Development of a Coldwater Benthic Index in Iowa

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Coldwater Benthic Index (CBI)

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Coldwater Benthic Index (CBI)

Introduction

Aquatic biological metric development for Iowa began in 1994 with the establishment of biological criteria sampling procedures for fish and macroinvertebrates (IDNR 2006). Metric development for macroinvertebrates resulted in a suite of 12 metrics which provided characterization of the community and stream health (Wilton 2004). During the development of Iowa's warmwater Benthic Macroinvertebrate Index of Biotic Integrity (WW BMIBI) the ecoregional differences within Iowa were evaluated. It was apparent that streams in the Paleozoic Plateau (Ecoregion 52b) differed most strongly from other regions of the state biologically and physically (Hubbard 2000; Wilton 2004). Differences in recent glacial history and geology and the consequent effect on hydrology resulted in clearer, coarse substrate dominated, higher gradient streams, including many streams characterized as coldwater. Additionally, the region contained Iowa's original native trout stocks and continues to support the vast majority of the state's trout streams.

Fish assemblages in coldwater streams are very different from those in warmwater streams. For example, in the upper Midwest a quality coldwater stream will have five or fewer fish species (Mundahl and Simon, 1999) while a similar sized warmwater (or thermally altered coldwater) stream might support 12-15 fish species. Consequently, many of the fish metrics effectively applied in warmwater settings are not appropriate for assessing coldwater streams (Lyons, 1992; Lyons et al., 1996) and this is undoubtedly the case with Iowa's warmwater fish IBI. As a result, biological metrics have been developed to characterize coldwater fish assemblages across the United States, including the adjacent states of Minnesota (Mundahl and Simon, 1999) and Wisconsin (Lyons et al., 1996).

Benthic macroinvertebrate assemblages do not appear to differ as markedly between coldwater and warmwater streams (in Iowa, at least) as do fish assemblages, and to our knowledge no parallel evaluation of benthic macroinvertebrate communities in coldwater streams has been conducted. What is known is that many taxa are exclusively collected in coldwater environments and are considered "rare" in Iowa from a biogeographical perspective. While the warmwater BMIBI has generally proven to work well as a diagnostic tool for Iowa's streams, the streams of Iowa's Paleozoic Plateau tend to group in the "excellent" to "good" qualitative rating categories. The streams of this area tend to be more ecologically intact than other areas of the state; however, there are some artifacts of the current warmwater BMIBI (most specifically metric scoring related to watershed size) that skew IBI values higher. Our objective is to develop a Coldwater Benthic Index (CBI) which will provide a more accurate assessment of streams classified, or potentially classifiable, as coldwater.

Methods

The sampling methods (Appendix A) used to obtain the benthic macroinvertebrate community data analyzed for this project have been standardized and have performed consistently in Iowa for over 15 years. Basically, sampling at each site involves the collection of three replicate standard-habitat (SH) samples and a multi-habitat (MH) sample. A physical habitat (phyhab) assessment usually accompanies each benthic macroinvertebrate site sampling and phyhab data were available for most sites. A combination of benthic macroinvertebrate and phyhab data from 58 sites comprised the dataset (Figure 1). Nearly all the sites were located on state-designated coldwater streams (Iowa's Surface Water Classification Document) and the several warmwater reaches included were known to support (often stocked) trout. The initial classification of sites as reference or impacted was quite conservative and subjective. Impacted sites had apparent thermal issues, or other stressors, and nearly all had been Total Maximum Daily Load (TMDL) monitoring sites reinforcing our observations of perceived impacts. Many of the reference sites had previously been sampled as "reference sites" but we focused on a subset that had been impressive in our own observations and were known to support self-sustaining trout populations. Potential metrics included the warmwater BMIBI and its 12 component metrics, as well as several others considered by Wilton (2004). We proposed several "new" metrics based on observations from both in-stream and at the bench (identifying specimens collected at the various sites). In addition, several metrics relating to coldwater taxa or coldwater proportions used by Mundahl and Simon (1999), although applied to coldwater fish assemblages, were included.

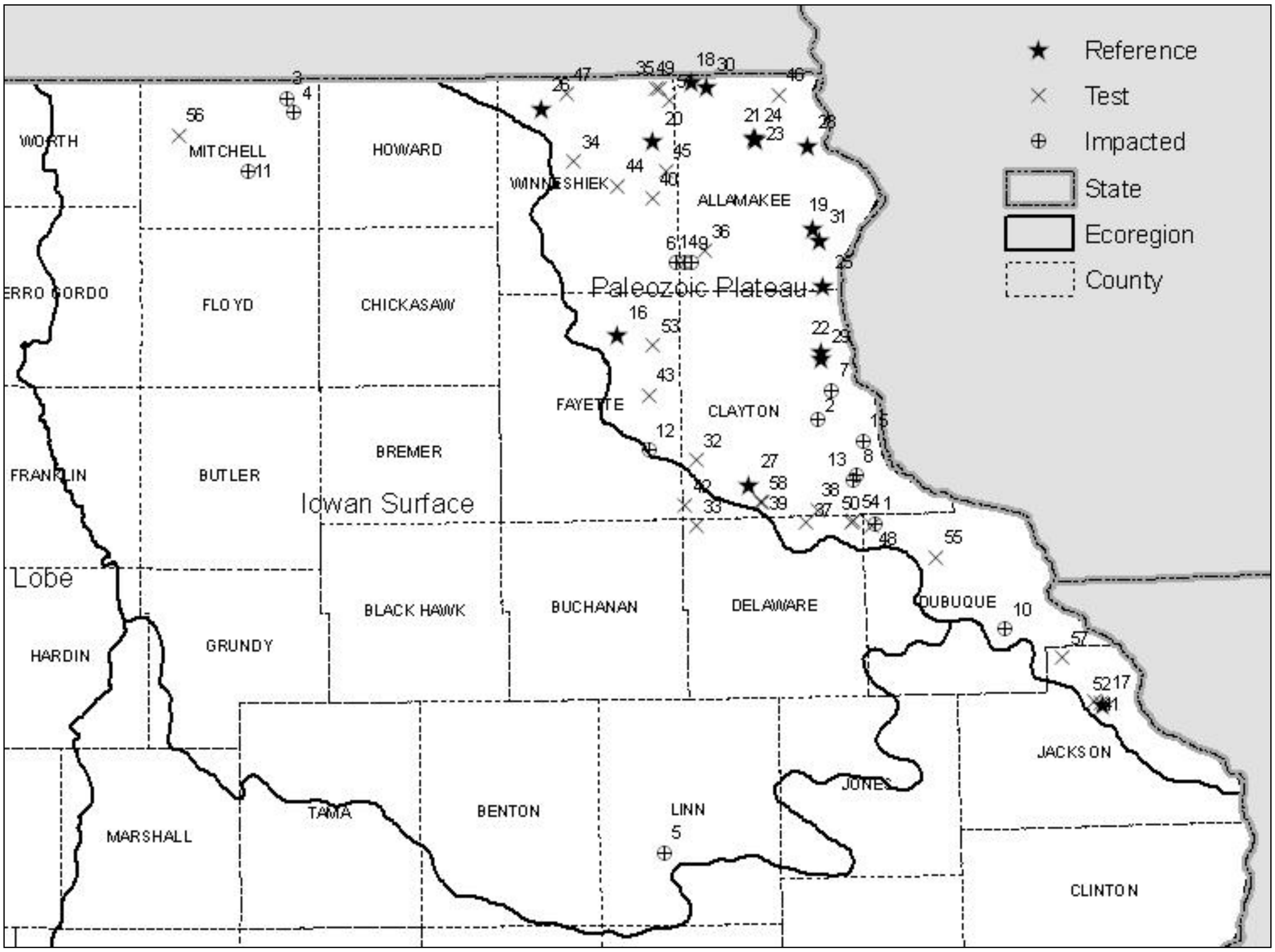

Figure 1. Map showing the location of 58 sampling sites in Northeast Iowa used in the development of the CBI. (Site numbers referenced in Table 5).

Recently, the Iowa Department of Natural Resources (IDNR) revised the classification process of streams designated as coldwater (IDNR 2004). During that process, the University of Iowa State Hygienic Laboratory (SHL) assisted in developing a list of obligate coldwater taxa for Iowa streams (Appendix B). The presence of these coldwater taxa was one of the tools that would allow for classification of a stream as coldwater. The obligate coldwater taxa designation provides a ready-made tool for inclusion in a multi-metric index.

While the construction of the warmwater BMIBI relied upon the log of the watershed size as the standardized comparison, our initial data analysis suggested mean stream width showed a stronger relationship with potential metrics. We suspect the correlation between stream flow and drainage area is less coupled due to larger sources of groundwater flow in the Paleozoic Plateau (e.g. large springs often originate in small surface watersheds). We decided to focus on stream width instead of the log of watershed size to evaluate potential metric relationships with stream size. Stream width has been used by others for coldwater system metric construction (Mundahl and Simon, 1999).

The Coefficient of Variation (CV) was used to compare the relative amount of sample variability among the potential metrics. CVs were calculated for each triplicate set of samples for SH metrics and across years for MH metrics (about 18 sites were sampled on multiple occasions).

Reference site criteria and site characterization

Several criteria were used to evaluate the initial 20 candidate coldwater reference sites. Initial criteria were chosen by the IDNR and required

- no wastewater treatment plant (WWTP) influence;
- minimal animal feeding influences;
- ecoregional appropriate in-stream habitat;
- natural riparian habitat;
- comparatively low row crop agriculture and urban development within the watershed;
- appropriate levels of *in situ* physical/chemical parameters (historical/present);
- limited alterations to the hydrologic regime;
- representative faunal assemblages;
- overall ecoregional representativeness

If a site met the thresholds for the aforementioned criteria, it was considered a reference site for our analyses. Four candidate reference sites that had criteria deficiencies, or were clearly influenced in one or more of these areas, were rejected and added to the "test" site pool. Test sites did not meet reference site criteria and/or had little or no thermal data. Impacted sites had obvious thermal and/or water quality issues (e.g. recent fish kills). Several of the impacted sites actually would have qualified as reference sites, based on the above criteria, but were prone to high summer water temperatures.

To evaluate the proposed metrics against a disturbance gradient, a select suite of measured variables was considered, but ultimately only three (% of temperature data points from data loggers exceeding 20^{o} C, % of fine sediment, trout reproductive rating) provided a broad enough spectrum of response on which site data could be evaluated. Temperature data were not available for all sites, but one-half of the total number of sites (29/58) had some temperature data available from SHL or IDNR Fisheries Bureau monitoring. Thermal logging generally occurred for two to three months and data points were collected every 15 minutes. The trout reproductive rating scale is modified from IDNR Fisheries' classification of trout reproduction (Appendix C) and each stream was assigned a value from one to four: 1 = no documented reproduction, 2 = inconsistent reproduction, 3 = consistent reproduction sustaining viable populations and 4 = consistent reproduction of brook trout sustaining viable populations. Using the three disturbance gradients, data were plotted on scatter plots (Appendices G, H, I) to provide insight into metric responses.

Box and whisker plots were used to compare reference versus impacted sites (Appendix J), with the strength of discrimination determined by degree of overlap of median values and interquartile ranges (Barbour et al. 1996, Wilton 2004). Discrimination between box plots of reference and impacted sites was scored 0 (poor), 1 (fair), 2 (good) and 3 (excellent). A value of three indicates no interquartile range (IQR) overlap; two represents IQR overlap but no median overlap of the opposing IQR; one represents IQR overlap of one box plot with the median of the other; zero represents the median of each box plot overlapping the opposing IQR. In addition, a correlation matrix for reference sites was constructed with all candidate metrics (as well as warmwater BMIBI and CBI) to evaluate relationships and the resultant level of redundancy (Table 5). Mundahl and Simon (1999) used a similar approach and excluded the metric with poorer discriminatory ability between reference and impacted sites if the correlation was $\geq$0.80.

Results and Discussion

Twenty-seven potential metrics, including the existing 12 WW BMIBI metrics and composite WW BMIBI, were characterized and evaluated for use in construction of the CBI (Table 1). The 27 metrics were characterized by their predicted response to: declining coldwater stream quality, metric variability (sampling error), impact site discriminatory power, stream gradient response range (Table 2), and redundancy (Table 3). One metric, MH taxa richness, exhibited a response contrary to the direction expected. Resultant *t*-tests (Table 2) and box plots (Appendix J) of reference versus impacted site comparisons yielded 13 metrics (including the WW BMIBI) that exhibited significant discriminatory power (Table 2).

Table 1. Candidate metrics of the CBI, metric type and expected response to coldwater stream degradation.

Metric	Type of metric	Expected response to declining CW stream quality
Existing WW BMIBI Metrics		
Multi-Habitat (MH) taxa richness	Richness/Diversity	decrease
Standard-Habitat (SH) taxa richness	Richness/Diversity	decrease
MH EPT richness	Richness/Diversity	decrease
SH EPT richness	Richness/Diversity	decrease
MH sensitive taxa richness	Tolerance	decrease
SH % 3-dominant taxa	Richness/Diversity and Balance	increase
SH Biotic Tolerance Index (BTI)	Tolerance	increase
SH % EPT	Richness/Diversity	decrease
SH % Chironomidae	Balance	increase
SH % Ephemeroptera	Balance	decrease
SH % Scrapers	Trophic	decrease
SH % Dominant functional feeding group (FFG)	Trophic	increase
WW BMIBI (composite of above 12 metrics)	Multimetric summation	decrease
Additional Proposed Metrics for Coldwater Benthic Index (CBI)		
SH CW taxa richness	Richness/Diversity and CW	decrease
MH CW taxa richness	Richness/Diversity and CW	decrease
SH Trichoptera taxa richness (no Hydropsychinae & Hydroptilidae)	Richness/Diversity	decrease
MH Trichoptera taxa richness (no Hydropsychinae & Hydroptilidae)	Richness/Diversity	decrease
SH % CW individuals	CW	decrease
SH % sensitive individuals	Tolerance	decrease
SH % Hydropsyche individuals	Balance	increase
SH % Hydropsychinae individuals	Balance	increase
SH % Isopoda & Turbellaria individuals	Balance	increase
SH % predator individuals	Trophic	decrease
SH % filterers/filter-collector individuals	Trophic	increase
SH % tolerant individuals	Tolerance	increase
MH Coleoptera taxa richness (no Elmidae & Dryopidae)	Richness/Diversity	decrease
MH % tolerant taxa	Tolerance	increase

Table 2. Summary of metric characterization, mean and (standard deviation) values at reference (n=16) and impacted (n=15) sites and results of t-tests between reference and impacted sites for candidate CBI metrics to be used in Iowa coldwater streams.

Data Metric	Amount of Metric Variability (sampling error)	Impacted Site Discriminatory Power	Stream Gradient Response Range	Reference sites	Impacted Sites	t	*p*
MH taxa richness	low	poor	undefinable	30.9 (6.6)	33.0 (9.2)	-0.7	0.461
SH taxa richness	low	fair	undefinable	12.8 (2.2)	11.3 (3.2)	1.6	0.129
MH EPT richness	low	poor	undefinable	11.0 (2.7)	10.4 (4.9)	0.5	0.653
SH EPT richness	low	poor	undefinable	6.3 (1.7)	5.9 (2.3)	0.5	0.605
MH sensitive taxa richness[1]	medium	excellent	broad	6.1 (1.9)	3.7 (2.9)	2.8	0.01
SH % 3-dominant taxa	low	fair	undefinable	68.4 (10.9)	73.7 (14.0)	-1.2	0.239
SH BTI[1]	low	good	broad	3.8 (0.7)	5.1 (0.7)	-5.3	<0.001
SH % EPT	low	poor	undefinable	56.0 (17.1)	49.3 (28.4)	0.8	0.414
SH % Chironomidae	medium	poor	narrow	14.7 (11.6)	26.0 (26.0)	-1.6	0.115
SH % Ephemeroptera	medium	fair	undefinable	25.5 (16.4)	17.8 (15.5)	1.4	0.185
SH % Scrapers	high	fair	undefinable	11.0 (6.8)	8.8 (10.2)	0.7	.474
SH % Dominant FFG[1]	low	good	narrow	55.6 (12.8)	66.2 (15.0)	-2.2	0.039
WW BMIBI (composite of above 12 metrics)	low	good	narrow	73.4 (9.7)	56.3 (16.7)	3.6	0.001
SH CW taxa richness	medium	excellent	moderate/ broad	1.8 (0.9)	0.04 (0.1)	7.4	<0.001
MH CW taxa richness[1]	medium	excellent	moderate/ broad	3.5 (1.5)	0.3 (0.6)	7.6	<0.001
SH Trichoptera taxa richness (no Hydropsychinae & Hydroptilidae)	high	excellent	broad	2.0 (1.0)	0.2 (0.3)	6.6	<0.001
MH Trichoptera taxa richness (no Hydropsychinae & Hydroptilidae) [1]	medium	excellent	broad	4.4 (1.5)	1.1 (1.1)	7.0	<0.001
SH % CW individuals[1]	high	excellent	moderate/ broad	17.2 (13.4)	0.05 (0.1)	5.0	<0.001
SH % sensitive individuals	medium	excellent	broad	40.7 (16.5)	11.4 (10.9)	5.9	<0.001
SH % Hydropsyche individuals[1]	high	good	moderate	0.3 (0.5)	2.5 (3.5)	-2.6	0.015
SH % Hydropsychinae individuals[1]	medium	excellent	broad	10.7 (9.5)	30.0 (20.2)	-3.5	0.001
SH % Isopoda & Turbellaria individuals	high	poor	narrow	1.8 (2.7)	9.8 (20.3)	-1.6	0.115
SH % predator individuals	high	poor	undefinable	0.8 (0.7)	1.0 (1.5)	-0.6	0.524
SH % filterers/filter-collector individuals	medium	poor	undefinable	34.9 (13.5)	32.6 (20.9)	0.4	0.710
SH % tolerant individuals	high	fair	undefinable	7.3 (5.5)	6.3 (10.6)	0.4	0.724
MH Coleotera taxa richness (no Elmidae & Dryopidae)	high	poor	undefinable	4.4 (3.0)	3.1 (1.8)	1.5	0.142
MH % tolerant taxa[1]	low	good	broad	18.1 (4.2)	25.3 (6.6)	-3.7	<0.001
[1] Metrics selected for the CBI							

Six of these 13 metrics were highly correlated with at least one other metric (Tables 3 & 7). In order to reduce metric redundancy, four metrics were eliminated: 1) SH % sensitive individuals, 2) SH Trichoptera taxa richness (no Hydropsychinae & Hydroptilidae), 3) SH CW taxa richness and 4) the WW BMIBI.

The SH % sensitive individuals metric was inversely correlated (r=-0.87) with the SH BTI metric. The SH BTI metric was retained because it displayed less variability.

Three other SH metrics were highly inter-correlated: the SH Trichoptera taxa richness (no Hydropsychinae & Hydroptilidae) metric was correlated with the SH % CW individuals (*r*=0.85) and SH CW taxa richness (*r*=0.94) metrics and the latter two metrics with each other (*r*=0.82). The SH % CW individuals metric was retained because it is not a measure of an aspect of taxa richness. The other two metrics were retained as MH metrics.

Though strongly correlated (*r*=0.87), the MH CW taxa richness and MH Trichoptera taxa richness (no Hydropsychinae & Hydroptilidae) metrics were both retained. We believe the MH Trichoptera taxa richness (no Hydropsychinae & Hydroptilidae) metric may assist in discriminating between streams that are thermally altered (or misclassified as coldwater) and streams with water quality or physical habitat deficiencies. We also hope that retaining the MH CW taxa richness metric may give the CBI some applicability if used in other seasons (primarily spring) when CW taxa not collected during the July-October period may be present.

The final metric to be eliminated was the overall WW BMIBI. The WW BMIBI is not an independent metric, but the summation of the 12 tested, and accepted, metrics used for wadeable, warmwater stream assessment. This metric retained good discriminatory power even though only three of its component metrics had significant reference vs. impacted site *t*-tests (Table 2). The discriminatory power of this metric may be partially driven by a strong inverse correlation between the WW BMIBI and BTI (*r*= -0.72) but may also reflect the inflating nature of the WW BMIBI in streams with small watersheds (Figure 2).

Table 3. Summary of the redundancy of seven (7) CBI metrics.

Data Metric	Redundancy (r >0.81)
SH % sensitive individuals	SH BTI
SH CW taxa richness	SH Trichoptera taxa richness (no Hydropsychinae & Hydroptilidae), SH % CW individuals
MH CW taxa richness	MH Trichoptera taxa richness (no Hydropsychinae & Hydroptilidae)
SH Trichoptera taxa richness (no Hydropsychinae & Hydroptilidae)	SH % CW individuals, SH CW taxa richness
MH Trichoptera taxa richness (no Hydropsychinae & Hydroptilidae)	MH CW taxa richness
SH % CW individuals	SH CW taxa richness, SH Trichoptera taxa richness (no Hydropsychinae & Hydroptilidae)
SH BTI	SH % sensitive individuals

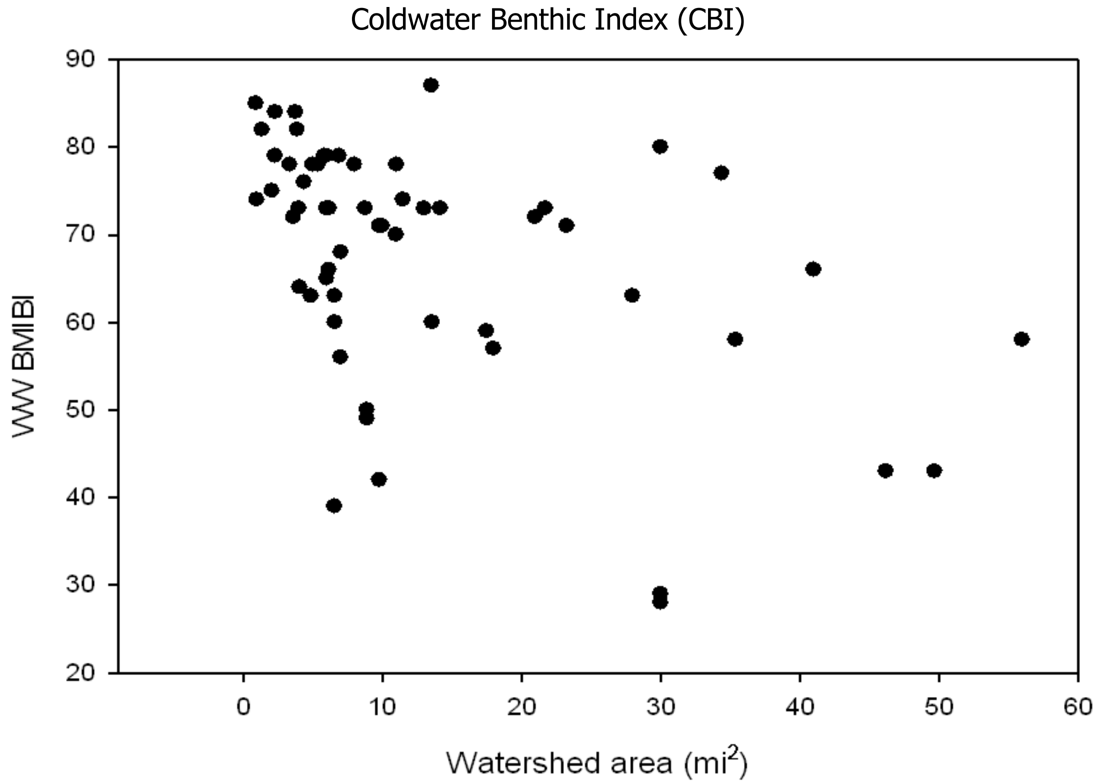

Figure 2. Scatter plot of warmwater BMIBI scores for coldwater sites against watershed area.

In the development of the warmwater BMIBI Wilton (2004) describes a subset of "core" metrics which display strong discriminatory power, low variability, and a broad response to environmental gradients. The nine metrics recommended here for use in the CBI do not demonstrate these core aspects as clearly (Table 2). The strongest discriminators also tend to show higher variability and often a "moderate" response to gradients. These responses would likely be considered narrow if plotted against a wider spectrum of gradients (all the streams in the dataset are relatively small: <40′ wide). However, we consider these nine metrics to be the most useful of those evaluated. They represent most aspects of a benthic macroinvertebrate community (aside from including a measure of trophic composition), adequately discriminate between reference and impacted conditions and display broad and narrow responses to the stream condition spectrum.

Metric Descriptions

Taxa richness metrics:

MH CW taxa richness is the number of CW designated taxa (Appendix B) handpicked from all the different types of benthic habitat in the sampling reach. Several CW taxa are not likely to be encountered in SH samples but should be collected in MH samples if present in the reach. This metric is strongly correlated with the MH Trichoptera taxa richness metric, and many of the CW taxa collected during the "standard" July – October field season are caddisflies. This metric may prove to be quite sensitive if applied to a springtime sampling (March-June).

MH Trichoptera taxa richness (no Hydropsychinae & Hydroptilidae) is the number of caddisfly taxa handpicked from all the different types of benthic habitat in the sampling reach, excluding the groups named in the metric. Observations during sampling suggested that several caddisfly taxa are commonly associated with quality coldwater streams (e.g. *Brachycentrus* spp and *Glossosoma* spp) and several others are less often encountered. We feel this metric (though redundant) may also be an indicator of coldwater stream habitat quality/diversity and could assist in discriminating between streams that are thermally altered (or misclassified as coldwater) and streams with water quality or physical habitat deficiencies. Hydropsychinae, a sub family of Hydropsychidae that includes the genera *Ceratopsyche* and *Hydropsyche*, tend to occur in nearly all streams are excluded from the metric. Hydroptilidae larvae, which cannot be identified below the generic level, are also excluded because they seem ubiquitous and are found in most Paleozoic Plateau streams.

MH sensitive taxa richness is the number of sensitive taxa handpicked from all the different types of benthic habitat in the sampling reach. Sensitive taxa are defined as those which have a BTI value of 3 or less (scale of 0-10 with increasing values reflecting increasing organic enrichment; see SH BTI metric description below). Most of the CW taxa are also sensitive, but this metric should assist in discriminating between streams that are thermally altered (or misclassified as coldwater) and streams with water quality or physical habitat deficiencies.

MH % tolerant taxa is the proportion of all taxa handpicked from all the different types of benthic habitat in the sampling reach that are tolerant taxa. Tolerant taxa are defined as those which have a BTI value of 7 or more (scale of 0-10 with increasing values reflecting increasing organic enrichment; see SH BTI metric description below). This metric showed a broader response to environmental gradients than was expected.

Proportional abundance metrics (calculated from SH samples only):

SH Benthic Tolerance Index (BTI) is adapted from the Hilsenhoff Biotic Index which was developed as an indicator of stream organic enrichment (Hilsenhoff, 1987). The scale ranges from 0-10 with increasing values reflecting increasing organic enrichment. This metric's effectiveness in both warmwater and coldwater streams is not surprising, as its development was driven by Hilsenhoff's dissatisfaction with how the diversity indices of his day underrated cold, pristine northern Wisconsin streams. The tolerance values are a combination of several regional resources (Hilsenhoff 1987, Hilsenhoff 1988, Huggins and Moffett 1988, Bode et al. 1991, Lenat 1993, Barbour et al. 1999, Bode et al. 2002) that were used to more accurately characterize the tolerance values of benthic macroinvertebrate communities within Iowa versus Hilsenhoff's Wisconsin focus.

SH % CW individuals is the proportion of the total number of organisms that belong to the CW designated taxa. This metric is strongly correlated with the SH CW taxa richness, which was eliminated due to redundancy. The decision of which metric to retain was partially based on our observation that in quality coldwater streams one CW taxa can often dominate the community. Mass emergences can quickly change benthic macroinvertebrate community composition and retaining this metric insures that coldwater dominated communities will be reflected temporally.

SH % Hydropsyche individuals is the proportion of the total number of organisms that belong to the Trichoptera genus *Hydropsyche*. *Hydropsyche betteni* is found in most small to medium sized streams in Iowa but its abundance seems to be notably limited in high quality coldwater streams. It is likely an indicator of both extremes in the coldwater stream condition spectrum.

SH % Hydropsychinae individuals is the proportion of the total number of organisms that belong to the Hydropsychidae sub-family Hydropsychinae, which includes the genera *Hydropsyche* (mentioned above) and *Ceratopsyche,* but not coldwater obligates, *Diplectrona modesta* and *Parapsyche apicalis* (both family Hydropsychidae). *Ceratopsyche and Hydropsyche spp.* have been collected in nearly all Paleozoic Plateau streams, but their abundances seem to be depressed in the Iowa reference coldwater streams. This metric displayed a broader response across the environmental gradients than the SH % Hydropsyche individuals metric alone.

SH % Dominant functional feeding group (FFG) is the proportion of the total number of organisms that belong to the numerically dominant FFG. As stream disturbance increases, one FFG tends to dominate the benthic macroinvertebrate assemblage and trophic diversity is reduced.

Metric scoring

Two methods were considered for scoring metrics: trisection and linear interpolation. The criteria for scoring each metric differs slightly, depending on the method, but both methods generate potential values of 0-10.

The trisection method is described by Mundahl and Simon (1999) and developed by Lyons (1992) and Lyons et al. (1996). Metric relationships with stream width were tenuous; especially considering the constrained stream width of our population (all streams had average widths <40 feet). Metric scoring was not linked with stream width, so scores were constructed based on a trisection of the population. Specifically, this is done by excluding the upper 5% of optimum values and the remaining (95%) range of values is divided into thirds. Values falling in the optimal third (or better) are scored 10, the middle third score 5, and the lower third (or lower) score 0.

The other option used in metric scoring, linear interpolation (Hughes et al. 1998), creates a continuous scale by using optimum (after excluding the upper 5%) and minimum metric values. The scoring range is continuous (between 0 and 10) and can include decimals (Table 4).

Following Mundahl and Simon (1999), the lower limit is defined by the lowest reference value. Other methods (e.g., Wilton, 2004) use the lowest value to be expected as the lower range limit. For example the MH CW taxa richness metric's lowest reference value was 1 and the highest (after excluding the upper 5%) was 5. The raw metric range would be from 1 to 5 (Mundahl and Simon, 1999) and 0 to 5 (Wilton, 2004). Ultimately, we decided our scoring methods should include the complete range to account for the total spectrum of potential scoring (e.g. sites that generate values greater than the lowest score, but under the reference condition threshold). This is the approach taken by Wilton (2004) for the warmwater BMIBI and seemed most appropriate and consistent.

Both methods of metric scoring were calculated for comparison (Table 5). With both methods, each metric is assigned a score of zero to ten and all nine proposed metrics are summed (to normalize CBI scores, they were multiplied by 1.111) to produce an overall CBI score of 0 to 100. With the linear interpolation method, it is possible to calculate a metric score that is less than zero or greater than ten; these values are adjusted to zero or ten, respectively.

Table 4. Scoring formulae for component CBI metrics resulting from the linear interpolation scoring method.

Metric	Metric Scoring Formula
SH % CW individuals	(raw value/37.28)*10
SH % Hydropsyche individuals	((14.86-raw value)/14.86)*10
SH % Hydropsychinae individuals	((66.19-raw value)/66.19)*10
SH % Dominant Functional Feeding Group (FFG)	((92.51-raw value)/53.2)*10
SH Benthic Tolerance Index (BTI)	((7.0-raw value)/3.98)*10
MH sensitive taxa richness	((raw value)/9)*10
MH Trichoptera taxa richness (no Hydropsychinae & Hydroptilidae)	((raw value)/6.46)*10
MH % tolerant taxa	((37.85-raw value)/30.16)*10
MH CW taxa richness	((raw value)/5.08)*10

Site scores for each method were generally very close. The linear interpolation method displays finer resolution due to its continuous scoring nature. Both CBI scoring methods, however, resulted in good discrimination between reference and impacted sites (Figure 3).

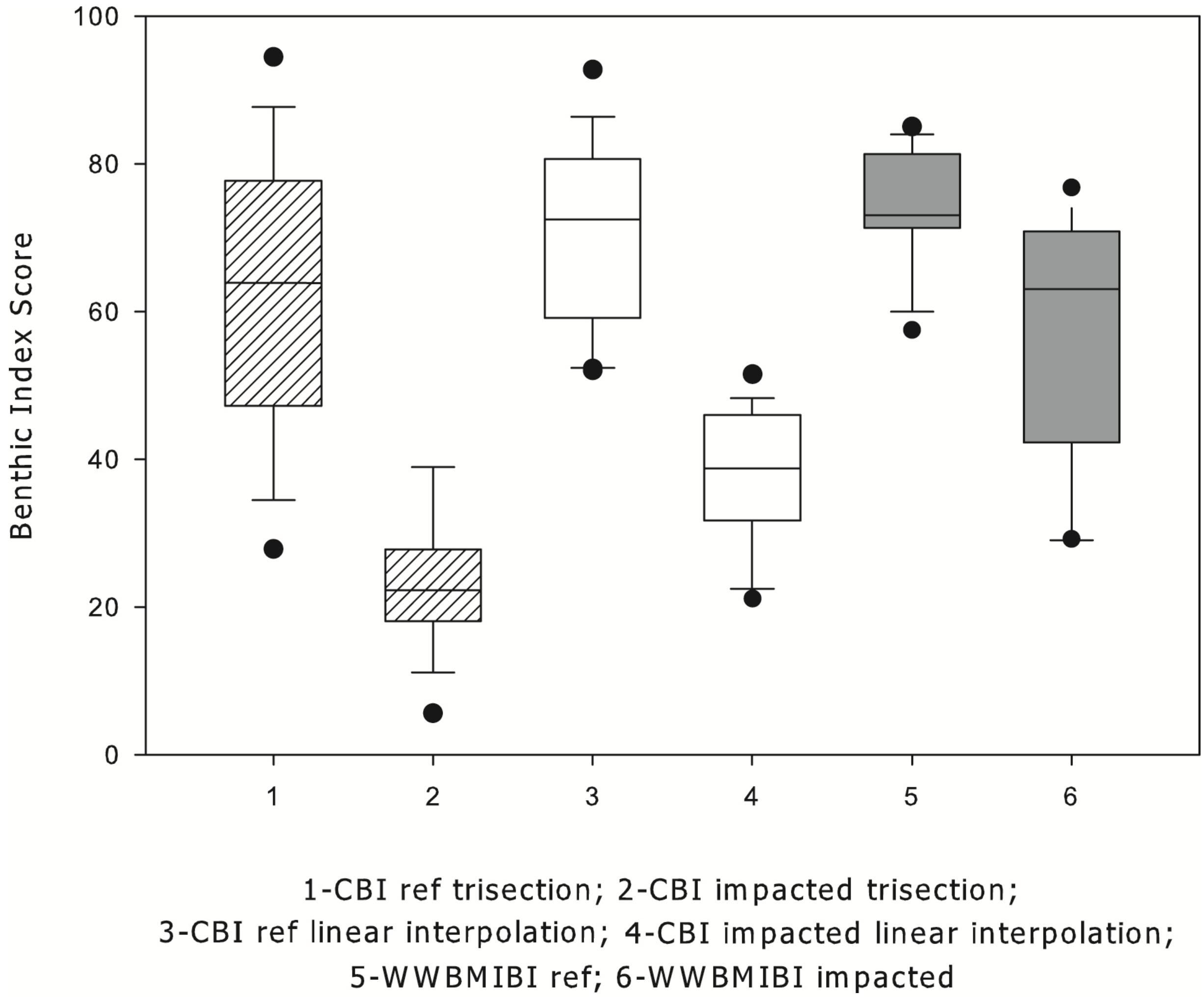

Figure 3. Box plots of CBI/warmwater BMIBI scores for reference (n=16) vs. impacted (n=15) sites. Two CBI scoring methods (tri-section and linear interpolation) and the WW BMIBI are illustrated.

As a final step in the metric evaluation process, a correlation analysis of the CBI with its nine selected metrics, as well as all the potential candidate metrics, was performed. All metrics were correlated in the correct (expected) direction. Most of the metrics were highly correlated to the CBI (Table 7), two exceeding the (r >0.80) level, suggesting excessive redundancy and dominance by these metrics. Mundahl and Simon (1999) also reported strong correlations between several individual metrics and their coldwater fish IBI.

Coldwater Benthic Index (CBI)

Table 5. Stream name, location, site type, and comparison of CBI scoring methods for the 58 sampling sites.

Map #	Bionet ID	Stream Name	Location	Site Type	0,5,10 Trisection Score	Linear Interpolation Score	Score Difference
1	370	White Pine Hollow Creek	Luxemburg - REMAP #58	Impacted	22.2	25.8	3.6
2	661	South Cedar Creek	Garnavillo - SoCed1	Impacted	38.9	47.2	8.3
3	58	Wapsipinicon River	Wapsipinicon SWMA - McIntire	Impacted	22.2	30.9	8.7
4	689	Wapsipinicon River	McIntire - Wap1	Impacted	27.8	36.9	9.1
5	675	McLoud Run	Cedar Rapids	Impacted	11.1	22.4	11.3
6	414	Yellow River	Castalia - REMAP #253	Impacted	22.2	34.1	11.9
7	660	Buck Creek	Garnavillo - BuCr1	Impacted	38.9	51.5	12.6
8	326	Peck Creek	Osterdock -- REMAP #230	Impacted	5.6	20.0	14.4
9	159	Yellow River	Site 3	Impacted	33.3	48.2	14.9
10	23	Catfish Creek	Swiss Valley Park	Impacted	22.2	40.2	18.0
11	28	Burr Oak Creek	Osage (Upstream)	Impacted	27.8	46.7	18.9
12	406	Brush Creek	Arlington	Impacted	22.2	41.4	19.2
13	570	Peck Creek	Osterdock- PeCr2	Impacted	16.7	36.1	19.4
14	157	Yellow River	Site 2	Impacted	22.2	43.8	21.6
15	269	Miners Creek	Guttenburg	Impacted	11.1	38.7	27.6
16	669	Duttons Creek	West Union - DuCr1	Reference	77.7	76.4	-1.3
17	664	South Big Mill Creek	Bellevue - SBigM1	Reference	77.7	76.5	-1.2
18	360	Waterloo Creek	Dorchester - REMAP #63	Reference	77.7	79.2	1.5
19	667	Little Paint Creek	Harpers Ferry - LiPaCr1	Reference	94.4	92.7	-1.7
20	673	South Pine Creek	Sattre - SoPCr1	Reference	77.7	79.5	1.8
21	539	French Creek (Main Branch)	Waukon	Reference	88.8	81.7	-7.1
22	10	North Cedar Creek	Public Access Area- Sny Magill	Reference	44.4	52.0	7.6
23	540	French Creek (West Branch)	Waukon	Reference	77.7	85.3	7.6
24	48	French Creek	French Creek SWMA - Waukon	Reference	72.2	81.7	9.5
25	352	Dousman Creek	Ion	Reference	50.0	59.6	9.6
26	18	Coldwater Creek	Coldwater Spring SWMA-Bluffton	Reference	55.5	68.3	12.8
27	408	Mossy Glen Creek	Edgewood - Mossy Glen Pr.	Reference	44.4	58.5	14.1
28	666	Clear Creek	Lansing - CCr1	Reference	50.0	67.8	17.8
29	474	North Cedar Creek	Clayton - REMAP #159	Reference	33.3	53.9	20.6
30	802	Waterloo Creek	Upstream of Dorchester	Reference	27.8	52.2	24.4
31	668	Little Paint Creek	Harpers Ferry - LiPaCr2	Reference	55.5	67.8	12.3
32	435	Hewitt Creek	Volga - REMAP #133	Test	50.0	53.6	3.6
33	671	Fenchel Cr. (Richmond Spr.)	Strawberry Point - FeCr1	Test	61.1	64.9	3.8
34	476	Ten Mile Creek	Decorah - REMAP #169	Test	55.5	60.6	5.1
35	361	Middle Bear Creek	Highlandville- REMAP #249	Test	55.5	64.5	9.0
36	160	Yellow River	Site 4-- W60 Bridge	Test	38.9	48.2	9.3
37	670	Twin Springs Creek	Colesburg - TwSpr1	Test	33.3	42.8	9.5
38	662	Brownfield Creek	Colesburg - BroCr1	Test	38.9	49.8	10.9
39	473	Bear Creek	Edgewood - REMAP #155	Test	50.0	61.2	11.2
40	49	Trout River	Trout R. Public Area - Decorah	Test	55.5	67.2	11.7
41	665	Storybook Hollow	Bellevue - StHol1	Test	44.4	56.4	12.0
42	672	Maquoketa River	Strawberry Point - Maq2	Test	38.9	51.0	12.1
43	614	Mink Creek	Wadena - REMAP #222	Test	77.7	75.9	-1.8
44	477	Trout Run Creek	Decorah - REMAP #178	Test	38.9	51.2	12.3
45	217	Coon Creek	Decorah	Test	38.9	53.8	14.9
46	521	Irish Hollow Creek	New Albin - REMAP #265	Test	11.1	26.0	14.9
47	375	East Pine Creek	Burr Oak - REMAP #80	Test	11.1	26.9	15.8
48	407	White Pine Hollow Creek	Luxemburg	Test	16.7	35.9	19.2
49	19	Middle Bear Creek	Highlandville	Test	44.4	63.6	19.2
50	632	Little Turkey River - US Site	UHL Special Project	Test	0.0	21.0	21.0
51	14	North Bear Creek	North Bear Creek Public Access	Test	33.3	54.7	21.4
52	663	Big Mill Creek	Bellevue - BigMil1	Test	11.1	32.7	21.6
53	424	Bell Creek	Clermont - REMAP #100	Test	22.2	44.2	22.0
54	631	Little Turkey River - DS Site	UHL Special Project	Test	5.6	27.9	22.3
55	419	MF Little Maquoketa River	Rickardsville - REMAP #261	Test	22.2	45.1	22.9
56	674	Turtle Creek	St Ansgar - TuCr1	Test	27.8	51.1	23.3
57	643	Tetes des Morts Creek	St. Donatus TDM2 (CW site)	Test	11.1	36.0	24.9
58	409	Bear Creek	Edgewood - Bixby Preserve	Test	11.1	41.9	30.8

Table 6. Summary statistics of CBI scoring methods and WW BMIBI.

Site Type	Scoring	n	Min	25%	Mean	SD	Median	75%	Max
Impacted	0,5,10 Tri-Section	15	5.6	19.5	23.0	9.6	22.2	27.8	38.9
Impacted	Linear Interpolation	15	20.0	32.5	37.6	9.6	38.7	45.3	51.5
Impacted	LI & 0,5,10 absolute difference	15	3.6	10.2	14.6	6.2	14.4	19.1	27.6
Impacted	WW BMIBI	15	28.0	42.3	56.3	16.7	63.0	70.8	78.0
Reference	0,5,10 Tri-Section	16	27.8	48.6	62.8	20.1	63.9	77.7	94.4
Reference	Linear Interpolation	16	52.0	59.3	70.8	12.7	72.4	80.1	92.7
Reference	LI & 0,5,10 absolute difference	16	1.2	1.8	9.4	7.2	8.6	13.1	24.4
Reference	WW BMIBI	16	57.0	71.3	74.4	8.0	73.0	81.3	85.0
Test	0,5,10 Tri-Section	27	0.0	13.9	33.5	19.7	38.9	47.2	77.7
Test	Linear Interpolation	27	21.0	39.0	48.4	14.1	51.0	58.5	75.9
Test	LI & 0,5,10 absolute difference	27	1.8	10.2	15.1	7.4	14.9	21.5	30.8
Test	WW BMIBI	27	43.0	63.3	70.1	10.7	73.0	78.0	87.0
All Sites	0,5,10 Tri-Section	58	0.0	22.2	38.9	23.4	38.9	54.1	94.4
All Sites	Linear Interpolation	58	20.0	39.1	51.8	17.8	51.2	64.3	92.7
All Sites	LI & 0,5,10 absolute difference	58	1.2	8.8	13.4	7.4	12.3	19.2	30.8
All Sites	WW BMIBI	58	28.0	60.0	67.6	13.7	72.0	78.0	87.0

Table 7. Correlation coefficient matrix of 26 candidate CBI metrics, WW BMIBI and CBI (n=58); r values >0.80 are indicated in bold.

	MHCWRich	MHTrichnoH/H	SHTrichnoH/H	MHColRichnoE/D	MHSensRich	SH%Chiro	SHBTI	SH%CWind	SH%Sensind	SH%Hpsyche	SH%Hpsychinae	SH%IsoTurb	SH%Pred	SH%Filterer	SH%Tolerant	MH%Tolerant	MHTaxaRich	SHTaxaRich	MHEPTRich	SHEPTRich	SH%Ephem	SH%EPT	SH%Scraper	SH%3Dom	SH%DomFFG	WW BMIBI	CBI
SHCWRich	.74	.66	**.94**	.08	.42	-.18	-.48	**.82**	.55	-.31	-.41	-.15	.02	.11	.07	-.48	-.11	.29	.02	.14	-.08	.06	.12	-.21	-.43	.36	.79
MHCWRich		**.87**	.63	.12	.60	-.16	-.44	.59	.54	-.28	-.40	-.17	-.11	.07	.09	-.56	-.06	.07	.16	-.12	.03	.00	-.07	-.06	-.17	.30	**.84**
MHTrichnoH/H			.66	.03	.62	-.24	-.48	.59	.55	-.25	-.29	-.15	-.04	.16	.08	-.54	.06	.10	.38	-.02	-.01	.08	.12	-.12	-.27	.32	**.83**
SHTrichnoH/H				-.02	.34	-.20	-.46	**.85**	.52	-.31	-.40	-.09	.16	.11	.08	-.43	-.14	.29	.02	.15	-.13	.04	.24	-.21	-.42	.32	.74
MHColRichnoE/D					-.01	-.01	-.02	.10	.11	.03	-.08	.02	-.17	.09	.16	.30	.46	.09	-.10	-.02	-.04	-.06	-.20	-.04	-.03	.09	-.05
MHSensRich						-.10	-.43	.39	.45	-.44	-.10	-.29	-.03	.18	-.07	-.60	.38	.19	.63	.19	.07	.17	.19	-.15	-.23	.26	.73
SH%Chiro							.47	-.17	-.38	-.08	-.26	-.11	-.19	-.50	.02	.26	.05	-.46	-.06	-.31	-.24	-.54	-.23	.45	.49	-.61	-.25
SHBTI								-.56	**-.87**	.26	.14	.58	-.21	-.32	.56	.48	-.02	-.21	-.27	-.26	-.56	-.63	-.12	.16	.38	-.72	-.66
SH%CWind									.65	-.28	-.42	-.14	.13	.21	-.01	-.32	-.03	.12	.02	.05	-.11	.11	.09	-.05	-.32	.25	.74
SH%Sensind										-.37	-.37	-.30	.18	.12	-.21	-.38	-.03	.11	.19	.11	.51	.43	-.07	.05	-.18	.52	.72
SH%Hpsyche											.58	.03	-.23	.36	-.04	.42	-.14	-.07	-.32	.02	-.23	.23	-.15	-.11	.11	-.07	-.57
SH% Hpsychinae												-.14	-.15	.65	-.25	.17	.31	.19	.24	.44	-.16	.55	.02	-.38	-.18	.08	-.50
SH%IsoTurb													-.03	-.32	.74	.37	-.12	-.05	-.31	-.25	-.36	-.49	-.06	.16	.24	-.48	-.25
SH%Pred														-.12	-.08	-.07	-.13	.18	-.03	.02	.11	.03	.32	-.01	-.11	.12	.11
SH%Filterer															-.35	-.13	.23	.18	.18	.36	-.18	.56	.01	-.35	-.40	.36	.03
SH%Tolerant																.19	-.06	.06	-.18	-.17	-.30	-.45	-.11	.08	.22	-.39	-.03
MH%Tolerant																	.01	-.16	-.43	-.11	-.14	-.17	-.23	.28	.39	-.45	-.72
MHTaxaRich																		.23	.63	.36	-.06	.17	.08	-.25	-.18	.03	.03
SHTaxaRich																			.22	.75	.12	.32	.50	-.79	-.65	.59	.22
MHEPTRich																				.47	.18	.36	.19	-.21	-.25	.23	.35
SHEPTRich																					.24	.62	.21	-.63	-.48	.49	.06
SH%Ephem																						.55	-.16	.10	.11	.47	.14
SH%EPT																							-.06	-.32	-.28	.59	.09
SH%Scraper																								-.54	-.62	.34	.22
SH%3Dom																									.74	-.55	-.17
SH%DomFFG																										-.68	-.41
WW BMIBI																											.47

Metric relationships with other variables

Correlative analysis of candidate metrics and various environmental indicators was conducted to examine the directionality and sensitivity of metric responses in relation to important environmental gradients. Specifically, basin drainage area, maximum temperature, minimum temperature, and average stream width were intra-correlated and compared to the candidate metrics (Appendix D). Additionally, land cover (Appendix E) and nutrients (Appendix F) were intra-correlated and compared with WW BMIBI and CBI, respectively. Pearson's correlation was used to explore the strength of linear dependence and provide insight into potential drivers of significance for components of the CBI.

These relationships are generally rather intuitive and (even if not statistically significant) in the direction expected. For example, the candidate CBI metric responses to maximum temperature were similar to those shown to temperatures exceeding 20^{o} C (scatter plots in Appendices G, H, I), with the metrics measuring CW taxa, sensitive taxa, and Trichoptera richness (no Hydropsychidae or Hydroptilidae) all showing fairly strong negative correlations with maximum temperature. Metric relationships with minimum temperature were lacking or weak, which is not surprising. The range of minimum temperatures was narrow (6.9 to 11.7^{o} C) and the duration of temperature logger deployment at sites was rather variable. Also, as expected, some of the WW BMIBI metrics showed positive responses to increasing drainage basin area. MH total taxa, EPT taxa richness, and sensitive taxa relationships to basin area were all driven by a few sites on larger streams like the Yellow, Maquoketa and Wapsipinicon rivers. On the wadeable stream spectrum, these "medium" sized streams typically exhibit a diverse coolwater assemblage containing many EPT taxa that are not found in coldwater streams, several of which are sensitive taxa. The EPT metrics were also positively correlated with stream width. Similarly, the correlation between basin area and Chironomidae % was also driven by three larger sites with very high SH midge abundance.

Correlations with the minor land use variables should be interpreted carefully, and probably disregarded. At both watershed and riparian scales the water/wetland and barren categories each comprised less than one percent of the basin areas and road/urban generally less than five percent. The three major land use classes (row crops, grass, woodlands) are all correlated with each other at both the landscape and riparian levels (as expected) and at the riparian level were correlated to one or both of the biological indices. We did not expect the overall CBI score to be negatively related to percent riparian grassland, though this may be an artifact of site selection. Most of the reference sites are on state property that already was or has been allowed to revert to woody vegetation, while some of the impacted sites are definitely affected by overgrazing (pasture=grassland). It also appears that row crops and grasslands were positively related at the riparian scale, suggesting that the borders of the row crop fields may have tended to be grassy and not wooded.

Total Kjeldahl Nitrogen (TKN) was positively correlated with both total and ortho phosphorus and negatively related to both biological indices. The moderately strong TKN-CBI relationship is promising, suggesting that the CBI may be sensitive to nutrient as well as thermal gradients.

Conclusions and Recommendations

The metric development process is certainly driven by the selection of appropriate/representative reference and impacted sites. We believe our analysis and the resulting CBI discriminates well between reference and impacted sites. It is particularly worth noting the increased level of discrimination that results from replacing the WW BMIBI with the CBI. It appears one of our initial hypotheses – inflated metric scores due to watershed size – is moderated with the incorporation of the CBI. As such we believe the CBI functions as a better tool to appropriately evaluate Iowa's coldwater streams in a comparative way. Furthermore, we believe that the CBI should be helpful, through the examination of component metrics, for discriminating test sites that might have thermal issues from those that have primarily habitat and/or water quality issues. Finally, the CBI might prove useful for looking at longitudinal changes (e.g. most specifically thermal degradation) in Iowa's coldwater streams.

There are issues with redundancy and dominance by several metrics that are worthy of further analysis, but these metrics were also the strongest discriminators. Areas in need of further exploration include: refining trophic feeding structure metrics, testing of sites in Iowa (possibly SE Minnesota and SW Wisconsin), and exploring temporal variability to further validate the model's utility.

Although we have not ascribed qualitative ratings (e.g. excellent, good, fair, etc.) to the CBI scores, we believe further testing and validation will provide logical breakpoints for assigning them. We have suspected, and even presumed, that reference quality streams in the southern third of the Paleozoic Plateau may score consistently lower than their northern Paleozoic Plateau counterparts. Natural benthic macroinvertebrate distributions, land use, physical habitat or other factors could be involved. CBI scores may require some type of alternate scoring, or equalizer, be employed, as is done with the warmwater FIBI in the Missouri drainage of Iowa.

Coldwater Benthic Index (CBI)

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Appendix A.

Sampling procedures for fish and macroinvertebrate collection (from Wilton, 2004).

In 1994, wadeable stream sampling procedures were established for biological sampling and physical habitat evaluation. Standard procedures ensure that sample data are consistent across sampling sites and years. The procedures were updated in 2001 to provide additional clarification (IDNR 2001). The biological sampling procedures describe methods for collecting and processing stream benthic macroinvertebrates and fish. The habitat evaluation procedures describe the collection and compilation of quantitative and qualitative habitat data. Biological sampling and habitat evaluation are conducted in a pre-defined stream reach ranging in length from 150-350 meters, depending on stream size and habitat repetition frequency. Following is a synopsis of the sampling procedures:

Benthic Macroinvertebrates

Two types of stream benthic macroinvertebrate samples are collected: standard-habitat and multi-habitat.

Standard-habitat samples are collected from wood or rock substrate in riffles or shallow runs. Either a modified-Hess sampler or Surber sampler is used in naturally occurring riffle/run habitats that are comprised of large gravel and cobble substrates. Hester-Dendy style artificial substrates are used in streams that lack riffles with coarse rock substrates. The artificial substrates are constructed of 8 wood plates separated by spacers and mounted on a steel rod, which is pushed into the stream bottom. The artificial substrates are allowed a 4-6 week colonization period before they are retrieved and processed. Three replicate standard-habitat samples are collected from each site. Subsamples of 100-organisms are randomly obtained in the laboratory from each replicate sample.

Multi-habitat samples are collected from a pre-defined stream length of 150-350 meters which includes at least two pool/riffle sequences or two major channel bends. Benthic macroinvertebrates are handpicked from all types of benthic habitat that are accessible. Common types of benthic substrates sampled include: silt, sand, muck, rock, detritus, wood, root wad, and vegetation. Organisms are collected both from depositional and erosional zones of the stream.

The objective of multi-habitat sampling is to maximize the number of taxa collected. Several (3-10) individuals of each visually unique taxa are collected to facilitate identification and differentiation of similar taxa. A combined sampling time of 90 minutes is divided among two or three collectors who cover the entire sampling reach. All of the organisms are combined in one sample for the stream reach. Macroinvertebrate sample contents are preserved in 10% formalin and transported to the University of Iowa State Hygienic Laboratory (SHL) for analysis. Organisms are identified to the lowest-practical taxonomic level. In most cases, the analysis endpoint is genus or species. Some problematic organisms (e.g., Chironomidae) are identified to family level. Factors that determine the taxonomic endpoint include: (1) life stage and maturity of the organism; (2) availability of dichotomous taxonomic keys; (3) time/cost required to make an accurate determination. Taxonomic determinations are confirmed by an outside expert for a subset of organisms.

Coldwater Benthic Index (CBI)

Appendix B.

List of coldwater benthic macroinvertebrates used in the development of the CBI.

Taxa	Order	IDNR 2004[1] CW protocol list	Used in CBI
Acerpenna macdunnoughi	Ephemeroptera (mayflies)	X	
Ephemerella excrucians (*E. inermis*)		X	X
Ephemerella subvaria		X	X
Eurylophella spp.		X	
*Paraleptophlebia debilis**		X	X
Dannella lita (*Timpanoga lita*)		X	
Nigronia serricornis	Megaloptera (fishflies)		X
Amphinemura linda	Plecoptera (stoneflies)	X	X
Clioperla clio			X
Leuctra spp.		X	X
Nemoura trispinosa		X	X
Soyedina vallicularia			X
Brachycentrus americanus	Trichoptera (caddisflies)	X	X
Brachycentrus lateralis		X	X
Brachycentrus occidentalis			X
Chimarra aterrima		X	
Diplectrona modesta		X	X
Frenesia missa		X	X
Glossosoma spp		X	X
Hesperophylax designatus		X	X
Lepidostoma libum			X
Limnephilus spp.			X
Micrasema gelidum			X
Neophylax concinnus			X
Parapsyche apicalis		X	X
Pseudostenophylax spp			X
Rhyacophila vibox		X	X

As indicated by the categories above, both the IDNR list of coldwater taxa and those considered coldwater in present and future applications of the CBI are in flux. Very few of these taxa had even been reported from the state 15 years ago and we are still documenting new state records almost annually. Additional sampling has indicated a wider distribution of coldwater taxa in NE Iowa and collections have occurred in both coldwater and warmwater streams.

**Paraleptophlebia* was not included as a coldwater obligate taxa at the genus level because we have found at least one species that occurs in warmwater streams. We have collected *Paraleptophlebia* in a number of quality coldwater streams but have generally not felt comfortable identifying them to the species level. If we are able to differentiate this coldwater species (we assume is *P. debilis*) from other Iowa species or show distributional separation, it is likely that *Paraleptophlebia* will be added to the coldwater list as a genus.

[1] IDNR 2004. Cold water use designation assessment protocol. Iowa Department of Natural Resources, December 15, 2004.

Coldwater Benthic Index (CBI)

Appendix C.

CLASSIFICATION OF TROUT NATURAL REPRODUCTION – Effective Date February 2010
Category I - Streams that exhibit fairly consistent natural reproduction and maintains a viable population of the listed species without any stocking.
Category II - Streams that exhibit recent, but inconsistent, reproductive success and are not capable of maintaining a viable population of the listed species at this time.

Stream	County	Category/Species		Length in Miles
		I	II	
Bankston (Upper)	Dubuque	Brown		1.5
Bear	Allamakee		Brown	0.7
Bigalk	Howard		Rainbow	1.5
Big Mill	Jackson	Brown		1.5
Bloody Run	Clayton	Brown		2.5
Brownfield	Clayton	Brown		0.5
Burr Oak	Mitchell		Brown	2.0
Casey Springs	Winneshiek		Brook	1.5
Catfish Creek (upper)	Dubuque		Brown	1.0
Chialk	Howard	Brown		1.5
Clear (near Lansing)	Allamakee	Brown		2.0
Coldwater	Winneshiek		Rainbow	3.0
Coldwater	Winneshiek	Brown		1.0
Coon	Winneshiek		Brown	2.0
Dousman	Allamakee	Brown		1.0
Duck	Allamakee	Brown		2.0
Dutton Cave	Fayette		Brook	1.0
Ensign	Clayton	Brown		1.0
Erickson Branch	Allamakee	Brown		0.5
Falling Springs	Fayette		Brook	0.2
Fountain Springs	Delaware		Brown	1.0
French Creek	Allamakee	Brown		5.3
French Creek (Upper)	Allamakee	Brook		0.6
French Creek West Branch	Allamakee	Brown		0.5
French Creek West Branch	Allamakee	Brook		0.5
Grannis	Fayette	Brown		1.5
Hickory Creek	Allamakee	Brown		1.5
Jones	Allamakee	Brown		0.5
Kleinlein	Clayton	Brown		2.0
Little Mill (Upper)	Jackson	Brown		1.0
Little Paint	Allamakee	Brown		2.0
Little Paint (Upper)	Allamakee		Brook	1.0
Little Turkey	Delaware		Brown	1.0
Ludlow Creek	Allamakee	Brown		1.2

Appendix C. continued

Stream	County	Category/Species		Length in Miles
		I	II	
Middle Bear	Winneshiek		Brook	0.3
Middle Bear	Winneshiek		Brown	1.0
Mossey Glen	Clayton	Brown		1.0
North Bear	Winneshiek	Brown		6.0
North Canoe	Winneshiek		Brown	0.5
North Cedar	Clayton		Brown	2.0
Paint	Allamakee		Brown	11.0
Pine (near Satre)	Winneshiek		Brown	1.0
Richmond Springs	Delaware	Brown		1.0
Saurs Cave Creek (fka. Winter)	Fayette	Brook		0.3
Sny Magill	Clayton		Brown	4.0
South Canoe	Winneshiek	Brook		0.4
South Fork Big Mill	Jackson	Brown		1.0
South Pine	Winneshiek	Brook		2.0
Spring Branch	Delaware		Brown	1.0
Spring Branch	Delaware		Brook	1.0
Spring Falls	Delaware	Brown		0.5
Storybook Hollow	Jackson	Brown		1.0
Trout River	Winneshiek	Brown		5.0
Trout River	Winneshiek		Rainbow	1.0
Trout Run	Allamakee	Brown		0.5
Turtle Cr	Mitchell		Brown	2.7
Twin Springs	Winneshiek		Brown	0.5
Twin Springs	Winneshiek		Brook	0.5
UNT to Spruce Creek	Jackson		Brook	0.5
Village	Allamakee	Brown		0.5
Village	Allamakee		Brown	7.5
Waterloo	Allamakee	Brown		10.5

Appendix D.

Pearson product moment correlation of basin drainage area, maximum temperature, minimum temperature and average stream width versus candidate CBI metrics.

CBI Candidate Metric	Basin Drainage Area	Maximum Temperature *C	Minimum Temperature *C	Average Stream Width
SH BTI	0.24	0.453*	0.383*	0.0788
MH Coleoptera taxa richness (no Elmidae & Dryopidae)	-0.161	-0.175	-0.32	-0.184
MH CW taxa richness	-0.182	-0.675**	-0.325	-0.235
MH EPT richness	0.391**	-0.0972	-0.155	0.309*
MH sensitive taxa richness	0.321*	-0.412*	-0.295	0.151
MH Trichoptera taxa richness (no Hydropsychinae & Hydroptilidae)	-0.132	-0.559**	-0.261	-0.148
MH % tolerant taxa	0.0815	0.251	0.135	0.0928
MH taxa richness	0.321*	0.0754	-0.105	0.179
SH % 3-dominant taxa	0.152	-0.0344	0.0764	-0.0826
SH % Chironomidae	0.268*	0.0933	0.211	0.0871
SH % CW individuals	-0.147	-0.589**	-0.293	-0.136
SH CW taxa richness	-0.229	-0.611**	-0.255	-0.192
SH % Dominant FFG	0.243	0.324	0.328	0.0742
SH % Ephemeroptera	-0.0892	0.0278	-0.0839	0.0926
SH % EPT	-0.0373	-0.125	-0.101	0.178
SH EPT richness	0.138	0.0302	-0.19	0.287*
SH % filterers/filter-collector individuals	-0.0569	-0.235	-0.166	0.00133
SH % Hydropsychinae individuals	0.16	0.342	0.162	0.242
SH % Hydropsyche individuals	-0.175	-0.0311	0.0246	0.00611
SH % predator individuals	-0.0589	0.454*	-0.344	-0.0111
SH % Scrapers	0.00729	0.182	-0.108	0.112
SH % sensitive individuals	-0.181	-0.549**	-0.416*	-0.132
SH % Isopoda & Turbellaria individuals	0.0822	0.148	0.222	-0.0402
SH Trichoptera taxa richness (no Hydropsychinae & Hydroptilidae)	-0.206	-0.521**	-0.216	-0.197
SH % tolerant individuals	0.116	-0.0352	0.128	-0.0126
SH taxa richness	-0.0824	0.0422	-0.279	0.0863
*p from 0.05 to 0.01; **p <0.01				

Four metrics exhibited significant positive relationships ($p \leq 0.05$) with basin drainage area. The metrics were: MH EPT richness, MH sensitive taxa richness, MH taxa richness, and SH % Chironomidae.

Nine metrics exhibited significant relationships ($p \leq 0.05$) with maximum temperature. The relationship was positive for SH BTI and SH % predator individuals; negative for SH CW taxa richness, MH CW taxa richness, MH Trichoptera taxa richness (no Hydropsychinae & Hydroptilidae), SH Trichoptera taxa richness (no Hydropsychinae & Hydroptilidae), SH % CW individuals, MH sensitive taxa richness, and SH % sensitive individuals.

Only two metrics exhibited significant relationships ($p \leq 0.05$) with minimum temperature. The metrics were SH BTI and SH % sensitive individuals. The relationship was positive for SH BTI and negative for SH % sensitive individuals.

Only two metrics exhibited positive significant relationships ($p \leq 0.05$) with average stream width. The metrics were MH EPT richness and SH EPT richness.

Appendix E.

Watershed and Riparian land use characteristics correlations with WW BMIBI and CBI.

	Watershed Land Cover					15 meter Riparian Land Cover							
	wood land	grass land	row crops	road/ urban	barren	water/ wetland	wood land	grass land	row crops	road/ urban	barren	WW BMIBI	CBI
WLC-water/wetland	0.04	0.04	-0.35**	0.74**	0.14	0.66**	-0.05	-0.11	-0.19	0.73**	-0.01	-0.01	0.29*
WLC-woodlands		0.33*	-0.79**	-0.14	-0.18	0.04	0.81**	-0.68**	-0.71**	-0.18	-0.14	0.17	0.16
WLC-grasslands			-0.70**	-0.17	-0.20	0.30*	0.40**	-0.11	-0.60**	-0.23	0.10	0.23	0.24
WLC-row crops				0.12	0.18	-0.38**	-0.68**	0.54**	0.84**	-0.15	0.05	-0.22	-0.25
WLC-road/urban					0.09	0.45**	-0.20	-0.06	-0.08	0.98**	-0.01	-0.05	0.14
WLC-barren						-0.11	-0.21	0.06	0.31*	0.11	0.19	-0.04	0.13
15M-water/wetland							0.04	-0.10	-0.25	0.43**	-0.08	0.01	0.30*
15M-woodlands								-0.88**	-0.79**	-0.28*	-0.11	0.34*	0.33*
15M-grasslands									0.52**	0.01	0.13	-0.24	-0.38**
15M-row crops										-0.003	0.06	-0.34*	-0.17
15M-road/urban											0.01	-0.12	-0.23
15M-barren												-0.15	-0.06
WW-BMIBI													0.46**

*p from 0.05 to 0.01; **p <0.01

Appendix F.

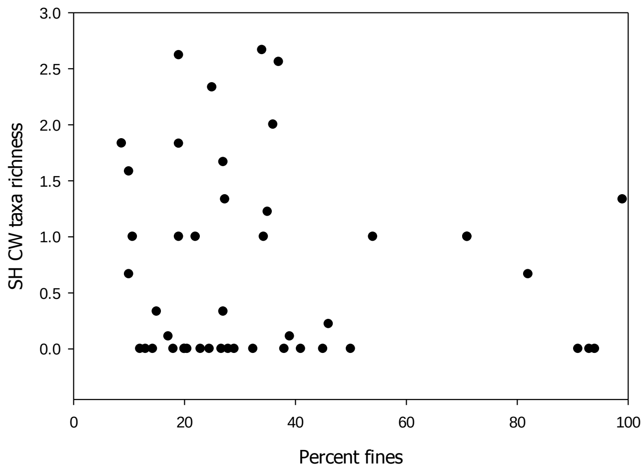
Nutrient water quality value correlations with WW BMIBI and CBI.

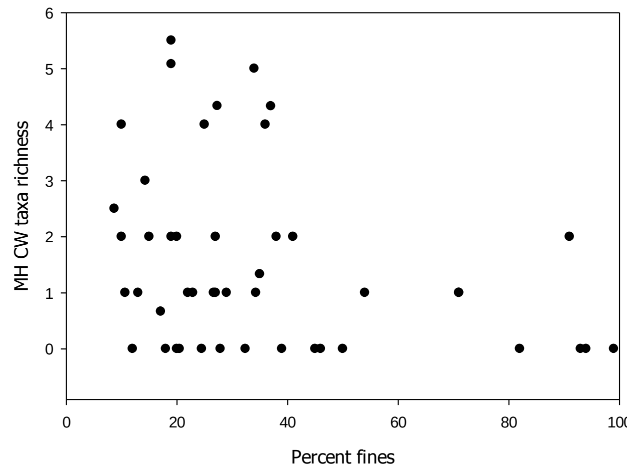
	NO3/NO2	TKN	Ortho-P	Total P	WW BMIBI	CBI
NH3	0.02	0.23	0.02	0.16	-0.28	-0.28
NO3/NO2		0.17	0.13	0.20	0.14	-0.12
TKN			0.69**	0.59**	-0.32*	-0.43**
Ortho-P				0.85**	-0.15	-0.05
Total P					-0.40**	-0.27

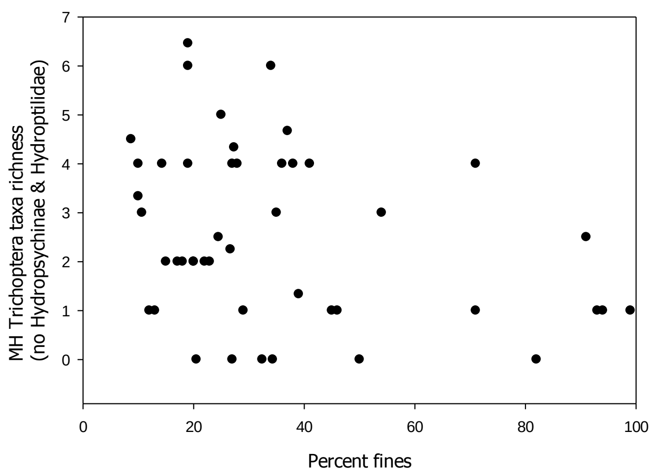
*p from 0.05 to 0.01; **p <0.01

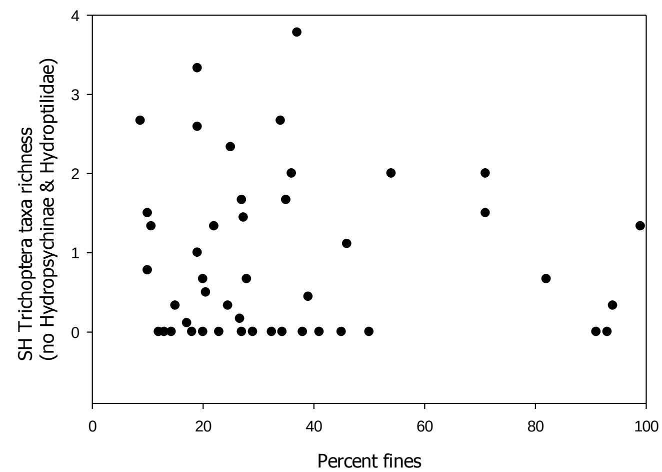
Appendix G.

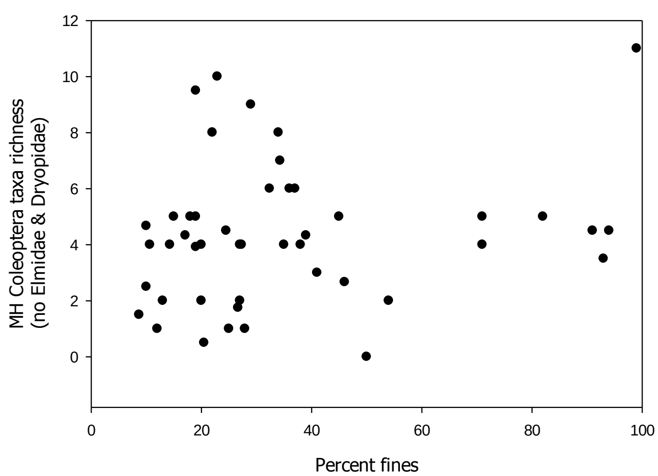
Scatter plots of CBI candidate metrics and the WW BMIBI versus percent fines (clay+silt+sand).

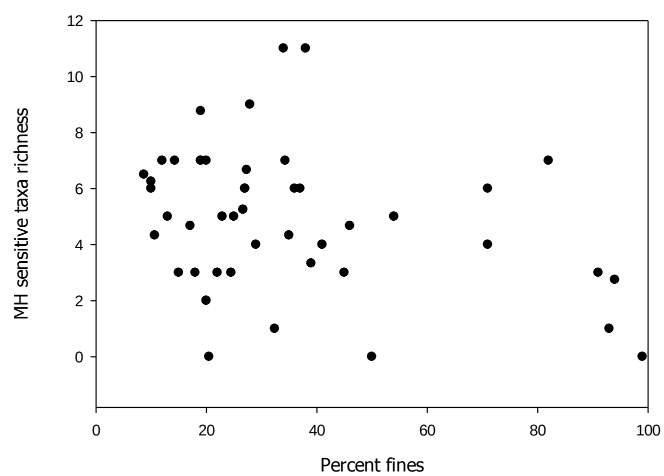






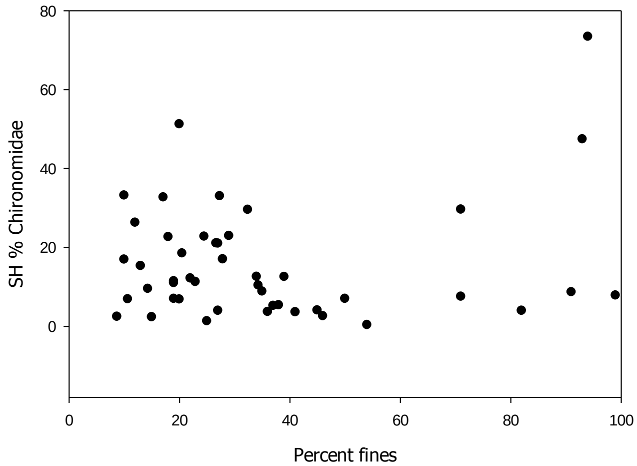


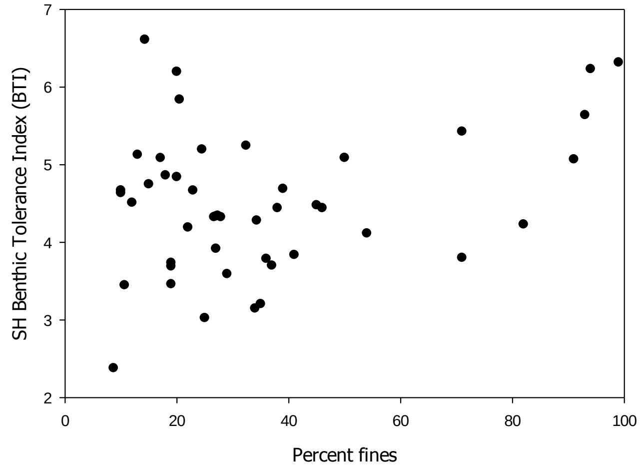


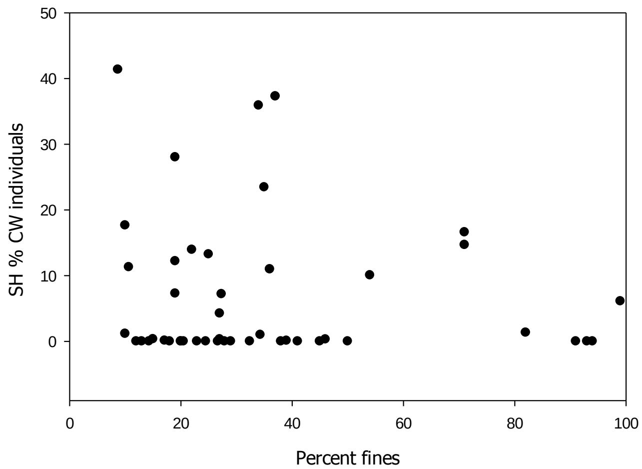


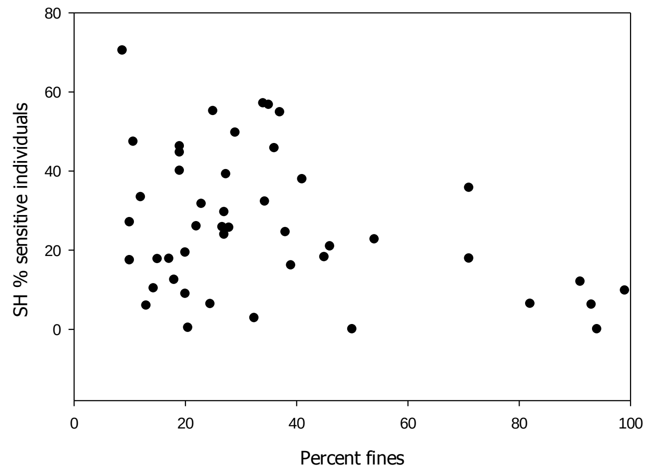
Coldwater Benthic Index (CBI)

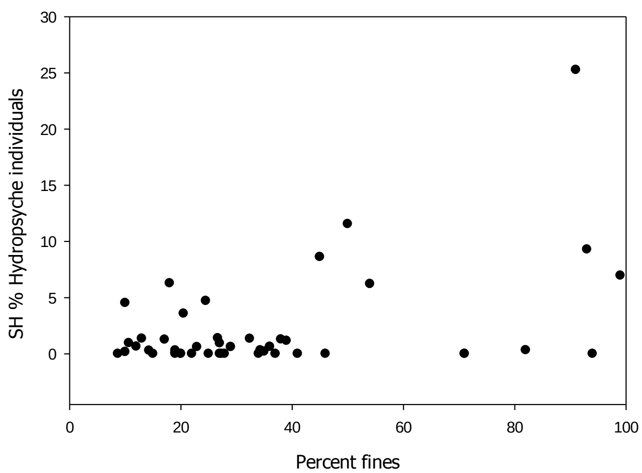
Appendix G. continued

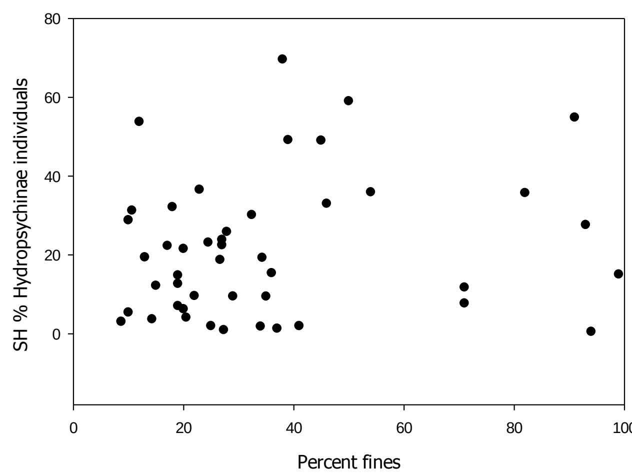




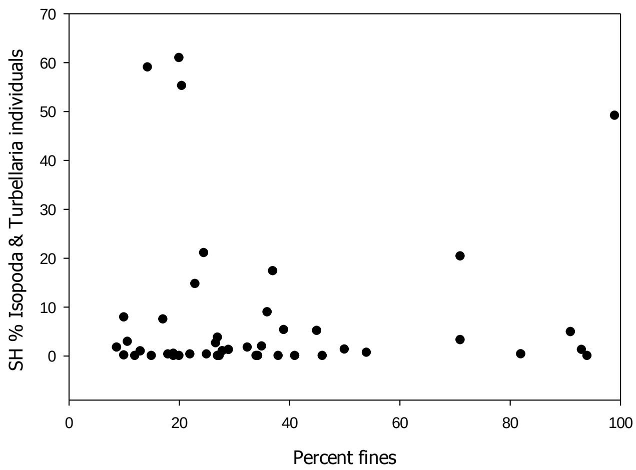


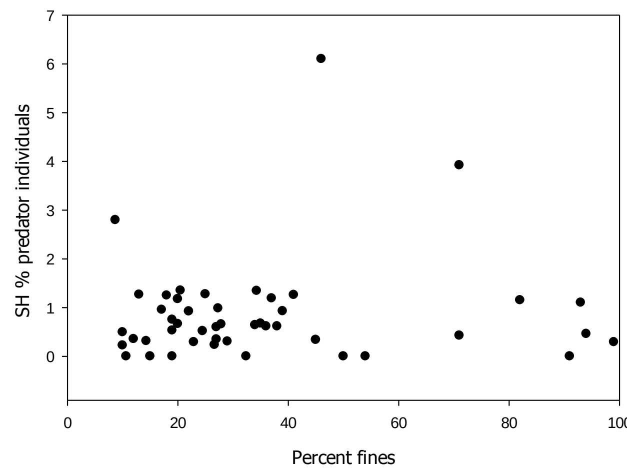


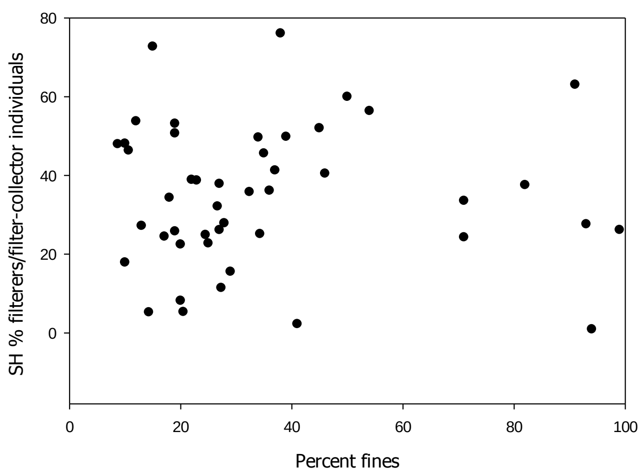


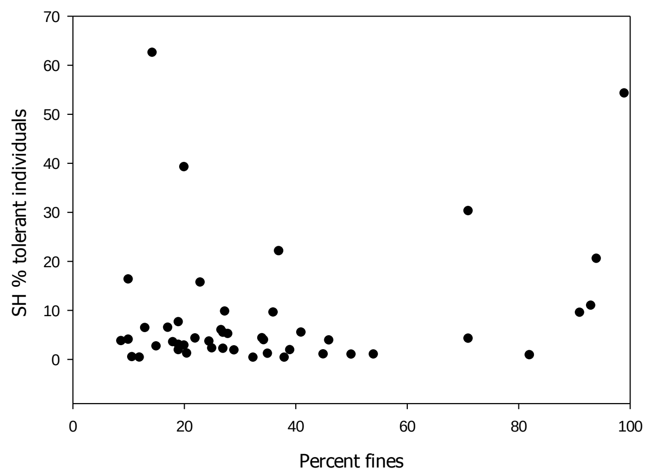


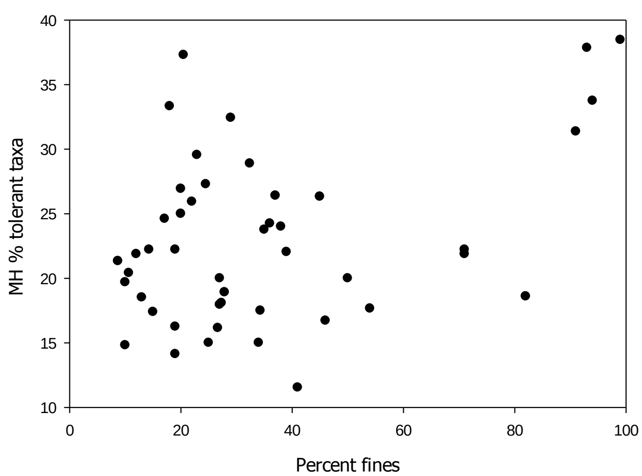
Appendix G. continued

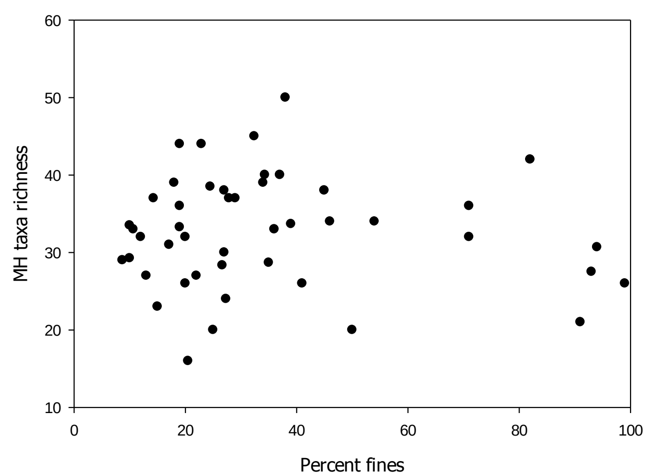




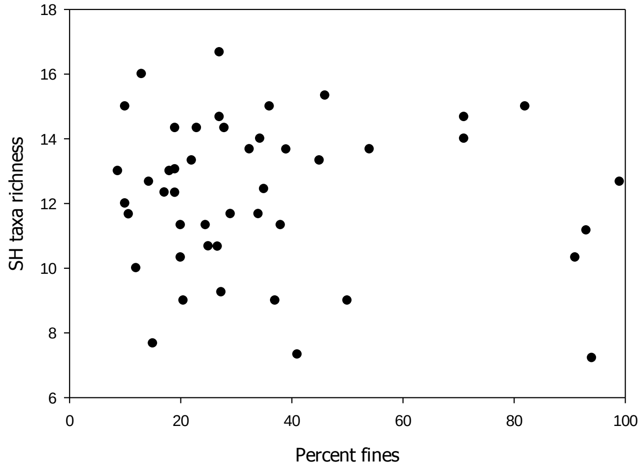


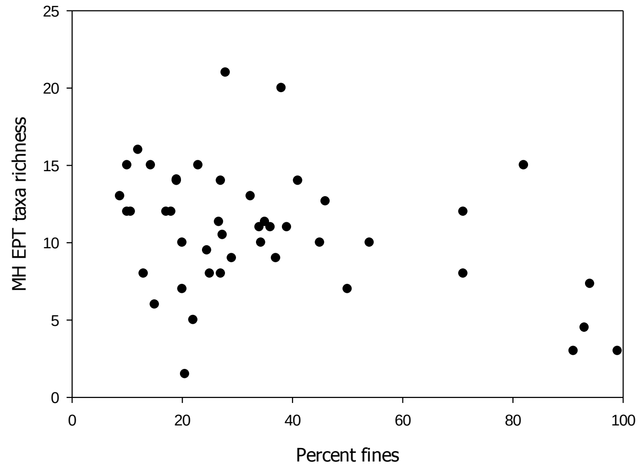


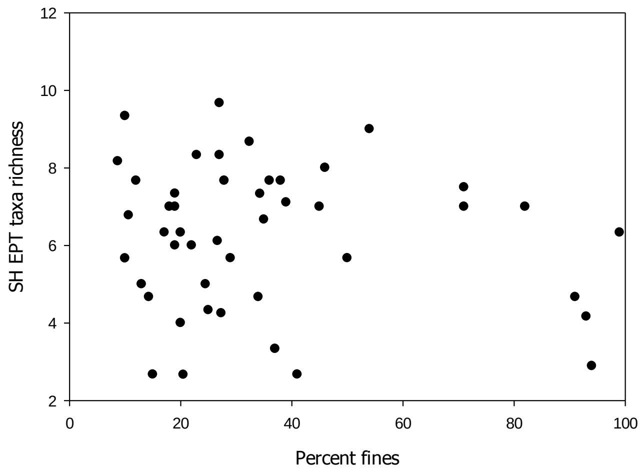


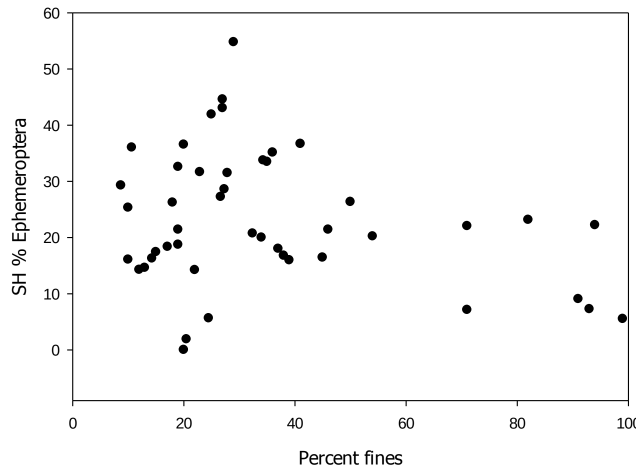


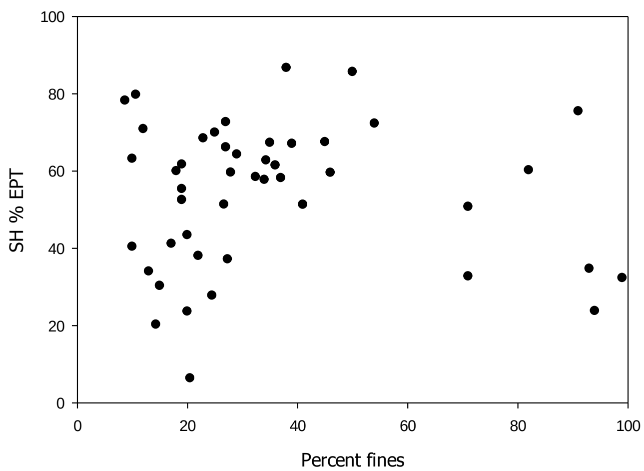
Coldwater Benthic Index (CBI)
Appendix G. continued

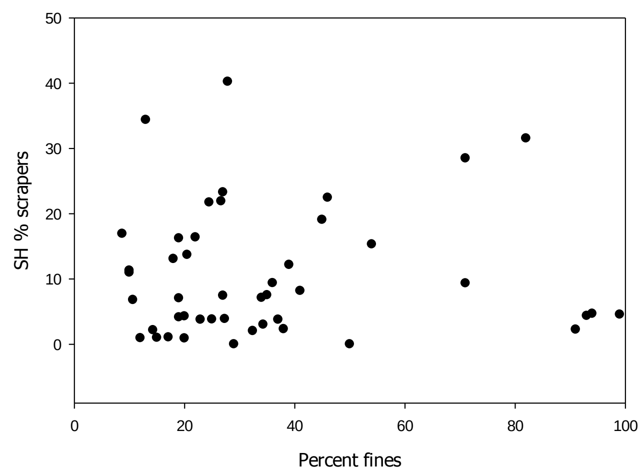












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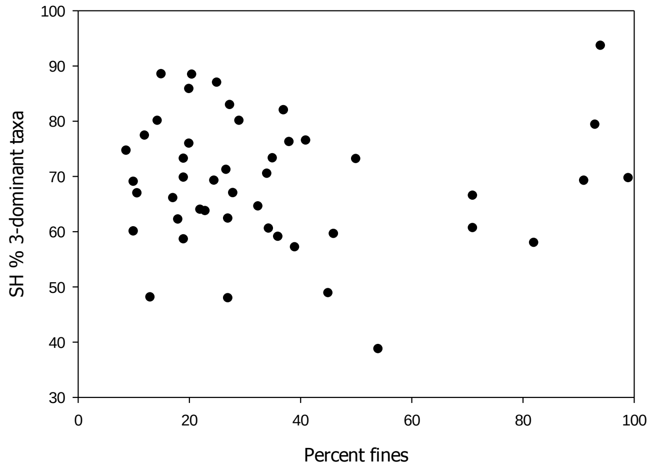
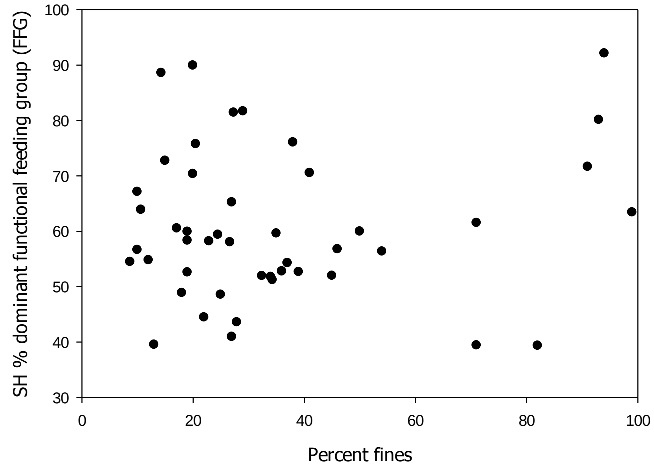
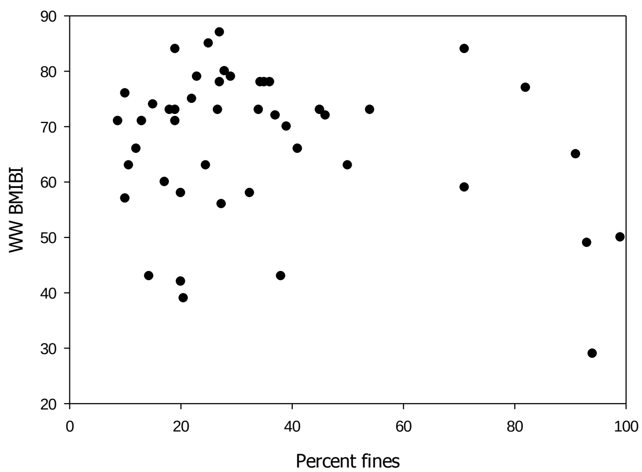
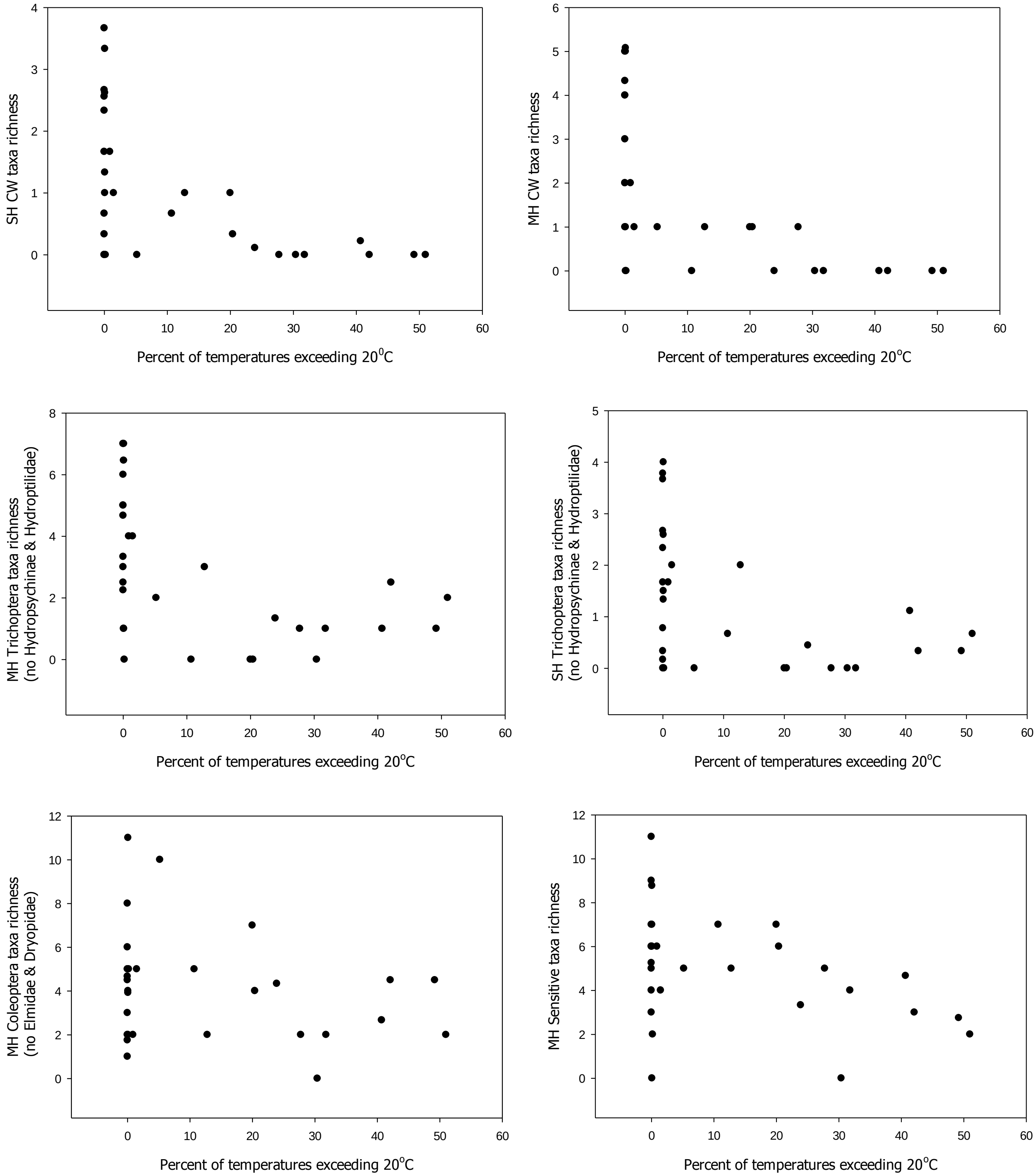




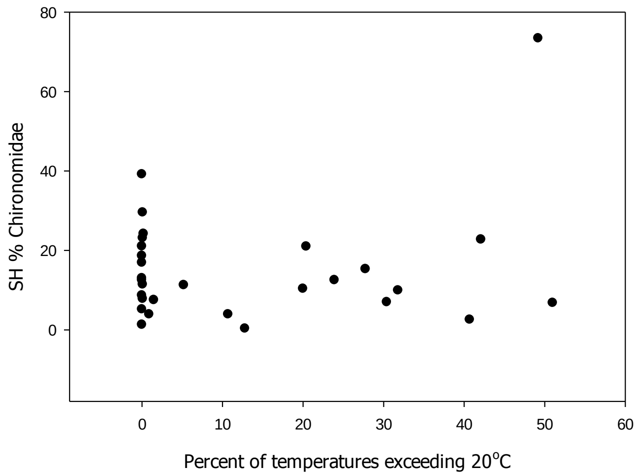


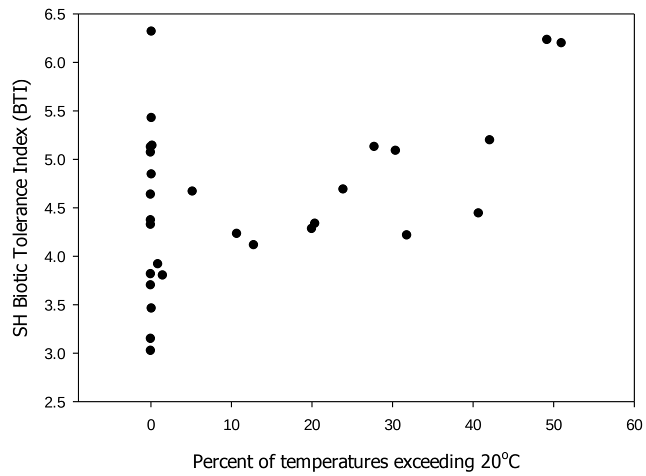
Appendix H.

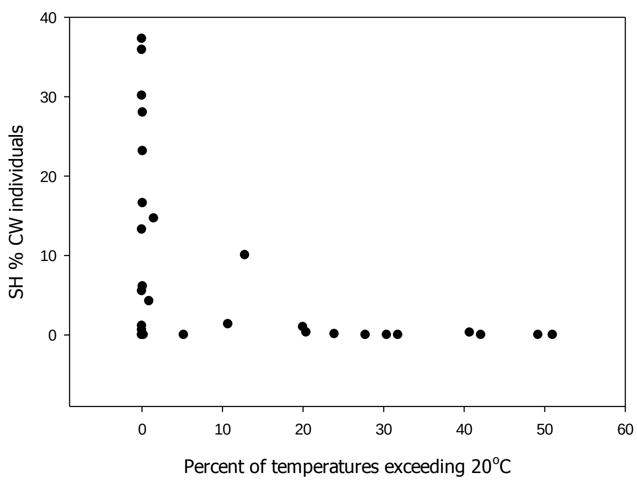
Scatter plots of candidate CBI metrics and the WW BMIBI versus the percent of temperature records exceeding 20°C.

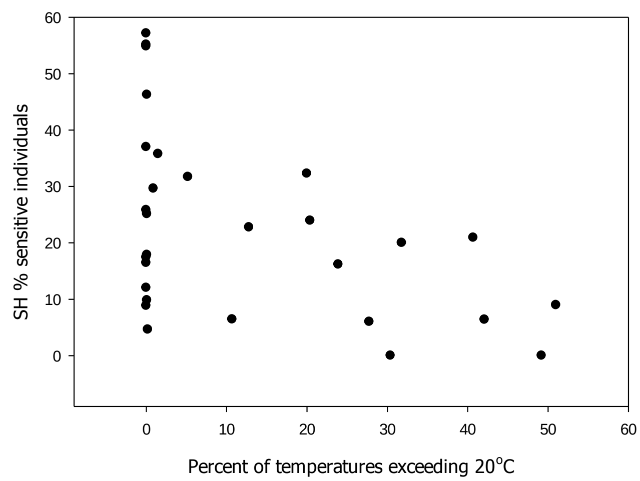


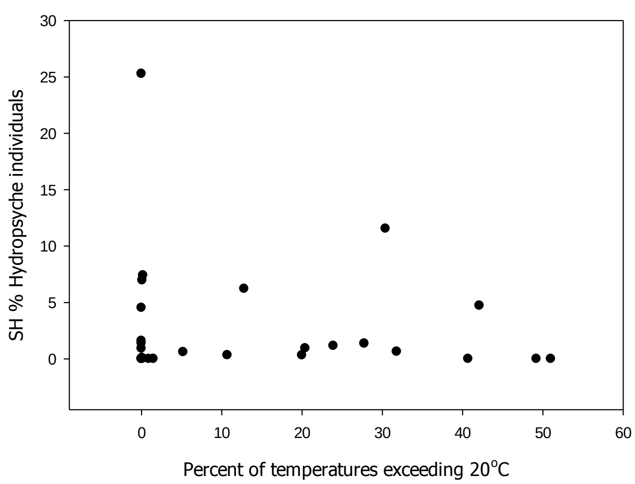
Appendix H. continued

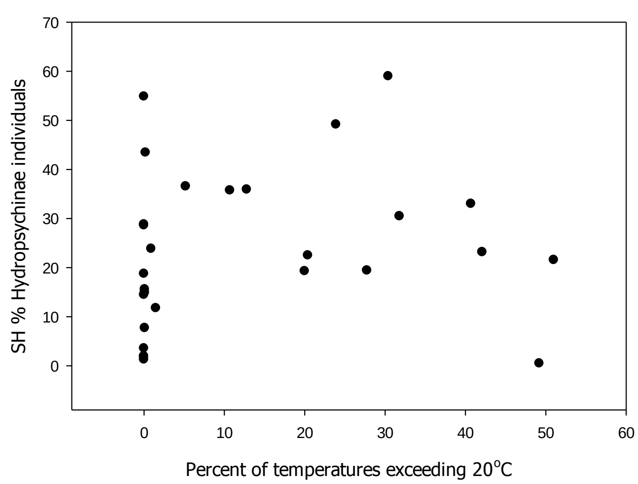












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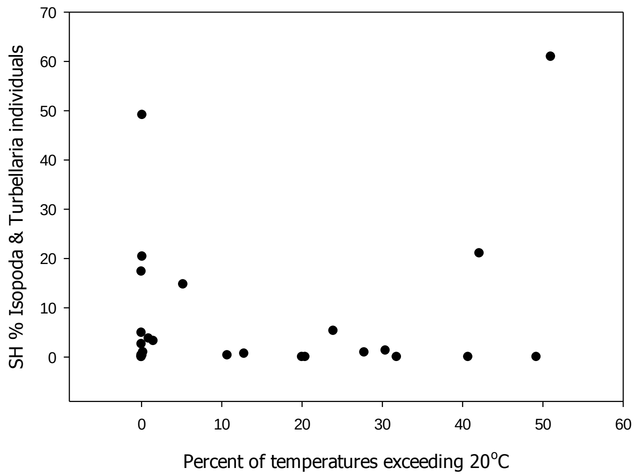

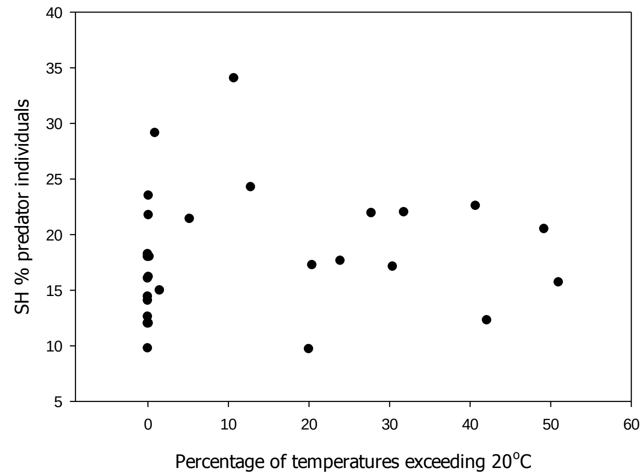

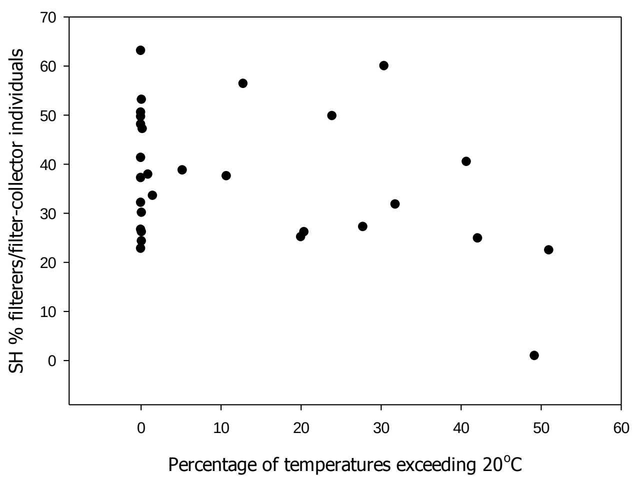

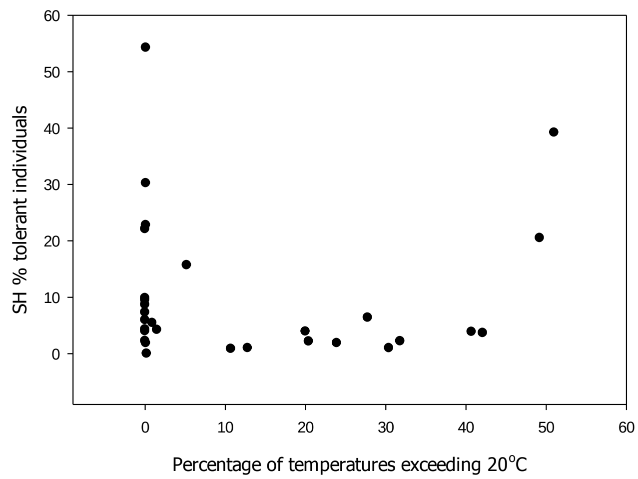

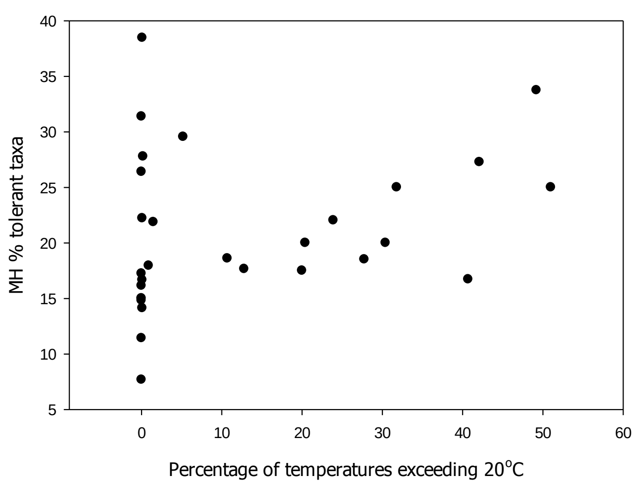

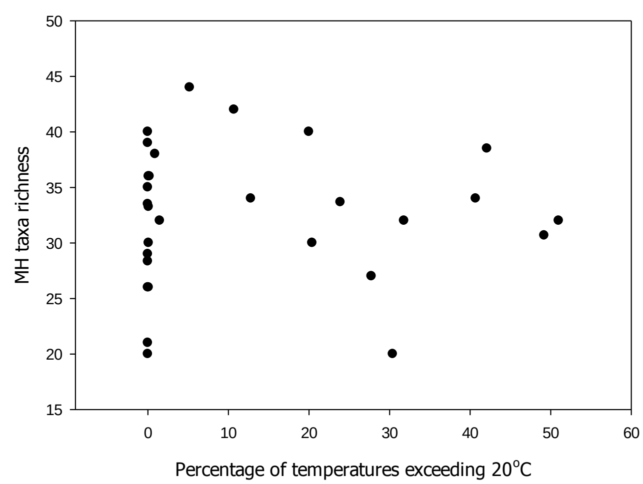

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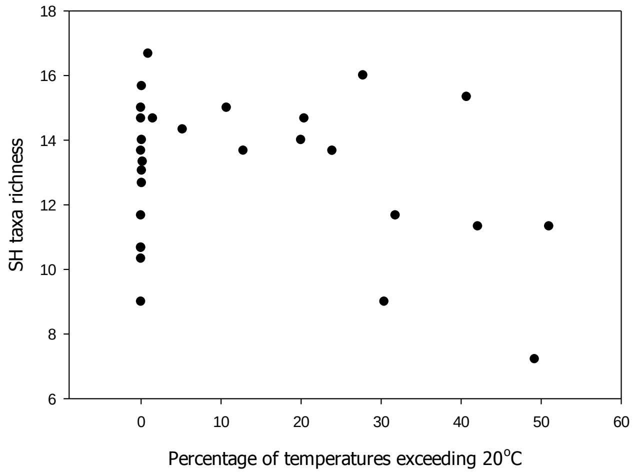
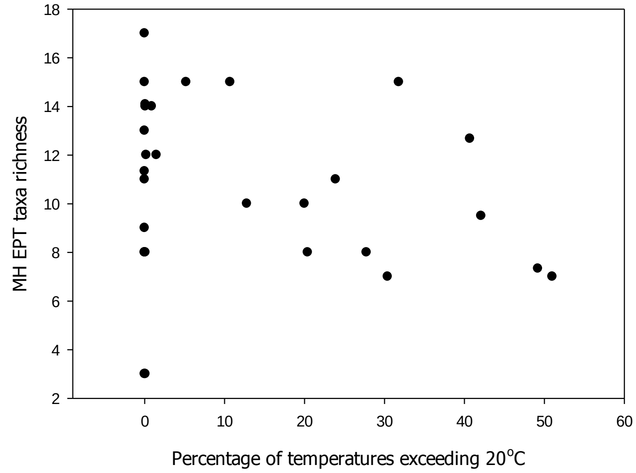
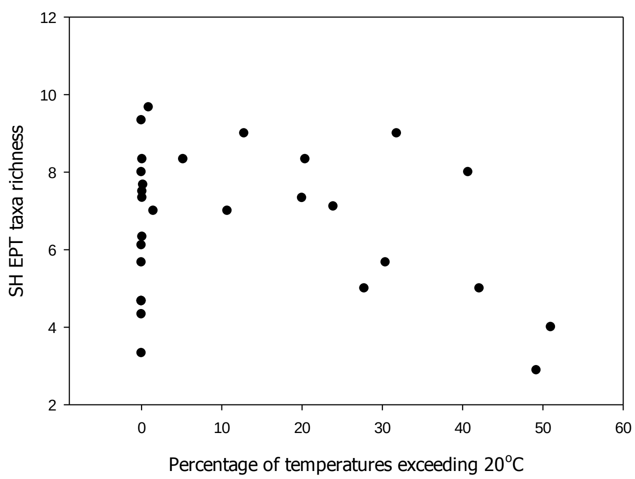
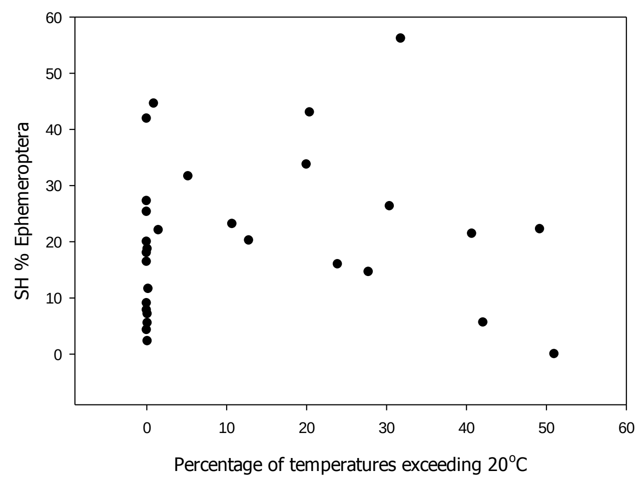
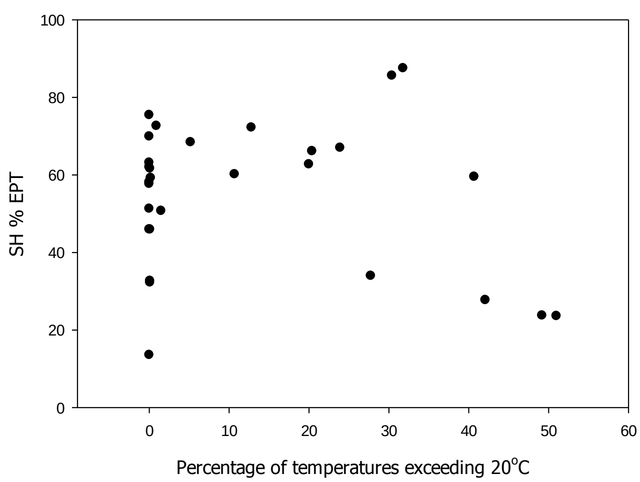
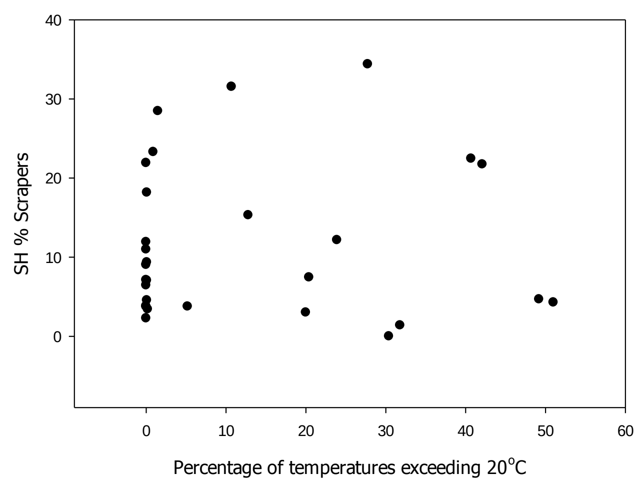




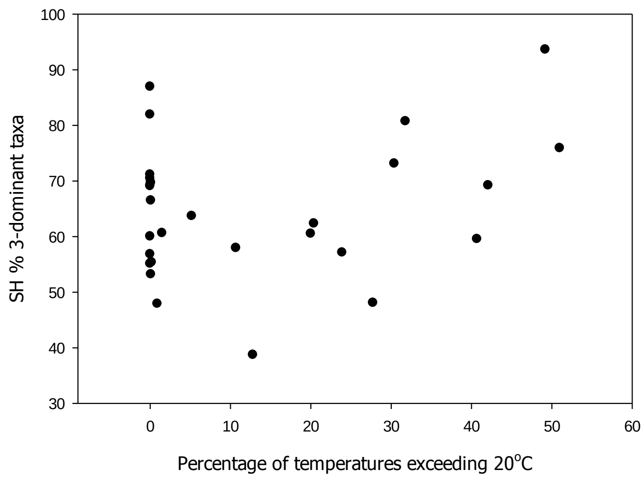


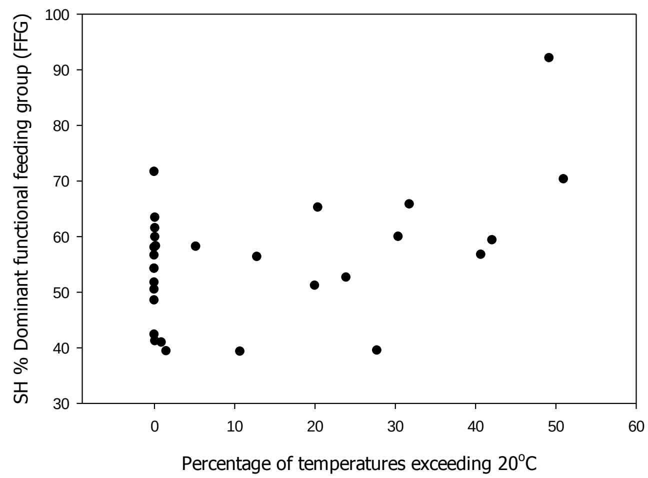


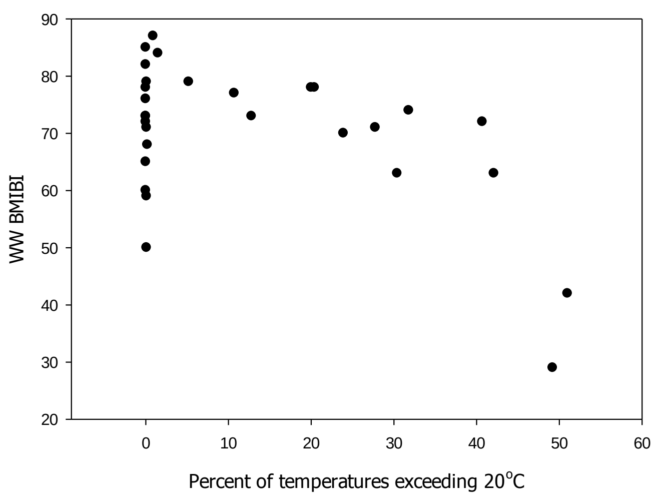




Appendix H. continued

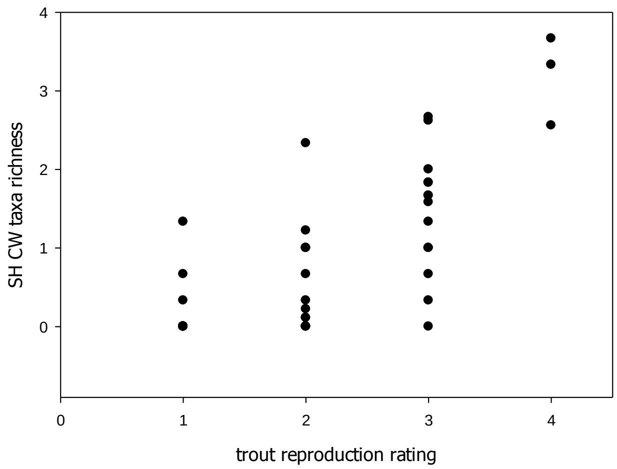


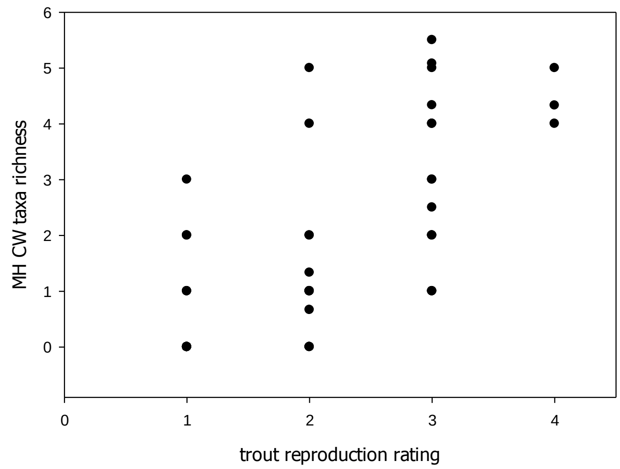


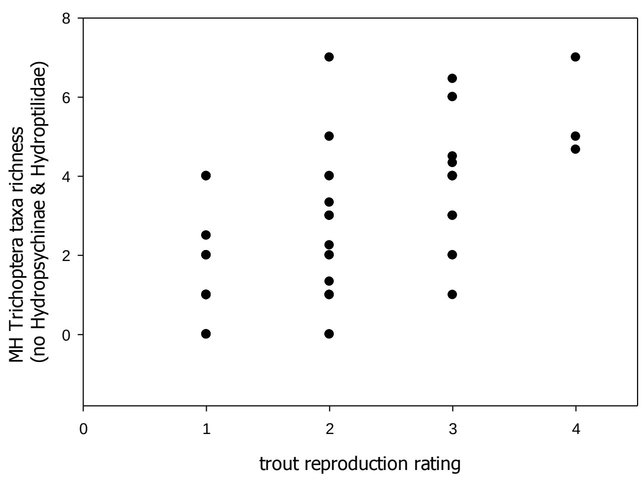


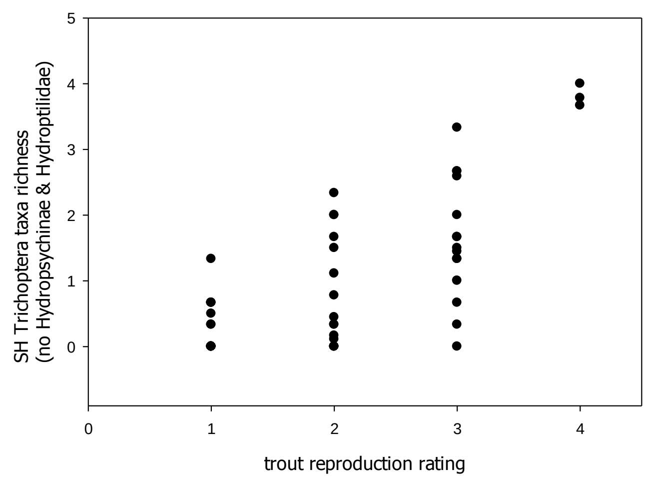
Appendix I.

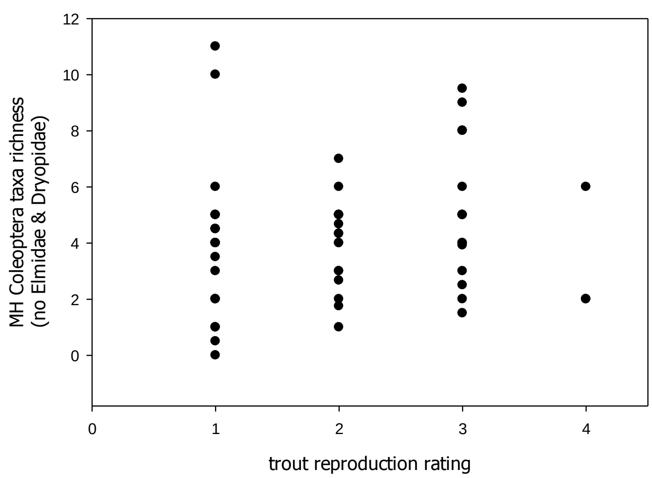
Scatter plots of CBI candidate metrics and the WW BMIBI versus the trout reproduction rating.

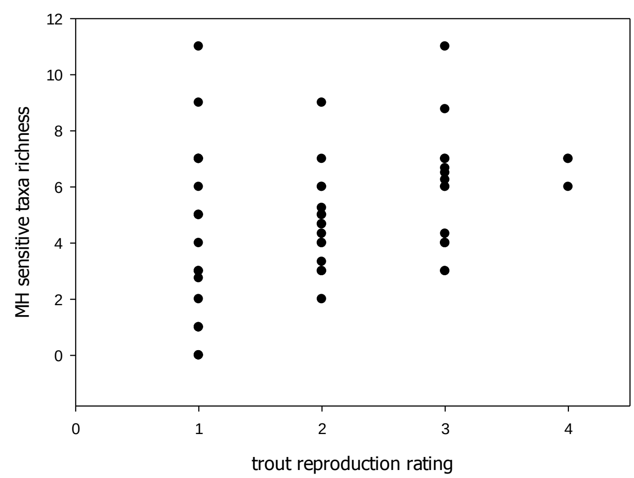




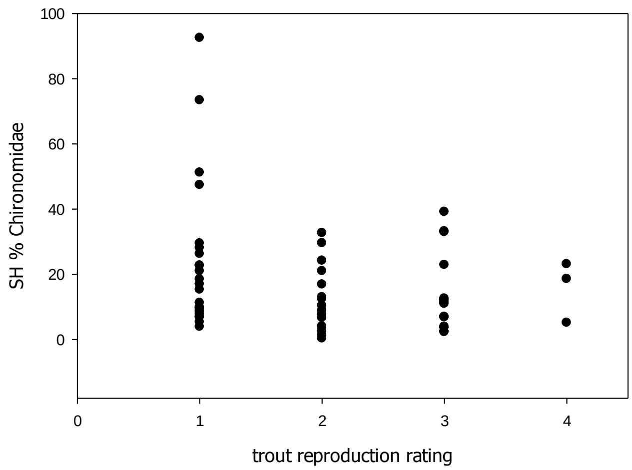


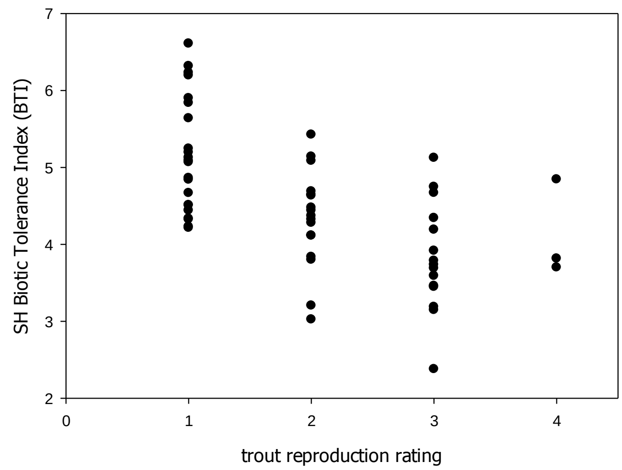


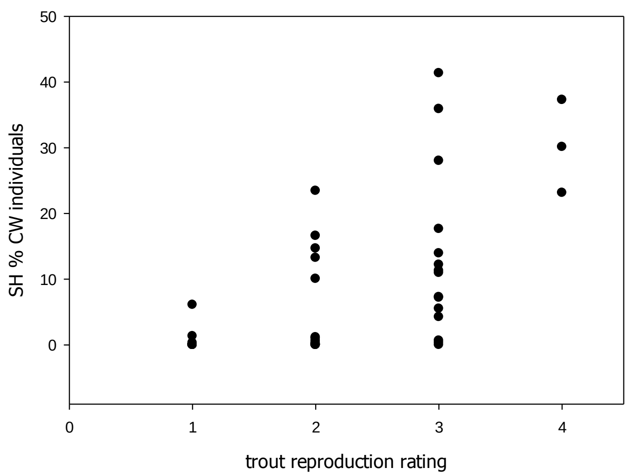


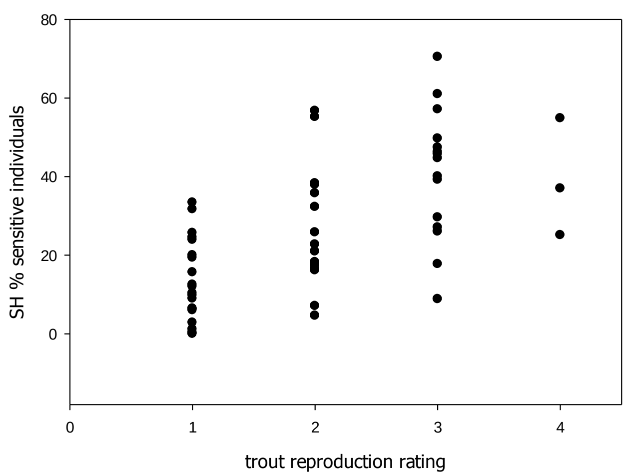


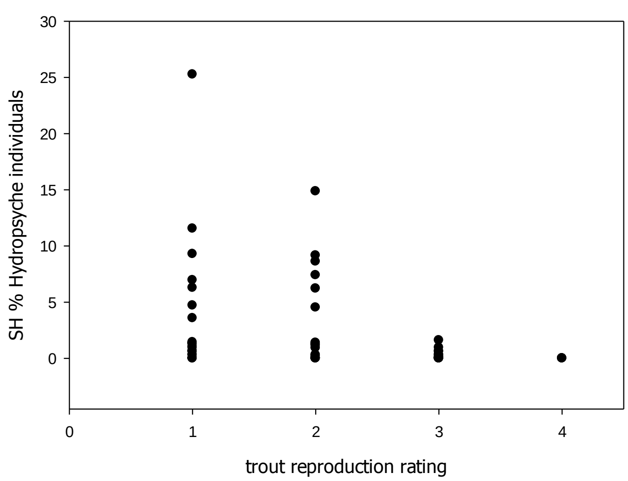
Coldwater Benthic Index (CBI)
Appendix I. continued

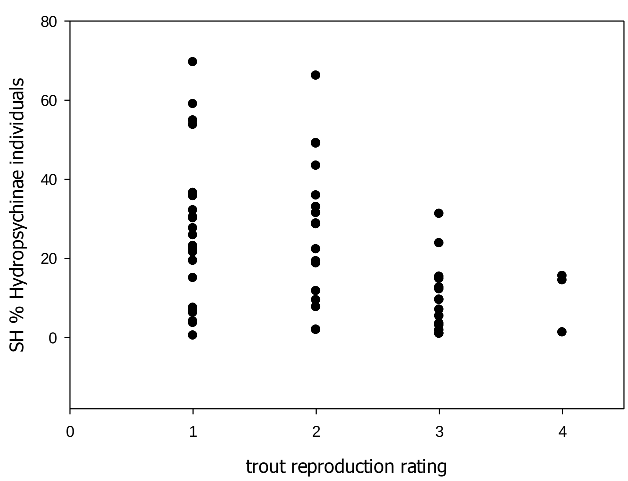




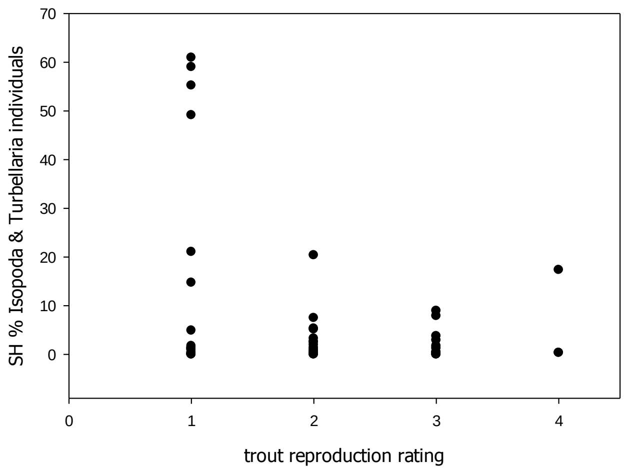


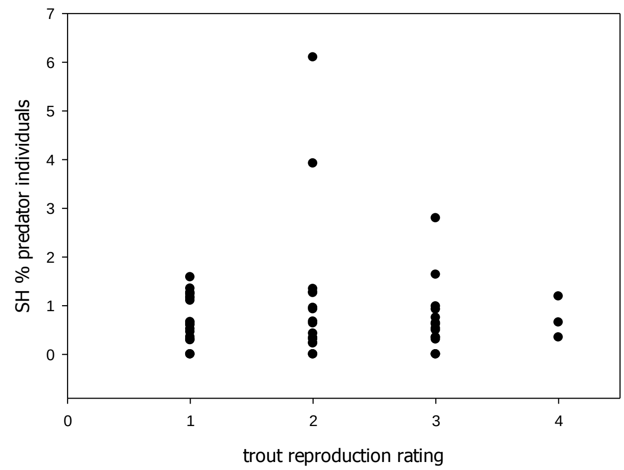


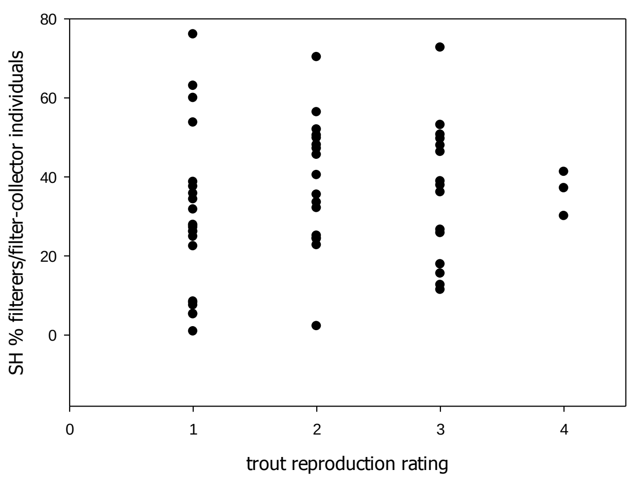


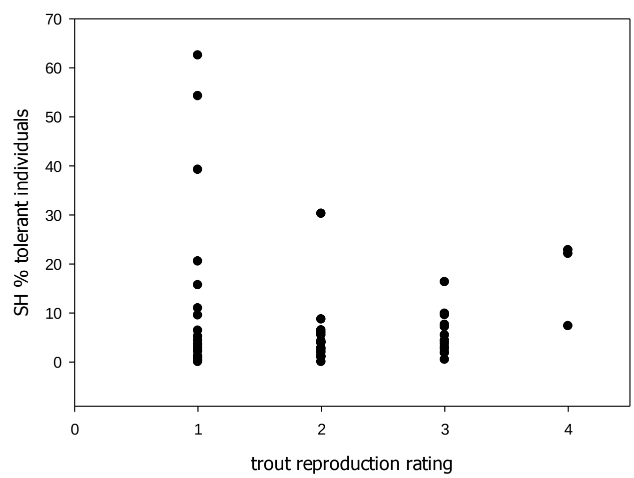


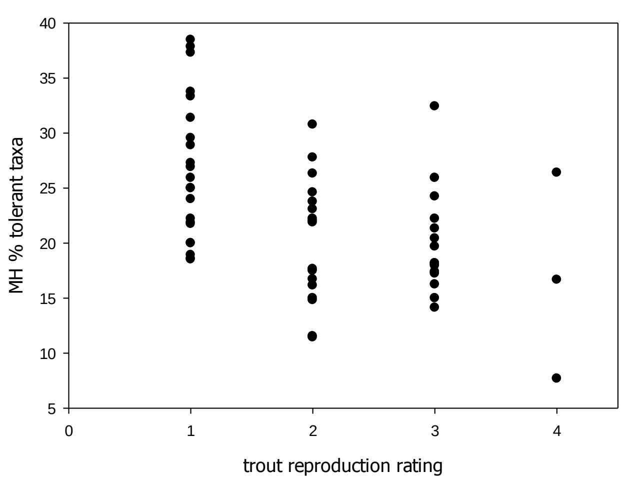
Appendix I. continued

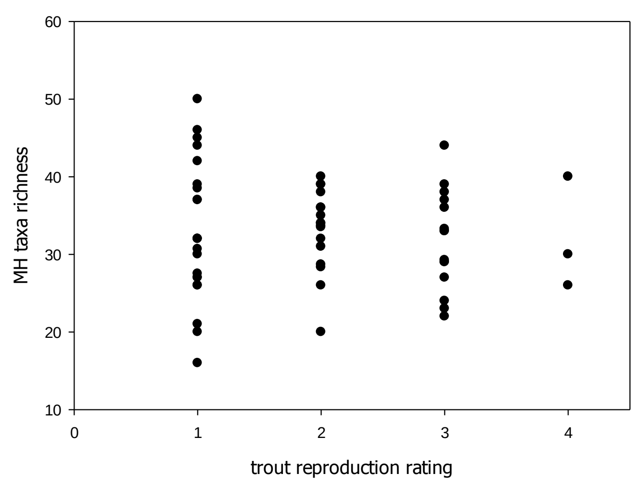




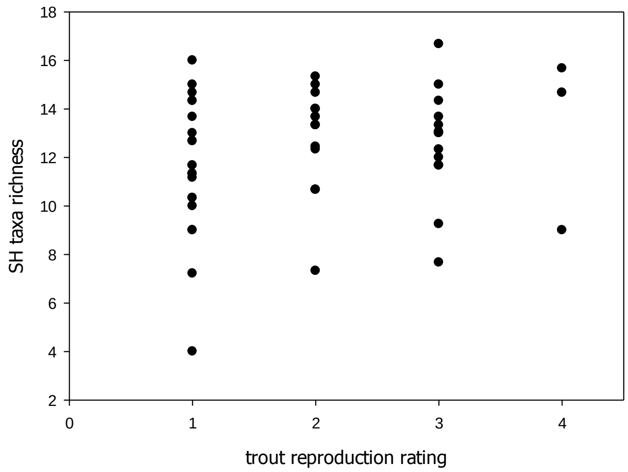


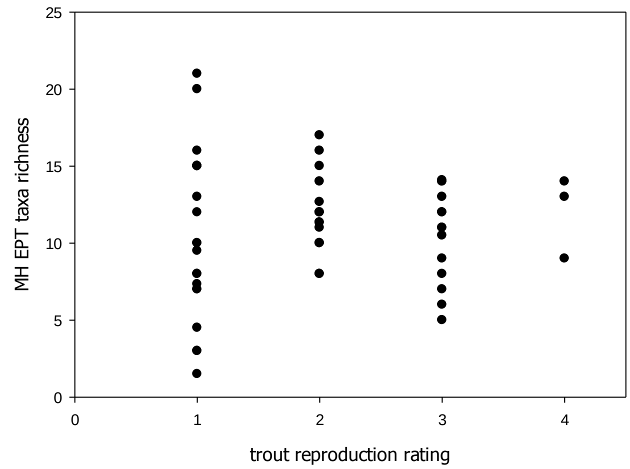


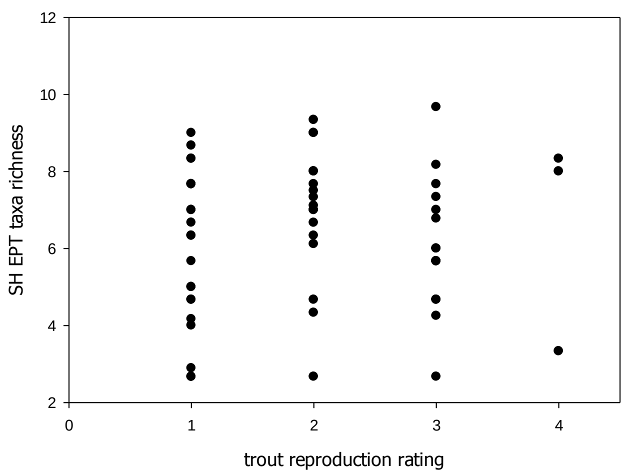


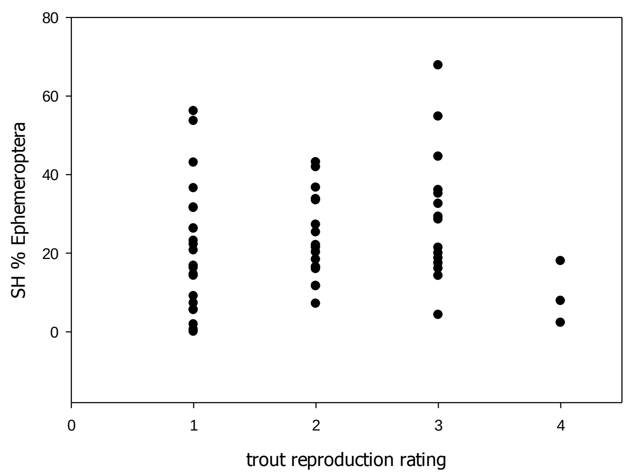


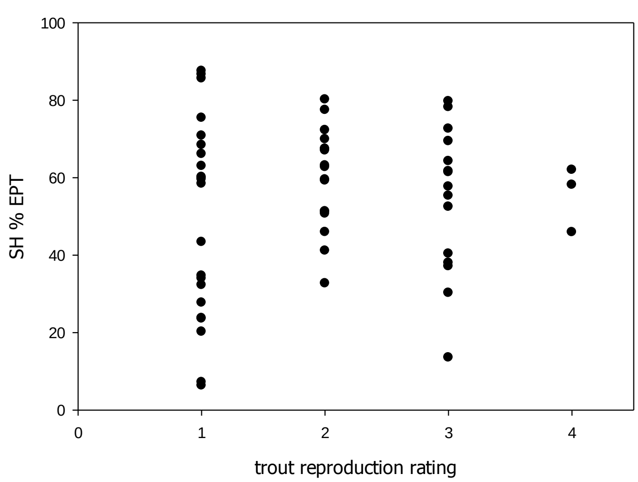
Appendix I. continued

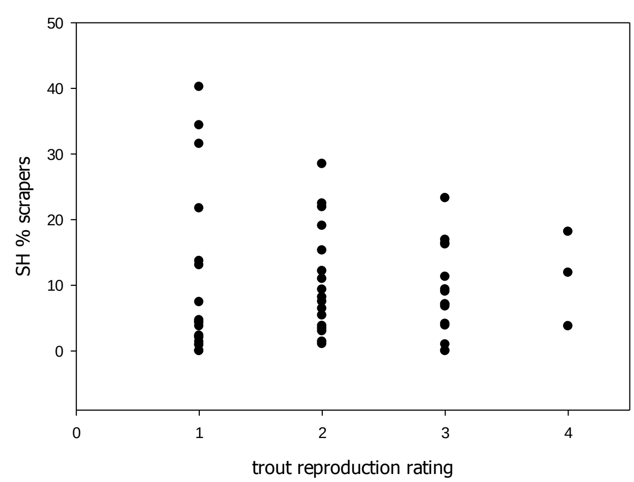




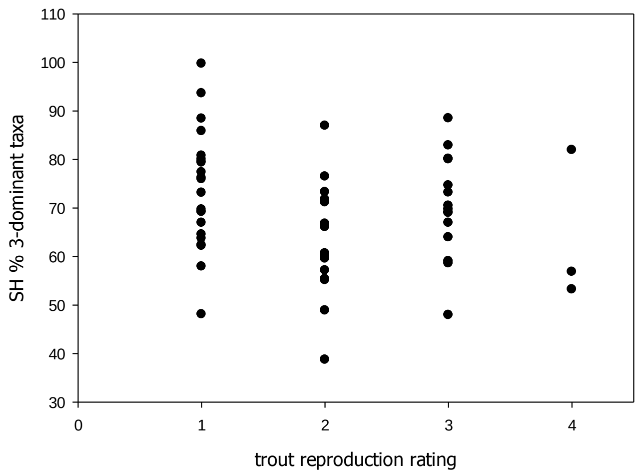


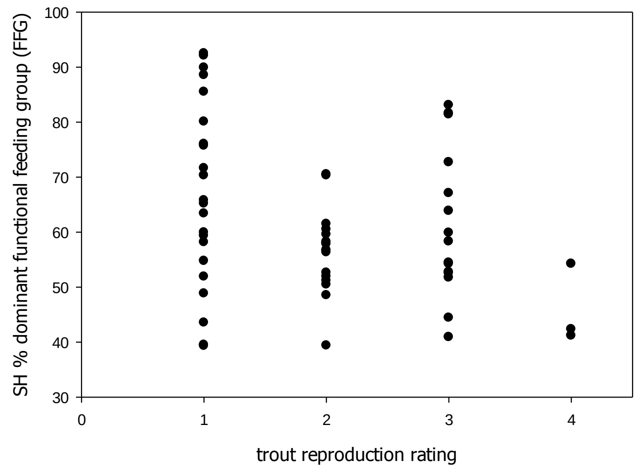


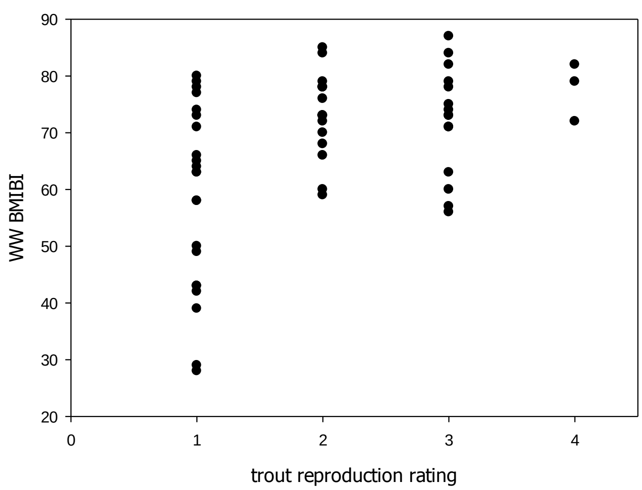




Appendix I. continued

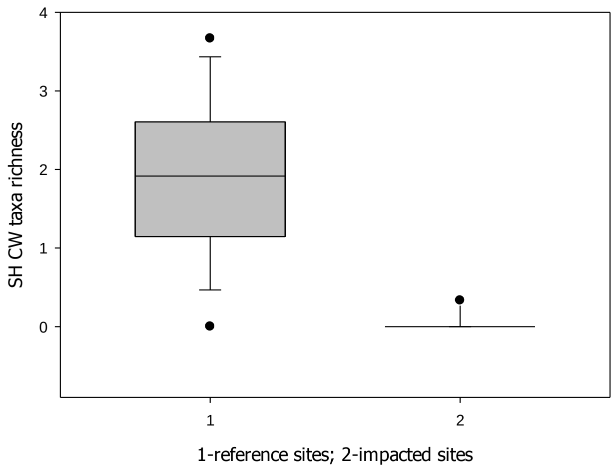


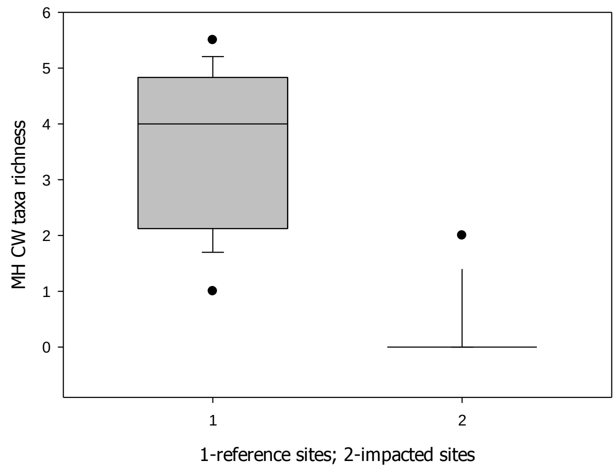


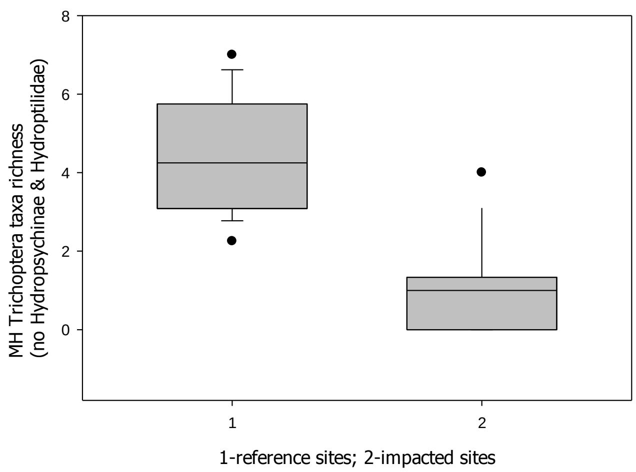


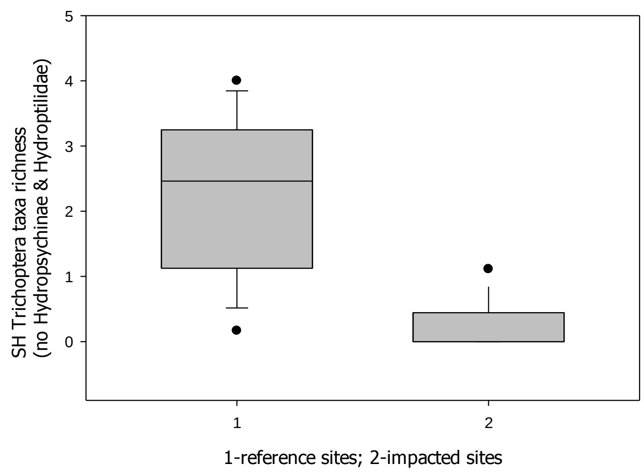
Appendix J.

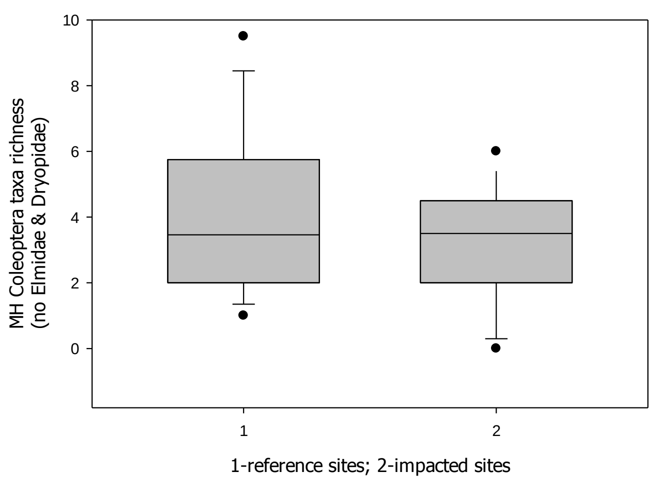
Box and whisker plots comparing coldwater reference and impacted site means for each candidate metric.

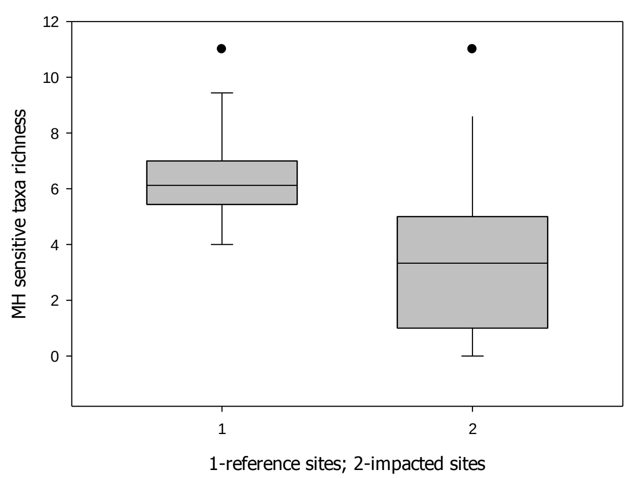






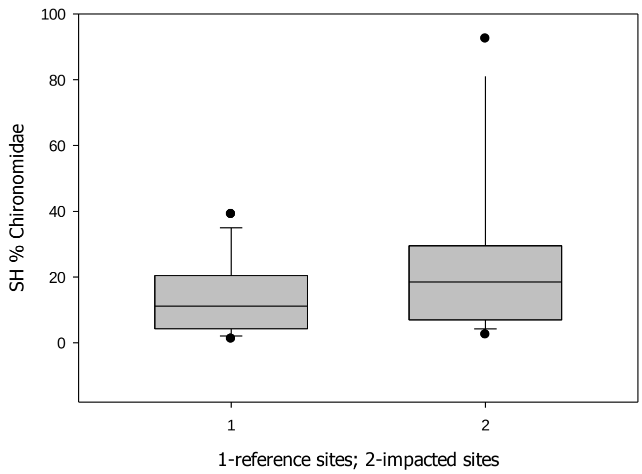


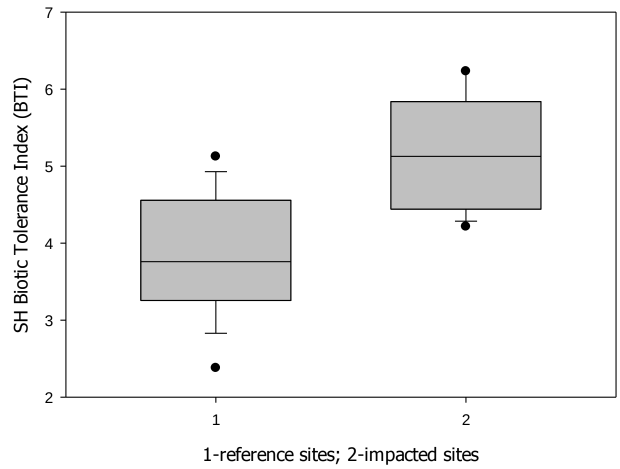


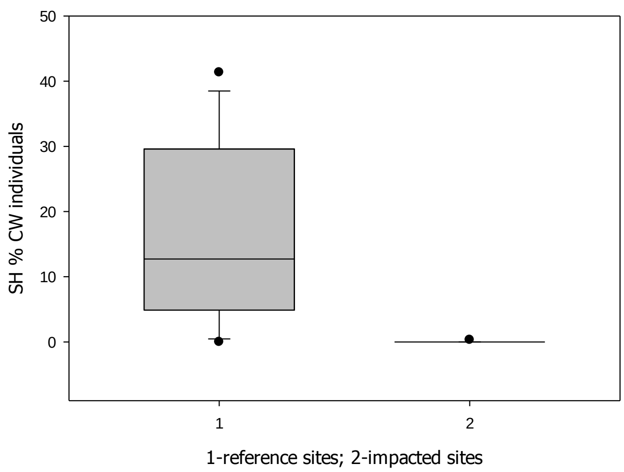


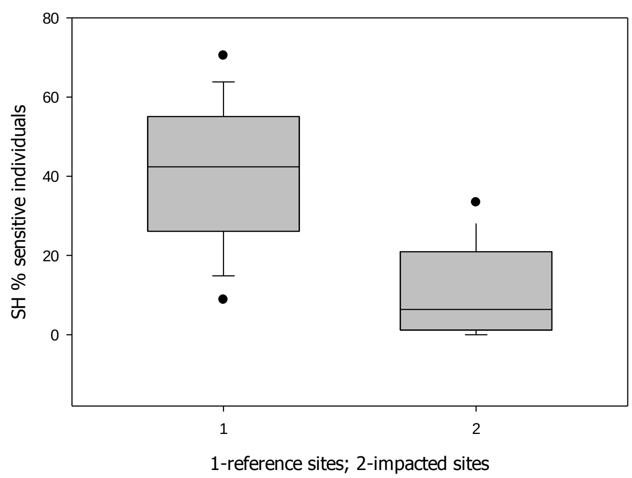
Coldwater Benthic Index (CBI)

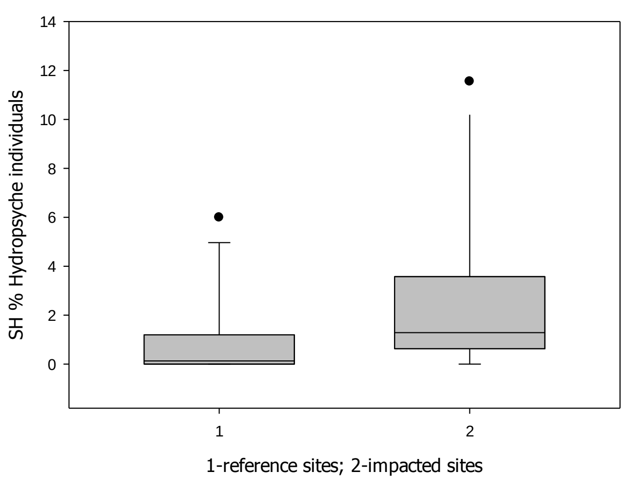
Appendix J. continued

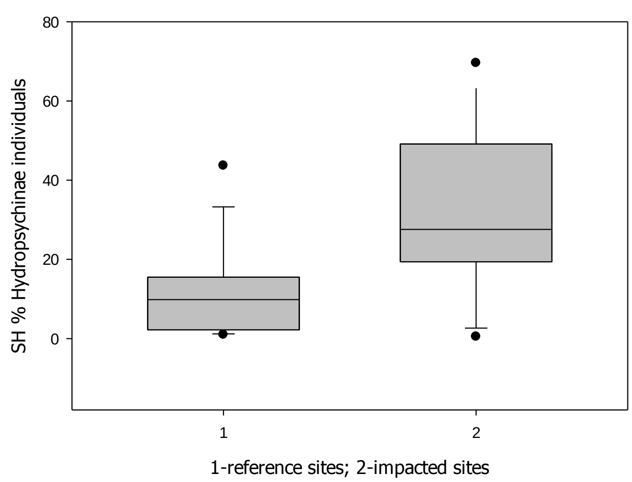




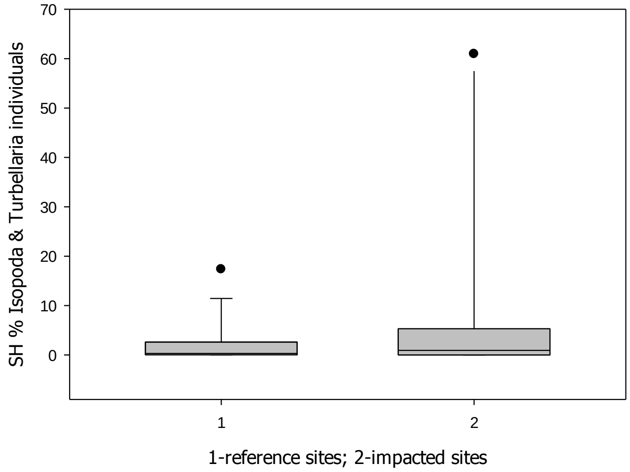


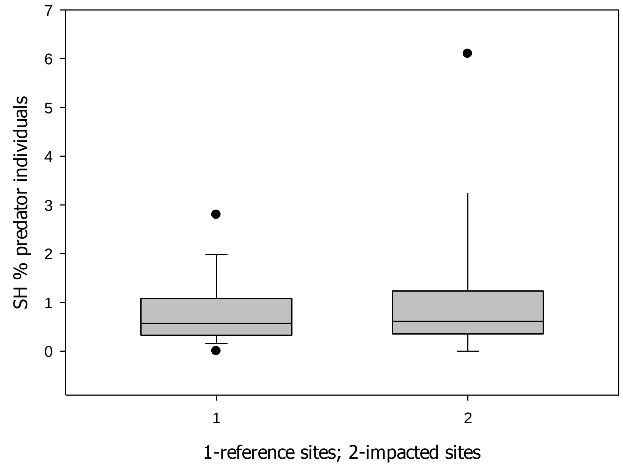


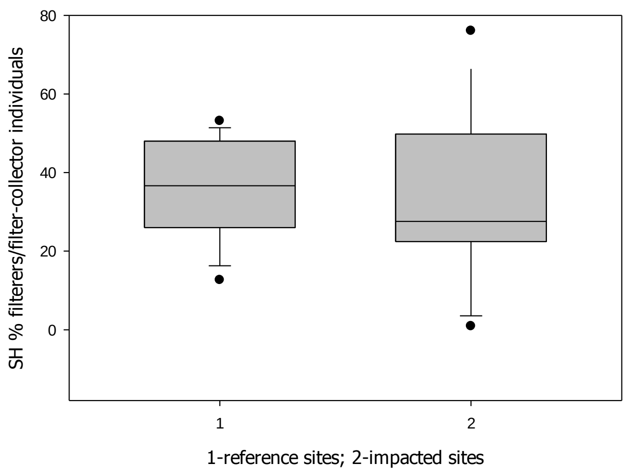


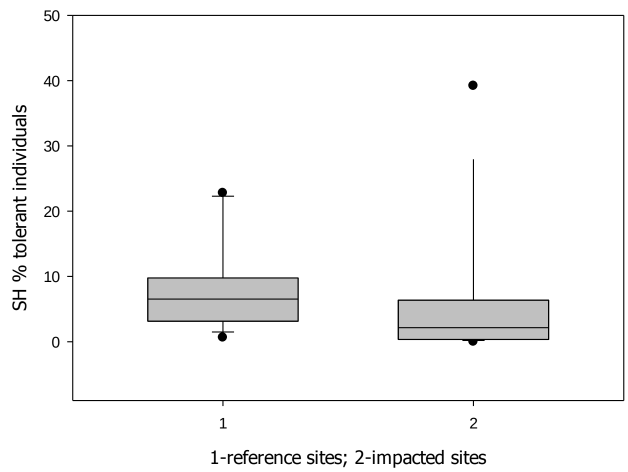


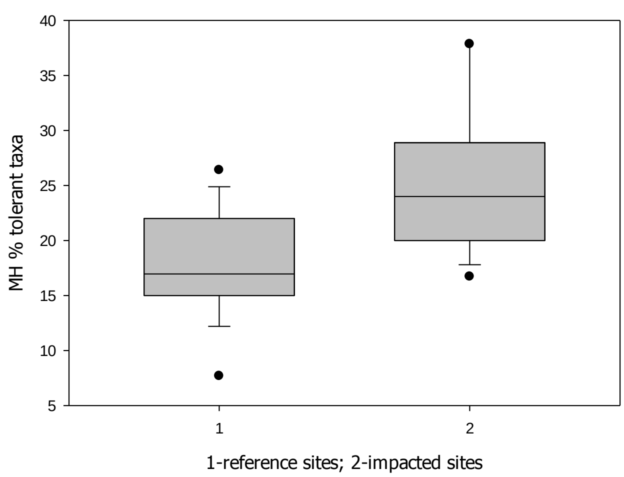
Appendix J. continued

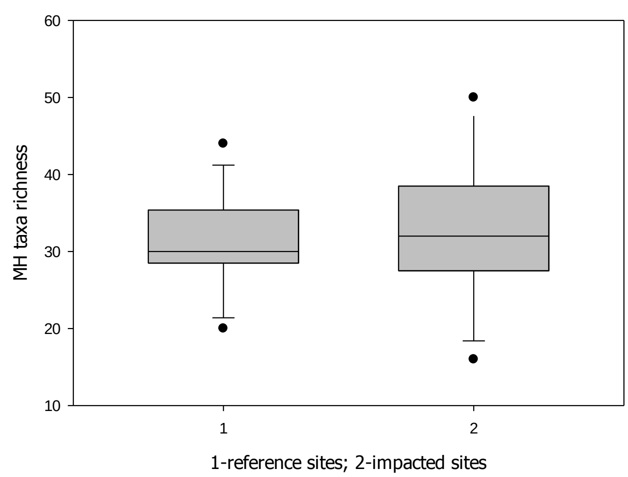




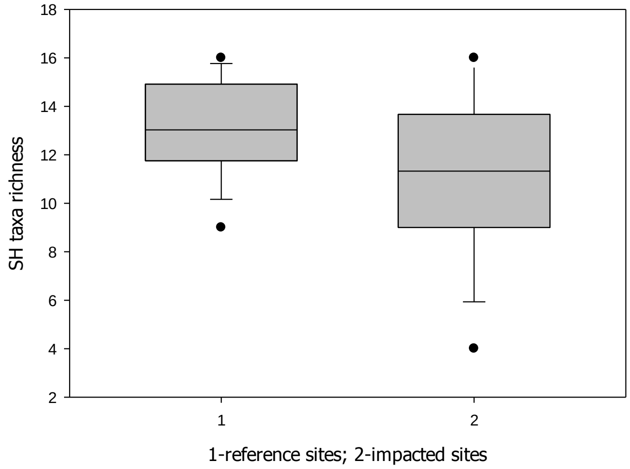


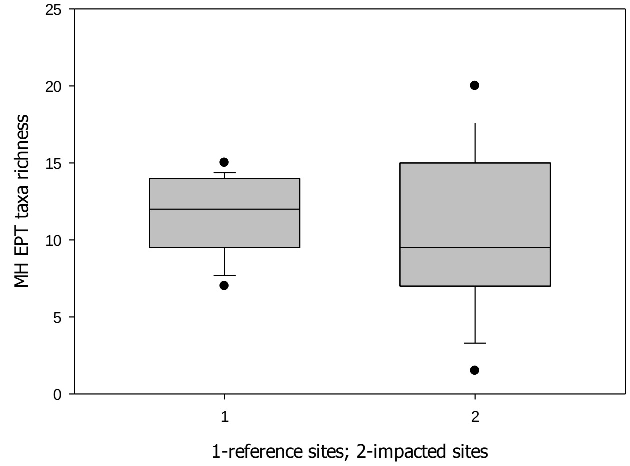


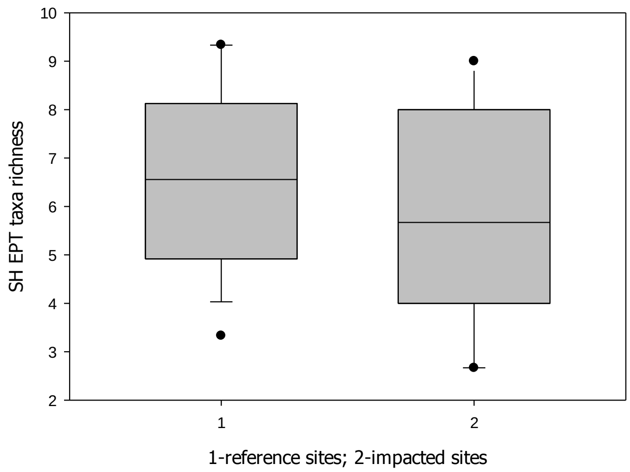


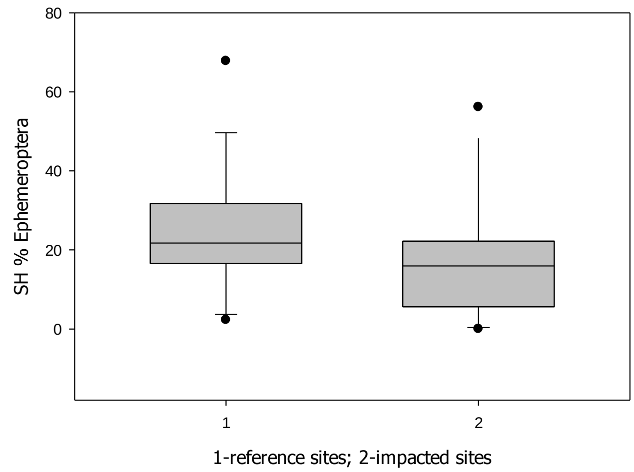


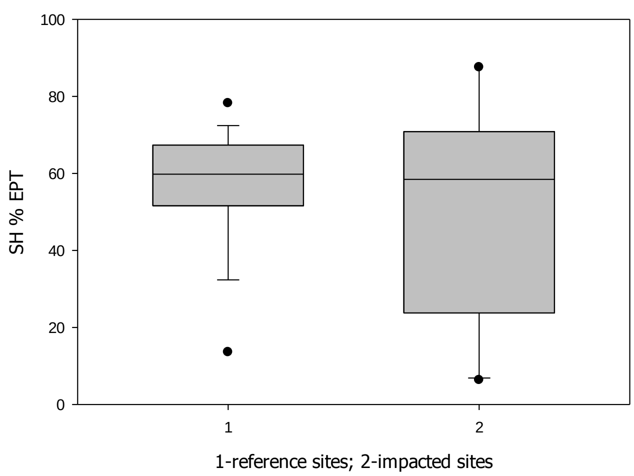
Appendix J. continued

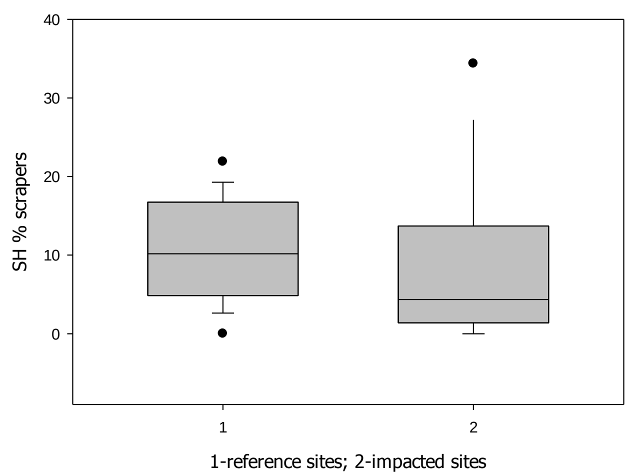












Appendix J. continued