

# **Recalibration of the Macroinvertebrate Multi-Metric Index for Colorado**

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## **Abstract**

The Colorado Department of Public Health and Environment (CDPHE) monitors streams throughout the state for assessment of water resource quality. The benthic macroinvertebrate assemblage can be associated with known gradients of natural conditions and of human disturbance. Association of biological communities with natural gradients allows classification by expected community characteristics, which should be recognized before trying to find relationships between biology and the disturbance gradient. Site classes were conceptualized by grouping sites with similar taxonomic composition and identifying distinctive environmental characteristics of the groups. Cluster analysis and ordination were used to define three site classes. Predicting site class membership was accomplished by developing classification rules with three environmental variables: ecoregion, site elevation, and stream slope. Within each site class, responses of 145 biological measures (metrics) were tested to find those that were indicative of differences among sites with minimal degradation (reference) and those with greater degrees of degradation (stressed). Several mathematical combinations of metrics were evaluated to find indices that were responsive to stress, ecologically meaningful, and both diverse and non-redundant in metric information. Three indices were identified, because metrics were not equally responsive to stress in each site class. The recommended MMIs apply to three specific site classes, contain 5-6 metrics each, and discriminate reference from stressed sites with minimal error (overall Discrimination Efficiency = 96.7%).

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# 1 Introduction

The State of Colorado is well-known for its natural beauty, often epitomized in images of clear mountain streams. These streams and all surface waters in Colorado should support a diverse and functional fauna. Land use activities such as mining, agriculture, silviculture, industrialization, and urbanization have contributed to water pollution and have often degraded the quality of the surface waters. It is the responsibility of Colorado's Department of Public Health and Environment (CDPHE) to maintain and protect the quality of the State's waters. In keeping with the Clean Water Act of 1972 (CWA, PL-92-500, and revisions of 1977, 1987) and technical guidance from the U. S. Environmental Protection Agency (USEPA), CDPHE has developed water quality standards for the protection of human and ecosystem health.

The Clean Water Act has as one of its primary goals the maintenance and restoration of biological integrity, which incorporates biological, physical, and chemical quality. This concept of biological integrity refers to the natural assemblage of indigenous organisms that inhabit a particular area that has not been affected by human activities (Frey 1977, Karr et al. 1986). The measurable definition of biological integrity is the *reference condition* (Barbour et al. 1995, 1999), which is characterized using data from minimally-disturbed sites within a region.

*Biological integrity is commonly defined as "the capability of supporting and maintaining a balanced, integrated, adaptive community or organisms having a species composition, diversity and functional organization comparable to that of the natural habitat of the regions" (Karr and Dudley 1981, Gibson et al. 1996)*

Biological metrics observed in the undisturbed reference sites become the standard to which biological conditions in other sites are compared. Therefore, proper identification of reference sites, characterization of the biological reference conditions, recognition of natural variability, and selection of the metrics that are most responsive to prevalent stressors are essential for making accurate comparisons and valid assessments.

Through the 303(d) and Total Maximum Daily Load (TMDL) framework outlined in the CWA, waters considered impaired and threatened must be identified and improved to meet their designated uses. Such uses almost always include aquatic life uses. *Impairment*, as defined by natural resource management or regulatory agencies in accordance with CWA provisions, is typically based on attainment or non-attainment of water quality criteria associated with a waterbody's designated use. If those criteria are not met (or attained) then the waterbody is considered to be impaired.

Resident aquatic biota in a stream can indicate environmental quality, responding to the effects of both episodic as well as cumulative pollution and habitat alteration. Thus, the assessment of ecosystem integrity is greatly enhanced through biomonitoring, or direct

survey of aquatic biota and comparison of meaningful and responsive measures of the samples to the regional ecological potential. In this exercise, we use the benthic macroinvertebrate assemblage to represent biological conditions.

The current investigation was framed in the context of previous indicator development processes, which were described by Paul et al. (2005). After applying the 2005 Multi-Metric Index (MMI), CDPHE recognized a need for recalibration of the index. The recalibration was expected to address perceived inadequacies of the 2005 indicator by incorporating new data, newly defined reference and stressed sites, and refinement of the stream classifications. The perceived inadequacies pertained to site classification, which did not address transition zones between ecoregion-defined classes.

#### *Biomonitoring in Colorado*

Colorado DPHE has established a biological monitoring program for streams throughout the State. The information collected in the biomonitoring program has been incorporated into Colorado's 305(b) report and its 303(d) list of impaired streams through application of indicators developed by Paul et al. (2005). The objective of this project was to recalibrate the multimetric biological index for interpreting the condition of Colorado streams. Recalibration is a logical improvement based on the addition of sample data since the last index development. The additional biological, chemical, and physical habitat data filled gaps noted in the spatial coverage during the previous indicator development effort. The specific questions investigated in this study were:

- Can site classification for assessing ecosystem condition across the diverse landscape and physiographic regions of Colorado be improved by considering additional data?
- Can site classification be developed so that it is sensitive to transitional areas that have characteristics of multiple site classes?
- Which metrics are most appropriate for use in a Colorado multimetric macroinvertebrate stream condition index?

#### *Concurrent Index Development Efforts*

As during the 2005 MMI calibration, a concurrent predictive model (Observed/Expected taxa) development process was performed. The two efforts were coordinated so that identical reference samples and environmental data would be considered. Otherwise, the indicator development processes were independent and we do not report on predictive model results here.

#### *Application Considerations*

The multimetric index development process was intended to recognize the highest degrees of sensitivity, accuracy, and precision in a biological indicator. This should allow CDPHE to apply the index with a high degree of confidence that they are assessing stream resources correctly. While we expect the MMI to indicate relative biological integrity, and can recommend possible application scenarios, we think the CDPHE is best qualified to decide on final index application protocols.

### *Index Development Process*

The MMI development process includes a series of data preparation and analytical steps.

1. Collect, Compile, & QC Data
2. Identify Reference and Stressed Conditions
3. Identify Natural Variability (Site Classification)
4. Calculate Metrics
5. Find Discriminating Metrics
6. Score Metrics
7. Combine Scores in an Index
8. Apply within the development context

These steps are generally linear, though reiteration can occur when a later step prompts revisions of earlier assumptions or questioning of earlier results. Most of the effort for steps 1 and 2 were accomplished by CDPHE and results were transferred to Tetra Tech for further analysis.

## **2 Data Description**

Colorado DPHE provided a robust dataset in the Ecological Data Application System (EDAS; an Access database) for use in this study. This dataset included benthic macroinvertebrate data. The dataset included data points obtained from other agencies. These additional datasets were of high quality and employed similar methods. The datasets used were Western EMAP and Southern Rockies Regional EMAP (US EPA Environmental Monitoring and Assessment Program), USU-STAR (Utah State University Western Center for Monitoring and Assessment of Freshwater Ecosystems Science to Achieve Results program), USFS (U.S. Forest Service), and NAWQA (US Geological Survey National Water Quality Assessment program).

Although different programs do not use the exact same methods, the protocols used by each program were relatively comparable (Paul et al. 2005). All the programs sampled in riffles, used kick or D-frame nets with similar mesh (425 - 600  $\mu\text{m}$ ), sampled a similar size area (0.25 – 1.0  $\text{m}^2$ ) using comparable kick methods, and identified organisms to a similar taxonomic level. The principal difference among the programs was the subsample size. Most programs enumerated the whole sample, while CDPHE identified a 300 count subsample. To standardize sample size, large samples were randomly subsampled to a 300 organism count before analysis.

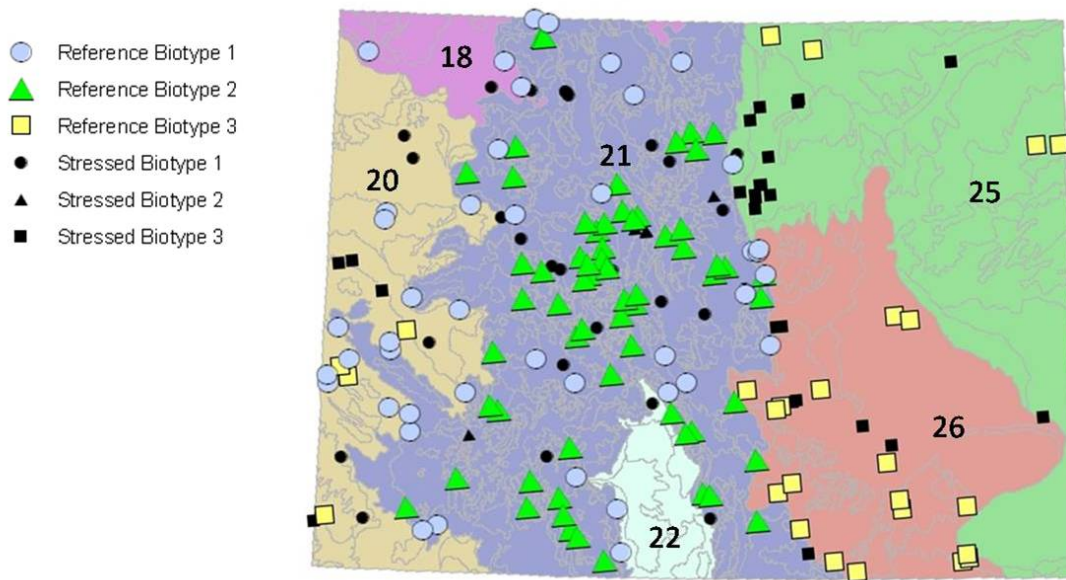
### *Reference and Stressed Site Identification*

The basis of biological assessment is comparison of conditions observed in a test site to conditions that are found in sites of the highest quality, the reference condition. CDPHE provided a list of reference sites, stressed sites, and sites that almost met the criteria for those categories. Sites that were not categorized were generally not used in index calibrations, except to illustrate final index distributions. The selection process for reference and stressed sites included remote sensing of land uses in the immediate and entire catchments of the sites. Sites were screened using Geographic Information System

(GIS) analysis and confirmed through inspection of aerial photography. The process is described in detail in **Appendix A**. As a result, 133 reference sites and 74 stressed sites were identified throughout Colorado (**Table 1, Figure 1**). In the stressed sites, 14 sites were not included because the samples had less than 150 organisms (resulting in 60 final stressed sites).

**Table 1.** Reference and stressed site tally by data source.

	CDPHE	WEMAP	REMAP	STAR	USFS	NAWQA
Reference	63	18	18	18	15	1
Stressed	48	5	1	0	0	6



**Figure 1.** Colorado map showing sample sites within ecoregions. Some stressed sites are obscured. Ecoregions are as follows: 18 = Wyoming Basin, 20 = Colorado Plateaus, 21 = Southern Rockies, 22 = Arizona/New Mexico Plateau, 25 = High Plains, 26 = Southwestern Tablelands.

### Data Treatments

For certain statistical analyses, the biological data were summarized, reduced, or filtered to conform to analytical requirements. These data treatments are listed in **Table 2** and described below. The final list of reference and stressed samples used in the analysis are in **Appendix B**.

**Table 2.** Data treatments for consistency in analysis.

Data treatment	Analytical justification
Elimination of rare taxa	Eliminates potential over-weighting of uncommon taxa in site classification
Condensing taxa to Operational Taxonomic Units (OTU's)	Eliminates potential confusion of identical taxa as unique, for site classification
Re-sampling samples to 300 organisms	Eliminates potential over-estimation of taxa richness in site classification and metric calculations
Elimination of samples with incomplete midge taxonomy	Standardizes taxonomy on genus level for midges
Elimination of samples with less than 150 individuals	Eliminates under-estimation of richness in index calibration
Selection of one sample per site	Reduces potential bias associated with pseudo-replication

Rare taxa can have undue influence on analyses that are used to detect biological site types. They were discounted from samples during cluster and ordination analyses only. Rare taxa were defined as those that occurred in less than 7 of the 133 reference samples.

The taxon identifications used for classification were called the operational taxonomic units (OTUs) and were the most common and confident taxonomic identification levels in the data set. Ambiguous taxa are those that are not identified to the targeted taxonomic levels because of damaged or undeveloped specimens. The site-by-taxon matrix was reduced to retain as much information as possible while excluding ambiguous taxa.

As mentioned above, the principal difference among the multiple sample collection procedures was the subsample size. Larger subsamples tend to contain more taxa than smaller subsamples. This uneven taxa representation can be misinterpreted in analyses of site classification or metric responsiveness to stress. Therefore, a standard subsample size was established as the smallest size of all the data sets, 300 organisms. All large samples were randomly reduced to a 300 organism count prior to classification or metric analysis.

The elimination of samples with incomplete midge taxonomy and less than 150 individuals were measures taken to standardize comparisons among samples. Some samples had midges (*Diptera: Chironomidae*) identified to the sub-family level. This level of identification was uncommon and the information from genus-level identifications could help in discriminating stressor effects. Therefore, the few samples with midges identified to sub-family were not used.

Samples with fewer than 150 organisms were not used because metrics and taxa lists are sensitive to such sample size. Interpretations of metrics from small sample are not always

straightforward, likely indicating stressed conditions, but not always. Since the target subsample size is 300 individuals, small samples were not used in index calibration.

Several samples were collected at some sites. However, including more than one sample from some sites and only one from other sites would over-weight the importance of those sites with multiple samples. Therefore, one sample per site was selected. Preferred samples were those that were collected recently, during the standard sampling period, and met all other analytical requirements. When several samples were of equal preference, selection was random.

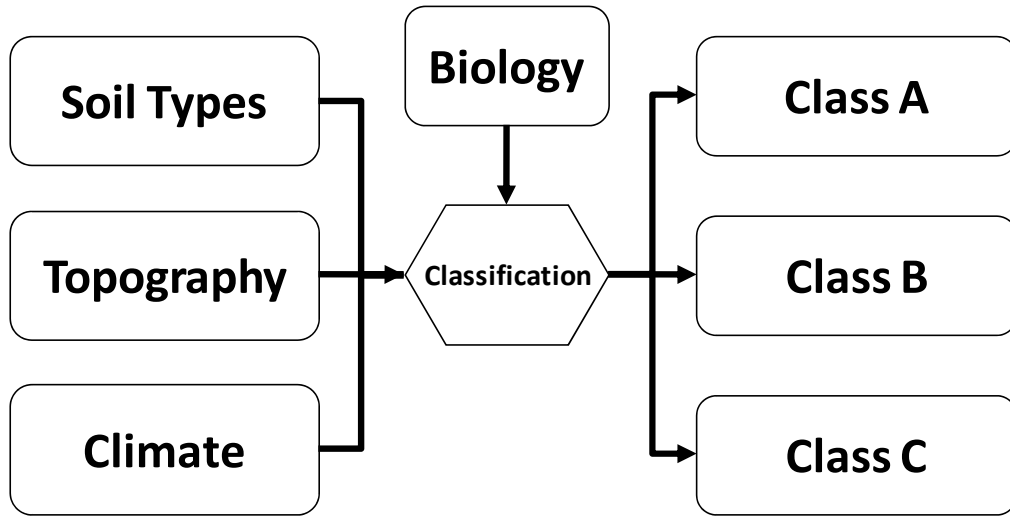
## **3 Analytical Methods**

### **3.1 Site Classification**

Reference conditions are expected to vary across the many natural settings in Colorado, due to environmental limitations or opportunities affecting the macroinvertebrates. When comparing conditions in test sites to the reference condition, the test sites must be of a similar site class, so that the biological community has the same potential regardless of stresses. Recognizing natural variability and evaluating sites according to their natural potential is extremely important to accurate bioassessment. In the site classification analysis we attempt to establish the distinctions among reference-quality macroinvertebrate community types across the state and to identify the environmental variables that are most important in determining the site classes.

Site classes can be defined using the reference biological samples to identify compositional similarity. Environmental variables are then used to determine the basis for the biological structure. Site classes can be conceptualized in terms of both biological and environmental characteristics (e.g., high elevation, cold water streams have more oxygen sensitive organisms, such as mayflies, stoneflies and caddisflies). For MMI development, sites are typically categorized into one of a set of possible classes in a discrete manner. While each site is unique, the site characteristics among site of a distinct site class are somewhat homogenous in those natural environmental factors that affect biological potential (**Figure 2**). For multimetric indices, classification simplifies biological index development, allowing an analytical focus on biological responses within one site type at a time (Gerritsen and Barbour 2000).

Ecoregions are commonly used as the primary classifying variable because they incorporate multiple environmental characteristics. They are often used to classify sites for bioassessment (Paul et al. 2005, Stribling et al. 2000). The ecoregion concept is a trusted method for identifying general ecological categories of site characteristics (Omernik and Bailey 1997).



**Figure 2.** Typical inputs and results in a discrete classification process. Inputs include natural environmental variables and biological similarities among sites. Resulting site classes are discrete bins, where a site can be placed in only one bin.

## Biological Groupings

### *Cluster Analysis*

In cluster analysis, sites are arranged in a branched hierarchy of similar groups by first calculating the taxonomic similarities between samples and then clustering sites with the flexible-beta algorithm (McCune and Grace 2002). Biologically distinct groups can be defined by breaking the dendrogram into several branches. Each group can then be associated with environmental factors through discriminant analysis and through display of the groups in the ordination diagram. We use the terms groups and clusters interchangeably.

A site-by-taxon matrix was compiled to reflect presence of each taxon in each site. Similarity among reference biological samples was determined using the Bray-Curtis similarity measure. The Bray-Curtis (BC) formula is sometimes written in shorthand as

$$BC = 1 - 2W / (A + B)$$

where  $W$  is the sum of shared abundances and  $A$  and  $B$  are the sums of abundances in individual samples. The analytical software (PC-Ord, McCune and Mefford 1999) calculates a site-by-site matrix of BC similarity from which the arrangement of samples in the ordination diagram is derived. Multiple dimensions are compressed into two or three dimensions that we can perceive. Taxon presence does not take abundance into account, so that a single individual or 50 individuals would be counted equally in this exercise. Clusters were also derived using relative abundance of taxa and calculated metrics.

Ordination diagrams and metric distributions are used to confirm the biological groupings indicated in the cluster analysis. When clusters have unique taxonomic composition, they

will appear in separate areas of the ordination diagram (see ordination details below). Significant overlap in the ordination diagram would suggest that the taxonomic composition in one group is similar to the overlapping group.

A biological metric is a numerical expression of a biological community attribute that responds to human disturbance in a predictable fashion. Because metrics are the building blocks of MMI's, it is important that the distributions of metric values among clusters are distinct. Metrics can be insensitive to the clustered groups depending on the constancy of taxonomic characteristics among taxa that are substituted across groups. In other words, the metrics may measure an assemblage characteristic or function that is independent of the specific taxa. Therefore, we used metric distributions in potential site groupings to confirm the proposed groupings and assure that the groupings are meaningful in the assessment context. In our analysis, those groups that show consistently overlapping metrics were considered for combination as a single group.

Metric distributions can also indicate when groupings are inadequate. Metric distributions that show considerable variability (broad ranges of values) within a single group suggest that the natural variability of the site grouping has not been accounted for. In these cases, we would look into the possibility of multiple refined groupings within the variable group or metric adjustments using linear regressions.

### **Discerning Environmental Factors**

The natural environmental settings of the sites were described using remotely sensed, sampled, observed, estimated, or calculated environmental data. Variables that might indicate site classes (predictor variables) were compiled among seven categories, including ecoregions, site location, site and watershed physical characteristics (including topography), geology, soil characteristics, climate, and flow (**Table 3**). Variables that were subject to alteration through human activity were relegated to lower analytical importance because we were seeking natural biological differences, not stressor induced differences. Stress levels should be minimal in the reference sites, but classifying sites based on stressor sensitive variables could possibly introduce errors in classification of non-reference sites.

We discerned the most important environmental determinants of biological groups using three techniques: 1) ordination overlays, 2) variable distributions, and 3) discriminant function analysis. These three techniques were used simultaneously in a weight of evidence approach, where results from one technique were used to inform and validate results from the others.

**Table 3.** Predictor variables listed categorically. This list does not include all the possible variations of each variable, which included statistical summaries by watershed or at the sampling point.

Site Location	Latitude Longitude Level 8 Hydrologic Unit Major Basin
Physical Characteristics	Catchment Area Relief Ratio Drainage Density Watershed Shape Factor Site Elevation Average Catchment Elevation Stream Slope <sup>1</sup>
Ecoregion	Dominant Level 3 Ecoregion in the site catchment Dominant Level 4 Ecoregion in the site catchment Site Level 3 Ecoregion Site Level 4 Ecoregion
Geology	Dominant Geology Site Geology Percent Granitic Geology Percent Sedimentary Geology Percent Volcanic Geology Geologic Chemical Activity Geologic Nutrient Activity Geologic Physical Activity Predicted Alkalinity Predicted Conductivity
Soil Characteristics	Soil Water Capacity Soil Density Soil Erodibility Soil Organic Content Soil Permeability Soil Depth Water Table Depth
Climate	Precipitation, Watershed Site Precipitation Number of Wet Days Average Temperature Maximum Summer Temperature Number of Frost Free Days Date of First Frost
Flow	Hydrologic Stability

<sup>1</sup> Stream slope was calculated the same way as USGS National Hydrography Dataset (NHD) slope but the distances of the stream segments were shorter resulting in differing values. Spatially mapped slope predictions will be made available to the public for estimating slope values.

### *Ordination Overlays*

Biological groups were explored using non-metric multidimensional scaling (NMS), a comparison of taxa within each sample (using a pair-wise Bray-Curtis similarity measure) and an arrangement of the samples so that similar samples plot closer together than dissimilar samples in multiple dimensions. Natural environmental variables can be associated with the biological groups through visual inspection of the ordination diagrams and correlations with the biologically defined axes of the NMS diagram. NMS is a robust method for detecting similarity and differences among ecological community samples and works as well with presence/absence data as with relative abundance data (McCune and Mefford 1999, Reynoldson et al. 1995).

The ordination software (PC-Ord; McCune and Mefford 1999) allows for the display of environmental variables associated with the biologically arranged samples. The display was used to discern those variables that were correlated with (continuous variables) or biased on (categorical variables) the ordination axes. Through rotation of the ordination axes, it is possible to show correlation or orthogonality among the environmental variables.

### *Variable Distributions*

Environmental variable distributions and comparisons are used to identify those variables that differ among groups. Variables with disparate distributions among biological groups are candidate determinants of the groupings. Box and whisker diagrams were used to identify breakpoints of the variable values among groups.

### *Discriminant Function Analysis*

Discriminant function analysis (DFA) is commonly used in predictive assessment tools to assign probabilities of class membership to sites. A discriminant function is created as a linear combination of independent variables. If DFA is effective, the model predicts correct groups for a high percentage of sites. The environmental variables that repeatedly appear as significant determinants in the DFA models are the ones that affect the natural biological community and may be valuable as classifying variables.

## **Predicting Site Classes**

We observed patterns in the environmental data distributions using graphic displays of environmental variables and cluster assignments. Mapping exercises displaying sites coded by potential groupings and overlain on landscape characteristics (such as ecoregion) were useful to conceptualize biological types (Biotypes). Rules for assigning sites to Biotypes were derived through an iterative process which included establishing preliminary rules, checking for correct assignment of sites to biological clusters, refining the rules through examination of assignment errors, and checking correct assignments after refining the rules. Rule establishment and refinement were based on biological-environmental relationships recognized in the ordination overlays, variable distributions, and DFA.

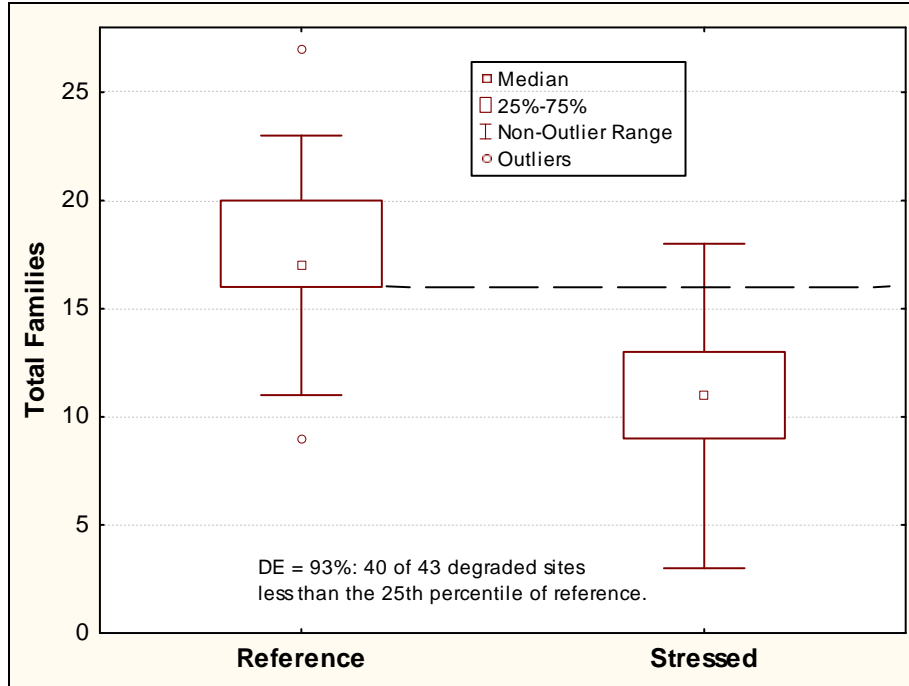
## 3.2 Metric Calculations

### Metric Calculation and Evaluation

Metrics were calculated as counts of taxa, relative percentages of individuals, or complex algorithms derived from the sample taxa lists. Several commonly applied, empirically proven, and theoretically responsive metrics were calculated for possible inclusion in a multimetric index. The calculations were made in the EDAS database. The metrics were organized into five categories: richness, composition, pollution tolerance, functional feeding group, and habit (mode of locomotion).

Discrimination efficiency (DE) is the capacity of the biological metric or index to detect stressed conditions. It is measured as the percentage of stressed sites that have values lower than the 25<sup>th</sup> percentile of reference values (Stribling et al. 2000, **Figure 3**). 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 5**). When there is no overlap of boxes representing reference and stressed sites, the DE is greater than 75%. A metric with a high DE thus has a greater ability to detect stress than metrics with low DEs. Metrics with DEs <25% do not discriminate and were not considered for inclusion in the index.

Metrics are based on some attribute of the taxa, as suggested by the metric categories. Several metrics are based on the sensitive insect orders Ephemeroptera, Plecoptera, and Trichoptera (EPT), commonly known as mayflies, stoneflies and caddisflies. Other metrics are based on pollution tolerance, as in the Hilsenhoff Biotic Index (HBI, Hilsenhoff 1987). The HBI was developed with organic pollution as the primary stressor, and the values may not be applicable in certain stressors in Colorado. For that reason, we analyzed indicator taxa for the stressors and samples in the current data set.



**Figure 3.** Illustration of metric discrimination efficiency (DE) between reference and stressed sites.

### Indicator Taxa

Indicator taxa are those that differ markedly in frequency of occurrence and relative abundance between groups of samples (Dufresne and Legendre 1997), such as those from reference and stressed sites. Frequency of occurrence was calculated as the percentage of sites in which each taxon occurred (by site category; reference or stressed). Relative abundance was the percentage of all individuals in a taxon that occurred in either reference or stressed samples. We grouped taxa at the family taxonomic level to simplify the analysis and resulting metrics. We considered responses in two site groupings, high elevation and low elevation, because of perceived differences in taxonomic distributions, sensitivities, and environmental characteristics between the two categories.

The indicator analysis included an empirical step and a subjective review step. During review, the indicator taxa that were more or less prevalent by reference category were scrutinized by the index development workgroup, which included biologists who were familiar with benthic taxa sensitivities. The workgroup removed some taxa from the lists of those empirically identified as indicators of stress. No taxa were added to the lists during the review process.

### Metric Adjustment to Environmental Conditions

Site classification with discrete class assignments can result in groupings of sites with similar biological characteristics. However, high biological metric variability within a class can persist. This can be controlled if relationships between metrics and environmental variables are recognized and adjustments are applied. Within reference

sites, correlations among metrics and environmental variables were analyzed. Where high correlations existed, metrics were adjusted to the environmental variable through linear regression. The ultimate metric used in metric testing and index development was then the residual of the metric value predicted by the regression equation.

### 3.3 Index Composition

A multimetric index is a combination of metric scores that indicates a degree of biological stress in the stream community (Barbour et al. 1999). Individual metrics are candidate for inclusion in the index if they:

- discriminate well between reference and stressed sites;
- are ecologically meaningful (mechanisms of responses can be explained);
- represent diverse types of community information (multiple metric categories); and
- are not redundant with other metrics in the index.

Metrics are scored on a common scale prior to combination in an index. The scale ranges from 0 to 100 and the optimal score is determined by the distribution of data. For metrics that decrease with increasing stress, the 95<sup>th</sup> percentile of all high gradient data was considered optimal and scored as 100 points. All other metric values were scored as a percentage of the 95<sup>th</sup> percentile value except those that exceeded 100, which were assigned a score of 100. Metrics that increase with stress are scored using the 5<sup>th</sup> percentile as the optimum. The 5<sup>th</sup> and 95<sup>th</sup> percentile values were selected as optimal instead of the maximum so that outlying values would not skew the scoring scale.

#### *Other Metric Considerations*

Index alternatives were evaluated based on index DE, a *z-score* to quantify reference and stressed differences, variability of reference scores, the number of metric categories included, and ecological meaningfulness of the included metrics. The DE for indices was calculated as described above for individual metrics. The *z-score* was calculated as the difference in mean index values between reference and stressed sites, divided by the standard deviation of reference index scores.

Ecologically meaningful metrics are those for which the assemblage response mechanisms are understandable and are represented by the calculated value. Ecological meaningfulness is a professional judgment based on theoretical or observed response mechanisms. Those metrics that respond according to expectations established in other studies are more defensible.

Metrics contribute information representative of integrity if they are from diverse metric categories. As many metric categories as practical should be represented in an index so that signals of various stressors can be integrated into the index. While several metrics should be included to represent biological integrity, those that are included should not be redundant with each other. Redundancy was evaluated using a Pearson Product-Moment correlation analysis.

For metrics to discriminate on a gradient of stress, they must have a sufficient range of values. Metrics with limited ranges (e.g., richness of taxa poor groups or percentages of rare taxa) may have good discrimination efficiency. However, small metric value changes will result in large and perhaps meaningless metric scoring changes.

## 4 Results

### 4.1 Site Classification

#### *Cluster Analysis*

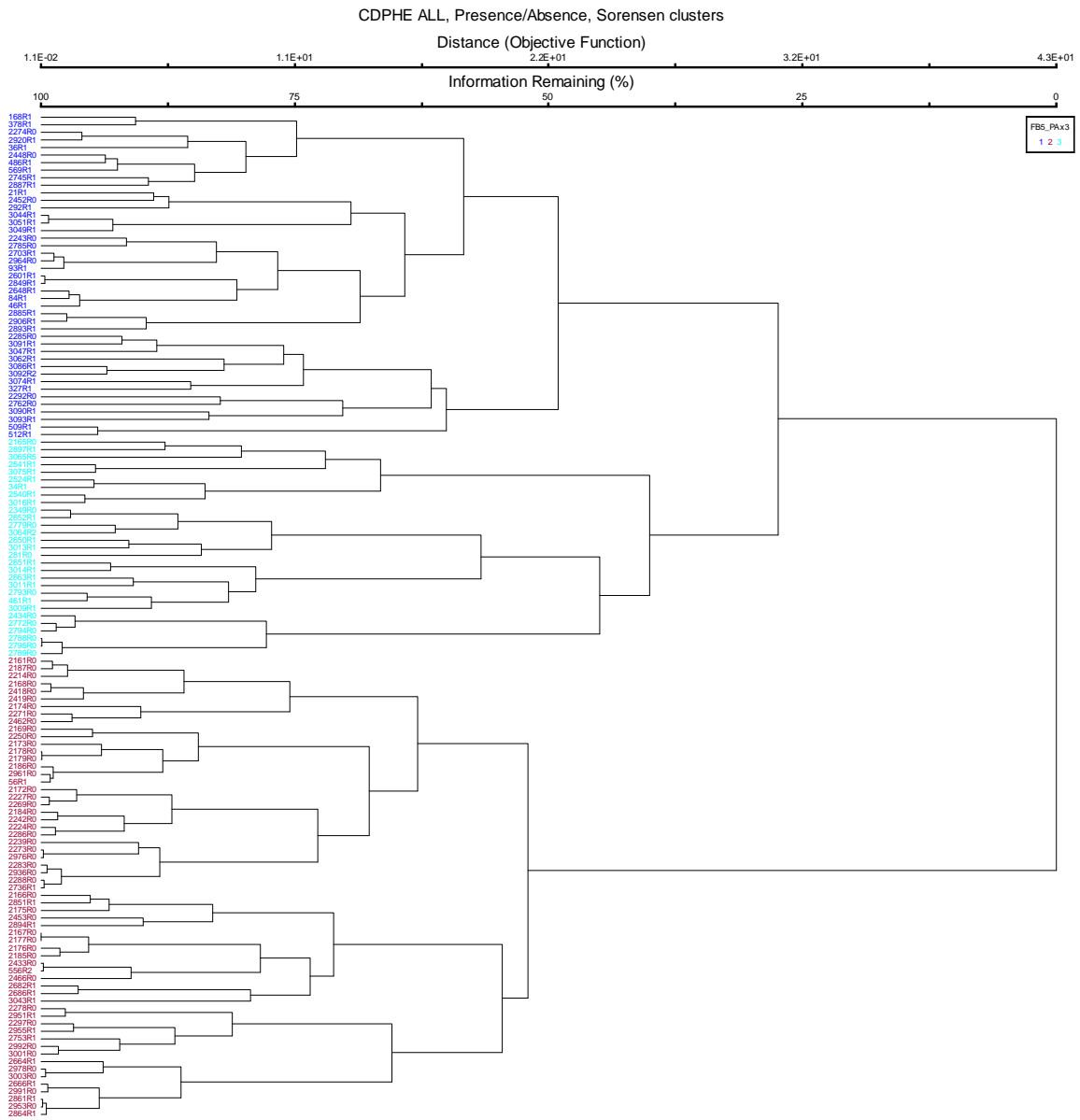
For our analytical data set, presence-absence data were more robust than relative abundance or metrics for identifying sample similarities. The test of robustness entailed iterative removal of a portion of sites during successive cluster analyses and checking on the consistency of assignments of sites to clusters in each run. We ultimately relied on the clusters indicated using presence and absence of taxa in all reference sites because the cluster assignments were relatively robust among iterations compared to methods using relative abundance or metrics. We suspect that any inconsistencies introduced through multiple sampling techniques would have less effect on taxa presence/absence than it would on taxa relative abundance. In addition, presence-absence data are commonly used in ordination and cluster analyses (e.g., RIVPACS analyses use presence-absence data exclusively).

In each of 20 iterations of the cluster analysis with presence/absence data, 10% of the sites were randomly removed. Site dendrograms and site cluster assignments were examined for each iteration. When more than 3 clusters were distinguished, sites moved among clusters in successive iterations. Based on this variability and results of other preliminary cluster analyses, we used only 3 cluster designations.

The cluster analysis of presence-absence data with flexible beta clustering technique resulted in a cluster dendrogram with minimal chaining when beta was set at -0.5. Chaining is the sequential addition of small groups to one or a few large groups. Dendrograms that are highly chained are undesirable as they are generally not helpful in defining subgroups. The beta value of -0.5 was selected based on visual inspection of alternative cluster dendrograms and consideration of the percent chaining statistic. Three groups were defined when using a cut line with 30% information remaining (**Figure 4**). The three groups were biologically distinct (**Figure 5**) and could be associated with conceptual sites types (**Table 4**).

**Table 4.** Site designations in three clusters.

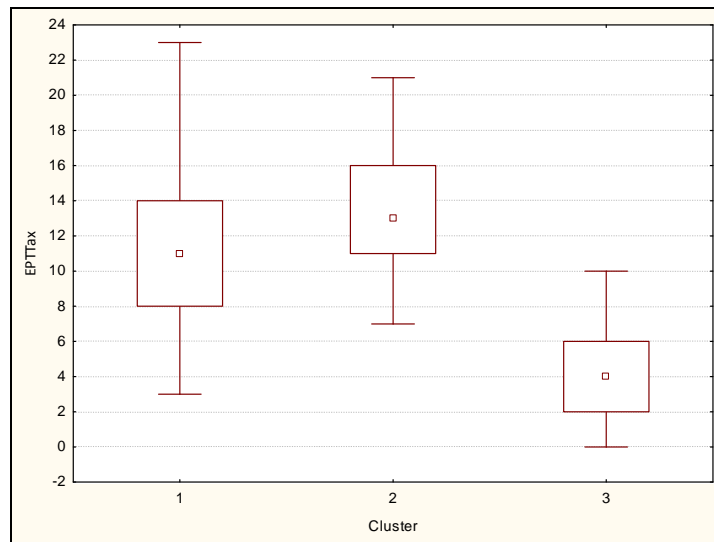
Cluster number and conceptual description	Number of Sites
1 (mid-level, intermediate temperatures)	43
2 (high elevation, cold water)	61
3 (low elevation, warm water)	29



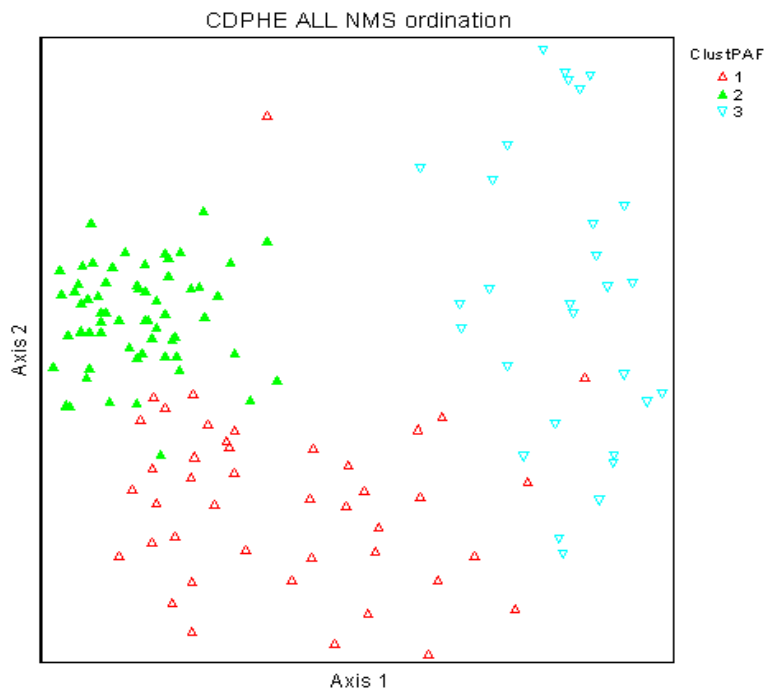
**Figure 4.** Cluster dendrogram showing reference sites grouped by similarities in the presence (and absence) of taxa in the samples.

*Ordination Analysis*

Ordination showed that groups defined through cluster analysis also showed separation in non-metric multidimensional scaling (**Figure 6**). The primary axis of the ordination diagram divided the mountainous sites on the left and the plains sites on the right. This axis was also associated with temperature, elevation, and precipitation. The second axis was strongly related to longitude, but this variable did not prove to be useful in separating the groups. Ecoregions were well separated on the ordination diagram, though some overlap among the clustered groups was obvious. The Southern Rockies did not overlap at all with the High Plains and Southwestern Tablelands ecoregions.



**Figure 5.** Distributions of EPT taxa richness in clusters.



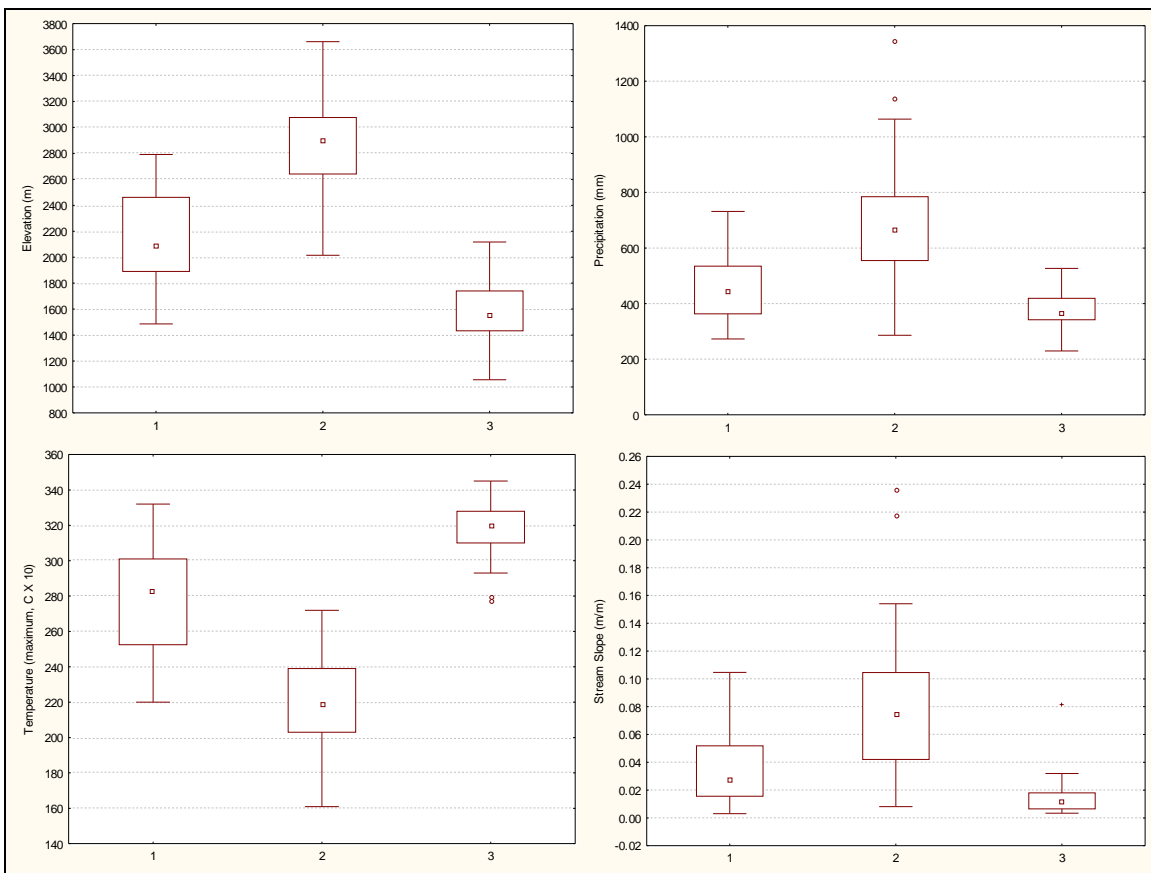
**Figure 6.** NMS ordination of taxa presence-absence with sites marked by groups resulting from cluster analysis.

Only two axes were needed in the ordination diagram to achieve a suitable arrangement (stress = 20.6). Ordination stress is a measure of the departure from the linear relationship between the dissimilarity (distance) in the original multi-dimensional space and distance in the reduced-dimensional ordination space. A target stress value for deciding on the number of ordination dimensions is 20. These ordination results were based on presence-

absence data. Ordinations using taxa relative abundance and metrics showed relationships that verified those described with presence-absence data.

*Variable Distributions*

The distributions of environmental variables in biological groups show that the environmental characteristics of the site clusters are somewhat distinctive (**Figure 7**). While groups 2 and 3 could be distinguished on elevation and temperature, overlapping ranges of values were evident in precipitation and stream slope and with the intermediate group, group 1. This figure includes only a subset of the environmental variables; those which showed the greatest distinctions among groups and were not redundant with each other. Other variables, such as catchment area and longitude, also showed distinctions among groups.



**Figure 7.** Distributions of environmental variables (elevation, precipitation, maximum air temperature, and stream slope) in the biologically-defined groups.

*Discriminant Analysis*

A forward stepwise DFA was conducted using the environmental variables. The DFA did not consider categorical variables such as ecoregion. The most accurate prediction of group membership used three variables in the model: elevation, slope, and precipitation. These were not the most significant variables to enter the model at each step, but they were forced into the model after eliminating variables that were relatively difficult to derive (e.g., average maximum summer temperature or average precipitation in the watershed) or that were conceptually non-linear (e.g., longitude). Elevation was the most significant determinant when summer temperature was removed, and the two were highly correlated. The model placed 76% of reference sites in the correct groups given equal probability of membership and 83% correct given probability weighted by group size.

*Conceptual Site Classes*

The groups defined by cluster analysis were indicative of three site types, which are conceptually described as:

1. Mid-elevation, semi-cold, low gradient, moist (Transitional)
2. High elevation, cold-water sites (Mountains)
3. Low elevation, warm-water, low gradient, dry (Plains)

The site types were in agreement with (and more refined than) the existing MMI assessment regions and represented groupings that could be conceptually associated with biological differences.

*Predicting Site Classes*

The process for assigning sites to classes included review of all analytical results, development and application of decision rules, and iteratively testing the results. The three variables used in the classification rules (**Table 5**) include site ecoregion, site elevation, and stream slope. These were decided upon because of corroborating results from ordination analysis, variable distribution evaluation, and DFA. Of the 133 sites used to derive the classification rules, 120 (90.2%) had predicted classes matching the biologically defined clusters. Class assignments are listed in **Appendix B**.

**Table 5.** Classification Rules.

<p><i>Group 3 prediction:</i>                  If ecoregion = 25 or 26 (except sub-ecoregion 25l and 26i), then Predicted Class = 3                  If elevation at the site &lt; 1550, the Predicted Class = 3</p>
<p><i>Group 2 prediction:</i>                  If sub-ecoregion (level 4) = 21a, 21b, 21e, or 21g, then Predicted Class = 2                  If sub-ecoregion (level 4) = 21c and slope &gt; 0.04, then Class = 2 (1 if shallower)                  If sub-ecoregion (level 4) = 21f and elevation &gt;2500, then Class = 2 (1 if lower)</p>
<p><i>Group 1 prediction:</i>                  If site is not predicted in classes 2 or 3, then Predicted Class = 1                  (e.g., If sub-ecoregion (level 4) = 25l, 26i, 21d, 21h, 21i, or 21j, then Predicted Class = 1)</p>
<p>Sub-ecoregions used in the rules                  Used in rules for group 3.                  26i = Pine-Oak Woodlands</p>

**Table 5 (continued)**

25l = Front Range Fans

Used in rules for group 2.

21a = Alpine Zone

21b = Crystalline Subalpine Forests

21c = Crystalline Mid-Elevation Forests (northern Front Range)

21e = Sedimentary Subalpine Forests

21f = Sedimentary Mid-Elevation Forests (primarily western slopes)

21g = Volcanic Subalpine Forests

Used in group 1 rules:

21d = Foothill Shrublands

21h = Volcanic Mid-Elevation Forests

21i = Sagebrush Parks

21j = Grassland Parks

## 4.2 Metric Development

### *Indicator Taxa*

Indicator taxa analysis using presence/absence family-level information revealed the taxa that were more common to reference or stressed samples. This analysis was performed separately in two sets of site groups: high elevation and low elevation. Because other tolerance measures were responsive in high elevation sites, indicator taxa were not as thoroughly pursued there compared to the low elevation sites. During a working meeting held in 2008, Colorado stakeholders scrutinized the analytical results for the low elevation sites and reduced the length of the lists based on professional experience (**Table 6**). These taxa were used to calculate metrics in the pollution tolerance metric category.

**Table 6.** Indicator taxa for the low elevation sites (groups 1 and 3).

Families Sensitive to Stress		Families Tolerant of Stress
Elmidae	Naucoridae	Tubificidae
Hydrophilidae	Gomphidae	Corixidae
Dytiscidae	Nemouridae	Naididae
Tipulidae	Limnephilidae	Erpobdellidae
Caenidae	Hydropsychidae	Enchytraeidae
Leptophlebiidae	Leptoceridae	

### *Metrics Adjustments to Environmental Variables*

In Biotype 1 reference sites, several metrics were correlated with elevation and maximum temperatures. For instance, the EPT taxa metric was correlated with elevation (Spearman  $r = 0.62$ ) and summer temperature (Spearman  $r = -0.68$ ). Our expectations were that EPT taxa would be more numerous in cooler and higher elevation reference sites. We adjusted metrics as residuals of regressions to eliminate the bias. Other relationships were noted in Biotype 1 and in the other biotypes. However, the relationships within other Biotypes were not strong. Metric variability was mostly accounted for by the classification scheme. Metric adjustment did not always improve the DE of the metrics.

*Metric Performance*

A total of 145 metrics were calculated using EDAS queries of the macroinvertebrate data with identifications reduced to the standard OTU level. This included 56 metrics calculated using environmental adjustments for elevation or summer temperature. The DE of each metric was calculated within the *predicted* site classes (**Appendix C**). Thirty-eight (38) metrics showed a similar discriminating trend in all three biotypes.

Metrics with limited ranges of values were not included in index trials. Metrics that quantify rare taxa can have limited ranges of values. Metrics with ranges of less than 5 taxa or percentages less than 10% were tested for responsiveness to stress. However, they were generally not included in index trials because small changes in metric values could result in large changes in metric scores and increased index variability.

In Biotype 1, metrics from each metric category had DEs greater than 50% and metrics in the richness, composition, and tolerance categories had DEs ranging to above 75%. The metrics with the highest DEs were Plecoptera taxa (adjusted for elevation) and percent Plecoptera.

Metrics from each category in Biotype 2 had DEs greater than 85%, including seven metrics in the richness composition and habit categories with DEs of 100%. In Biotype 3, richness and specialized tolerance metrics performed best. Tolerance values ranged upwards to 92%. Except for % Coleoptera, composition metrics did not have DEs greater than 72%, and feeding group metrics did not have any DEs greater than 64%.

**4.3 Index Performance**

One hundred and four (104) index alternatives were calculated and tested using reference and stressed sites predicted in the three site classes (**Appendix D**). The index alternatives that performed best were reconsidered and the following indices were recommended.

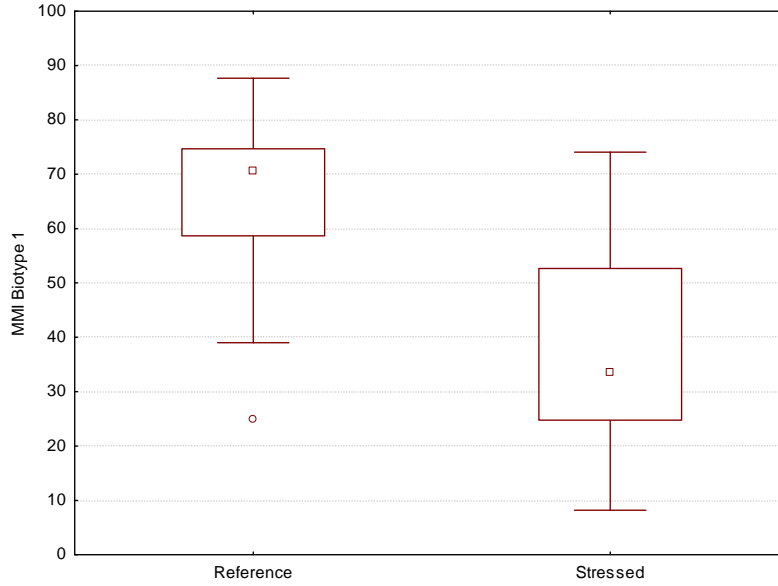
In Biotype 1, 18 of 59 alternatives had DEs greater than 85%, including three with the highest DE of 92.9%. The alternative selected as the MMI for 1 (**Table 7, Figure 8**) included six metrics from all five metric categories and had the lowest standard deviation in reference sites. Two of the metrics were sensitive to site elevation and required adjustment before scoring.

**Table 7.** Metrics included in the Biotype 1 MMI with adjustment and scoring formulae.

Metric (X)	Metric Adjustment (Xa <sup>2</sup> )	Scoring Formulae <sup>3</sup>
% non-insect taxa		100*(28.09375-X)/ 28.0937
EP Taxa	(X-(-4.803+ (0.00583)*Elev))	100*(Xa+6.481)/ (2.870+6.481)
% Chironomidae		100*(66.839- X)/ (66.839-1.308)
% Sens. Fam. Plains		100*X/ 61.812
Predator, Shredder taxa		100*X/ 14
Clinger taxa	(X -(-3.262+ (0.00694)*Elev))	100*(Xa +8.615)/ (3.136+8.615)

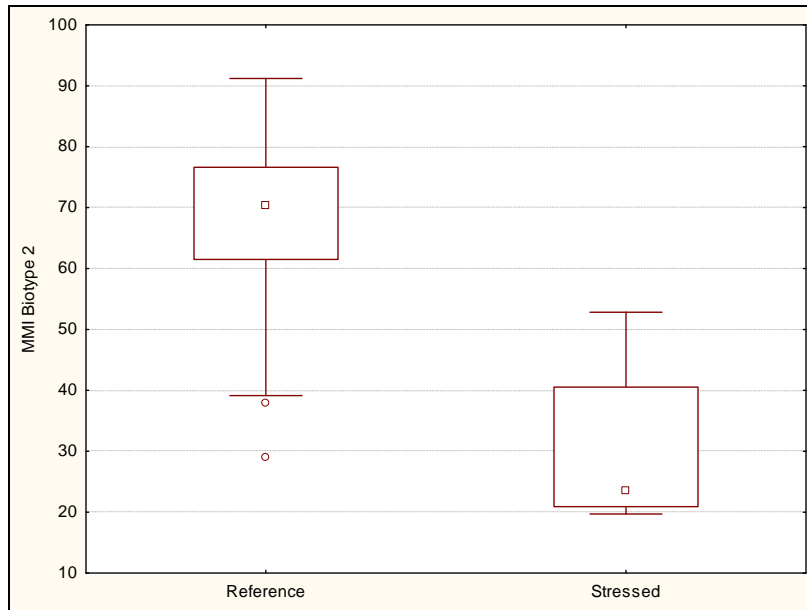
<sup>2</sup> Xa = Elevation adjusted metric

<sup>3</sup> Scoring formulae are rounded



**Figure 8.** Distribution of index scores in reference and stressed sites in Biotype 1.

In Biotype 2, 16 of 19 alternatives had DEs of 100%. Therefore, other index evaluation factors were important in selecting the final MMI. The alternative selected as the MMI for Biotype 2 (**Table 8, Figure 9**) included five metrics, one from each of the five metric categories. It had the lowest variability in reference sites of those indices that had 100% DE.



**Figure 9.** Distribution of index scores in reference and stressed sites in Biotype 2.

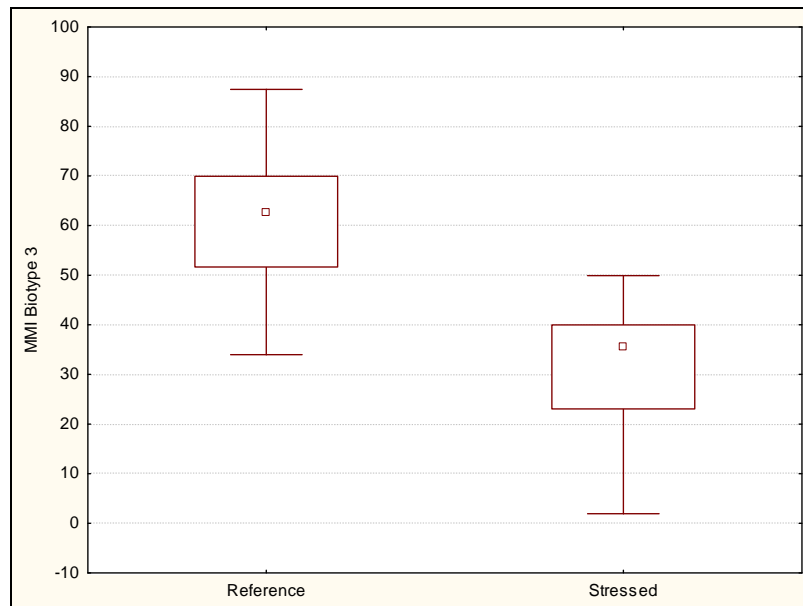
**Table 8.** Metrics included in the Biotype 2 MMI with scoring formulae.

Metric (X)	Scoring Formulae
Total taxa	$100 * X / 36$
% Ephemeroptera	$100 * X / 72.090$
Predator, Shredder taxa	$100 * X / 14$
Beck's Biotic Index	$100 * X / 33$
Clinger taxa	$100 * X / 17$

In Biotype 3, 16 of 26 alternatives had DEs of 100%. Therefore, other index evaluation factors were important in selecting the final MMI. The alternative selected as the MMI for Biotype 3 (**Table 9, Figure 10**) included six metrics, representing each of the five metric categories. Some index alternatives included metrics with low ranges of values (e.g., Coleoptera taxa). These alternatives were not selected because such metrics could introduce high variability to the index.

**Table 9.** Metrics included in the Biotype 3 MMI with scoring formulae.

Metric (X)	Scoring Formulae
Insect taxa	$100 * X / 26$
% non-insect taxa	$100 * (44.444 - X) / (44.444 - 1.977)$
% Sens. Fam. Plains	$100 * X / 57.739$
% dominant taxon	$100 * (79.590 - X) / (79.590 - 18.138)$
Predator, Shredder taxa	$100 * X / 10$
% sprawler	$100 * X / 50.625$



**Figure 10.** Distribution of index scores in reference and stressed sites in Biotype 3.

## 4.4 Index Application

Application of a typical MMI is straightforward; calculate the index for the appropriate site class and compare it to an established threshold. Thresholds will be determined by CDPHE.

Index application should proceed as follows:

- 1) Enter the biological and predictor variable data into the analytical database.
- 2) Determine the site class of the site using the classification rules.
- 3) Calculate index metrics and scores (**Table 7, 8, or 9**) and average as an MMI.
- 4) Compare the resulting MMI value to impairment thresholds for the appropriate site class.
- 5) Include MMI performance statistics when reporting assessments (**Table 10**).

**Table 10.** MMI performance statistics.

Site Class	DE	25 <sup>th</sup> %ile of reference scores <sup>4</sup>
Biotype 1, Transitional	92.9	58.7
Biotype 2, Mountains	100	61.5
Biotype 3, Plains	100	51.7
All Biotypes	96.7	

## 6 Conclusions and Recommendations

The proposed classification scheme and multi-metric indices gives CDPHE a system in which sites can be recognized in three unique site classes and assessed with specific index formulations. Sites were grouped by biological similarities. Cluster analysis, ordination analysis, and metric distributions corroborated the biological groups. Environmental variables, that helped to classify sites, included ecoregions, elevation, and stream slope. These determinants and the way they were applied in classification rules were derived through multiple analyses, including DFA. One measure of error associated with site classification is the percentage of reference sites in which the classification rules assign a class that does not match the biological cluster. That error rate is 9.8%.

The MMIs were selected among several alternatives. All final MMIs included metrics from each of five metric categories and had a combined DE of 96.7%. This high DE and low classification error indicate that assessments of Colorado stream benthic condition, made using the MMIs, will be accurate.

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<sup>4</sup> In EPA guidelines, the 25<sup>th</sup> percentile of reference is often used as an impairment threshold

## 7 References Cited

Barbour, M. T., J. B. Stribling, and J. R. Karr. 1995. The multimetric approach for establishing biocriteria and measuring biological condition. Pp. 63-76 in W. S. Davis and T. P. Simon, editors. *Biological Assessment and Criteria: Tools for Water Resource Planning and Decision Making*. Lewis Publishers, Ann Arbor, Michigan.

Barbour, M. T., J. Gerritsen, B. D. Snyder, and J. B. Stribling. 1999. *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish*. Second edition. EPA/841-B-99-002. U.S. EPA, Office of Water, Washington, DC.

Dufrene, M. and P. Legendre. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological Monographs* 67:345-366.

Frey, D. G. 1977. Biological integrity of water – an historical approach. Pages 127-140 in R.K. Ballantine and L. J. Guarraia (editors). *The Integrity of Water*. Proceedings of a Symposium, March 10-12, 1975, U. S. Environmental Protection Agency, Washington, DC.

Gerritsen, J. and M. Barbour. 2000. Apples, oranges, and ecoregions: on determining pattern in aquatic assemblages. *Journal of the North American Benthological Society* 19(3): 487–496.

Gibson, G.R., M.T. Barbour, J.B. Stribling, J. Gerritsen, J.R. Karr (1996) *Biological Criteria and Technical Guidance for Streams and Small Rivers*. EPA 822-B-96-001. USEPA, Office of Science and Technology, Health and Ecological Criteria Division, Washington, DC

Hilsenhoff, W. L. 1987. An improved biotic index of organic stream pollution. *Great Lakes Entomologist* 20:31-39.

Karr, J. R., K. D. Fausch, P. L. Angermeier, P. R. Yant, and I. J. Schlosser. 1986. *Assessment of biological integrity in running waters: A method and its rationale*. Illinois Natural History Survey, Champaign, Illinois. Special Publication 5.

Karr, JR, and D.R. Dudley. 1981. Ecological Perspective on Water Quality Goals. *Environmental Management* 5:11.

McCune, B., and J.B. Grace. 2002. *Analysis of ecological communities*. MjM Software Design, Gleneden Beach, OR.

McCune, B. and M.J. Mefford. 1999. *Multivariate Analysis of Ecological Data*, Version 4.10. MJM Software, Gleneden Beach, Oregon.

Omernik, J.M., and R.G. Bailey. 1997. Distinguishing between watersheds and ecoregions. *Journal of the American Water Resources Association* 33(5):935-949.

Paul M.J., J. Gerritsen, C. Hawkins, and E. Leppo. 2005. Development of Biological Assessment Tools for Colorado. Prepared for the Colorado Department of Public Health and Environment, Water Quality Control Division – Monitoring Unit, Denver, CO.

Reynoldson, T.B., R.C. Bailey, K.E. Day, and R.H. Norris. 1995. Biological guidelines for freshwater sediment based on Benthic Assessment of Sediment (the BEAST) using multivariate approach for predicting biological state. *Australian Journal of Ecology* (1995) 20: 198-219.

Stribling, J. B., B. K. Jessup, J. Gerritsen. 2000. Development of Biological and Physical Habitat Criteria for Wyoming Streams and Their Use in the TMDL Process. Prepared for U. S. EPA Region 8, Denver, CO.

## **Appendix A**

### **Reference and Stressed Site Selection Process**

## **Appendix A: Reference and Stressed Site Selection Process**

Reference sites were selected using a GIS based approach that focused on using mapped human disturbance categories, delineated watershed area polygons and delineated 5 km polygons upstream from each station. GIS was then used to calculate percent (%) disturbed area or count within the total watershed area (“far field”) or the 5 km radius “near field” area. Candidate reference sites were further screened by reviewing Global Explorer images at EPA Region VIII to confirm reference status.

As recommended by EPA, the WQCD (“Division”) decided to avoid issues of circularity further down the road during nutrient criteria development by dropping water chemistry and habitat criteria all together. Nitrogen and phosphorus parameters were dropped outright due to concern over circularity (i.e. nutrient criteria development conflict). The Division plans on establishing nutrient thresholds by plotting MMI scores versus nutrient values and then finding a cutoff where MMI scores are low when nutrient values are high.

Other water chemistry criteria were dropped due to three reasons:

- Lacked screening power
- Lacked sufficient amount of data across all candidate sites
- Difficulty setting thresholds (what values are appropriate for distinguishing between ideal and unacceptable?)

Habitat parameters were also dropped for the following reasons:

- Lacked sufficient amount of data across all candidate sites
- Difficulty setting thresholds (what values are appropriate for distinguishing between ideal and unacceptable?)

Using the GIS-based approach allowed each candidate site to be considered in the reference screening process. In other words, all sites started off on equal footing in this process. The issue of data gaps could be avoided all together.

### **GIS Based Approach**

The Division used the following human disturbance parameters in the screening process:

- Irrigated agriculture (%)
- Dryland agriculture (%)
- Urban (%)
- Permitted Point Sources - outfalls (count)
- Diversions – headgated only (count)
- Road density (average)
- Abandoned mines (count)
- Oil and gas facilities (count)
- CAFO’s (count)

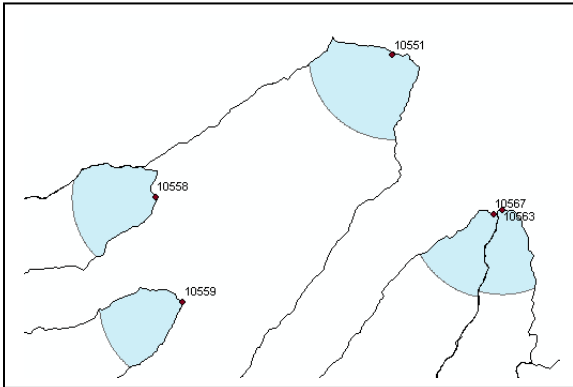
**Sources of GIS Information**

Irrigated agriculture	CO Division of Wildlife Aquatic GAP land use/land cover
Dryland agriculture	CO Division of Wildlife Aquatic GAP land use/land cover
Urban	CO Division of Wildlife Aquatic GAP land use/land cover
Permitted point sources	WQCD GIS shapefile (updated summer 2007)
Headgated diversions	CO Division of Water Resources (updated fall 2007)
Roads	U.S. Census Bureau 2007 TIGER/Line shapefile
Abandoned mines	U.S. Geological Survey Abandoned Mines Land Initiative
Oil and Gas facilities	CO Oil and Gas Conservation Commission GIS Online
CAFO's	WQCD GIS shapefile

**Total Watershed Area vs. 5 km Radius**

Total watershed area is a calculation of the total area that drains to a station. These areas were calculated using Utah State University's Multi-Watershed Delineation Tool.

The 5 km radius was created by first setting a 5 km buffer around each station. Then a buffer polygon was intersected with a total watershed area for each given station. The area where the two intersect is considered the 5 km radius area above the station. The intersections are illustrated in blue below.



**GIS Calculation**

Irrigated agriculture	km <sup>2</sup> per total watershed area (km <sup>2</sup> )
Dryland agriculture	km <sup>2</sup> per total watershed area (km <sup>2</sup> )
Urban	km <sup>2</sup> per total watershed area (km <sup>2</sup> )
Permitted point sources	# of PPS's within 5 km radius
Headgated diversions	# of diversions within 5 km radius
Roads	linear miles (mi.) per total 5 km radius area (mi. <sup>2</sup> )
Abandoned mines	# of abandoned mines within total watershed area
Oil and Gas facilities	# of oil and gas facilities within 5 km radius area
CAFO's	# of CAFO's within 5 km radius area

Human disturbance values for 1032 stations in CO-EDAS were calculated on GIS or on Excel spreadsheets. Results were stored on a master spreadsheet and then moved to a final spreadsheet that could provide a platform for selecting thresholds or cutoffs.

The process for selecting thresholds was iterative and involved the best professional judgment of WQCD Standards Unit staff. For the mountains bioregion, values for each human disturbance parameter were plotted. Each plot was reviewed and a threshold was selected that best partitioned values that were deemed ideal and those deemed marginally ideal for a reference status in the mountains. Once this was completed the threshold values were manually input into the same spreadsheet where a formula calculated the number of mountain sites that would qualify for reference status given the thresholds provided. The iterative process involved striking a balance between an adequate number of candidate reference sites and maintaining a sensible partition between ideal and marginally ideal. This process continued until the Division felt there was an adequate set of candidates that could then continue forward in the screening process.

The same process was used in selecting candidate reference sites in the plains and xeric bioregions. This was much more difficult since more human activity occurs in these bioregions. The partition between ideal and marginally ideal shifted to a partition between acceptable and undesirable. The threshold result often granted an allowance for some minimum amount of human disturbance.

The thresholds selected for each bioregion are found in the following table:

	Irrigated Agriculture	Dryland Agriculture	Urban	Permitted Pt. Sources	Diversions	Road Density	Abandoned Mines	O & G	CAFO's
	% of WSA	% of WSA	% of WSA	#/km <sup>2</sup>	#/km <sup>2</sup>	mi/mi <sup>2</sup>	#/km <sup>2</sup>	#/km <sup>2</sup>	#/km <sup>2</sup>
Mnts	0.0	-	0.0	0	1	0.6	0.03	-	-
Plains	6	50	1	2	30	3	0.002	3	2
Xeric	5	2	0.05	0	10	2	0.05	0	-

The outcome of this process yielded 110 candidate reference sites in the mountains bioregion, 60 in the plains bioregion and 64 in the xeric bioregion.

### **Site Duplication Removal**

The next phase removed multiple sites from the same waterbody. For instance, the Division did not want to have 6 reference sites on one given stream. The Division established a “rule of thumb” for removing duplication sites. If adjacent sites on the same waterbody had a greater than 20% increase in watershed area from the upstream site

to the downstream site then both sites were retained for further analysis. If the difference was <20% then the Division selected the site with the most recent sample year. 2005 would supersede 2004 and so forth. However, there were two exceptions to the rule. The first exception was if the data was collected in 2002. This was a drought year and this data was avoided all-together. For example, if data were collected in 2001 and 2002, then 2001 would supersede 2002. The second exception was if one of the two stations was below a significant hydrological modification(s), such as below an impoundment or clustering of upstream diversion. The minimally impacted site would supersede the most impacted site.

The outcome of this process yielded 100 candidate reference sites in the mountains bioregion, 48 in the plains bioregion and 60 in the xeric bioregion.

### **Aerial Imagery Review**

This phase of the reference screening process involved reviewing aerial images on Global Explorer at EPA Region VIII – Denver. EPA staff assisted Division staff in queuing up images on Global Explorer through a GIS routine. Then one at a time each station and a 3 km distance upstream was scrutinized for human activity and scored on a 0 to 10 scale. The source of the scoring scale is the Rapid Screening Disturbance Scoring (RSDS) instruction manual (draft 12/10/03) developed by EPA. Assignment of numeric disturbance scores was based upon visual interpretation of aerial orthophotos. Stations with obvious signs of human perturbation were removed from further consideration. Images were converted to PDF files and saved.

The outcome of this process yielded 88 reference sites in the mountains bioregion, 34 in the plains bioregion and 28 in the xeric bioregion.

After the aerial image review the Division mapped the final list of candidate reference points on GIS and examined the spatial distribution across the state. It was determined that it was necessary to fill spatial gaps particularly in the NE plains, the “core” sections of the xeric bioregion and the North Platte basin. Sites that were removed during the aerial image review were re-examined and further scrutinized. For the mountains bioregion, two sites were added to the reference list. These two sites were initially screened out simply because they fell too close to the mountains and plains bioregion interface. For the plains bioregion, five sites were added to the reference list. These sites primarily filled spatial gaps in the NE quadrant of the Colorado plains. For the xeric bioregion, 11 sites were added to the reference list. These sites primarily filled spatial gaps on big rivers and “core” portions of the xeric bioregion.

The outcome of this process yielded 90 reference sites in the mountains bioregion, 39 in the plains bioregion and 39 in the xeric bioregion. The total number of reference sites was 168. These reference sites were forwarded to Utah State to kick start the recalibration of the RIVPACS style OE macroinvertebrate model. During the stressed site screening process 3 xeric reference sites were removed thereby knocking the total number of reference sites in the xeric bioregion down to 36 and the grand total to 165. These sites will be provided to Tetra Tech for the recalibration of the Colorado MMI.

## **W OCD Stressed Site Screening Process**

The stressed site screening process involved using the same human disturbance values derived during the reference site screening process and aerial image review on Google Earth. The goal was to take the remaining EDAS stations not selected as reference sites and run these sites through a screening process that would result in true, “bookend” stressed sites. To this end, the Division focused on avoiding any stressed sites that might be considered marginally stressed, an issue that likely impacted the first version of the CO multimetric index in 2005.

For each bioregion, 85<sup>th</sup> percentile values were calculated for each human disturbance parameter (i.e. irrigated agriculture, CAFO’s, etc). Eighty-fifth percentile values varied across all human disturbance parameters and across all three bioregions. This was expected as human disturbance increases significantly in both the plains and xeric bioregions. The goal in this exercise was to flag values that exceeded acceptable conditions and could be best described as “unacceptable”. Values that exceeded the 85<sup>th</sup> percentile were assigned a score of “1”. Values that fell below the 85<sup>th</sup> percentile were assigned a score of “0”. Scores were tallied on a spreadsheet for each site. The aggregated scores were stored in a spreadsheet and then sorted highest to lowest thus revealing sites that had “stressed” potential. The maximum aggregated score potential was 9 and the lowest 0. However, the highest score turned out to be 5, which occurred on several occasions. Scores that ranged from 3-5 were further examined in the aerial image phase.

To supplement the stressed candidate list, each human disturbance parameter was sorted from highest to lowest, thus revealing the upper echelon of values for each disturbance type. This was done in the event that one human disturbance parameter value was extremely high but other parameter values were negligible for any given site. For instance, at a particular site an irrigated agriculture value was 95% but all other parameters were okay. This site would have an aggregated score of 1 and would not be flagged as having cumulative disturbance. However, it would be flagged in this supplemental screening and reviewed further. There was no set system for how many sites were selected from each human disturbance parameter, but typically it ranged from the top 10 to the top 20.

Sites with cumulative high scores or with one obviously high value were reviewed by examining Google Earth aerial images on a desktop PC with internet access. Each site and an upstream section of the waterbody (approximately 2-3 km) were examined for clear characteristics of stressed condition, such as high concentration of diversions, impoundments, road crossings, near stream grazing, mining, adjacent agriculture and riparian instability, to name a few. Only one person performed this initial image review. This provided a consistent and repetitive approach. Images were saved to PDF files and stored on a network drive.

On occasion, when a stressed determination was difficult to make from aerial orthophotos alone, the reviewer would examine certain tolerance and diversity metrics to help make sense of things, sort of a reality check. This was used sparingly and only as backstop to

prevent true, “bookend” stressed sites from being unnecessarily removed from the process. The initial set of candidate stressed sites were not selected using metric values, only human disturbance values and aerial orthophotos. Metric values were only used to substantiate a handful of candidate sites in the xeric bioregion where finding stressed sites was fairly onerous. While the Division knew that examining biological data is frowned upon in bioassessment tool circles, the inability to utilize water chemistry and habitat data as criteria left the Division with few screening options.

Once a list of candidate stressed sites was assembled the reviewer stepped away from the process for 3 weeks. After 3 weeks the reviewer took a fresh look at each candidate stressed site. Google images and human disturbance values were re-examined in order to fine-tune the final list of stressed sites. This was an iterative and image-intensive venture.

This process yielded 31 stressed sites in the mountains bioregion, 23 sites in the plains bioregion and 20 sites in the xeric bioregion. These sites will be used for the recalibration of the Colorado MMI.

## **Appendix B**

### **Reference and Stressed Sites Used in Analysis**

## Appendix B: Reference and Stressed Samples

Station	Site Name	Sample_Replicate	Collection Date	Latitude	Longitude	Ref-Biotype
<u>Reference sites</u>						
WCOP99-0502	Adams Fork Conejos River	2418_0	8/5/2000	37.3296	-106.6896	R-2
EPA01-247	Agate Creek	2173_0	7/17/2001	38.4510	-106.3410	R-2
USFS Avalanche	Avalanche Creek	2936_0	9/7/2005	39.2311	-107.1992	R-2
CO151M	Badger Creek	2285_0	8/22/1995	38.5942	-105.8364	R-1
7630	Bear Creek	3016_1	5/2/2007	37.6138	-104.7738	R-3
CO125M	Beaver Creek	2274_0	8/21/1995	37.9997	-108.1939	R-1
USFSPIKE4	Beaver Creek	2666_1	11/6/2003	39.0287	-104.9448	R-2
10262	Beaver Creek	3044_1	8/1/2007	38.5525	-107.0530	R-1
EPA01-246	Big Blue Creek	2172_0	7/16/2001	38.1760	-107.3870	R-2
10666	Billy Creek	3051_1	7/16/2007	38.2948	-107.7020	R-1
7285	Boggs Creek	327_1	3/21/2000	38.2294	-104.7462	R-3
9716A	Box Canyon Creek	2851_1	7/11/2006	37.4489	-108.2078	R-2
7164	Browns Creek	2682_1	7/27/2005	38.6690	-106.1610	R-2
5341	Cache La Poudre River	486_1	5/9/2002	40.7083	-105.7266	R-1
11206	Carr Creek	2885_1	9/13/2006	39.5665	-108.5017	R-1
USFS Cataract	Cataract Creek	2951_1	9/26/2006	39.8285	-106.3272	R-2
WCOP01-0777	Chacuaco Creek	2772_0	4/22/2003	37.4942	-103.6313	R-3
7512	Chico Creek	3013_1	4/30/2007	38.3577	-104.3873	R-3
WCOP03-R003	Chief Creek	2779_0	7/10/2003	40.1033	-102.3225	R-3
5775	Cook Creek	3064_2	8/16/2007	39.1817	-104.8968	R-1
EPA01-248	Cottonwood Creek	2174_0	7/18/2001	38.0567	-105.5790	R-2
7997b	Cottonwood Creek	2651_1	4/7/2004	37.1109	-103.0744	R-3
USFS Cross 4	Cross Creek	2953_0	10/2/2006	39.4841	-106.5039	R-2
USFS Cross 1	Cross Creek	2992_0	9/15/2005	39.5413	-106.4334	R-2
WCOP99-0633	Crystal River	2762_0	6/18/2003	38.7239	-106.6725	R-2
Cuchara	Cucharas River	461_1	4/5/2000	37.6797	-104.6520	R-3
EPA01-259	Deeds Creek	2184_0	8/8/2001	39.2979	-106.6590	R-2
7595	Del Agua Arroyo	3014_1	5/29/2007	37.3472	-104.5742	R-3
10700	Dolores River	34_1	10/19/1994	38.3711	-108.8020	R-3
WCOP99-0597	Dyer Creek	2453_0	5/21/2002	38.5928	-107.4472	R-2
7997a	East Carrizo Creek	2650_1	4/7/2004	37.1354	-103.0157	R-3
7999a	East Carrizo Creek	2652_1	4/7/2004	37.1685	-103.0345	R-3
USFS EFH Upper	East Fork Homestake Creek	2955_1	9/18/2006	39.3559	-106.4540	R-2
WCOP03-R009	East Fork Piedra River	2785_0	8/8/2003	37.4817	-107.0971	R-2
11765	East Miller Creek	2906_1	8/7/2006	39.8768	-107.7649	R-2
5779B	East Plum Creek	3043_1	8/14/2007	39.1843	-104.9307	R-2
WCOP99-0595	East Rifle Creek	2452_0	8/2/2001	39.6431	-107.7104	R-1
EPA01-242	El Rito Azul	2169_0	7/11/2001	37.2743	-106.6190	R-2
10551	Escalante Creek	2524_1	9/28/2004	38.7178	-108.2686	R-3

<b>Station</b>	<b>Site Name</b>	<b>Sample_Replicate</b>	<b>Collection Date</b>	<b>Latitude</b>	<b>Longitude</b>	<b>Ref-Biotype</b>
EPA01-168	Fawn Creek	2161_0	8/11/2001	39.8525	-107.3150	R-2
CO162M	Fernleaf Gulch	2292_0	8/25/1995	38.4069	-105.6389	R-1
CO153M	Fourmile Creek	2286_0	8/24/1995	38.9661	-106.1397	R-2
5772A	Garber Creek	3062_1	8/16/2007	39.3552	-105.0272	R-1
CO116M	Garner Creek	2271_0	8/28/1995	38.1897	-105.7764	R-2
EPA01-250	Goose Creek	2176_0	7/22/2001	39.1856	-105.3910	R-2
USFS Gore 1	Gore Creek	3001_0	9/27/2004	39.6277	-106.2710	R-2
7226	Hardscrabble Creek	3009_1	5/30/2007	38.3434	-105.0682	R-3
7130	Hayden Creek	2736_1	9/14/2005	38.3356	-105.8022	R-1
CO03RS	Hope Creek	2250_0	9/21/1995	37.5531	-106.8022	R-2
EPA01-241	Huerfano River	2168_0	7/10/2001	37.6138	-105.4720	R-2
12961	Illinois River	84_1	9/23/1997	40.4624	-106.1770	R-1
5771	Jackson Creek	509_1	5/30/2003	39.3457	-104.9812	R-1
EPA01-262	Jefferson Fork	2187_0	8/11/2001	39.4626	-105.8580	R-2
CO072M	Junction Creek	2243_0	8/1/1994	37.3336	-107.9094	R-1
10570	Kannah Creek	3049_1	7/19/2007	38.9612	-108.2297	R-1
8337	La Jara Creek	2745_1	9/28/2005	37.1775	-106.2119	R-1
8715	La Manga Creek	2753_1	9/29/2005	37.1164	-106.3778	R-2
9680	La Plata River	2601_1	3/30/2005	37.2920	-108.0416	R-1
10906	La Sal Creek	3091_1	9/12/2007	38.3205	-108.9770	R-1
EPA01-261	Lake Fork	2186_0	8/10/2001	39.2853	-106.4490	R-2
10415	Leroux Creek	21_1	11/5/1996	38.8811	-107.7858	R-1
CO070M	Lime Creek	2242_0	8/4/1994	37.6770	-107.7509	R-2
12832	Little Bear Creek	2920_1	8/16/2006	40.6888	-107.4345	R-1
CO04RS	Little Cimarron Creek	2224_0	8/16/1994	38.2100	-107.4636	R-2
7385	Little Fountain Creek	2849_1	4/12/2006	38.6817	-104.8581	R-1
11408	Little Snake River	46_1	10/7/1998	40.9970	-107.1508	R-1
6753400	Lonetree Creek	2349_0	7/20/1994	40.8983	-104.8680	R-3
11535	Lost Creek	292_1	6/7/2000	40.0506	-107.4687	R-1
WCOP99-0503	Lost Man Creek	2419_0	7/27/2000	39.1595	-106.5718	R-2
12785A	Lost Man Creek	2864_1	9/10/2006	39.1292	-106.6180	R-2
CO051M	Lottis Creek	2227_0	8/17/1994	38.7725	-106.6225	R-2
USFS Meadow	Meadow Creek	3003_0	9/11/2003	39.5954	-106.1237	R-2
CO155M	Middle Fork Brush Creek	2288_0	9/6/1995	38.9542	-106.8583	R-2
10559	Middle Fork Escalante Creek	3075_1	7/18/2007	38.5812	-108.4059	R-1
WCOP99-0578	Middle Fork Little Snake R.	2448_0	8/14/2001	40.9715	-107.0190	R-1
CO038M	Middle Fork North Crestone	2214_0	8/11/1994	38.0361	-105.6425	R-2
10834	Naturita Creek	3086_1	9/11/2007	38.1591	-108.4031	R-1
EPA01-249	Newlin Creek	2175_0	7/19/2001	38.2662	-105.1898	R-2
WCOP99-0649	Noname Creek	2466_0	7/23/2002	39.5817	-107.2881	R-1
10558	North Fork Escalante Creek	3074_1	7/18/2007	38.6369	-108.4272	R-1
CO142M	North Anthracite Creek	2283_0	9/13/1995	38.9817	-107.1911	R-2

<b>Station</b>	<b>Site Name</b>	<b>Sample_Replicate</b>	<b>Collection Date</b>	<b>Latitude</b>	<b>Longitude</b>	<b>Ref-Biotype</b>
EPA01-253	North Cottonwood Creek	2179_0	7/27/2001	38.8708	-106.2660	R-2
10917	North Fork Mesa Creek	3065_5	9/10/2007	38.5032	-108.7904	R-1
NPlatte	North Platte River	378_1	11/4/1997	40.6973	-106.4125	R-1
CO113M	North St. Vrain Creek	2269_0	9/14/1995	40.2047	-105.4061	R-2
USFS N Tenmile	North Tenmile Creek	2961_0	9/8/2005	39.5730	-106.1722	R-2
11462	Oliver Creek	2893_1	10/3/2006	40.8771	-107.0871	R-2
11463	Oliver Creek	2894_1	10/4/2006	40.8641	-107.0674	R-2
WCOP99-0518	Ouzel Creek	2433_0	7/10/2000	40.1998	-105.6258	R-2
USFSPIKE1	Pine Creek	2663_1	11/3/2003	39.2406	-105.2826	R-2
7170	Pine Creek	2686_1	7/26/2005	38.9988	-106.2318	R-2
USFS Piney 2	Piney River	2964_0	9/28/2005	39.7504	-106.4713	R-1
EPA01-238	Purgatoire River	2165_0	7/6/2001	37.5584	-103.6520	R-3
10350	Razor Creek	3047_1	8/2/2007	38.3846	-106.6733	R-1
USFS Ripple	Ripple Creek	2976_0	8/12/2004	40.0746	-107.3028	R-2
11208	Roan Creek	2887_1	9/13/2006	39.5096	-108.5248	R-1
12182	Roaring Fork Arapaho Creek	56_1	9/9/1997	40.1308	-105.7675	R-2
10980	Roc Creek	3093_1	9/10/2007	38.4418	-108.8774	R-3
EPA01-252	Rock Creek	2178_0	7/26/2001	39.3746	-105.6840	R-2
EPA01-260	Rock Creek	2185_0	8/9/2001	39.2169	-106.3980	R-2
7284	Rock Creek	3011_1	5/1/2007	38.2080	-104.7931	R-3
8395	Rock Creek	93_1	4/15/1993	37.4903	-106.2589	R-1
USFSPIKE2	Rough and Tumbling Creek	2664_1	11/4/2003	39.0360	-106.1159	R-2
5971c1	Rule Creek	2648_1	10/25/2001	39.0477	-105.0983	R-1
WCOP04-R008	Rush Creek	2794_0	6/9/2004	40.0994	-102.0974	R-3
7571	San Francisco Creek	2541_1	8/26/2004	37.1206	-104.2614	R-3
10805	San Miguel River	36_1	10/19/1994	38.1260	-108.2077	R-1
CO174M	Scott Gomer Creek	2297_0	9/11/1995	39.5081	-105.7047	R-2
CO067M	Silver Creek	2239_0	8/1/1994	37.4289	-106.7589	R-2
USFS Snowmass	Snowmass Creek	2978_0	9/8/2004	39.1801	-107.0220	R-2
EPA01-239	South Apache Creek	2166_0	7/7/2001	37.8522	-104.9850	R-2
5588	South Boulder Creek	569_1	6/24/2003	39.9805	-105.2208	R-1
12759	South Fork Fryingpan River	2861_1	9/12/2006	39.2372	-106.5900	R-2
CO122M	South Fork Saguache Creek	2273_0	8/23/1995	37.9192	-106.7153	R-2
WCOP99-0563	South Rush Creek	2434_0	5/17/2001	38.8867	-103.6830	R-3
AR0097	South Rush Creek	281_0	12/19/2000	38.8507	-103.5394	R-3
5548	South St. Vrain Creek	556_2	6/24/2003	40.0788	-105.5778	R-2
WCOP04-R009	Timpas Creek	2795_0	7/6/2004	37.8271	-103.7730	R-3
7560	Trinchera Creek	2540_1	8/26/2004	37.0446	-104.0510	R-3
CO133M	Trout Creek	2278_0	8/17/1995	37.6661	-107.0700	R-2
WCOP04-R003	Two Butte Creek	2789_0	5/19/2004	37.5134	-103.0267	R-3
WCOP99-0634	Ute Creek	2462_0	8/13/2002	37.5955	-105.3989	R-2
10922	Ute Creek	3092_2	9/13/2007	38.7237	-108.9097	R-1

<b>Station</b>	<b>Site Name</b>	<b>Sample_Replicate</b>	<b>Collection Date</b>	<b>Latitude</b>	<b>Longitude</b>	<b>Ref-Biotype</b>
11485	Vermillion Creek	2897_1	8/15/2006	40.7197	-108.7518	R-1
EPA01-240	Wahatoya Creek	2167_0	7/9/2001	37.4123	-104.9640	R-2
8116	West Alder Creek	2703_1	8/22/2005	37.7050	-106.6478	R-1
USFS West Lake	West Lake Creek	2991_0	9/27/2006	39.5395	-106.6160	R-2
10905G	West Paradox Creek	3090_1	9/12/2007	38.3827	-108.9960	R-1
5777A	West Plum Creek	512_1	5/30/2003	39.3692	-104.9655	R-1
EPA01-251	Wigwam Creek	2177_0	7/25/2001	39.2470	-105.3580	R-2
WCOP04-R001	Willow Creek	2788_0	7/29/2004	40.8003	-104.4593	R-3
12802	Yampa River	168_1	8/28/2001	40.5013	-107.2643	R-1
WCOP04-R007	Yellow Jacket Creek	2793_0	6/3/2004	37.3642	-108.9511	R-3

#### Stressed Sites

7701	Apishapa River	477_1	6/11/2002	38.0958	-103.9847	S-3
7186A	Arkansas River	2828_1	4/22/2006	39.2024	-106.3530	S-1
5762	Bear Creek	3082_1	9/17/2007	39.6432	-105.3072	S-1
WCOP01-0756	Big Dry Creek	2787_0	7/15/2003	40.0321	-104.8729	S-3
5417	Big Thompson River	275_0	5/21/2001	40.3943	-104.9643	S-3
12304b	Blue River	2570_1	5/12/2004	39.4865	-106.0463	S-2
5575	Boulder Creek	2692_1	8/12/2005	40.0511	-105.1810	S-1
12761B	Brush Creek	2862_1	9/7/2006	39.2247	-106.9210	S-1
AR0018	Buffalo Creek	287_0	12/20/2000	38.1487	-102.3110	S-3
12893	Burgess Creek	436_1	8/29/2001	40.4517	-106.8102	S-1
5306	Cache La Poudre River	359_1	6/17/2002	40.4227	-104.5980	S-3
12715	Castle Creek	388_1	10/23/2001	39.1958	-106.8329	S-1
6713500	Cherry Creek	2320_0	7/18/2002	39.7425	-105.0000	S-3
34	Clear Creek	2563_1	5/10/2005	39.8276	-104.9521	S-3
5605	Clear Creek	2587_1	5/10/2005	39.7742	-105.1413	S-3
10320	Cochetopa Creek	2520_1	10/13/2004	38.5171	-106.7862	S-1
12102	Colorado River	3094_1	10/15/2007	40.1083	-106.0036	S-1
WCOP99-0594	Colorado River	2451_0	8/9/2001	39.1817	-108.9331	S-3
12731	Crystal River	64_1	8/18/1998	39.4081	-107.2183	S-1
10908B	Dolores River	2720_1	9/28/2005	37.7938	-108.8181	S-1
12852	Dry Creek	518_0	4/16/2003	40.4942	-107.2675	S-1
9149480	Dry Creek	2394_0	8/5/1998	38.6458	-108.0490	S-1
12870	Fish Creek	298_0	8/29/2001	40.4665	-106.8247	S-1
7390	Fountain Creek	2850_1	4/13/2006	38.2853	-104.6034	S-3
12191	Fraser River	3030_1	5/31/2007	39.9903	-105.8297	S-1
9046530	French Gulch	2371_0	8/4/1998	39.4930	-106.0447	S-2
WCOP99-0648	Gunnison River	2465_0	8/26/2002	39.0050	-108.5064	S-3
WCOP99-0622	Hartman Draw	2456_0	6/19/2002	37.3659	-108.5933	S-1
8650	Kerber Creek	410_1	3/24/2000	38.2500	-105.9498	S-1
7187G	Lake Fork	2836_1	4/22/2006	39.2512	-106.3754	S-2

<b>Station</b>	<b>Site Name</b>	<b>Sample_Replicate</b>	<b>Collection Date</b>	<b>Latitude</b>	<b>Longitude</b>	<b>Ref-Biotype</b>
5454	Little Thompson River	2612_1	6/14/2004	40.2945	-105.0588	S-3
6753990	Lonetree Creek	2354_0	8/6/2002	40.4425	-104.5888	S-3
CO050M	Noname Creek	2226_0	8/18/1994	38.7831	-106.4822	S-1
133	North Fork Clear Creek	2575_1	5/27/2004	39.7479	-105.3993	S-2
11601	Piceance Creek	2898_1	8/8/2006	39.9643	-108.2682	S-1
7580	Purgatoire River	154_1	6/24/1999	37.1842	-104.4882	S-3
9153290	Reed Wash	2398_0	11/12/1997	39.2114	-108.8037	S-3
12851	Sage Creek	498_1	4/16/2003	40.4833	-107.1705	S-1
7309	Sand Creek	2840_1	4/12/2006	38.8101	-104.7480	S-3
5212a	Sand Creek	2582_1	7/7/2004	39.7611	-104.8576	S-3
8375	Sangre de Cristo Creek	2751_1	9/26/2005	37.4282	-105.3949	S-1
11191b	South Canyon Creek	226_1	3/8/2000	39.5619	-107.4081	S-1
5965	South Fork South Platte River	2819_1	7/28/2006	38.9835	-105.8834	S-1
5009	South Platte River	552_1	5/7/2003	40.7161	-103.1257	S-3
5164	South Platte River	2579_1	7/7/2004	39.8313	-104.9480	S-3
6714000	South Platte River	2325_0	9/9/1998	39.7597	-105.0033	S-3
5951a	South Platte River	2815_1	7/29/2006	38.9001	-105.4668	S-1
WCOP99-0671	South Platte River	2766_0	5/13/2003	39.6631	-105.0038	S-3
7336	Spring Creek	2845_1	4/11/2006	38.8078	-104.7954	S-3
12336	Tenmile Creek	60_1	9/23/1998	39.5028	-106.1403	S-2
7501	Timpas Creek	2695_1	8/8/2005	37.9606	-103.7185	S-3
5971a3	Trout Creek	2639_1	10/28/2003	39.0881	-105.0969	S-1
10609	Uncompahgre River	27_1	8/21/1996	37.9897	-107.6475	S-2
12337	West Tenmile Creek	492_1	5/8/2003	39.5020	-106.1467	S-2
7293	Wildhorse Creek	372_1	3/21/2000	38.2732	-104.6365	S-3
8105	Willow Creek	406_1	3/23/2000	37.8484	-106.9245	S-1
12804	Yampa River	420_1	8/28/2001	40.4993	-107.5532	S-1
12806F	Yampa River	455_1	8/29/2001	40.4832	-106.8323	S-1
11732A	Yellow Creek	2904_1	8/9/2006	40.1181	-108.3609	S-1
9830	Yellow Jacket Creek	2625_1	3/17/2005	37.3280	-109.0447	S-3

## **Appendix C**

### **Metric Descriptions and Discrimination Efficiencies**

## Appendix C: Metric Descriptions and Discrimination Efficiencies

<b>Metric Category</b>	<b>Metric</b>	<b>Biotype 1</b>	<b>Biotype 2</b>	<b>Biotype 3</b>	<b>Trend*</b>
Richness	ChiroTax	UnResp	71.4	76.0	--
Richness	ChiroTaxaSmr	UnResp	NA	NA	0
Richness	ColeoTax	64.3	71.4	68.0	---
Richness	ColeoTax_aSmr	NA	71.4	NA	-
Richness	CrMolTax	53.6	UnResp	40.0	+/-
Richness	DipTax	50.0	85.7	72.0	---
Richness	DipTaxaSmr	UnResp	NA	NA	0
Richness	EphemTax	53.6	100.0	28.0	---
Richness	EphemTax_aElv	75.0	NA	NA	-
Richness	EphemTaxaSmr	71.4	NA	NA	-
Richness	EPtax	67.9	71.4	28.0	---
Richness	EPtax_aElv	78.6	NA	NA	-
Richness	EPTTax	64.3	71.4	36.0	---
Richness	EPTTax_aElv	67.9	NA	NA	-
Richness	EPTTaxaSmr	60.7	NA	NA	-
Richness	InsectTax	78.6	100.0	92.0	---
Richness	InsectTax_aElv	75.0	NA	NA	-
Richness	InsectTaxaSmr	67.9	NA	NA	-
Richness	NonInsPT	78.6	57.1	64.0	+++
Richness	OligoTax	35.7	28.6	52.0	+++
Richness	OligoTax_aSmr	NA	NA	76.0	+
Richness	PlecoTax	78.6	71.4	UnResp	--
Richness	PlecoTax_aElv	82.1	NA	NA	-
Richness	PlecoTaxaSmr	75.0	NA	40.0	-
Richness	TanytTax	UnResp	85.7	56.0	--
Richness	TotalTax	64.3	100.0	88.0	---
Richness	TotalTax_aElv	64.3	NA	NA	-
Richness	TotalTaxaSmr	50.0	NA	NA	-
Richness	TrichTax	46.4	71.4	UnResp	--
Richness	TrichTax_aElv	57.1	NA	NA	-
Richness	TrichTaxaSmr	50.0	NA	NA	-
Richness	WormlikeTax	50.0	UnResp	UnResp	+
Composition	AmphPct	42.9	UnResp	UnResp	+
Composition	AmphPct_aElv	NA	NA	52.0	-
Composition	BivalPct	UnResp	UnResp	UnResp	0
Composition	ChironominiPct	50.0	UnResp	44.0	++
Composition	ChironominiPct_aSmr	NA	NA	52.0	+
Composition	ChiroPct	50.0	57.1	UnResp	++
Composition	ColeoPct	53.6	71.4	96.0	---
Composition	ColeoPct_aSmr	NA	71.4	NA	-
Composition	CorbPct	UnResp	UnResp	UnResp	0

<b>Metric Category</b>	<b>Metric</b>	<b>Biotype 1</b>	<b>Biotype 2</b>	<b>Biotype 3</b>	<b>Trend*</b>
Composition	CrCh2ChiPct	UnResp	UnResp	44.0	+
Composition	CrMolPct	67.9	UnResp	40.0	+/-
Composition	D_Mg	71.4	100.0	68.0	---
Composition	DiamesPct	35.7	42.9	UnResp	++
Composition	DiamesPctaSmr	32.1	NA	40.0	++
Composition	DipPct	46.4	57.1	UnResp	++
Composition	EphemPct	50.0	71.4	44.0	---
Composition	EPTPct	57.1	57.1	44.0	---
Composition	Evenness	67.9	85.7	52.0	---
Composition	GastrPct	53.6	UnResp	UnResp	+
Composition	GastrPct_aElv	NA	NA	40.0	-
Composition	GastrPct_aSmr	NA	NA	36.0	-
Composition	IsoPct	UnResp	UnResp	UnResp	0
Composition	NonInPct	64.3	57.1	52.0	+/-
Composition	NonInPct_aElv	NA	42.9	NA	-
Composition	OdonPct	UnResp	UnResp	UnResp	0
Composition	OdonPctaSmr	35.7	NA	NA	-
Composition	OligoPct	53.6	42.9	56.0	+++
Composition	Orth2ChiPct	32.1	42.9	28.0	+/-
Composition	Orth2ChiPct_aSmr	NA	NA	UnResp	0
Composition	PentanPct	UnResp	UnResp	68.0	-
Composition	PercDom01	50.0	42.9	64.0	+++
Composition	PlecoPct	82.1	57.1	UnResp	--
Composition	PlecoPct_aElv	NA	NA	40.0	+
Composition	PlecoPct_aSmr	NA	NA	44.0	-
Composition	Shan_2	67.9	100.0	56.0	---
Composition	SimpsonD	50.0	71.4	72.0	+++
Composition	TanytPct	UnResp	100.0	56.0	--
Composition	TanytPct_aSmr	NA	NA	52.0	-
Composition	Tnyt2ChiPct	UnResp	85.7	56.0	--
Composition	Tnyt2ChiPct_aElv	NA	NA	56.0	-
Composition	Tnyt2ChiPct_aSmr	NA	NA	52.0	-
Composition	TrichPct	53.6	57.1	UnResp	--
Composition	WCSpct	71.4	42.9	48.0	+++
Feeding	ClctPct	UnResp	UnResp	UnResp	0
Feeding	ClctTax	39.3	UnResp	UnResp	-
Feeding	FiltrPct	50.0	42.9	56.0	+/-
Feeding	FiltrPct_aElv	NA	42.9	NA	+
Feeding	FiltrPctaSmr	39.3	UnResp	NA	-
Feeding	FiltrTax	UnResp	UnResp	48.0	-
Feeding	FiltrTax_aSmr	NA	42.9	NA	-
Feeding	PredPct	39.3	57.1	64.0	---
Feeding	PredTax	53.6	85.7	64.0	---

<b>Metric Category</b>	<b>Metric</b>	<b>Biotype 1</b>	<b>Biotype 2</b>	<b>Biotype 3</b>	<b>Trend*</b>
Feeding	PreShrTax	60.7	85.7	64.0	---
Feeding	PreShrTaxaSmr	75.0	NA	NA	-
Feeding	ScrapPct	46.4	57.1	60.0	---
Feeding	ScrapTax	UnResp	57.1	56.0	--
Feeding	ScrapTax_aElv	53.6	NA	NA	-
Feeding	ShredPct	50.0	57.1	44.0	+/-
Feeding	ShredPctaSmr	UnResp	NA	NA	0
Feeding	ShredTax	57.1	42.9	36.0	---
Feeding	ShredTaxaSmr	71.4	NA	NA	-
Habit	BrrwrPct	42.9	57.1	32.0	---
Habit	BrrwrTax	UnResp	42.9	44.0	--
Habit	ClmbrPct	35.7	42.9	36.0	+/-
Habit	ClmbrPctaSmr	32.1	NA	NA	-
Habit	ClmbrTax	UnResp	UnResp	UnResp	0
Habit	ClmbrTaxaSmr	46.4	NA	NA	-
Habit	ClngrPct	57.1	71.4	56.0	---
Habit	ClngrPct_aSmr	UnResp	NA	60.0	-
Habit	ClngrTax	67.9	100.0	40.0	---
Habit	ClngrTax_aElv	75.0	NA	NA	-
Habit	ClngrTaxaSmr	60.7	NA	NA	-
Habit	ClnSprPct	50.0	UnResp	76.0	--
Habit	SprwlPct	35.7	71.4	72.0	+/-
Habit	SprwlTax	42.9	28.6	76.0	---
Habit	SwmmrPct	60.7	UnResp	56.0	--
Habit	SwmmrPctaSmr	UnResp	NA	NA	0
Habit	SwmmrTax	UnResp	42.9	UnResp	+/-
Tolerance	BeckBI	64.3	85.7	28.0	---
Tolerance	BeckBI_aElv	78.6	NA	NA	-
Tolerance	BeckBIaSmr	67.9	NA	NA	-
Tolerance	GeneraSensChir	28.6	57.1	88.0	---
Tolerance	GeneraSensChirPLNS	UnResp	42.9	76.0	--
Tolerance	GeneraSensFamPlns	67.9	42.9	84.0	---
Tolerance	GeneraTolChir	35.7	42.9	28.0	+/-
Tolerance	GeneraTolChir_aElv	UnResp	NA	NA	0
Tolerance	GeneraTolChiraSmr	39.3	NA	NA	+
Tolerance	GeneraTolChirPLNS	32.1	UnResp	48.0	++
Tolerance	GenTolerFamPLNS	32.1	UnResp	60.0	++
Tolerance	IntolTax	60.7	71.4	36.0	---
Tolerance	IntolTax_aElv	78.6	NA	NA	-
Tolerance	IntolTaxaSmr	64.3	NA	NA	-
Tolerance	newHBI	71.4	71.4	56.0	+++
Tolerance	PctSensChir	53.6	71.4	92.0	---
Tolerance	PctSensChirPLNS	39.3	57.1	88.0	---

<b>Metric Category</b>	<b>Metric</b>	<b>Biotype 1</b>	<b>Biotype 2</b>	<b>Biotype 3</b>	<b>Trend*</b>
Tolerance	PctSensFamPlns	64.3	42.9	88.0	+/-
Tolerance	PctTolChir	46.4	57.1	56.0	+++
Tolerance	PctTolChiraSmr	UnResp	NA	NA	0
Tolerance	PctTolChirPLNS	32.1	UnResp	72.0	++
Tolerance	PctTolChirPLNS_aSmr	NA	NA	52.0	+
Tolerance	PctTolerFamsPLNS	53.6	42.9	60.0	+++
Tolerance	PercBaetEph	39.3	42.9	32.0	+/-
Tolerance	PercBaetEph_aElv	NA	NA	32.0	+
Tolerance	PercBaetEph_aSmr	NA	NA	32.0	+
Tolerance	PercHydr2Tri	39.3	42.9	UnResp	+/-
Tolerance	PercHydr2TriaSmr	UnResp	NA	NA	0
Tolerance	PercIntol	71.4	71.4	72.0	---
Tolerance	PercTol	64.3	UnResp	48.0	++
Tolerance	PercTol_aSmr	NA	NA	40.0	+
Tolerance	TolerPT	71.4	57.1	52.0	+/-
Tolerance	TolerPT_aSmr	NA	NA	56.0	+
Tolerance	TolerTax	46.4	57.1	UnResp	+/-

\*Trend symbols:

- +++ = metric value increases with increasing stress in all three Biotypes
- ++ = metric value increases with increasing stress in two of three Biotypes
- + = metric value increases with increasing stress in one of three Biotypes
- +/- = mixed metric responsiveness, by Biotype
- 0 = not responsive in any Biotype where the metric was tested
- = metric value decreases with increasing stress in one of three Biotypes
- = metric value decreases with increasing stress in two of three Biotypes
- = metric value decreases with increasing stress in all three Biotypes

# **Appendix D**

## **Index Alternatives**



## Biotype 1 cont.

DE_1		85.7	78.6	78.6	82.1	82.1	78.6	78.6	89.3	75.0	82.1
Z-score		1.99	2.09	1.97	2.15	2.06	1.98	1.91	2.09	2.02	1.99
Ref SD		15.53	15.14	14.26	14.85	15.05	15.75	15.87	15.50	15.96	15.98
# of Categories		3 of 5	4 of 5	5 of 5	4 of 5	5 of 5	3 of 5	4 of 5	2 of 5	3 of 5	2 of 5
Redundancy		<0.85	<0.85	<0.85	0.86	0.86	0.86	0.86	<0.80	<0.80	<0.80
Remarks											
Metric		lx1-21	lx1-22	lx1-23	lx1-24	lx1-25	lx1-26	lx1-27	lx1-28	lx1-29	lx1-30
Rch	InsectTax_aElv	21	22	23	24	25	26	27	28	29	30
Rch	NonInsPT	21	22	23	24	25	26	27	28	29	30
Rch	EPTax_aElv				24	25	26	27	28	29	
Rch	EphemTax_aElv	21	22	23							30
Rch	PlecoTax_aElv	21	22	23							30
Cmp	EPTPct	21	22	23	24	25	26	27	28	29	30
Cmp	ChiroPct	21	22	23	24		26	27			
Tol	BeckBI_aElv	21	22		24	25					
Tol	newHBI			23			26	27		29	
FFG	ScrapPct			23		25		27			
Hab	ClngrTax_aElv		22	23	24	25					

DE_1		82.1	78.6	82.1	85.7	92.9	89.3	82.1	78.6	89.3	82.1
Z-score		1.97	1.89	2.16	2.21	2.23	2.10	2.04	2.20	2.33	2.44
Ref SD		16.94	15.85	15.20	14.85	16.02	17.14	17.31	15.47	14.40	14.61
# of Categories		3 of 5	4 of 5	5 of 5	4 of 5	4 of 5	5 of 5	5 of 5	5 of 5	5 of 5	4 of 5
Redundancy		<0.85	<0.85	<0.85	<0.85	0.93	0.93	0.88	<0.80	<0.85	<0.85
Remarks				Summer Adjust						Concept Redund.	Concept Redund.
Metric		lx1-31	lx1-32	lx1-33	lx1-34	lx1-35	lx1-36	lx1-37	lx1-38	lx1-39	lx1-40
Rch	InsectTax_aElv	31	32	33	34	35					40
Rch	NonInsPT	31	32	33	34	35				39	40
Rch	EPTTax_aElv					35					
Rch	EPTax_aElv						36	37	38		
Rch	EphemTax_aElv	31	32								
Rch	PlecoTax_aElv	31	32								
Cmp	EPTPct	31	32								
Cmp	PlecoPct			33	34	35					
Cmp	D_Mg			33	34						
Cmp	NonInPct						36	37	38	39	40
Cmp	WCSpct			33	34	35					
Tol	BeckBI_aElv	31	32	33	34	35	36			39	40
Tol	IntolTax_aElv							37			
Tol	newHBI								38		
FFG	ScrapPct		32								
FFG	PreShrTax						36	37	38	39	
FFG	PreShrTaxaSmr			33							
Hab	ClngrTax_aElv			33	34	35	36	37	38	39	40

## Biotype 1 cont.

DE_1		85.7	89.3	89.3	78.6	82.1	82.1	85.7	78.6	85.7	82.1
Z-score		2.13	2.24	2.26	2.02	2.16	2.04	2.15	2.34	2.06	2.11
Ref SD		16.23	15.66	16.34	17.00	17.63	15.07	15.57	13.81	13.21	15.25
# of Categories		5 of 5	5 of 5	4 of 5	4 of 5	4 of 5	5 of 5	5 of 5	5 of 5	4 of 5	5 of 5
Redundancy		<0.85	0.86	0.86	0.86	0.86	<0.85	0.86	<0.80	<0.80	0.86
Remarks			Concept Redund.	Concept Redund.				Summer Adjust			
Metric		lx1-41	lx1-42	lx1-43	lx1-44	lx1-45	lx1-46	lx1-47	lx1-48	lx1-49	lx1-50
Rch	InsectTax_aElv	41				45					
Rch	NonInsPT		42	43	44		46	47	48	49	50
Rch	EPTax_aElv		42	43	44	45		47	48		50
Cmp	EPTPct				44		46				
Cmp	ChiroPct							47	48	49	50
Cmp	NonInPct	41	42	43							
Tol	BeckBI_aElv	41	42	43	44	45	46	47			50
Tol	PercTol								48		
FFG	PreShrTax	41	42				46		48	49	50
FFG	PreShrTaxaSmr							47			
Hab	CIngrTax_aElv	41	42	43	44	45	46	47	48	49	50

DE_1		78.6	78.6	85.7	82.1	92.9	92.9	85.7	78.6	85.7
Z-score		1.90	1.93	2.12	2.06	2.19	2.25	2.24	2.29	2.14
Ref SD		17.09	16.52	14.17	14.16	14.04	13.21	13.55	14.40	14.66
# of Categories		5 of 5	5 of 5	5 of 5	5 of 5	5 of 5	5 of 5	5 of 5	5 of 5	5 of 5
Redundancy		0.86	0.86	<0.85	<0.80	<0.80	<0.80	<0.80	<0.80	<0.80
Remarks							CDPHE choice			
Metric		lx1-51	lx1-52	lx1-53	lx1-54	lx1-55	lx1-56	lx1-57	lx1-58	lx1-59
Rch	InsectTax				54					
Rch	NonInsPT	51	52	53	54	55	56	57	58	59
Rch	EPTax_aElv	51	52		54	55	56	57	58	59
Cmp	Shan_2		52							
Cmp	EPTPct				54			57	58	59
Cmp	ChiroPct			53	54	55	56			
Cmp	D_Mg	51								
Tol	BeckBI_aElv	51	52	53	54					
Tol	PercTol								58	
Tol	GenSensFamPlns					55				59
Tol	PctSensFamPlns						56	57		
FFG	ScrapPct				54					
FFG	PreShrTax	51	52	53		55	56	57	58	59
Hab	CIngrTax_aElv	51	52	53	54	55	56	57	58	59

## Biotype 2

DE_1		100	100	100	85.71	85.71	100	100	100	100	100
Z-score		3.27	2.83	2.94	2.97	2.91	2.89	2.92	2.93	2.88	2.79
Ref SD		13.46	14.21	14.28	13.05	13.11	14.55	13.45	13.19	12.86	13.13
# of Categories		3 of 5	4 of 5	4 of 5	3 of 5	4 of 5	4 of 5	5 of 5	5 of 5	5 of 5	5 of 5
Redundancy		<0.80	<0.80	0.92	0.92	0.92	<0.80	<0.80	0.92	<0.80	<0.80
Remarks											
Metric		lx2-1	lx2-2	lx2-3	lx2-4	lx2-5	lx2-6	lx2-7	lx2-8	lx2-9	lx2-10
Rch	TotalTax	1	2	3			6	7	8	9	10
Rch	InsectTax				4	5					
Rch	NonInsPT				4	5					
Rch	EPTTax			3	4	5			8		
Rch	EphemTax		2				6	7		9	10
Rch	TrichTax		2				6	7		9	10
Rch	PlecoTax		2								
Cmp	EphemPct							7	8	9	10
FFG	PreShrTax		2	3		5	6	7	8	9	10
Tol	BeckBI	1	2	3	4	5	6	7	8		10
Tol	newHBI									9	
Hab	ClngrTax	1	2	3	4	5	6	7	8	9	
Hab	ClngrPct										10

DE_1		100	100	100	100	100	100	100	85.7	100
Z-score		2.99	3.13	3.06	2.98	2.95	3.20	3.08	2.78	3.20
Ref SD		14.01	13.91	12.86	12.75	12.88	12.90	13.77	12.58	12.84
# of Categories		4 of 5	3 of 5	4 of 5	5 of 5	5 of 5	5 of 5	5 of 5	5 of 5	5 of 5
Redundancy		<0.80	0.92	0.92	<0.80	<0.80	<0.80	<0.80	<0.85	<0.80
Remarks					CDPHE choice					Concept Redund.
Metric		lx2-11	lx2-12	lx2-13	lx2-14	lx2-15	lx2-16	lx2-17	lx2-18	lx2-19
Rch	TotalTax	11	12	13	14					19
Rch	InsectTax					15	16	17	18	
Rch	EPTTax		12	13						
Cmp	EphemPct			13	14	15			18	
Cmp	Shan_2*						16			19
Cmp	D_Mg*							17		
FFG	PreShrTax	11			14	15	16	17	18	19
Tol	BeckBI	11	12	13	14	15	16	17		19
Tol	newHBI								18	
Hab	ClngrTax	11	12	13	14	15	16	17	18	19

### Biotype 3

	DE_1	96.0	100.0	96.0	100.0	96.0	100.0	96.0	96.0	96.0	96.0
	Z-score	1.62	2.09	2.20	2.11	2.29	2.18	2.11	2.25	2.36	2.46
	Ref SD	14.34	12.09	11.09	12.16	11.37	12.13	12.77	11.13	11.04	10.49
	# of Categories	3 of 5	4 of 5	4 of 5	5 of 5	4 of 5	4 of 5	4 of 5	4 of 5	4 of 5	4 of 5
	Redundancy	<.8	<.8	<.8	<.8	<.8	<.8	<.8	0.84	0.84	0.84
	Remarks				Col metric						
	Metric	lx3-1	lx3-2	lx3-3	lx3-4	lx3-5	lx3-6	lx3-7	lx3-8	lx3-9	lx3-10
Rch	TotalTax	1	2	3	4				8		
Rch	InsectTax					5	6	7		9	10
Rch	NonInsPT					5					10
Rch	DipTax								8	9	10
Tol	PctSensFamPlns	1	2	3	4	5	6	7	8	9	10
Tol	TolerPT			3							
Cmp	ColeoPct				4						
FFG	PredPct	1	2	3	4	5	6	7	8	9	10
Hab	SprwlPct		2	3	4	5	6		8	9	10
Hab	ClnSprPct							7			

	DE_1	96.0	100.0	100.0	100.0	96.0	100.0	100.0	100.0	100.0	100.0
	Z-score	2.75	3.23	3.16	3.01	2.38	2.51	3.21	2.63	2.14	2.56
	Ref SD	9.51	8.75	9.15	9.34	12.06	10.18	8.75	10.05	14.12	10.47
	# of Categories	5 of 5	4 of 5	4 of 5	4 of 5	4 of 5	5 of 5	5 of 5	5 of 5	5 of 5	5 of 5
	Redundancy	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	<.8	<.8
	Remarks		Col metric	Col metric	Col metric		% Pred	Col metric	Redund.		% Pred
	Metric	lx3-11	lx3-12	lx3-13	lx3-14	lx3-15	lx3-16	lx3-17	lx3-18	lx3-19	lx3-20
Rch	TotalTax				14	15	16	17			
Rch	InsectTax	11	12	13					18	19	20
Rch	NonInsPT	11	12								
Rch	DipTax	11	12	13	14	15	16	17	18		
Rch	ColeoTax		12	13	14			17			
Tol	PctSensFamPlns	11	12	13	14		16	17	18	19	20
Tol	GenSensFamPlns					15					
Cmp	PercDom01	11					16	17	18	19	20
FFG	PredPct	11	12	13	14	15	16	17	18		20
FFG	PreShrTax									19	
Hab	SprwlPct	11	12	13	14	15	16	17	18	19	20

**Biotype 3 cont.**

		100.0	92.0	100.0	100.0	100.0	100.0
	Z-score	2.33	2.13	2.28	2.68	2.36	2.25
	Ref SD	13.12	13.97	12.84	9.86	12.53	14.36
	# of Categories	5 of 5	5 of 5	5 of 5	5 of 5	5 of 5	5 of 5
	Redundancy	<.8	0.81	<.8	<.8	<.8	<.8
Remarks				<b>CDPHE choice</b>	% Pred		
Metric		lx3-21	lx3-22	lx3-23	lx3-24	lx3-25	lx3-26
Rch	InsectTax	21	22	23	24	25	26
Rch	NonInsPT			23	24	25	26
Tol	PctSensFamPlns	21	22	23	24	25	
Tol	GenSensFamPlns						26
Cmp	PercDom01	21	22	23	24	25	26
FFG	PredPct				24		
FFG	PreShrTax	21	22	23		25	26
Hab	SprwlPct			23	24		26
Hab	SprwlTax		22				
Hab	ClnSprPct	21				25	