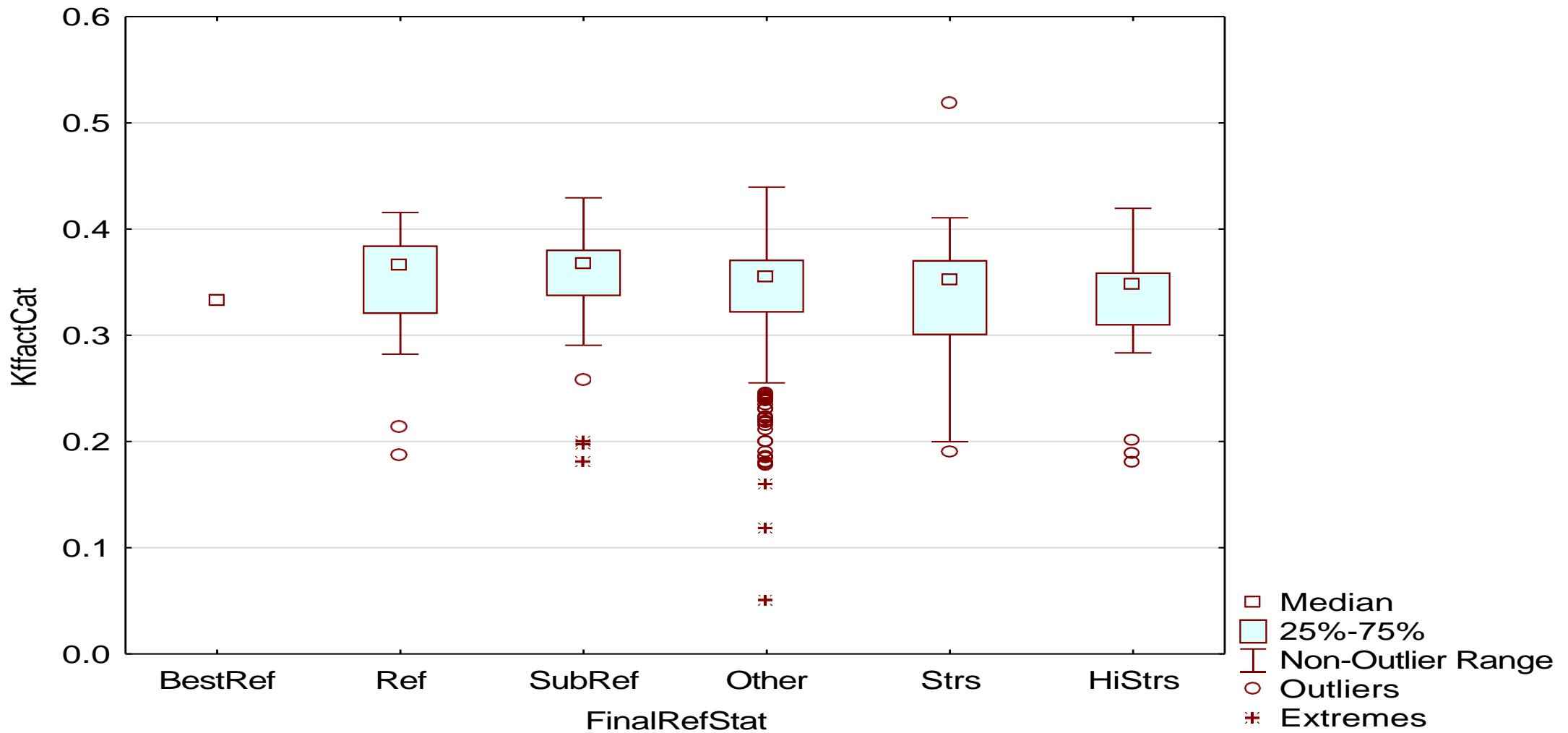
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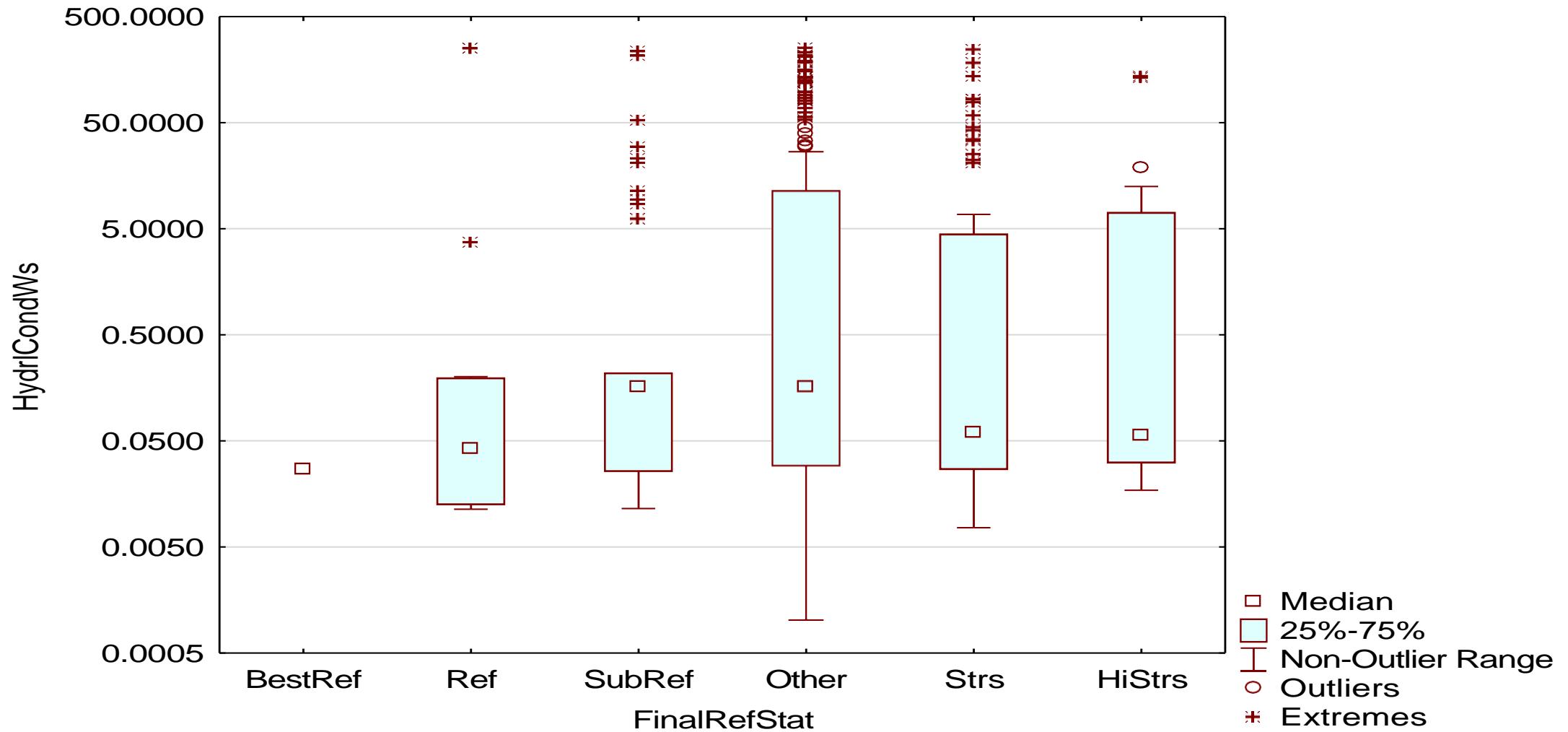
Box Plot of KffactWs grouped by FinalRefStat  
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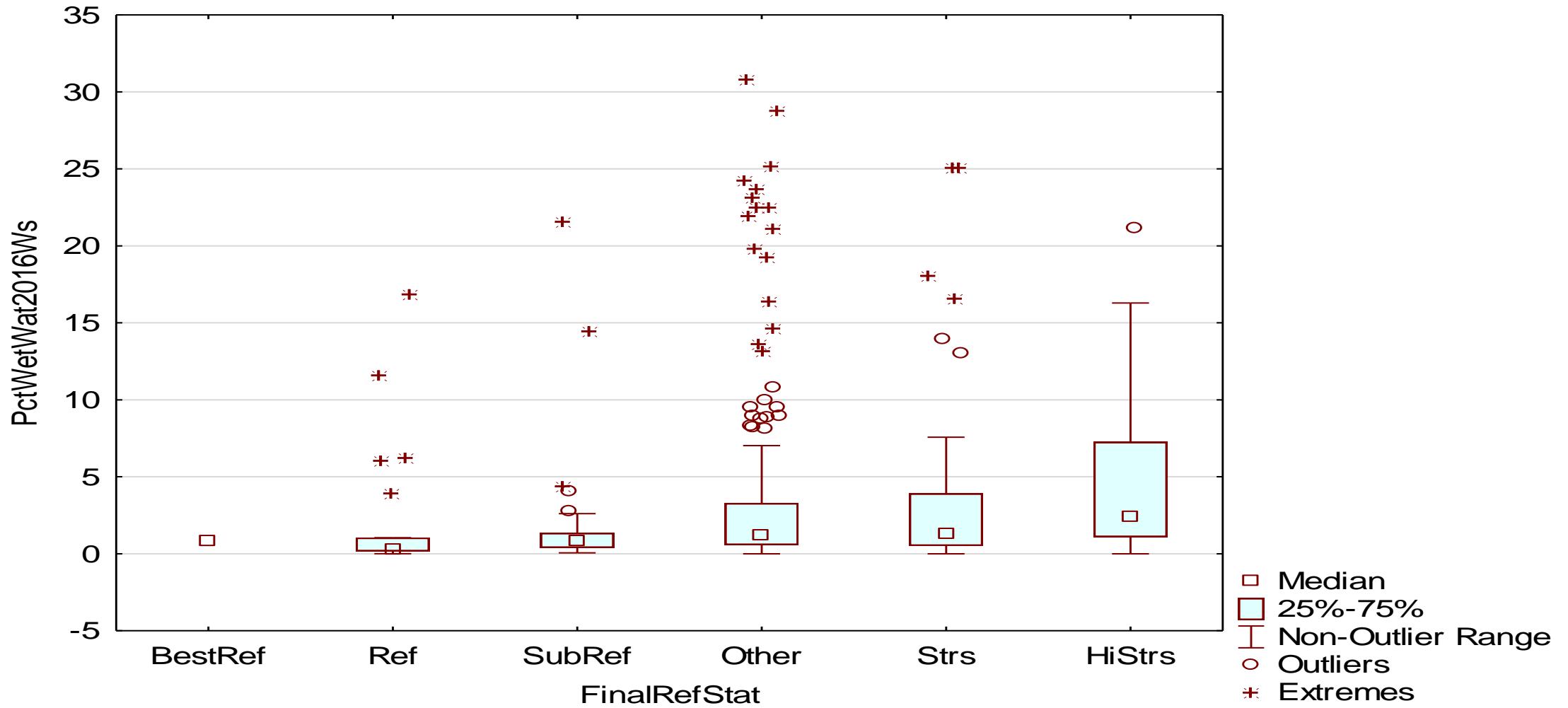
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Box Plot of HydrlCondWs grouped by FinalRefStat  
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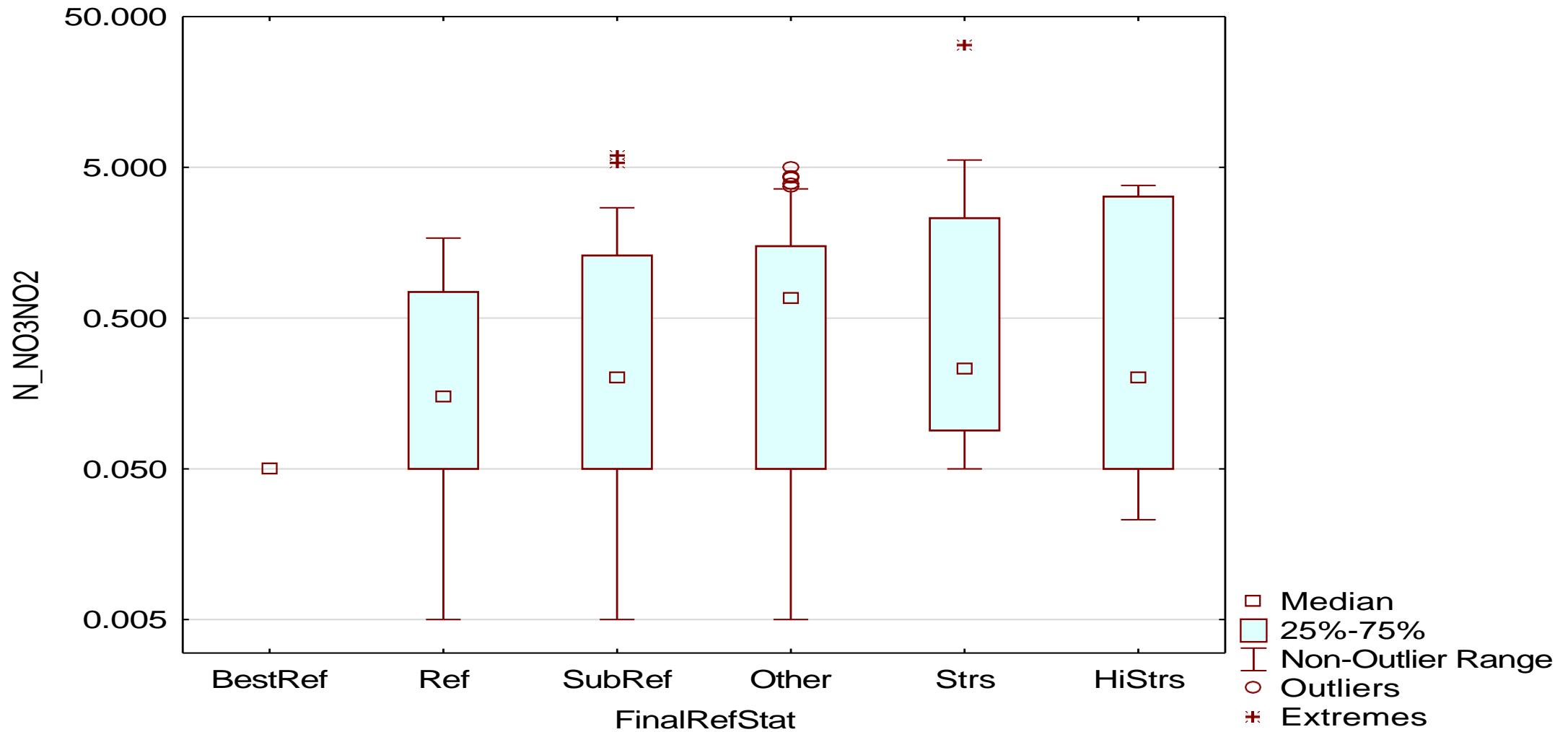
Box Plot of PctWetWat2016Ws grouped by FinalRefStat  
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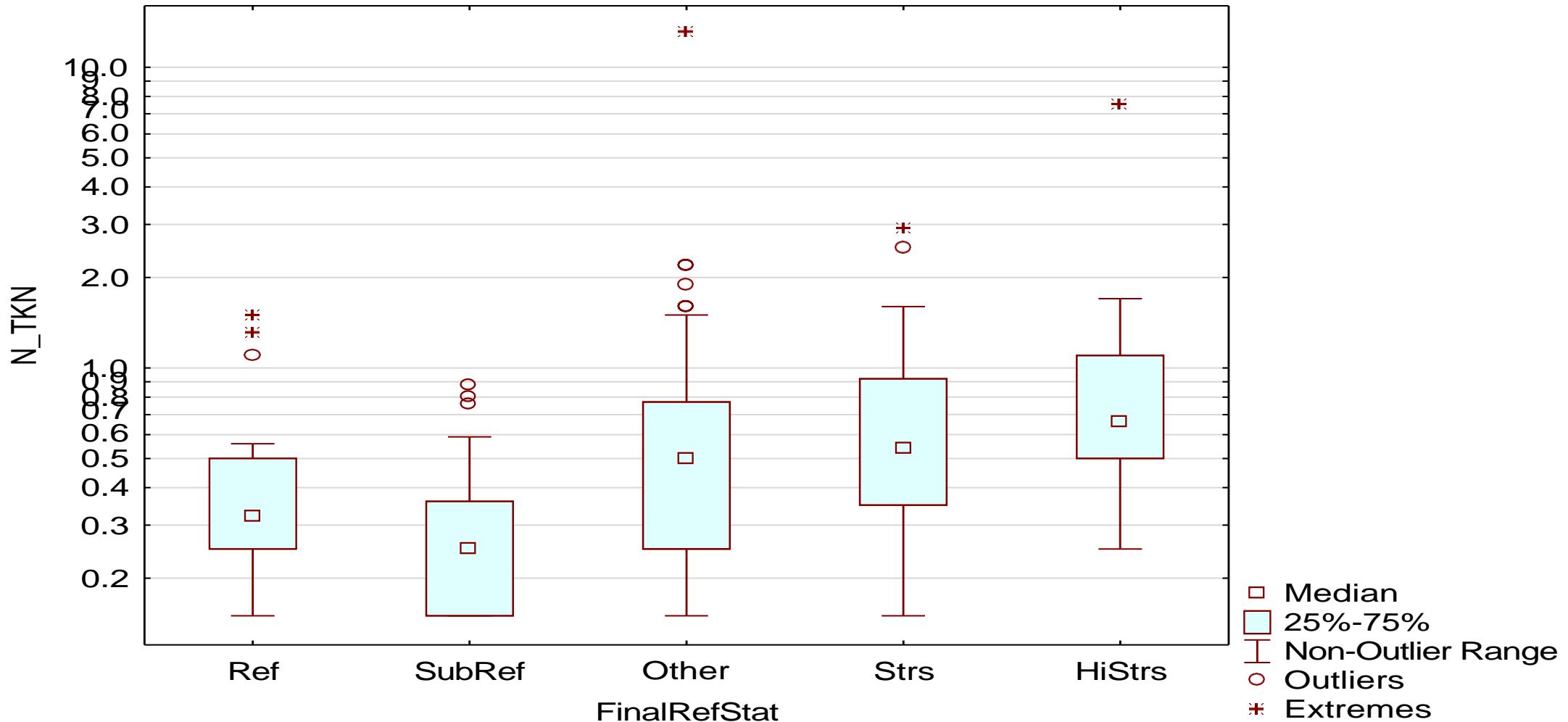


# Nutrients (measured)

Box Plot of N\_NO3NO2 grouped by FinalRefStat  
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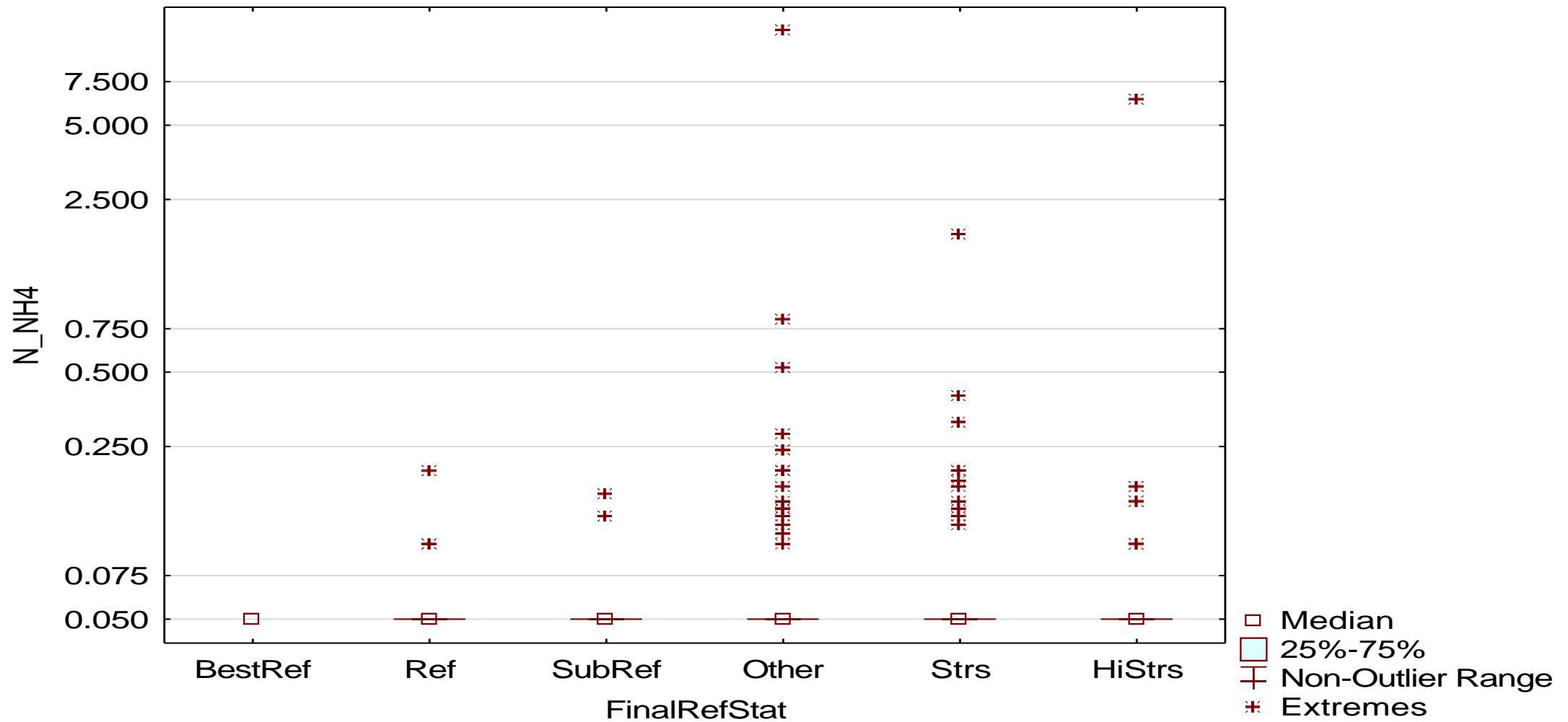
Box Plot of N\_TKN grouped by FinalRefStat  
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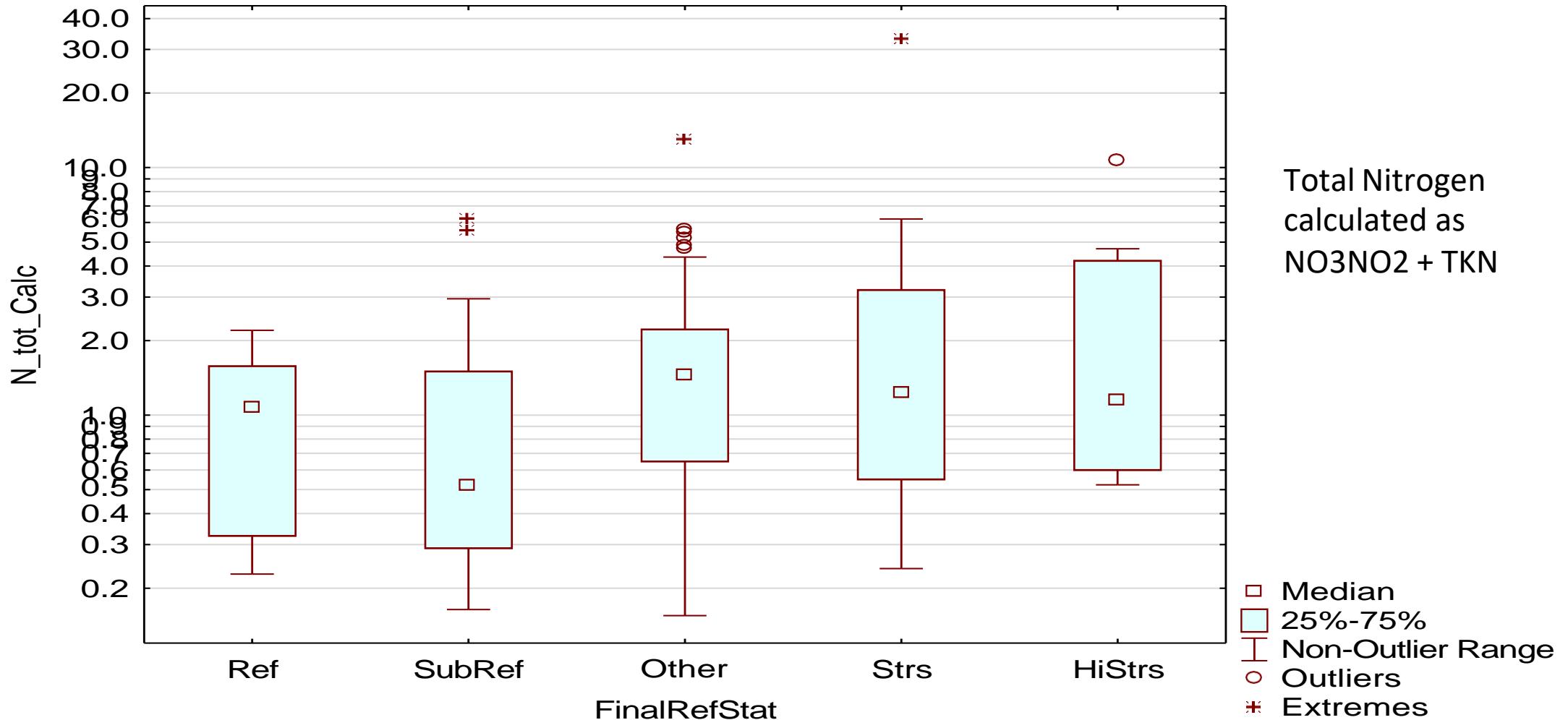
Box Plot of N\_NH4 grouped by FinalRefStat

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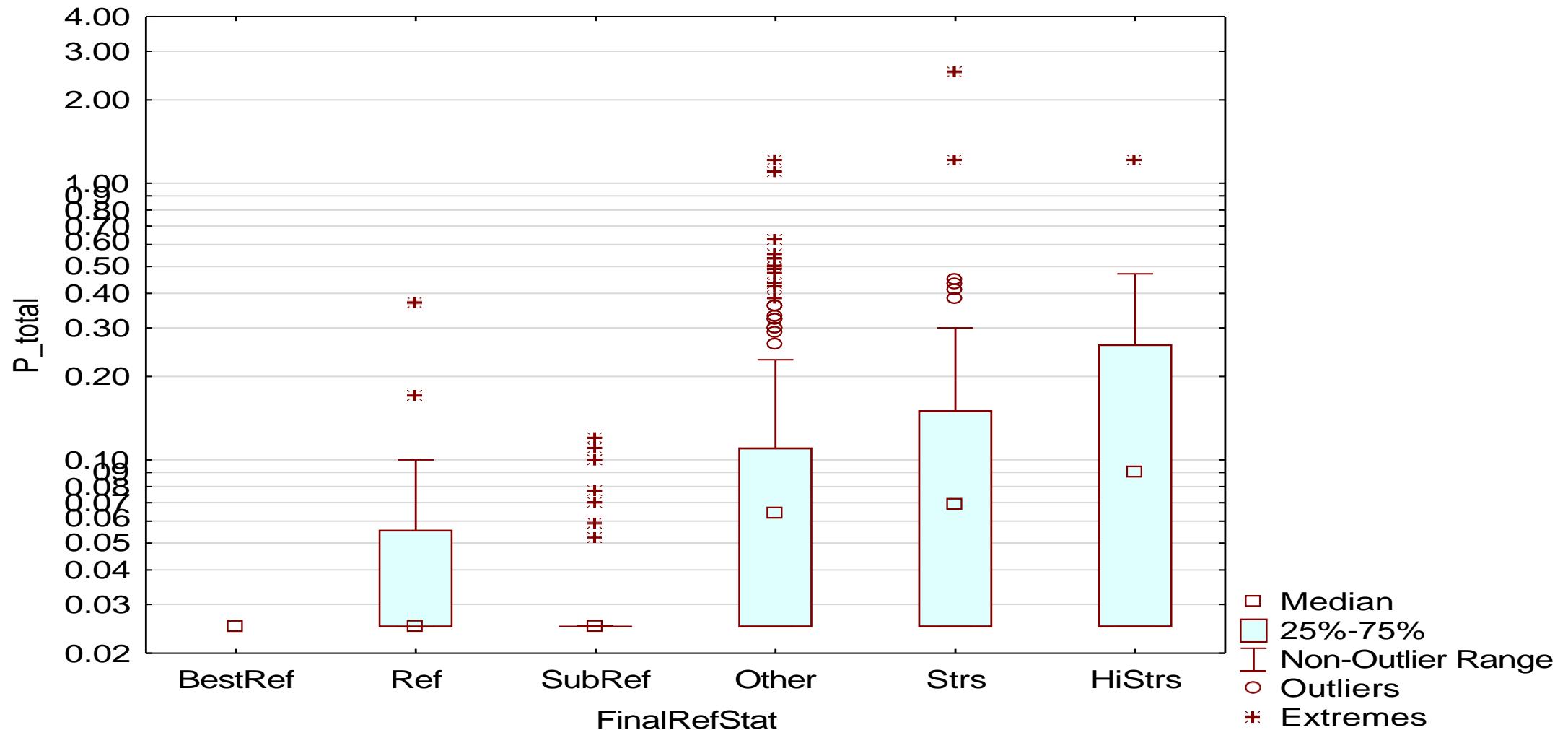
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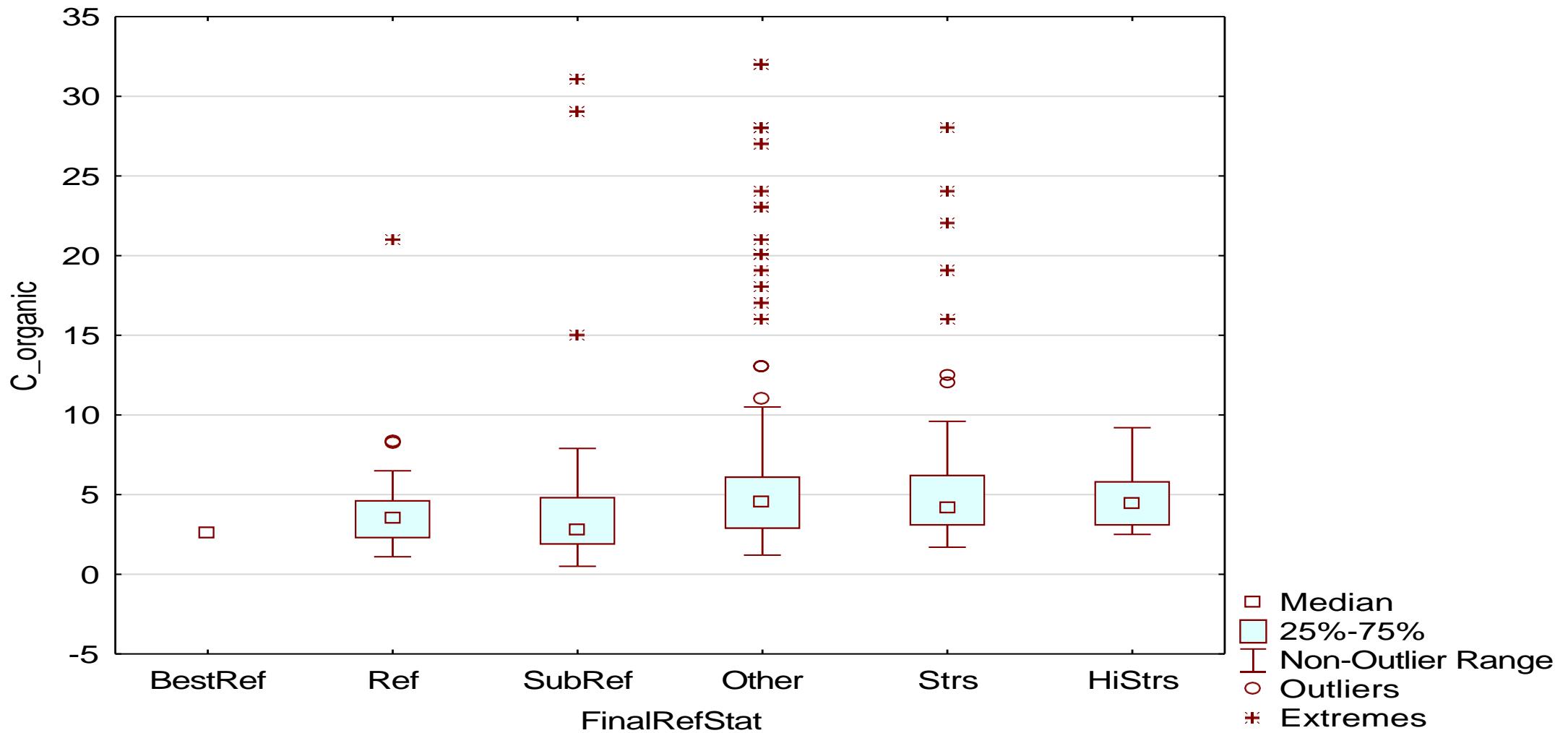
Box Plot of N\_tot\_Calc grouped by FinalRefStat  
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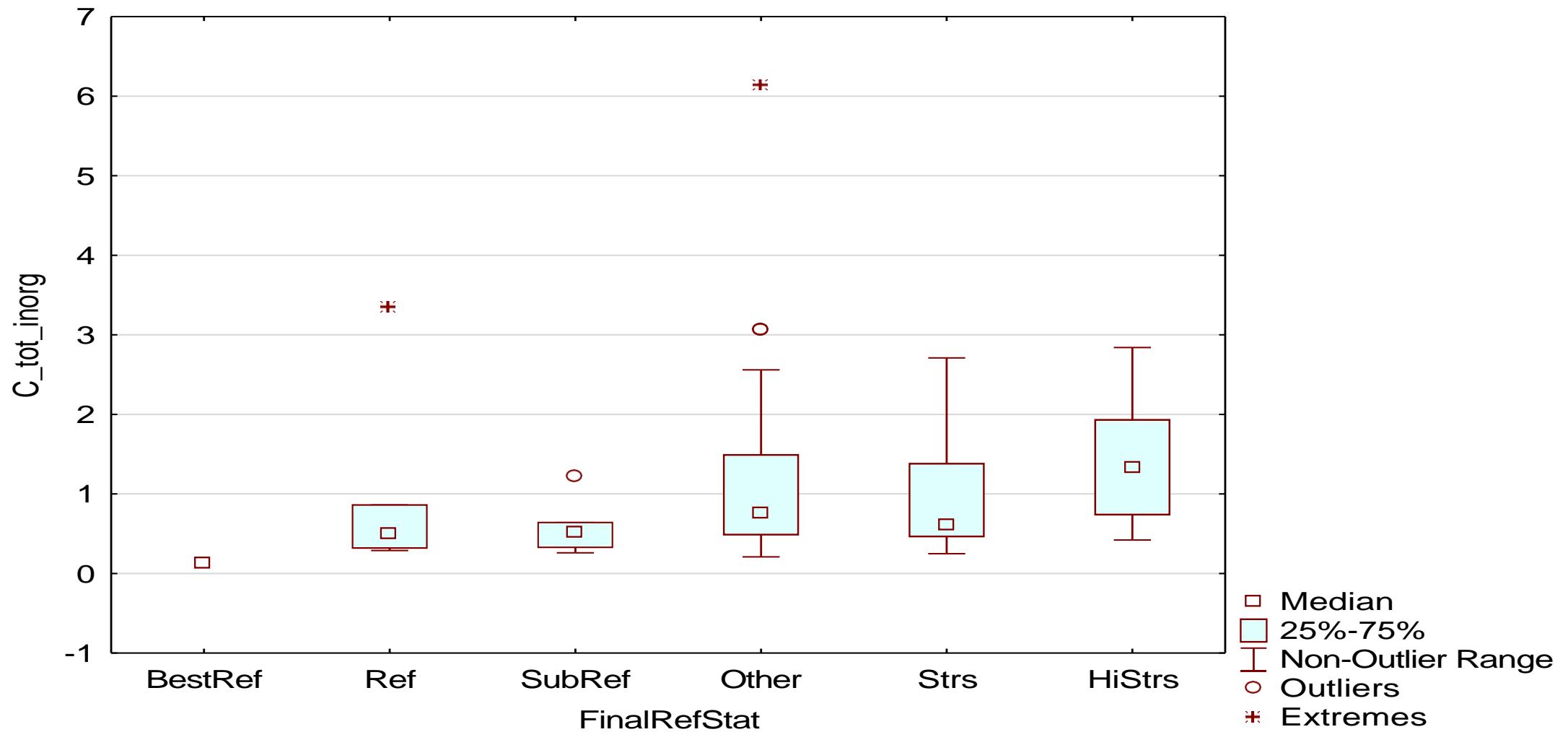
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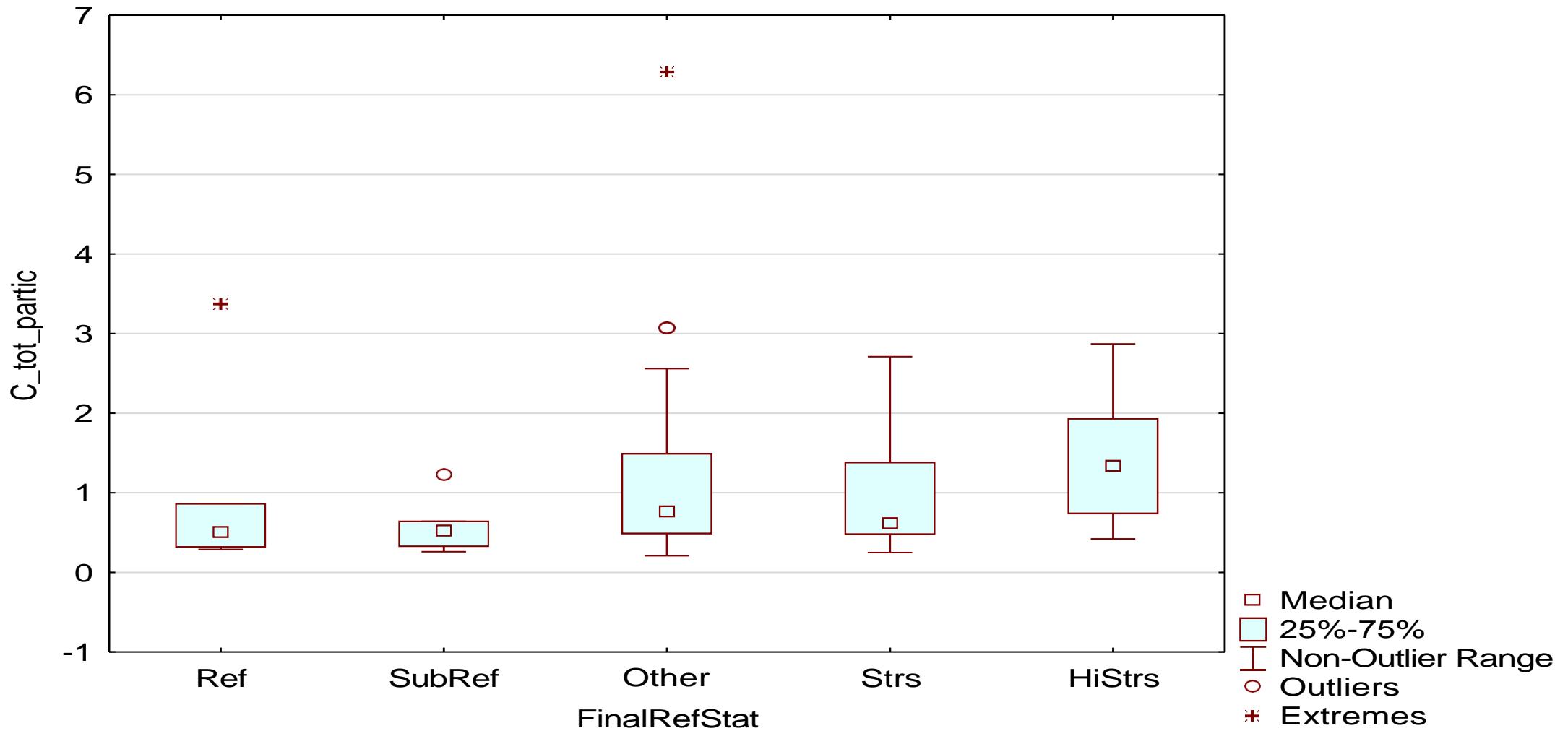
Box Plot of C\_organic grouped by FinalRefStat  
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Box Plot of C\_tot\_inorg grouped by FinalRefStat  
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Box Plot of C\_tot\_partic grouped by FinalRefStat  
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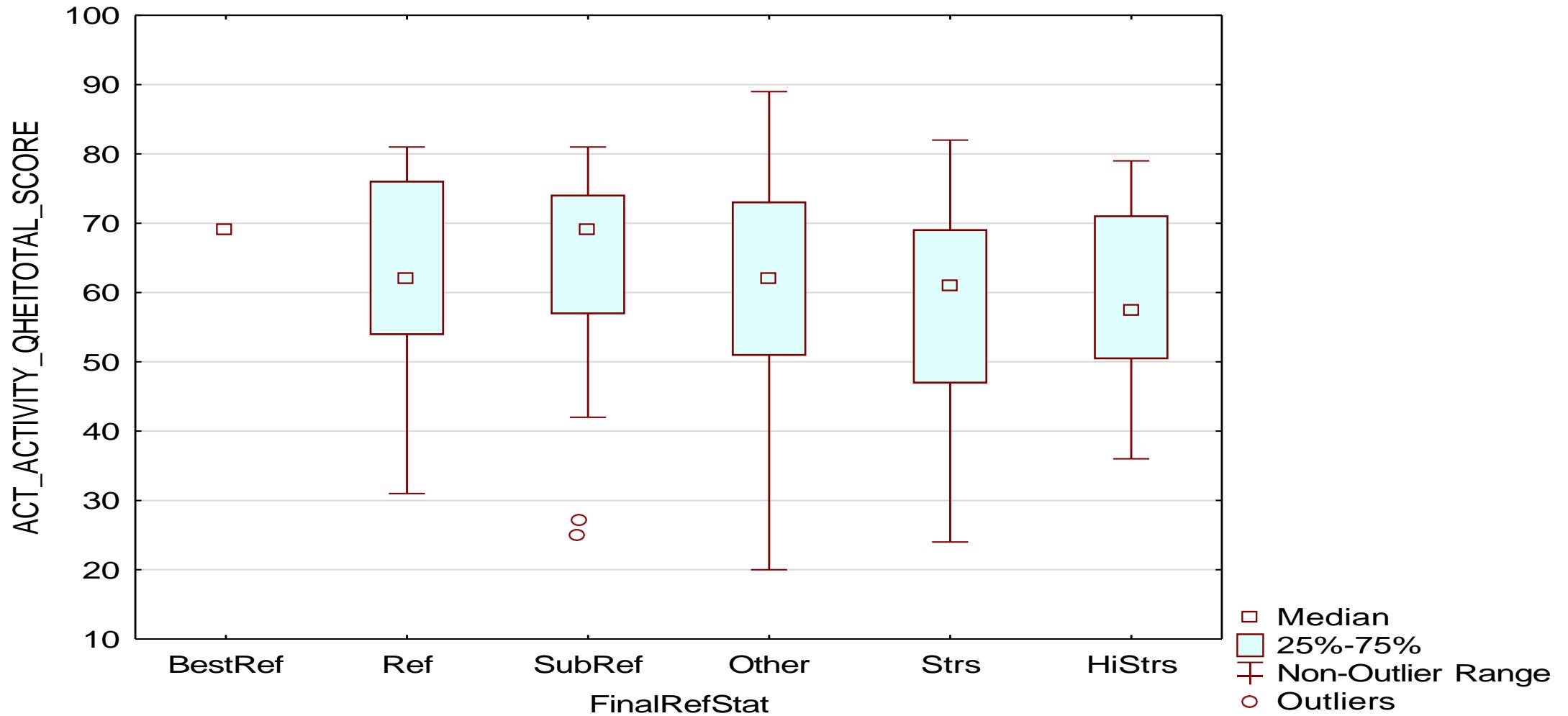


# Habitat (QHEI)

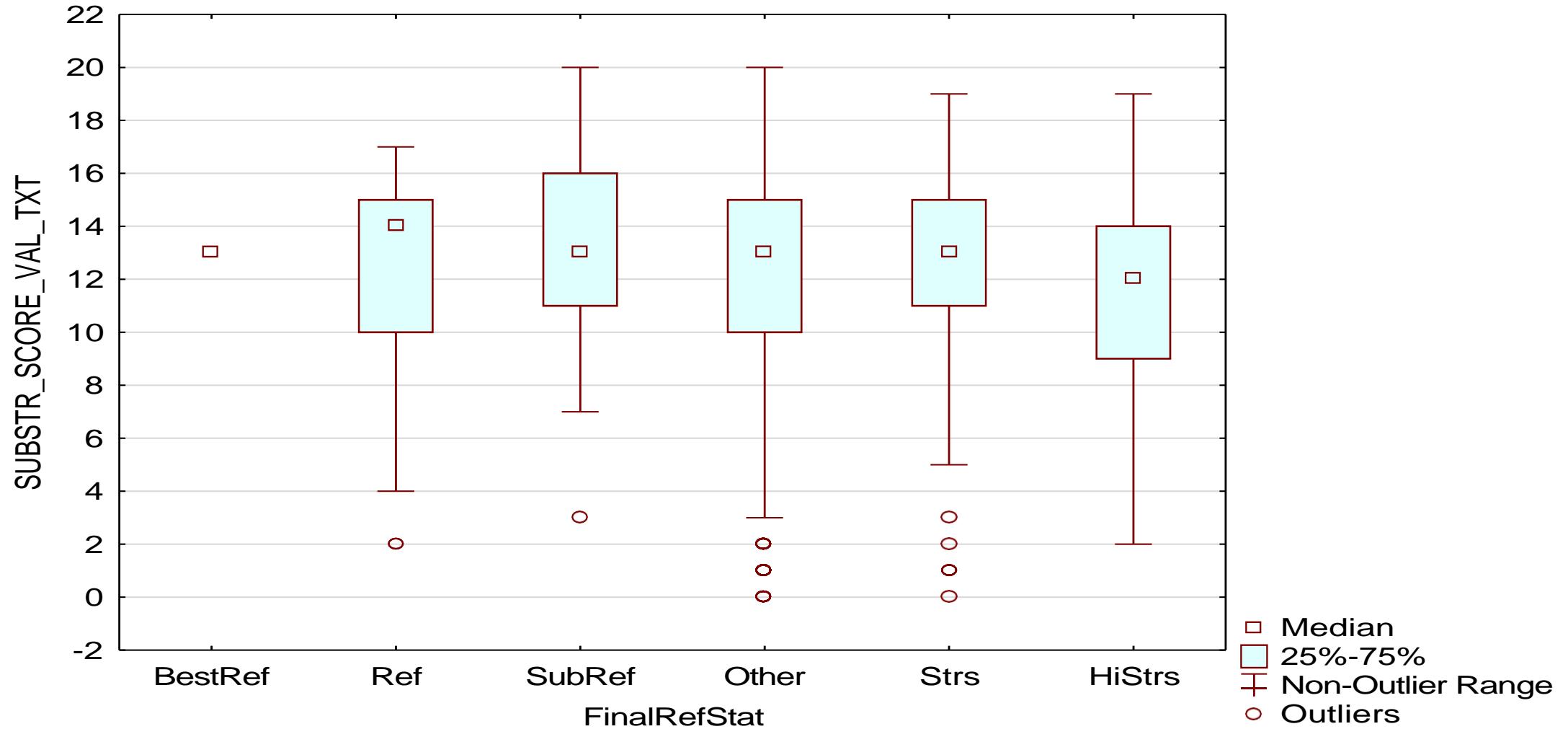
Box Plot of ACT\_ACTIVITY\_QHEITOTAL\_SCORE grouped by FinalRefStat

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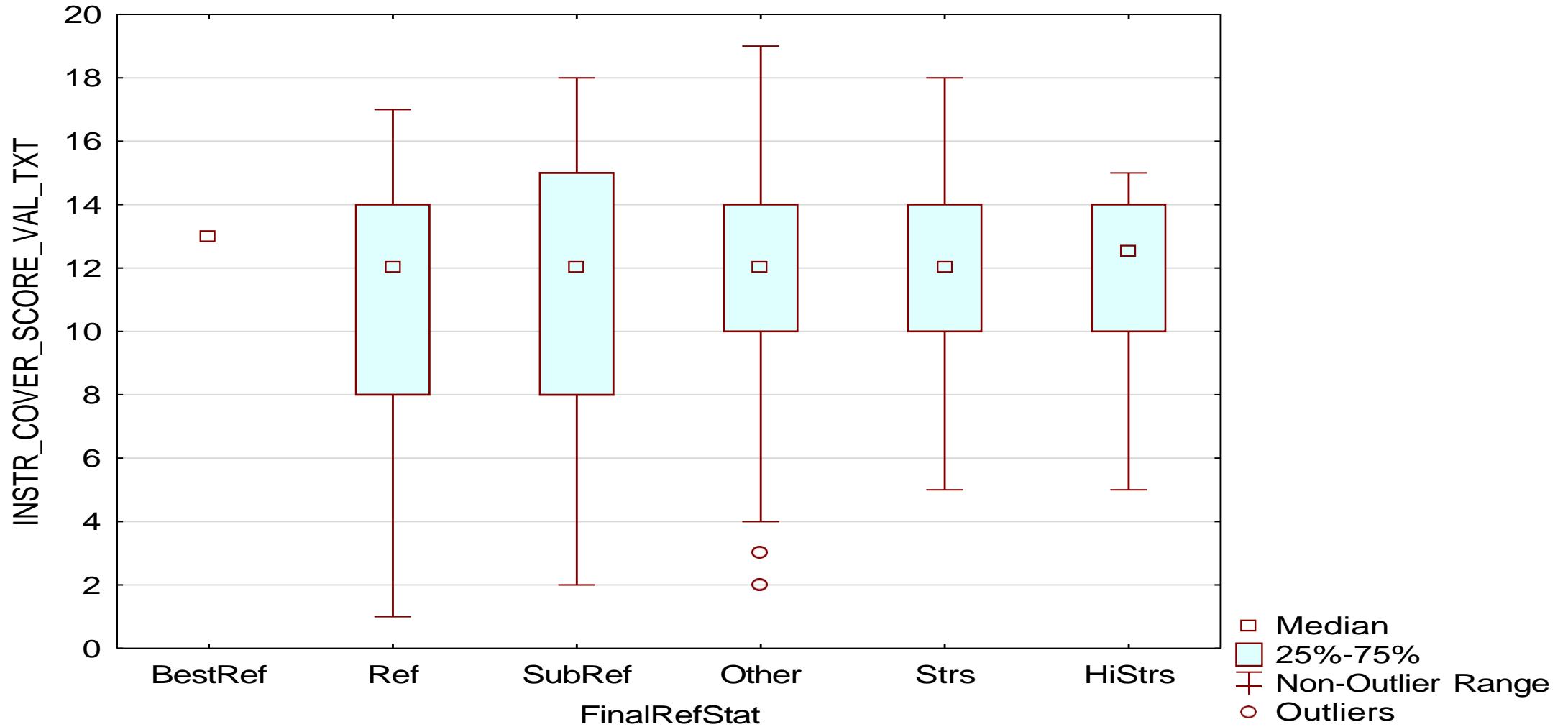
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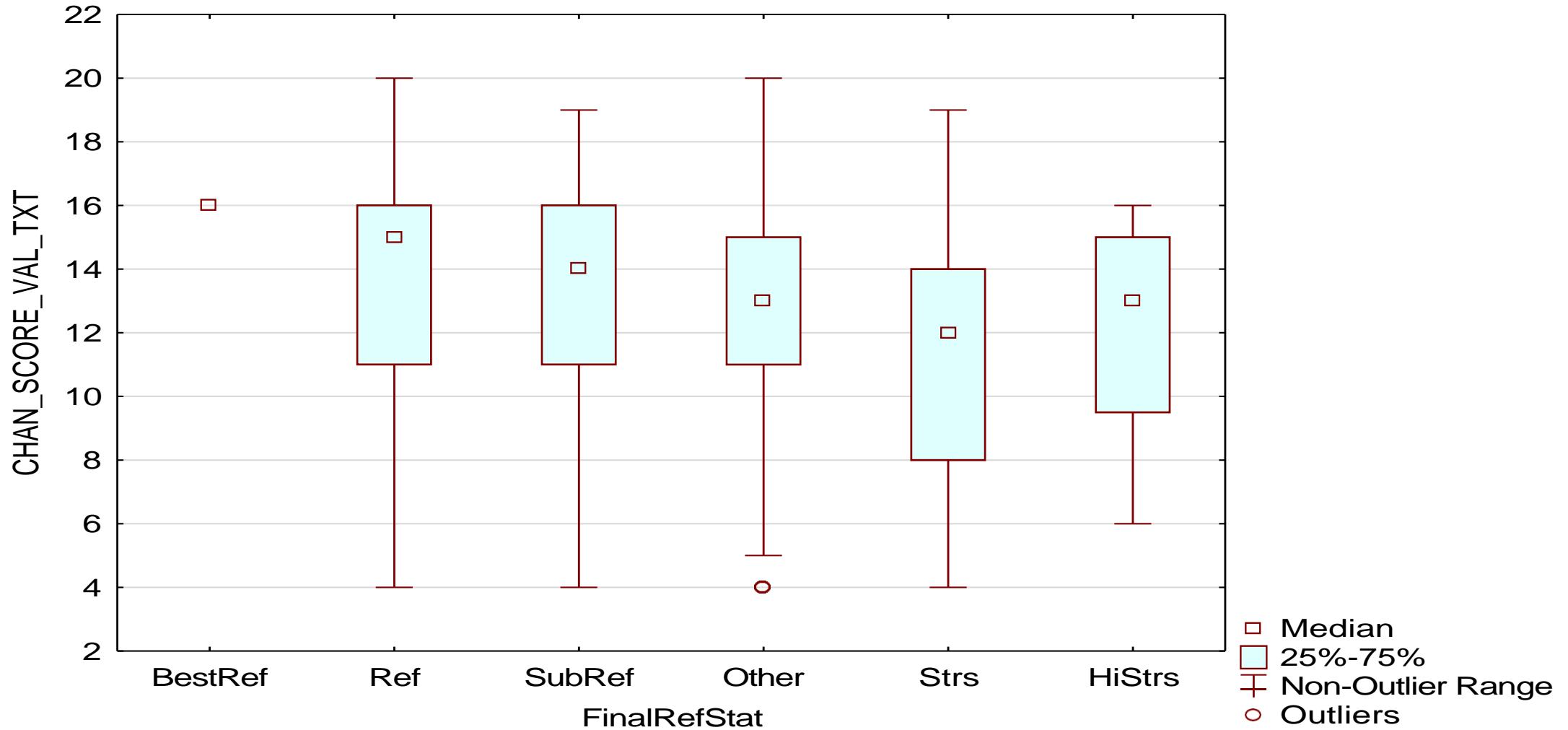
Box Plot of SUBSTR\_SCORE\_VAL\_TXT grouped by FinalRefStat  
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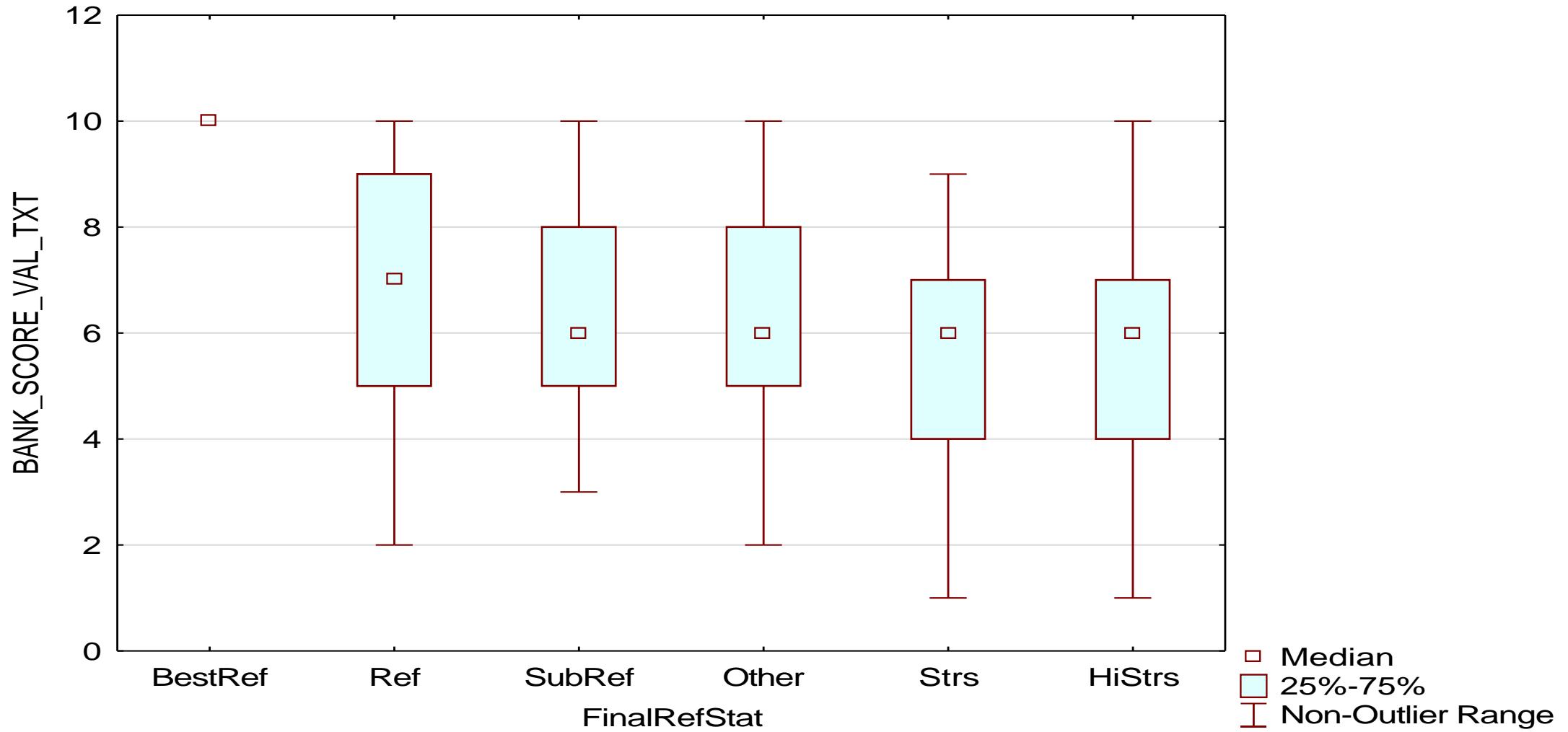
Box Plot of INSTR\_COVER\_SCORE\_VAL\_TXT grouped by FinalRefStat  
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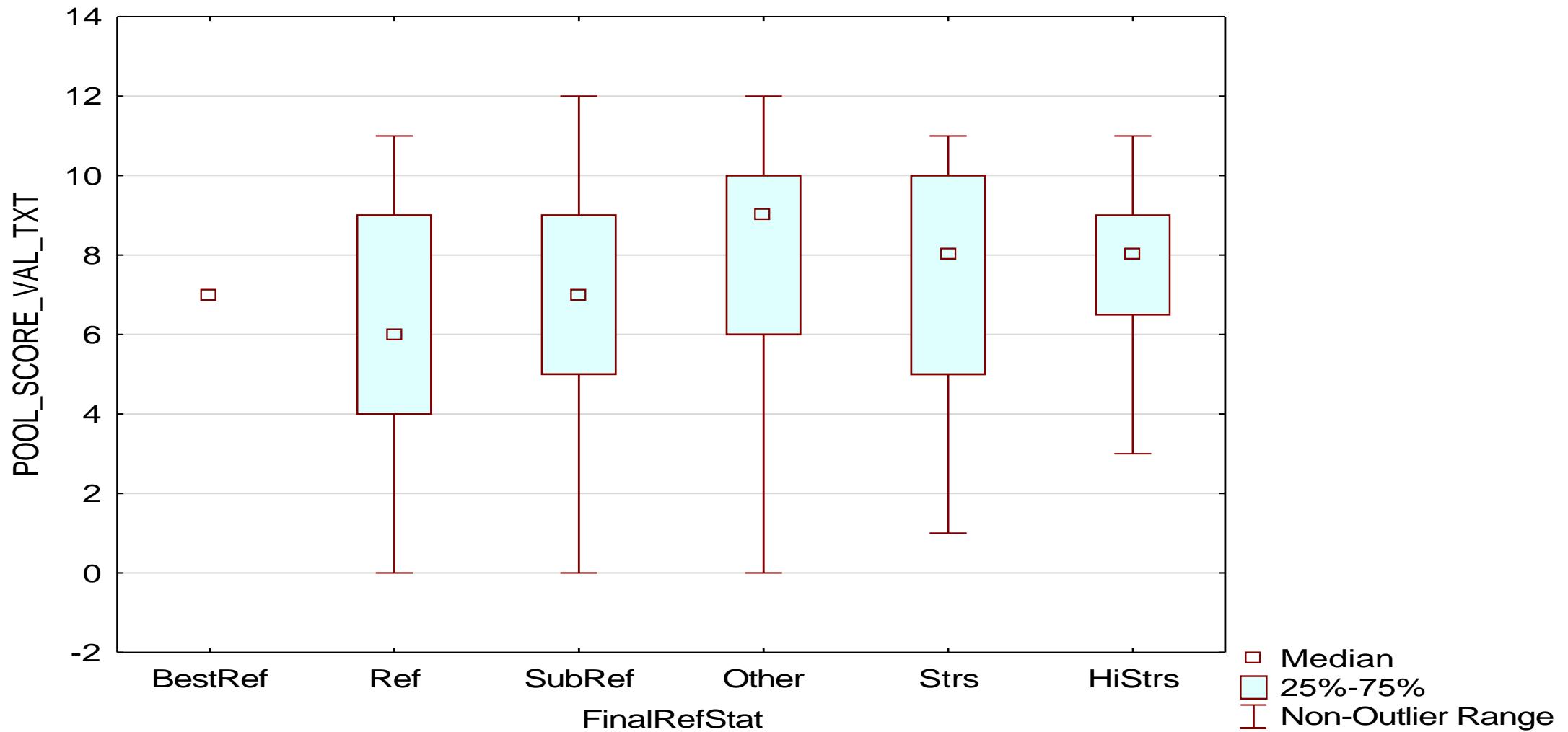
Box Plot of CHAN\_SCORE\_VAL\_TXT grouped by FinalRefStat  
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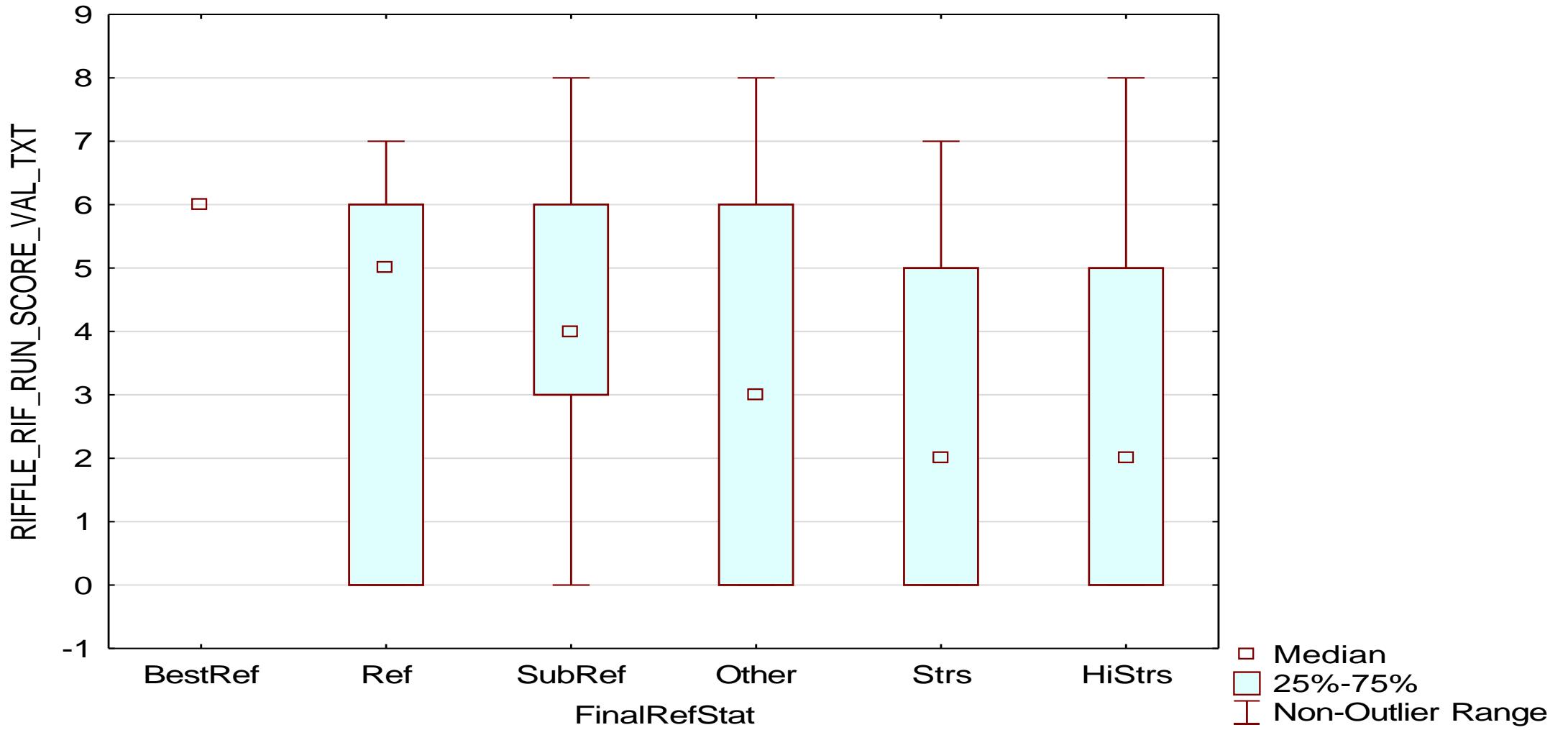
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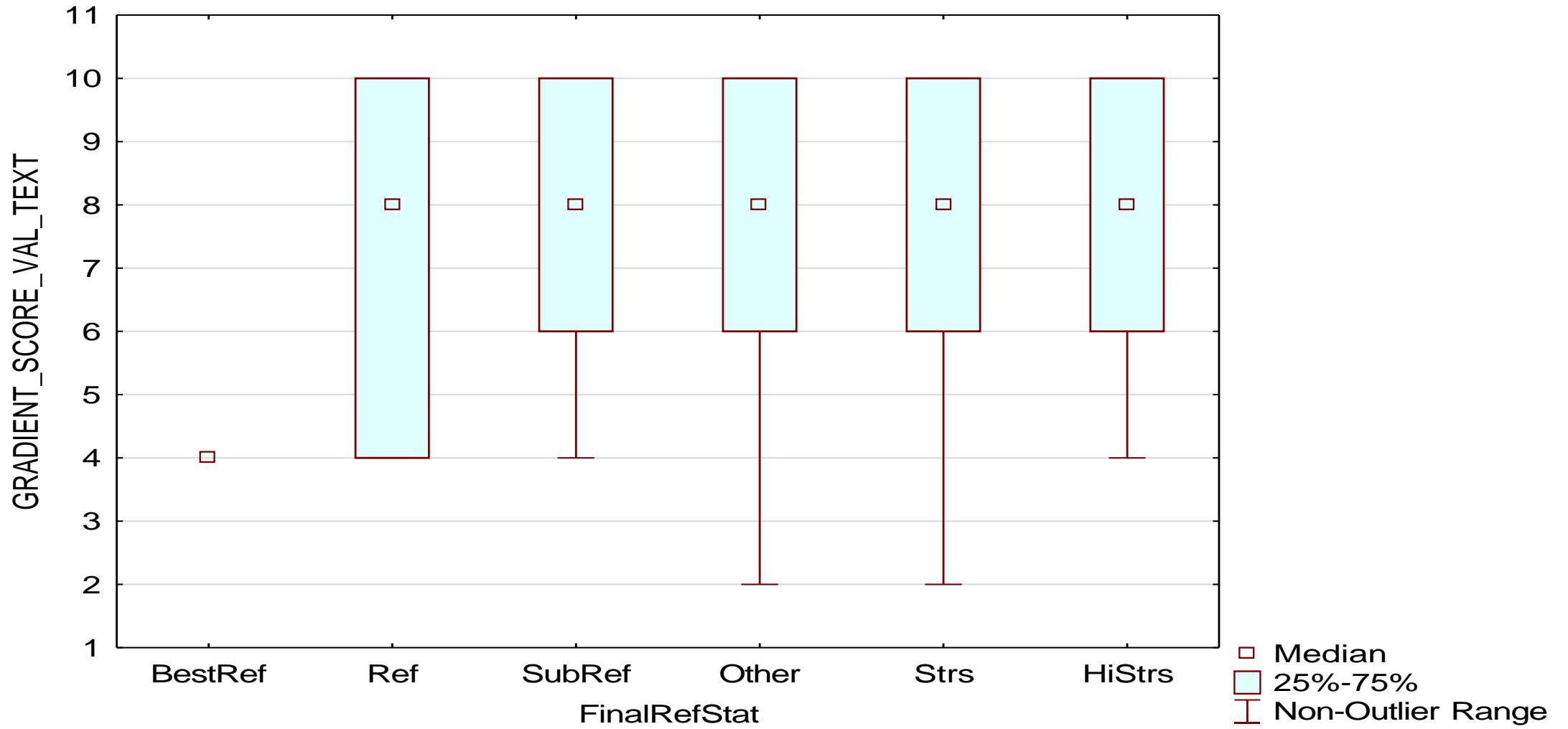
Box Plot of POOL\_SCORE\_VAL\_TXT grouped by FinalRefStat  
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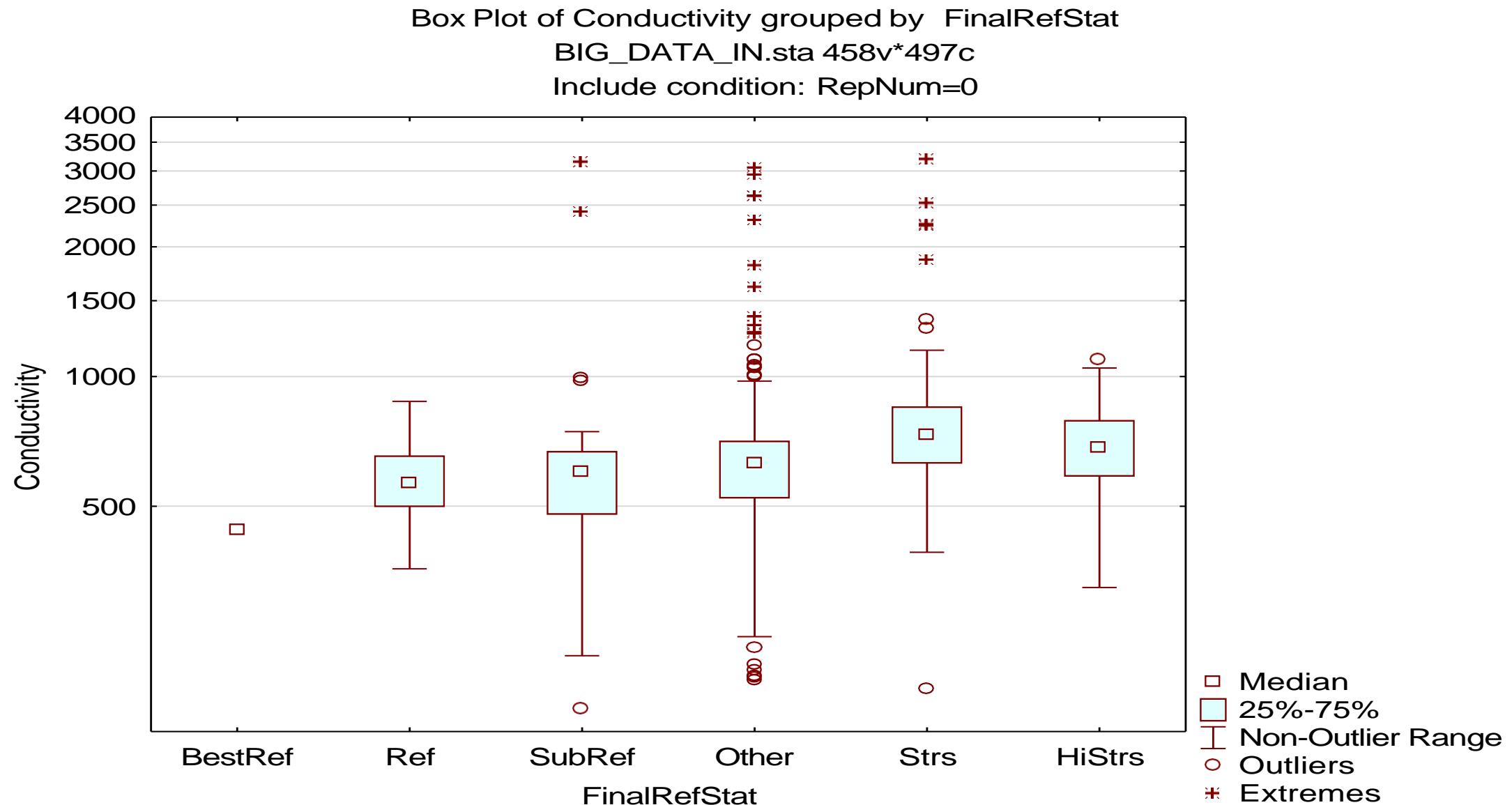
Box Plot of RIFFLE\_RIF\_RUN\_SCORE\_VAL\_TXT grouped by FinalRefStat  
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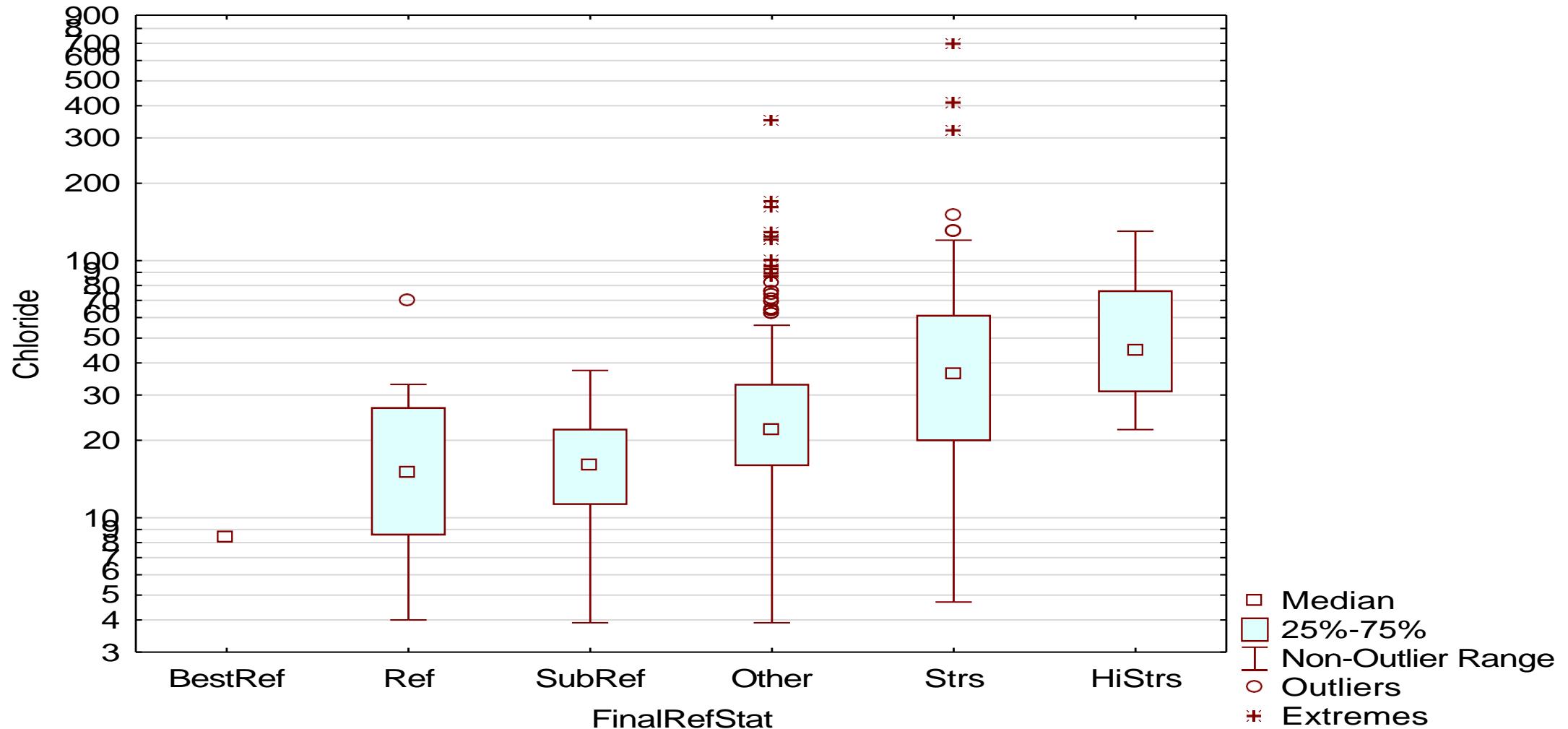
Box Plot of GRADIENT\_SCORE\_VAL\_TEXT grouped by FinalRefStat  
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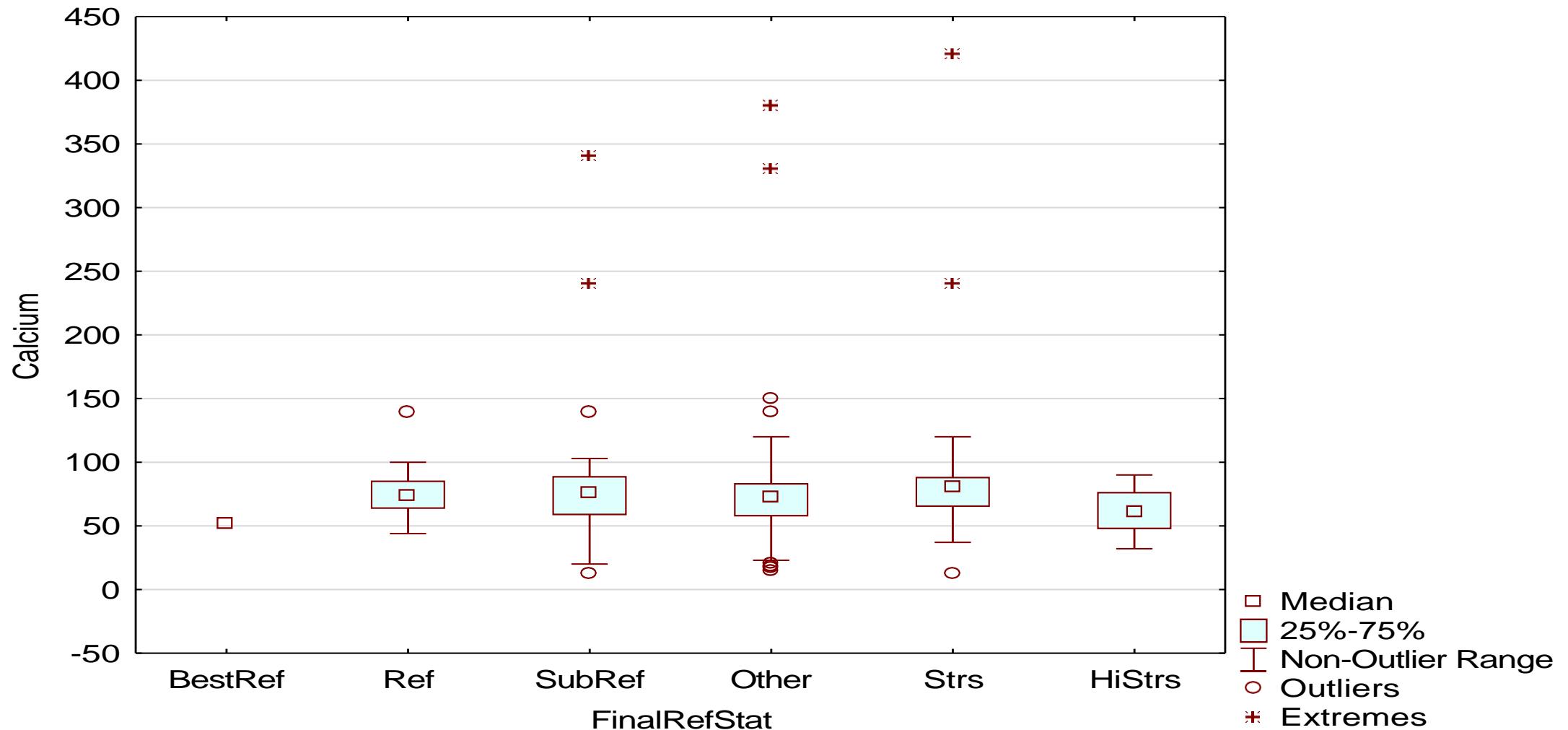
# Salts



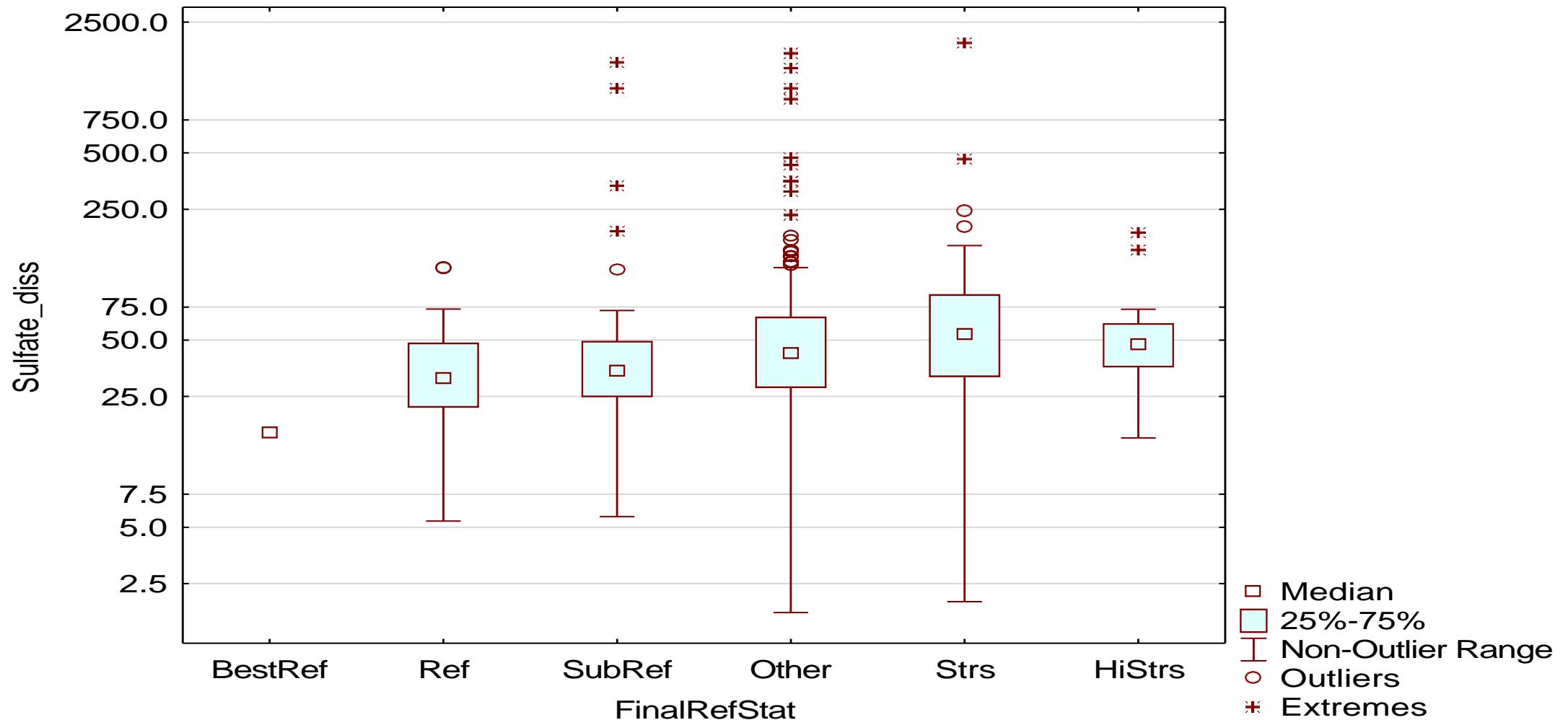
Box Plot of Chloride grouped by FinalRefStat  
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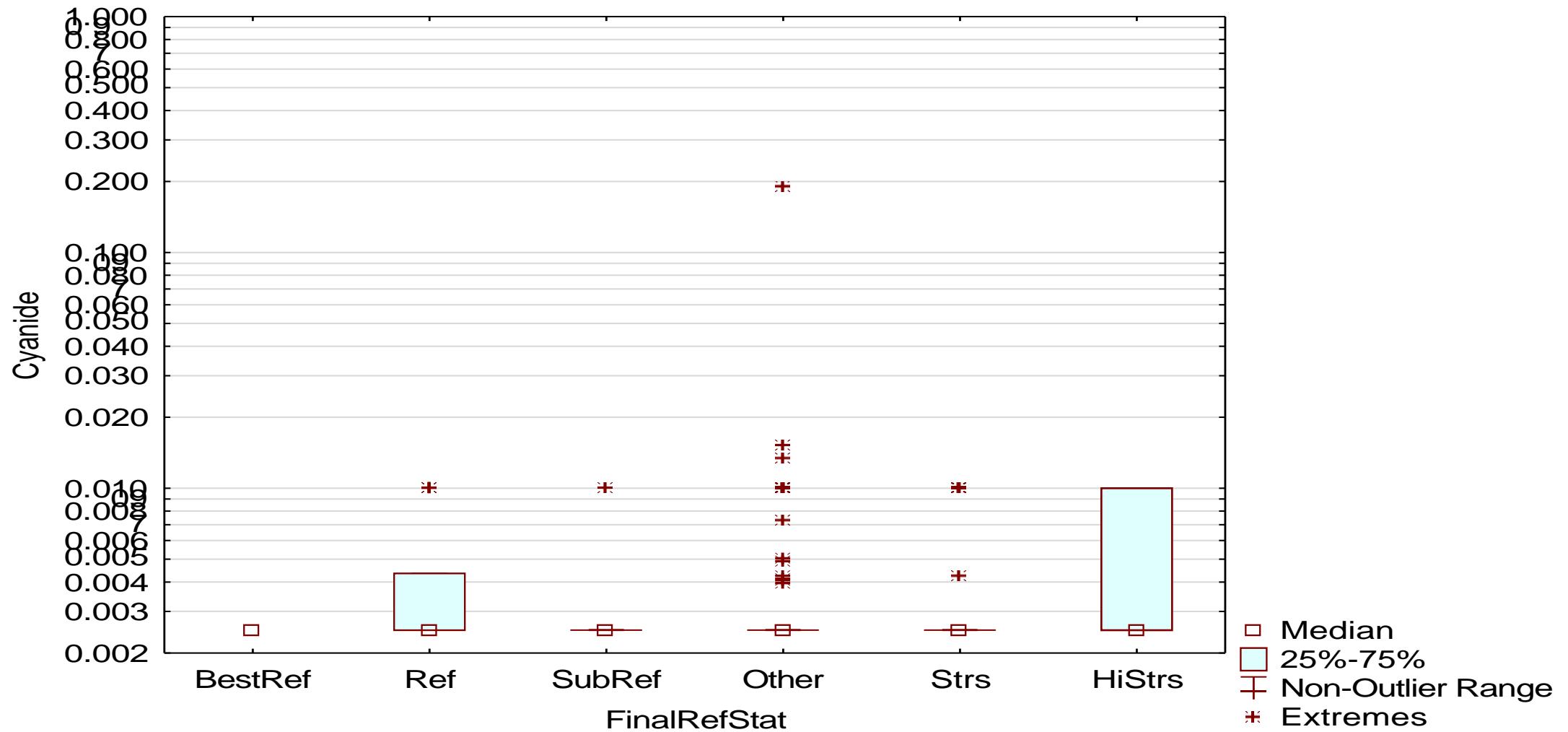
Box Plot of Calcium grouped by FinalRefStat  
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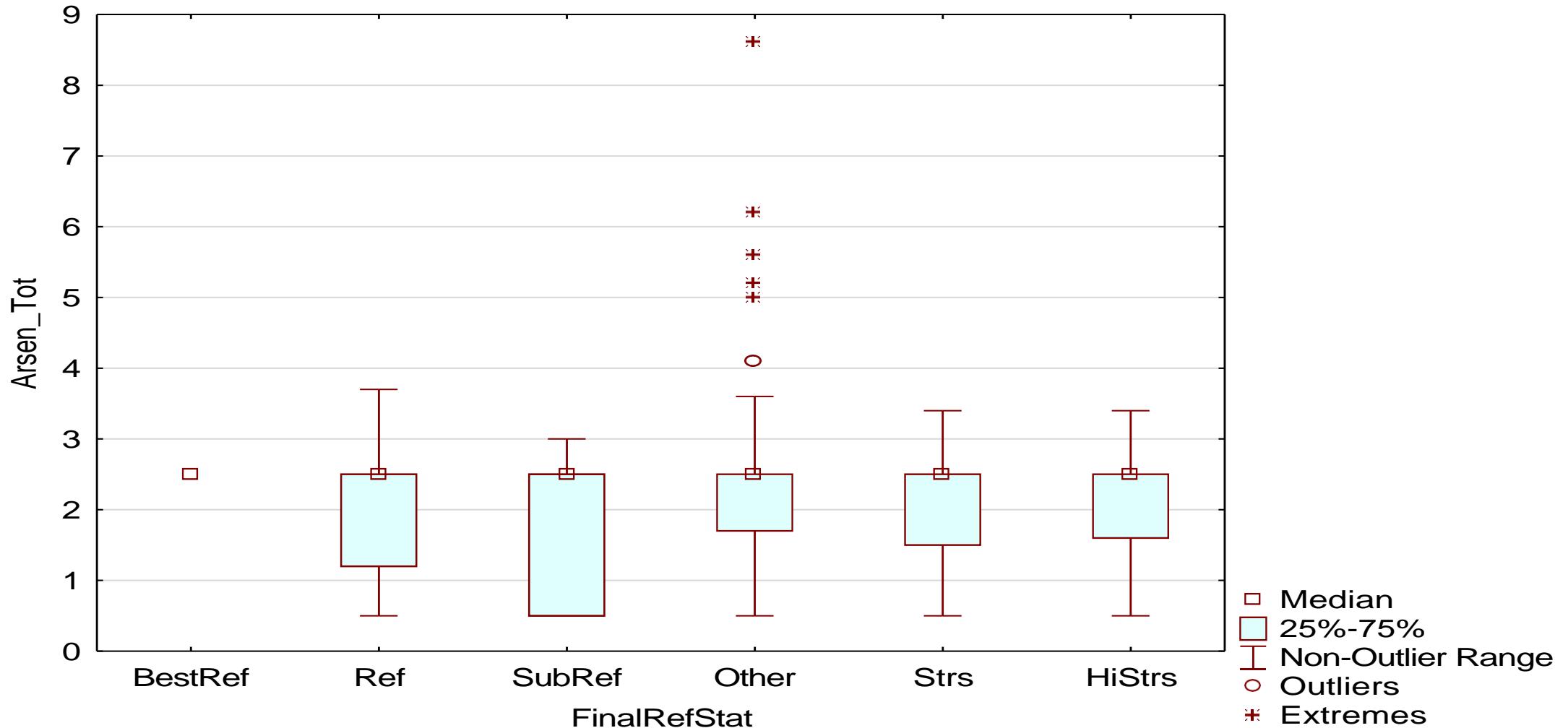
Box Plot of Sulfate\_diss grouped by FinalRefStat  
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Box Plot of Cyanide grouped by FinalRefStat  
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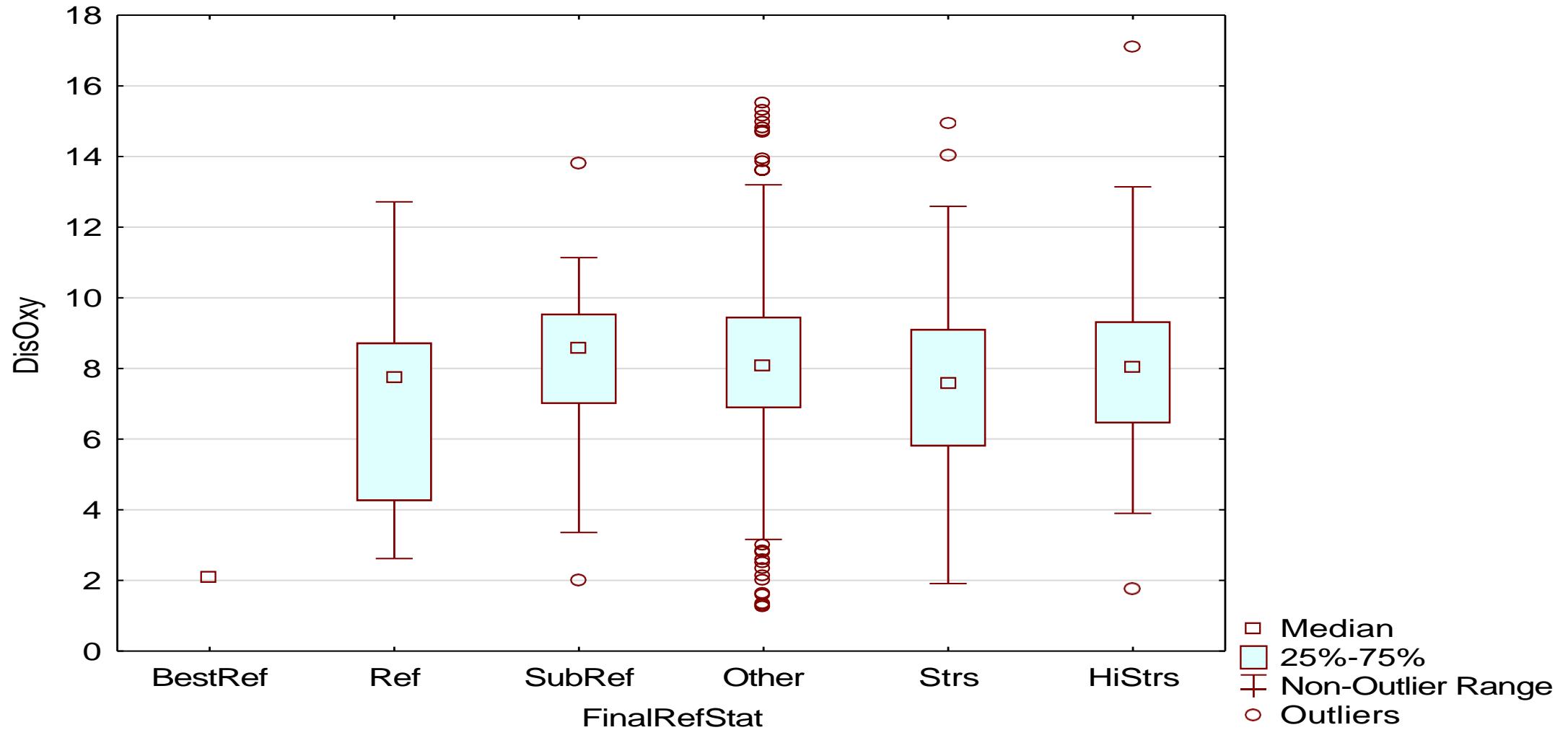


Box Plot of Arsen\_Tot grouped by FinalRefStat  
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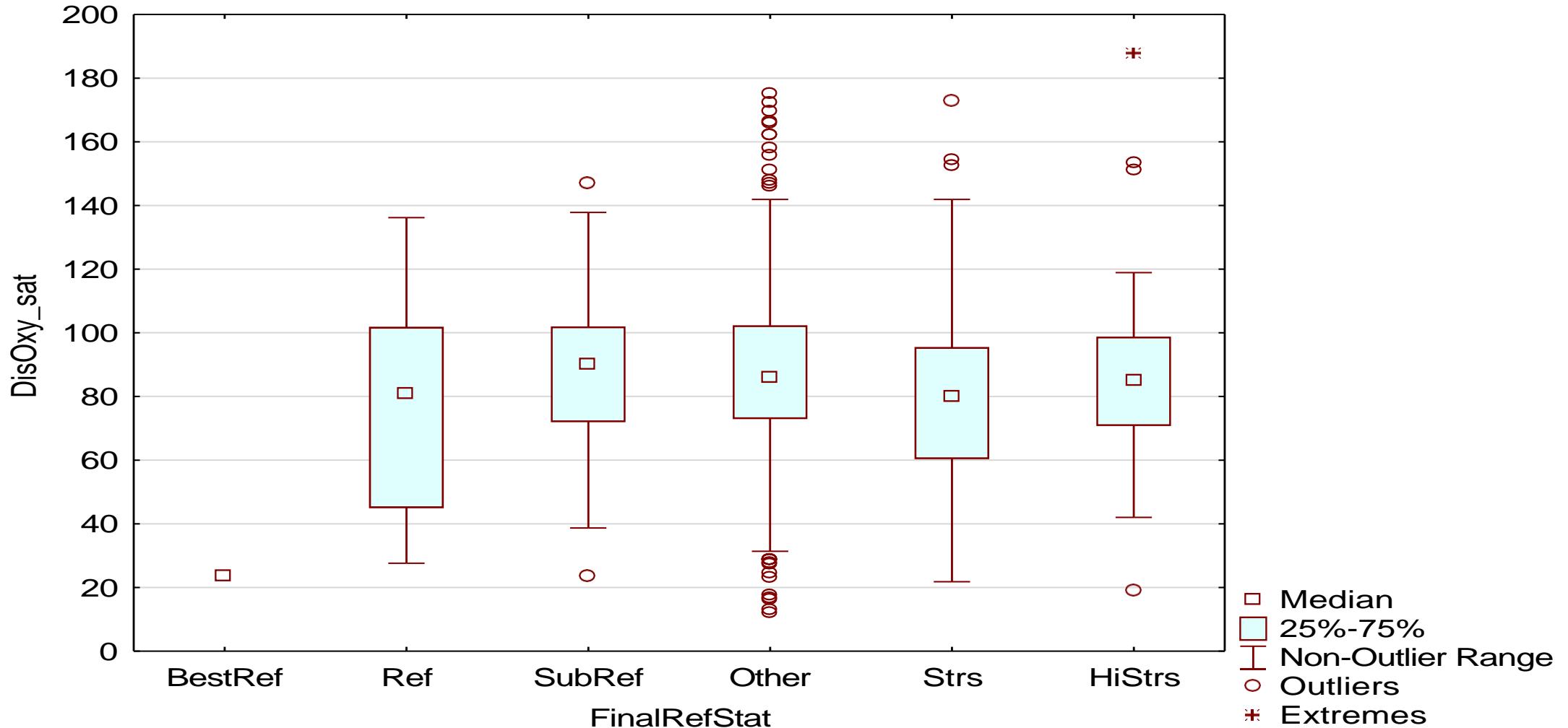


# Water Properties

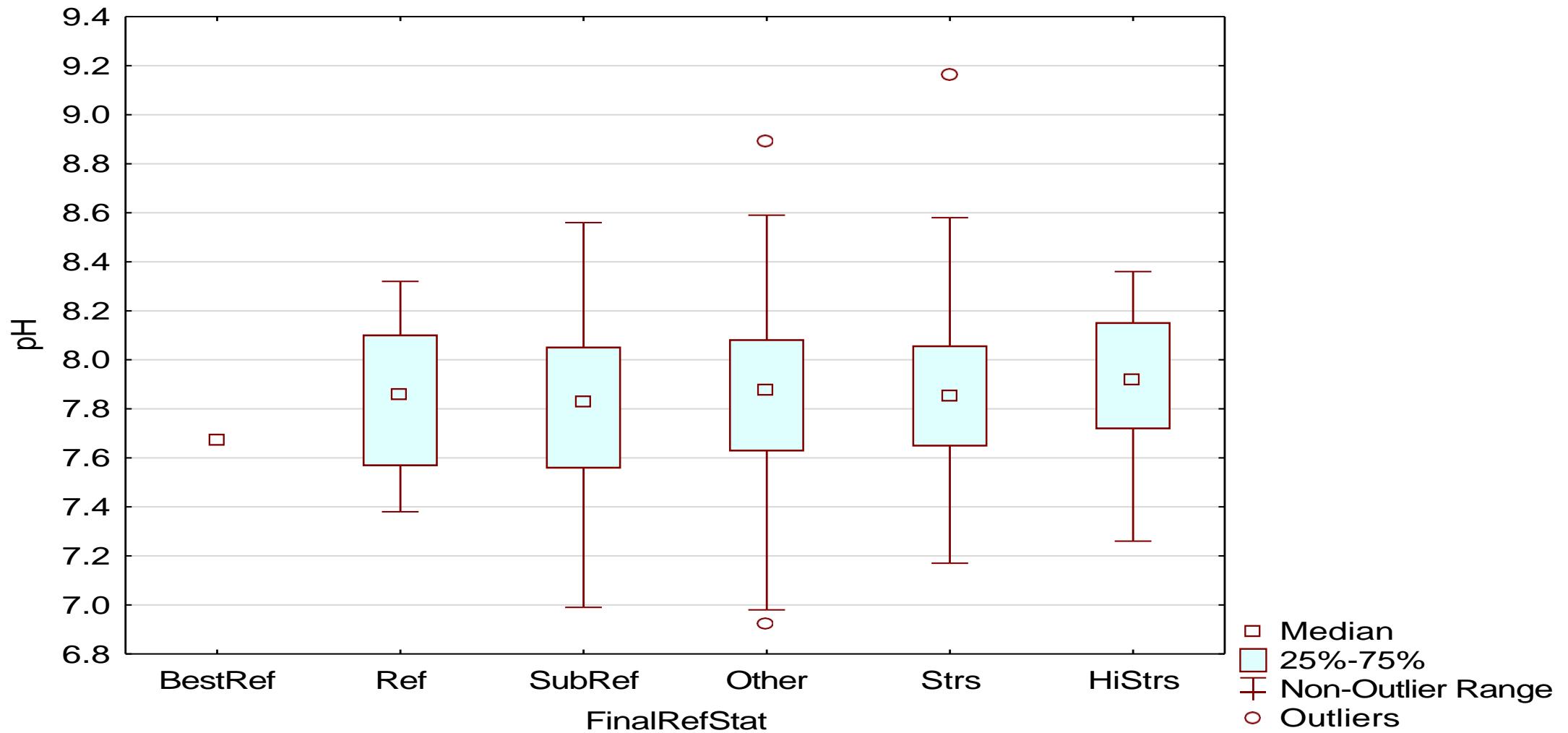
Box Plot of DisOxy grouped by FinalRefStat  
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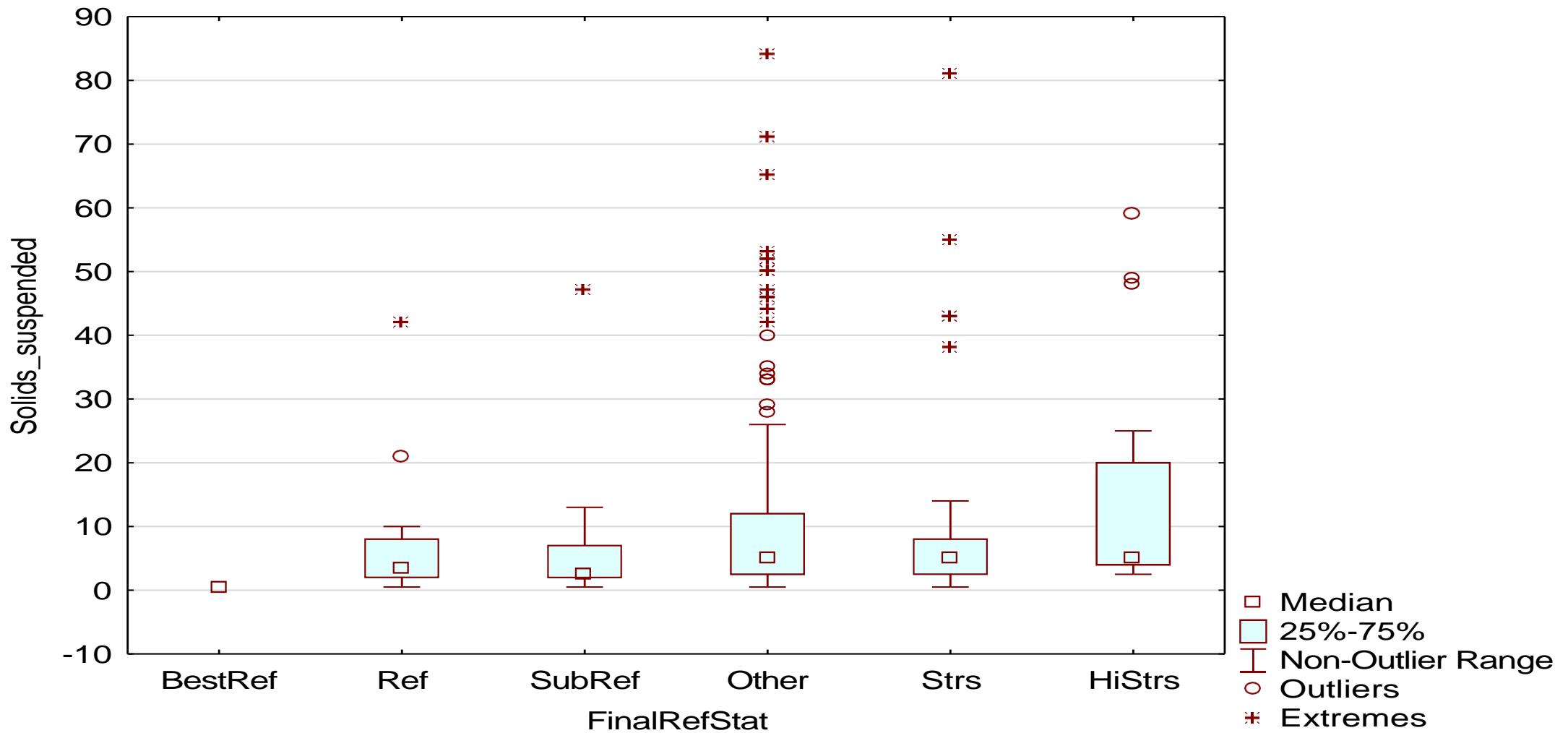
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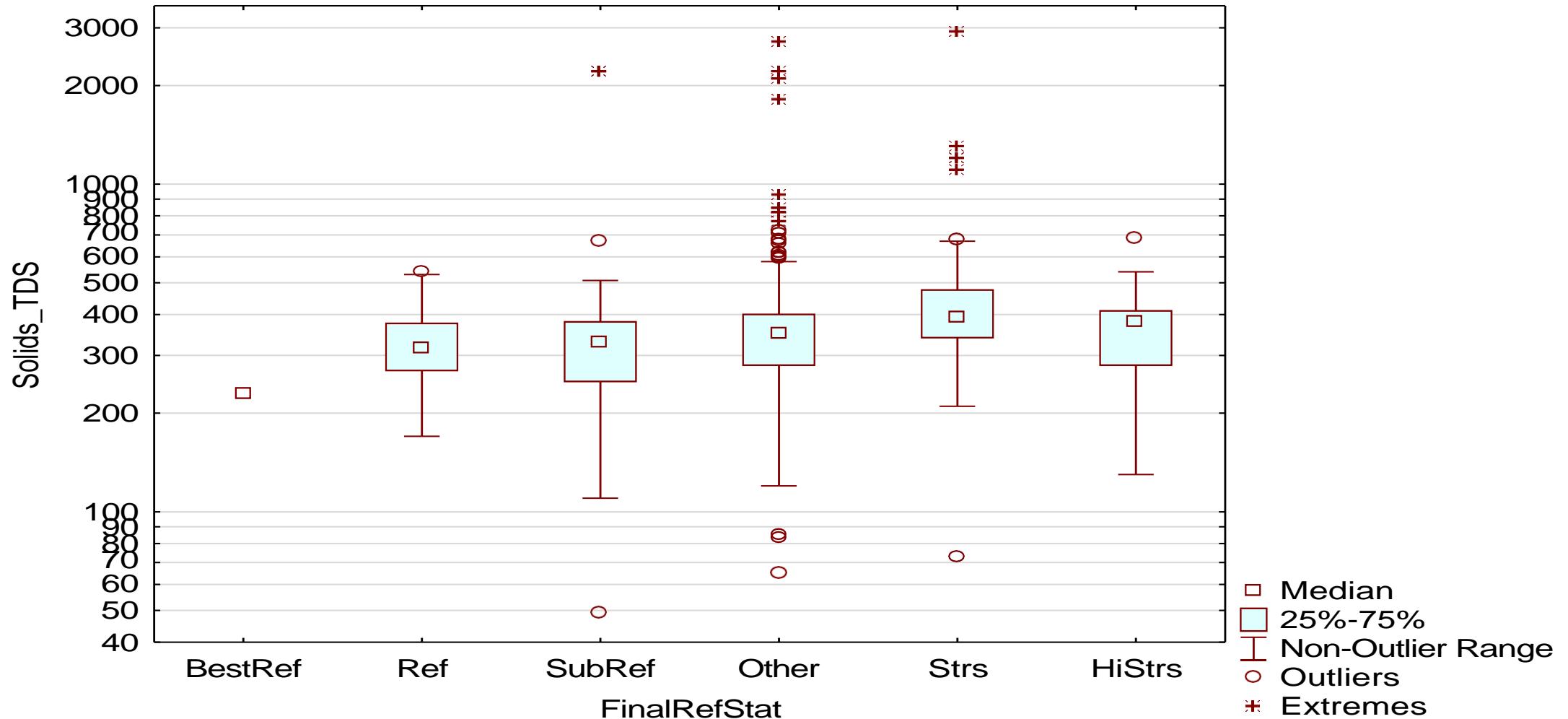
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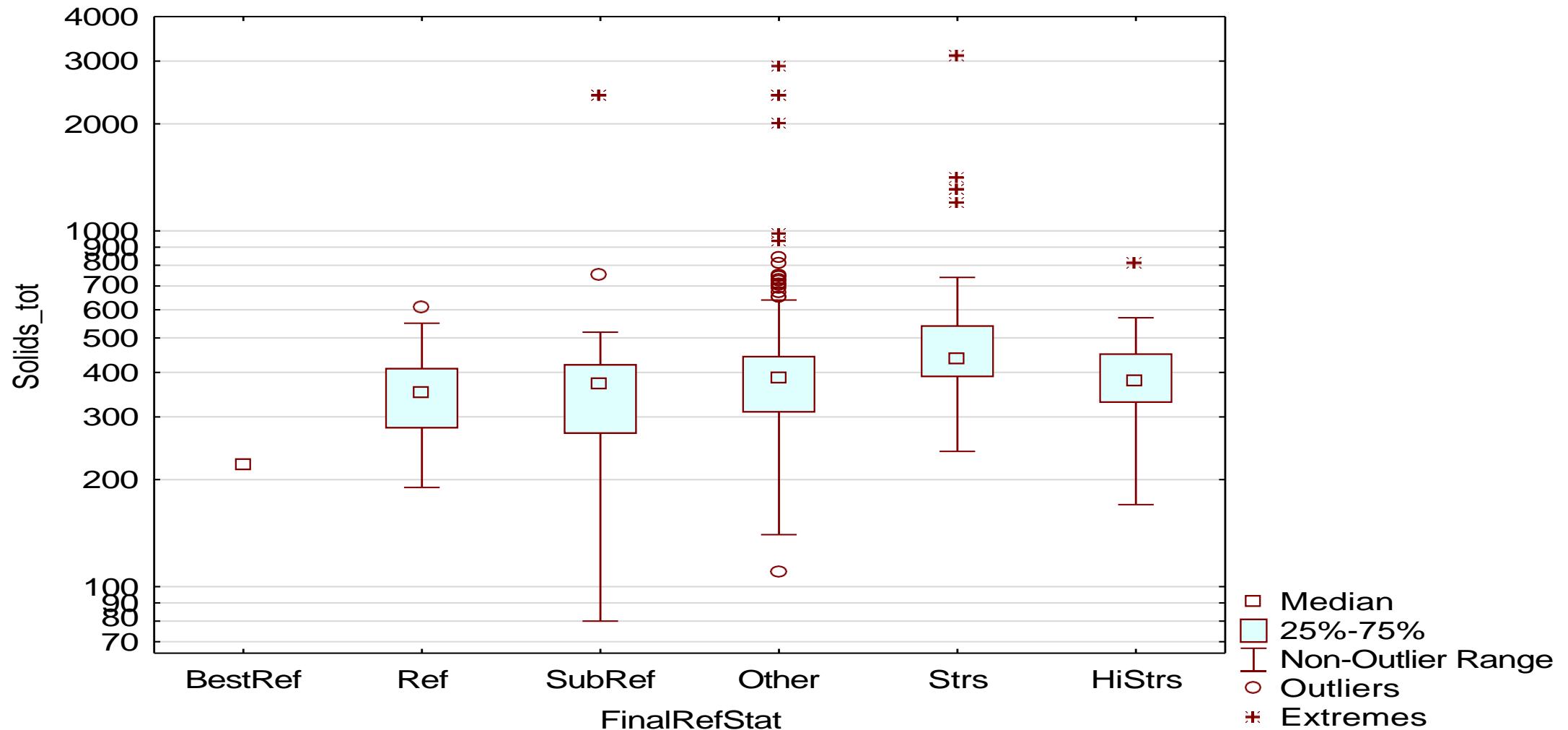
Box Plot of Solids\_suspended grouped by FinalRefStat  
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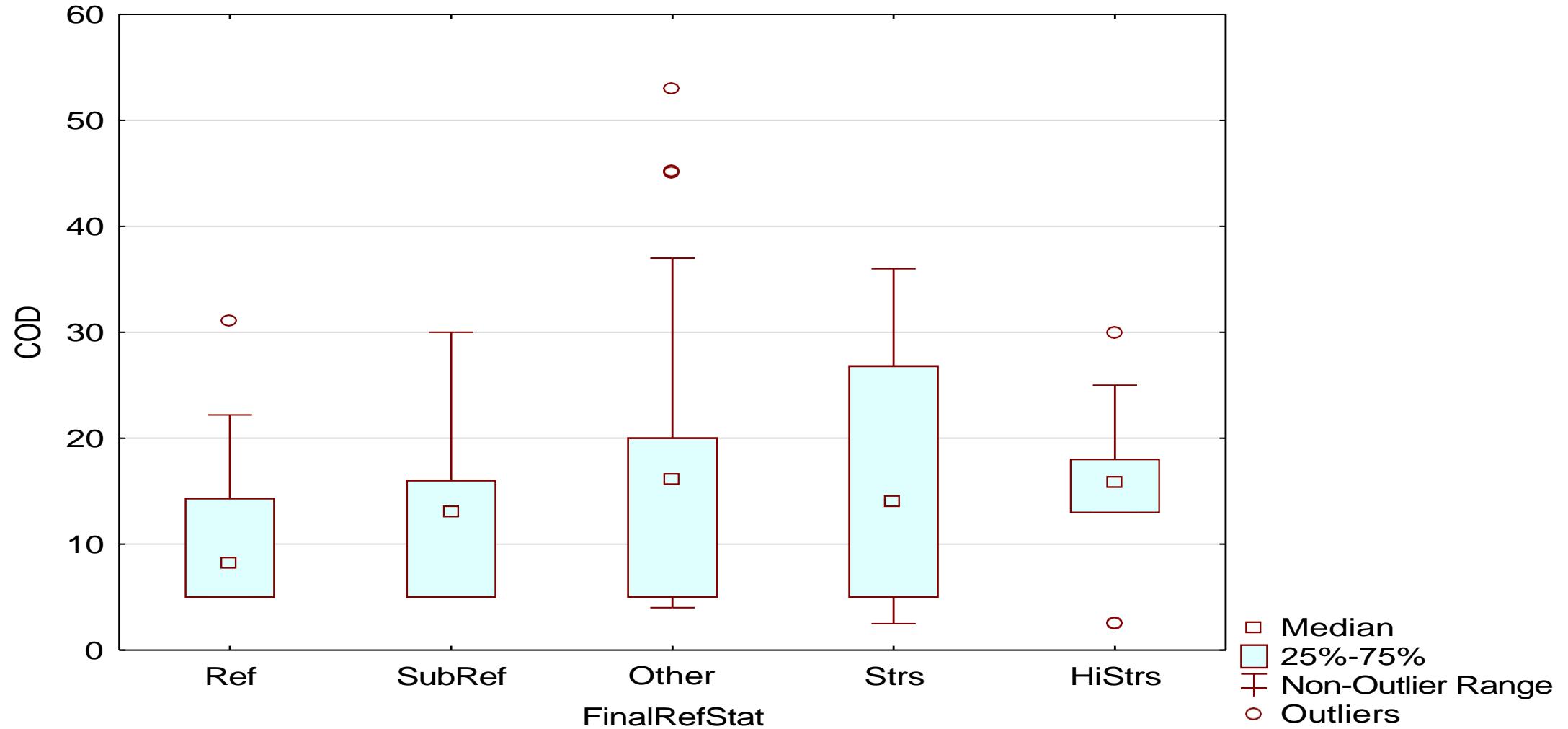
Box Plot of Solids\_TDS grouped by FinalRefStat  
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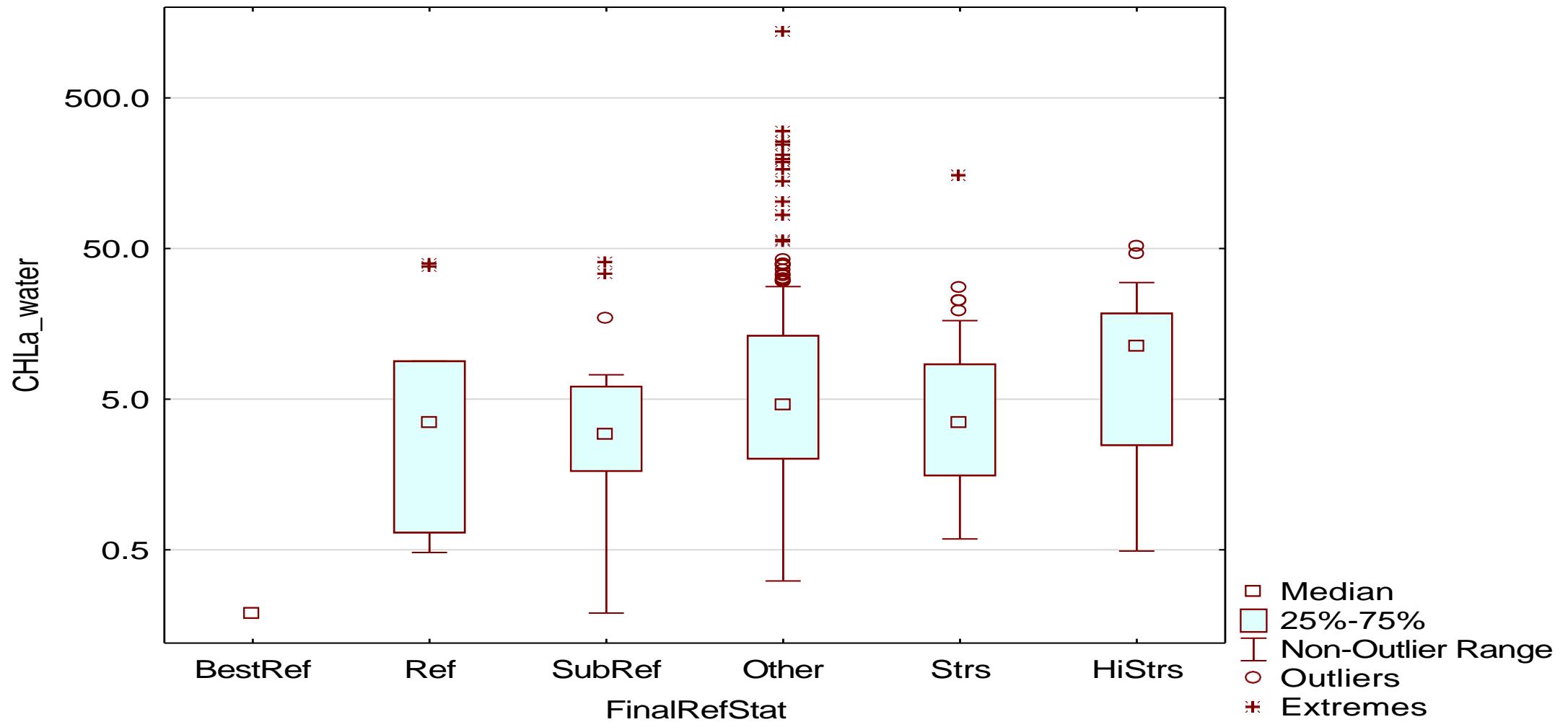


Box Plot of COD grouped by FinalRefStat  
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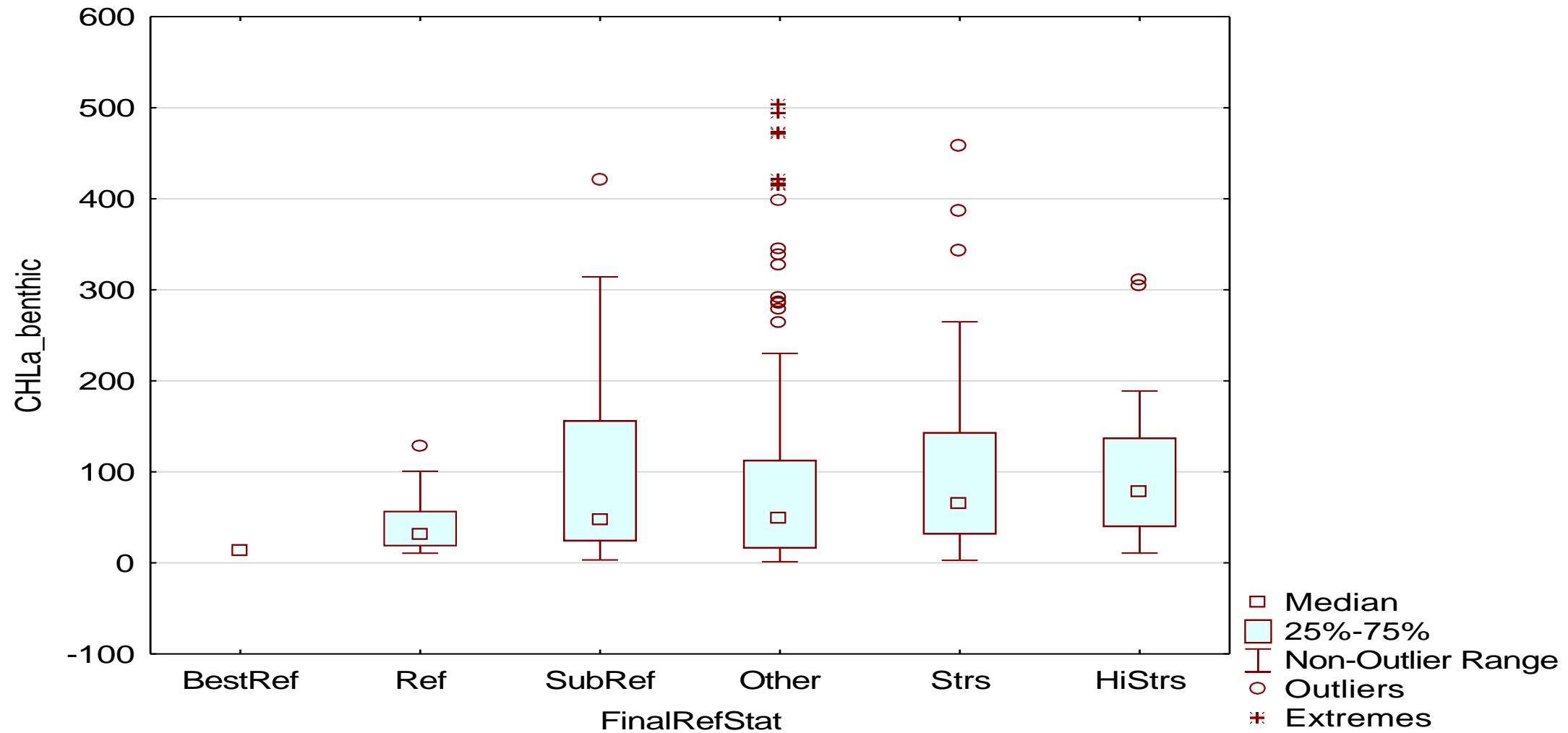


# Chlorophyll

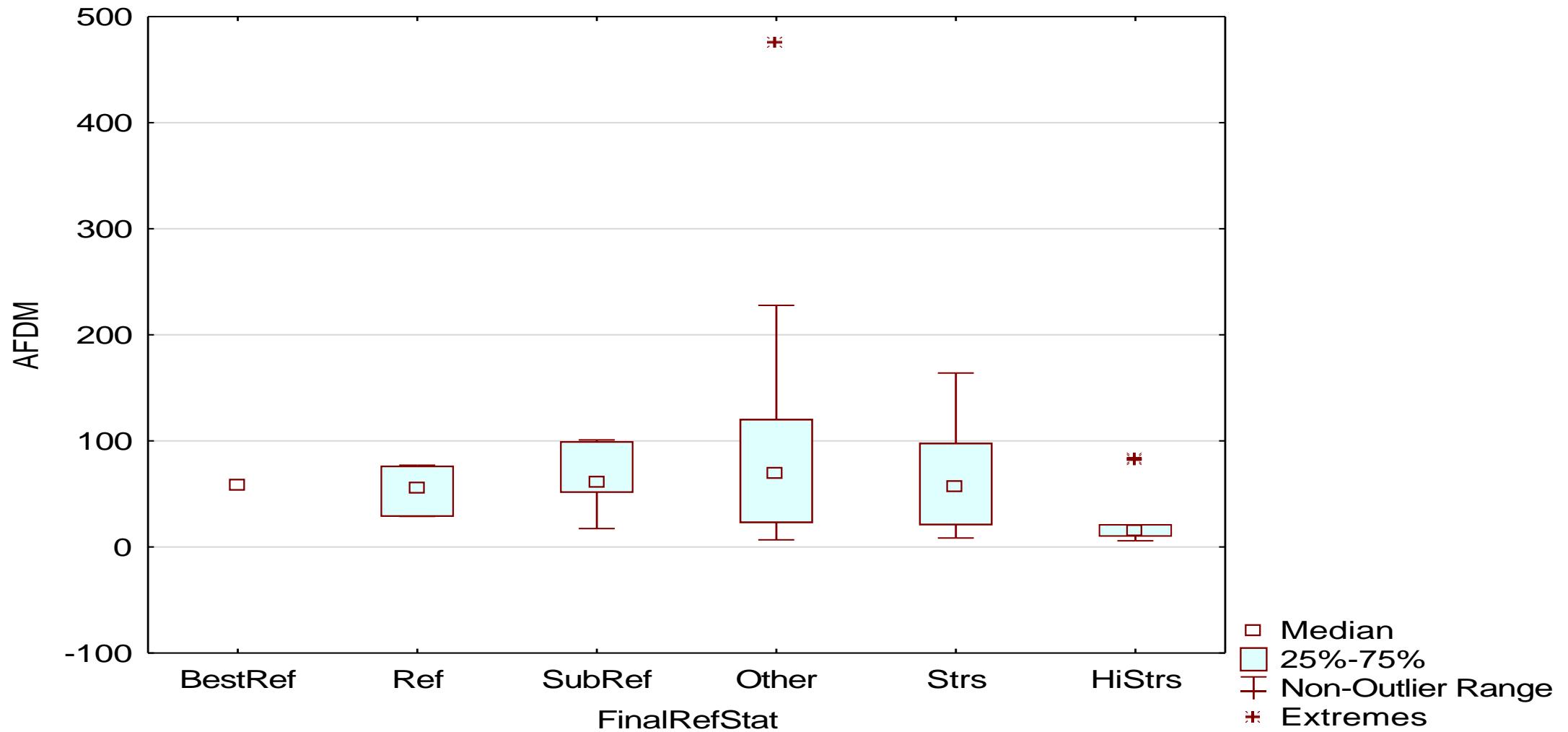
Box Plot of CHLa\_water grouped by FinalRefStat  
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Box Plot of CHLa\_benthic grouped by FinalRefStat  
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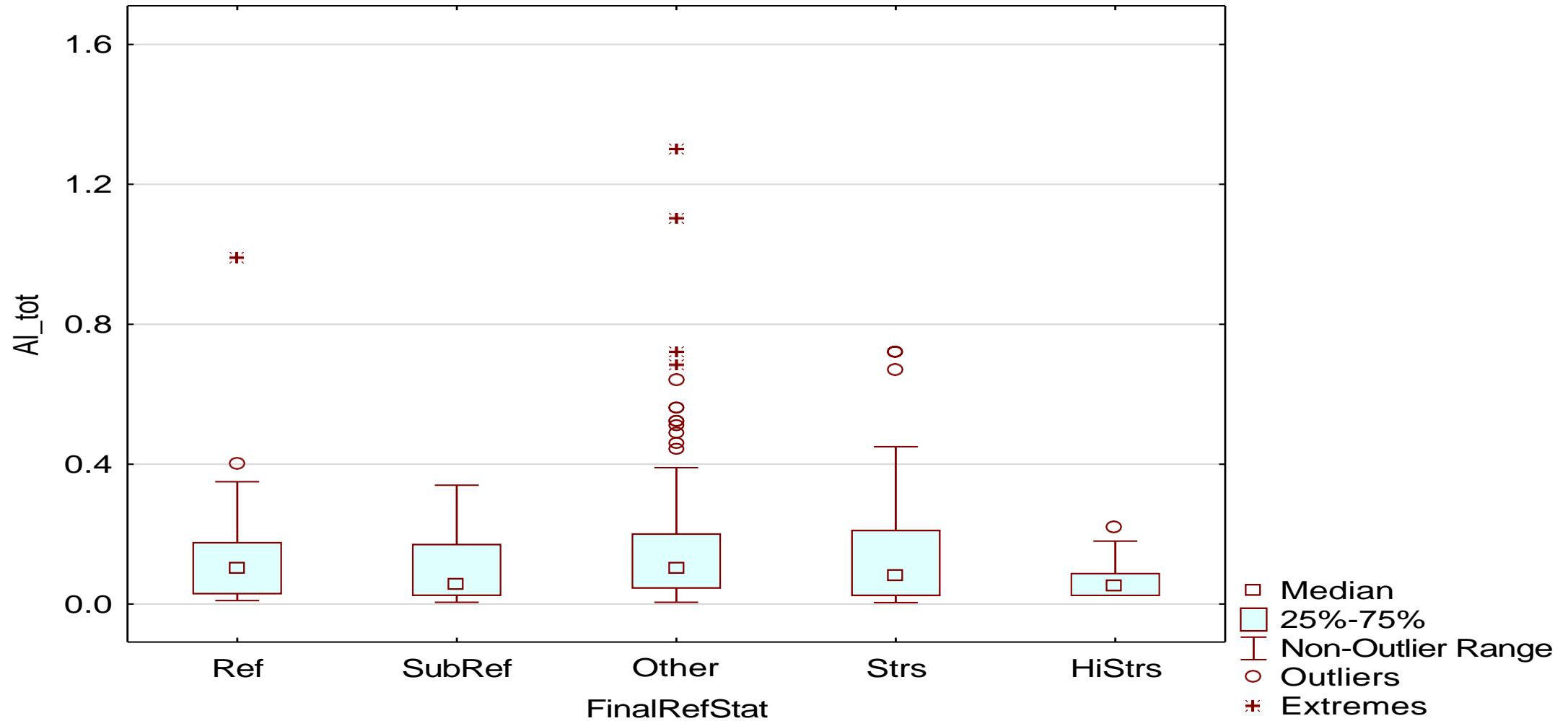
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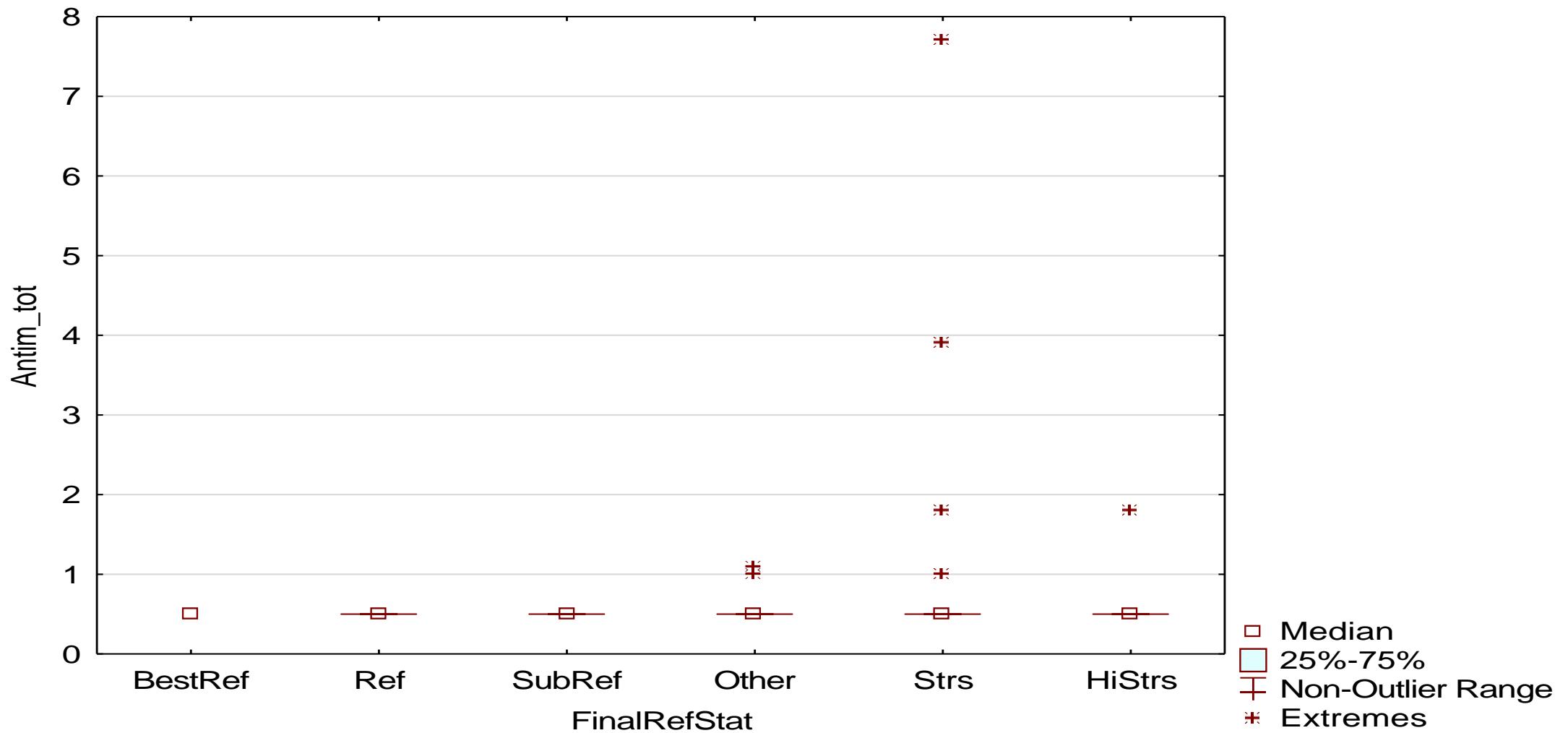
# Metals

Box Plot of AI\_tot grouped by FinalRefStat  
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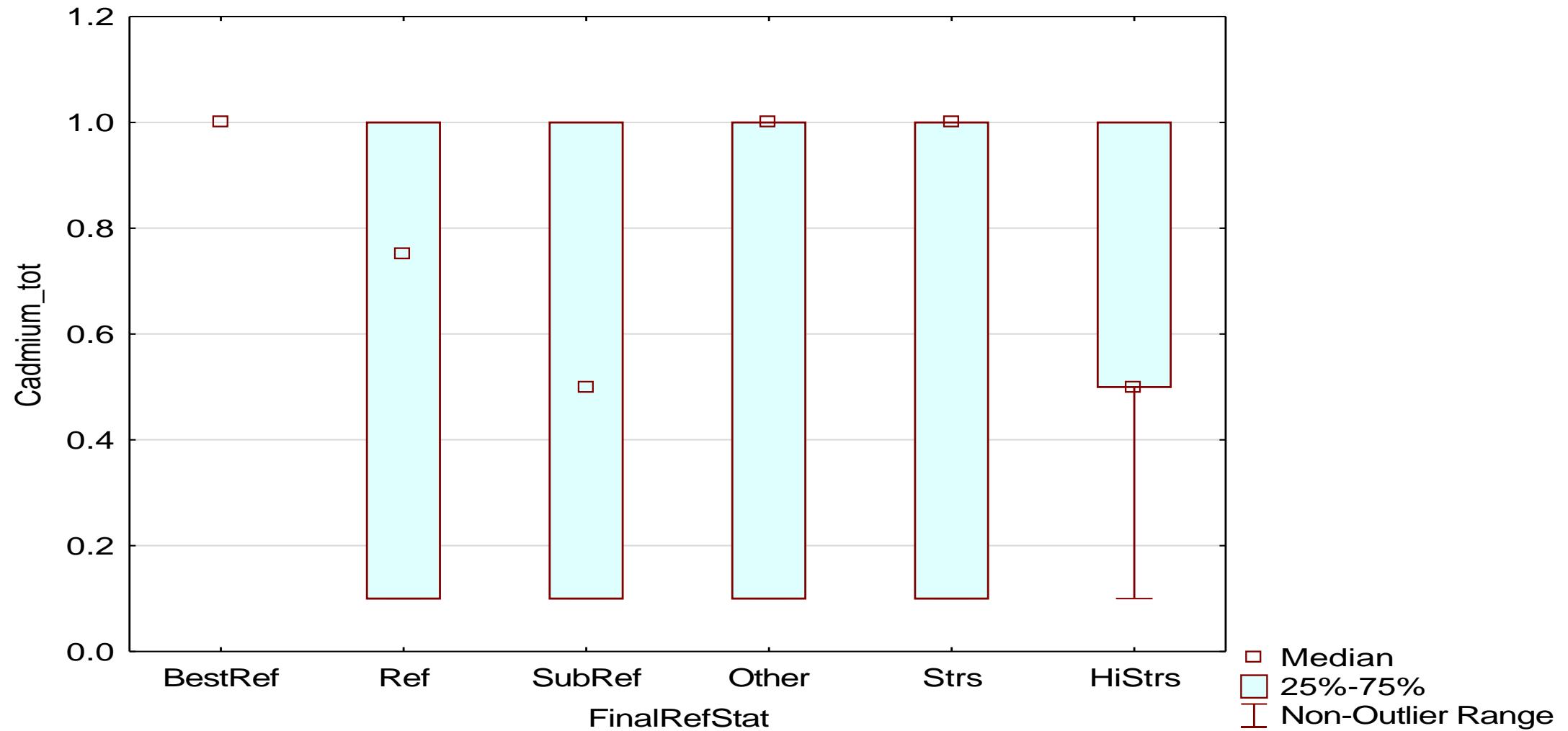
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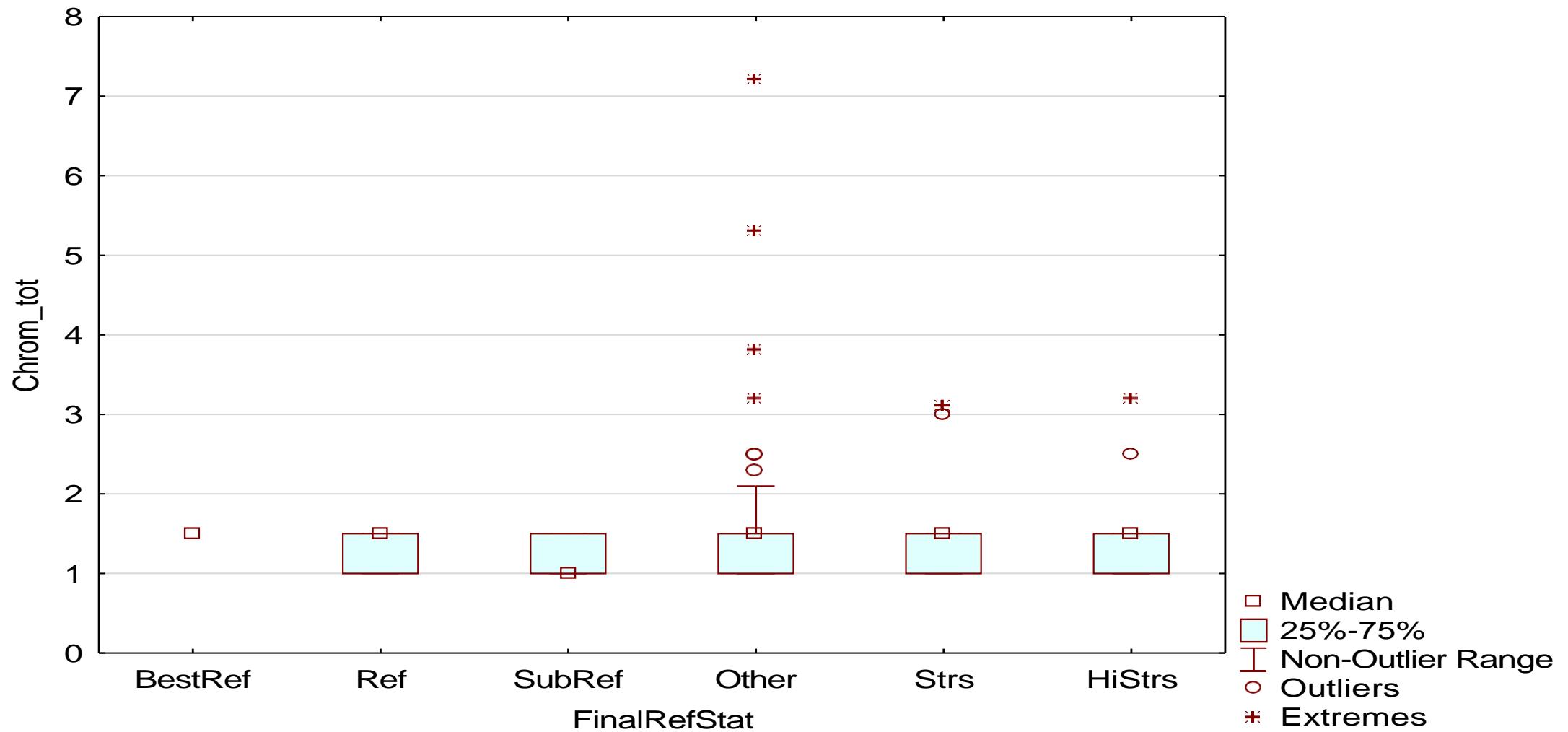
Box Plot of Antim\_tot grouped by FinalRefStat  
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Box Plot of Cadmium\_tot grouped by FinalRefStat  
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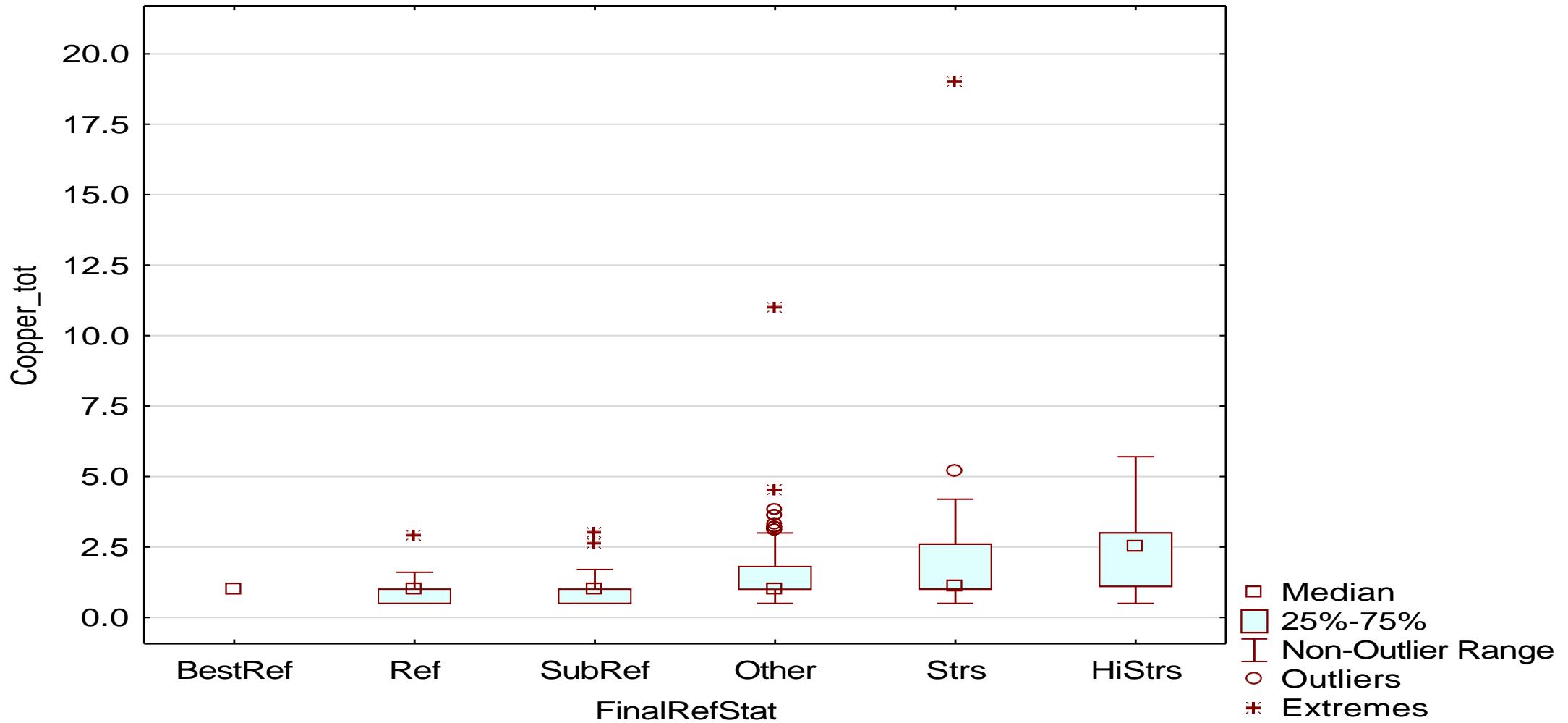


Box Plot of Chrom\_tot grouped by FinalRefStat  
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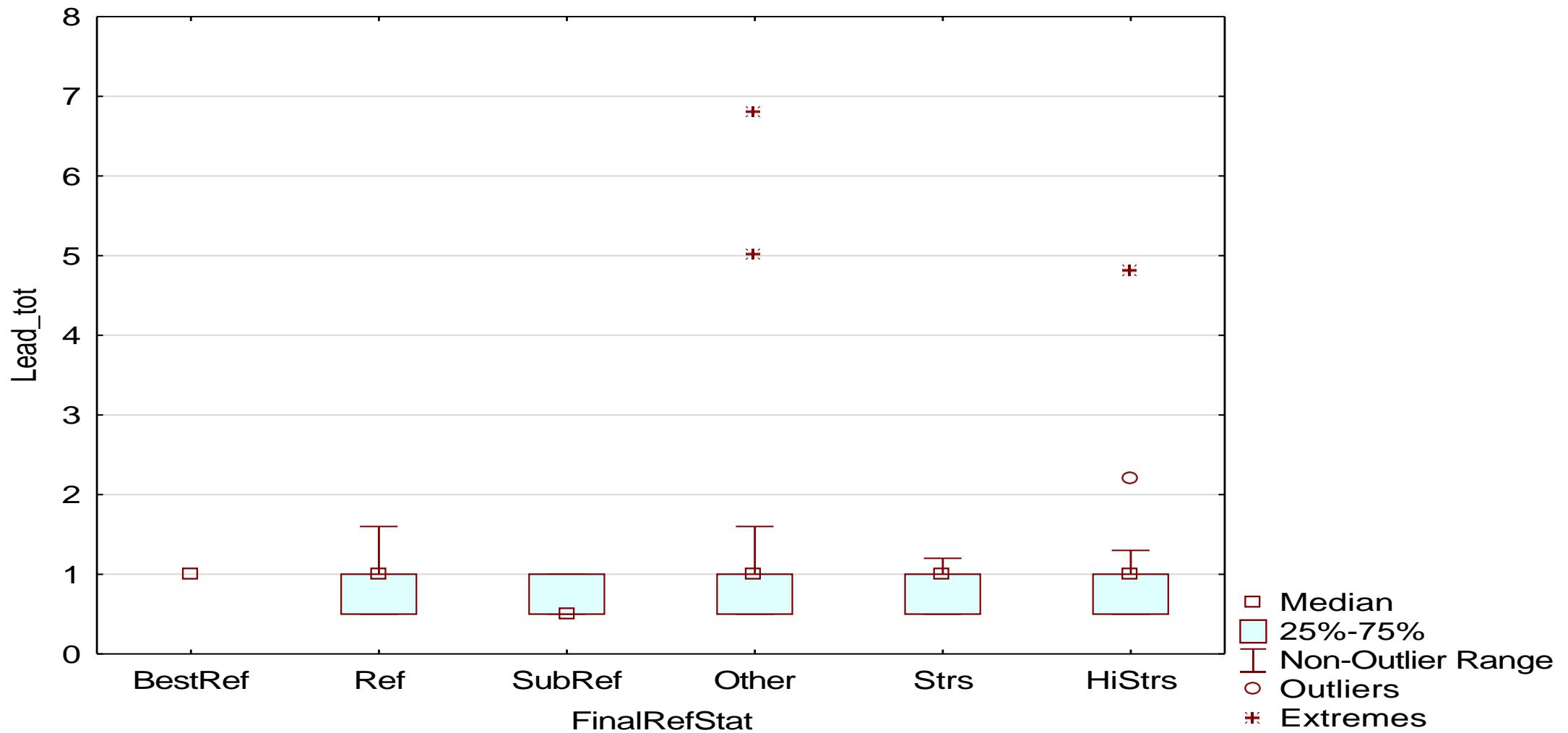


Box Plot of Copper\_tot grouped by FinalRefStat  
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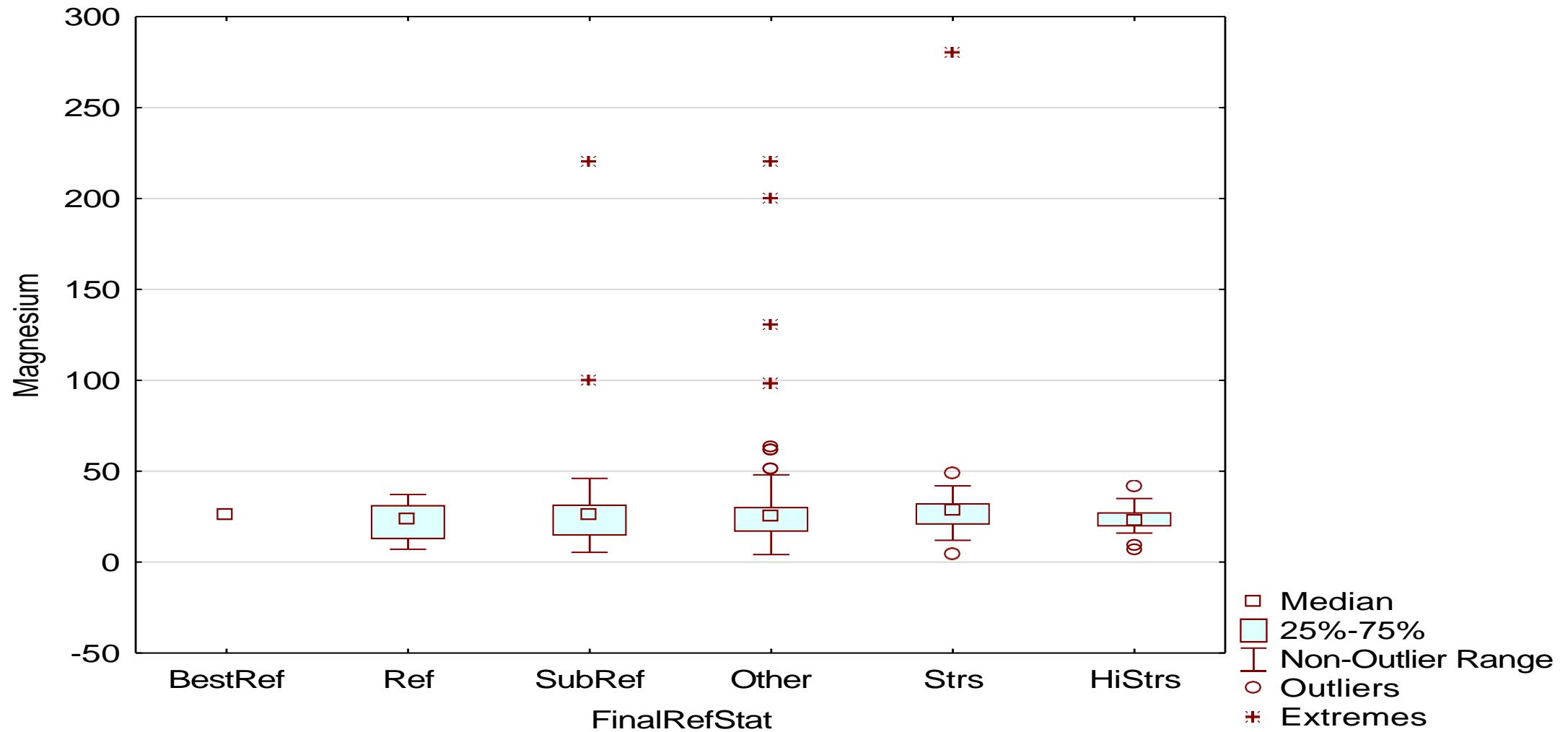
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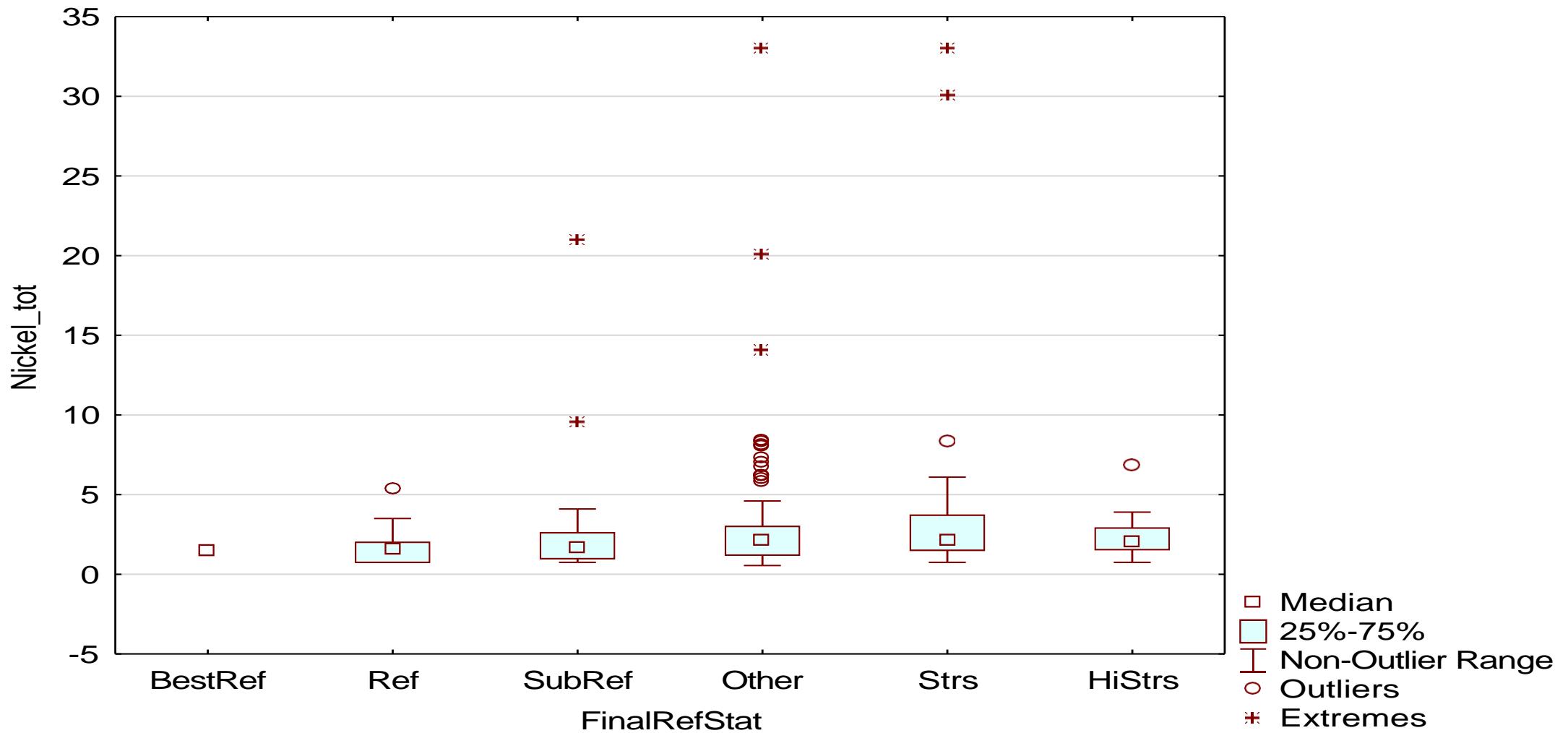
Box Plot of Lead\_tot grouped by FinalRefStat  
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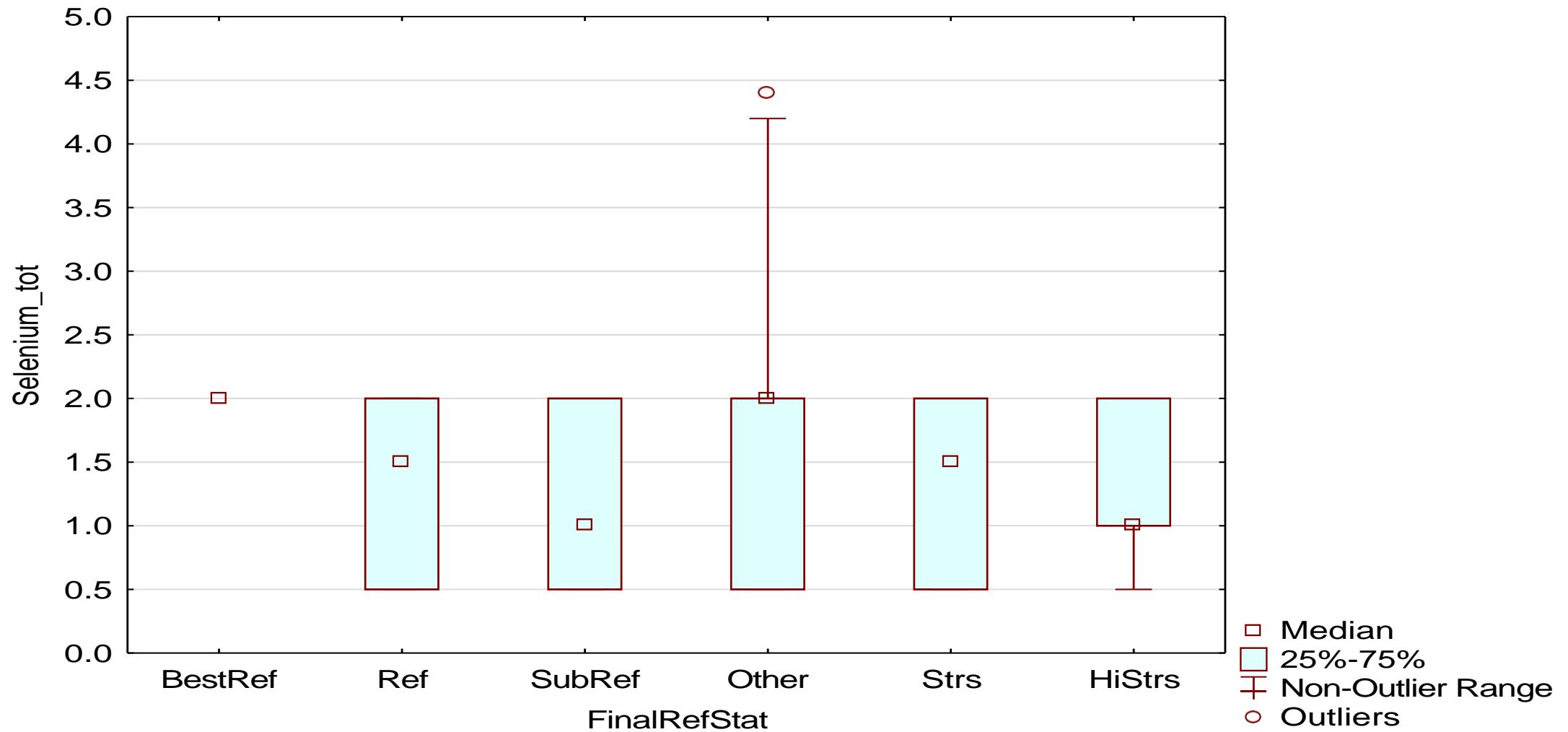
Box Plot of Magnesium grouped by FinalRefStat  
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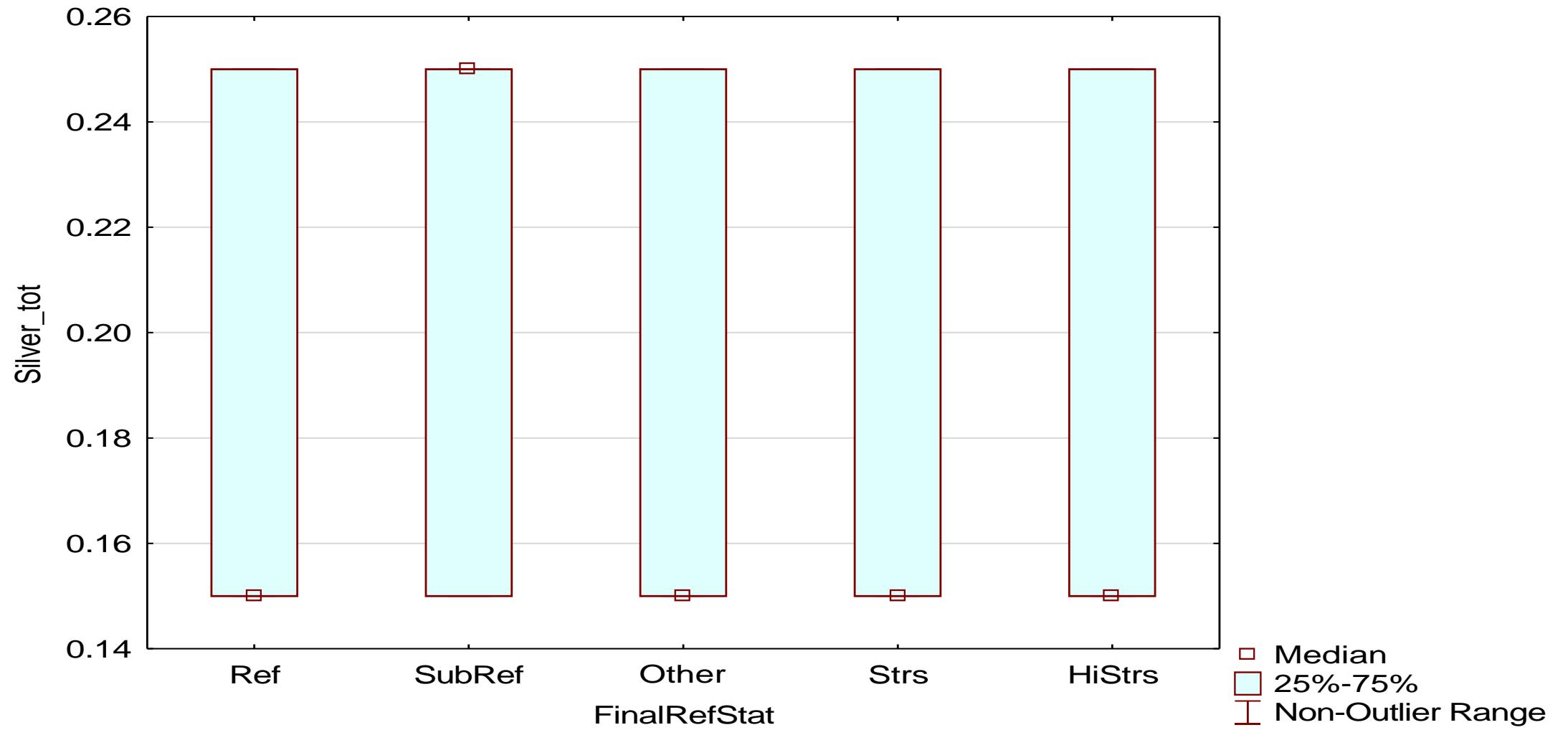
Box Plot of Nickel\_tot grouped by FinalRefStat  
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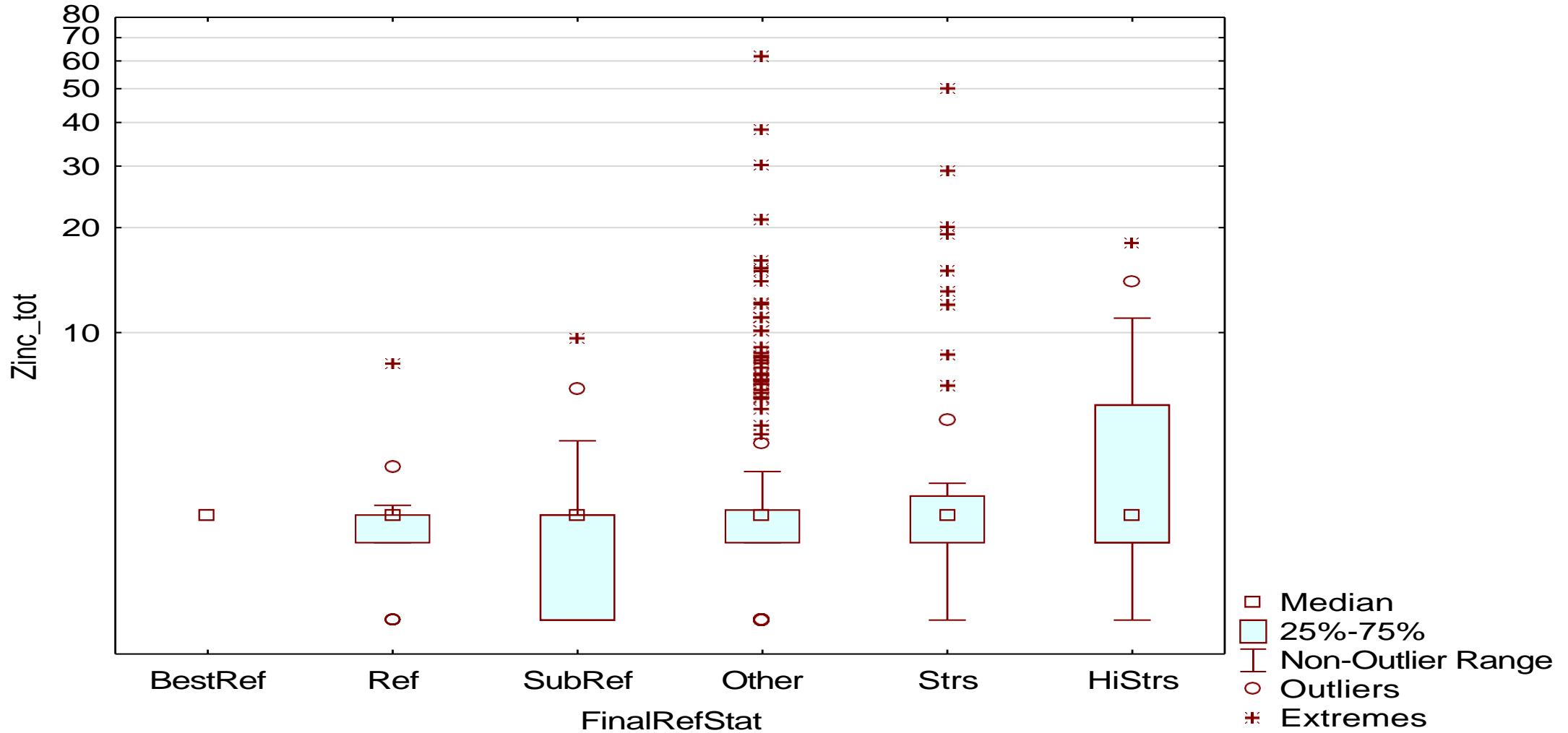
Box Plot of Selenium\_tot grouped by FinalRefStat  
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Include condition: RepNum=0



Box Plot of Silver\_tot grouped by FinalRefStat  
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Include condition: RepNum=0



Box Plot of Zinc\_tot grouped by FinalRefStat  
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Include condition: RepNum=0



## Appendix D - Site Locations

SITE	Station @ Location	Data Set	Final Ref Status	N Class	Latitude	Longitude
GMW-01-0003	Whitewater River @ Main Street	3_Strs_Cal	Strs	HiN	39.78496	-85.1515
GMW-01-0004	Whitewater River @ SR 1	2_Mid	Other	HiN	39.79349	-85.1494
GMW-01-0005	Martindale Creek @ Charles Road	1_Ref_Val	SubRef	HiN	39.97167	-85.1034
GMW-01-0006	Martindale Creek @ Fox Road	2_Mid	Other	HiN	39.91704	-85.1069
GMW-01-0012	Whitewater River @ Lacy Road	3_Strs_Cal	Strs	HiN	39.96306	-85.1449
GMW-02-0001	Greens Fork @ Carlos Road	2_Mid	Other	HiN	39.91655	-85.0288
GMW-02-0002	Greens Fork @ Round Barn Road	2_Mid	Other	HiN	39.99378	-84.9676
GMW-02-0003	Greens Fork @ Carlos Road	2_Mid	Other	HiN	39.92188	-85.0244
GMW-02-0004	Whitewater River @ Pennville Road	1_Ref_Cal	SubRef	HiN	39.74233	-85.1166
GMW-02-0014	Greens Fork @ Smoky Row Road	2_Mid	Other	HiN	39.91067	-85.033
GMW-03-0007	Centeral Run @ Willow Grove Road	3_Strs_Cal	Strs	LoN	39.77081	-85.0302
GMW-03-0008	Nolands Fork @ Nolands Fork Road	1_Ref_Cal	SubRef	HiN	39.86882	-84.9825
GMW-03-0009	Nolands Fork @ Fountain City Cemetary	2_Mid	Other	HiN	39.95105	-84.9155
GMW-03-0015	Nolands Fork @ Willow Grove Road	2_Mid	Other	HiN	39.79426	-85.0319
GMW-04-0002	Whitewater River @ SR 121	2_Mid	Other	HiN	39.60249	-85.144
GMW-04-0003	Whitewater River @ SR 121	2_Mid	Other	HiN	39.57973	-85.1573
GMW-04-0004	Whitewater River @ Eastern Avenue	3_Strs_Cal	Strs	HiN	39.63593	-85.1391
GMW-04-0005	Bear Creek @ Little Bear Road	2_Mid	Other	LoN	39.54788	-85.1207
GMW-04-0008	North Branch Garrison Creek @ S CR 390 W	2_Mid	Other	LoN	39.56659	-85.2158
GMW-04-0012	Whitewater River @ SR 121	2_Mid	Other	HiN	39.53152	-85.1679
GMW-04-0013	South Branch Garrison Creek @ Coletrane Road	3_Strs_Cal	Strs	LoN	39.57131	-85.26
GMW-04-0019	Bear Creek @ Little Bear Road	1_Ref_Cal	Ref	LoN	39.54109	-85.13
GMW-05-0001	Little Salt Creek @ Stipps Hill Road	1_Ref_Cal	SubRef	LoN	39.43579	-85.194
GMW-05-0002	Bull Fork @ Bullfork Road	1_Ref_Cal	Ref	LoN	39.40167	-85.2129
GMW-05-0003	Salt Creek @ Giesting Road	3_Strs_Cal	Strs	LoN	39.36381	-85.2652
GMW-06-0002	Whitewater River @ St. Mary Road	2_Mid	Other	HiN	39.43039	-85.033

SITE	Station @ Location	Data Set	Final Ref Status	N Class	Latitude	Longitude
GMW-06-0003	McCarty's Run @ St. Mary Road	1_Ref_Cal	Ref	LoN	39.40016	-85.0681
GMW-06-0004	Whitewater River @ Silver Creek Road	2_Mid	Other	HiN	39.44513	-85.1124
GMW-06-0005	Whitewater River @ Pennington Road	2_Mid	Other	HiN	39.4408	-85.1404
GMW-06-0006	Walnut Fork @ Walnut Fork Road	1_Ref_Val	Ref	LoN	39.39194	-85.1329
GMW-07-0009	East Fork Whitewater River @ Philomath Road	2_Mid	Other	LoN	39.65794	-85.0104
GMW-07-0010	Tributary of East Fork Whitewater River @ Beelor Road	2_Mid	Other	HiN	39.77791	-84.9413
GMW-07-0011	East Fork Whitewater River @ Abington Pike	2_Mid	Other	HiN	39.78705	-84.9282
GMW-07-0012	Silver Creek @ CR 275 N	2_Mid	Other	LoN	39.67128	-84.9181
GMW-07-0014	Middle Fork East Fork Whitewater River @ Whitewater Road	2_Mid	Other	HiN	39.94247	-84.821
GMW-07-0015	West Fork East Fork Whitewater River @ Friends Fellowship Community	3_Strs_Cal	Strs	HiN	39.85839	-84.8977
GMW-07-0017	Hanna Creek @ Cr. 50 N	1_Ref_Cal	Ref	LoN	39.63946	-84.8973
GMW-07-0024	East Fork Whitewater River @ Lake Road	1_Ref_Cal	SubRef	HiN	39.84308	-84.8198
GMW-08-0001	Blue Creek @ County Line Road	2_Mid	Other	LoN	39.28526	-85.0503
GMW-08-0003	Logan Creek @ Covered Bridge Road	3_Strs_Cal	Strs	LoN	39.27795	-84.9043
GMW-08-0005	Logan Creek @ Higher Ground Lane	2_Mid	Other	LoN	39.27774	-84.8925
GMW-08-0006	East Fork Blue Creek @ Blue Creek Road	1_Ref_Cal	SubRef	LoN	39.34486	-85.0316
GMW-08-0013	Whitewater River @ River Road	2_Mid	Other	HiN	39.38413	-84.9837
GMW010-0044	Morgan Creek @ Gilmer Road	1_Ref_Cal	SubRef	HiN	39.90749	-85.0852
GMW020-0035	Whitewater River @ CR 450 N	2_Mid	Other	HiN	39.70784	-85.1176
GMW040-0040	Little Williams Creek @ Williams Road	2_Mid	Other	HiN	39.61978	-85.1716
GMW060-0021	Jim Run @ Jim Run Road	1_Ref_Cal	Ref	LoN	39.49004	-85.1262
GMW060-0022	Pipe Creek @ Pipe Creek Road	2_Mid	Other	LoN	39.36478	-85.1189
GMW070-0117	Silver Creek @ Stout Road	1_Ref_Cal	Ref	LoN	39.68426	-84.9086
LEA120-0009	Flatrock Creek @ Lincoln Highway E	3_Strs_Cal	Strs	LoN	41.0121	-84.8509
LEJ050-0066	Fish Creek @ CR 775 S	1_Ref_Cal	SubRef	HiN	41.53569	-84.8615
LEJ050-0068	Fish Creek @ CR 18	2_Mid	Other	HiN	41.46369	-84.8113
LEJ060-0015	Big Run @ CR 28	3_Strs_Cal	Strs	HiN	41.42985	-84.8463

SITE	Station @ Location	Data Set	Final Ref Status	N Class	Latitude	Longitude
LEJ070-0028	Saint Joseph River @ St. Joe Rd	2_Mid	Other	HiN	41.19518	-85.0302
LEJ080-0016	Leins Ditch @ CR 16	2_Mid	Other	HiN	41.47286	-85.105
LEJ080-0017	W Smith Ditch @ CR 43	2_Mid	Other	HiN	41.40316	-84.989
LEJ090-0040	Peckhart Ditch @ CR 36A	2_Mid	Other	HiN	41.38797	-85.0857
LEJ090-0041	Black Creek @ CR 9	2_Mid	Other	HiN	41.28244	-85.1425
LEJ100-0026	Saint Joseph River @ St. Joe Rd	3_Strs_Cal	HiStrs	HiN	41.1461	-85.1003
LEM010-0046	Maumee River @ Irving Rd	2_Mid	Other	HiN	41.13301	-84.9214
LEM010-0050	Hamm Ditch @ Springfield Center Rd	3_Strs_Cal	Strs	HiN	41.22917	-84.8506
LEM010-0051	Maumee River @ Parent Rd	3_Strs_Cal	Strs	HiN	41.10093	-84.9813
LEM010-0052	Maumee River @ SR 930	3_Strs_Cal	HiStrs	HiN	41.07599	-85.0818
LES030-0002	Saint Marys River @ N Piqua Rd	1_Ref_Cal	Ref	HiN	40.76416	-84.8098
LES040-0104	Koos Ditch @ Jackson St	3_Strs_Cal	HiStrs	LoN	40.83613	-84.9167
LMG-04-0039	East Branch Little Calumet River @ Otis Road	2_Mid	Other	HiN	41.61247	-86.9061
LMG-05-0015	Deep River @ Clay Street	3_Strs_Cal	Strs	HiN	41.44734	-87.2776
LMG030-0028	Main Beaver Dam Ditch @ 101st Ave	2_Mid	Other	HiN	41.43963	-87.4397
LMG030-0029	Main Beaver Dam Ditch @ 100th Ave	3_Strs_Cal	HiStrs	HiN	41.43717	-87.3416
LMG040-0012	Deep River @ Riverside Dr	3_Strs_Cal	HiStrs	HiN	41.5671	-87.2549
LMG050-0121	Beauty Creek @ SR 130	3_Strs_Cal	Strs	HiN	41.47647	-87.0847
LMG060-0040	Coffee Creek @ 1100 N	3_Strs_Cal	HiStrs	HiN	41.59395	-87.0405
LMG060-0041	East Arm Little Calumet River @ CR 450 E	3_Strs_Val	Strs	HiN	41.62314	-86.9821
LMG070-0035	East Branch of Trail Creek @ CR 700 N	1_Ref_Cal	SubRef	HiN	41.70659	-86.7705
LMG070-0038	Trail Creek @ Karwick Rd	3_Strs_Cal	HiStrs	HiN	41.70817	-86.8567
LMG100-0009	Tributary of Spring Creek @ CR 1000 N	1_Ref_Val	Ref	HiN	41.7529	-86.5603
LMJ110-0124	Pigeon Creek @ Bill Deller Rd	2_Mid	Other	HiN	41.59714	-84.9719
LMJ110-0125	Tributary of Turkey Creek @ CR 425 S	2_Mid	Other	HiN	41.58358	-85.2188
LMJ110-0128	Pigeon Creek @ CR 200	2_Mid	Other	HiN	41.67343	-85.2052
LMJ110-0129	Pigeon Creek @ 600 W	2_Mid	Other	HiN	41.62755	-85.1021
LMJ120-0042	Pigeon River @ N 675 W	2_Mid	Other	HiN	41.74064	-85.5579
LMJ120-0048	Pigeon River @ CR 100 W	2_Mid	Other	HiN	41.71501	-85.4501
LMJ130-0008	Saint Joseph River @ Antone Rd	2_Mid	Other	HiN	41.74345	-85.8031

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LMJ140-0118	Little Elkhart River @ CR 35	2_Mid	Other	HiN	41.71707	-85.736
LMJ140-0119	Tributary of Little Elkhart River @ CR 300S	1_Ref_Cal	Ref	HiN	41.59696	-85.5568
LMJ150-0023	Saint Joseph River @ Sunnyside Rd	3_Strs_Cal	HiStrs	HiN	41.70596	-85.8905
LMJ170-0080	Middle Br Elkhart River @ Northport Rd	3_Strs_Cal	Strs	HiN	41.50637	-85.3949
LMJ180-0049	South Branch Elkhart River @ CR 50 W	2_Mid	Other	HiN	41.36998	-85.4392
LMJ180-0052	Rimmell Branch @ 500 E	1_Ref_Cal	Ref	HiN	41.38458	-85.337
LMJ190-0031	Elkhart River @ CR 127	2_Mid	Other	HiN	41.5011	-85.7799
LMJ190-0032	Solomon Creek @ Sparta Lake Rd	2_Mid	Other	HiN	41.39578	-85.5739
LMJ200-0060	Turkey Creek @ CR 56	2_Mid	Other	HiN	41.43248	-85.7731
LMJ210-0030	Elkhart River @ Oxbow Dr.	3_Strs_Cal	Strs	HiN	41.64484	-85.9126
LMJ220-0014	Cobus Creek @ David Dr.	3_Strs_Cal	Strs	HiN	41.70228	-86.0533
LMJ240-0046	Bowman Creek @ Ireland Rd	3_Strs_Cal	Strs	HiN	41.62996	-86.2763
OBS090-0008	Indian Creek @ SR 135	2_Mid	Other	LoN	38.27924	-86.1071
OBS090-0009	Indian Creek @ Water St.	3_Strs_Val	Strs	LoN	38.2165	-86.1294
OBS100-0010	Indian Creek @ Five Oaks Rd. SW	2_Mid	Other	LoN	38.15785	-86.2354
OBS120-0015	Mill Creek @ SR 56	2_Mid	Other	LoN	38.5767	-86.1645
OBS120-0016	Blue River @ Becks Mill Rd	2_Mid	Other	LoN	38.46062	-86.1635
OBS130-0009	South Fork Blue River @ Misty Hollow	2_Mid	Other	LoN	38.52208	-85.9308
OBS140-0010	Blue River @ Lonesome Valley Rd	2_Mid	Other	LoN	38.41944	-86.2299
OBS150-0024	Blue River @ Harrison Spring Rd	2_Mid	Other	LoN	38.22907	-86.2252
OBS150-0025	Blue River @ SR 62	2_Mid	Other	LoN	38.22384	-86.2676
OBS150-0027	Blue River @ Mt. Lebanon Rd	2_Mid	Other	LoN	38.2998	-86.2648
OBS180-0022	Camp Fork Creek @ Temple Rd	1_Ref_Val	SubRef	LoN	38.3434	-86.4404
OBS180-0025	Little Blue River @ Beechwood Rd	1_Ref_Val	Ref	LoN	38.17735	-86.414
OHP020-0026	Tributary of Pigeon Creek @ CR 175 E	3_Strs_Cal	Strs	HiN	38.26385	-87.5333
OHP030-0026	Pigeon Creek @ Heim Rd	2_Mid	Other	HiN	38.03842	-87.4042
OHP030-0027	Pigeon Creek @ New Harmony Rd	2_Mid	Other	HiN	38.09657	-87.3992
OLP070-0064	Anderson River @ CR 1300 N	2_Mid	Other	HiN	38.07812	-86.7805
OLP080-0010	Crooked Creek @ CR 1450 N	3_Strs_Val	Strs	HiN	38.09567	-86.896
OLP110-0001	Honey Cr @ SR 231	2_Mid	Other	HiN	37.95295	-87.0396

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OLP140-0098	Little Pigeon Creek @ SR 62	2_Mid	Other	HiN	38.05989	-87.0975
OLP140-0099	Little Pigeon Creek @ Gentryville Rd	2_Mid	Other	HiN	38.1323	-87.0306
OLP140-0100	East Fork Little Pigeon Creek @ US 231	2_Mid	Other	HiN	38.03379	-87.0374
OML030-0015	West Fork Tanners Creek @ Villa Lane	2_Mid	Other	LoN	39.17397	-84.9396
OML040-0008	South Hogan Creek @ CR 50 N.	2_Mid	Other	LoN	39.07571	-85.1177
OML040-0012	Little Hogan Creek @ Union Ridge Road	2_Mid	Other	LoN	39.11134	-84.9904
OML040-0014	Allen Branch @ Stitts Hill Rd	1_Ref_Cal	Ref	LoN	39.05177	-84.9946
OML060-0017	Tub Creek @ CR 1000 E	2_Mid	Other	LoN	39.27261	-85.3008
OML060-0019	Laughery Creek @ N CR 75 E	2_Mid	Other	LoN	39.16017	-85.2525
OML070-0021	Laughery Creek @ CR 300 S	2_Mid	Other	LoN	39.02698	-85.222
OML200-0004	Indian Creek @ Posten Road	2_Mid	Other	LoN	38.77638	-85.0787
OML200-0018	Indian Creek @ Posten Rd	2_Mid	Other	LoN	38.77965	-85.08
OSK-02-0016	Indian Kentuck Creek @ Brooksburg Manville Rd	1_Ref_Cal	SubRef	LoN	38.7585	-85.2477
OSK030-0017	Indian Kentuck Creek @ Lonnis Hill Rd	1_Ref_Val	SubRef	LoN	38.844	-85.2594
OSK030-0019	West Fork Indian Kentuck Creek @ N China Manville Rd	2_Mid	Other	LoN	38.80934	-85.3168
OSK060-0001	Bull Creek @ Blue Ridge Rd	1_Ref_Cal	BestRef	LoN	38.48112	-85.5135
OSK070-0018	Fourteenmile Creek @ New Market Rd	1_Ref_Val	SubRef	HiN	38.52636	-85.6169
OSK100-0001	Lancassange Creek @ Parker Pl	3_Strs_Cal	Strs	LoN	38.31233	-85.6875
OSK140-0040	Blue Lick Creek @ Biggs Rd	1_Ref_Val	SubRef	LoN	38.50265	-85.7783
OSK140-0041	Silver Creek @ Potters Ln/Lapping Memori	3_Strs_Cal	Strs	HiN	38.34305	-85.7777
OSK140-0042	Silver Creek @ Killen Rd	3_Strs_Cal	Strs	HiN	38.4567	-85.7615
OSK140-0043	Sinking Fork @ Hansberry Rd	2_Mid	Other	HiN	38.49438	-85.7288
OSK150-0005	Falling Run @ Pimlico Drive	3_Strs_Cal	HiStrs	LoN	38.3226	-85.8315
UMI-02-0014	Slough Creek @ US 231	2_Mid	Other	HiN	40.8916	-87.155
UMI-02-0015	Carpenter Creek @ CR 600 E	2_Mid	Other	LoN	40.70549	-87.2083
UMI-02-0016	Bice Ditch @ CR 280 W	2_Mid	Other	HiN	40.8786	-87.0999
UMI-02-0017	Keefe Ditch @ CR 900 S	2_Mid	Other	HiN	40.87709	-87.0624
UMI-03-0008	Ryan Ditch @ CR 230 E	3_Strs_Cal	Strs	LoN	40.98242	-86.9917

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UMI-03-0010	Iroquois River @ CR 100 S	2_Mid	Other	HiN	40.99332	-87.14
UMI-04-0019	Iroquois River @ SR 55	2_Mid	Other	HiN	40.87094	-87.3046
UMI-04-0022	Iroquois River @ CR 700 S	2_Mid	Other	HiN	40.90484	-87.2103
UMI-05-0004	Kent Ditch @ Graham Street	3_Strs_Val	HiStrs	HiN	40.76915	-87.4423
UMI-05-0005	Thompson Ditch @ CR 300 W	3_Strs_Cal	Strs	HiN	40.85885	-87.4496
UMI-05-0006	Whaley Ditch @ CR 1125 S	3_Strs_Val	Strs	HiN	40.83075	-87.5118
UMI-07-0001	Upper Sugar Creek @ CR 100 E	2_Mid	Other	HiN	40.68643	-87.3101
UMI-07-0002	Sugar Cr @ SR 71	2_Mid	Other	LoN	40.66125	-87.4819
UMI-07-0003	Mud Creek @ Division Road	2_Mid	Other	HiN	40.60566	-87.5236
UMI-13-0003	Carlson Ditch @ CR 650 W	3_Strs_Cal	Strs	HiN	40.93837	-87.5181
UMI030-0044	Carpenter Creek @ CR 1300 S	3_Strs_Cal	Strs	HiN	40.82274	-87.176
UMK-02-0014	Mill Creek @ CR 400 S	2_Mid	Other	HiN	41.54937	-86.5463
UMK-02-0017	Geyer Ditch @ Tamarack Road	2_Mid	Other	HiN	41.62473	-86.4188
UMK-03-0036	Yellow River @ Centennial Park	3_Strs_Cal	Strs	HiN	41.3575	-86.294
UMK-04-0009	Kankakee River @ CR 1000 S	2_Mid	Other	HiN	41.46315	-86.6133
UMK-04-0010	Kankakee River @ Kingsbury FWA	2_Mid	Other	HiN	41.49469	-86.5668
UMK-04-0011	Kankakee River @ SR 104	2_Mid	Other	HiN	41.5161	-86.5365
UMK-04-0012	Mill Creek @ Long Lane	1_Ref_Cal	Ref	HiN	41.45668	-86.748
UMK-05-0016	Yellow River @ Yellow River Road	2_Mid	Other	HiN	41.29421	-86.7652
UMK-05-0017	Yellow River @ Redwood Road	2_Mid	Other	HiN	41.27028	-86.3824
UMK-05-0018	Yellow River @ CR 200 E	3_Strs_Cal	Strs	HiN	41.30633	-86.6729
UMK-05-0019	Yellow River @ Yellow River Road	2_Mid	Other	HiN	41.30641	-86.7404
UMK-05-0022	Harry Cool Ditch @ SR 17	2_Mid	Other	HiN	41.29993	-86.4205
UMK-08-0001	Kankakee River @ Dunn's Bridge	2_Mid	Other	HiN	41.24185	-87.0122
UMK-08-0002	Kankakee River @ CR 1100 W	2_Mid	Other	HiN	41.24848	-86.9098
UMK-10-0007	Kankakee River @ CR 625 W	2_Mid	Other	HiN	41.26818	-87.1837
UMK-10-0009	Sandy Hook Ditch @ CR 900 S	3_Strs_Val	Strs	HiN	41.30243	-87.095
UMK-10-0014	Sandy Hook Ditch @ CR 250 W	2_Mid	Other	HiN	41.28584	-87.1172
UMK-11-0001	Kankakee River @ Whitcomb Street	2_Mid	Other	HiN	41.17267	-87.3802
UMK-11-0002	Kankakee River @ Clay Street	2_Mid	Other	HiN	41.21194	-87.2902

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UMK-12-0001	Kankakee River @ LaSalle FWA	2_Mid	Other	HiN	41.17036	-87.4695
UMK-13-0013	West Creek @ Magoun Street	2_Mid	Other	HiN	41.35445	-87.4964
WAE-02-0002	Eel River @ Carroll Road	2_Mid	Other	HiN	41.18825	-85.2836
WAE-04-0001	Swank Creek @ East Street	1_Ref_Cal	SubRef	HiN	41.03916	-85.7681
WAE-06-0003	Flowers Creek @ Broadway Street	2_Mid	Other	HiN	40.86191	-86.0258
WAE-07-0002	Eel River @ CR 400 N	2_Mid	Other	HiN	40.81674	-86.1696
WAE-07-0003	Eel River @ Eel River Road	2_Mid	Other	HiN	40.79778	-86.2564
WAW-01-0001	Mud Creek @ SR 26	2_Mid	Other	HiN	40.42274	-85.9044
WAW-03-0036	South Fork Wildcat Creek @ Ripple Creek Drive	2_Mid	Other	HiN	40.42969	-86.7721
WAW-03-0037	Kilmore Creek @ Gas Line Road	2_Mid	Other	HiN	40.32838	-86.6179
WAW-04-0002	Wildcat Creek @ CR 350 S	2_Mid	Other	HiN	40.48647	-86.4378
WAW-04-0003	Wildcat Creek @ CR 750 W	2_Mid	Other	LoN	40.4721	-86.2683
WAW040-0018	South Fork Wildcat Creek @ CR 200 N	2_Mid	Other	HiN	40.31495	-86.5436
WAW040-0043	South Fork Wildcat Creek @ SR 26	2_Mid	Other	HiN	40.41824	-86.7682
WAW040-0065	South Fork Wildcat Creek @ CR 600 W	2_Mid	Other	HiN	40.32081	-86.6181
WAW040-0080	South Fork Wildcat Creek @ W Mulberry-Jefferson Rd	2_Mid	Other	HiN	40.32919	-86.6474
WBU-04-0005	North Branch Otter Creek @ Hayne Road	2_Mid	Other	HiN	39.54642	-87.2865
WBU-04-0006	Otter Creek @ Private Road 1275 N	1_Ref_Cal	SubRef	HiN	39.56978	-87.1097
WBU-05-0002	Wolf Creek @ N Arms Place	2_Mid	Other	HiN	39.51544	-87.4732
WBU-06-0001	Wabash River @ US 41	2_Mid	Other	HiN	39.54035	-87.416
WBU-07-0002	Tributary of Honey Creek @ McDaniel Road	3_Strs_Val	Strs	HiN	39.3741	-87.3614
WBU-11-0001	Wabash River @ CR 600 N	2_Mid	Other	HiN	39.17334	-87.6198
WBU030-0060	North Branch Otter Creek @ Fontanet Rd	1_Ref_Cal	SubRef	HiN	39.56949	-87.2428
WDE-01-0003	Galbreath Ditch @ CR 250 N	2_Mid	Other	LoN	40.80015	-86.537
WDE-01-0004	Wabash River @ Georgetown Road	2_Mid	Other	HiN	40.74194	-86.4905
WDE-03-0001	Pleasant Run @ CR 550 N	1_Ref_Cal	SubRef	HiN	40.62659	-86.6832
WDE-05-0007	Little Deer Creek @ CR 200 N	3_Strs_Cal	Strs	HiN	40.5782	-86.4605
WDE050-0025	Deer Creek @ Cemetery Rd	2_Mid	Other	LoN	40.60056	-86.5474
WDE050-0031	Deer Creek @ CR 1100 S	2_Mid	Other	LoN	40.60793	-86.3706

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WED-01-0006	Big Blue River @ Garner St	2_Mid	Other	HiN	39.94459	-85.3789
WED-01-0008	Buck Creek @ US 40	2_Mid	Other	HiN	39.80555	-85.4293
WED-02-0003	Little Blue River @ CR 400 W	2_Mid	Other	HiN	39.70783	-85.514
WED-03-0001	Brandywine Creek @ W 650 N	2_Mid	Other	HiN	39.61833	-85.8003
WED-04-0001	Sugar Cr @ E CR 1000 N	2_Mid	Other	HiN	39.93141	-85.699
WED-04-0003	Sugar Cr @ CR 1000 N	2_Mid	Other	HiN	39.92798	-85.6428
WED-05-0001	Doe Creek @ Vandergriff Rd	2_Mid	Other	HiN	39.71407	-85.9542
WED-07-0001	Sugar Cr @ River Rd	2_Mid	Other	HiN	39.36952	-85.9955
WED-07-0003	Sugar Cr @ CR 700 E	2_Mid	Other	HiN	39.45254	-85.9681
WED-08-0004	Sixmile Cr @ CR 900 E	2_Mid	Other	HiN	39.73091	-85.633
WEF-01-0001	Flatrock River @ CR 400 S	2_Mid	Other	HiN	39.87918	-85.3473
WEF-02-0001	Little Flatrock River @ CR 175 W	2_Mid	Other	LoN	39.48794	-85.4757
WEF-06-0001	Flatrock River @ CR 400 N	2_Mid	Other	HiN	39.2601	-85.922
WEF-06-0002	Flatrock River @ CR 900 N	2_Mid	Other	HiN	39.33197	-85.8613
WEF-06-0003	Flatrock River @ Sleepy Hollow Rd	2_Mid	Other	HiN	39.39632	-85.7157
WEL-01-0003	Rush Creek @ N Cox Ferry Rd	2_Mid	Other	LoN	38.70965	-86.2081
WEL-04-0001	South Fork Salt Creek @ Maumee Rd	2_Mid	Other	LoN	39.05023	-86.2826
WEL-04-0002	Little Salt Creek @ Buffalo Pike	1_Ref_Cal	SubRef	LoN	39.01517	-86.1866
WEL-09-0001	Spring Creek @ SR 58	2_Mid	Other	LoN	38.91403	-86.6484
WEL-09-0004	Indian Creek @ E SR 54	1_Ref_Cal	Ref	LoN	38.96139	-86.6982
WEL-10-0002	East Fork White River @ Port Williams Rd	2_Mid	Other	HiN	38.78049	-86.5977
WEL-11-0001	Boggs Creek @ CR 147	1_Ref_Cal	SubRef	HiN	38.73593	-86.8444
WEL-13-0023	Lost River @ CR 3	2_Mid	Other	LoN	38.53699	-86.8089
WEL-13-0024	Lost River @ W CR 350 N	2_Mid	Other	LoN	38.61131	-86.5893
WEL-13-0025	Lost River @ CR 375 N	2_Mid	Other	LoN	38.60918	-86.5965
WEL-13-0026	French Lick Creek @ SR 145	2_Mid	Other	LoN	38.51055	-86.6144
WEL-14-0001	East Fork White River @ CR 55	1_Ref_Cal	Ref	HiN	38.62121	-86.8431
WEL-15-0001	East Fork White River @ CR 1250 E	2_Mid	Other	HiN	38.50504	-86.9332
WEM-02-0001	Little Graham Creek @ CR 700 E	1_Ref_Cal	Ref	LoN	38.93868	-85.4766
WEM-02-0002	Graham Creek @ CR 500 S	2_Mid	Other	LoN	38.90615	-85.6064

SITE	Station @ Location	Data Set	Final Ref Status	N Class	Latitude	Longitude
WEM-06-0016	Muscatatuck River @ CR 1150 S	2_Mid	Other	LoN	38.80706	-85.7867
WEM-07-0002	Vernon Fork Muscatatuck River @ CR 400 S	2_Mid	Other	HiN	38.82584	-85.8806
WEM-07-0004	Vernon Fork Muscatatuck River @ CR 25 W	2_Mid	Other	LoN	38.97454	-85.6141
WEM-07-0005	Mutton Creek Ditch @ US 31	2_Mid	Other	HiN	38.91438	-85.8305
WEM-08-0003	Honey Run @ Park Rd	3_Strs_Cal	HiStrs	LoN	38.70089	-85.7936
WEM-09-0005	Cammie Thomas Ditch @ E Mount Eden Rd	3_Strs_Cal	Strs	LoN	38.75698	-86.0089
WEM020-0036	Graham Creek @ CR 230 S	2_Mid	Other	LoN	38.95666	-85.4946
WEM050-0048	Vernon Fork Muscatatuck River @ CR 740 E	1_Ref_Cal	SubRef	LoN	39.13581	-85.4753
WEU-01-0001	Fall Fork @ CR 850 W	2_Mid	Other	LoN	39.27018	-85.6444
WEU-02-0001	East Fork White River @ CR 1000 S	2_Mid	Other	HiN	39.06389	-85.8509
WEU-03-0034	Sand Creek @ CR 220 W	2_Mid	Other	LoN	39.2688	-85.5307
WEU-03-0035	Muddy Fork Sand Creek @ CR 280 W	3_Strs_Cal	HiStrs	LoN	39.31115	-85.5236
WEU-04-0002	White Creek @ CR 930 S	2_Mid	Other	LoN	39.05763	-85.9712
WEU-05-0001	East Fork White River @ CR 1000 E	2_Mid	Other	HiN	39.02801	-85.8585
WEU-06-0001	East Fork White River @ SR 235	2_Mid	Other	HiN	38.82864	-86.1365
WEU010-0039	Clifty Creek @ CR 420 W	2_Mid	Other	LoN	39.38693	-85.5602
WEU010-0040	Clifty Creek @ CR 50 N	3_Strs_Cal	Strs	LoN	39.20855	-85.8733
WLV-01-0080	Wea Creek @ US 231	3_Strs_Cal	HiStrs	HiN	40.36952	-86.9204
WLV-01-0081	Wea Creek @ CR 100 E	3_Strs_Cal	Strs	HiN	40.27732	-86.8803
WLV-02-0030	Cole Ditch @ CR 150 W	2_Mid	Other	HiN	40.49557	-86.9376
WLV-04-0002	Big Pine Creek @ CR 50 W	2_Mid	Other	HiN	40.37036	-87.3225
WLV-04-0006	Big Pine Creek @ CR 850 E	2_Mid	Other	HiN	40.60934	-87.1664
WLV-05-0110	Flint Creek @ CR 510 S	1_Ref_Val	SubRef	HiN	40.34097	-87.0671
WLV-06-0004	Big Shawnee Creek @ CR 70 W	2_Mid	Other	LoN	40.24768	-87.2791
WLV-07-0006	North Fork Coal Creek @ SR 341	2_Mid	Other	LoN	40.18205	-87.1531
WLV-08-0001	Wabash River @ Water Street	2_Mid	Other	HiN	40.04909	-87.4304
WLV-09-0001	Graham Creek @ Mud Creek Road	2_Mid	Other	HiN	40.12657	-87.3519
WLV-12-0004	Big Raccoon Creek @ SR 234	2_Mid	Other	HiN	39.91697	-86.781
WLV-12-0005	Cornstalk Creek @ Cornstalk Creek Road	2_Mid	Other	LoN	39.8815	-86.8538
WLV-13-0011	South Fork Little Raccoon Creek @ CR 900 E	2_Mid	Other	LoN	39.81868	-87.0696

SITE	Station @ Location	Data Set	Final Ref Status	N Class	Latitude	Longitude
WLV-13-0013	Williams Creek @ CR 225 E	3_Strs_Val	Strs	HiN	39.72068	-87.199
WLV-15-0001	Leatherwood Creek @ 10 Oclock Road	2_Mid	Other	HiN	39.81607	-87.2974
WLV-15-0002	Rock Run @ CR 325 W	1_Ref_Cal	SubRef	HiN	39.65902	-87.2965
WLV-15-0003	Rocky Run @ CR 420 W	1_Ref_Cal	SubRef	HiN	39.77094	-87.3254
WLV-16-0002	Wabash River @ CR 251	2_Mid	Other	HiN	39.93647	-87.4323
WLV-16-0003	Mill Creek @ CR 1120 S	2_Mid	Other	HiN	39.96715	-87.3357
WLV-16-0004	Mill Creek @ CR 800 S	3_Strs_Val	Strs	HiN	40.01092	-87.2323
WLV010-0022	Burnett Creek @ Prophet St	2_Mid	Other	HiN	40.50864	-86.845
WLV040-0003	Big Pine Creek @ SR 55	2_Mid	Other	HiN	40.45242	-87.2544
WLV070-0013	Big Shawnee Creek @ Green Bay Rd	2_Mid	Other	LoN	40.25532	-87.1986
WLV080-0015	Opossum Run @ Browns Hill Rd	1_Ref_Cal	Ref	HiN	40.19305	-87.4597
WLV110-0006	Prairie Creek @ CR 170 W	1_Ref_Cal	Ref	HiN	40.03491	-87.2981
WLV160-0013	Big Raccoon Creek @ US 231	1_Ref_Cal	SubRef	HiN	39.85441	-86.8868
WLV160-0019	Big Raccoon Creek @ CR 500S	2_Mid	Other	HiN	39.96684	-86.6839
WLV160-0020	Big Raccoon Creek @ CR 775	1_Ref_Cal	SubRef	HiN	39.94098	-86.7569
WLV160-0038	Cornstalk Creek @ CR 1150 S	1_Ref_Cal	SubRef	LoN	39.87295	-86.8608
WLW-02-0001	River Deshee @ Beal Road	3_Strs_Cal	Strs	HiN	38.51969	-87.6275
WLW-03-0002	Wabash River @ CR 1500 W	2_Mid	Other	HiN	38.33361	-87.8279
WLW-07-0002	Little Creek @ Number 6 School Road	1_Ref_Cal	SubRef	HiN	38.05453	-87.6674
WLW-07-0003	Fun Creek @ Smith School Road	1_Ref_Cal	Ref	HiN	38.03024	-87.9259
WLW-09-0001	Wabash River @ Spencer Ditch Road	2_Mid	Other	HiN	37.88061	-88.0765
WMI-01-0008	Mississinewa River @ CR 300 E	2_Mid	Other	HiN	40.28486	-84.9126
WMI-02-0021	Mississinewa River @ CR 900 E	2_Mid	Other	HiN	40.28956	-85.2211
WMI-05-0018	Mississinewa River @ CR 700 S	2_Mid	Other	HiN	40.45756	-85.535
WMI-05-0019	Mississinewa River @ Cardinal Drive	2_Mid	Other	HiN	40.40742	-85.5024
WMI-05-0020	Tippey Ditch @ CR 600 E	3_Strs_Val	Strs	HiN	40.57321	-85.5547
WMI-06-0006	Mississinewa River @ Peru Circus Lane	2_Mid	Other	HiN	40.75369	-86.0214
WPA-01-0009	Patoka River @ CR 475 E	1_Ref_Cal	Ref	LoN	38.48916	-86.3601
WPA-01-0010	Hogs Defeat Creek @ SR 37	2_Mid	Other	LoN	38.44462	-86.463
WPA-02-0001	Straight River @ CR 100 S	2_Mid	Other	HiN	38.34984	-86.9182

SITE	Station @ Location	Data Set	Final Ref Status	N Class	Latitude	Longitude
WPA-02-0002	Straight River @ SR 162	2_Mid	Other	HiN	38.35442	-86.8997
WPA-02-0003	Flat Creek @ CR 500 E	2_Mid	Other	HiN	38.30296	-86.8215
WPA-02-0004	Straight River @ CR 100 W	2_Mid	Other	HiN	38.35192	-86.9225
WPA-02-0005	Grassy Fork @ Santine Road	1_Ref_Cal	SubRef	HiN	38.3407	-86.8067
WPA-02-0006	Flat Creek @ St. Anthony Road W	2_Mid	Other	HiN	38.34457	-86.8737
WPA-02-0007	Straight River @ SR 162	3_Strs_Cal	Strs	HiN	38.35505	-86.8943
WPA-03-0001	Hunley Creek @ CR 850 S	2_Mid	Other	HiN	38.25241	-86.9023
WPA-03-0006	Hunley Creek @ CR 660 S	2_Mid	Other	HiN	38.27539	-86.9052
WPA-03-0007	Indian Creek @ Old SR 162	2_Mid	Other	HiN	38.28012	-86.8608
WPA-03-0009	Bruner Creek @ SR 64	2_Mid	Other	HiN	38.30298	-86.9281
WPA-03-0011	Hunley Creek @ CR 660 S	2_Mid	Other	HiN	38.28512	-86.9048
WPA-03-0012	Bruner Creek @ 1st Street	2_Mid	Other	HiN	38.2901	-86.9284
WPA-03-0013	Tributary of Short Creek @ CR 900 S	2_Mid	Other	HiN	38.24722	-86.9799
WPA-04-0003	Patoka River @ CR 600 W	2_Mid	Other	HiN	38.36456	-87.0054
WPA-04-0004	Patoka River @ CR 150 S	2_Mid	Other	HiN	38.35329	-86.9883
WPA-04-0005	Patoka River @ CR 175 E	2_Mid	Other	HiN	38.44758	-86.8718
WPA-04-0006	Patoka River @ CR 175 E	2_Mid	Other	HiN	38.42244	-86.872
WPA-04-0007	Polson Creek @ Celestine Road	1_Ref_Val	SubRef	HiN	38.43233	-86.7945
WPA-04-0008	Polson Creek @ Jasper Dubois Road	2_Mid	Other	HiN	38.43415	-86.8042
WPA-04-0010	Buffalo Stream @ Lake Road	2_Mid	Other	HiN	38.42149	-86.9179
WPA-04-0012	Elli Creek @ Duff Road SE	2_Mid	Other	HiN	38.31436	-87.0029
WPA-04-0013	Altar Creek @ CR 620 W	2_Mid	Other	HiN	38.38312	-87.0194
WPA-04-0014	Teder Creek @ Jasper Dubois Road	2_Mid	Other	HiN	38.41598	-86.8254
WPA-04-0015	Elli Creek @ CR 650 W	1_Ref_Val	SubRef	HiN	38.30526	-87.029
WPA-05-0001	Flat Creek @ CR 50 S	2_Mid	Other	HiN	38.42074	-87.1176
WPA-05-0002	Flat Creek @ CR 50 N	2_Mid	Other	HiN	38.39257	-87.0596
WPA-05-0004	Lick Creek @ CR 725 E	2_Mid	Other	HiN	38.43307	-87.1338
WPA-06-0001	Patoka River @ CR 350 S	2_Mid	Other	HiN	38.37461	-87.1785
WPA-06-0003	Patoka River @ Meridian Road	2_Mid	Other	HiN	38.38267	-87.2911
WPA-06-0005	Patoka River @ Line/Meridian Road	2_Mid	Other	HiN	38.3828	-87.2364

SITE	Station @ Location	Data Set	Final Ref Status	N Class	Latitude	Longitude
WPA-06-0006	Patoka River @ CR 650 E	2_Mid	Other	HiN	38.35331	-87.1355
WPA-06-0007	Patoka River @ CR 350 S	2_Mid	Other	HiN	38.37397	-87.1863
WPA-07-0001	Rough Creek @ CR 925 S	3_Strs_Val	Strs	HiN	38.28788	-87.2425
WPA-07-0002	South Fork Patoka River @ CR 25 S	2_Mid	Other	HiN	38.34772	-87.32
WPA-07-0003	South Fork Patoka River @ South Fork Patoka River Road	1_Ref_Val	SubRef	HiN	38.25112	-87.193
WPA-07-0004	Hat Creek @ Field Road	2_Mid	Other	HiN	38.32389	-87.2615
WPA-08-0007	Patoka River @ SR 65	2_Mid	Other	HiN	38.39686	-87.5366
WPA-08-0008	Patoka River @ CR 250 N	2_Mid	Other	HiN	38.39922	-87.6884
WPA-08-0009	Patoka River @ CR 200 W	2_Mid	Other	HiN	38.3867	-87.6227
WPA-08-0010	West Fork Keg Creek @ CR 125 S	2_Mid	Other	HiN	38.33278	-87.4015
WPA-08-0011	Patoka River @ CR 250 N	2_Mid	Other	HiN	38.39792	-87.6945
WSA-02-0003	Salamonie River @ CR 550 N	2_Mid	Other	LoN	40.51417	-85.194
WSA010-0012	Little Salamonie River @ Boundary Pike	1_Ref_Cal	SubRef	HiN	40.40698	-84.9614
WSU-01-0010	Sugar Cr @ Frankfort Road	2_Mid	Other	HiN	40.14496	-86.5704
WSU-04-0003	Honey Cr @ SR 47	2_Mid	Other	LoN	40.1093	-86.7674
WSU-05-0001	Sugar Mill Cr @ Thomas Road	2_Mid	Other	HiN	39.94483	-87.2082
WSU-06-0010	Sugar Cr @ CR 550 W	2_Mid	Other	HiN	39.84905	-87.3587
WSU-06-0012	Sugar Cr @ Turkey Run SP	2_Mid	Other	HiN	39.88626	-87.2144
WSU-06-0014	Sugar Cr @ CR 225 W	3_Strs_Cal	HiStrs	HiN	40.04168	-86.9392
WSU010-0010	Spring Creek @ SR 47 E of US 52	2_Mid	Other	HiN	40.13117	-86.5443
WSU040-0020	Sugar Cr @ CR 275 E	1_Ref_Cal	Ref	HiN	40.08773	-86.8523
WTI-02-0021	Deeds Creek @ Van Ness Road	3_Strs_Val	Strs	HiN	41.21279	-85.731
WTI-03-0014	Tippecanoe River @ Park Schram Road	2_Mid	Other	HiN	41.24074	-85.9288
WTI-04-0017	Deer Creek @ 15th Road	1_Ref_Cal	SubRef	HiN	41.26163	-86.1675
WTI-05-0015	Tippecanoe River @ CR 375 N	2_Mid	Other	HiN	41.10628	-86.2517
WTI-06-0008	Tippecanoe River @ CR 550 N	1_Ref_Cal	SubRef	HiN	41.13379	-86.3962
WTI-06-0010	Tippecanoe River @ CR 200 E	2_Mid	Other	HiN	41.15841	-86.5611
WTI-08-0004	Indian Creek @ CR 1000 S	1_Ref_Cal	SubRef	HiN	40.91119	-86.6274
WTI-10-0012	Hansell Ditch @ SR 14	2_Mid	Other	HiN	41.03391	-86.9077

SITE	Station @ Location	Data Set	Final Ref Status	N Class	Latitude	Longitude
WTI-12-0004	Hoagland Ditch @ CR 600 W	3_Strs_Val	Strs	HiN	40.74847	-86.9944
WTI-13-0002	Pike Creek @ SR 39	2_Mid	Other	HiN	40.78088	-86.7442
WUW-07-0014	Mossburg Ditch @ CR 550 W	2_Mid	Other	LoN	40.72139	-85.3275
WUW-08-0006	Wabash River @ SR 201	2_Mid	Other	HiN	40.72537	-85.1292
WUW-10-0001	Seegar Ditch @ Eme Road	2_Mid	Other	HiN	41.09829	-85.2913
WUW-10-0002	Little River @ Gundy Road	3_Strs_Cal	HiStrs	HiN	40.98239	-85.3549
WUW-11-0004	Flat Creek @ Mayne Road	1_Ref_Val	SubRef	LoN	40.91142	-85.3685
WUW-14-0002	Treaty Creek @ CR 50 E	2_Mid	Other	HiN	40.74271	-85.7774
WUW-15-0001	Honey Cr @ CR 400 N	2_Mid	Other	LoN	40.53925	-85.9459
WUW-15-0002	Pipe Creek @ US 31	3_Strs_Cal	Strs	LoN	40.68087	-86.1302
WUW-16-0002	Wabash River @ River Road	2_Mid	Other	HiN	40.74522	-86.1463
WWE-02-0002	Little Walnut Creek @ CR 75 S	3_Strs_Cal	Strs	LoN	39.65145	-86.9356
WWE-04-0003	Big Walnut Creek @ 480 E	2_Mid	Other	HiN	39.72377	-86.768
WWE-04-0006	Big Walnut Creek @ CR 480 E South of historical site	2_Mid	Other	HiN	39.71882	-86.7714
WWE-05-0005	Mill Creek Ditch @ 550 S	2_Mid	Other	LoN	39.5795	-86.6545
WWE-06-0001	Birch Creek @ 200 N	2_Mid	Other	HiN	39.40841	-87.1073
WWE-07-0001	Jordan Creek @ 875 E	2_Mid	Other	HiN	39.40493	-86.9429
WWE-07-0003	Hog Creek @ 375 E	2_Mid	Other	HiN	39.38133	-87.0448
WWE-07-0004	Eel River @ River Rd	2_Mid	Other	HiN	39.34499	-87.0871
WWE-07-0005	Croys Creek @ 800 N	2_Mid	Other	HiN	39.50514	-87.0176
WWE-08-0003	Eel River @ SR 59	2_Mid	Other	HiN	39.19763	-87.1179
WWL-01-0003	Beanblossom Creek @ Gatesville Rd	2_Mid	Other	LoN	39.26118	-86.2417
WWL-01-0004	Beanblossom Creek @ Mt Tabor Rd	2_Mid	Other	LoN	39.30655	-86.6181
WWL-02-0003	Rattlesnake Creek @ Hyden Rd	1_Ref_Val	SubRef	LoN	39.28223	-86.806
WWL-03-0003	Plummer Creek @ Plankeshaw Trail	1_Ref_Cal	Ref	LoN	38.97113	-86.8427
WWL-04-0001	Miller Creek @ 300 N	1_Ref_Val	SubRef	HiN	39.06782	-87.0628
WWL-05-0001	White River @ Baseline Rd	2_Mid	Other	HiN	38.98349	-86.9533
WWL-07-0002	Prairie Creek @ SR 57	3_Strs_Val	Strs	HiN	38.7179	-87.1651
WWL-08-0003	White River @ CR 150 N	2_Mid	Other	HiN	38.68895	-87.2707

SITE	Station @ Location	Data Set	Final Ref Status	N Class	Latitude	Longitude
WWL-08-0004	White River @ SR 358	2_Mid	Other	HiN	38.81238	-87.2427
WWL-10-0004	White River @ Smithville Rd	2_Mid	Other	HiN	38.50649	-87.5466
WWL-10-0005	White River @ River Rd	3_Strs_Val	Strs	HiN	38.52775	-87.3309
WWU-01-0003	White River @ SR 32	1_Ref_Cal	SubRef	HiN	40.1759	-84.8902
WWU-01-0006	White River @ Sciscoe Rd	2_Mid	Other	HiN	40.15779	-85.3318
WWU-03-0001	White River @ Lincolnshire Dr	3_Strs_Val	HiStrs	HiN	40.18139	-85.4885
WWU-03-0002	White River @ 2nd St	3_Strs_Cal	HiStrs	HiN	40.11578	-85.702
WWU-04-0001	Pipe Creek @ 600 N	2_Mid	Other	HiN	40.19045	-85.8184
WWU-05-0001	Duck Creek @ 281st St	2_Mid	Other	LoN	40.20013	-85.8771
WWU-07-0001	White River @ Strawtown Ave	2_Mid	Other	HiN	40.1321	-85.9521
WWU-07-0002	West Fork White River @ Prairie View Golf Club	3_Strs_Val	Strs	HiN	39.98054	-86.0333
WWU-08-0002	Lick Creek @ Lick Cr Rd	3_Strs_Cal	Strs	HiN	39.95017	-85.8093
WWU-08-0004	Deer Creek @ 650 W	3_Strs_Cal	Strs	HiN	39.98357	-85.5045
WWU-09-0002	Fall Creek @ 10th St	3_Strs_Val	HiStrs	HiN	39.78044	-86.1872
WWU-09-0003	Fall Creek @ Fall Cr Pkwy N Dr	3_Strs_Val	HiStrs	HiN	39.86384	-86.0517
WWU-09-0004	Mud Creek @ SR 38	2_Mid	Other	LoN	40.01895	-85.849
WWU-10-0002	Carmel Creek @ Gould Dr	3_Strs_Cal	HiStrs	HiN	39.93705	-86.1033
WWU-11-0004	Little Eagle Creek @ Georgetown Rd	3_Strs_Cal	HiStrs	HiN	39.86598	-86.2454
WWU-11-0005	Eagle Creek @ Belmont Ave	3_Strs_Val	HiStrs	HiN	39.72428	-86.1961
WWU-13-0001	West Fork White Lick Creek @ Cartersburg Rd	3_Strs_Cal	Strs	HiN	39.74936	-86.5069
WWU-13-0003	White Lick Creek @ Carol Ln	3_Strs_Val	Strs	HiN	39.59109	-86.3678
WWU-13-0006	West Fork White Lick Creek @ 550 E	3_Strs_Cal	Strs	HiN	39.86084	-86.4335
WWU-15-0001	West Fork White River @ SR 37	3_Strs_Cal	Strs	HiN	39.39873	-86.4791
WWU-15-0002	West Fork White River @ Blue Bluff Rd	3_Strs_Cal	Strs	HiN	39.49042	-86.4144
WWU-17-0003	White River @ Sand College Rd	2_Mid	Other	HiN	39.34861	-86.6396
WWU010-0039	West Fork White River @ CR 200 E	1_Ref_Val	SubRef	HiN	40.1862	-84.9369
WWU100-0040	Fall Creek @ C.R. 650W	3_Strs_Cal	Strs	HiN	39.97075	-85.7962
WWU100-0064	Fall Creek @ CR 750 W	2_Mid	Other	HiN	39.96437	-85.815
WWU100-0078	Fall Creek @ Brown St	3_Strs_Cal	Strs	HiN	40.01594	-85.6922
WWU100-0098	Fall Creek @ CR 700 N	2_Mid	Other	HiN	40.03386	-85.5479

SITE	Station @ Location	Data Set	Final Ref Status	N Class	Latitude	Longitude
WWU100-0106	Fall Creek @ Mechanicsburg Rd	2_Mid	Other	HiN	40.02523	-85.5571

# Appendix E

## Preliminary Site Classification

Initial and preliminary site classification proceeded using non-metric multidimensional scaling (NMS) and principal components analysis (PCA) ordinations to organize reference samples by taxonomic similarity and then to associate the ordinated axes with natural environmental factors. The objective was to find distinct categorical classes of similar biological groups defined by a common landscape factor (e.g. bug classes, fish classes, ecoregions) or a threshold of a continuous variable, thus associating community types with natural factors to improve biological sensitivity to disturbance responses.

Sixty-five (65) reference samples were reduced to comparable taxa and metrics. For the NMS ordination diatom taxa were generally lumped to species level identifications, ignoring varieties and family taxonomic levels. Because uncommon taxa are generally un-informative, taxa with less than 4 occurrences were lumped to genus or eliminated. The ordination used Bray-Curtis similarities on taxa presence data.

Twenty-nine (29) metrics were used in the PCA ordination; selected to represent several components of the samples (Table 1). Metrics were selected for the PCA if they showed response along the disturbance gradient, were used in the USGS MMI, or represented an otherwise underrepresented metric category. Besides the ordination, a cluster analysis was performed followed by classification and regression trees (CART) analysis. Metrics were normalized to the mean and standard deviation for the analysis.

Table 1. See metric descriptions in Attachment 1.

NRSA metrics	Responsive	Representing Categories
MMI_USGS	pt_HIGH_P	pi_Diat_CL_2
LOW_P.r.res	pi_HIGH_P	x_Kelly_TDI_2008
pt_BC_4	pi_SAP_345	pt_TROPHIC_456
nt_BC_4	pt_O_345	nt_MOD_HI_MOTILE
pt_HIGH_N	pi_O_45	nt_STALKED
nt_NON_MOTILE	pi_PT_45	nt_sm_vsm
pt_NON_MOTILE	WA_PT_USGS	nt_ACHNANTHIDIAE
pt_SESTONIC_HABIT	pt_SALINITY_34	
pt_BENTHIC_HABIT	nt_BC_12	
nt_SALINITY_3	pt_BC_12	
pt_NAVICULA	pt_BC_45	

The environmental variables were selected to represent natural conditions that might affect diatom communities. They included factors related to location, waterbody type, environmental setting, climate, watershed characteristics, geology, hydrology, and predicted natural chemistry

(Appendix F). Measured chemistry was also considered, though these variables might not be natural factors.

Correlations of environmental variables on the NMS axes were related to landscape slope, predicted chemistry, and climate (Table 2). An example of an ordination diagram of an environmental variable that was correlated with the axis is the base flow index (BFI) in the watershed (Figure 1). The BFI was correlated with axis 1 and had higher values in the southern Michigan northern Indiana ecoregion. However, if a BFI threshold was selected for classification, the resulting classes would not show separation in the diagram. With this and other correlated variables, there was not an identifiable threshold for site classification.

Table 2.

<b>Axis 1 correlations (r)</b>		<b>Axis 2 correlations (r)</b>		<b>Axis 3 correlations (r)</b>	
CaOWs	-0.468	PrecipWs	0.498	BFICat	-0.453
CaOCat	-0.425	PrecipCat	0.49	BFIWs	-0.450
SiO2Ws	0.416	TmeanWs	0.482	SCat	0.435
BFIWs	0.412	TmaxWs	0.474	TmeanCat	0.410
SLOPE_segm	-0.411	TminWs	0.468	SWs	0.409
BFICat	0.411	TmeanCat	0.449	TmaxCat	0.400
CompStrgthWs	-0.404	TmaxCat	0.445		
CompStrgthCa	-0.4	TminCat	0.421		
		Latitude	-0.419		
		Elev_m_site	-0.42		
		Longitude	-0.405		

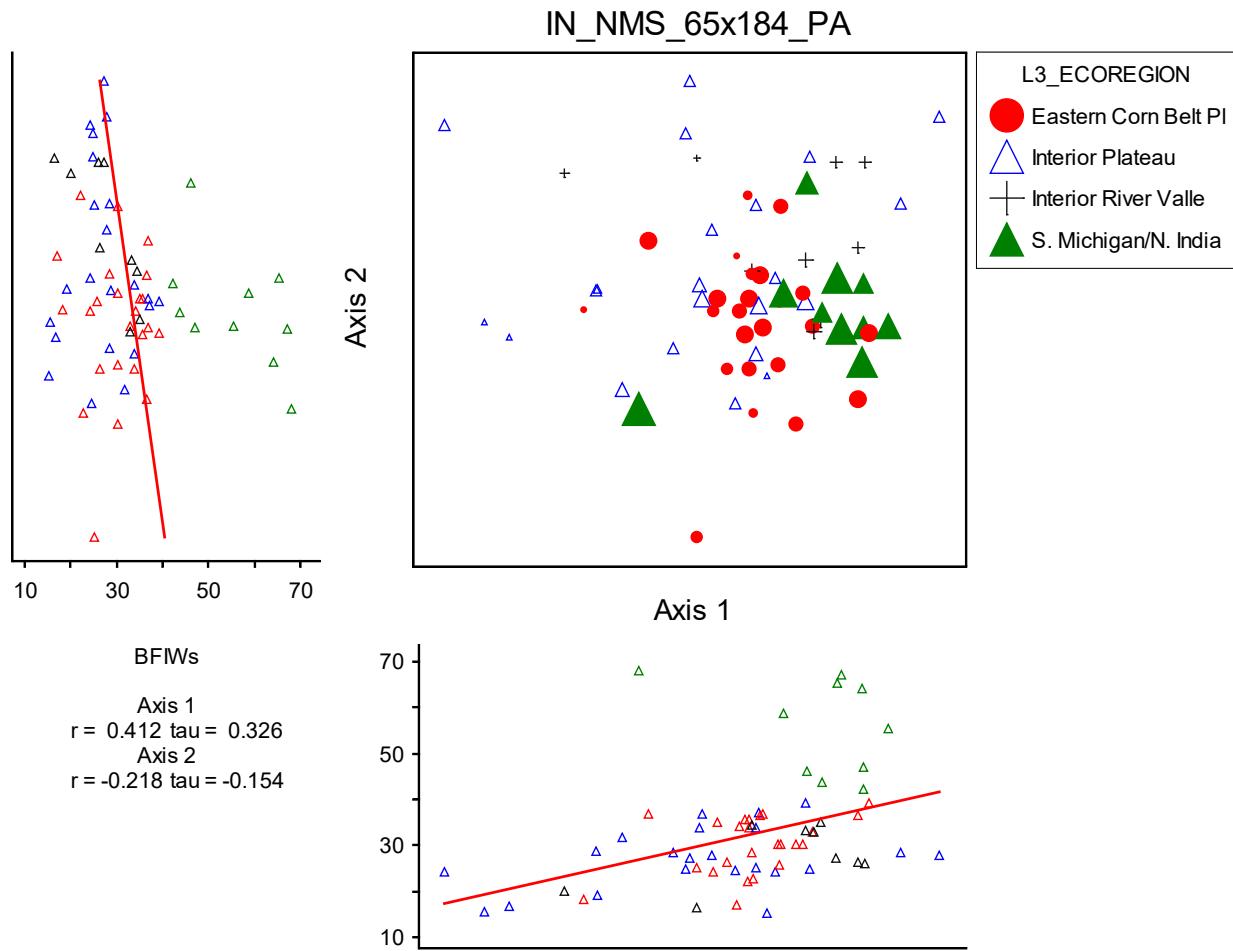


Figure 1.

The cluster analysis was used to identify four groups of similar reference samples (Figure 2). CART analysis on these clusters could predict three groups, combining two similar clusters. The largest cluster (#1) was predicted by river basin and included the Great Lakes tributaries, the Kankakee, the Lower Wabash, the Potoka, the East Fork White River, and the White River. The remaining sites were separated by high (#2 and #4) and low (#3) precipitation (threshold – 1,078 mm/yr).

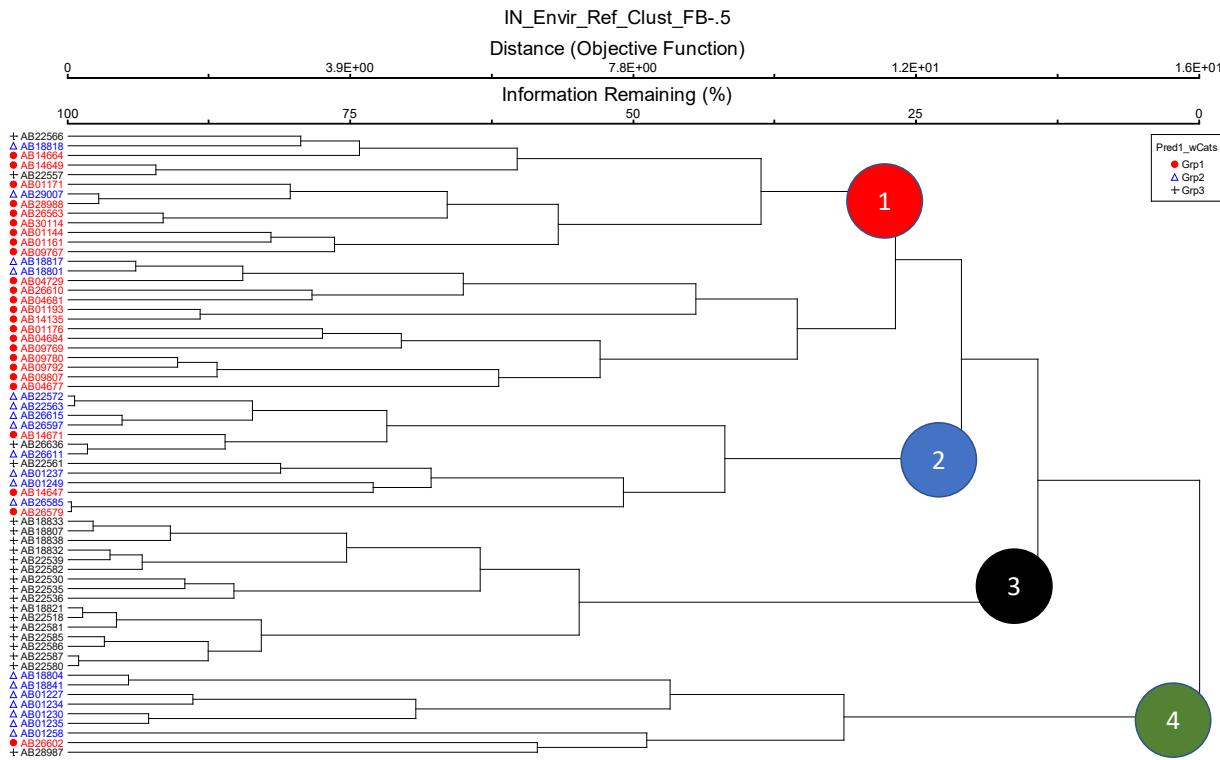


Figure 2.

In the PCA of metrics, the eigenvalues suggested that 4 axes were important (with 67% cumulative variance explained), but only three axes were displayed and analyzed. The PCA axes suggested that the natural factors affecting diatom metrics were related to wetness and slope on the first axis, groundwater and temperature on the second axis, and wetness, slope, and elevation on the third axis (Table 3).

Table 3.

Axis 1 correlations (r)	Axis 2 correlations (r)	Axis 3 correlations (r)
WetIndexCat*	0.437	BFICat
CaOWs	-0.389	TminCat
WetIndexWs	0.372	BFIWs
PrecipWs	-0.323	TminWs
CaOCat	-0.322	SCat
PrecipCat	-0.317	TmeanCat
SLOPE_segment	-0.308	
Al2O3Ws	0.304	

\* WetIndex: High index value indicates high potential of water accumulated due to low slope.

### ***Conclusions***

The ordinations and cluster analysis did not show distinctive site classes for diatoms. While some natural factors were suggested, no categories or thresholds were identified. Upon presentation to the IDEM staff, lack of classification for the diatom assemblage was not surprising. They were asked to consider whether diatom communities should be naturally different and based on what factors. The factors considered included basins, precipitation, slope (wet index), BFI, and temperature. In general, the inconclusive analyses lead to consideration of other classification approaches, including adjusting metrics individually by environmental variables and classification based on a preliminary index.

## Appendix F

### Variable codes and descriptions

Variable Code	Description
Location	Site location
Latitude	Site latitude
Longitude	Site longitude
COMID_Final	NHDPlus v2 COMID associated with the NHD stream segment nearest the site
AUID	Assessment Unit identification number
COUNTY_NAME	Name of county
Waterbody Type	Type of waterbody the site is located at
Ref_Status	Disturbance designation
<b><u>Environmental Setting</u></b>	
Basins	Indiana state basins - based on major drainages
HUC4 – HUC14	Hydrologic Unit Code (HUC) at various levels
NATURAL_REGION	Designated by IDEM
L3_ECOREGION	U.S. EPA level III ecoregion number
L3_ECOREGION_NAME	U.S. EPA level III ecoregion name
US_L4CODE	U.S. EPA level ILLI ecoregion number
US_L4NAME	U.S. EPA level ILLI ecoregion name
BUG_SITE_CLASS	The macroinvertebrate site class that a given site is assigned to
FISH_SITE_CLASS	The fish site class that a given site is assigned to
<b><u>Climate</u></b>	
PrecipCat	PRISM climate data - 30-year normal mean precipitation (mm): Annual period: 1981-2010 within the catchment
TmaxCat	PRISM climate data - 30-year normal maximum temperature (°C): Annual period: 1981-2010 within the catchment
TmeanCat	PRISM climate data - 30-year normal mean temperature (°C): Annual period: 1981-2010 within the catchment
TminCat	PRISM climate data - 30-year normal minimum temperature (°C): Annual period: 1981-2010 within the catchment
PrecipWs	PRISM climate data - 30-year normal mean precipitation (mm): Annual period: 1981-2010 within the watershed
TmaxWs	PRISM climate data - 30-year normal maximum temperature (°C): Annual period: 1981-2010 within the watershed
TmeanWs	PRISM climate data - 30-year normal mean temperature (°C): Annual period: 1981-2010 within the watershed
TminWs	PRISM climate data - 30-year normal minimum temperature (°C): Annual period: 1981-2010 within the watershed
<b><u>Watershed Characteristics</u></b>	
Elev_m_site	Elevation (meters) of a given site

PctWetWat2016Cat	Combined land cover metric that consists of catchment estimates of the following: PctOw2016Cat (open water), PctHbWet2016Cat (herbaceous wetland), and PctWdWet2016Cat (woody wetland)
PctWetWat2016Ws	Combined land cover metric that consists of watershed estimates of the following: PctOw2016Ws (open water), PctHbWet2016Ws (herbaceous wetland), and PctWdWet2016Ws (woody wetland)
DRAINAGE	Drainage area in IDEM database stations table
GRADIENT	stream gradient in IDEM database stations table
<u>Geology and Hydrology</u>	
CompStrgthCat	Mean lithological uniaxial compressive strength (megaPascals) content in surface or near surface geology within catchment
CompStrgthWs	Mean lithological uniaxial compressive strength (megaPascals) content in surface or near surface geology within watershed
KffactCat	Mean soil erodibility (Kf) factor (unitless) of soils within catchment. The Kf factor is used in the Universal Soil Loss Equation (USLE) and represents a relative index of susceptibility of bare, cultivated soil to particle detachment and transport by rainfall.
KffactWs	Mean soil erodibility (Kf) factor (unitless) of soils within watershed. The Kf factor is used in the Universal Soil Loss Equation (USLE) and represents a relative index of susceptibility of bare, cultivated soil to particle detachment and transport by rainfall.
BFIcat	Baseflow is the component of streamflow that can be attributed to ground-water discharge into streams. The Baseflow Index (BFI) is the ratio of baseflow to total flow, expressed as a percentage, within catchment.
BFIWs	Baseflow is the component of streamflow that can be attributed to ground-water discharge into streams. The Baseflow Index (BFI) is the ratio of baseflow to total flow, expressed as a percentage, within watershed.
HydrlCondCat	Mean lithological hydraulic conductivity (micrometers per second) content in surface or near surface geology within catchment
HydrlCondWs	Mean lithological hydraulic conductivity (micrometers per second) content in surface or near surface geology within watershed
WetIndexCat	Mean Composite Topographic Index (CTI) [Wetness Index] within catchment
WetIndexWs	Mean Composite Topographic Index (CTI) [Wetness Index] within watershed
<u>Predicted background chemistry</u>	
Al2O3Cat	Mean % of lithological aluminum oxide (Al2O3) content in surface or near surface geology within catchment
CaOCat	Mean % of lithological calcium oxide (CaO) content in surface or near surface geology within catchment
Fe2O3Cat	Mean % of lithological ferric oxide (Fe2O3) content in surface or near surface geology within catchment
K2Ocat	Mean % of lithological potassium oxide (K2O) content in surface or near surface geology within catchment
MgOCat	Mean % of lithological magnesium oxide (MgO) content in surface or near surface geology within catchment
Na2Ocat	Mean % of lithological sodium oxide (Na2O) content in surface or near surface geology within catchment

P2O5Cat	Mean % of lithological phosphorous oxide (P2O5) content in surface or near surface geology within catchment
SCat	Mean % of lithological sulfur (S) content in surface or near surface geology within catchment
SiO2Cat	Mean % of lithological silicon dioxide (SiO2) content in surface or near surface geology within catchment
Al2O3Ws	Mean % of lithological aluminum oxide (Al2O3) content in surface or near surface geology within watershed
CaOWs	Mean % of lithological calcium oxide (CaO) content in surface or near surface geology within watershed
Fe2O3Ws	Mean % of lithological ferric oxide (Fe2O3) content in surface or near surface geology within watershed
K2OWs	Mean % of lithological potassium oxide (K2O) content in surface or near surface geology within watershed
MgOWs	Mean % of lithological magnesium oxide (MgO) content in surface or near surface geology within watershed
Na2OWs	Mean % of lithological sodium oxide (Na2O) content in surface or near surface geology within watershed
P2O5Ws	Mean % of lithological phosphorous oxide (P2O5) content in surface or near surface geology within watershed
SWs	Mean % of lithological sulfur (S) content in surface or near surface geology within watershed
SiO2Ws	Mean % of lithological silicon dioxide (SiO2) content in surface or near surface geology within watershed
NCat	Mean % of lithological nitrogen (N) content in surface or near surface geology within catchment
NWs	Mean % of lithological nitrogen (N) content in surface or near surface geology within watershed

#### Measured chemistry

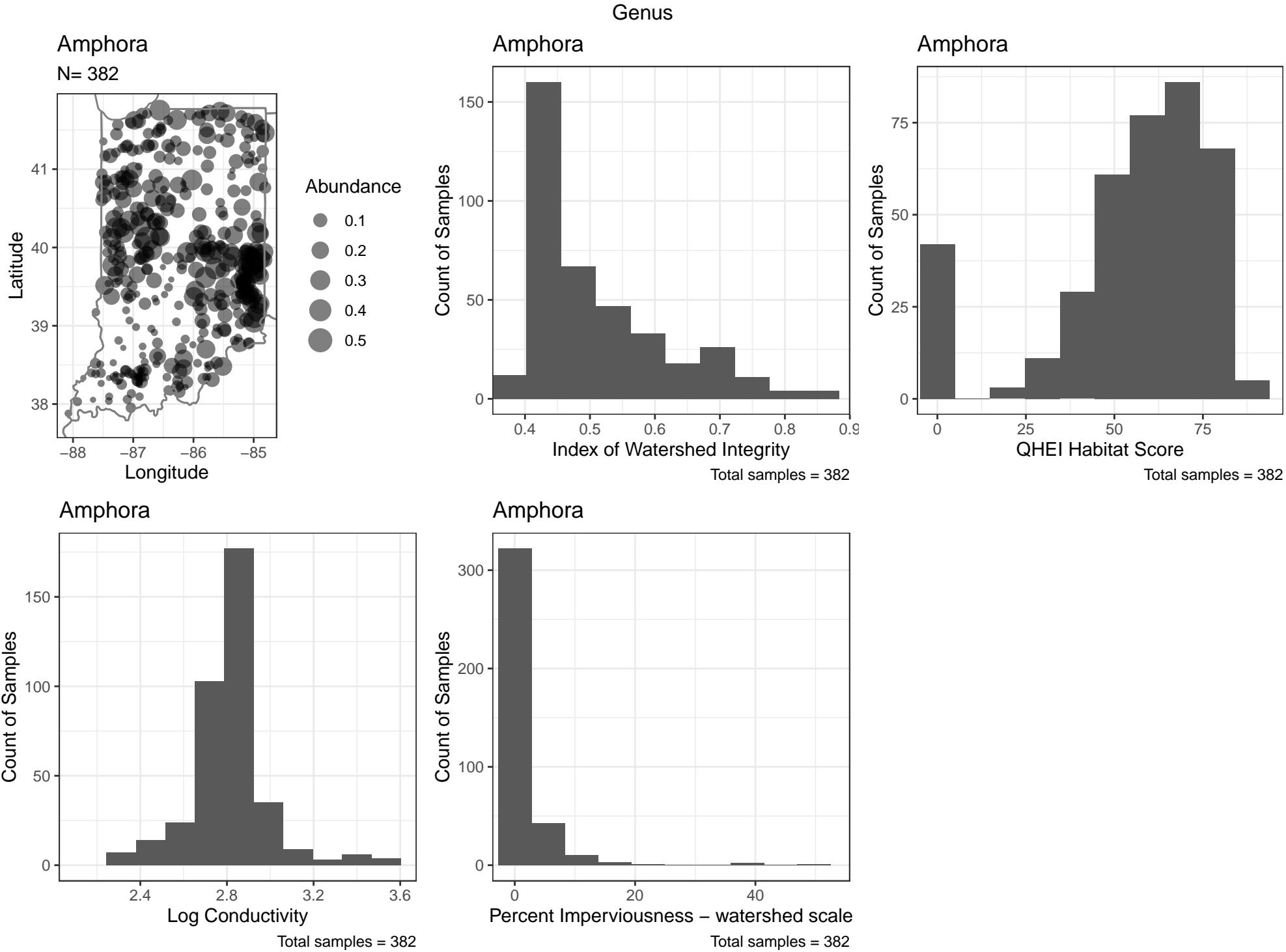
Al_diss	Aluminum measure - dissolved
Al_tot	Aluminum measure - total
Arsen_Diss	Arsenic measure - dissolved
Arsen_Tot	Arsenic measure - total
C_organic	Organic carbon measure
Cadmium_diss	Cadmium measure - dissolved
Cadmium_tot	Cadmium measure - total
Calcium	Calcium measure
CHLa_benthic	Benthic chlorophyll-a measure
Pheophyt_benthic	Benthic pheophytin measure
Chrom_diss	Chromium measure - dissolved
Chrom_tot	Chromium measure - total
COD	Chemical oxygen demand
Copper_diss	Copper measure - dissolved
Copper_tot	Copper measure - total
Lead_diss	Lead measure - dissolved
Lead_tot	Lead measure - total

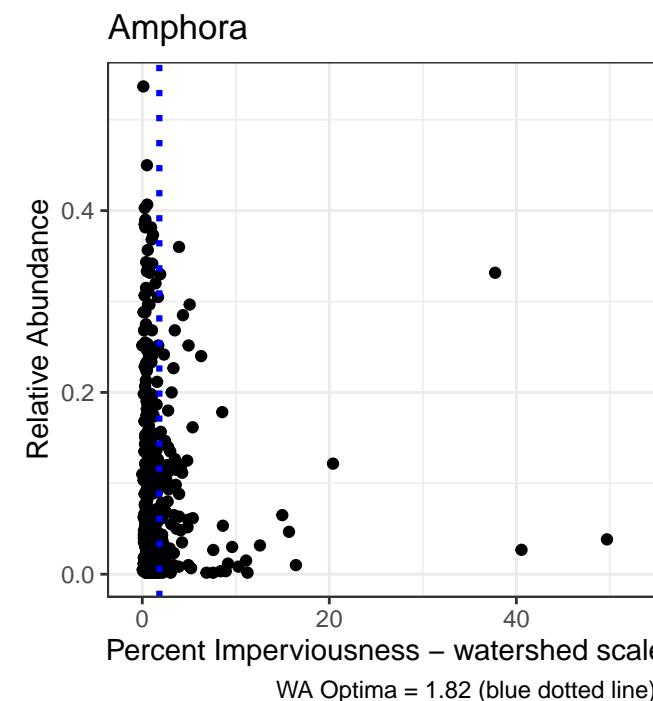
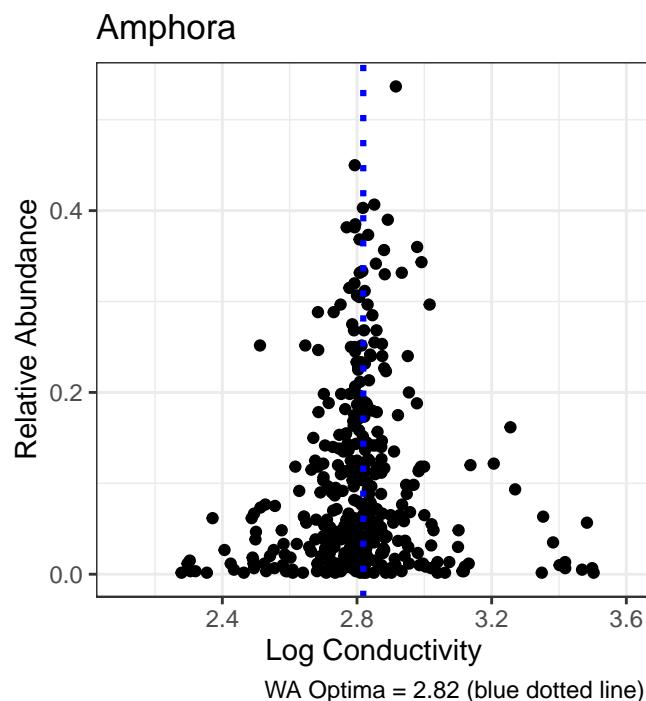
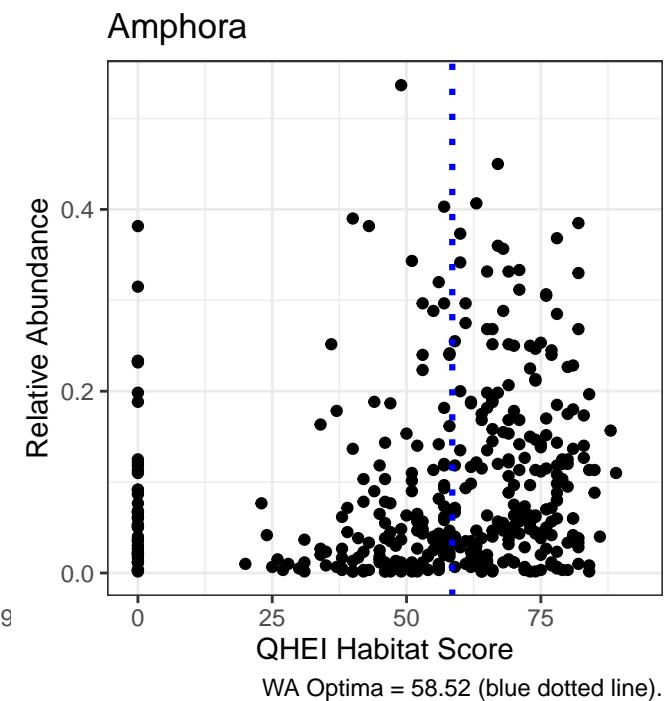
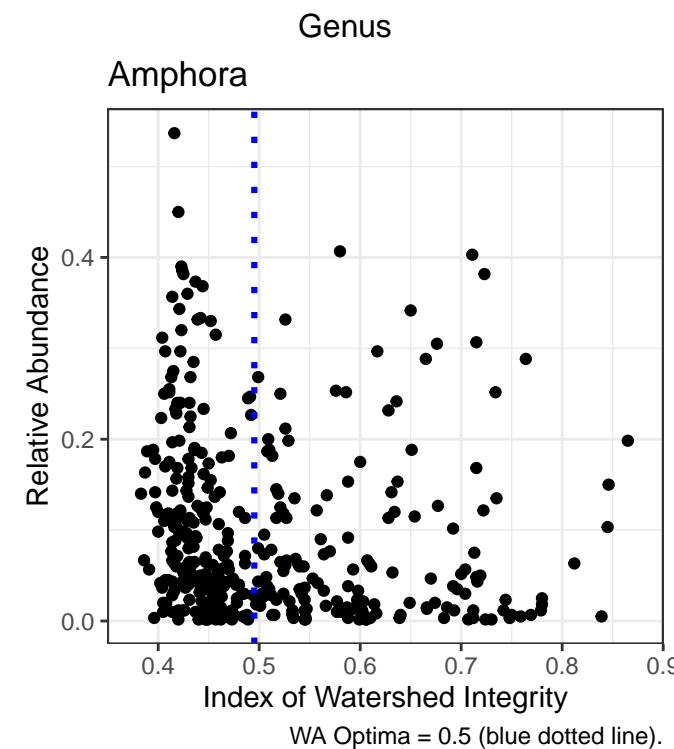
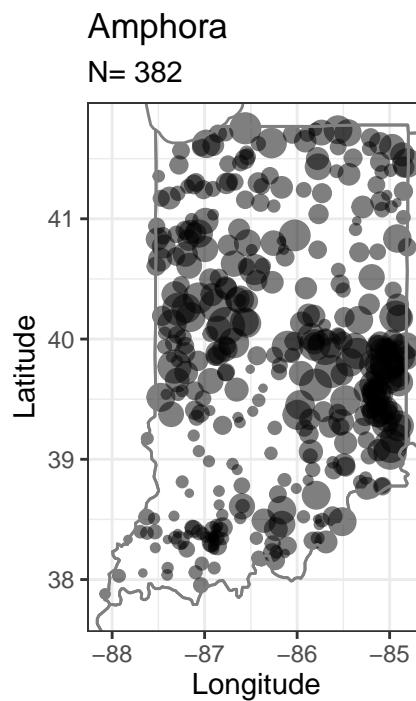
Magnesium	Magnesium measurer
N_NH4	Ammonium measure
N_NO3NO2	Nitrate/Nitrite measure
N_TKN	Total Kjeldahl nitrogen measure
Nickel_diss	Nickel measure - dissolved
Nickel_tot	Nickel measure - total
P_total	Phosphorus measure - total
Zinc_diss	Zinc measure - dissolved
Zinc_tot	Zinc measure - total
<hr/>	
<u>Disturbance</u>	
PopDens	Site estimate: population density
W_pcUrban	% urban land cover within the catchment
k5_pcUrban	% urban land cover within 5 km upstream of site
k1_pcUrban	% urban land cover within 1 km upstream of site
W_pcAg	% agricultural land cover within the catchment
k5_pcAg	% agricultural land cover within 5 km upstream of site
k1_pcAg	% agricultural land cover within 1 km upstream of site
W_pcImp	% impervious land cover within the catchment
k5_pcImp	% impervious land cover within 5 km upstream of site
k1_pcImp	% impervious land cover within 1 km upstream of site
Wd_RdCross	# of road crossings per 100 km/2 upstream of site
k5_RdCross	# of road crossings with 5 km upstream of site
k1_RdCross	# of road crossings with 1 km upstream of site
W_RdDens	Road density (km/km2) within the catchment
k5_RdDens	Road density (km/km2) within 5 km upstream of site
k1_RdDens	Road density (km/km2) within 1 km upstream of site
W_pc_CanalPipe	% of NHD stream segments designated as canals/ditch or pipeline within the catchment
k5_pc_CanalPipe	% of NHD stream segments designated as canals/ditch or pipeline within 5 km upstream of site
k1_pc_CanalPipe	% of NHD stream segments designated as canals/ditch or pipeline within 1 km upstream of site
Wd_Mines	# of mines per 100 km/2 upstream of site
k5_Mines	# of mines within 5 km upstream of site
k1_Mines	# of mines within 1 km upstream of site
Wd_NPDES	# of NPDES sites per 100 km/2 upstream of site
k5_NPDES	# of NPDES sites within 5 km upstream of site
k1_NPDES	# of NPDES sites within 1 km upstream of site
Wd_CERCLIS	# of CERCLIS sites per 100 km/2 upstream of site
k5_CERCLIS	# of CERCLIS sites within 5 km upstream of site
k1_CERCLIS	# of CERCLIS sites within 1 km upstream of site
Wd_CAFO	# of CAFO sites per 100 km/2 upstream of site
k5_CAFO	# of CAFO sites within 5 km upstream of site
k1_CAFO	# of CAFO sites within 1 km upstream of site
Wd_TRI	# of TRI sites per 100 km/2 upstream of site

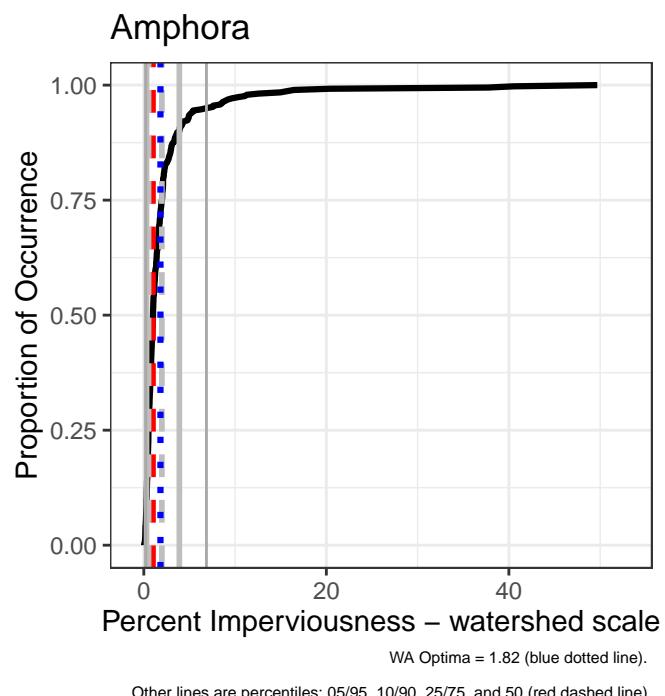
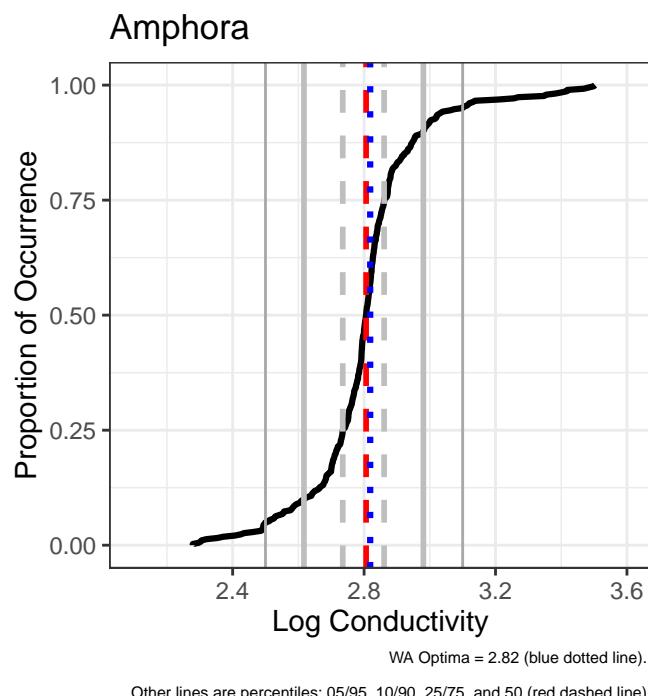
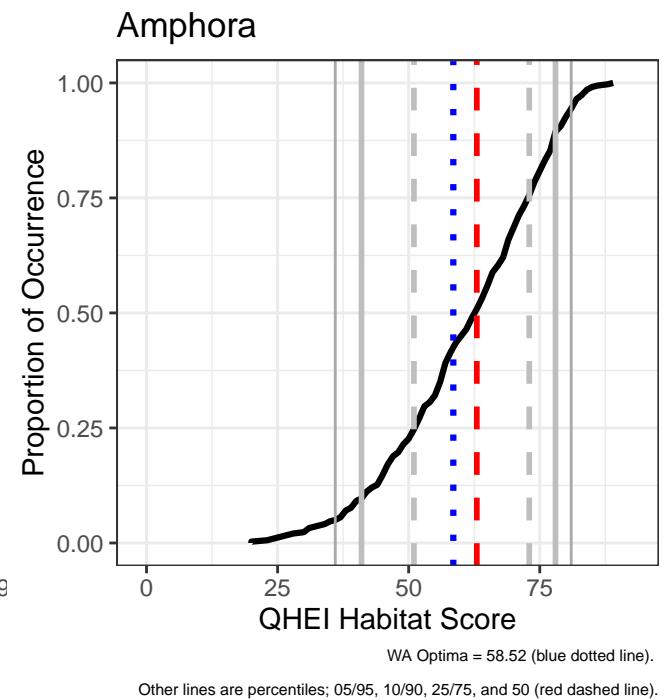
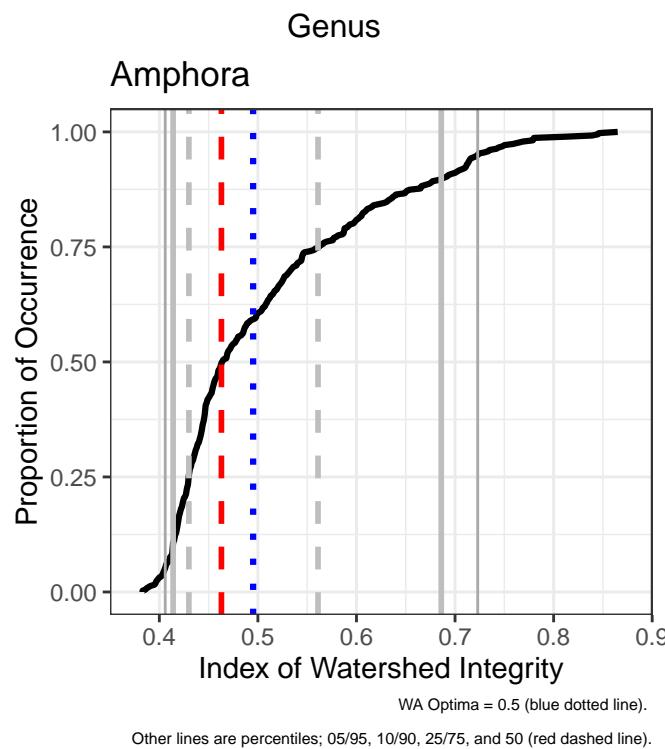
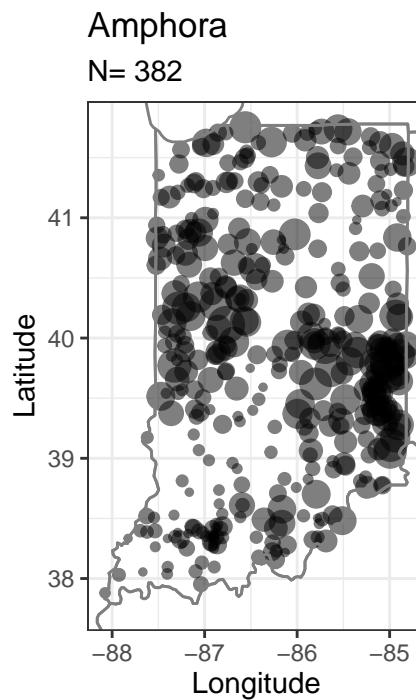
k5_TRI	# of TRI sites within 5 km upstream of site
k1_TRI	# of TRI sites within 1 km upstream of site
Wd_DAMS	# of dams per 100 km/2 upstream of site
k5_DAMS	# of dams within 5 km upstream of site
k1_DAMS	# of dams within 1 km upstream of site
ICI	Index of catchment integrity
CHYD	Hydrologic regulation component score calculated using catchment metrics
CCHEM	Regulation of water chemistry component score calculated using catchment metrics
CSED	Sediment regulation component score calculated using catchment metrics
CCONN	Hydrologic connectivity component score calculated using catchment metrics
CTEMP	Temperature regulation component score calculated using catchment metrics
CHABT	Habitat provision component score calculated using catchment metrics
IWI	Index of watershed integrity
WHYD	Hydrologic regulation component score calculated using watershed metrics
WCHEM	Regulation of water chemistry component score calculated using watershed metrics
WSED	Sediment regulation component score calculated using watershed metrics
WCONN	Hydrologic connectivity component score calculated using watershed metrics
WTEMP	Temperature regulation component score calculated using watershed metrics
WHABT	Habitat provision component score calculated using watershed metrics

#### Habitat

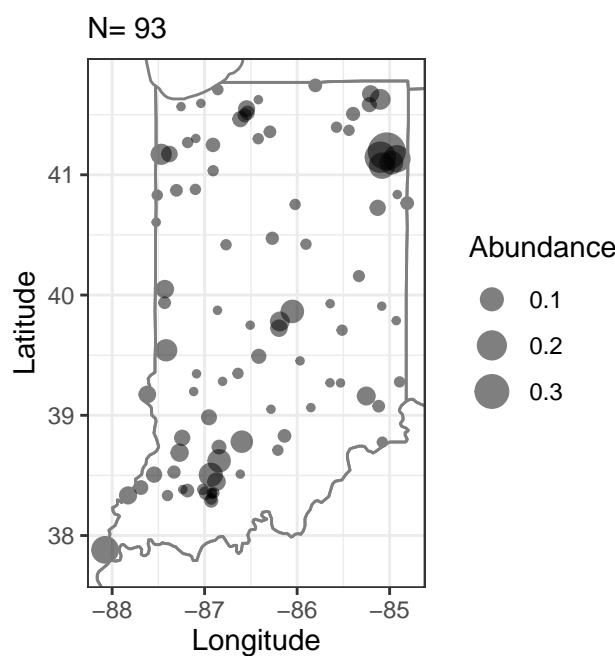
ACT_ACTIVITY_	QHEI score - calculated from component QHEI variables
QHEITOTAL_SCORE	
SUBSTR_SCORE_	Substrate score - Assigned to site by IDEM field crew
VAL_TXT	
INSTR_COVER_SCORE_	Instream cover score - Assigned to site by IDEM field crew
VAL_TXT	
CHAN_SCORE_	Channelization score - Assigned to site by IDEM field crew
VAL_TXT	
BANK_SCORE_	Bank stability score - Assigned to site by IDEM field crew
VAL_TXT	
POOL_SCORE_	Pool quality score - Assigned to site by IDEM field crew
VAL_TXT	
RIFFLE_RIF_RUN_	Riffle/Run quality score - Assigned to site by IDEM field crew
SCORE_VAL_TXT	
GRADIENT_SCORE_	Gradient score - Assigned to site by IDEM field crew
VAL_TEXT	





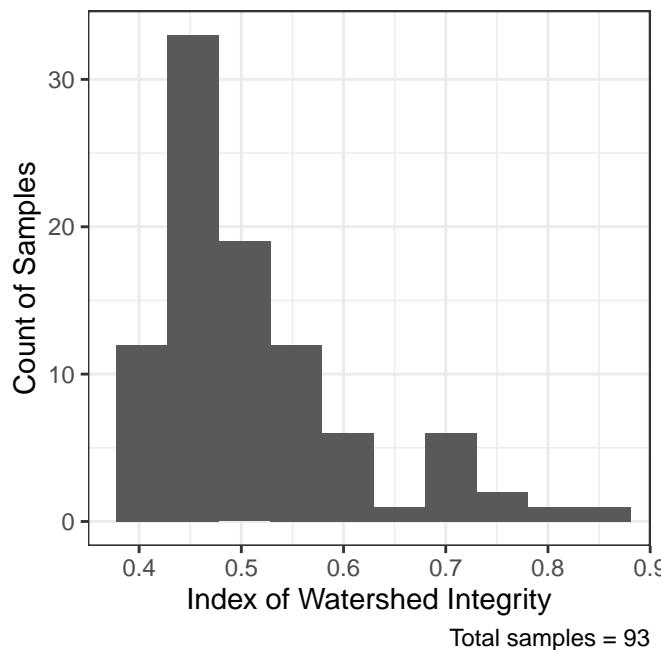


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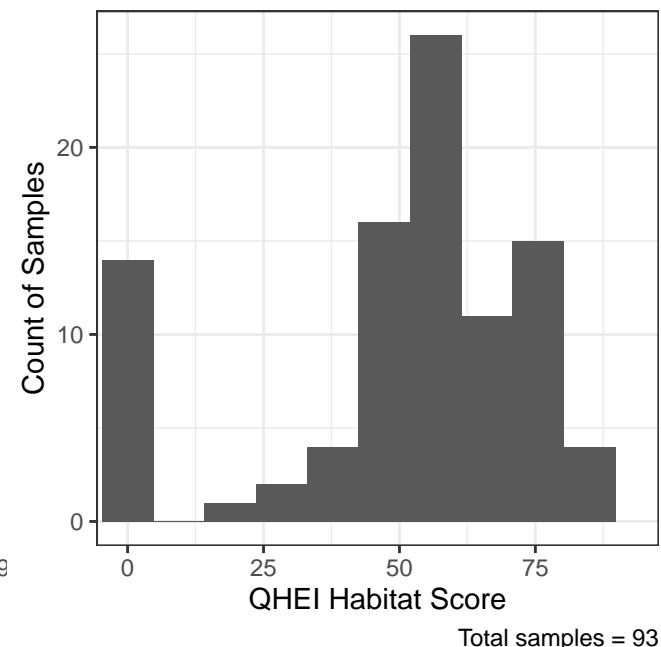


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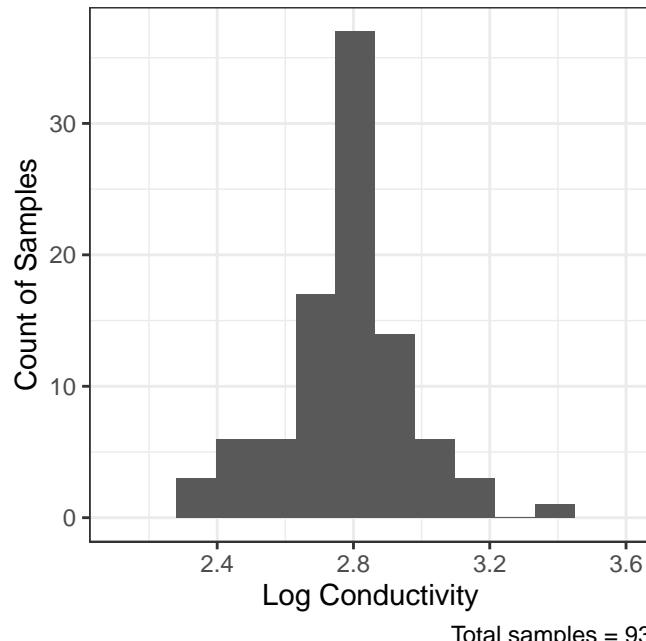
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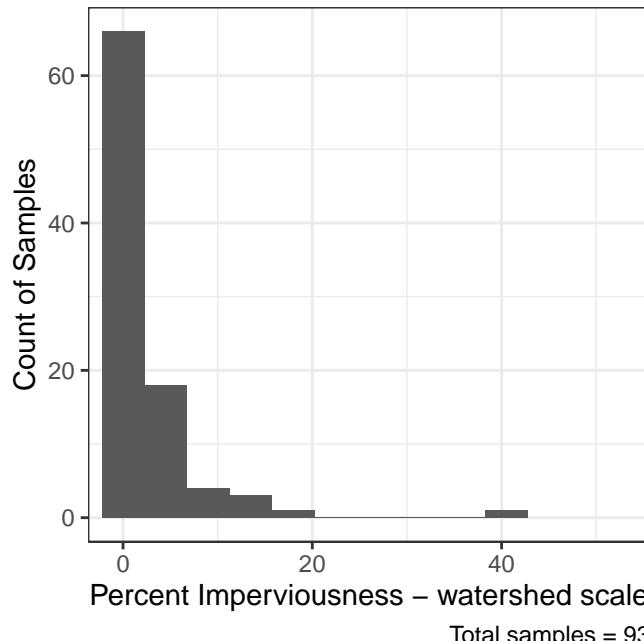
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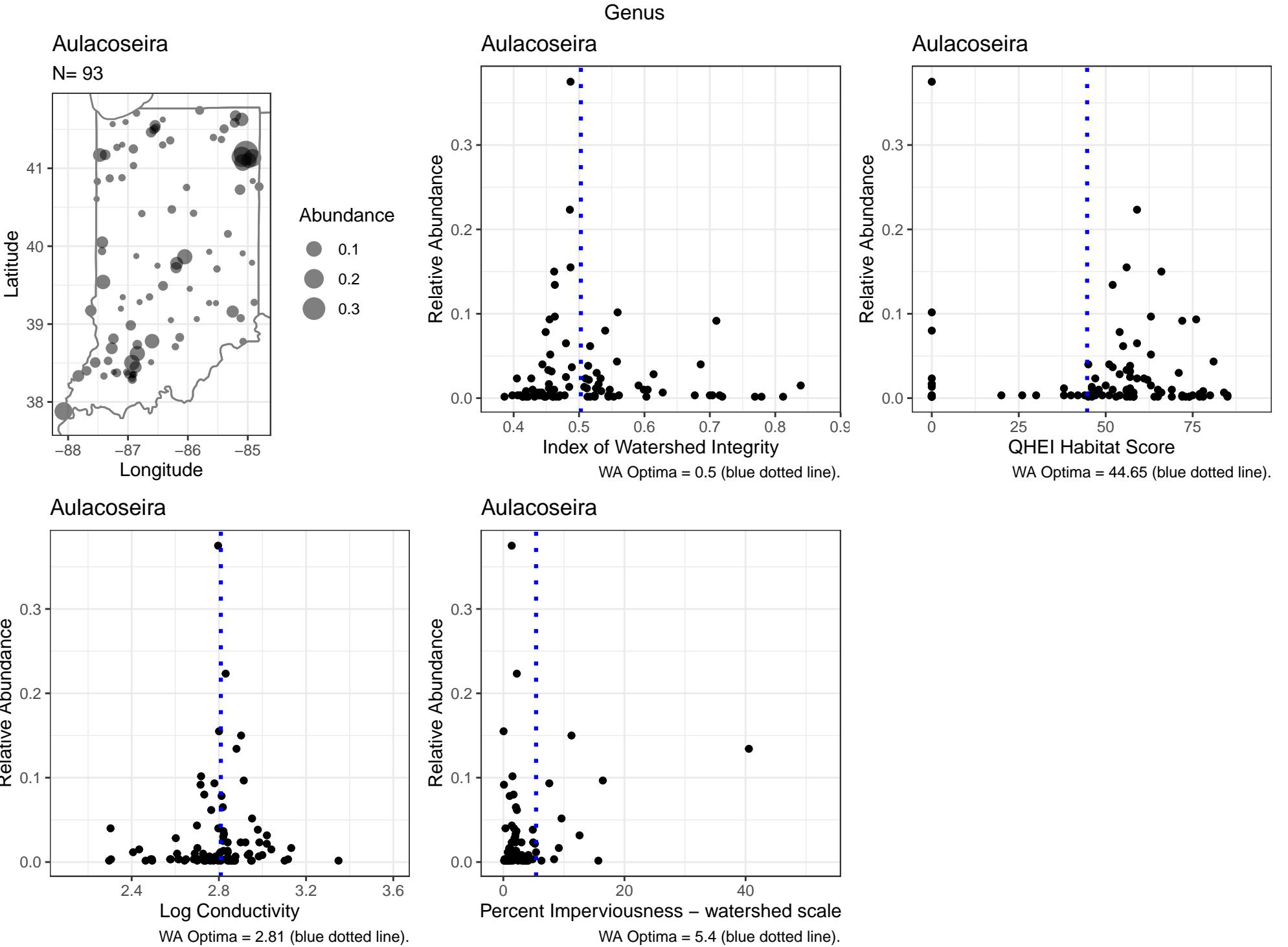


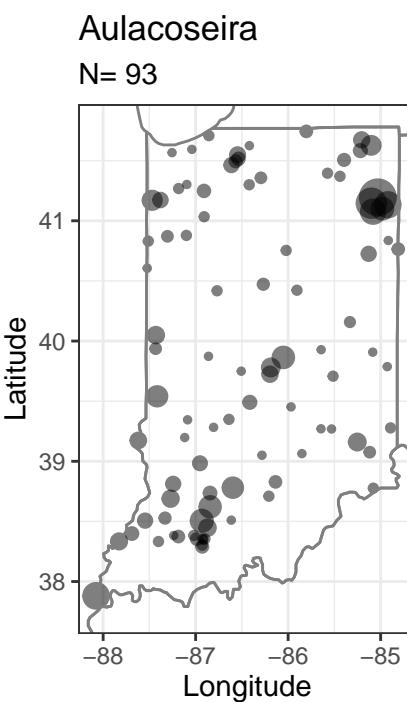
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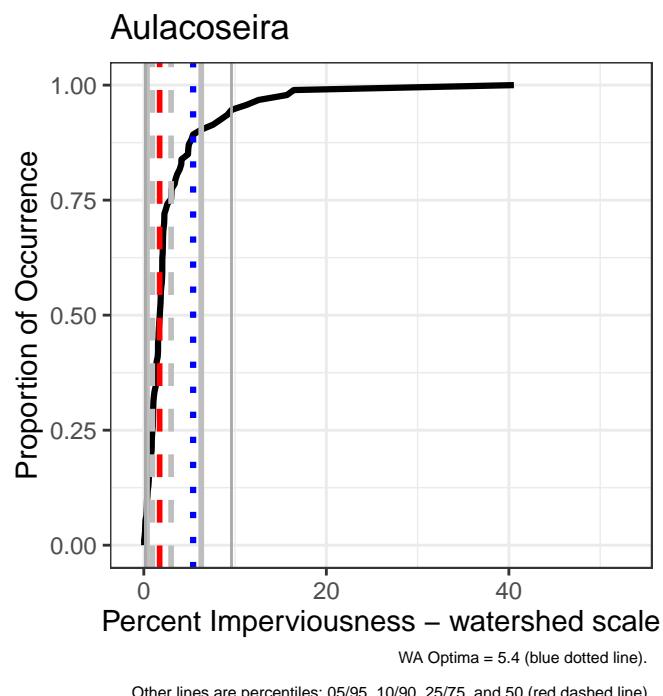
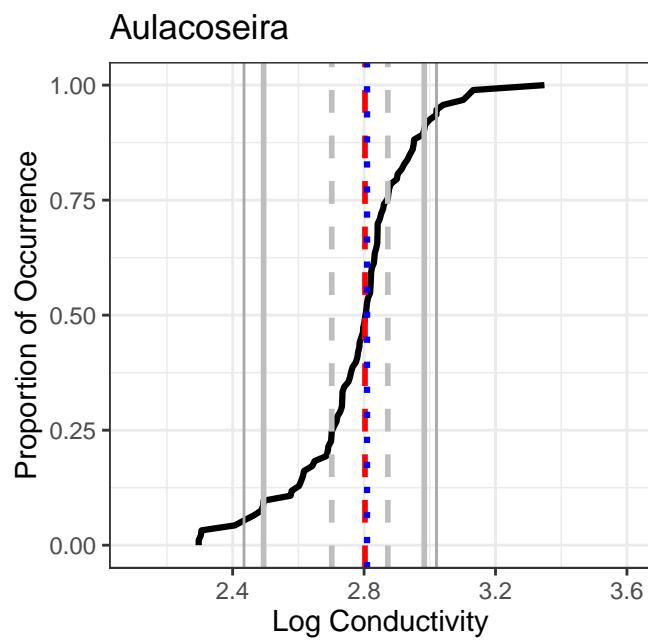
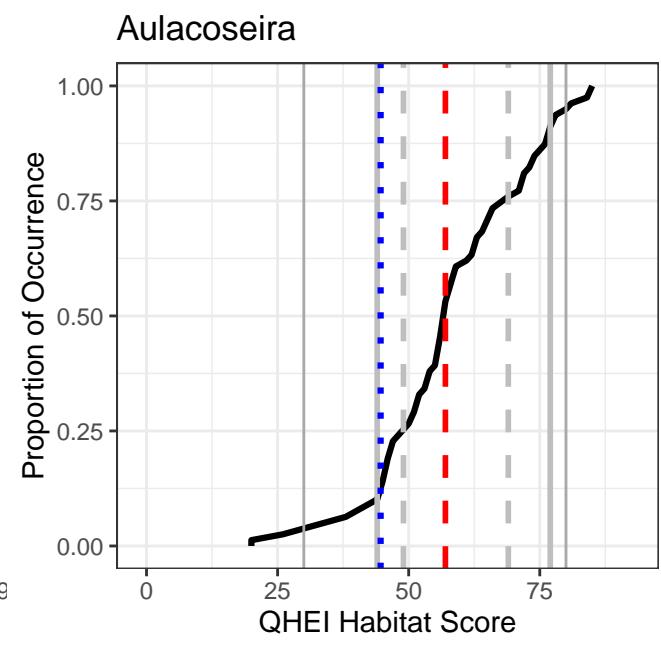
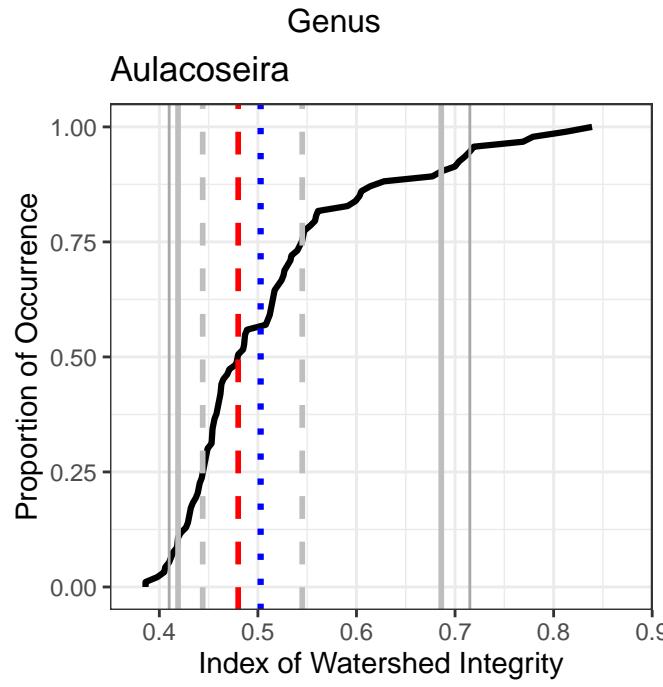


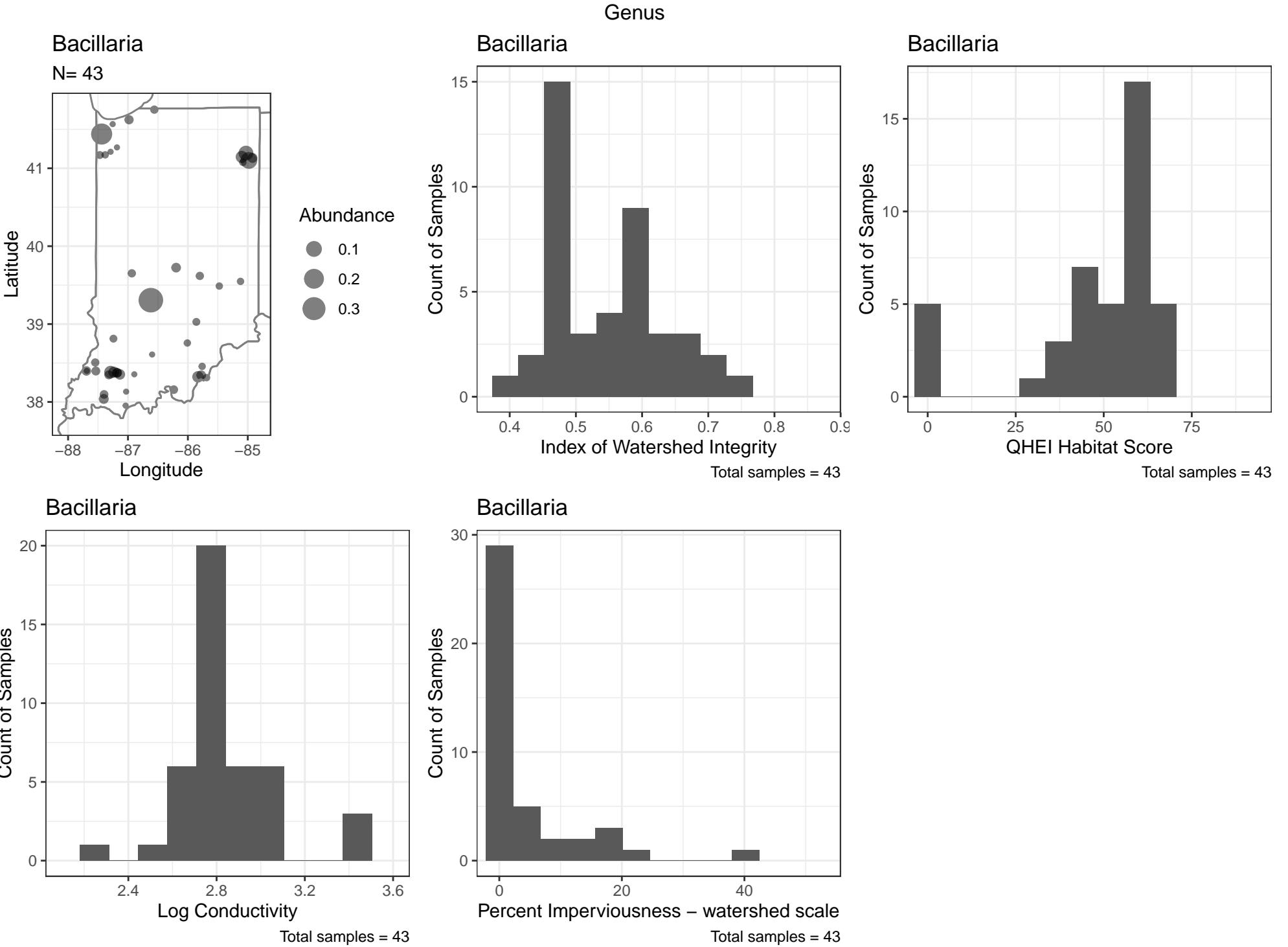


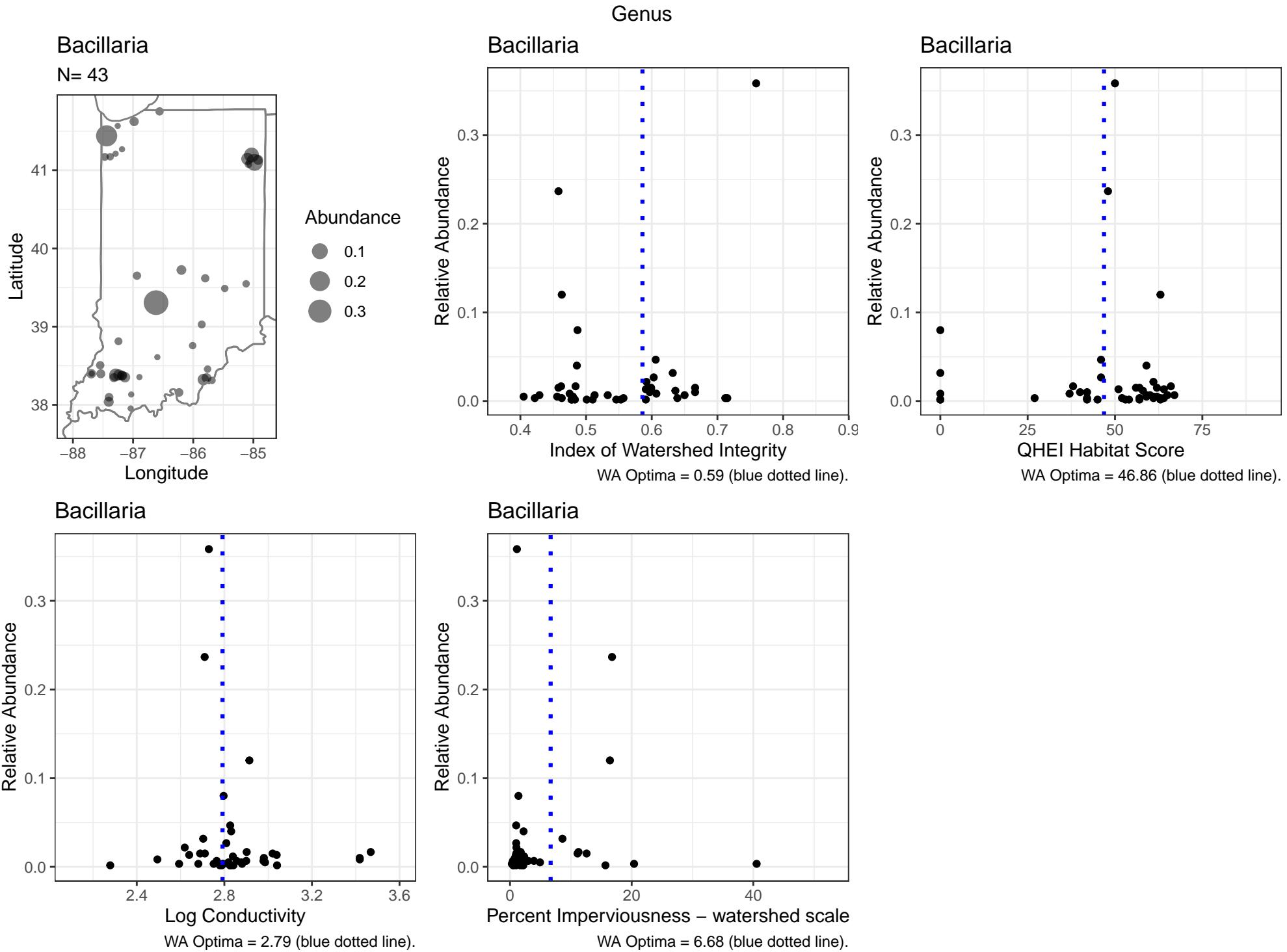


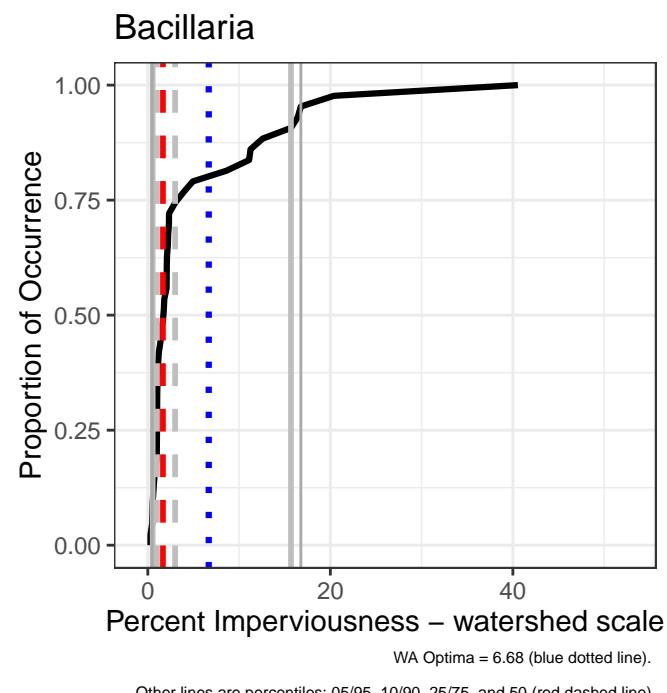
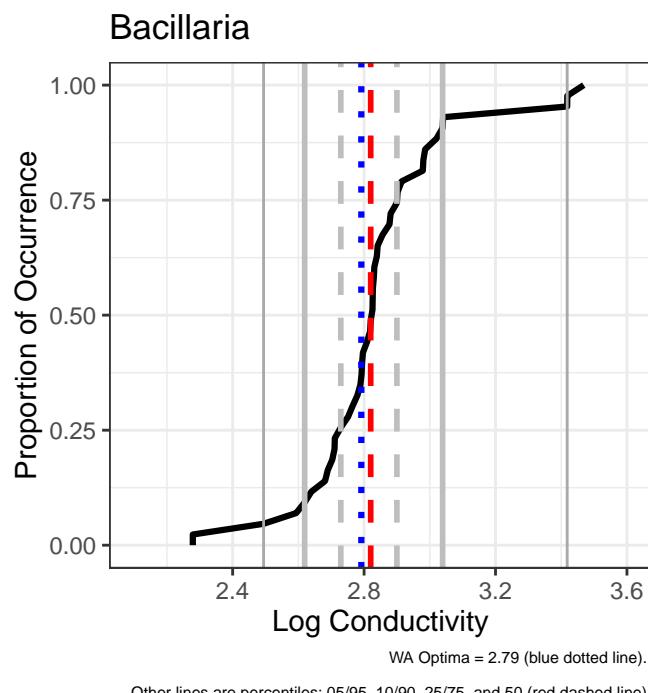
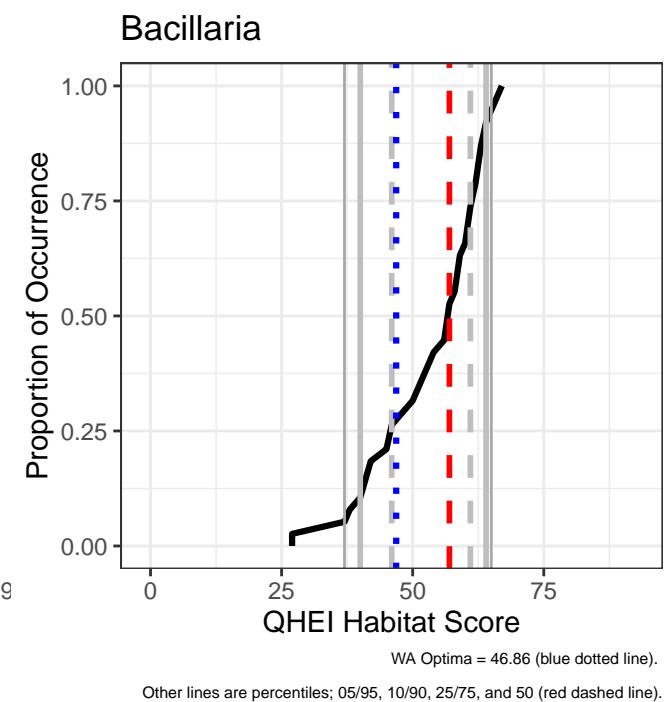
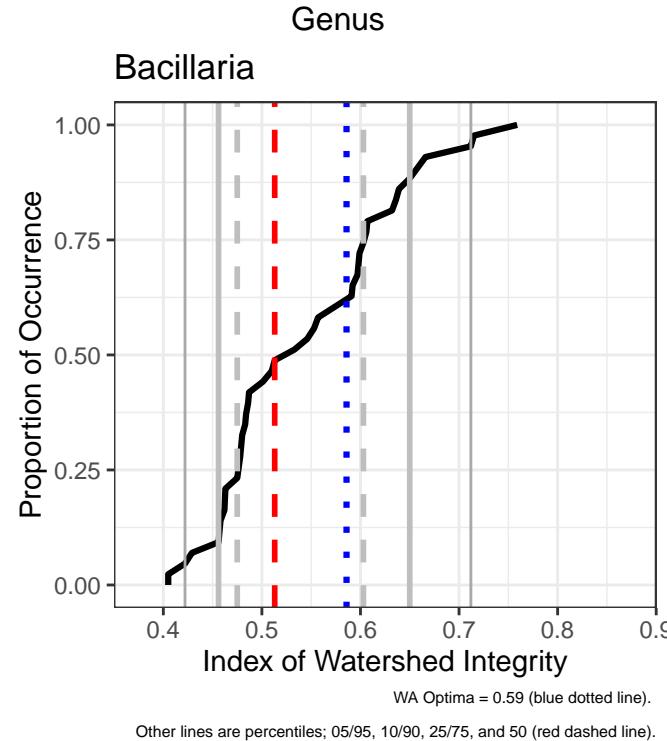
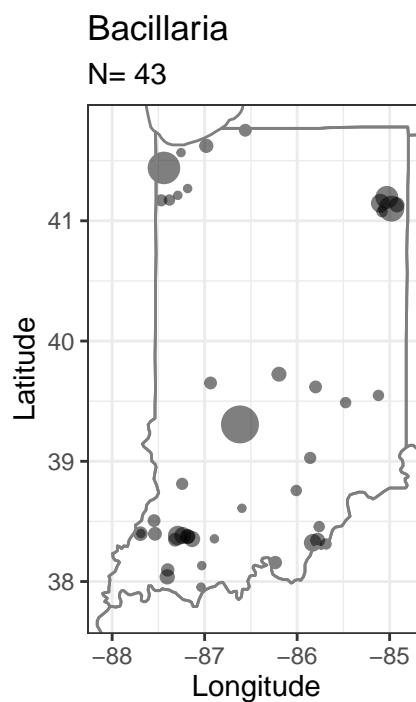
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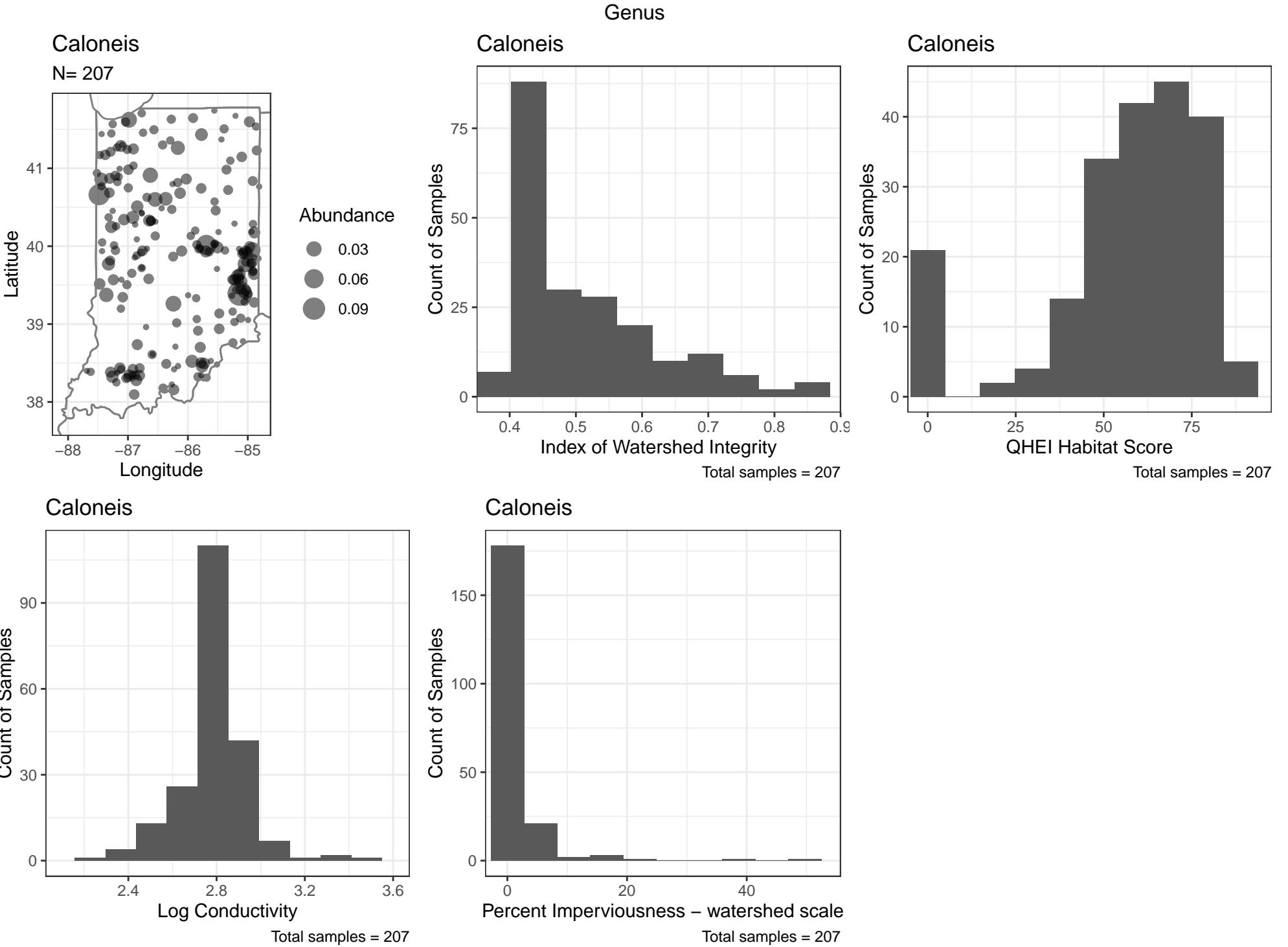
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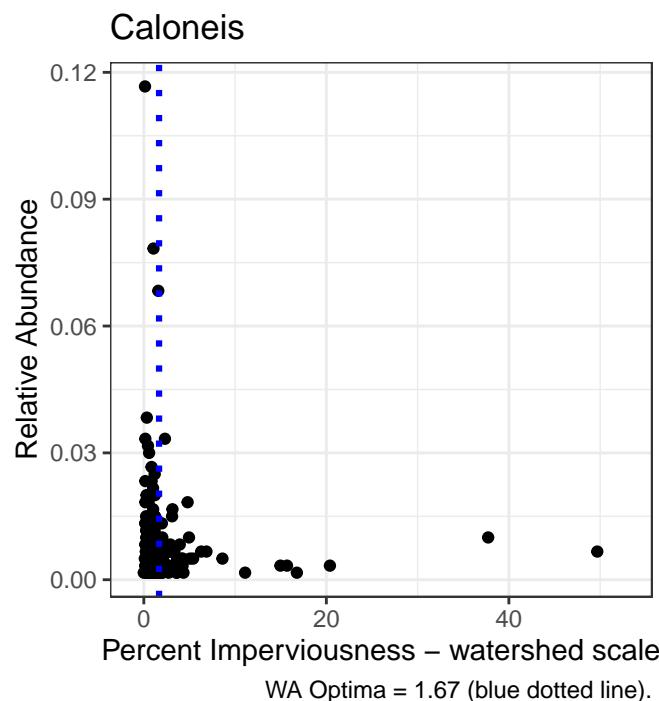
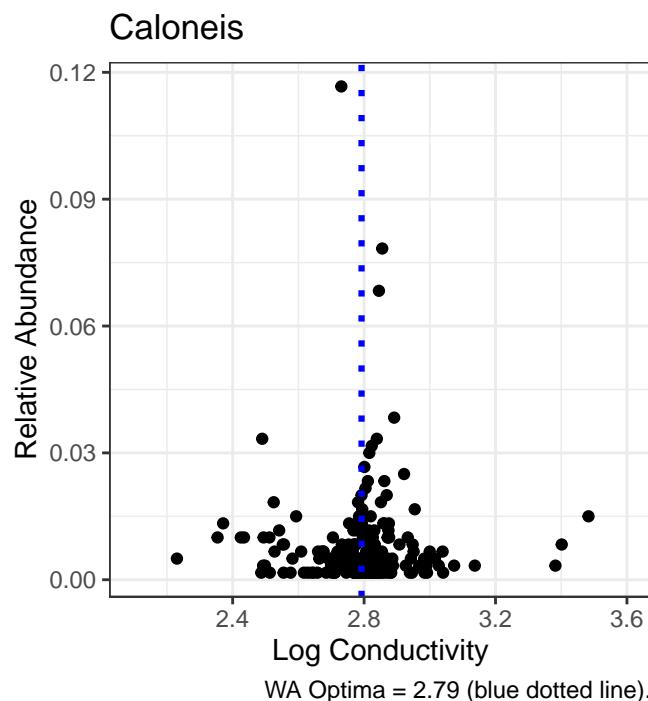
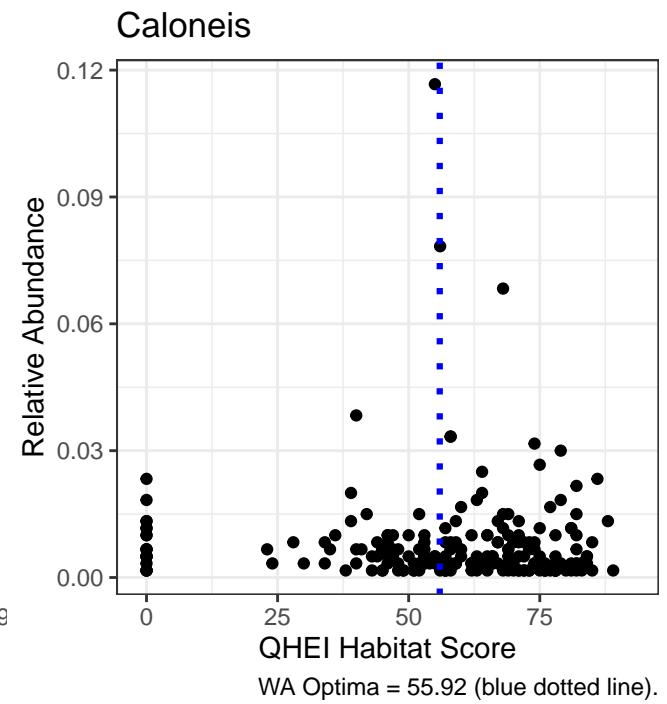
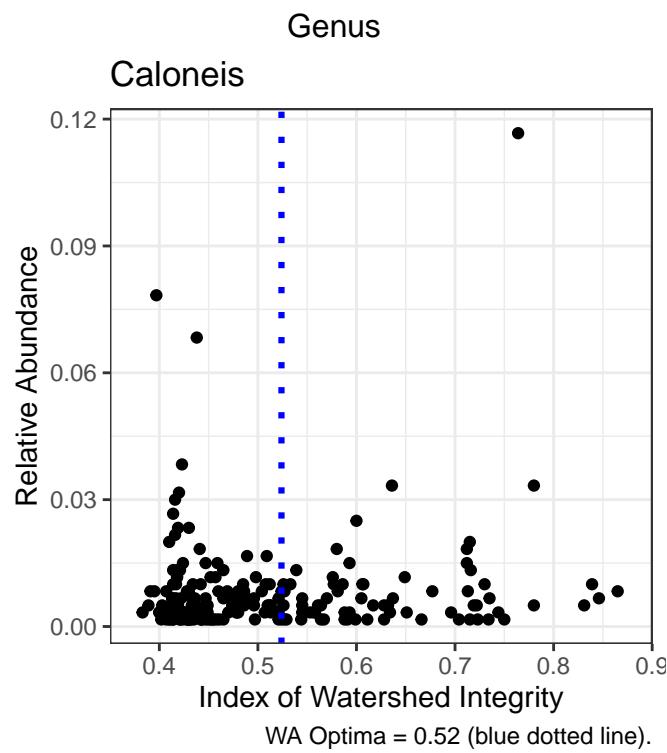
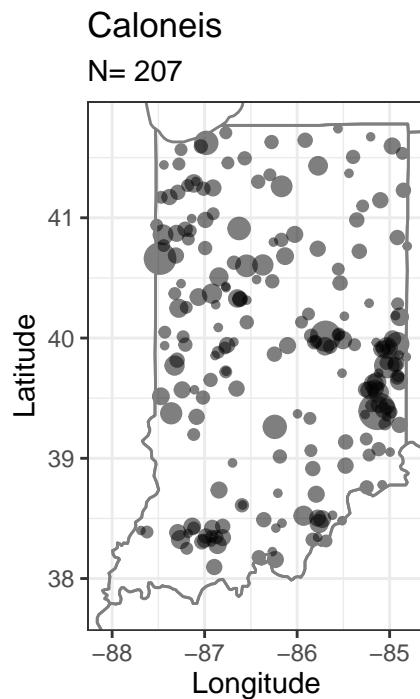


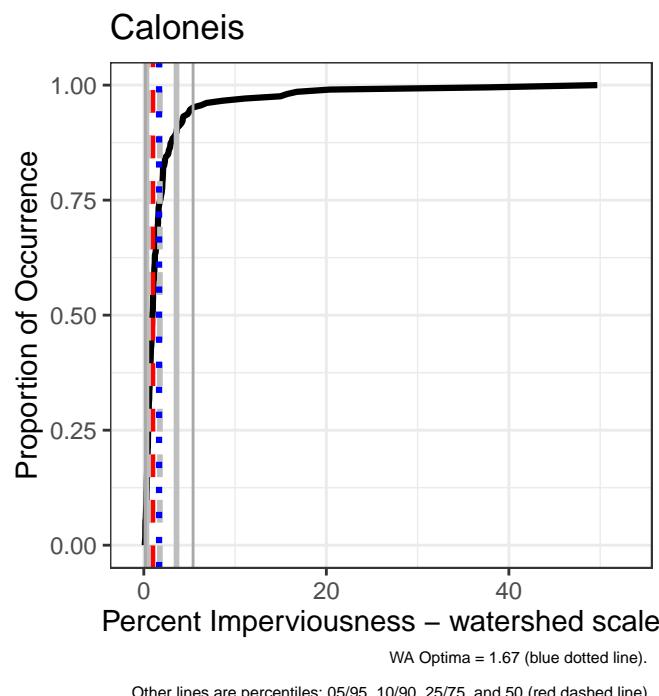
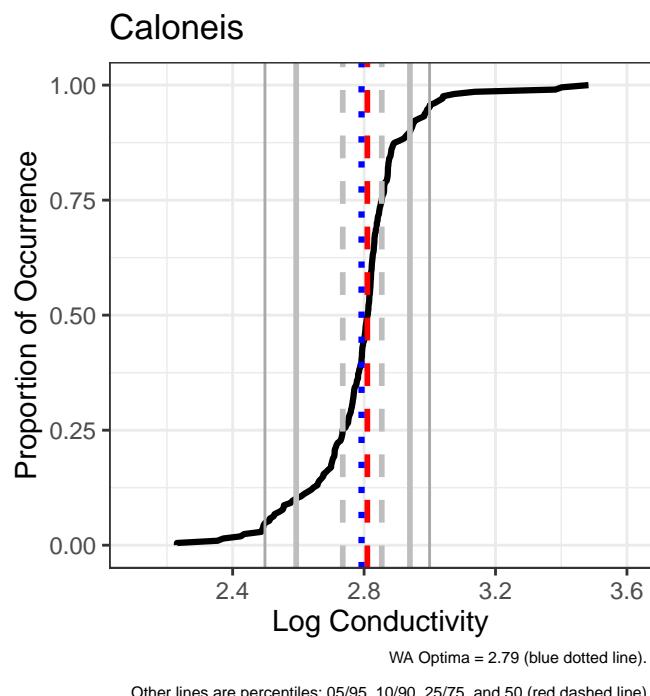
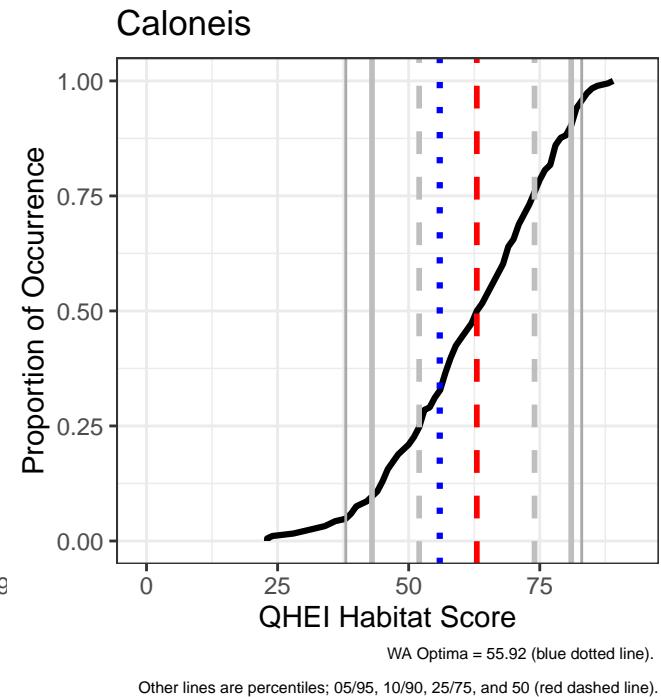
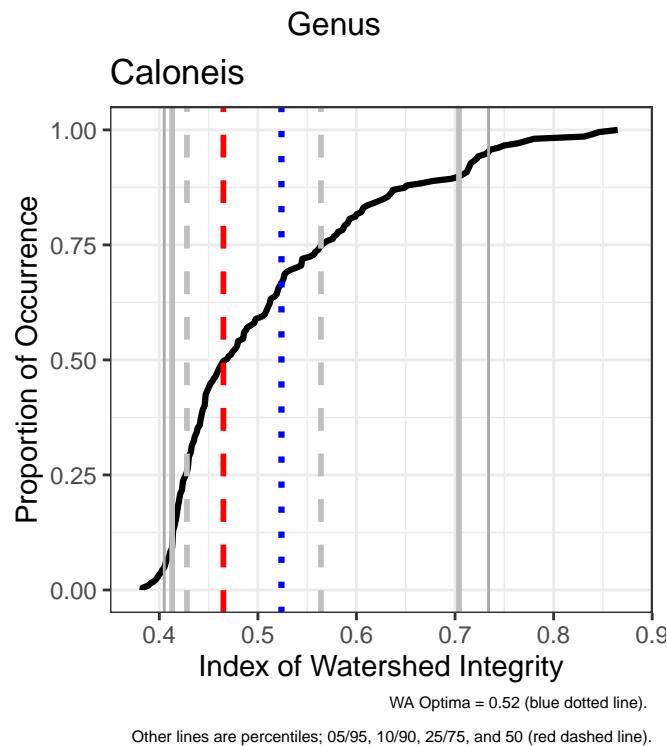
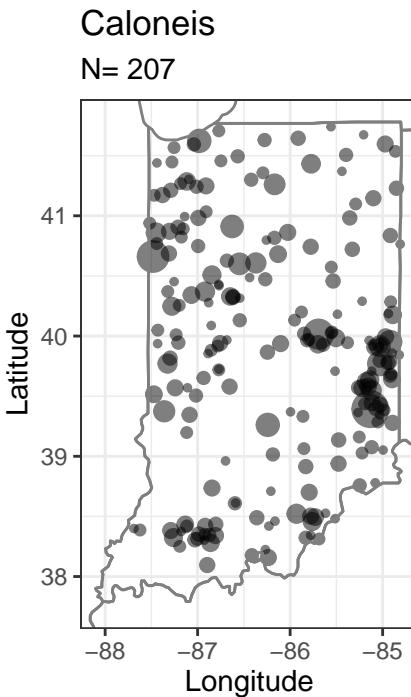


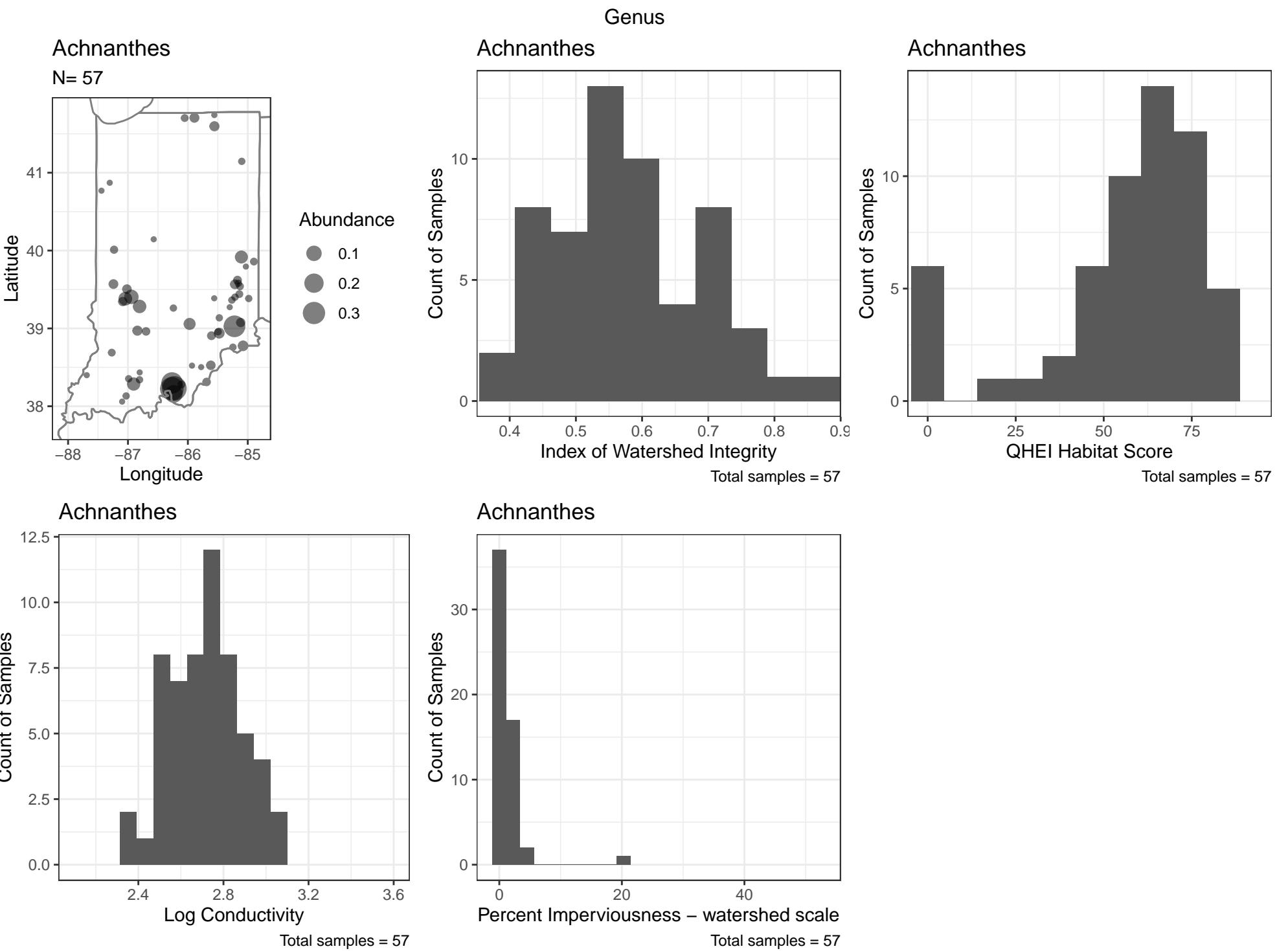


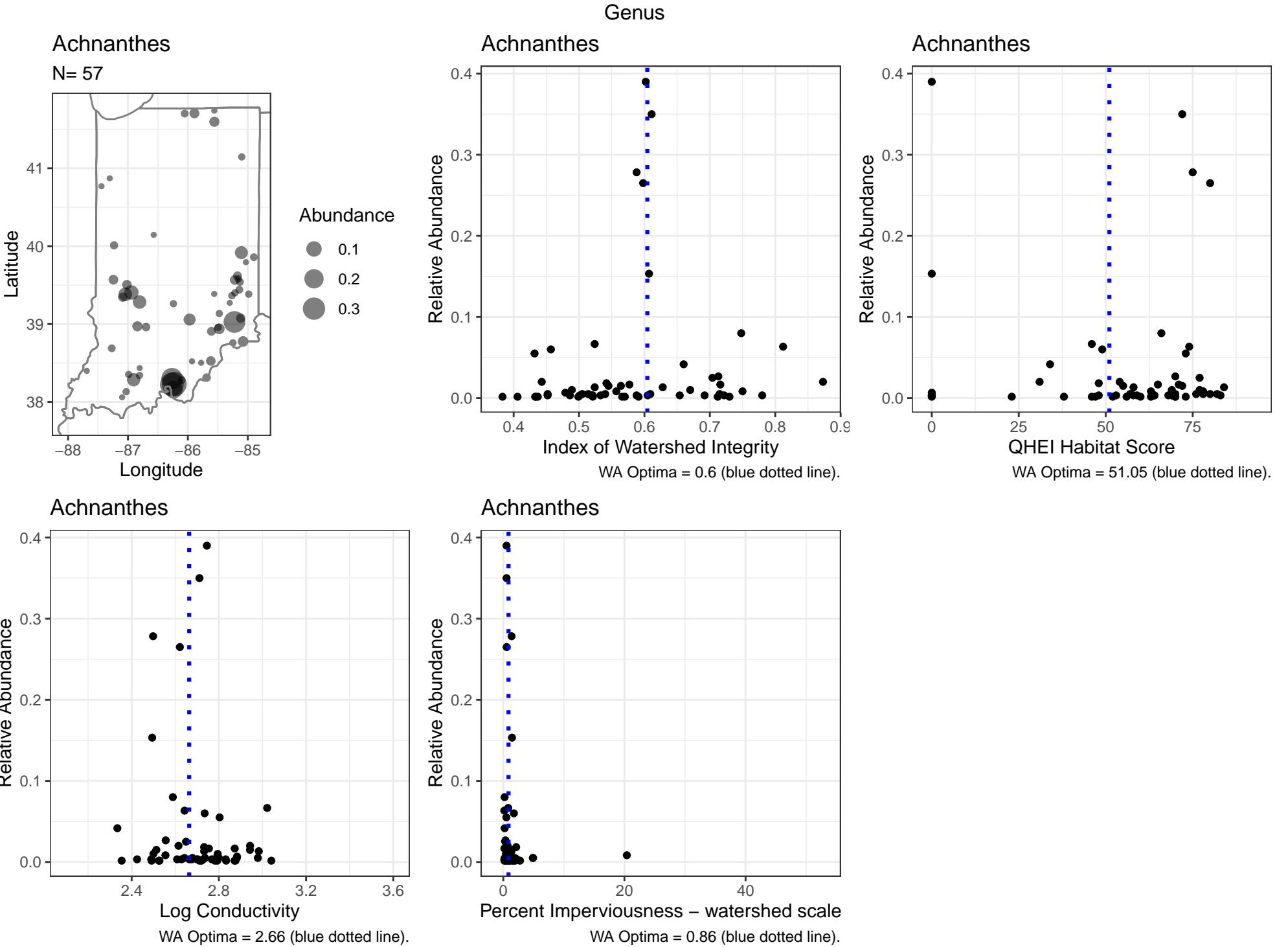


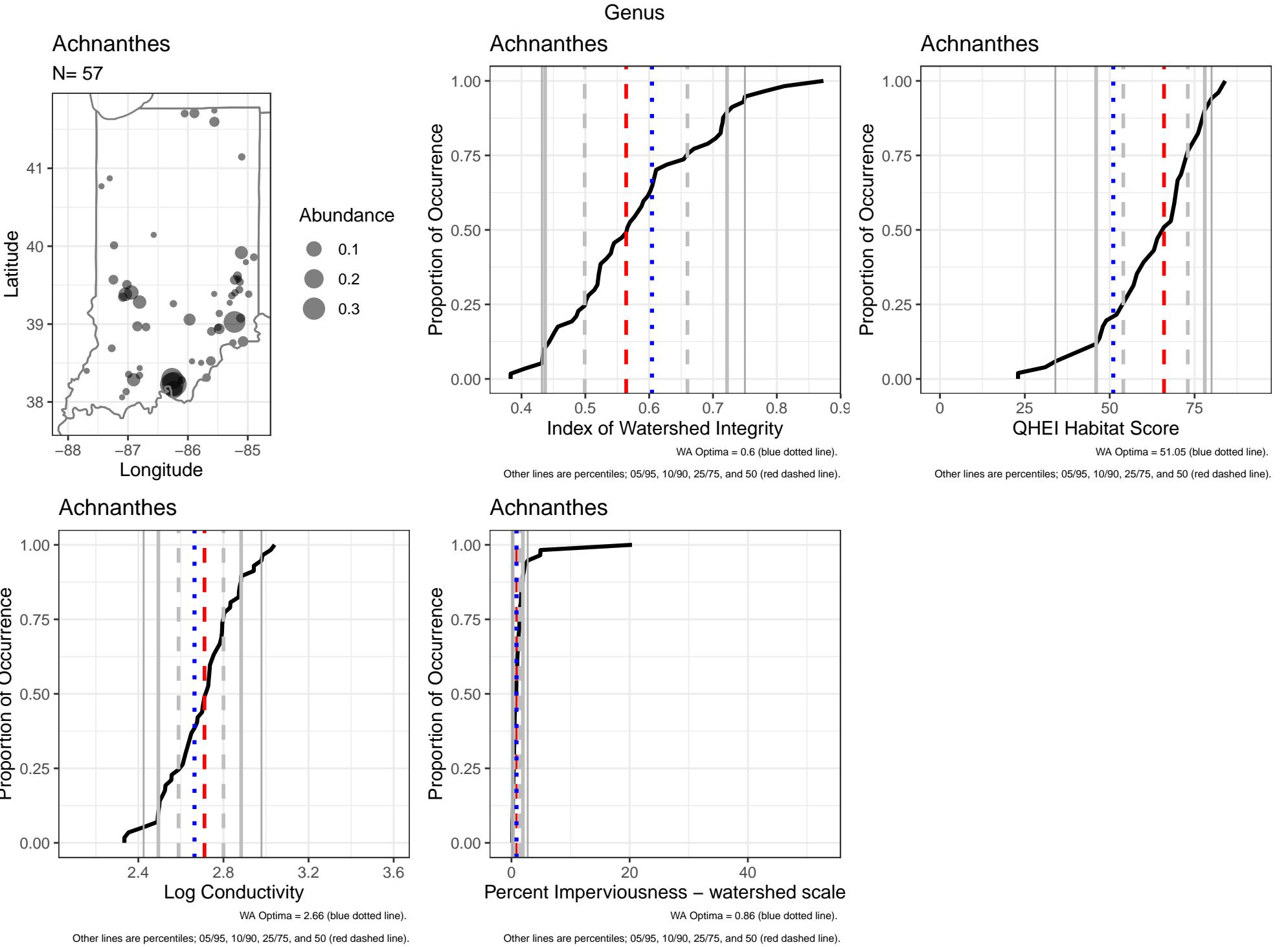


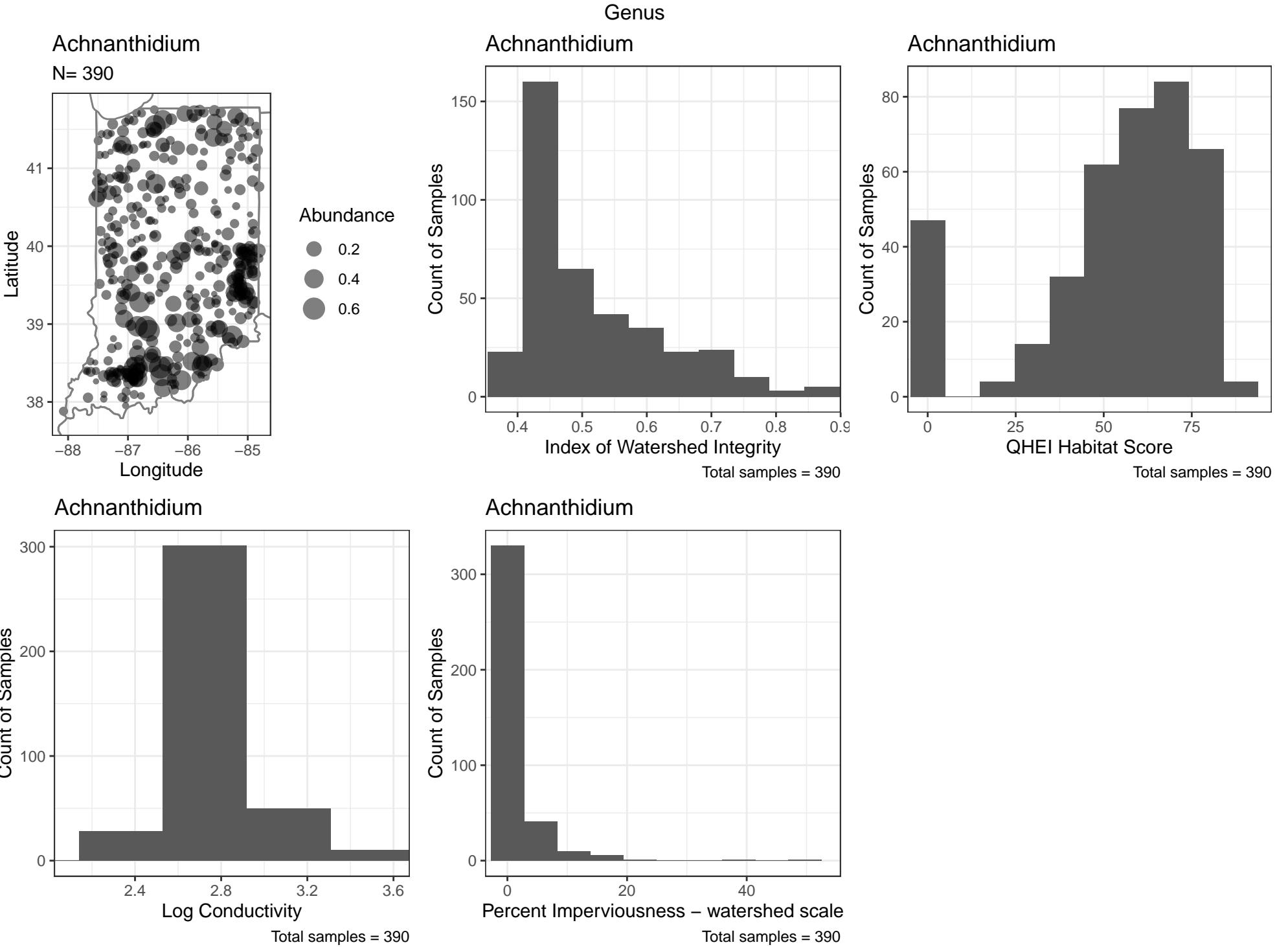


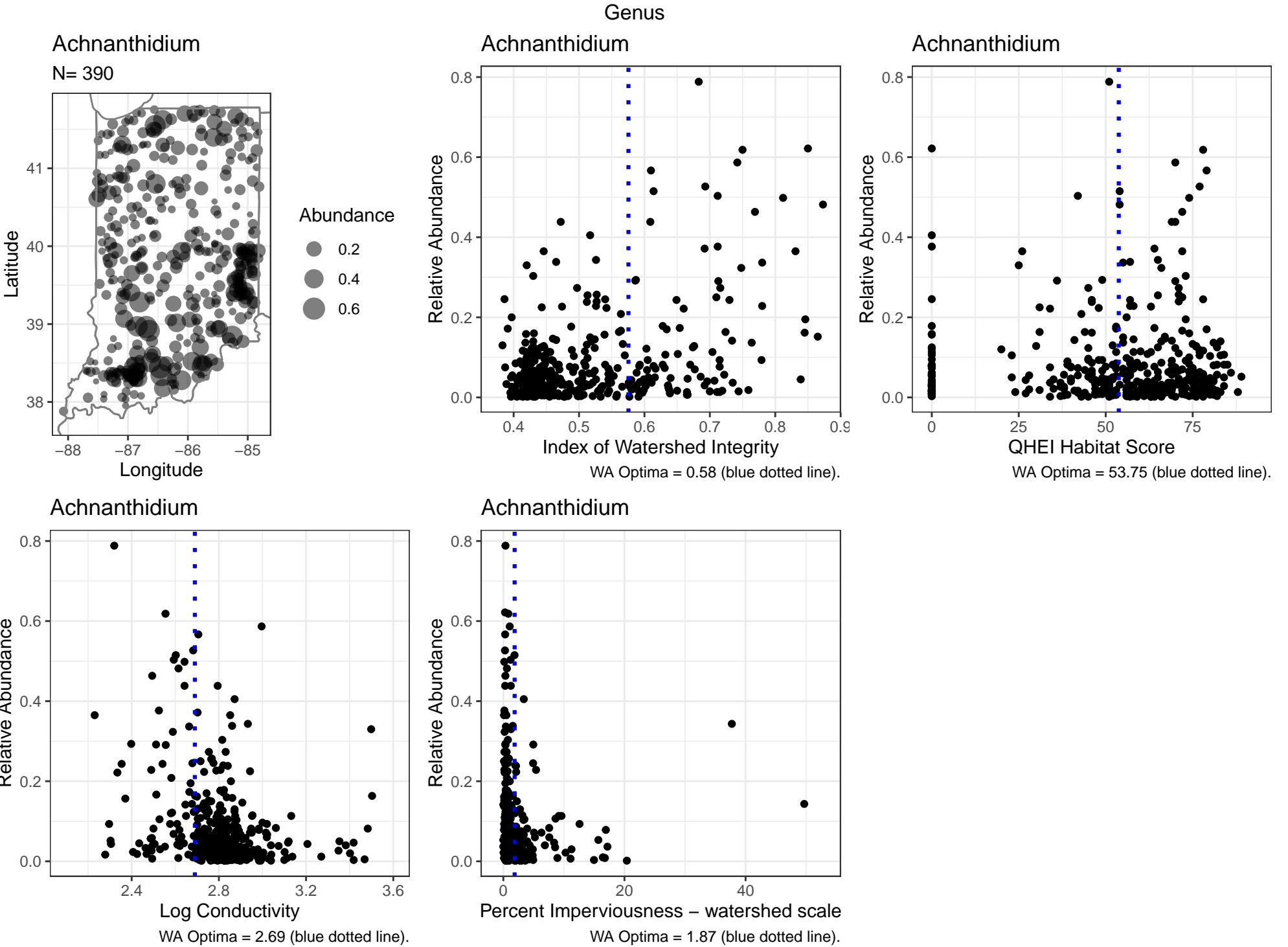


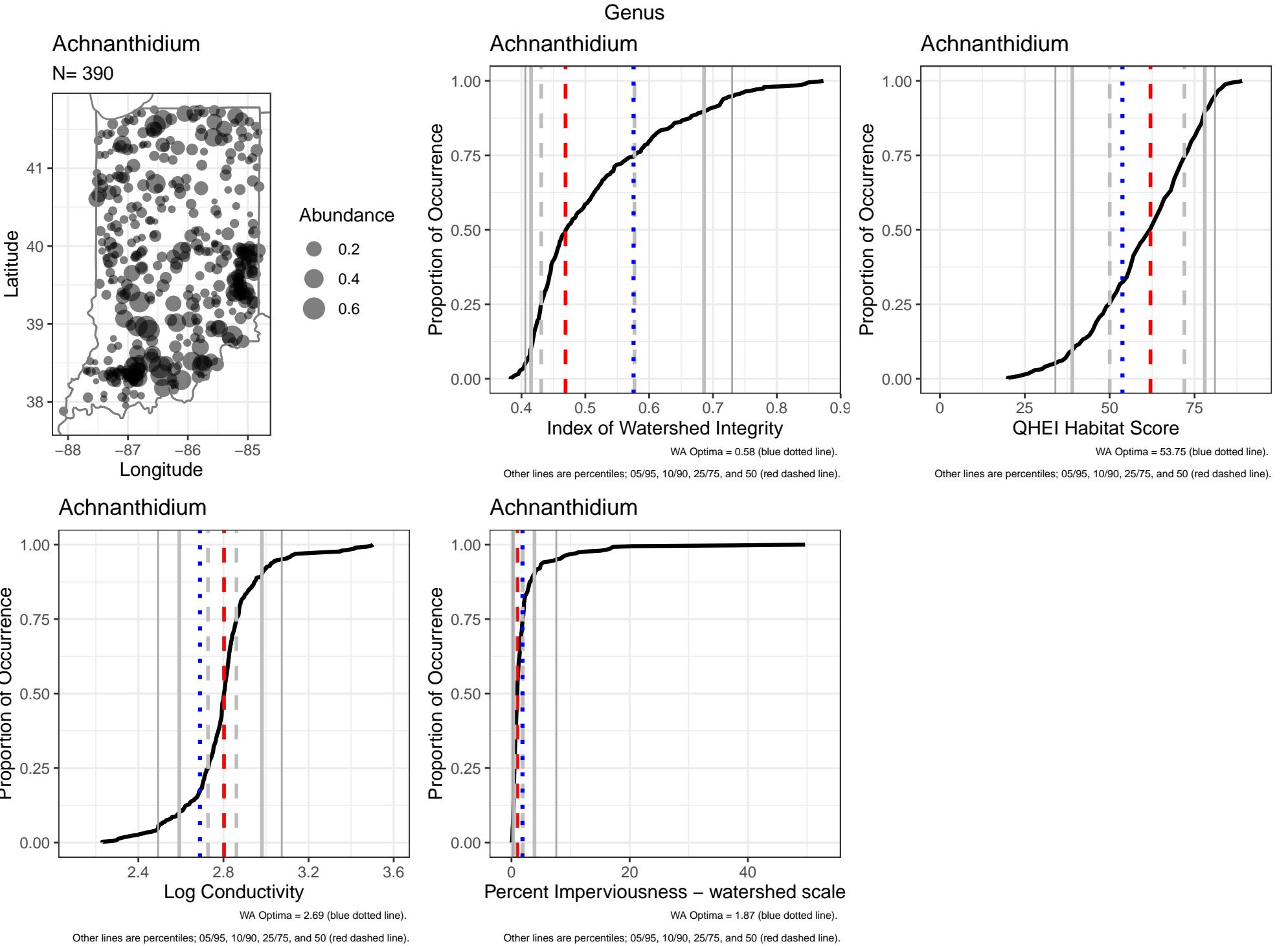


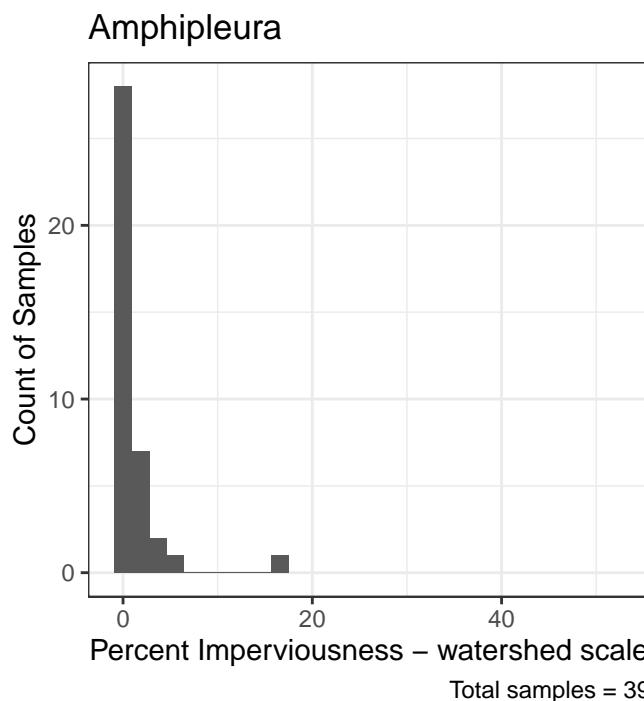
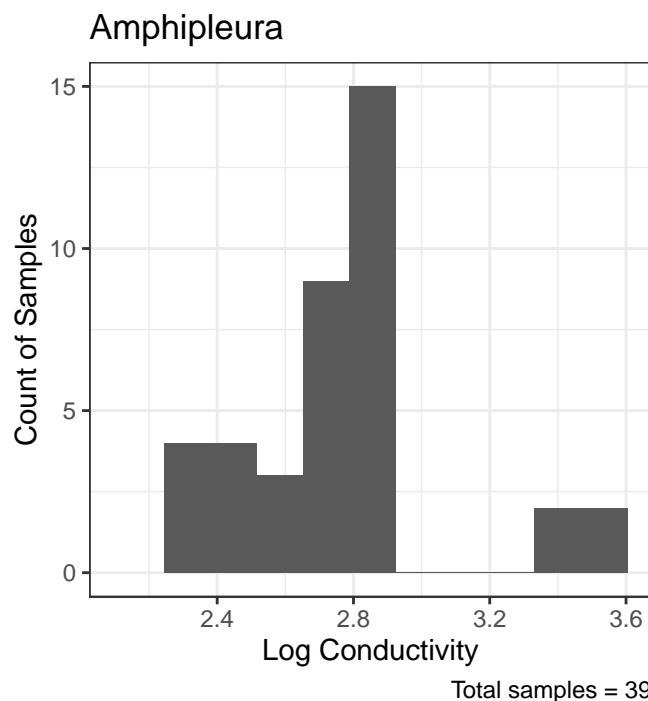
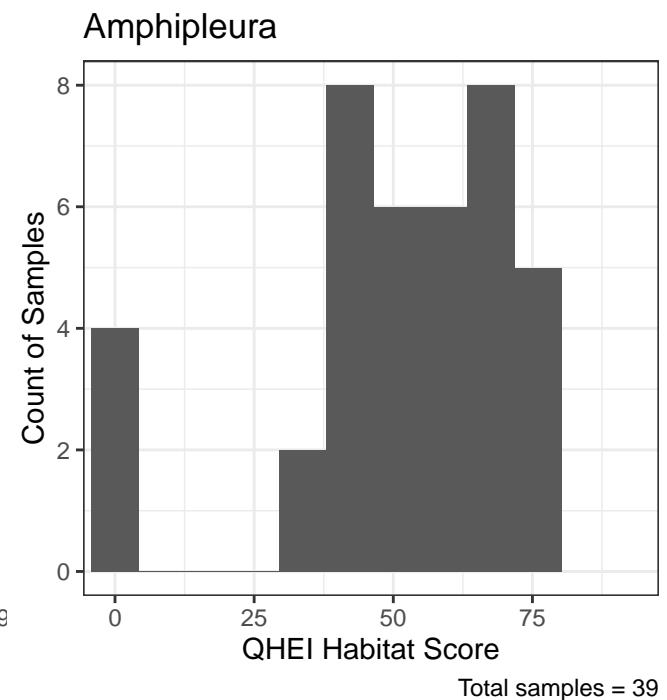
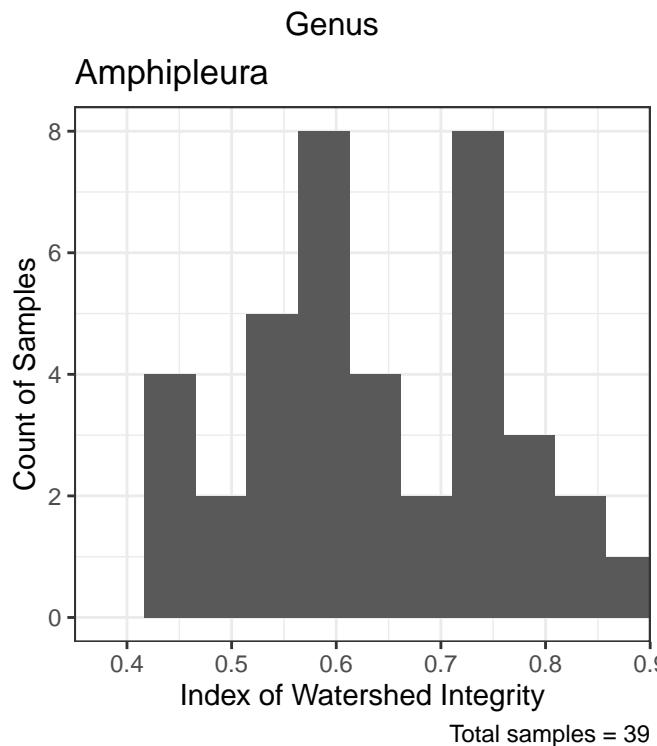
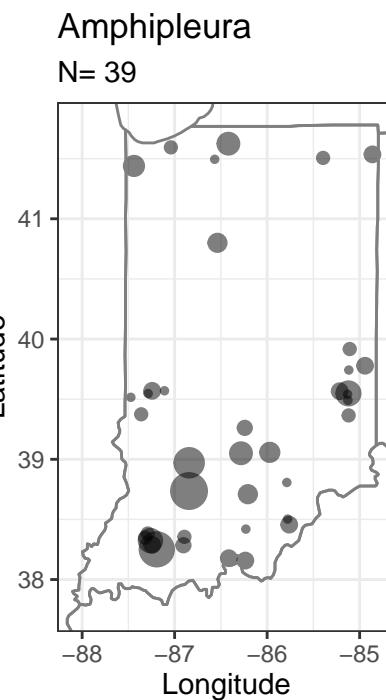




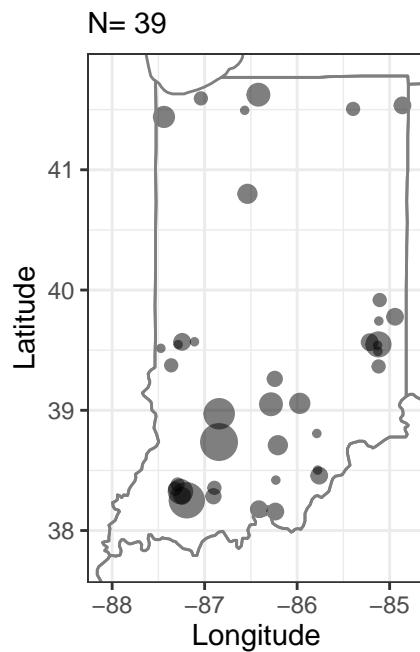






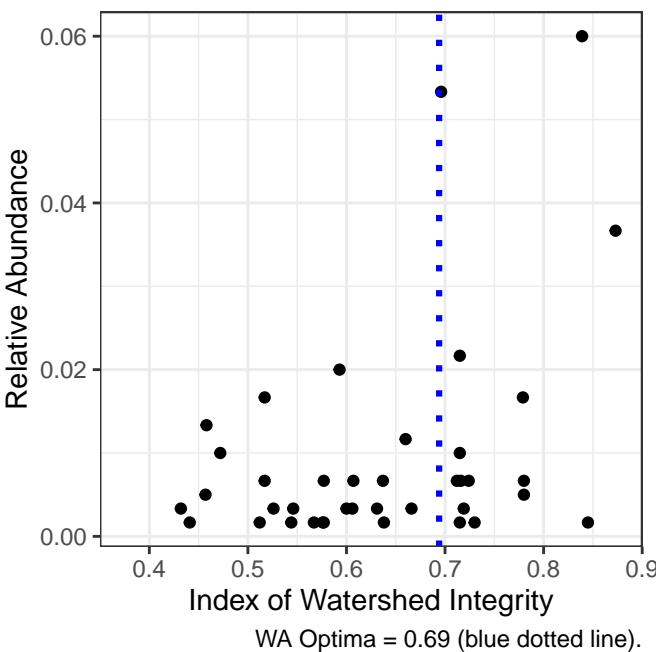


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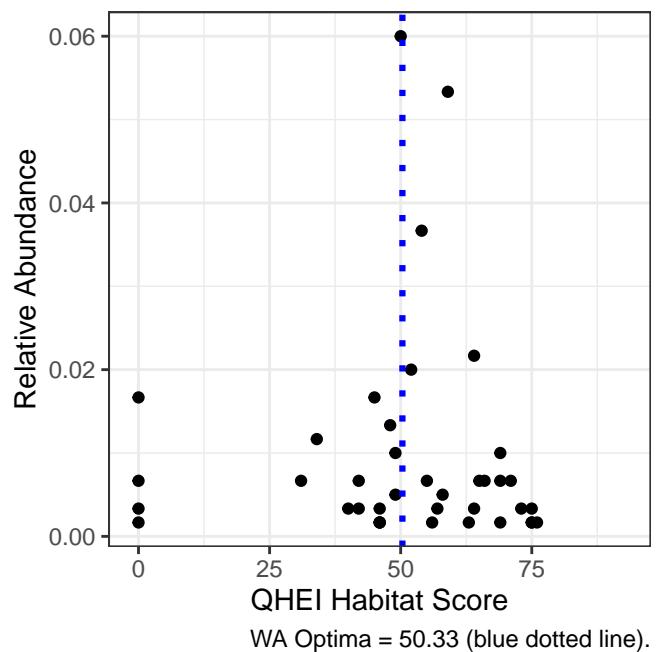


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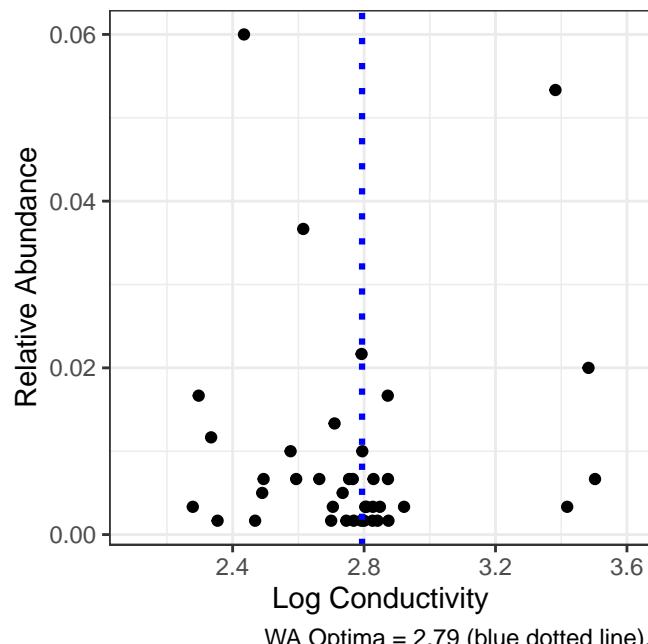
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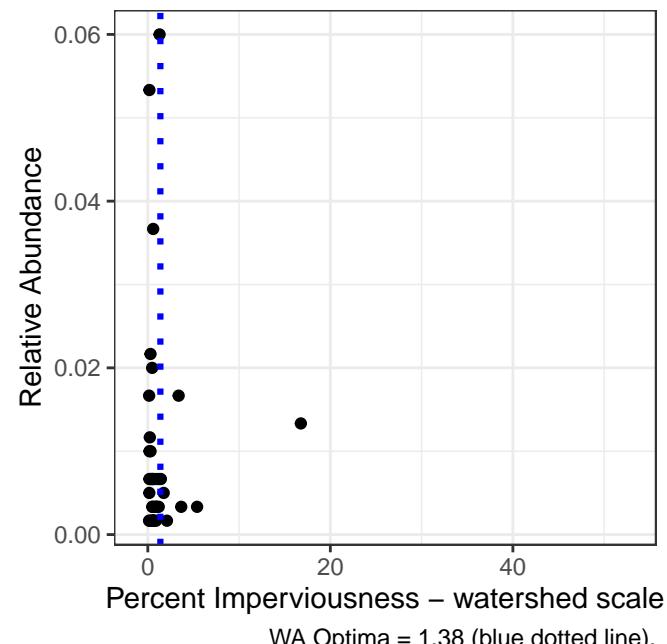
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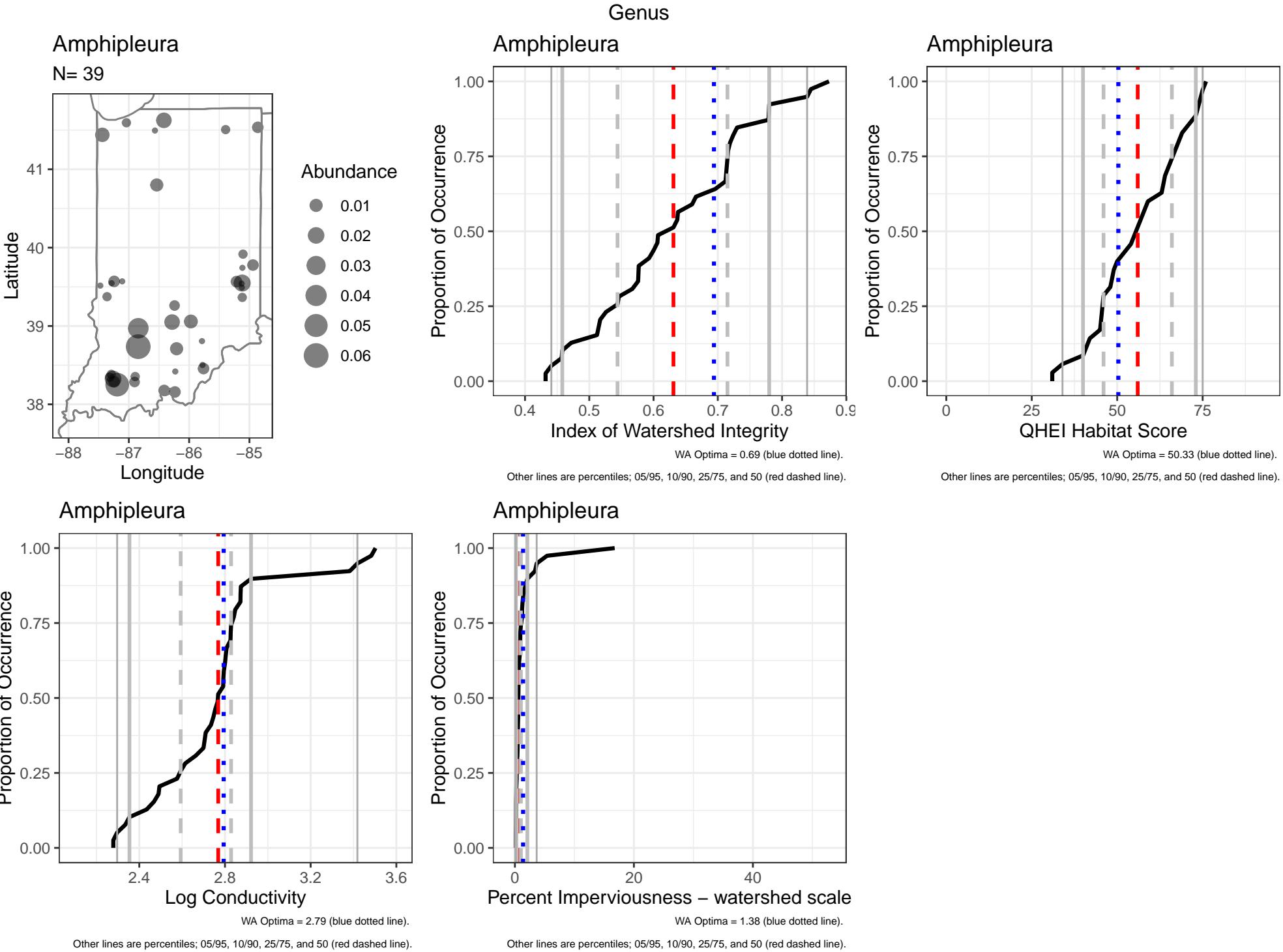


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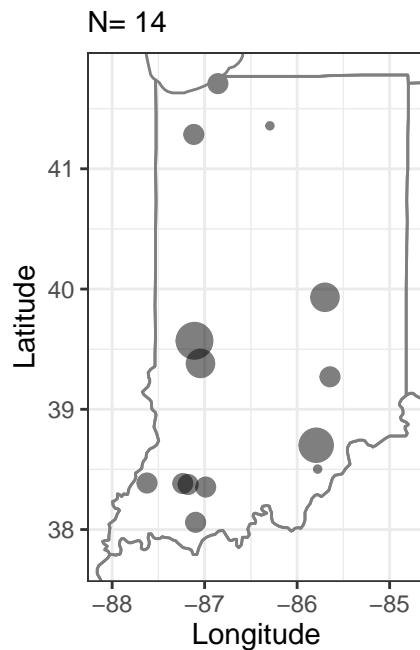


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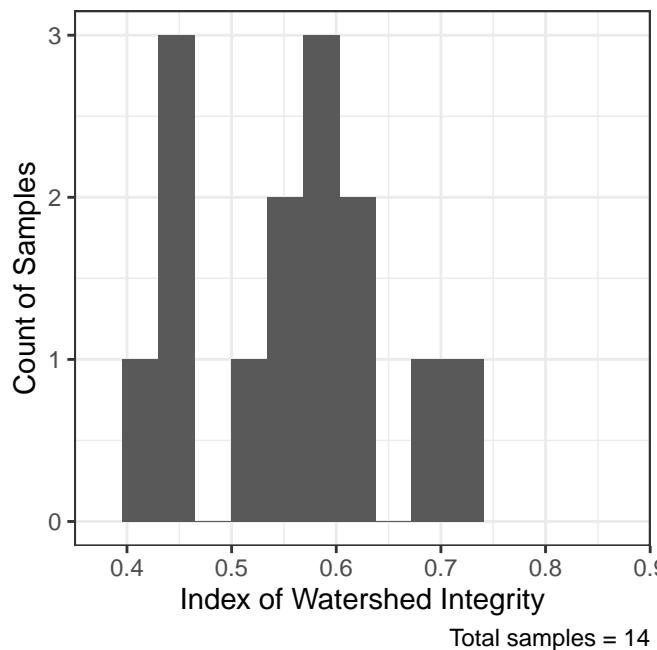


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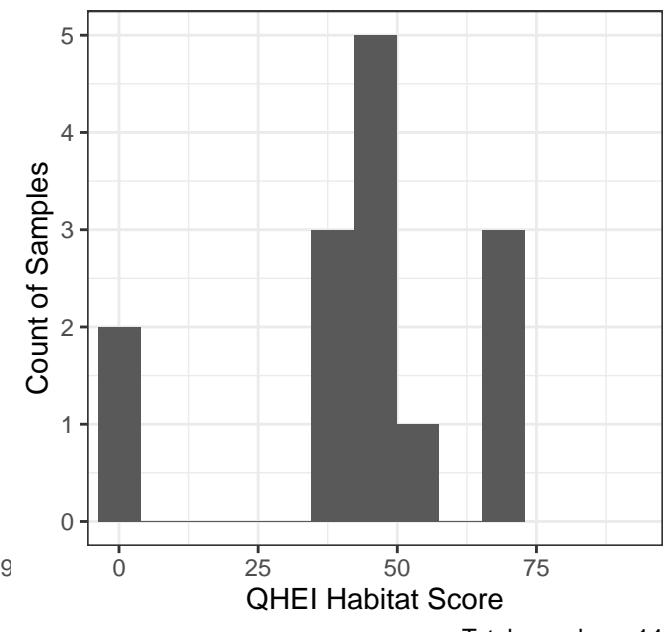


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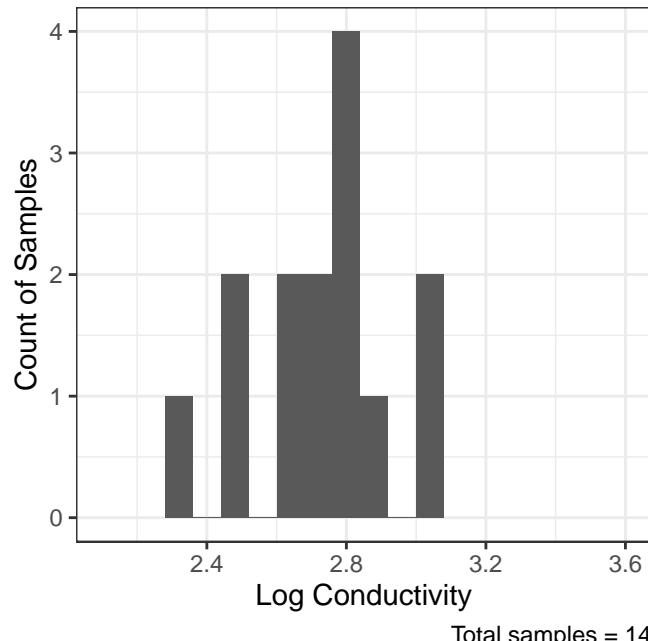
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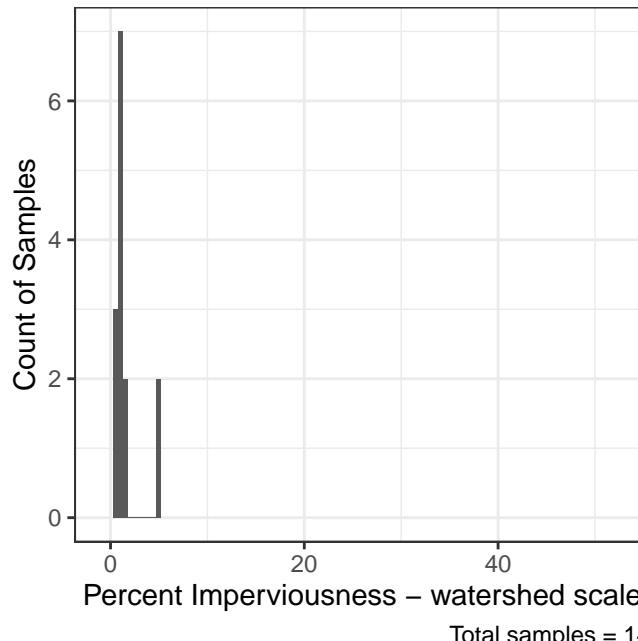
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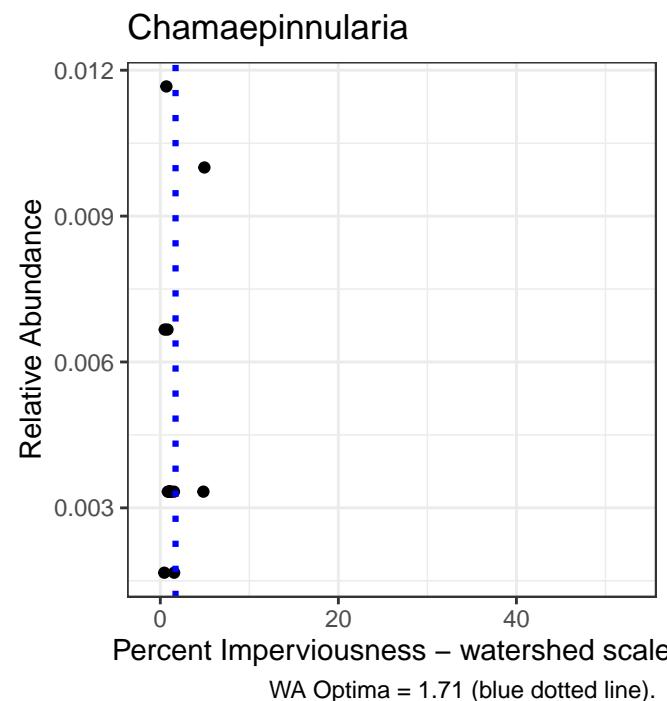
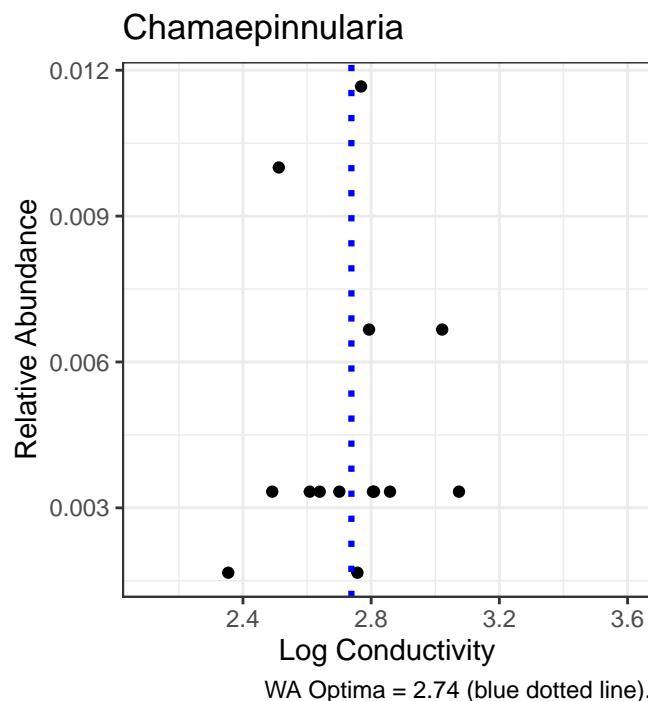
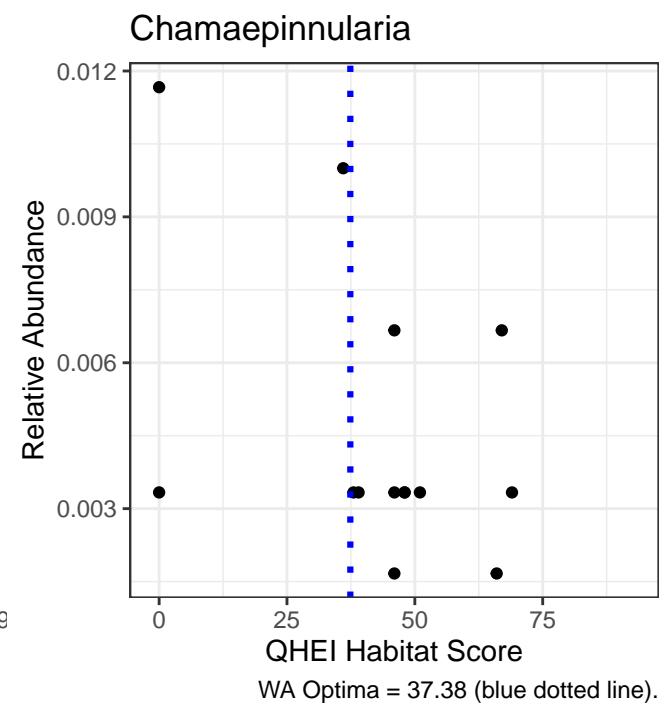
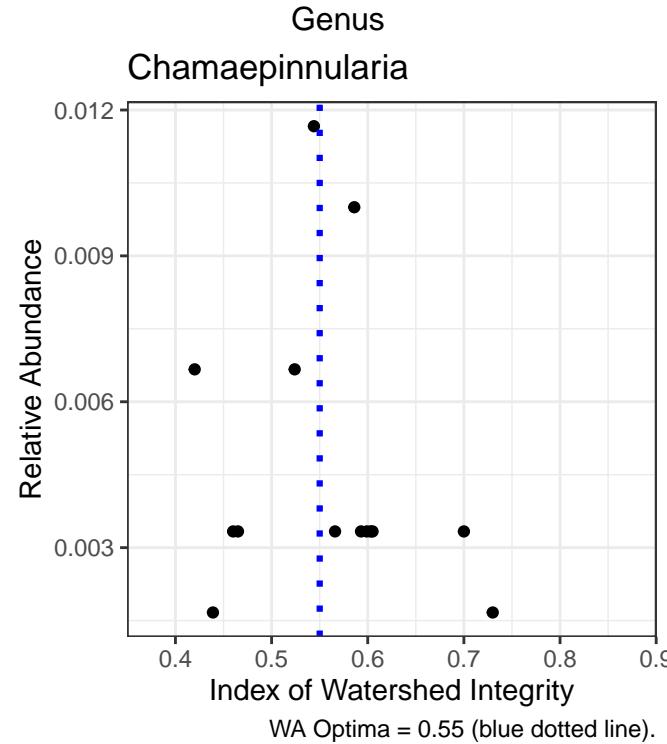
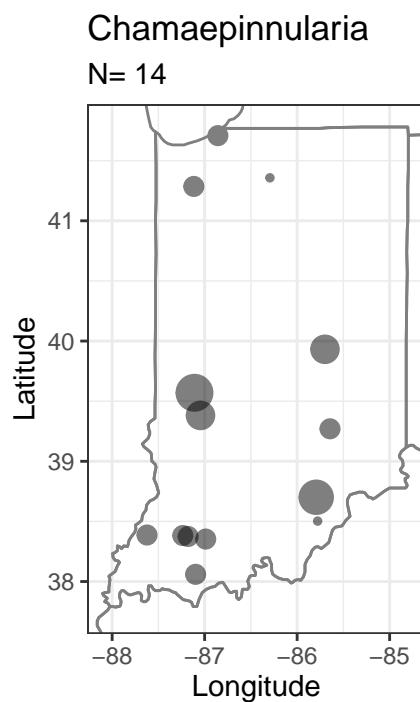


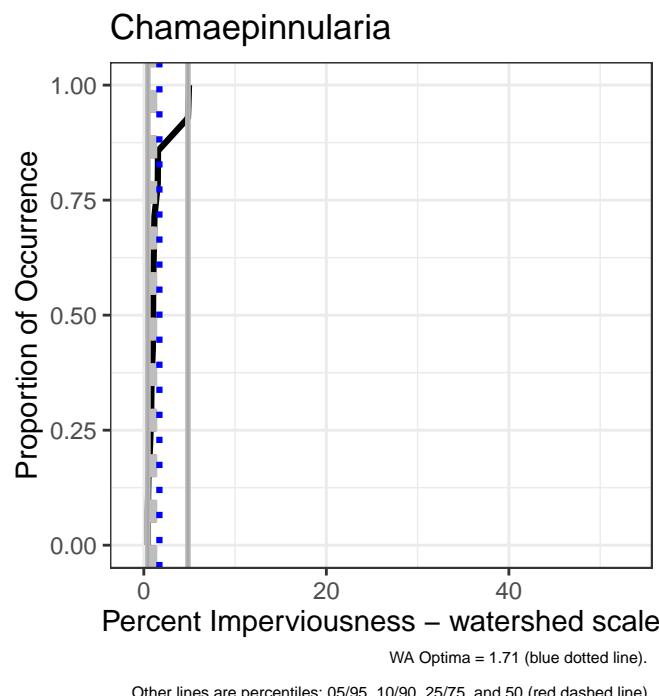
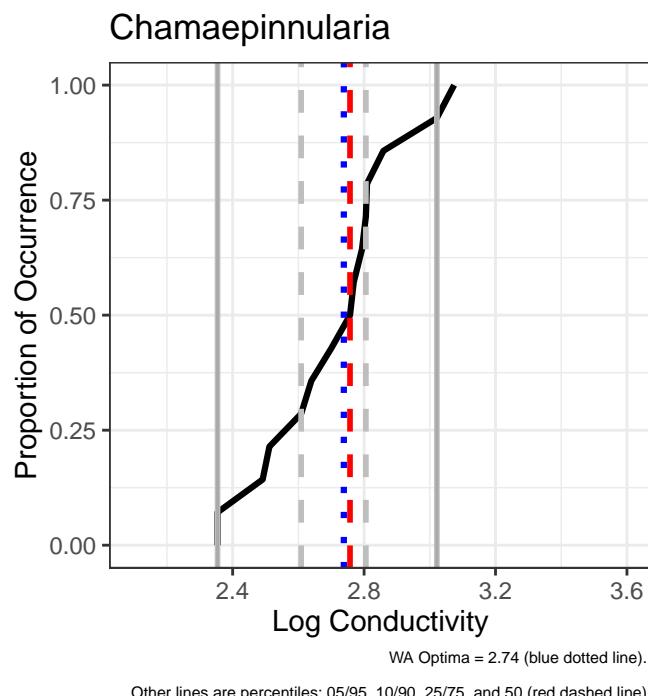
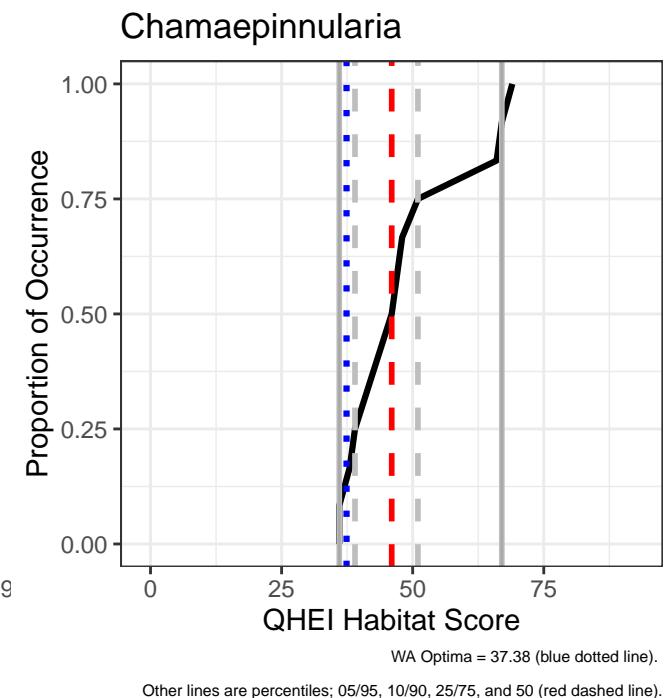
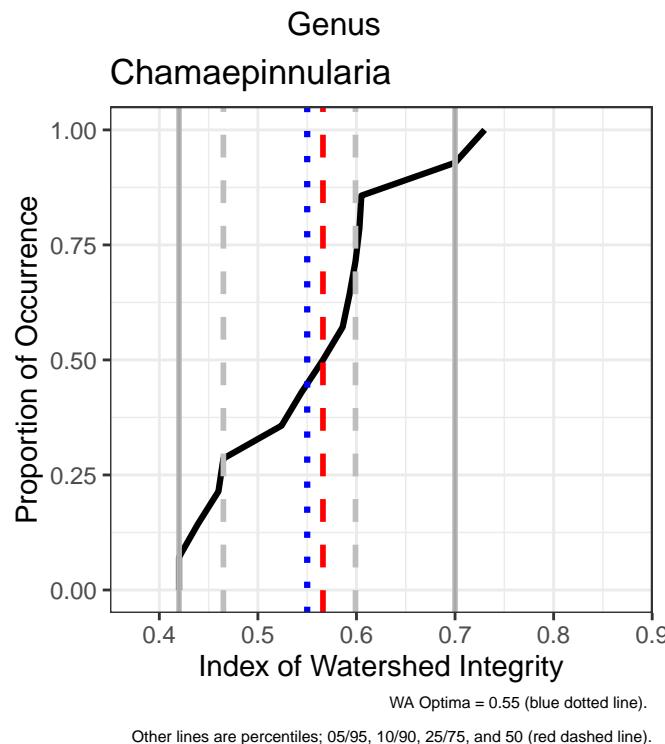
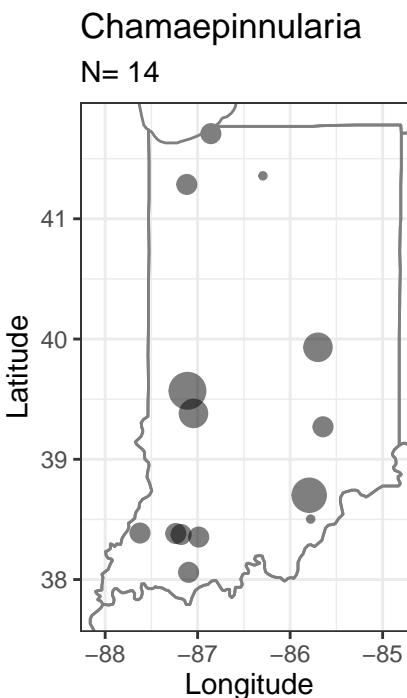
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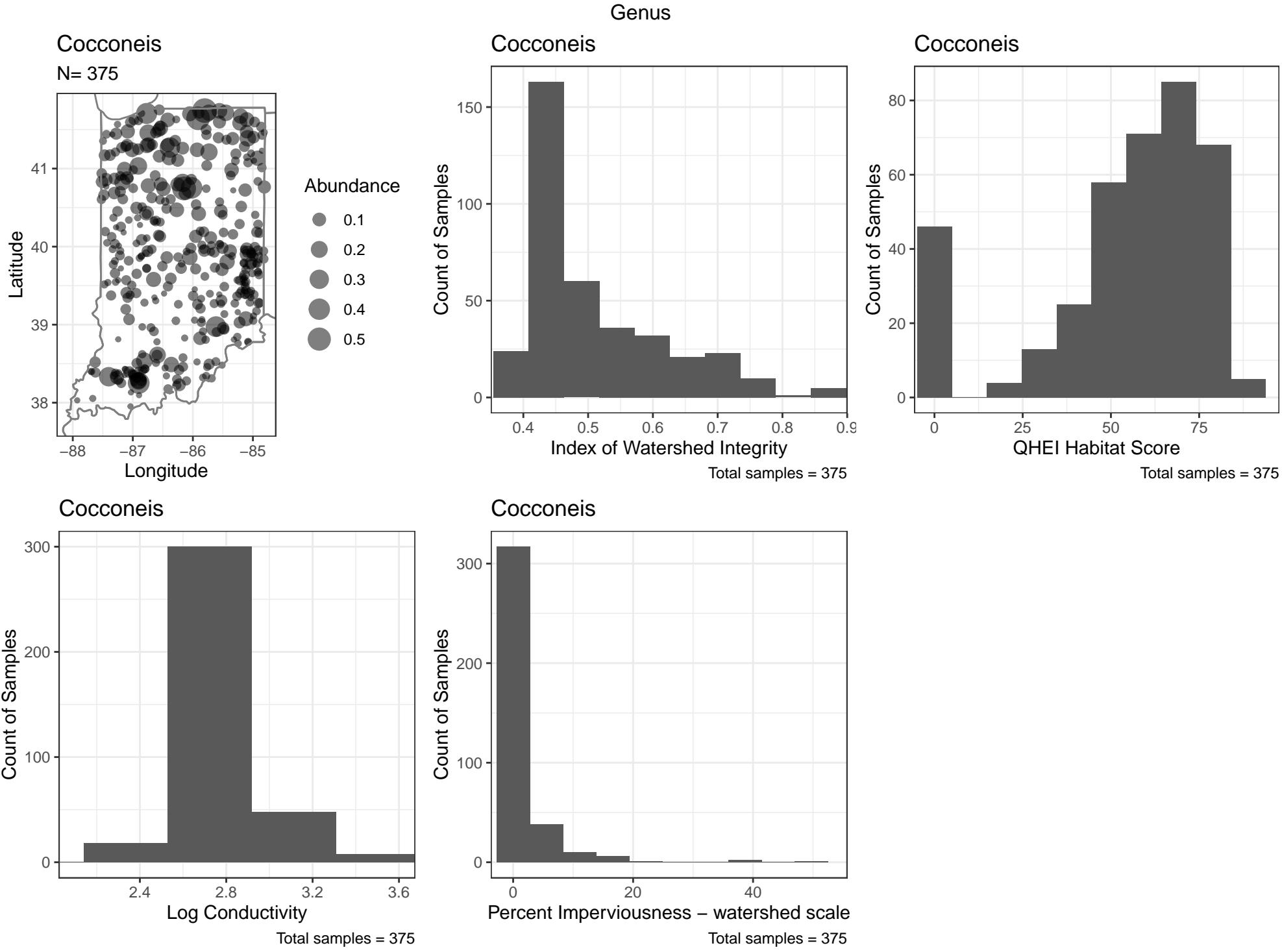


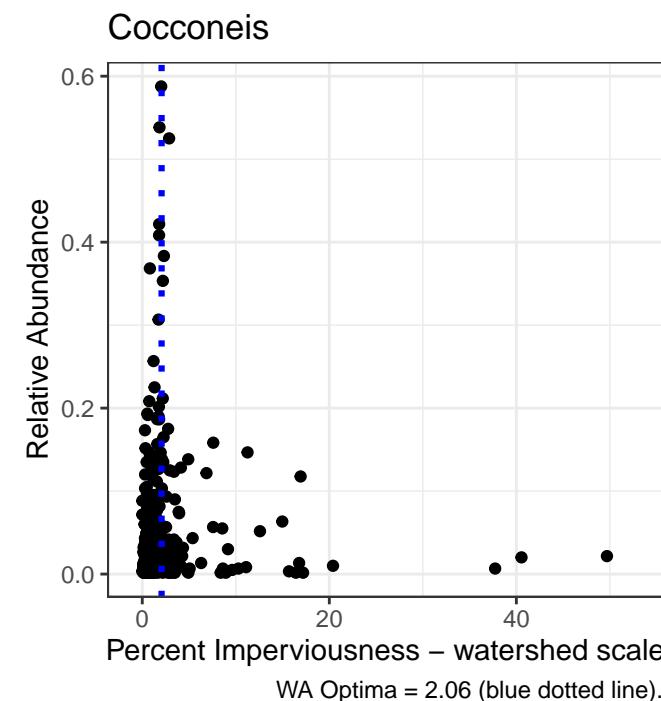
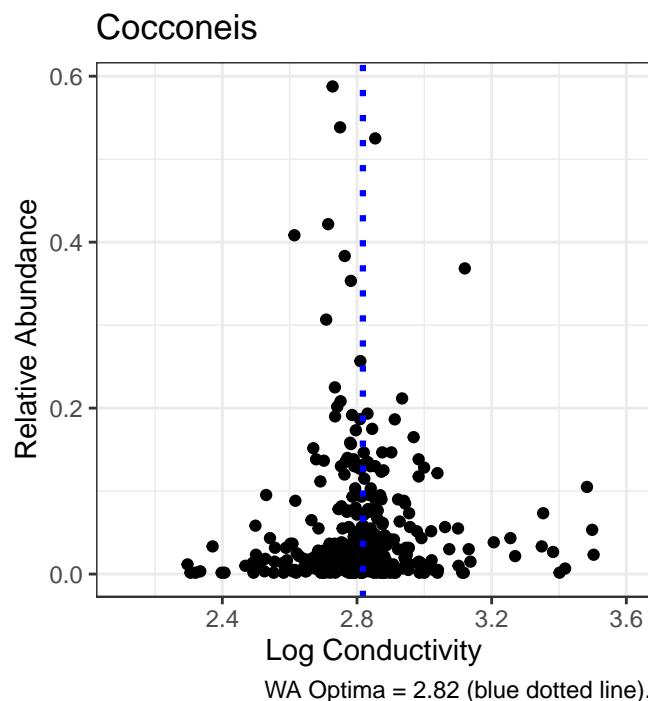
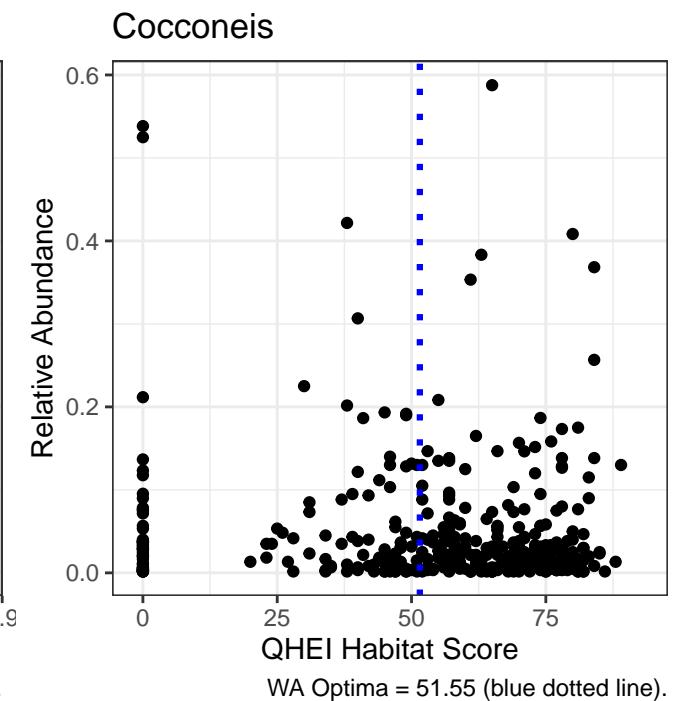
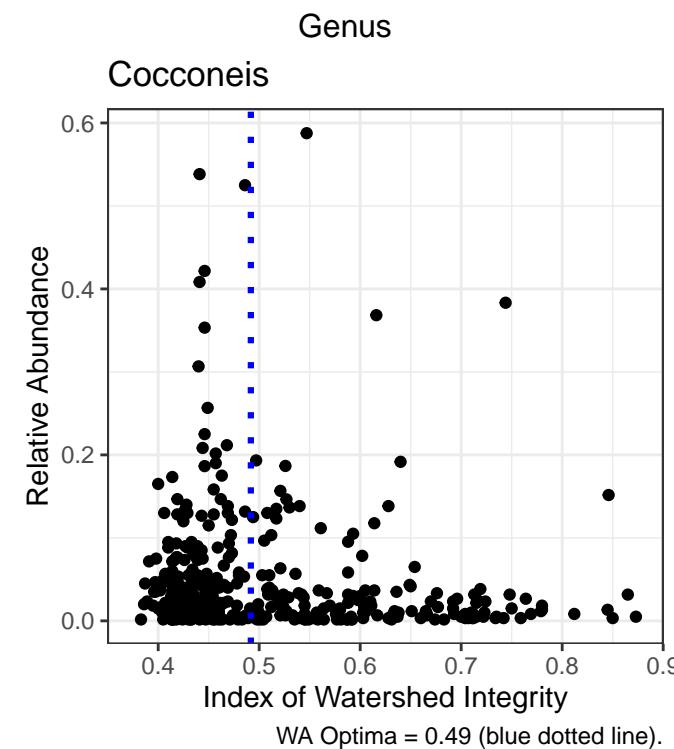
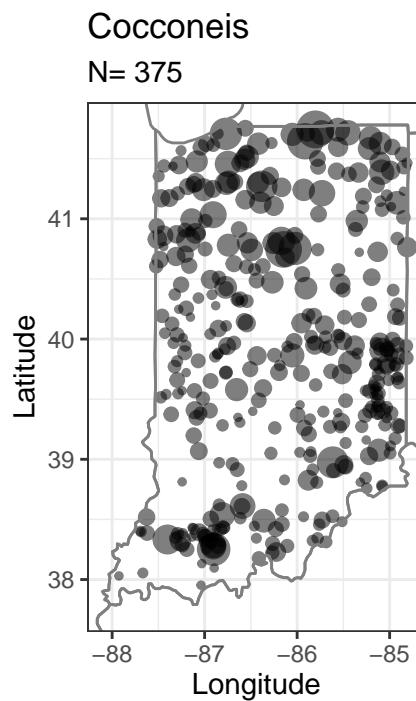
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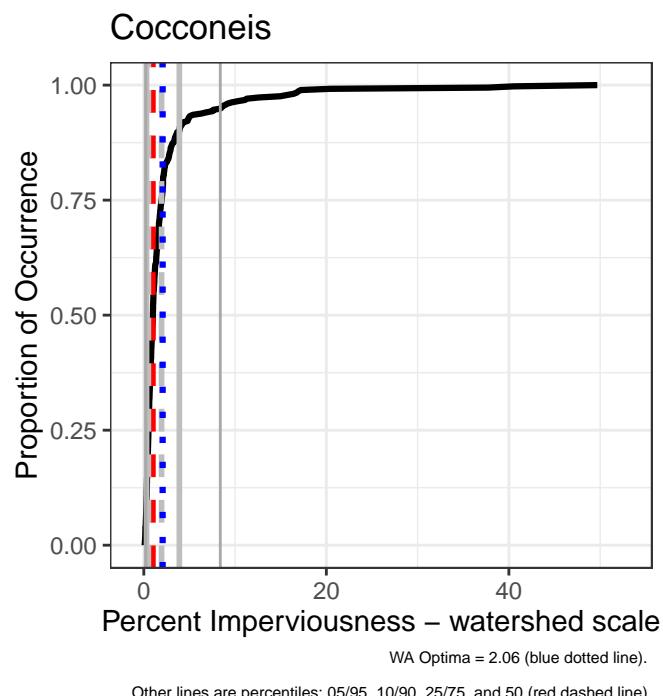
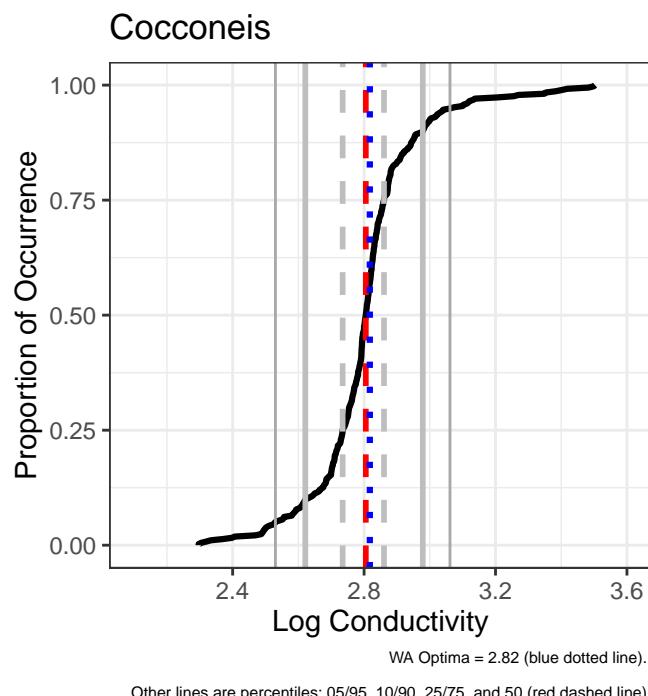
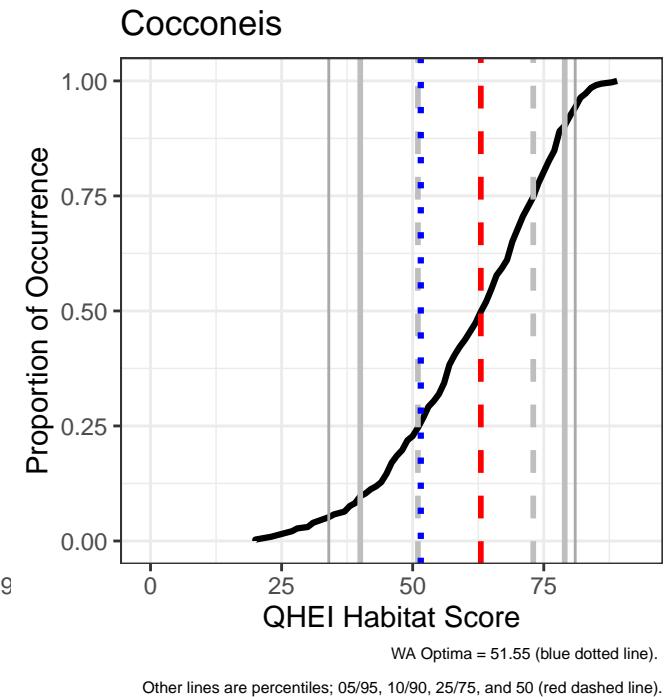
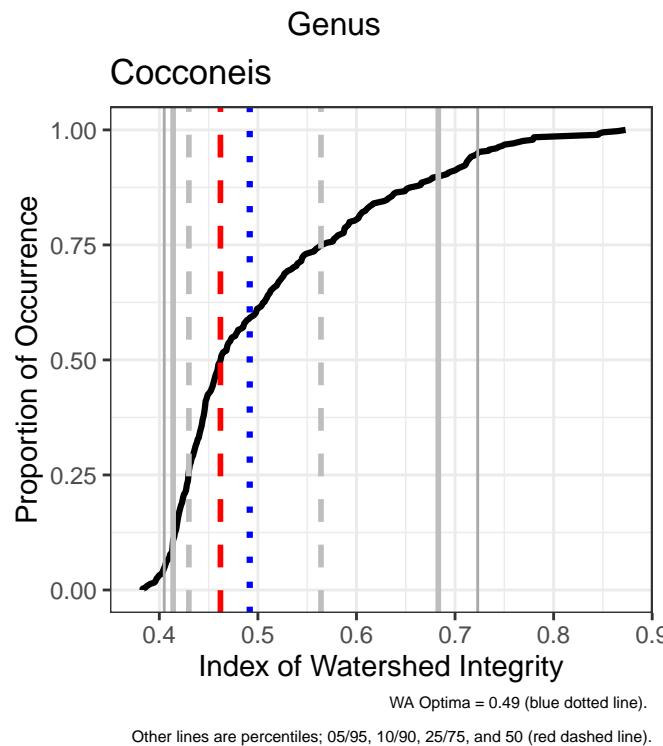
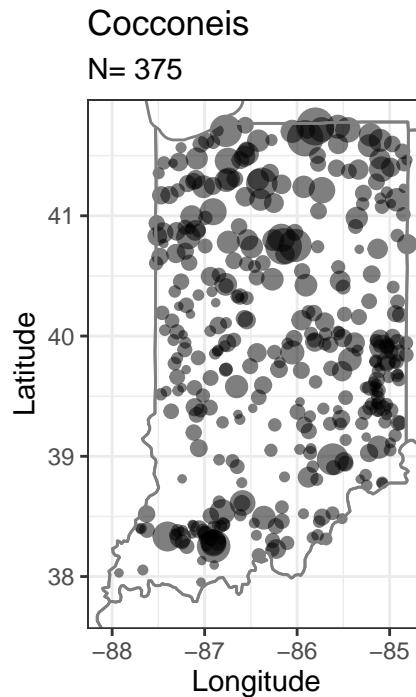


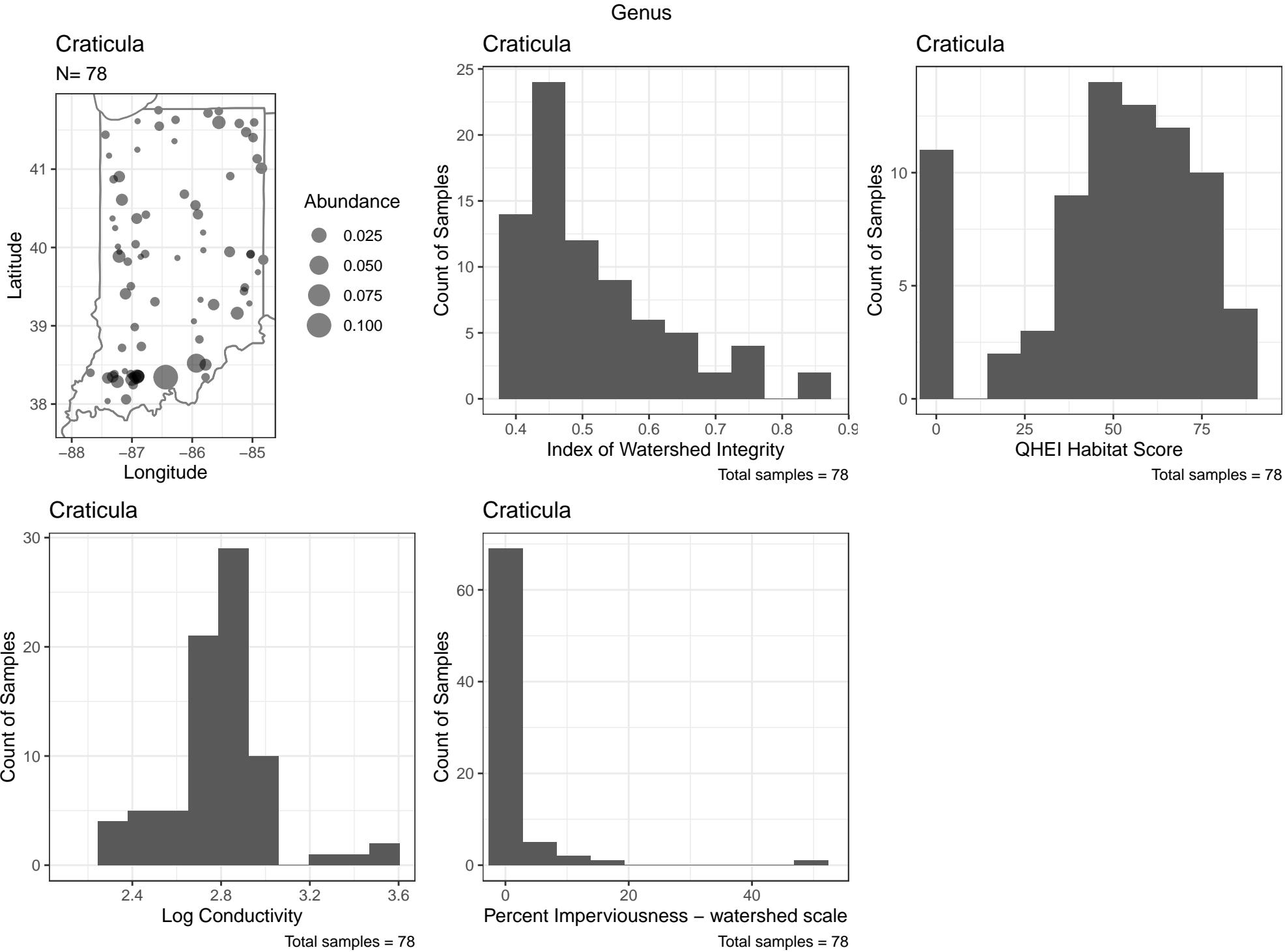


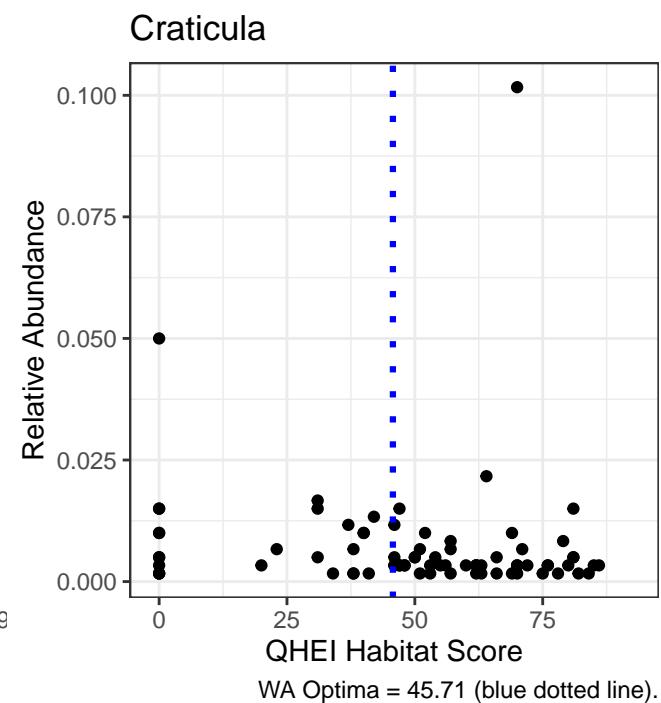
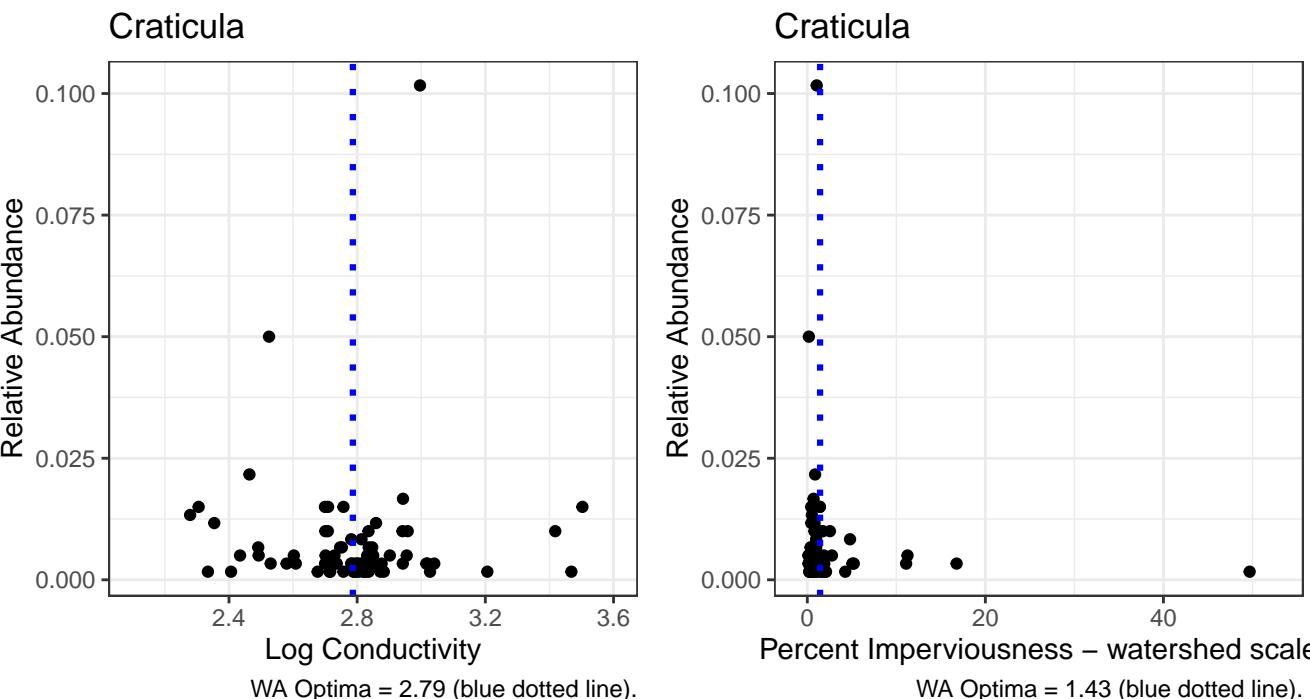
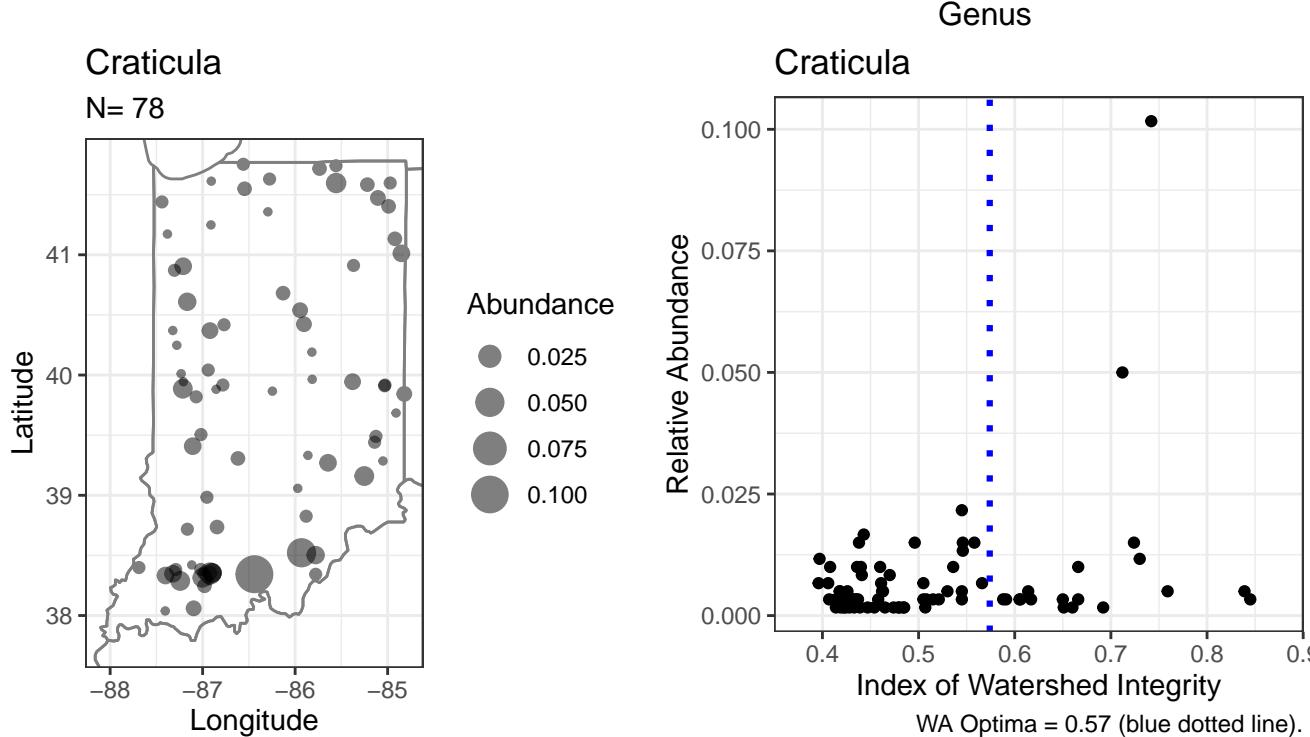
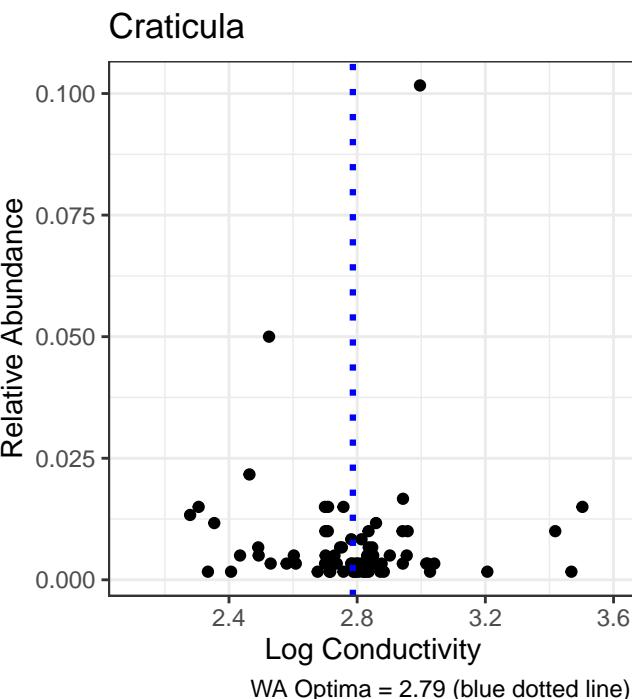
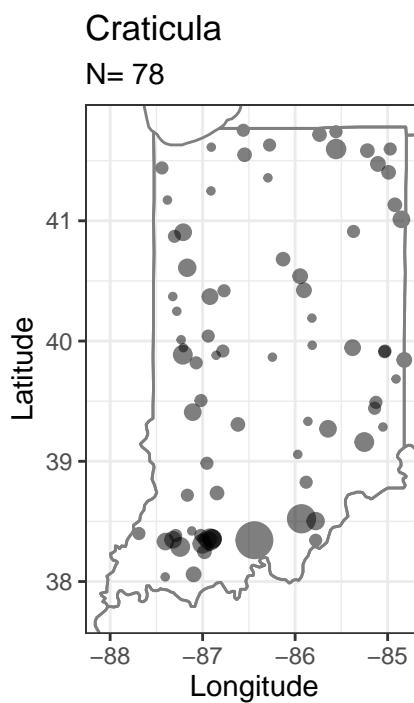


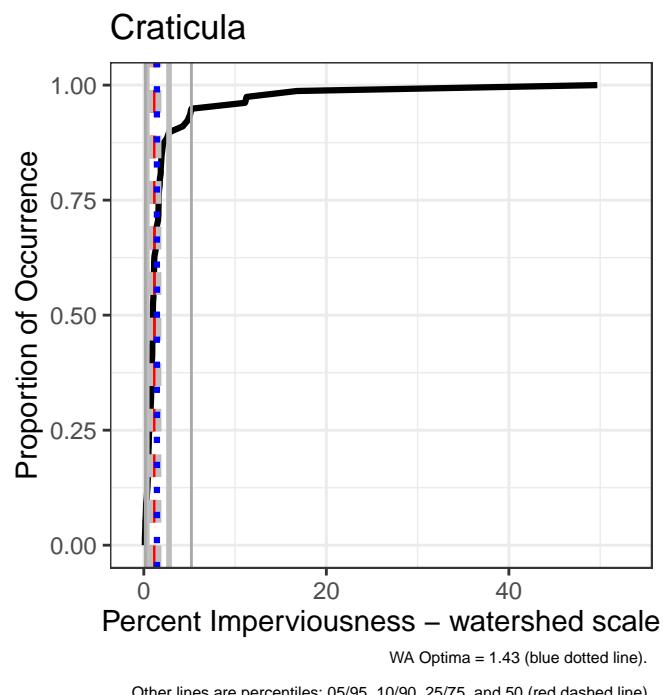
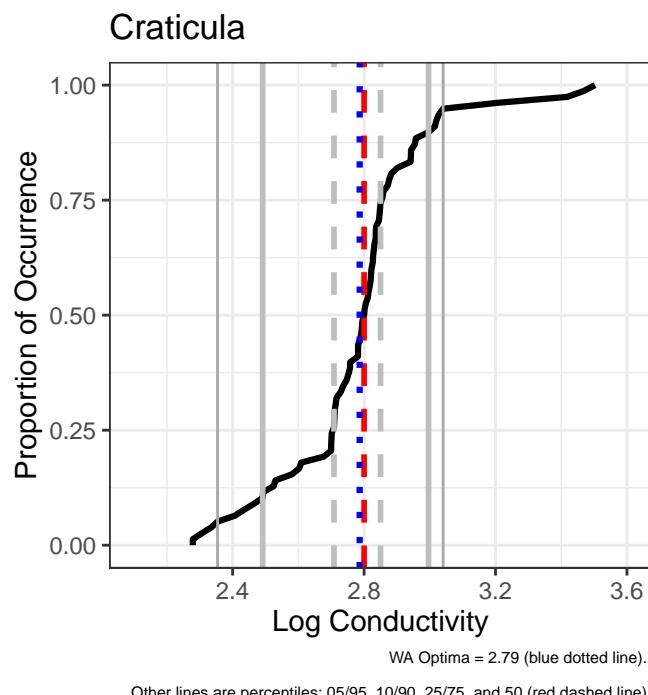
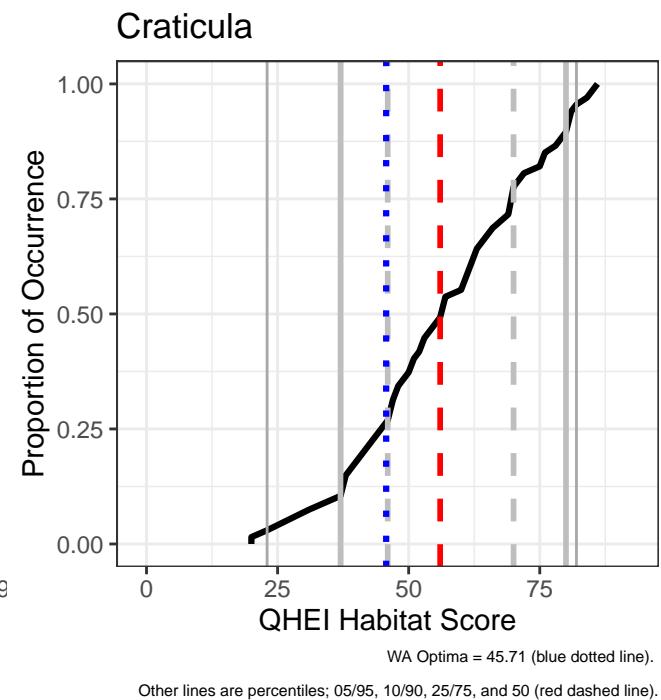
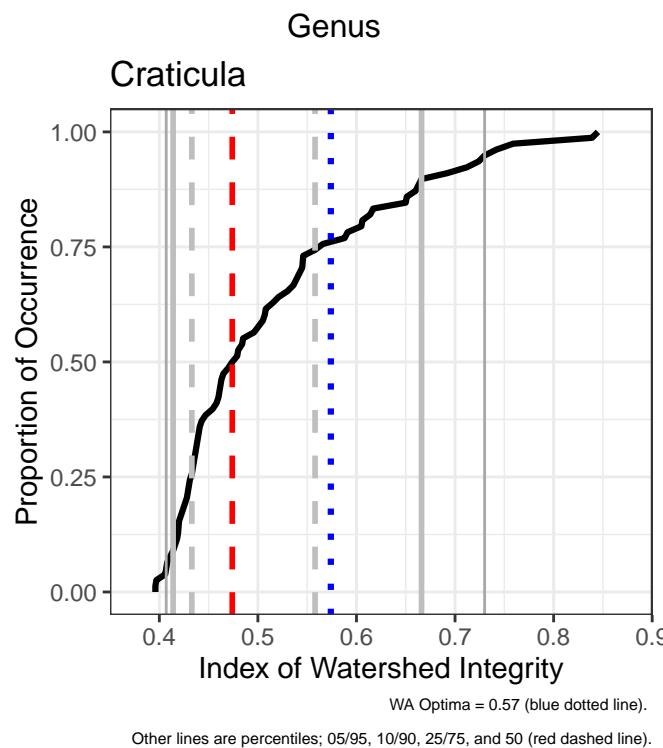
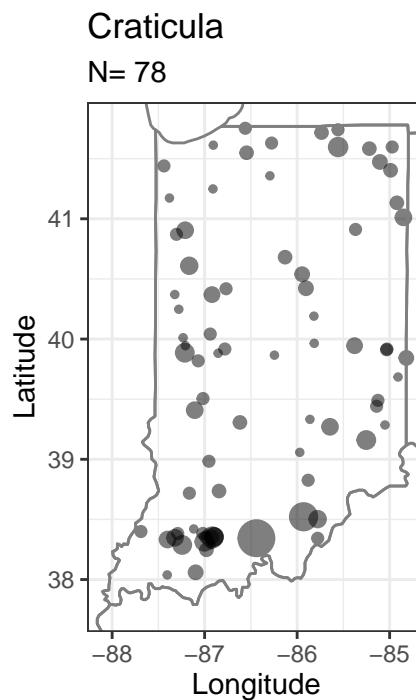


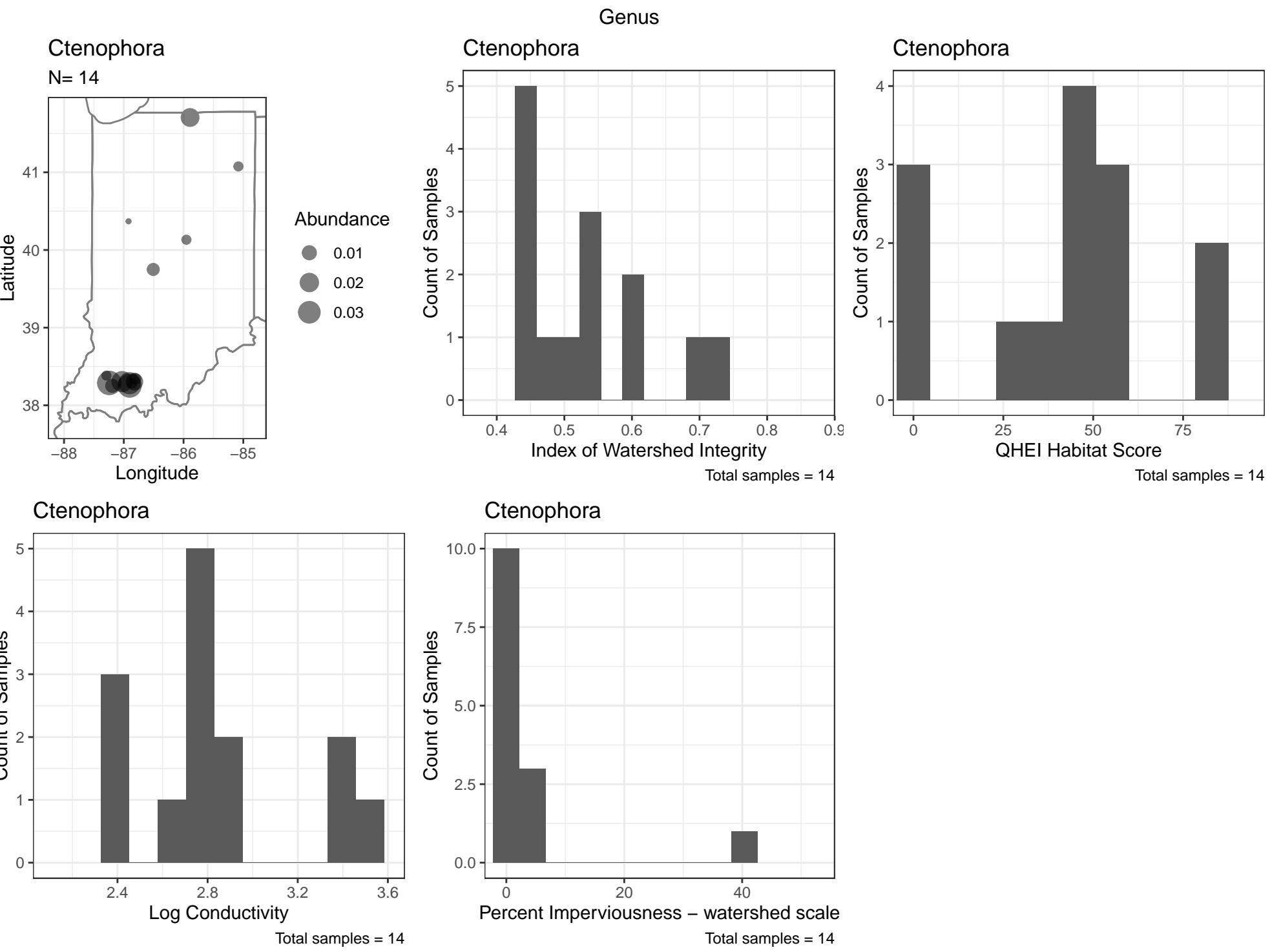


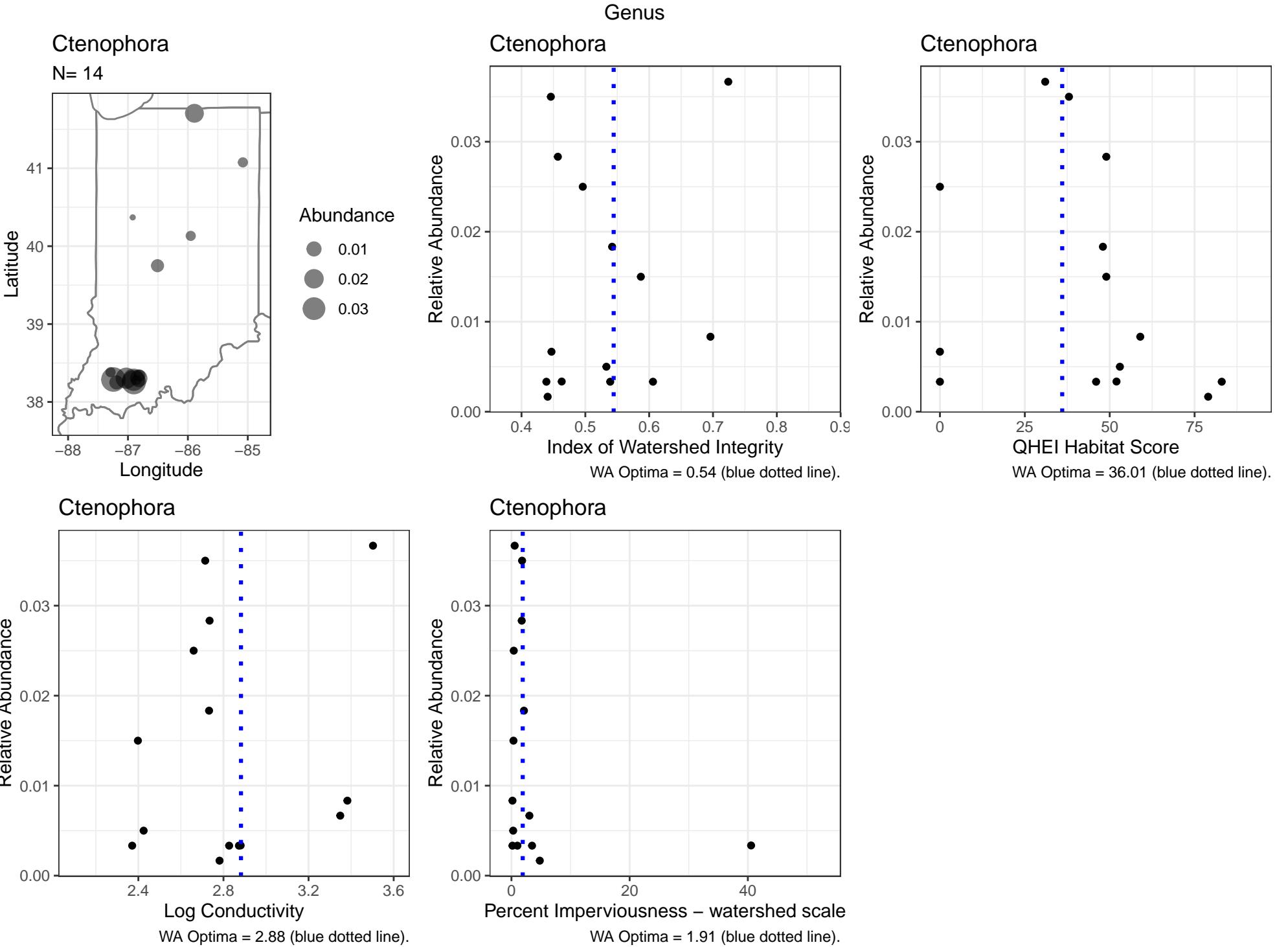


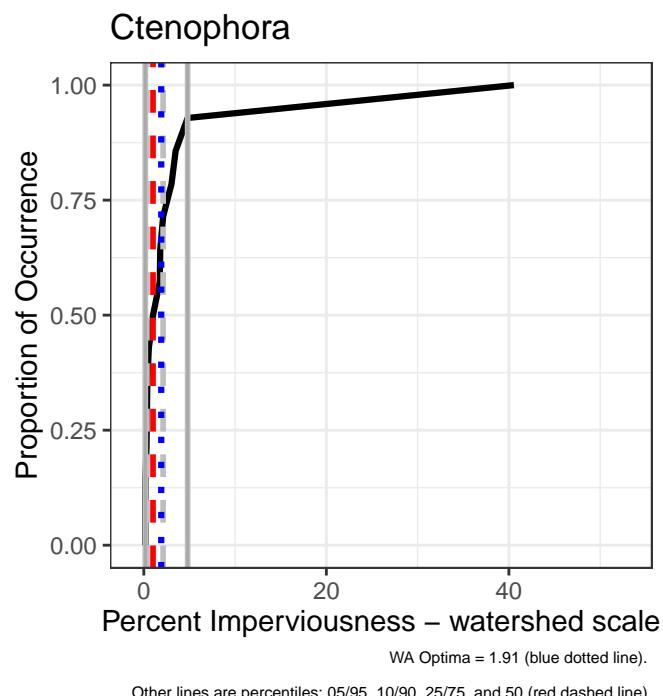
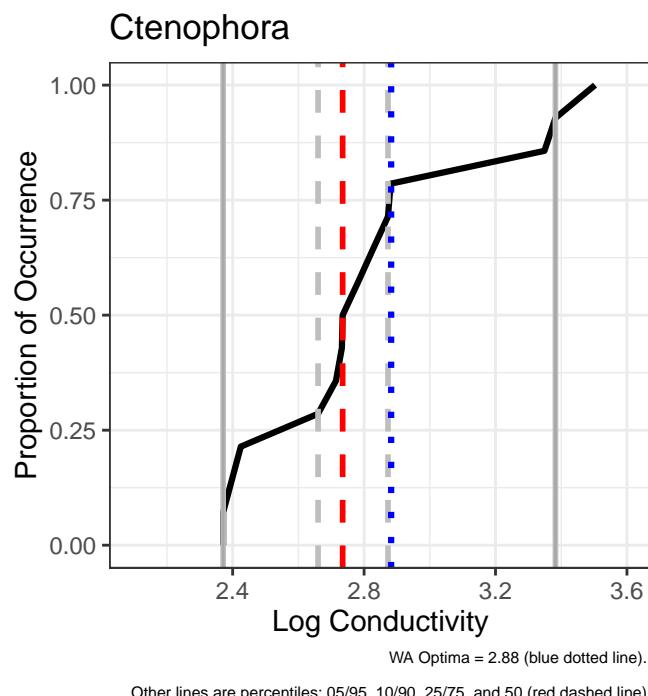
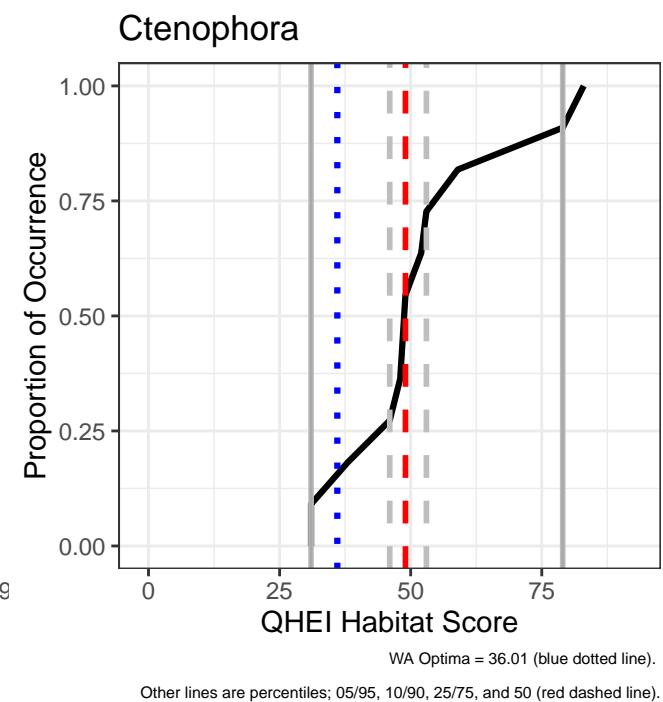
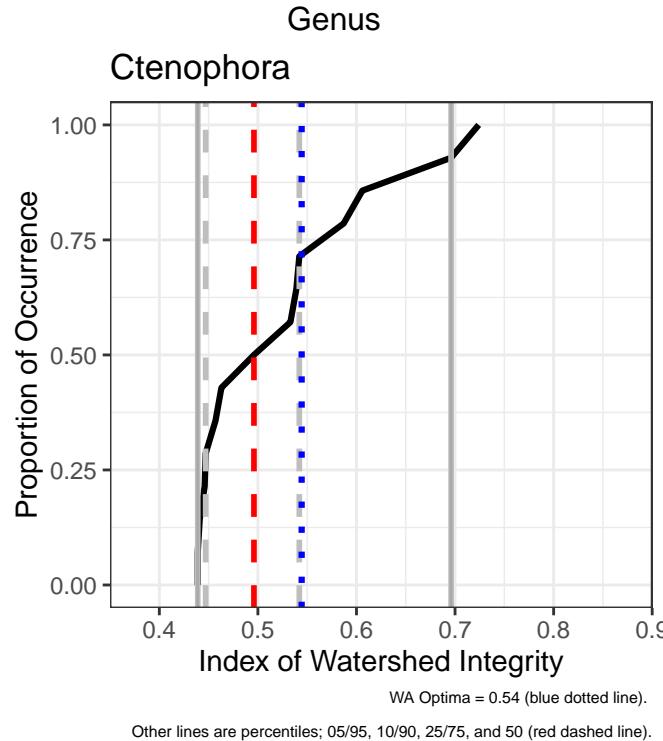
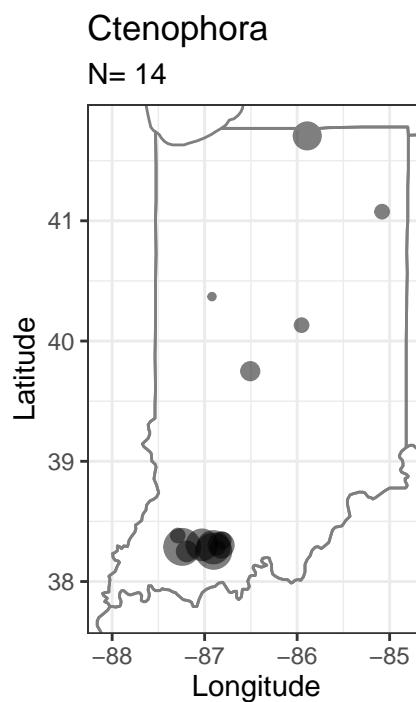




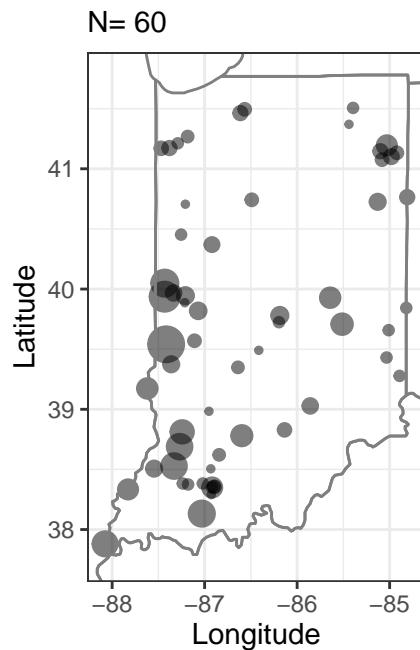






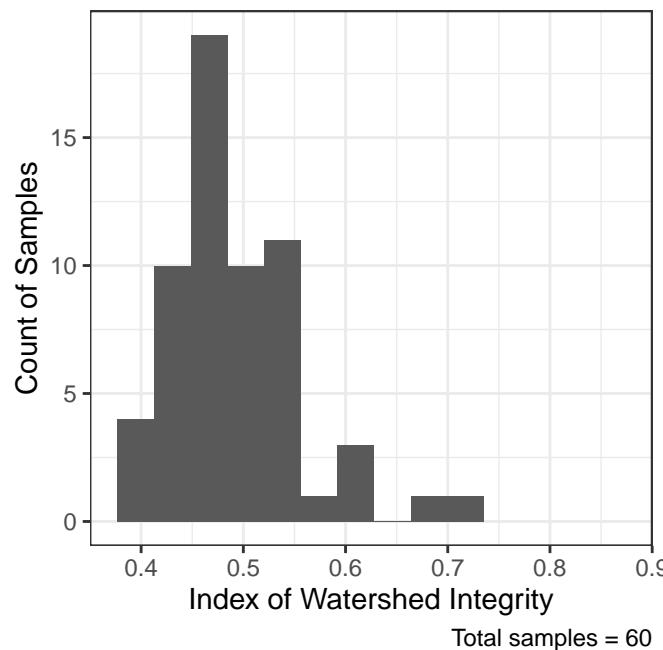


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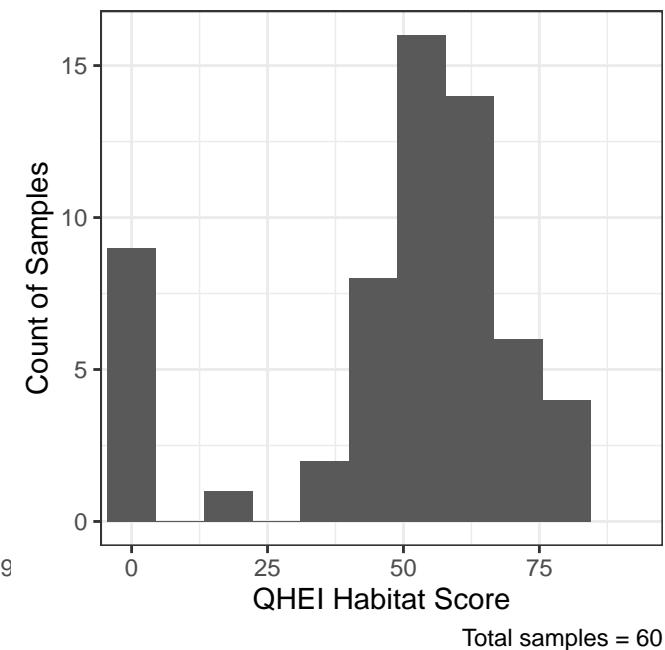


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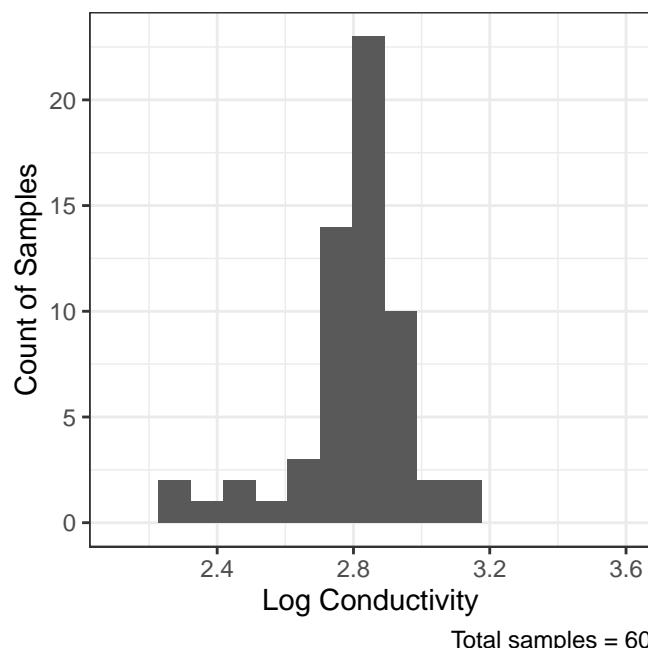
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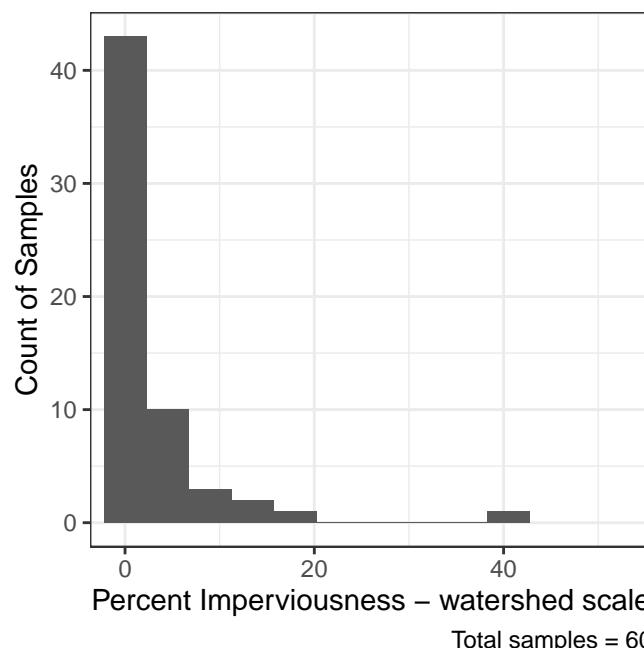
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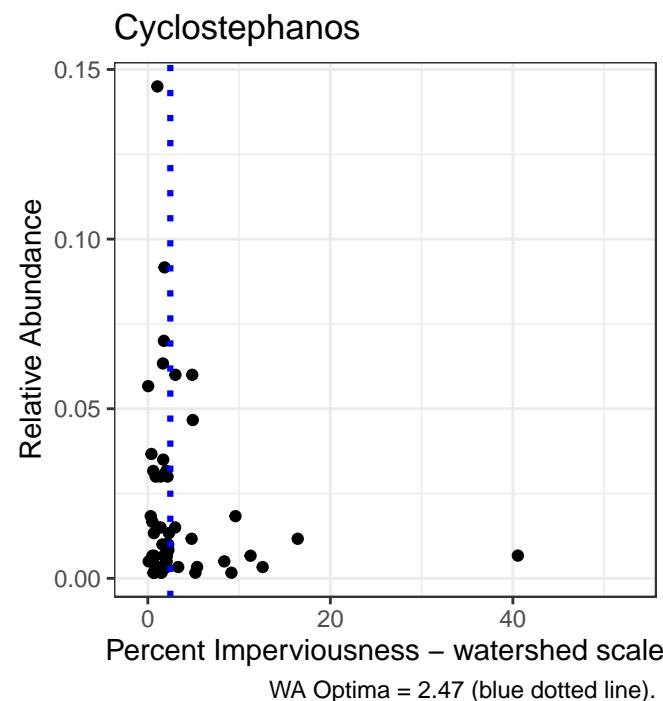
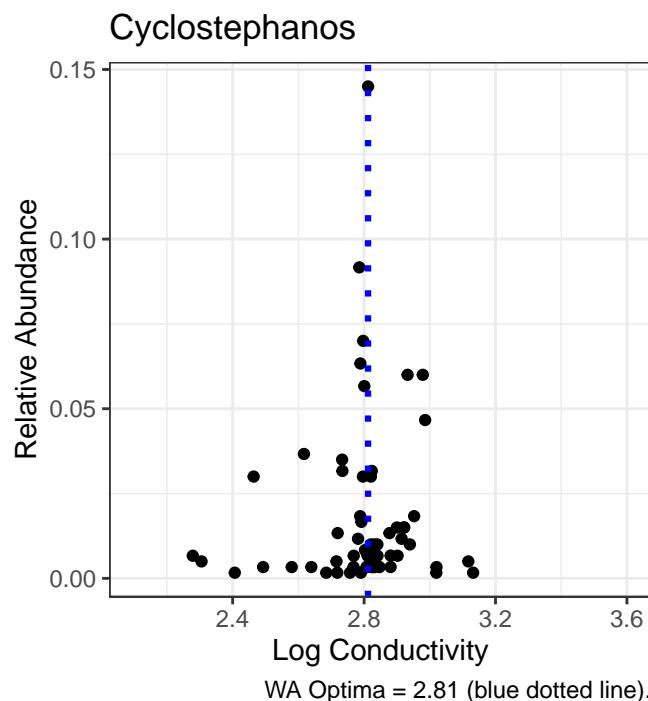
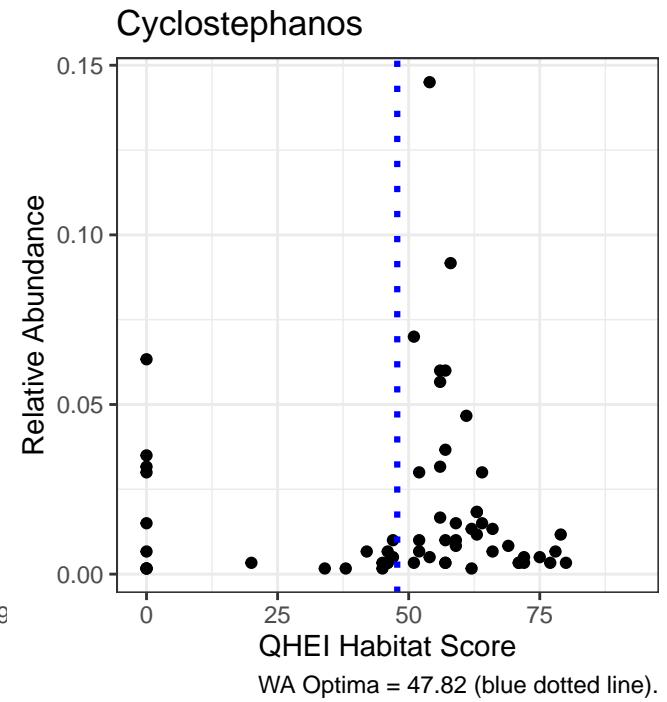
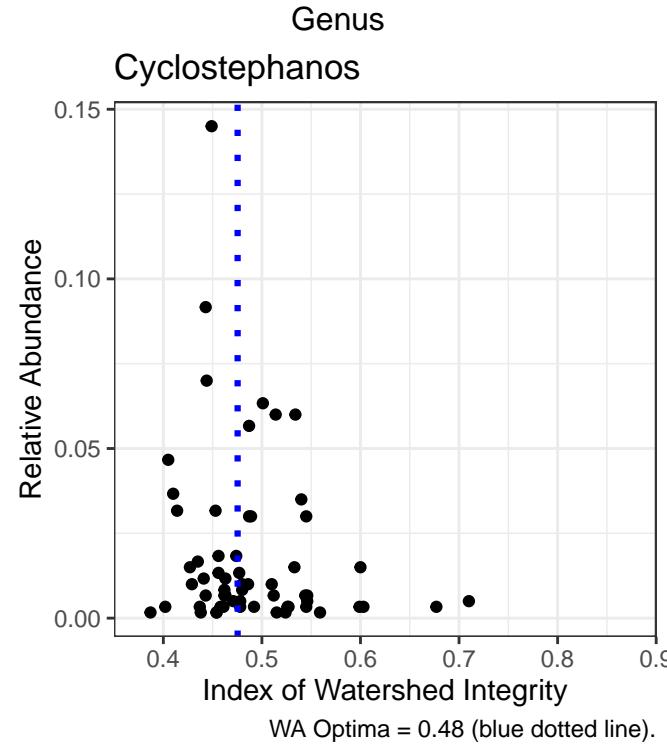
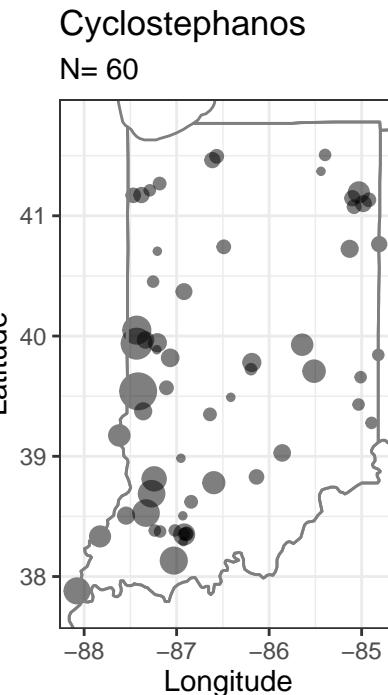


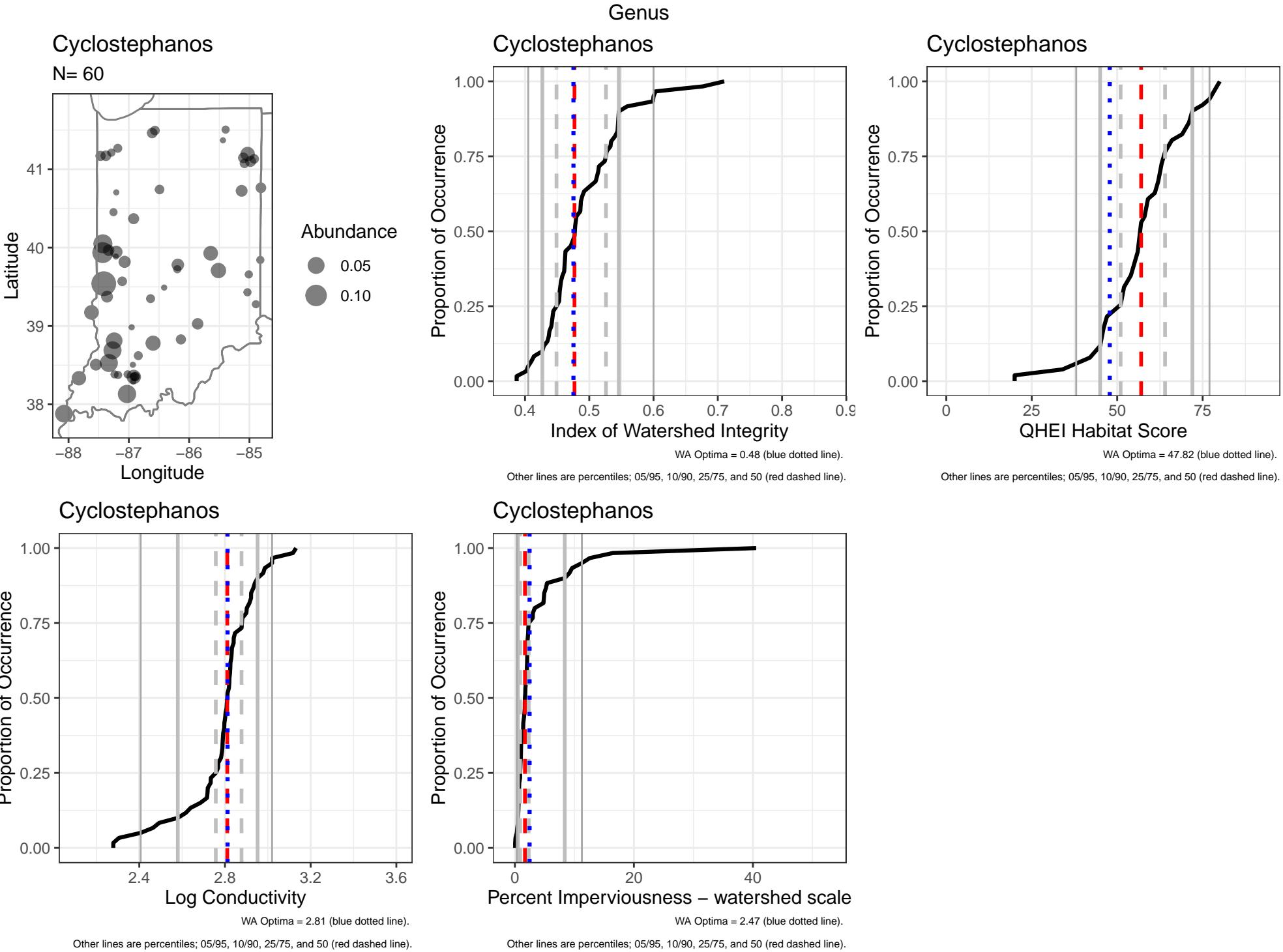
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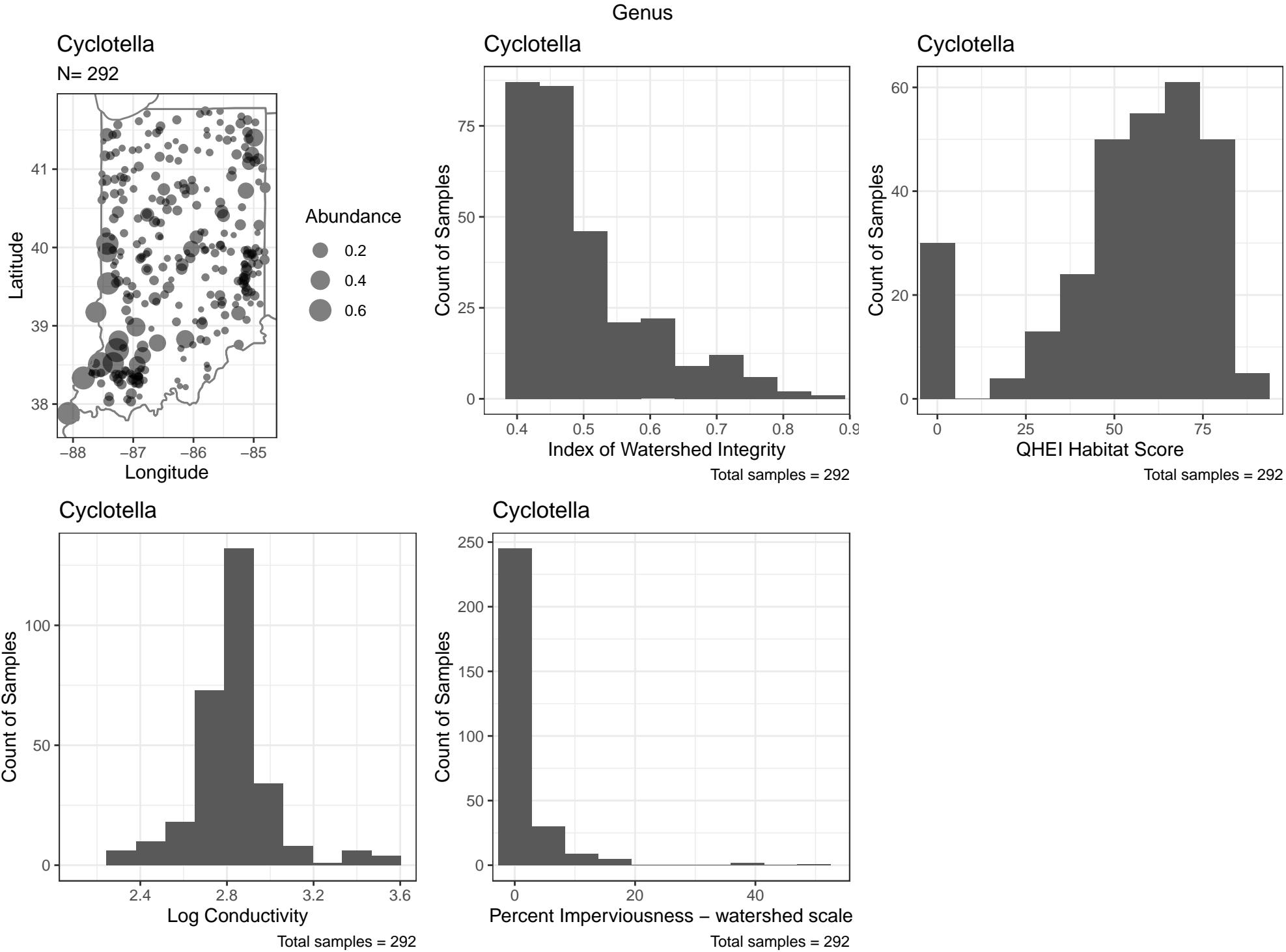


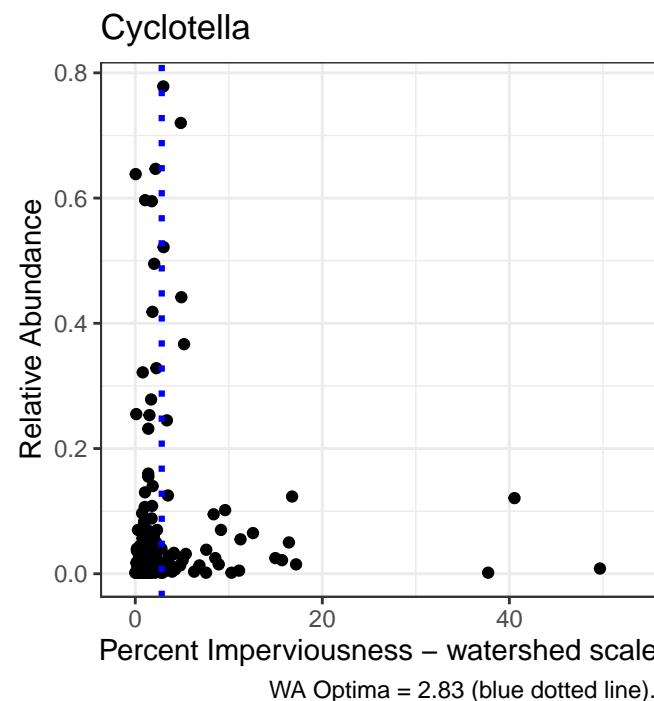
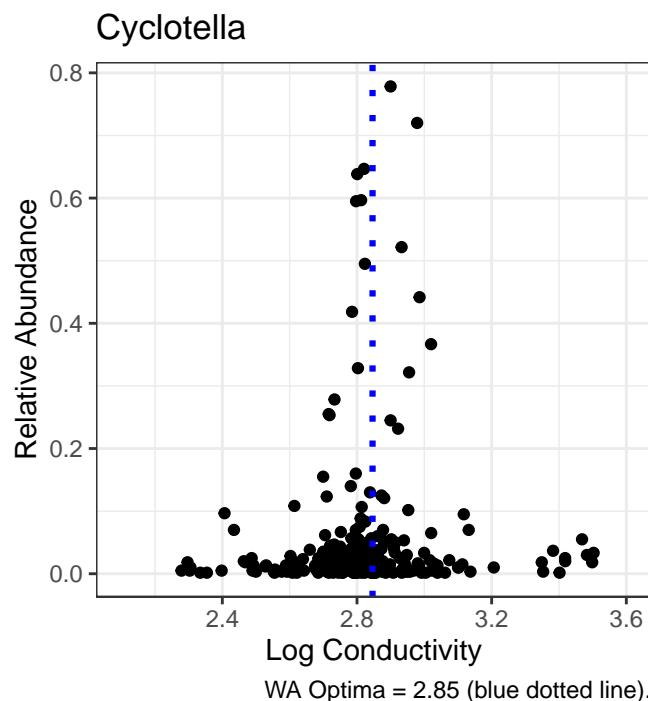
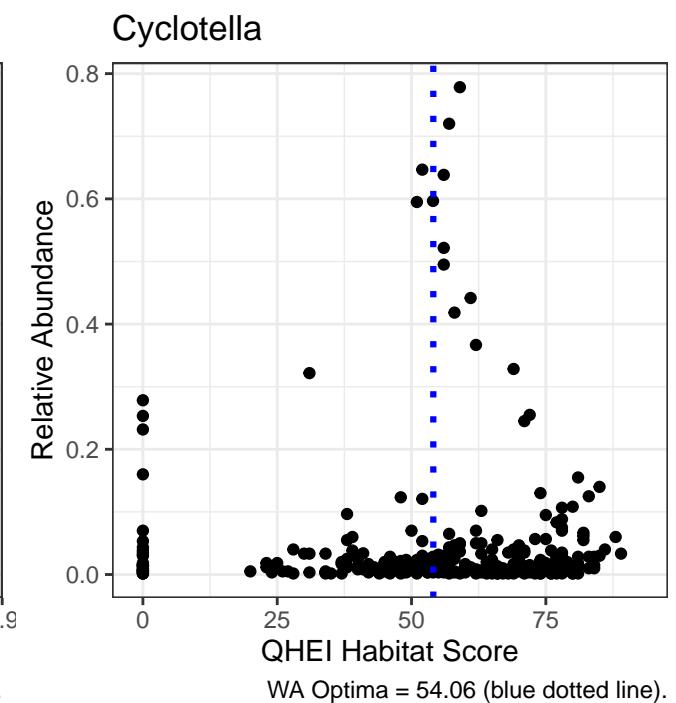
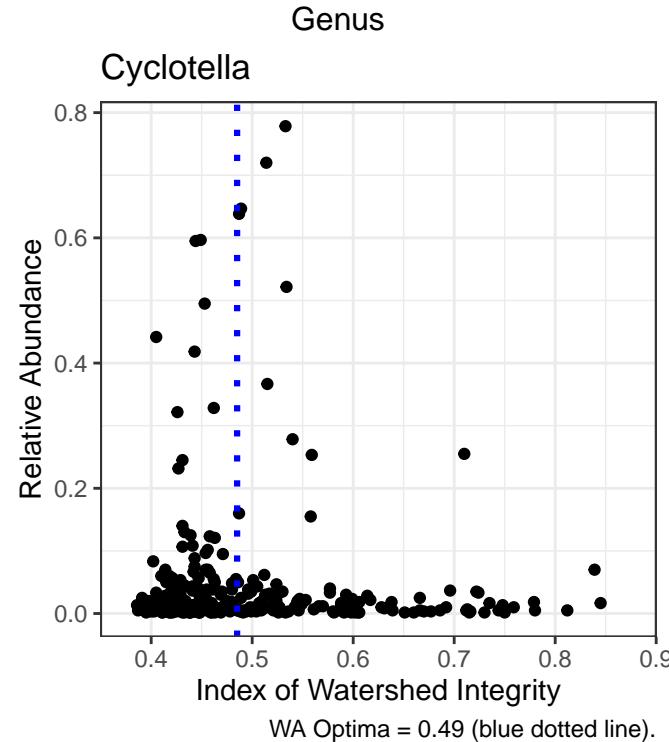
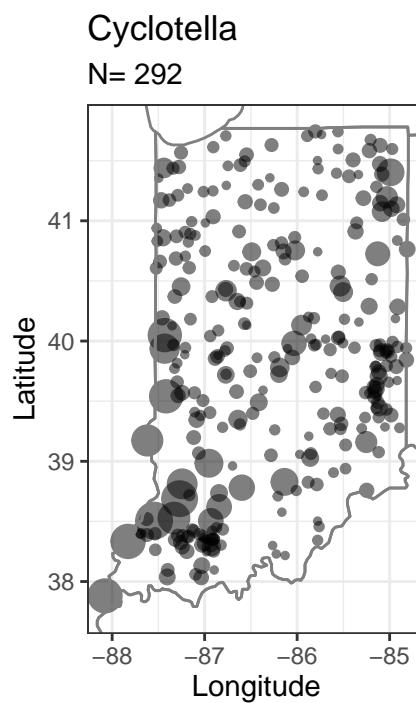
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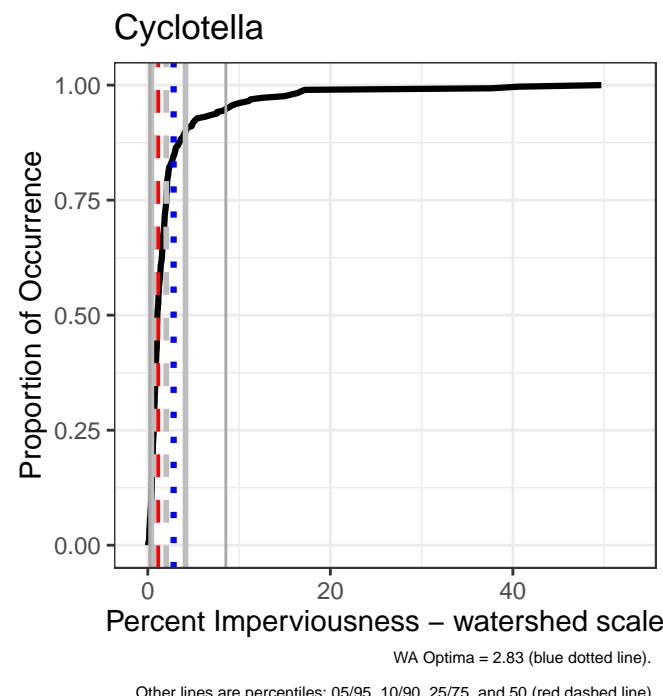
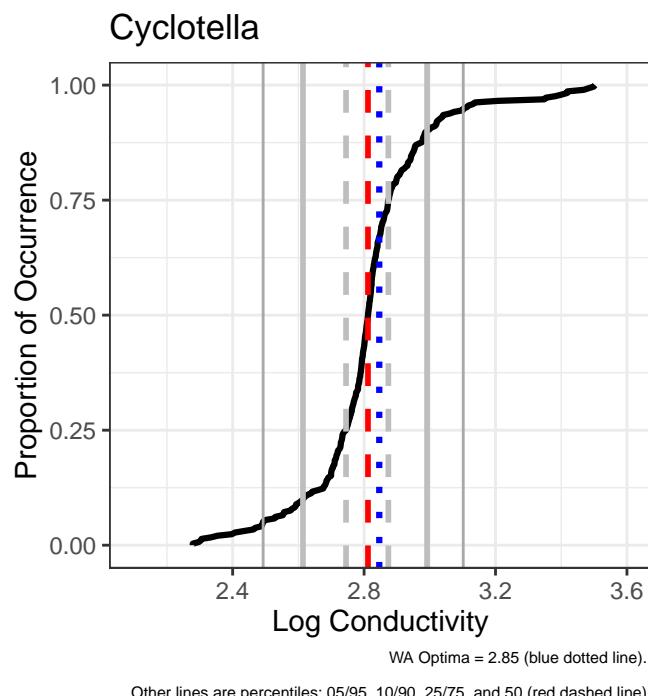
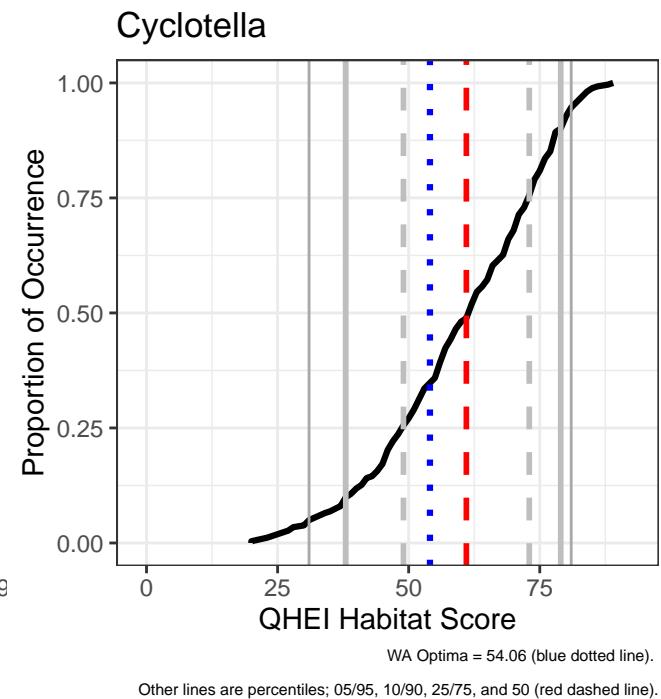
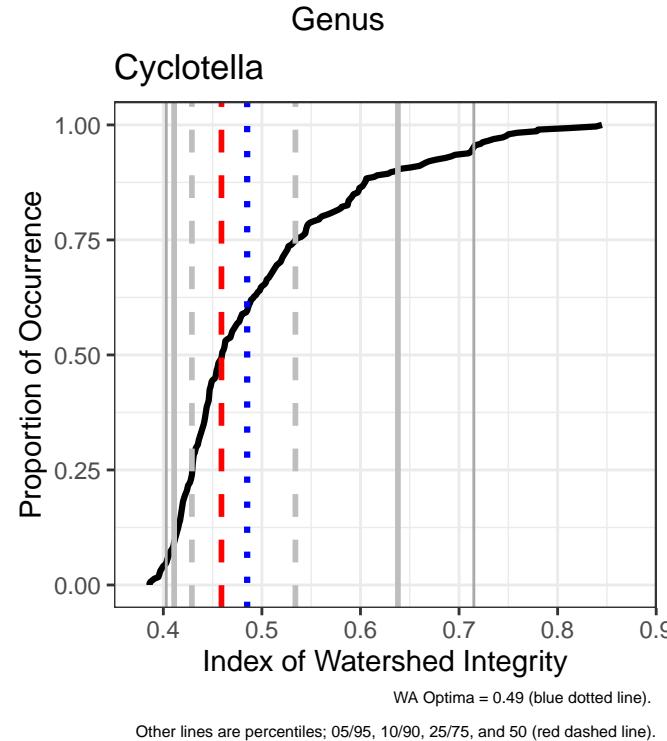
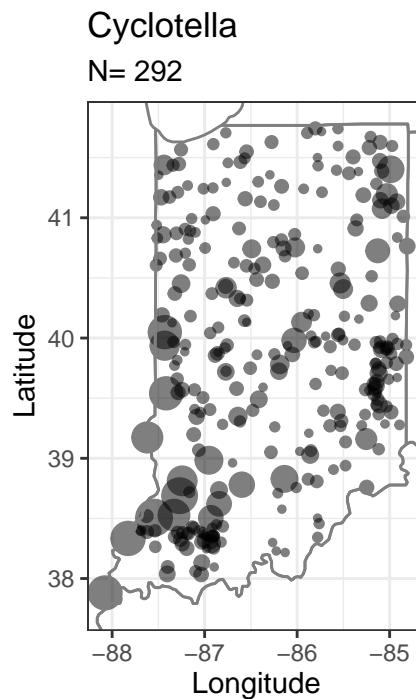




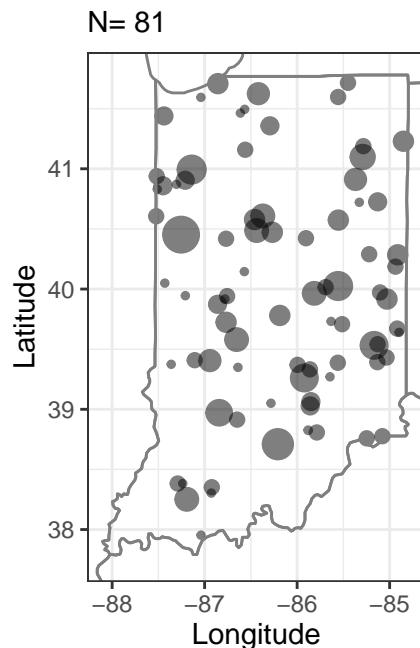






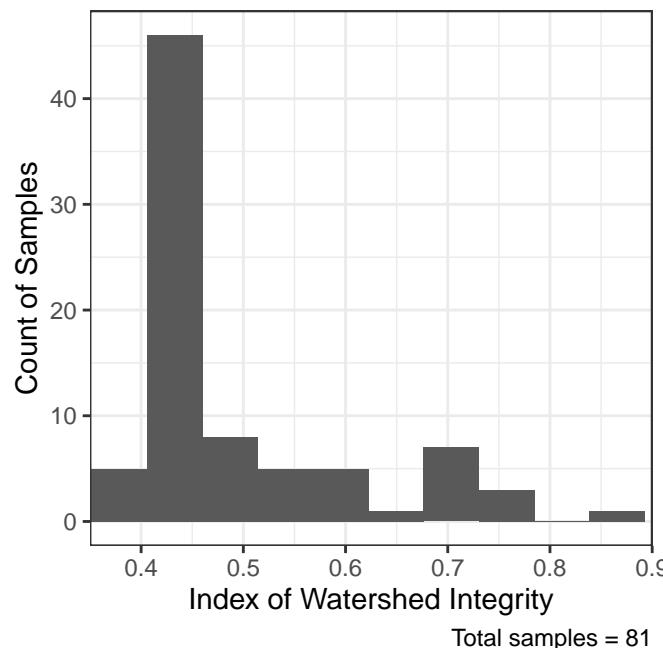


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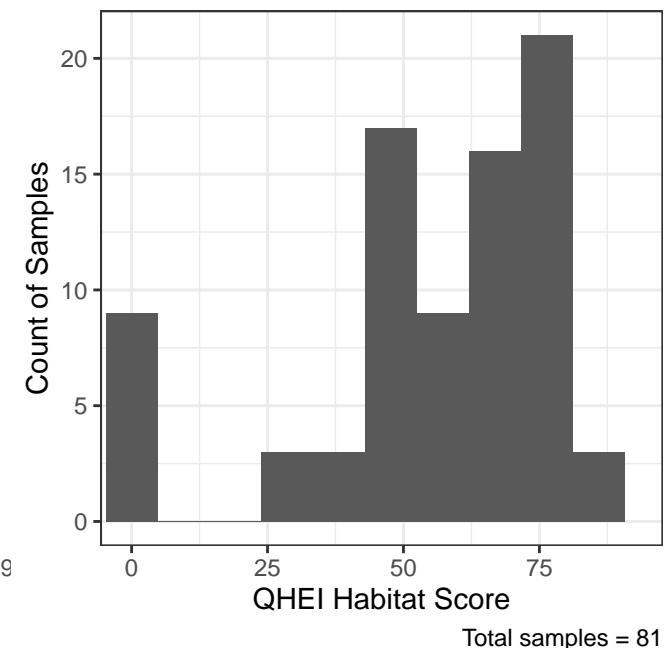


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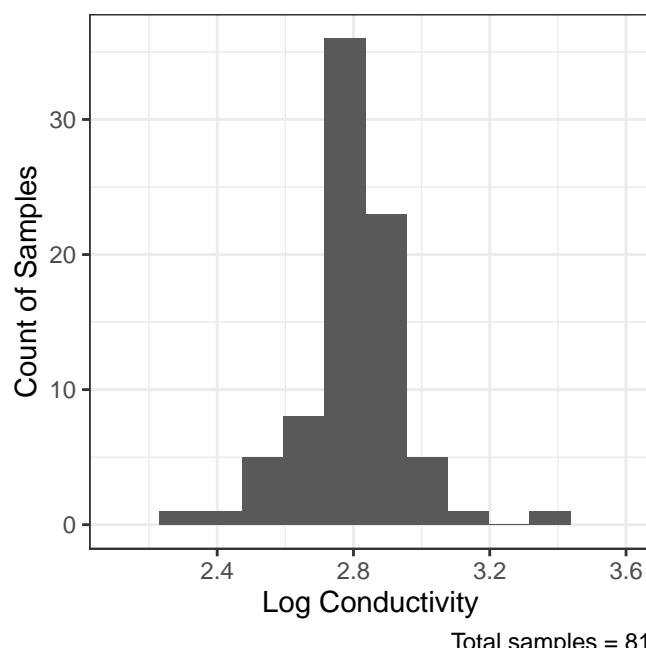
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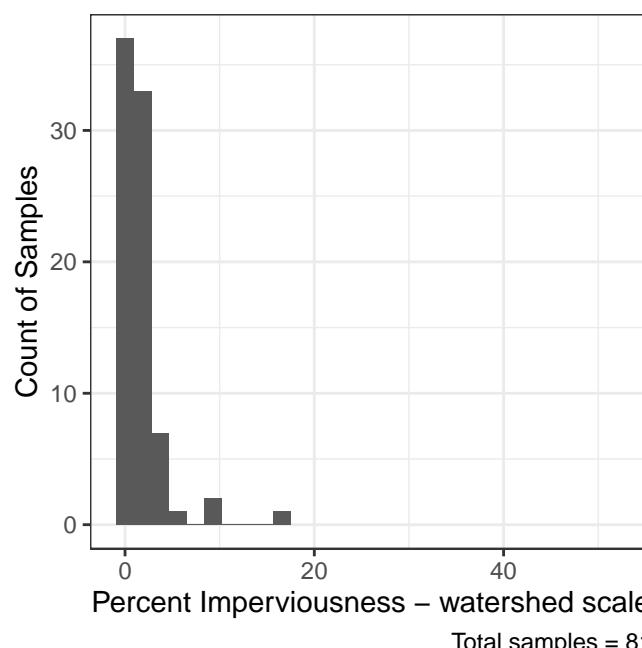
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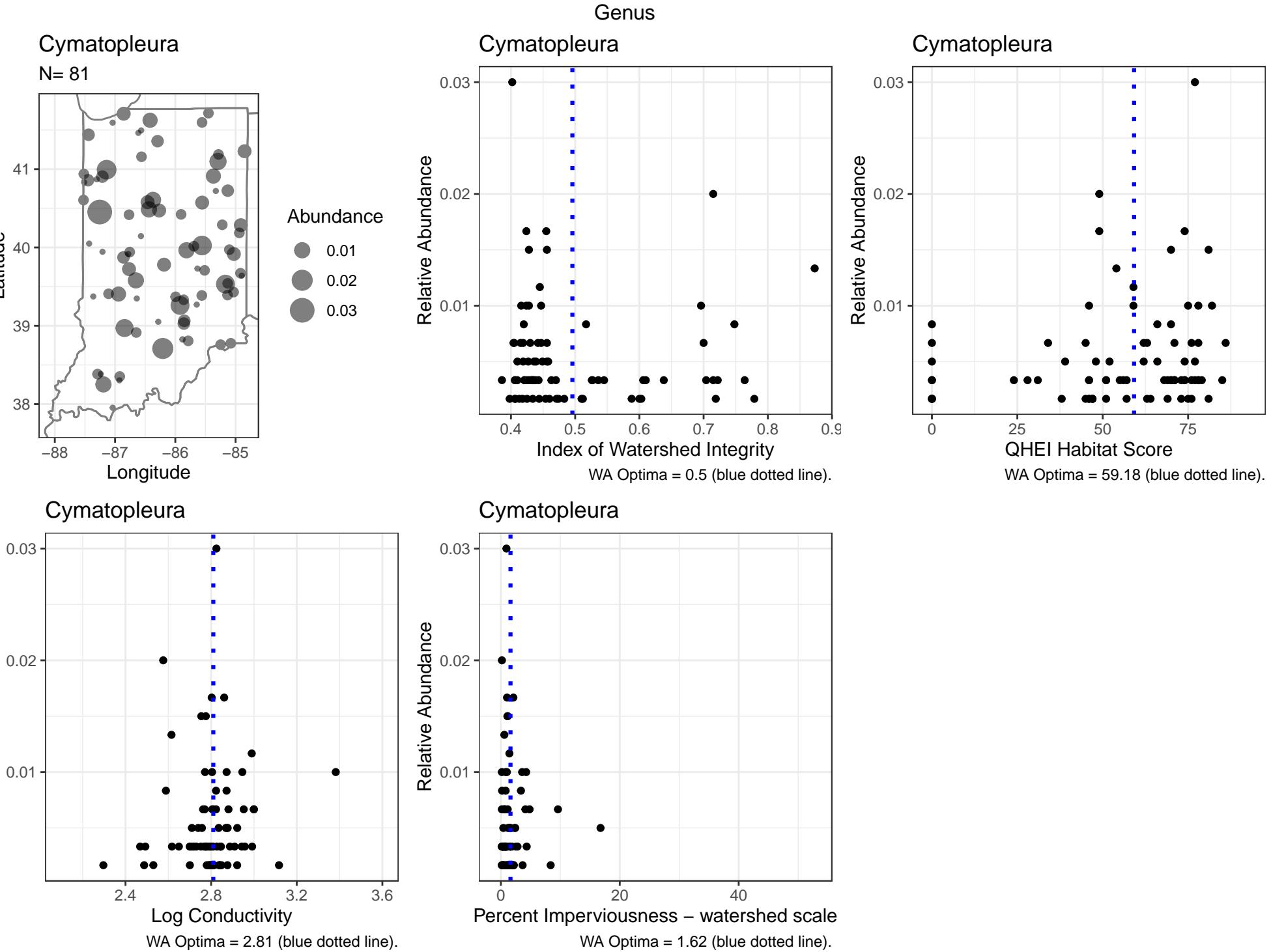


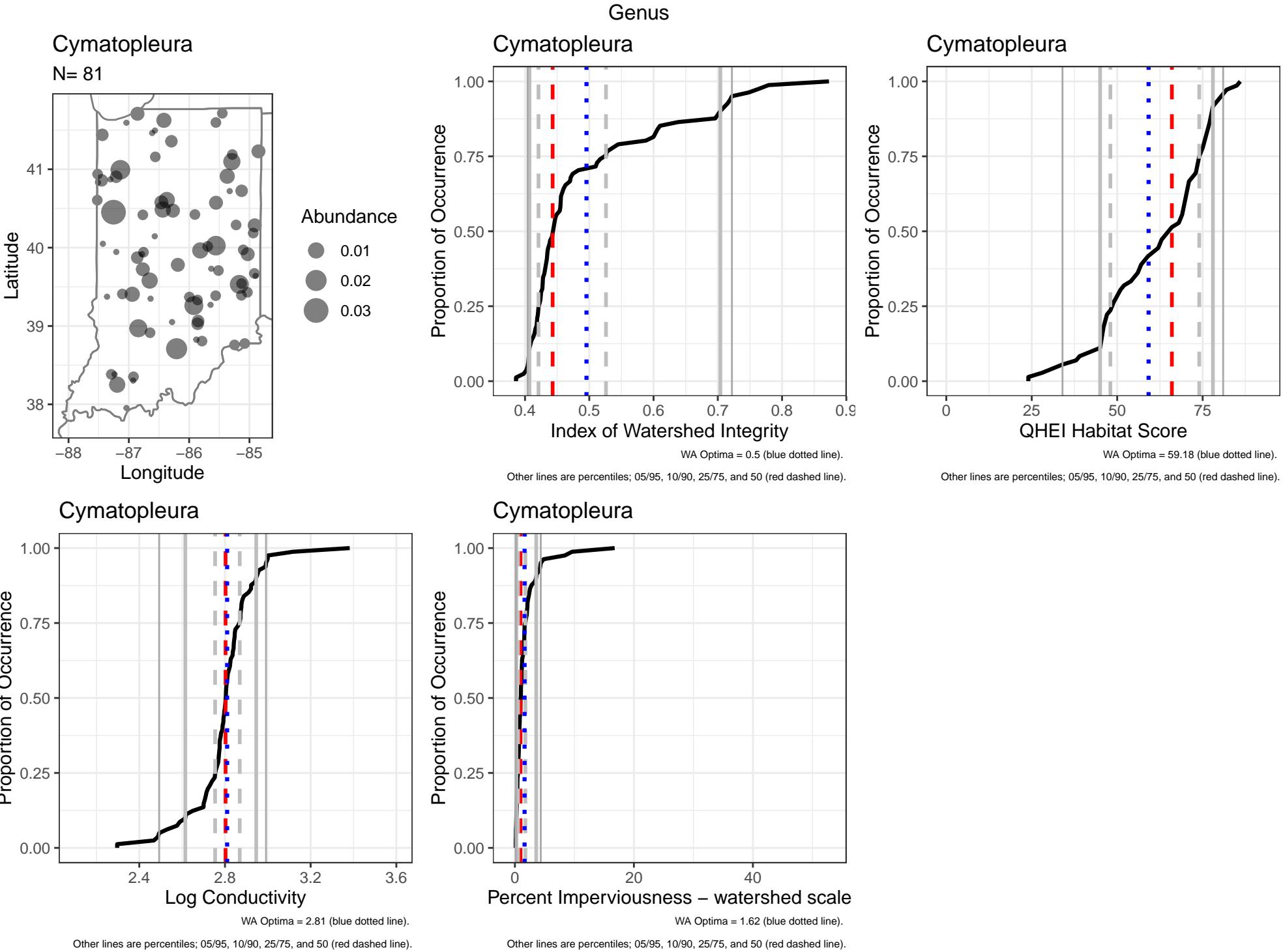
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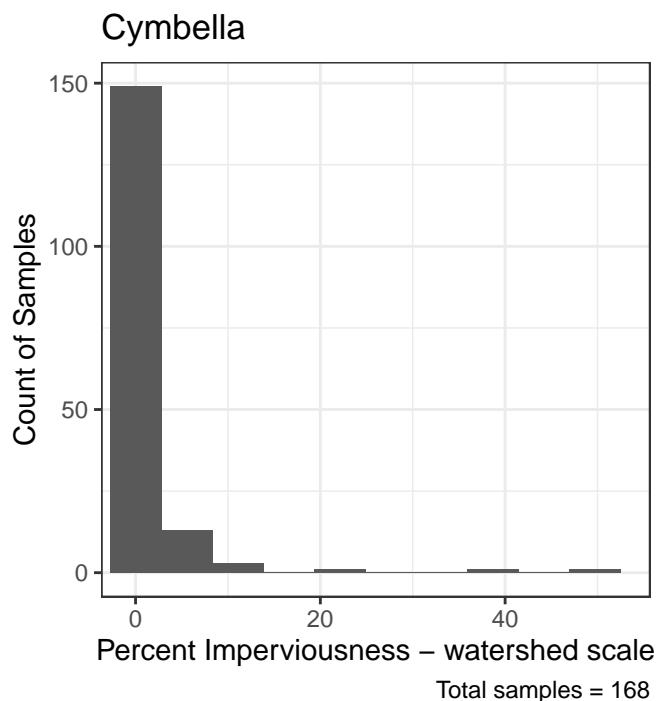
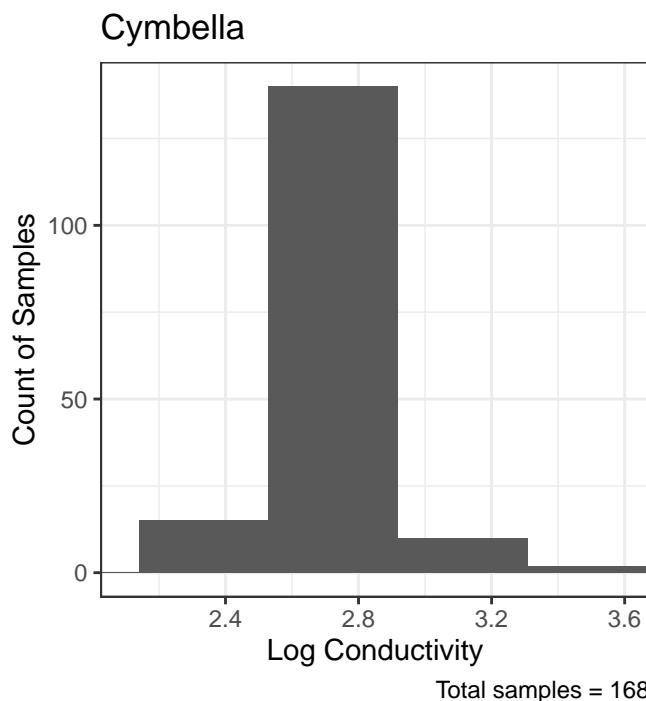
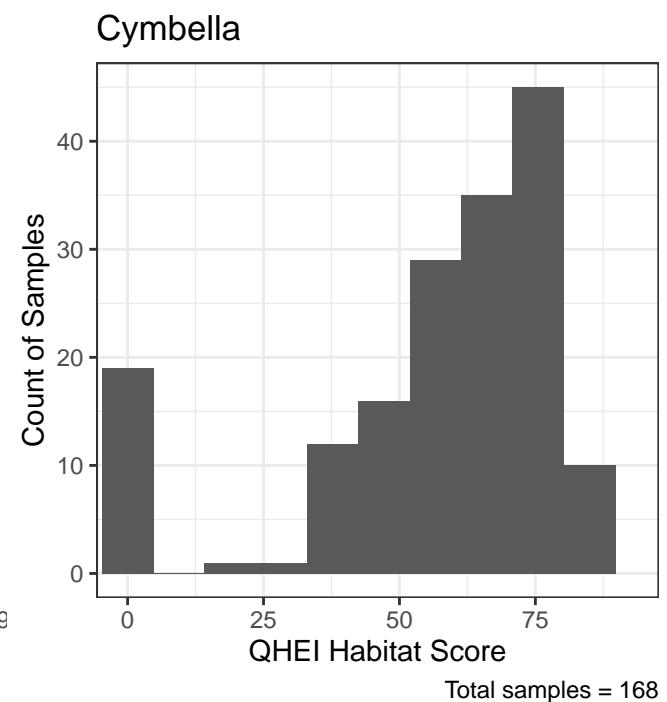
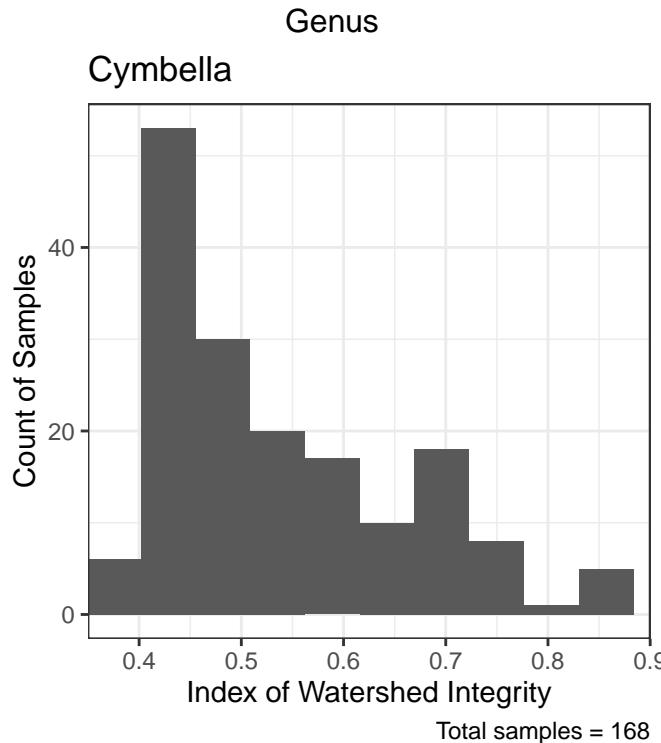
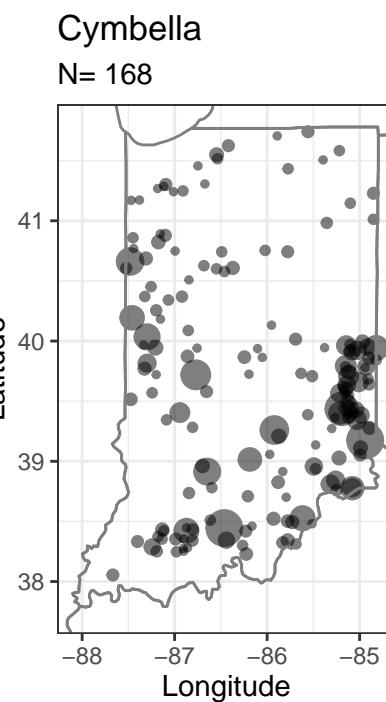


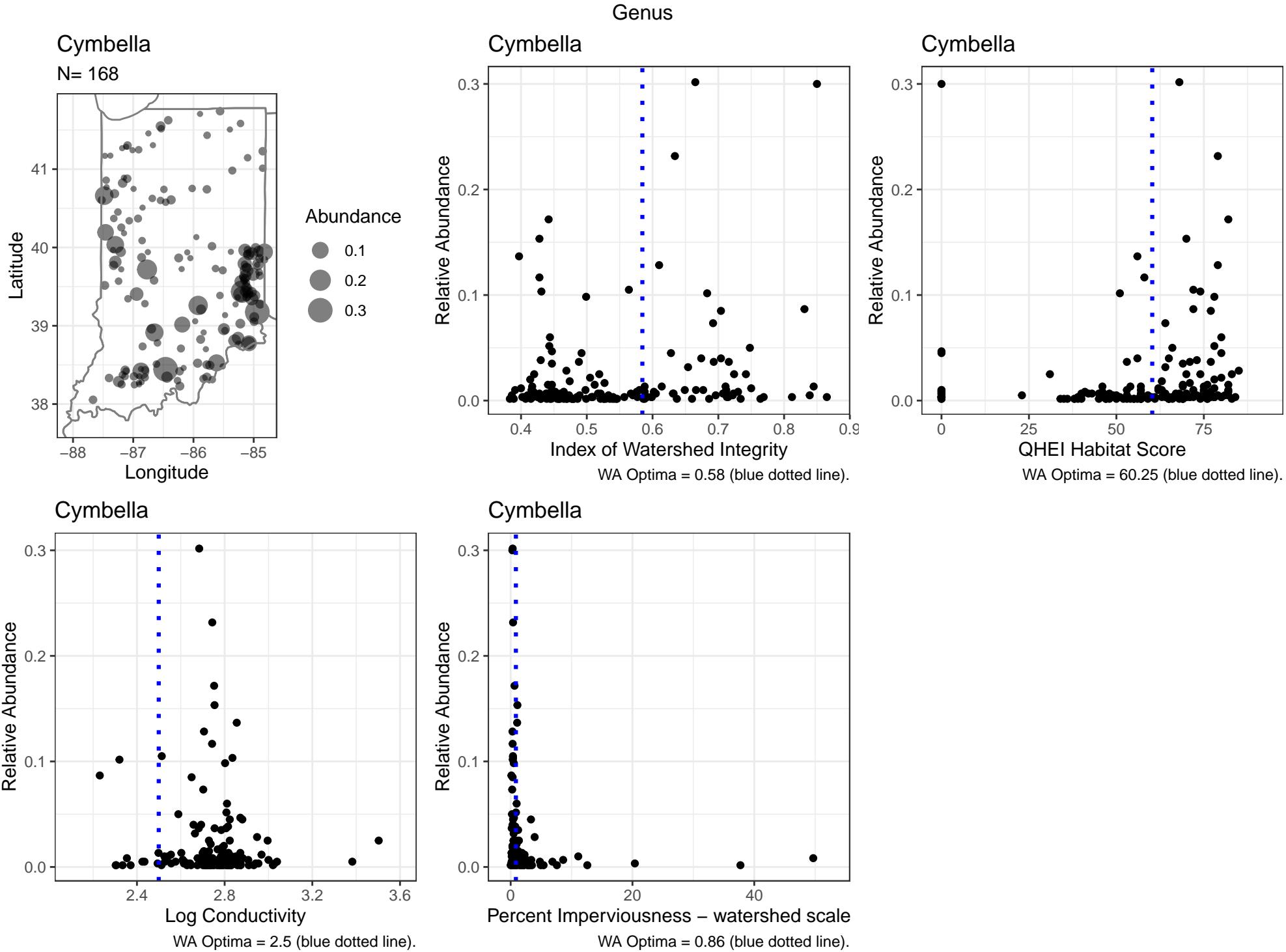
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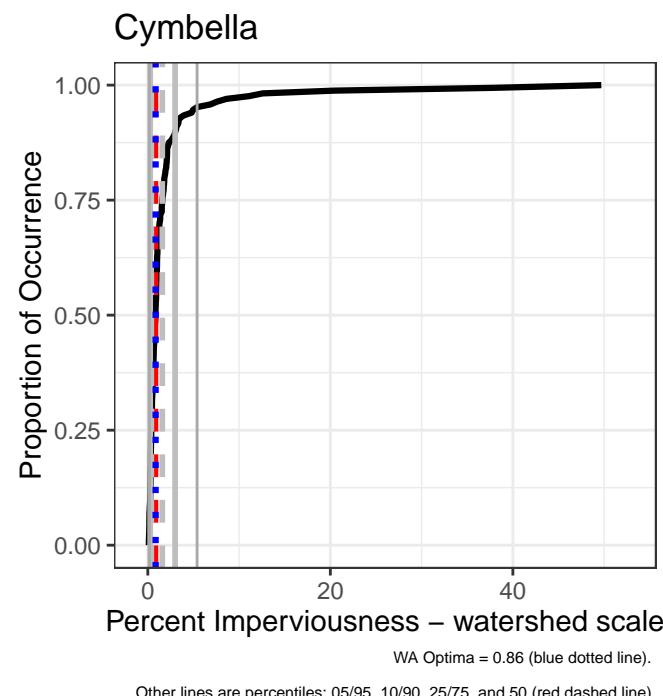
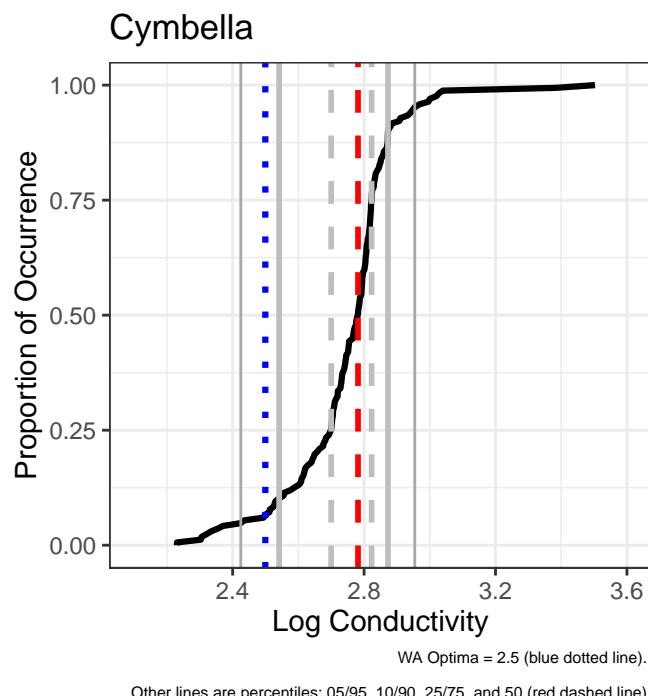
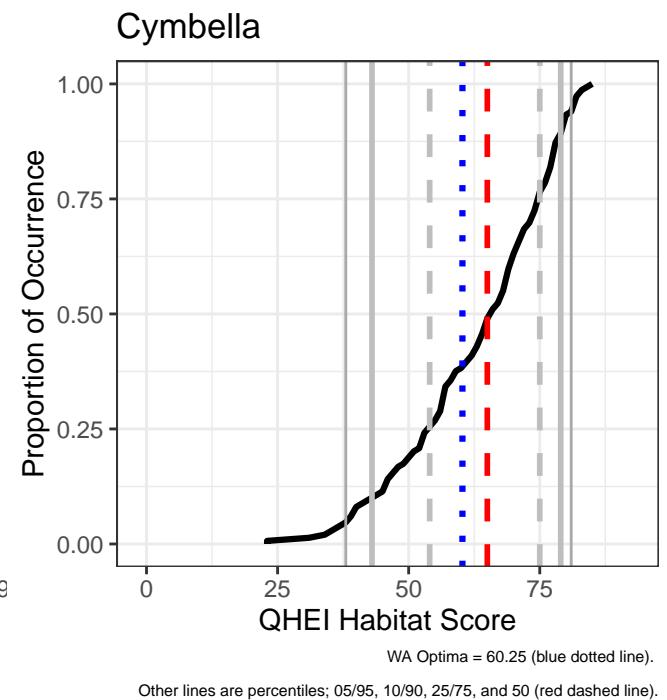
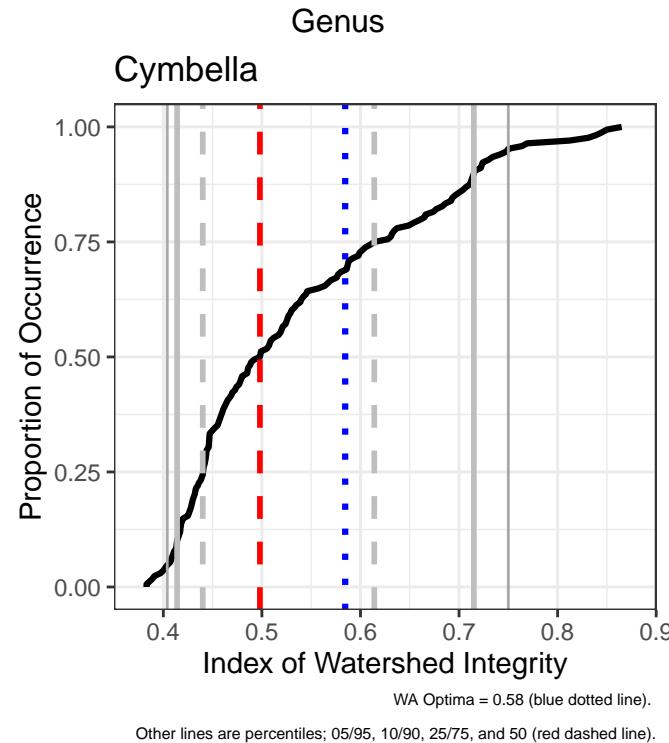
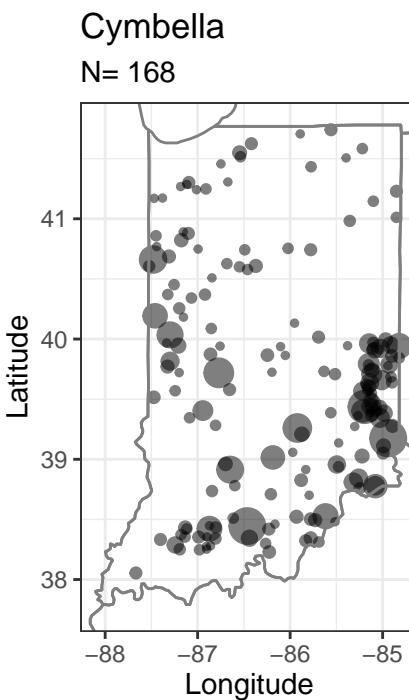




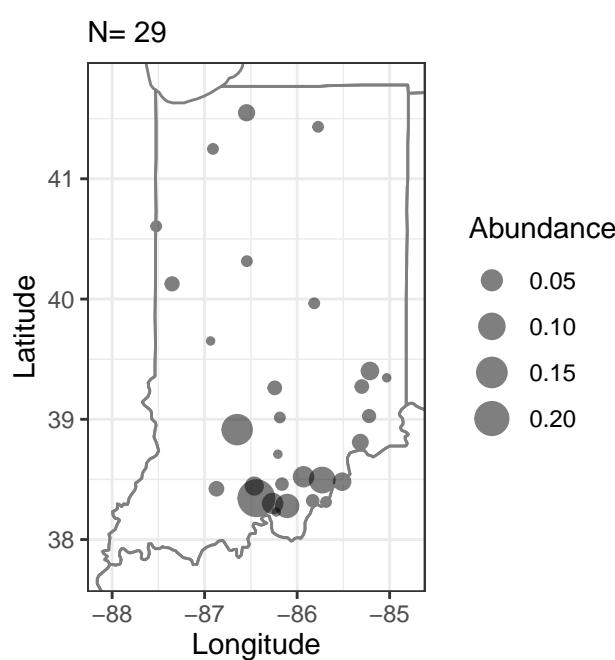






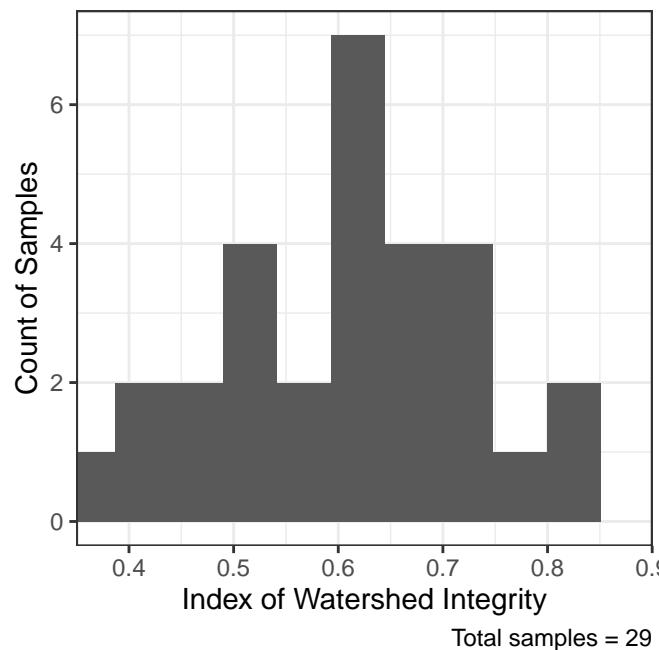


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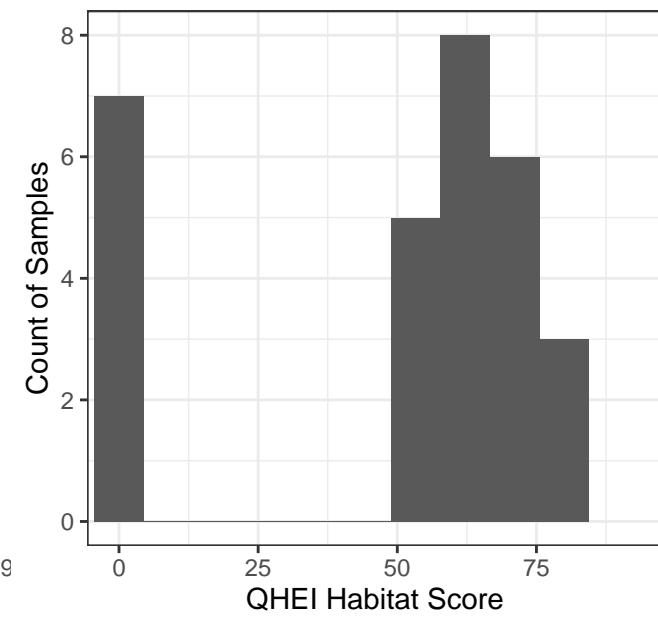


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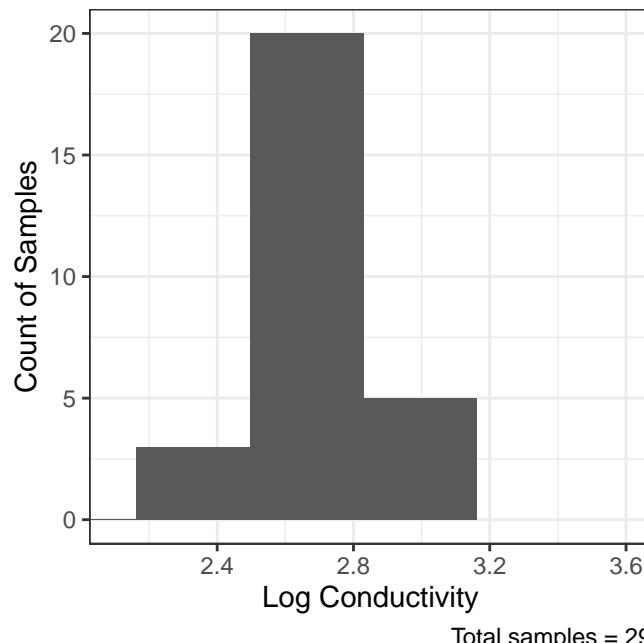
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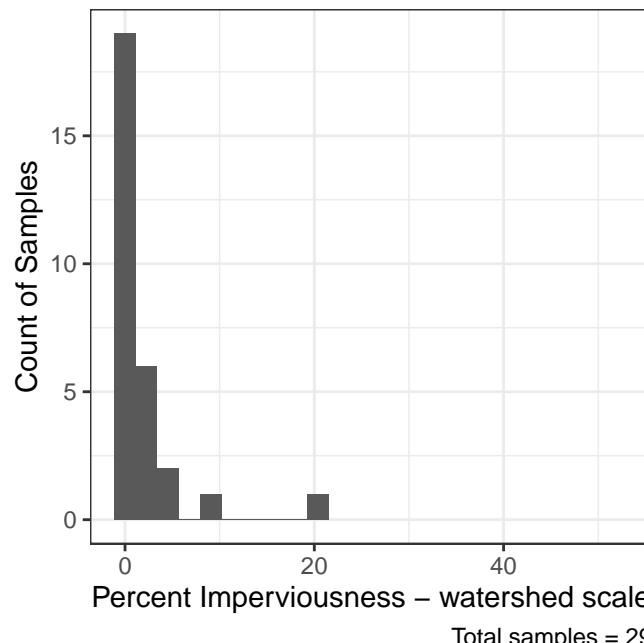
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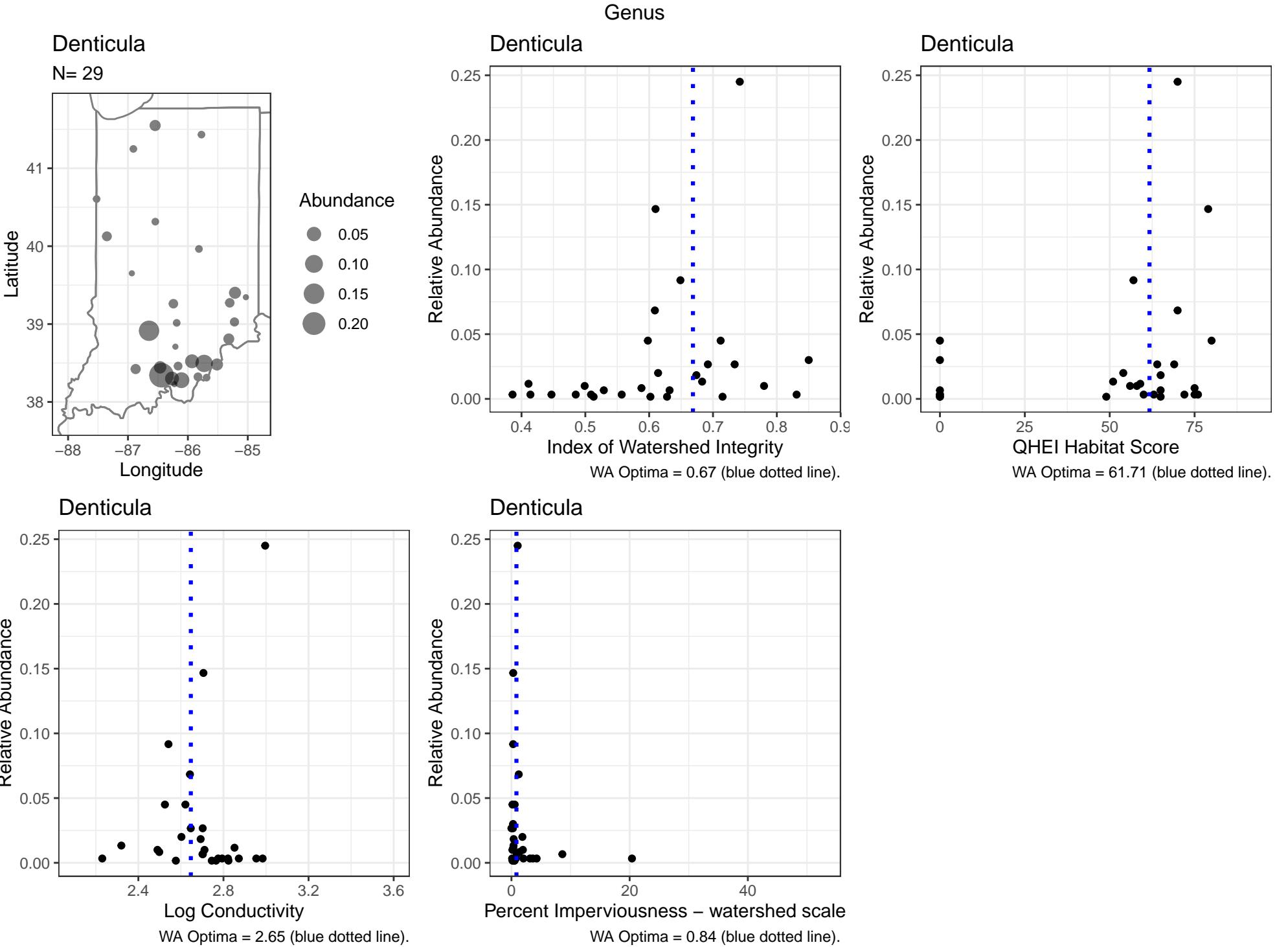


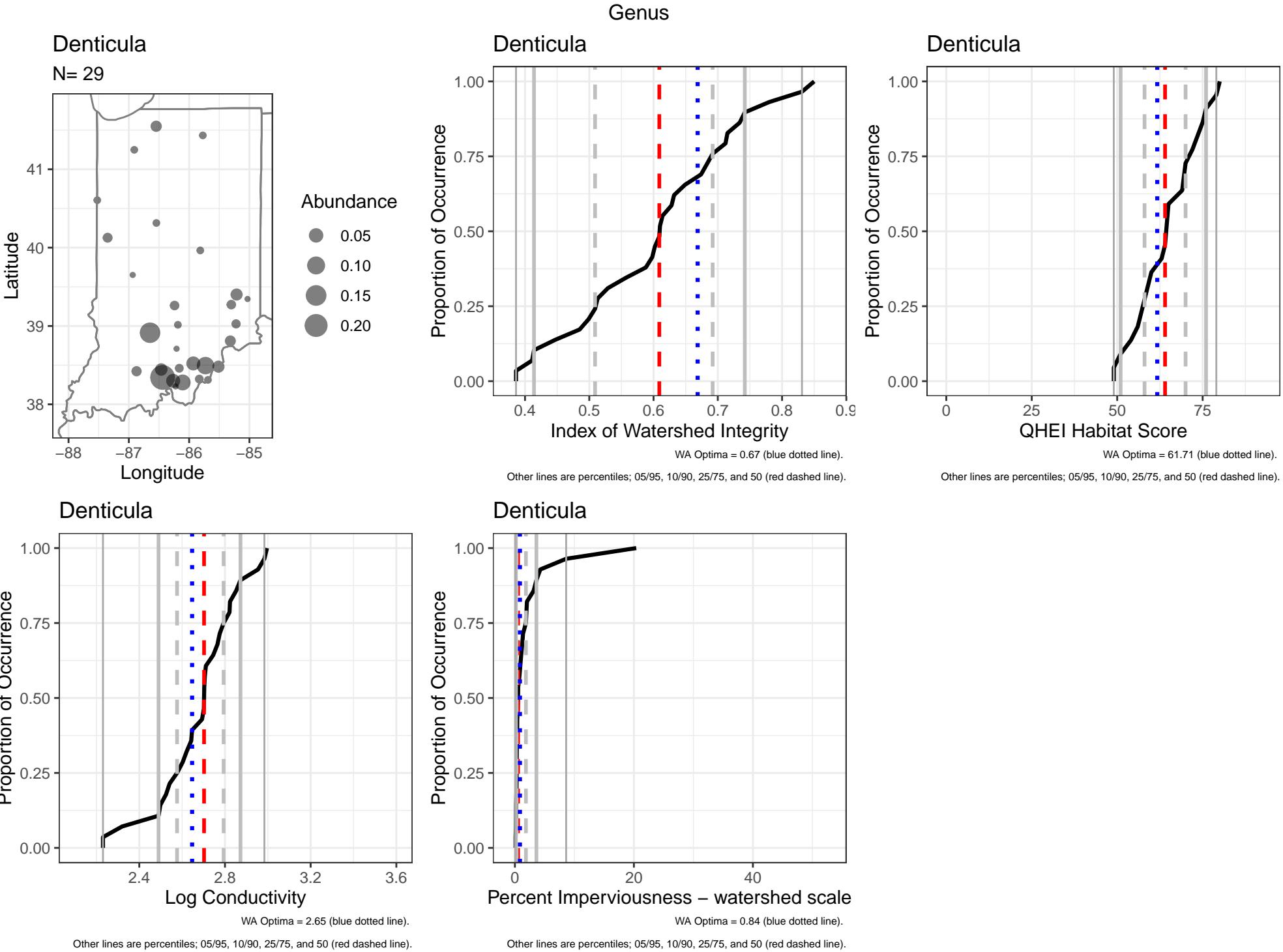
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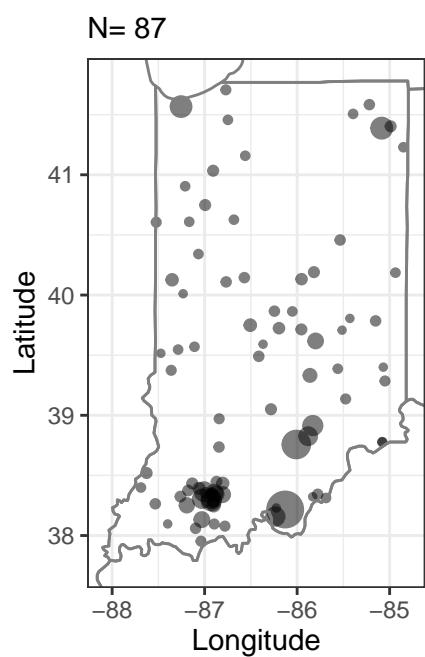
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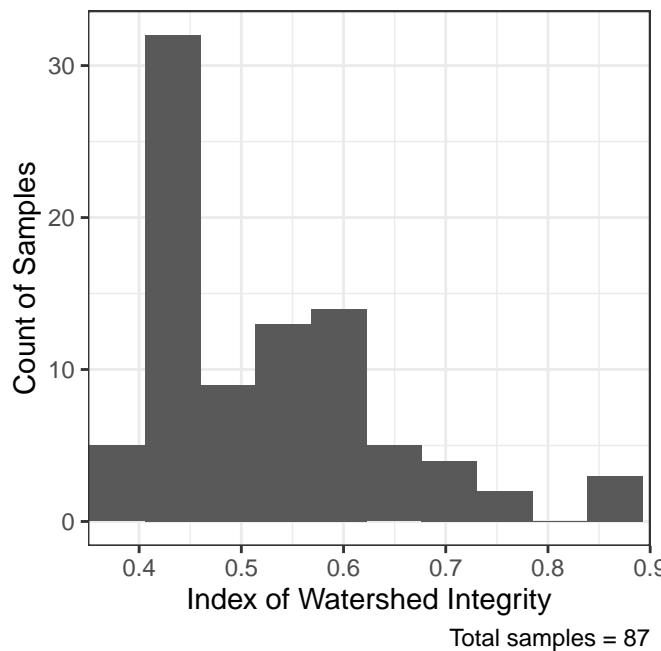


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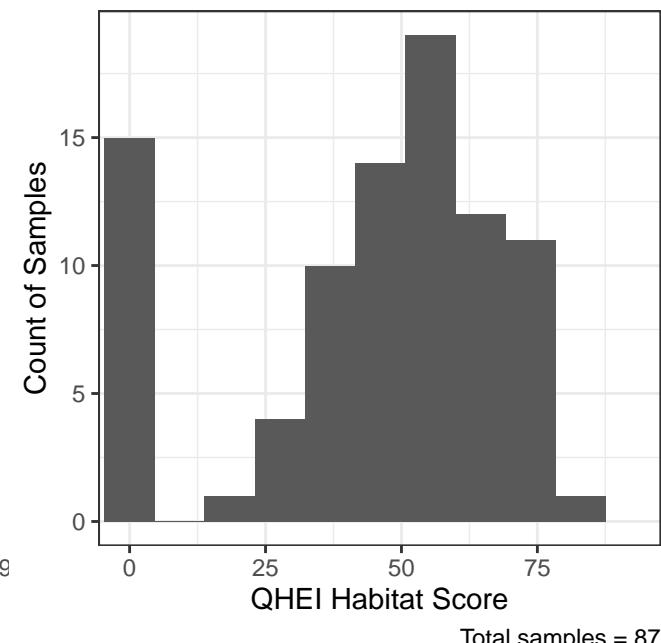


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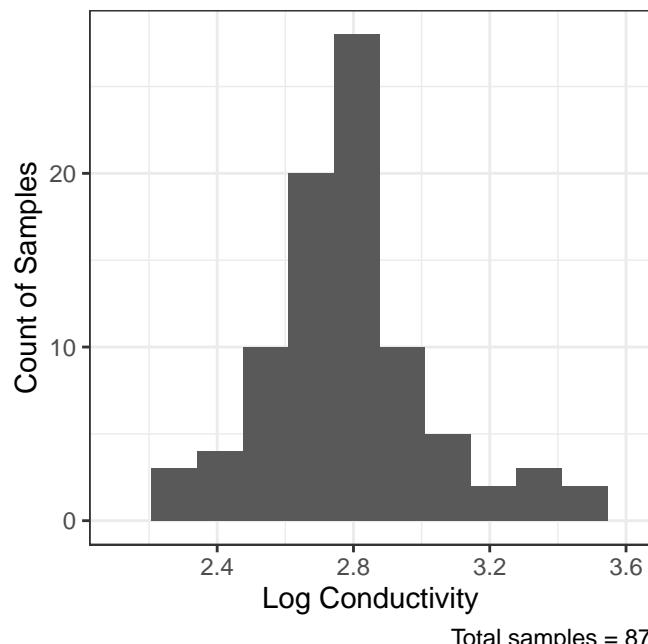
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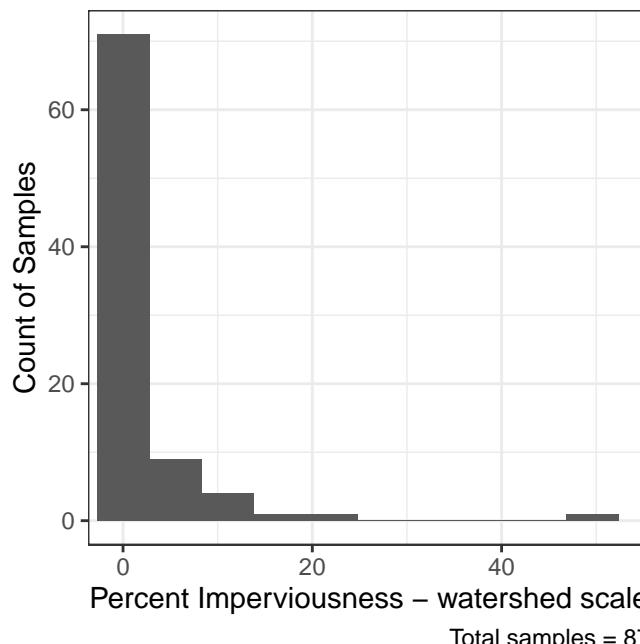
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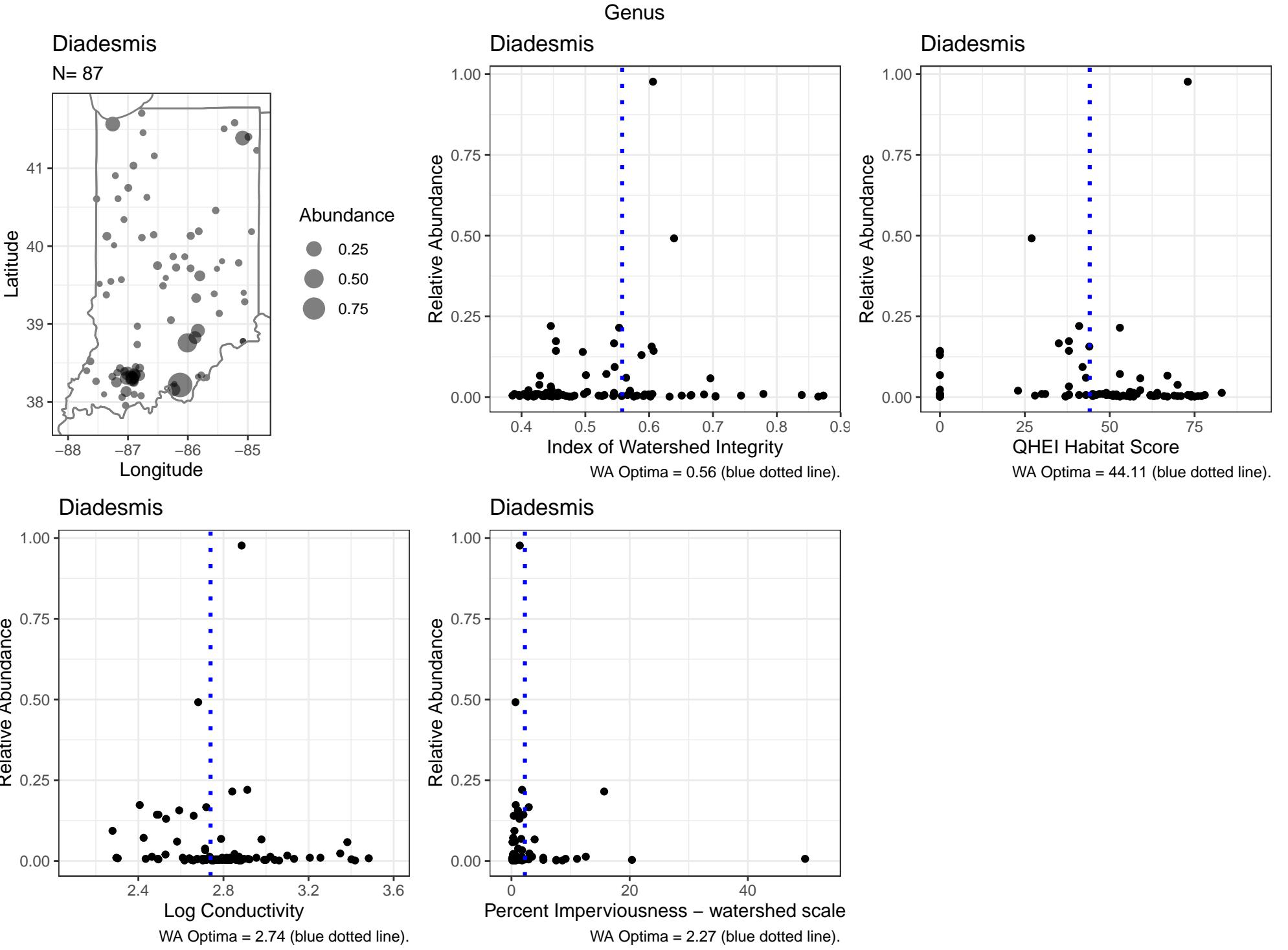


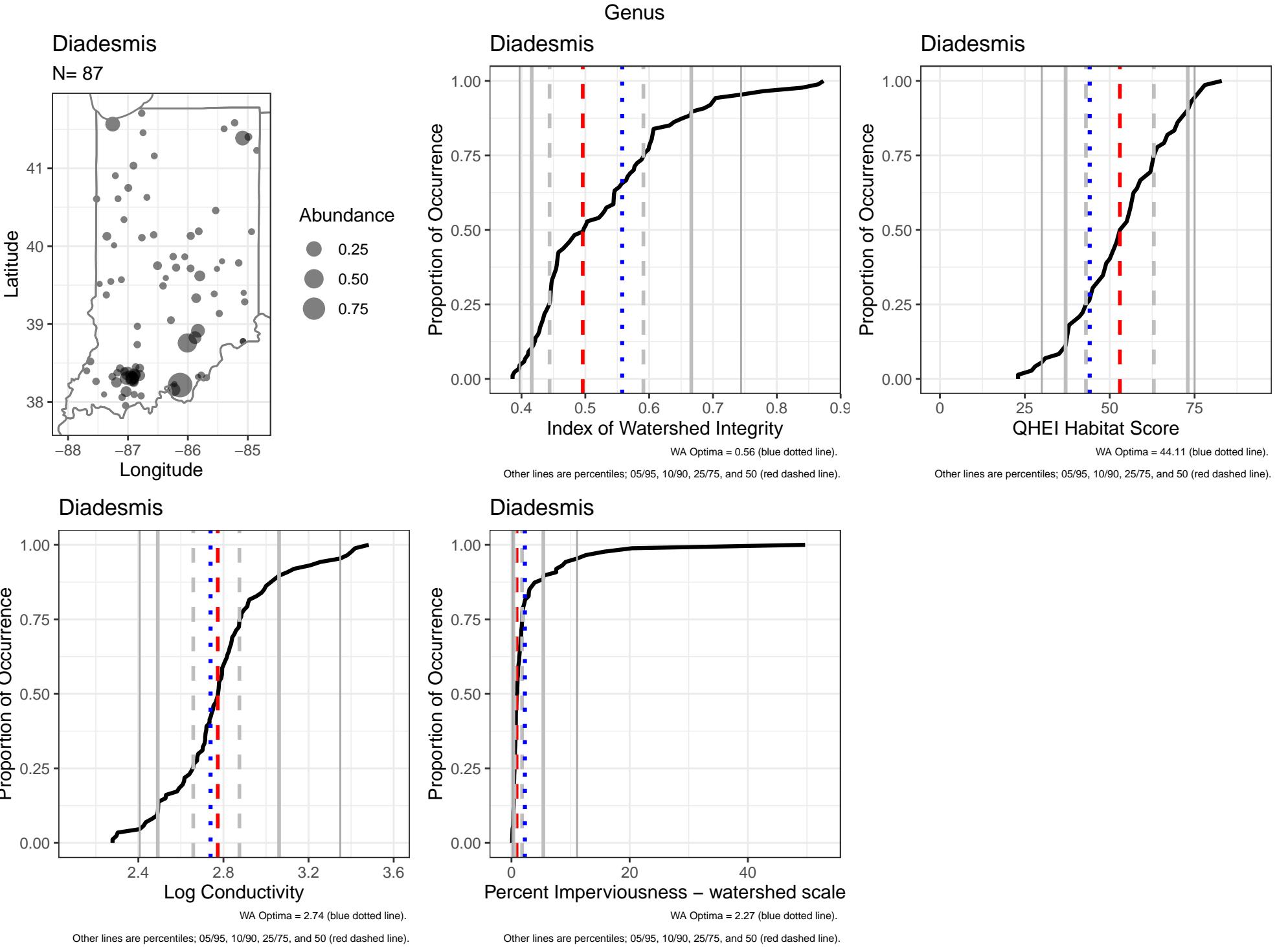
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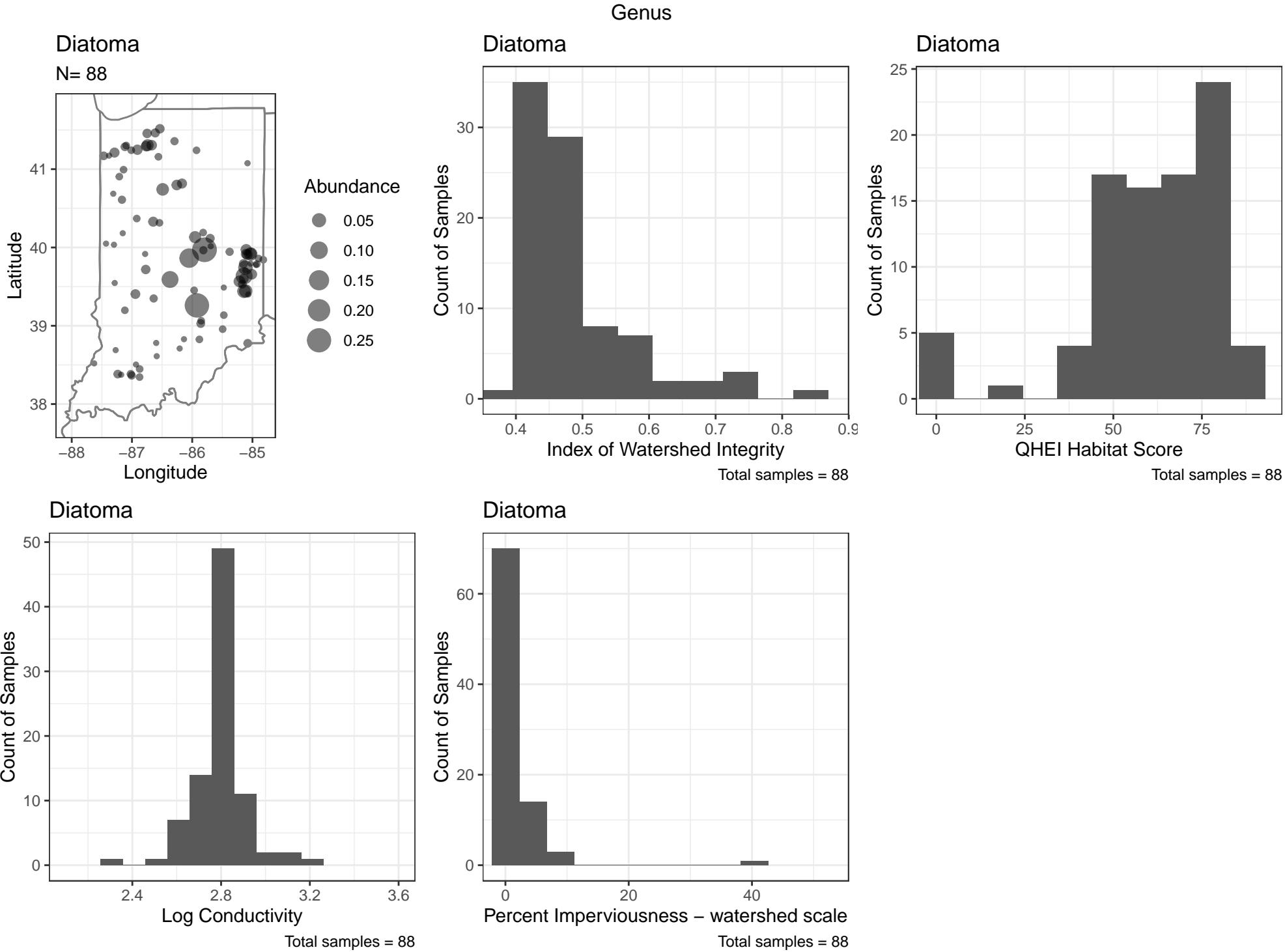


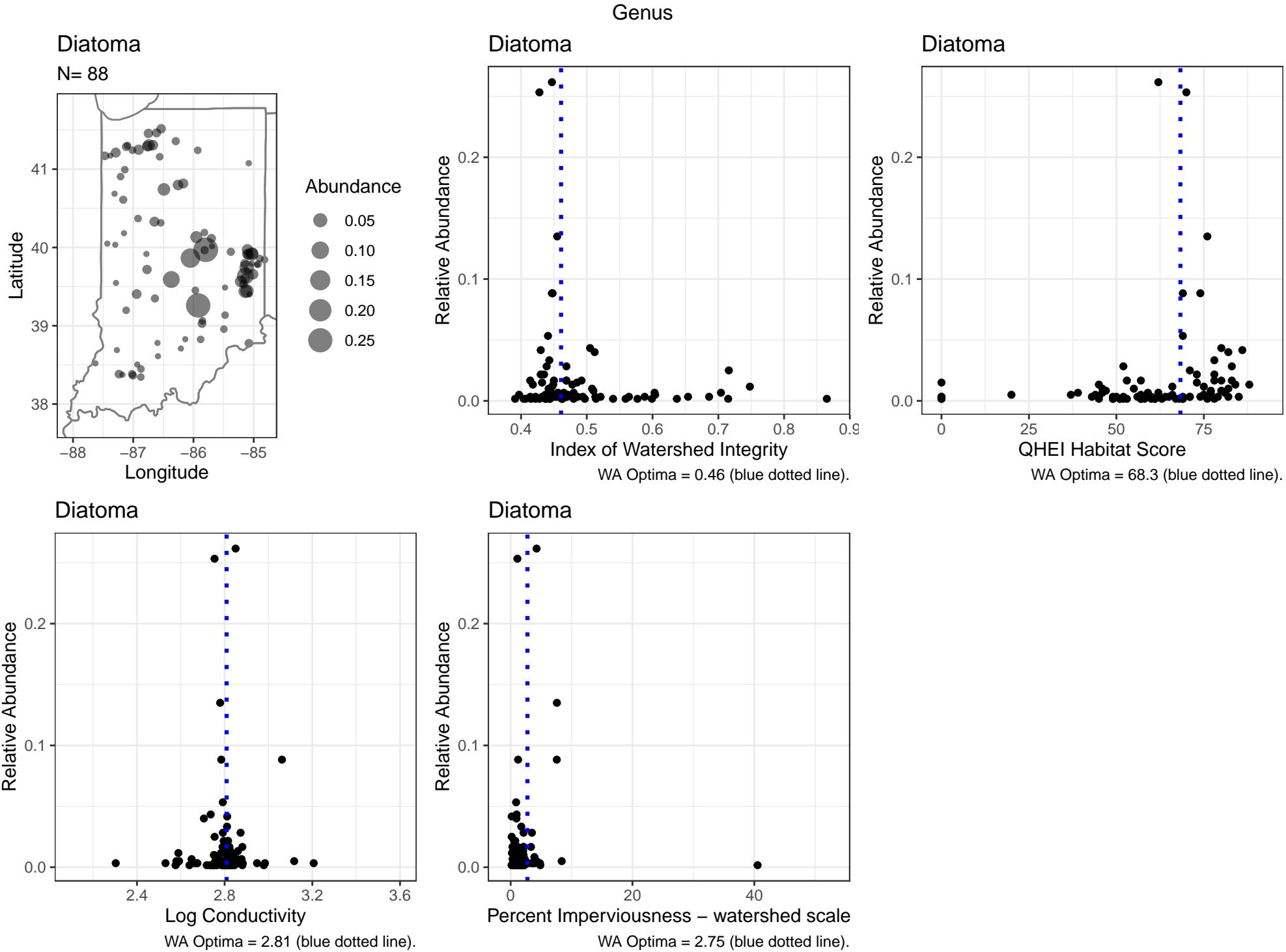
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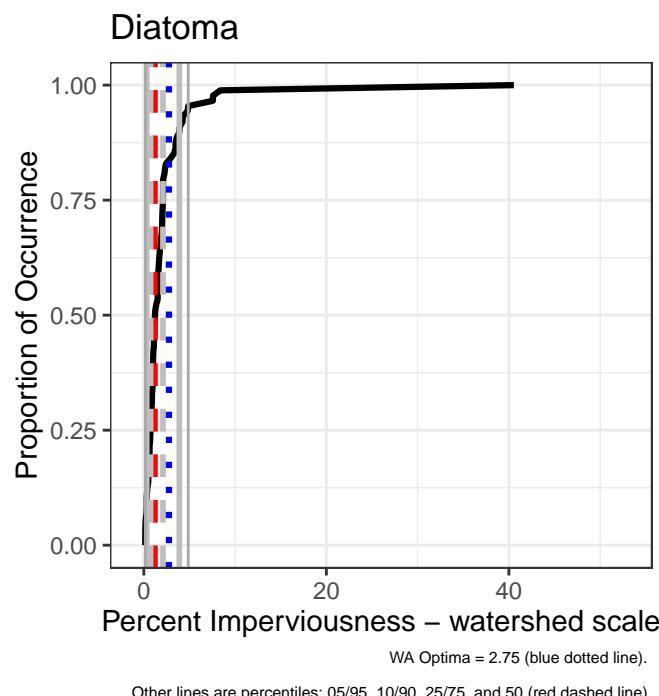
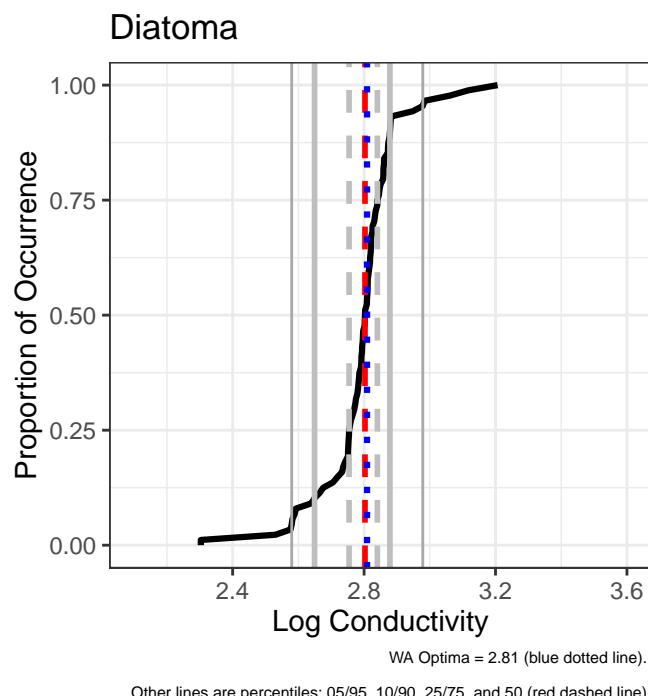
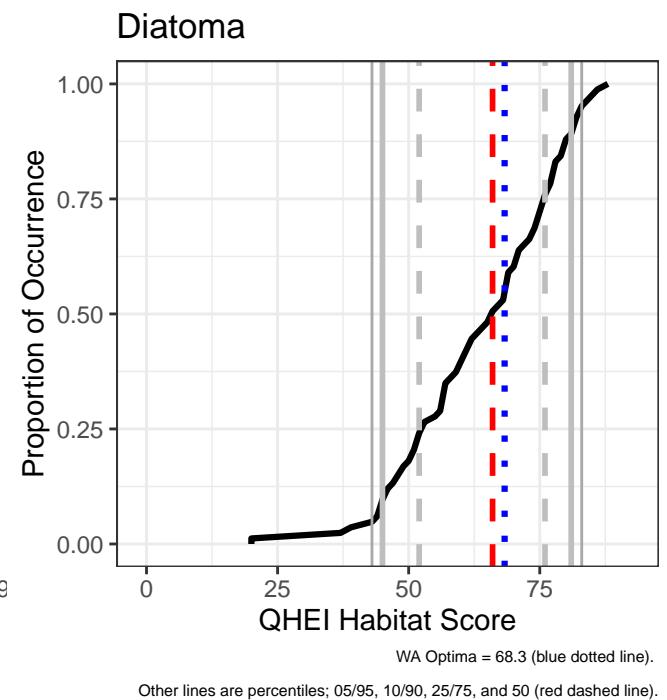
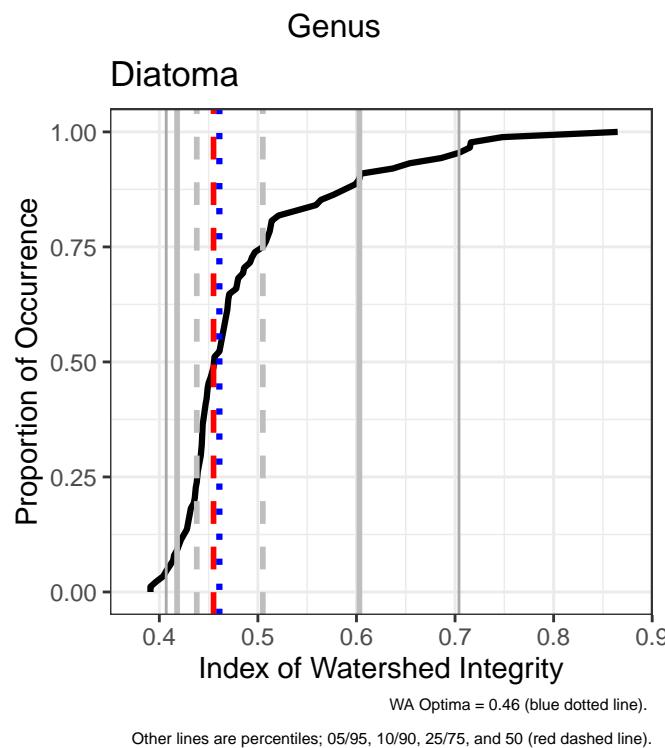
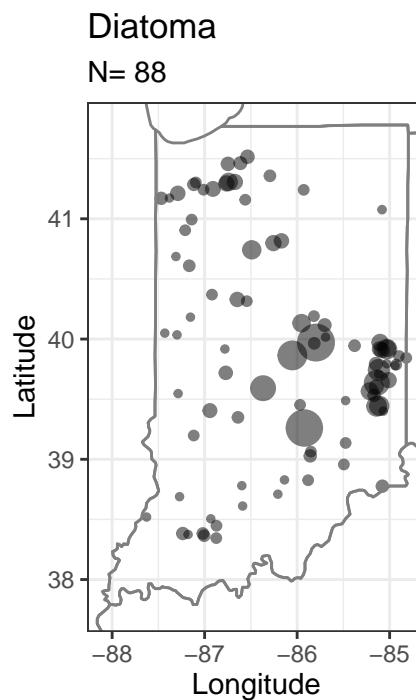


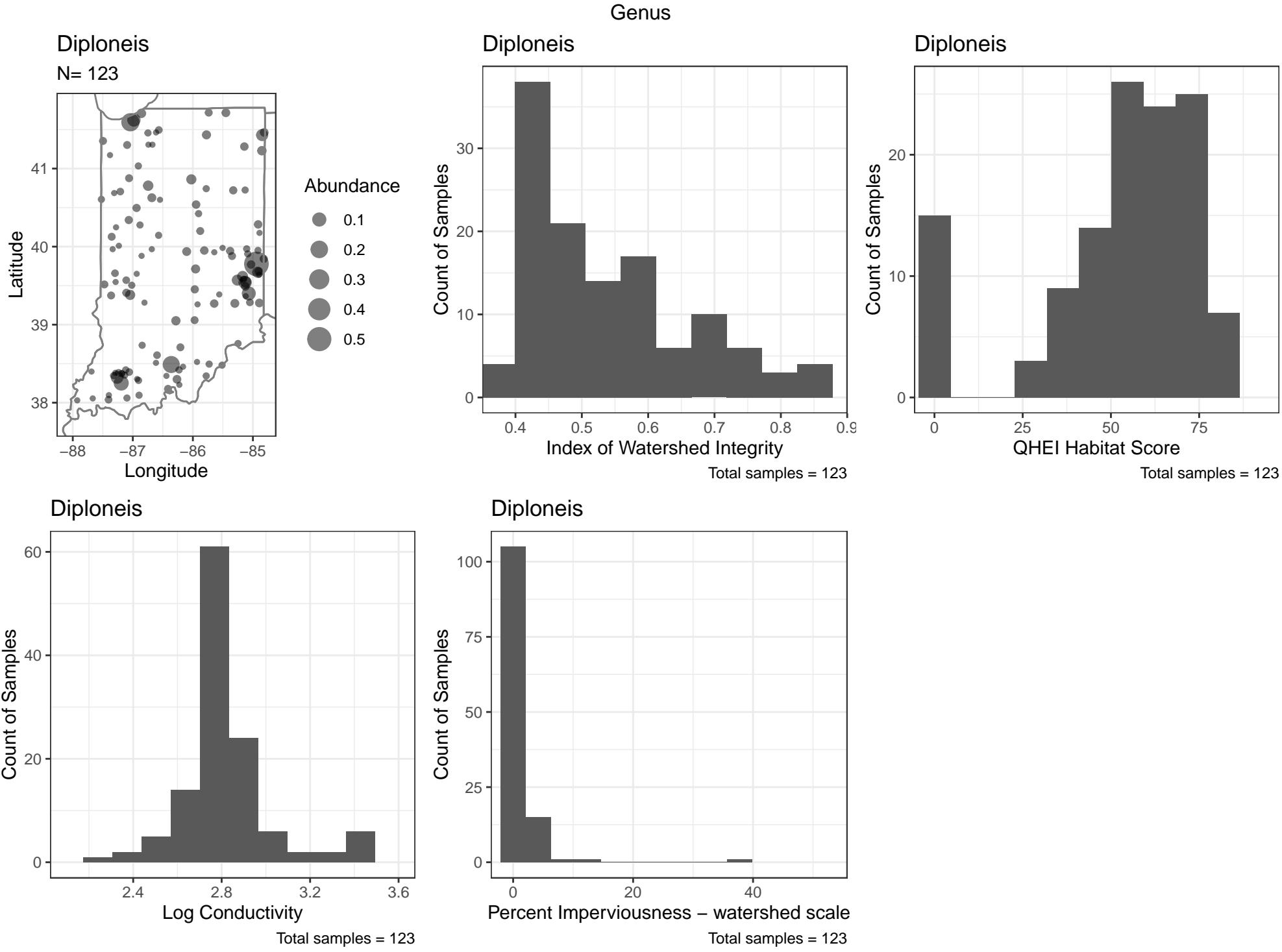


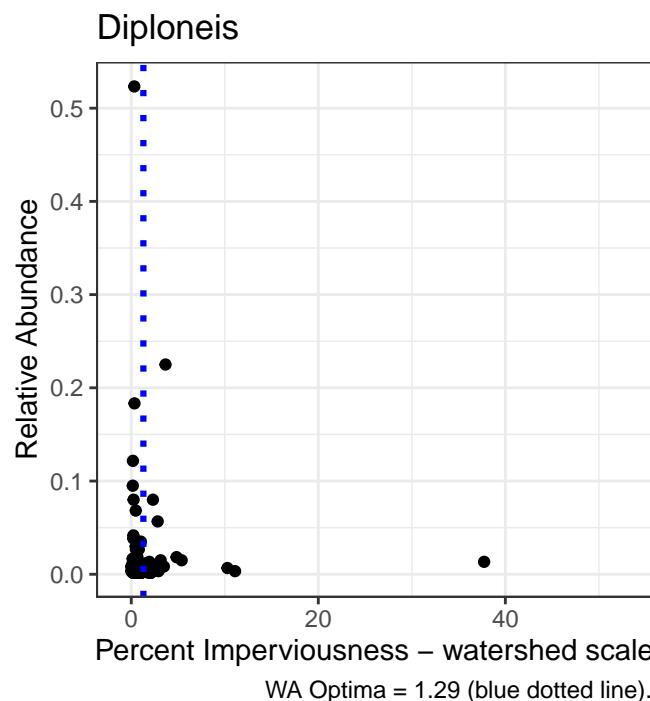
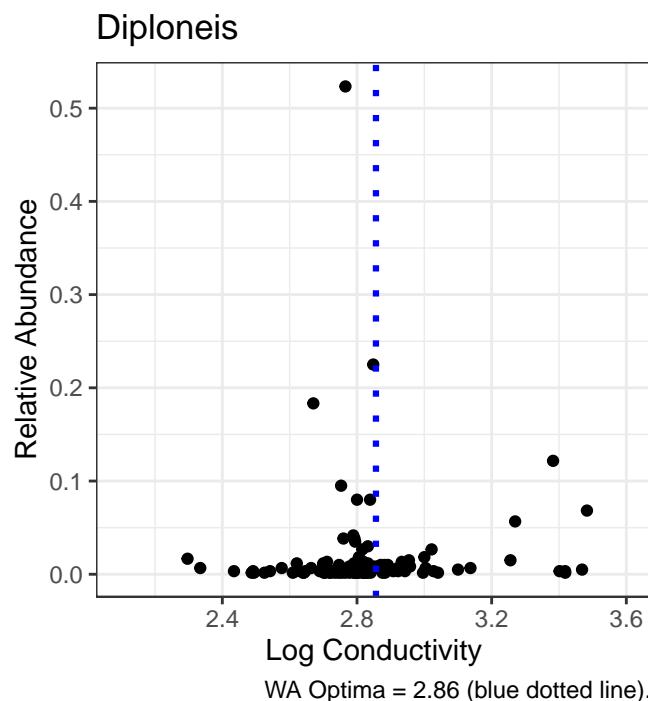
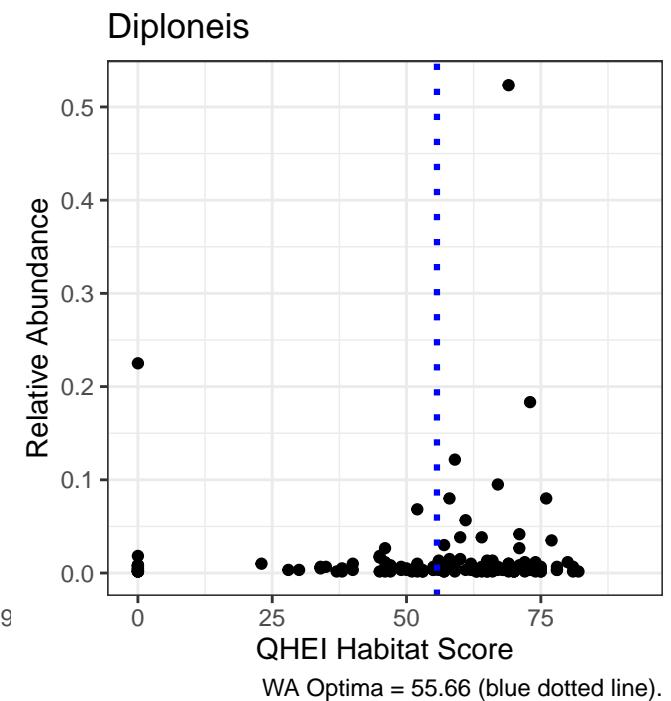
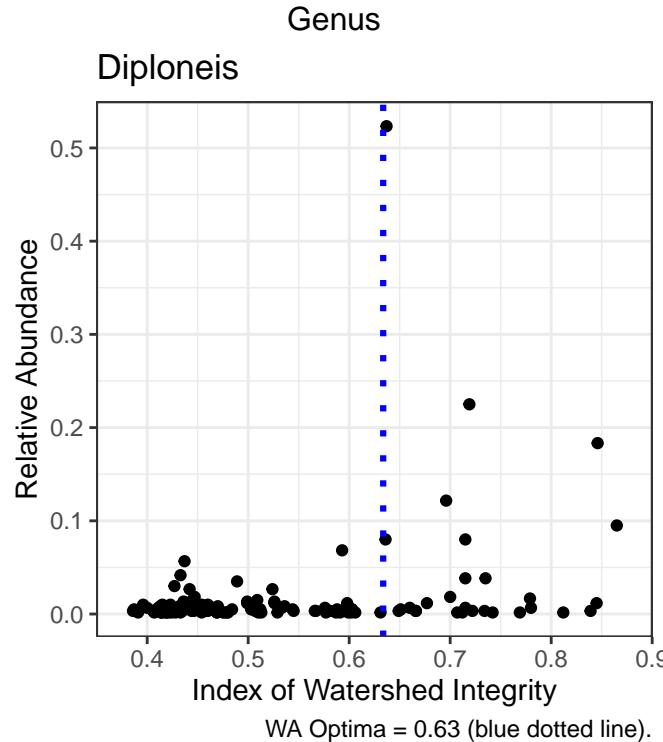
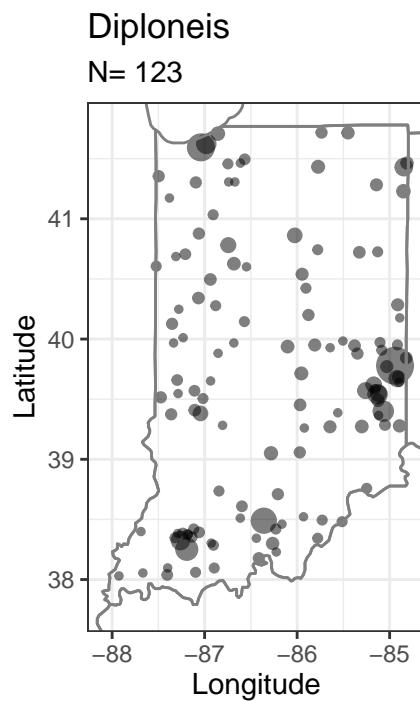


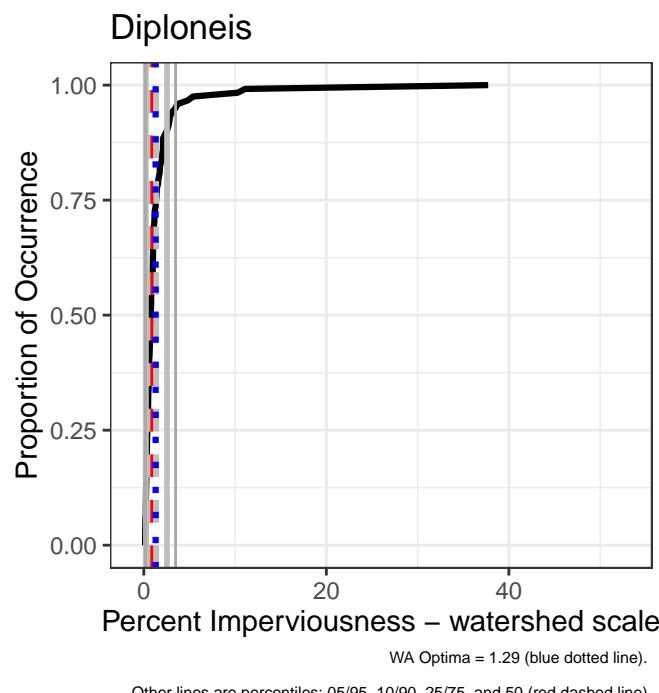
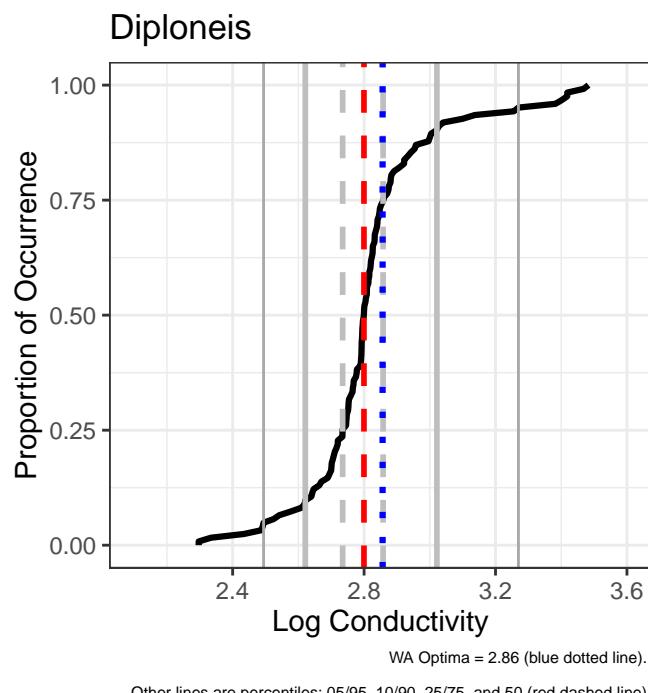
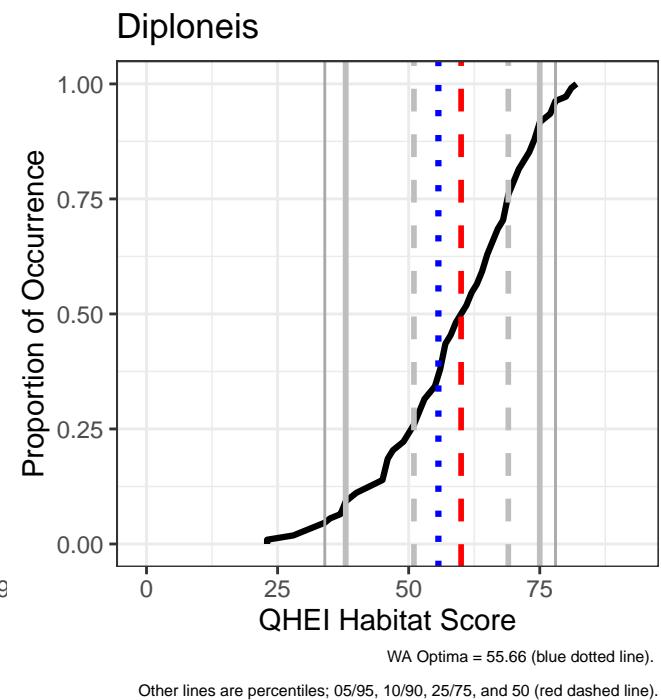
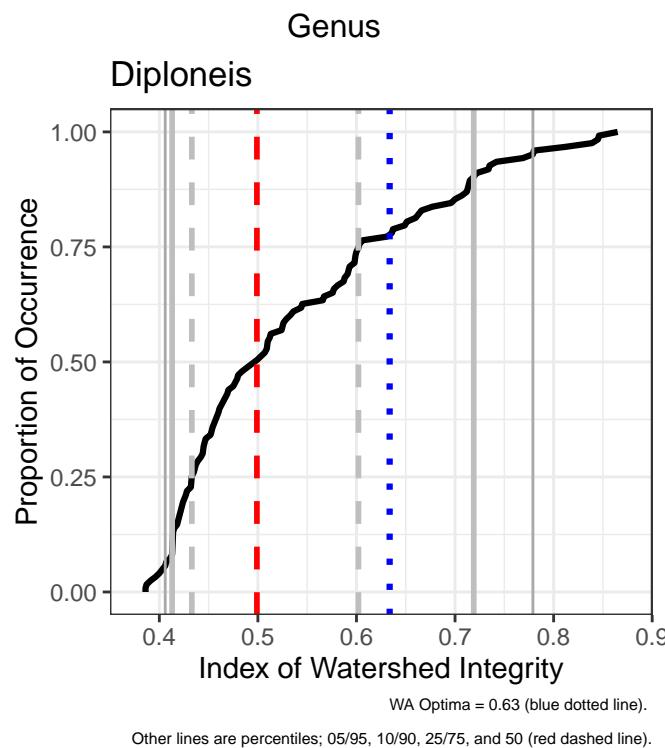
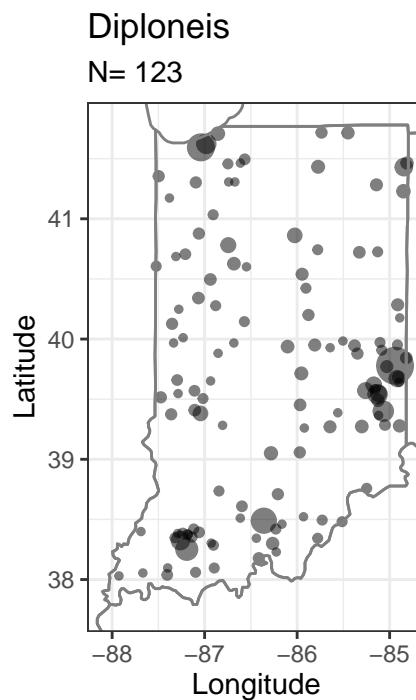




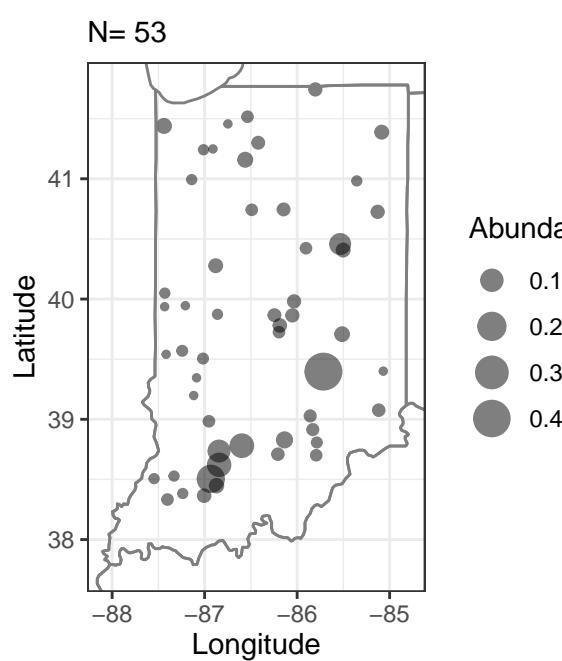






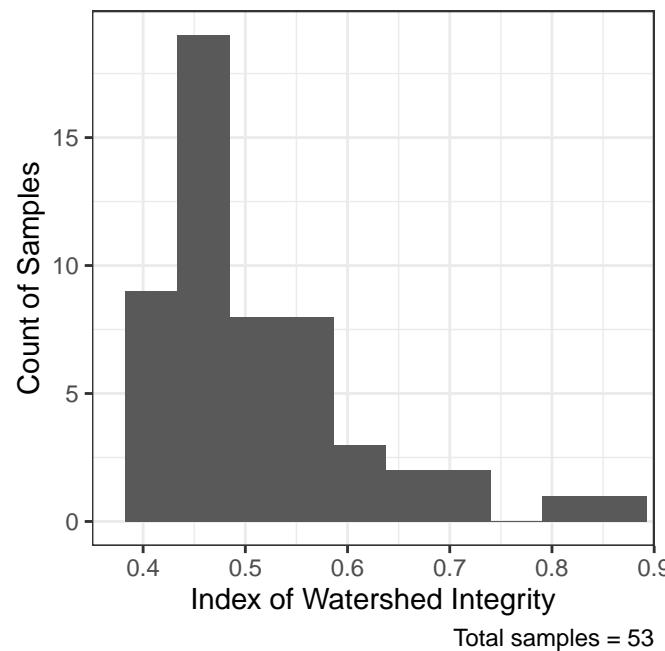


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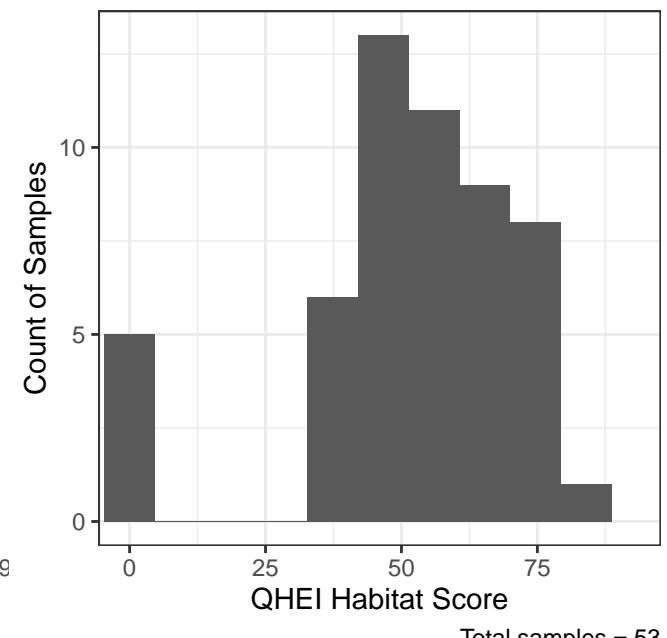


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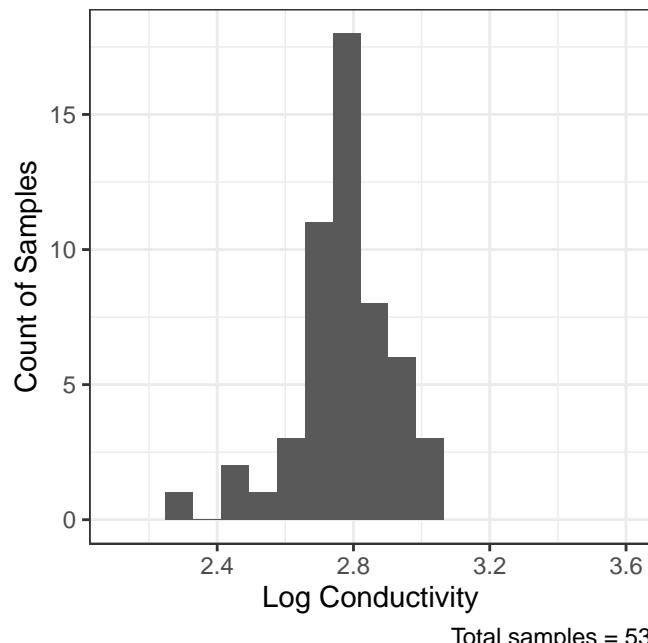
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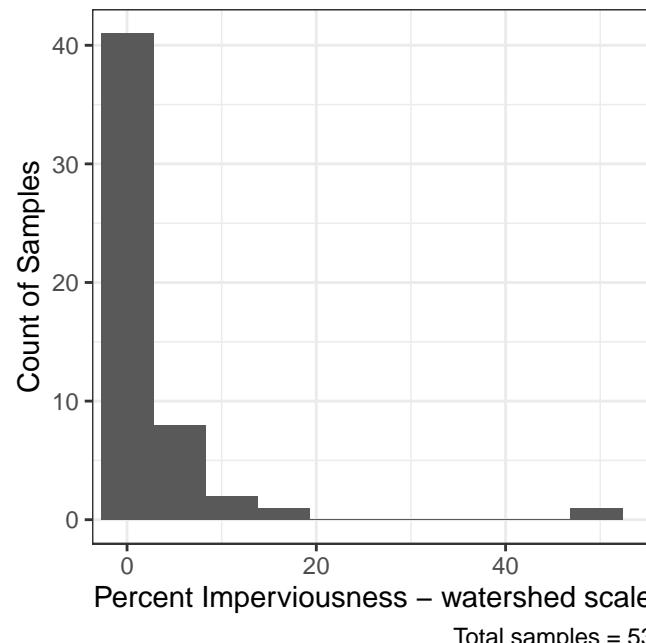
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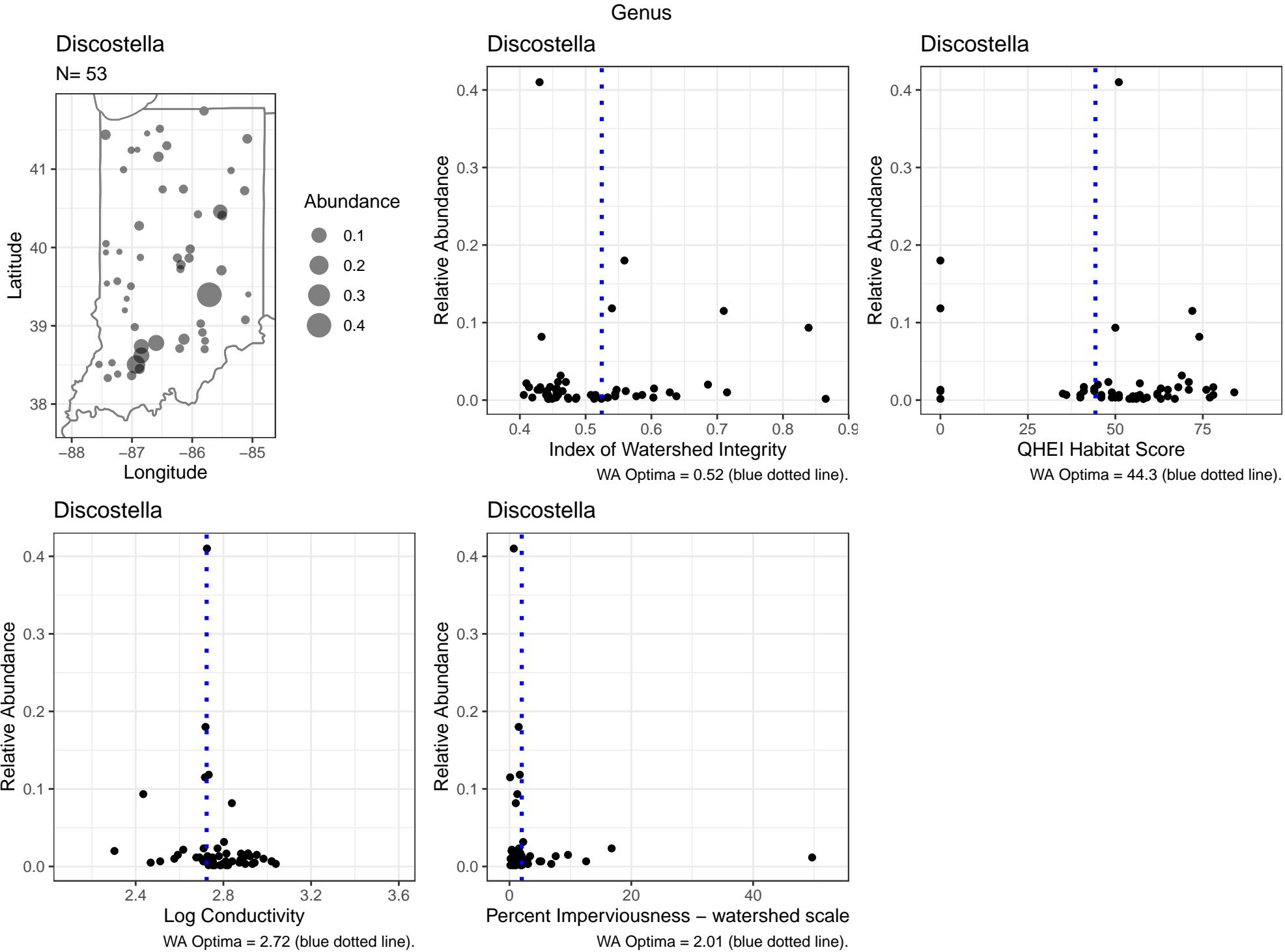


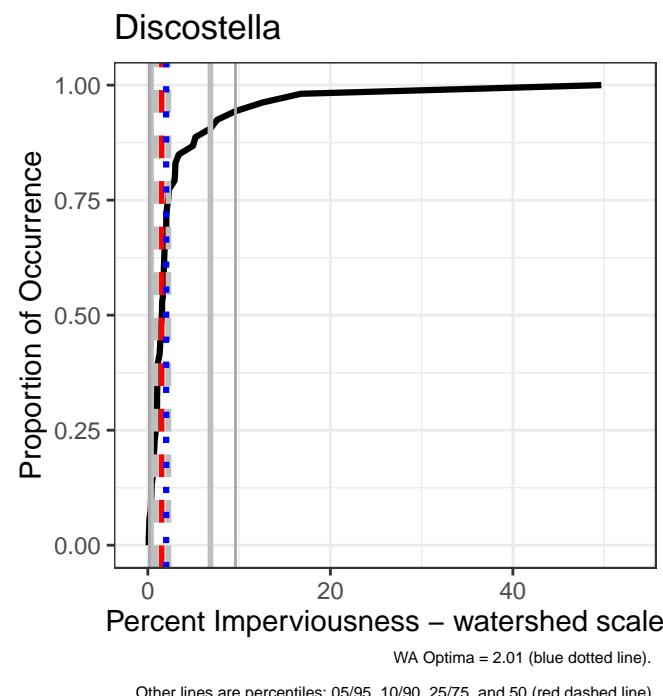
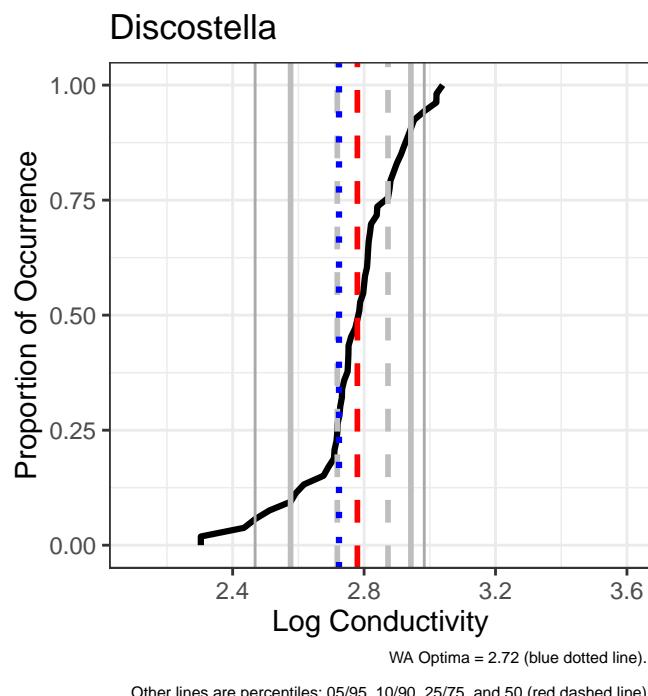
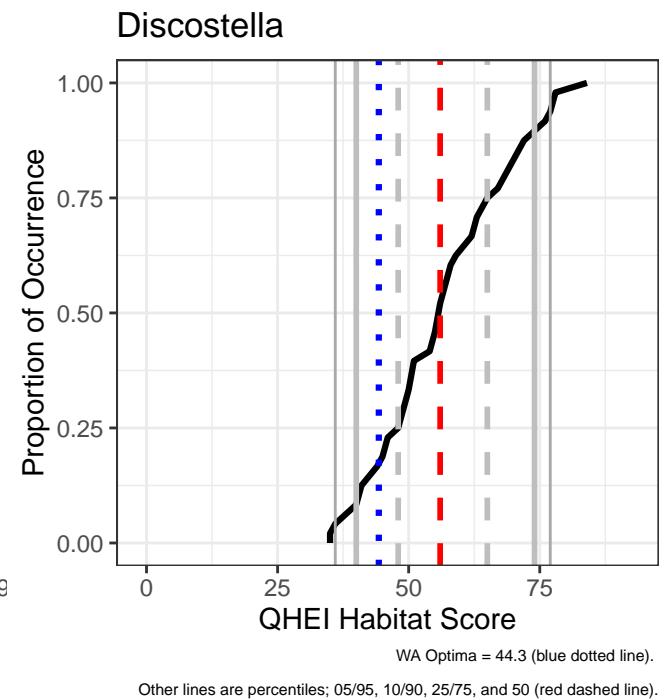
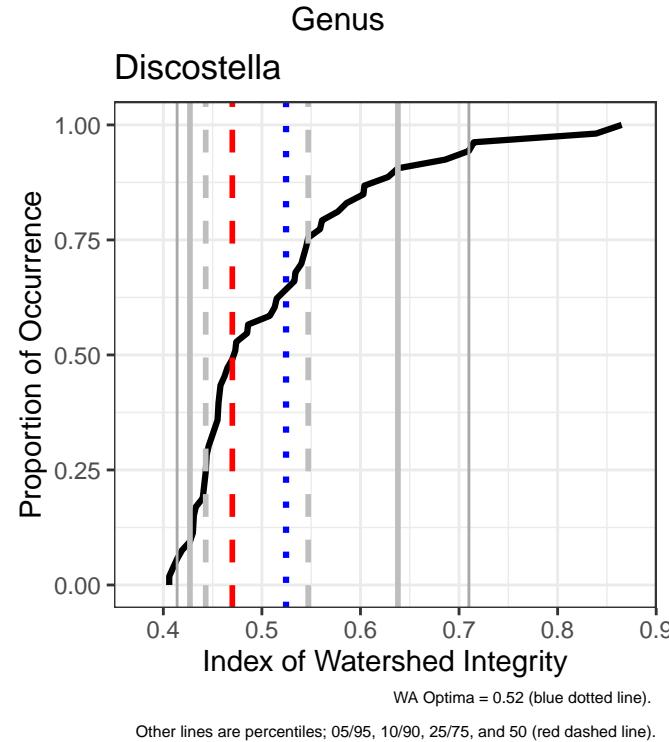
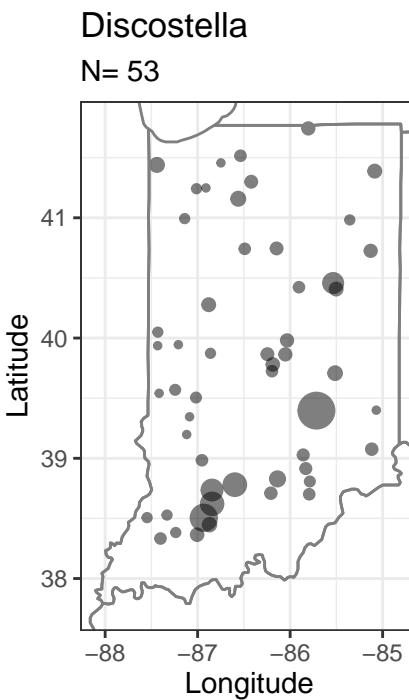
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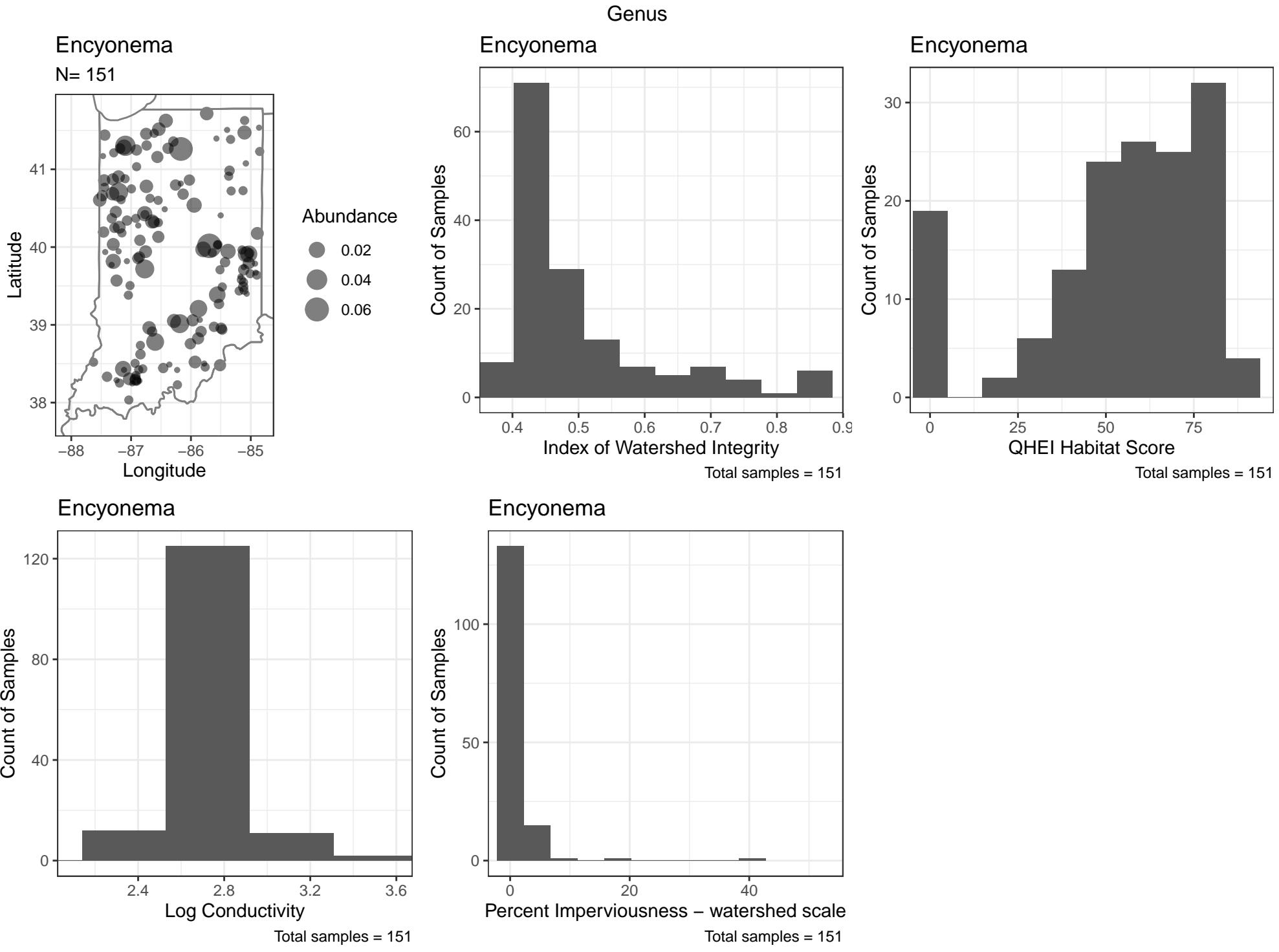


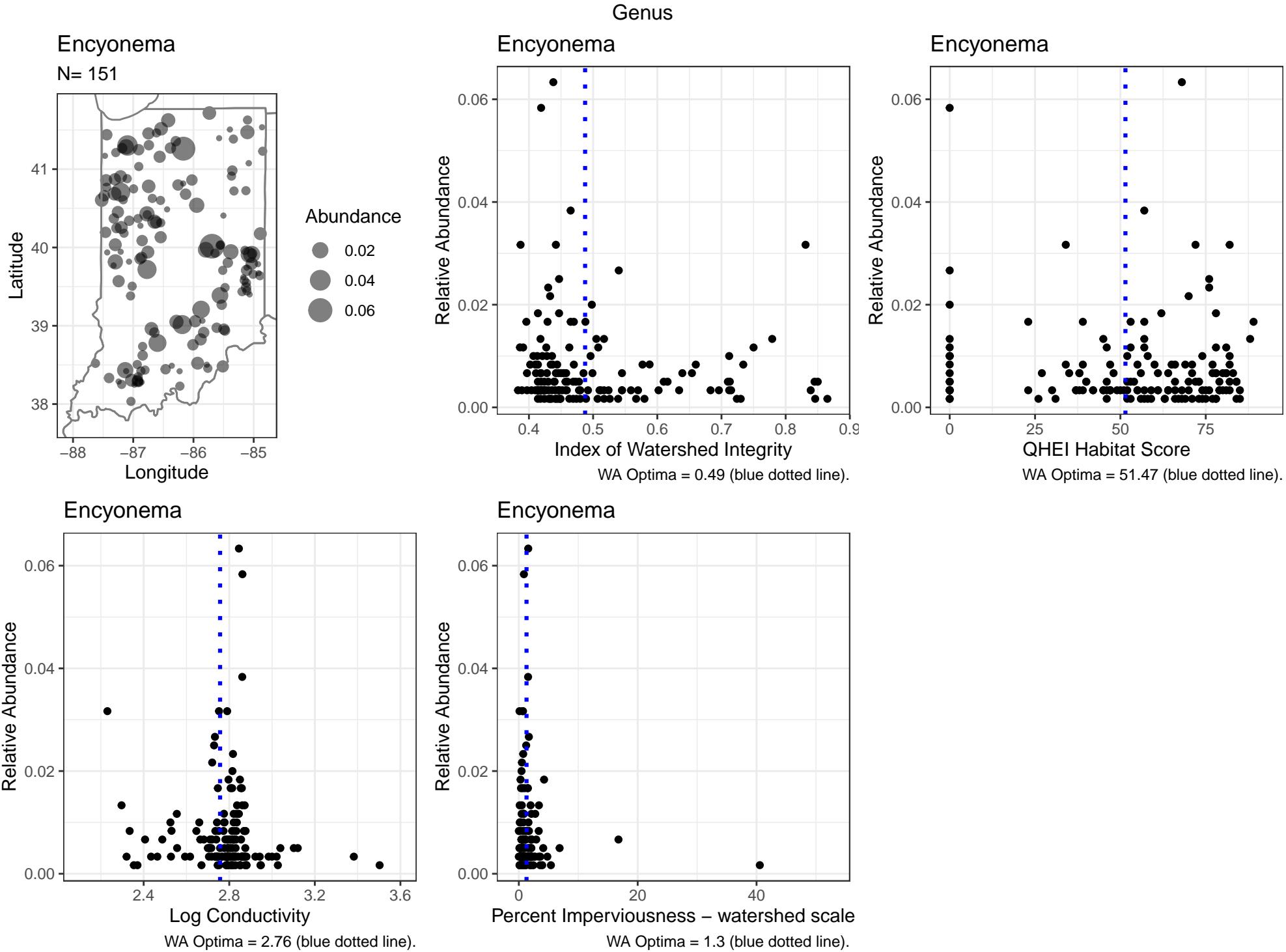
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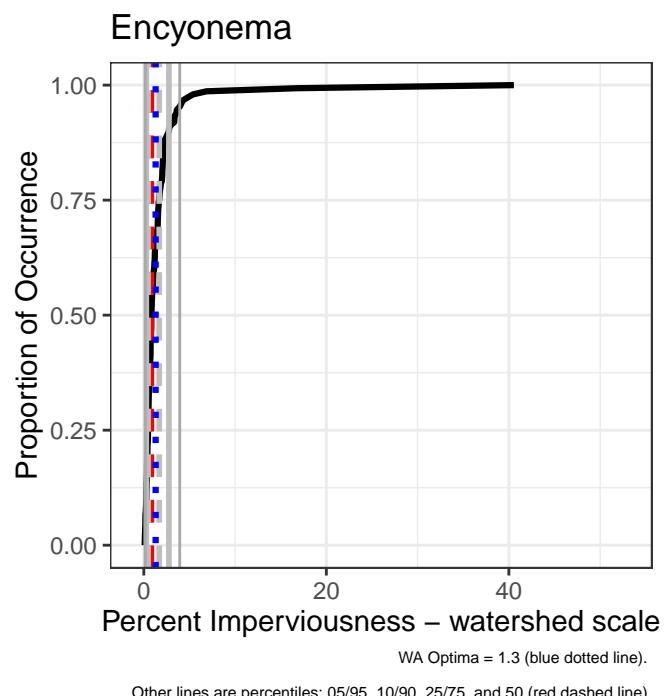
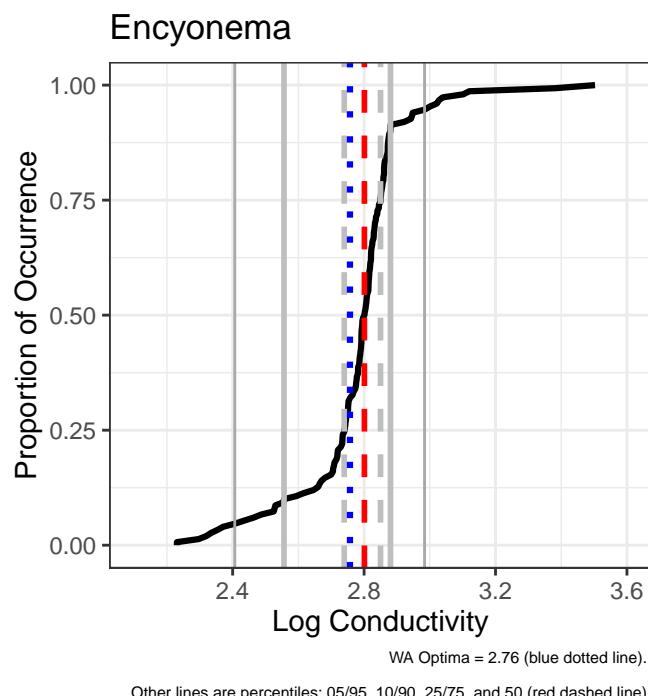
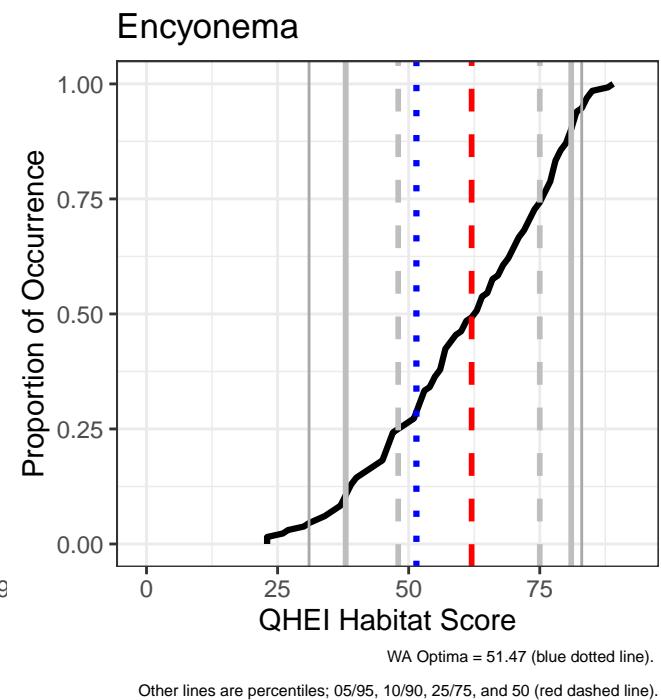
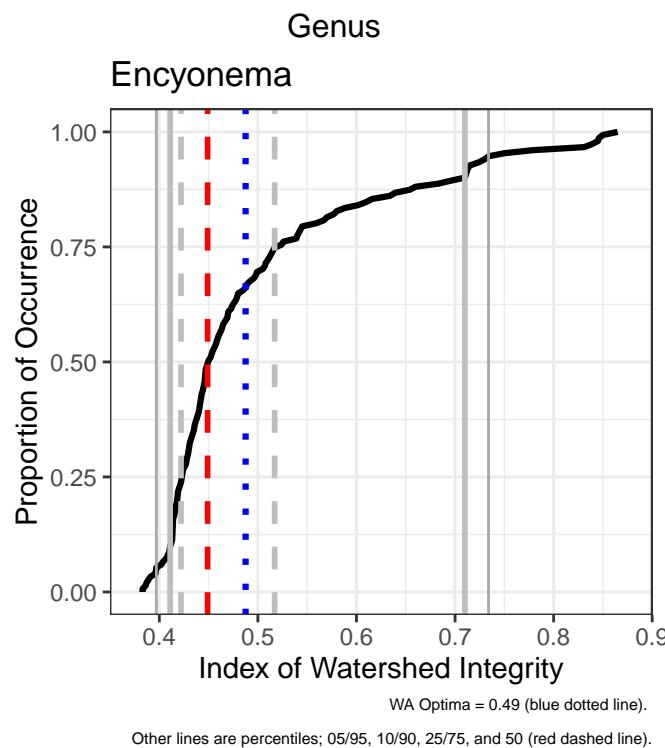
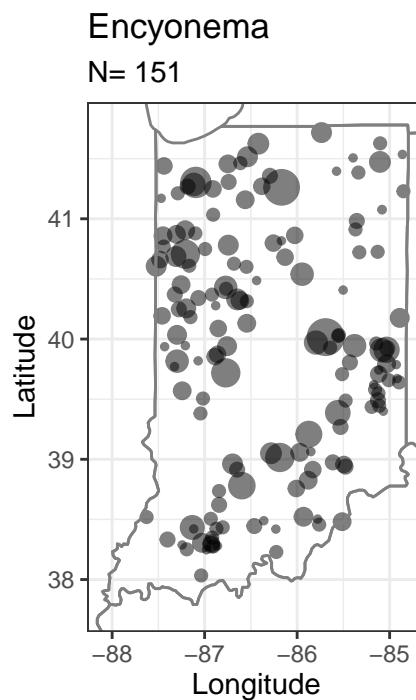


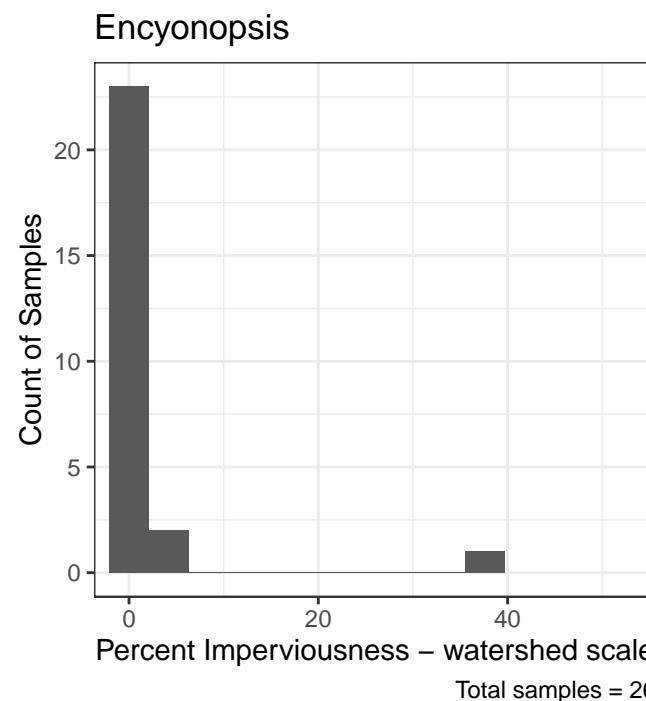
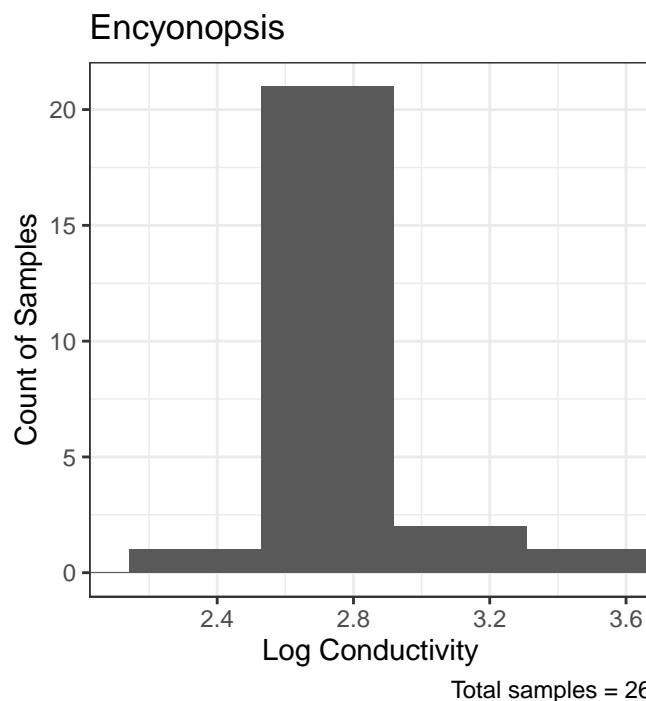
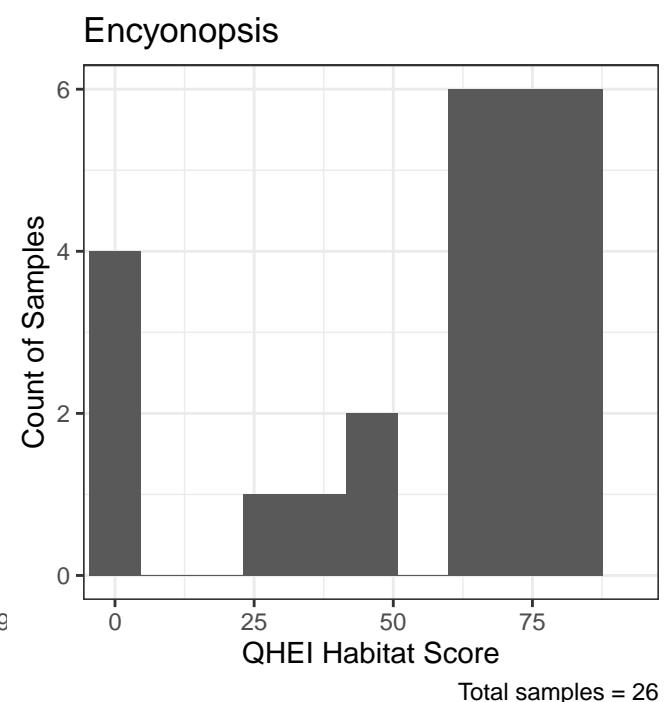
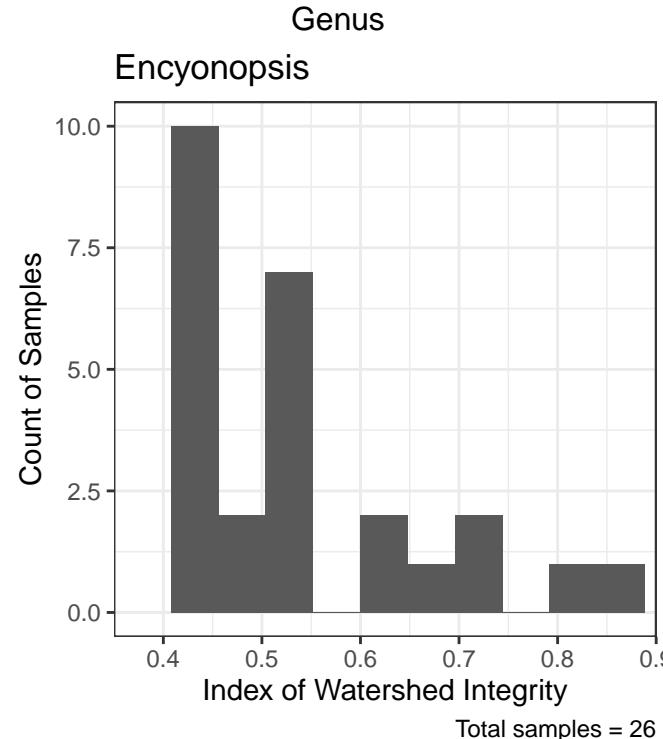
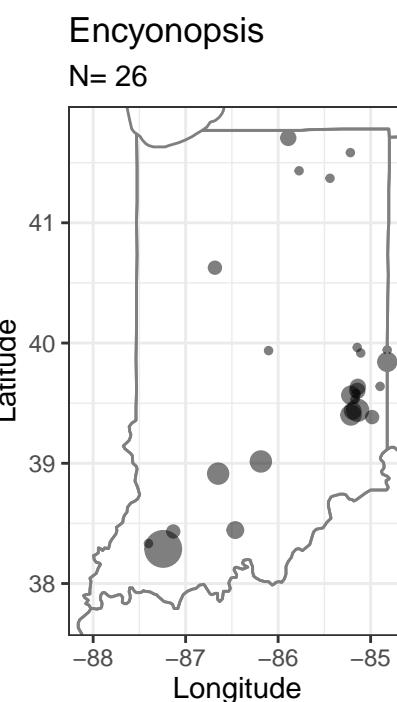


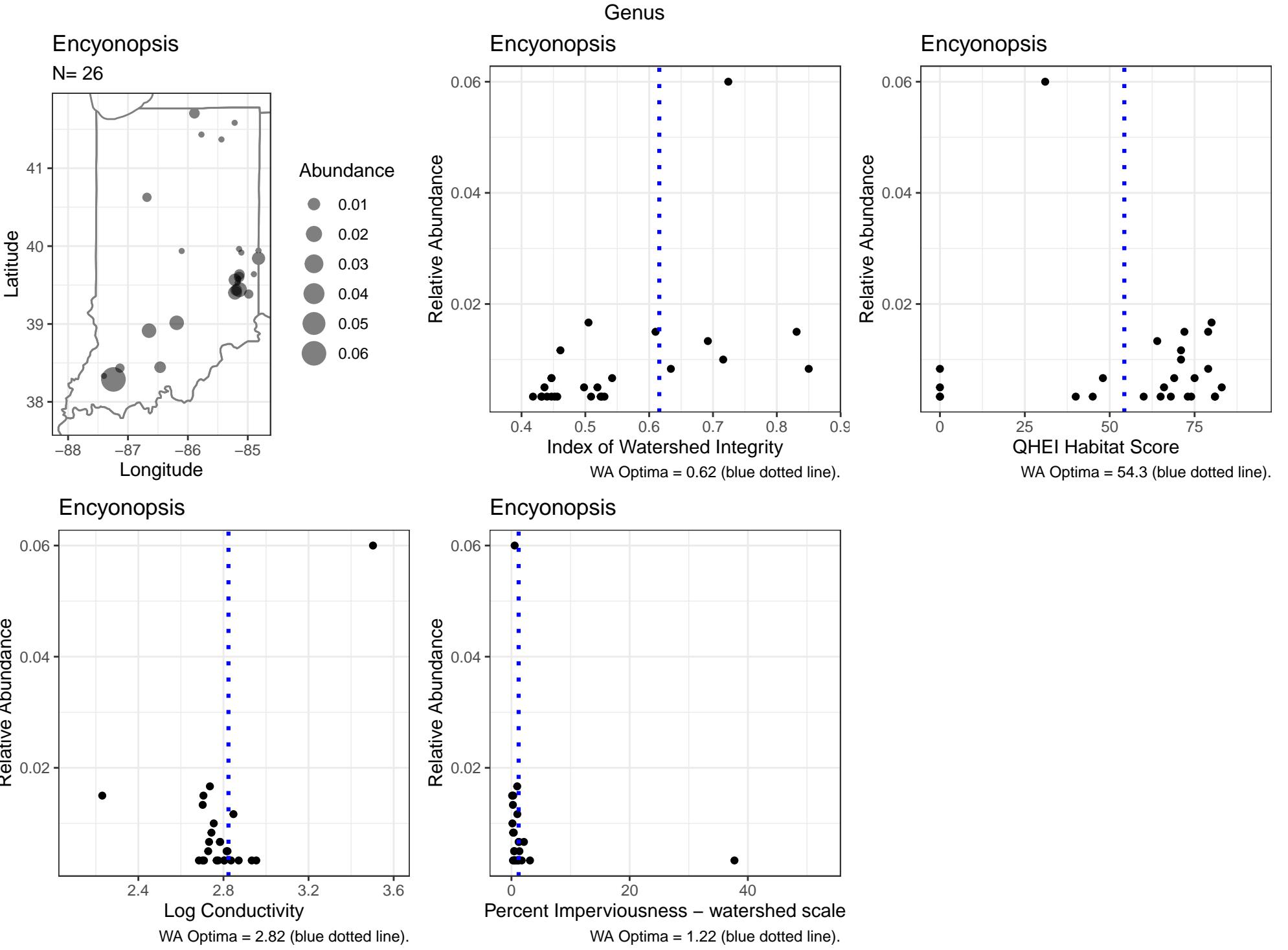


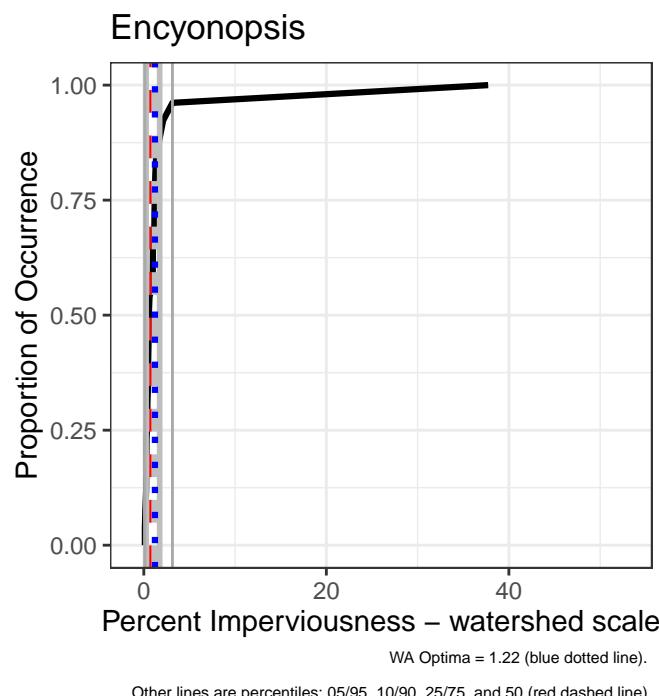
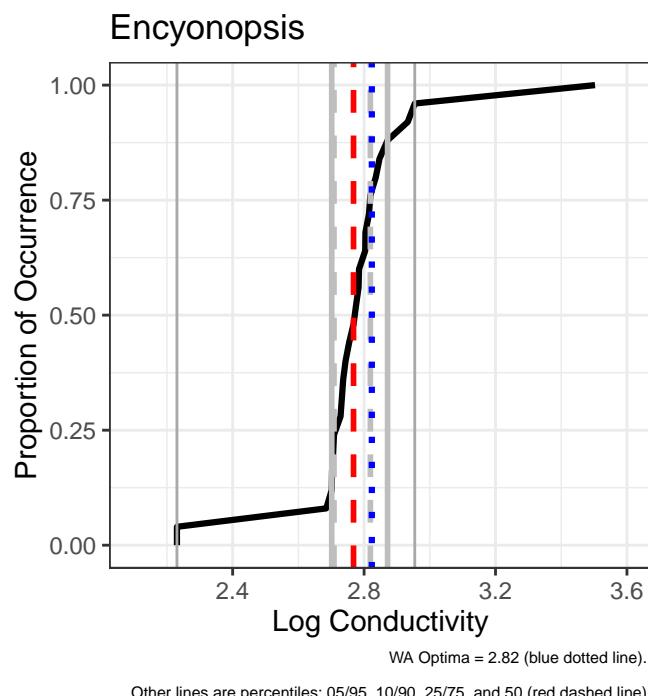
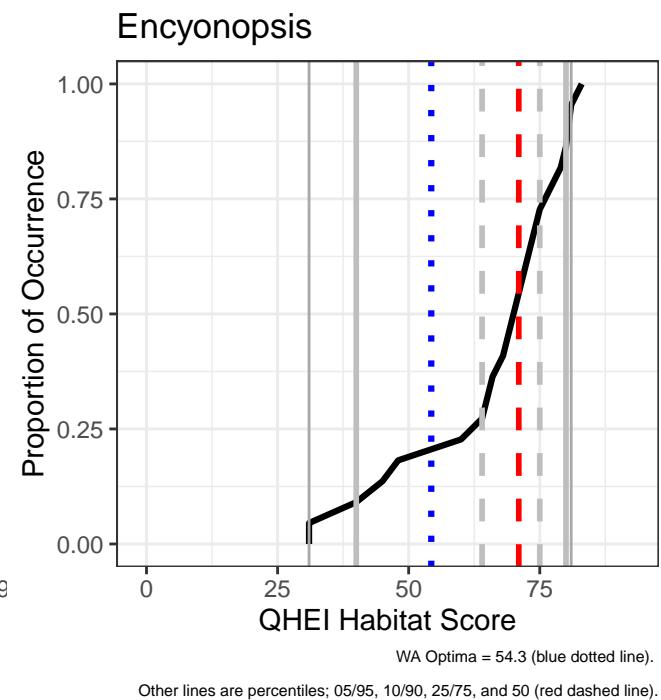
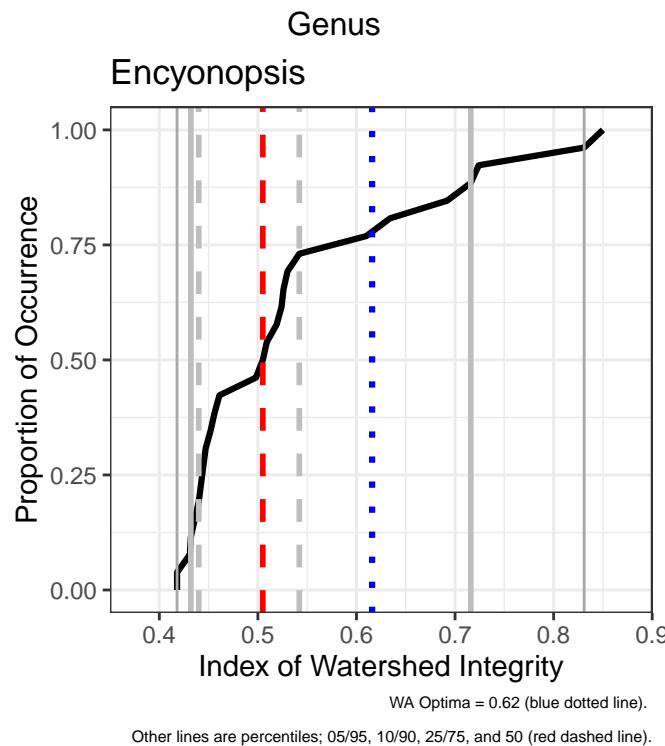
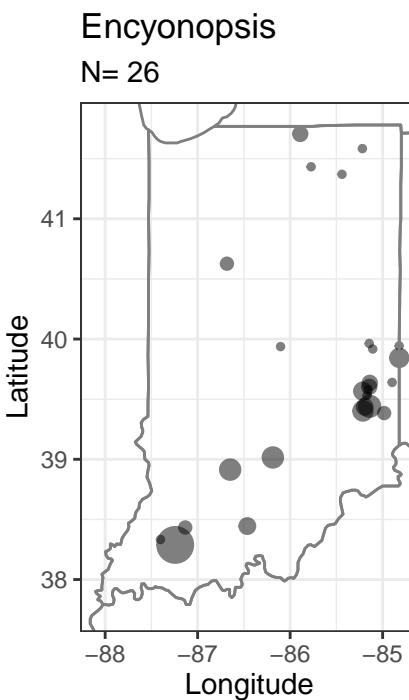


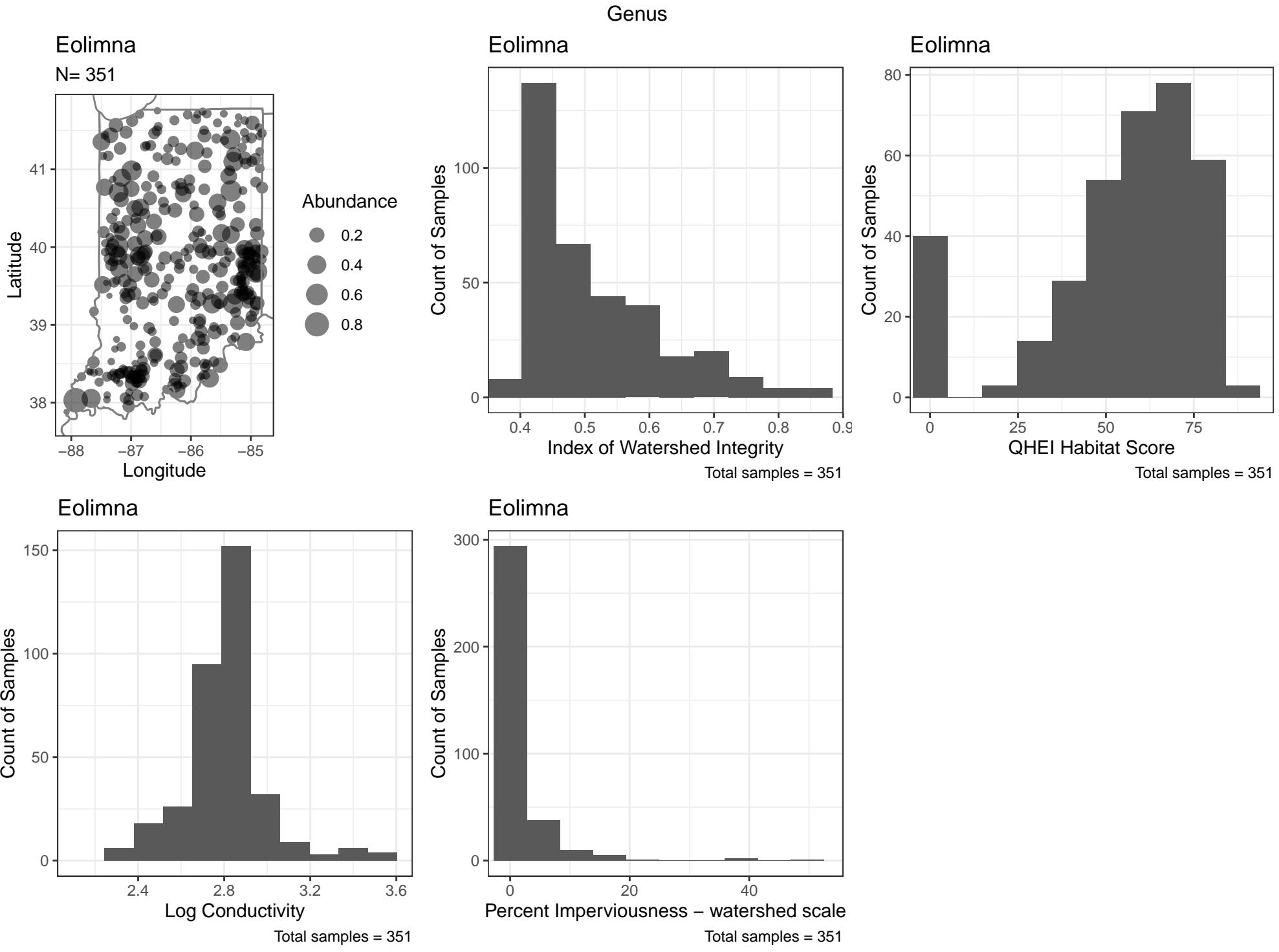


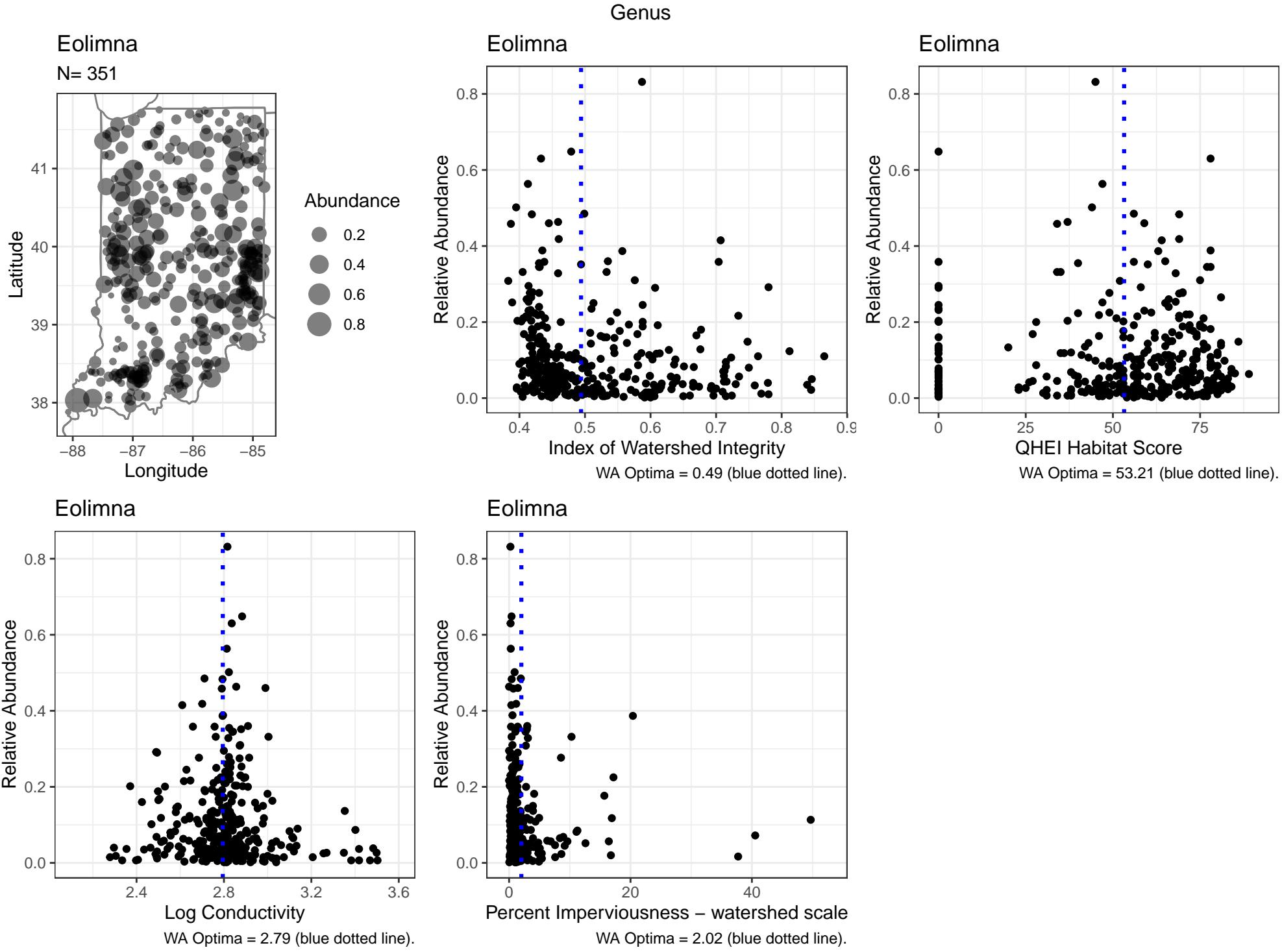


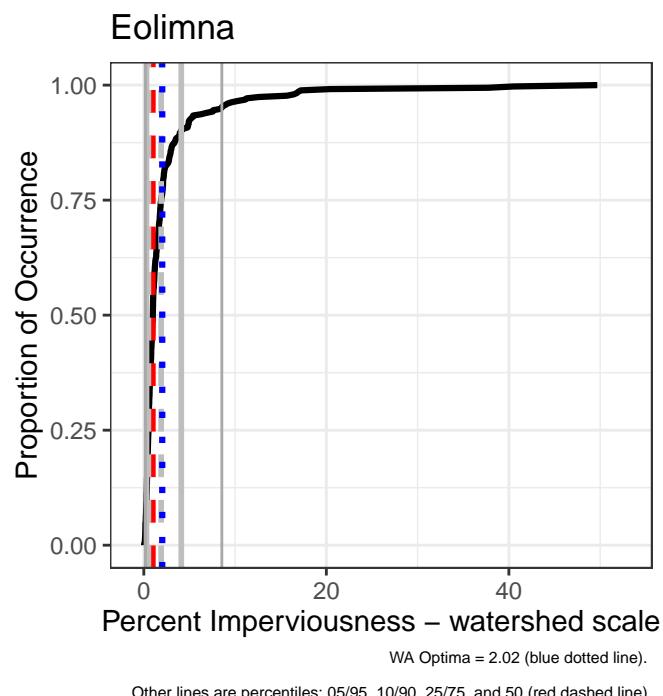
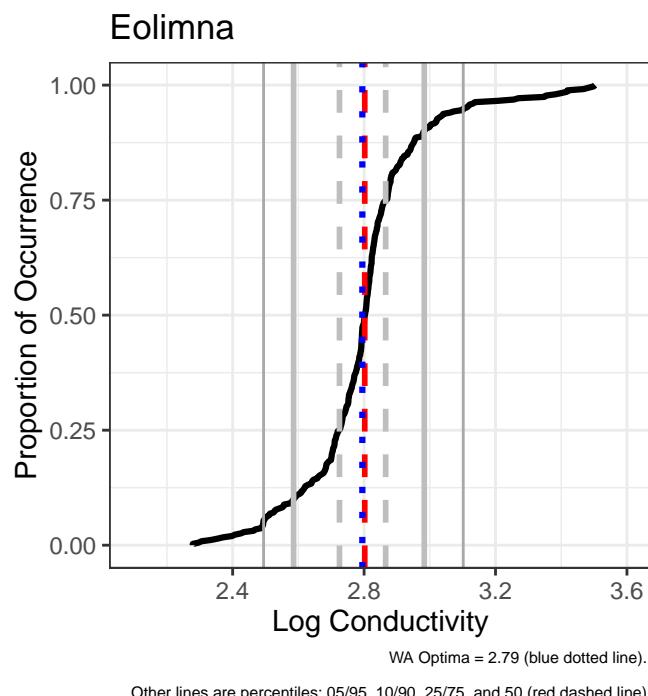
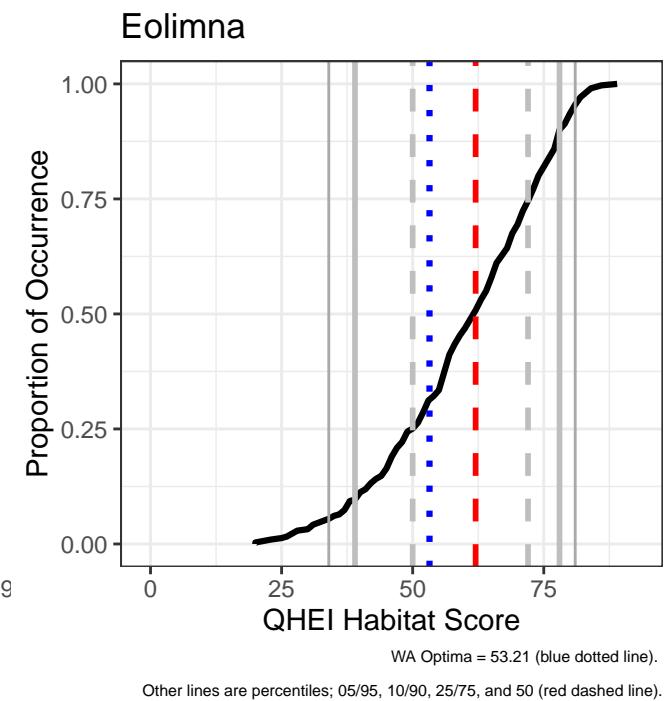
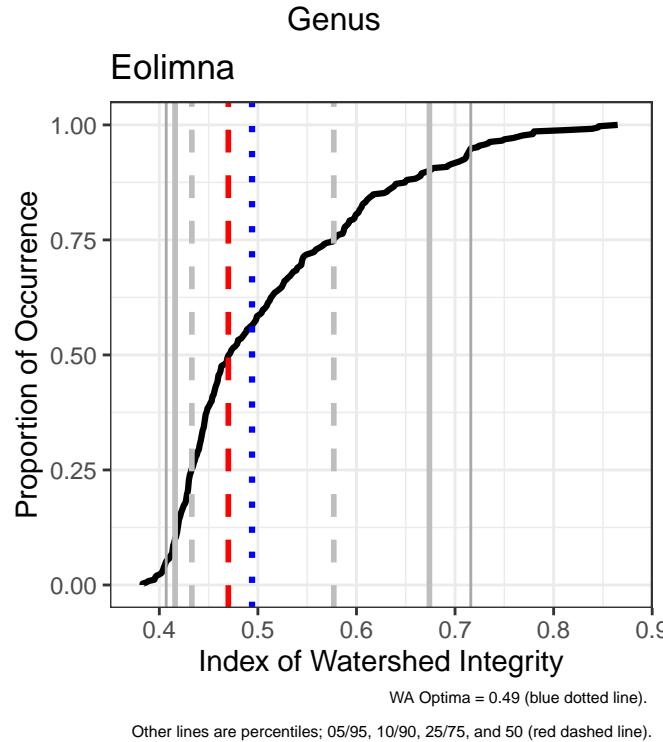
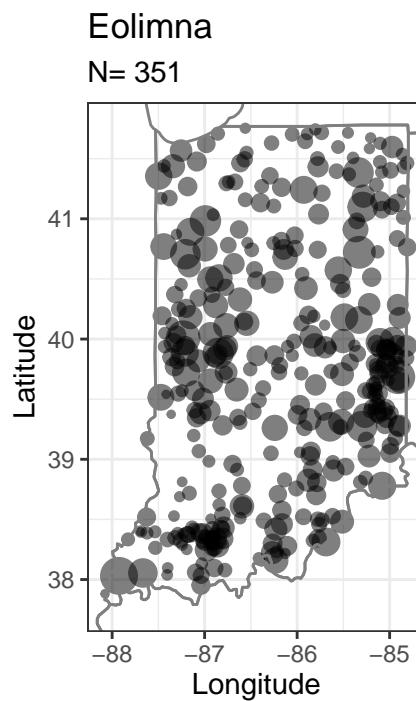


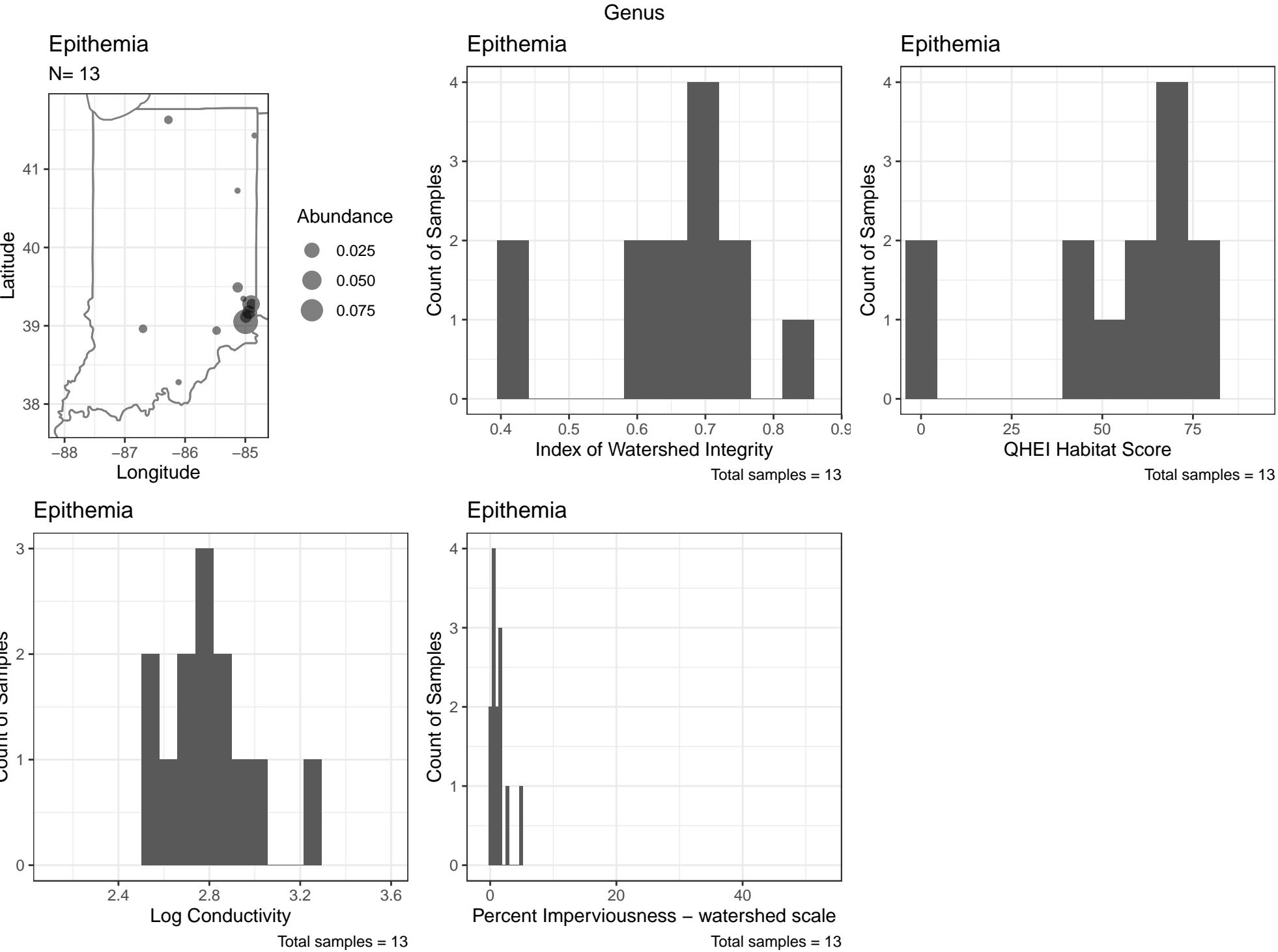


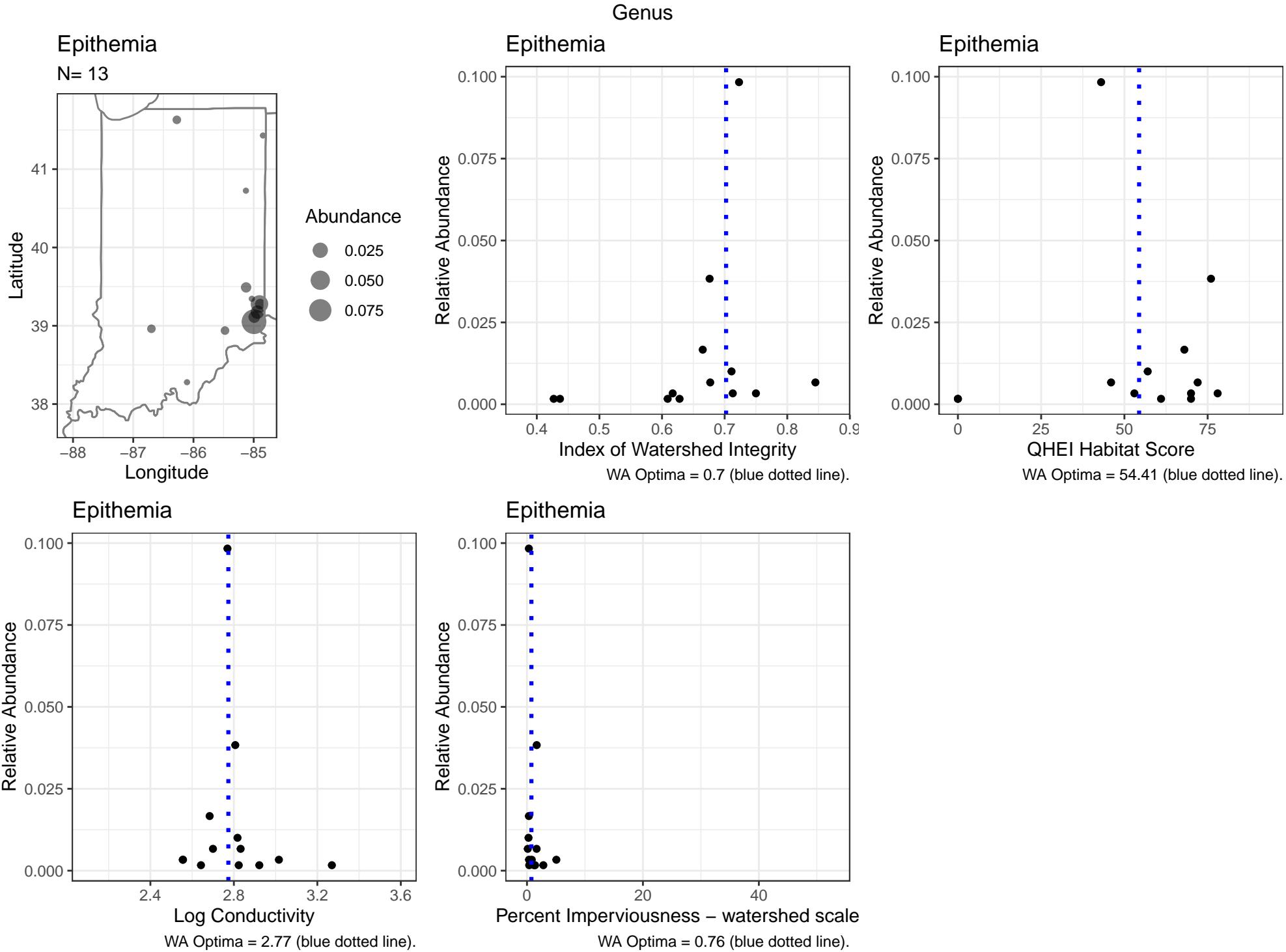


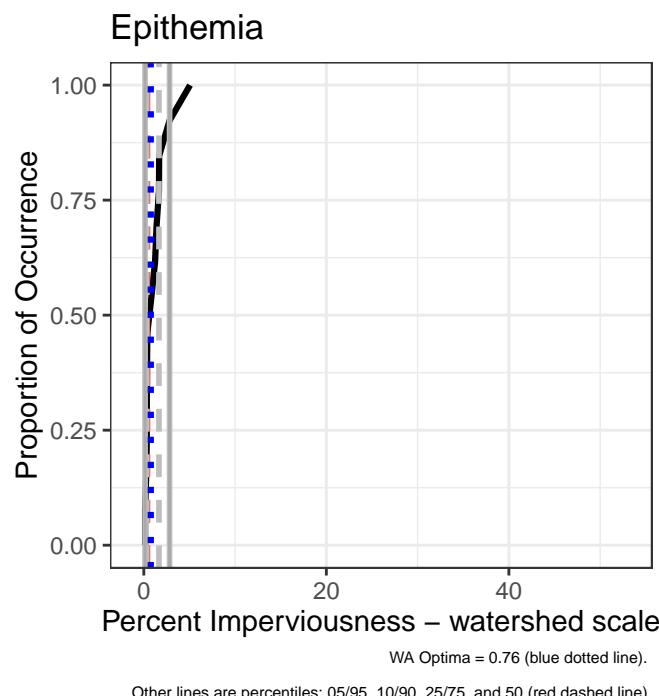
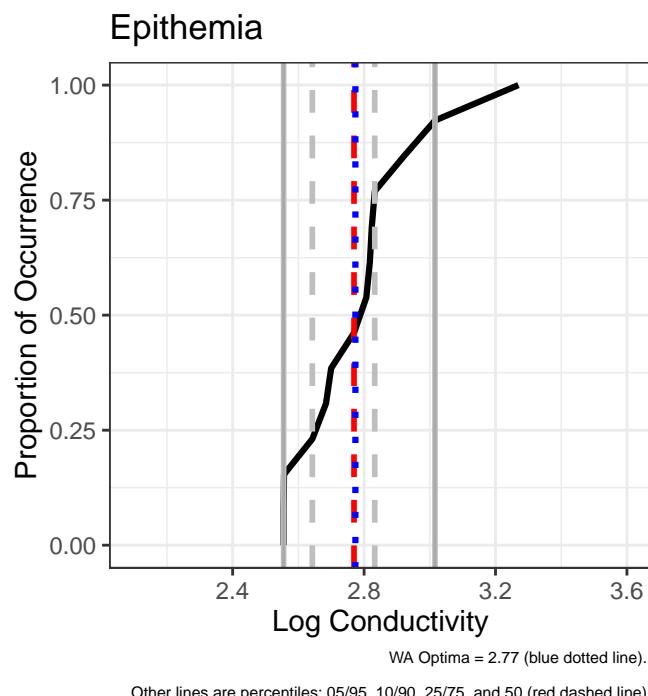
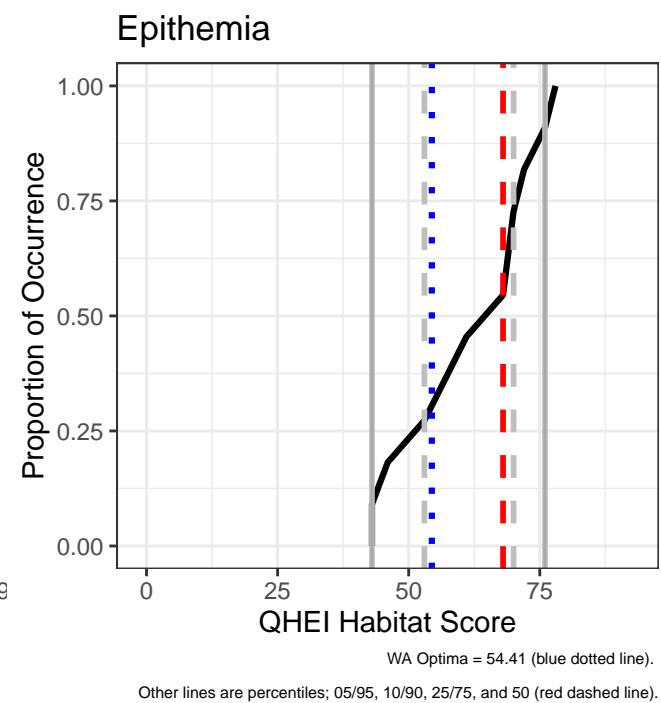
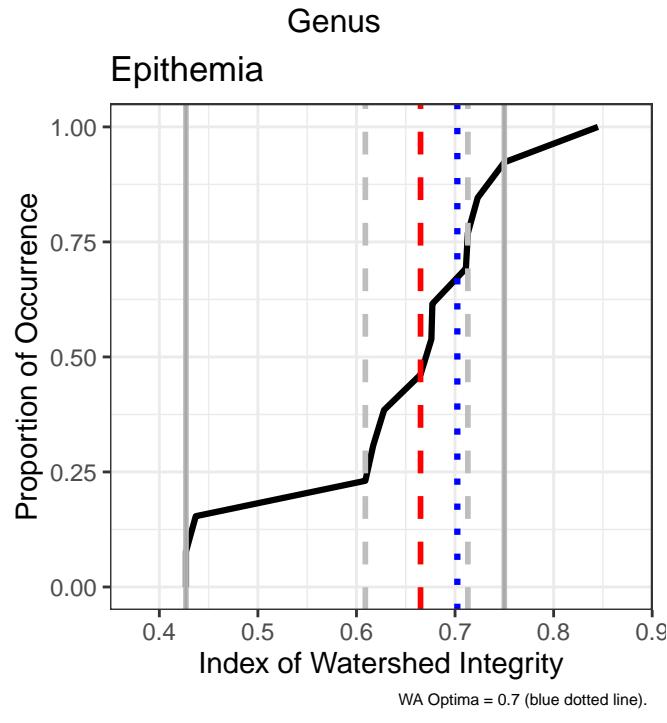
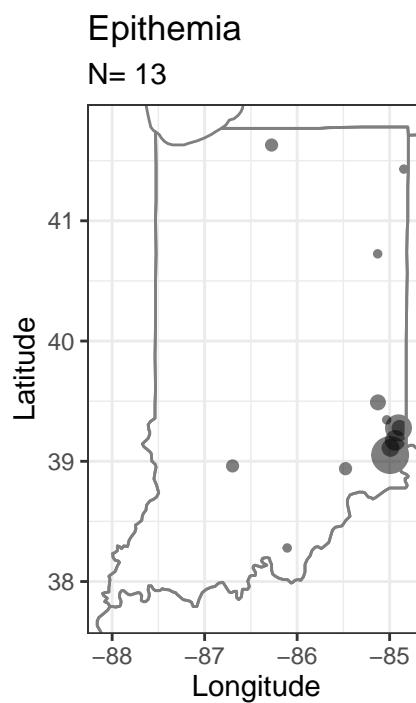


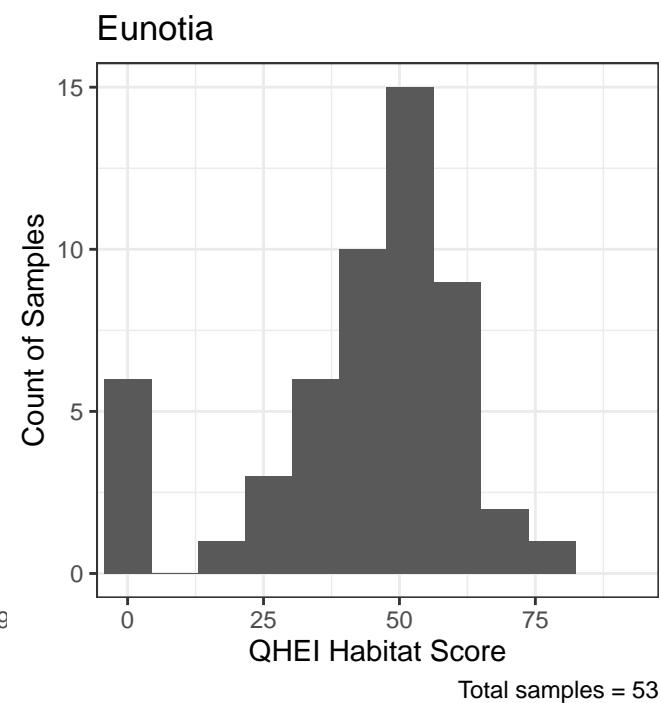
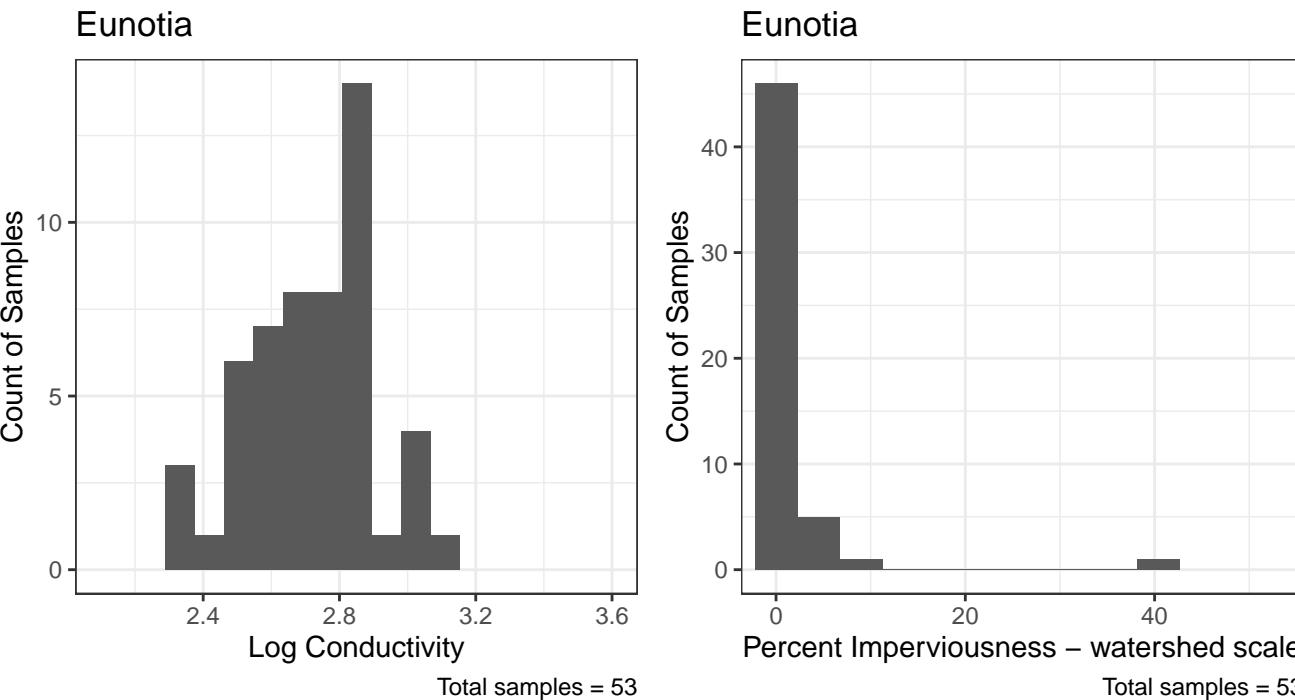
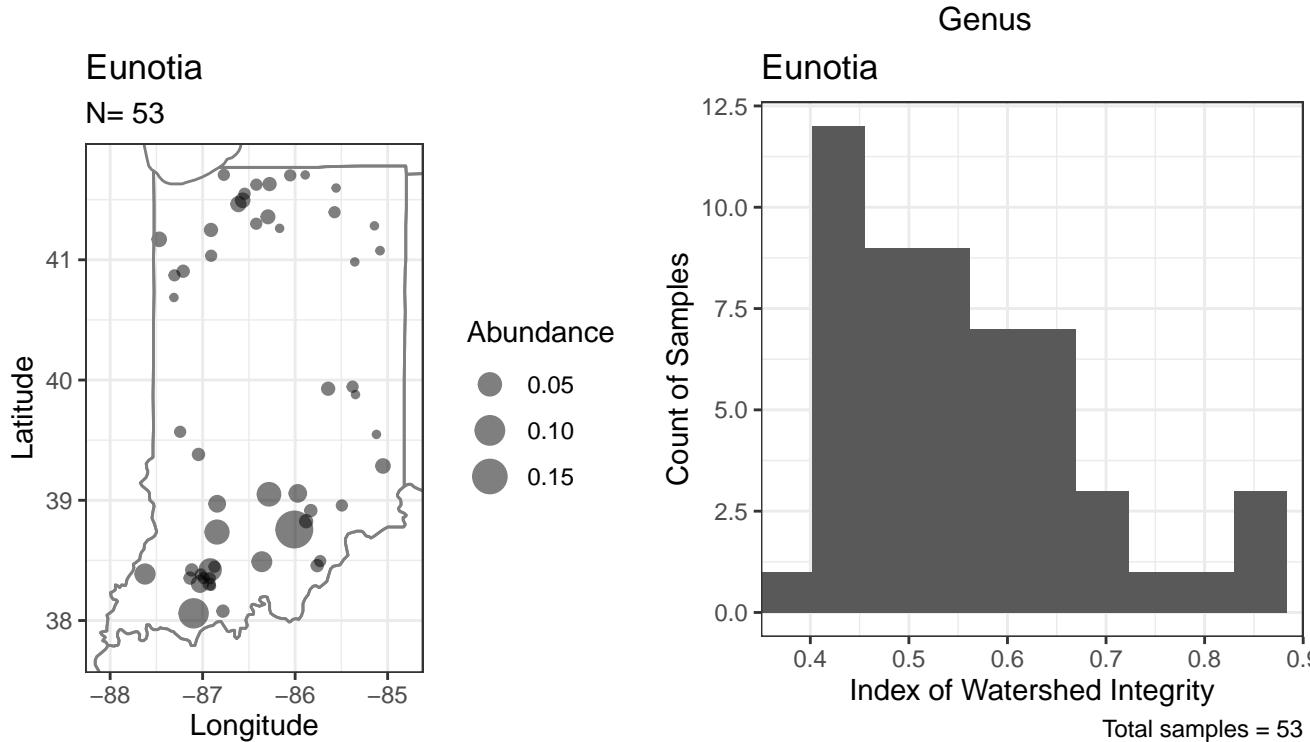
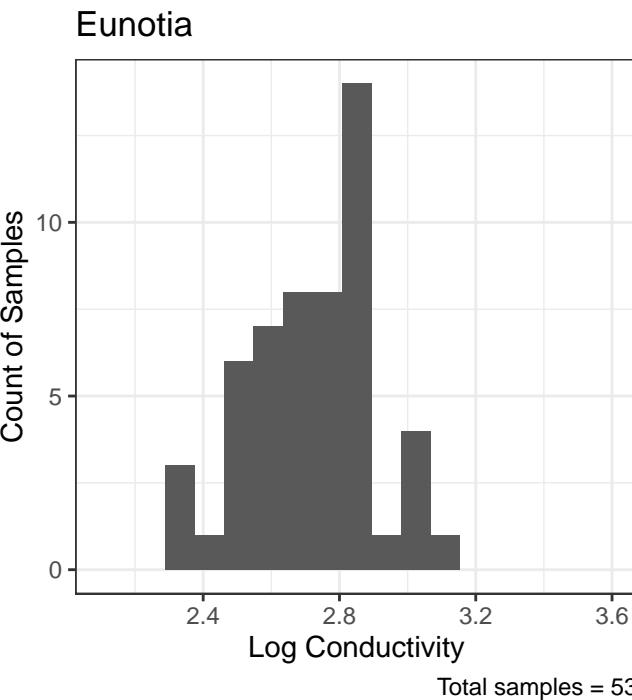
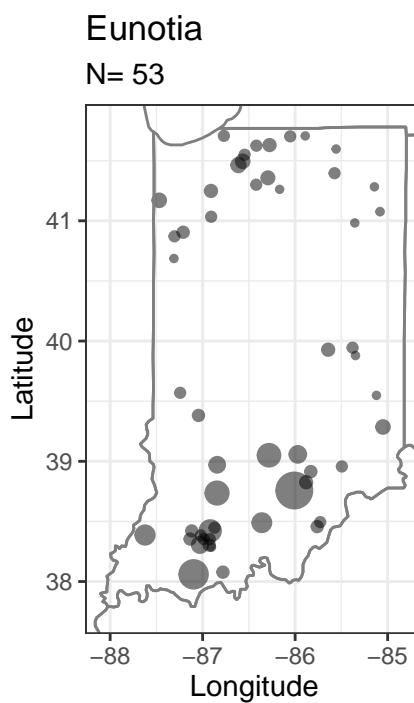


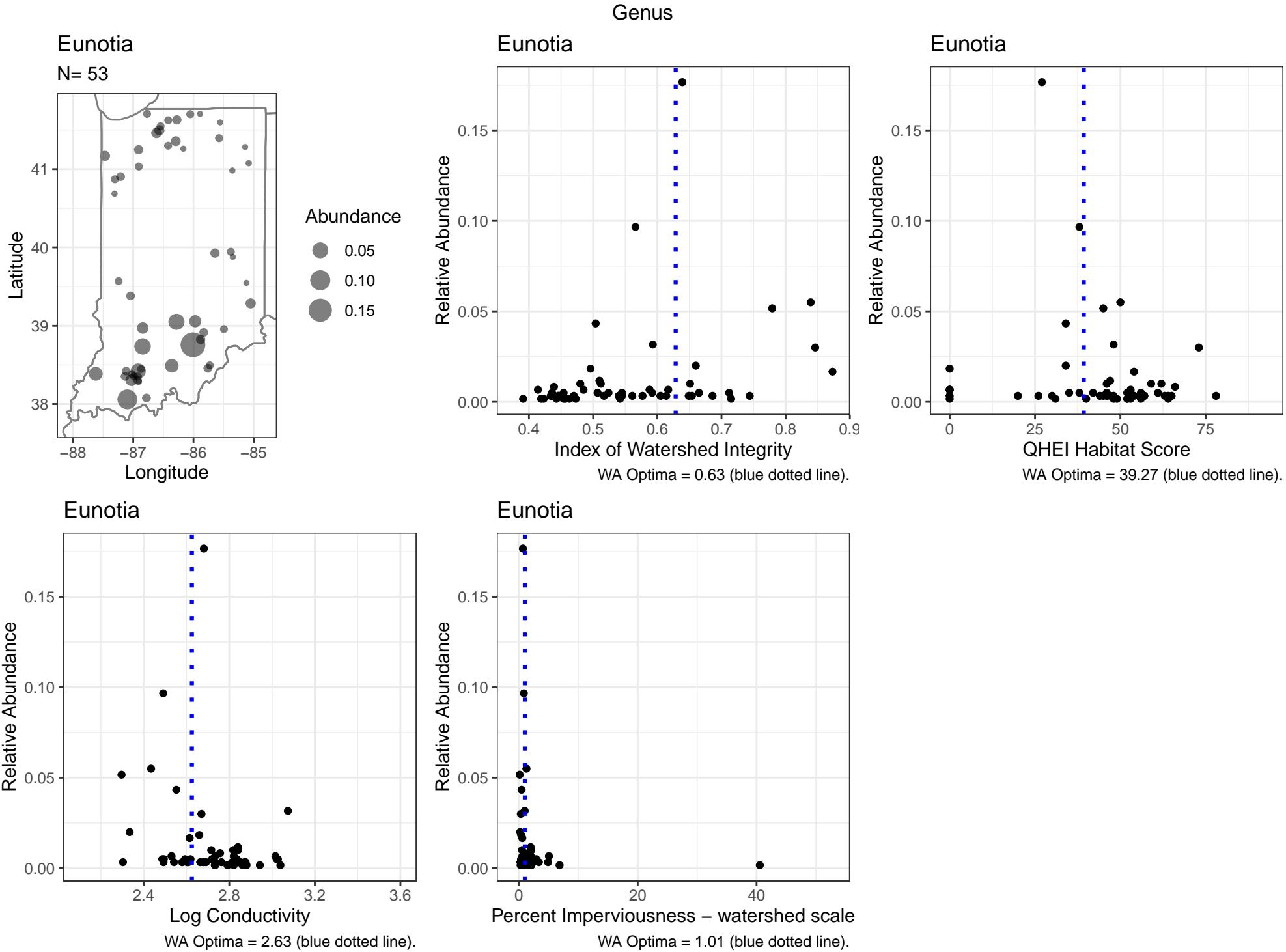


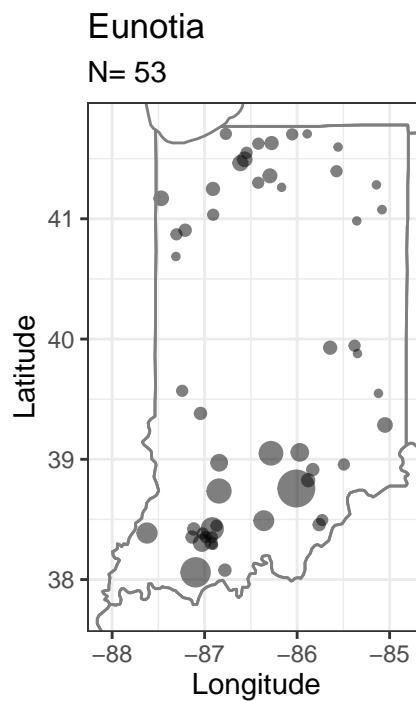






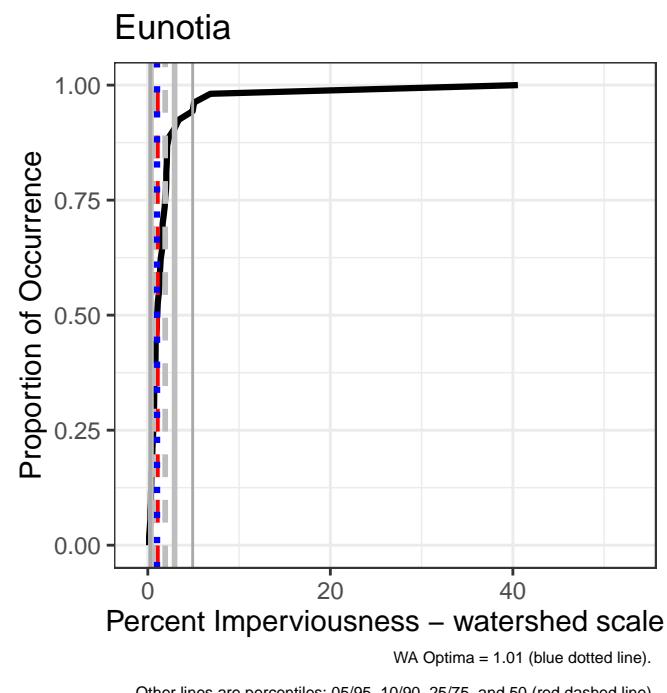
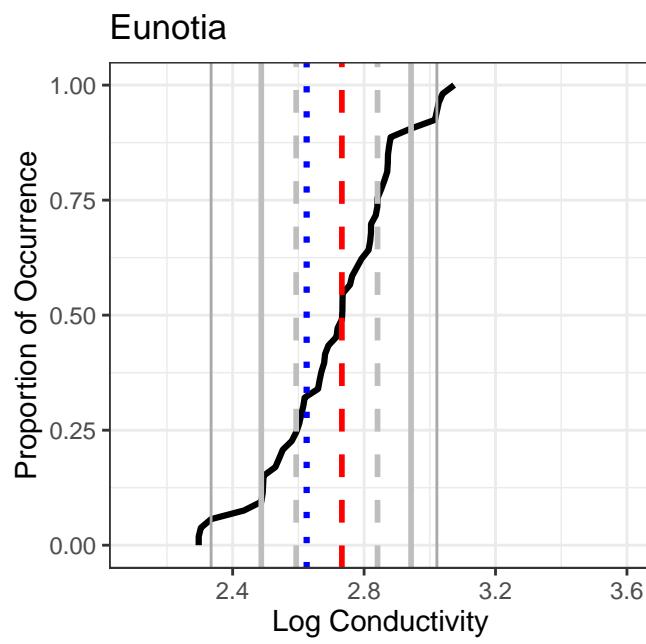
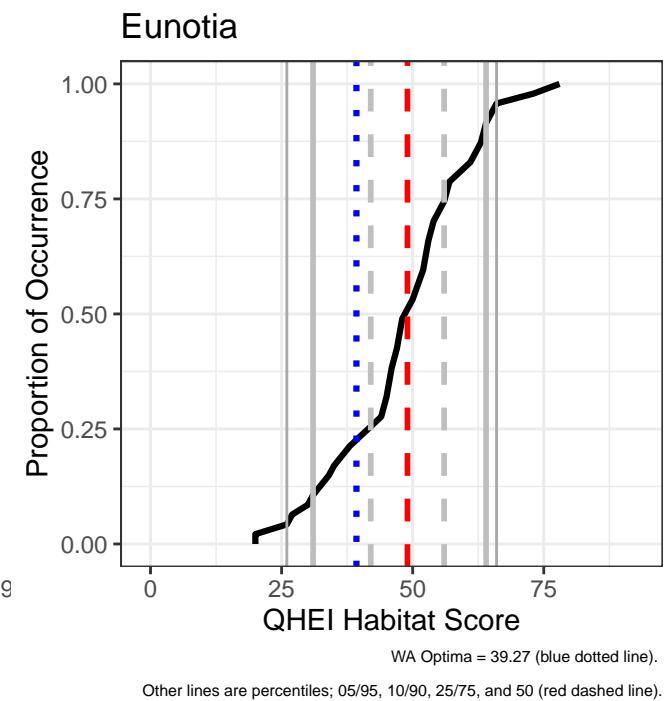
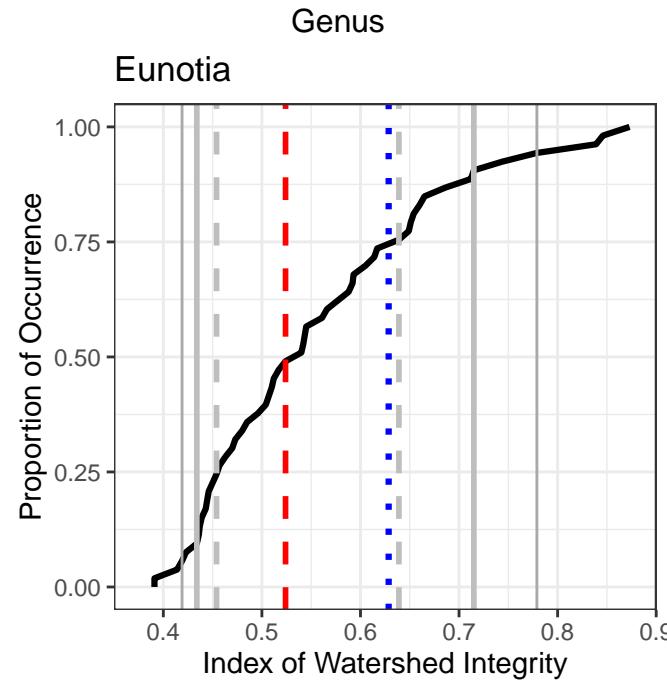


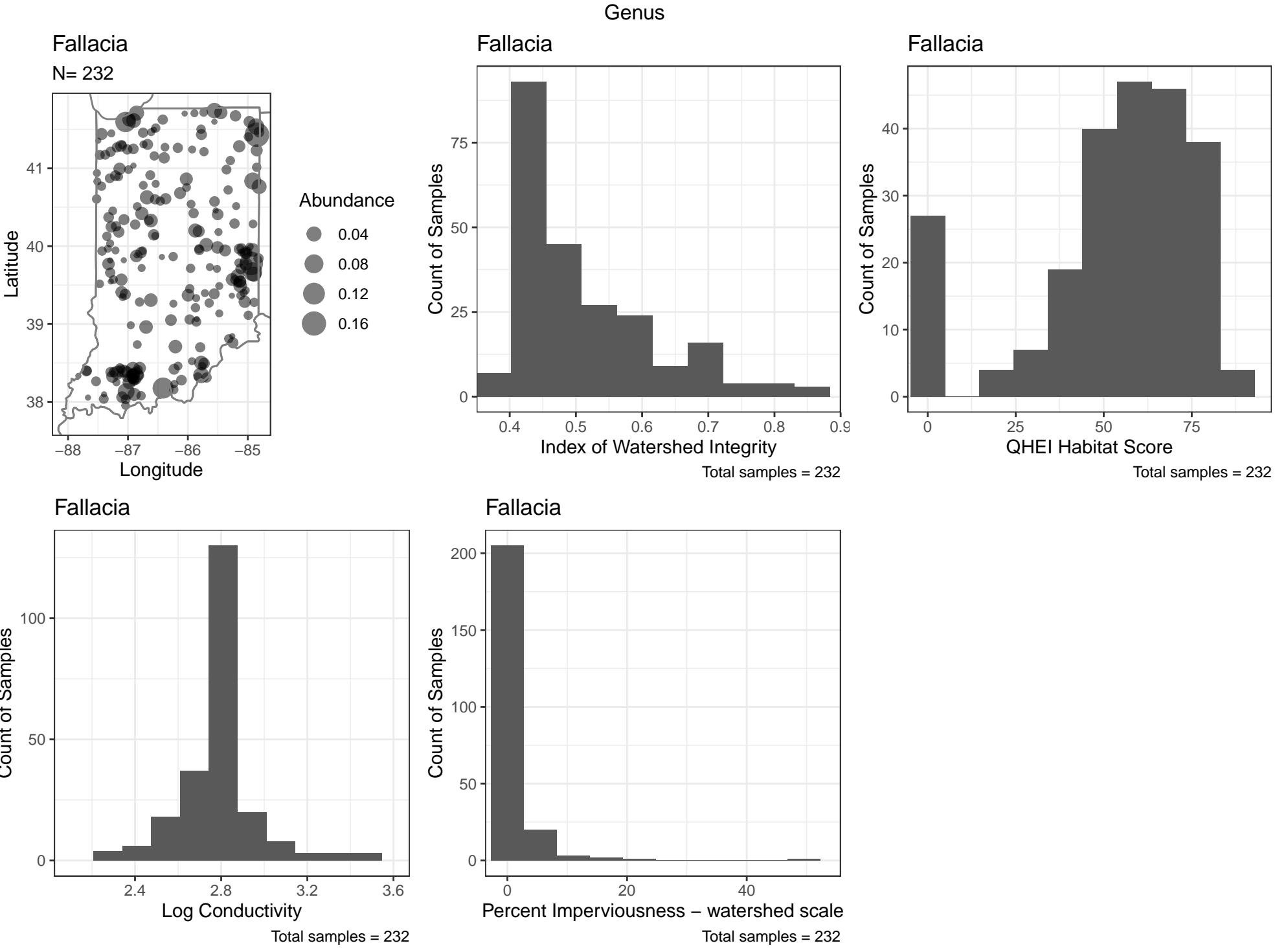


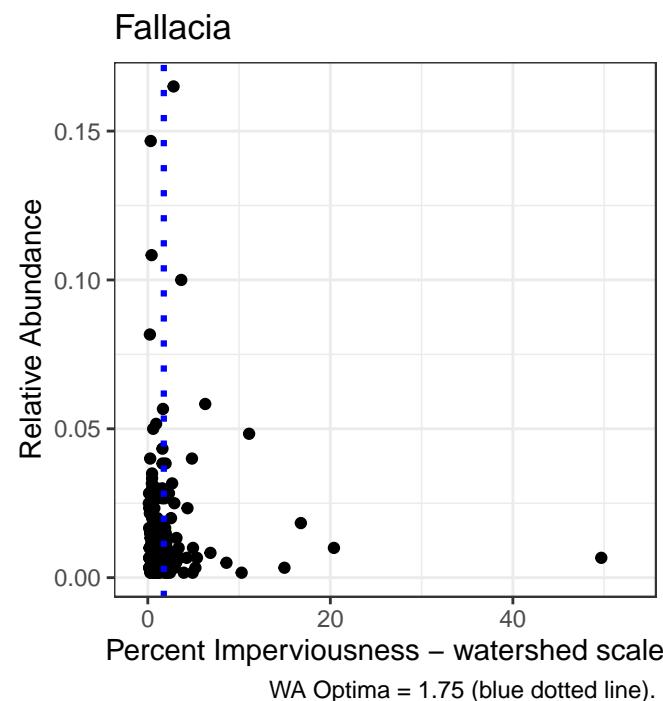
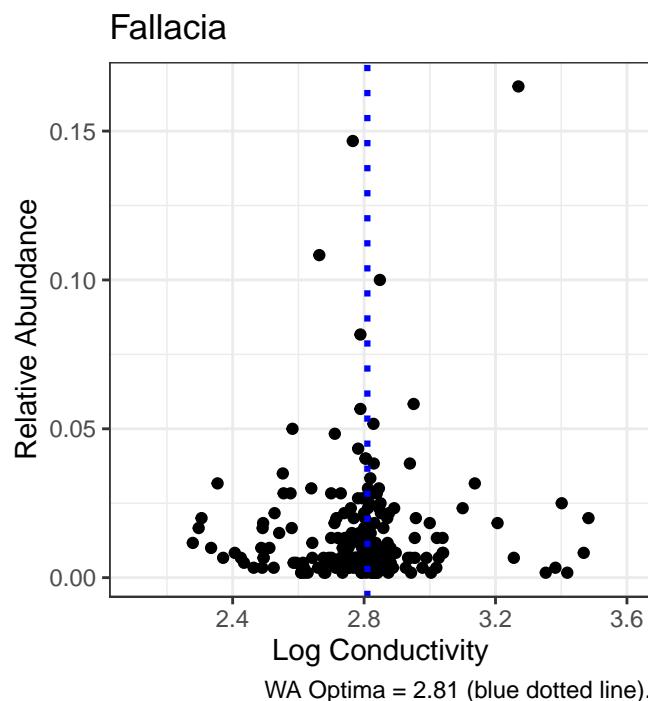
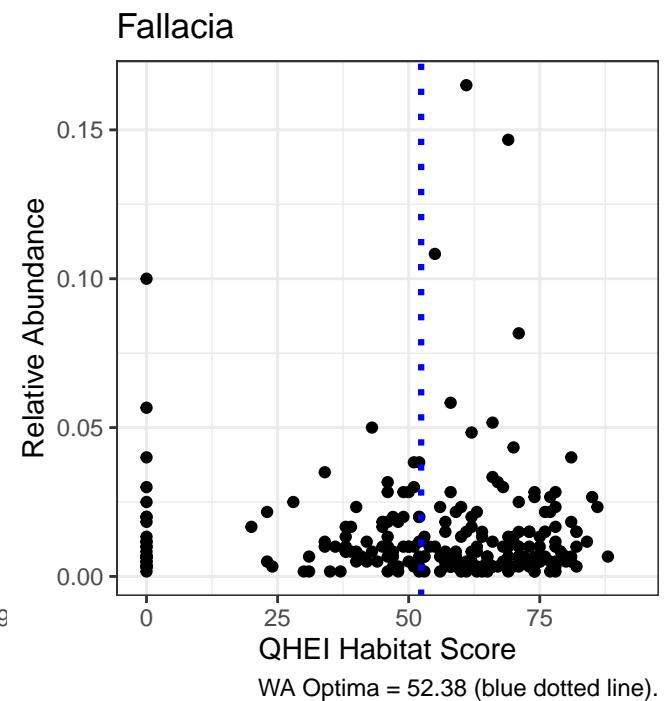
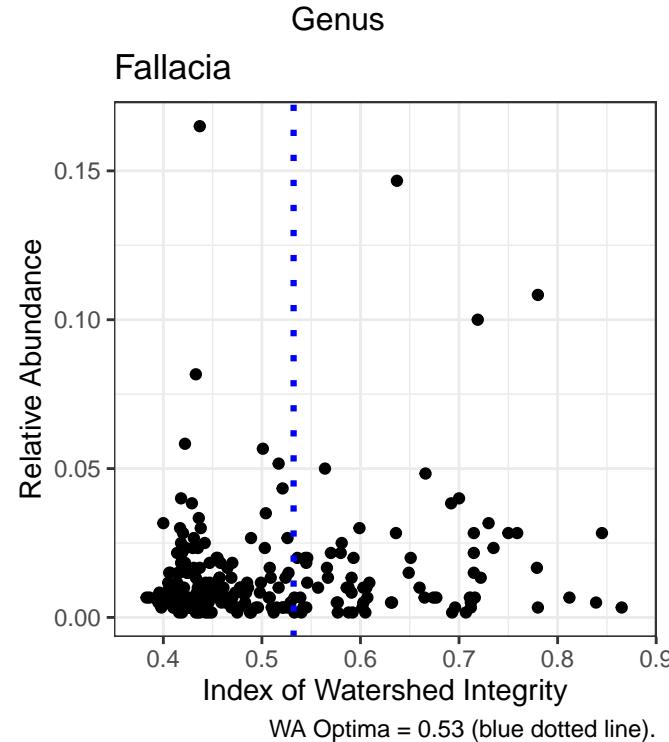
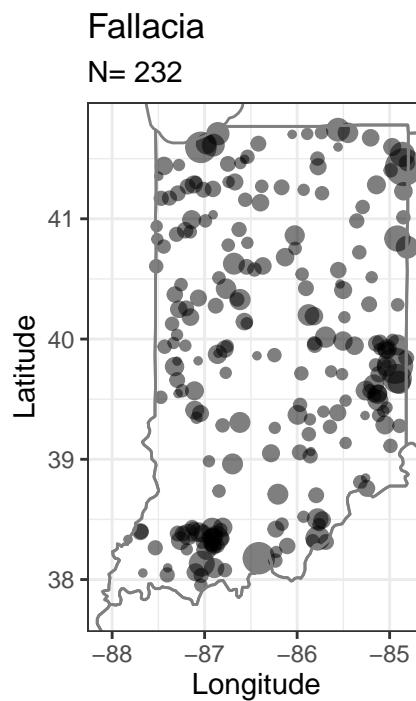


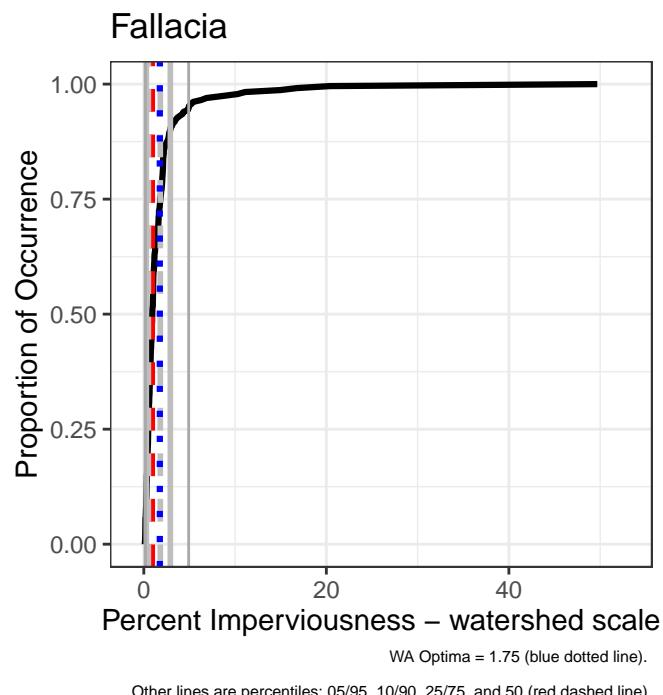
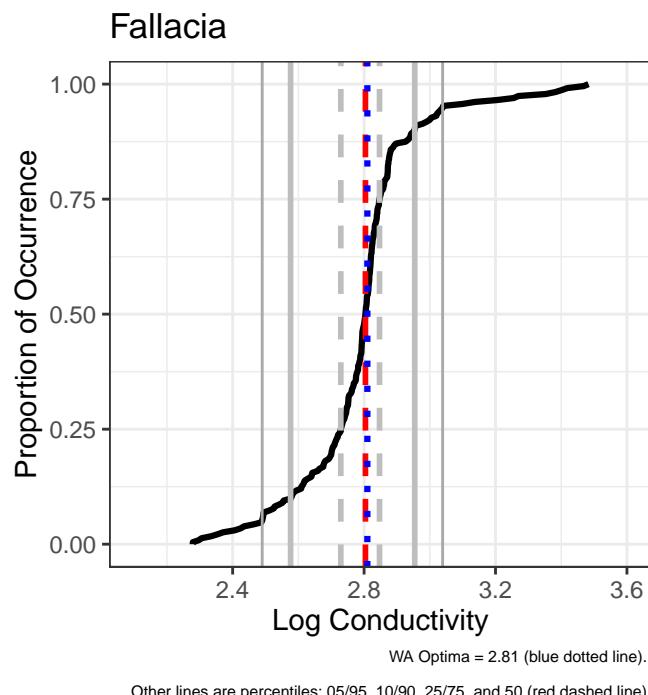
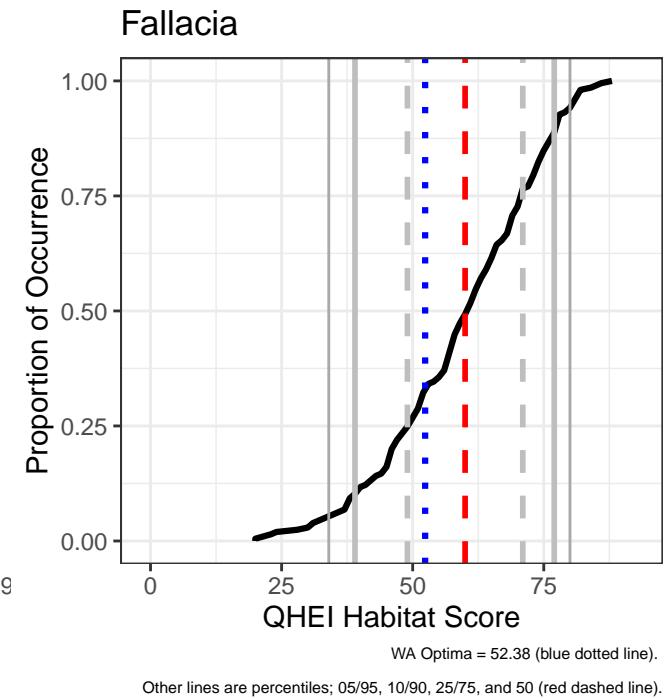
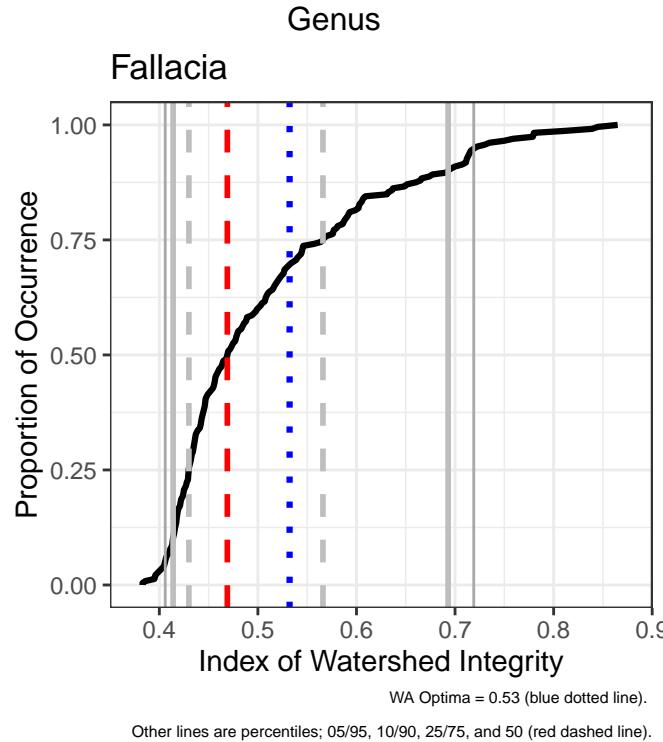
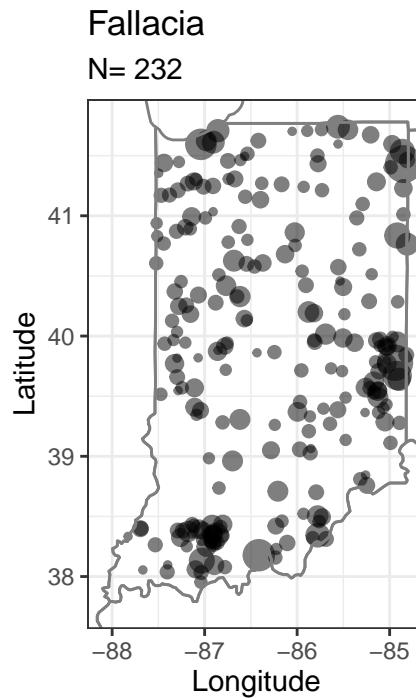
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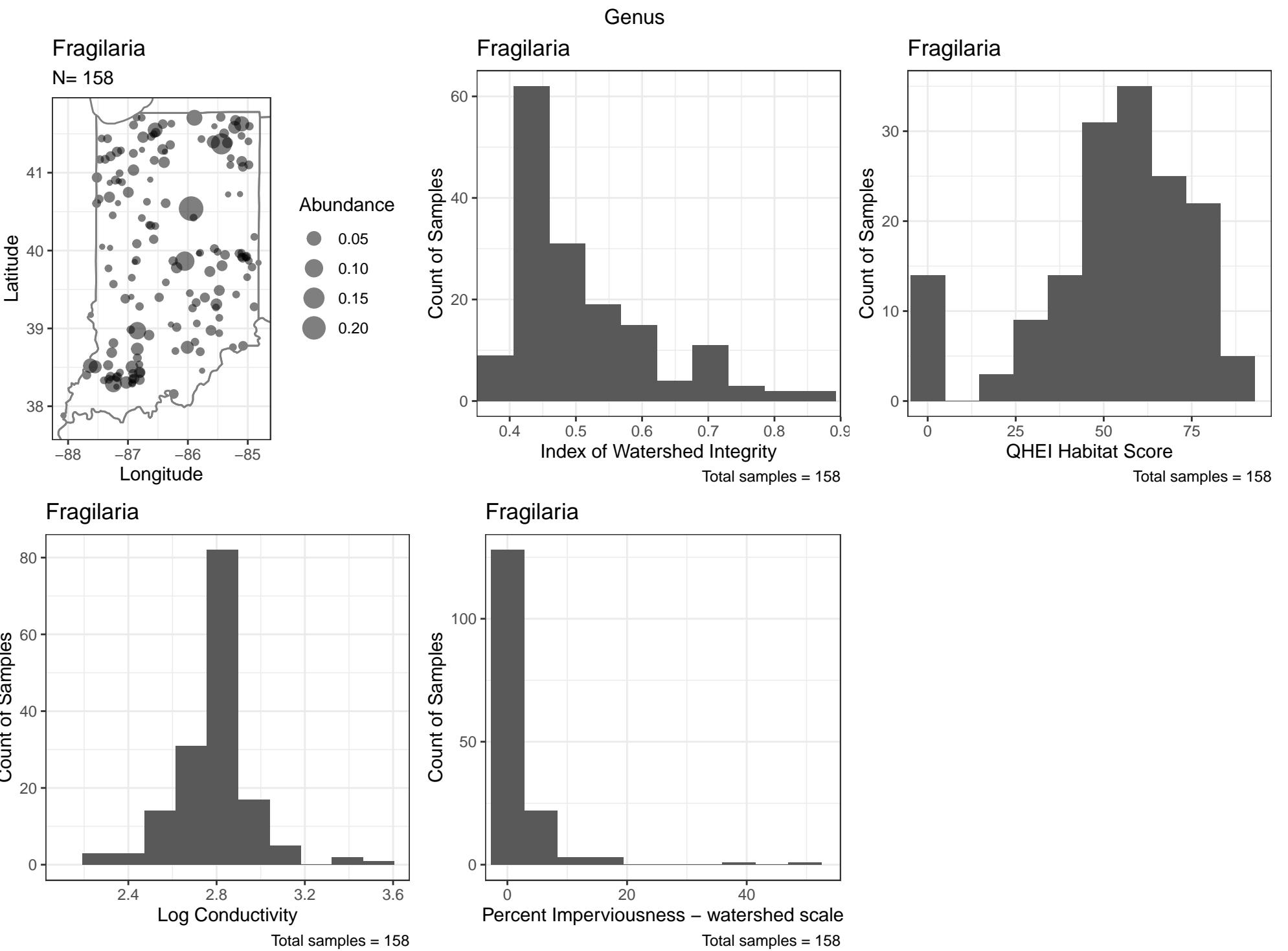
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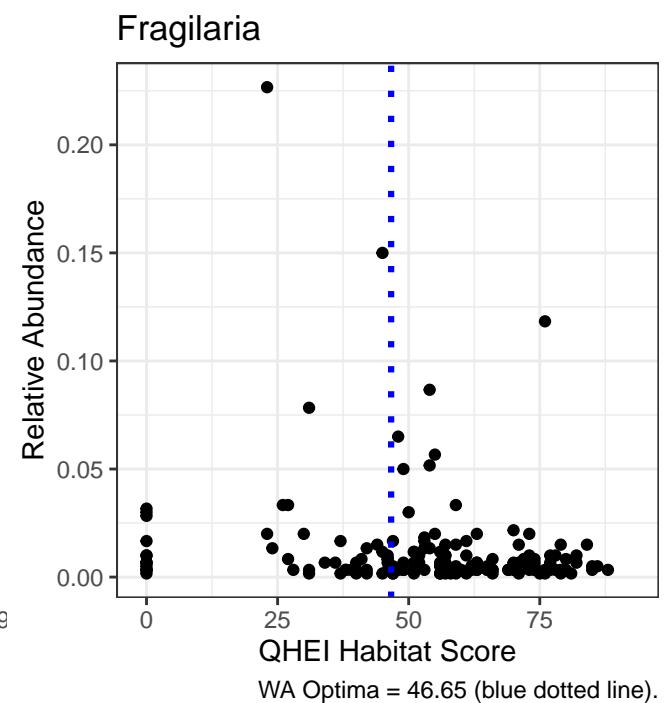
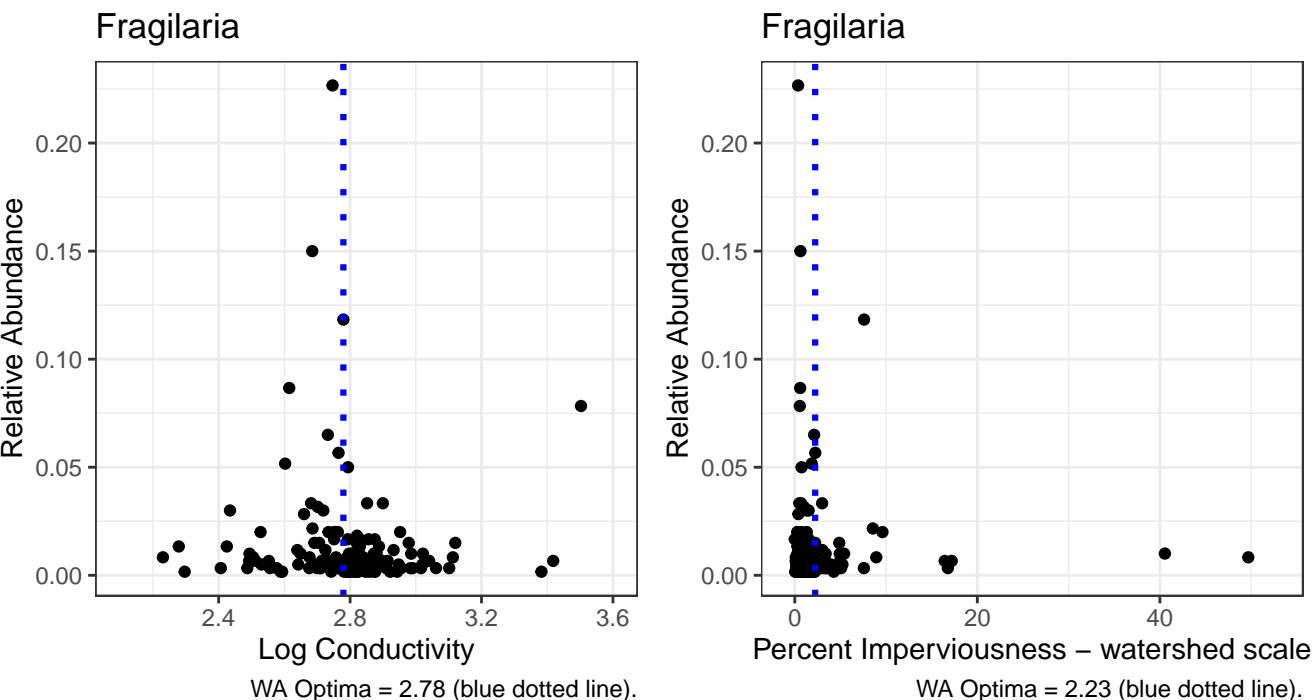
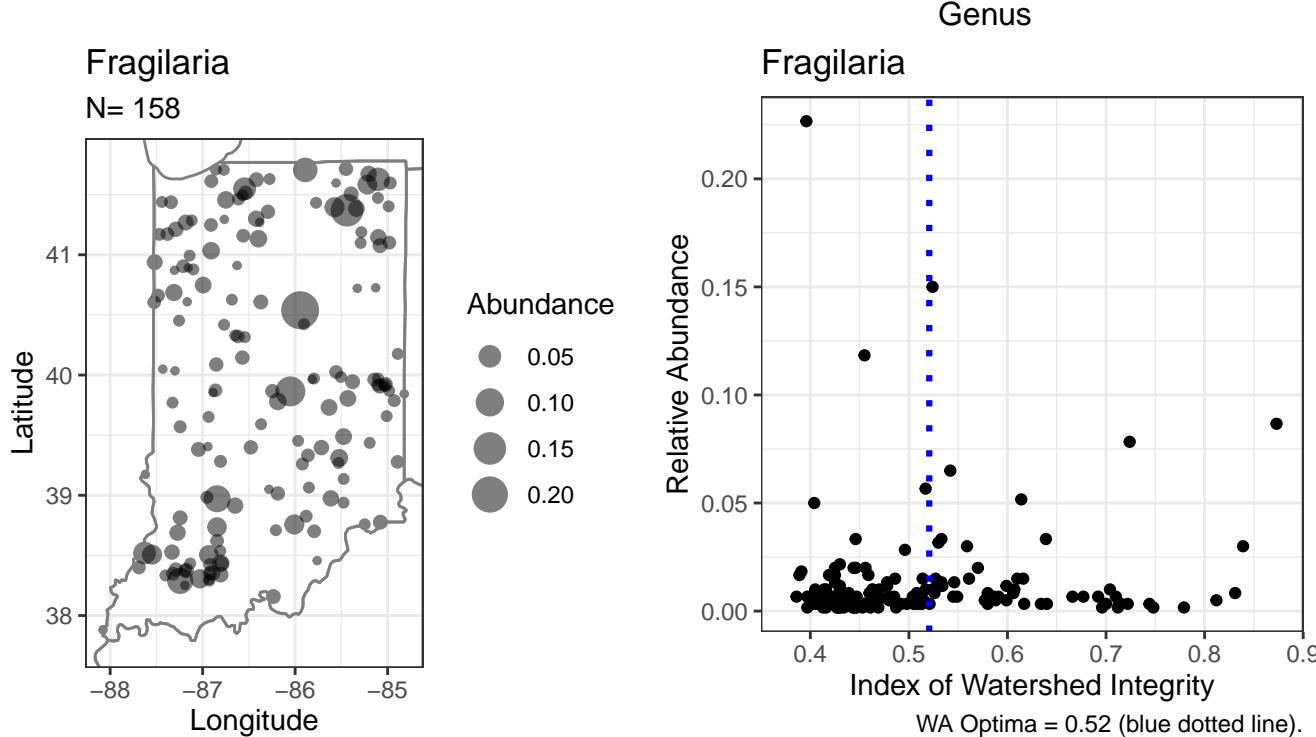
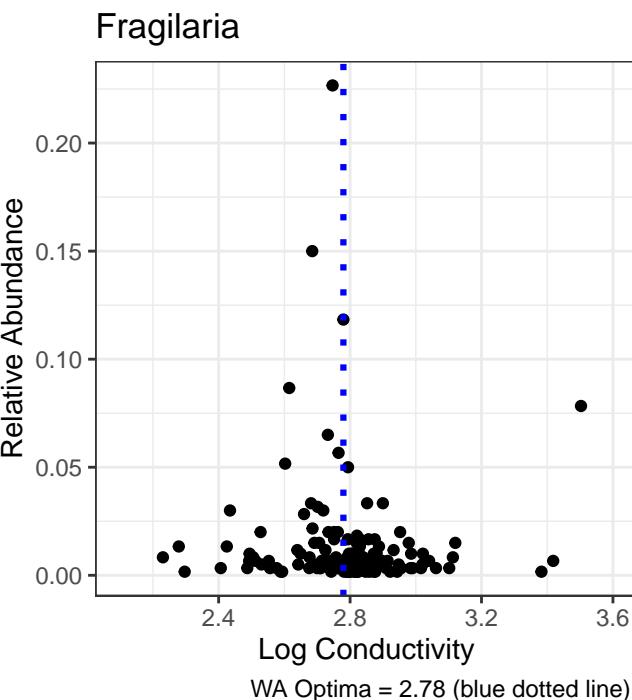
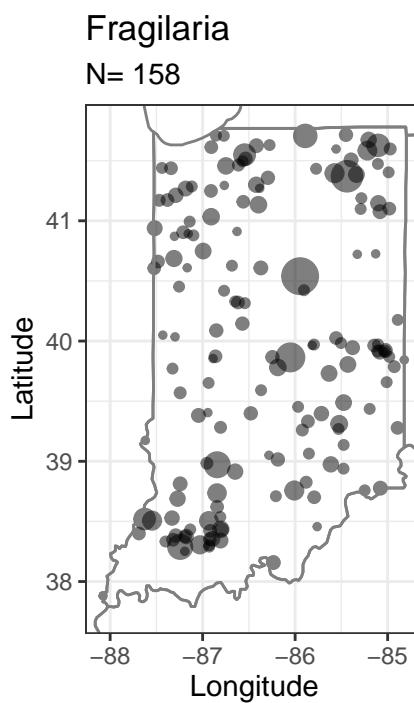


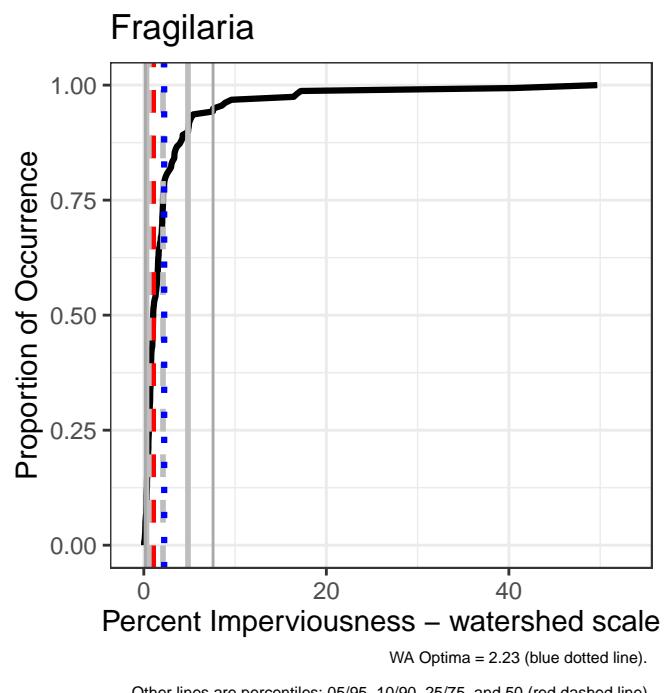
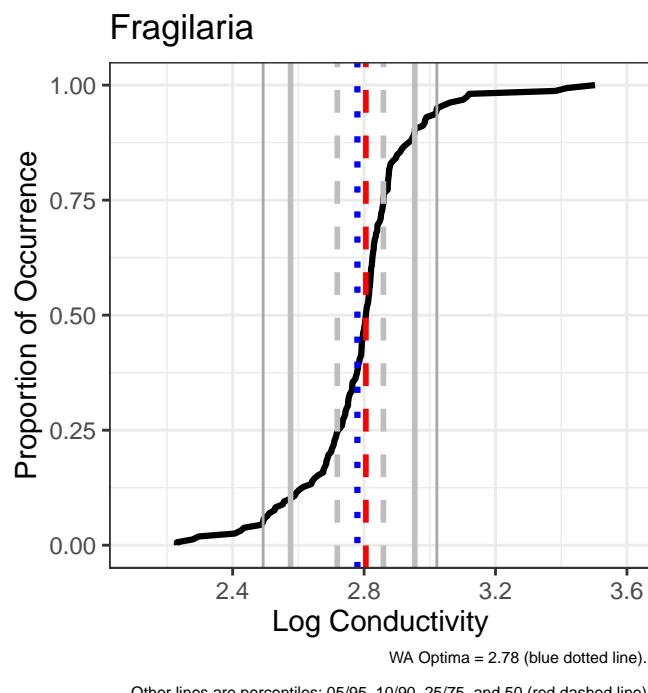
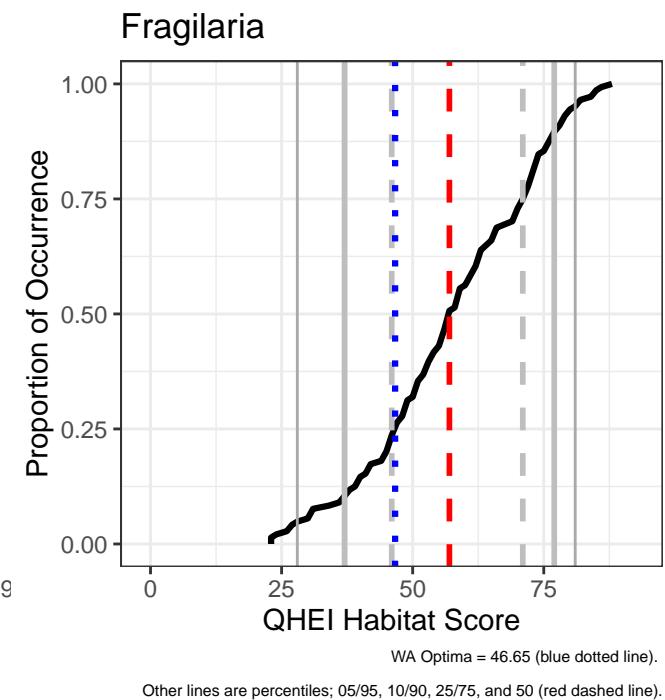
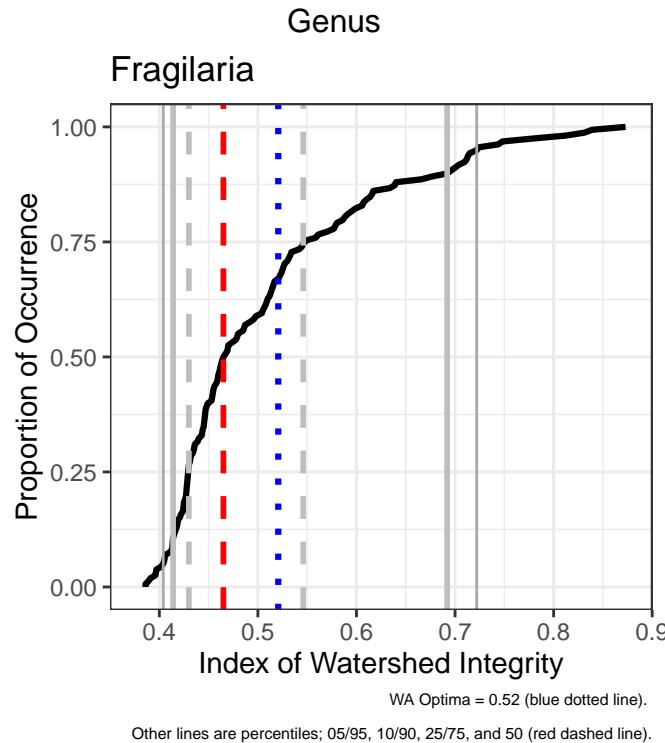
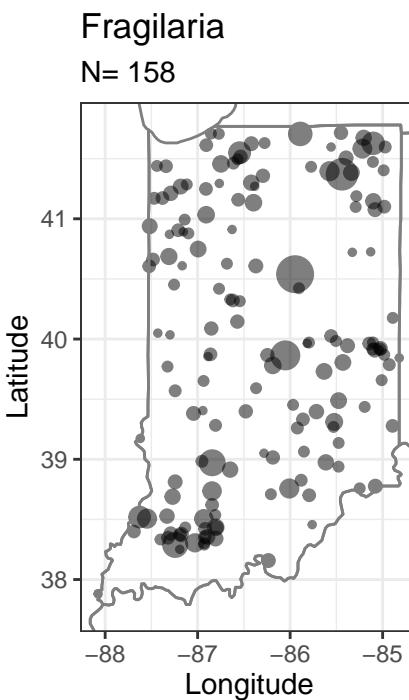


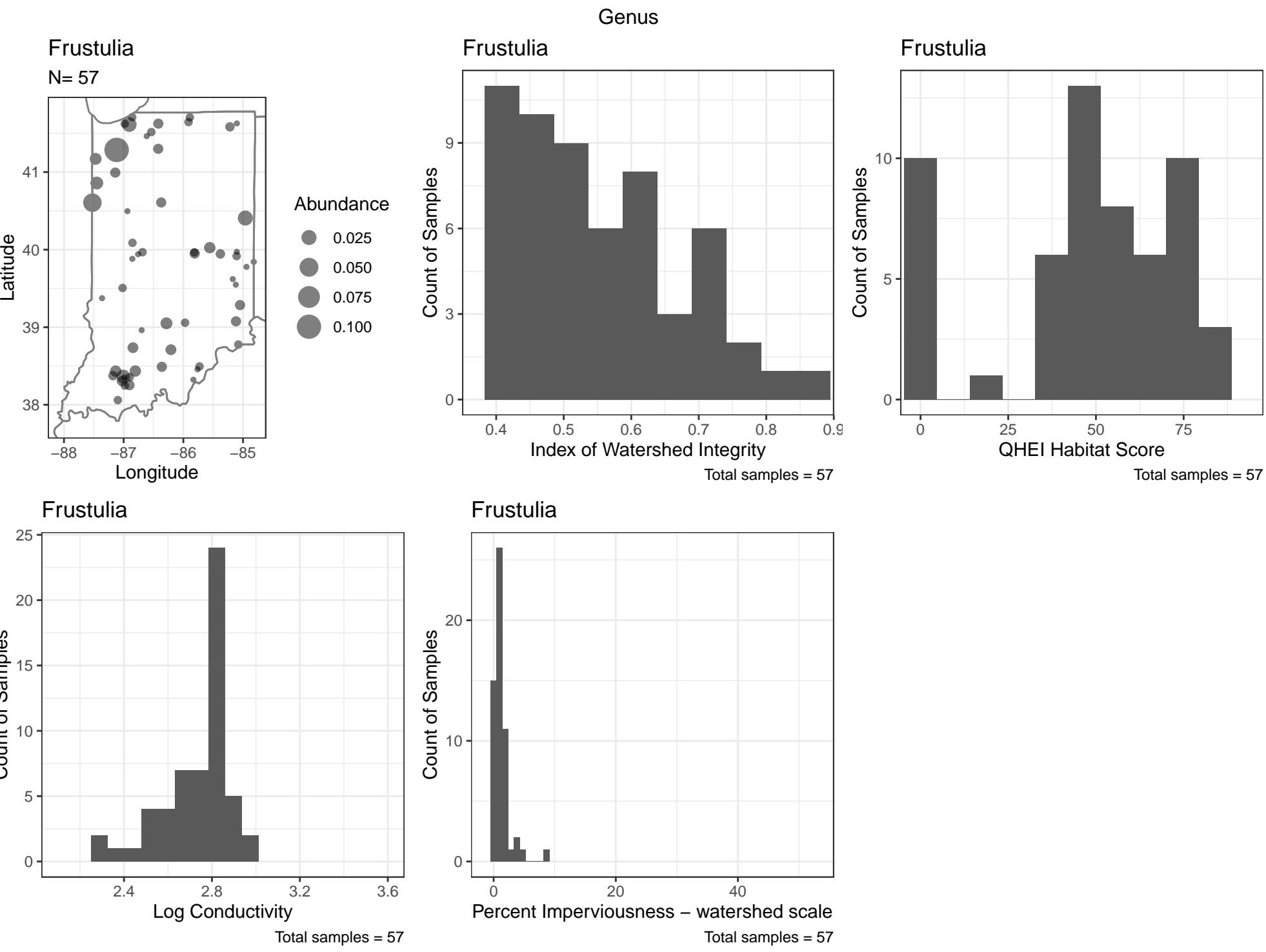


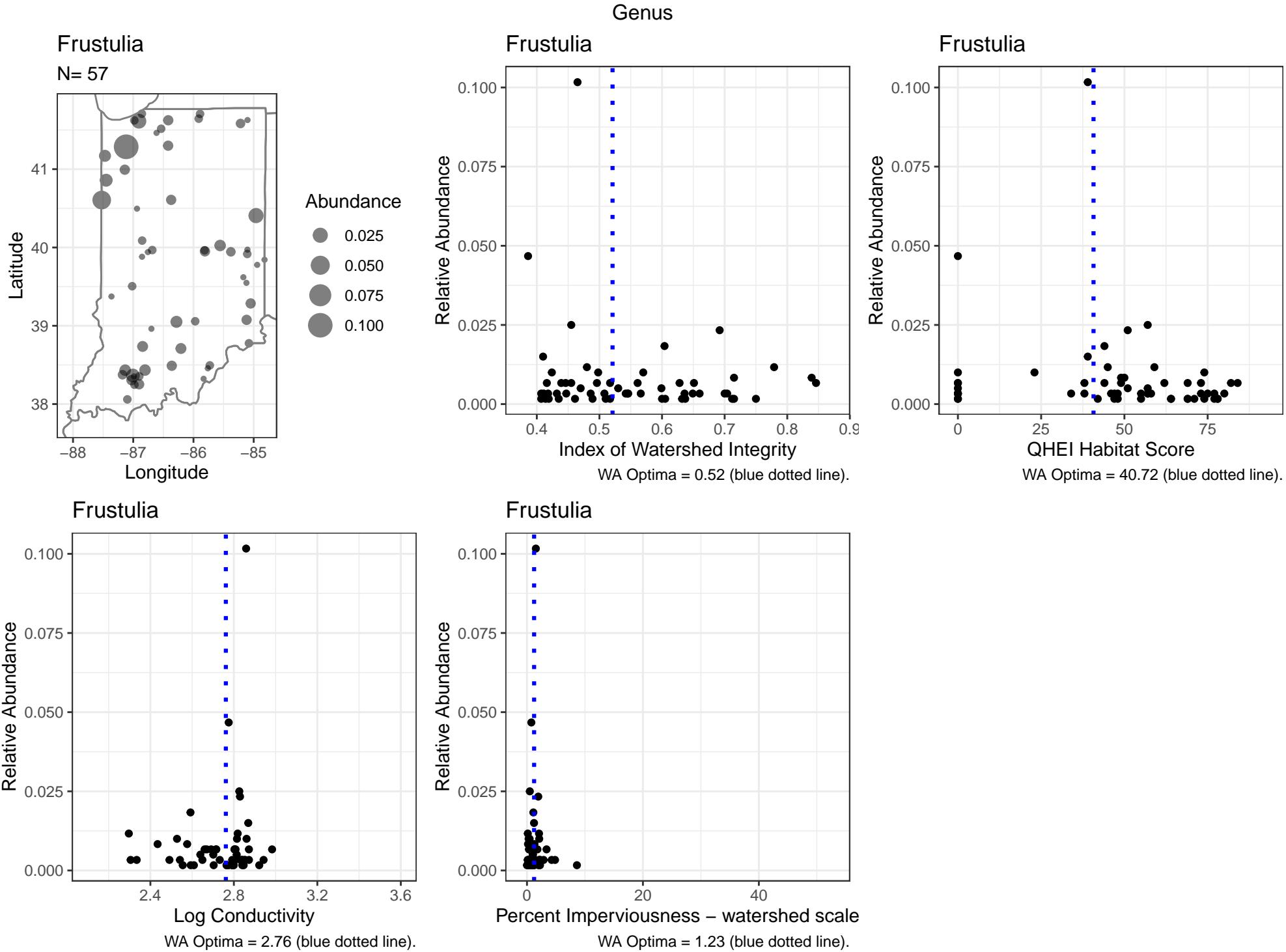


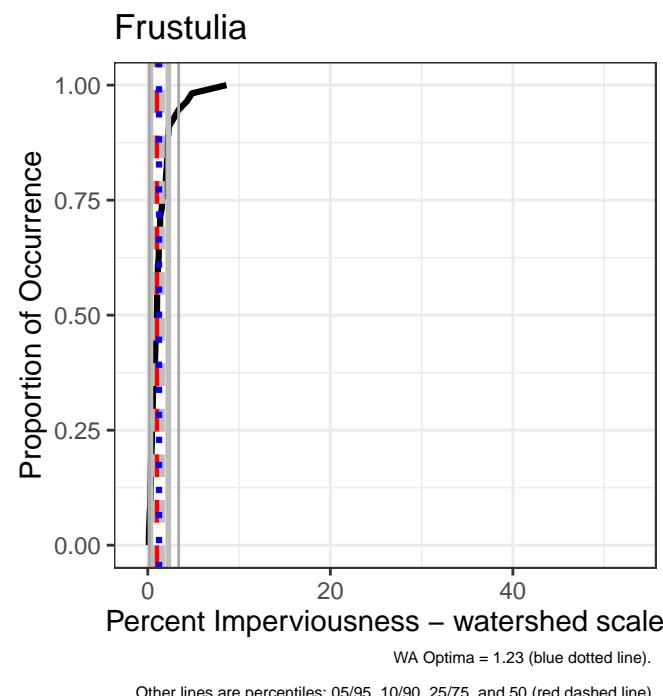
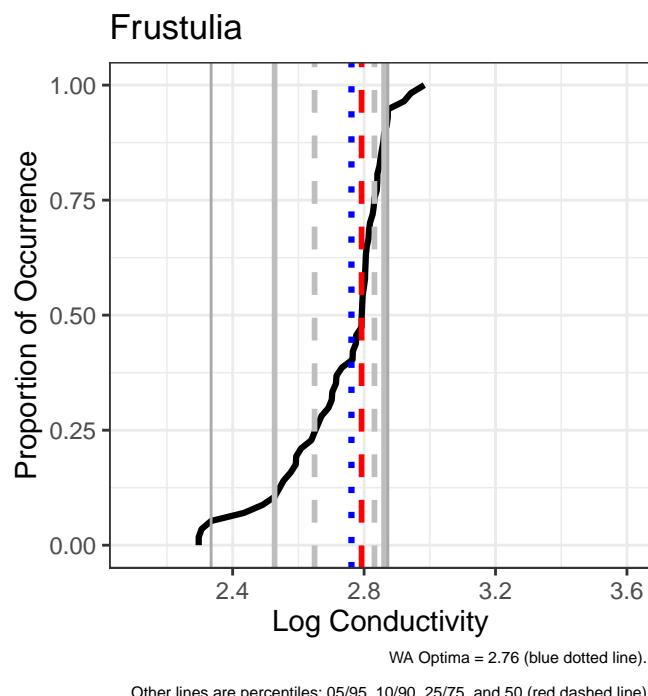
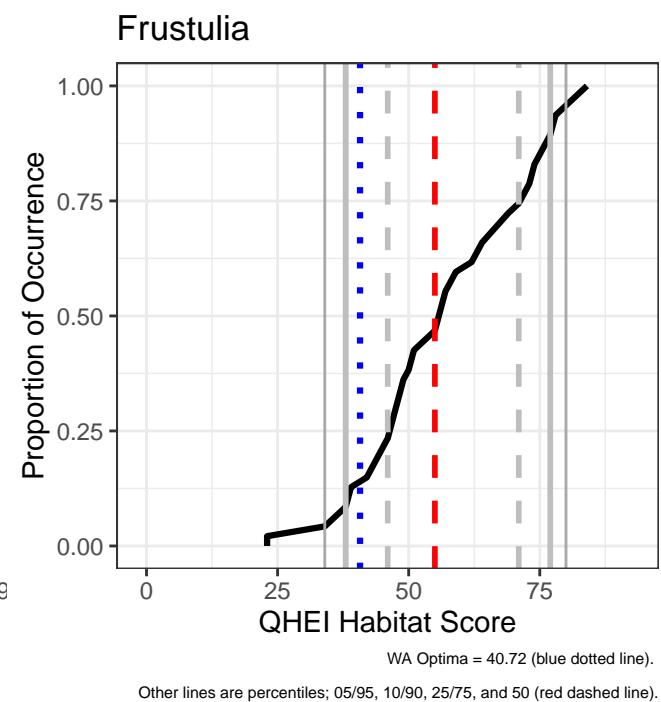
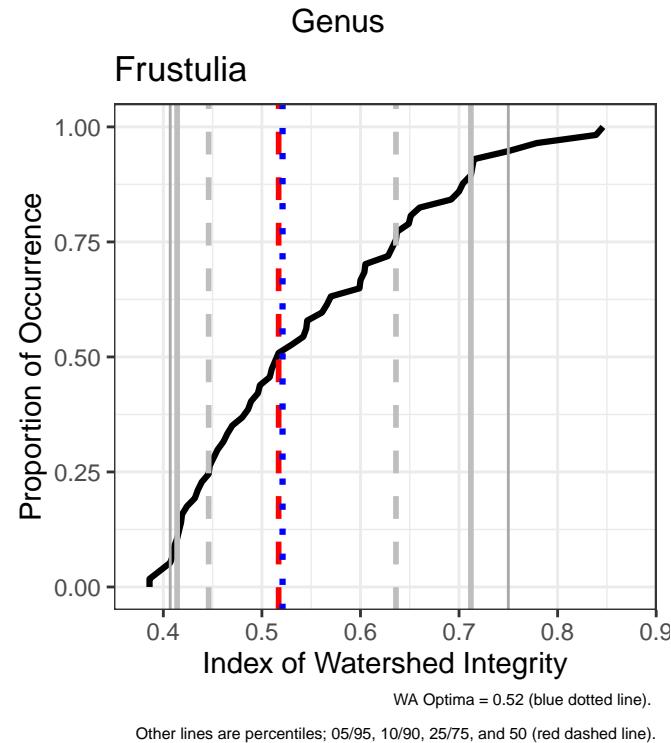
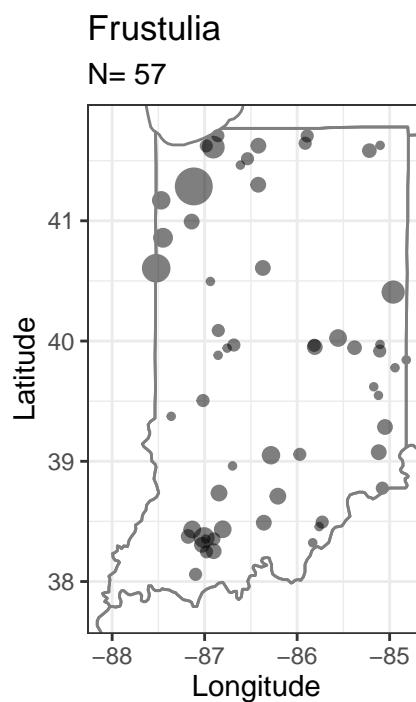


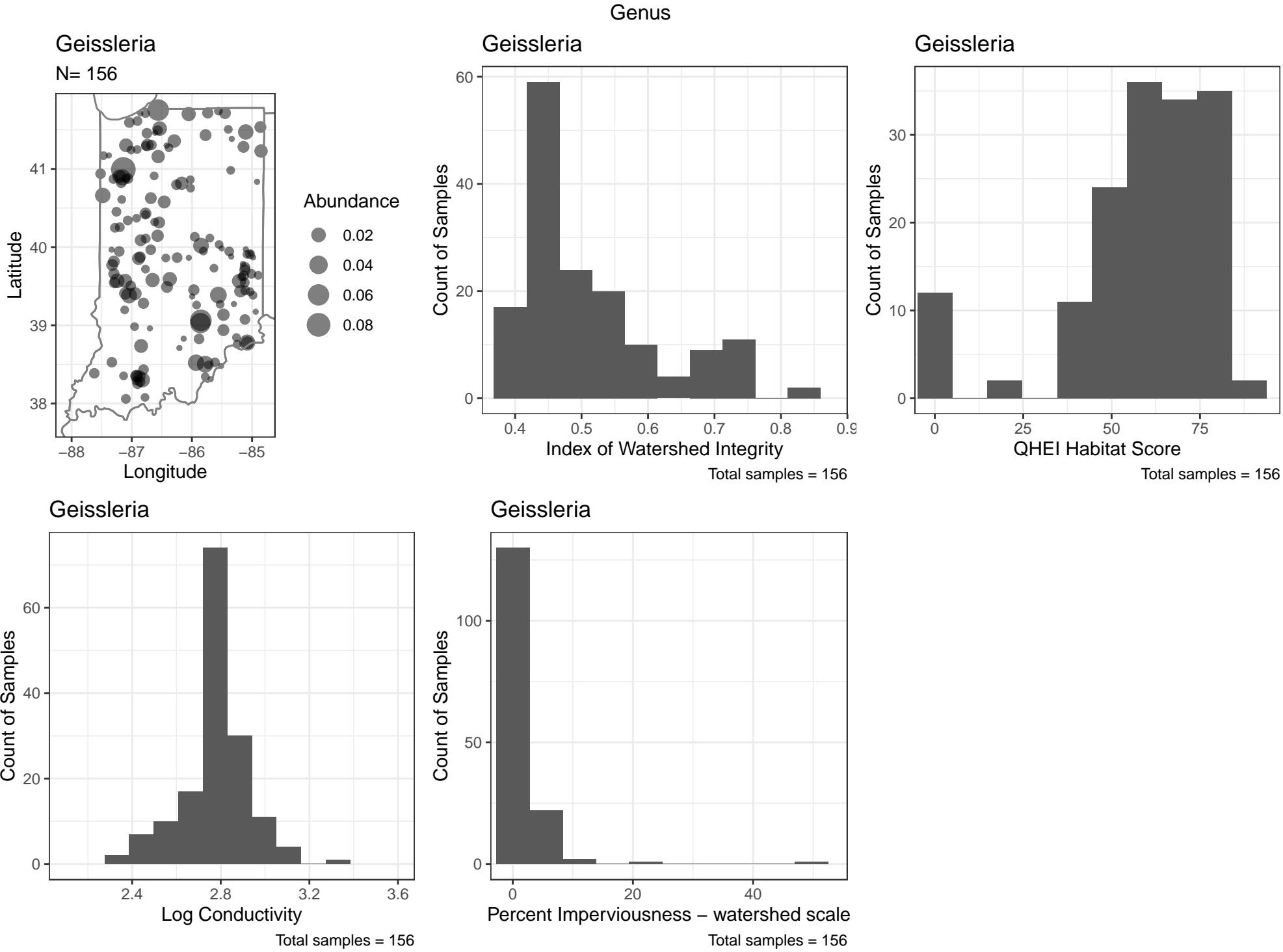


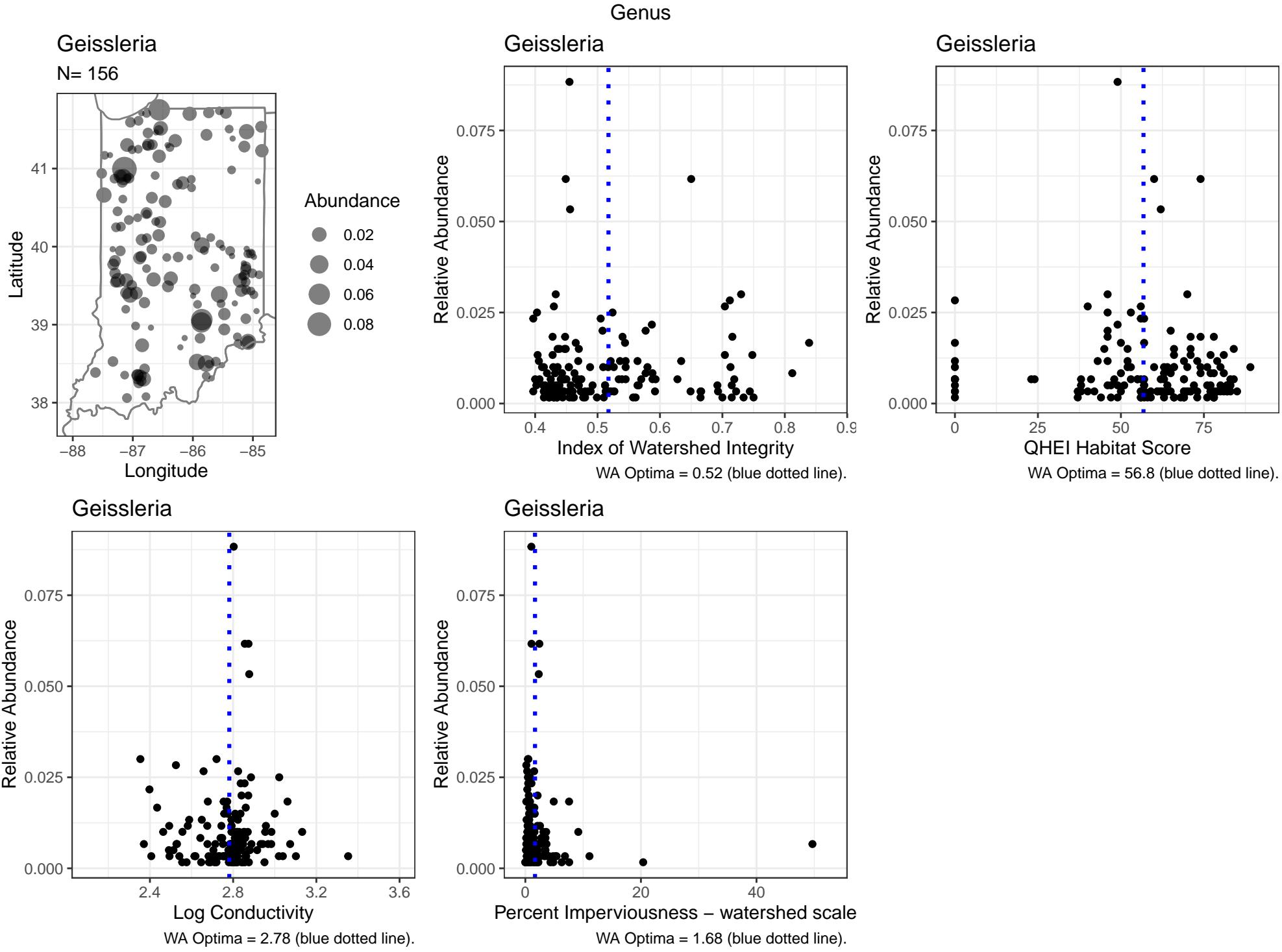


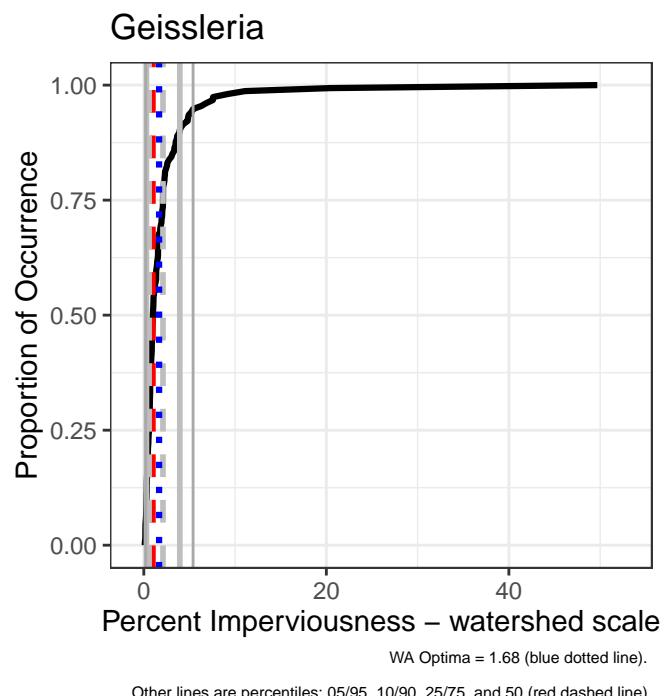
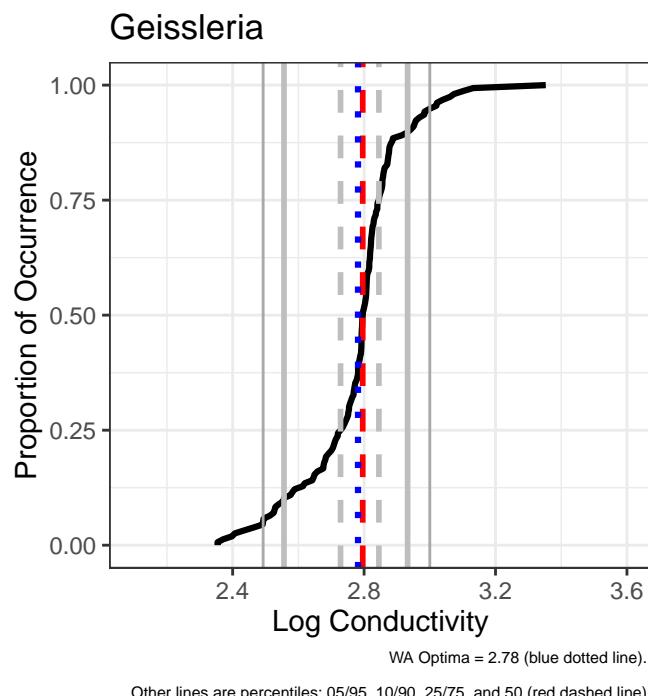
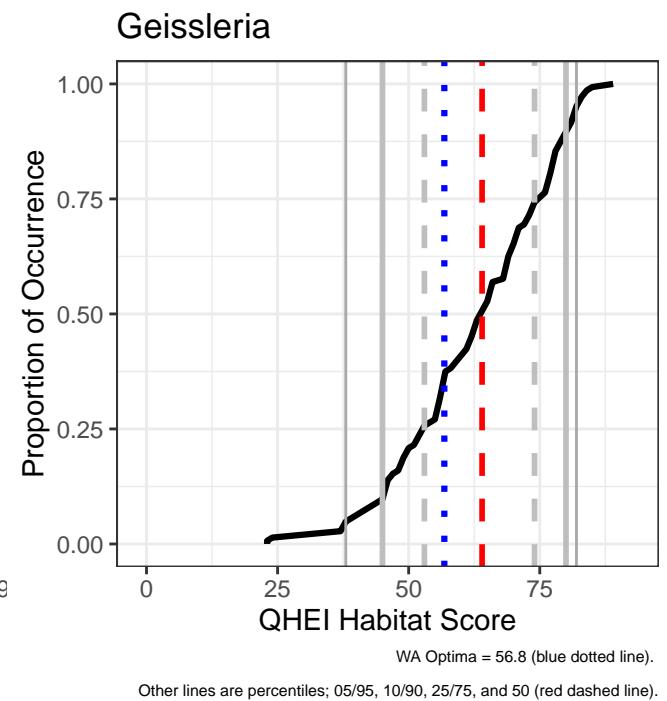
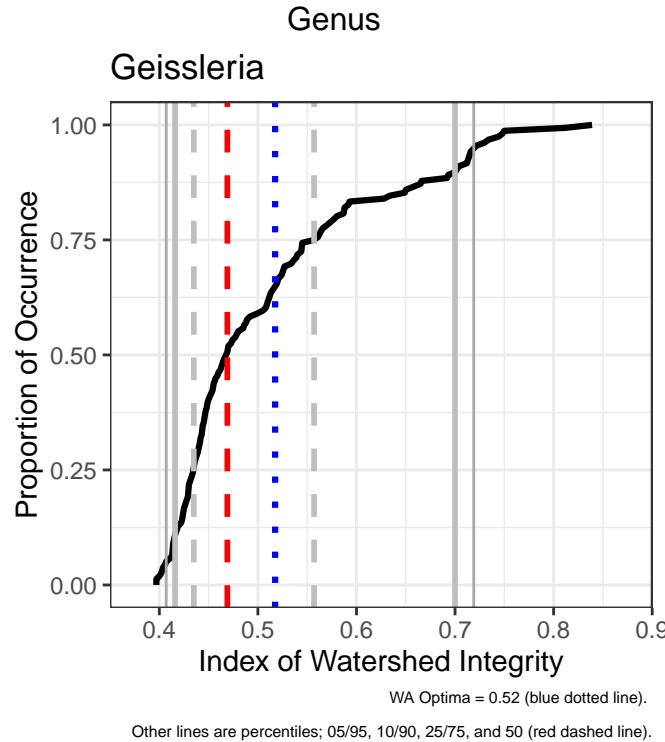
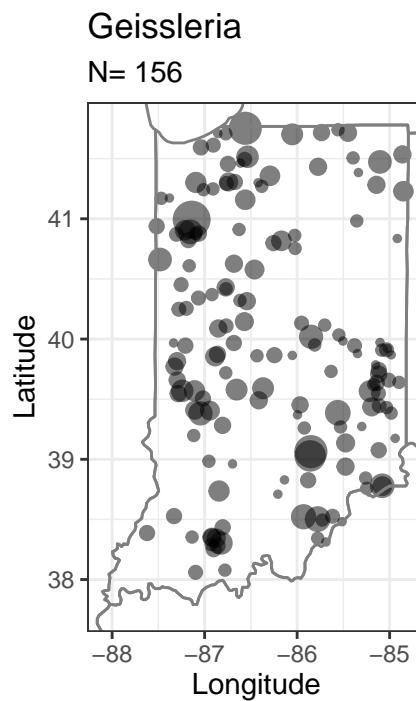




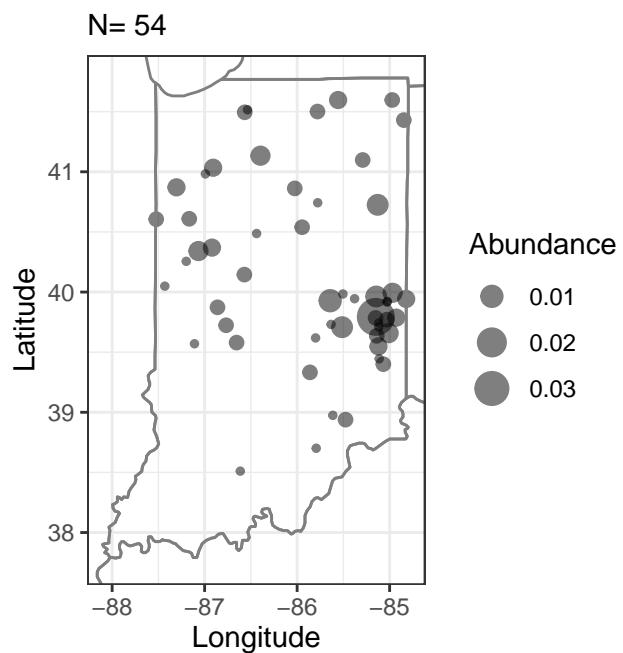






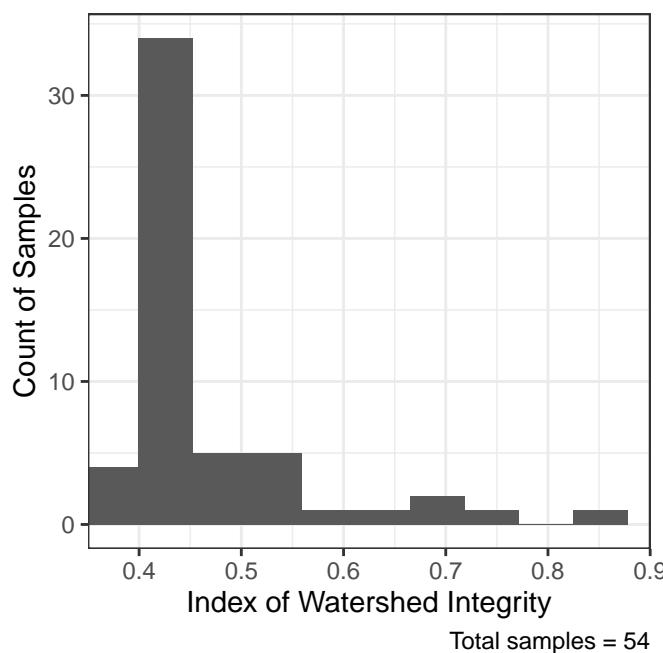


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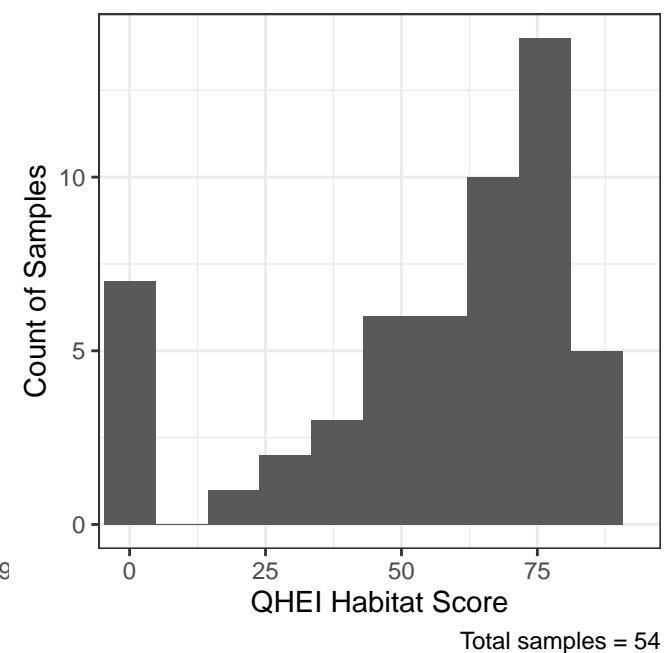


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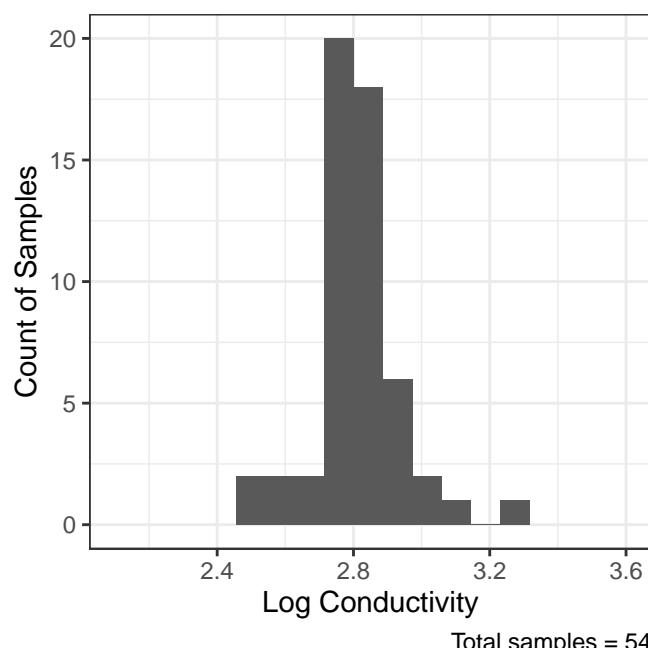
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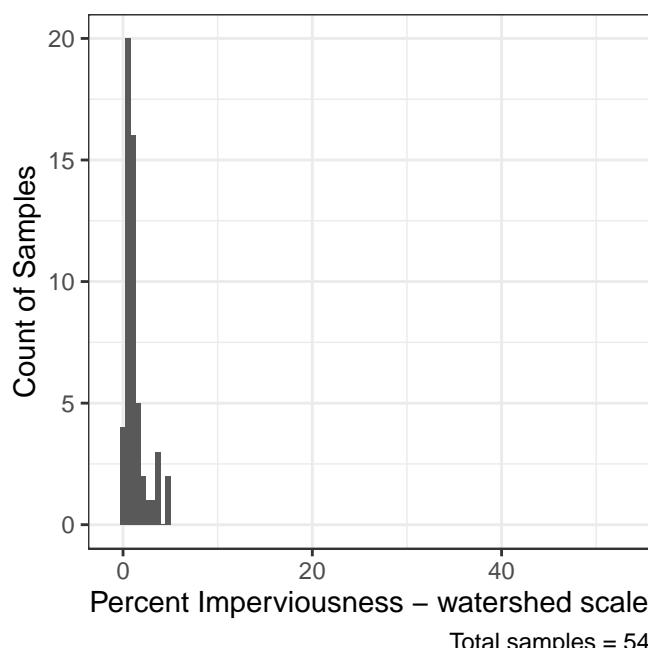
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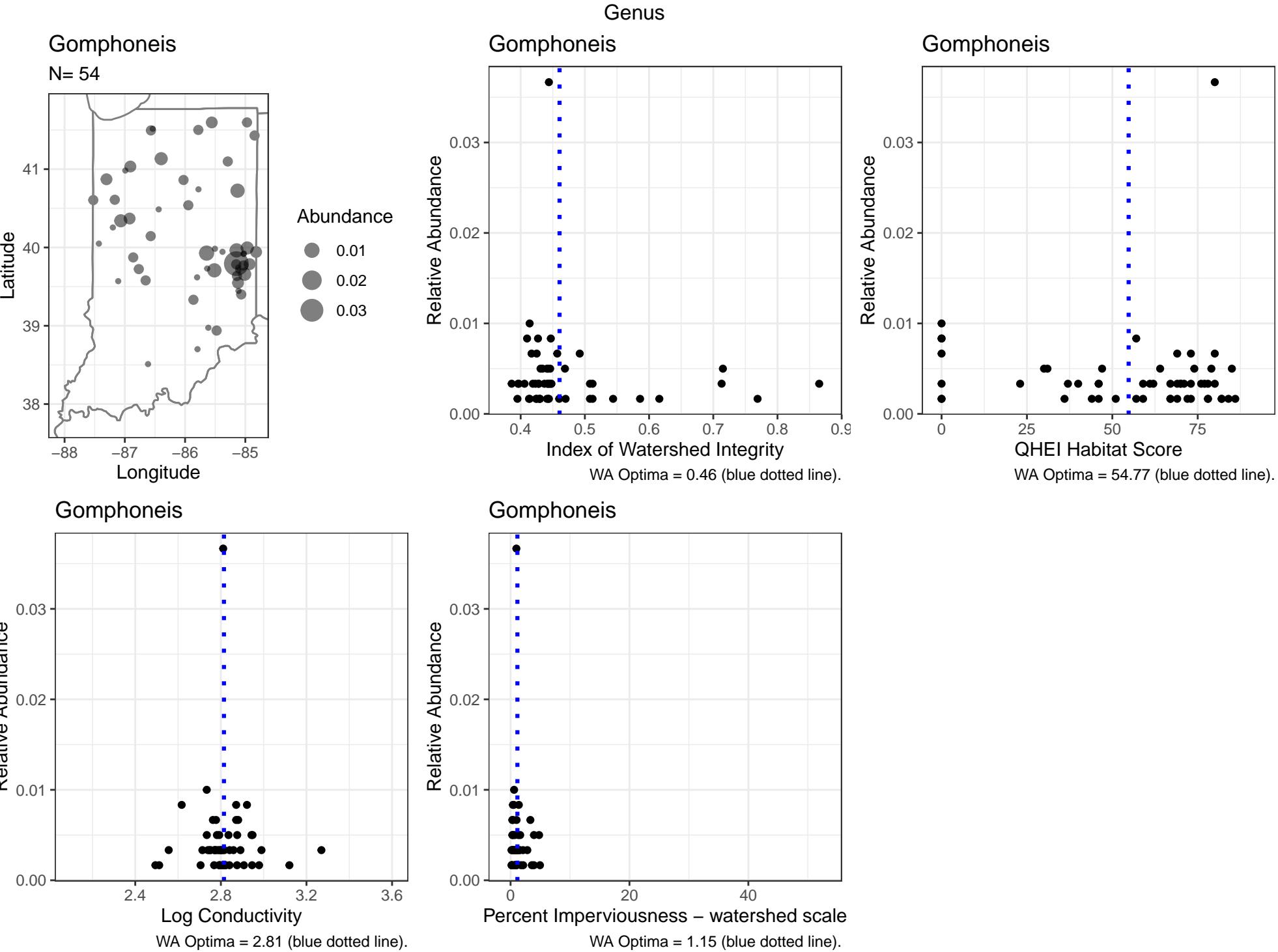


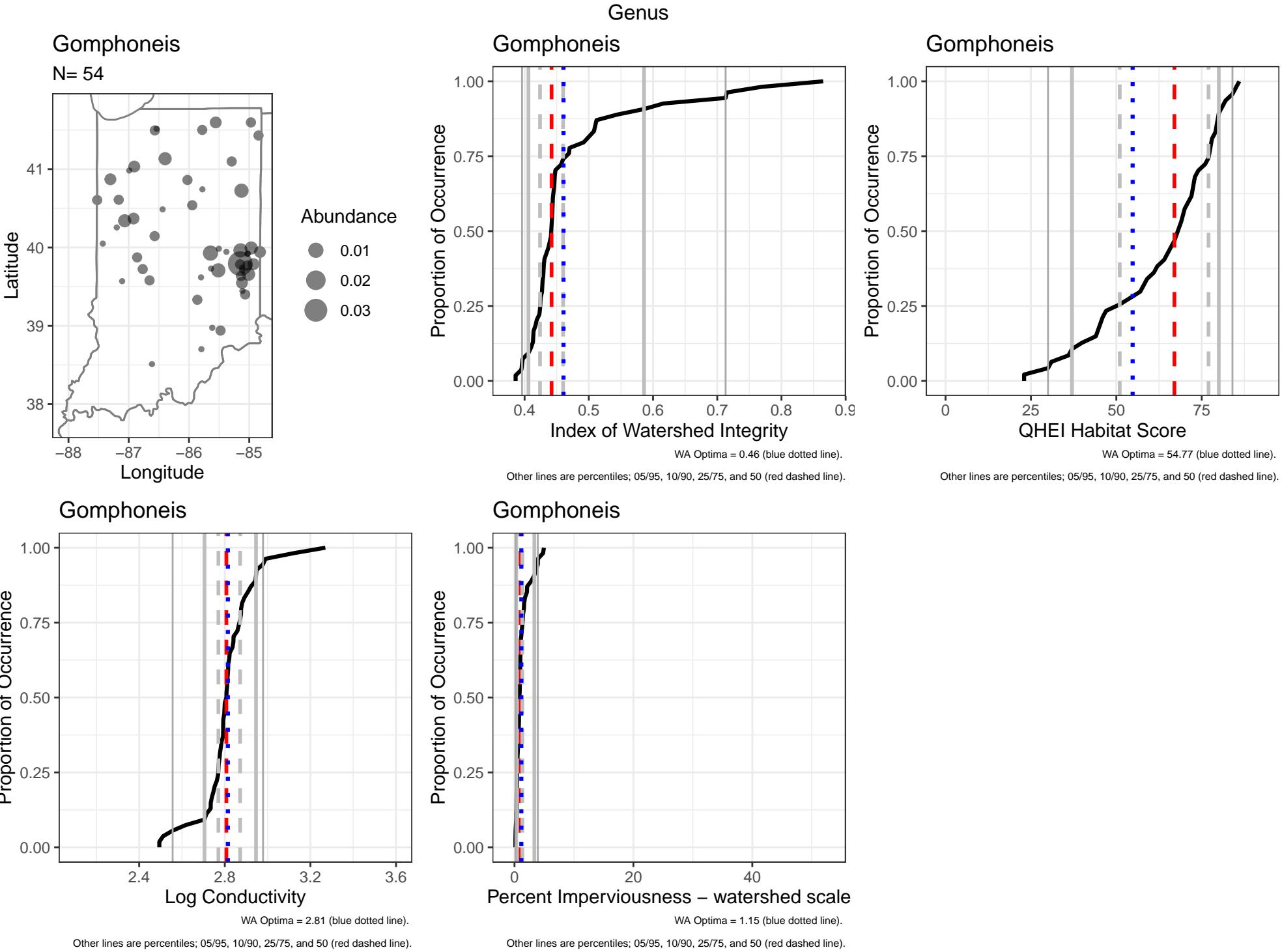
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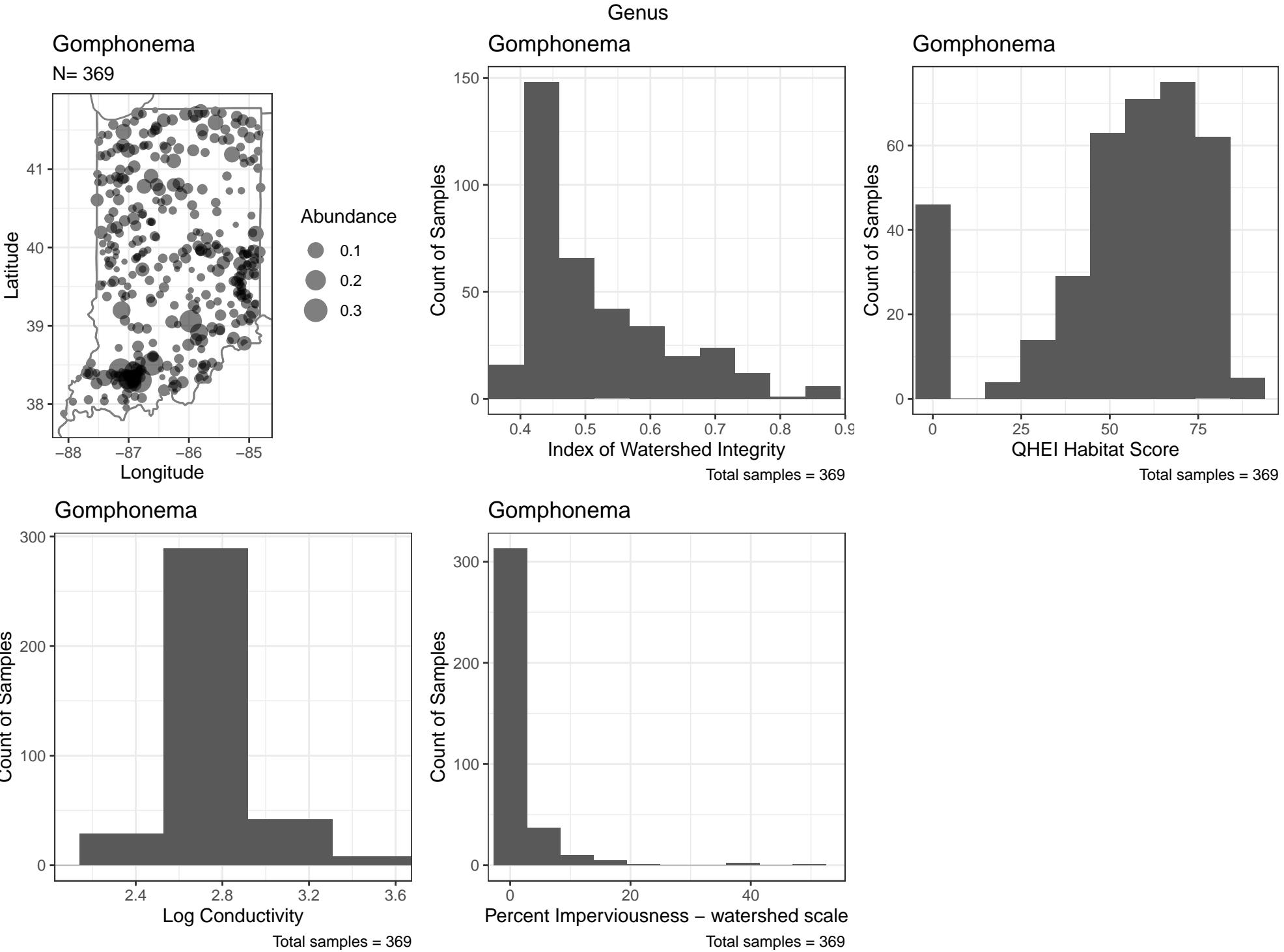


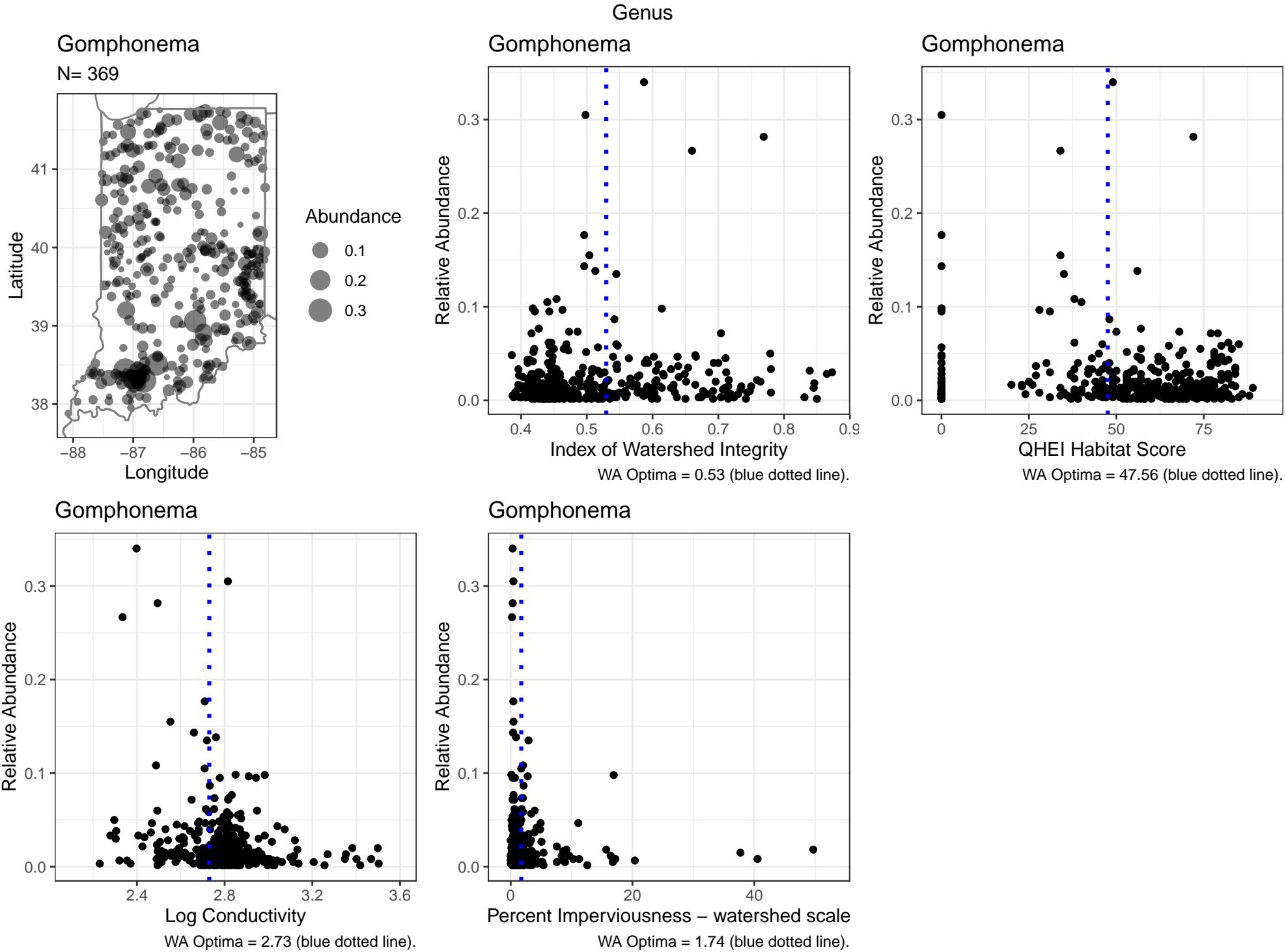
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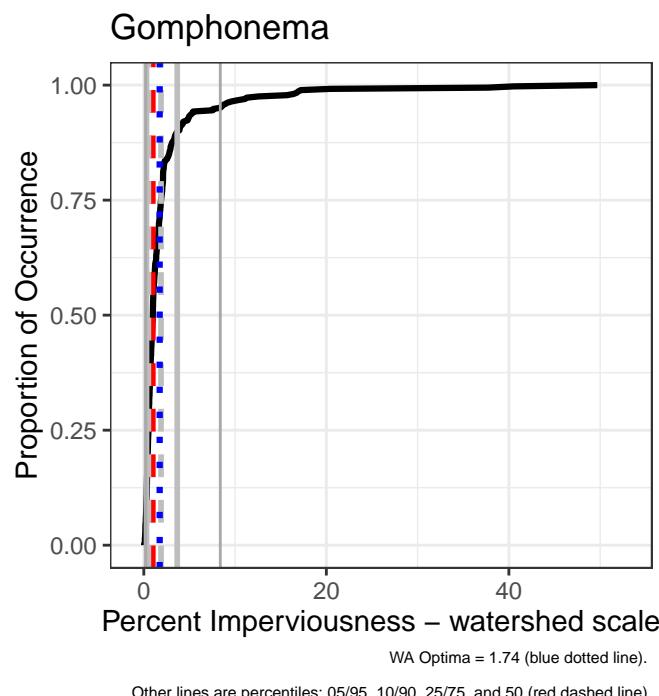
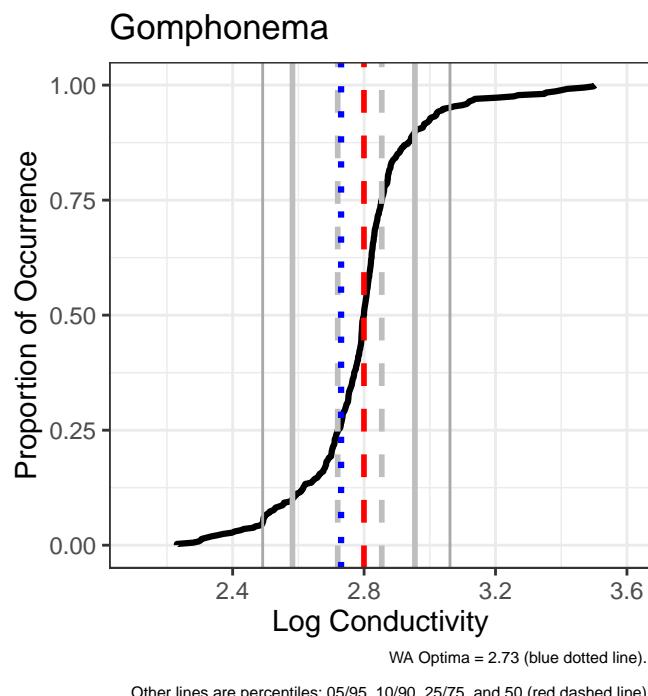
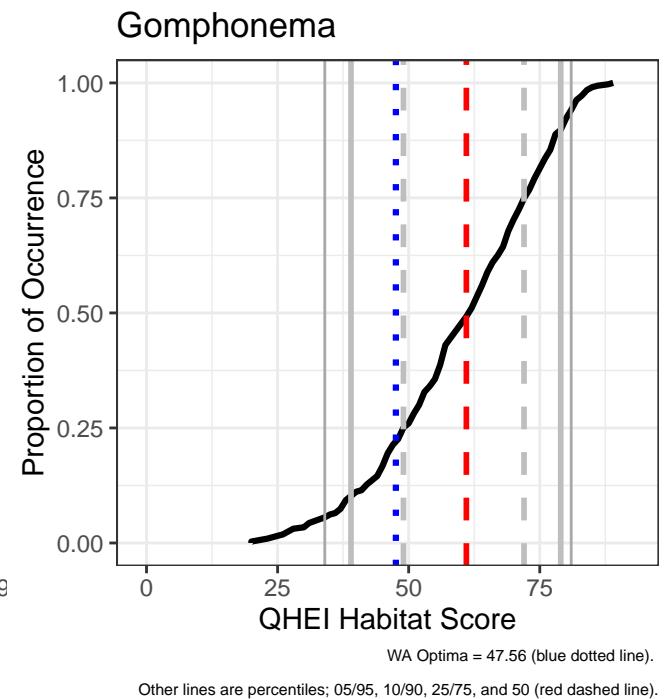
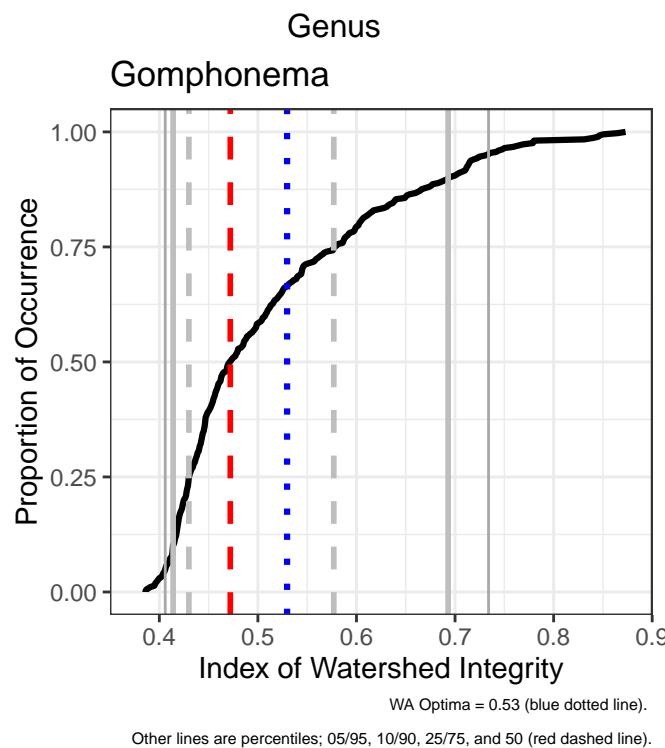
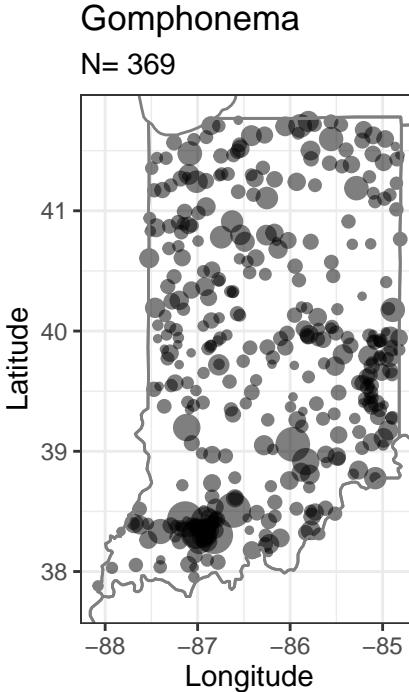




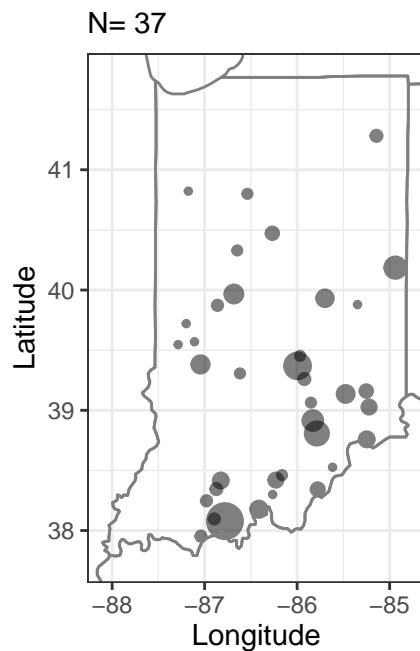






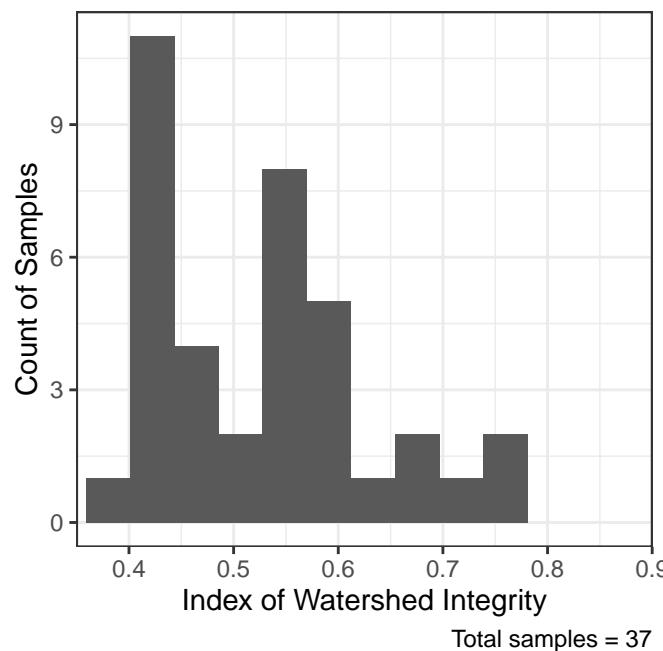


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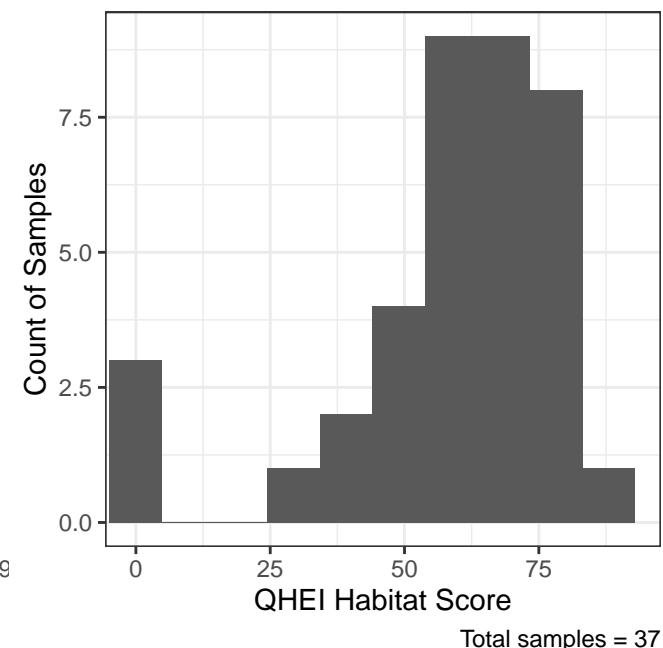


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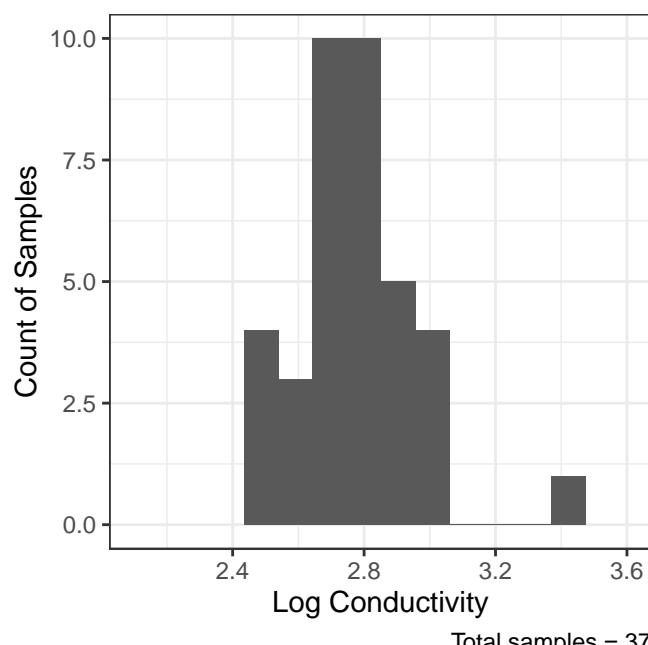
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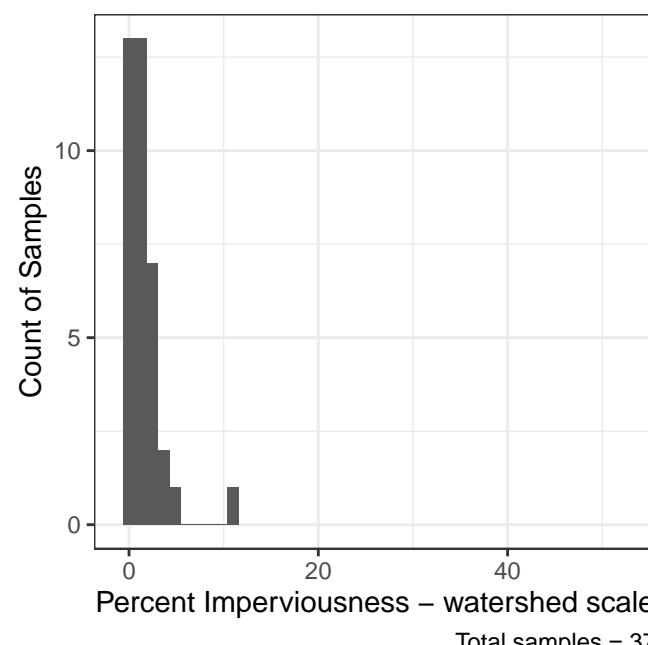
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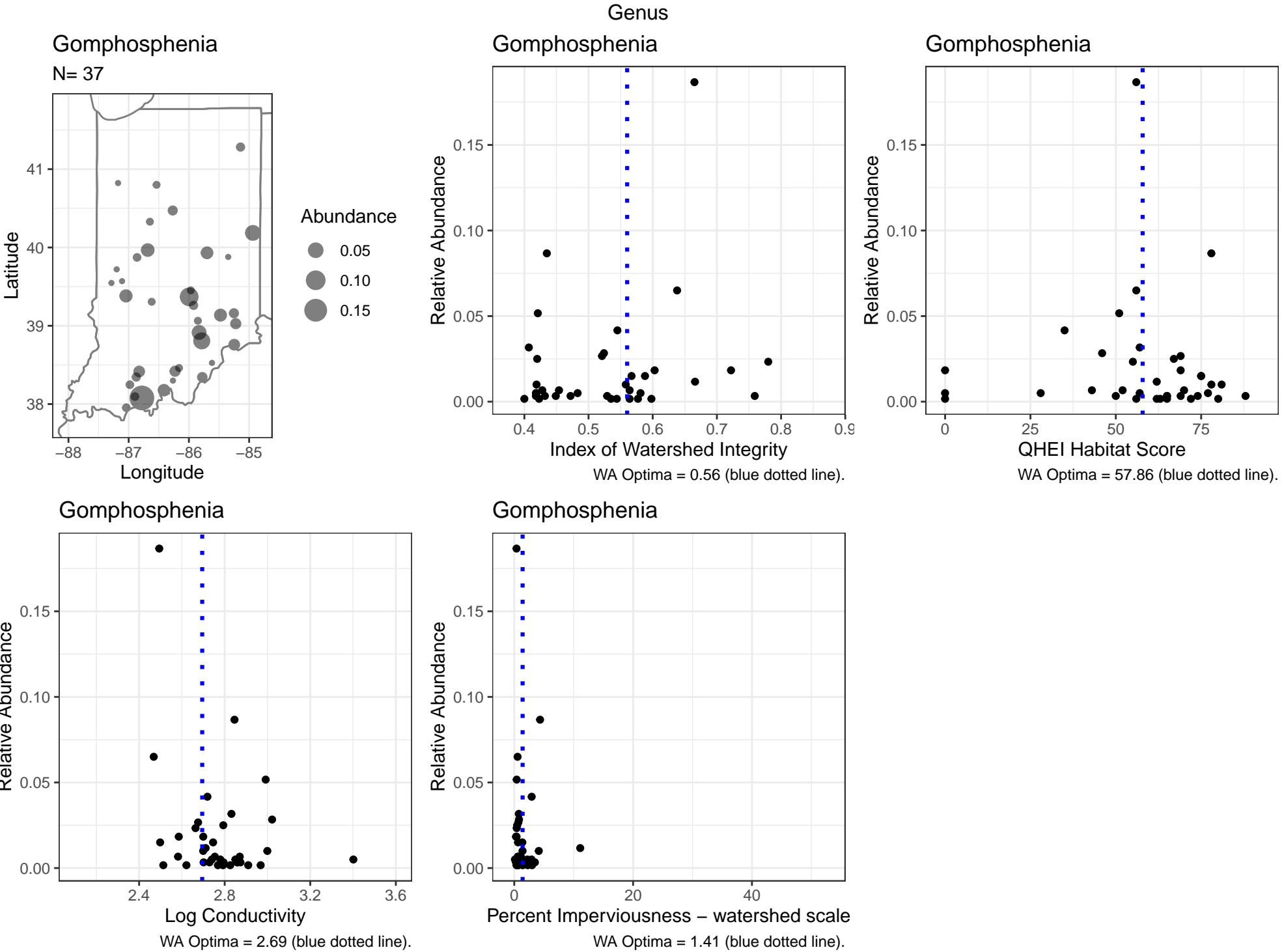


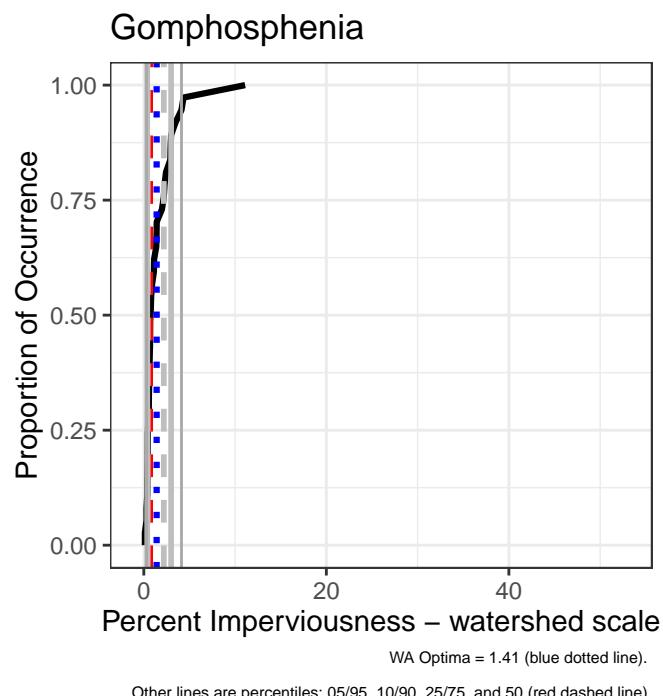
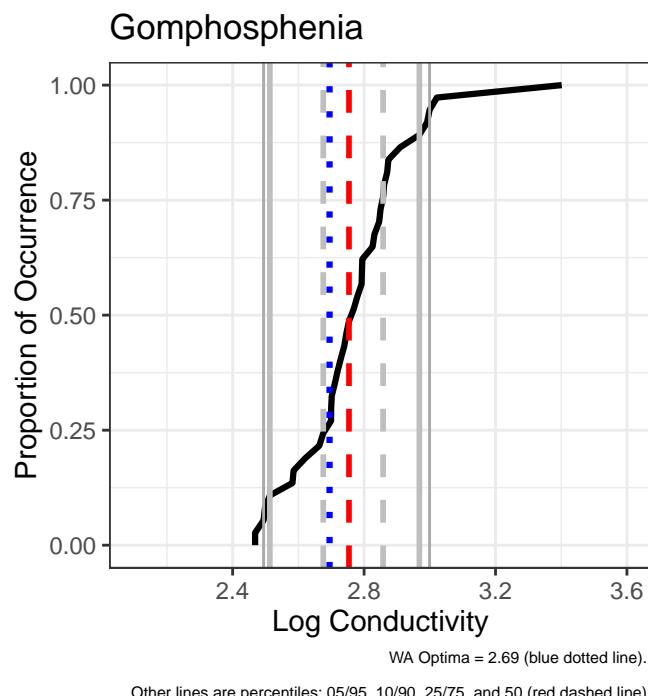
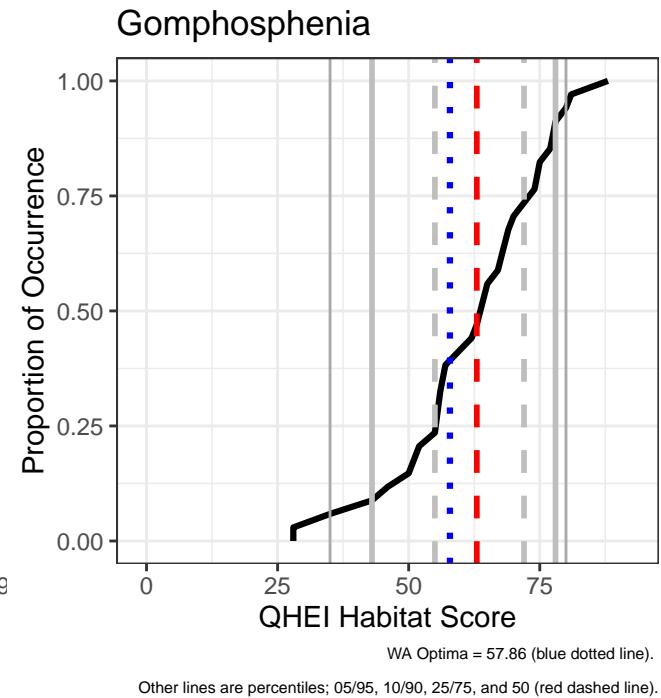
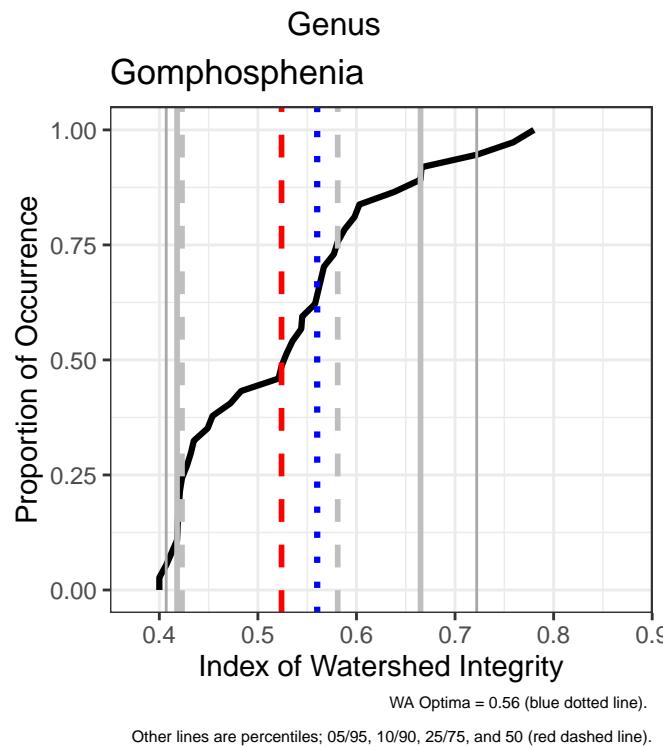
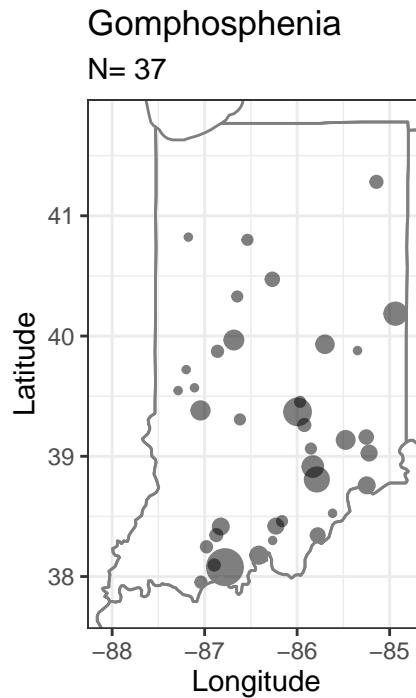
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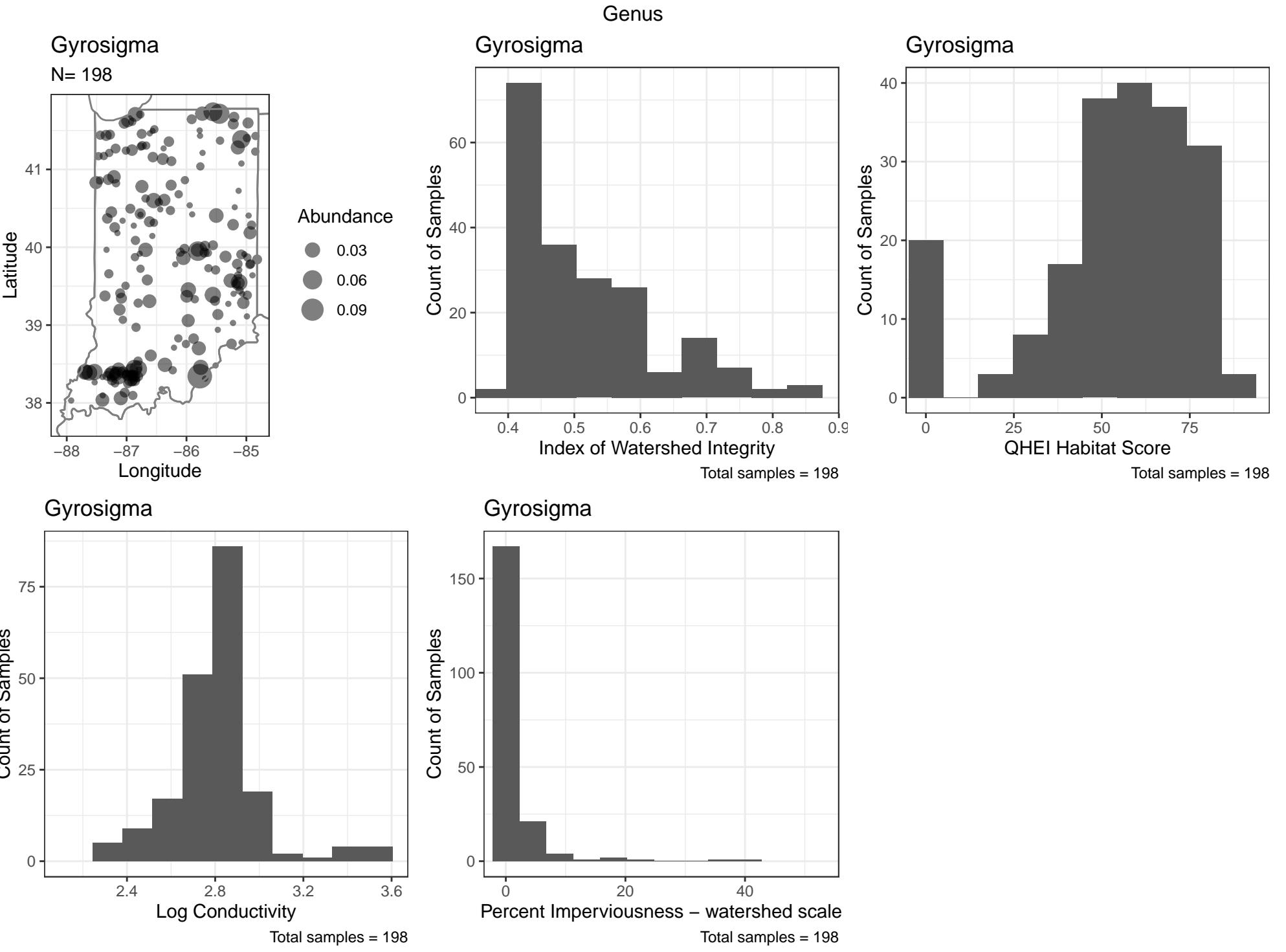


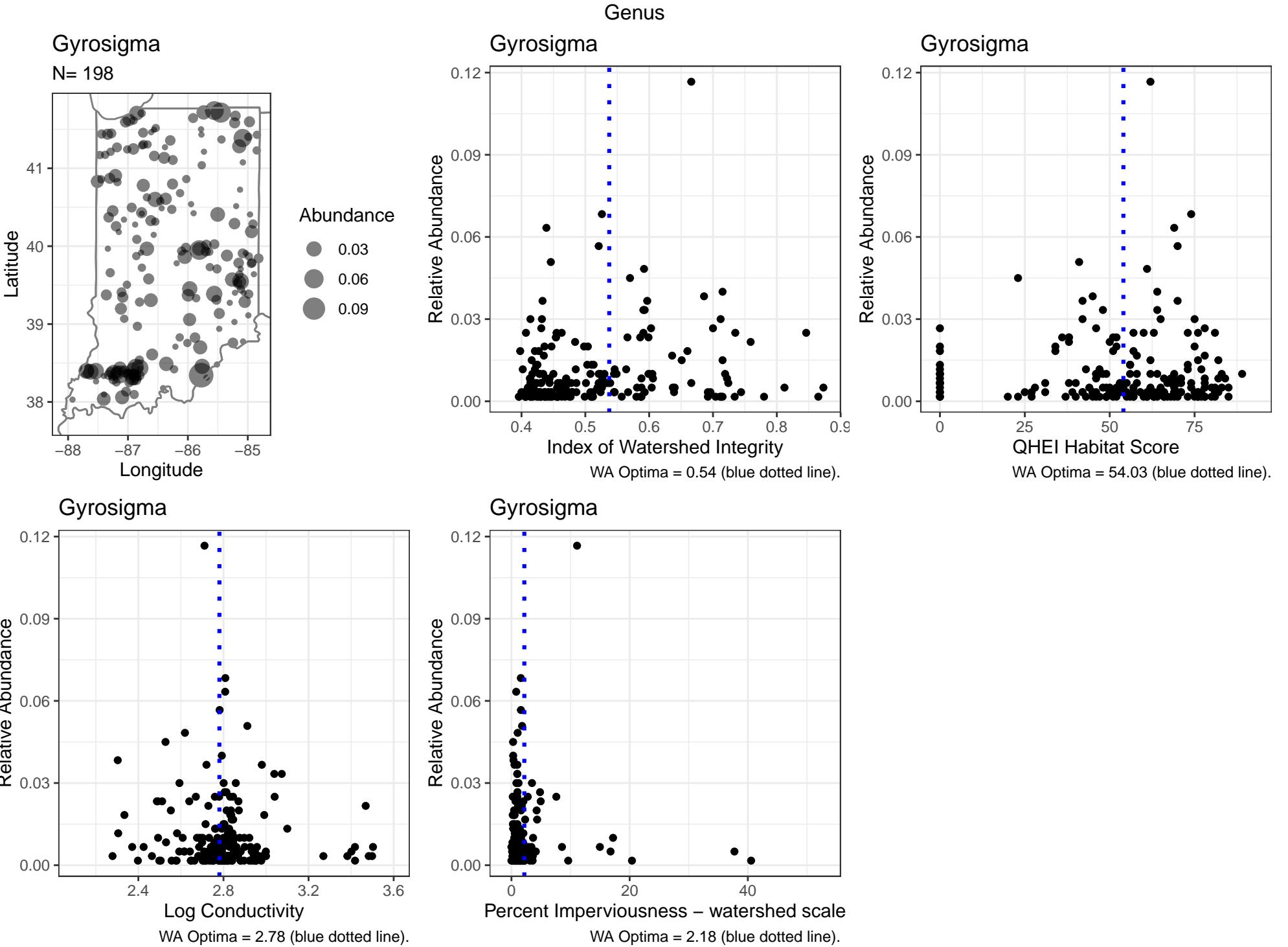
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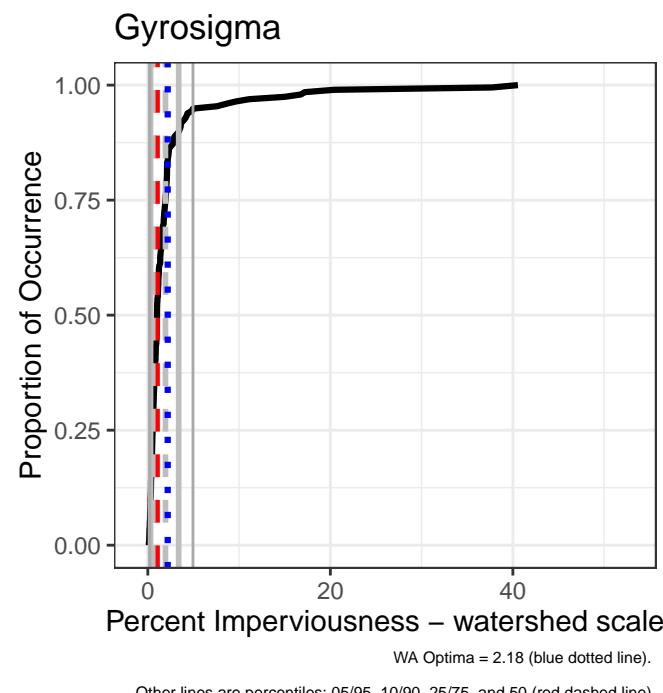
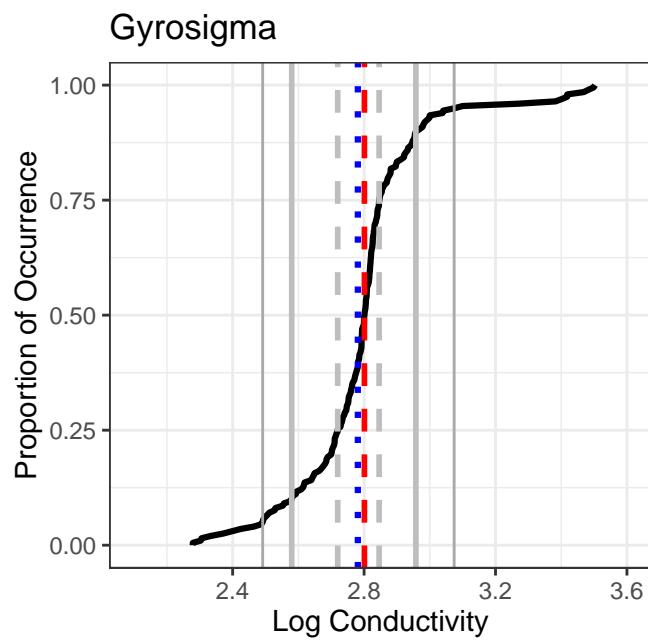
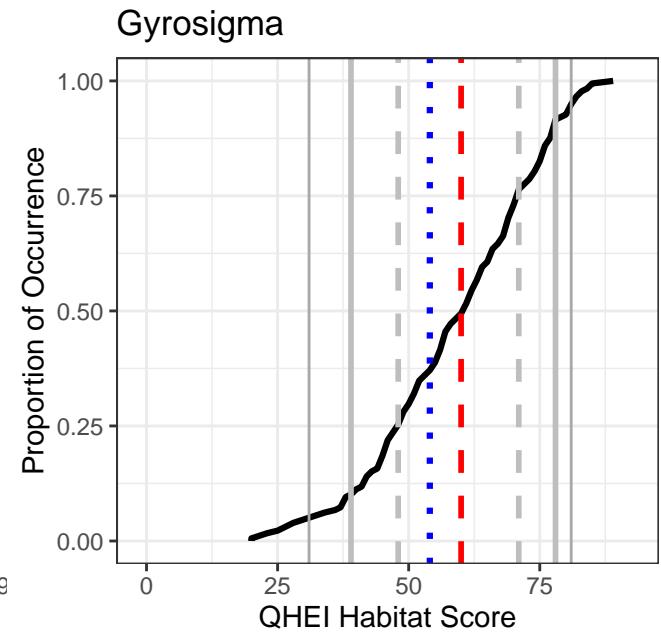
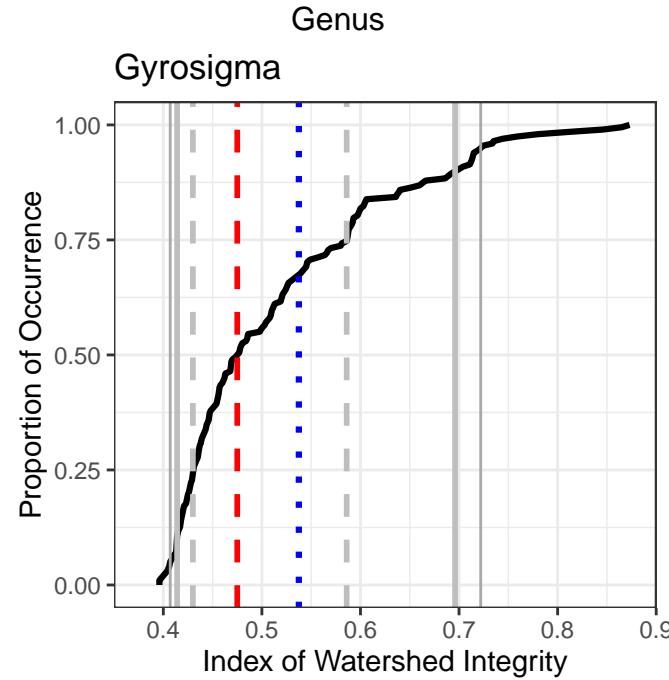
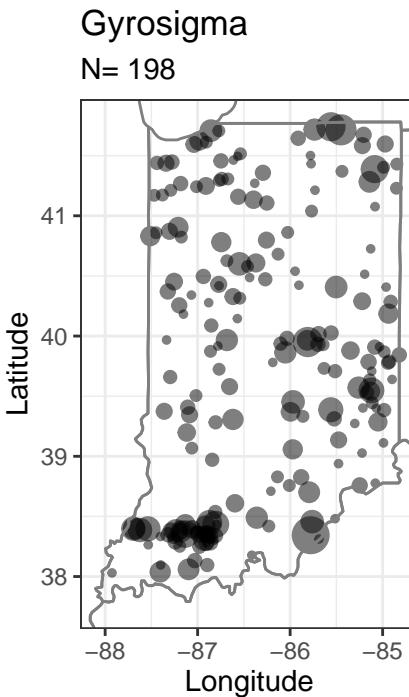




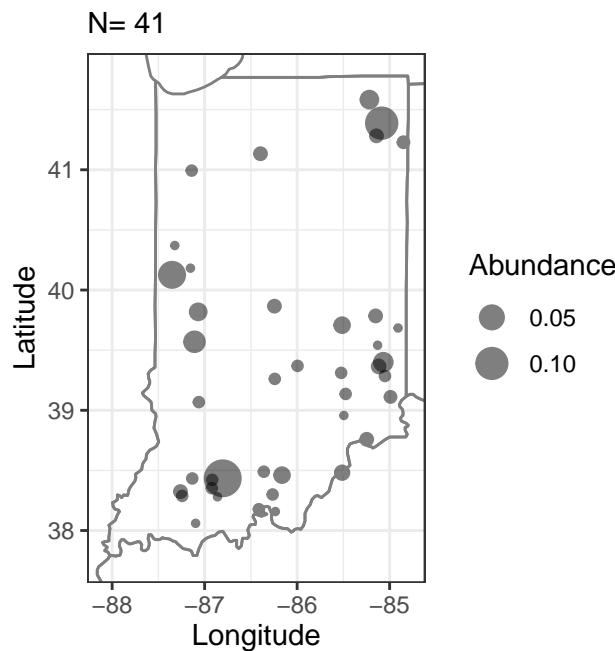




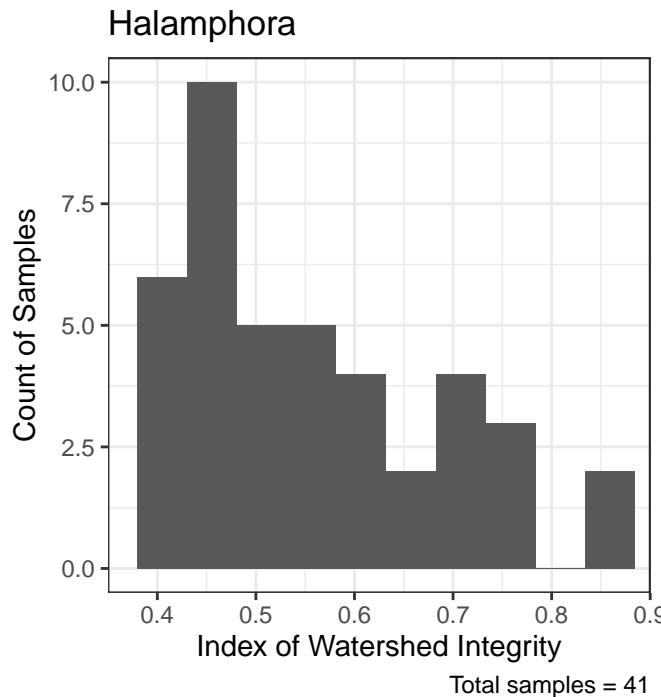




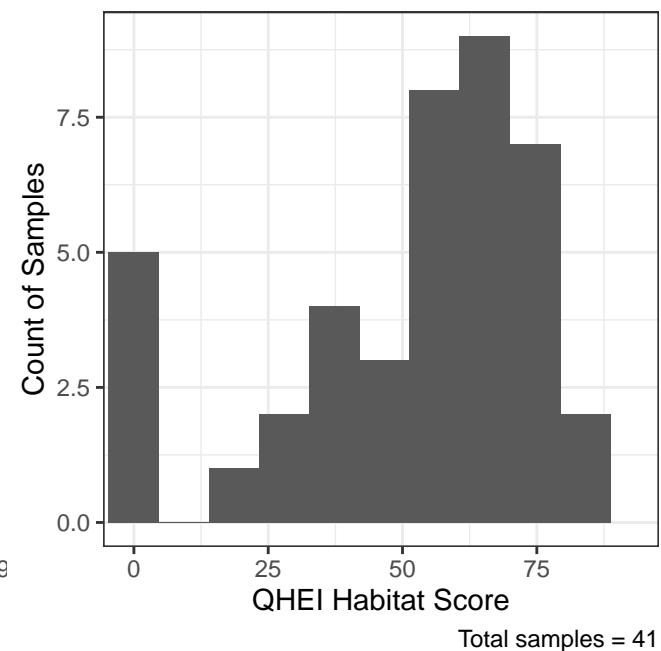
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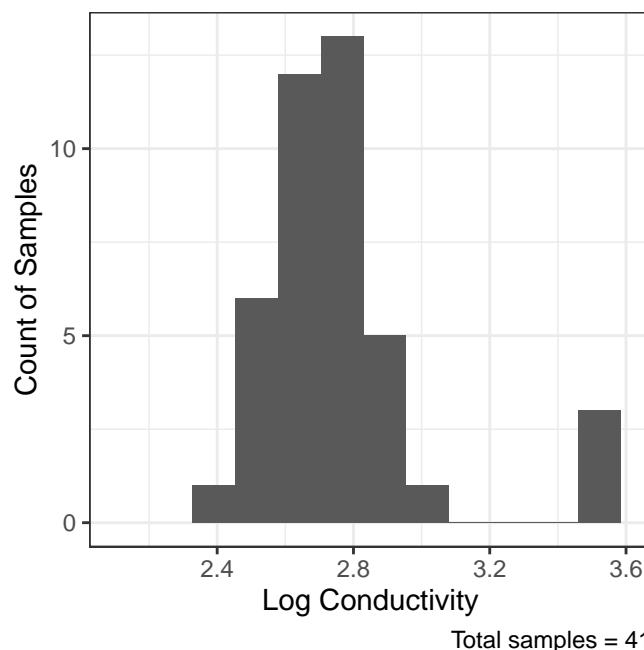
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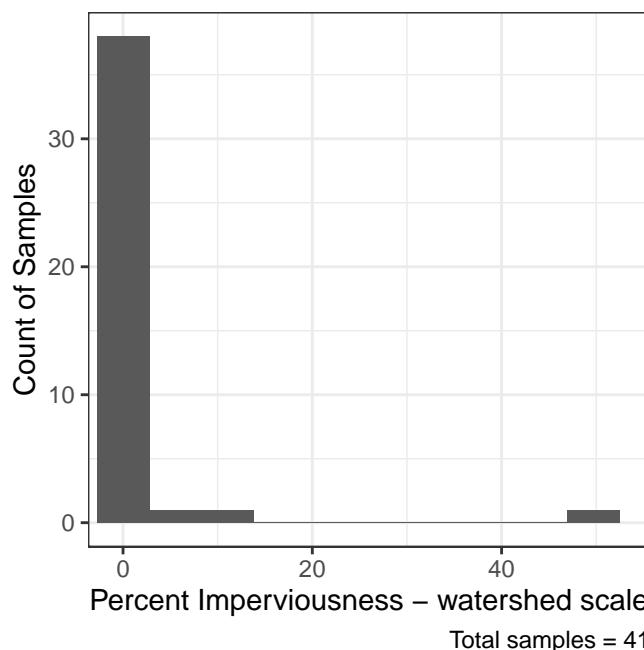
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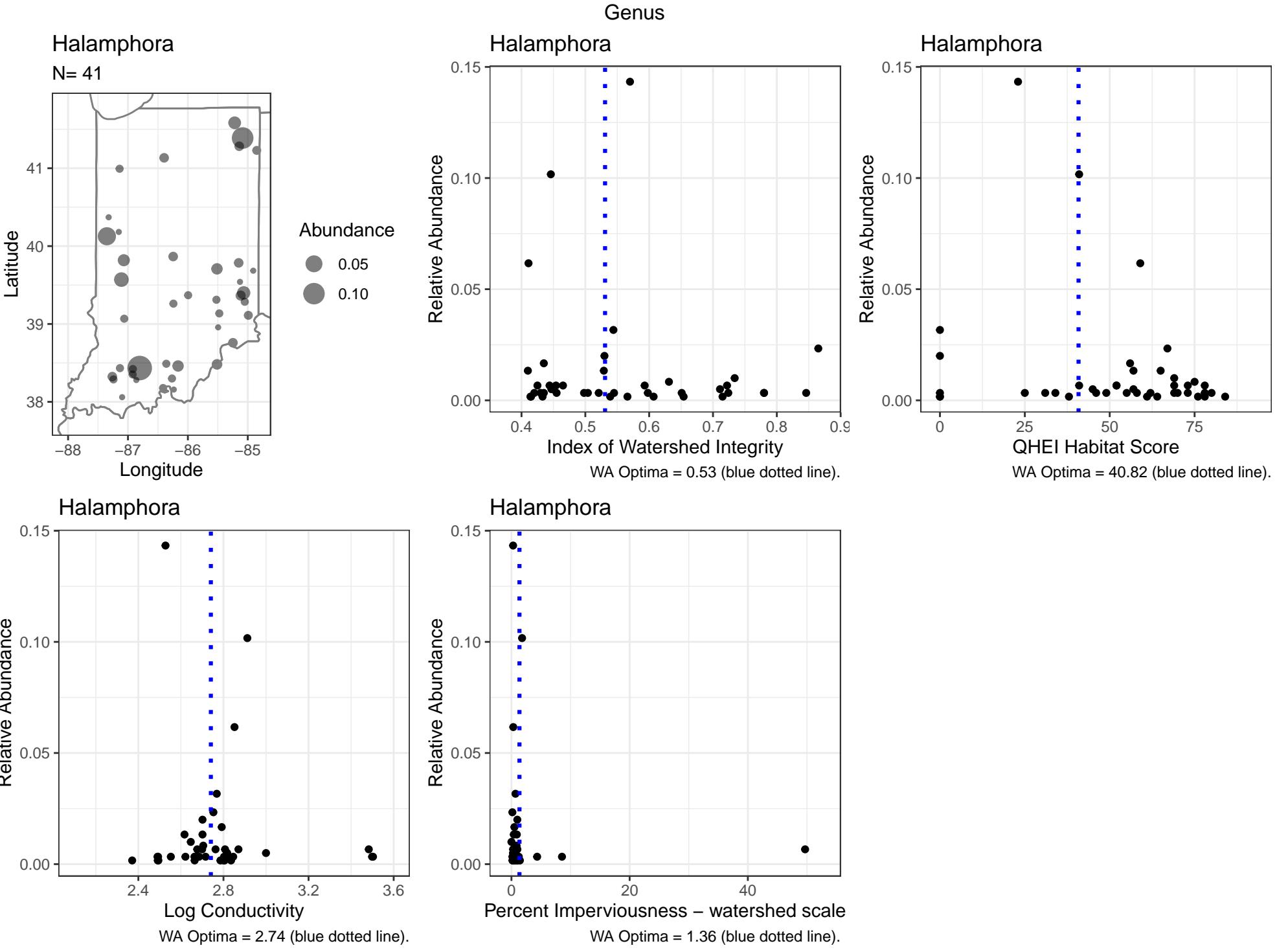


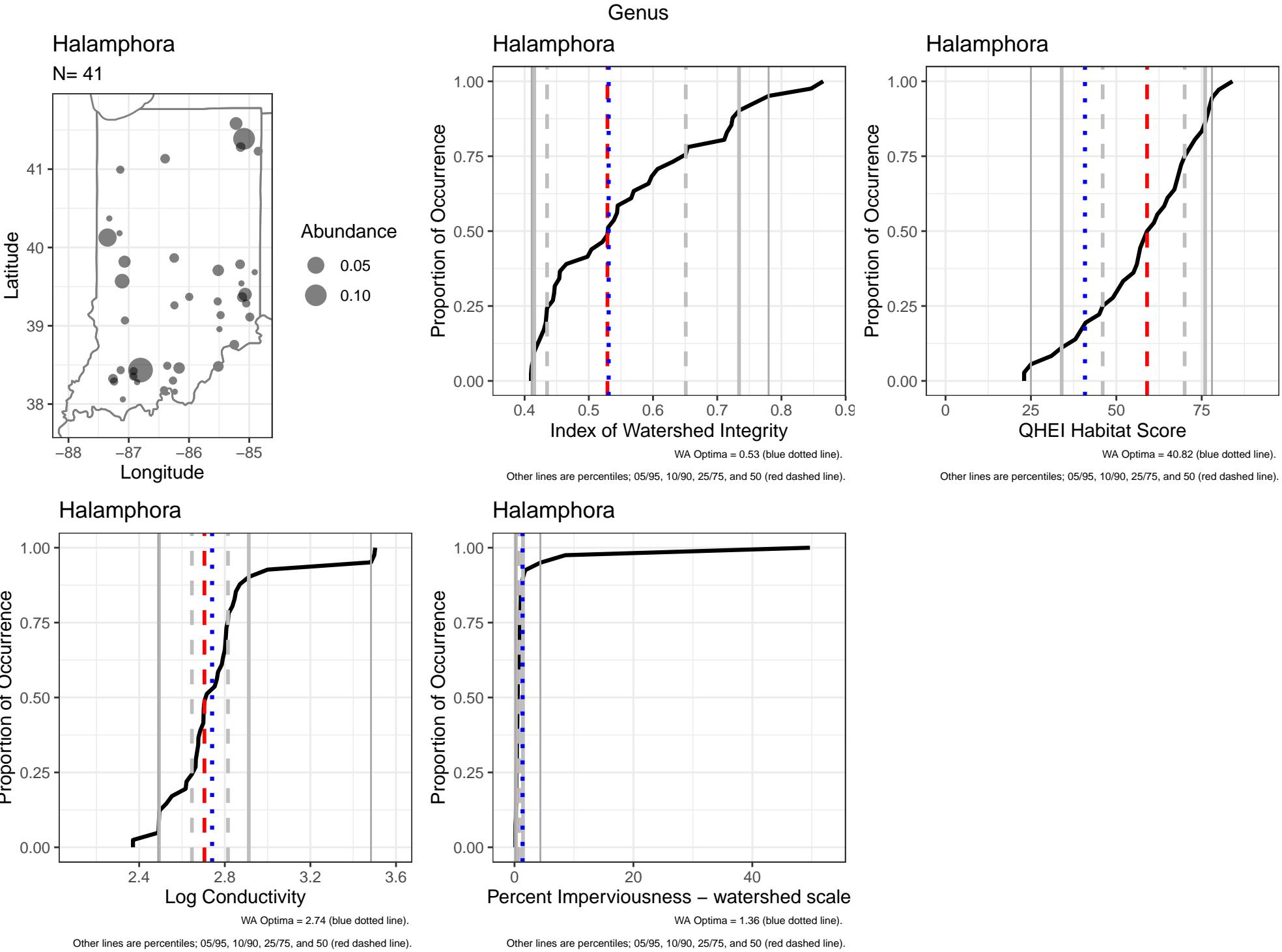
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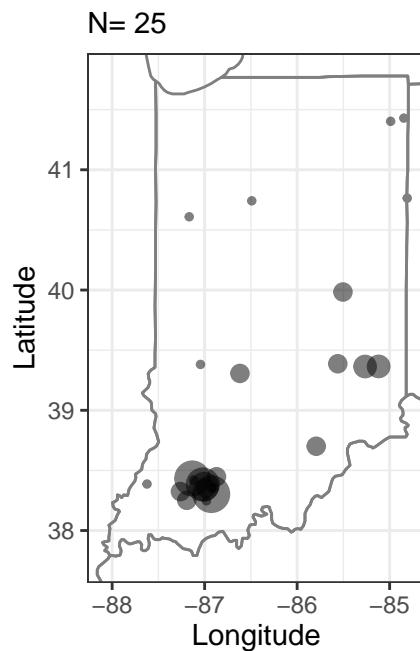
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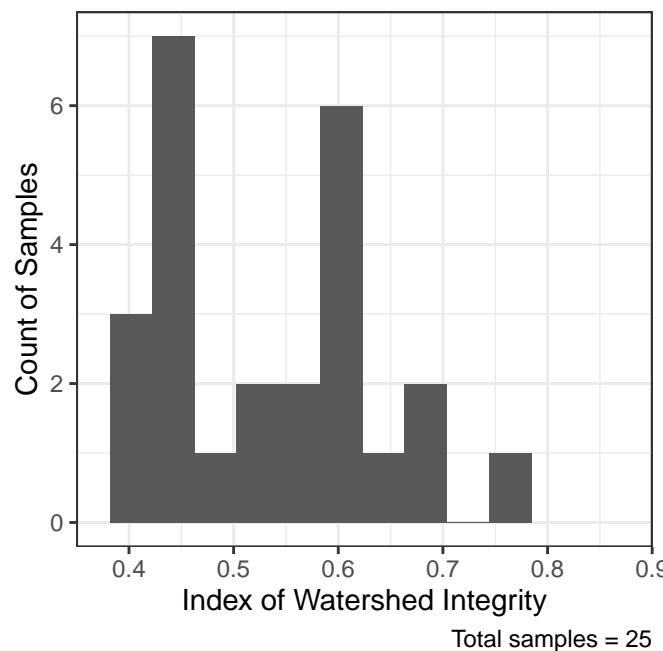


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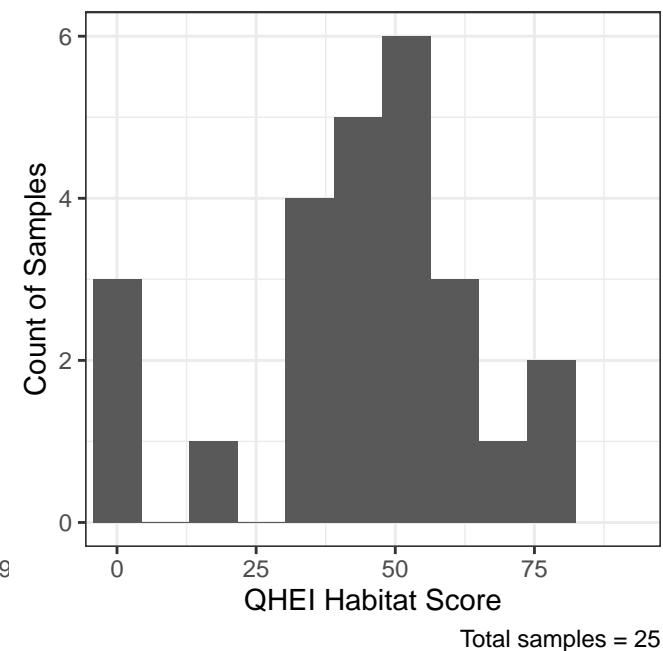


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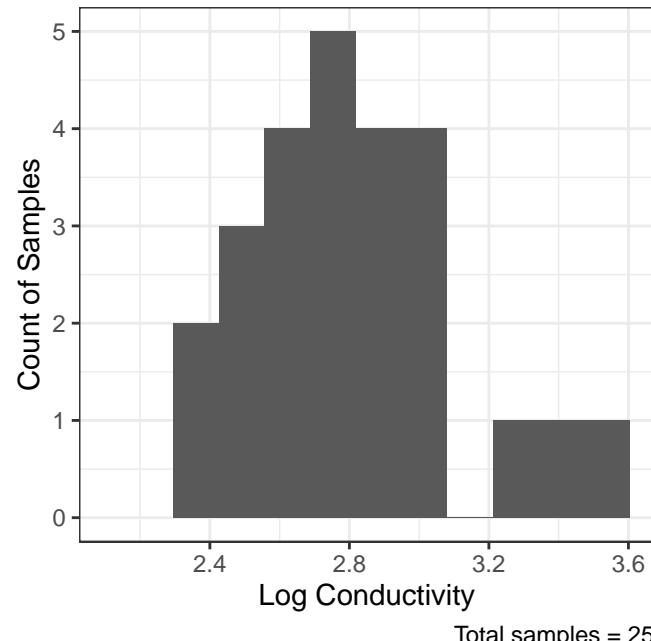
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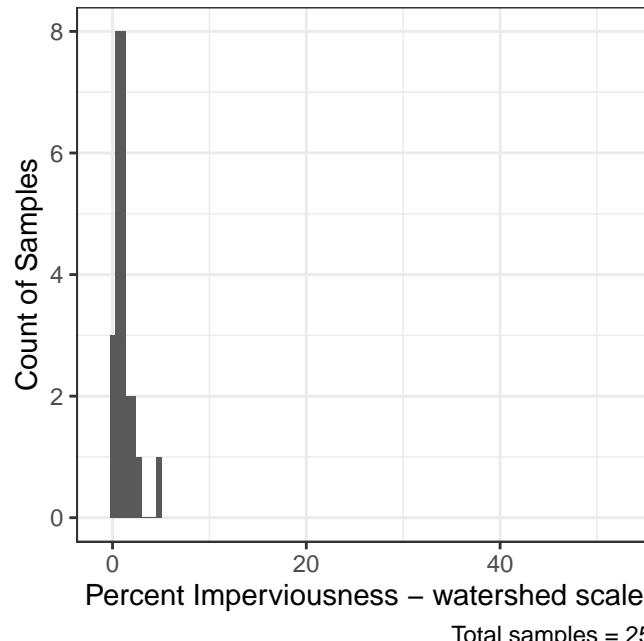
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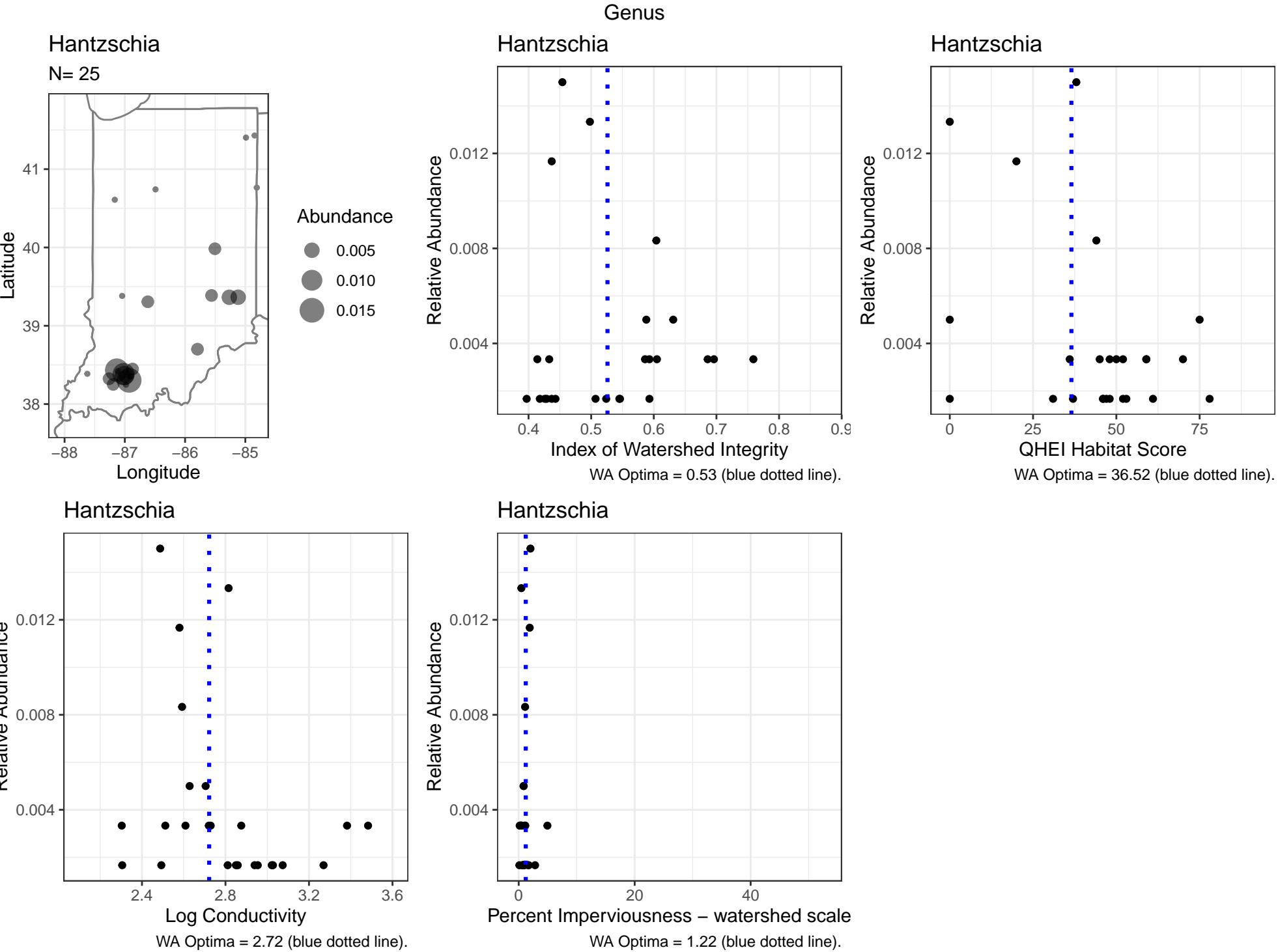


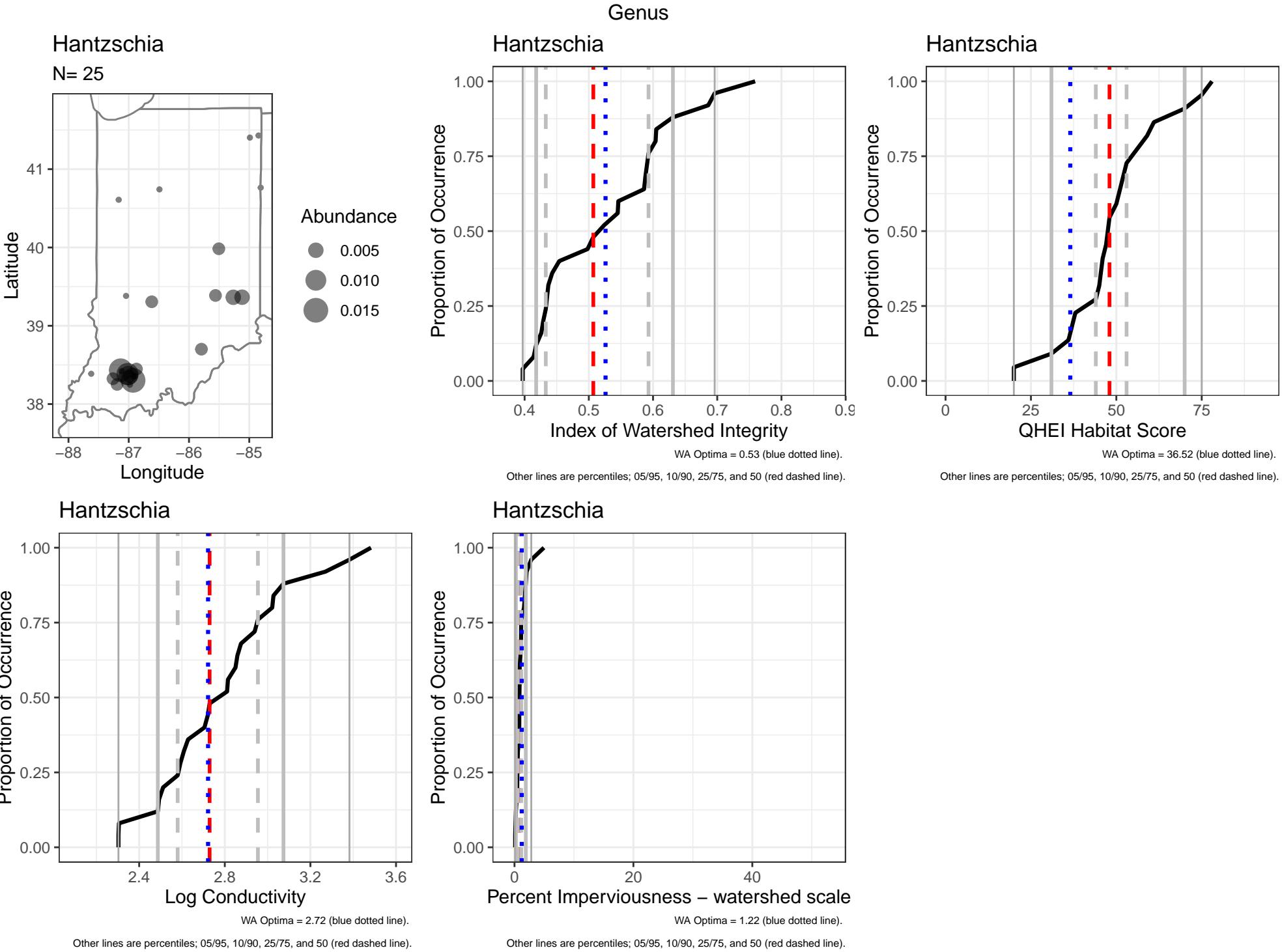
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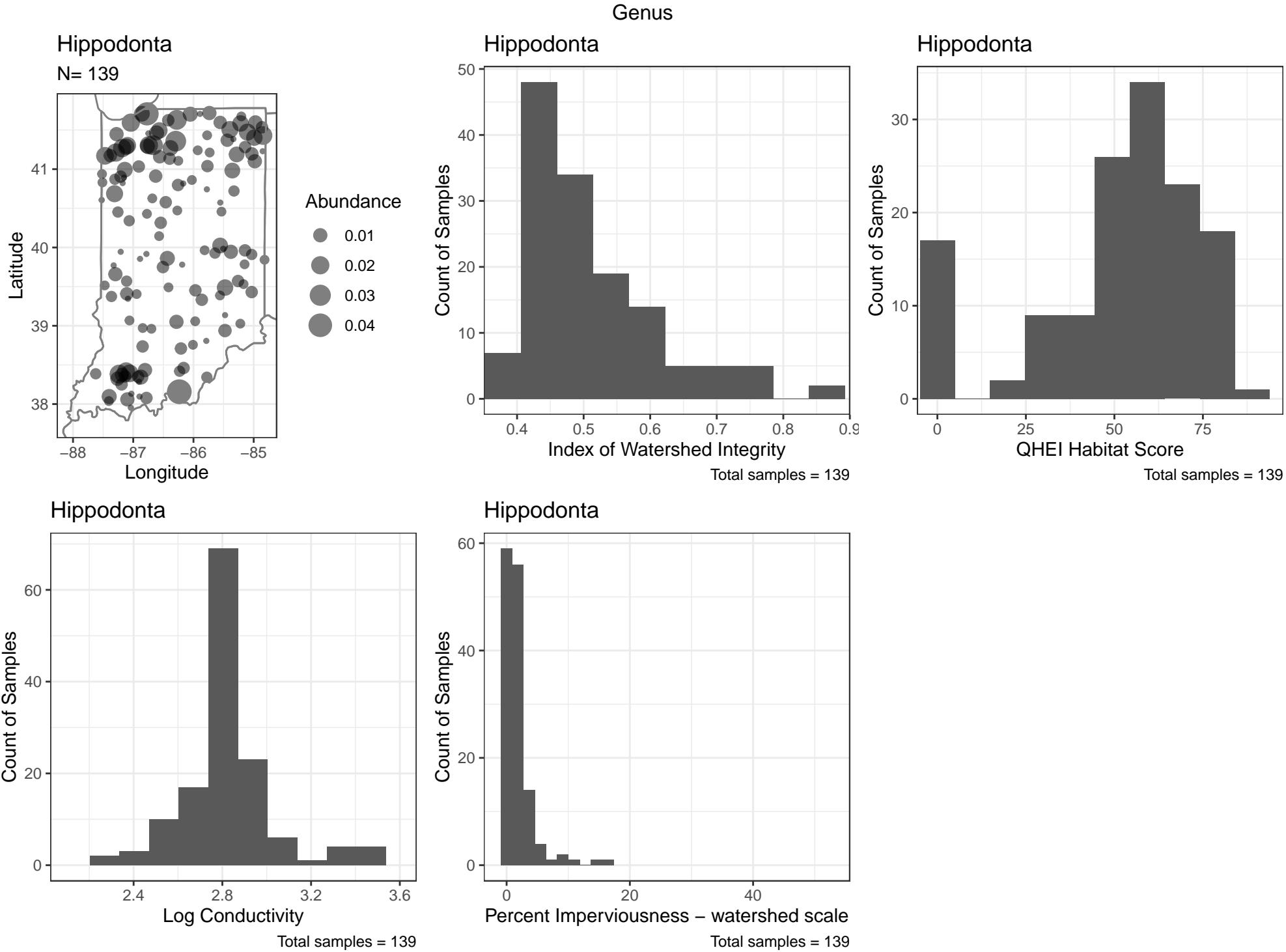


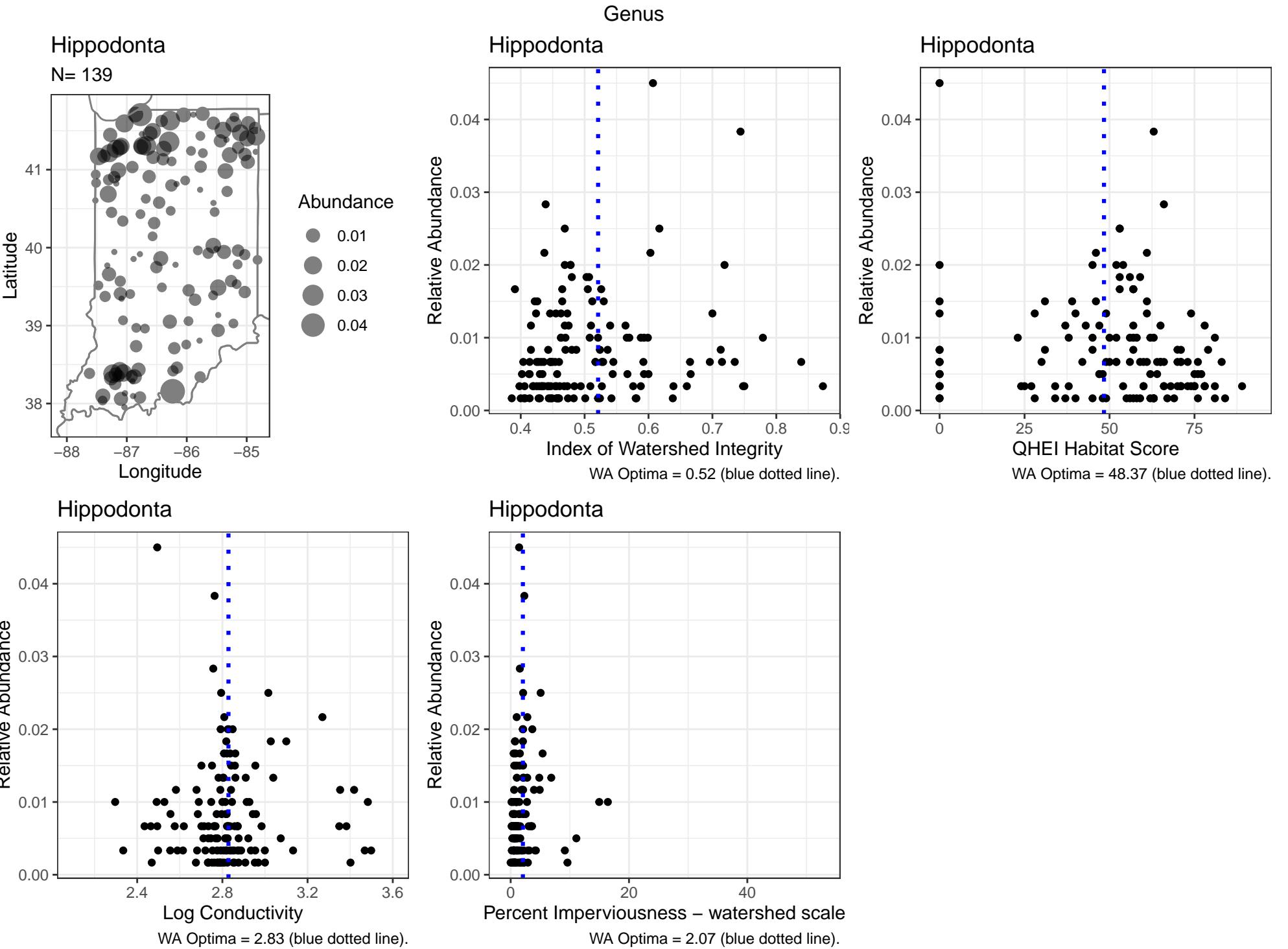
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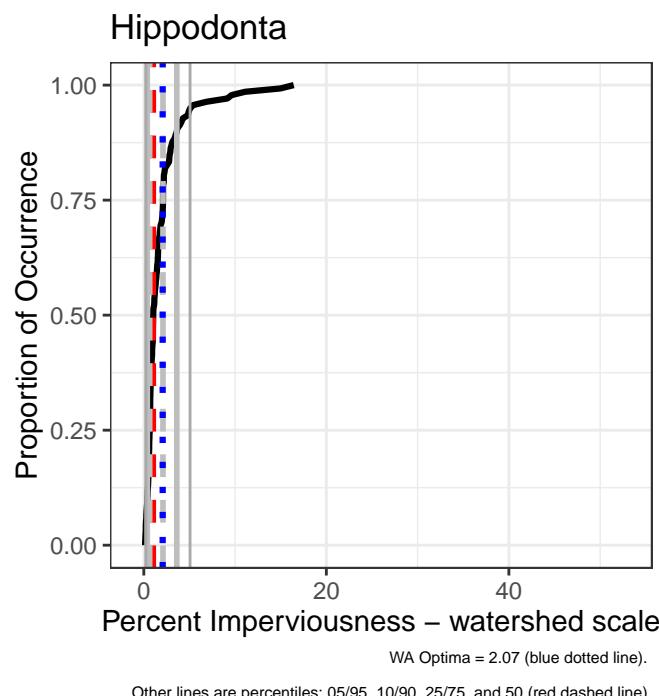
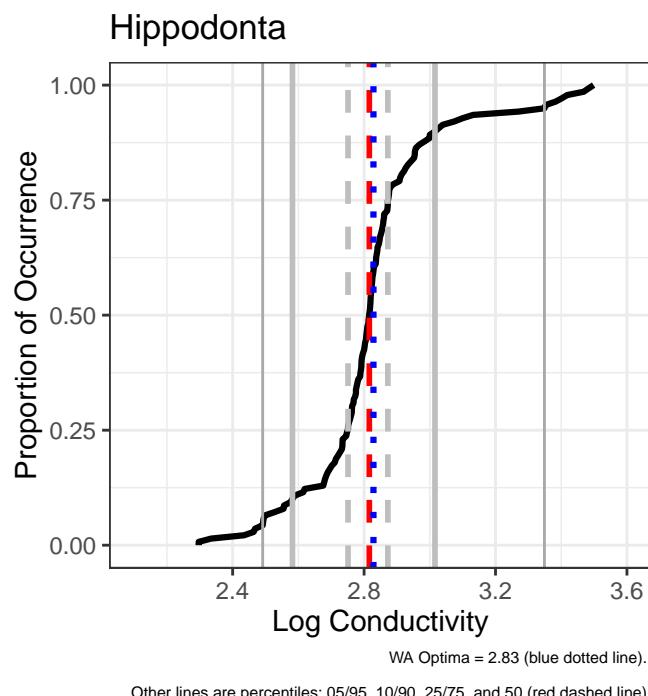
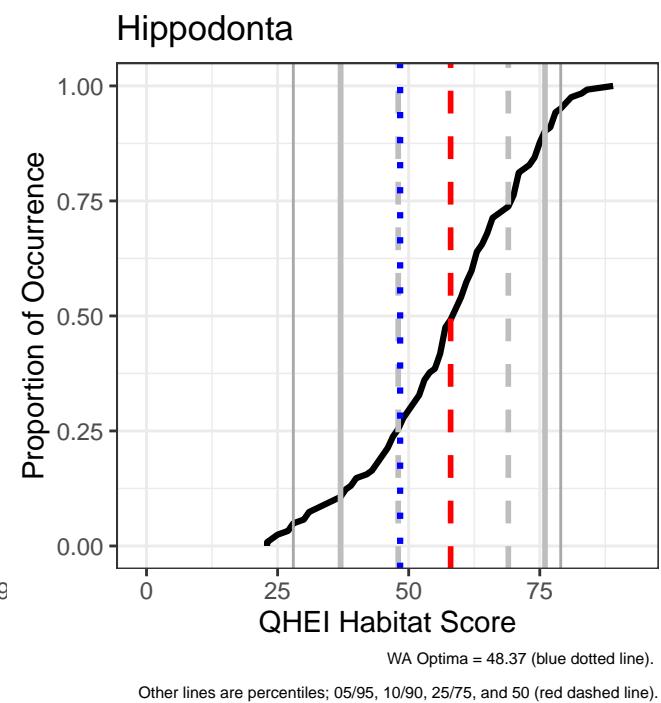
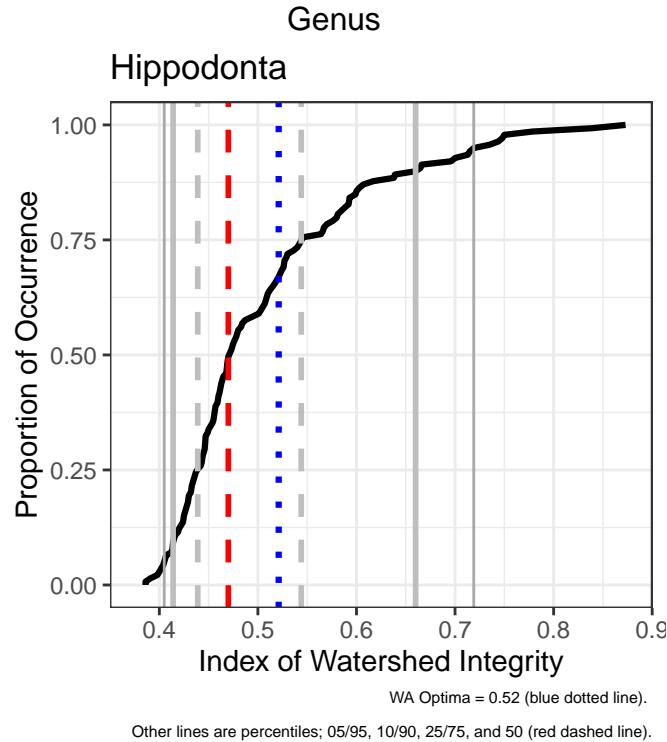
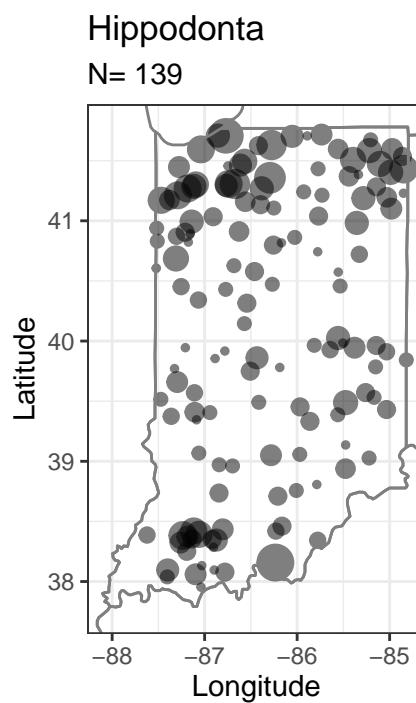




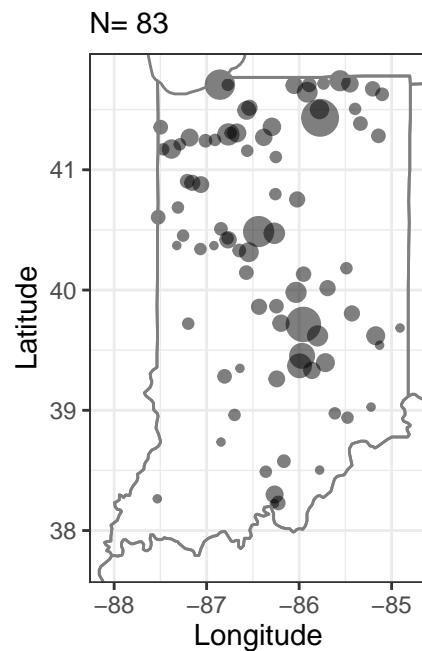






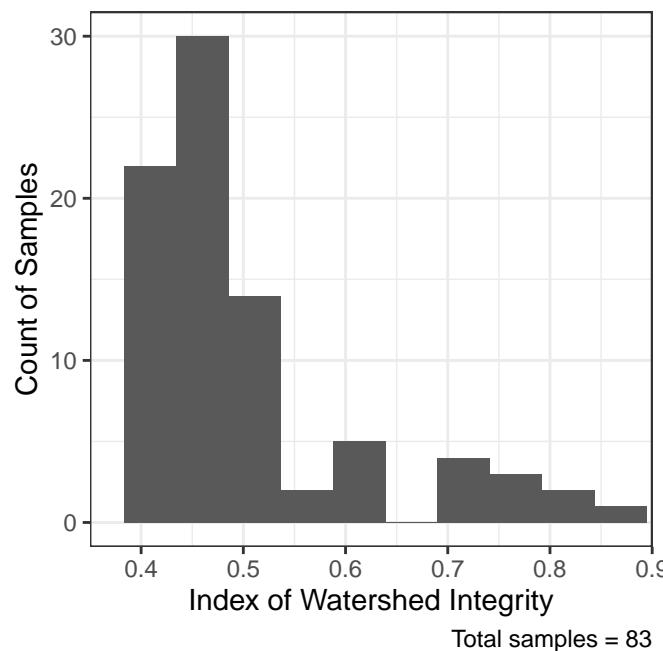


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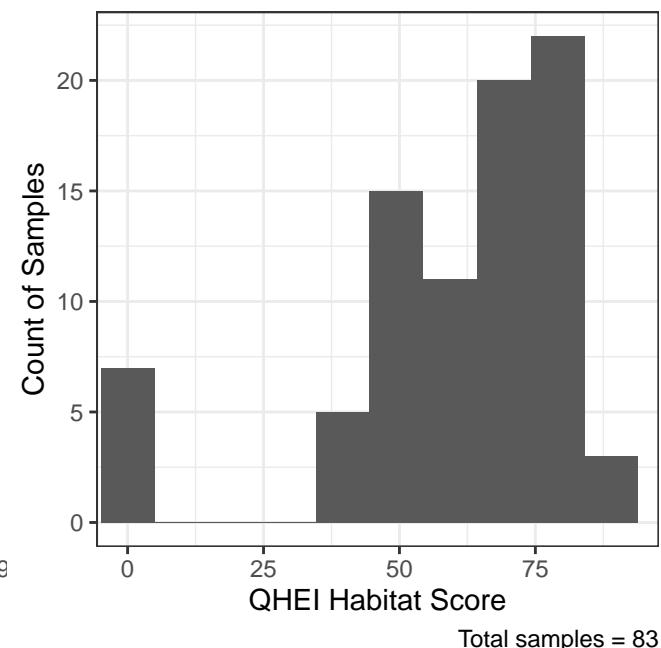


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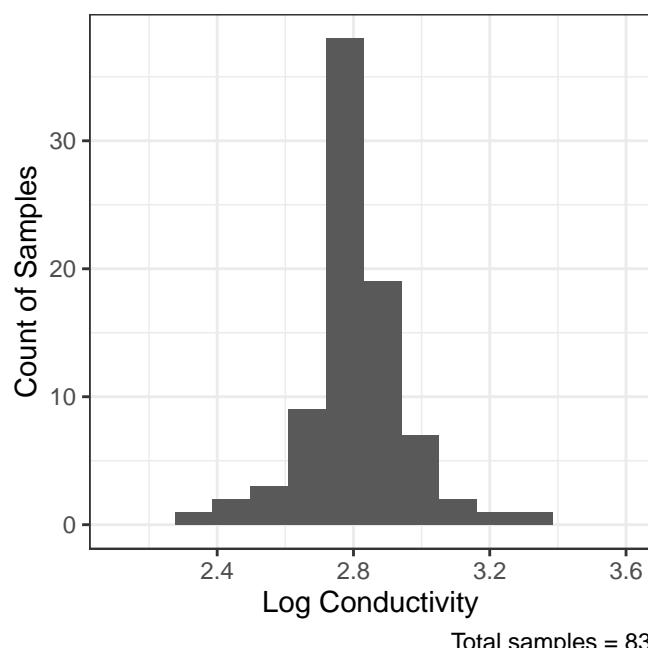
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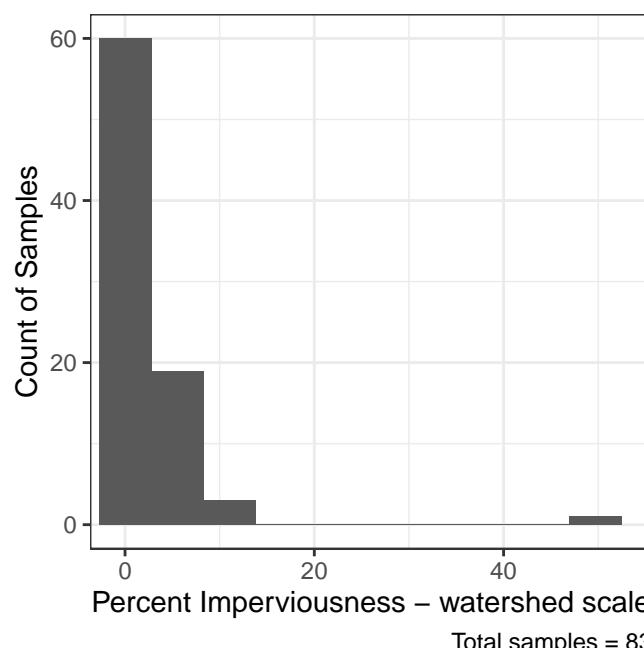
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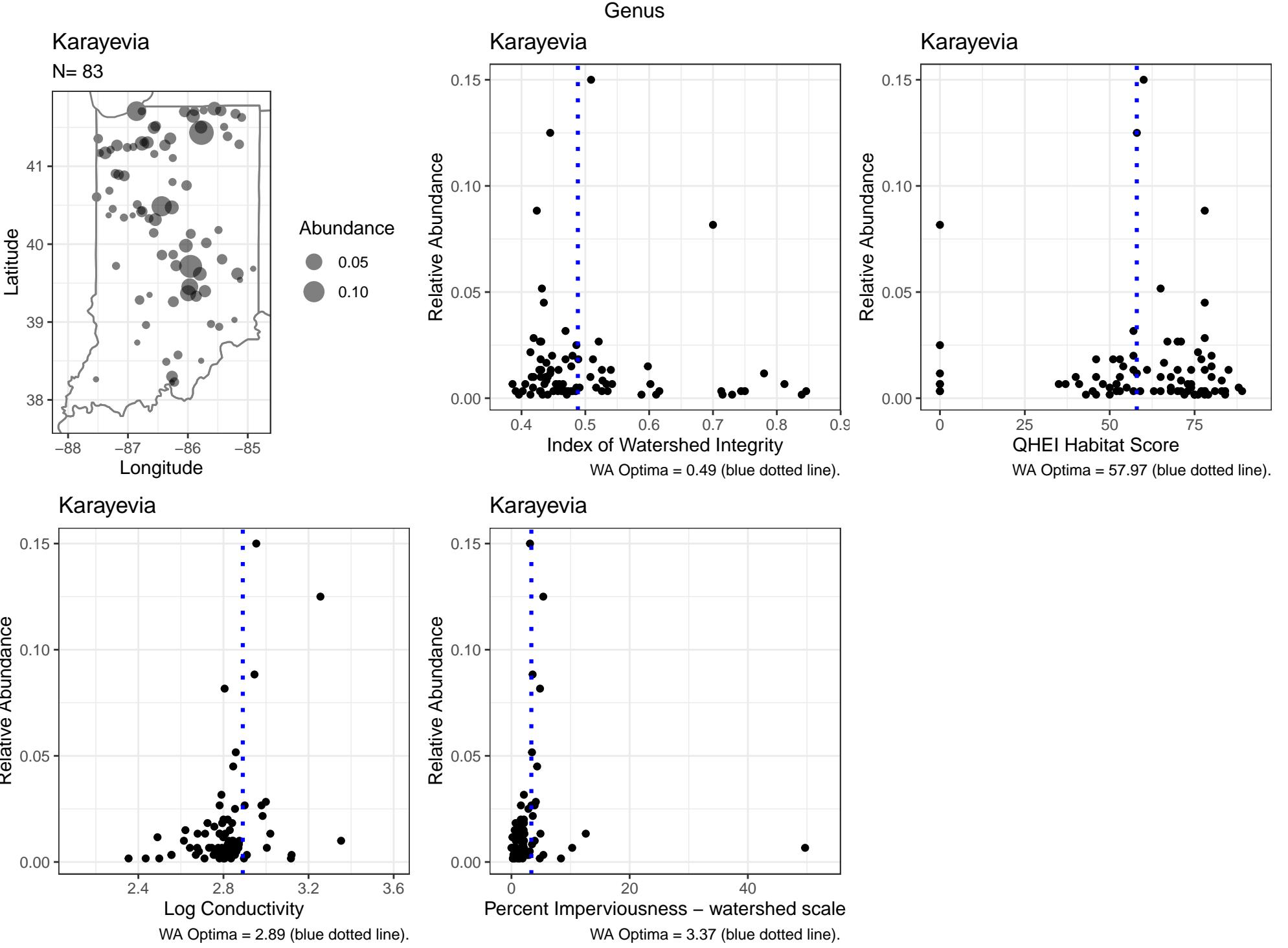


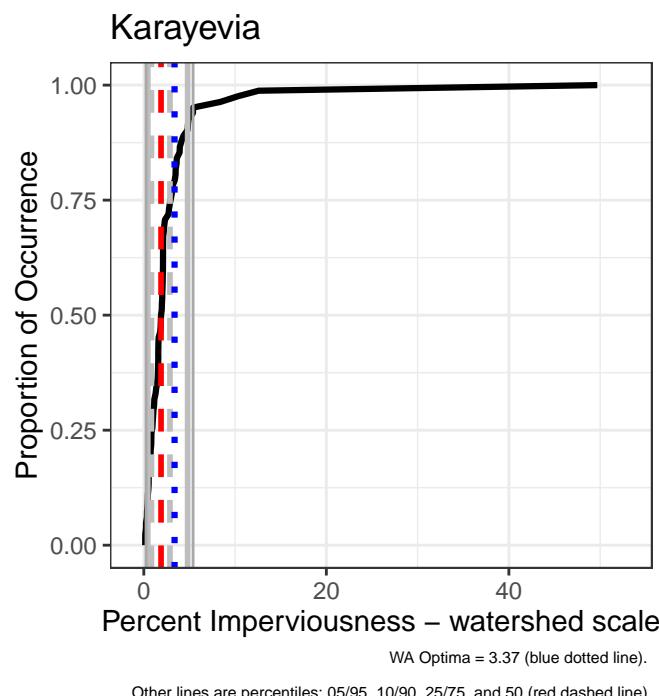
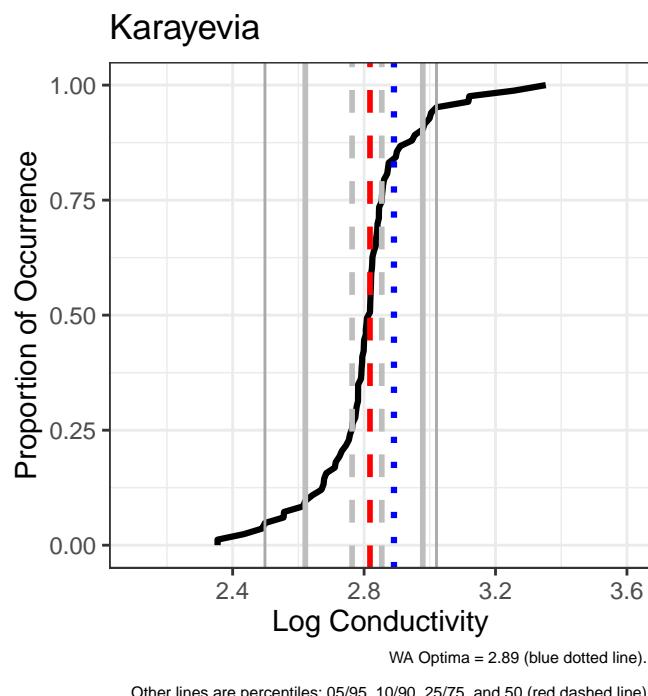
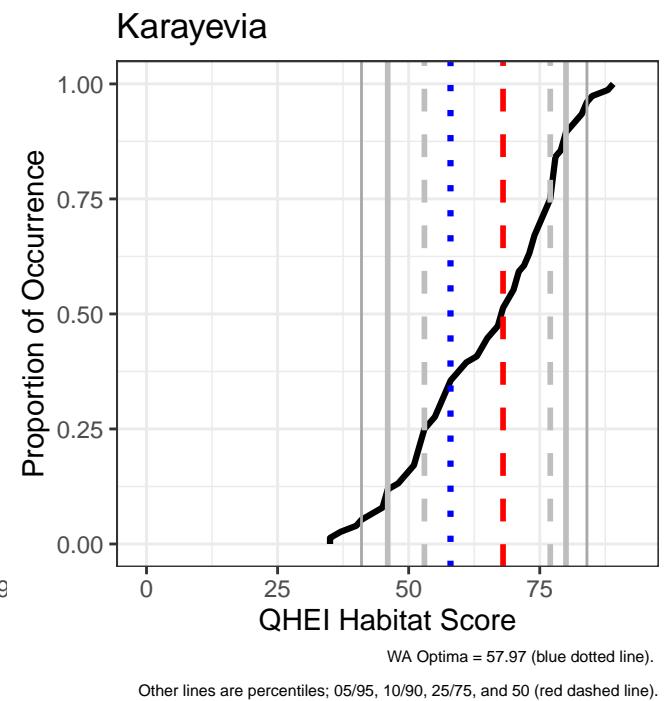
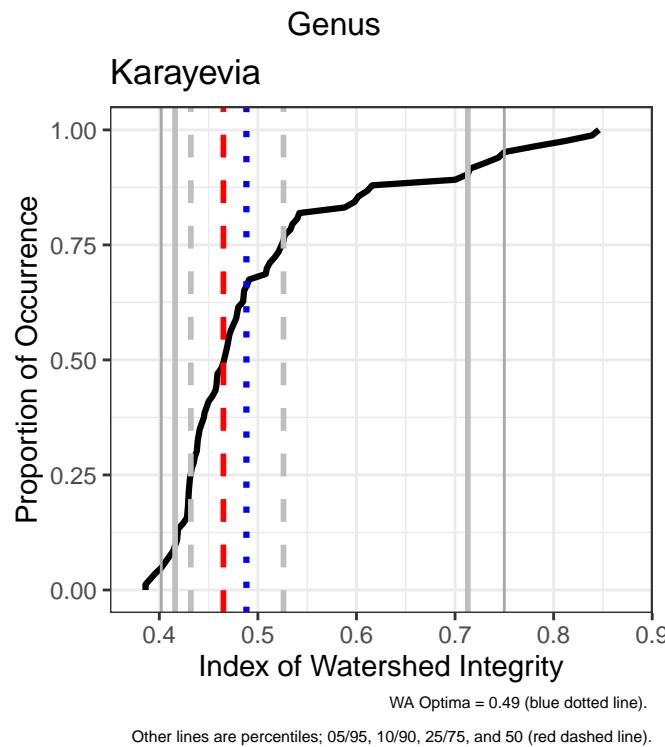
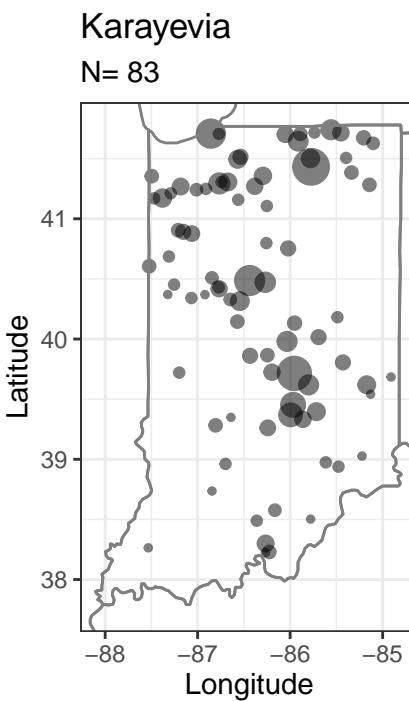
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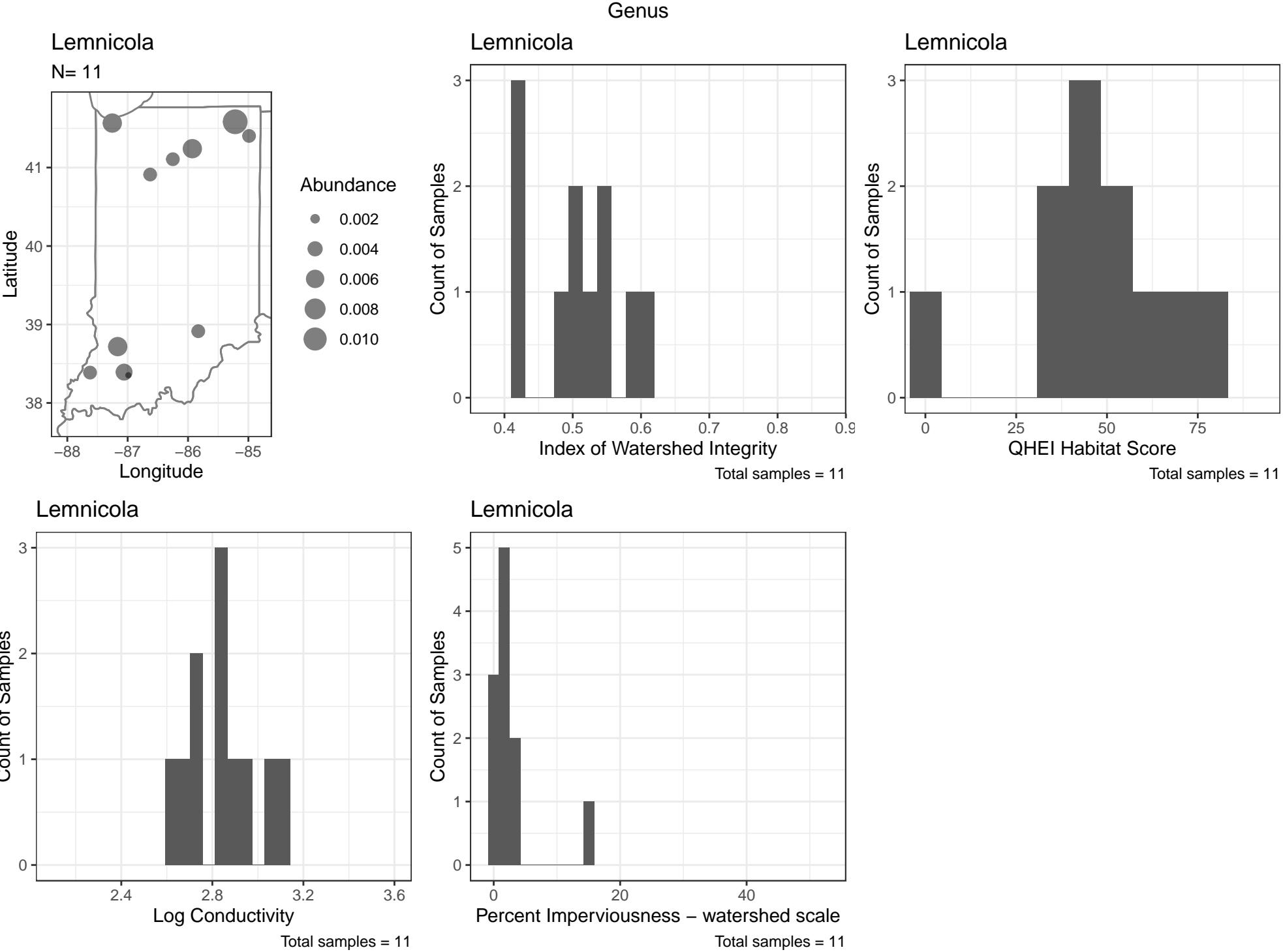


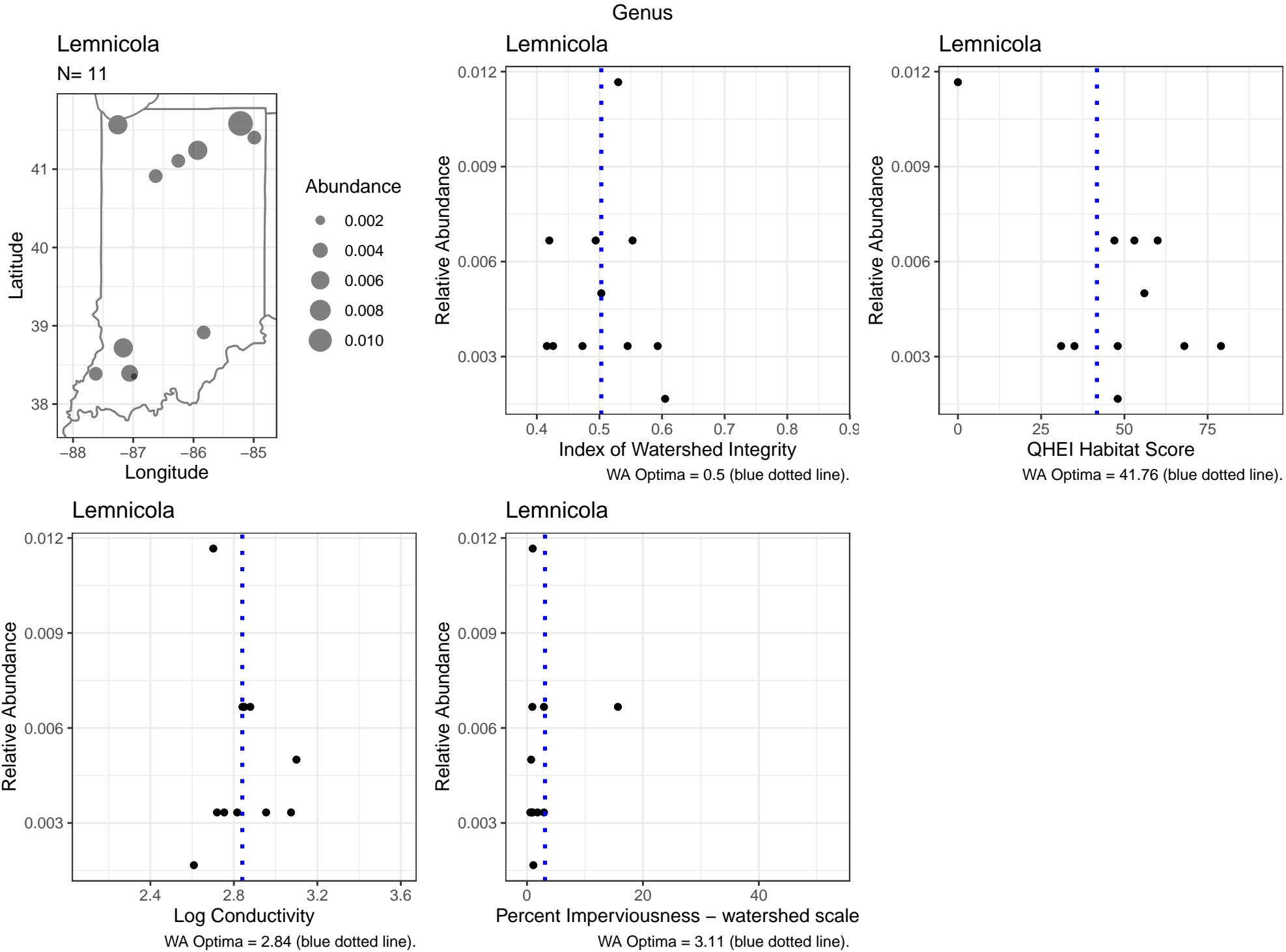
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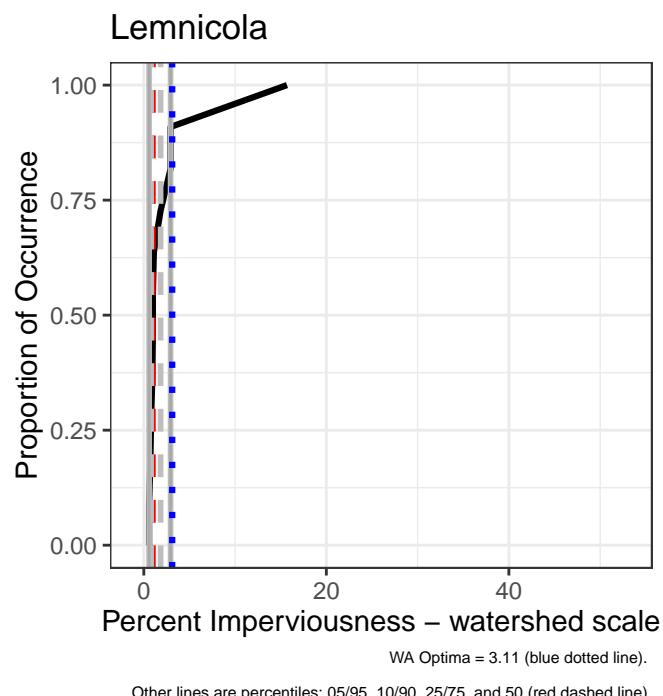
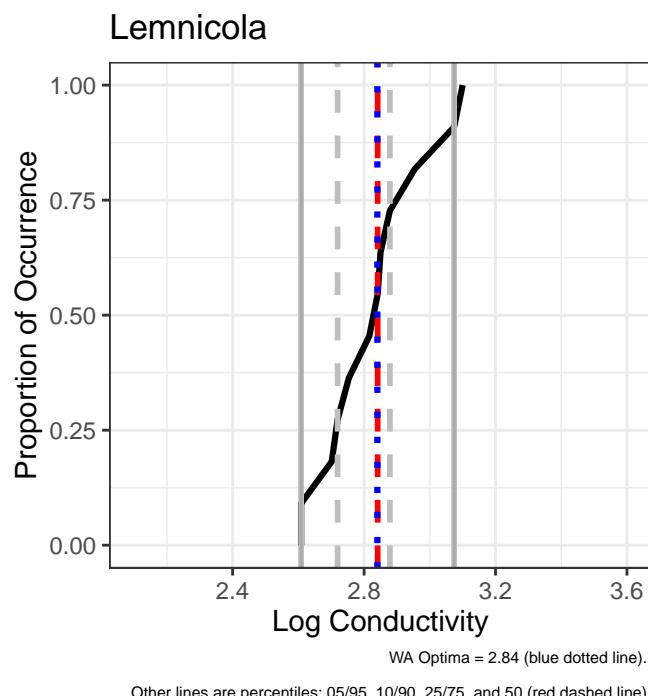
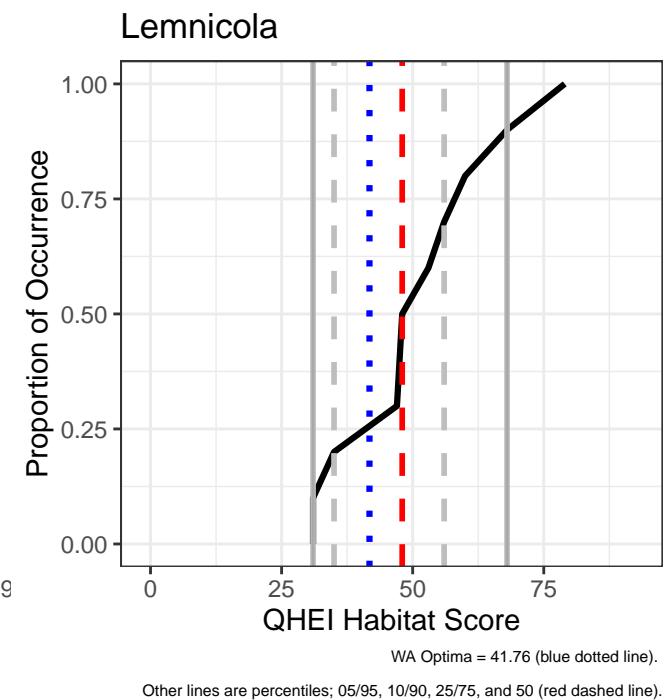
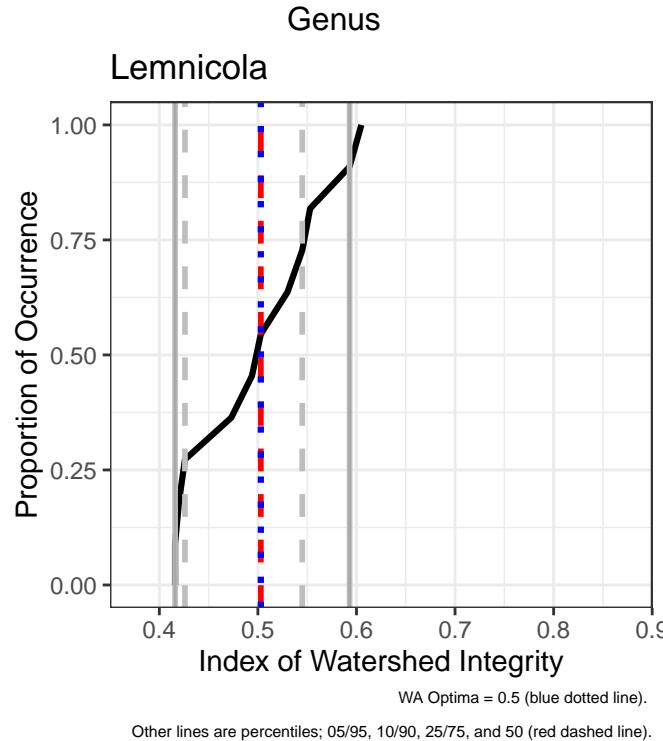
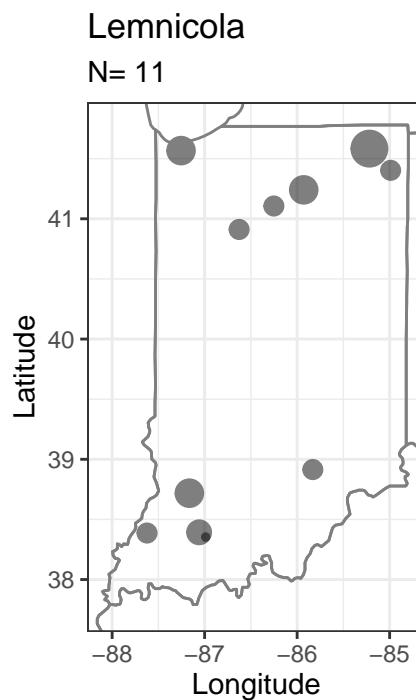


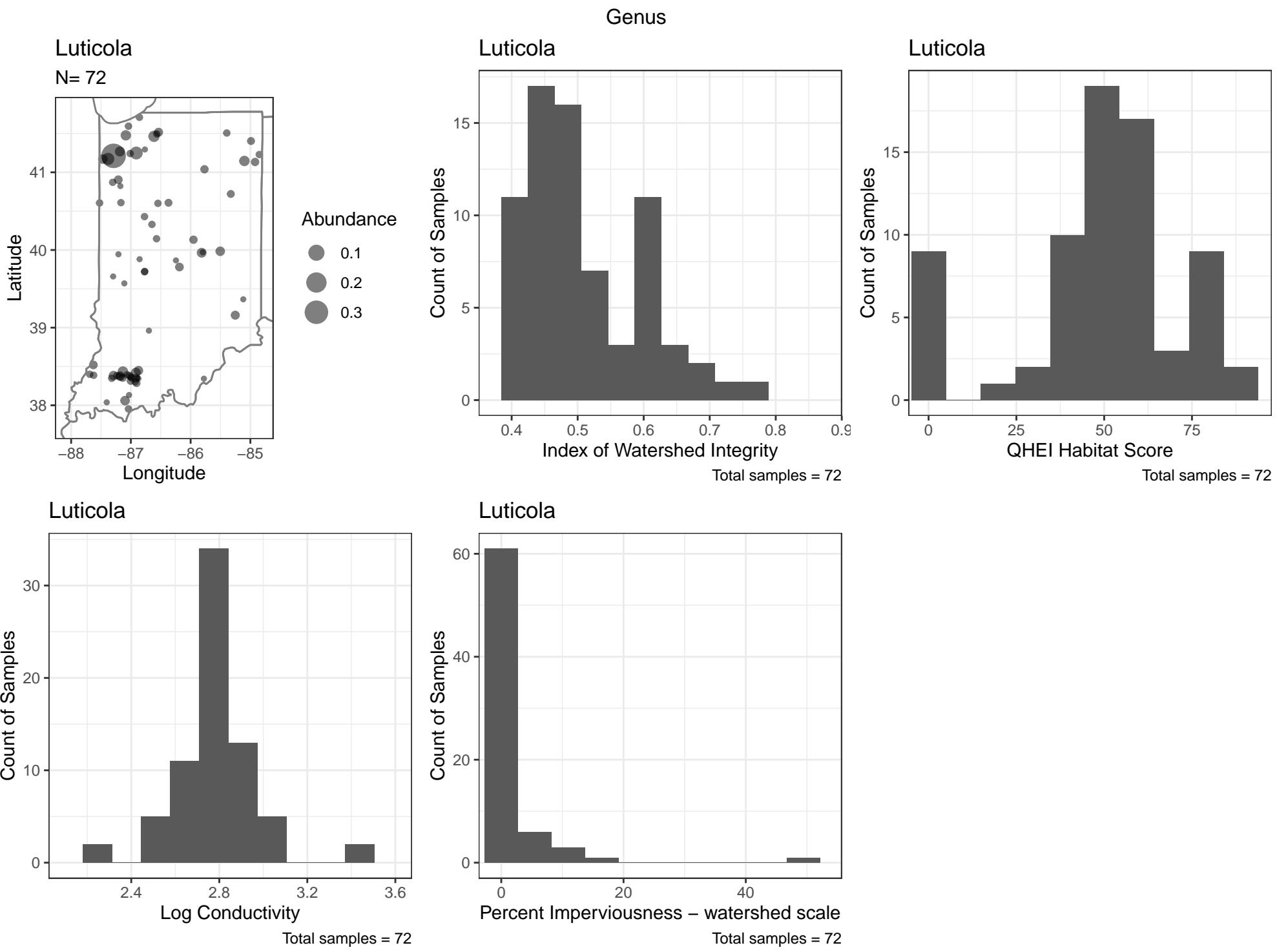


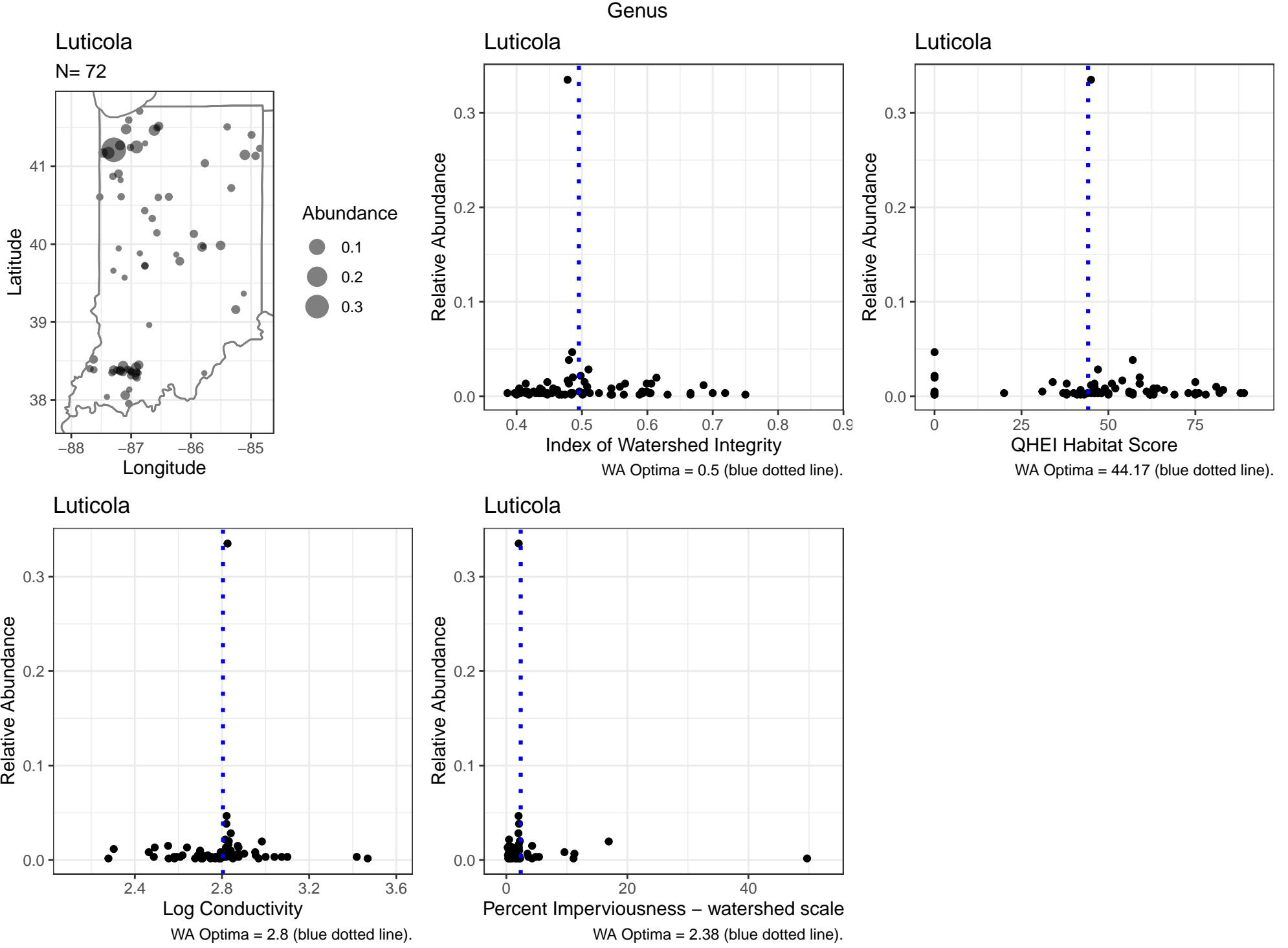


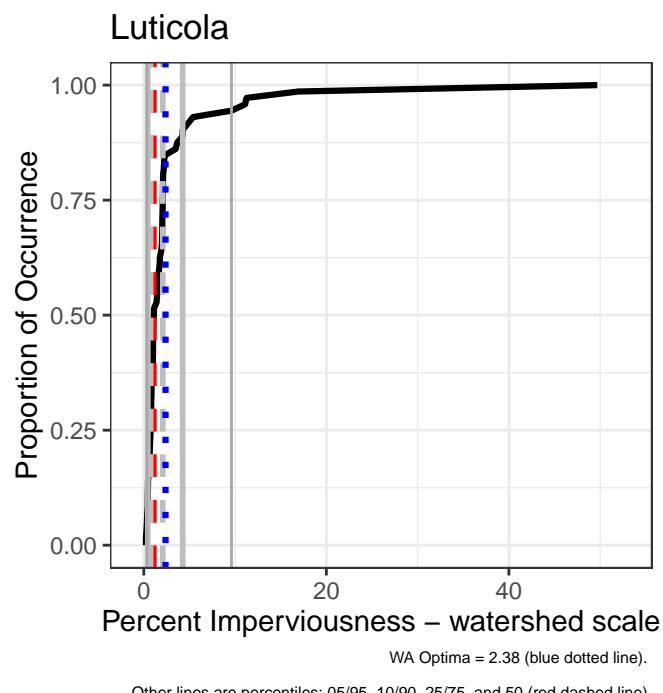
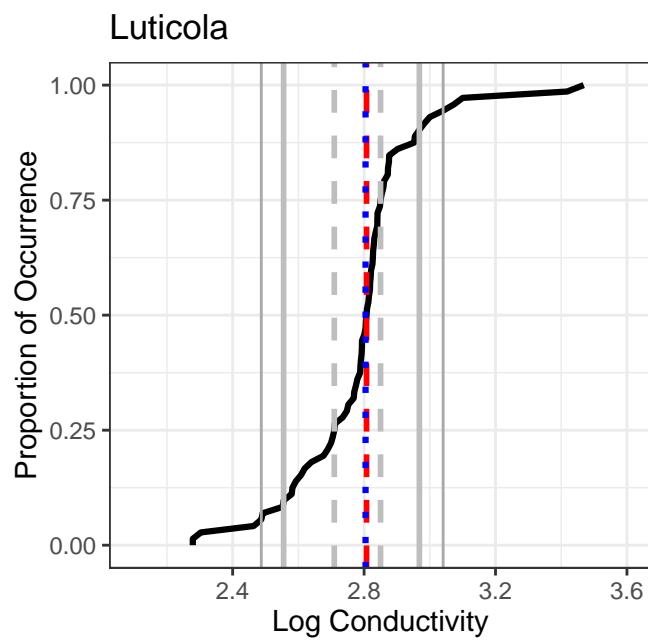
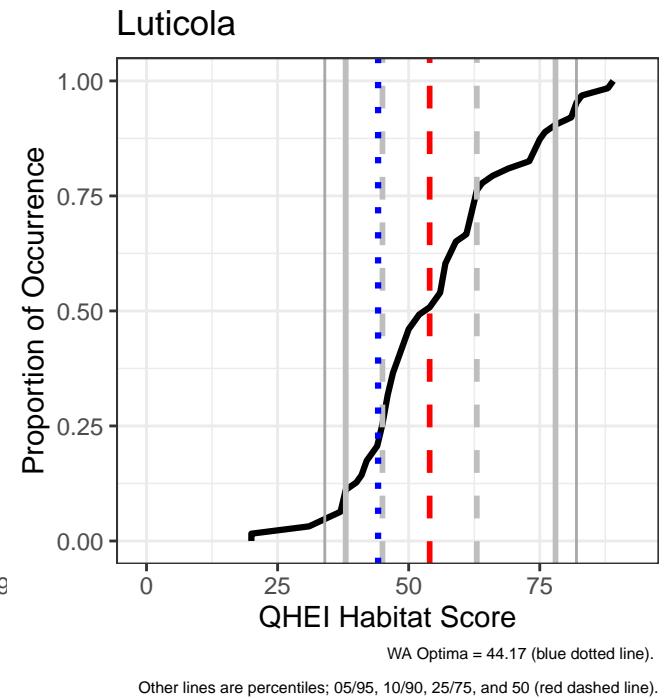
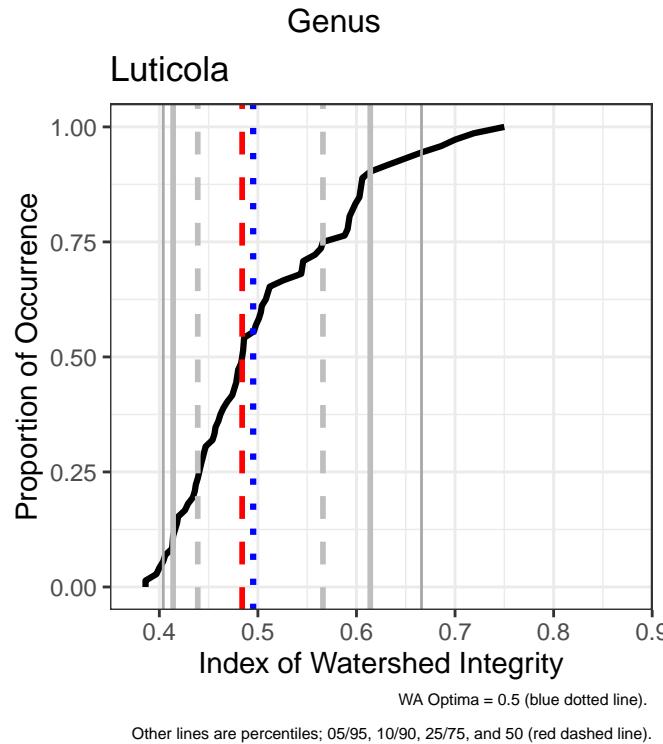
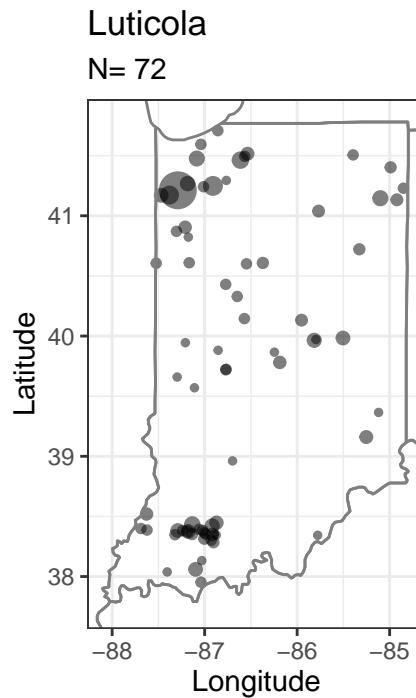


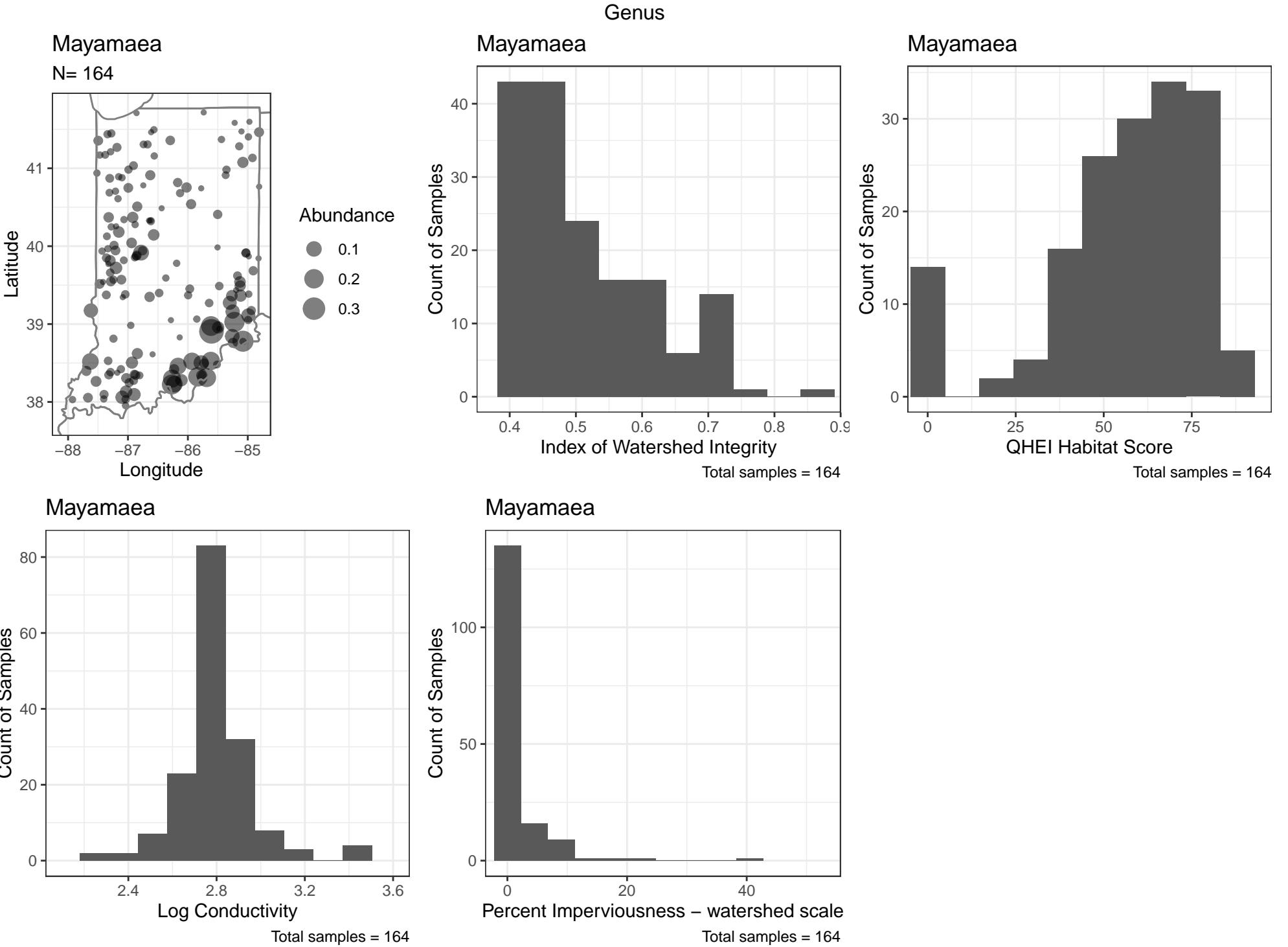


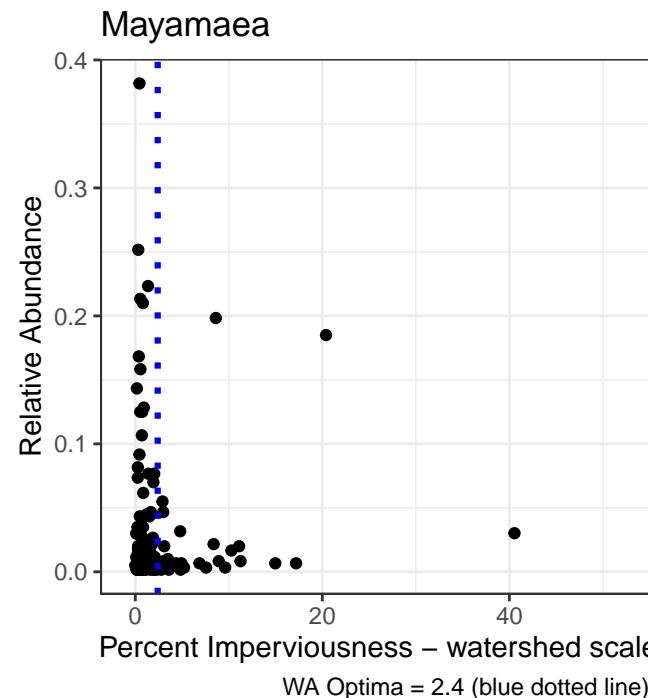
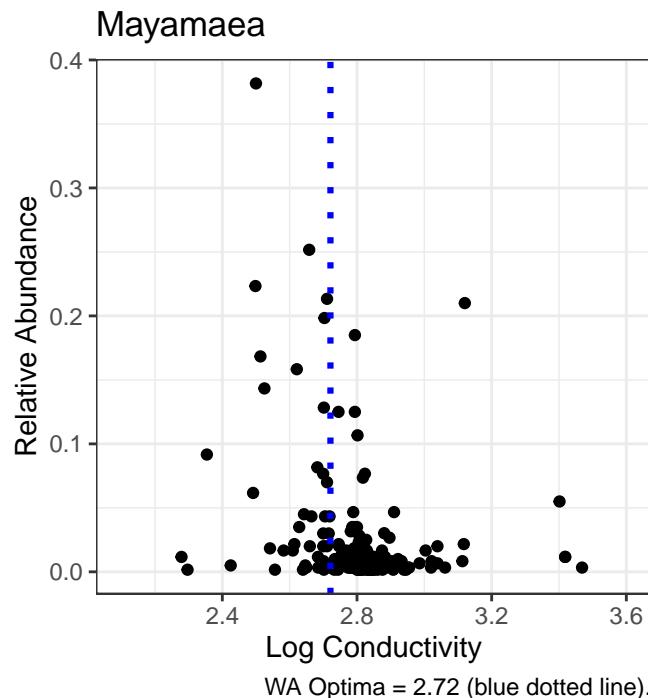
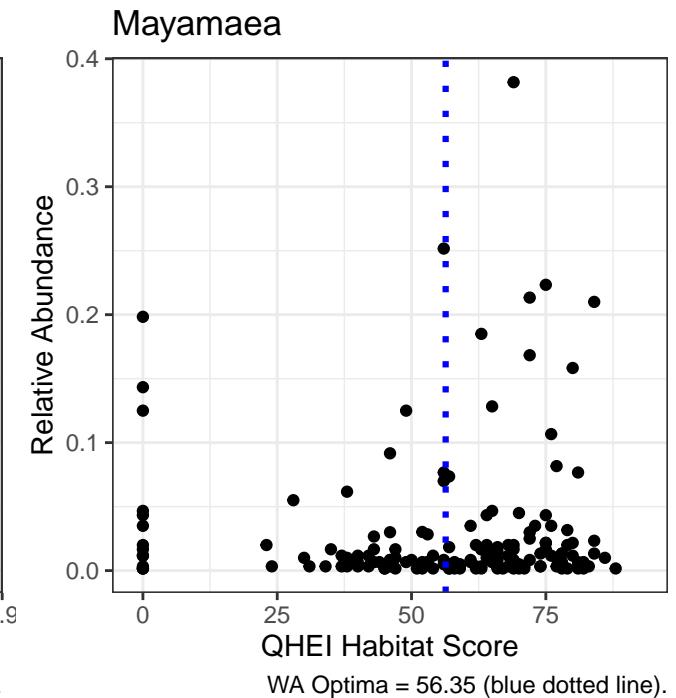
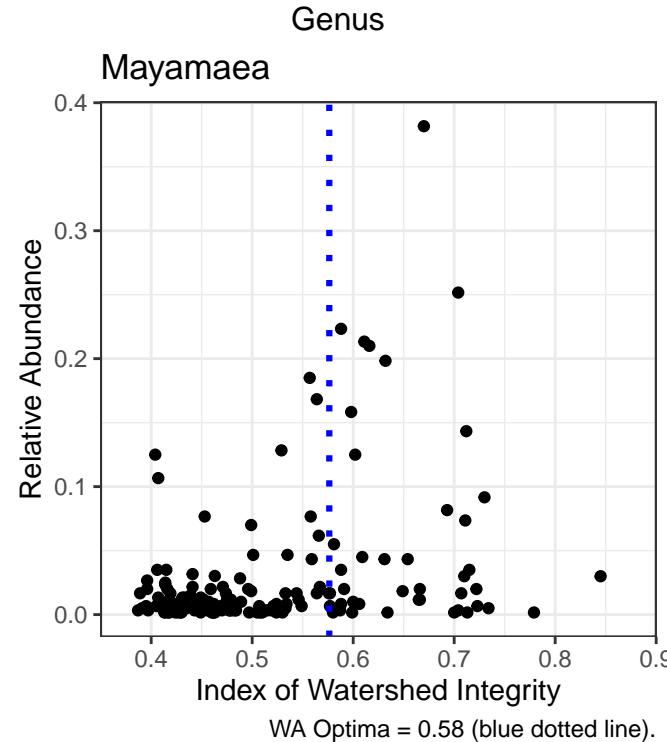
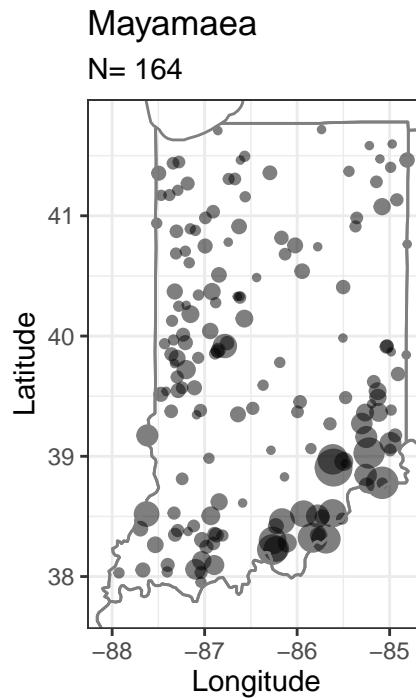


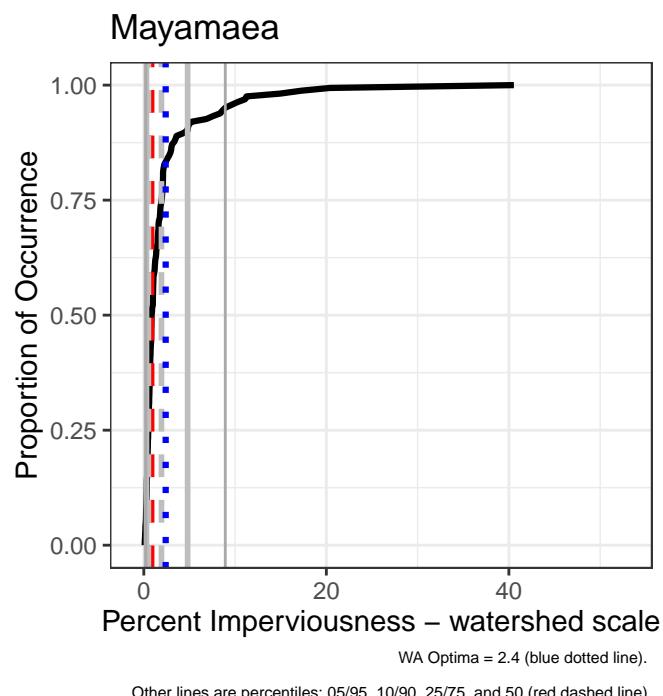
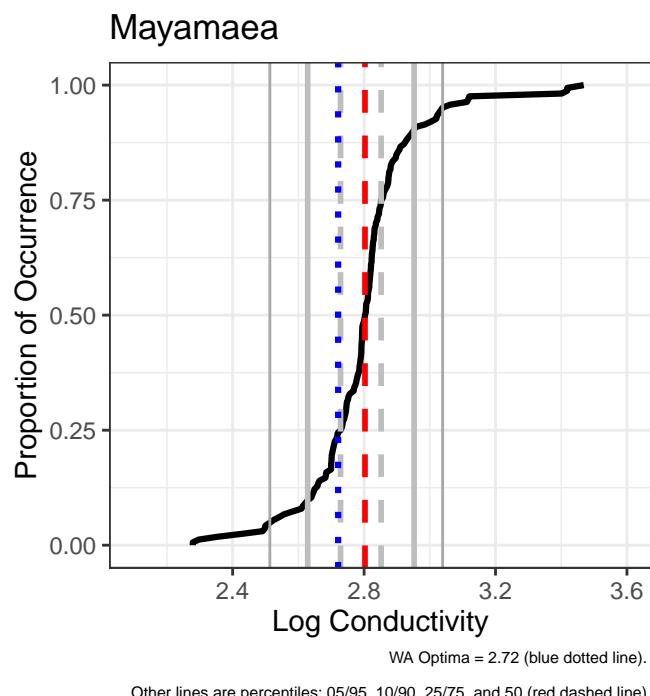
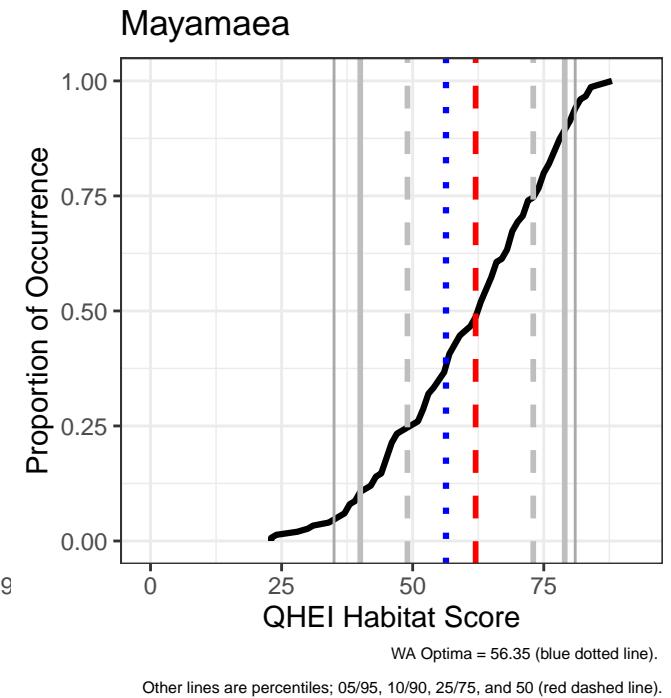
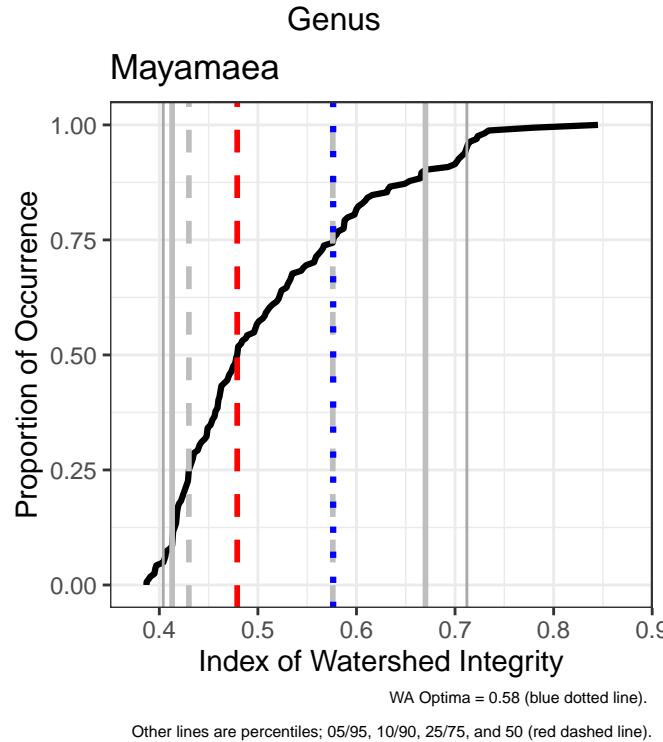
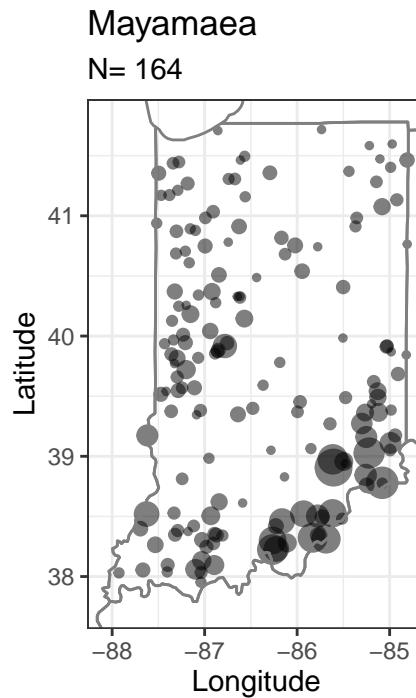


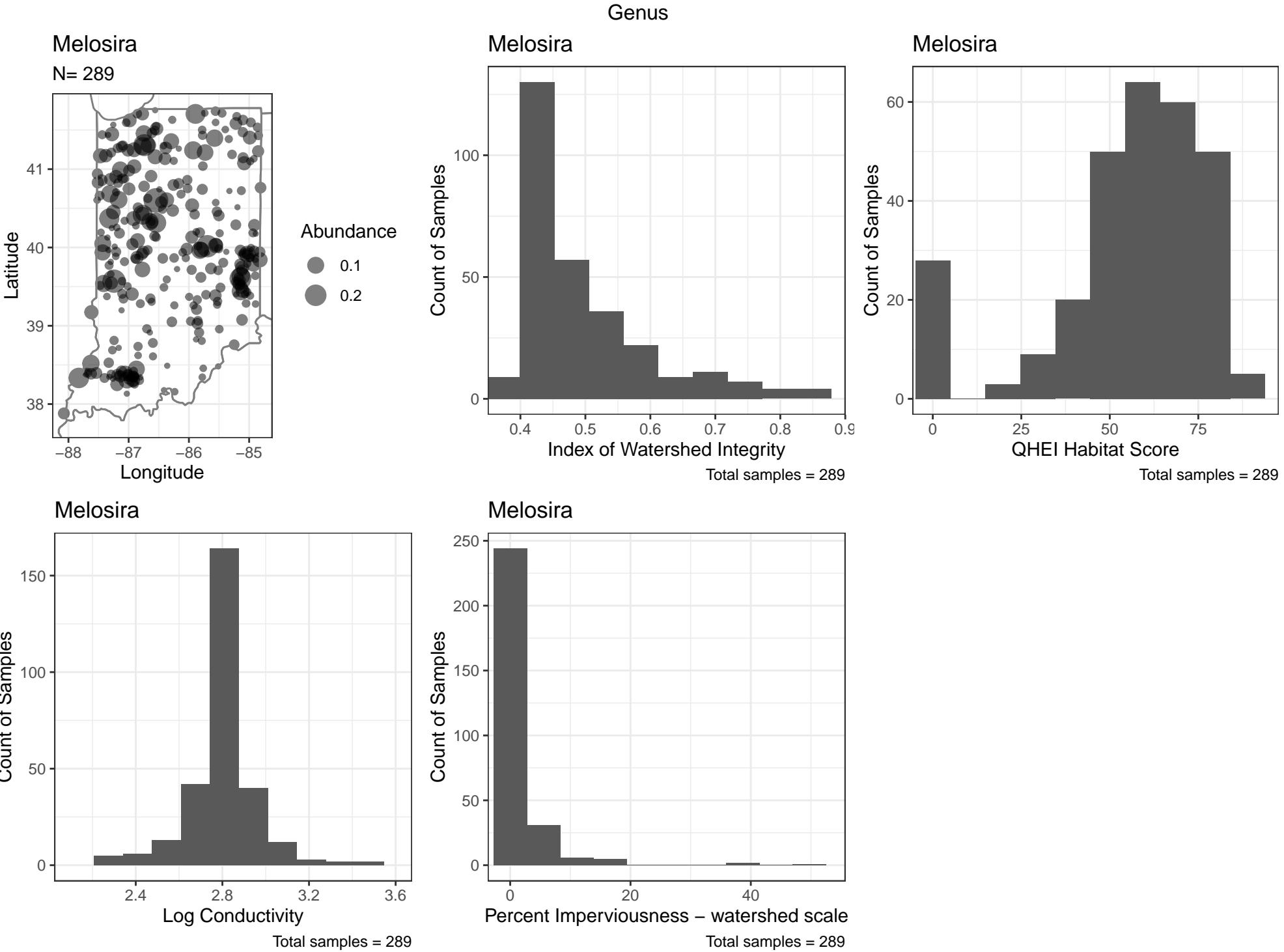


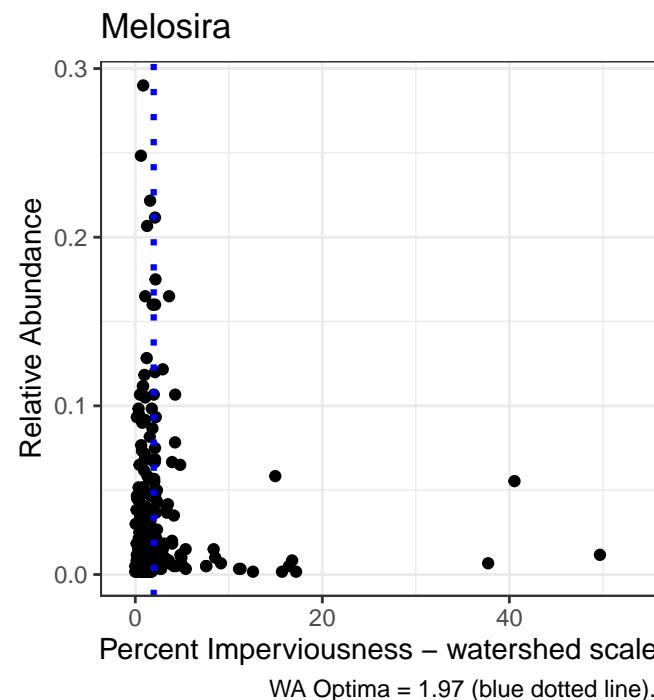
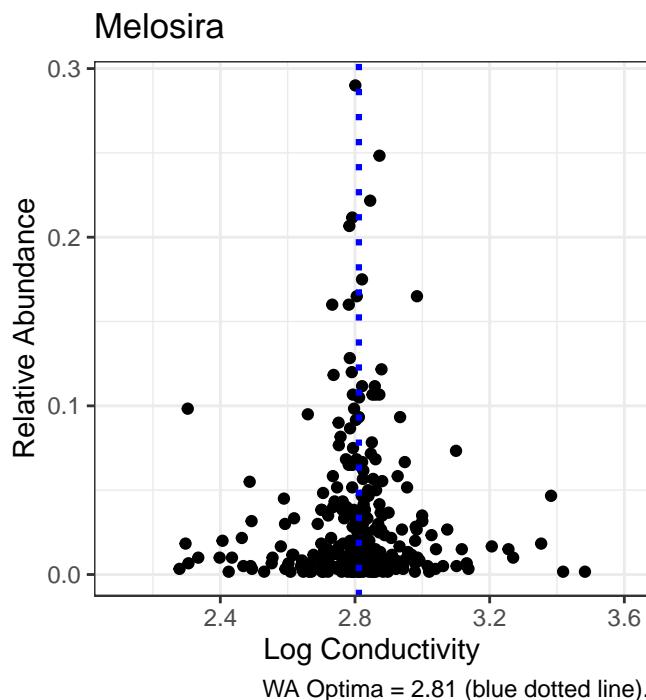
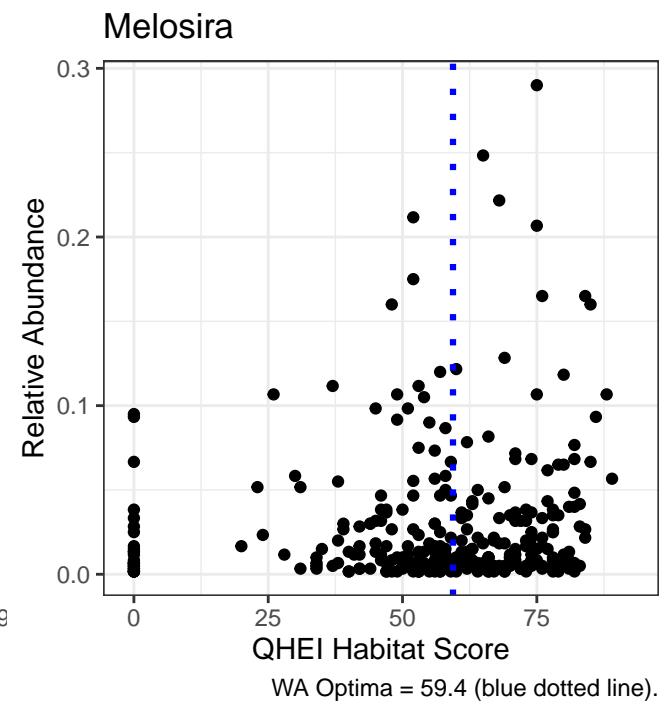
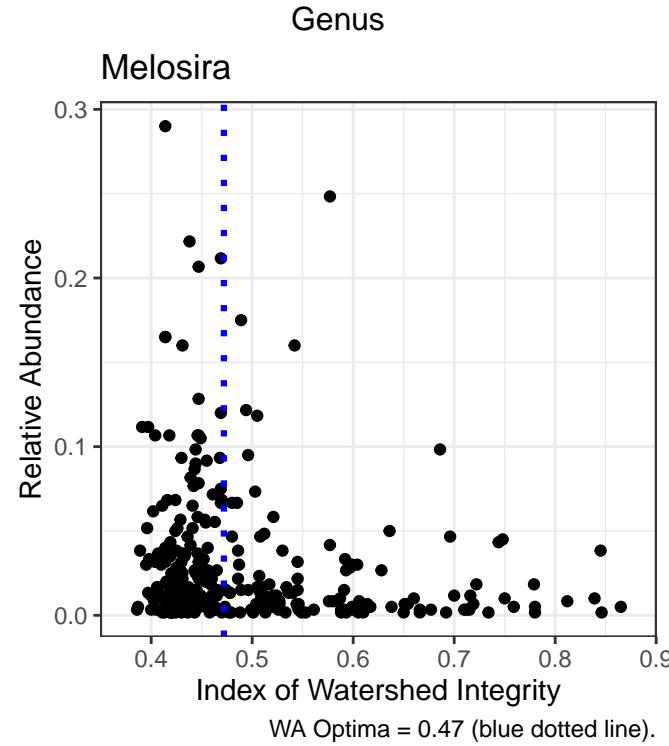
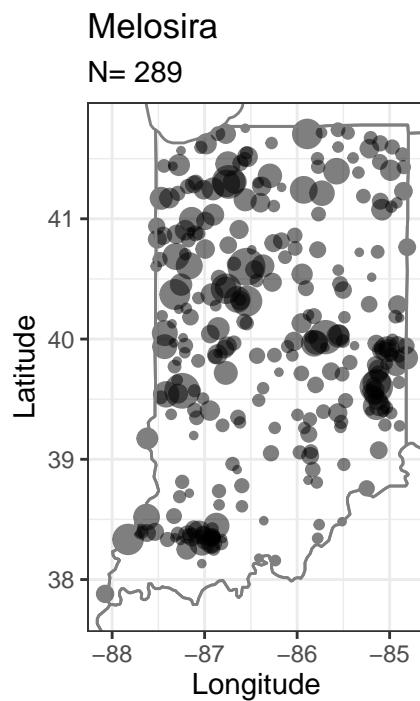


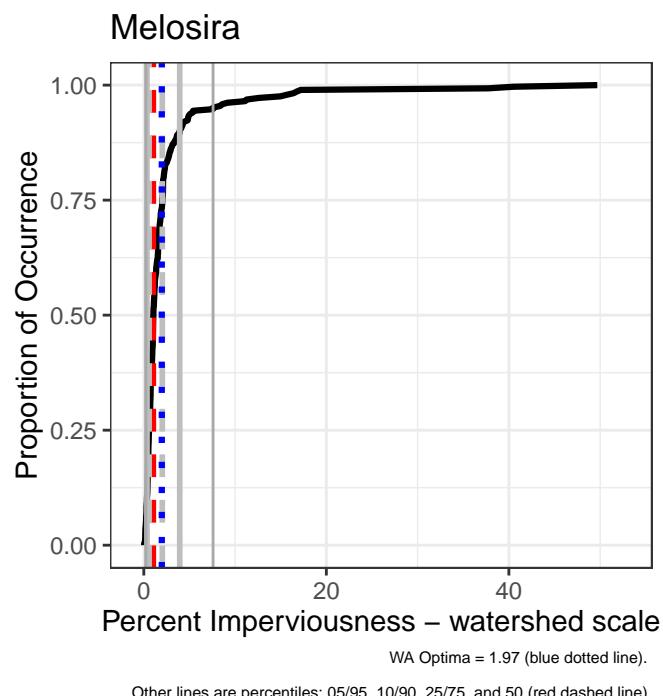
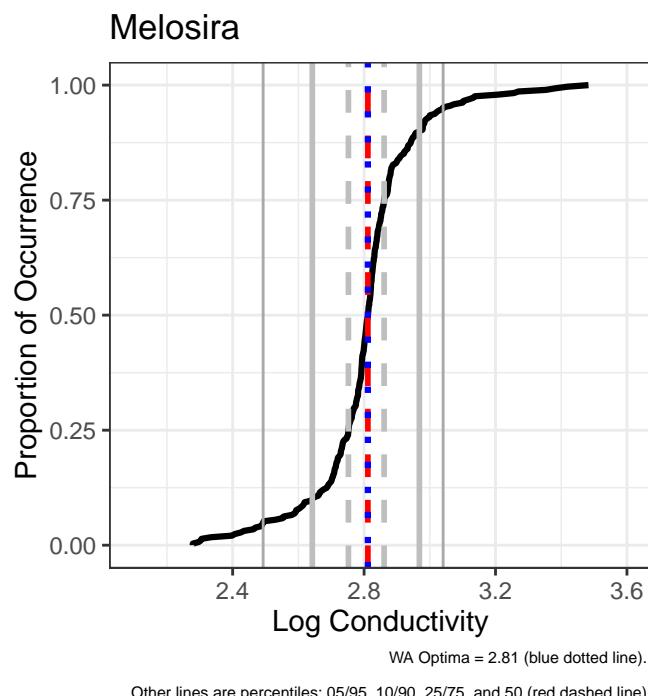
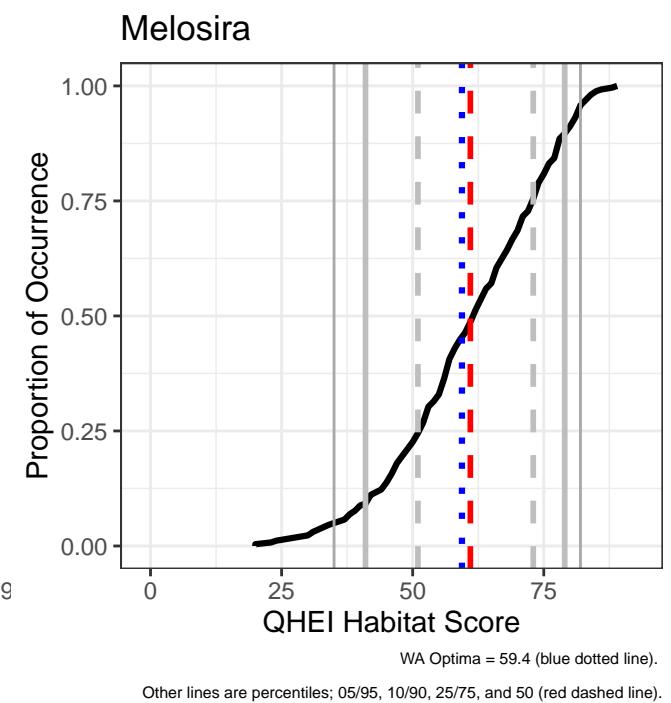
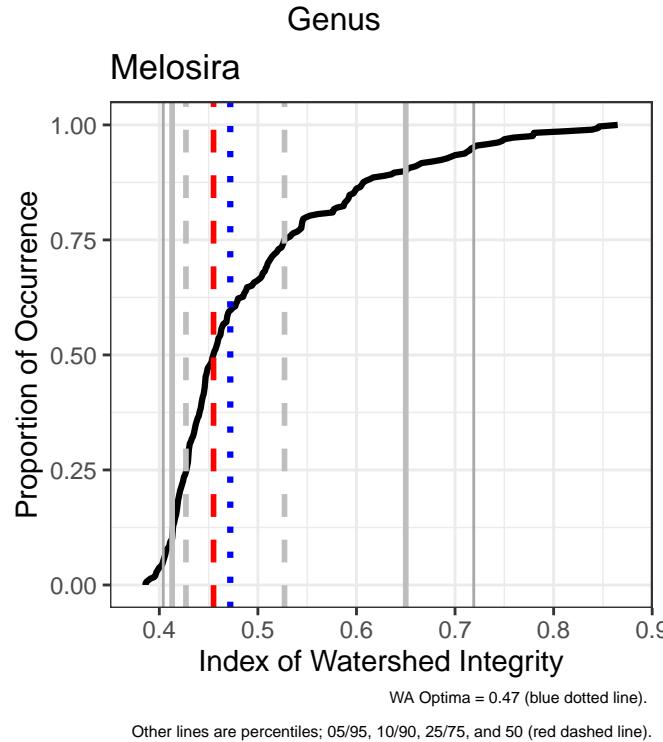
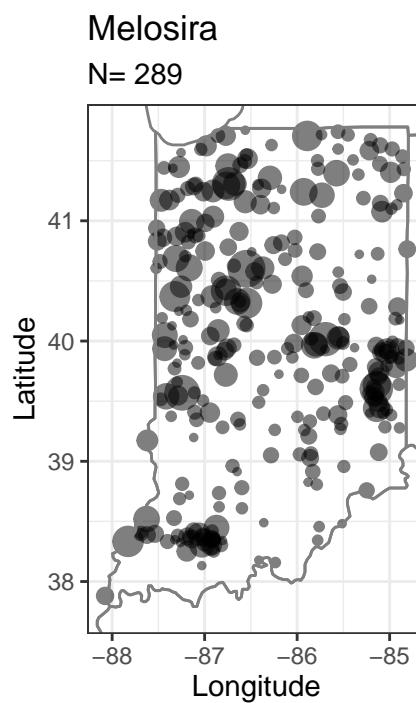


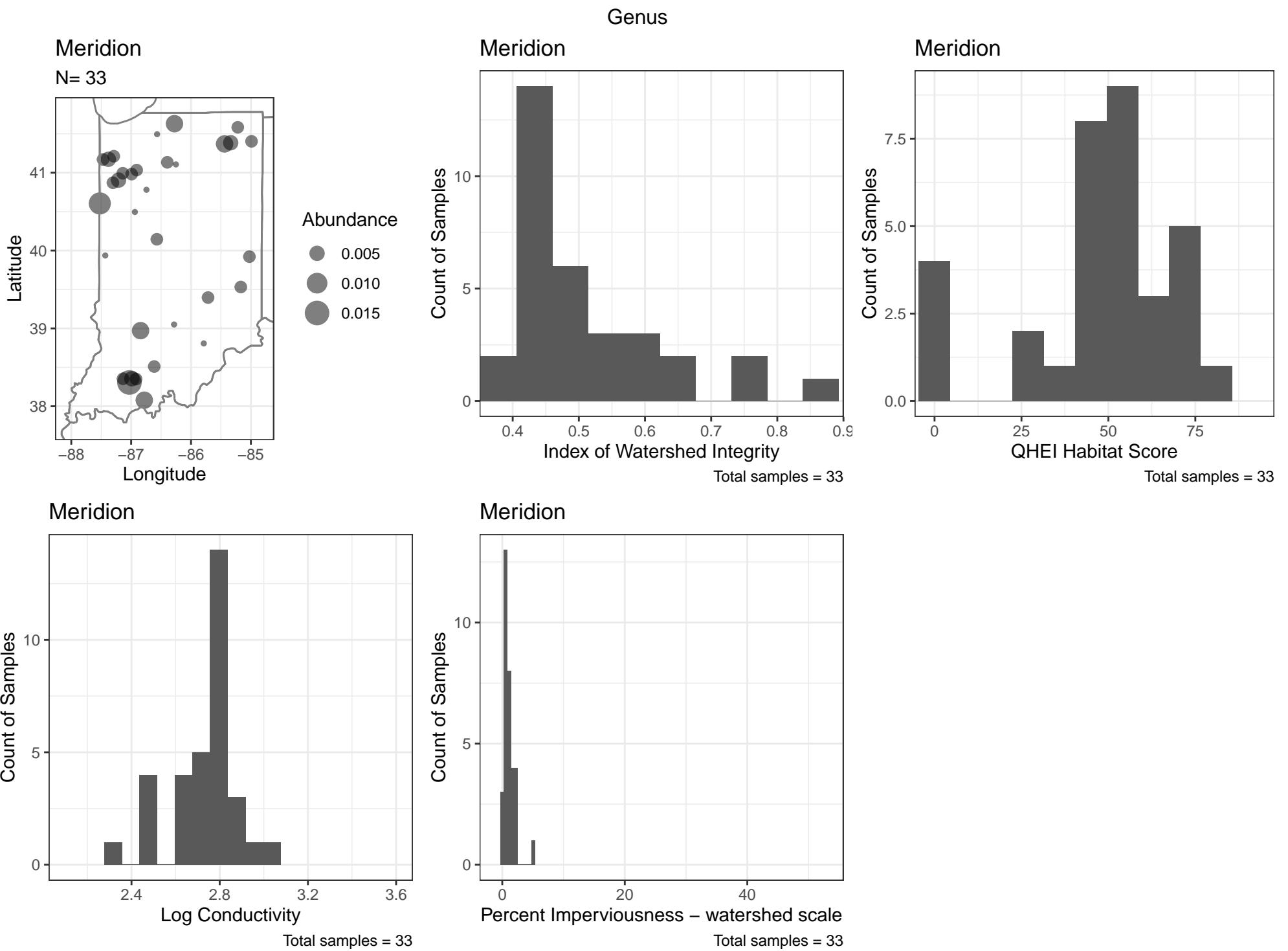


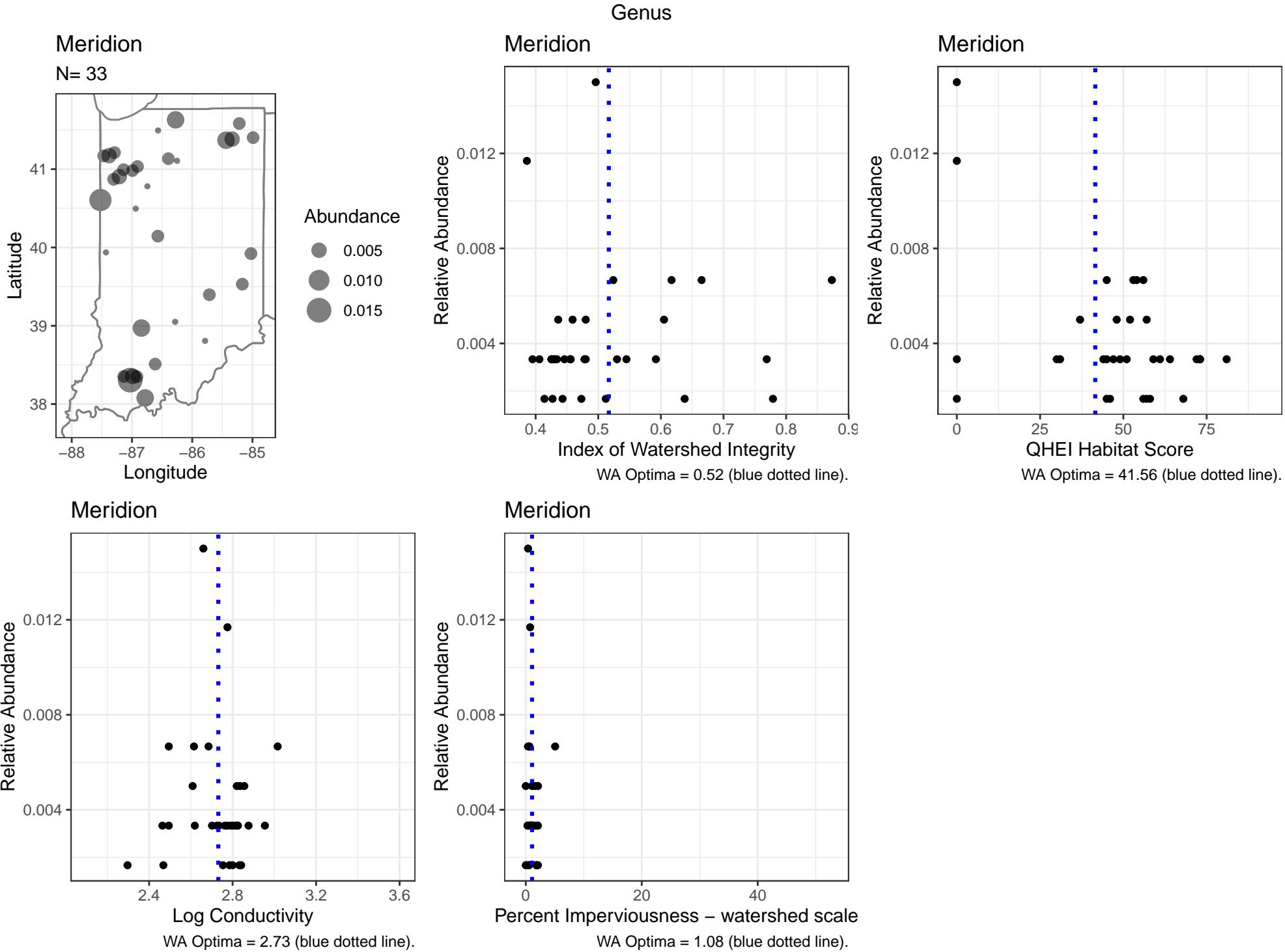


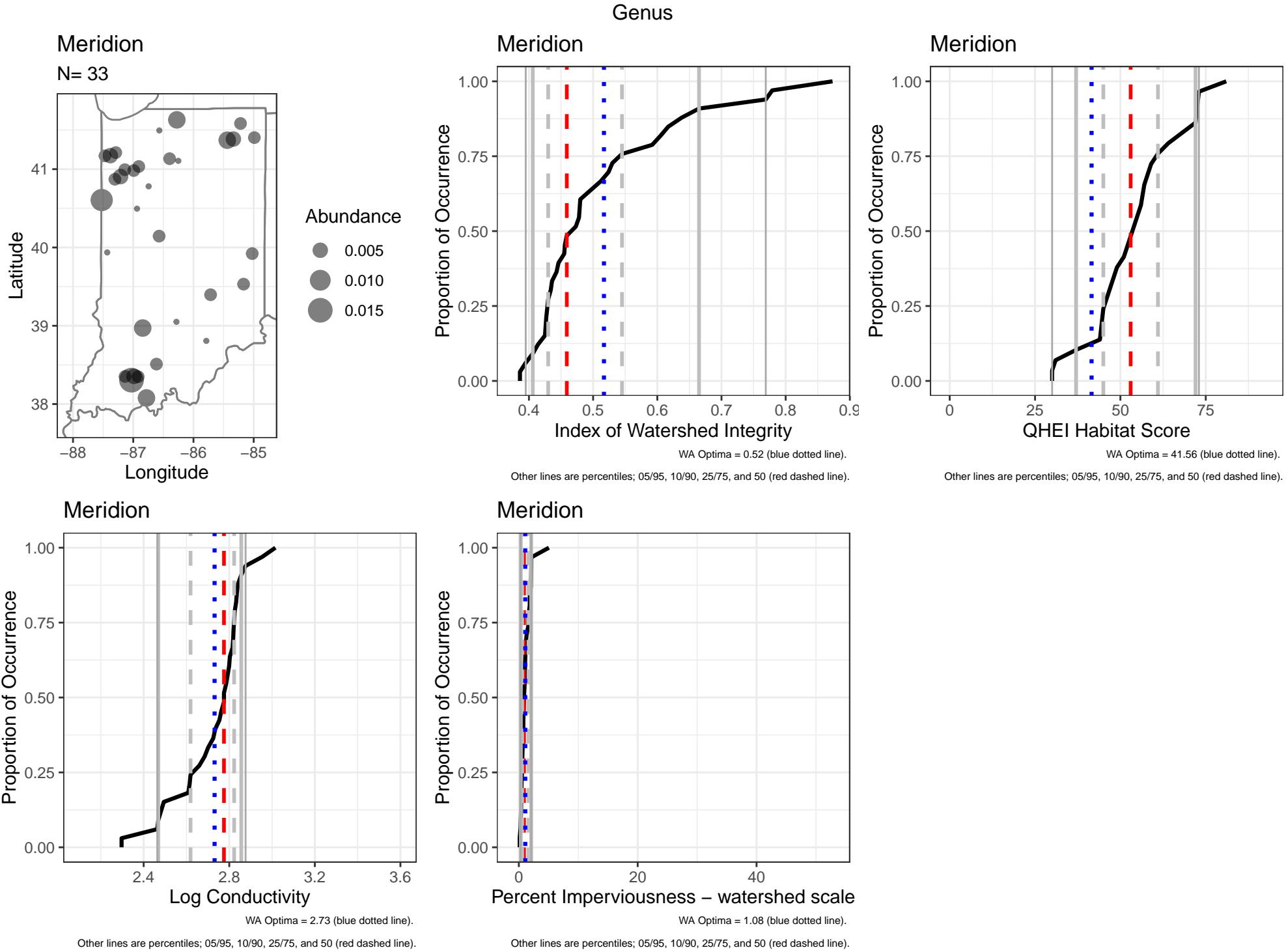


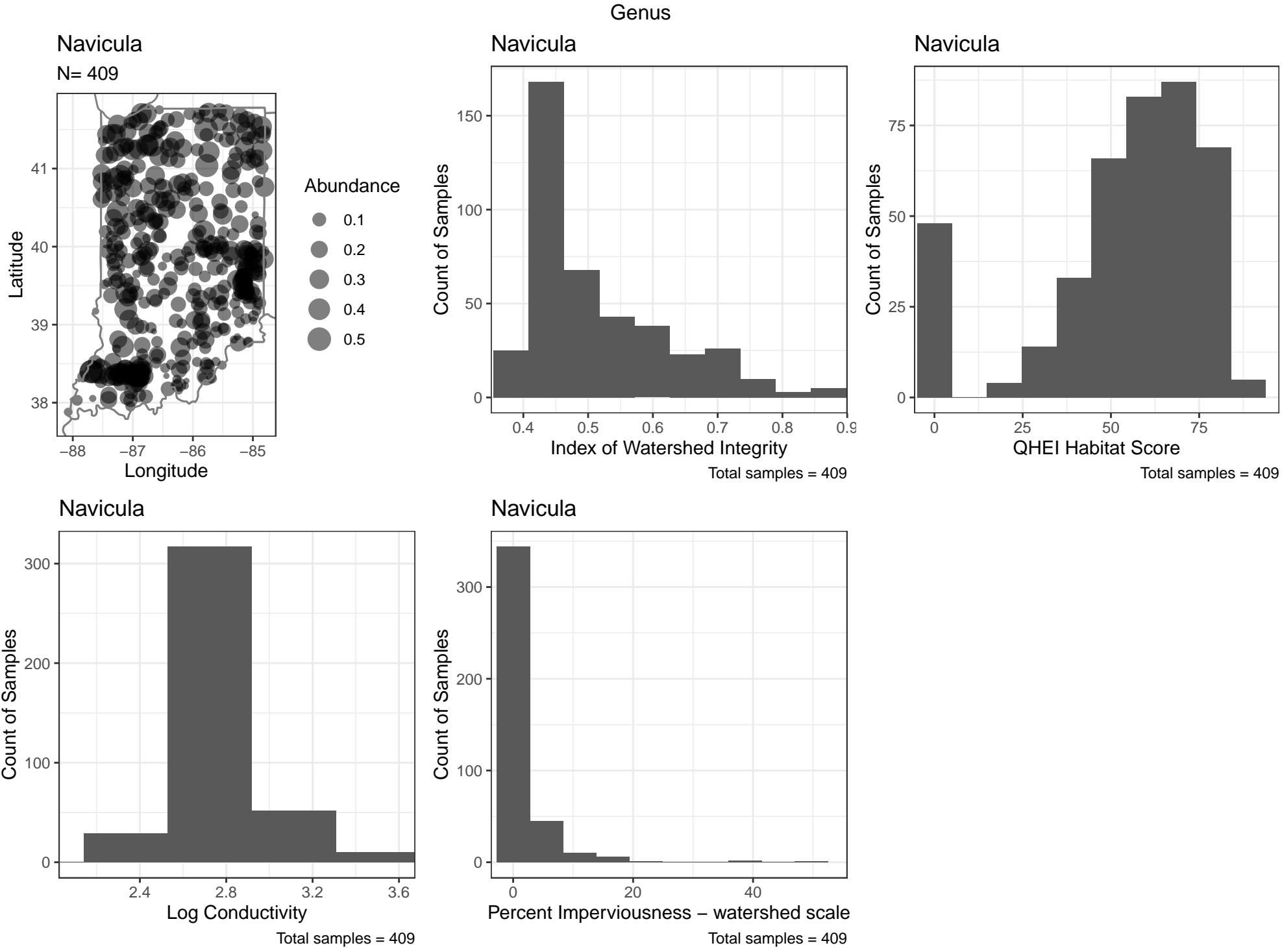


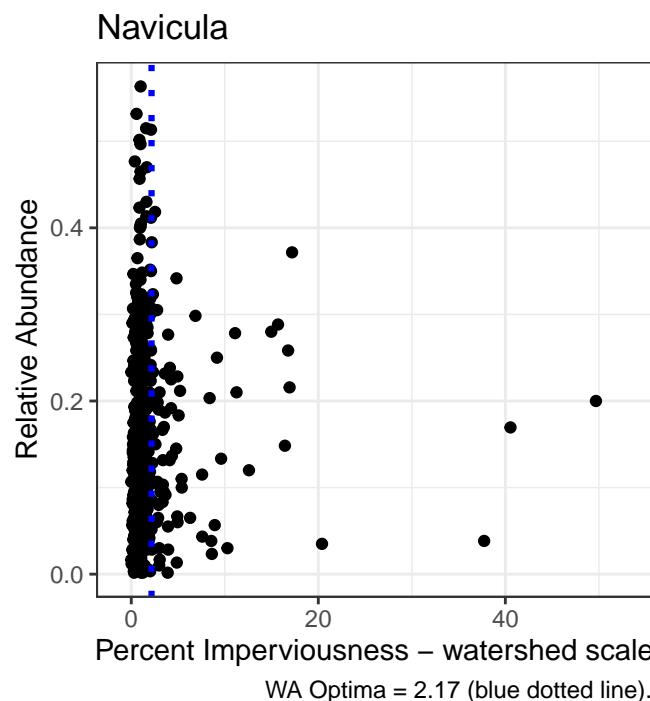
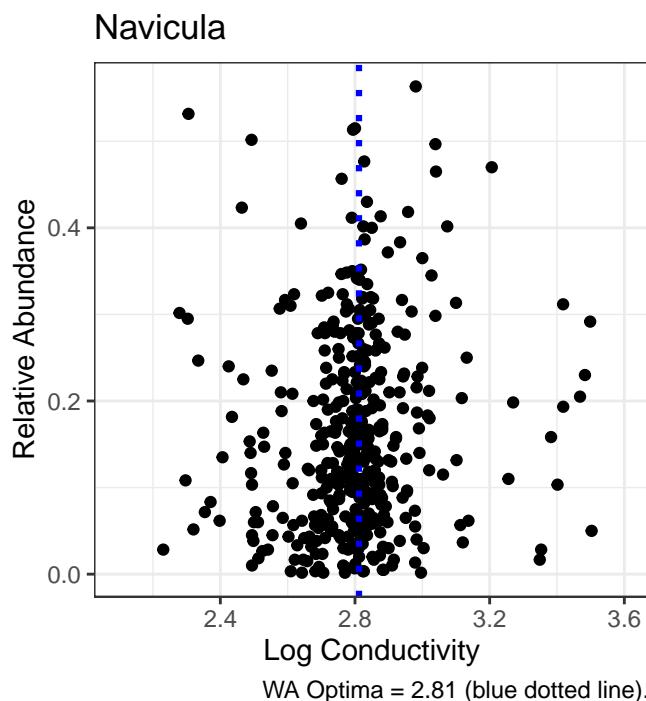
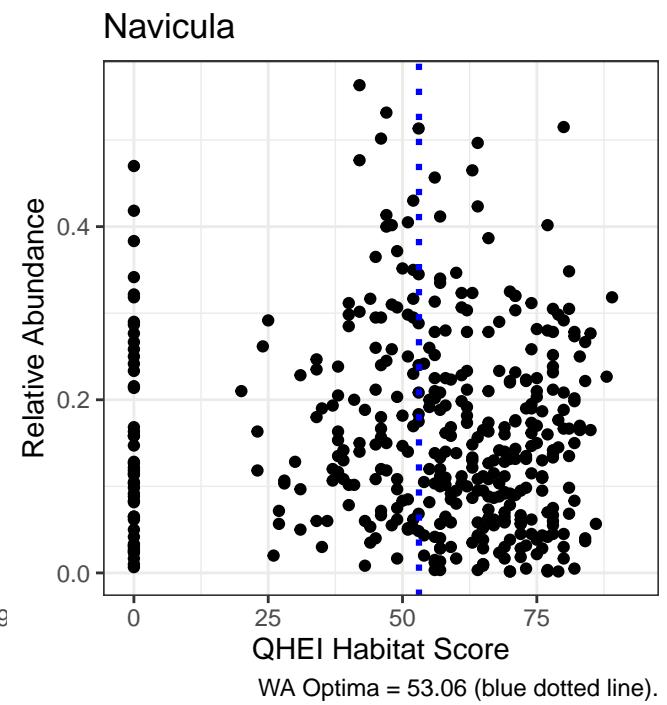
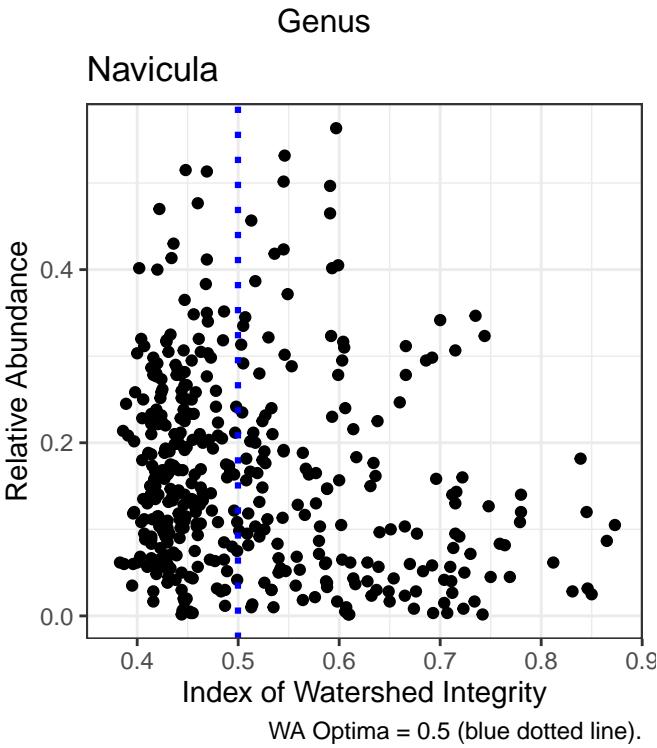
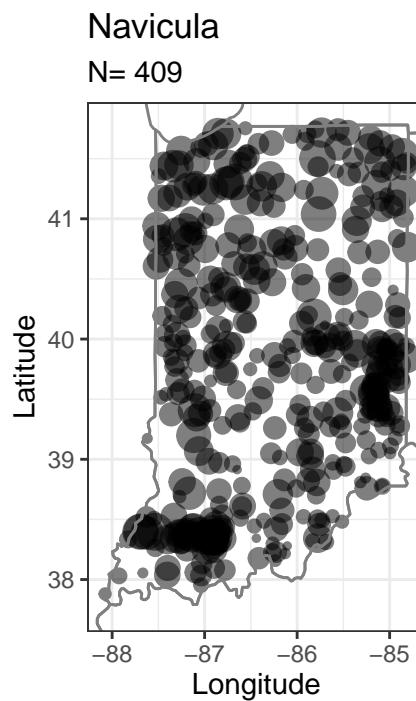


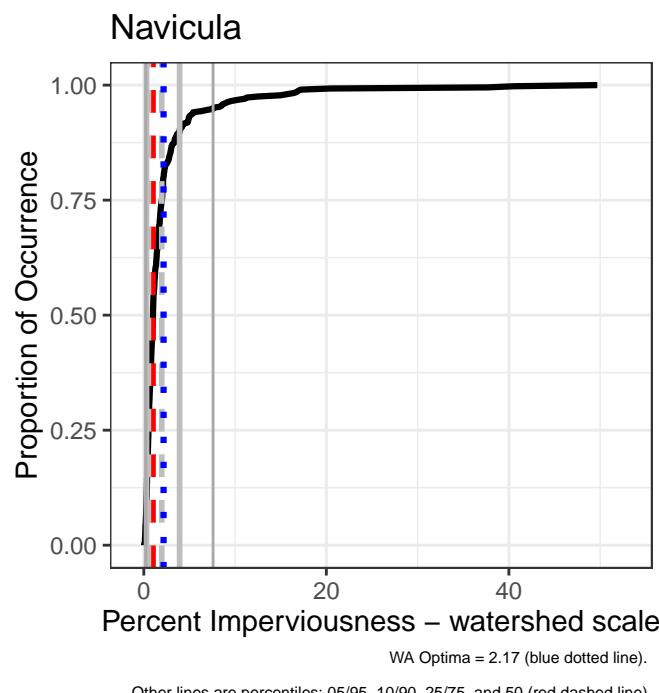
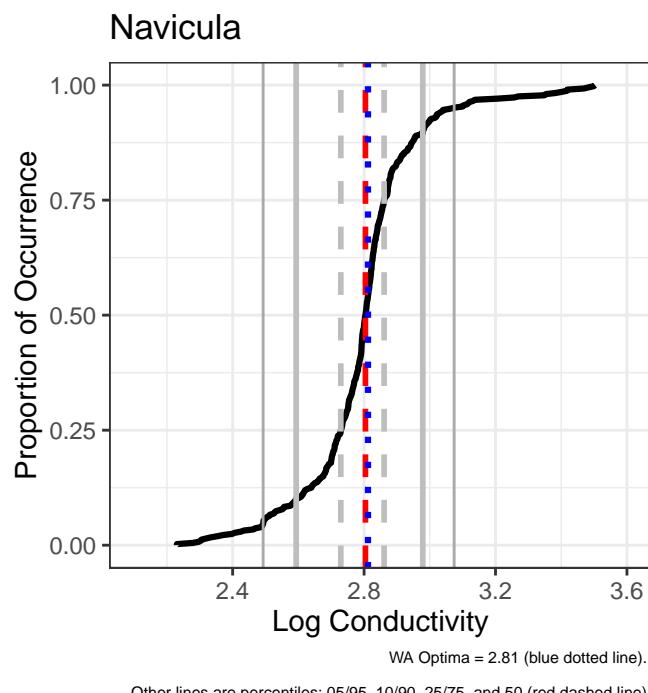
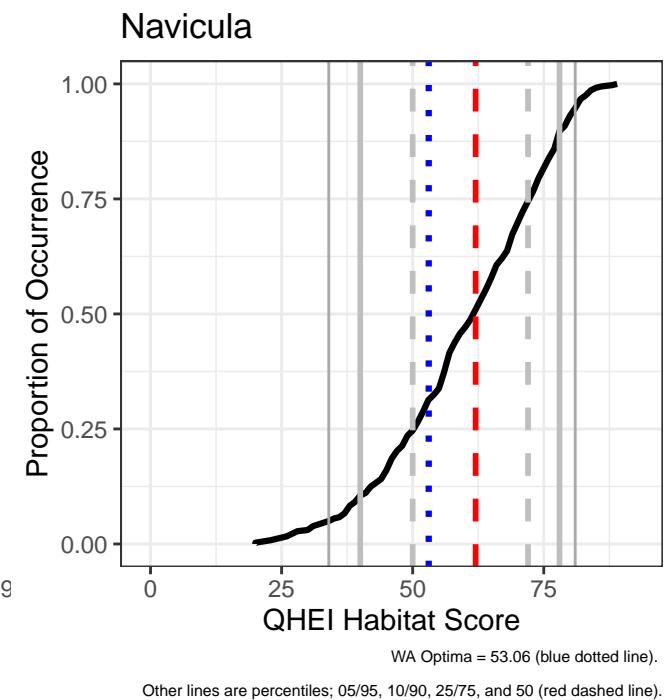
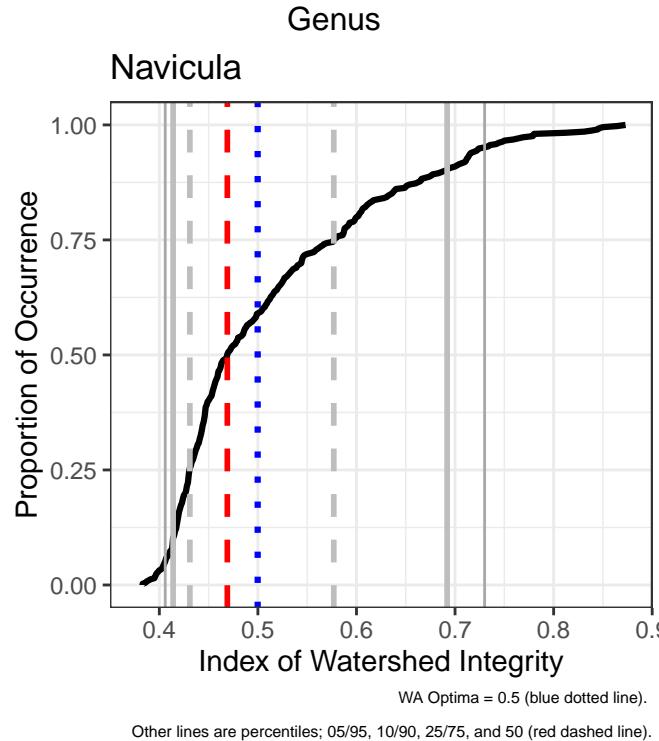
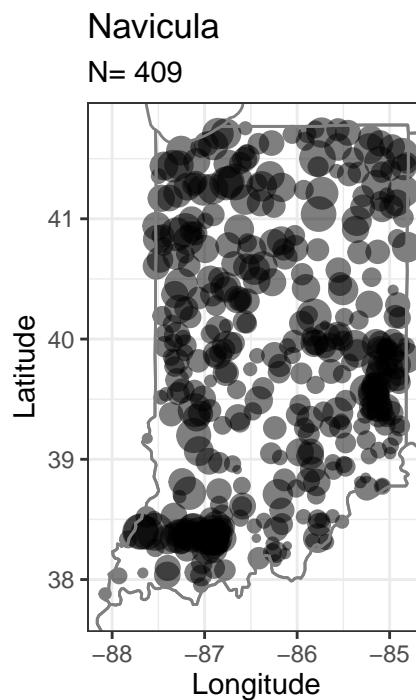


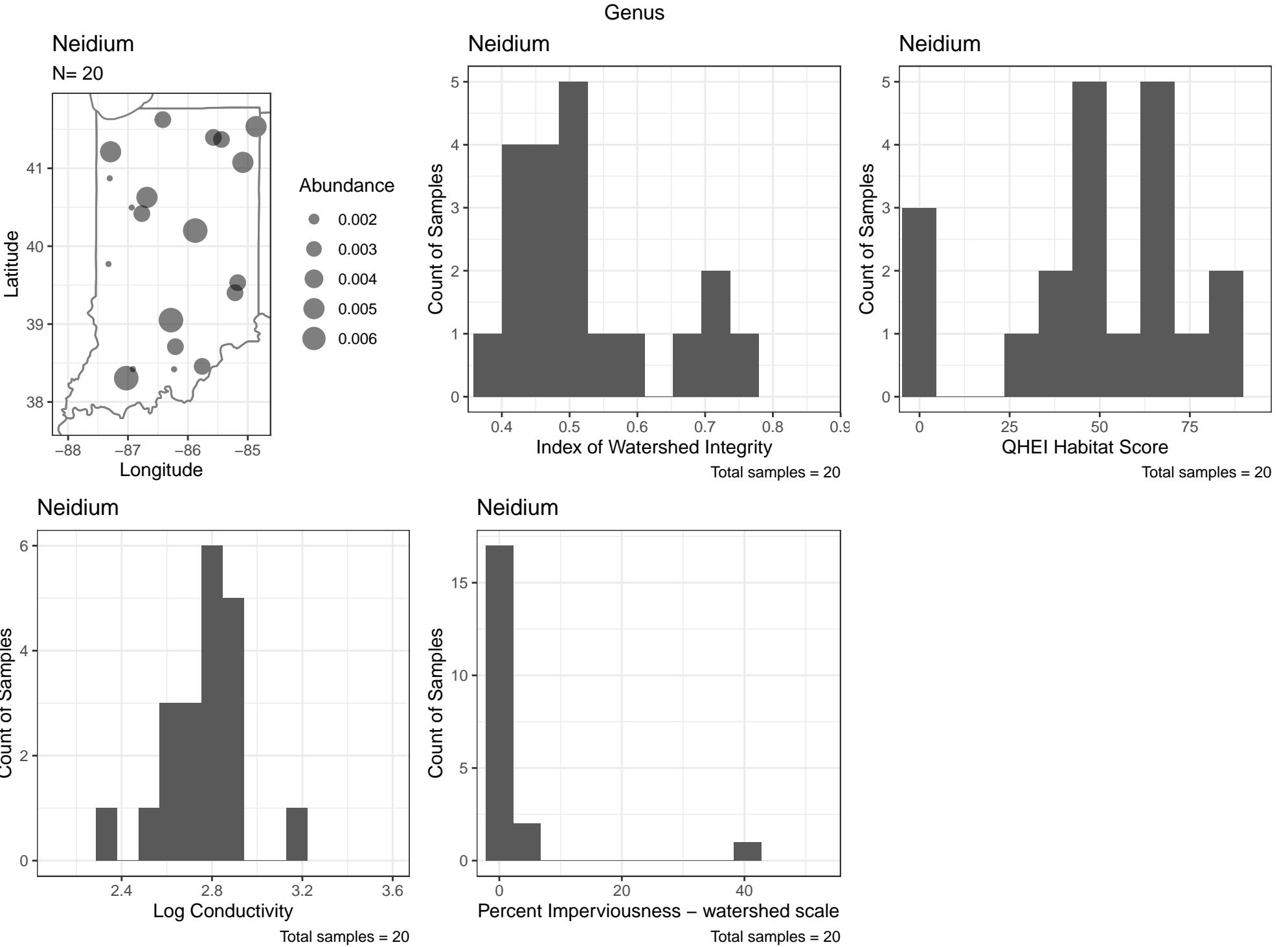


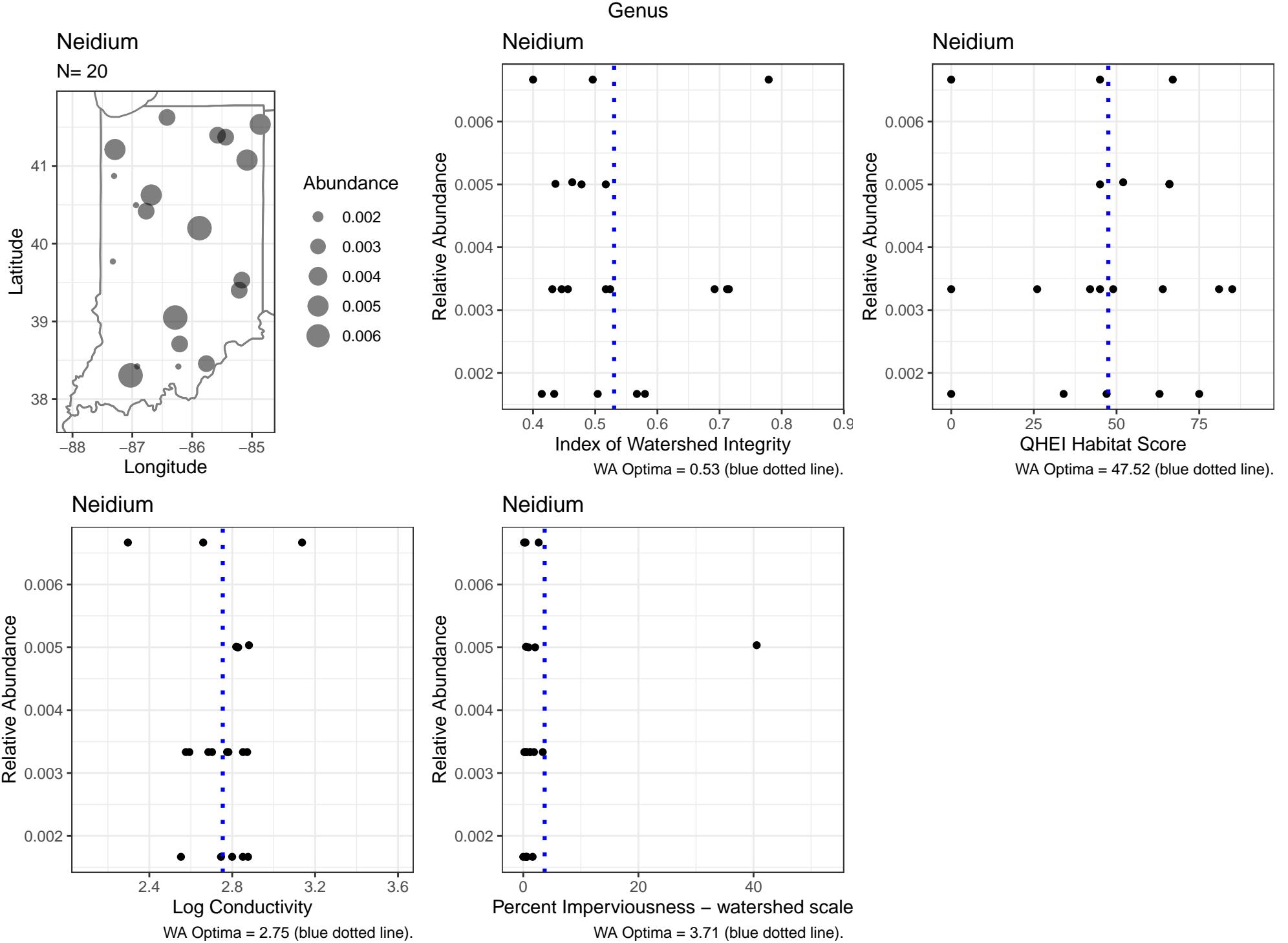


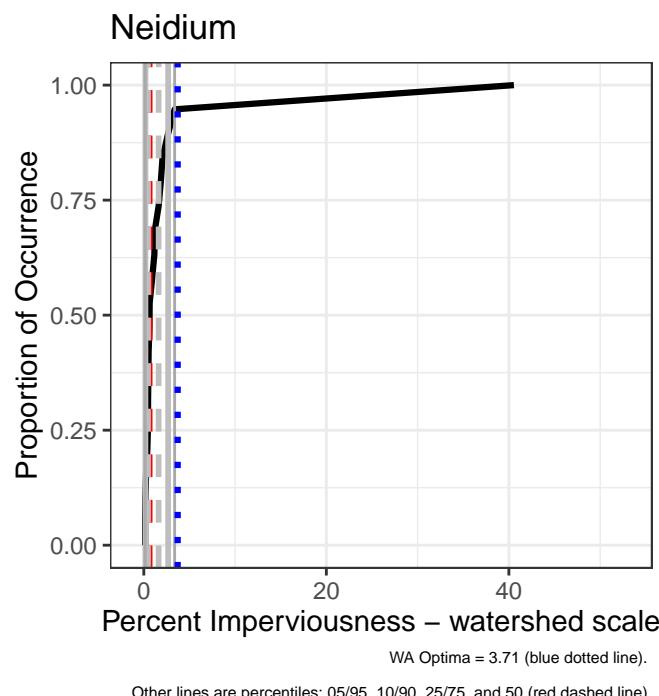
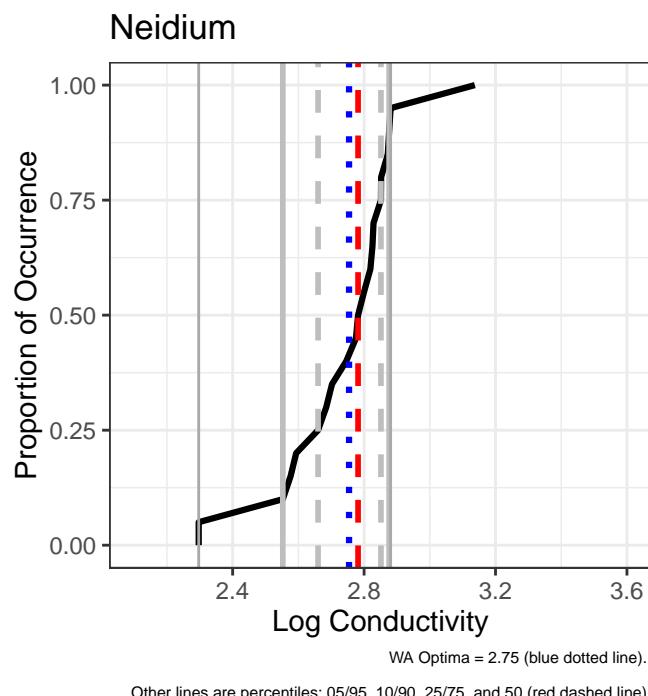
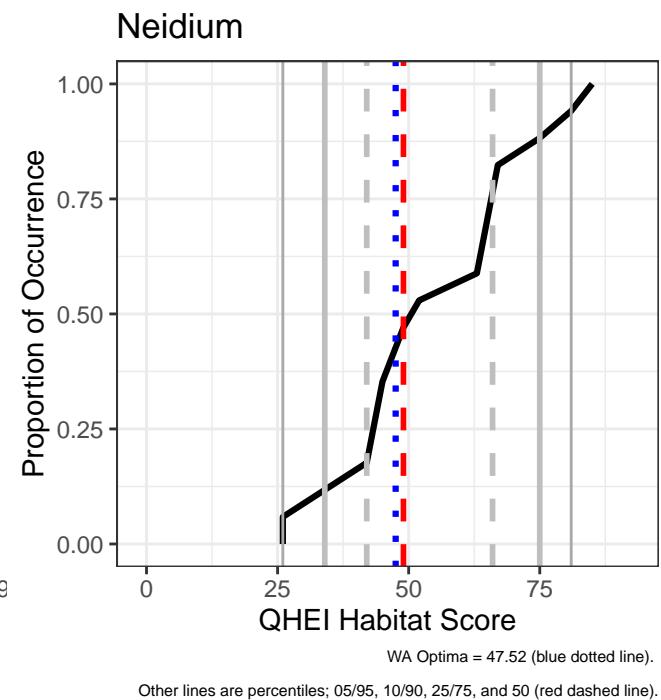
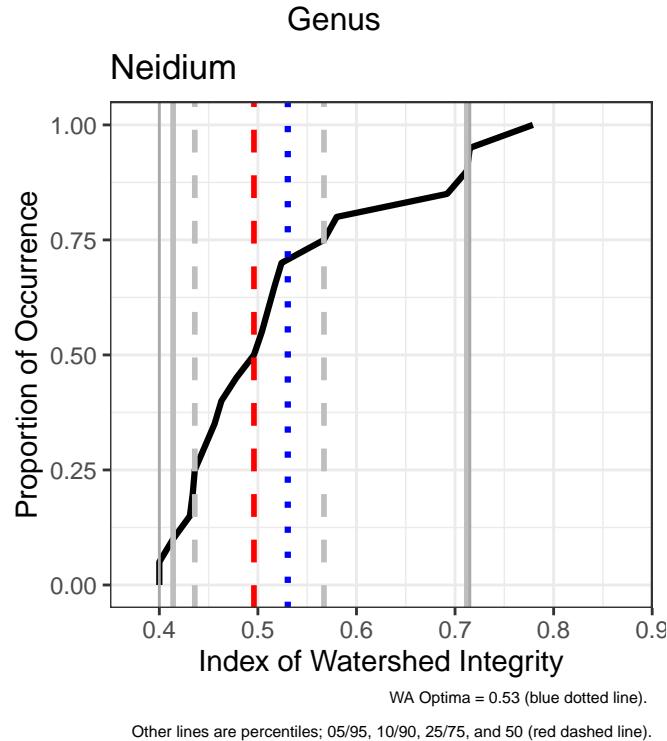
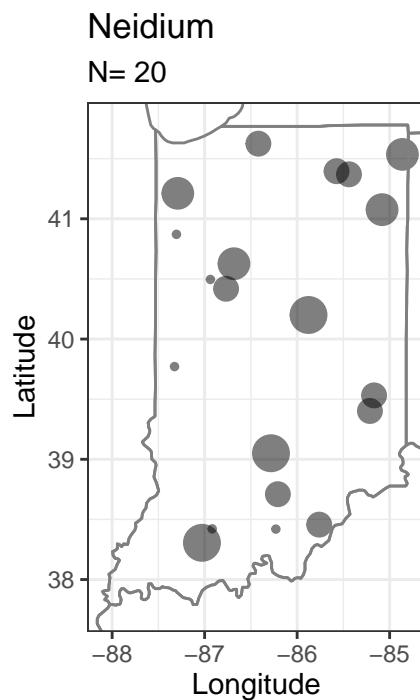


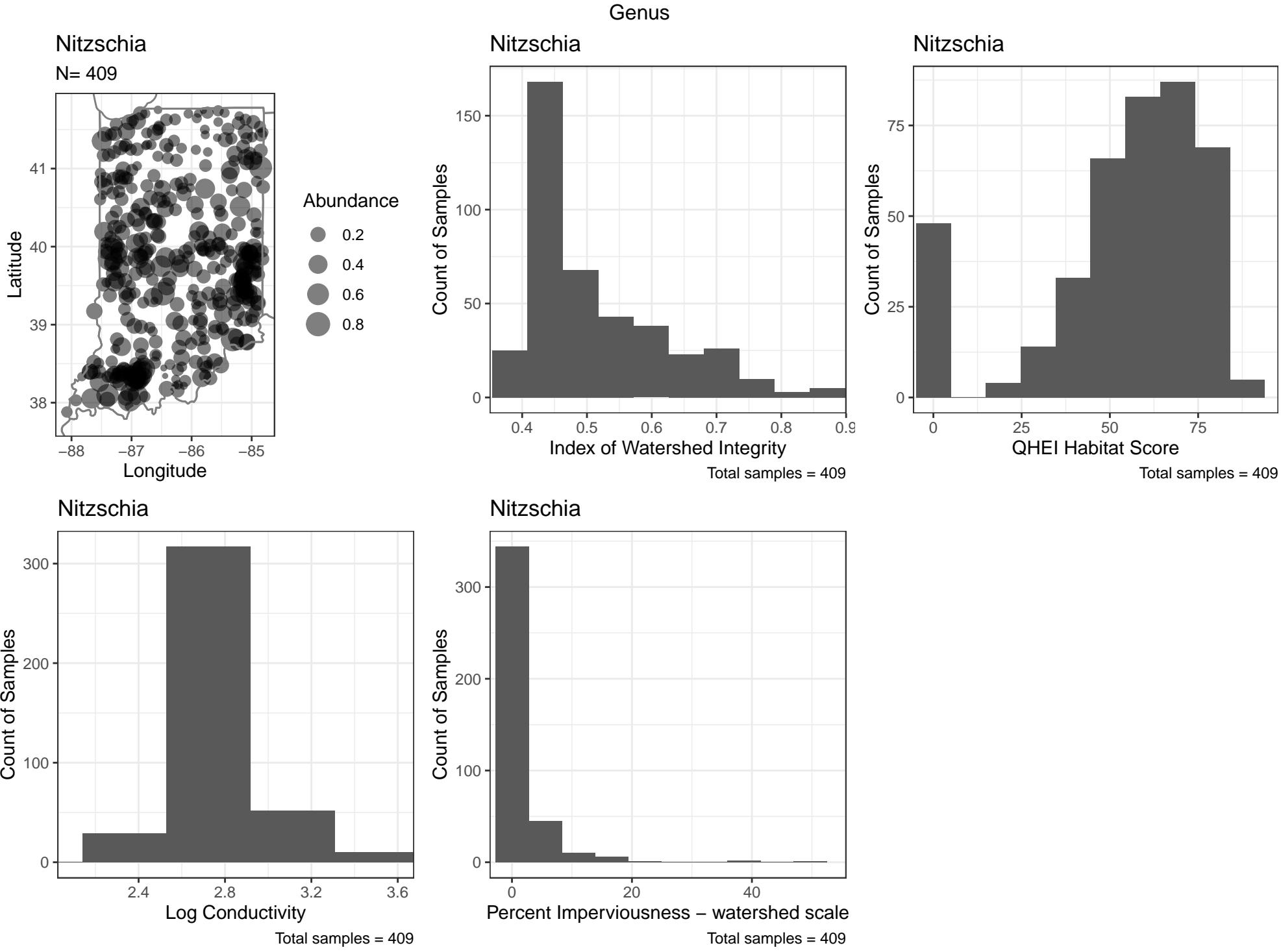


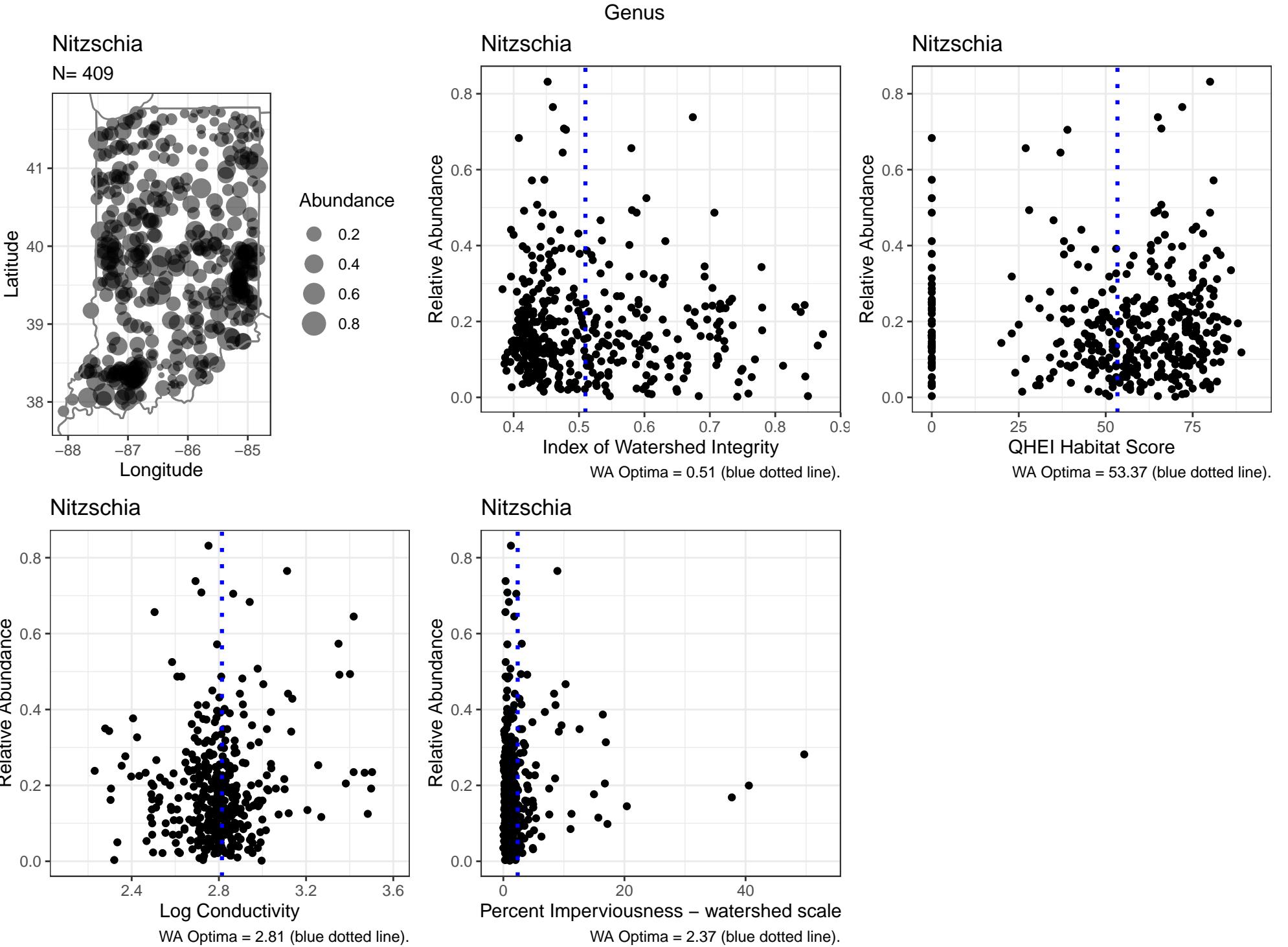


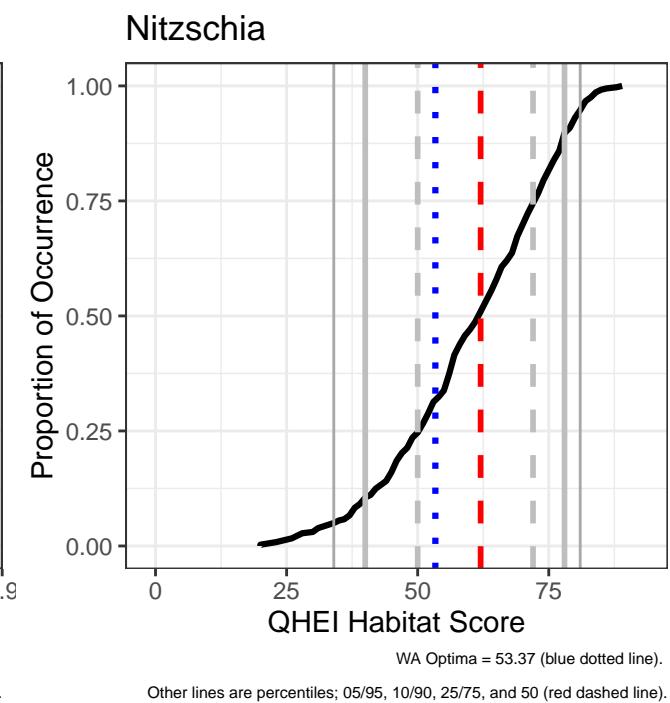
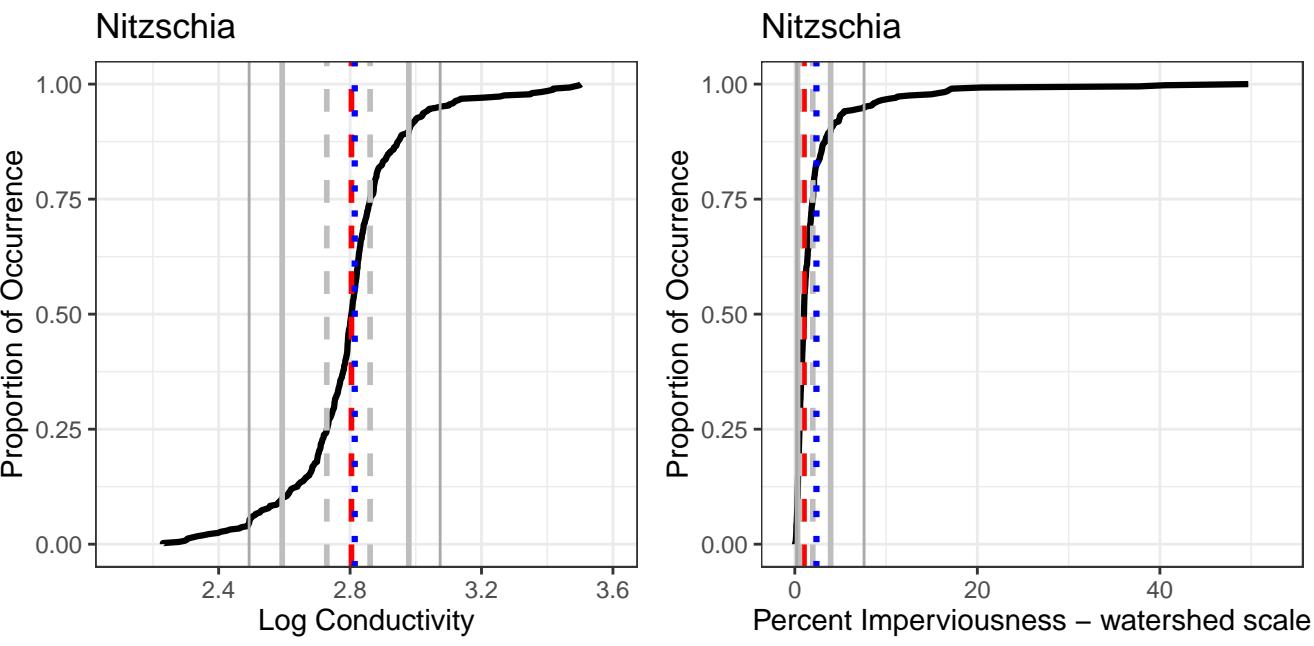
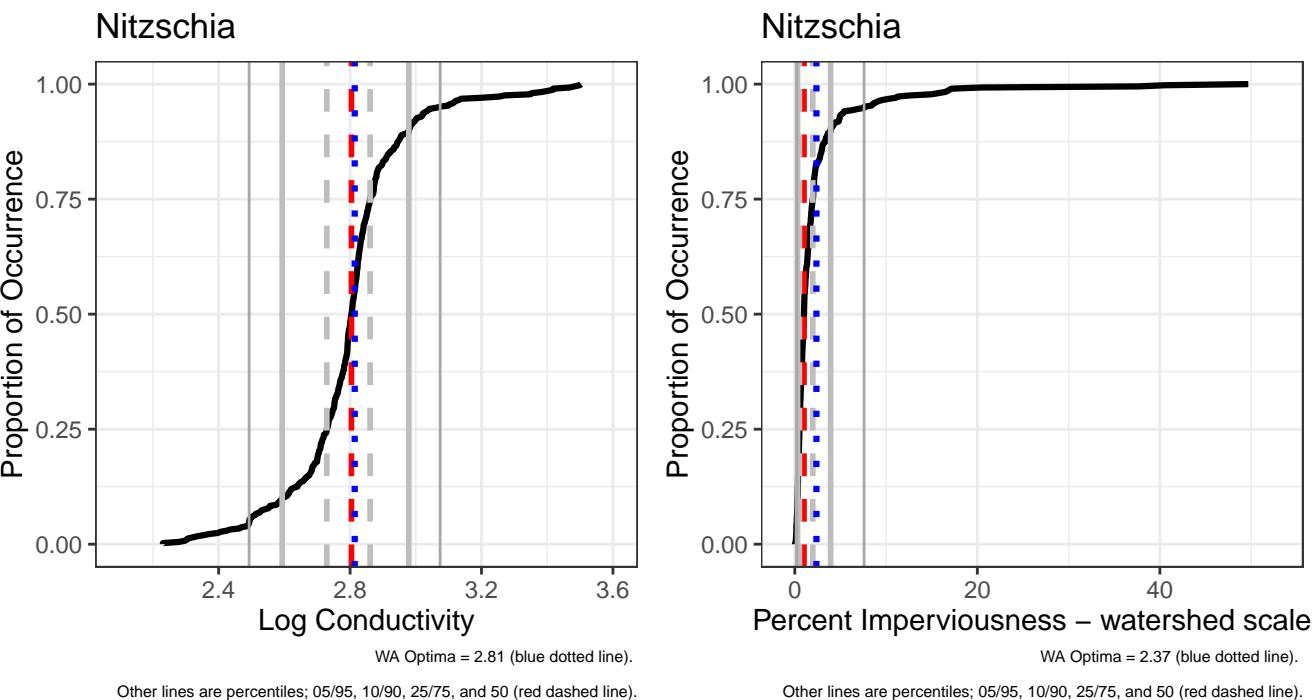
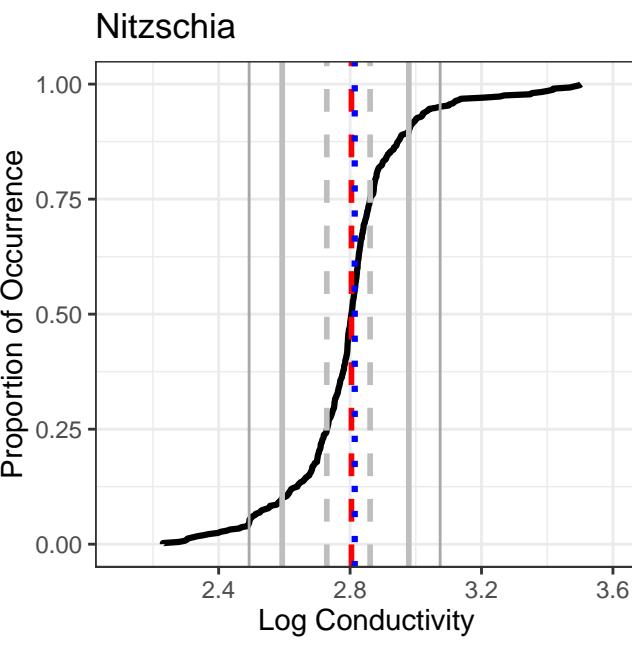
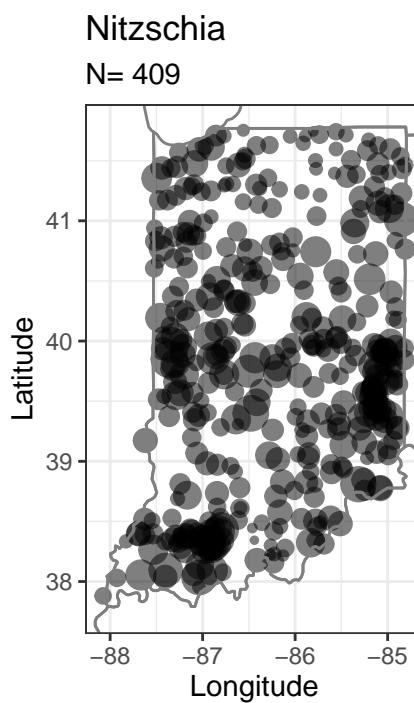


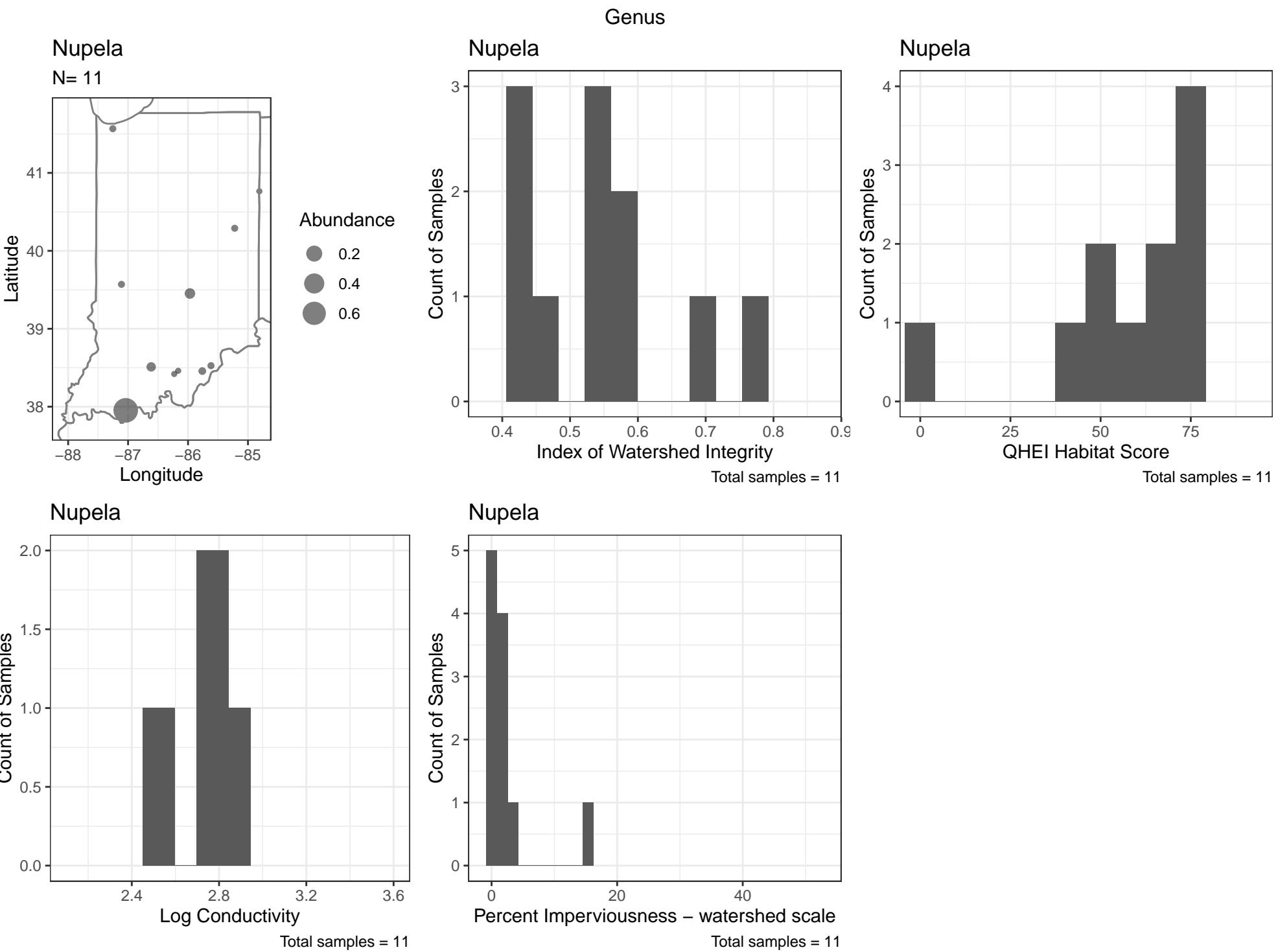


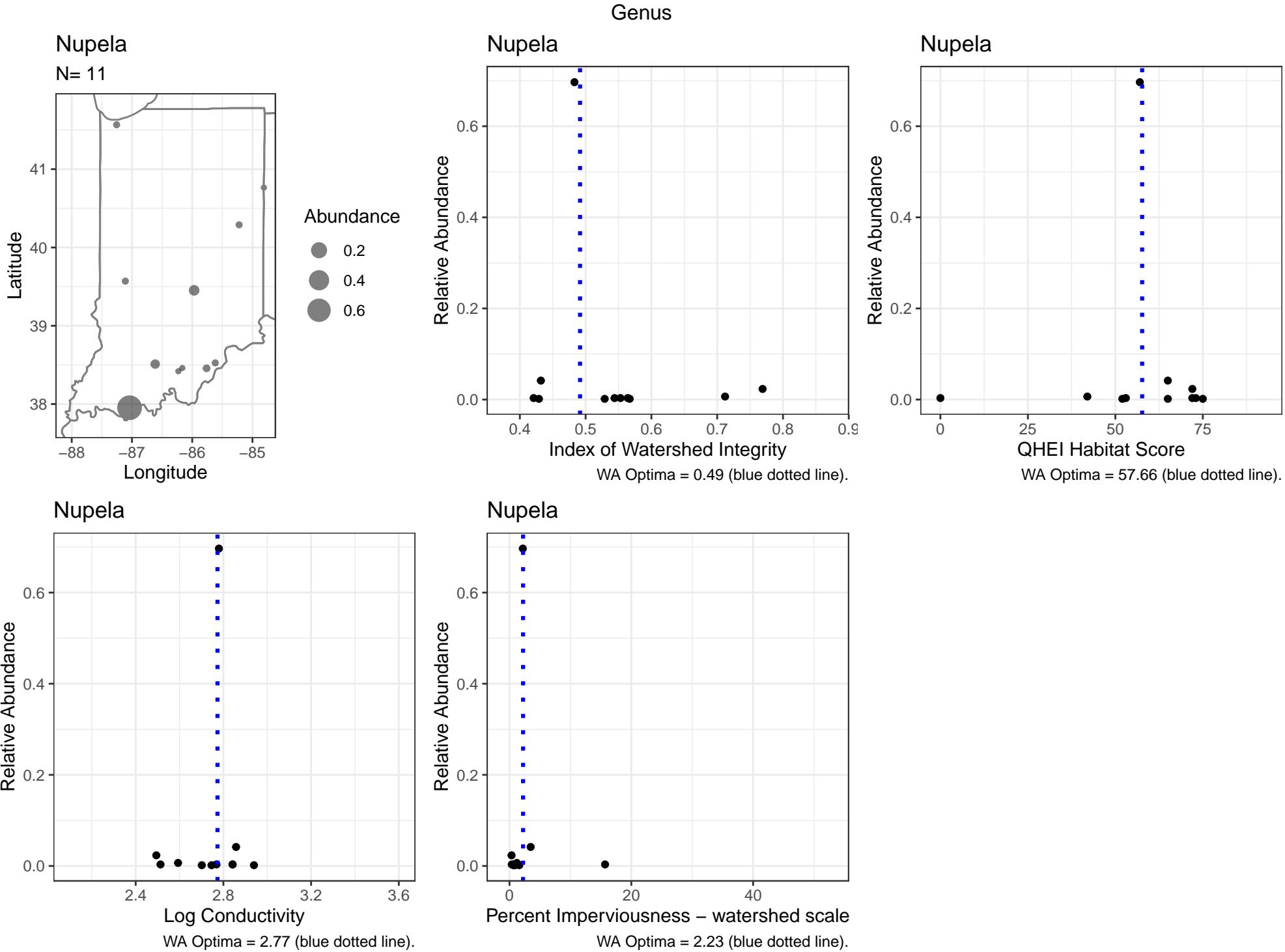


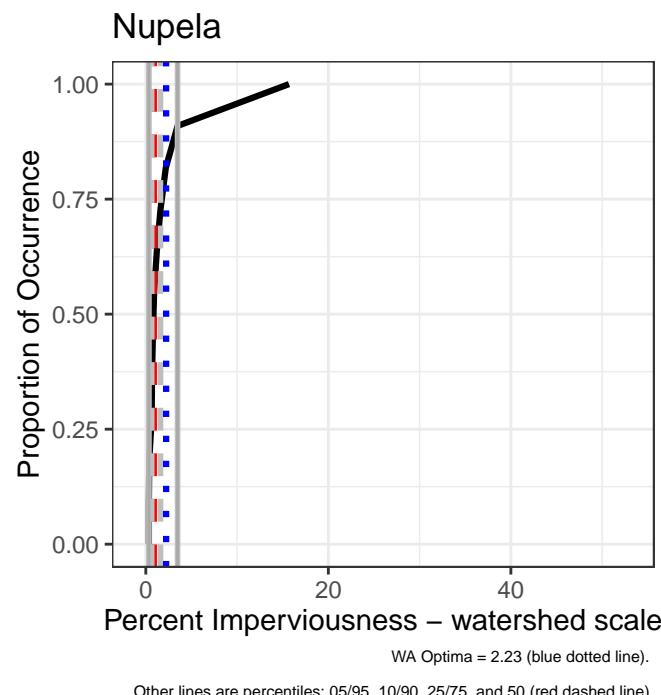
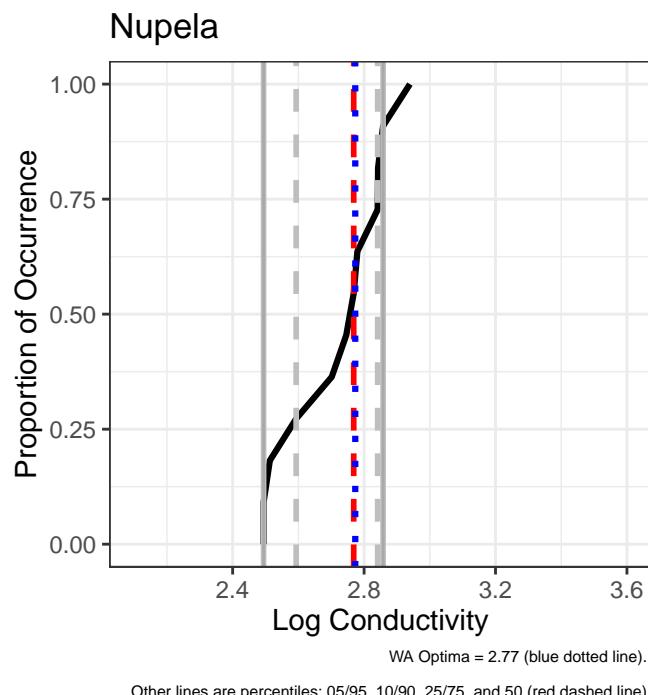
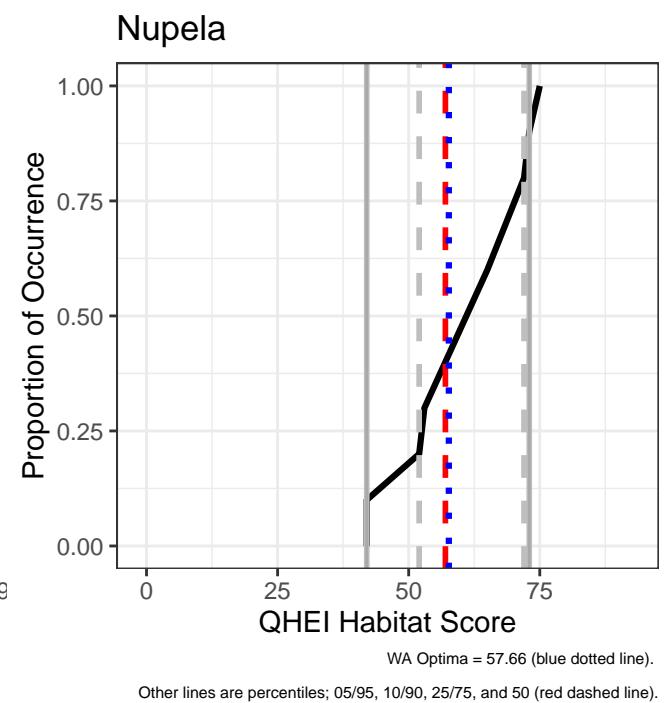
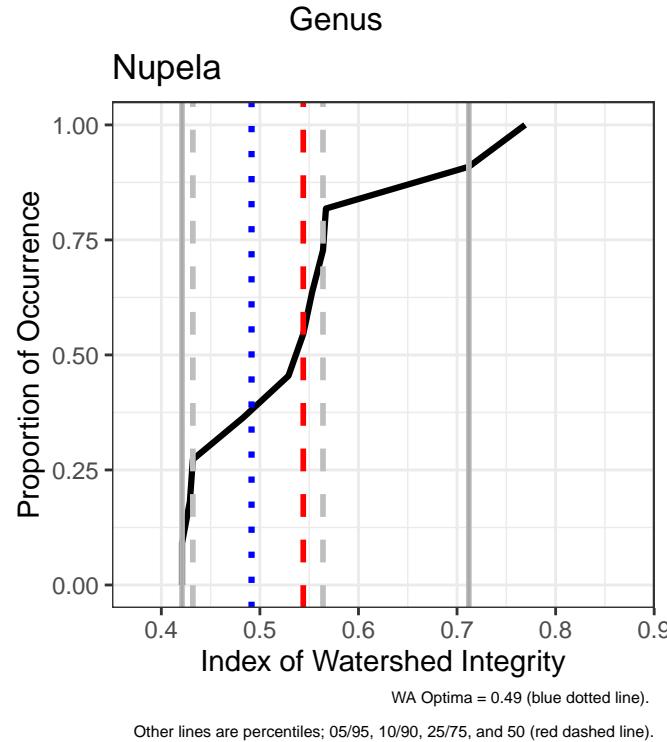
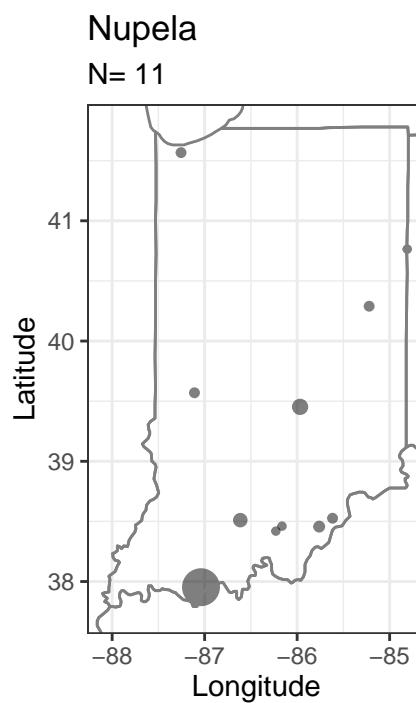




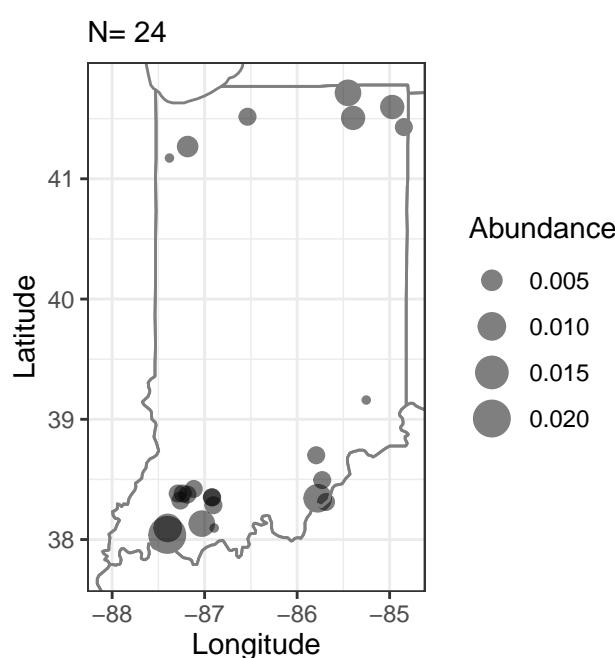




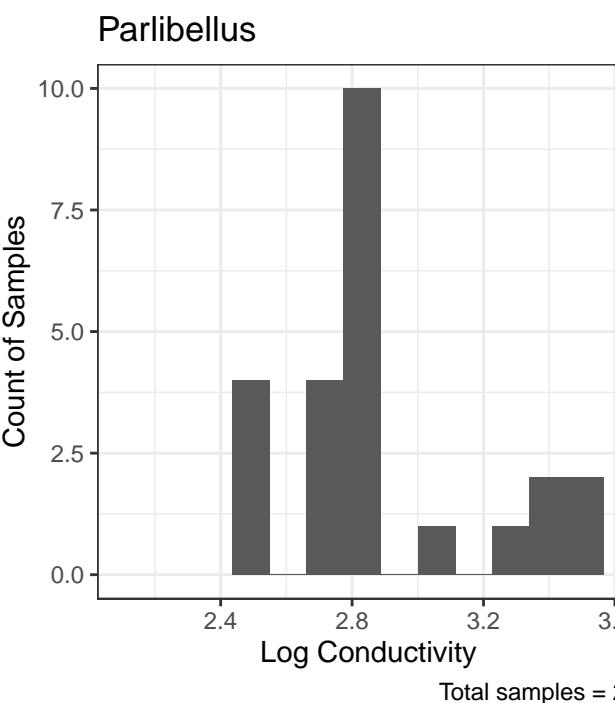




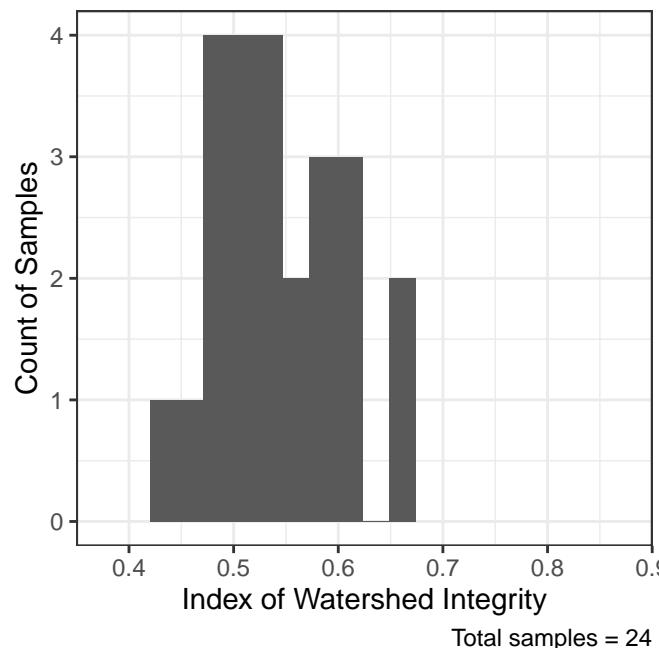
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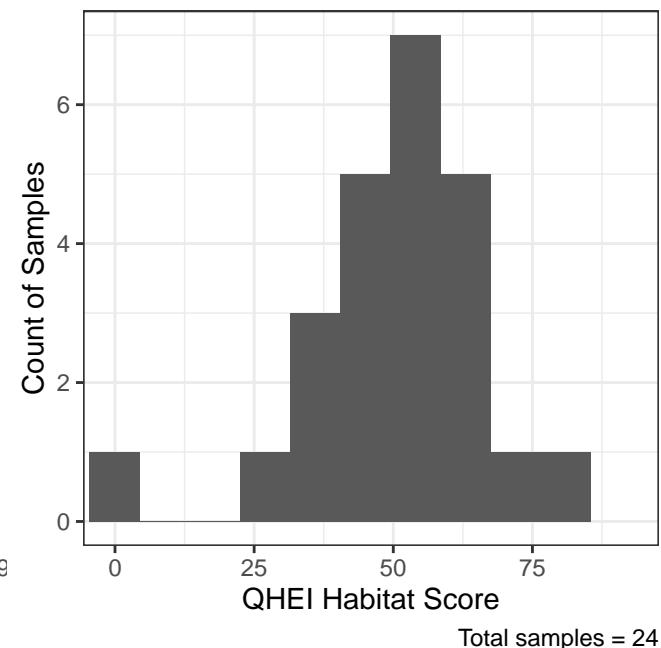
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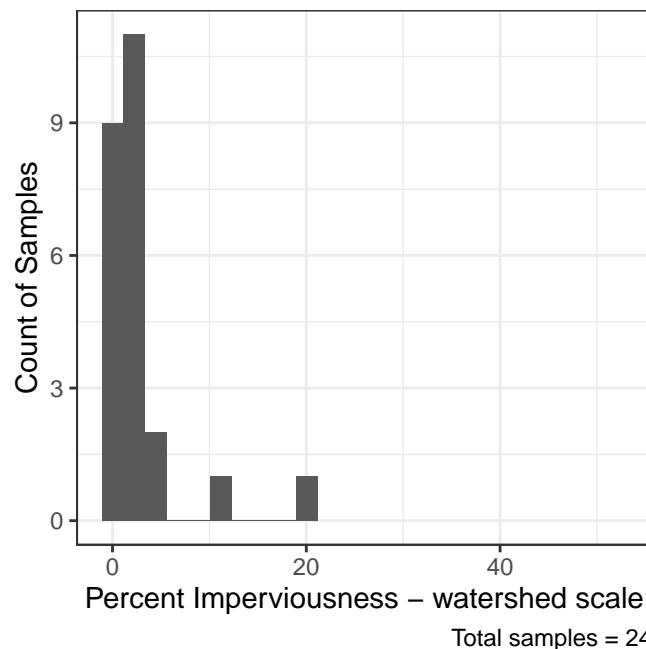
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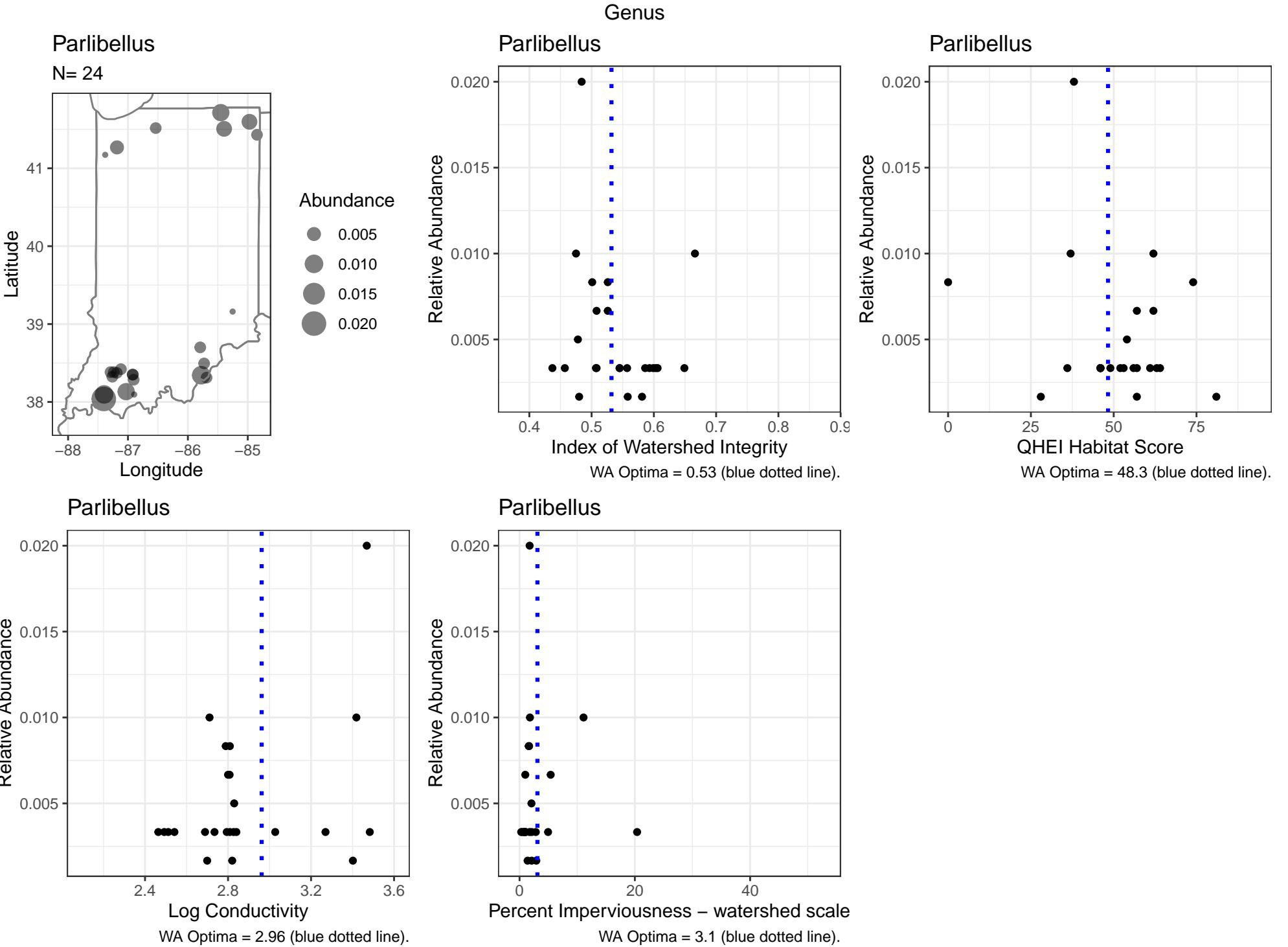


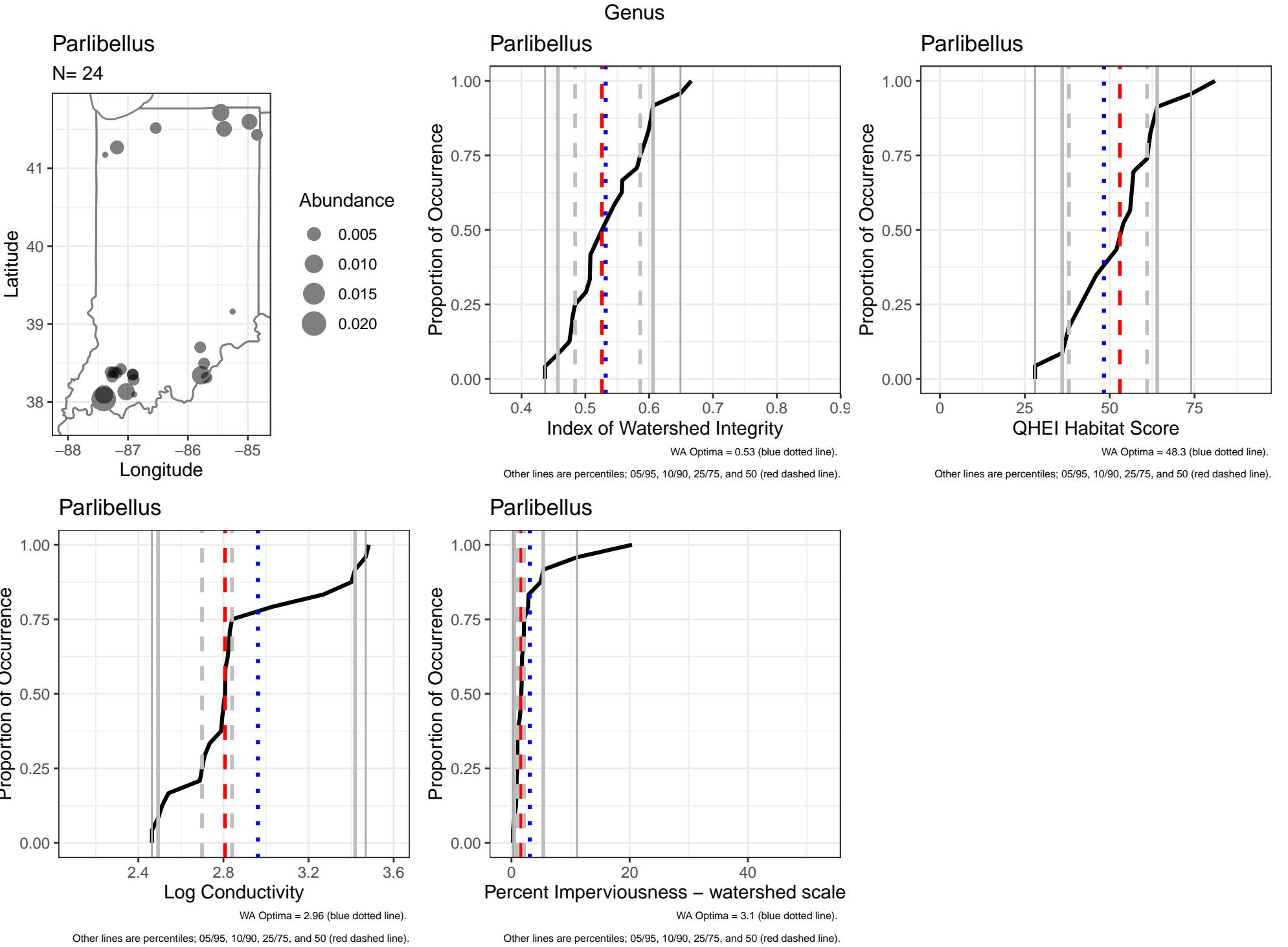
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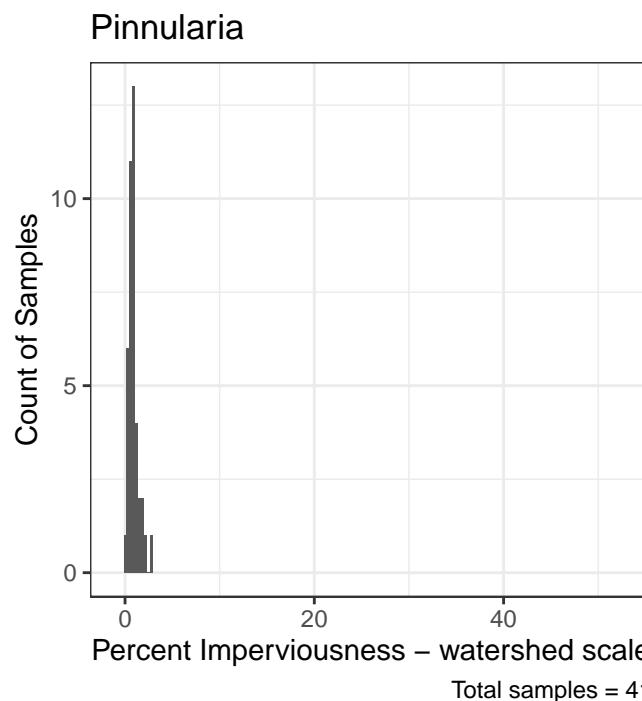
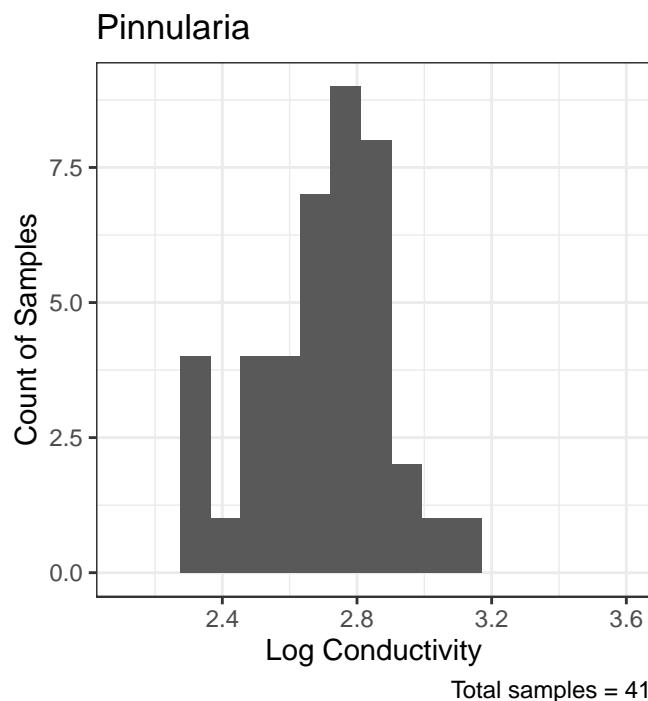
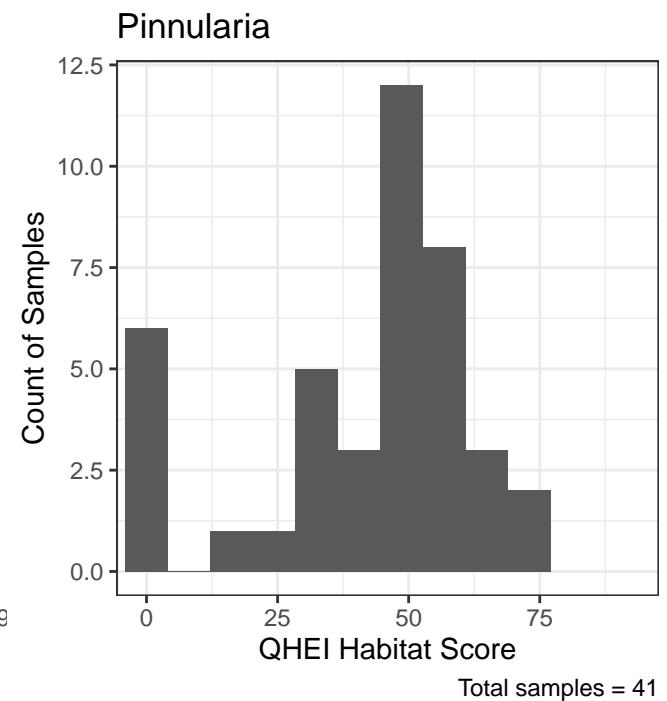
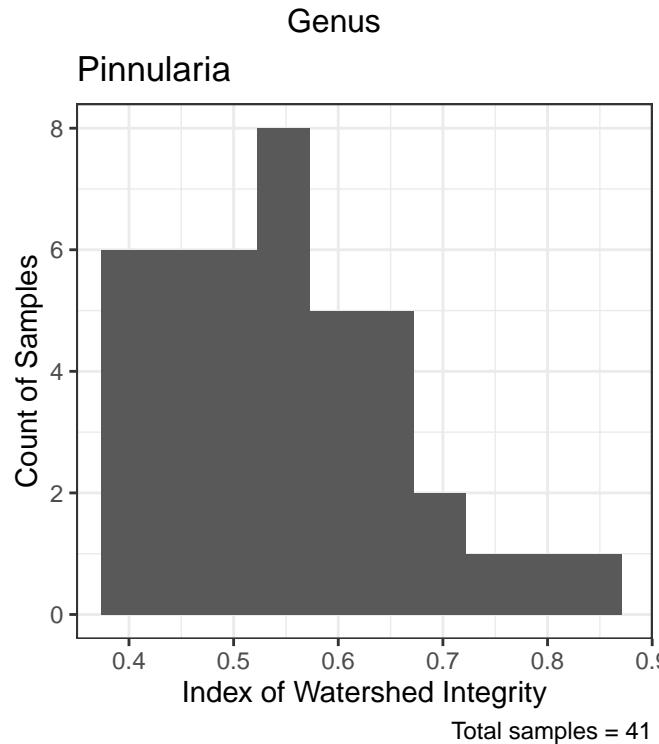
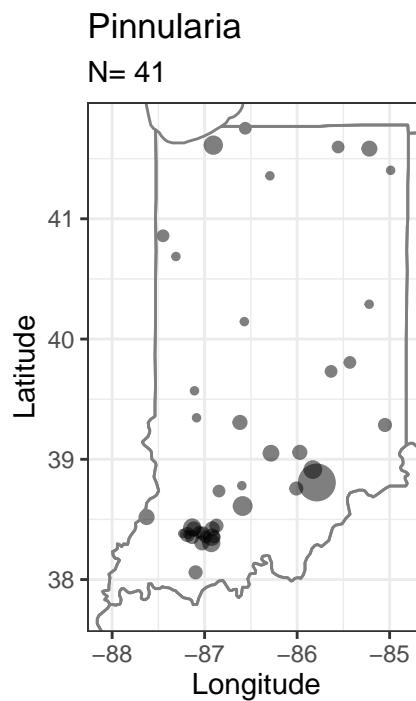


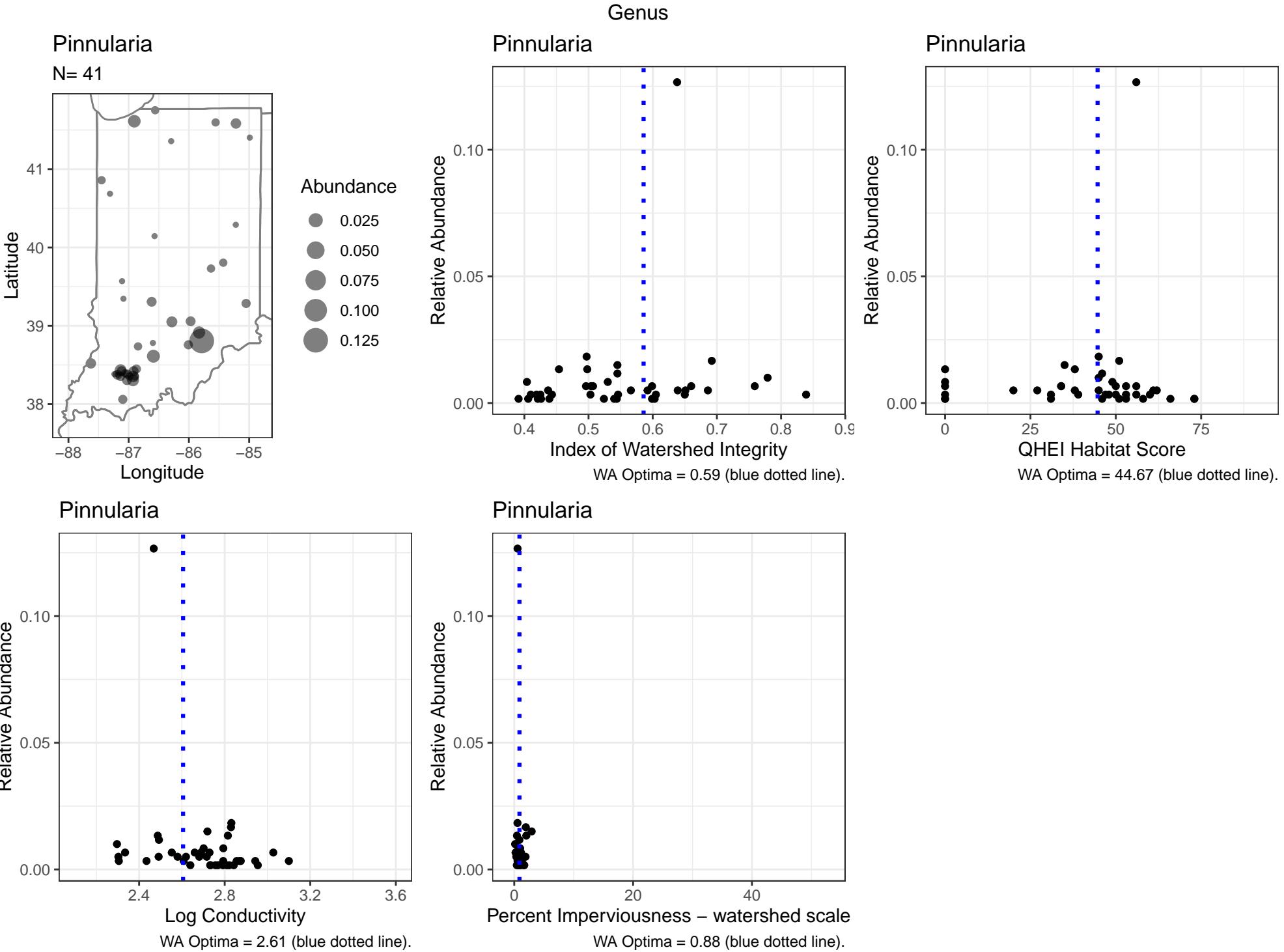
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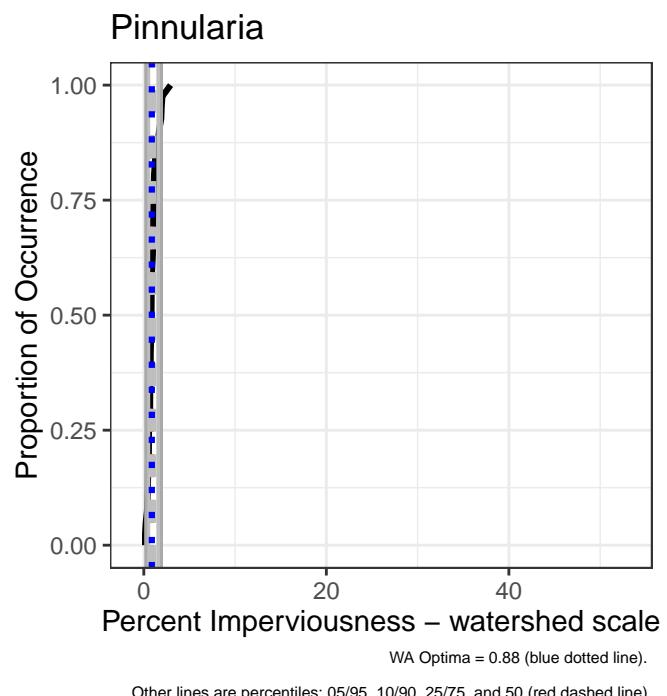
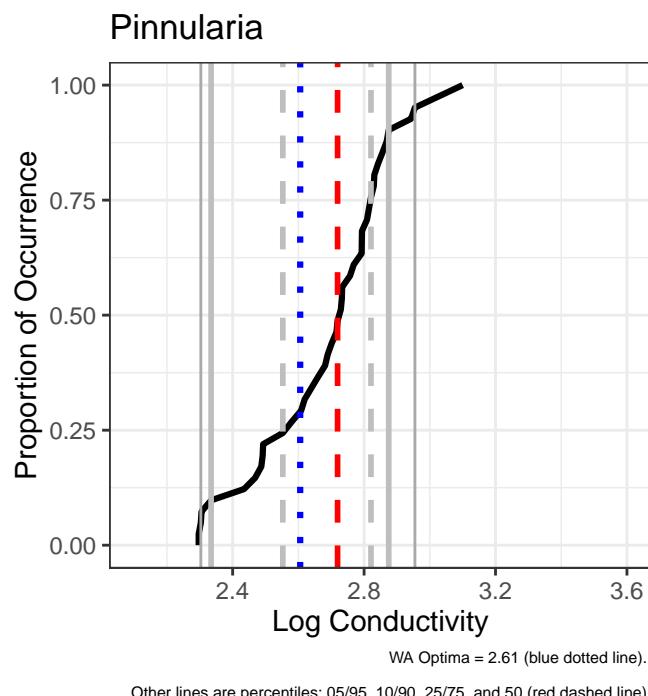
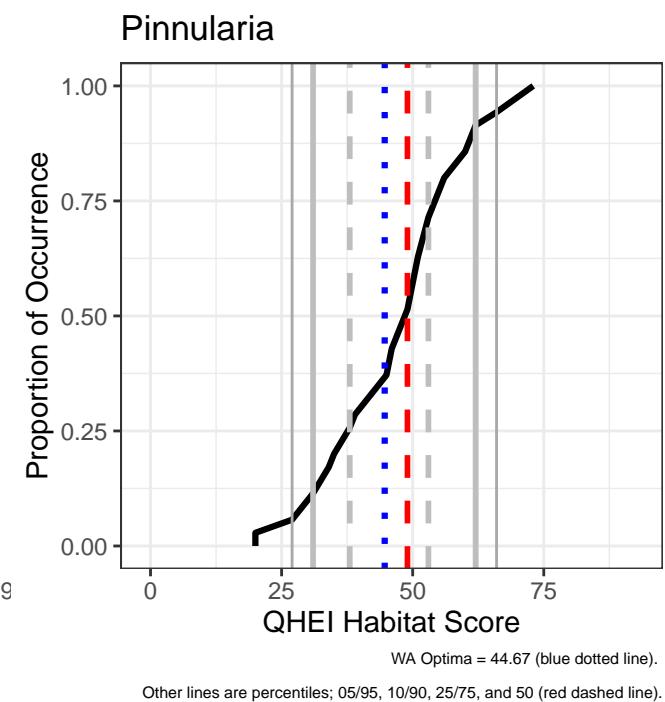
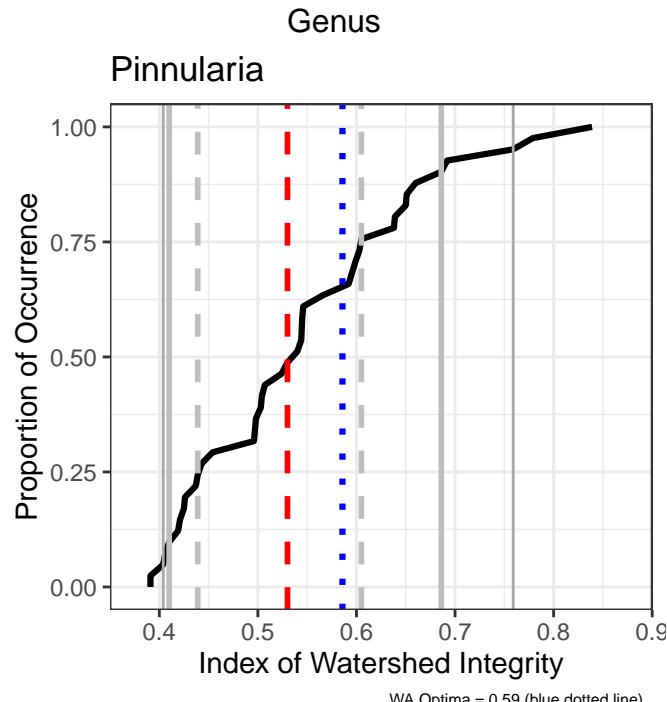
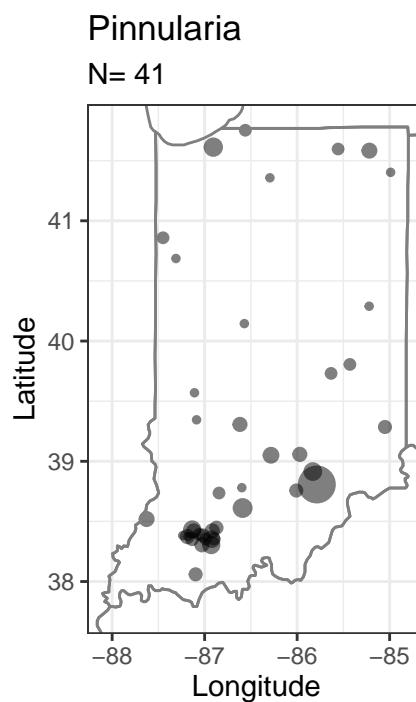




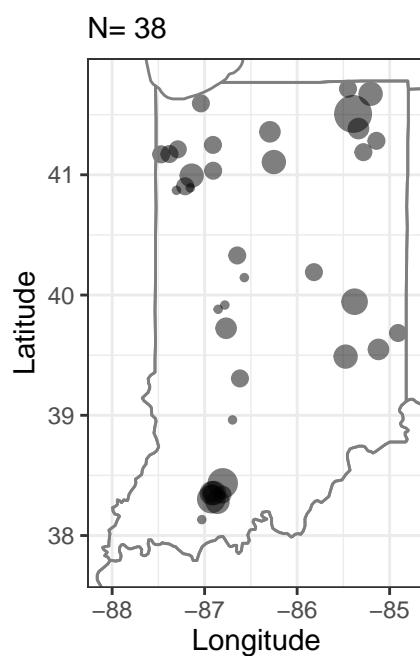






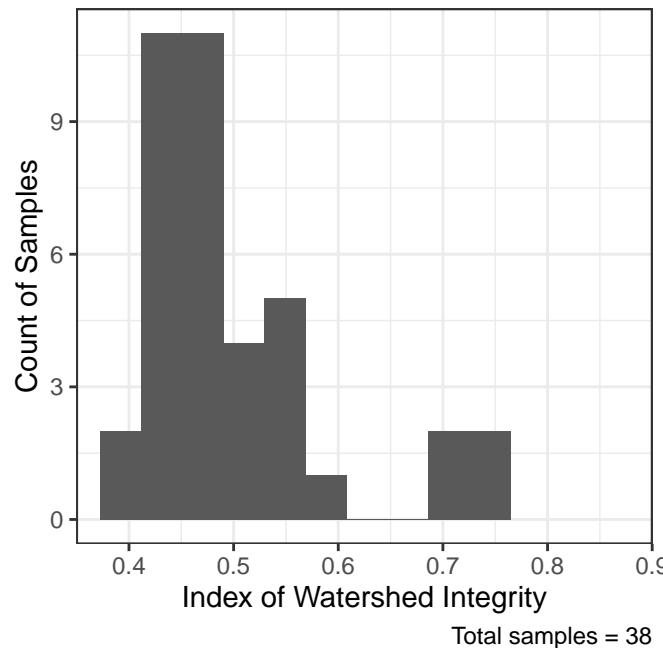


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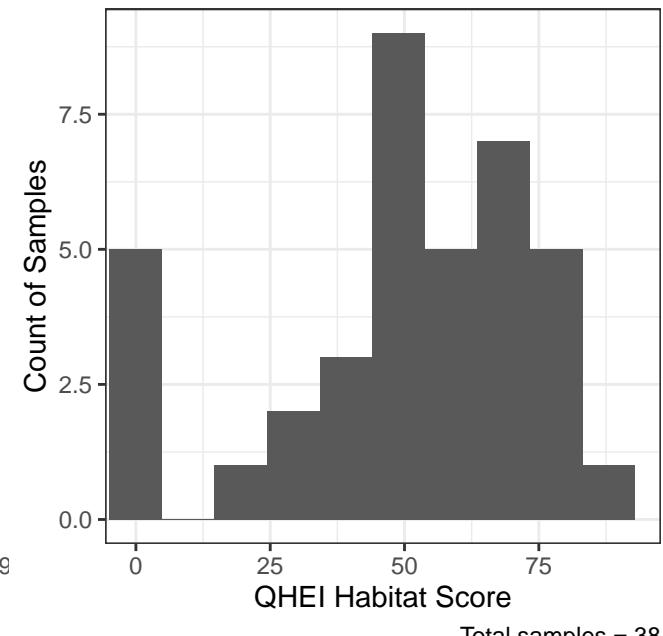


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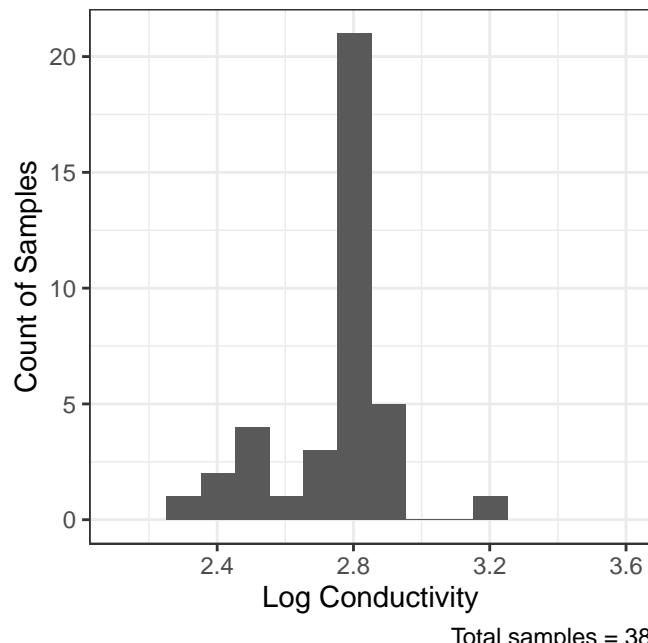
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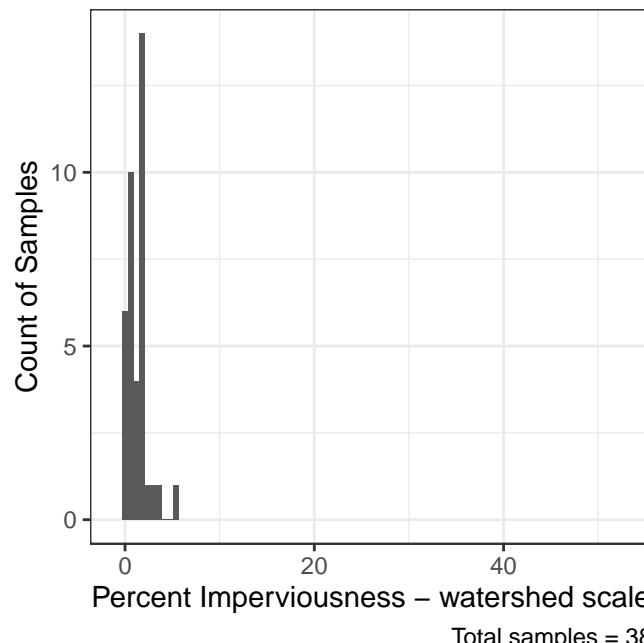
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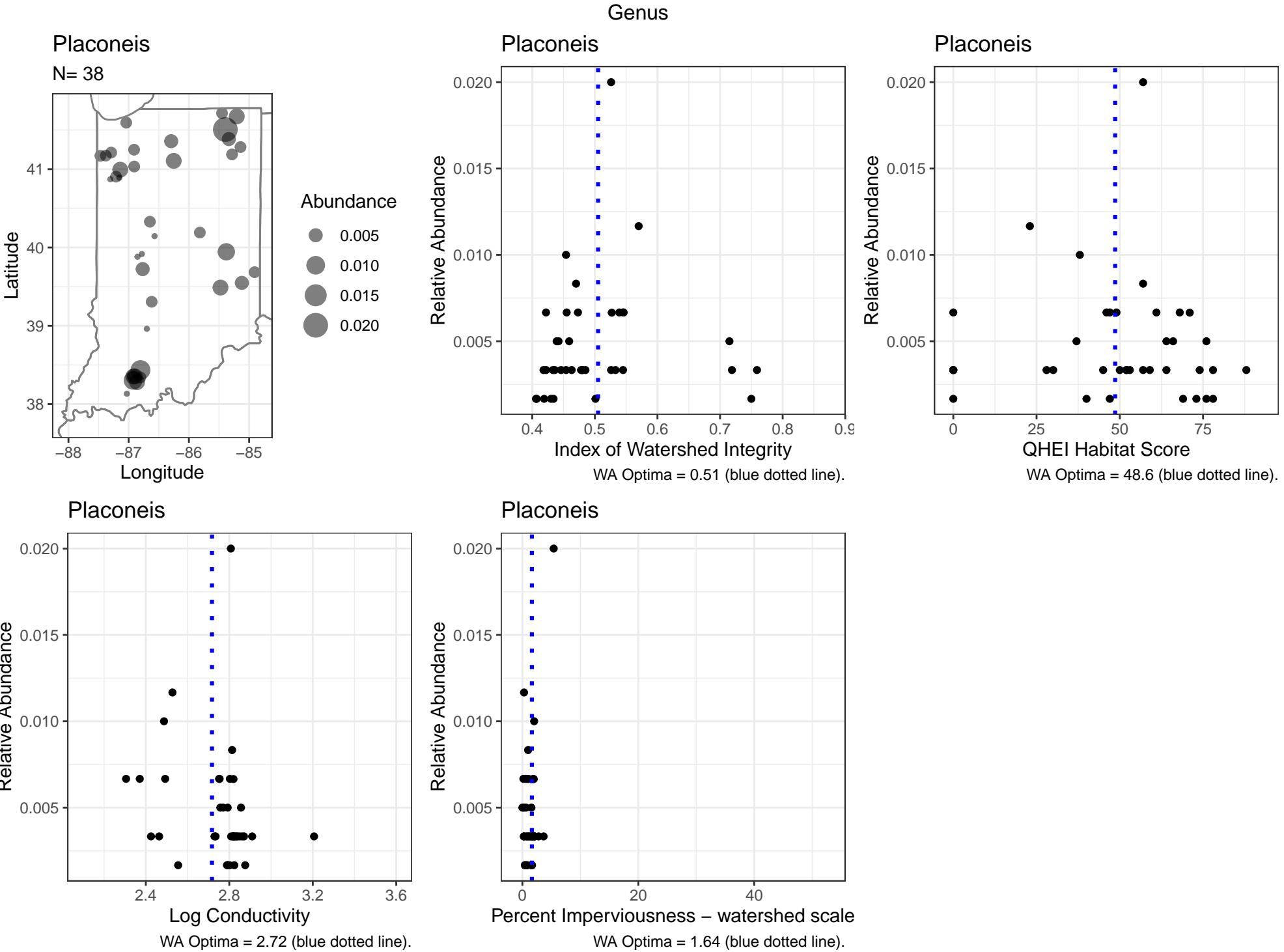


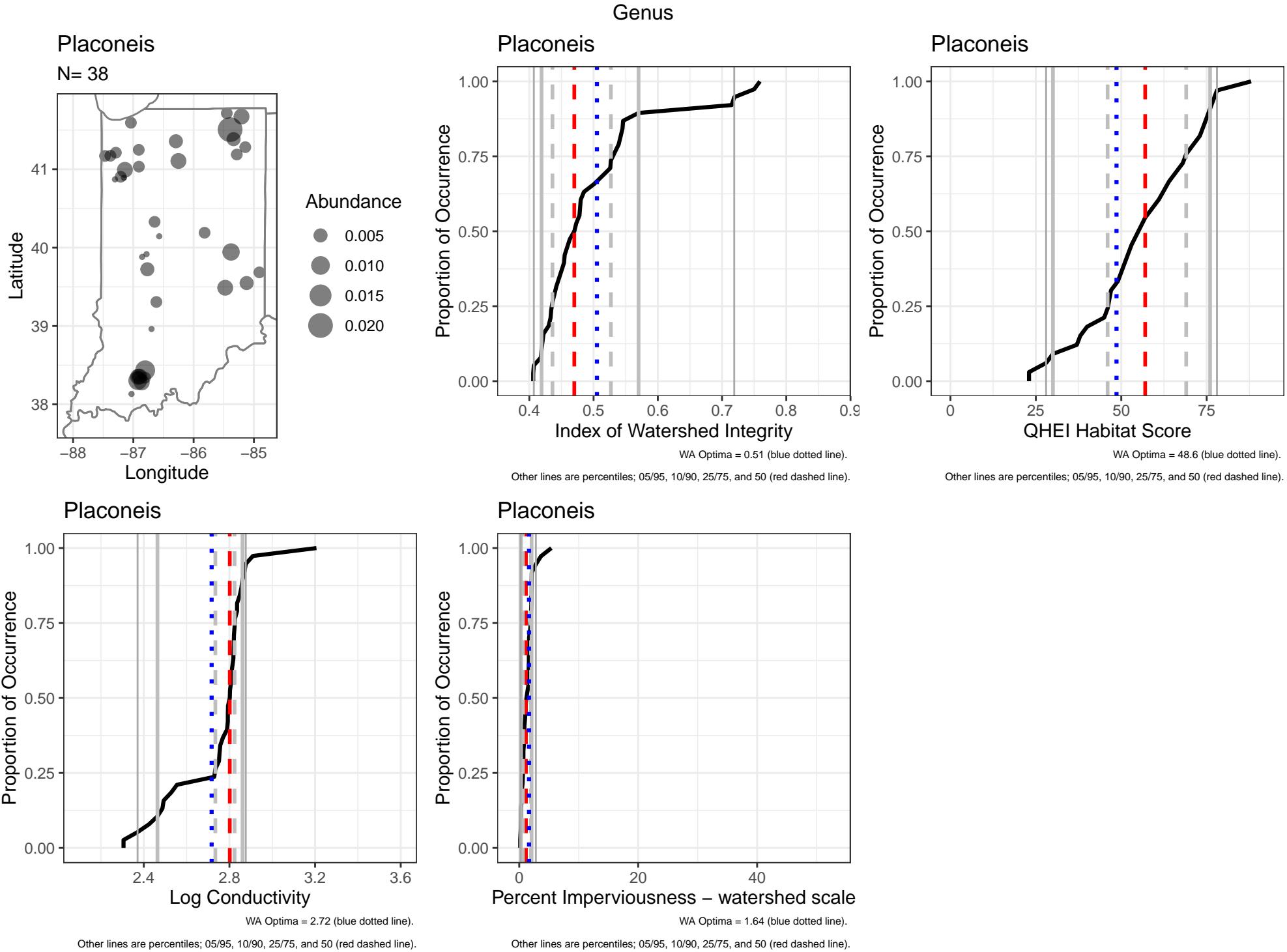
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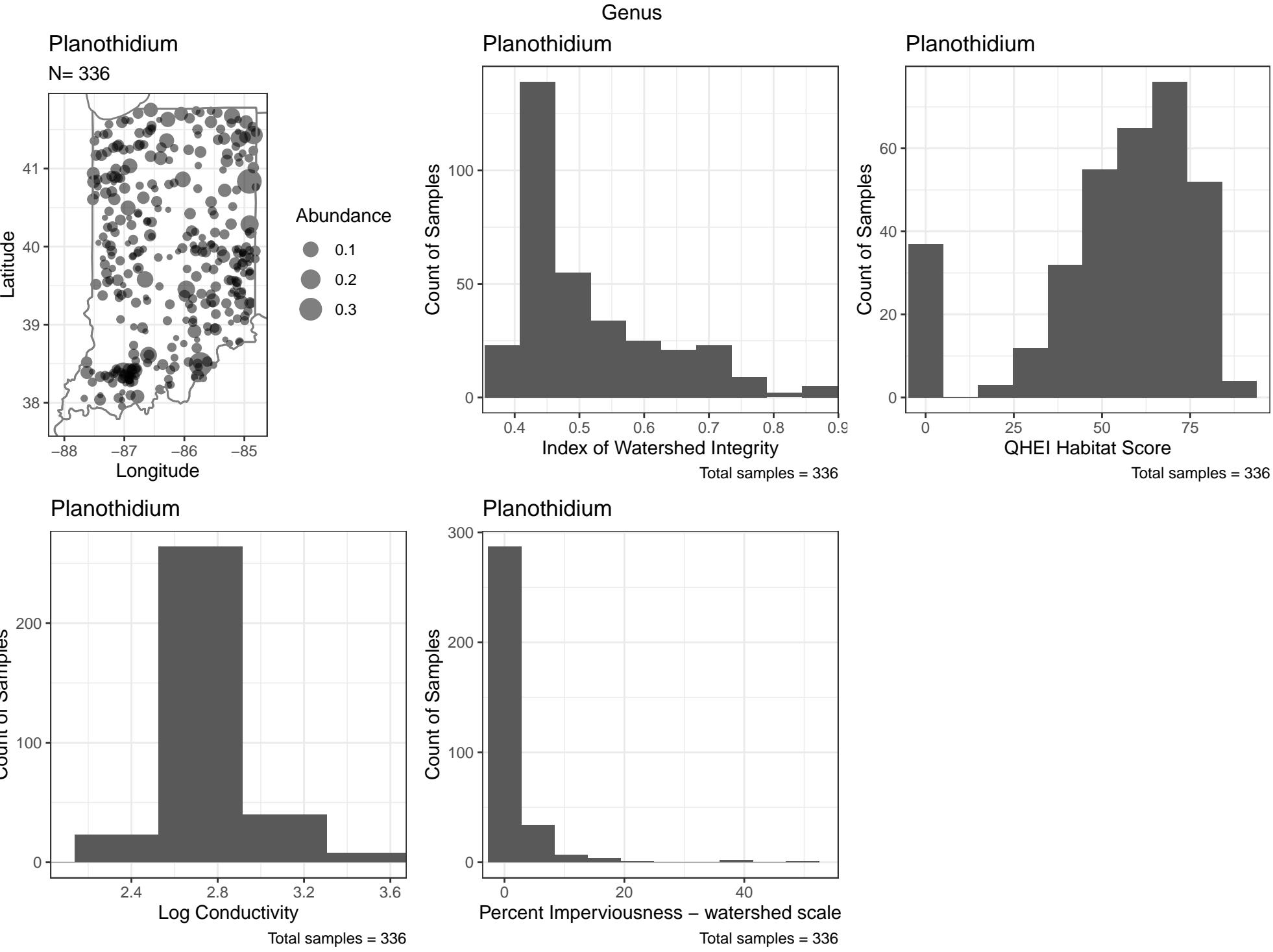


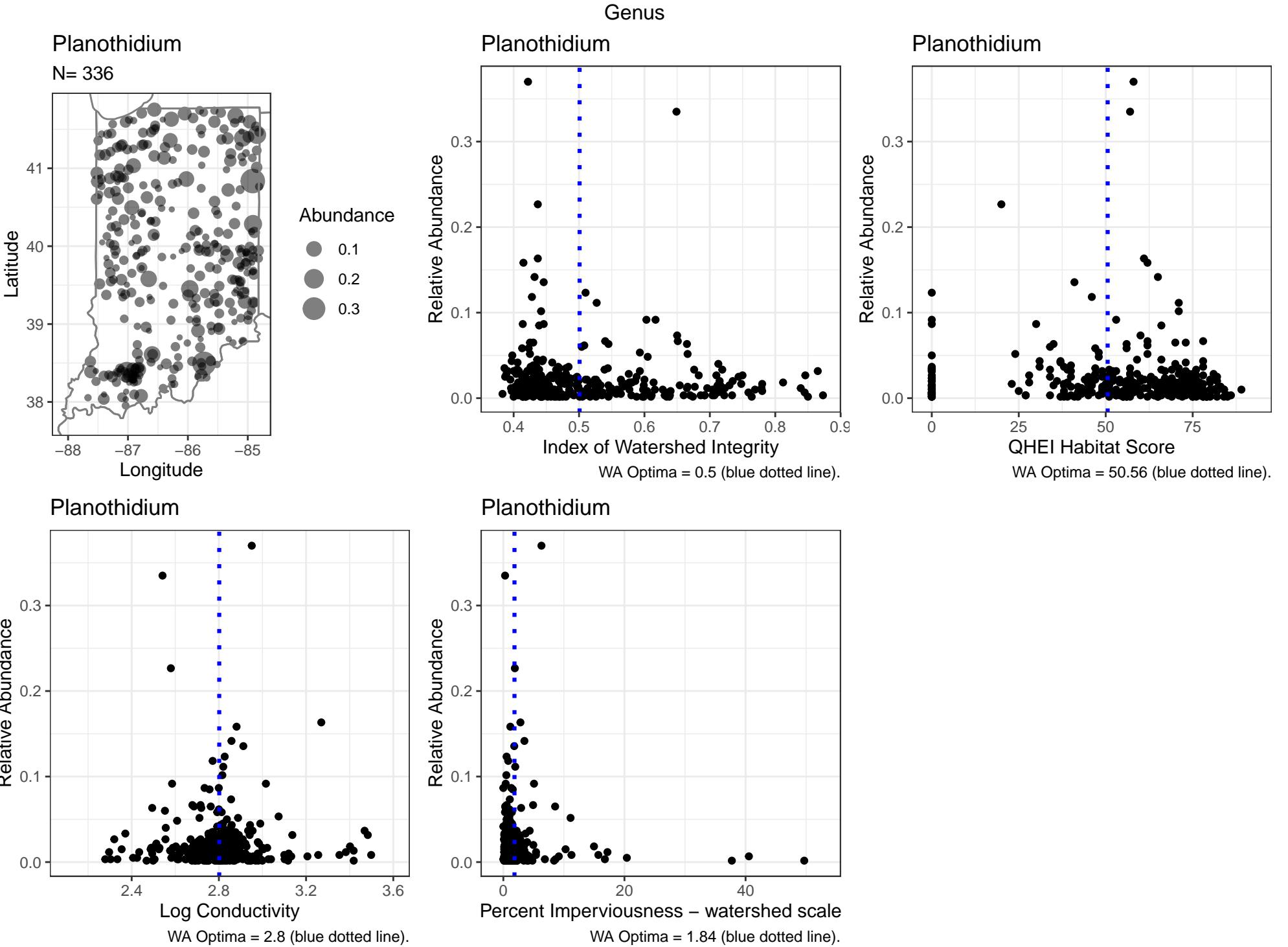
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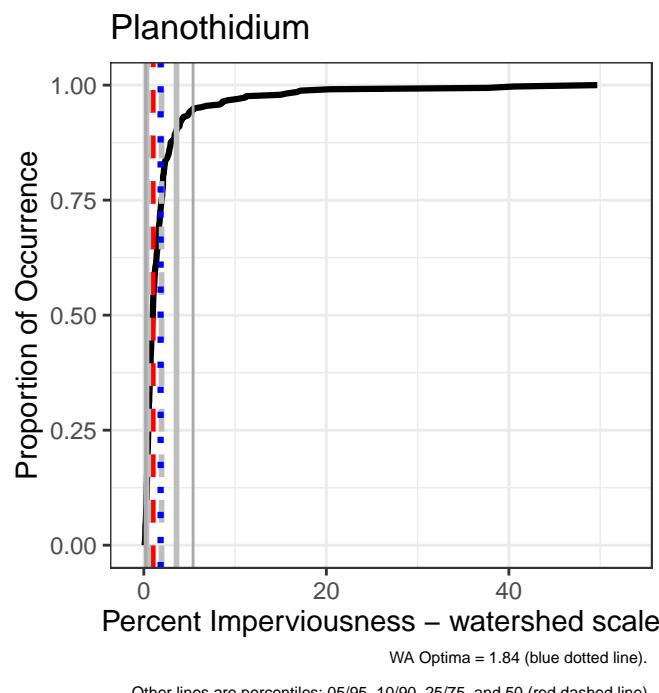
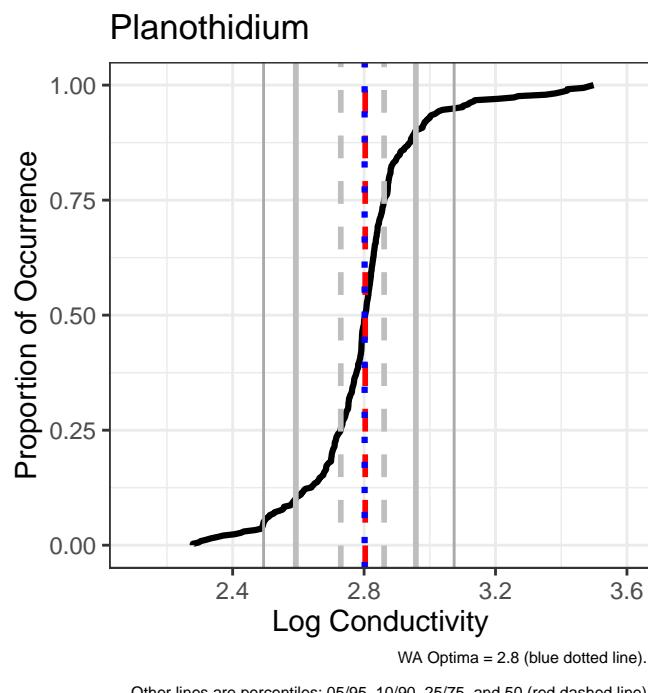
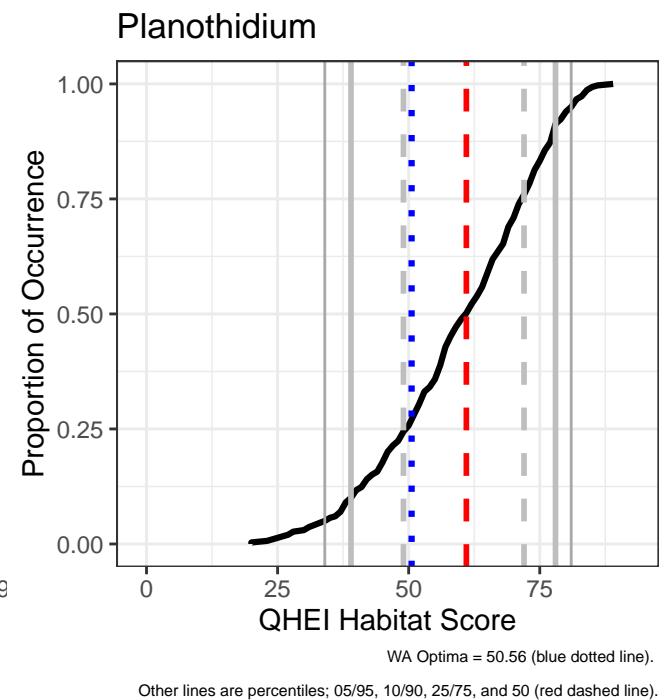
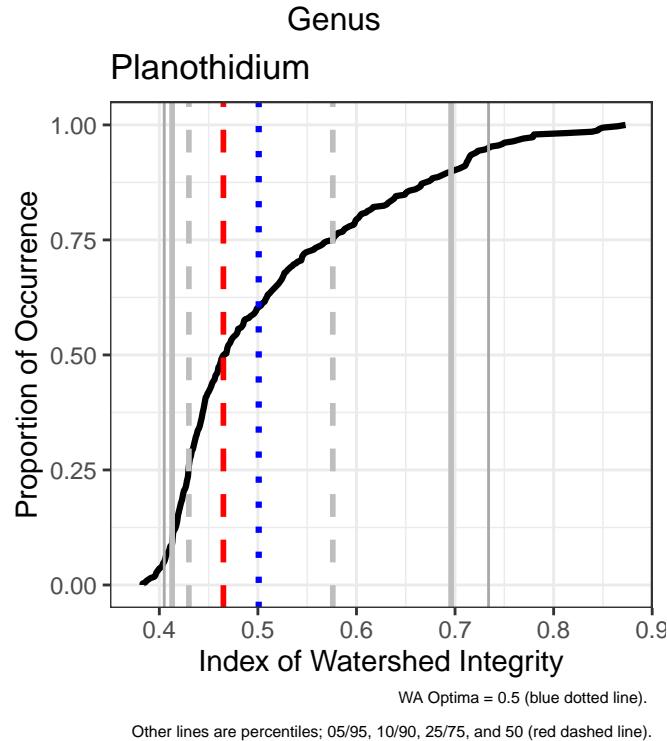
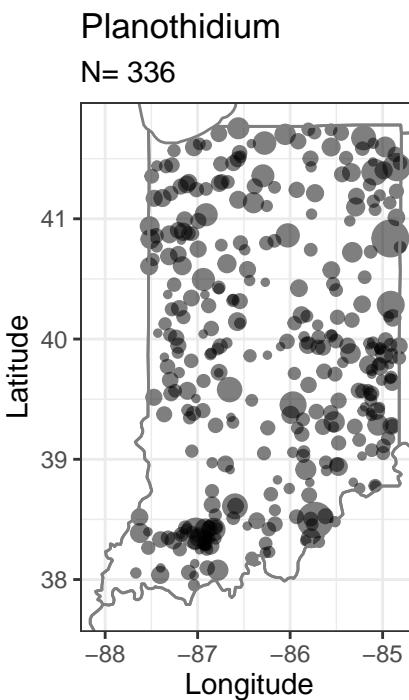


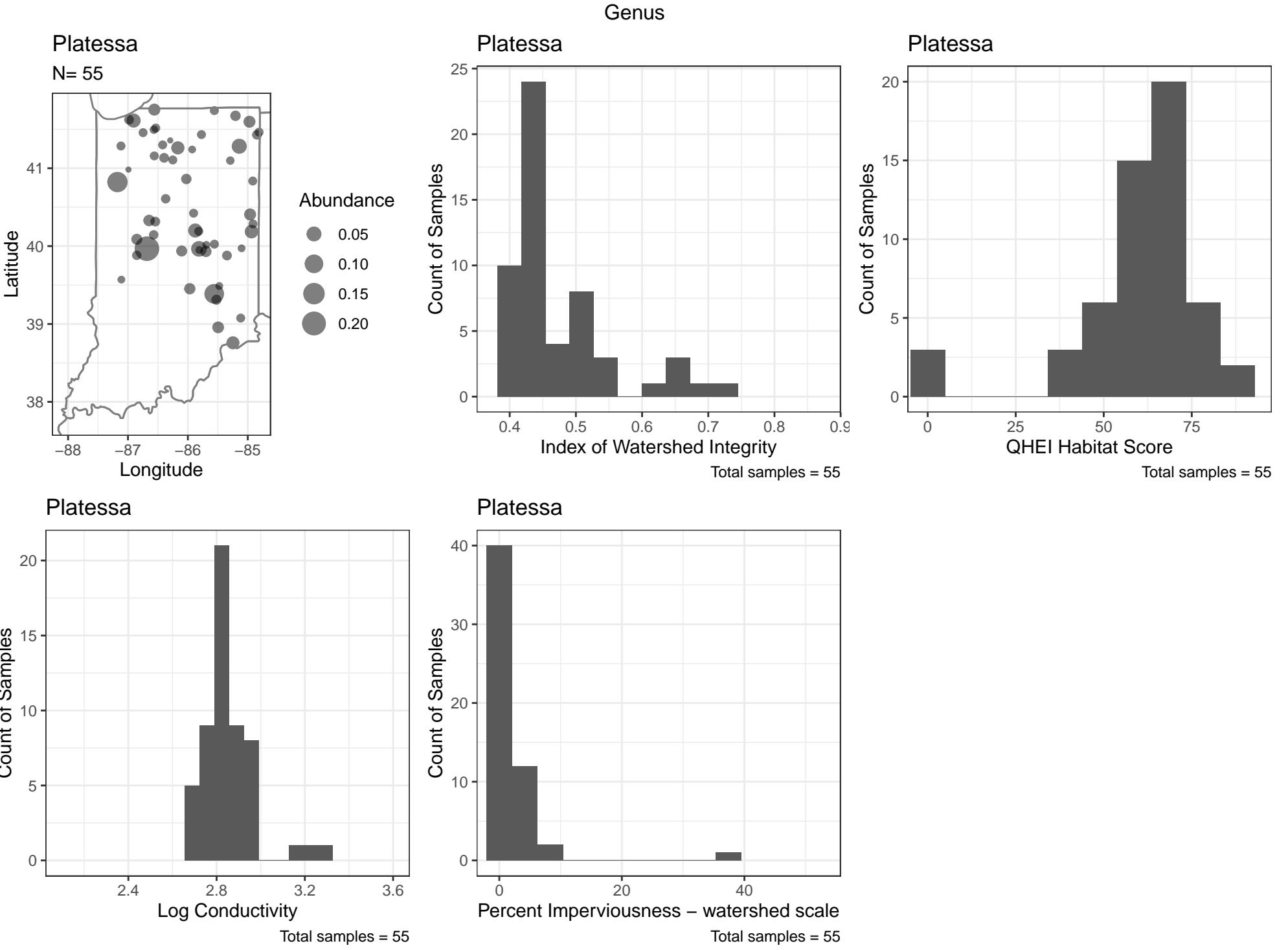


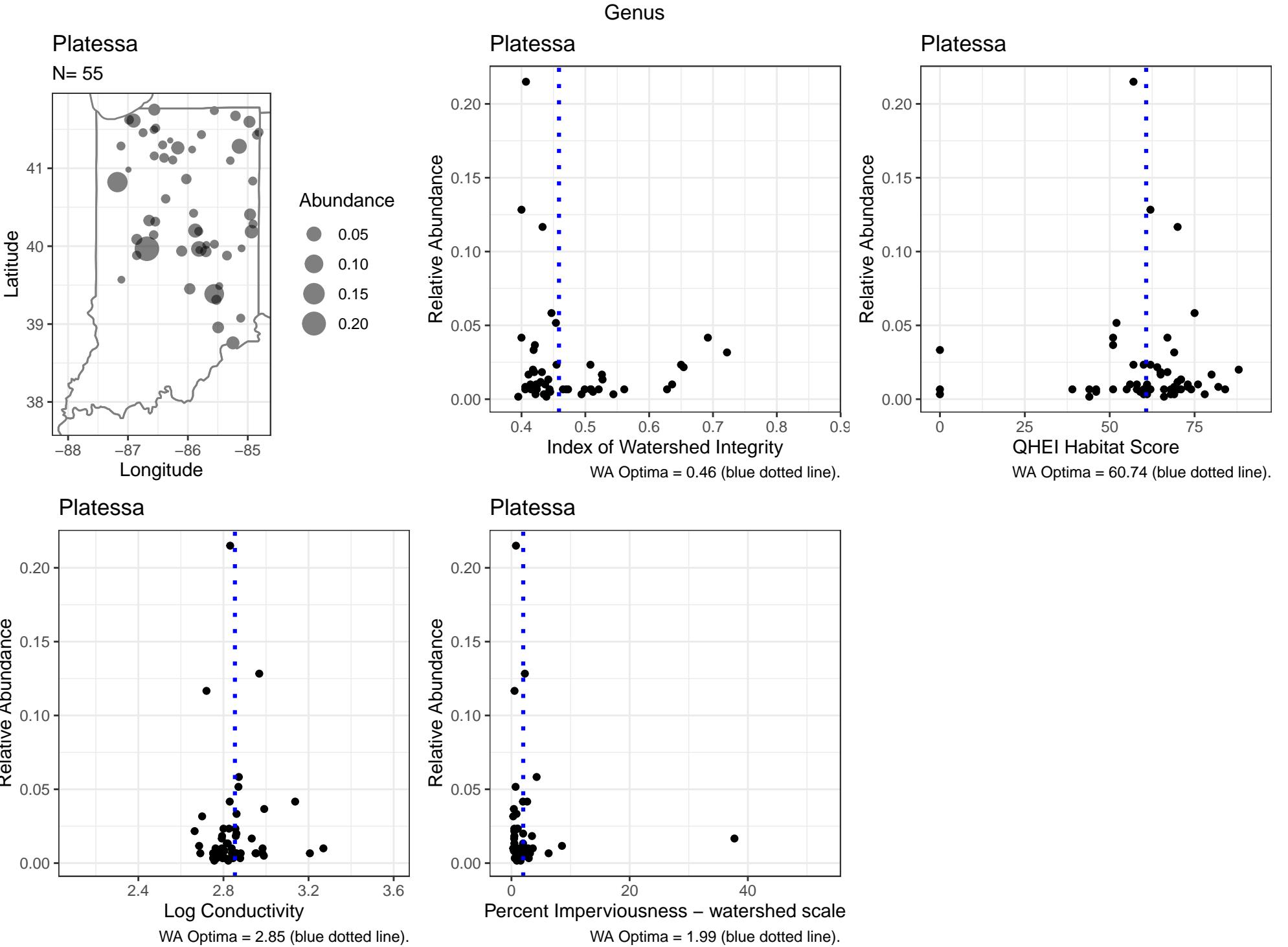


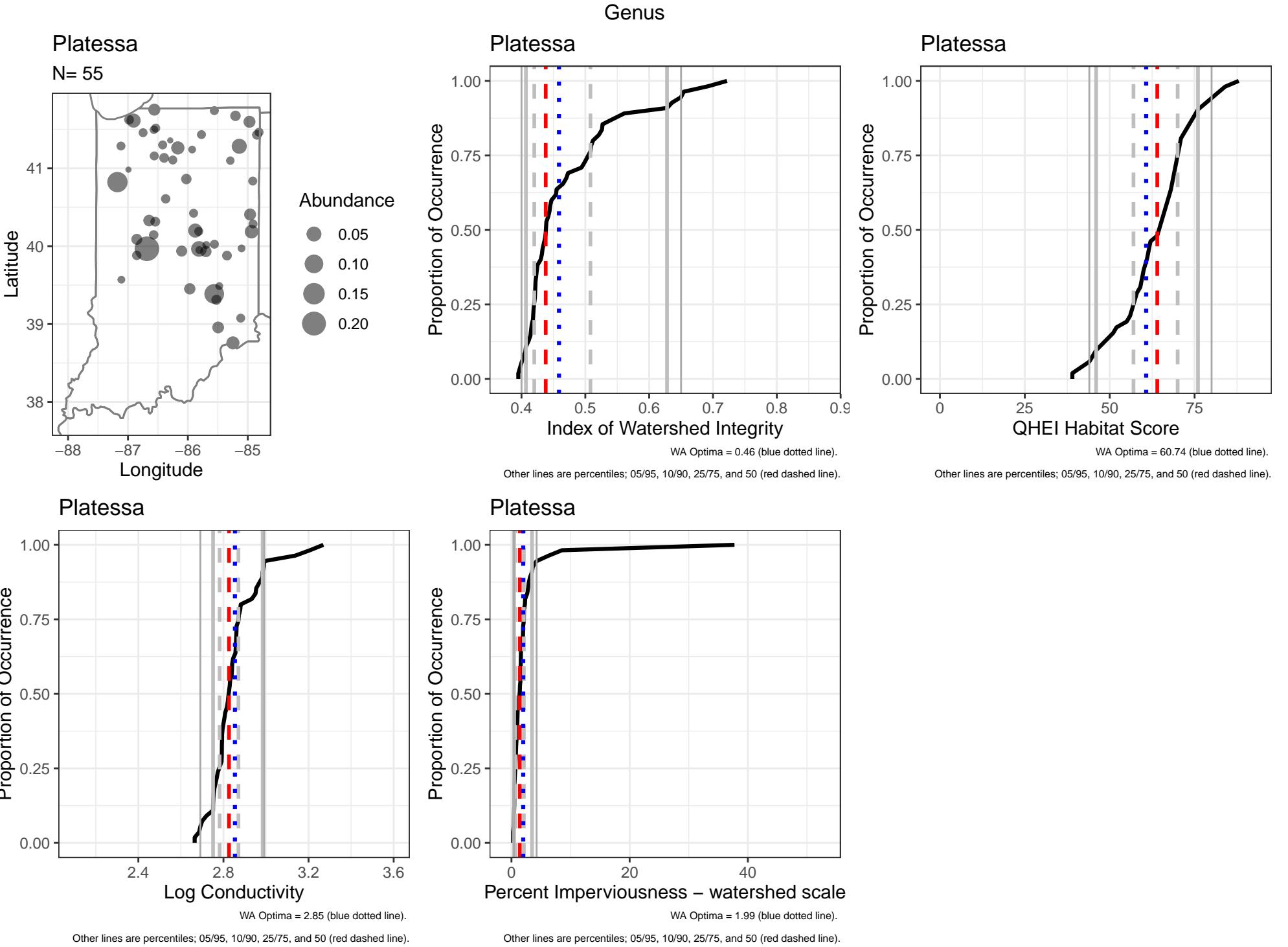




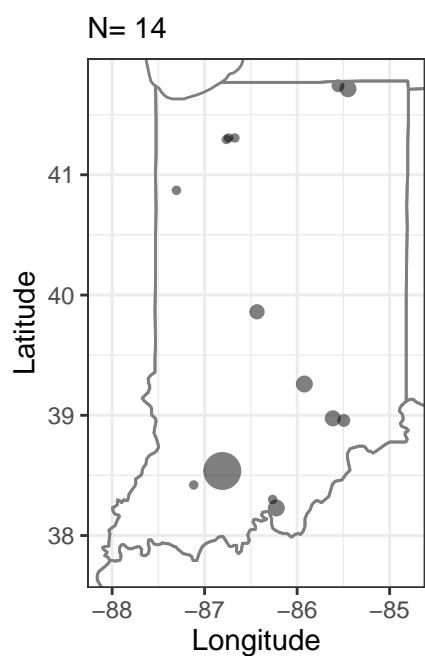






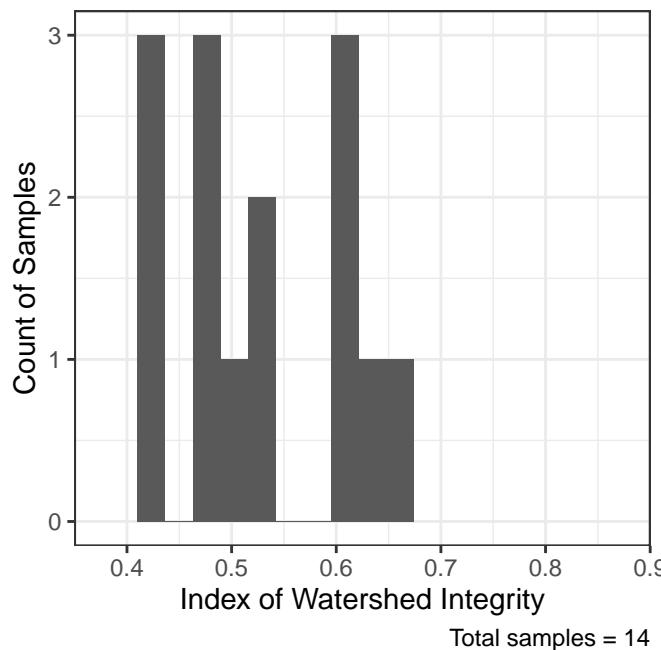


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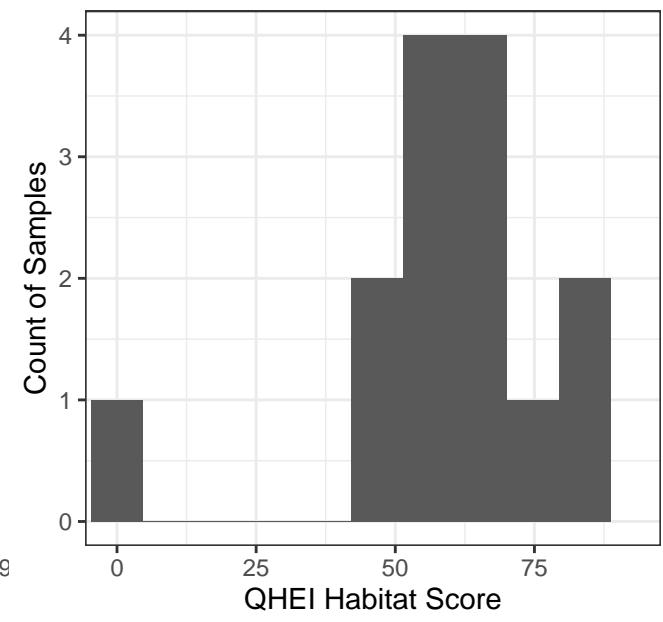


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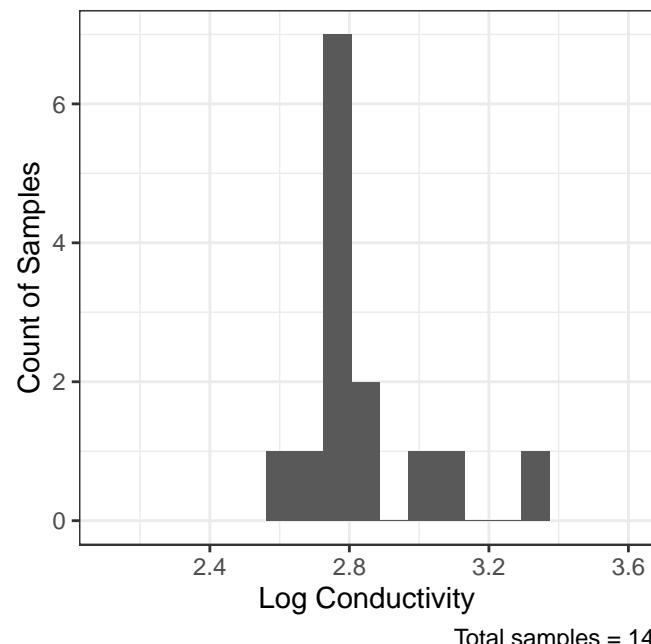
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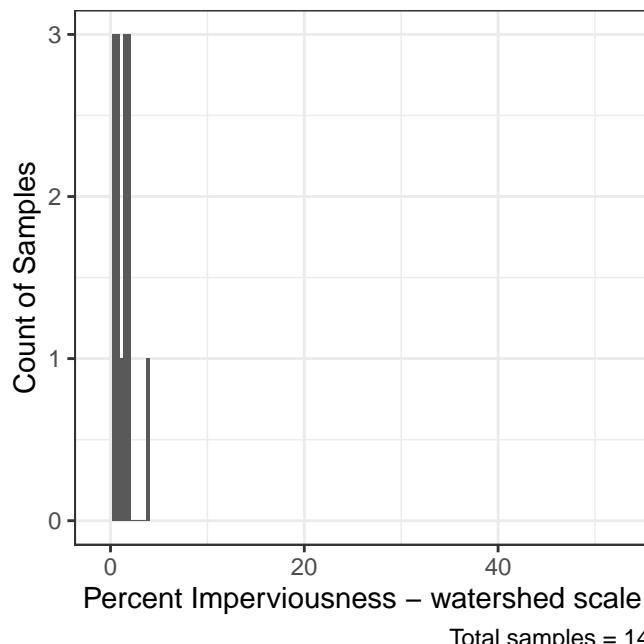
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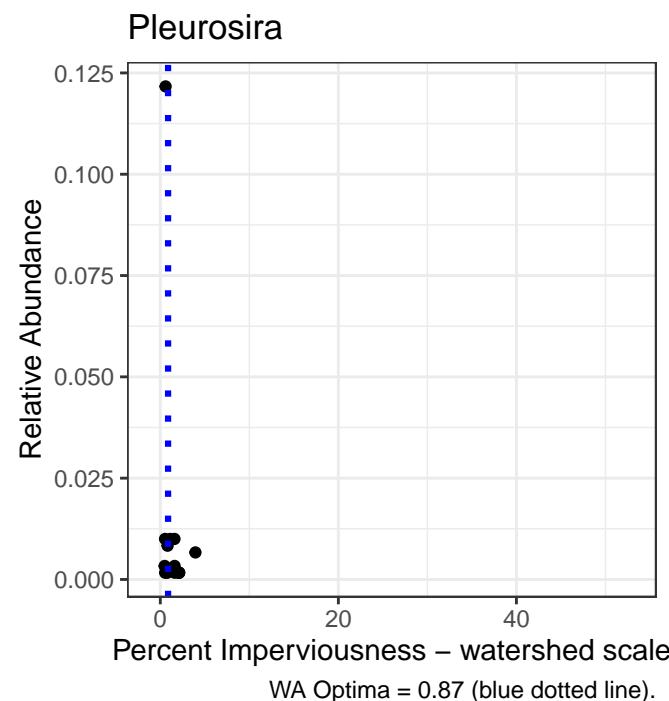
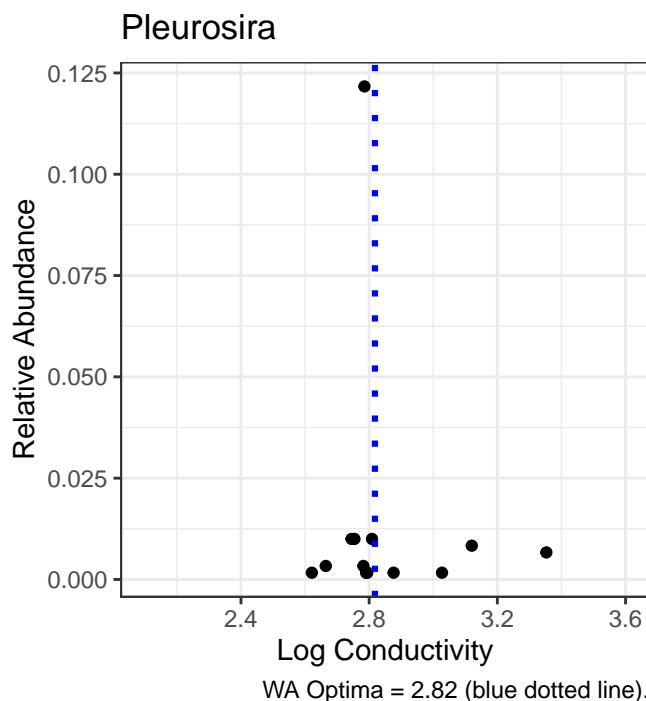
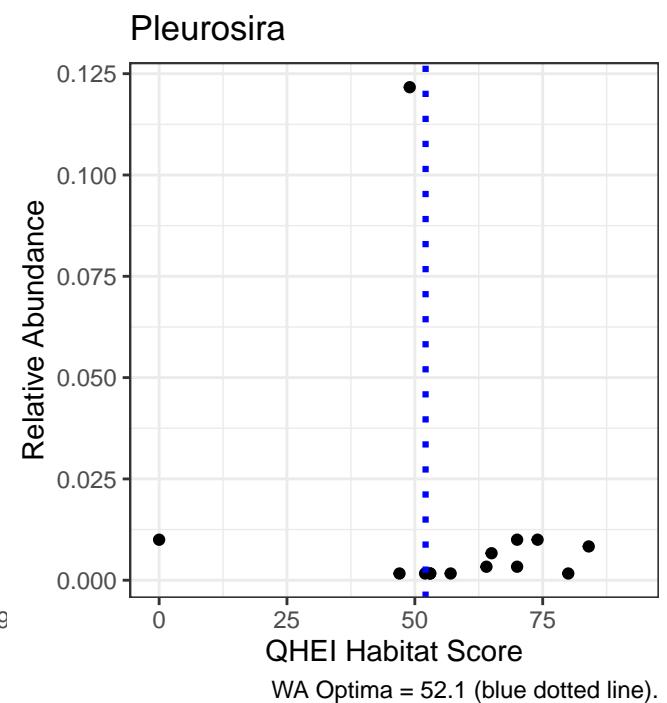
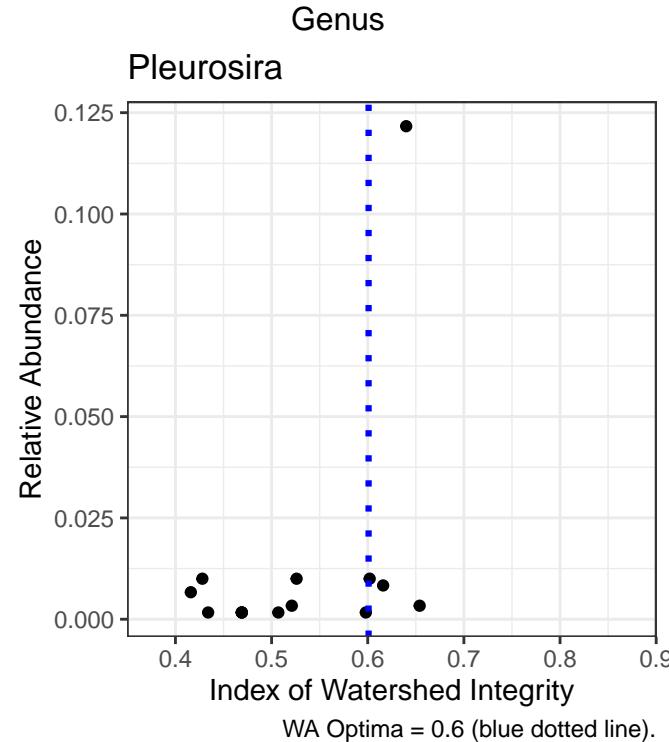
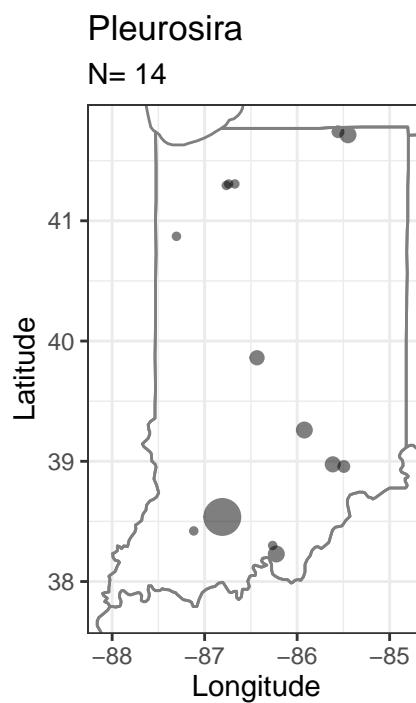


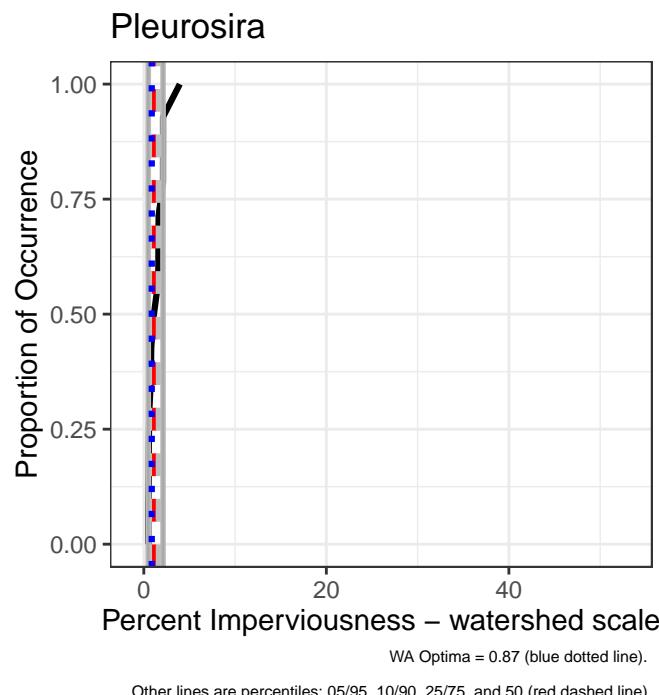
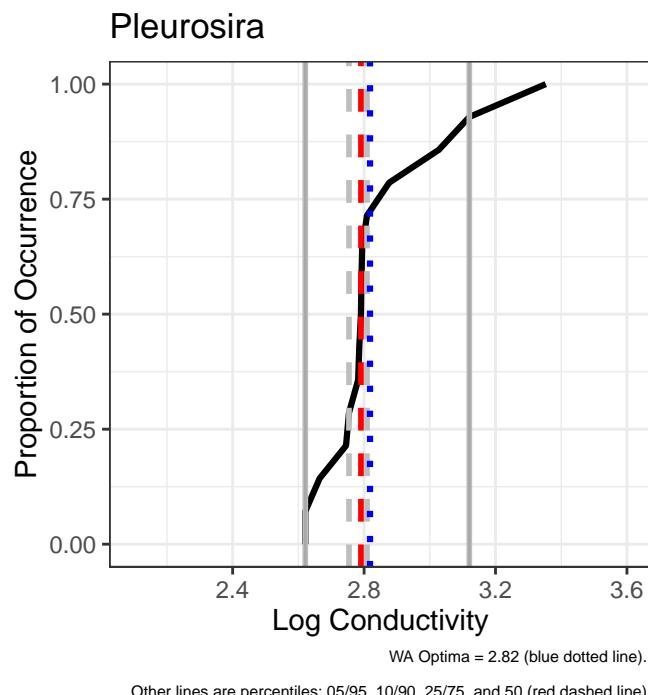
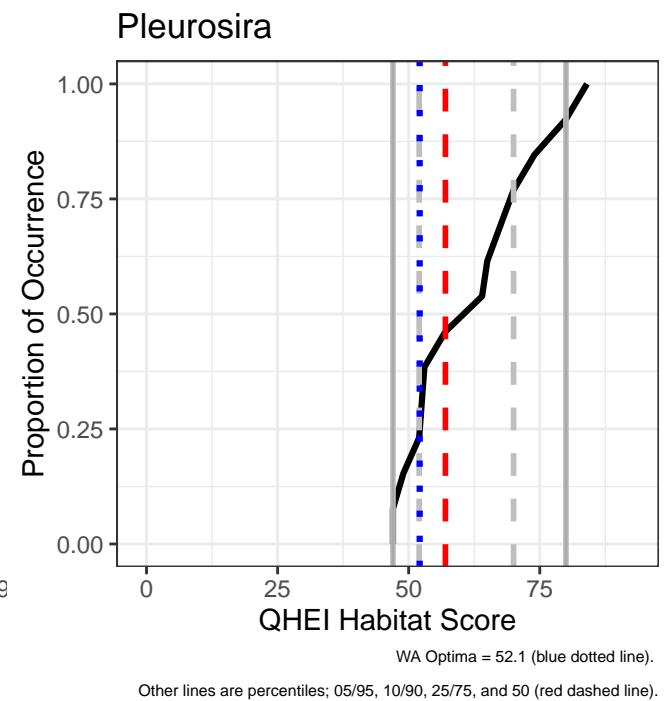
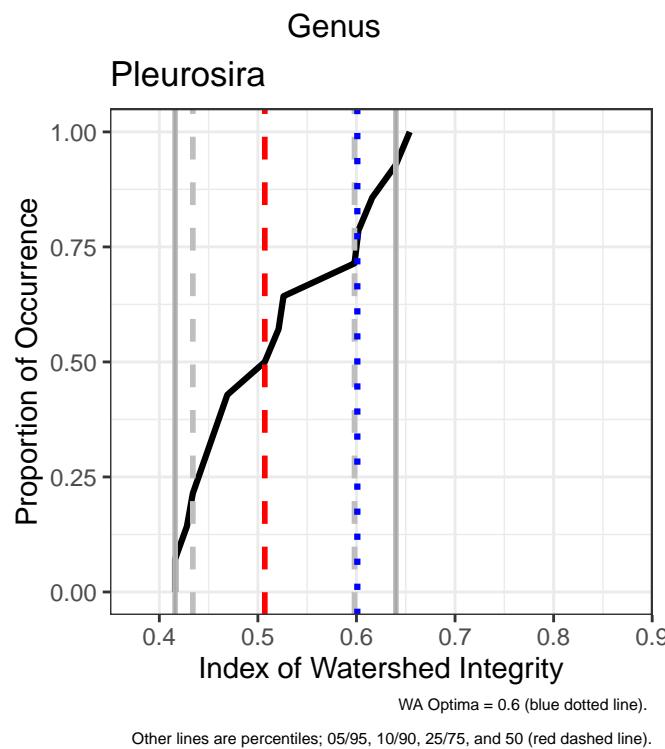
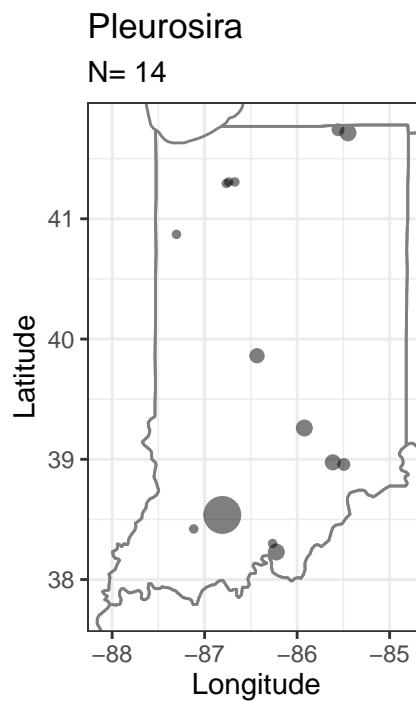
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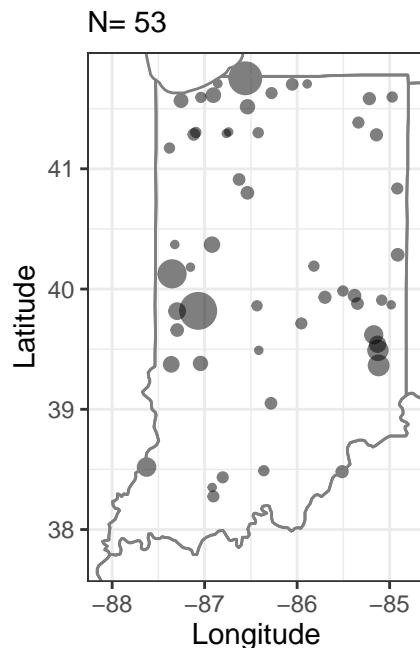
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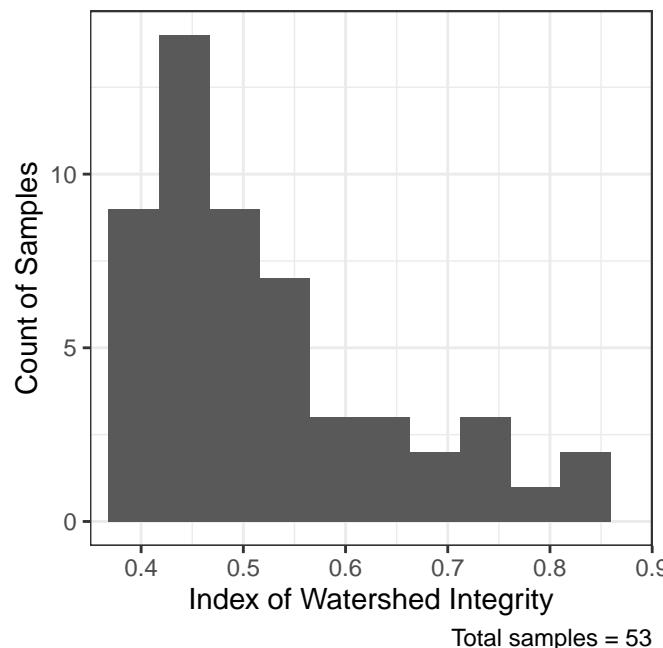


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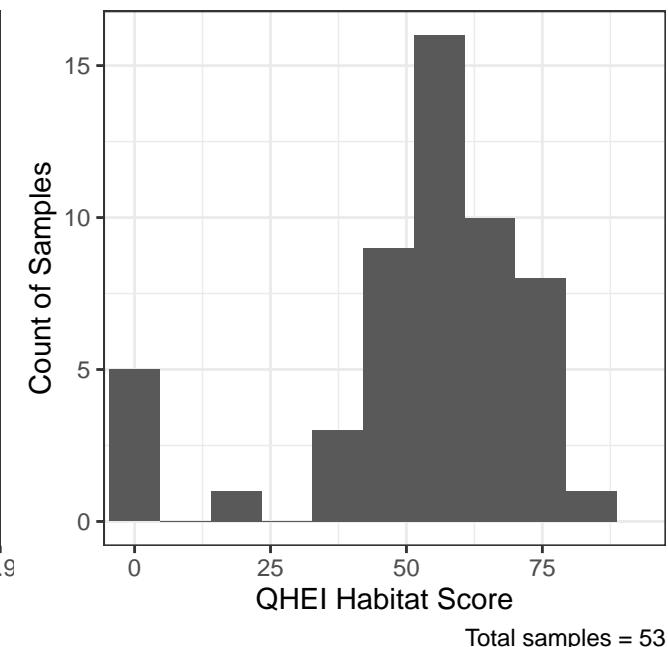


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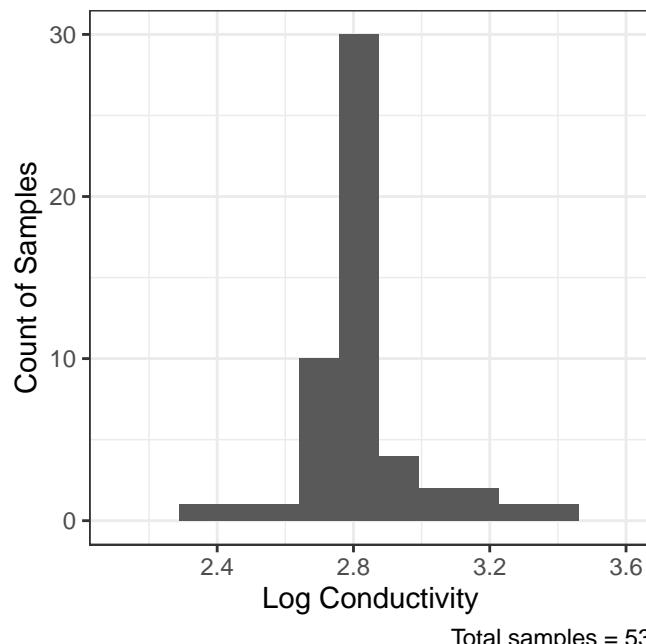
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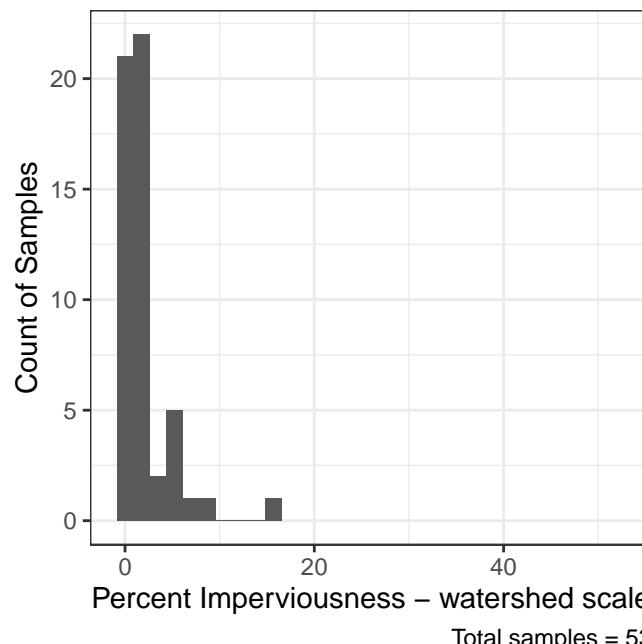
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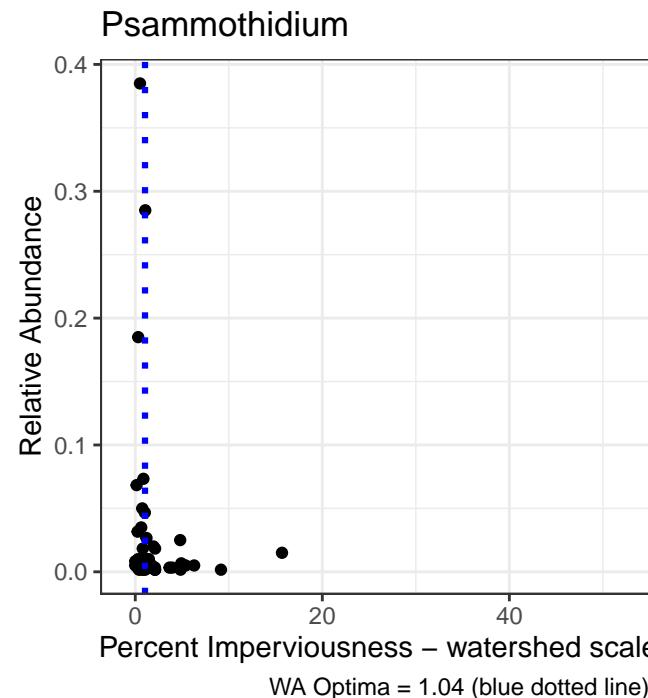
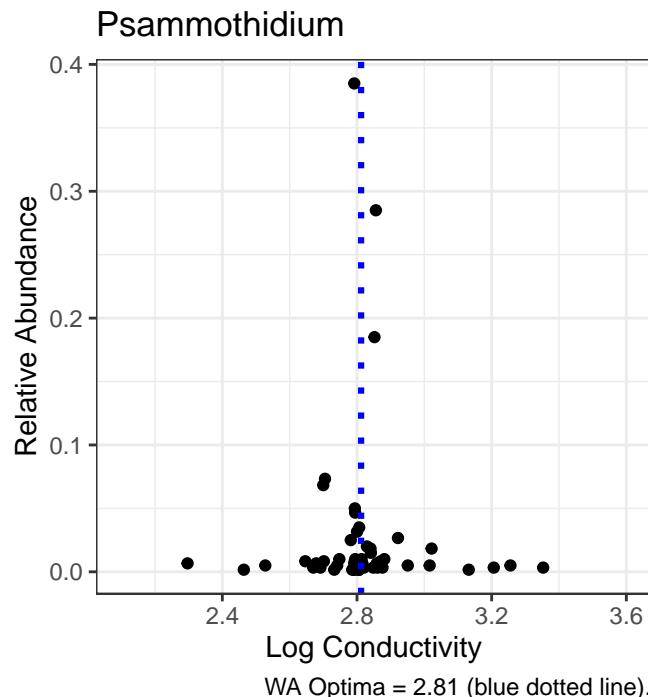
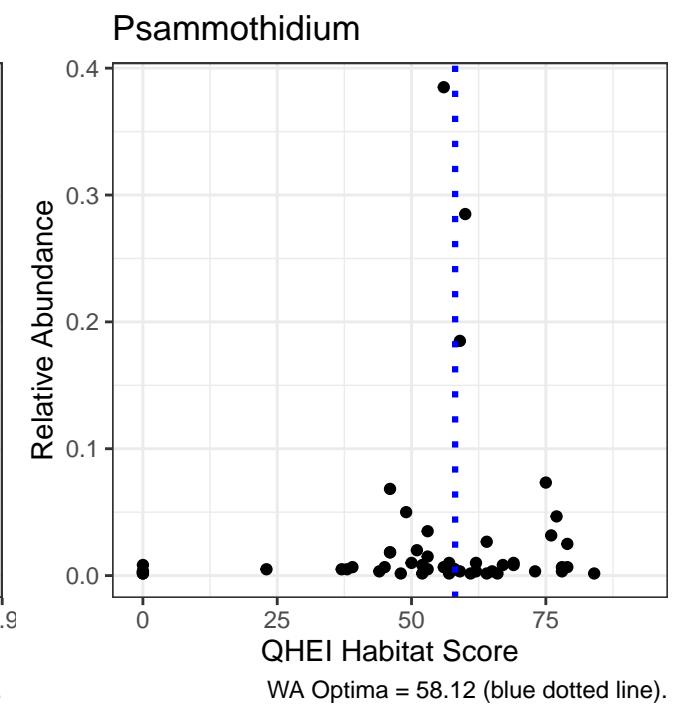
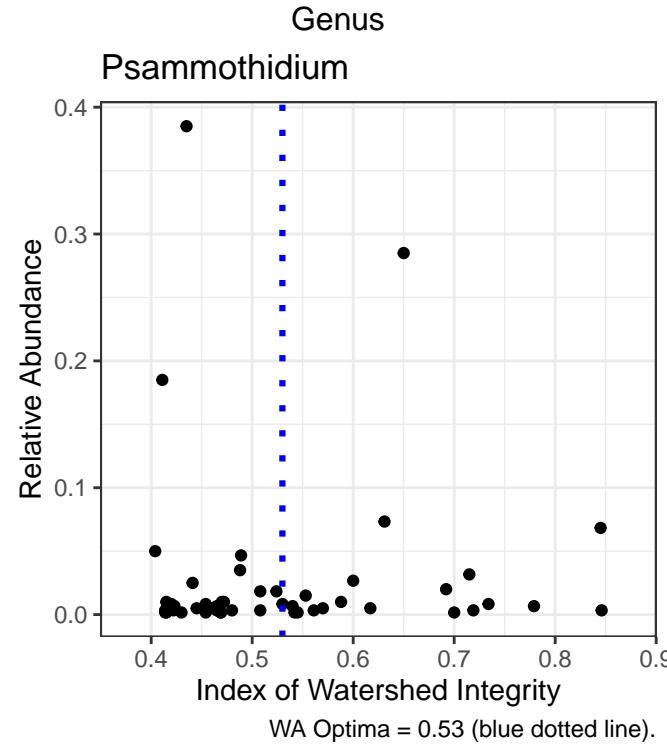
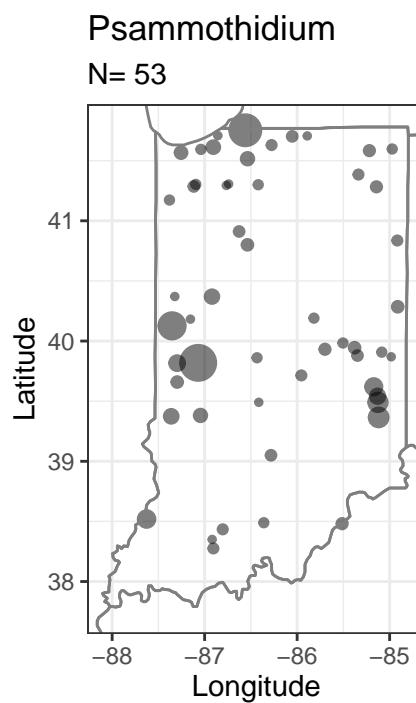


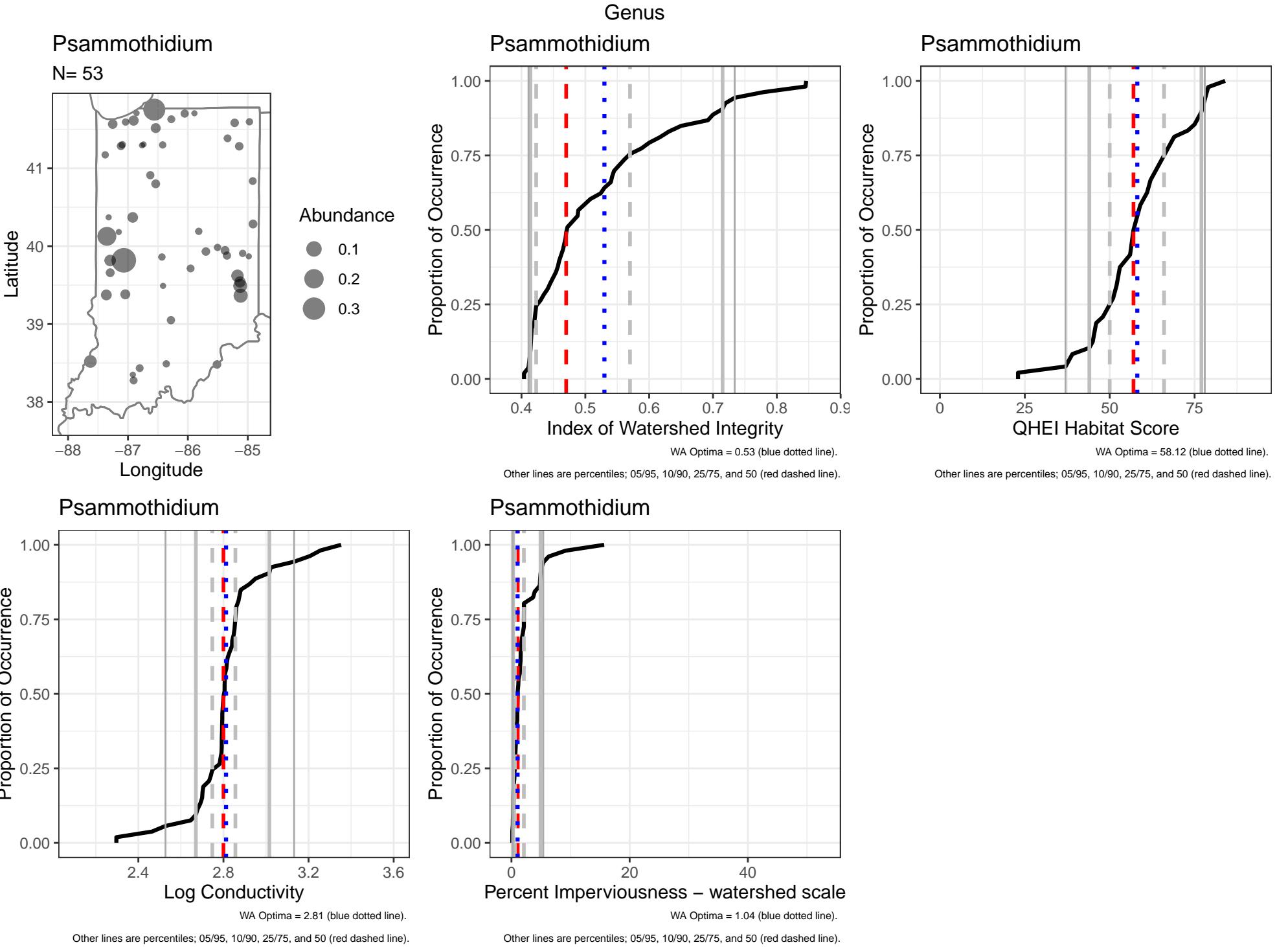
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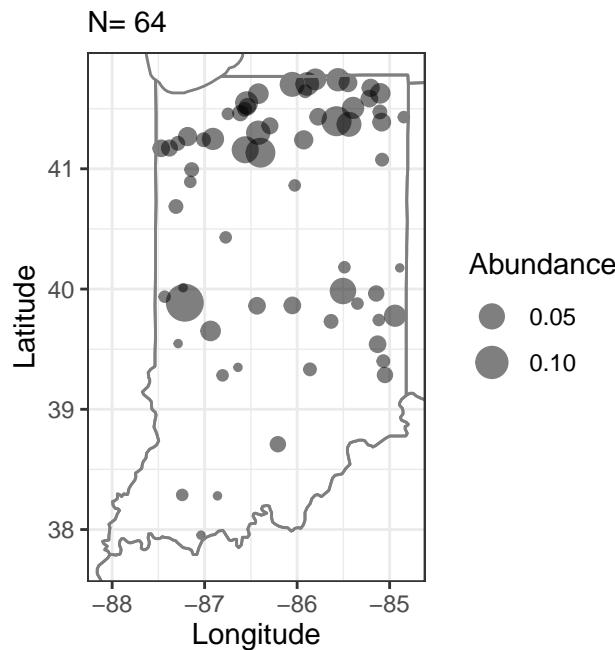
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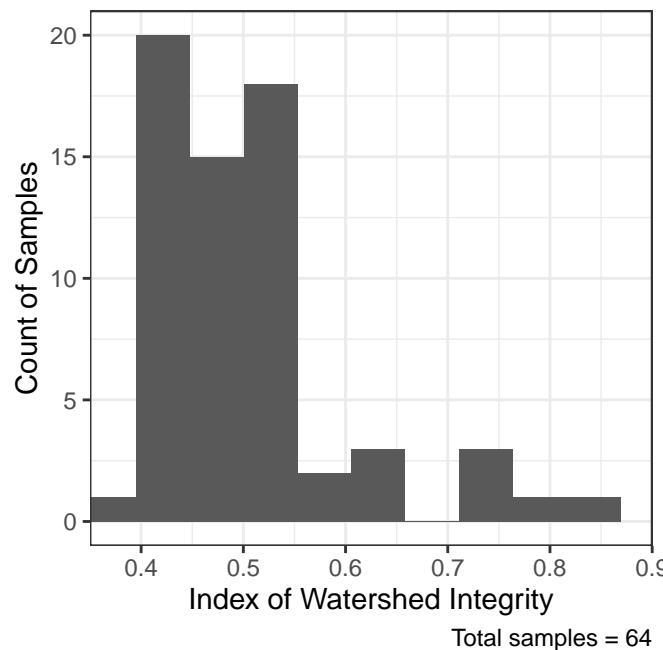


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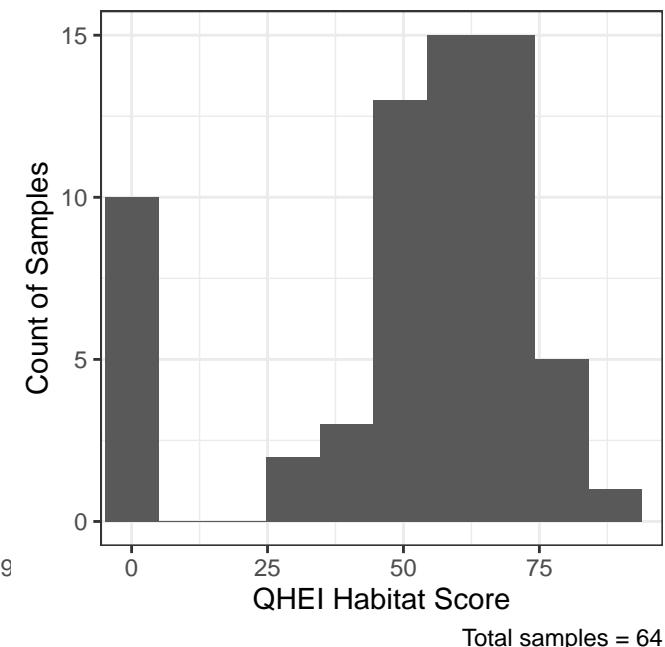


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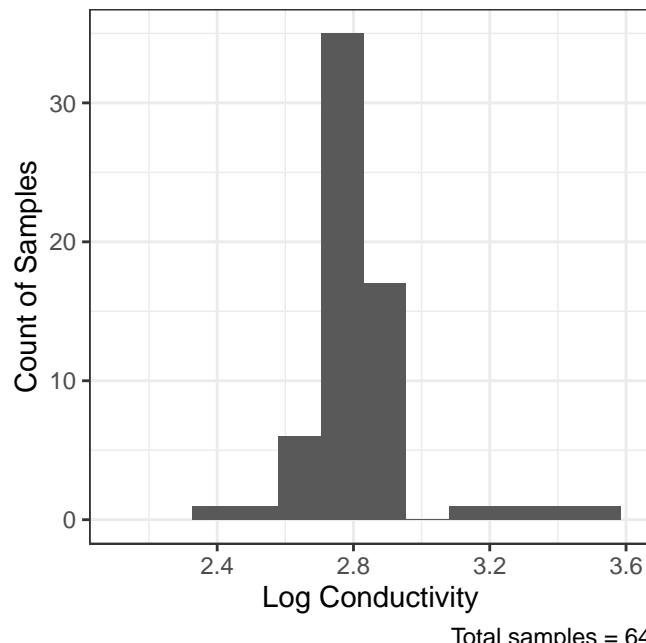
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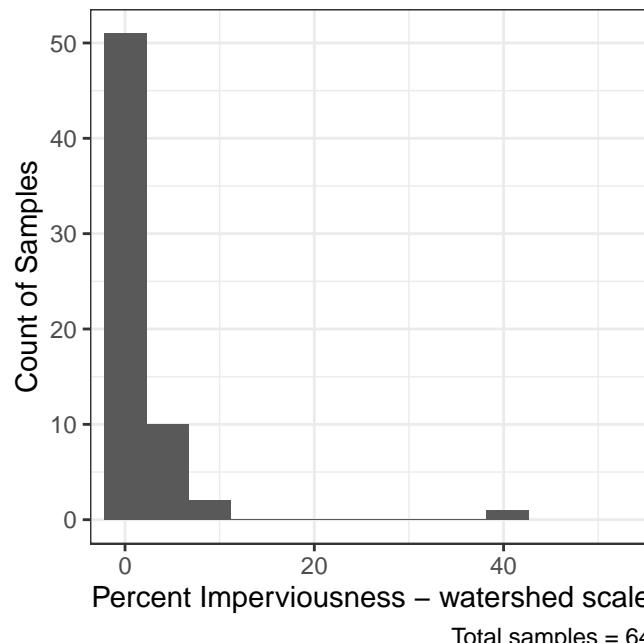
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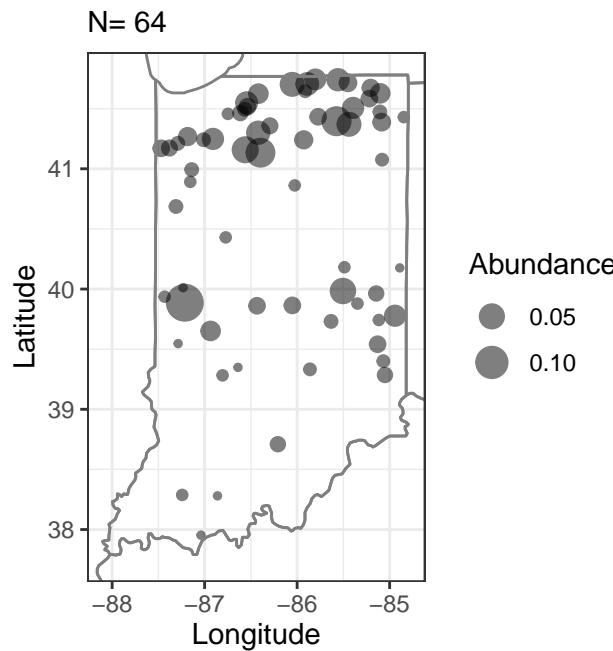
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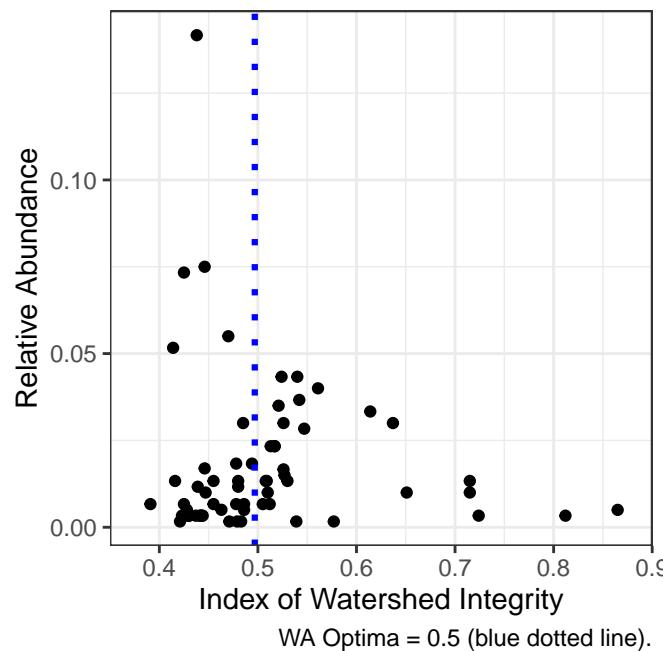


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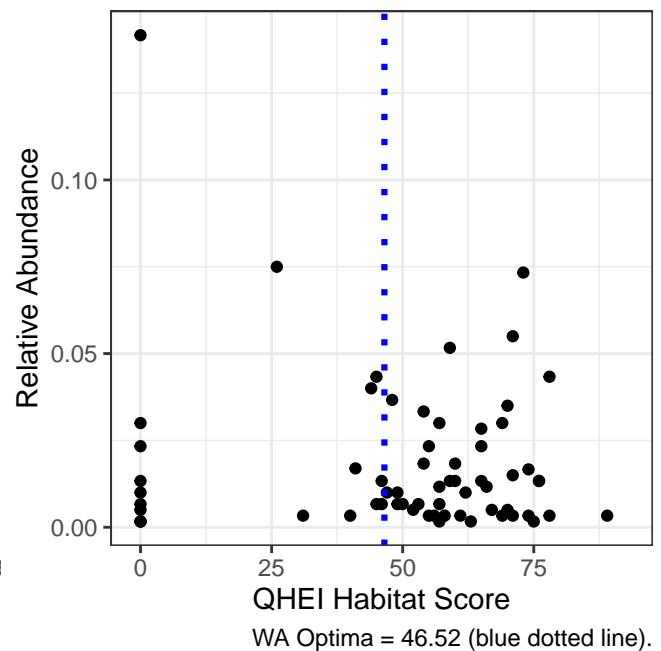


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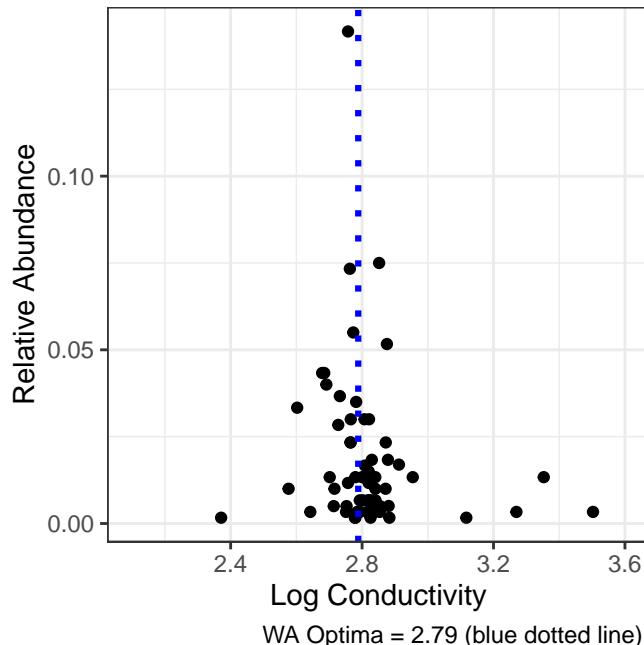
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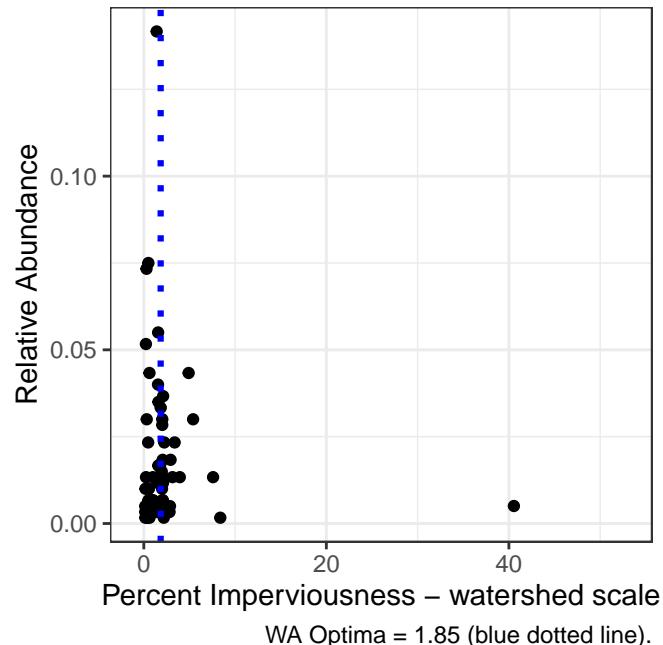
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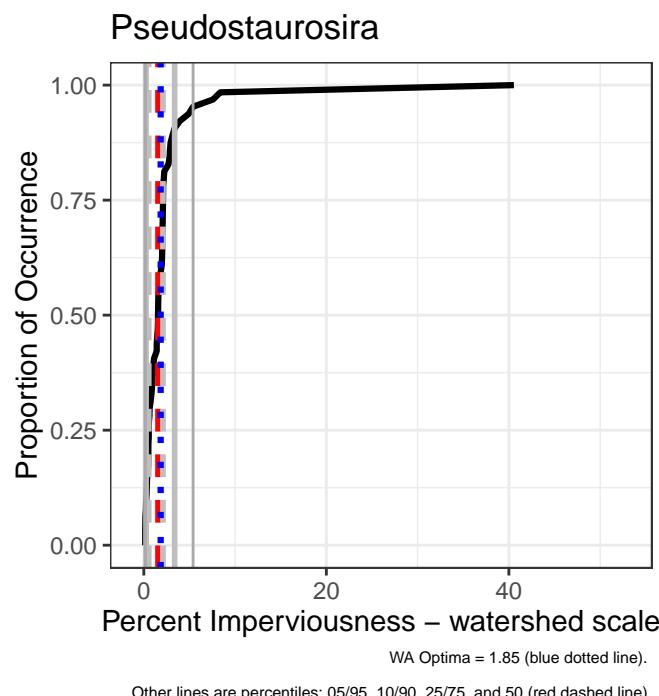
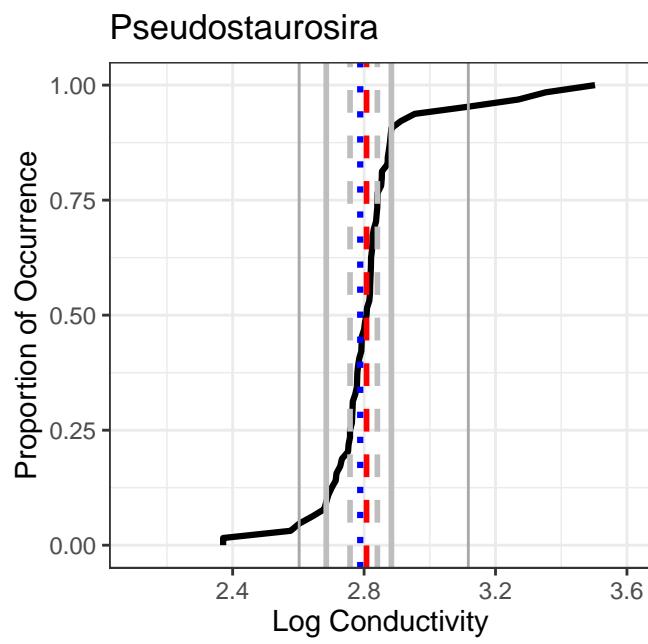
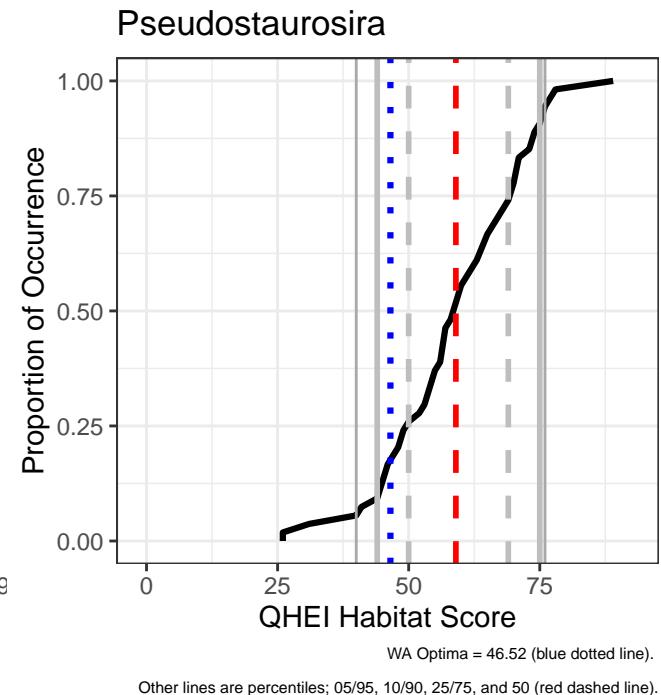
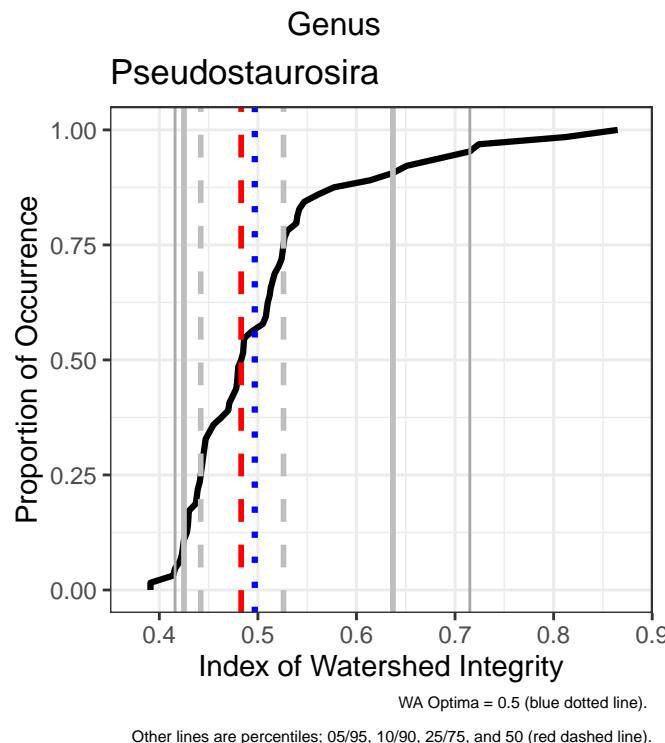
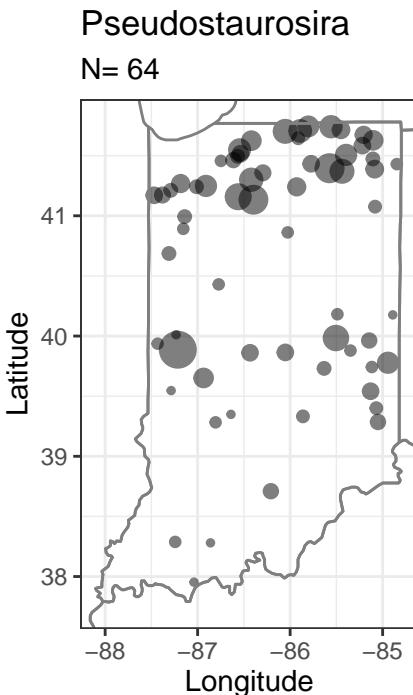


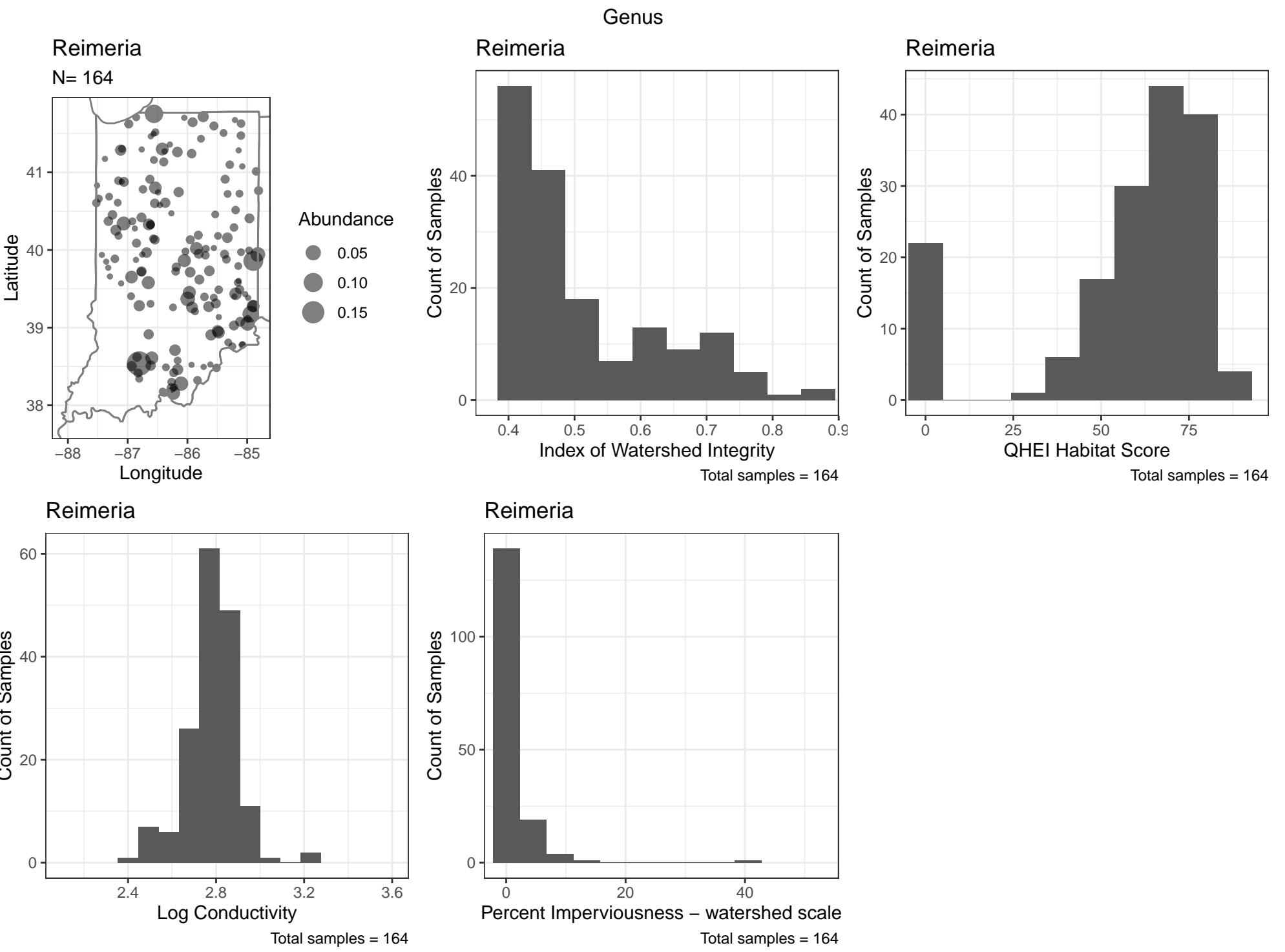
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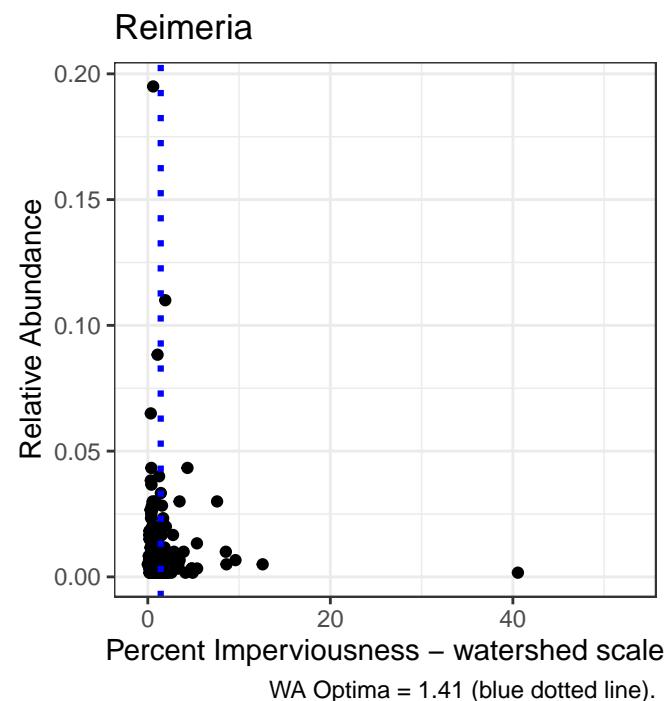
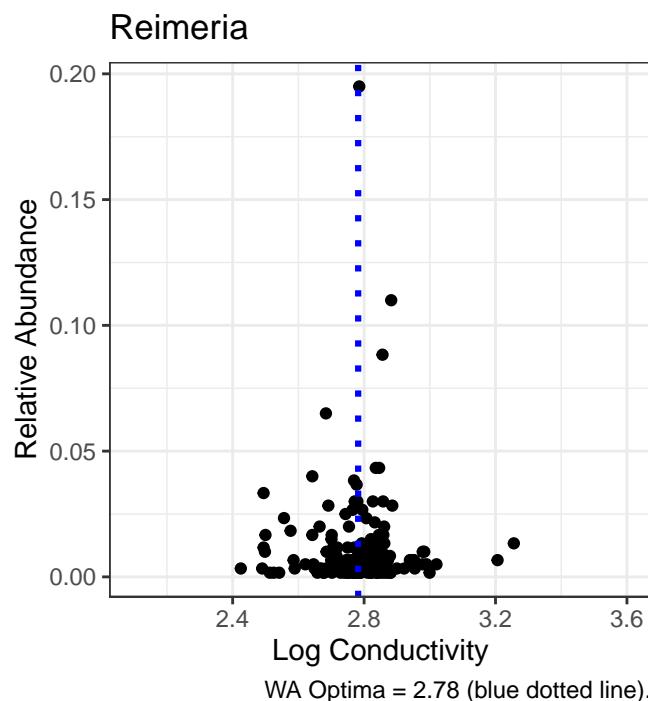
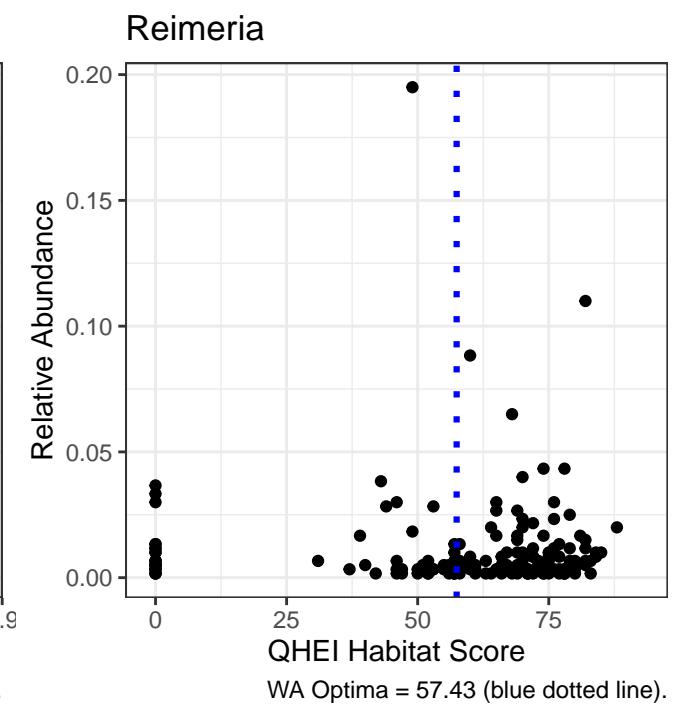
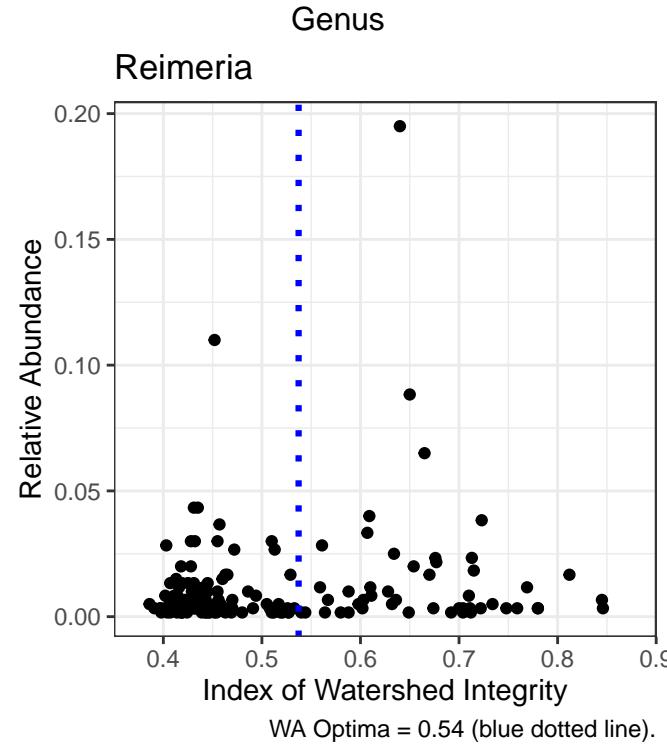
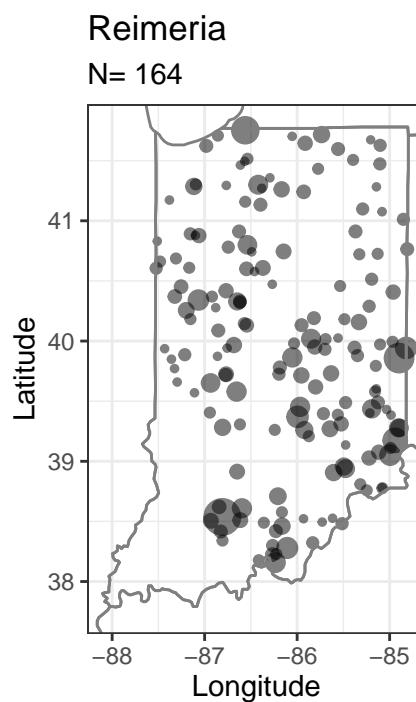


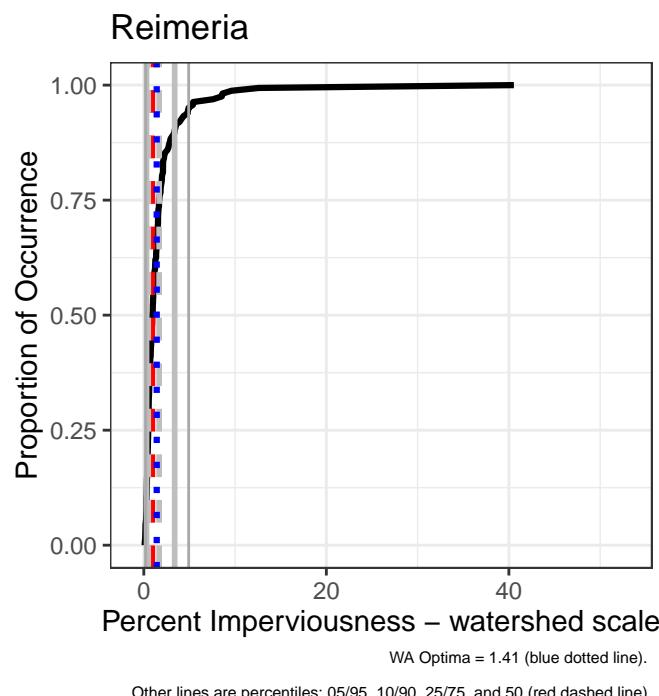
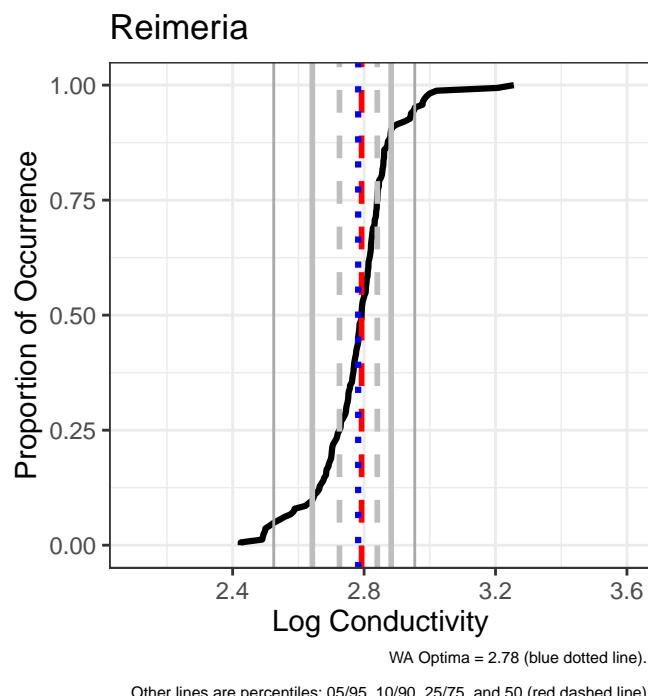
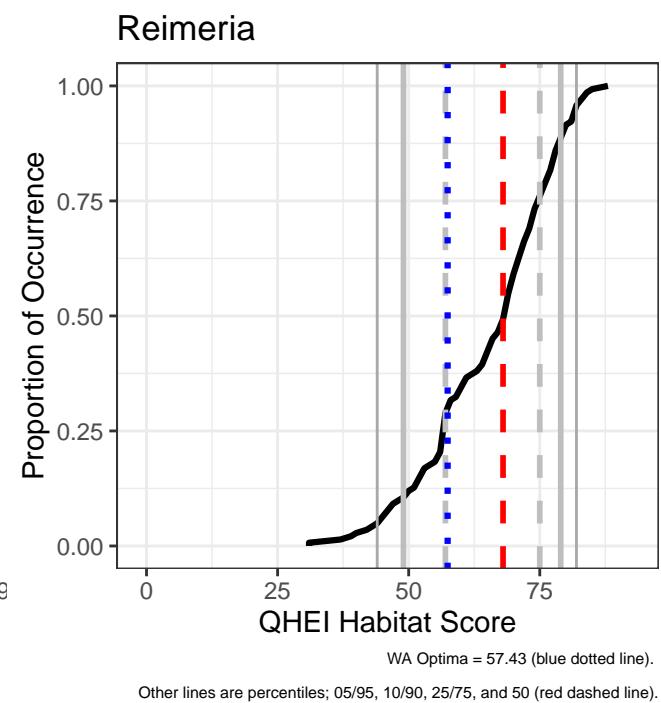
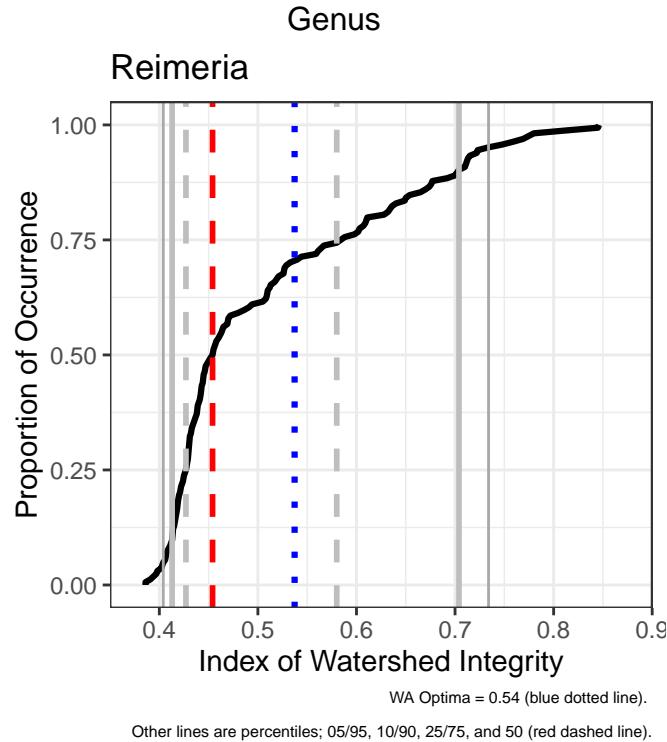
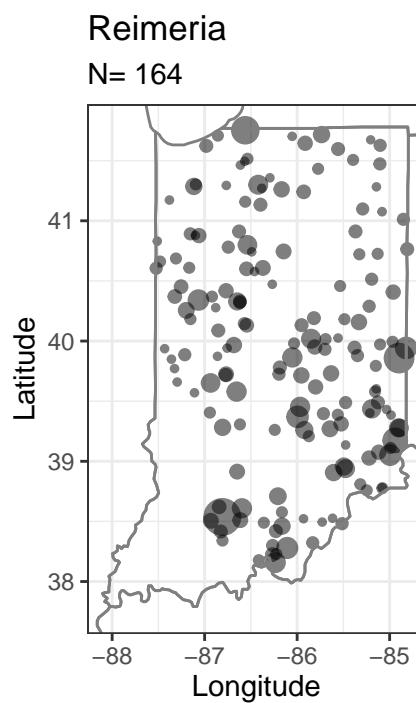
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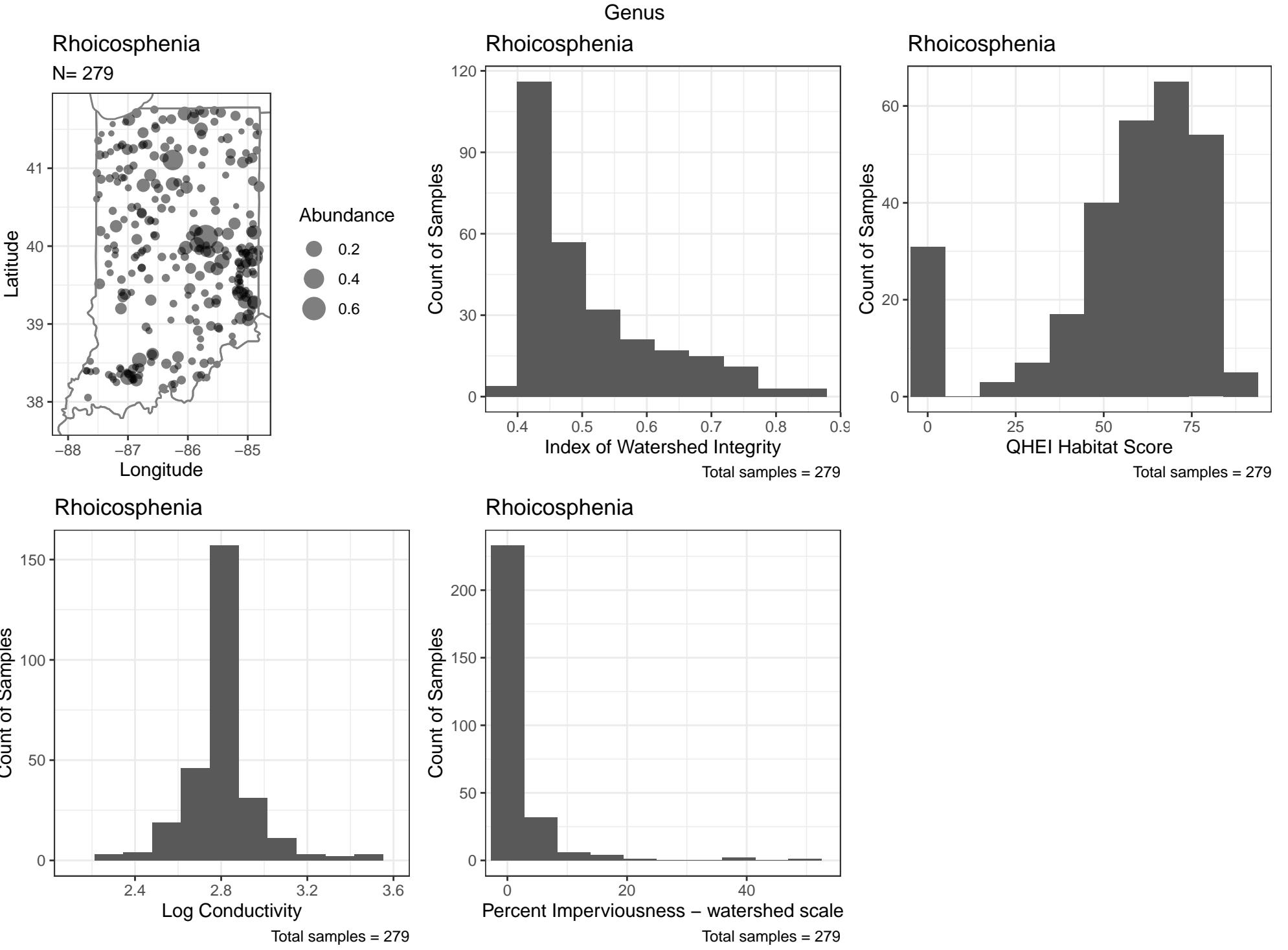


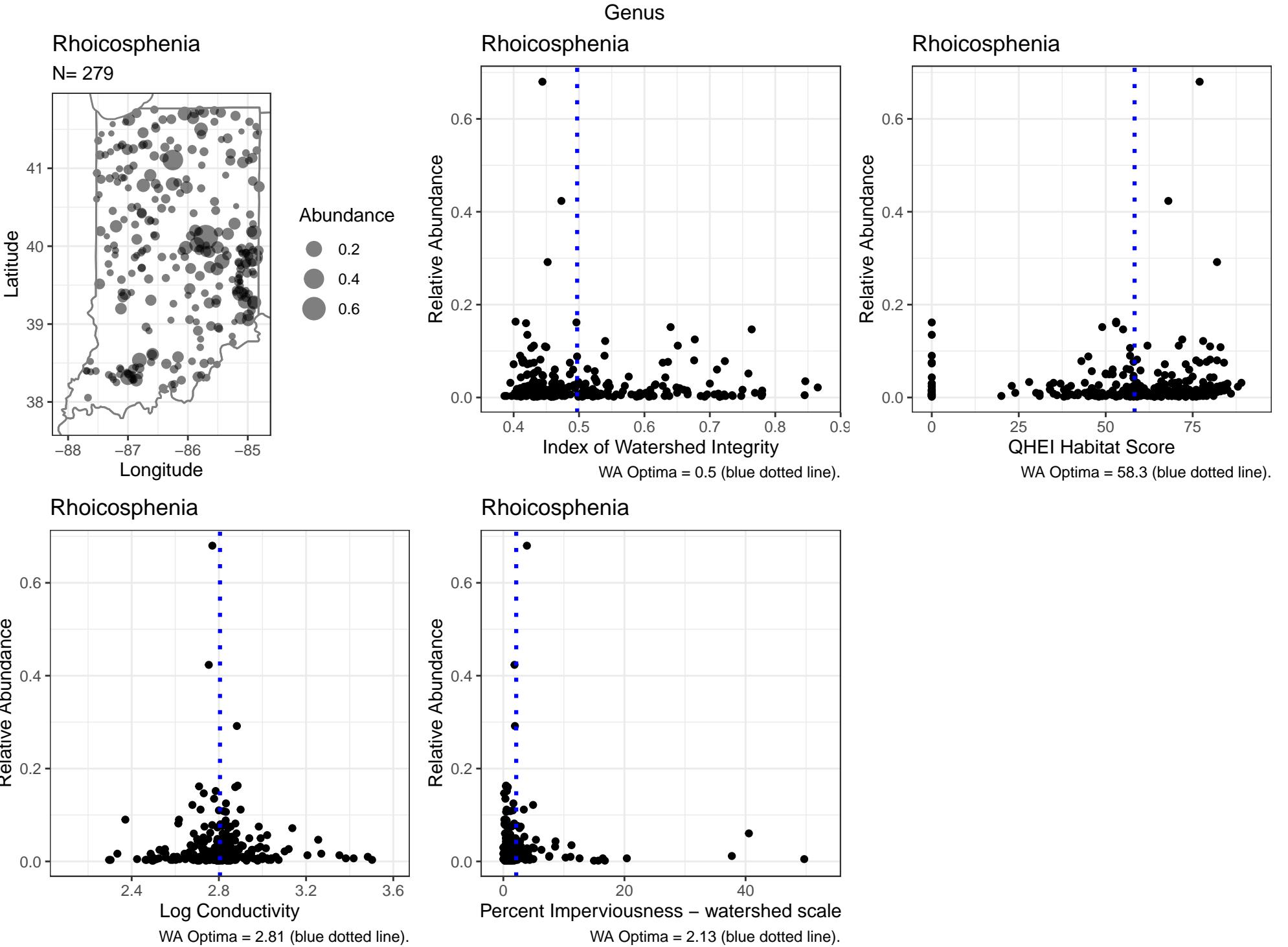


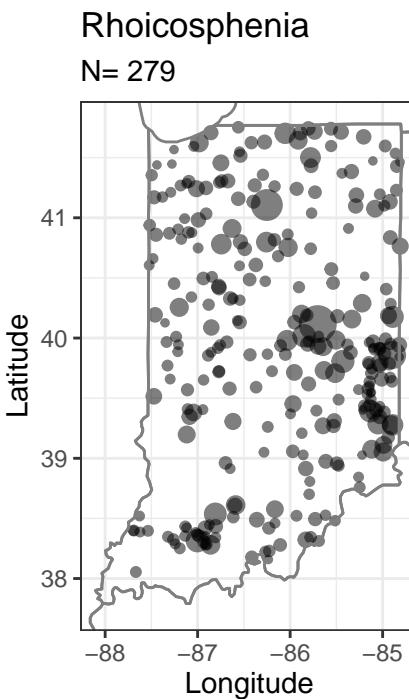




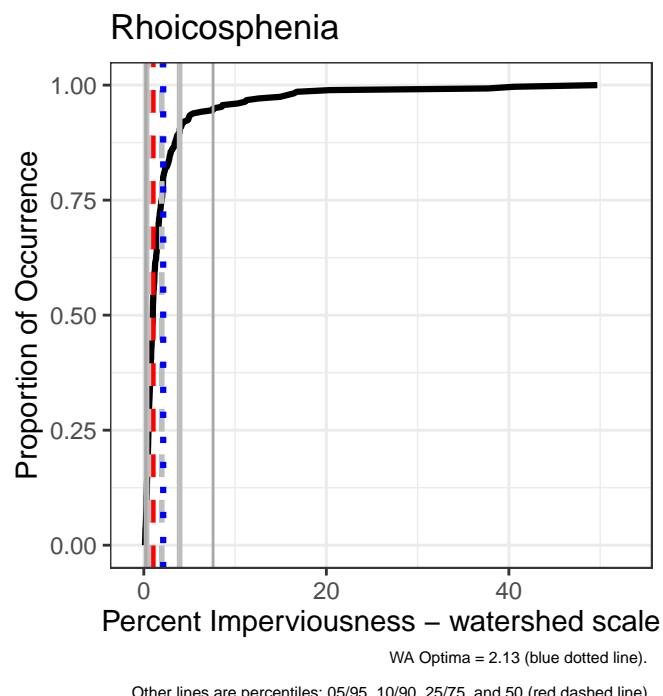
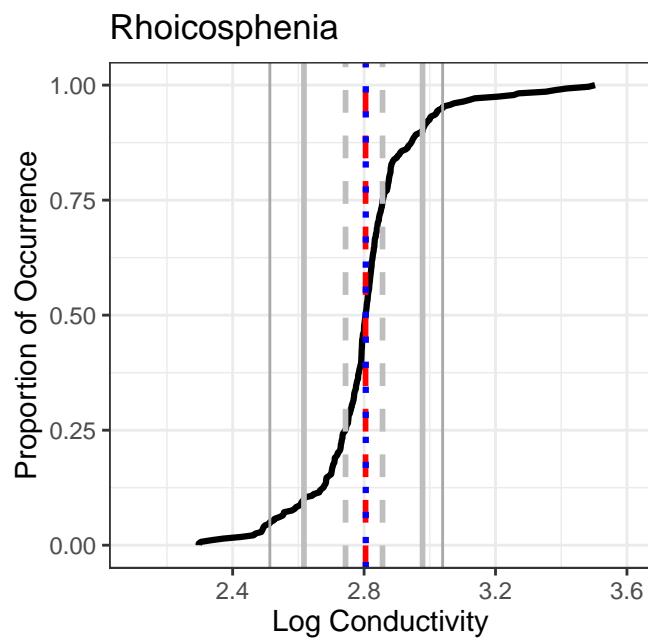
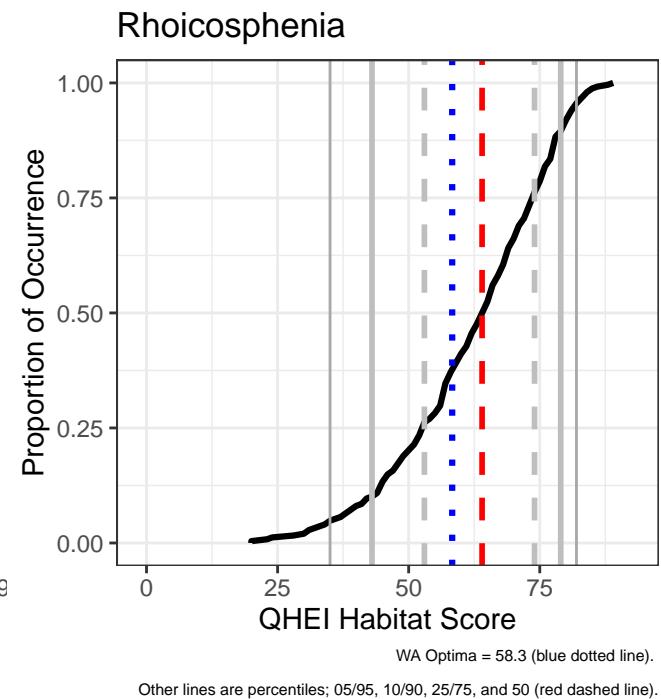
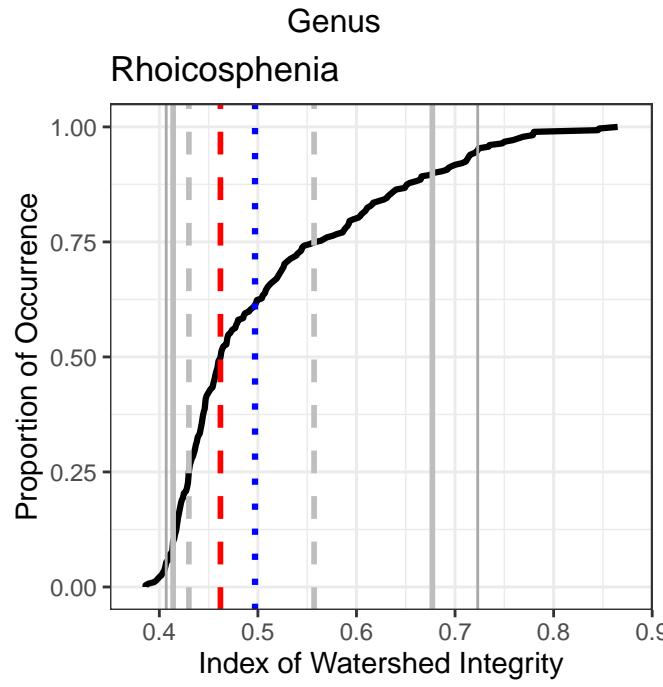


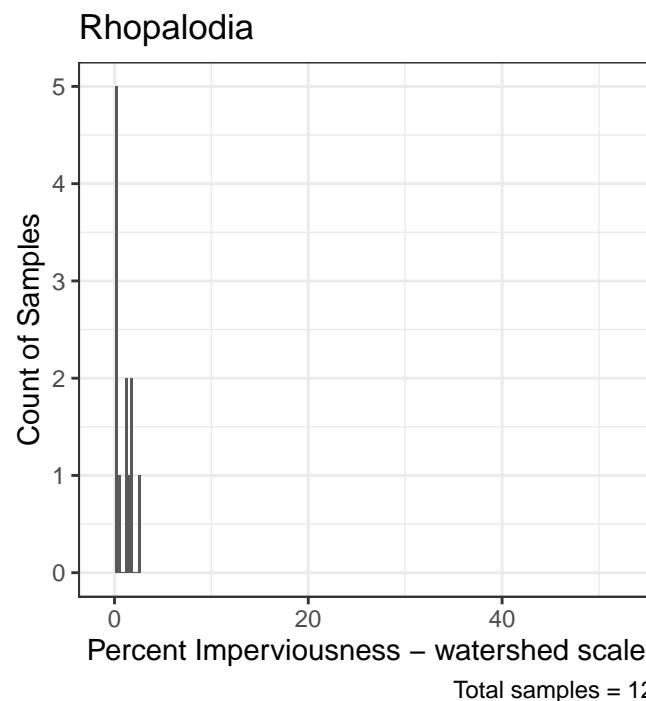
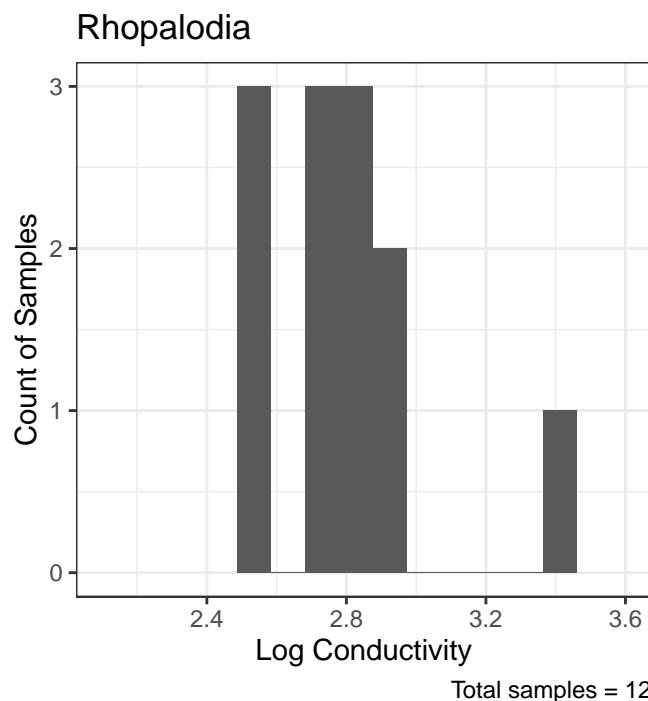
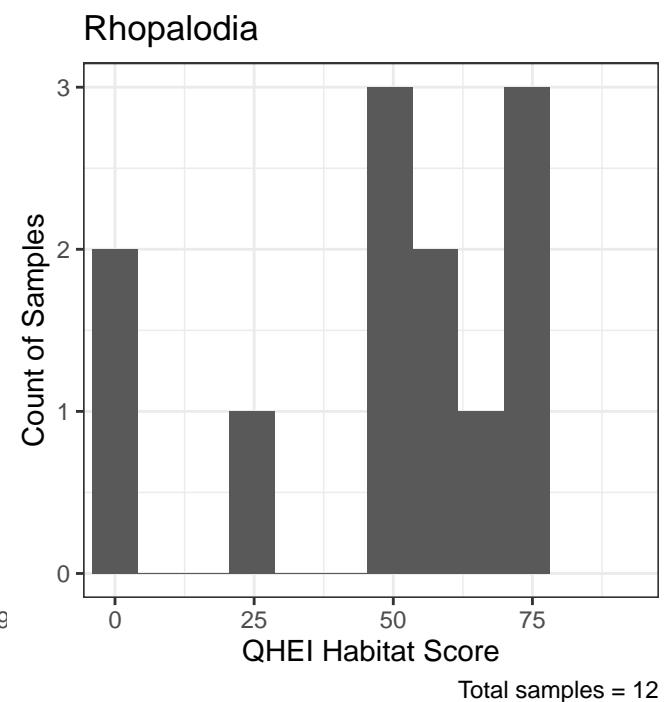
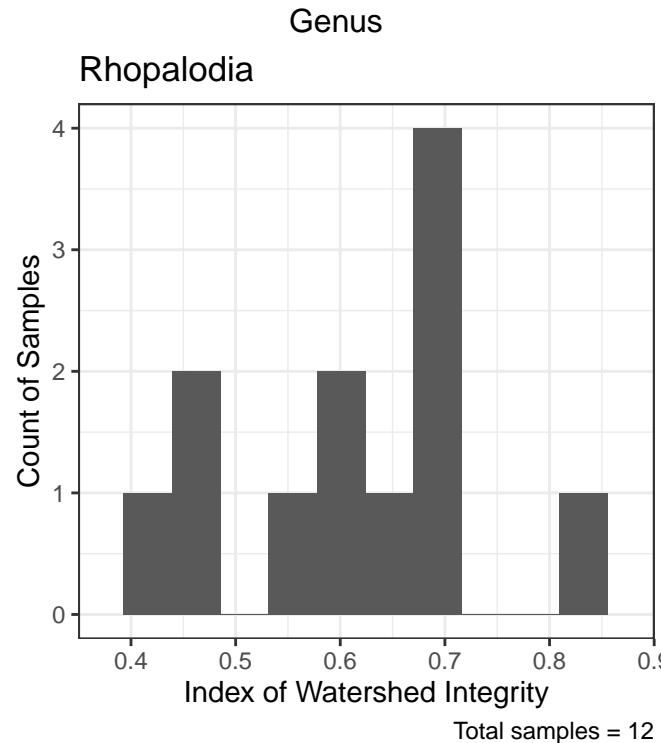
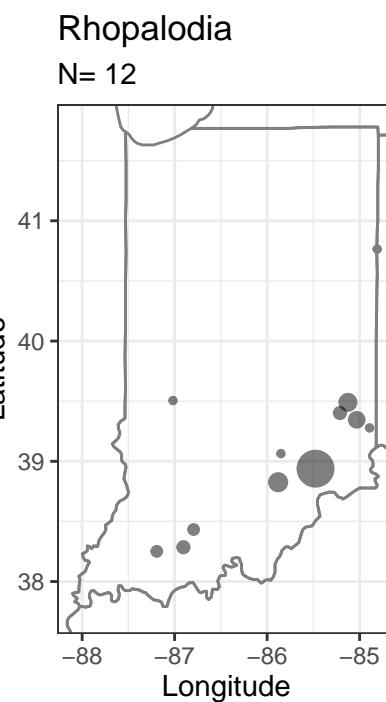


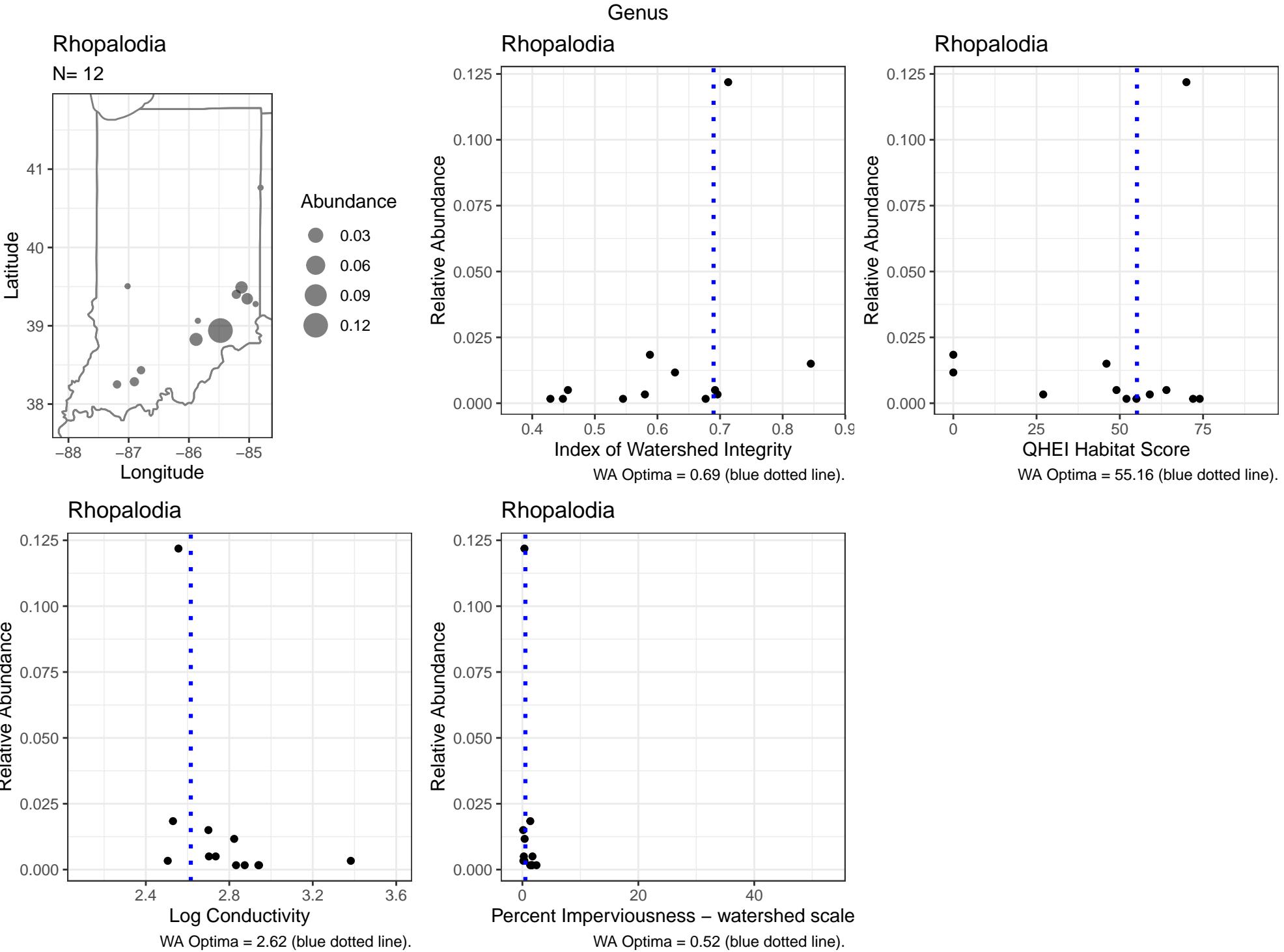


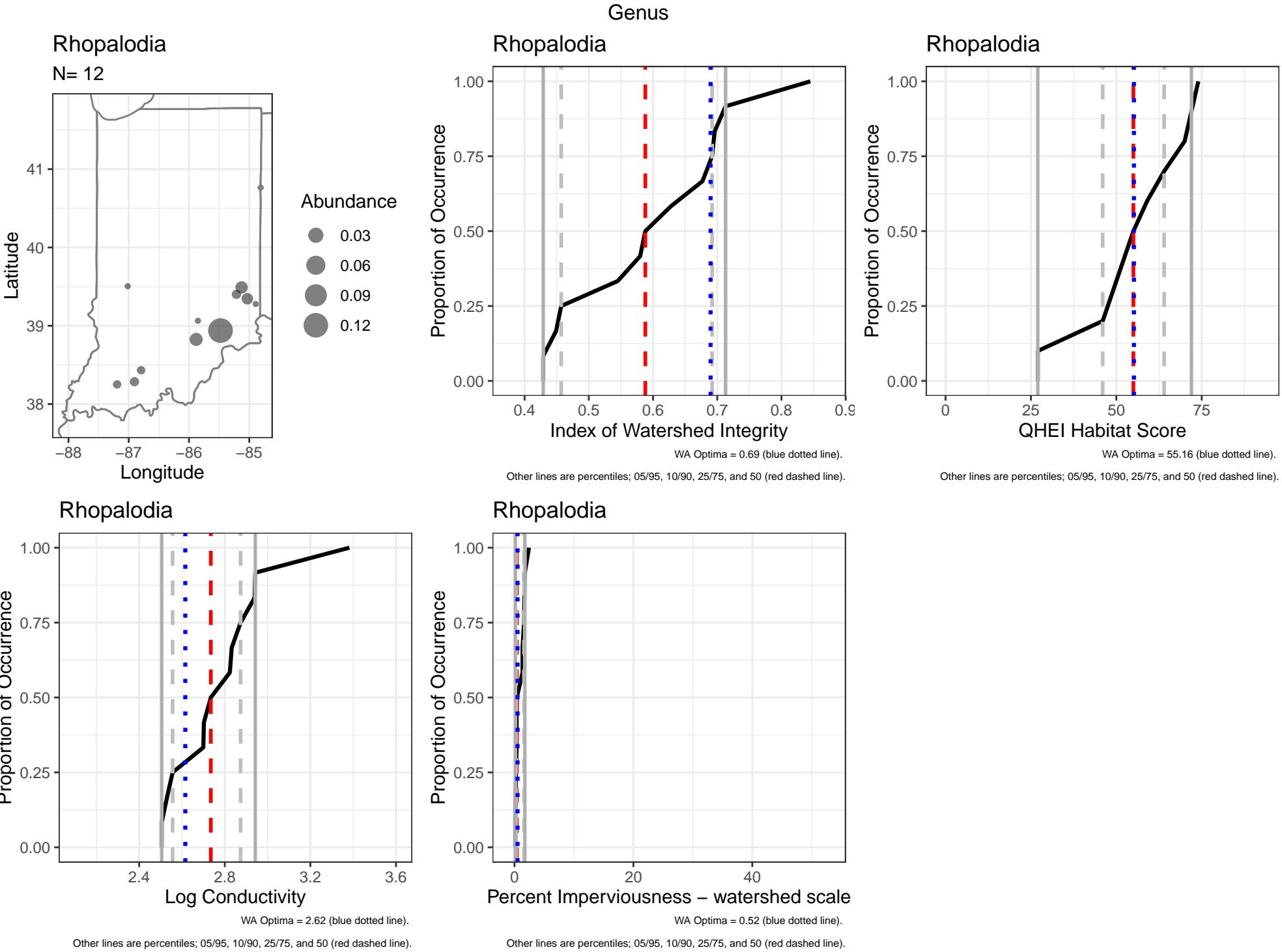


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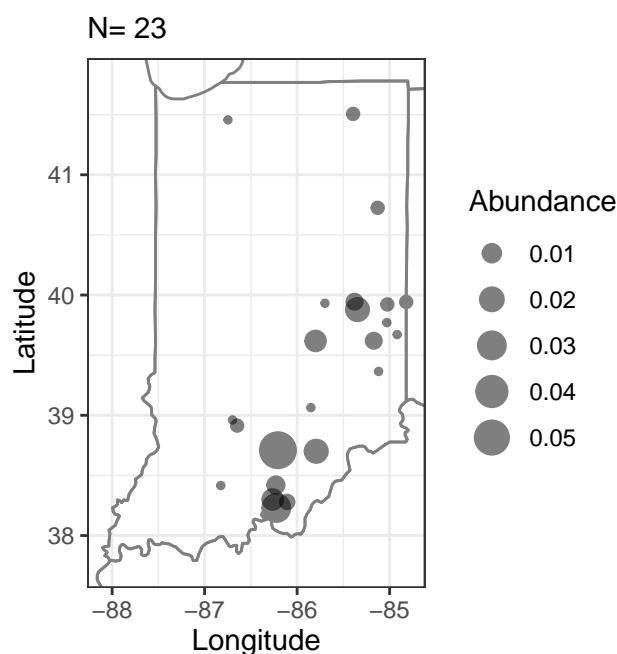






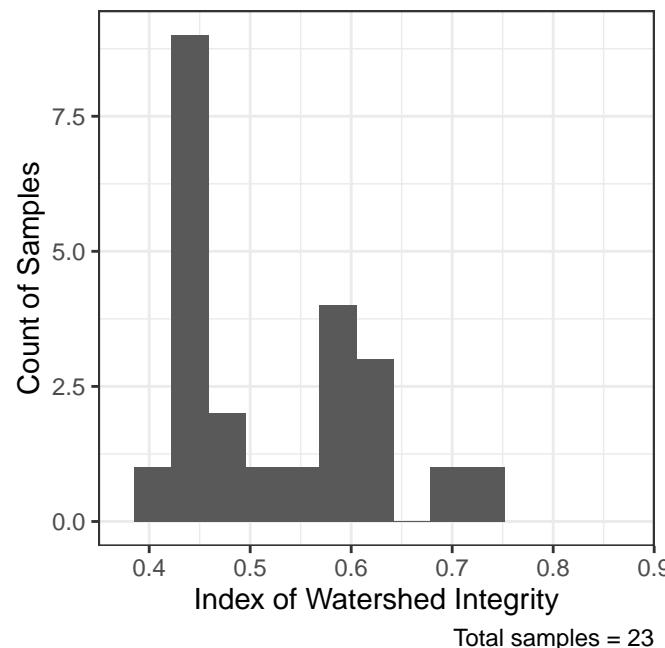


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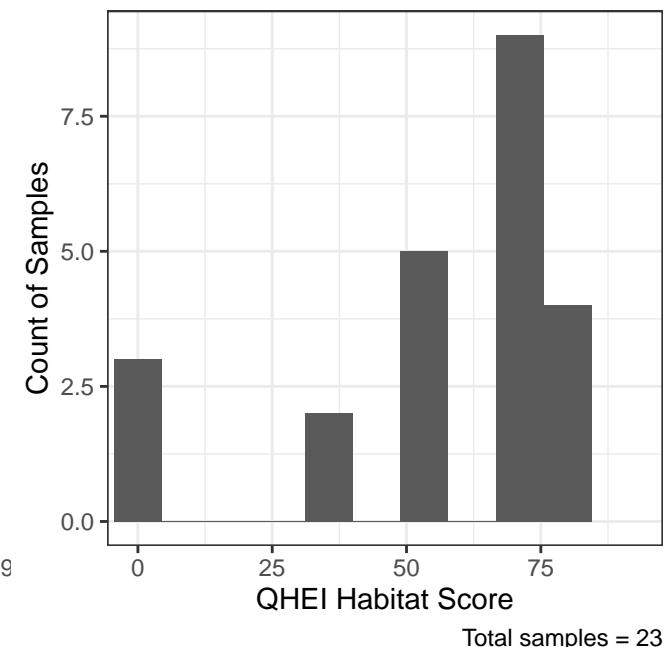


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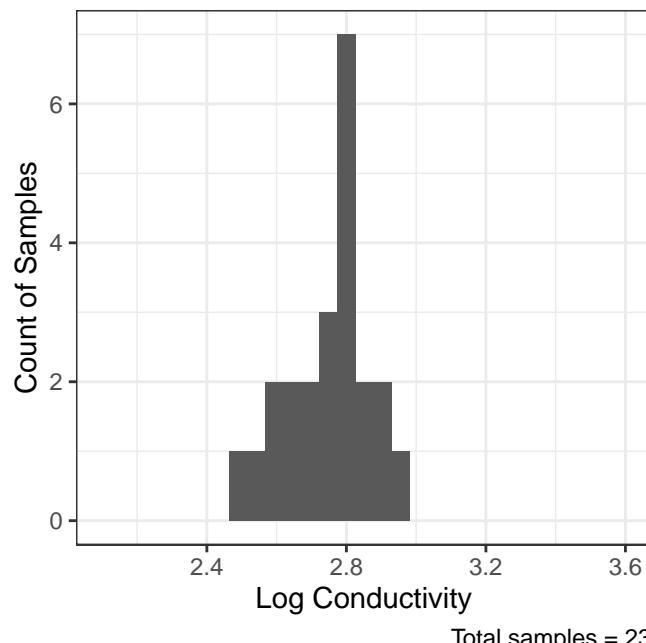
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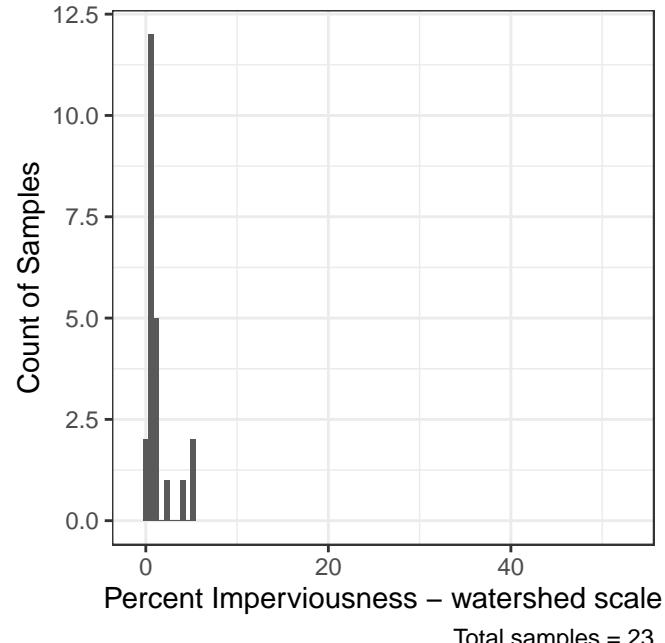
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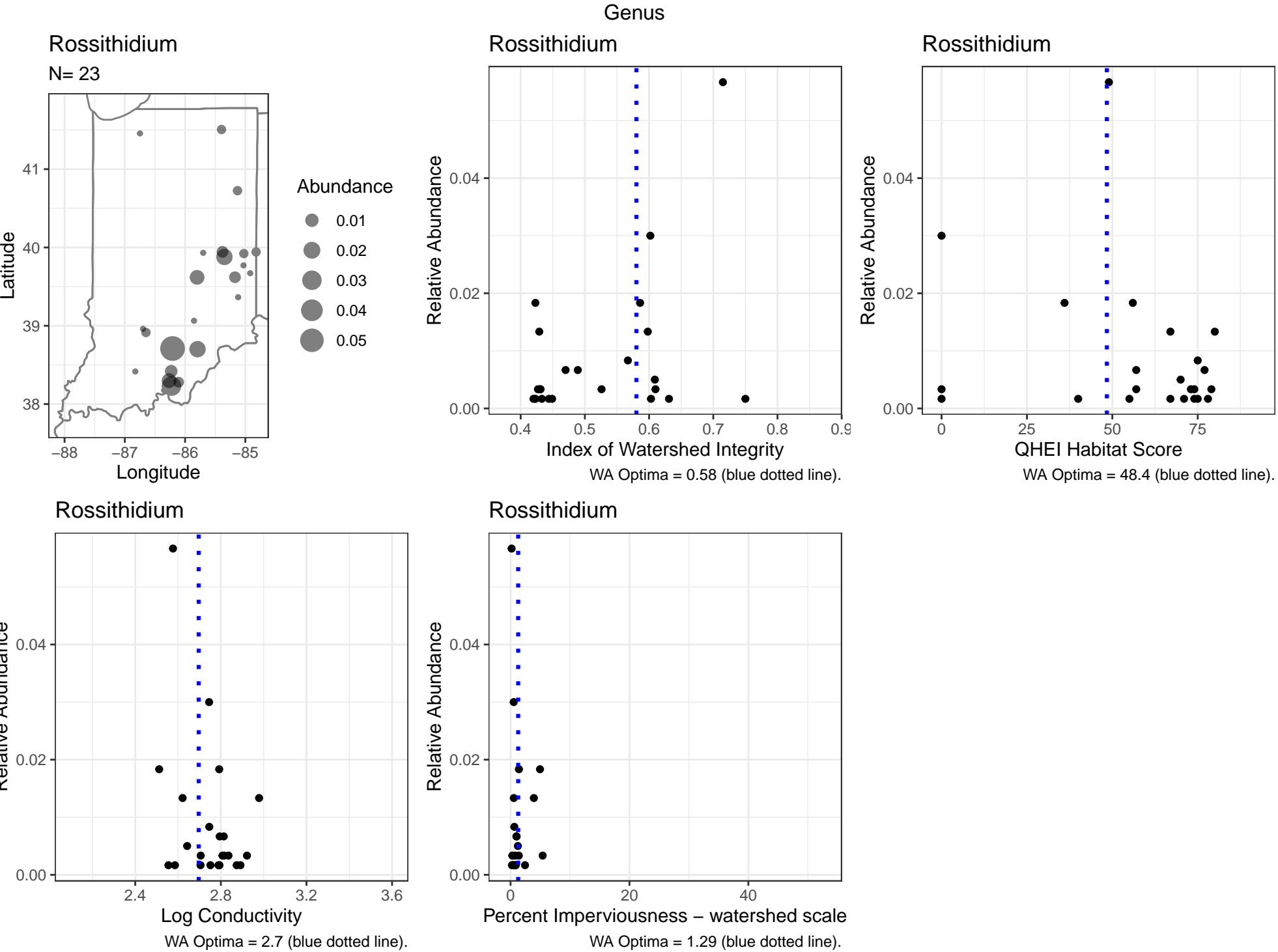


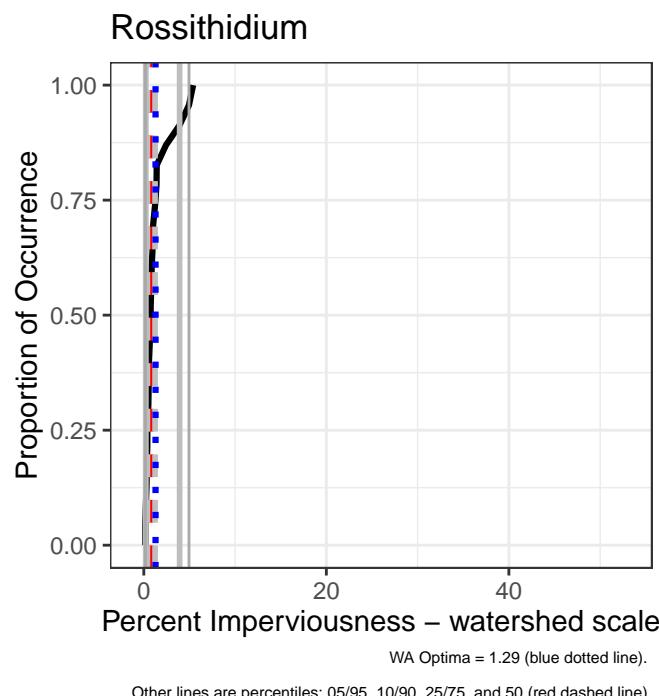
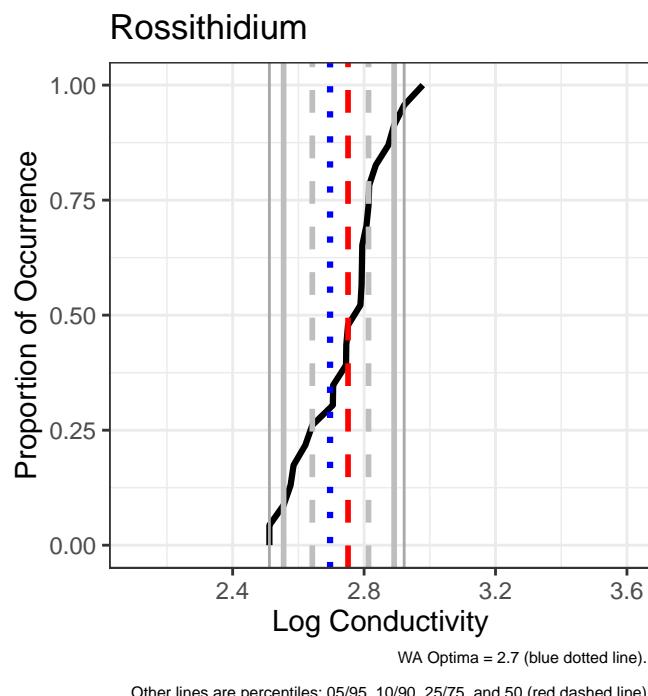
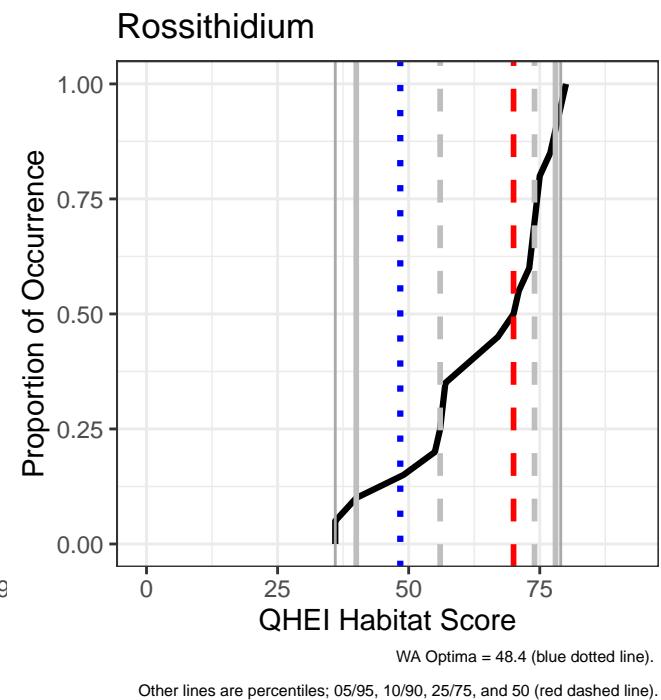
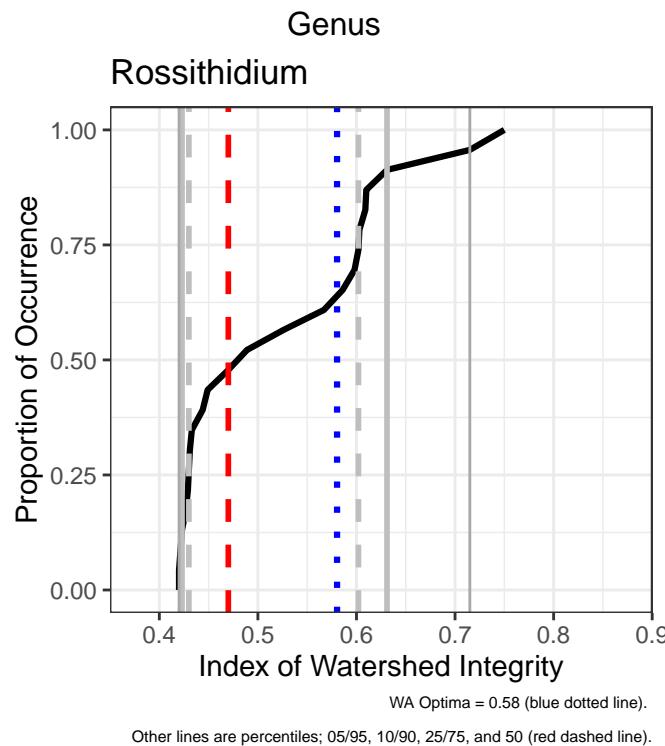
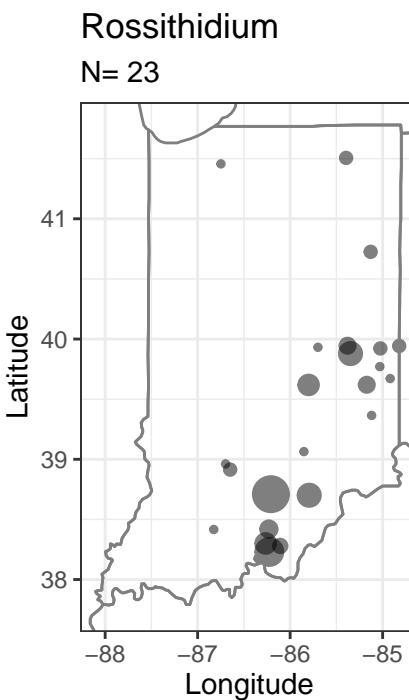
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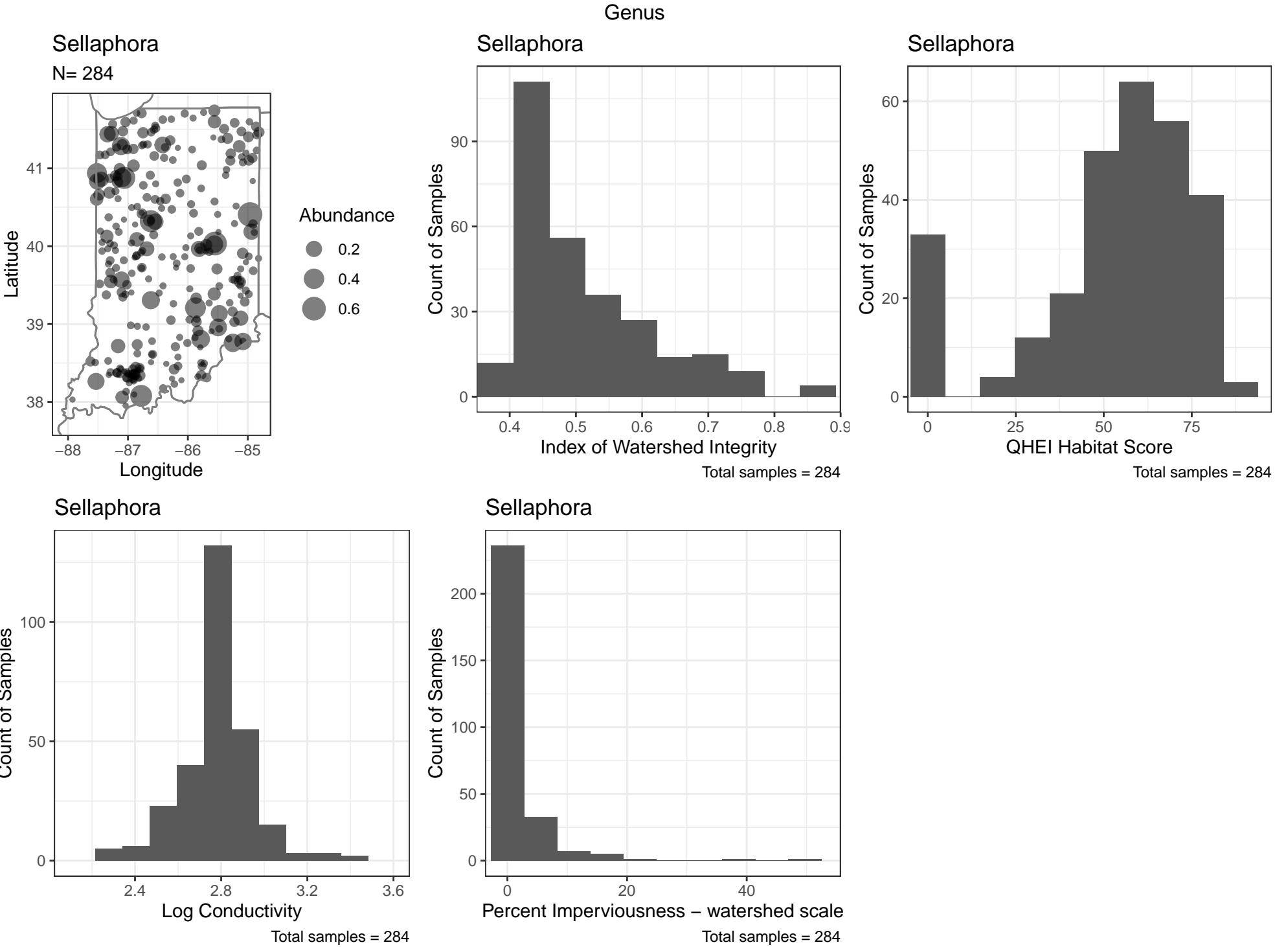


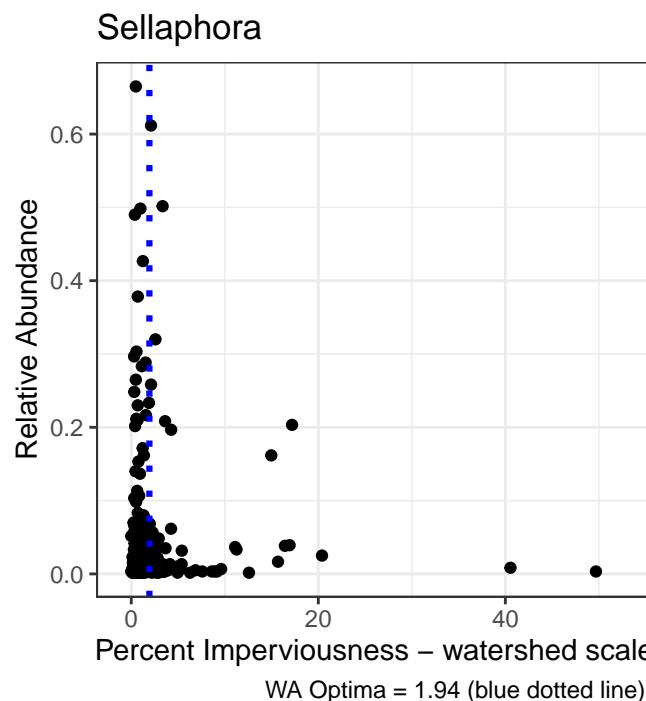
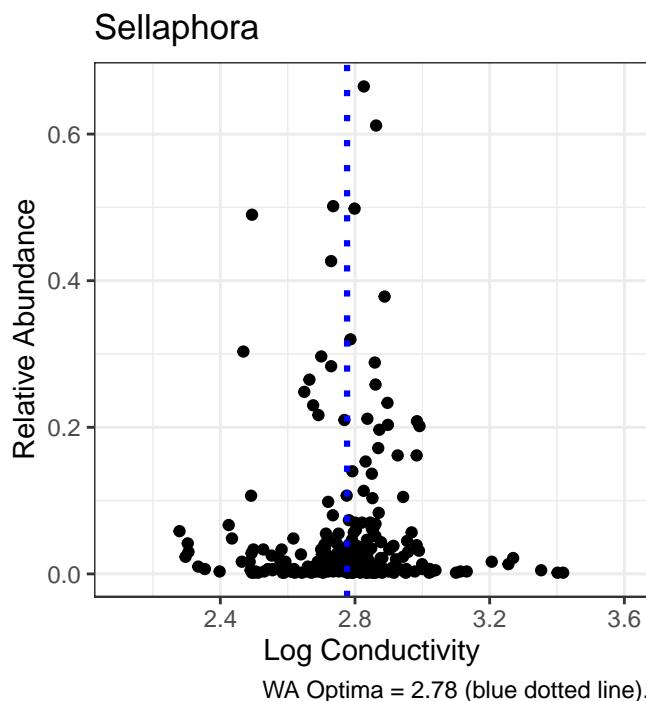
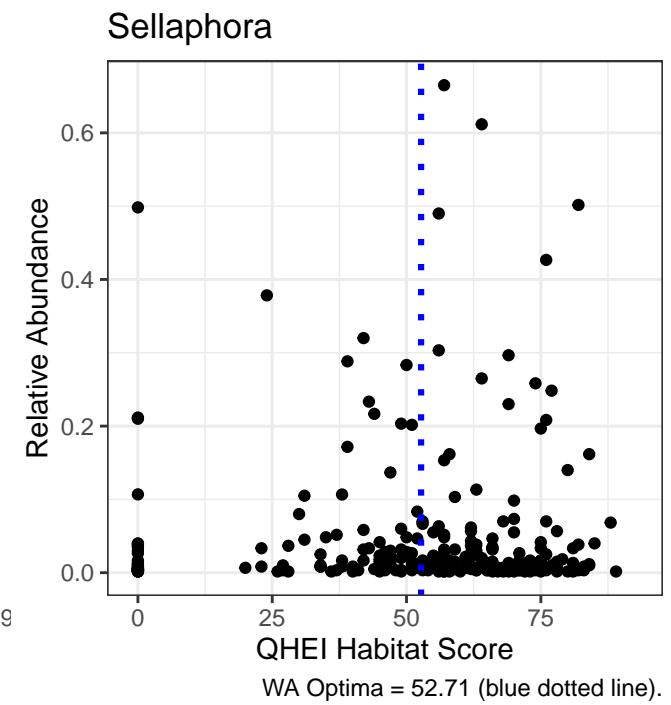
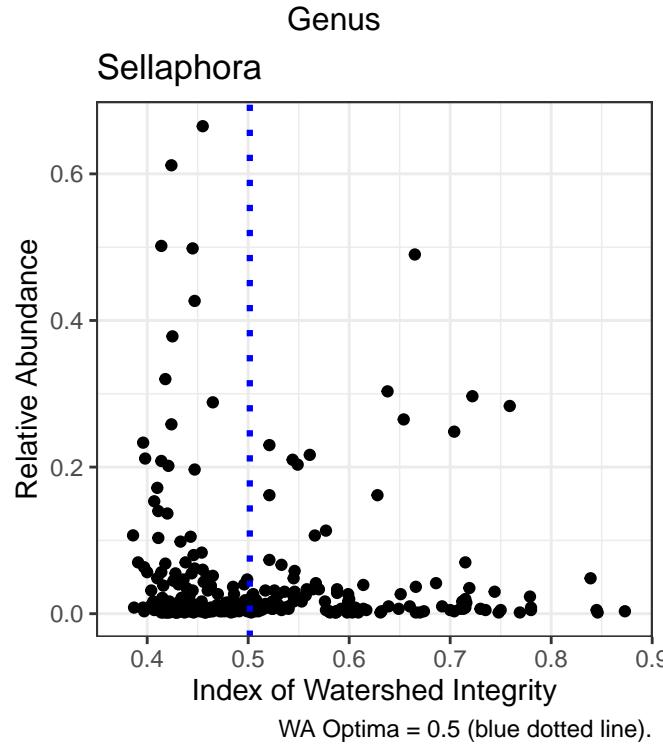
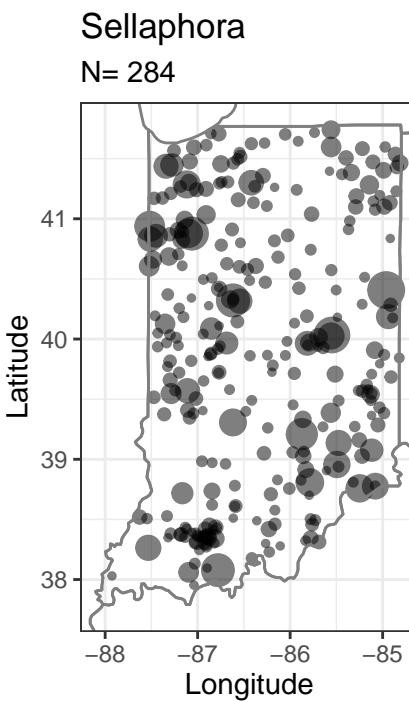
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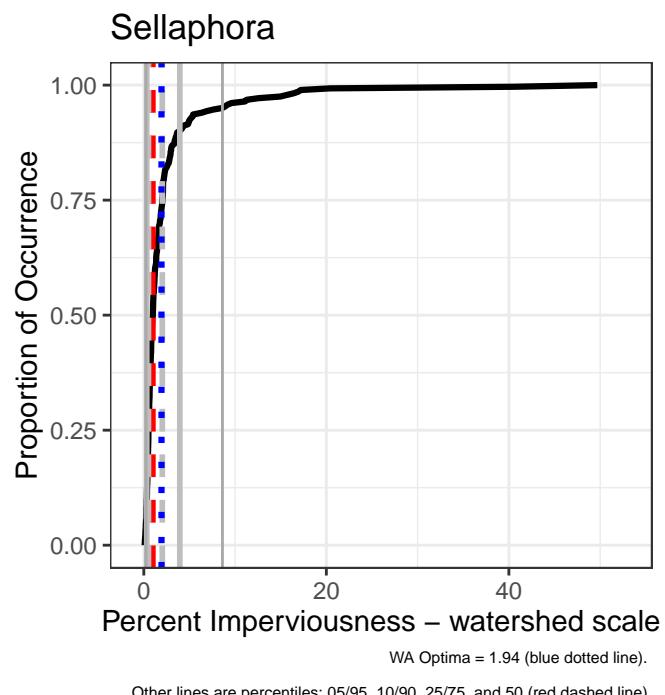
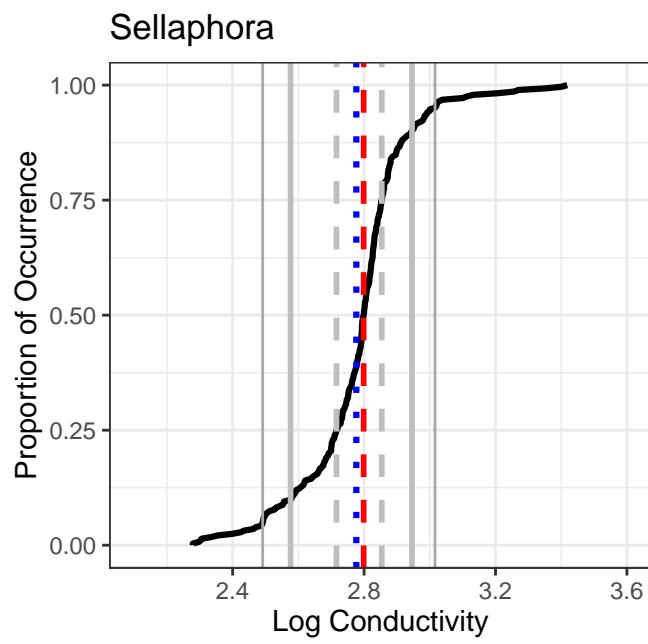
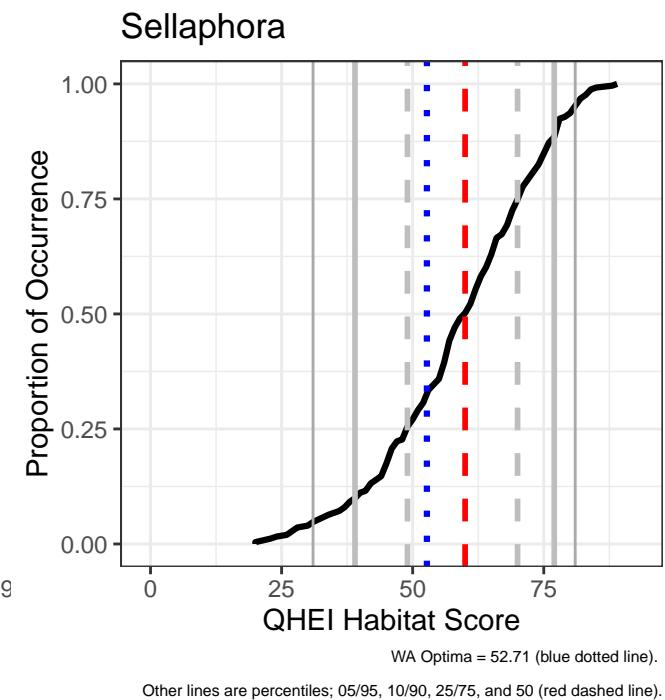
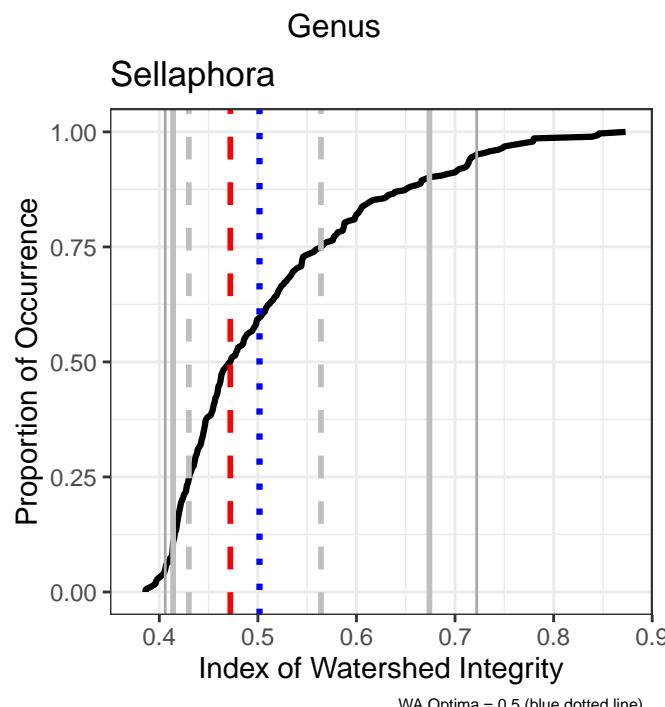
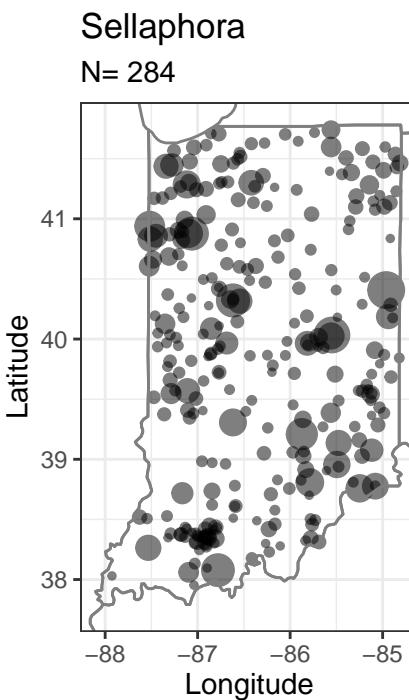


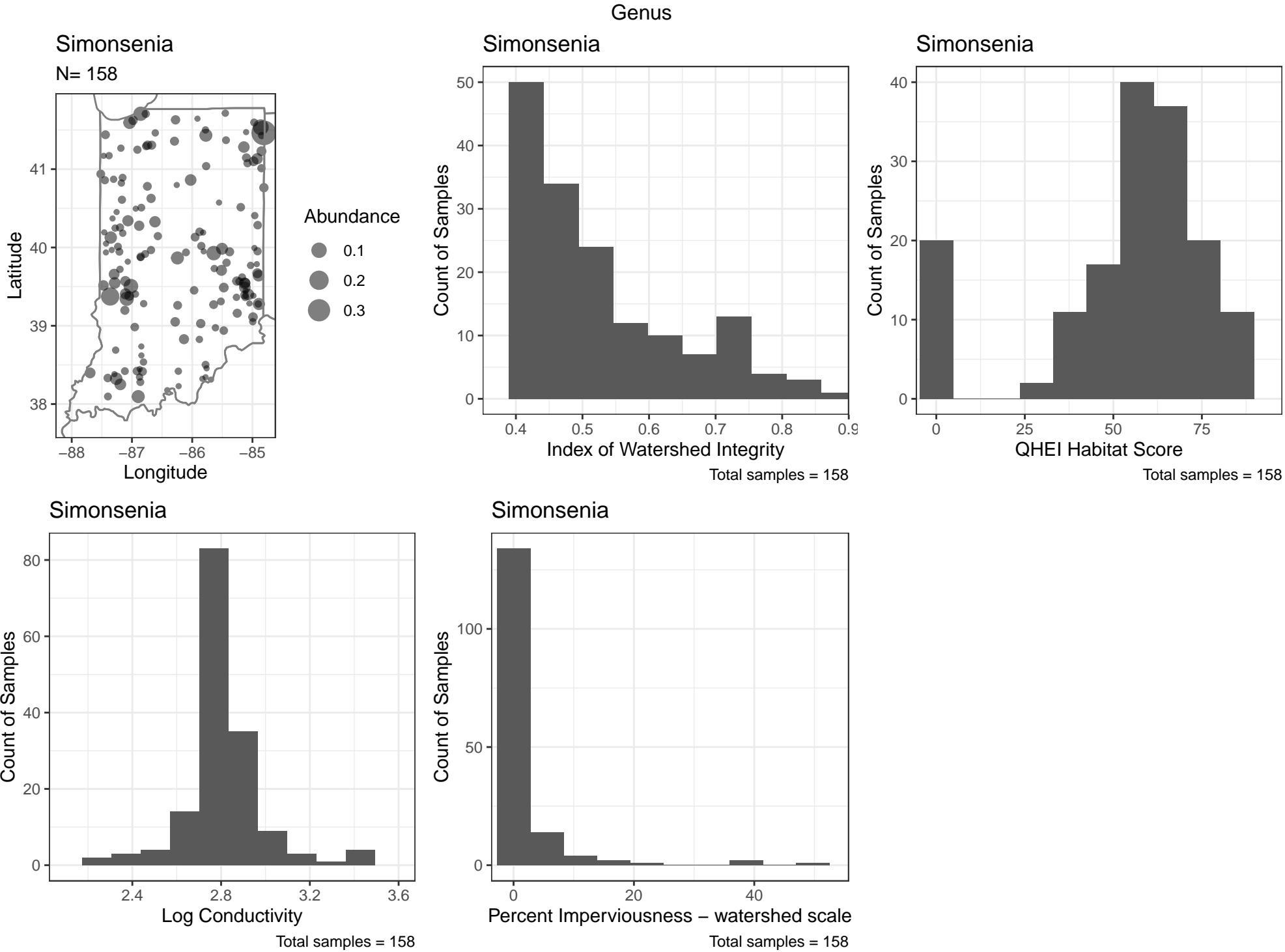


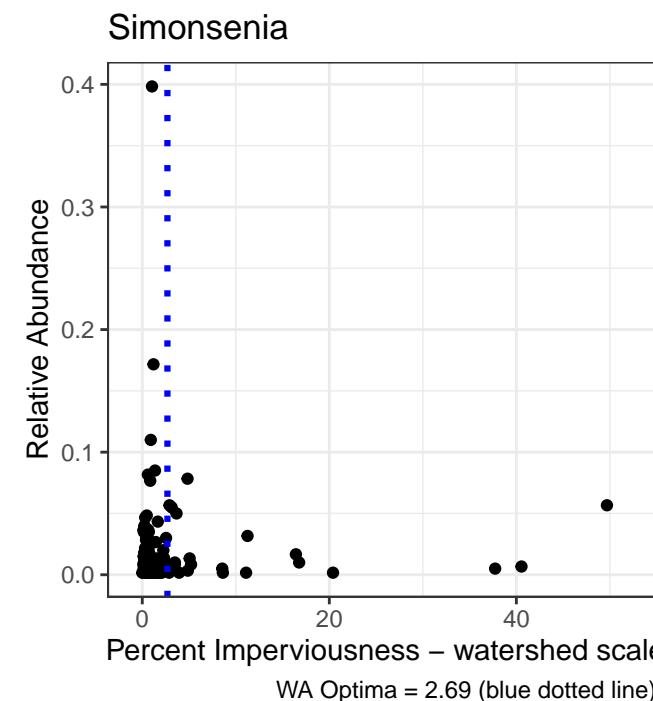
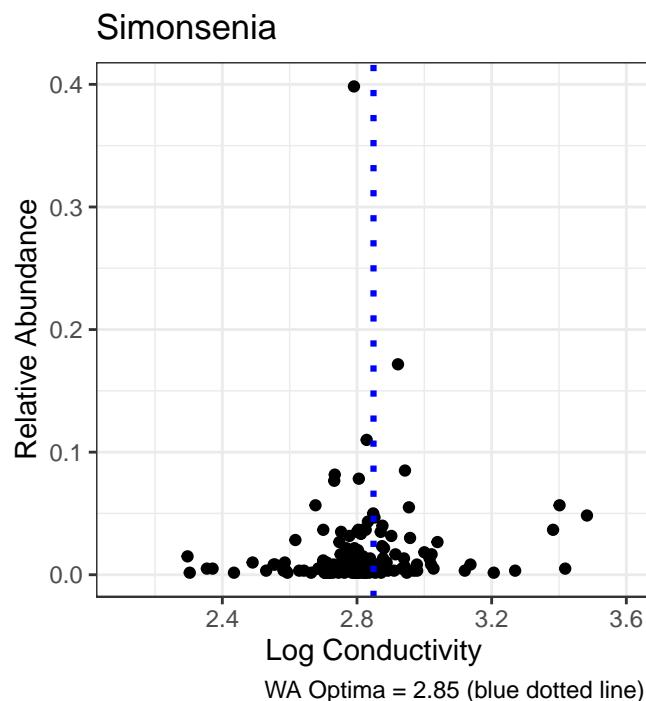
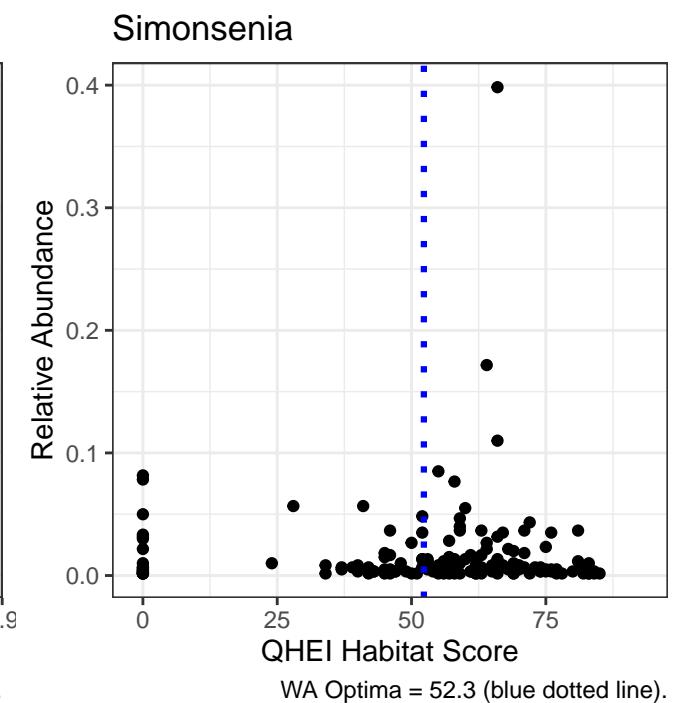
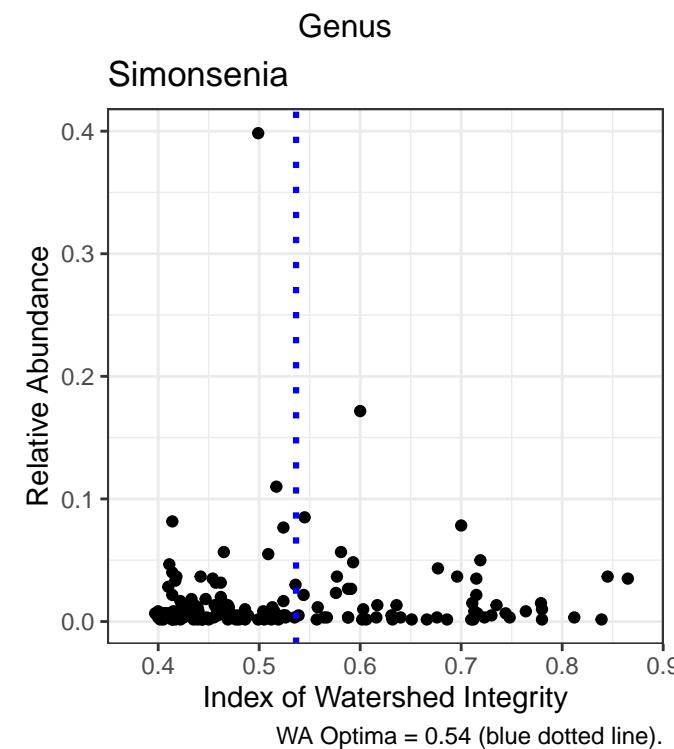
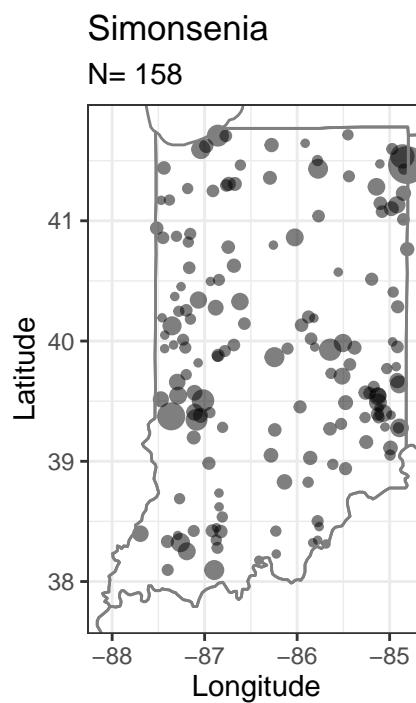


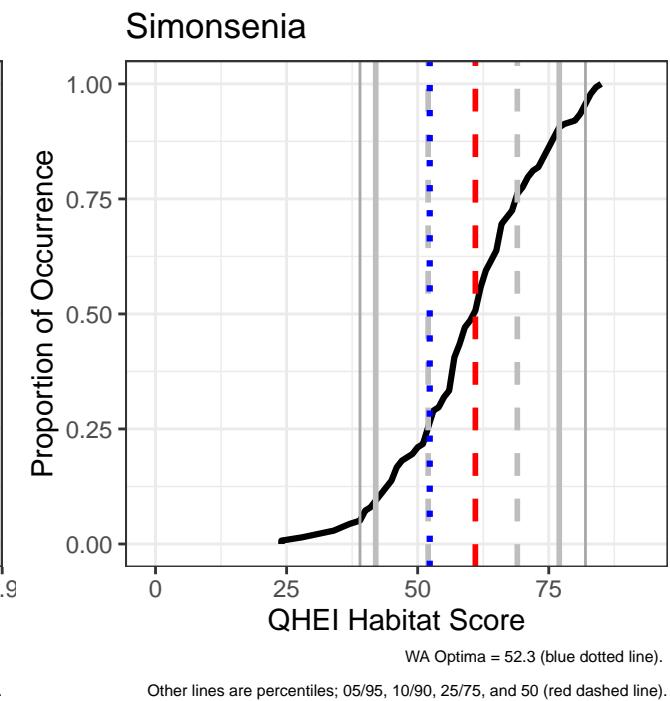
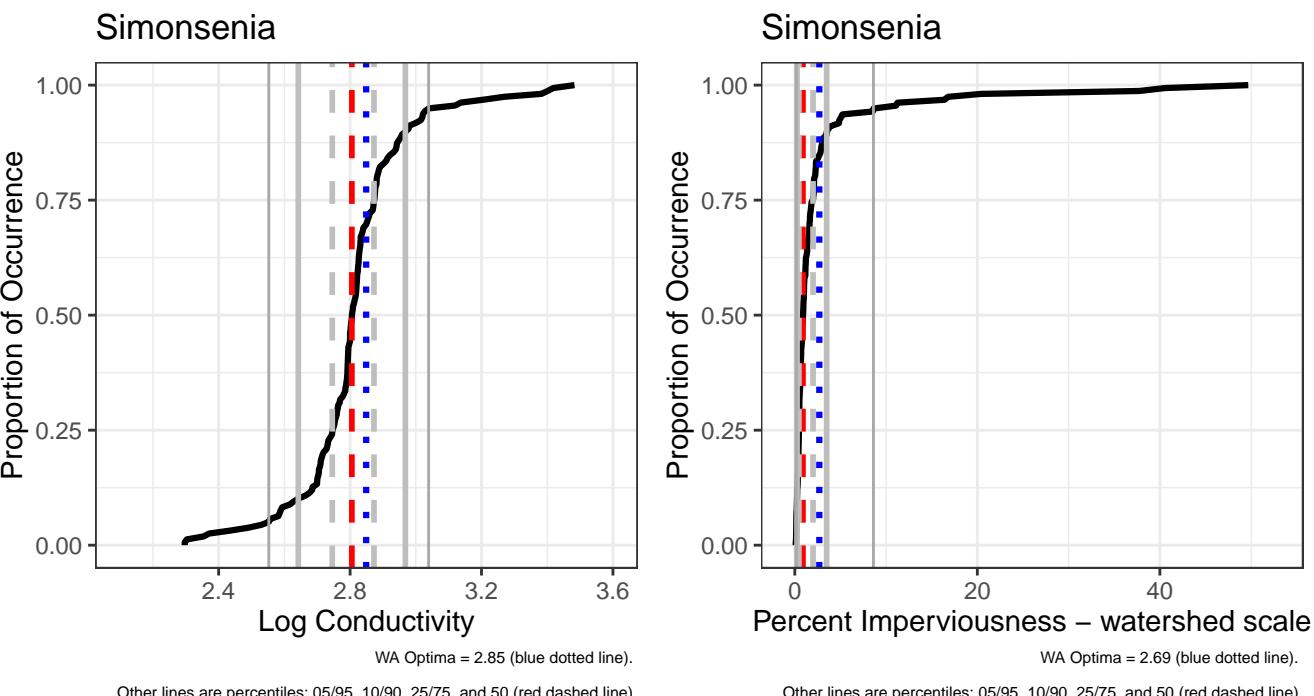
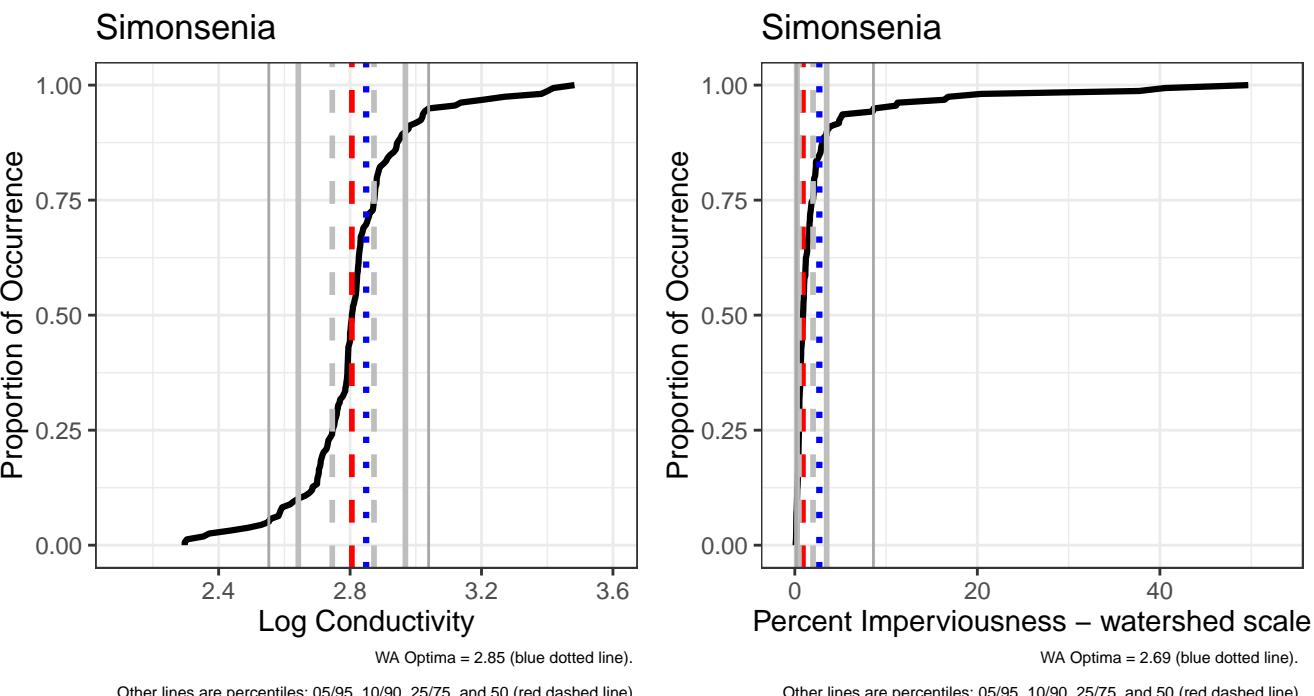
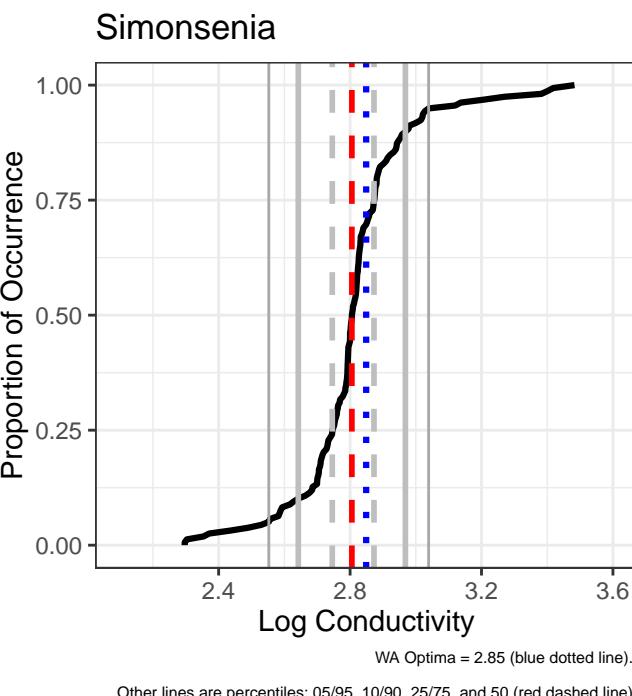
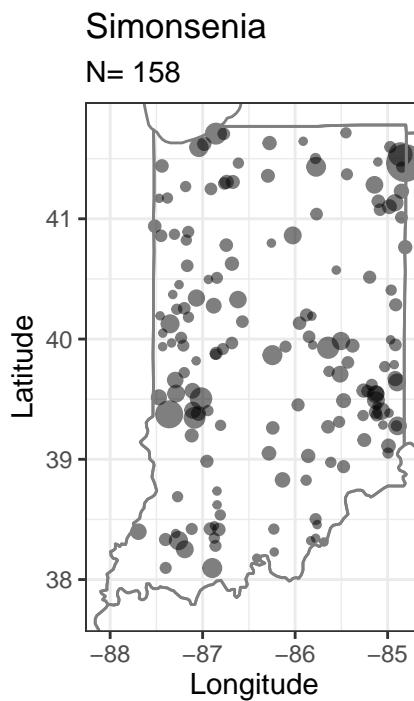


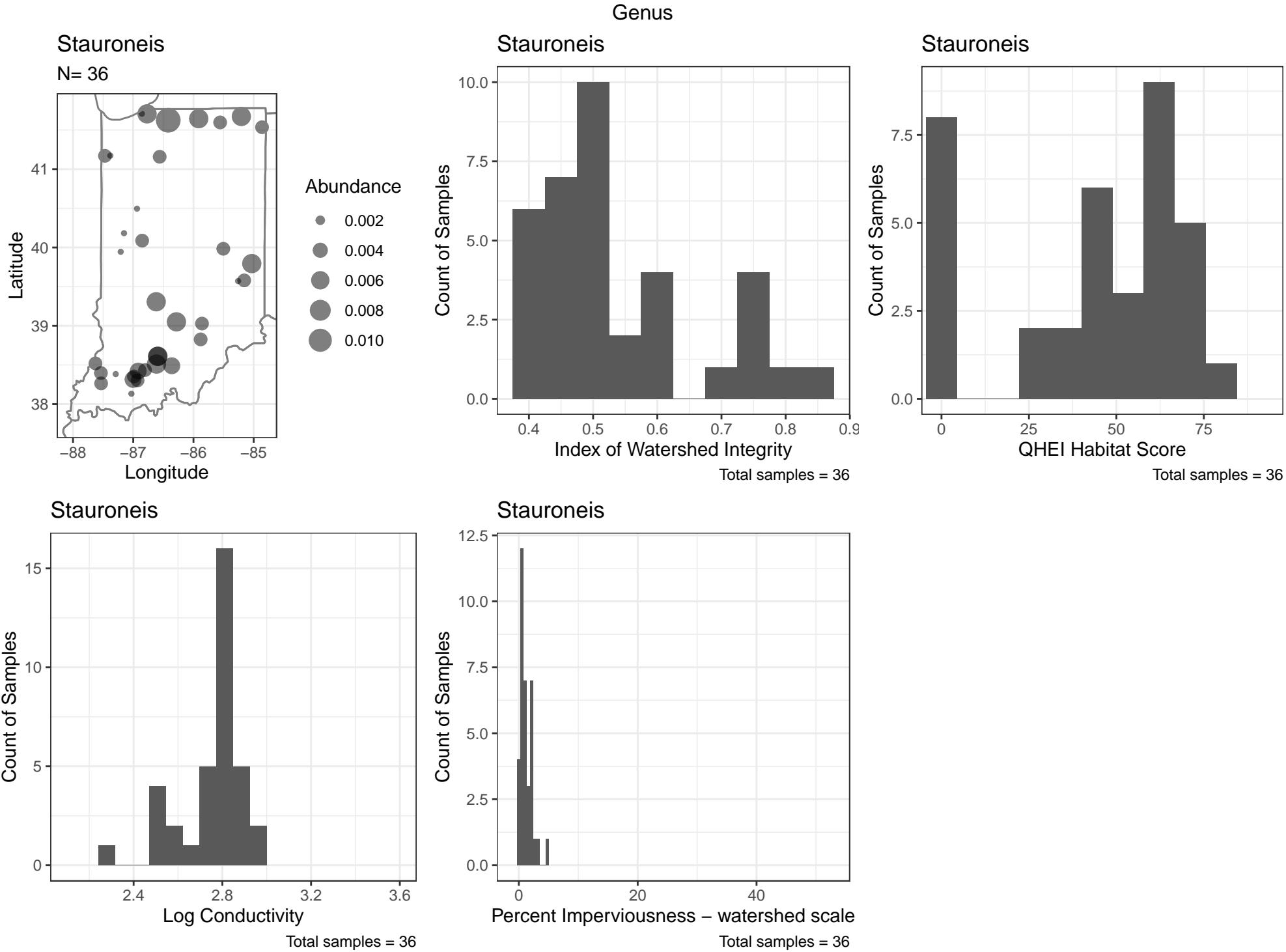


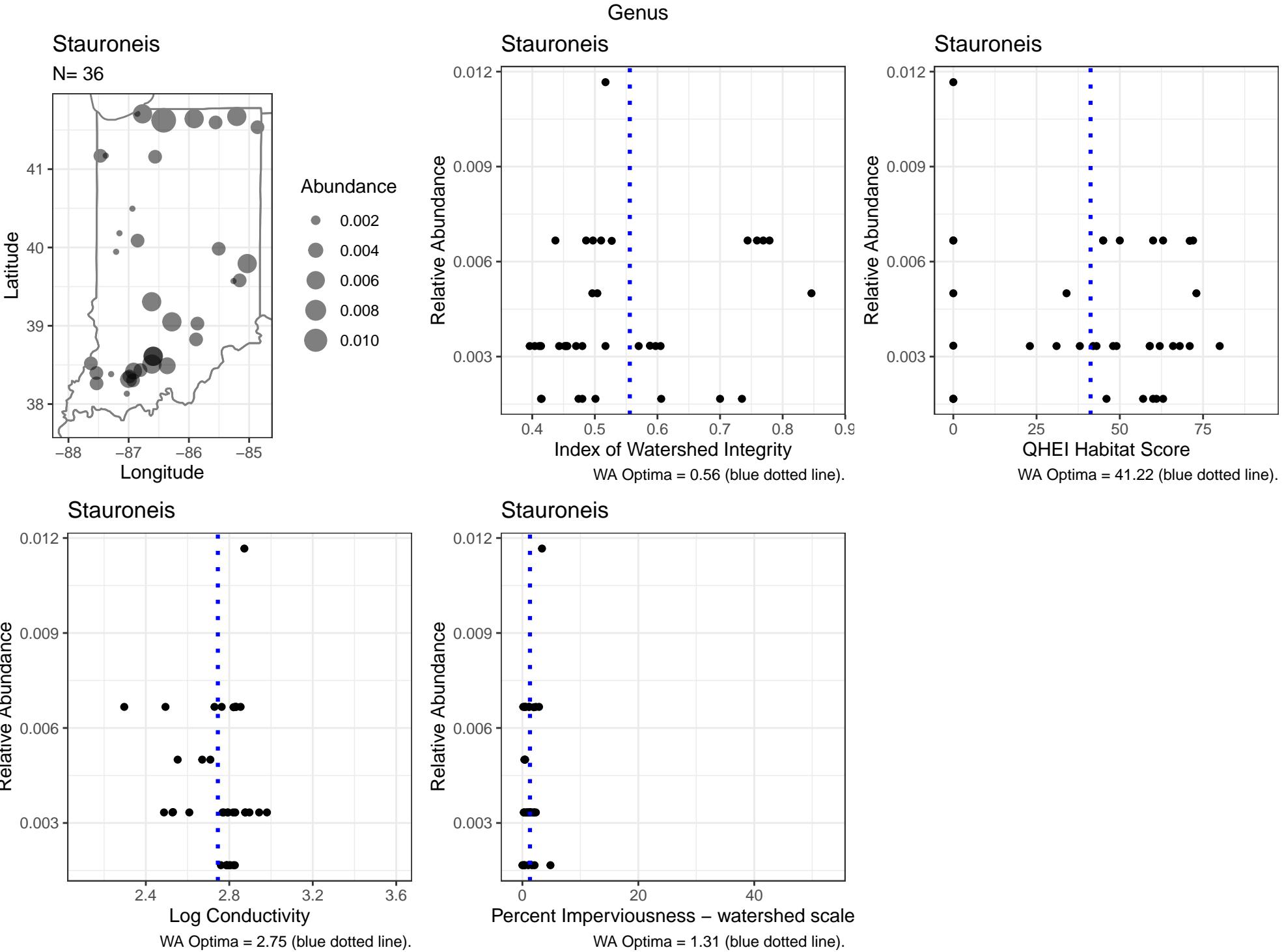


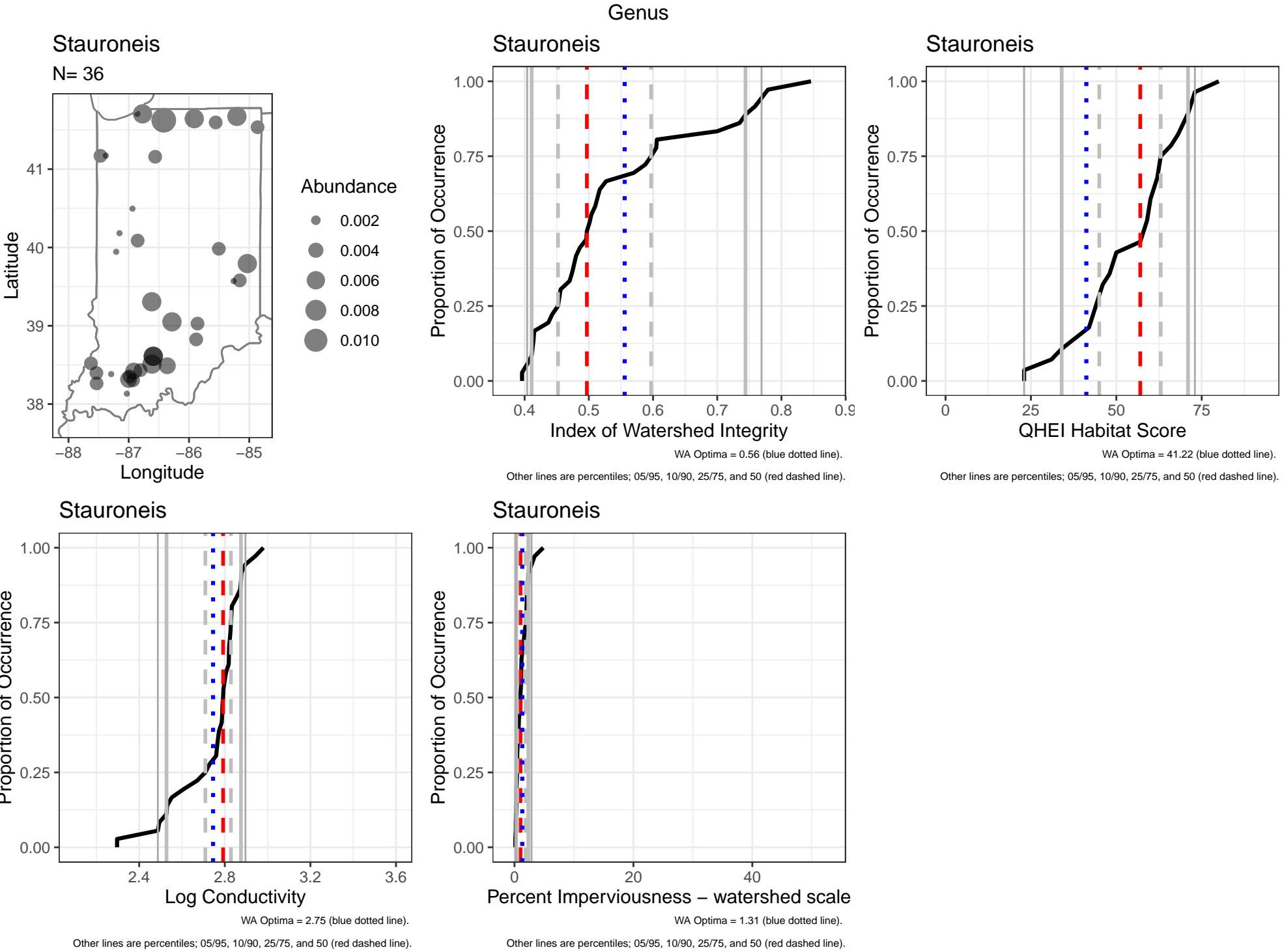


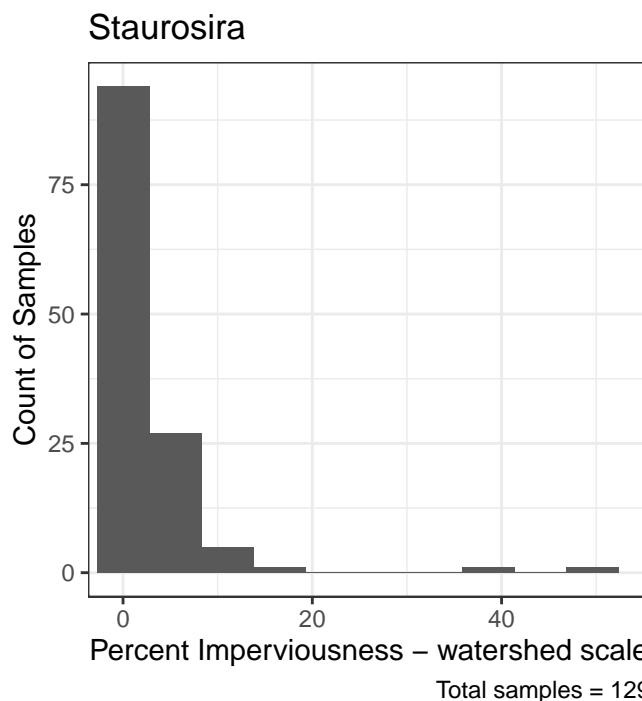
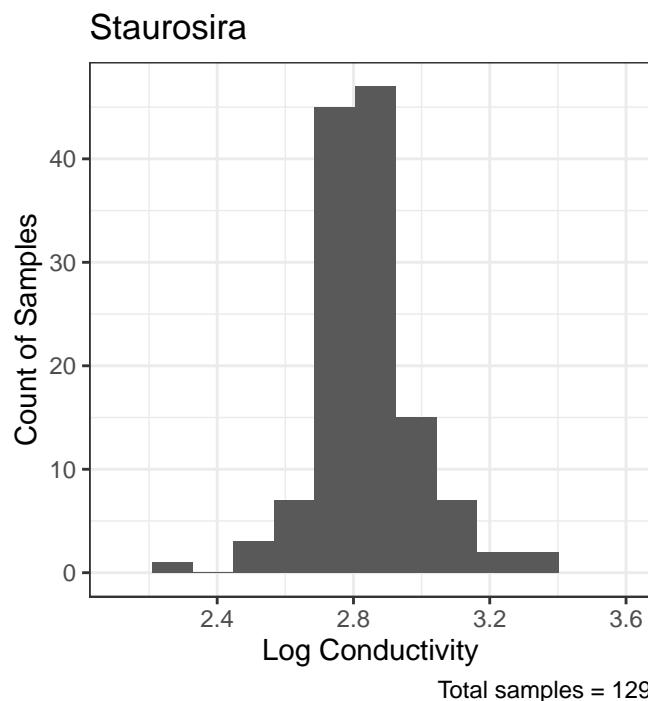
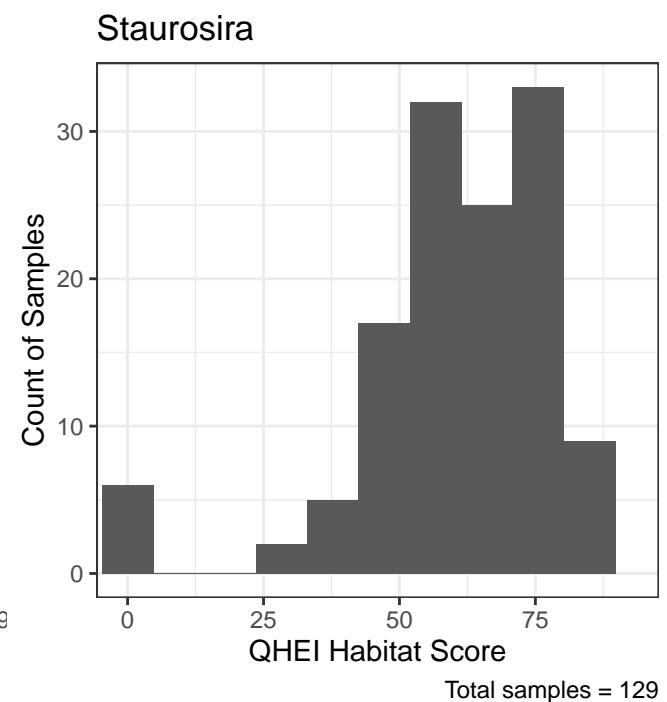
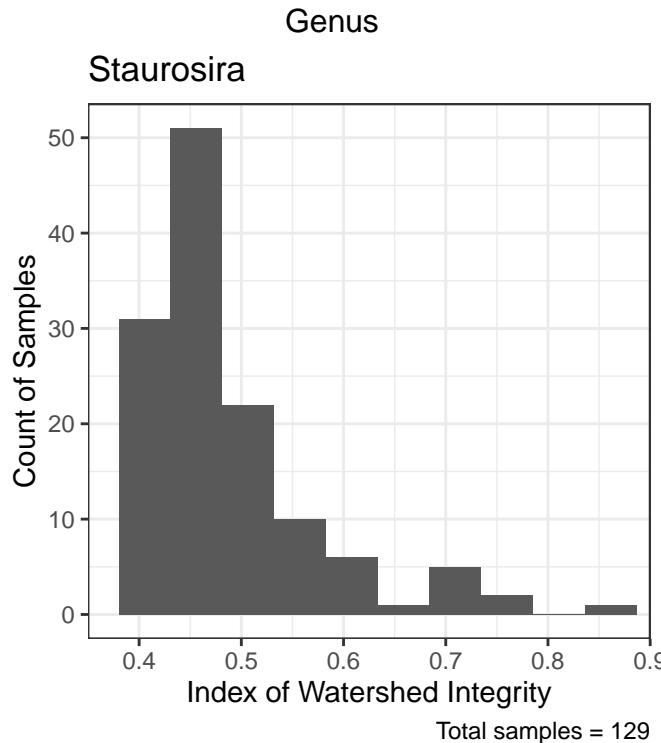
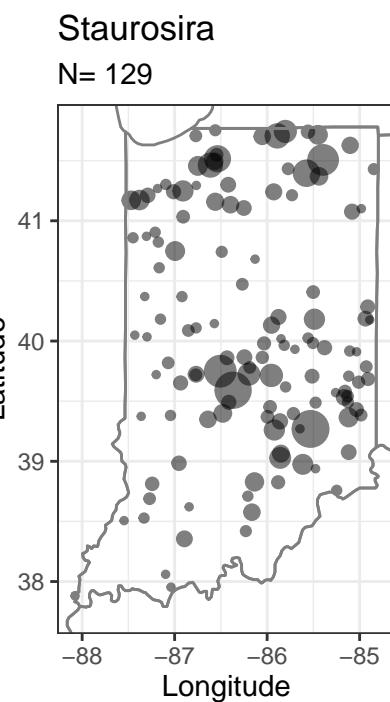


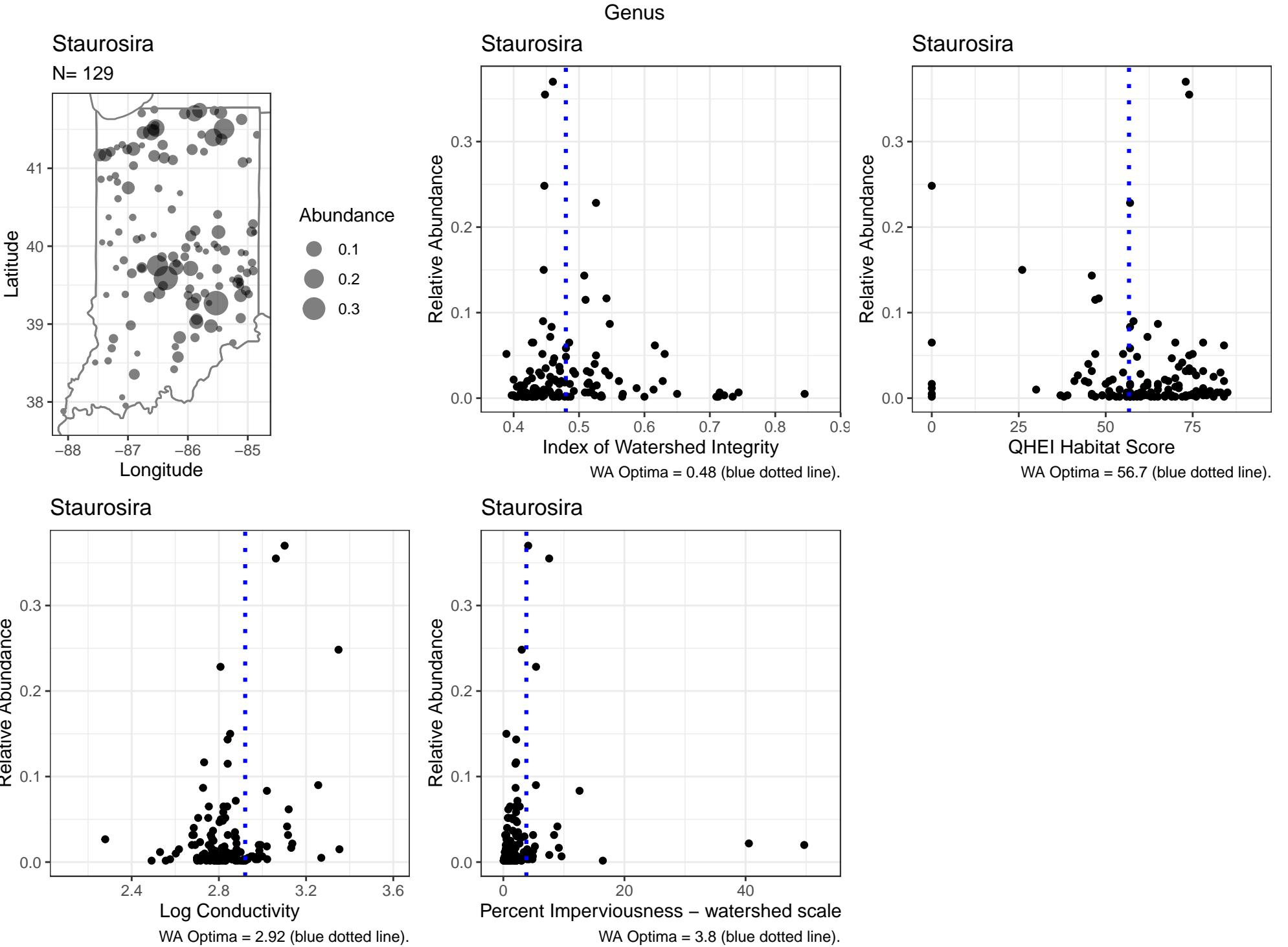


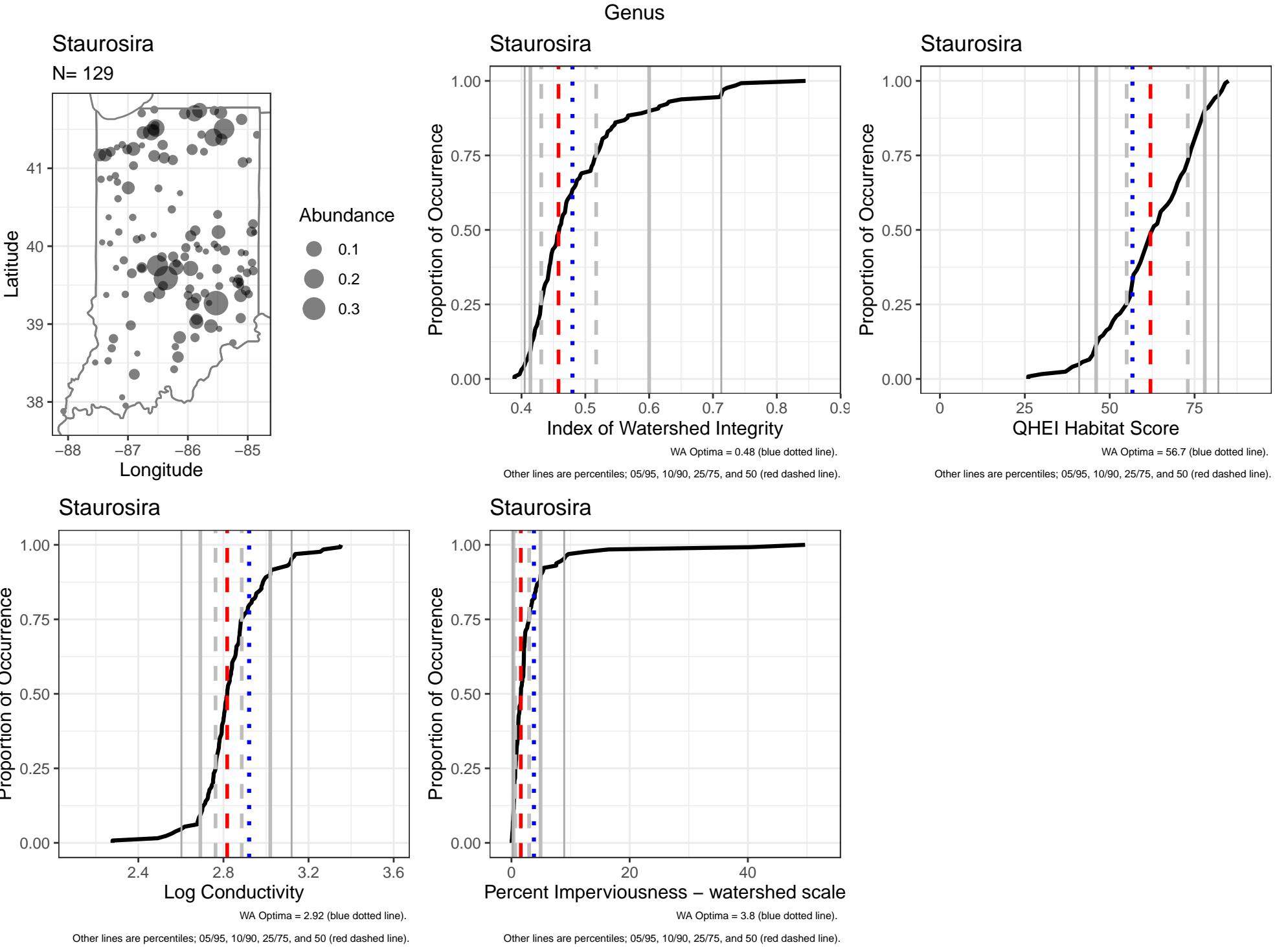




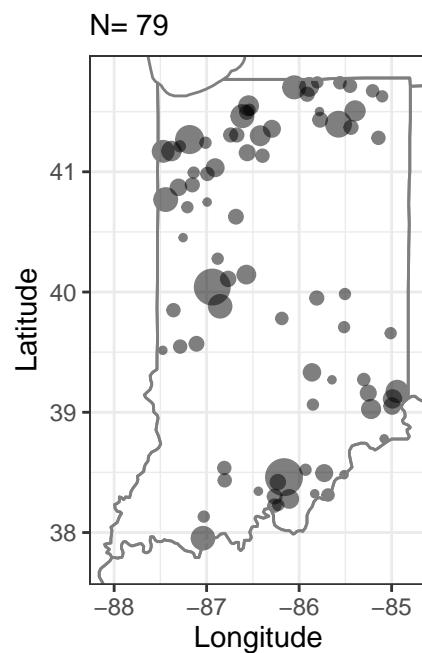






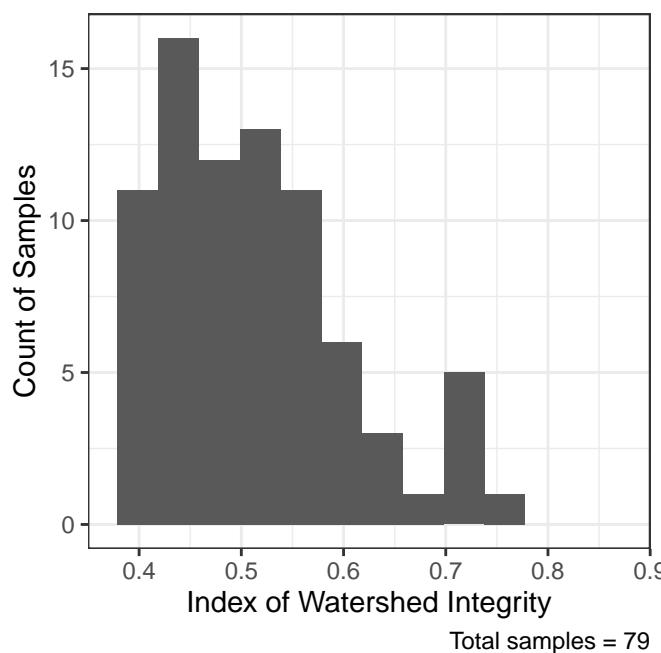


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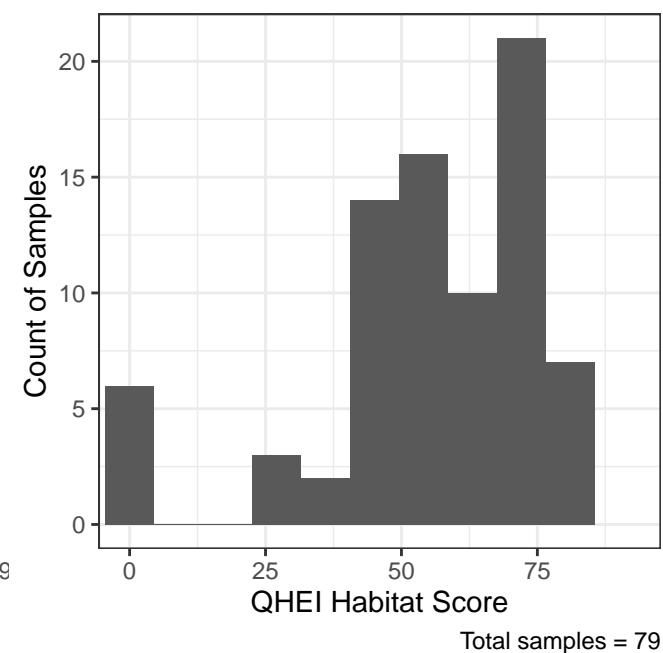


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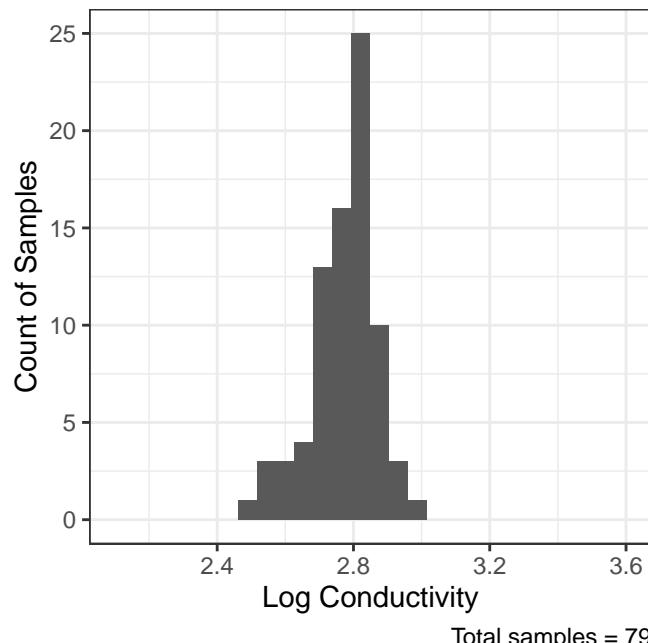
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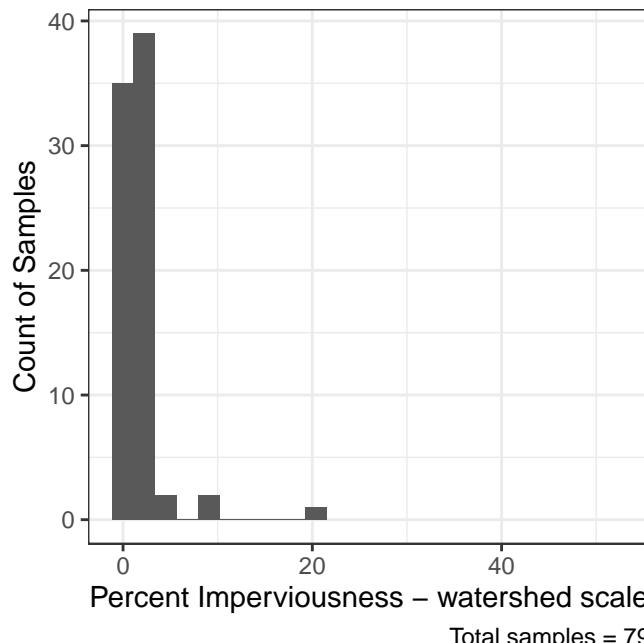
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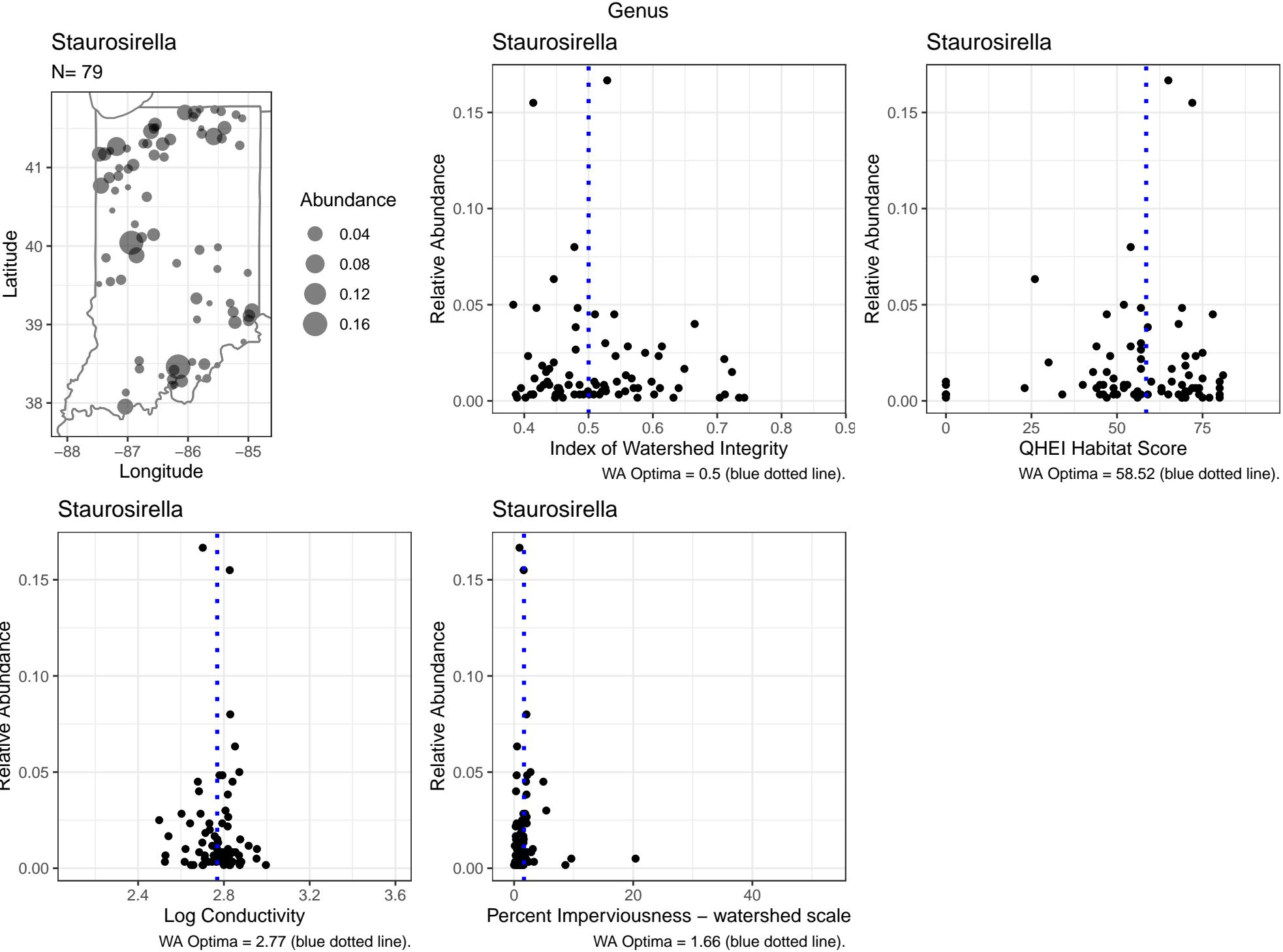


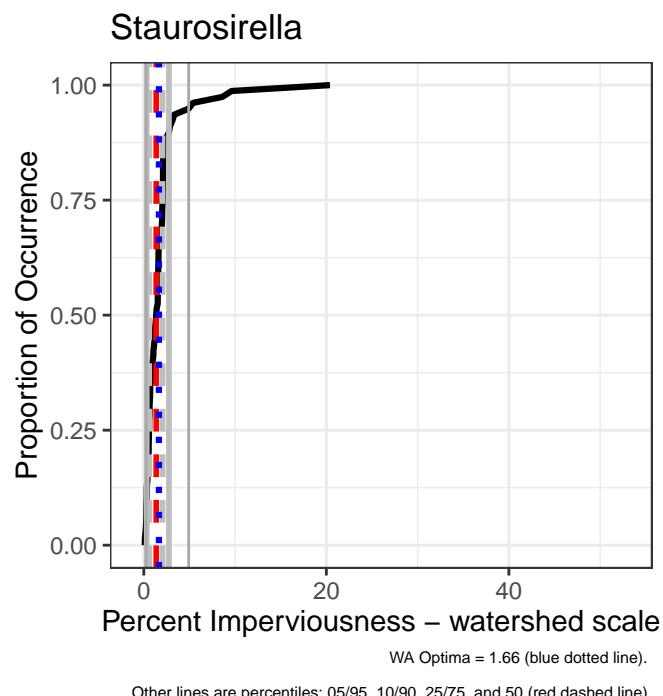
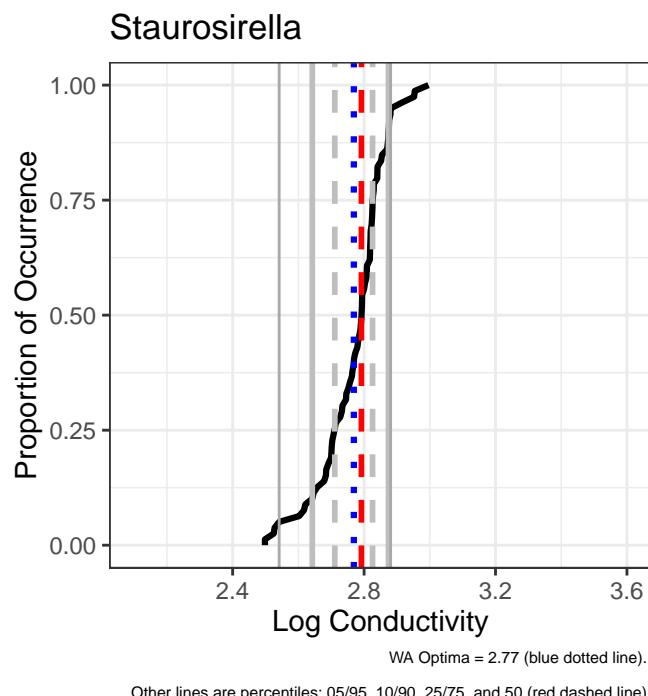
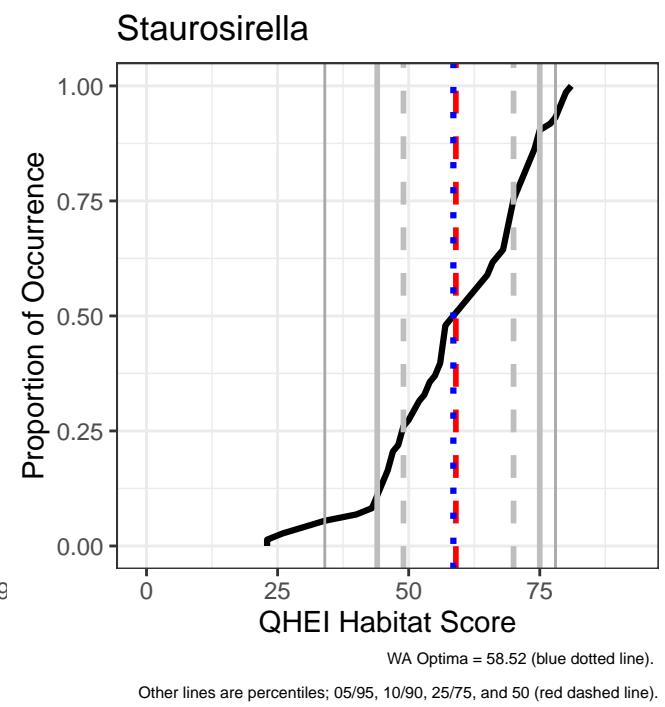
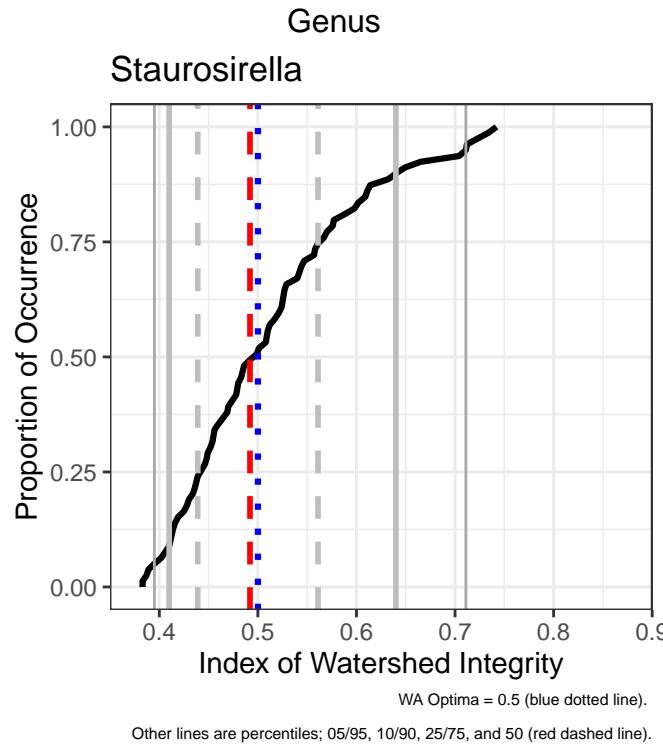
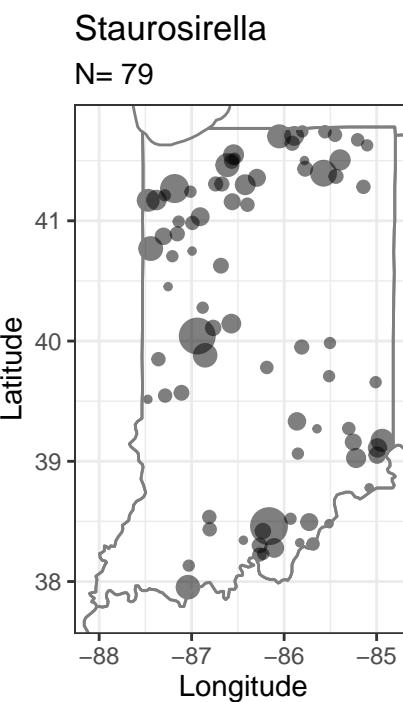
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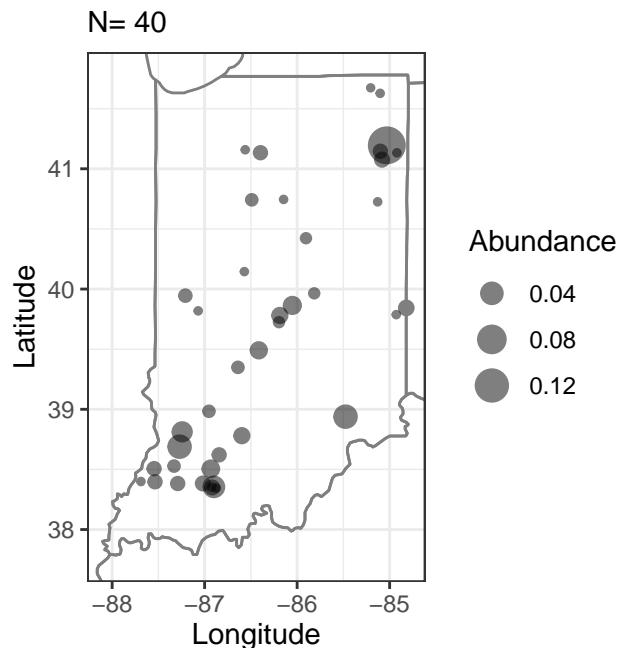
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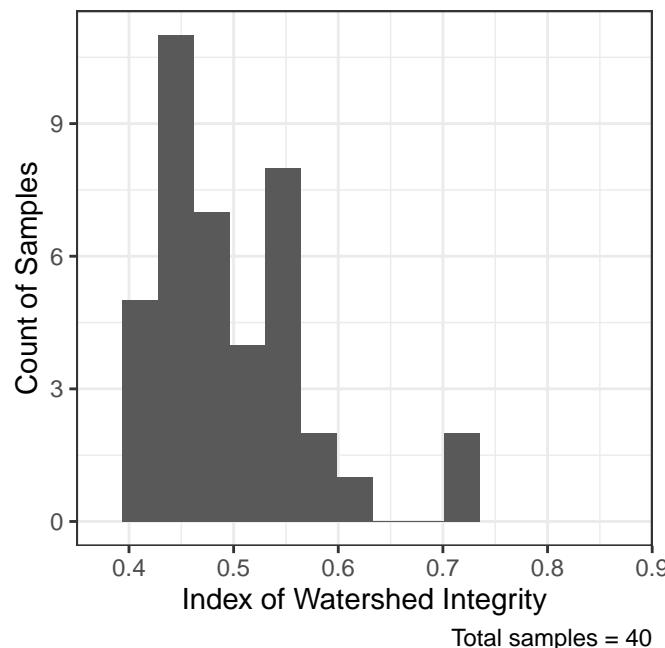


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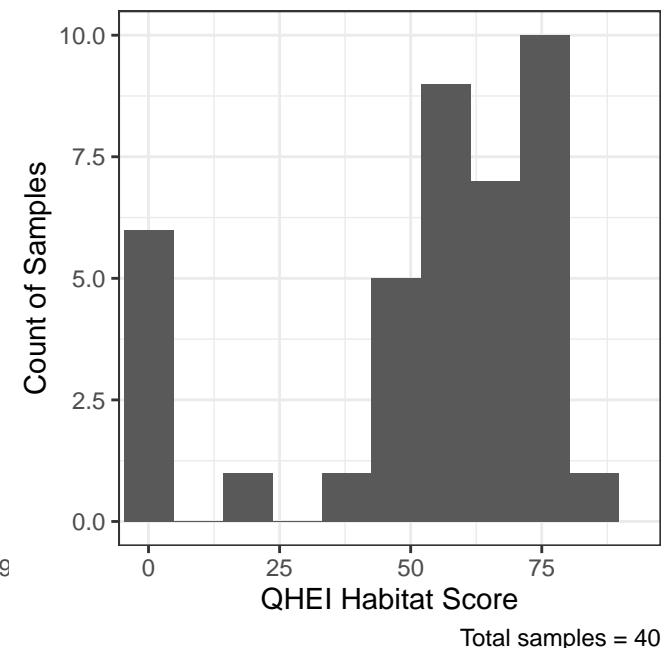


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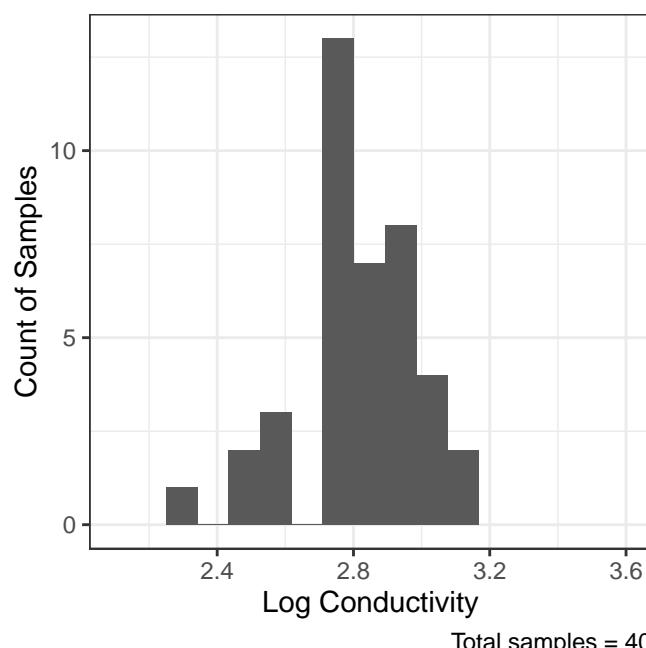
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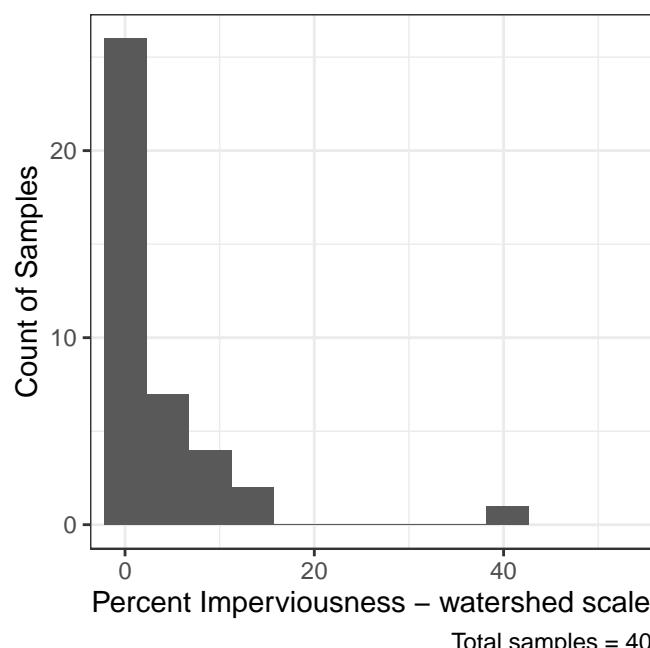
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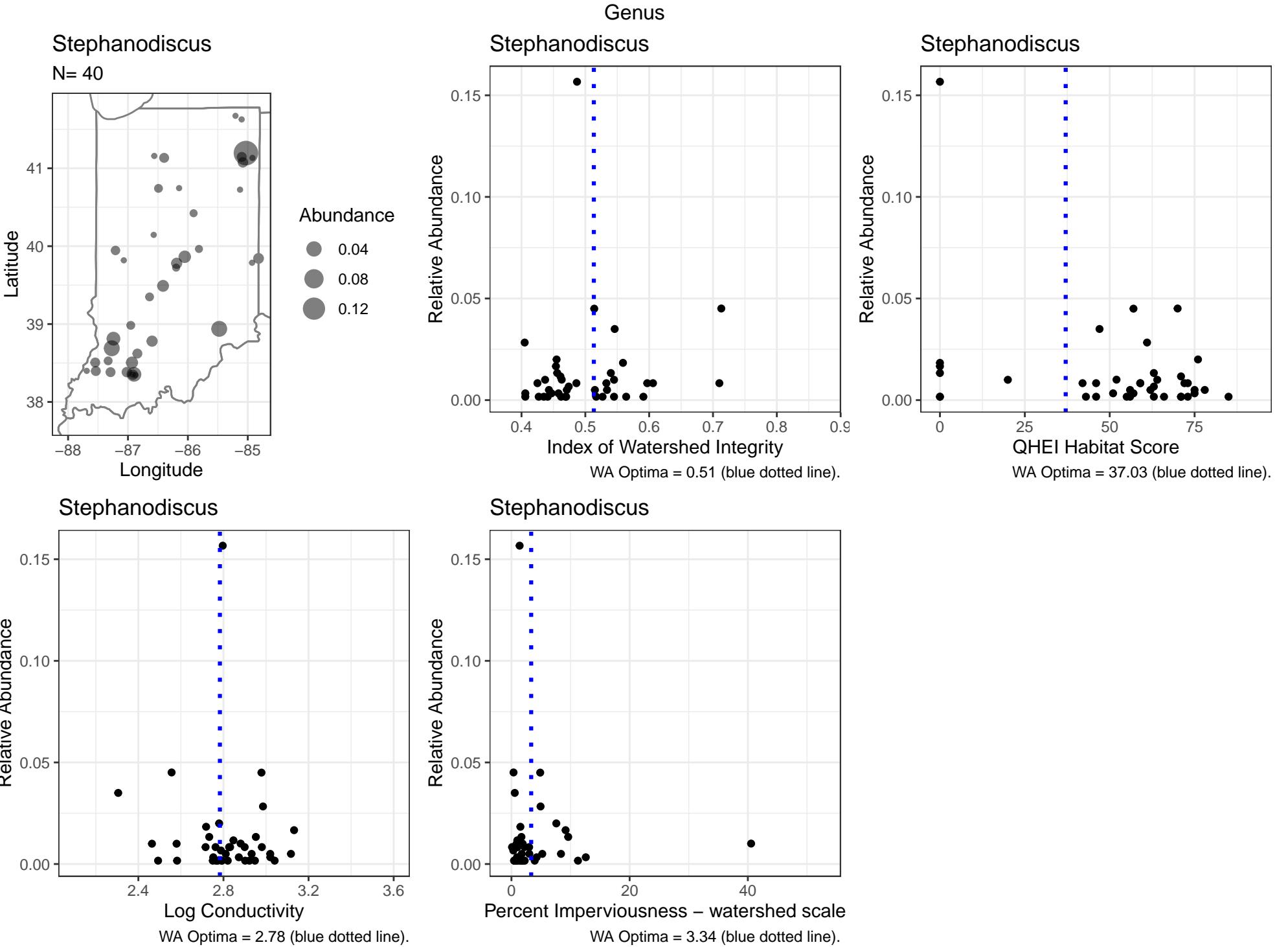


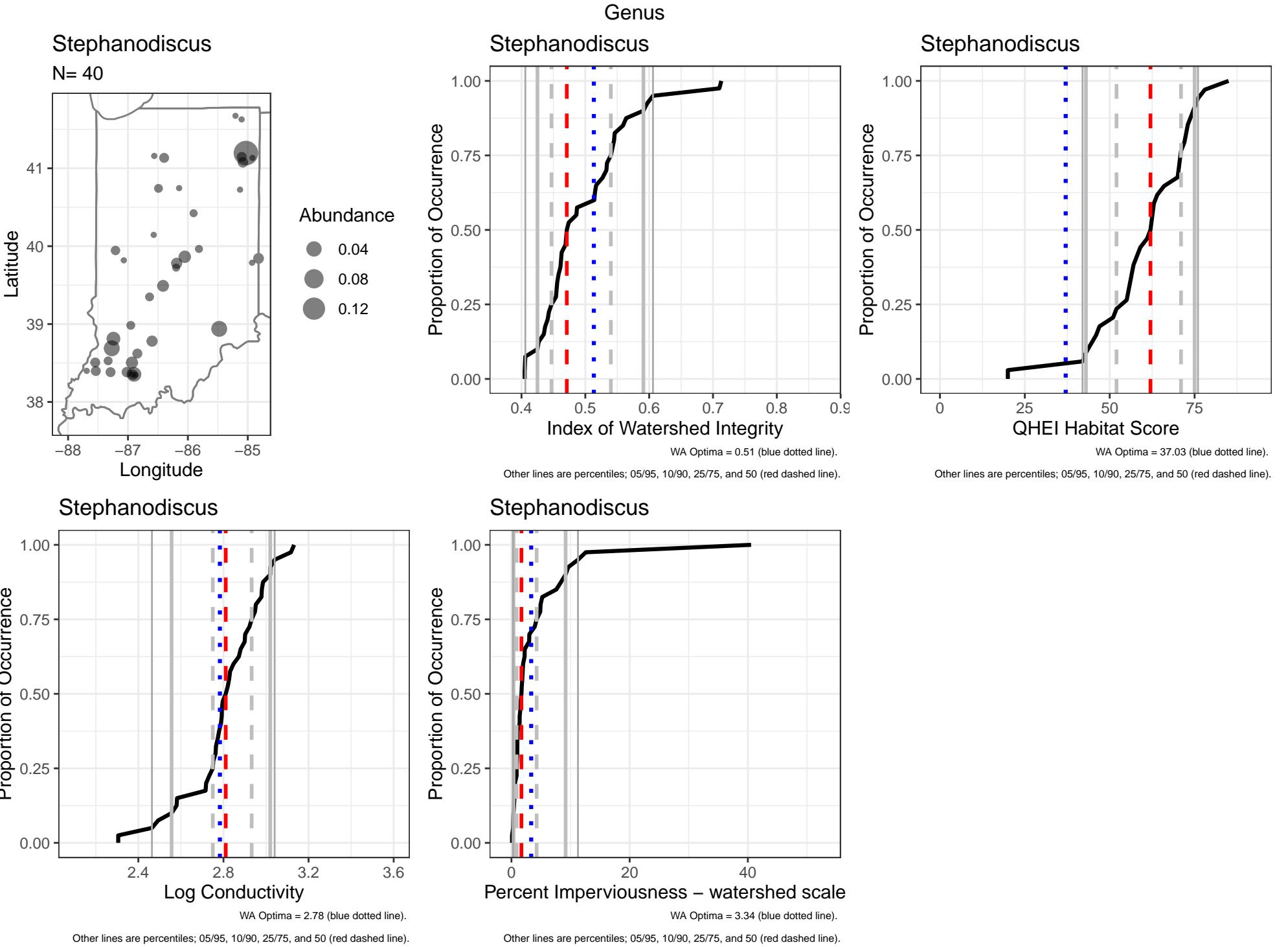
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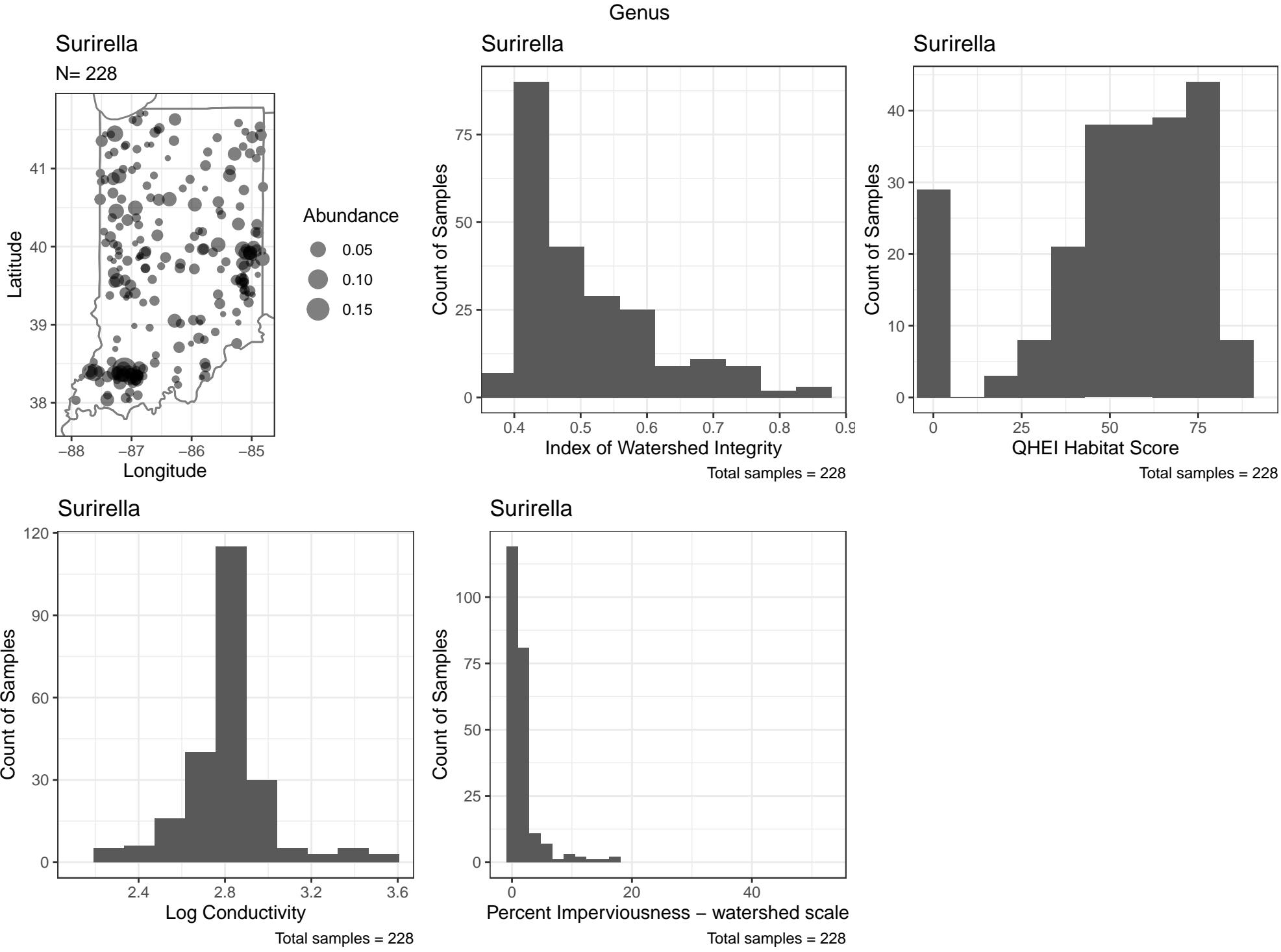


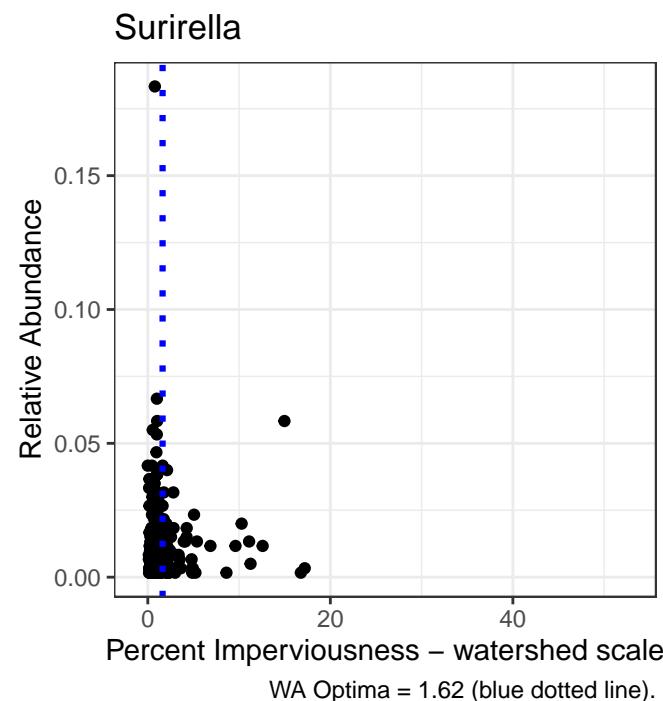
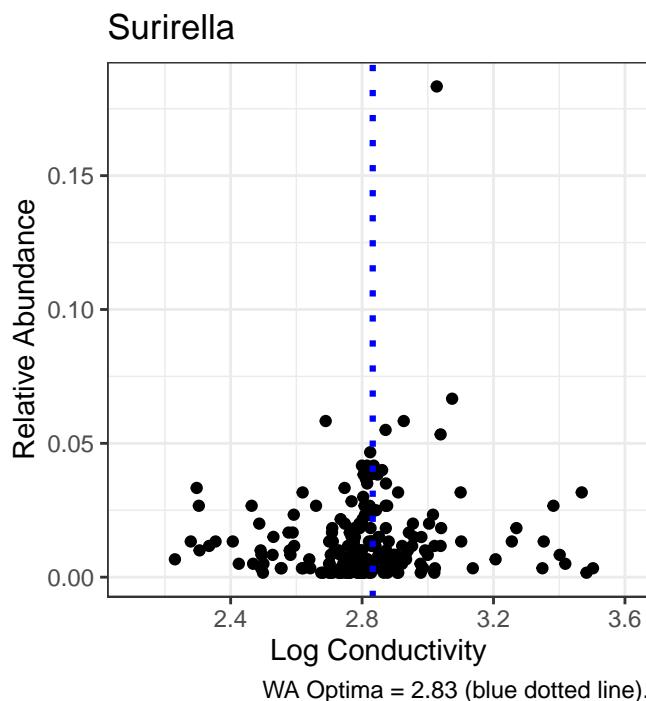
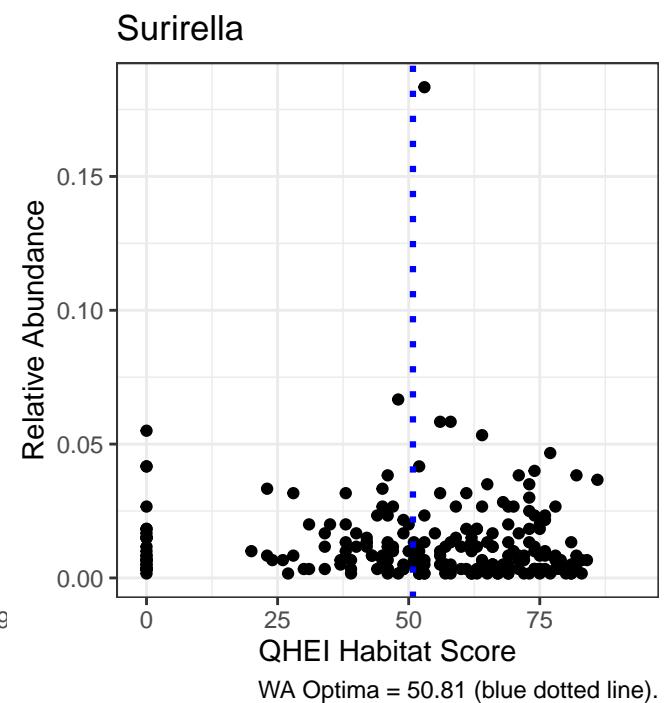
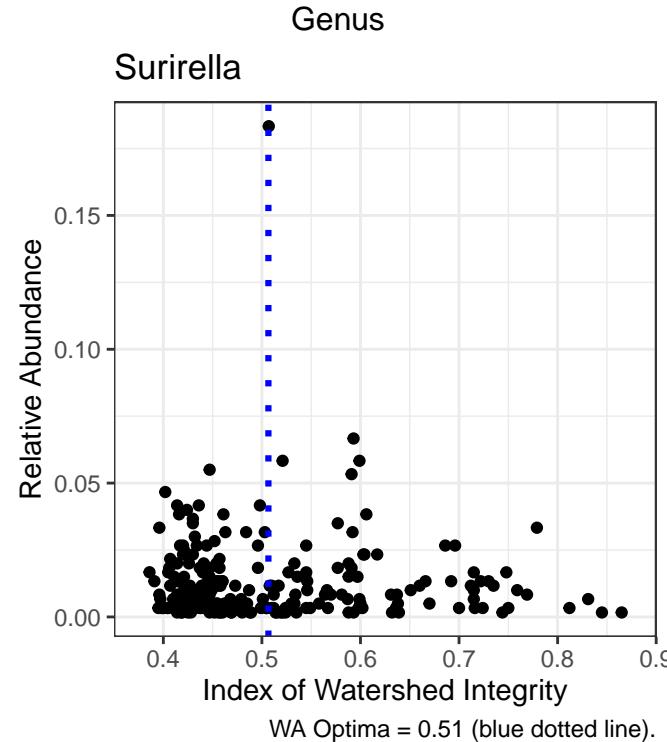
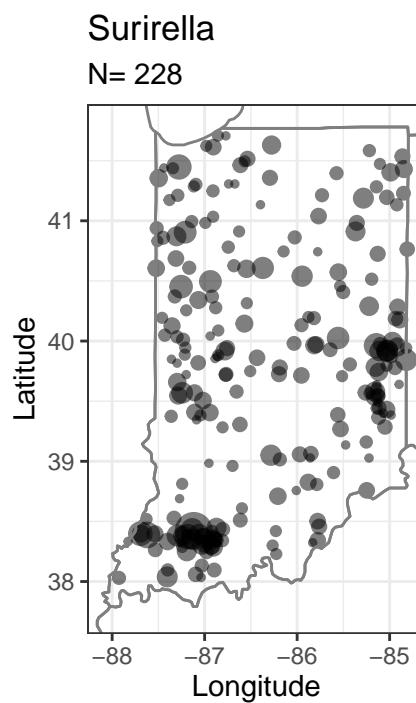
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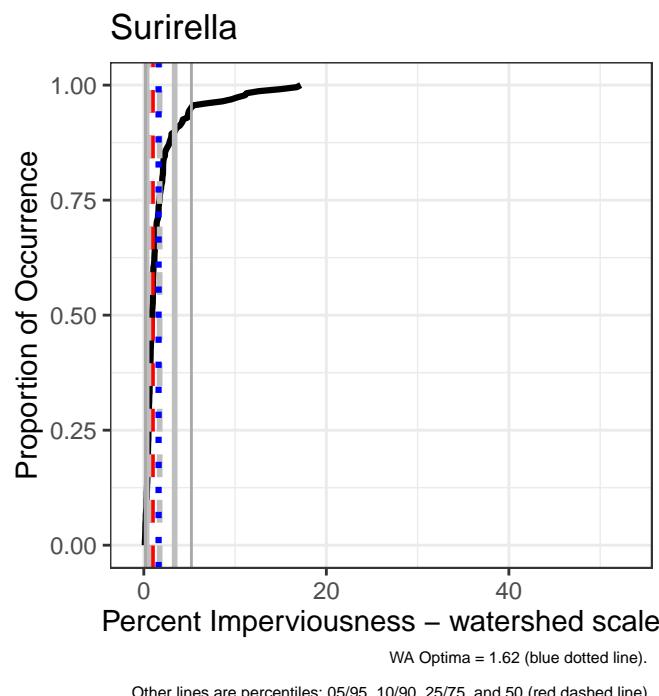
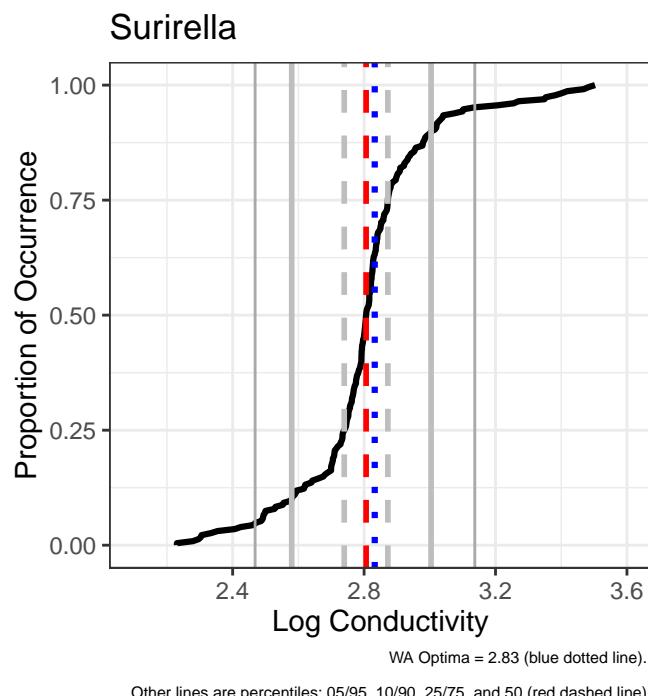
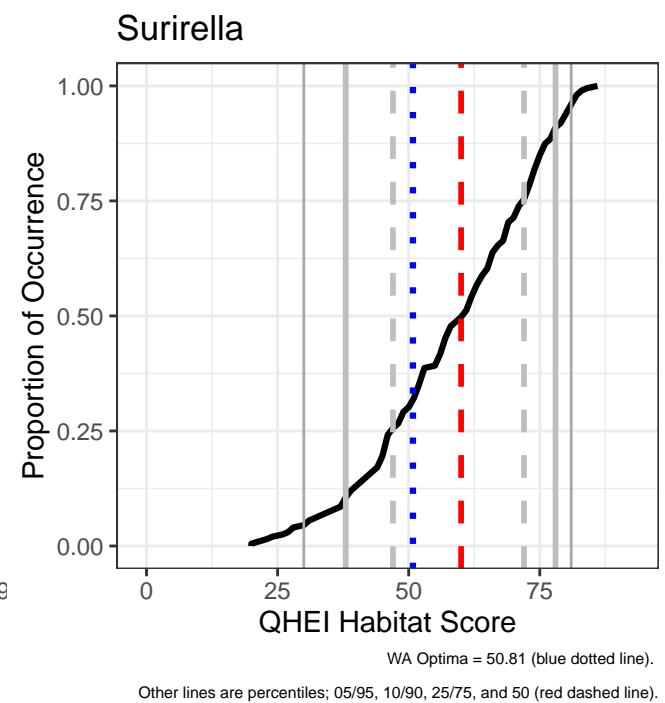
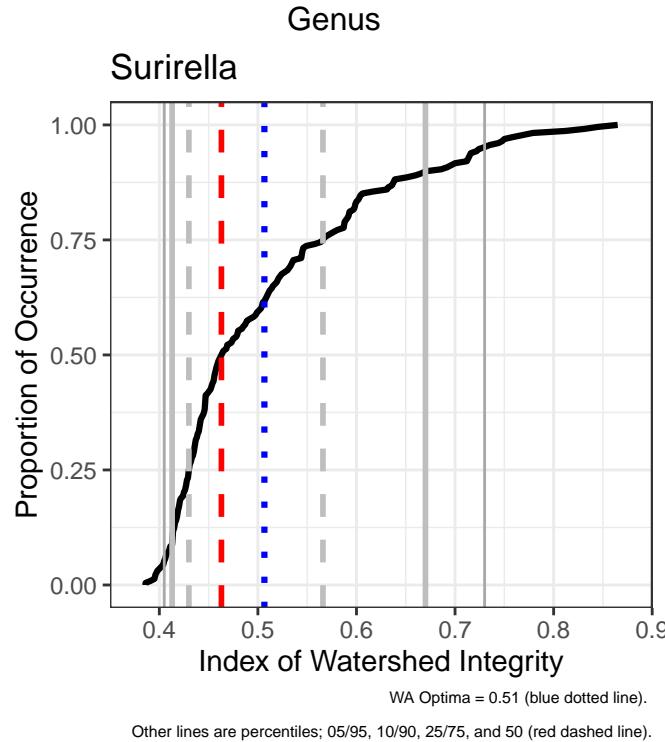
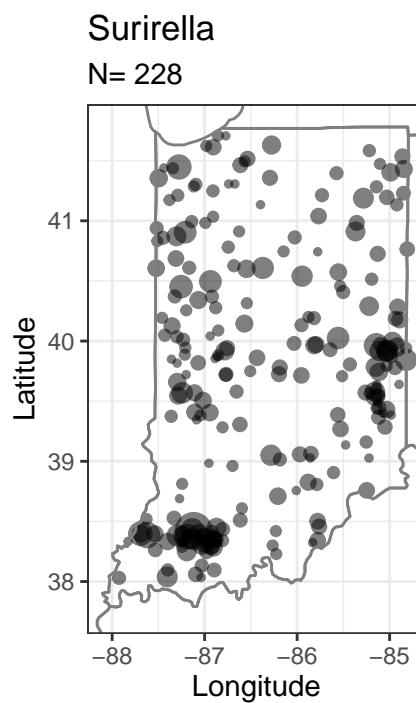


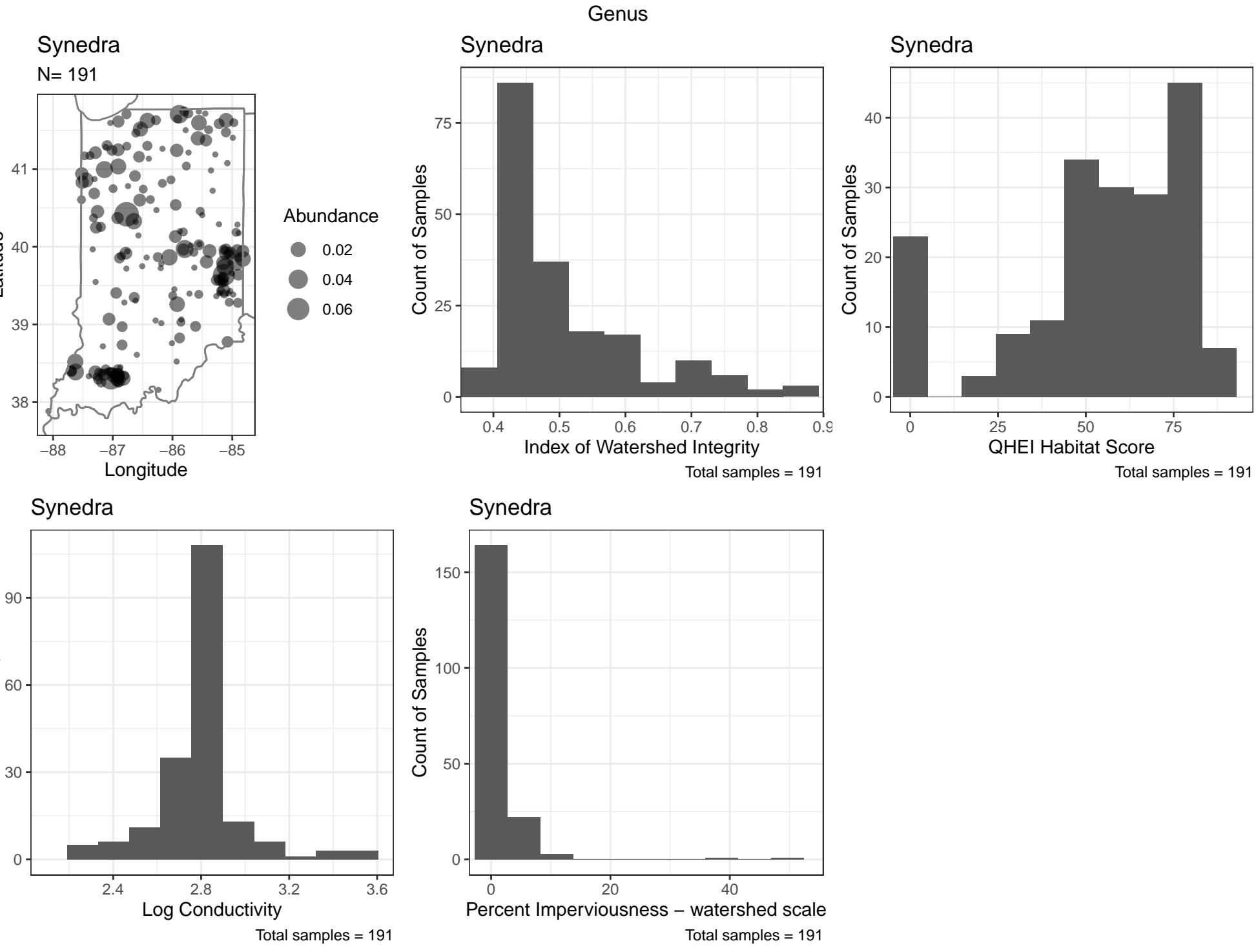


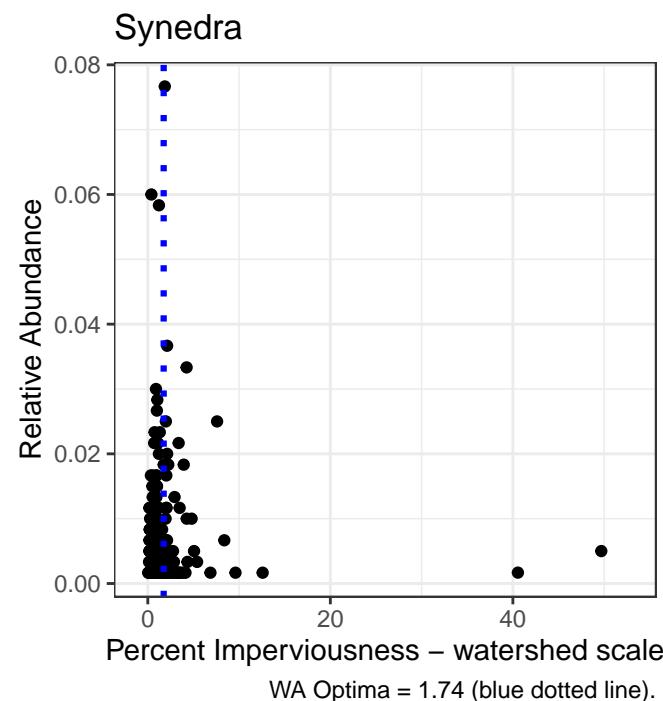
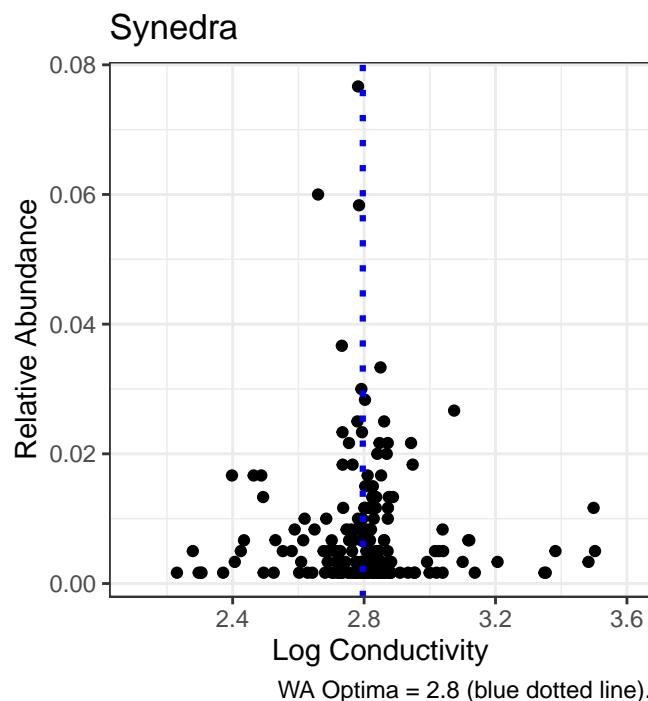
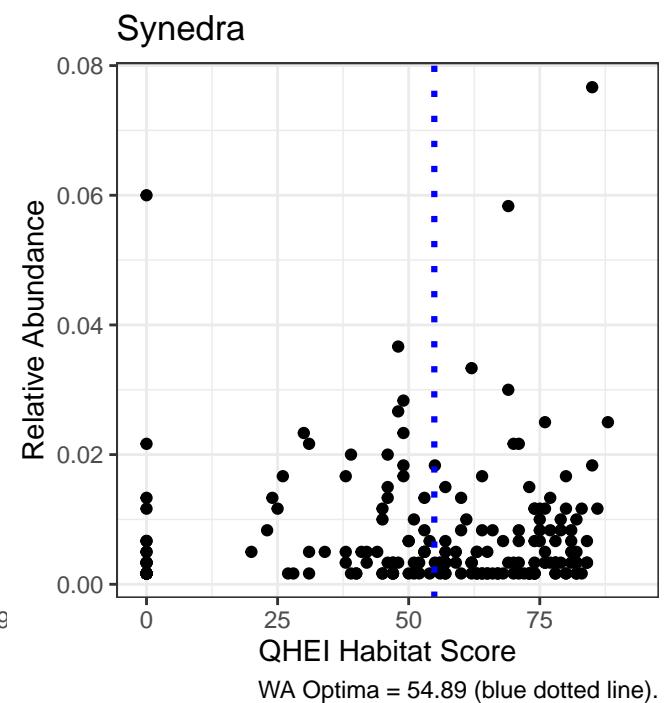
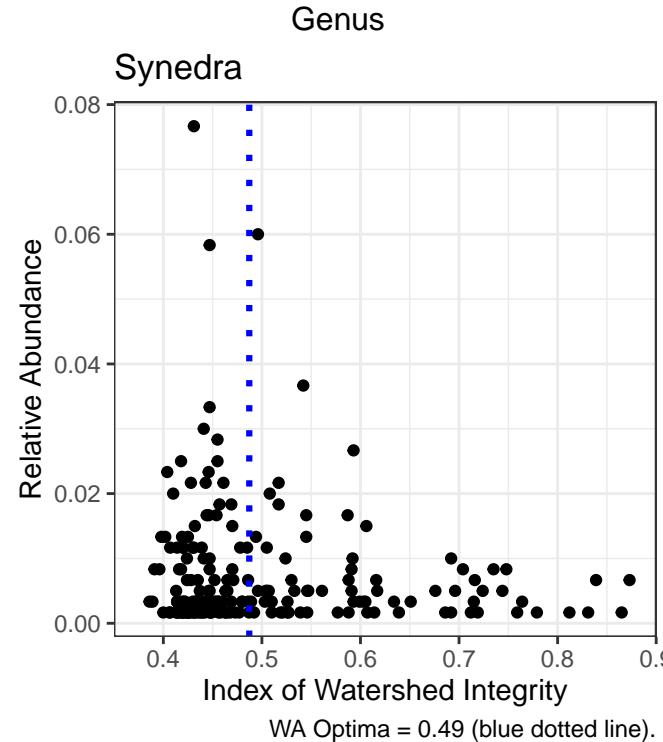
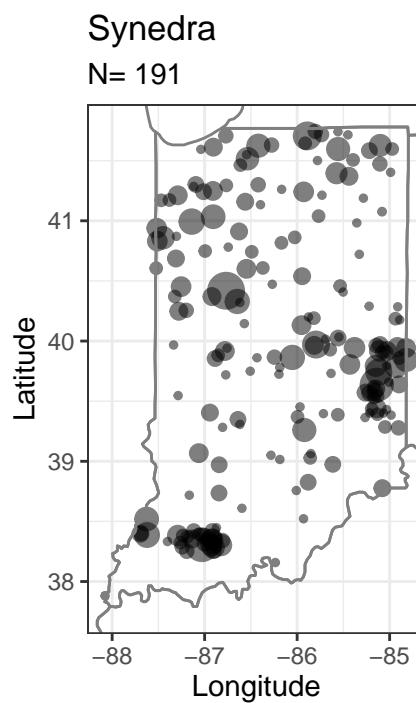


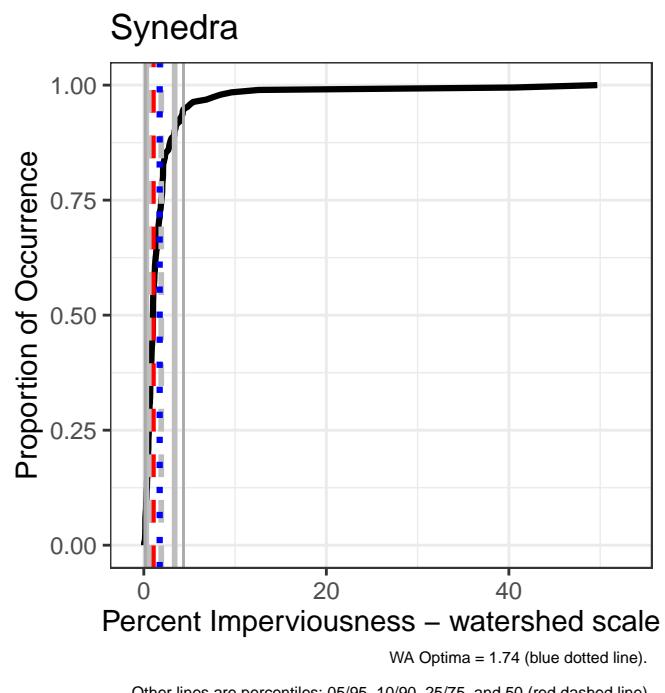
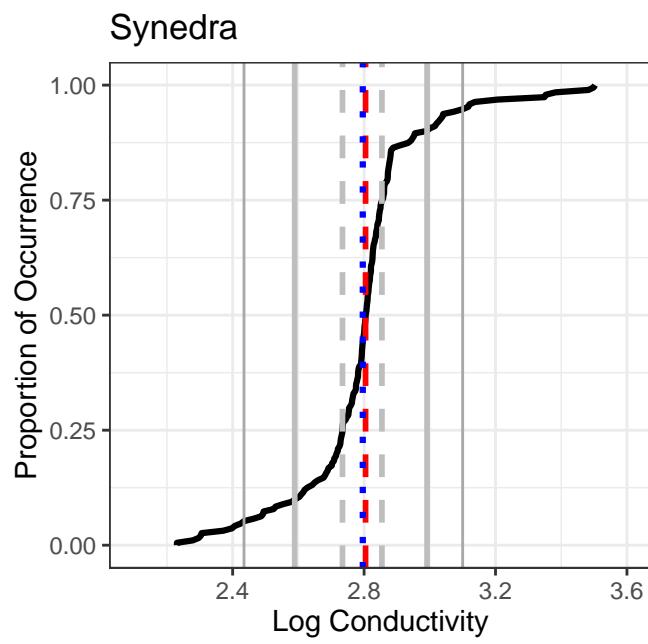
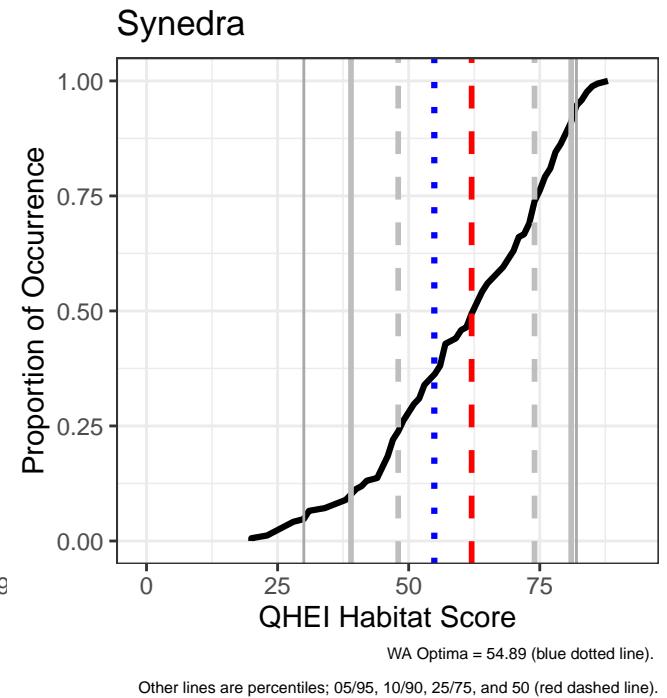
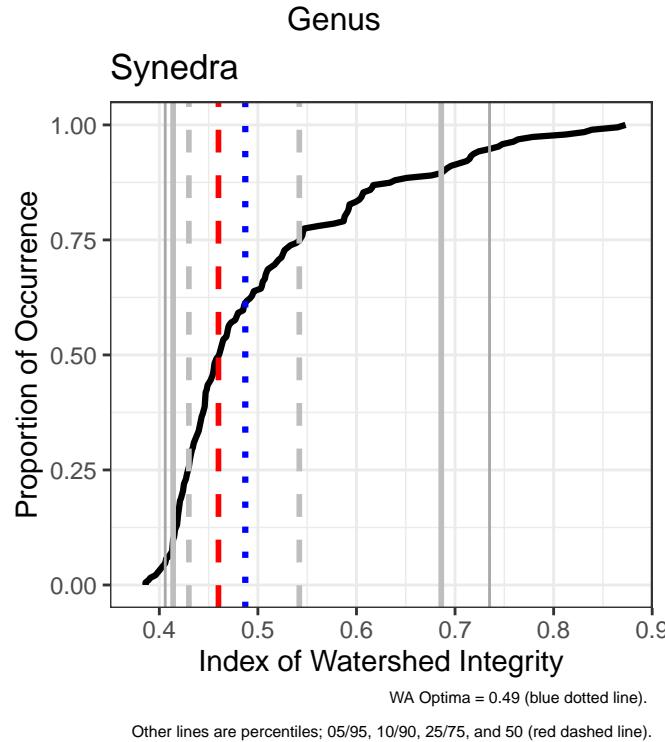
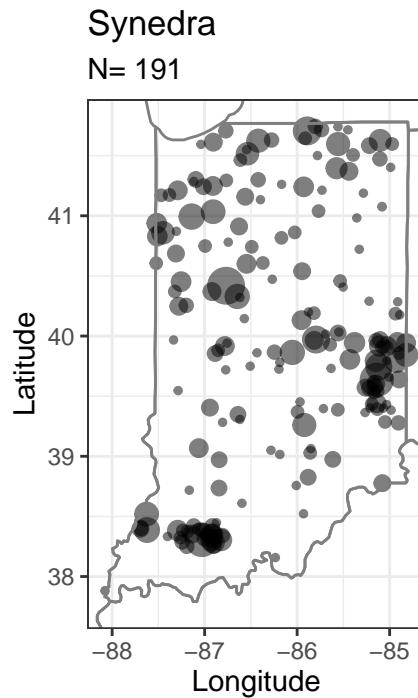


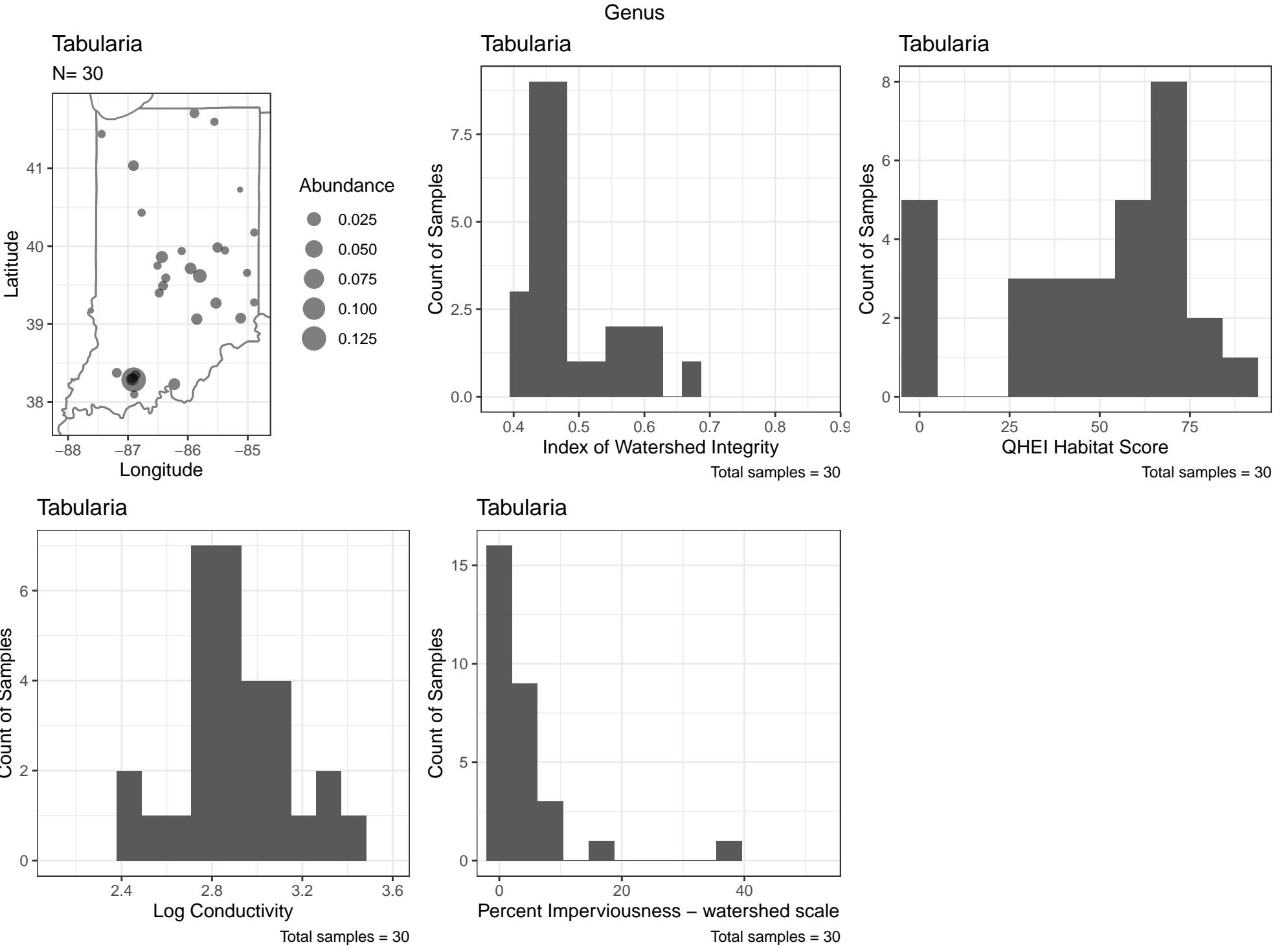


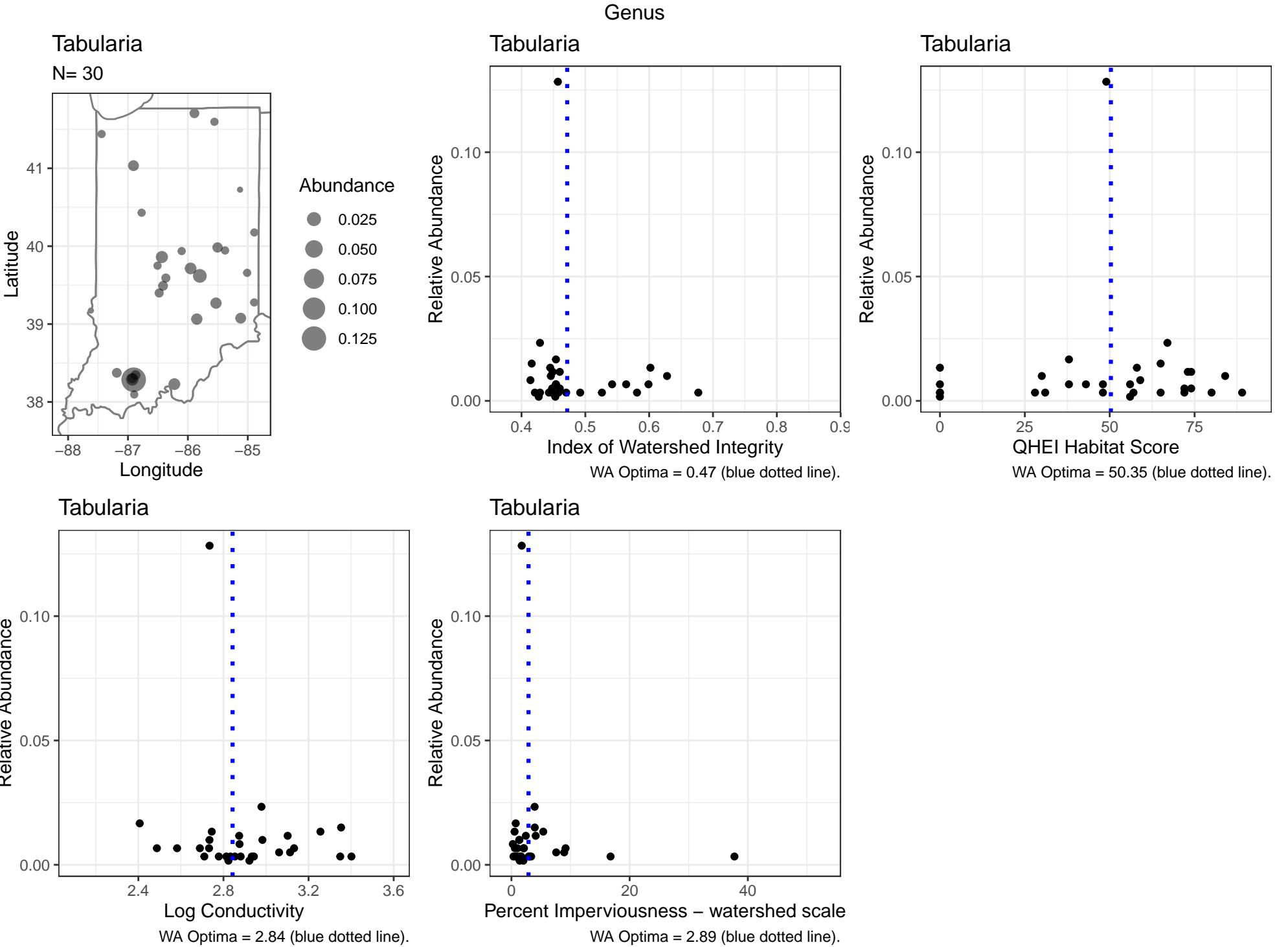


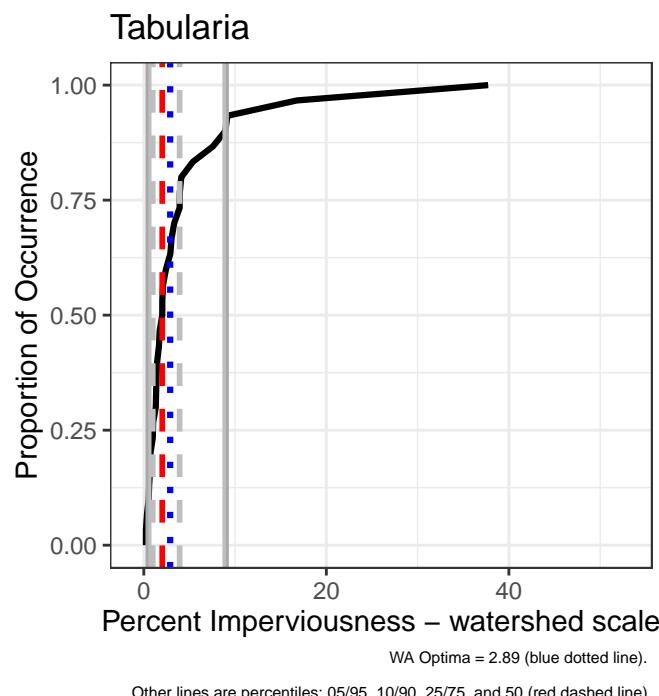
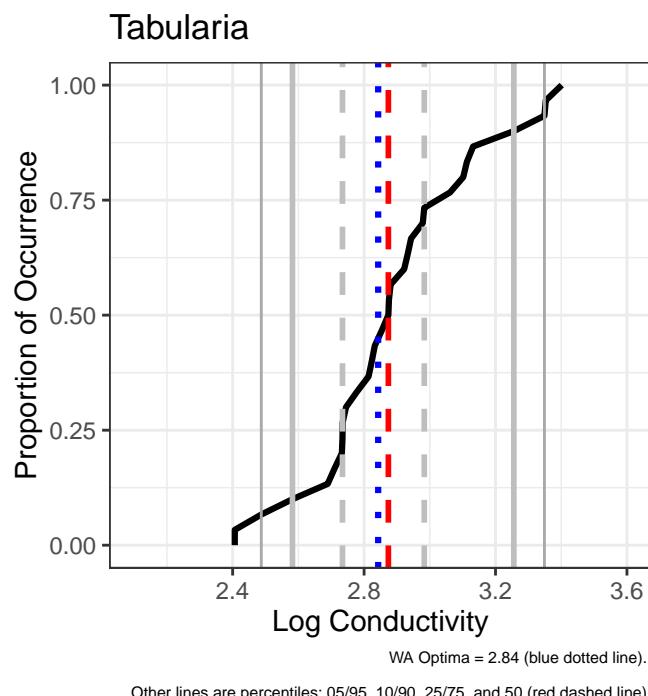
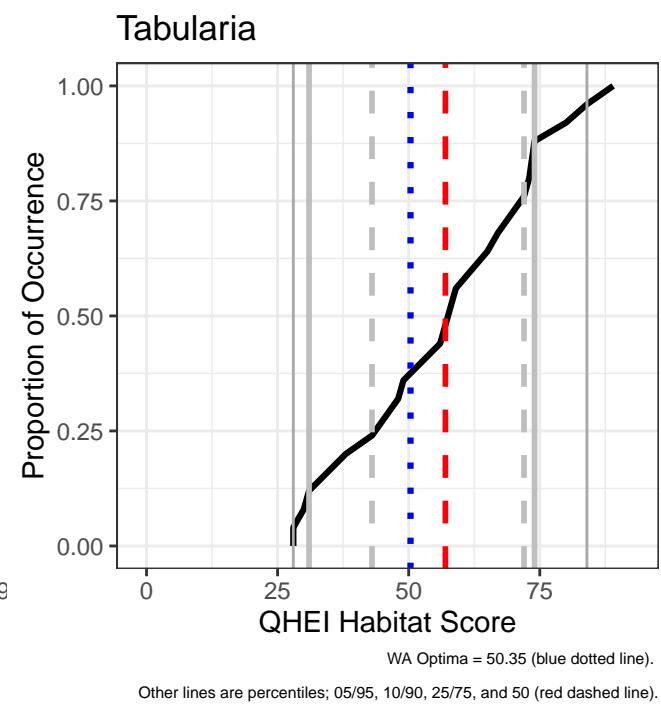
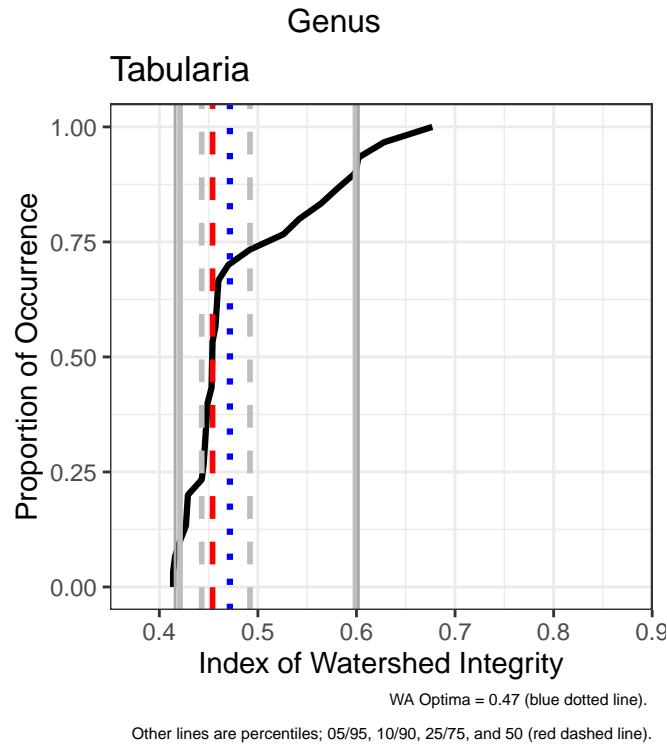
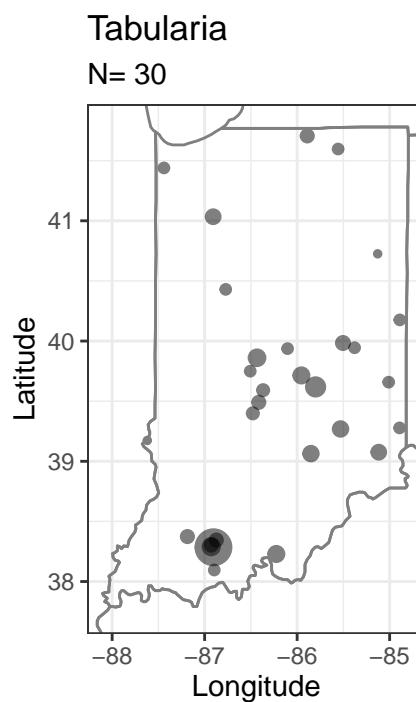


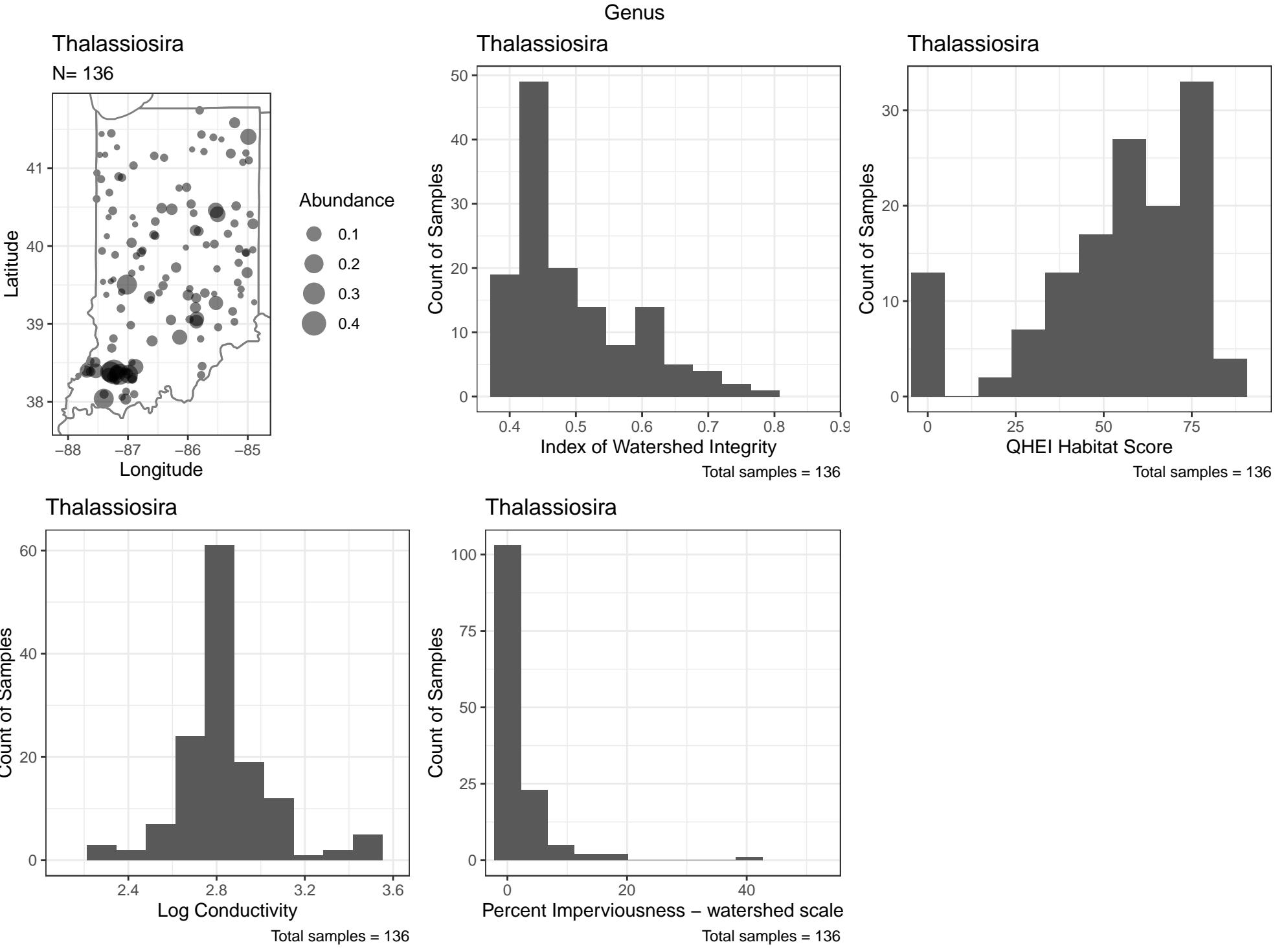


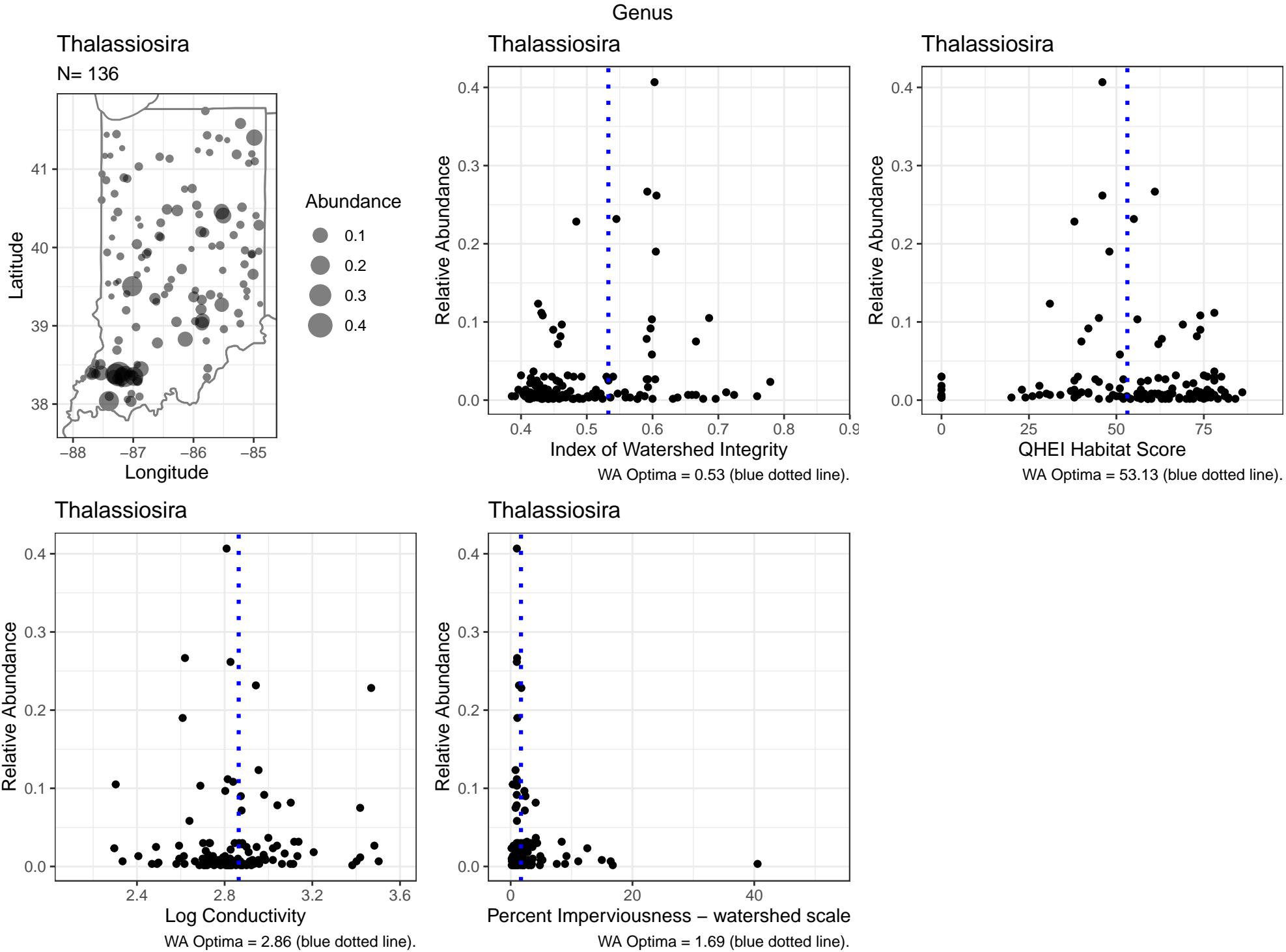


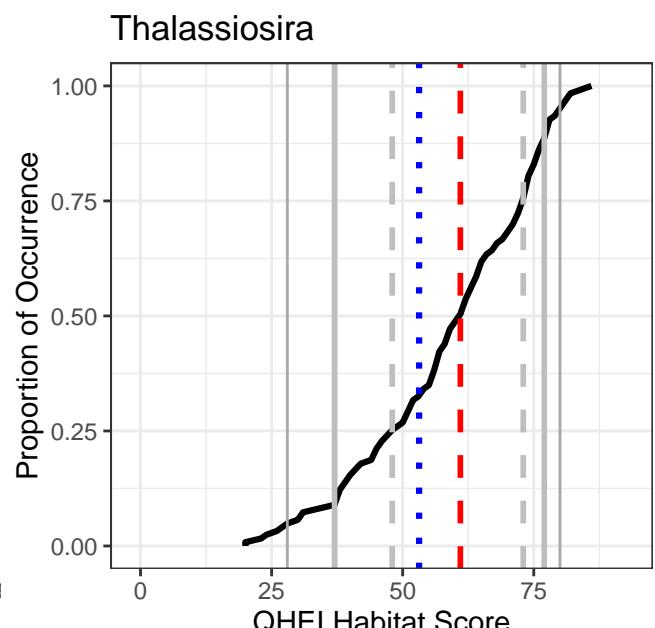
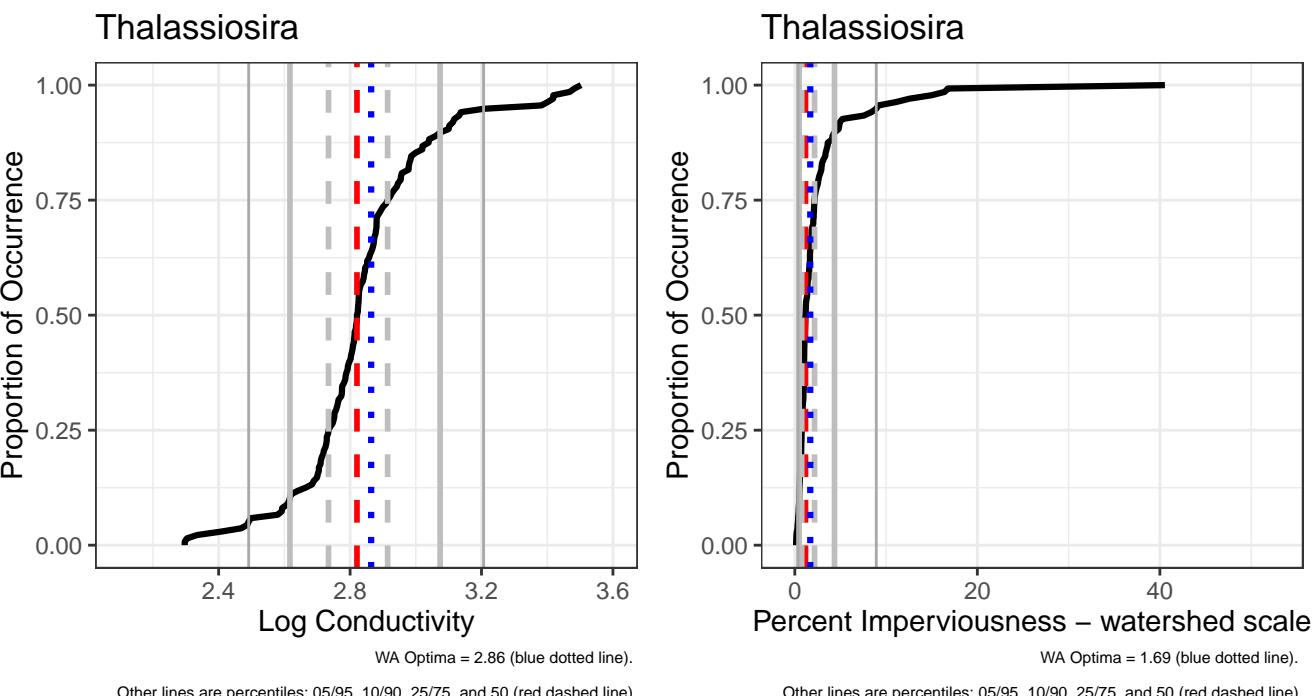
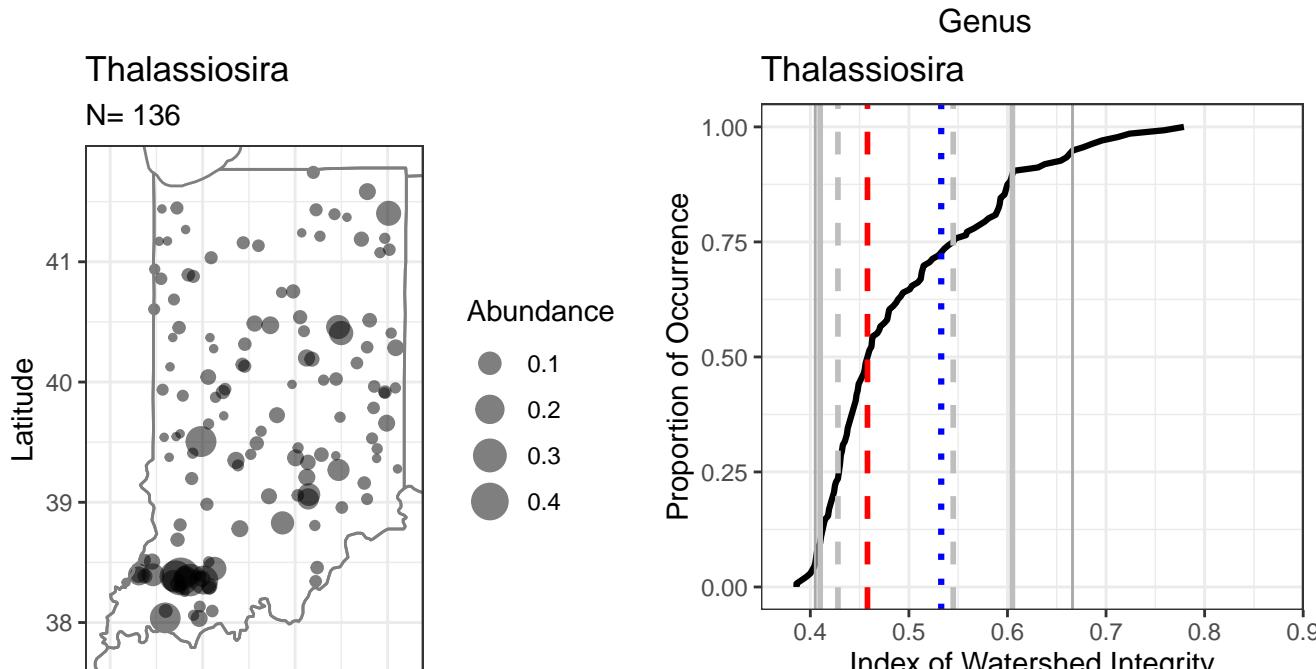
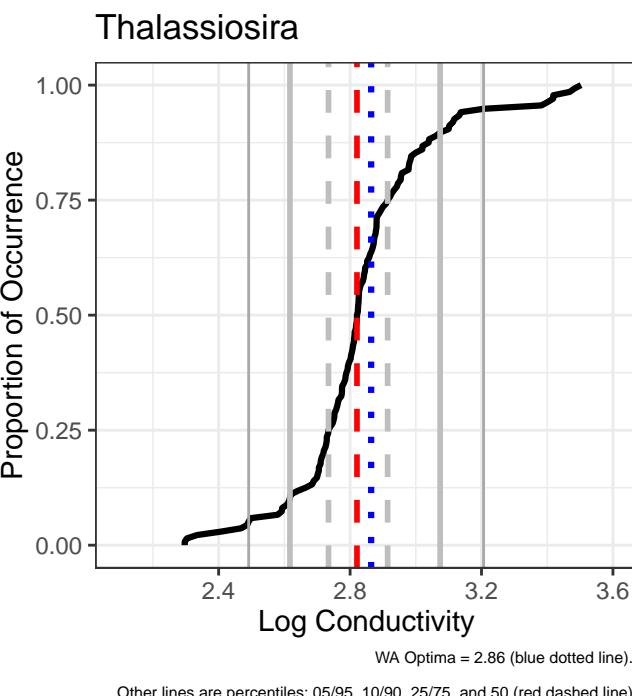
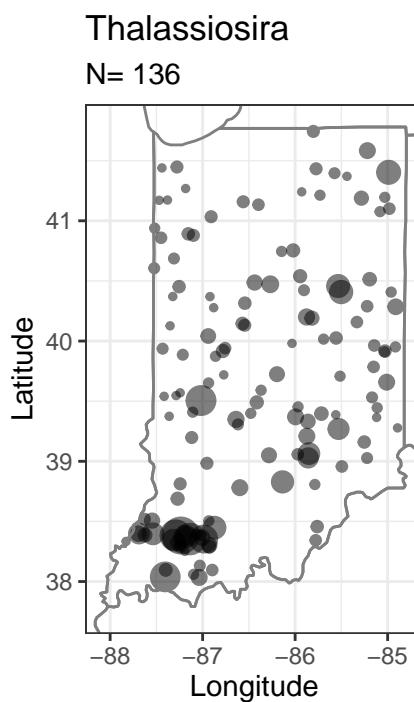


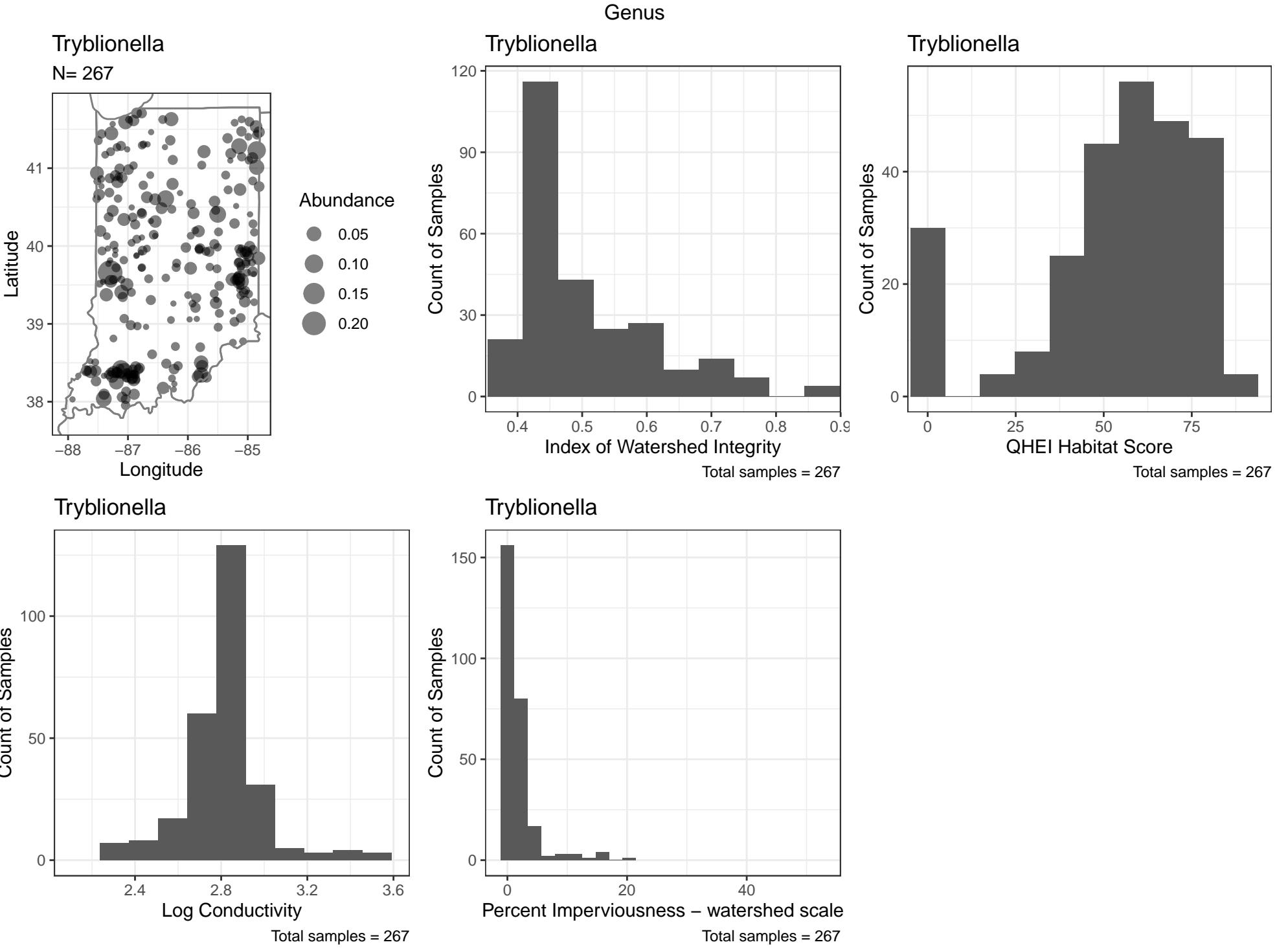


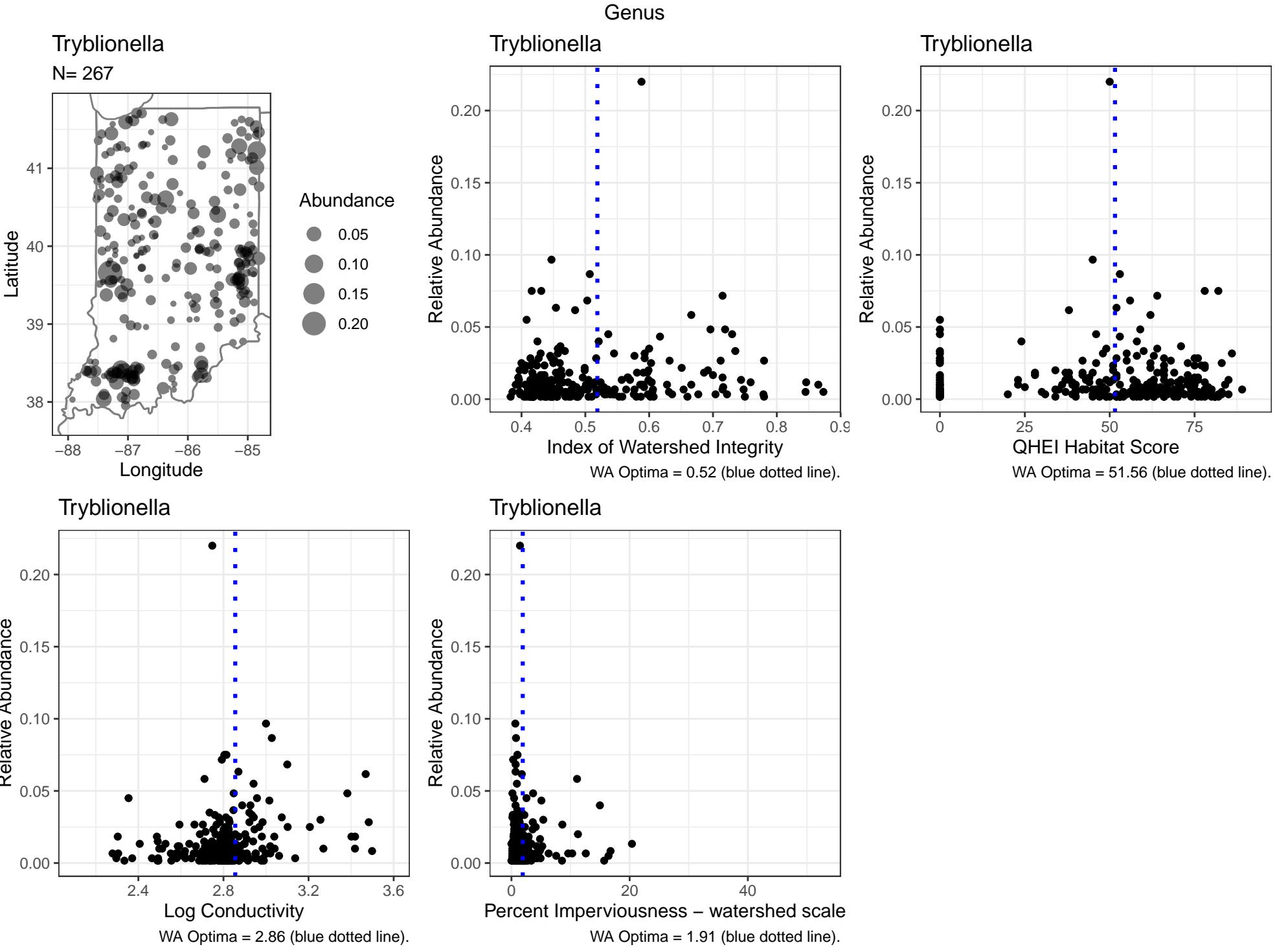


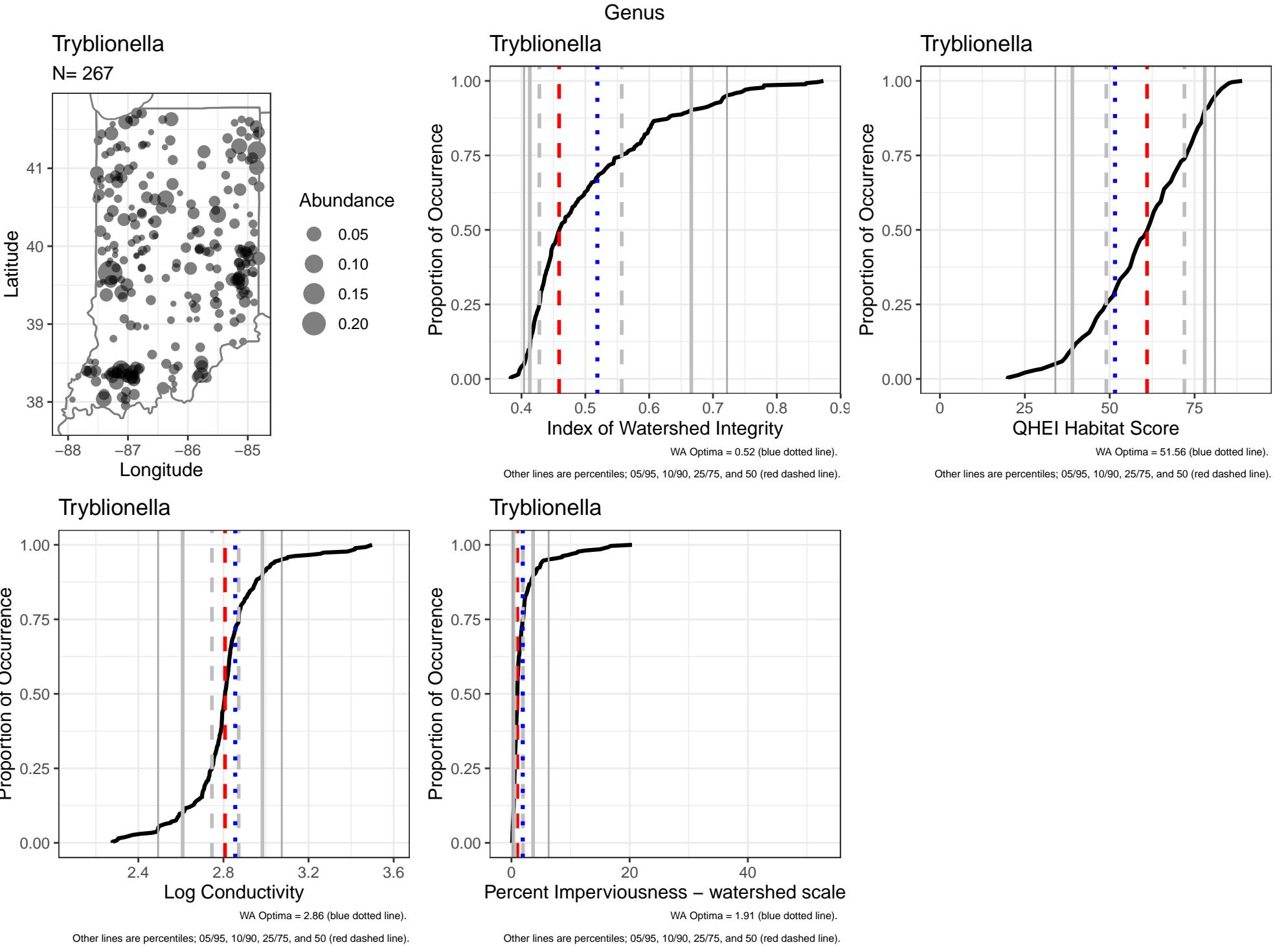




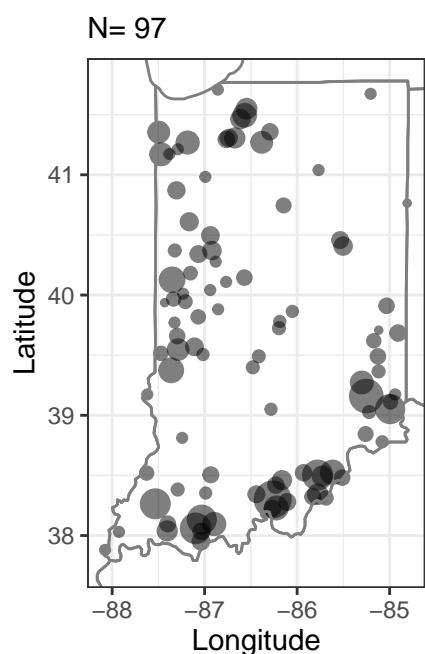






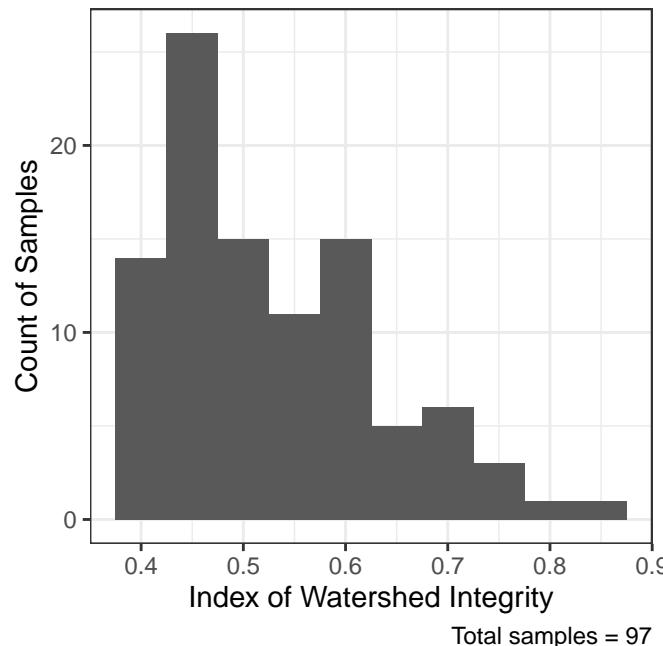


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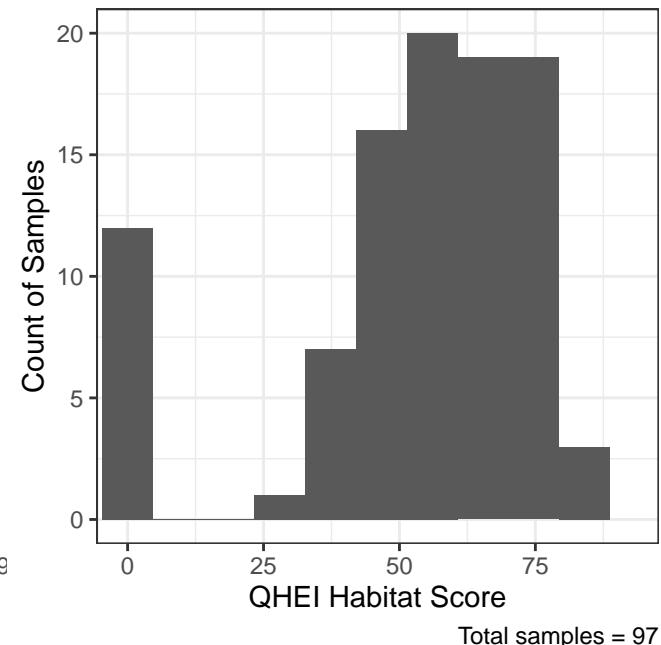


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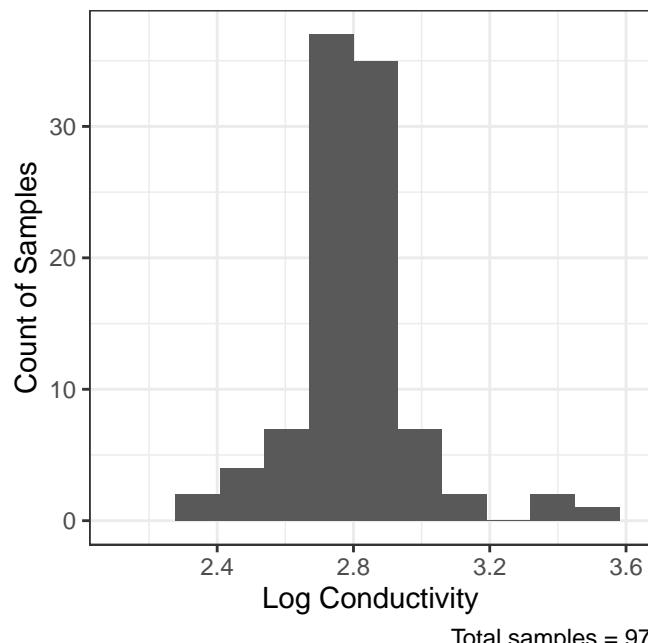
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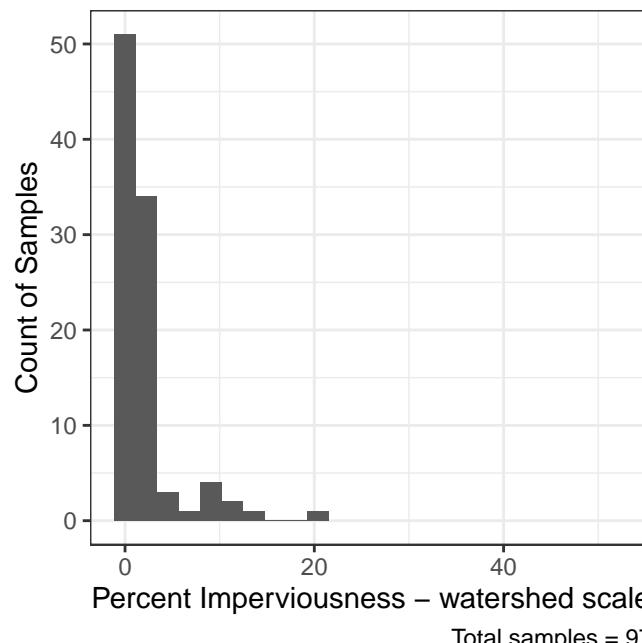
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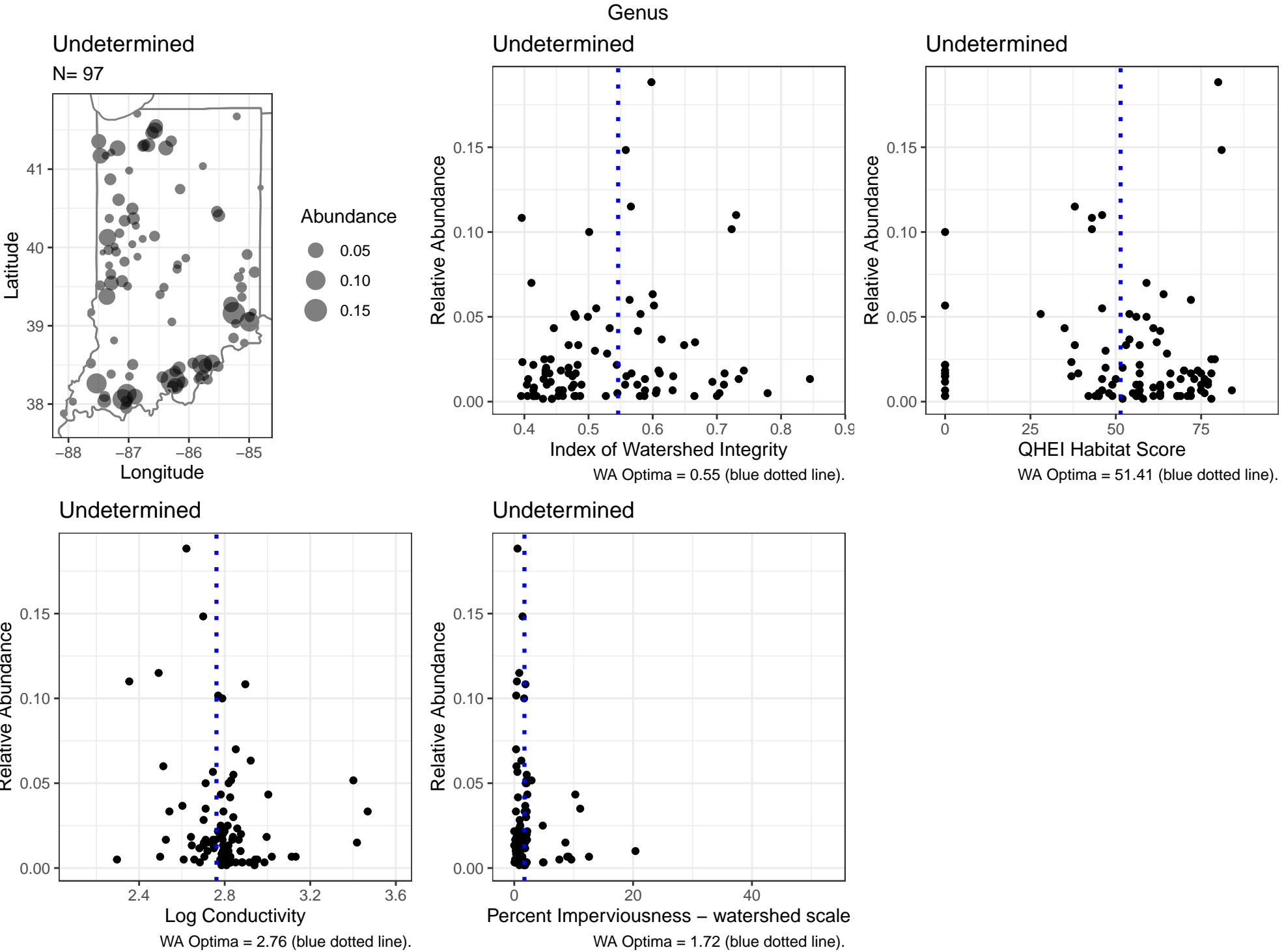


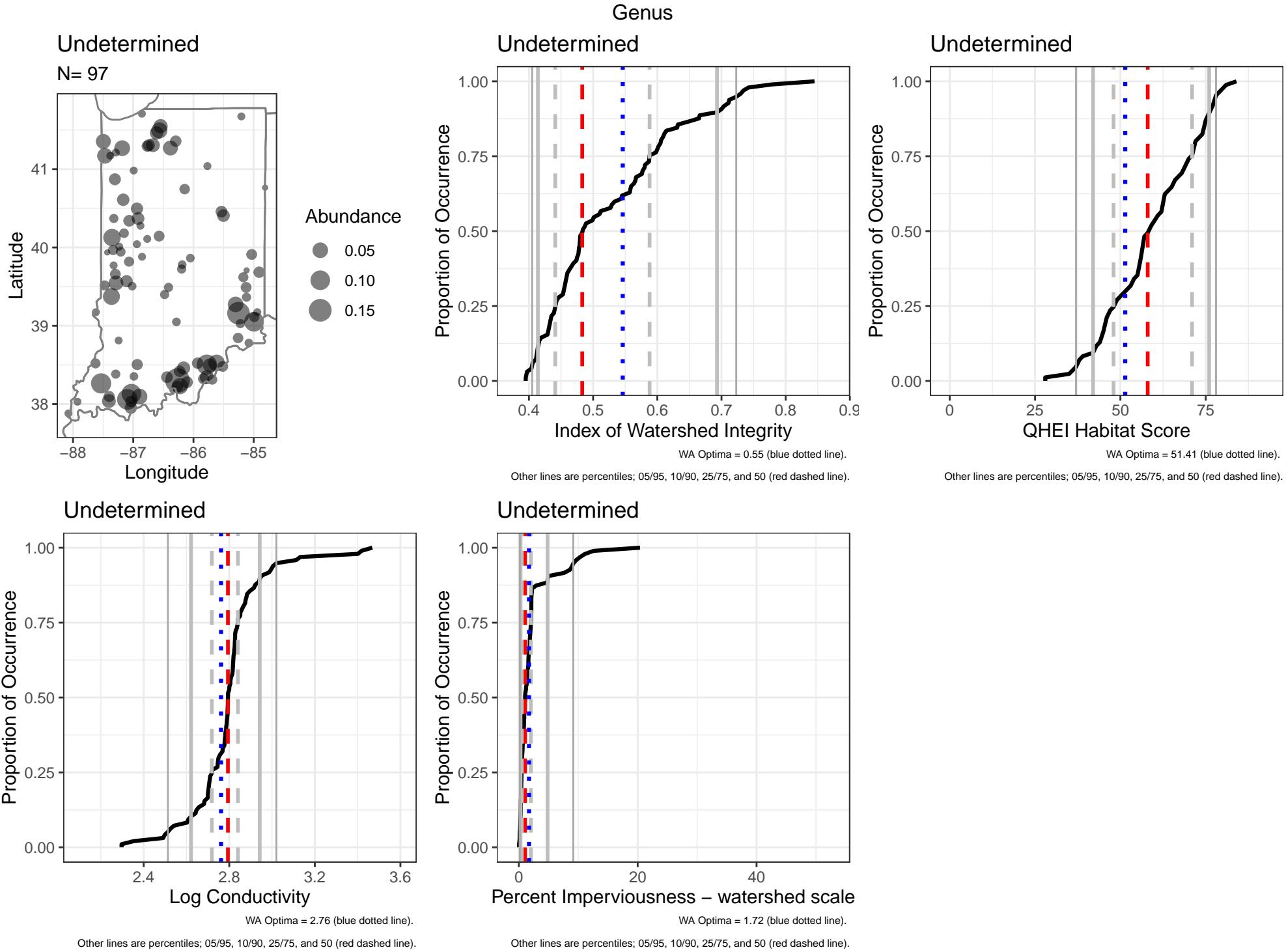
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# Appendix H

## Metric Discrimination and Precision

Calibration discrimination statistics (DE and Z-score in site classes)

Replicate precision statistics (CV = Coefficient of Variation from same-day samples)

NR No Response (DE <25)

WR Weak Response (DE <40)

MR Mixed Response (equal + and - trend)

DEC Decreasing trend with stress

INC Increasing trend with stress

MetCat	DataSet	Hi_N	Hi_N	Lo_N	Lo_N	CV%
		DE_25	Z_score	DE (trend)	Z_score	
Acidity	wa_OptCat_pH	50 (DEC)	0.82	45.5 (INC)	-0.25	0.4
Acidity	wa_pH	41.3 (INC)	-0.34	WR	-0.05	4.6
BC	nt_BC_1	NR	0.27	81.8 (DEC)	1.49	41.8
BC	nt_BC_12	NR	0.35	90.9 (DEC)	1.91	27.3
BC	nt_BC_2	NR	0.21	81.8 (DEC)	1.54	40.1
BC	nt_BC_3	NR	-0.03	WR	0.18	19.3
BC	nt_BC_4	MR	-0.03	45.5 (DEC)	0.44	15.0
BC	nt_BC_45	45.7 (INC)	-0.14	WR	0.26	12.4
BC	nt_BC_5	43.5 (INC)	-0.23	WR	0.02	19.8
BC	pi_BC_1	NR	-0.09	63.6 (DEC)	0.75	99.2
BC	pi_BC_12	MR	-0.64	90.9 (DEC)	0.89	59.1
BC	pi_BC_2	41.3 (DEC)	-1.01	81.8 (DEC)	1.28	63.2
BC	pi_BC_3	NR	-0.13	NR	0.52	35.6
BC	pi_BC_4	NR	0.04	WR	-0.24	24.3
BC	pi_BC_45	WR	0.25	45.5 (INC)	-0.52	11.0
BC	pi_BC_5	NR	0.10	45.5 (INC)	-0.53	29.5
BC	pt_BC_1	41.3 (DEC)	0.36	90.9 (DEC)	1.26	45.6
BC	pt_BC_12	50 (DEC)	0.55	81.8 (DEC)	1.43	26.2
BC	pt_BC_12_adj	54.3 (DEC)	0.60			NA
BC	pt_BC_2	45.7 (DEC)	0.30	72.7 (DEC)	0.96	38.6
BC	pt_BC_3	MR	-0.17	WR	-0.72	17.9
BC	pt_BC_4	WR	0.13	45.5 (INC)	-0.43	11.8
BC	pt_BC_45	50 (INC)	-0.12	54.5 (INC)	-0.73	7.3
BC	pt_BC_5	WR	-0.25	54.5 (INC)	-0.67	17.1
BC	WA_BC	NR	0.16	45.5 (INC)	-0.35	5.4

MetCat	DataSet	Hi_N	Hi_N	Lo_N	Lo_N	CV%
		DE_25	Z_score	DE (trend)	Z_score	
DO	nt_O_1	NR	-0.22	45.5 (DEC)	0.76	25.6
DO	nt_O_2	NR	0.11	WR	0.20	21.1
DO	nt_O_3	NR	-0.03	NR	0.33	16.2
DO	nt_O_4	43.5 (INC)	-0.41	WR	-0.25	26.9
DO	nt_O_45	wr	-0.38	45.5 (INC)	-0.28	23.5
DO	nt_O_5	NR	-0.11	NR	-0.26	41.0
DO	pi_O_1	NR	-0.35	90.9 (DEC)	0.84	36.8
DO	pi_O_12	WR	-0.35	45.5 (DEC)	0.63	23.6
DO	pi_O_2	NR	-0.19	45.5 (INC)	-0.65	29.1
DO	pi_O_3	47.8 (DEC)	0.41	45.5 (INC)	-0.78	25.9
DO	pi_O_4	WR	0.15	45.5 (INC)	-0.42	45.7
DO	pi_O_45	43.5 (DEC)	0.16	45.5 (INC)	-0.42	40.6
DO	pi_O_5	NR	0.03	WR	0.02	99.7
DO	pt_O_1	WR	-0.40	45.5 (DEC)	0.50	22.0
DO	pt_O_12	MR	-0.17	45.5 (DEC)	0.05	14.5
DO	pt_O_2	MR	0.19	WR	-0.51	20.5
DO	pt_O_3	NR	-0.09	54.5 (INC)	-0.78	13.5
DO	pt_O_345	NR	-0.23	72.7 (INC)	-1.15	11.7
DO	pt_O_345_adj	45.7 (INC)	-0.29	72.7 (INC)	-1.15	NA
DO	pt_O_345_Ngrp_adj	WR	-0.24			NA
DO	pt_O_4	NR	-0.25	54.5 (INC)	-0.76	27.3
DO	pt_O_5	NR	-0.20	63.6 (INC)	-0.80	43.0
DO	WA_O_USGS	52.2 (DEC)	0.35	72.7 (INC)	-0.71	13.3
DO	wa_OxyTol	MR	0.30	54.5 (INC)	-0.80	8.3
Habit	nt_ADNATE	NR	0.16	45.5 (DEC)	0.61	25.2
Habit	nt_BENTHIC_HABIT	WR	0.00	63.6 (DEC)	0.63	10.8
Habit	nt_SESTONIC_HABIT	NR	-0.38	WR	0.03	30.8
Habit	nt_STALKED	NR	0.05	NR	0.13	30.3
Habit	pi_ADNATE	MR	-0.09	WR	0.48	48.8
Habit	pi_Ben_Ses_1	NR	0.47	WR	-0.42	3.4
Habit	pi_Ben_Ses_2	NR	-0.27	WR	0.20	24.7
Habit	pi_BENTHIC_HABIT	NR	0.31	NR	-0.20	2.1
Habit	pi_SESTONIC_HABIT	NR	-0.25	NR	0.15	26.1
Habit	pi_STALKED	NR	-0.20	WR	-0.21	51.0
Habit	pt_ADNATE	NR	0.14	45.5 (DEC)	0.08	24.2
Habit	pt_BENTHIC_HABIT	47.8 (DEC)	0.59	WR	0.38	2.4
Habit	pt_SESTONIC_HABIT	54.3 (INC)	-0.65	54.5 (INC)	-0.45	30.7
Habit	pt_SESTONIC_HABIT_adj	MR	-0.20	WR	-0.04	NA
Habit	pt_STALKED	NR	0.00	45.5 (INC)	-0.24	31.4
Habit	pt2_SESTONIC_HABIT_adj	52.2 (INC)	-0.61	45.5 (INC)	-0.43	NA
Habit	wa_OptCat_PctFN	41.3 (INC)	-0.03	WR	-0.57	4.1
Habit	wa_OptCat_XEMBED	WR	-0.09	WR	-0.52	3.4

MetCat	DataSet	Hi_N	Hi_N	Lo_N	Lo_N	CV%
		DE_25	Z_score	DE (trend)	Z_score	
Indicators	NRSA_MM1	WR	0.36	54.5 (DEC)	0.92	11.9
Indicators	nt_RefIndicators	41.3 (DEC)	0.51	100 (DEC)	2.12	35.1
Indicators	pi_RefIndicators	50 (DEC)	0.05	72.7 (DEC)	0.85	50.4
Indicators	pi_RefIndicators_adjSc			81.8 (DEC)	0.92	NA
Indicators	PMA	41.3 (DEC)	0.47	45.5 (INC)	0.00	9.9
Indicators	pt_RefIndicators	NR	0.51	100 (DEC)	1.41	32.0
Indicators	pt_RefIndicators_adj	52.2 (DEC)	0.47	100 (DEC)	1.37	NA
Moisture	wa_Moisture	NR	0.06	72.7 (DEC)	0.71	6.2
Motility	nt_HIGHLY_MOTILE	MR	-0.21	WR	0.36	16.9
Motility	nt_HIGHLY_MOTILE.1	MR	-0.01	45.5 (DEC)	0.64	14.0
Motility	nt_MOD_HI_MOTILE	MR	0.00	45.5 (DEC)	0.57	12.6
Motility	nt_MODERATELY_MOTILE	NR	0.17	45.5 (DEC)	0.67	15.4
Motility	nt_NON_MOTILE	NR	-0.29	WR	0.19	25.4
Motility	nt_SLIGHTLY_MOTILE	NR	0.08	54.5 (DEC)	0.65	22.3
Motility	nt_WEAKLY_MOTILE	NR	0.11	NR	-0.01	29.9
Motility	pi_HIGHLY_MOTILE	NR	-0.10	45.5 (DEC)	-0.07	26.1
Motility	pi_HIGHLY_MOTILE.1	MR	0.13	63.6 (DEC)	0.50	18.3
Motility	pi_MODERATELY_MOTILE	WR	0.21	45.5 (DEC)	0.27	30.9
Motility	pi_Motility_1	WR	-0.17	54.5 (DEC)	-0.31	17.9
Motility	pi_Motility_2	NR	-0.27	WR	0.24	17.3
Motility	pi_NON_MOTILE	43.5 (INC)	-0.55	WR	0.30	24.8
Motility	pi_SLIGHTLY_MOTILE	WR	0.24	45.5 (INC)	-0.03	26.1
Motility	pi_WEAKLY_MOTILE	NR	-0.19	63.6 (INC)	-1.33	64.3
Motility	pt_HIGHLY_MOTILE	45.7 (INC)	-0.24	WR	-0.15	14.8
Motility	pt_HIGHLY_MOTILE.1	WR	0.16	45.5 (DEC)	0.54	6.9
Motility	pt_HIGHLY_MOTILE_adj	WR	-0.29	WR	0.13	NA
Motility	pt_MODERATELY_MOTILE	41.3 (DEC)	0.50	45.5 (DEC)	0.62	11.4
Motility	pt_NON_MOTILE	43.5 (INC)	-0.49	WR	-0.18	21.8
Motility	pt_SLIGHTLY_MOTILE	MR	0.23	WR	0.05	18.9
Motility	pt_WEAKLY_MOTILE	MR	0.01	45.5 (INC)	-0.43	30.9
Motility	WA_Motility_USGS	NR	0.29	45.5 (DEC)	0.15	5.3
Nutrients	nt_HIGH_N	WR	-0.09	WR	-0.04	11.8
Nutrients	nt_HIGH_P	47.8 (INC)	-0.31	WR	-0.12	13.7
Nutrients	nt_LOW_N	43.5 (DEC)	0.33	72.7 (DEC)	1.35	31.6
Nutrients	nt_LOW_P	52.2 (DEC)	0.34	81.8 (DEC)	1.73	32.6
Nutrients	nt_LOW_P_adj	52.2 (DEC)	0.29	81.8 (DEC)	1.39	NA
Nutrients	nt_LOW_P_Ngrp_adj			90.9 (DEC)	2.13	NA
Nutrients	nt_N_FIXER	NR		NR	0.56	611.9
Nutrients	nt_NON_N_FIXER	NR	-0.04	63.6 (DEC)	0.57	10.7
Nutrients	pi_Diatas_TN_1	WR	-0.50	WR	-0.42	21.9
Nutrients	pi_Diatas_TN_2	WR	-0.05	72.7 (DEC)	0.78	52.5
Nutrients	pi_Diatas_TP_1	50 (INC)	-0.54	WR	-0.95	15.2

MetCat	DataSet	Hi_N	Hi_N	Lo_N	Lo_N	CV%
		DE_25	Z_score	DE (trend)	Z_score	
Nutrients	pi_Diatas_TP_2	NR	-0.04	72.7 (DEC)	0.79	48.6
Nutrients	pi_HIGH_N	NR	-0.20	45.5 (INC)	-0.60	14.0
Nutrients	pi_HIGH_P	50 (INC)	-0.59	WR	-0.90	15.0
Nutrients	pi_LOW_N	WR	-0.13	72.7 (DEC)	0.73	44.2
Nutrients	pi_LOW_P	43.5 (DEC)	-0.03	72.7 (DEC)	0.74	49.8
Nutrients	pi_N_FIXER	NR	#DIV/0!	WR	0.25	611.6
Nutrients	pi_NAWQA_TN_1	WR	-0.50	WR	-0.42	21.9
Nutrients	pi_NAWQA_TN_2	WR	-0.05	72.7 (DEC)	0.78	52.5
Nutrients	pi_NAWQA_TP_1	43.5 (INC)	-0.49	WR	-0.99	19.7
Nutrients	pi_NAWQA_TP_2	41.3 (DEC)	-0.13	72.7 (DEC)	0.81	51.8
Nutrients	pi_NF_1	NR		WR	0.25	611.9
Nutrients	pi_NF_2	NR	0.49	NR	-0.42	0.3
Nutrients	pi_NON_N_FIXER	NR		NR	-0.25	0.0
Nutrients	pi_TPReqMA97_0	MR	0.04	45.5 (DEC)	0.58	28.0
Nutrients	pi_TPReqMA97_1	41.3 (DEC)	0.16	WR	-0.35	52.2
Nutrients	pi_TPSENSMA97_0	MR	0.10	WR	-0.03	16.9
Nutrients	pi_TPSENSMA97_1	MR	0.10	WR	0.35	70.8
Nutrients	pt_HIGH_N	NR	-0.04	90.9 (INC)	-2.01	10.1
Nutrients	pt_HIGH_P	NR	-0.19	72.7 (INC)	-1.91	11.9
Nutrients	pt_LOW_N	NR	0.35	54.5 (DEC)	0.67	29.0
Nutrients	pt_LOW_P	43.5 (DEC)	0.34	72.7 (DEC)	0.92	33.2
Nutrients	pt_N_FIXER	NR		WR	0.44	631.5
Nutrients	pt_NON_N_FIXER	NR		NR	-0.44	0.4
Nutrients	wa_OptCat_L1Ptl	WR	0.00	WR	-0.53	4.2
Nutrients	wa_OptCat_LNtl	MR	-0.05	WR	-0.51	2.1
Nutrients	wa_OptCat_NutMMI	MR	-0.02	WR	-0.52	29.7
Nutrients	wa_Org_N	NR	0.11	54.5 (INC)	-0.74	6.0
Nutrients	wa_TPCATMA97	MR	0.16	WR	-0.46	6.5
Nutrients	wa_TPOPTMA97	41.3 (INC)	-0.01	WR	-0.33	2.8
PollToler	nt_Bahls_1	41.3 (INC)	-0.45	WR	-0.11	28.8
PollToler	nt_Bahls_2	MR	0.07	WR	0.49	15.3
PollToler	nt_Bahls_3	43.5 (DEC)	0.12	54.5 (DEC)	0.70	16.0
PollToler	nt_PT_1	45.7 (INC)	-0.61	WR	0.22	30.1
PollToler	nt_PT_2	NR	0.19	WR	0.21	26.7
PollToler	nt_PT_3	NR	-0.14	WR	0.09	39.7
PollToler	nt_PT_4	NR	0.16	WR	-0.11	16.3
PollToler	nt_PT_5	NR	0.21	45.5 (DEC)	0.74	43.6
PollToler	nt_Sens_810	MR	0.12	81.8 (DEC)	1.36	27.7
PollToler	nt_Tol_13	47.8 (INC)	-0.28	WR	-0.19	19.6
PollToler	pi_Bahls_1	41.3 (DEC)	0.20	54.5 (INC)	-0.47	47.6
PollToler	pi_Bahls_2	NR	0.38	45.5 (INC)	-0.39	22.2
PollToler	pi_Bahls_3	WR	-0.42	72.7 (DEC)	0.72	19.5

MetCat	DataSet	Hi_N	Hi_N	Lo_N	Lo_N	CV%
		DE_25	Z_score	DE (trend)	Z_score	
PollToler	pi_PT_1	NR	0.25	54.5 (INC)	-0.43	56.8
PollToler	pi_PT_12	NR	0.18	54.5 (INC)	-0.43	32.7
PollToler	pi_PT_123	NR	0.23	54.5 (INC)	-0.46	28.5
PollToler	pi_PT_2	MR	-0.21	WR	0.06	49.8
PollToler	pi_PT_3	NR	0.29	54.5 (INC)	-0.46	44.9
PollToler	pi_PT_4	WR	-0.08	WR	0.24	29.5
PollToler	pi_PT_45	41.3 (DEC)	-0.05	45.5 (DEC)	0.29	27.0
PollToler	pi_PT_5	NR	0.18	WR	0.29	44.5
PollToler	pi_Sens_810	MR	-0.08	81.8 (DEC)	0.88	29.1
PollToler	pi_Tol_13	52.2 (INC)	-1.07	54.5 (INC)	-1.43	28.8
PollToler	pt_Bahls_1	52.2 (INC)	-0.60	54.5 (INC)	-0.63	27.1
PollToler	pt_Bahls_2	NR	0.19	45.5 (INC)	-0.14	12.0
PollToler	pt_Bahls_3	41.3 (DEC)	0.08	WR	0.02	14.9
PollToler	pt_PT_1	WR	-0.63	WR	-0.03	31.2
PollToler	pt_PT_12	50 (INC)	-0.18	63.6 (INC)	-0.50	23.5
PollToler	pt_PT_2	NR	0.28	45.5 (INC)	-0.67	29.5
PollToler	pt_PT_3	WR	-0.14	45.5 (INC)	-0.30	38.8
PollToler	pt_PT_4	WR	0.06	63.6 (INC)	-0.82	21.2
PollToler	pt_PT_45	45.7 (DEC)	0.18	54.5 (INC)	-0.37	18.9
PollToler	pt_PT_5	NR	0.23	54.5 (DEC)	0.67	43.3
PollToler	pt_Sens_810	WR	0.27	81.8 (DEC)	1.68	23.6
PollToler	pt_Tol_13	NR	-0.37	54.5 (INC)	-1.29	18.6
PollToler	wa_AVGTSIC	50 (DEC)	0.48	45.5 (INC)	-0.48	4.3
PollToler	WA_Bahls_USGS	WR	-0.16	54.5 (DEC)	0.50	10.3
PollToler	wa_FTSIC	NR	0.12	WR	-0.33	5.6
PollToler	wa_FTSIC2	NR	0.11	WR	-0.32	5.6
PollToler	wa_FTSIC3	MR	0.05	54.5 (INC)	-0.11	6.5
PollToler	wa_KY_PTI	MR	0.12	WR	0.47	7.2
PollToler	wa_MAIATSIC	50 (DEC)	0.48	45.5 (INC)	-0.48	4.3
PollToler	wa_MT_Tol	MR	0.14	45.5 (INC)	-0.11	4.5
PollToler	wa_NEWTSIC	47.8 (DEC)	0.65	63.6 (INC)	-0.61	4.9
PollToler	wa_OptCat_DisTotMMI	MR	-0.07	WR	-0.49	30.3
PollToler	wa_OptCat_L1DisTot	MR	-0.16	WR	-0.41	10.2
PollToler	wa_Poll_Class	43.5 (INC)	-0.36	72.7 (DEC)	0.83	6.1
PollToler	wa_Poll_Tol	41.3 (DEC)	-0.01	72.7 (DEC)	0.75	11.3
PollToler	WA_PT_USGS	WR	0.04	WR	0.16	18.8
PollToler	wa_SensitivityIN	NR	0.37	81.8 (DEC)	1.13	5.8
PollToler	x_Kelly_TDI_2008	NR	-0.23	WR	-0.45	5.1
PollToler	x_Kelly_TDI_95_	NR	-0.26	WR	-0.34	5.9
PollToler	partAttributes	NR	-0.26	WR	-0.34	5.9
PollToler	x_Kelly_WMS_2008	NR	-0.23	WR	-0.45	3.9
PollToler	x_Kelly_WMS_95	NR	-0.26	WR	-0.34	4.5
Richness	nt_total	MR	-0.04	63.6 (DEC)	0.60	10.7

MetCat	DataSet	Hi_N	Hi_N	Lo_N	Lo_N	CV%
		DE_25	Z_score	DE (trend)	Z_score	
Richness	nt_total	MR	-0.06	63.6 (DEC)	0.63	10.7
Richness	x_Shan_2	NR	-0.08	WR	0.44	8.0
Salts	nt_SALINITY_1	NR	-0.22	81.8 (DEC)	0.81	41.4
Salts	nt_SALINITY_12	NR	0.00	54.5 (DEC)	0.70	12.5
Salts	nt_SALINITY_2	NR	0.12	54.5 (DEC)	0.55	12.7
Salts	nt_SALINITY_3	NR	-0.47	45.5 (INC)	-0.47	19.0
Salts	nt_SALINITY_4	WR	-0.37	54.5 (INC)	-0.58	74.6
Salts	pi_Diat_CA_1	NR	-0.09	45.5 (INC)	-0.56	23.1
Salts	pi_Diat_CA_2	NR	-0.50	WR	-0.13	546.4
Salts	pi_Diat_CL_1	52.2 (INC)	-2.49	WR	-1.72	202.5
Salts	pi_Diat_CL_1_ASSR	52.2 (INC)	-1.20	WR	-0.54	82.7
Salts	pi_Diat_CL_2	NR	0.06	WR	0.63	27.7
Salts	pi_Diat_CL2_AdjSc			54.5 (DEC)	-0.10	NA
Salts	pi_Diat_Cond_1	43.5 (INC)	-0.39	WR	-1.43	60.1
Salts	pi_Diat_Cond_2	NR	0.08	90.9 (DEC)	0.98	177.1
Salts	pi_NAWQA_CL_1	52.2 (INC)	-2.49	WR	-1.72	202.5
Salts	pi_NAWQA_CL_2	NR	0.06	WR	0.63	27.7
Salts	pi_NAWQA_Cond_1	43.5 (INC)	-0.39	WR	-1.43	60.1
Salts	pi_NAWQA_Cond_2	NR	0.08	90.9 (DEC)	0.98	177.1
Salts	pi_SALINITY_1	45.7 (INC)	-0.36	72.7 (DEC)	0.74	66.6
Salts	pi_SALINITY_2	43.5 (DEC)	0.18	45.5 (INC)	-0.03	11.0
Salts	pi_SALINITY_3	NR	0.13	54.5 (INC)	-0.98	31.4
Salts	pi_SALINITY_34					30.8
Salts	pi_SALINITY_34	NR	0.04	54.5 (INC)	-1.02	30.8
Salts	pi_SALINITY_4	47.8 (INC)	-1.27	54.5 (INC)	-0.89	126.7
Salts	pt_SALINITY_1	NR	-0.29	63.6 (DEC)	0.52	39.2
Salts	pt_SALINITY_2	43.5 (DEC)	0.29	MR	0.02	7.5
Salts	pt_SALINITY_3	NR	-0.38	81.8 (INC)	-2.01	19.0
Salts	pt_SALINITY_34	WR	-0.50	90.9 (INC)	-2.04	19.5
Salts	pt_SALINITY_4	50 (INC)	-0.40	54.5 (INC)	-1.16	82.1
Salts	wa_OptCat_LCond	MR	0.16	WR	-0.53	2.5
Salts	wa_Salinity	MR	0.13	54.5 (INC)	-1.06	4.0
Salts	WA_Salinity_USGS	WR	0.15	63.6 (INC)	-0.62	9.2
Saprobity	nt_SAP_1	NR	0.20	45.5 (DEC)	0.55	35.8
Saprobity	nt_SAP_2	WR	0.02	WR	0.37	13.9
Saprobity	nt_SAP_3	MR	-0.04	NR	0.28	17.2
Saprobity	nt_SAP_4	WR	-0.35	WR	0.08	28.3
Saprobity	nt_SAP_5	WR	-0.06	63.6 (DEC)	0.61	48.9
Saprobity	pi_SAP_1	wr	-0.52	54.5 (DEC)	0.69	81.9
Saprobity	pi_SAP_2	MR	-0.17	WR	0.45	21.4
Saprobity	pi_SAP_3	WR	0.25	WR	-0.89	34.3
Saprobity	pi_SAP_345	47.8 (DEC)	0.34	54.5 (INC)	-0.73	23.5

MetCat	DataSet	Hi_N	Hi_N	Lo_N	Lo_N	CV%
		DE_25	Z_score	DE (trend)	Z_score	
Saprobity	pi_SAP_4	43.5 (DEC)	0.29	54.5 (INC)	-0.44	49.4
Saprobity	pi_SAP_45	NR	0.22	54.5 (INC)	-0.41	45.1
Saprobity	pi_SAP_5	50 (INC)	-1.14	63.6 (DEC)	0.45	126.6
Saprobity	pt_SAP_1	MR	0.18	45.5 (DEC)	0.36	34.6
Saprobity	pt_SAP_12	41.3 (DEC)	0.05	WR	-0.24	11.1
Saprobity	pt_SAP_2	41.3 (DEC)	-0.07	54.5 (INC)	-0.51	12.4
Saprobity	pt_SAP_3	WR	0.06	WR	-0.65	17.0
Saprobity	pt_SAP_4	WR	-0.47	WR	-0.32	26.6
Saprobity	pt_SAP_5	WR	0.12	63.6 (DEC)	0.57	52.8
Saprobity	WA_SAP_USGS	45.7 (DEC)	0.28	63.6 (INC)	-0.52	10.9
Saprobity	wa_Saprofic	WR	0.22	63.6 (INC)	-0.63	6.8
Size	nt_BIG	NR	0.10	54.5 (DEC)	0.65	34.5
Size	nt_MEDIUM	NR	-0.06	WR	0.42	21.5
Size	nt_sm_vsm	NR	-0.03	WR	0.71	11.8
Size	nt_SMALL	NR	0.18	NR	0.53	13.2
Size	nt VERY_BIG	NR	-0.18	NR	0.76	109.1
Size	nt VERY_SMALL	43.5 (INC)	-0.38	54.5 (DEC)	0.82	18.2
Size	pi_BIG	NR	0.16	WR	0.12	45.3
Size	pi_MEDIUM	WR	-0.07	NR	0.63	32.2
Size	pi_SMALL	41.3 (INC)	-0.24	WR	-0.75	20.2
Size	pi VERY_BIG	NR	-2.08	WR	0.51	108.1
Size	pi VERY_SMALL	MR	0.04	45.5 (INC)	0.04	21.6
Size	pt_BIG	NR	0.15	54.5 (DEC)	0.66	34.4
Size	pt_MEDIUM	NR	-0.03	WR	-0.03	21.5
Size	pt_SMALL	50 (DEC)	0.48	WR	-0.28	8.9
Size	pt VERY_BIG	NR	-0.06	WR	0.59	118.3
Size	pt VERY_SMALL	WR	-0.33	54.5 (DEC)	0.40	15.2
Size	WA_Size_USGS	NR	-0.09	WR	0.46	8.5
TaxaGroup	nt_Achnan_Navic	45.7 DEC)	0.20	81.8 (DEC)	1.10	17.8
TaxaGroup	nt_ACHNANTHIDIAE	NR	0.01	72.7 (DEC)	0.98	33.6
TaxaGroup	nt_ACHNANTHIDIUM	NR	0.21	100 (DEC)	1.59	37.1
TaxaGroup	nt_BACILLARIACEAE	41.3 (INC)	-0.27	WR	0.10	16.4
TaxaGroup	nt_NAVICULA	41.3 (DEC)	0.19	72.7 (DEC)	0.68	19.7
TaxaGroup	pi_Achnan_Navic	WR	0.10	72.7 (DEC)	0.90	25.3
TaxaGroup	pi_ACHNANTHIDIAE	41.3 (INC)	-0.39	WR	0.58	60.3
TaxaGroup	pi_ACHNANTHIDIUM	43.5 (DEC)	-0.26	45.5 (DEC)	0.74	59.6
TaxaGroup	pi_BACILLARIACEAE	NR	-0.03	WR	-0.06	28.4
TaxaGroup	pi_NAVICULA	43.5 (DEC)	0.20	72.7 (DEC)	0.28	28.2
TaxaGroup	pt_Achnan_Navic	45.7 DEC)	0.50	81.8 (DEC)	1.50	15.7
TaxaGroup	pt_ACHNANTHIDIAE	WR	0.08	45.5 (DEC)	0.60	31.4
TaxaGroup	pt_ACHNANTHIDIUM	MR	0.22	72.7 (DEC)	1.09	36.4
TaxaGroup	pt_BACILLARIACEAE	WR	-0.34	WR	-0.78	16.0

MetCat	DataSet	Hi_N	Hi_N	Lo_N	Lo_N	CV%
		DE_25	Z_score	DE (trend)	Z_score	
TaxaGroup	pt_NAVICULA	43.5 (DEC)	0.33	WR	0.64	17.3
Trophic	nt_TROPHIC_1	NR	0.24	NR	0.10	43.2
Trophic	nt_TROPHIC_123	NR	-0.06	45.5 (DEC)	0.47	32.8
Trophic	nt_TROPHIC_2	NR	0.03	72.7 (DEC)	0.65	117.0
Trophic	nt_TROPHIC_3	WR	-0.25	45.5 (DEC)	0.29	47.8
Trophic	nt_TROPHIC_4	NR	0.08	72.7 (DEC)	1.16	30.0
Trophic	nt_TROPHIC_5	WR	-0.12	NR	0.07	12.6
Trophic	nt_TROPHIC_56	MR	-0.17	NR	0.13	12.8
Trophic	nt_TROPHIC_6	50 (INC)	-0.34	63.6 (DEC)	0.59	43.5
Trophic	nt_TROPHIC_7	NR	0.15	WR	0.06	25.4
Trophic	pi_TROPHIC_1	43.5 (INC)	-0.55	54.5 (DEC)	0.32	73.8
Trophic	pi_Trophic_12	47.8 (INC)	-0.22	54.5 (DEC)	0.49	123.6
Trophic	pi_TROPHIC_2	NR	0.12	72.7 (DEC)	0.60	472.1
Trophic	pi_TROPHIC_3	45.7 (INC)	-0.77	45.5 (DEC)	0.02	91.3
Trophic	pi_TROPHIC_4	41.3 (DEC)	-0.09	45.5 (DEC)	0.65	49.4
Trophic	pi_TROPHIC_456	MR	-0.07	WR	-0.43	12.2
Trophic	pi_TROPHIC_5	MR	0.03	45.5 (INC)	-0.44	14.2
Trophic	pi_Trophic_56	MR	-0.16	WR	-0.55	15.5
Trophic	pi_TROPHIC_6	52.2 (INC)	-1.39	72.7 (DEC)	-0.41	107.7
Trophic	pi_TROPHIC_7	MR	-0.15	NR	0.52	41.2
Trophic	pt_TROPHIC_1	NR	0.17	45.5 (INC)	-0.14	49.1
Trophic	pt_TROPHIC_2	NR	-0.02	72.7 (DEC)	0.65	120.7
Trophic	pt_TROPHIC_3	WR	-0.37	45.5 (DEC)	0.09	49.0
Trophic	pt_TROPHIC_4	MR	0.03	72.7 (DEC)	1.42	30.8
Trophic	pt_TROPHIC_456	NR	-0.15	63.6 (INC)	-0.54	8.4
Trophic	pt_TROPHIC_5	NR	-0.12	81.8 (INC)	-0.95	10.2
Trophic	pt_TROPHIC_6	MR	-0.11	63.6 (DEC)	0.59	42.7
Trophic	pt_TROPHIC_7	NR	0.20	45.5 (INC)	-0.26	24.6
Trophic	wa_Trophic	WR	0.18	WR	0.22	5.0
Trophic	WA_Trophic_USGS	WR	-0.22	WR	0.32	10.5

## Appendix I: Index Plots with Stressors

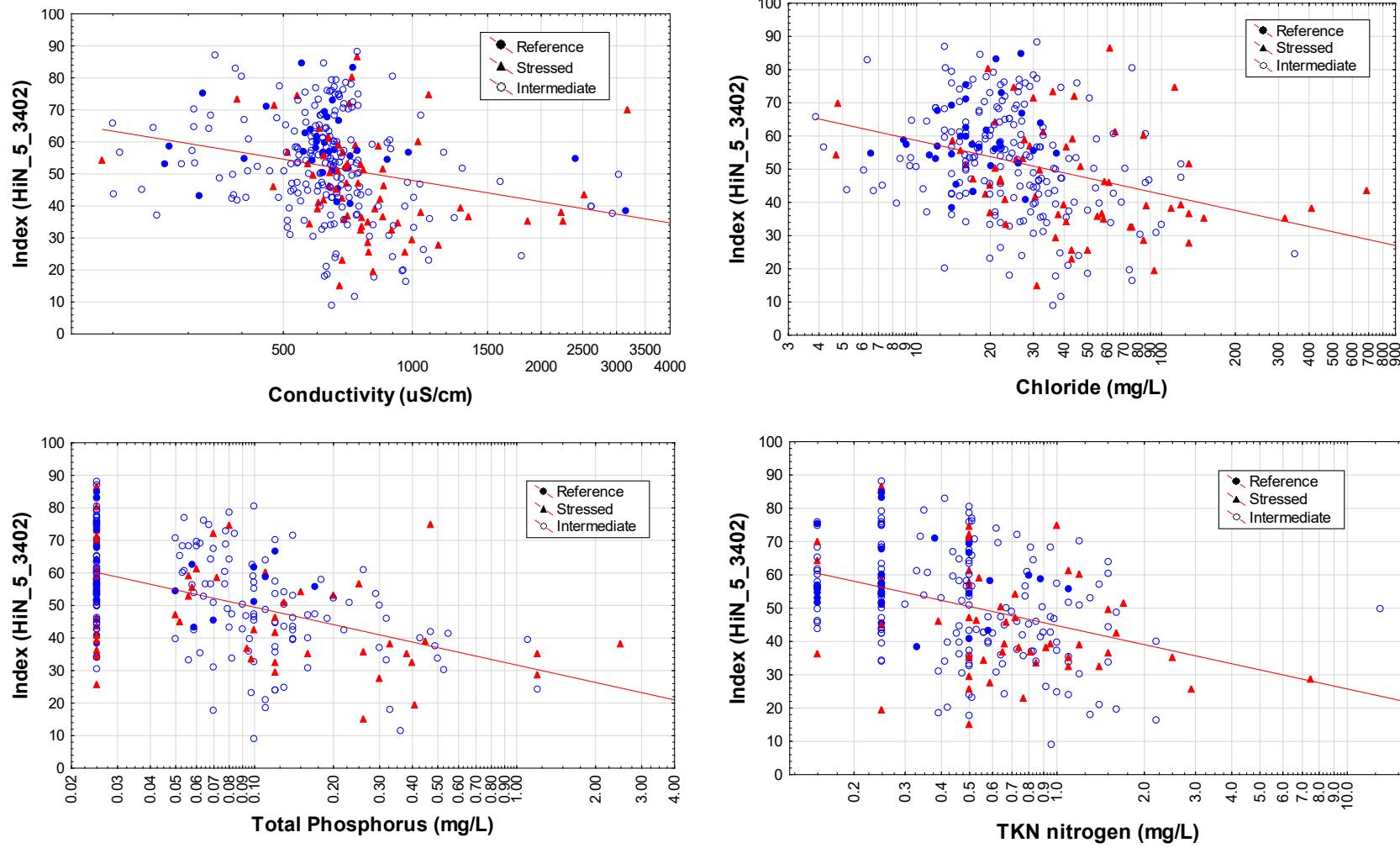


Figure 1. HiN diatom index in relation to stressors: conductivity, chloride, total phosphorus, and total Kjeldahl nitrogen.

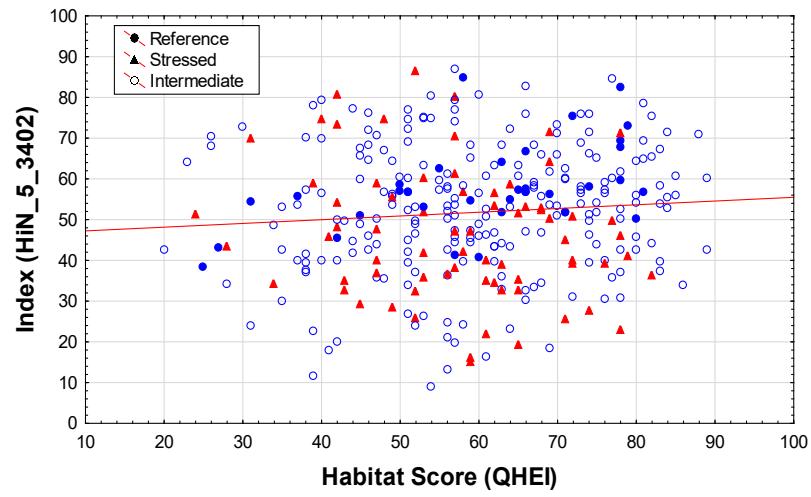
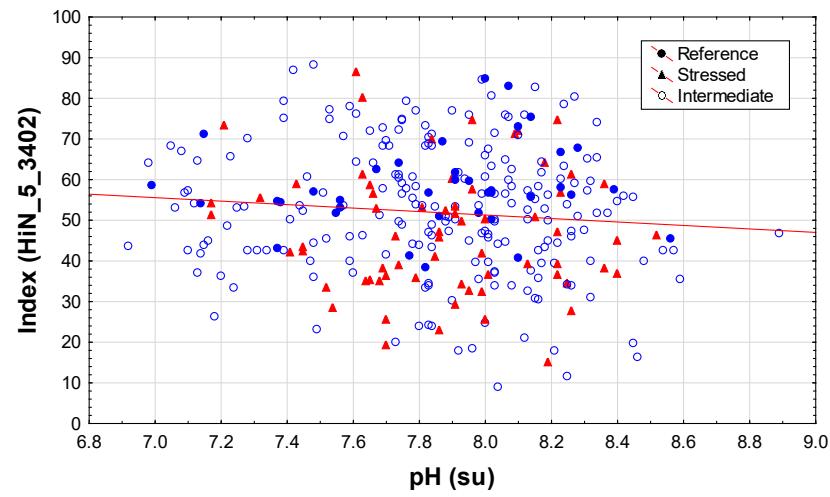
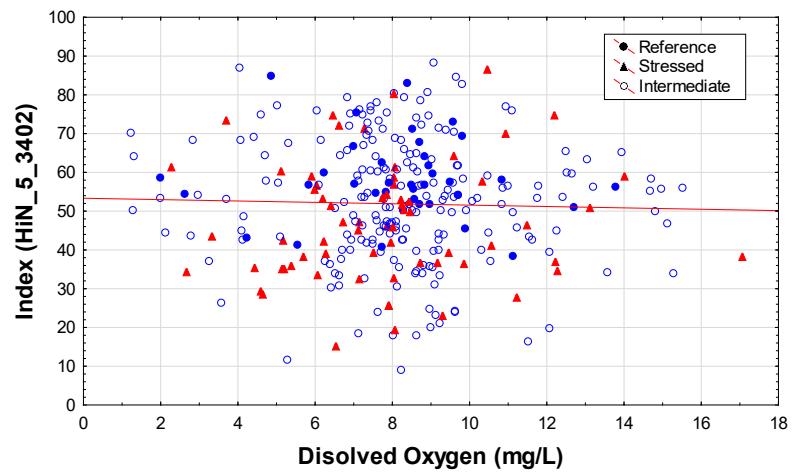
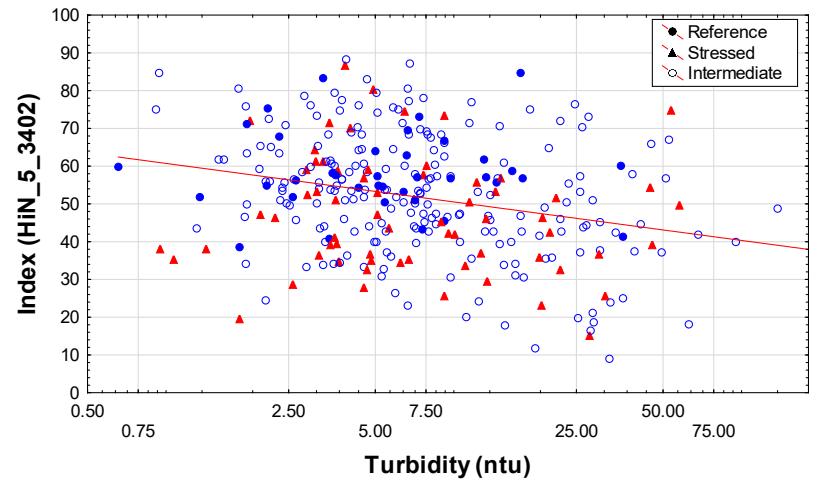


Figure 2. HiN diatom index in relation to stressors: turbidity, dissolved oxygen, pH, and the QHEI habitat score.

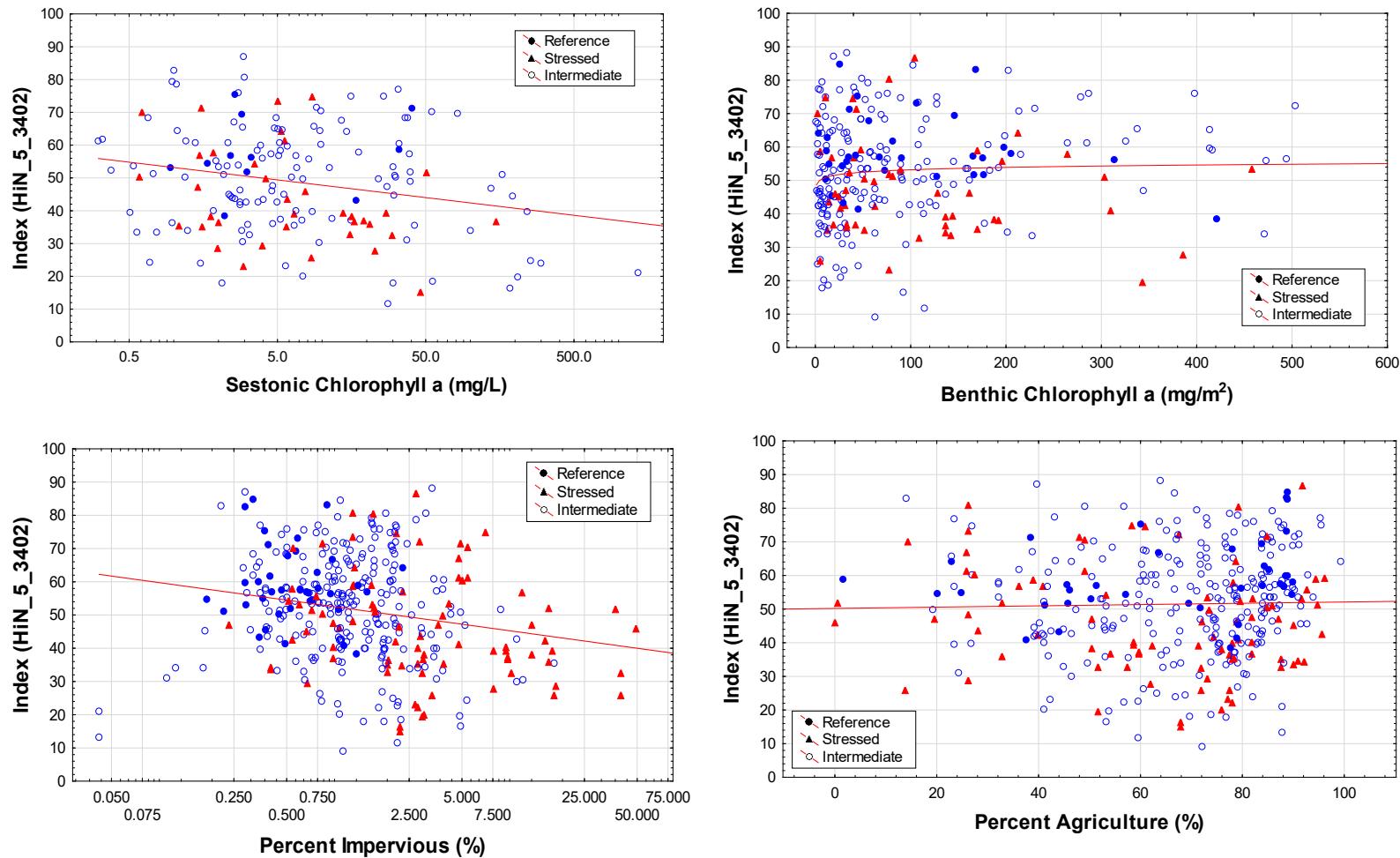


Figure 3. HiN diatom index in relation to sestonic chlorophyll a, benthic chlorophyll a, percent impervious land cover, and percent agriculture in the watershed.

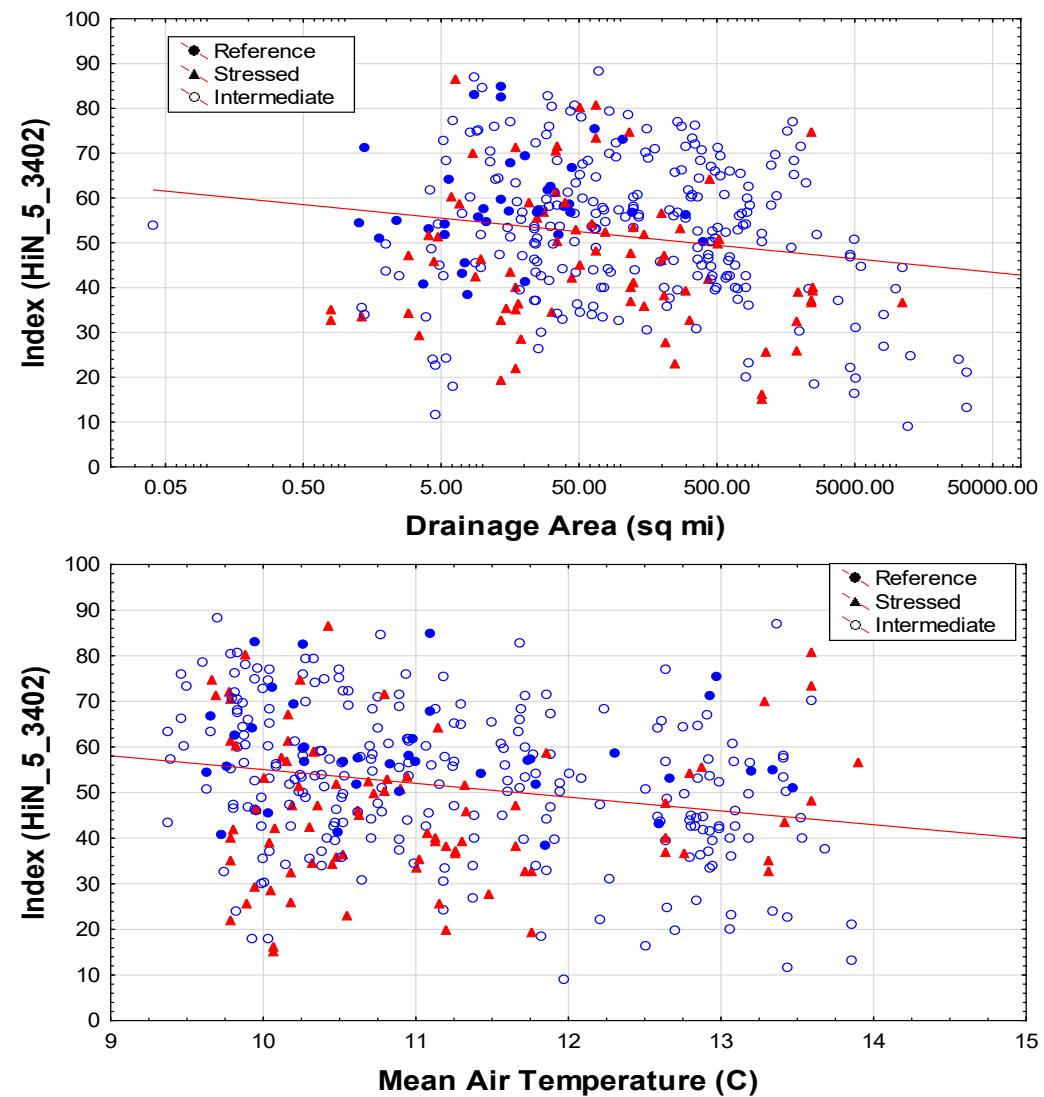


Figure 4. HiN diatom index in relation to drainage area and mean air temperature.

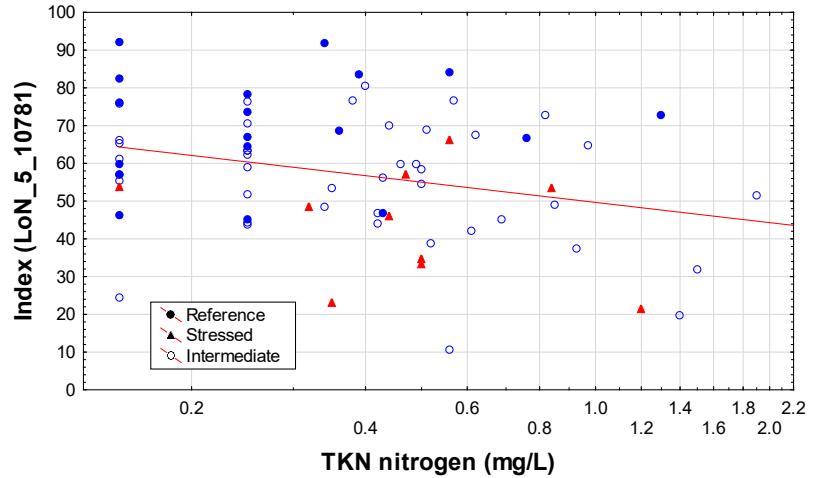
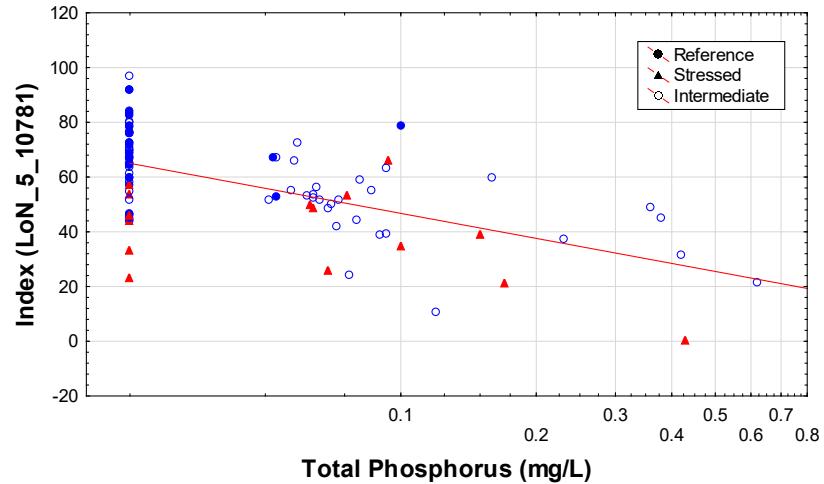
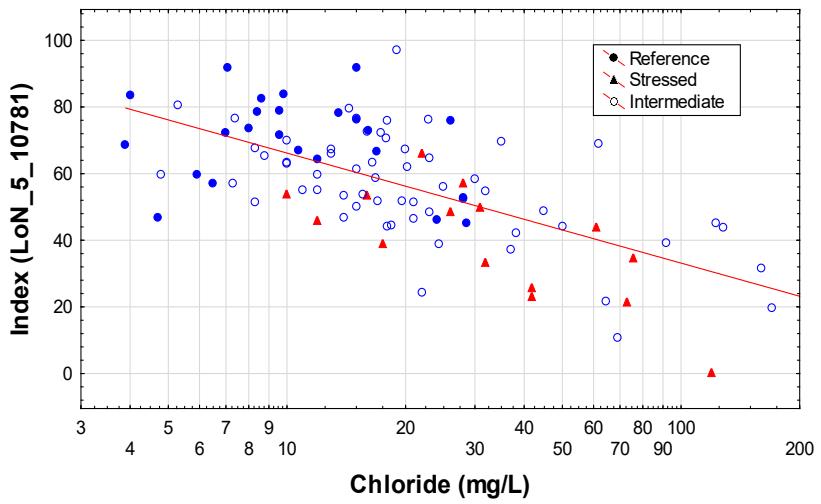
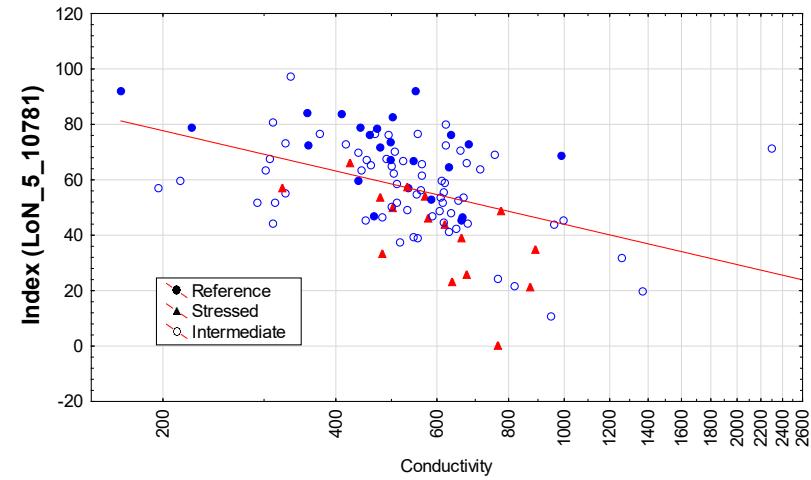


Figure 5. LoN diatom index in relation to stressors: conductivity, chloride, total phosphorus, and total Kjeldahl nitrogen.

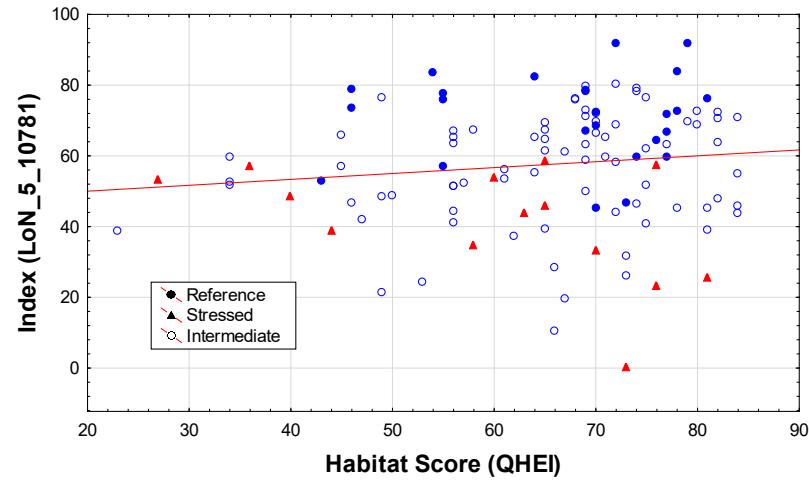
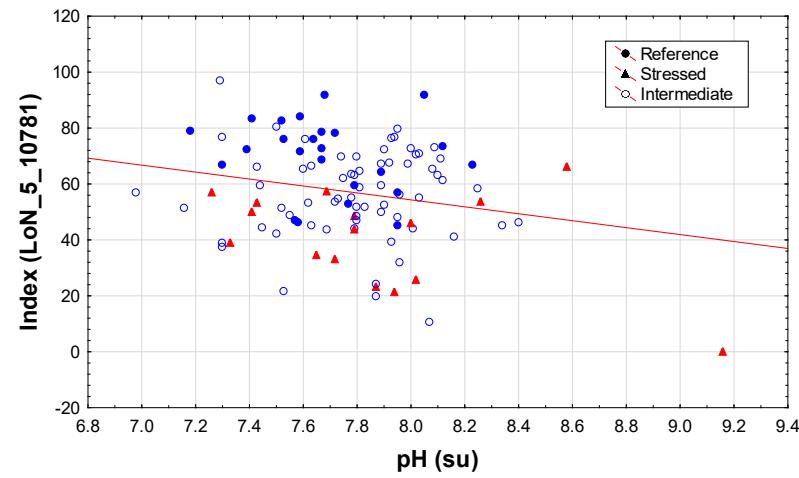
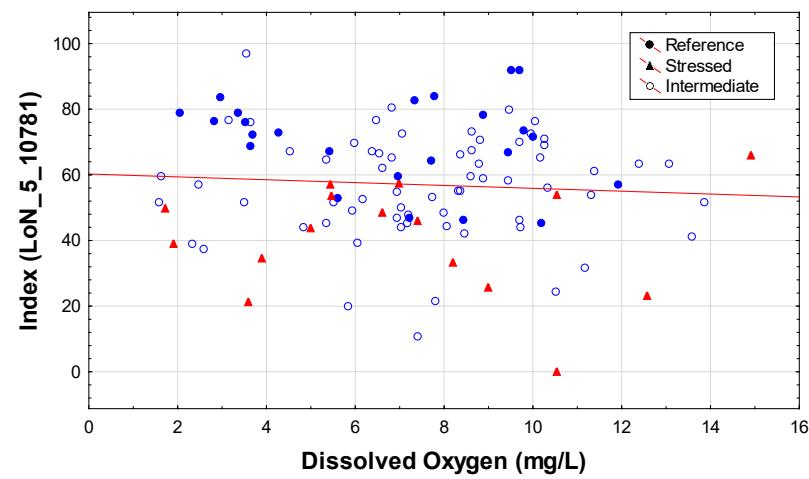
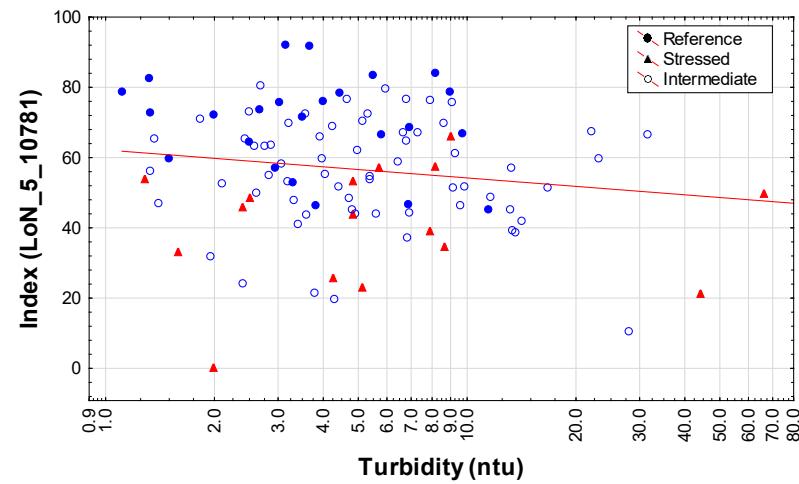


Figure 6. LoN diatom index in relation to stressors: turbidity, dissolved oxygen, pH, and the QHEI habitat score.

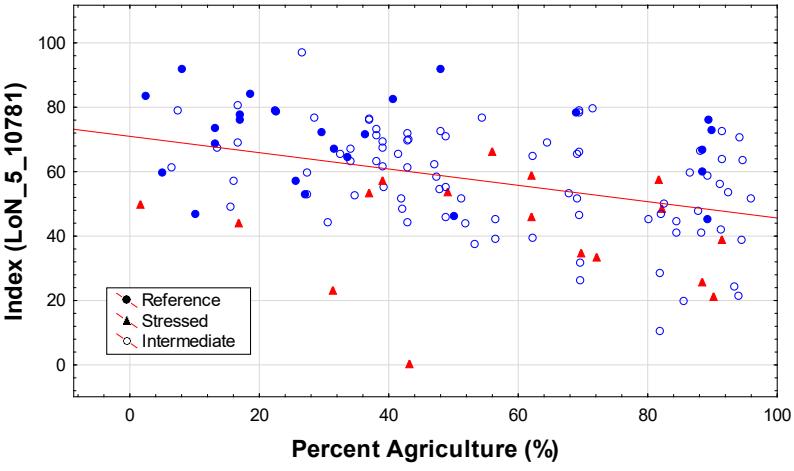
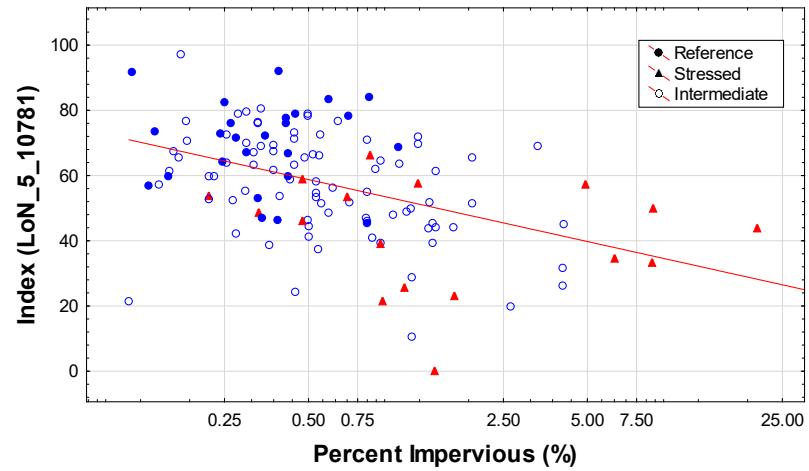
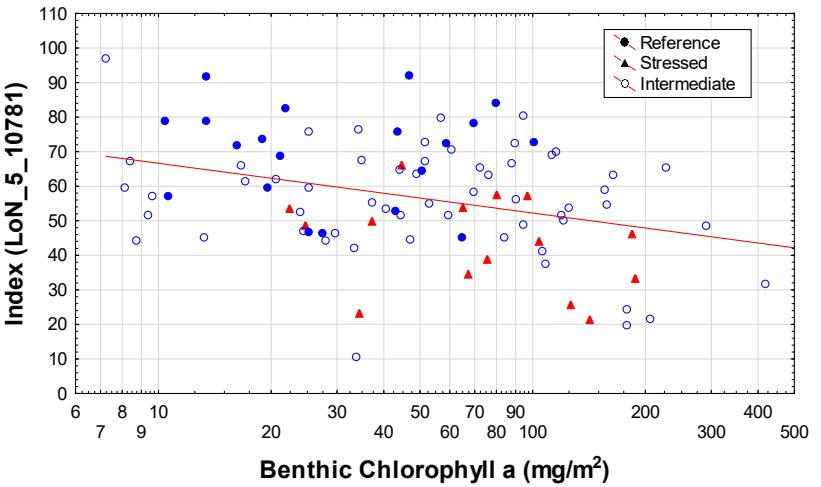
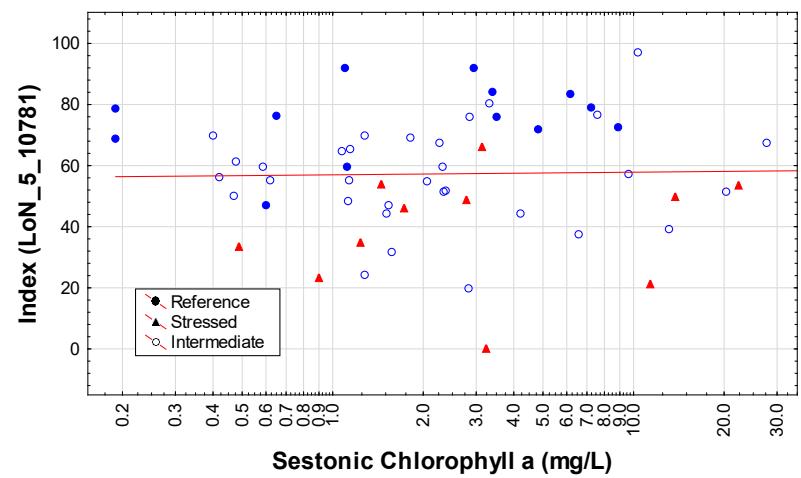


Figure 7. LoN diatom index in relation to sestonic chlorophyll a, benthic chlorophyll a, percent impervious land cover, and percent agriculture in the watershed.

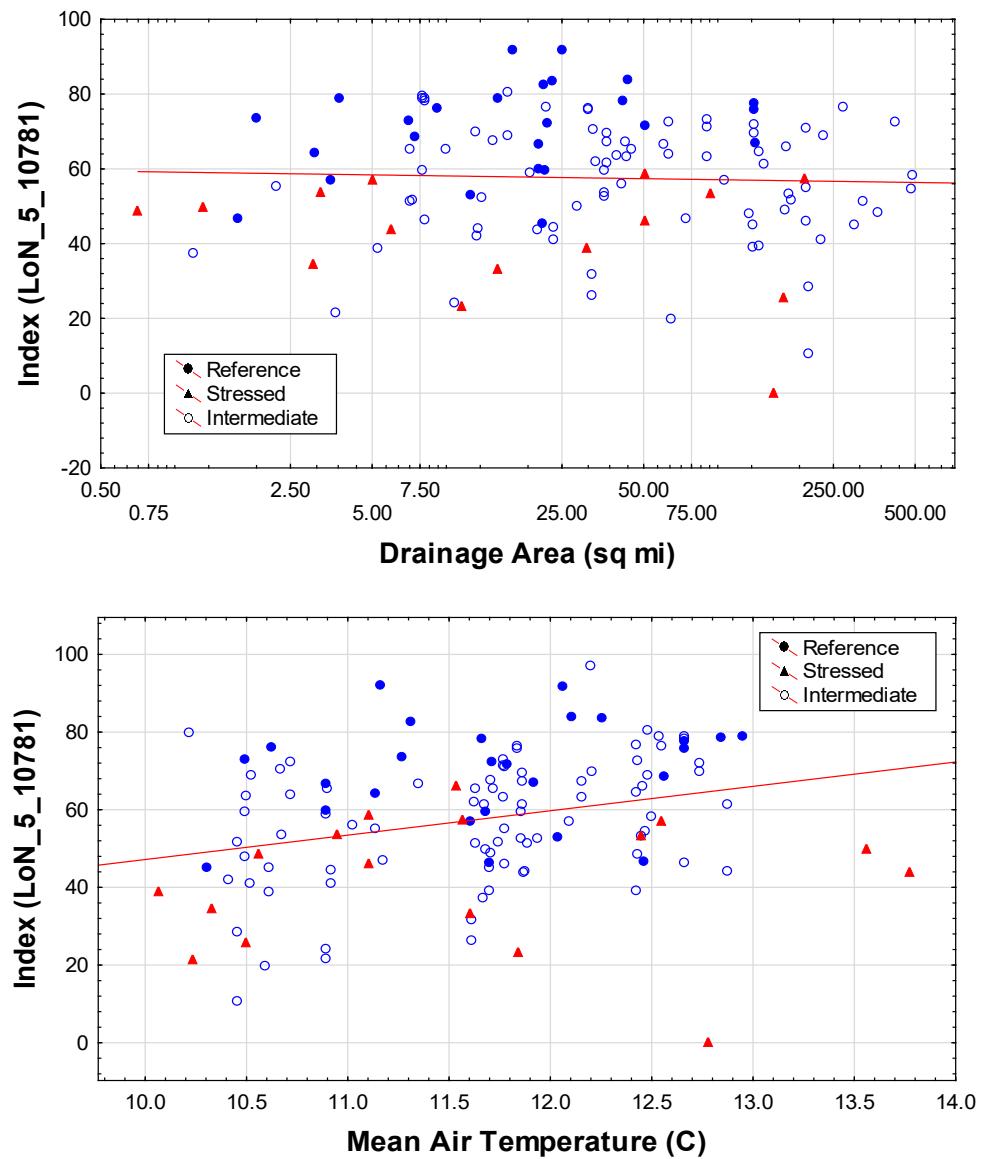


Figure 8. HiN diatom index in relation to drainage area and mean air temperature.

# Indiana Diatoms Project – Diatom Metric Discrepancies Explanation

Ben Block

June 30, 2021

## Purpose

The purpose of this document is to highlight discrepancies that Tetra Tech (Ben Block and Ben Jessup) found in diatom taxa attribute assignments after initial IBI calibration. These discrepancies were identified during metric calculation quality control procedures using BioMonTools (see *Metric calculation R code* section). Overall, we think that the changes made to the diatom taxa attributes and metric calculation formulae are justified and important for future application of the IN Diatom IBI (and other IBIs that rely on diatom data).

## Causes of discrepancies

Throughout this section, I describe the causes of discrepancies and have ordered the causes from most to least influential on the discrepancies observed. In general, the major contributor to discrepancies was how metrics that used USGS traits were calculated. Less important discrepancies were related to quality control of the TaxaMasterPeriphyton table which was outdated compared to the tables used for USGS metric calculations. In other words, the tables used to calculate metrics based on the USGS traits were more recent than the traits in the TaxaMasterPeriphyton table. Through the changes described below, we have alleviated all of the discrepancies.

### Metric calculation R code

Discrepancies in metric values were found for metrics calculated by Ben Jessup using the USGS diatom traits R code (S. A. Spaulding, USGS) – hereafter referred to as USGS code – and metrics calculated using the BioMonTools R Package (E. Leppo, Tetra Tech). BioMonTools is an R package that Tetra Tech has developed to calculate metric values from input taxa lists. We currently use BioMonTools to calculate metrics within all our R Shiny apps.

To ensure that BioMonTools is correctly calculating diatom metrics, I compared the metric value results to those originally generated by Ben Jessup using the USGS code. We identified the major issue to be that the **USGS code removes taxa without associated traits prior to metric calculation while BioMonTools retains these taxa**. Although we are confident in our initial results using the USGS strategy, we think that retaining all taxa for metric calculations is a better option because 1) the removal of taxa dismisses the total number of taxa within a sample (see Box 1), and 2) the removal of unrepresented taxa is not applied consistently. We believe the removal is inconsistent because only taxa that are not matched between the sample taxa list and the traits taxa list are removed. There are also many taxa within the traits taxa list with unknown trait assignments because they were not encountered in the original trait studies. These were given a default trait assignment of “0” (not having the trait), though in reality the trait characteristics were not identified. If removal of unmatched taxa is justified, then removal of taxa with uncertain trait assignments is also warranted. The alternative, retaining all

taxa even when a trait is not assigned (for either reason), is the standard used in other metric calculation examples (in Tetra Tech's broad experience). Ultimately, we are confident in the IBI calibration results derived using the USGS code; however, we would like to maintain the continuity of BioMonTools and move forward by calculating metrics using all taxa found in a sample.

Box 1. Example of metric calculation by USGS code and BioMonTools

$$\text{Metric} = \text{pt\_BC\_1 (\% BC\_1 taxa)}$$

$$\text{BioMonTools: \% BC 1 taxa} = \frac{(\# \text{ taxa with BC 1 attribute})}{(\text{total } \# \text{ taxa in sample})}$$

$$\text{USGS Code: \% BC 1 taxa} = \frac{(\# \text{ taxa with BC 1 attribute})}{(\text{total } \# \text{ taxa in sample}) - (\# \text{ taxa w/o USGS diatom attributes})}$$

### Achnanthidium and Navicula taxa

A common QC issue was found when calculating the nt\_Achnan\_Navic metric (# Achnanthidium and Navicula taxa per sample). BioMonTools uses the 'Genus' field of an input dataset to identify taxa that fit into one of these genera, whereas, the USGS code has unique fields titled 'Achnanthidium' and 'Navicula' on which the metrics are calculated. A natural inclination is to assume that all taxa with 'Achnanthidium' and 'Navicula' in the 'Genus' field would also have the appropriate fields filled out in the unique USGS field. However, this is not the case. There are multiple instances where the 'Genus' field is filled by 'Navicula' yet the USGS trait signifies that the taxa are in fact not 'Navicula'. Also, Achnanthes subhudsonis is technically considered part of 'Achnanthidium' according to USGS. We reached out to S. Spaulding from USGS and they recommended we keep the attributes because of recent taxa revisions, reassessments, and retired names. Therefore, the 'Genus' field was populated in the TaxaMasterPeriphyton table to accommodate these changes, using the USGS traits.

### Updated 'Taxon\_forUSGS\_traits' field

The 'Taxon\_forUSGS\_traits' field was used to link the TaxaMasterPeriphyton table to the original diatom taxa traits table provided by USGS. The field was used to list taxa synonyms that matched the USGS traits table during the taxa harmonization process. There were instances where we found that the original iteration of the TaxaMasterPeriphyton had trait associations that were not a perfect match to the 'COMMON\_NAME' field and therefore would not produce the same results as the data run through the USGS code (which does not rely on the TaxaMasterPeriphyton table but rather the built-in diatom traits table). Thus, we made the following edits to reconcile the differences:

Changed Sellaphora\_rexii/Sellaphora\_meridionalis to Sellaphora\_rexii

- Sellaphora\_rexii/Sellaphora\_meridionalis not in USGS diatom traits table; probably used Sellaphora\_rexii initially

Changed Nitzschia\_lanceola\_var.\_minutula to Nitzschia\_lanceola

- Nitzschia\_lanceola\_var.\_minutula not in USGS diatom traits table; probably used Nitzschia\_lanceola initially

Changed *Fragilaria\_capucina\_var.\_mesolepta* to *Fragilaria\_mesolepta*

- Now the ‘Taxon\_forUSGS\_traits’ field matches the ‘COMMON\_NAME’ field. Going back to the original taxa attribute associations, we found that *Fragilaria\_mesolepta* is likely the correct name to use (recommended by S. Spaulding, USGS). Therefore, we updated the traits to be associated with the new name:
  - BC\_3 trait added
  - BC\_4, SALINITY\_2, BAHLS\_2, LOW\_P, and LOW\_N traits removed

Changed *Achnanthidium\_exiguum* to *Achnanthes\_exigua\_var.\_elliptica*

- Now the ‘Taxon\_forUSGS\_traits’ field matches the ‘COMMON\_NAME’ field. In the USGS diatoms traits table, there is an entry titled ‘*Achnanthidium\_exiguum/Achnanthes\_exigua\_var.\_elliptica/Achnanthidium\_exiguum\_var.\_constrictum*’ which was used for this taxon. We decided to use the ‘*Achnanthes\_exigua\_var.\_elliptica*’ entry instead. Therefore, we updated the traits to be associated with the new name:
  - BC\_4, TROPHIC\_7, SAP\_2, O\_1, and SALINITY\_2 were removed

### Small changes

- Added USGS traits for COMMON\_NAME == “*Delicata delicatula*”
  - Unclear why traits were missing from the original copy of TaxaMasterPeriphyton
- Removed TROPHIC\_5 designation from COMMON\_NAME == “*Amphora ovalis*”. Unclear why it was there to begin with because the USGS diatom traits table did not have TROPHIC\_5 as a trait for this taxon
- Updated traits for COMMON\_NAME == “*Navicula permitis*” which has been equated to “*Mayamaea\_permitis*” in the ‘Taxon\_forUSGS\_traits’ field. Unclear how the original traits came to be as the values were updated by matching to the USGS diatom traits table.
- Added PT\_3 designation to COMMON\_NAME == “*Pseudostaurosira parasitica* var. *constricta*” which has been equated to “*Pseudostaurosira\_parasitica*” in the ‘Taxon\_forUSGS\_traits’ field. Unclear how the original traits came to be as the values were updated by matching to the USGS diatom traits table.
- Changed ‘Bahls’ designation for COMMON\_NAME == “*Navicula schroeteri* var. *escambia*” from BAHLS\_2 to BAHLS\_3. Unclear how the original traits came to be as the values were updated by matching to the USGS diatom traits table.
- Added BAHLS\_3 designation to COMMON\_NAME == “*Staurosira construens* var. *constricta*” which has been equated to “*Staurosira\_construens*” in the ‘Taxon\_forUSGS\_traits’ field. Unclear how the original traits came to be as the values were updated by matching to the USGS diatom traits table.
- Removed LOW\_N designation from COMMON\_NAME == “*Gomphonema olivaceoides*”. Unclear how the original traits came to be as the values were updated by matching to the USGS diatom traits table.
- Removed HIGH\_N designation from COMMON\_NAME == “*Navicula pseudoventralis*”. Unclear how the original traits came to be as the values were updated by matching to the USGS diatom traits table.

## Results on index scores and site narratives

We determined the effects that the changes to the TaxaMasterPeriphyton table and metric calculation formulae had on index performance by scoring individual samples, comparing subsequent changes in index scores and narratives, and examining the overall effect on index discrimination efficiency (DE). Overall, these changes had relatively minor effects on index performance. Of the 497 samples, 418 had index scores within 1 point of their previous value. The remaining 79 samples had larger index score differences that were all less than 5 points. Using the impairment thresholds derived in the report, index narratives were assigned to each sample. Of the 497 samples, only nine samples had index scores change sufficiently to cause a change in the index narrative condition category. All but one of the nine samples had a lower score from BioMonTools than the USGS code. Five of these samples switched between the major condition categories of ‘satisfactory’ and ‘moderately degraded’, which are draft assignments until the thresholds are confirmed and approved by IDEM. Ultimately, these changes resulted in a decrease in DE in the HiN site class but there was no difference in DE between calculation methods in the LoN site class (Table 1).

*Table 1. Discrimination Efficiency (%) of the Indiana Diatom IBIs for both the HiN and LoN site classes depending on the scoring methodology. BioMonTools uses R to calculate and score metrics while the USGS code relies on R for metric calculation and MS Excel for metric scoring.*

	HiN Site Class	LoN Site Class
USGS Code	71.7	100
BioMonTools	67.4	100