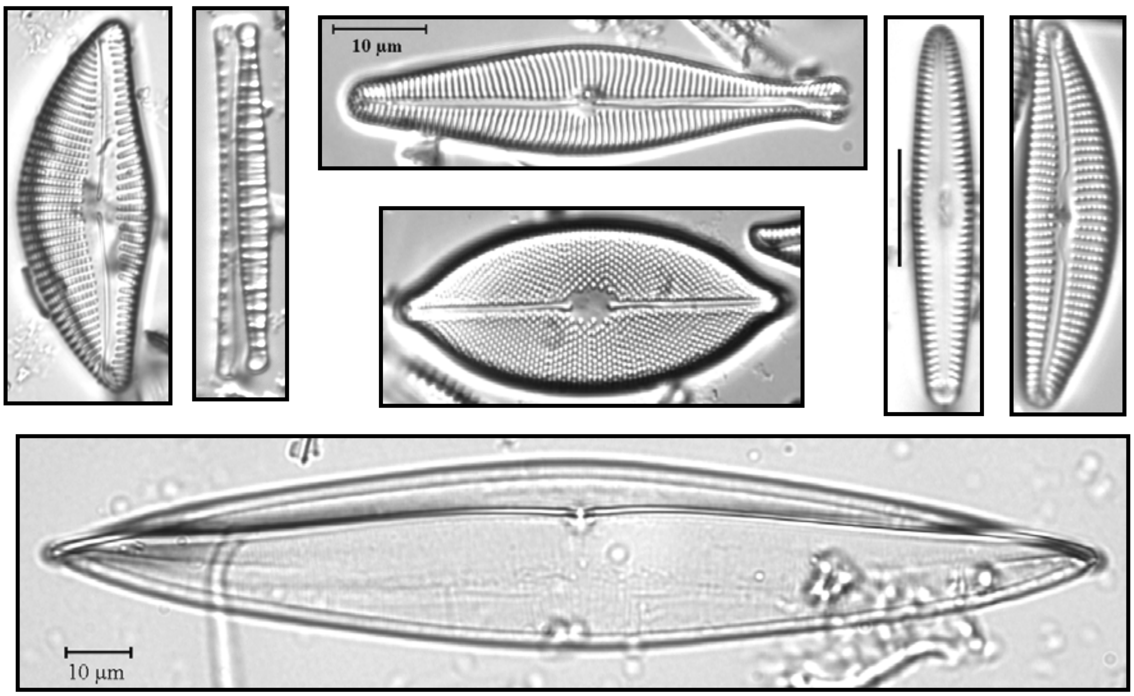
STATISTICAL EVALUATION OF PERIPHYTON SAMPLES FROM MONTANA REFERENCE STREAMS



Prepared By:

Mark Teply
Larix Systems, Inc.

Loren Bahls
Hannaea

Prepared For:

The Montana Department of Environmental Quality.
Water Quality Monitoring Section. Rosie Sada. Project Manager.
1520 E 6th Avenue. Helena, MT 59620.

February 5, 2007

Cover. Some uncommon native diatom species from Montana restricted to higher quality waters that are typical of reference streams. Top (left to right): *Encyonema yellowstonianum* from headwaters of the Missouri River in the Middle Rockies Ecoregion; *Distrionella incognita* from the Canadian Rockies Ecoregion; *Gomphoneis septa* from rivers east and west of the Continental Divide; *Gomphonema sinestigma* from the Northern Rockies Ecoregion; *Cymbella subturgidula* from west of the Continental Divide. Center: *Decussata placenta* from headwater streams in the mountains on both sides of the Continental Divide; Bottom: *Plagiotropis arizonica* from the Northwestern Great Plains Ecoregion.

Statistical Evaluation of Periphyton Samples from Montana Reference Streams

Prepared by

Mark Teply[1] and Loren Bahls[2]

February 2007

[1] **Larix Systems, Inc., 44 No. Last Chance Gulch, No. 4, Helena, MT 59601. (406) 458-0045.**

[2] **Hannaea, 1032 12th Street, Helena, MT 59601. (406) 443-2196.**

Table of Contents

List of Tables

List of Figures

Introduction

Since 2000, Montana Department of Environmental Quality (MDEQ) has been intensively sampling and analyzing periphyton communities from streams throughout Montana considered representative of natural biological, physical, and chemical integrity of the region. This supplements reference site sampling used to support biocriteria developed by Bahls (1993) and other incidental sampling of reference sites in the interim. It also represents additional reference sample results not available during development of revised periphyton metrics by Teply and Bahls (2005). A full discussion of reference streams and criteria used to screen them can be found in Suplee et al. (2005). Through 2005, over 100 reference streams had been sampled throughout the state, enabling meaningful statistical analysis. This report summarizes these analyses, specifically addressing four separate but related investigations:

1. Evaluate the extent of inter- and intra- annual variability on metric values;
2. Evaluate the similarity of floristic communities among reference sites; and,
3. Characterize the distribution of metric values calculated from these samples;
4. Validate the classification accuracy of Teply and Bahls (2005) for these samples.

The results of each investigation will directly support MDEQ's use of periphyton sample data in conducting water quality investigations.

Inter- and Intra-annual Variability

Sampling of reference sites did not take place with an express experimental design based on specific *a priori* hypotheses. Consequently, our analyses are exploratory and based on sampling that supports *a posteriori* hypotheses about the relative effect of inter- and intra-annual variability on metric values. Overall, only a limited number of reference sites had samples collected on successive occasions (i.e., within and/or between years) in a manner addressing such hypotheses (see **Appendix A**). Furthermore, sites with samples between years are not always the same as those with samples within years; and vice versa. Power of the available data is further reduced by conducting investigations separately for samples collected from cold-water fisheries

v. those from warm waters. Comparisons that could be supported by the available data therefore include the following:

- Cold Water Reference Sites
  - August 2003 v. September 2003 (n=6)
  - July 2004 v. August 2004 (n=4)
  - July 2004 v. September 2004 (n=4)
  - August 2004 v. September 2004 (n=4)
  - July/August 2004 v. July/August 2005 (n=3)

- Warm Water Reference Sites
  - August 2003 v. September 2003 (n=7)
  - July 2004 v. August 2004 (n=5)
  - July 2004 v. September 2004 (n=5)
  - August 2004 v. September 2004 (n=5)
  - August 2003 v. August 2004 (n=3)
  - September 2003 v. September 2004 (n=3)

Univariate comparisons utilized the Wilcoxon Match-Pairs Signed-Ranks Test (Sheskin 1997) to test the null hypothesis that the difference in metric values (within or between years) equals zero. The six "original" metrics presented by Bahls (1993) were evaluated. If a significant difference is evident, this indicates a high likelihood that two different populations are represented by the different sample events. All tests were two-sided, conducted using an *a priori* level of significance of 5 percent.

Results from univariate analyses indicated that among cold water sites, only one significant difference was evident – Siltation Index between 2004 and 2005. Among warm water sites, several significant differences existed within 2004 samples. July results for Shannon's Diversity Index, Percent Dominant Species, and Disturbance Index were significantly different than values obtained in August and September. Between 2003 and 2004 samples, two significant differences resulted – Pollution Index in August and Disturbance Index in September. Otherwise, there were no significant results among or within years.

Multivariate comparisons were also conducted utilizing Blocked Multi-response Permutation Procedures (McCune and Grace 2002) available in PC-ORD (Version 4.0). This tested the null hypothesis of no difference between taxa assemblages. Standardized relative abundance values were used as the basis of comparison; Euclidean distance measures were used to construct similarity matrices. If a significant difference is evident, this indicates a high likelihood that two different populations are represented by the different sample events. All tests were two-sided, conducted using an *a priori* level of significance of 5 percent.

Results from multivariate analyses indicated that the effects of site location tend to be stronger than potential temporal effects. In other words, the taxa assemblage found at any given site varied little over time – within or among years; variation site to site was observed to be greater. Among the temporal comparisons outlined above, only one was significant: warm-water sites, August 2003 v. September 2003; otherwise, there were no significant differences among or within years for either stream group.

Interpretations that can be drawn from these results are limited given the limited number of sites. Nevertheless, some consistent results are evident and are as expected. Overall, the effect of site location tends to be greater than the month or year sampled. Among cold-water sites, temporal effects are limited and mostly not significant. Among warm-water sites, some temporal effects are evident within years, several of which are significant; the latest sampling event (i.e., September) tends to yield taxa composition and metric values that are different than the earlier sampling events. It is posited that this reflects the successional development of diverse diatom communities in warmer, slower velocity streams compared to the lower diversity and limited successional development found in colder, higher velocity streams in the mountains.

In terms of use of periphyton sample data in conducting water quality investigations, these findings have two key implications. First, the similarity found year-to-year at any given site indicates that repeated sampling at any site would likely yield a serially correlated data set. In other words, results yielded would be similar and could be considered dependent thereby violating assumptions of many inferential statistical methods. Several statistical remedies exist to deal with this, but practically, consideration could be given to sampling reference sites at longer intervals; i.e., other than year-to-year. Second, the dissimilarity found within-year at any given warm-water site indicates that time of sample collection may be important. Variability of a sample data set that combines early and late samples may therefore be greater than if the data set considered exclusively either early or late samples. From an ecological standpoint, these results suggest that sampling conducted later in the season may be more representative for warm-water sites. No such cautions are evident among results from cold-water sites; early and late sampling appears to yield comparable results.

Stream Grouping

Potential stream grouping criteria were evaluated using available sample results from sites meeting reference screening criteria outlined in Suplee et al. (2005). Where more than one sample existed for a site, either within and/or between years, one sample was selected following the recommendations above. For cold-water fisheries sites, one sample was randomly selected from the entire period of record available for each site. For warm-water sites, one sample was randomly selected from the period of record, with preference given to samples collected later in the season. Sampling in this manner produced an independent data set representative of reference stream conditions found in the state. In total, eighty-five (85) samples were available (see **Appendix B**).

Potential stream grouping criteria were evaluated using an approach adapted from Van Sickle et al. (2006). This is an all-possible-subsets procedure used to identify variables, via a discriminant function model, that best predict group membership based on taxonomic composition of reference sites. As implemented by Van Sickle et al. (2006), every combination of "predictor variables" and taxonomic group (i.e. all-possible-subsets) is evaluated to choose that which is most accurate and precise. Predictor variables are descriptive site observations (e.g., ecoregion, watershed area, or stream gradient) theorized to explain the taxa assemblage one might observe at a site. Taxonomic groups are identified by hierarchical cluster analysis techniques. Criteria used by Van Sickle et al. (2006) to choose the best model are specific to RIVPACS-type models.

Our adaptation chooses predictor variables that yield stream grouping criteria with the best overall classification accuracy among the sample data set.

In our analyses, we considered predictor that could be remotely observed for any given site, could be reliably observed for any given site, and had an ecological basis for discriminating taxa assemblages at any given site. This included the following:

- River Basin (i.e., Columbia, Missouri, or Yellowstone);
- Latitude and Longitude (decimal degrees, NAD83);
- MDEQ Fisheries Classification (i.e., cold- or warm- water fishery);
- Predominant Level III Ecoregion (Woods et al. 1999);
- Total Alkalinity of Surface Waters (Omernik and Griffith 1986);
- Vascular Plant Region (Dorn 1984);
- Vegetation Provinces (Nesser et al. 1997);
- Number of 6th Code HUC's Drained (derived from Montana NRCS 2006);
- Stream Order (derived from the National Hydrography Dataset);
- Elevation (observed from 7.5' USGS quadrangles);
- Stream Gradient (observed from 7.5' USGS quadrangles);
- Annual Mean Daily Maximum Temperature (Daly et al. 2004); and,
- Annual Precipitation (Daly et al. 2004).

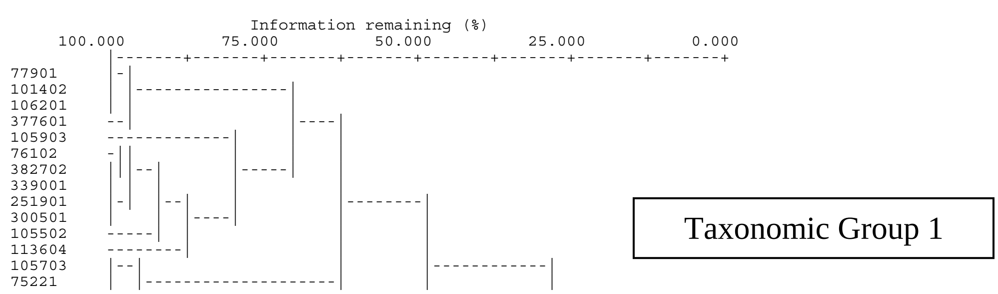
Other variables were considered (e.g., total alkalinity and mean percent fine substrates) but not carried forward in analysis because they required on-site observation of water chemistry and physical habitat – information not available for all samples.

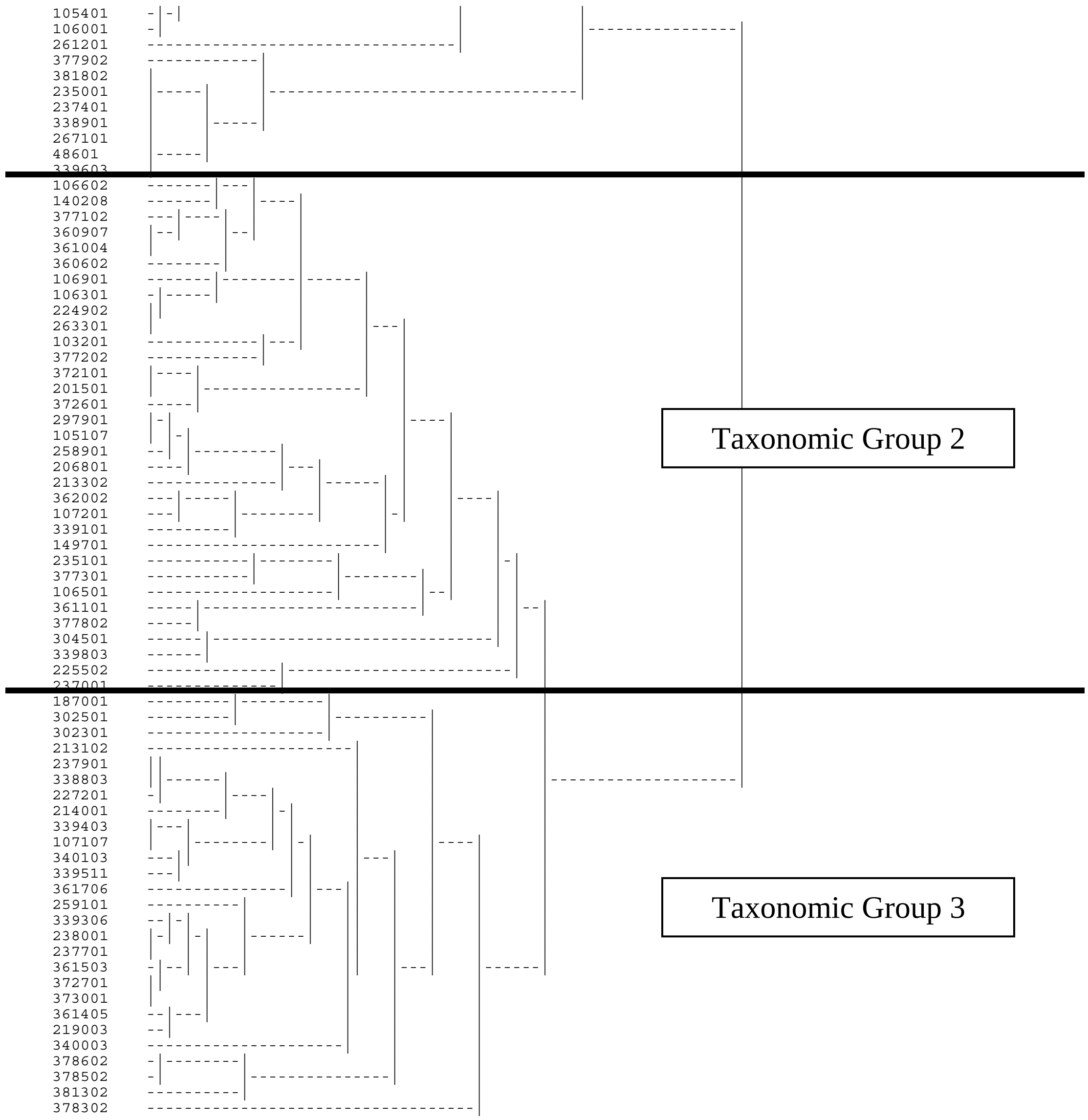
The MDEQ Fisheries Classification used in the analyses was developed for reference sites and was derived from Omernik's Level III ecoregions of Montana (Woods et al. 1999). It is also described on page 11 of Suplee et al. (2005). Briefly, streams sites falling within ecoregions 15 (Northern Rockies), 16 (Idaho Batholith), 17 (Middle Rockies) and 41 (Canadian Rockies) are considered cold-water fisheries, whereas stream sites located in ecoregions 42 (Northwestern Glaciated Plains), 43 (Northwestern Great Plains) and 18 (Wyoming Basin) are considered warm-water fisheries. Minor exceptions may exist at boundary situations.

Hierachical cluster analyses (dendrograms) were constructed using PC-ORD (Version 4.0). All taxa counted in a sample were considered in clustering; clustering was based on relativized percent relative abundance. The linkage method employed was Ward's Method based on Euclidean distance measures. Dendrograms were interpreted, or "pruned", to identify distinct groups of taxonomic composition among reference sites. Stepwise discriminant function analysis was then used to evaluate the ability of predictor variables to predict group membership. Discriminant function analysis was conducted using SYSTAT (Version 11). Discriminant functions were evaluated primarily for statistical significance and classification accuracy; preference was given to functions that used the least number of predictor variables. The above was applied progressively to the sample data; looking for the combination of predictor variables and taxonomic groups that yielded stream grouping criteria with the best overall classification accuracy.

In the analysis of this data set, resulting models were quite clear in identifying those variables that best explained taxonomic composition. At each step in the process, only one or two variables would emerge as significant and providing acceptable classification accuracy. **Figure 1** depicts the hierarchical cluster analysis using all 85 samples. Three distinct groups are evident, demarcated by a heavy black line in the dendrogram. No predictor variable was able to accurately discriminate among the three groups. However, by combining the first two groups, one predictor variable – MDEQ Fisheries Classification – was significant in predicting taxa composition based on whether it was considered a cold-water fishery or a warm-water fishery. Overall classification accuracy

Figure 1. Dendrogram from hierarchical cluster analysis of all samples.

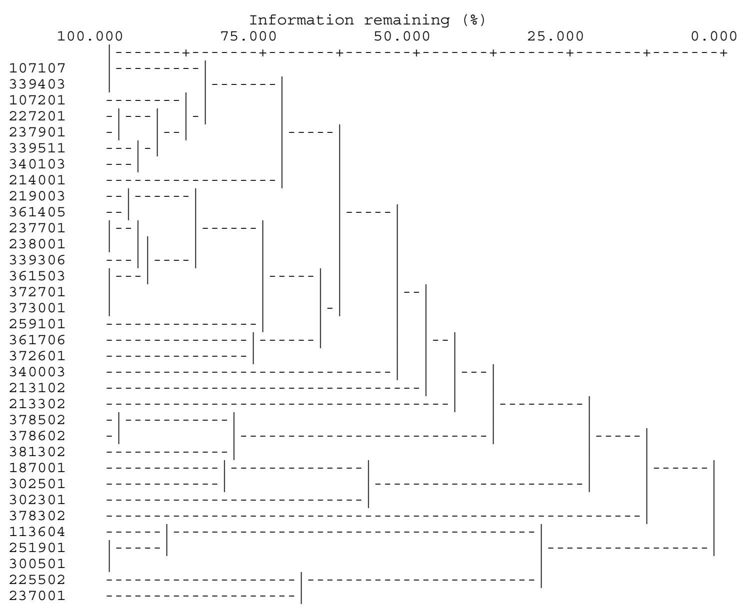



105401
106001
261201
377902
381802
235001
237401
338901
267101
48601
339603
106602
140208
377102
360907
361004
360602
106901
106301
224902
263301
103201
377202
372101
201501
372601
297901
105107
258901
206801
213302
362002
107201
339101
149701
235101
377301
106501
361101
377802
304501
339803
225502
237001
187001
302501
302301
213102
237901
338803
227201
214001
339403
107107
340103
339511
361706
259101
339306
238001
237701
361503
372701
373001
361405
219003
340003
378602
378502
381302
378302
Taxonomic Group 2
Taxonomic Group 3

was 89%; 98% of cold-water sites could be found among the first two taxonomic groups in Figure 1; 76% of warm-water sites are in the third group.

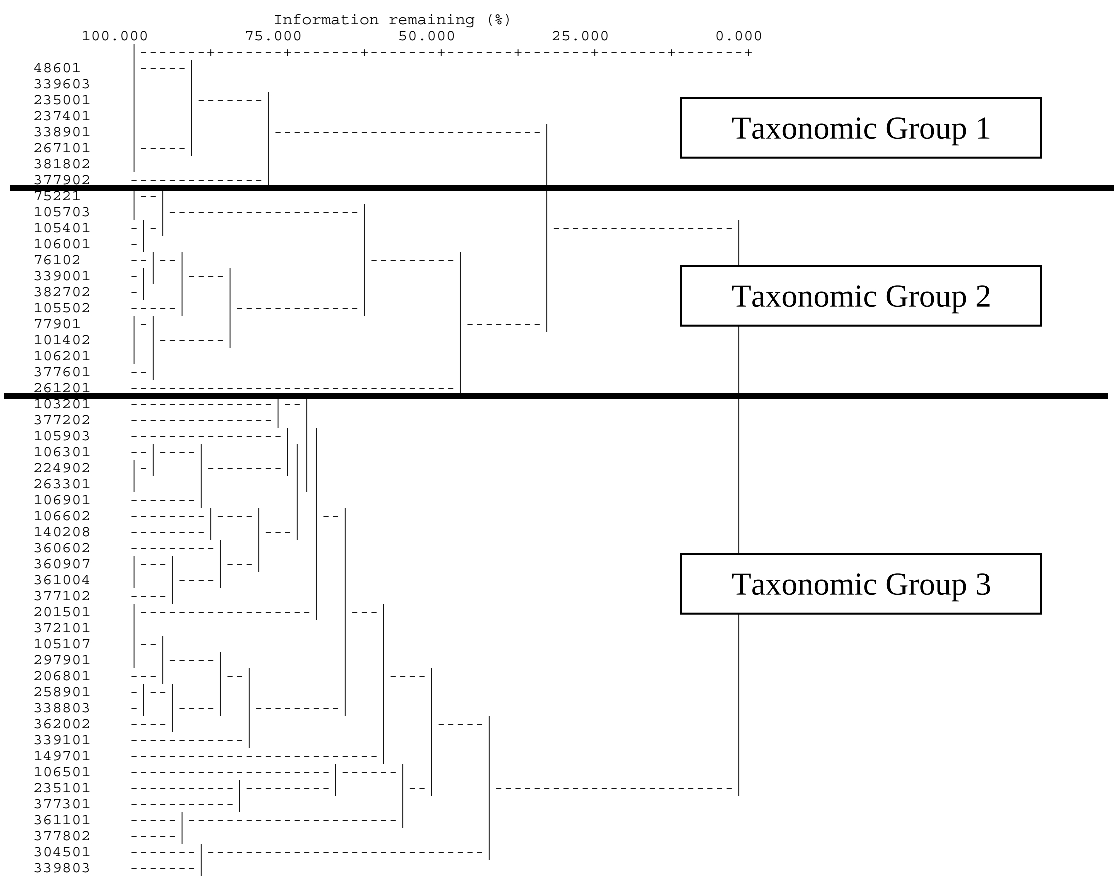
Using the MDEQ Fisheries Classification as a first order subsetting criterion, analysis then proceeded to identify meaningful models among samples from cold-water sites and those from warm-water sites. In doing so, hierarchical cluster analysis was re-evaluated among samples within each fisheries group. Among samples from warm-water sites, the resulting dendrogram (see **Figure 2**) indicated a high level of dissimilarity in taxa composition among sites; in fact, we have already observed that about a quarter of warm-water sites have taxa assemblages that are more similar to cold-water sites than among other warm-water sites. Pruned groups were not visually evident and discriminant analysis did not reveal any significant predictor variables at any level of grouping indicated by the dendrogram. This level of dissimilarity is not unexpected and has been previously observed by Teply and Bahls (2005 and 2006).

Figure 2. Dendrogram from hierarchical cluster analysis of warm-water sites.



Among samples from cold-water sites, three distinct groups are evident, demarcated by a heavy black line in the dendrogram (see **Figure 3**). No predictor variable was able to accurately discriminate among the three groups. However, by combining the first two groups, two predictor variables – River Basin and Predominant Level III Ecoregion – were each significant in predicting taxa composition. Based on whether the site was found in the Missouri River Basin, taxonomic grouping could be predicted with an overall classification accuracy of 69%. Based on whether the site was found in the Middle Rockies Ecoregion, taxonomic grouping could be accurately predicted about 71% of the time. For each predictor variable, error was distributed equally among taxonomic groups. Consequently, both were more or less comparable.

Figure 3. Dendrogram from hierarchical cluster analysis of cold-water sites.



When River Basin and Predominant Level III Ecoregion were combined, predictive ability improved. Residual analysis indicated that sites within the Canadian Rockies Ecoregion within the Missouri River Basin were systematically misclassified; these sites were more similar to cold-water sites in the Northern Rockies Ecoregion. Otherwise, errors were distributed more or less evenly, geographically. When Canadian Rockies sites were excluded from the Missouri River Basin, classification accuracy improved to 89%. Among remaining sites, classification accuracy fell to 56%; therefore, subsequent analysis was conducted to determine if meaningful predictor variable/taxonomic group combinations existed that better discriminated these sites. These would be cold-water sites in the Columbia River Basin, Yellowstone River Basin, or in the Canadian Rockies Ecoregion (including the portion within the Missouri River Basin).

Among the remaining cold-water sites, two taxonomic groups could be pruned from the hierarchical cluster analysis (see **Figure 4**). Only one variable – Percent Stream Gradient – was significant and accurate in discriminating these taxonomic groups. Using a stream gradient break of 5.5%, taxonomic grouping could be accurately predicted for about 75% of these samples. High and low gradient streams were correctly classified at more or less the same rate. Errors were generally evenly distributed geographically and this criterion appeared to account for previous misclassification error observed across the region. Subsequent analysis within high and low stream gradient groups indicated no meaningful predictor variable/taxonomic group combinations existed that would better discriminate these sites. At this point, the search for stream grouping variables ended.

Based on the analysis presented above, two alternative sets of stream grouping criteria emerge for consideration in supporting MDEQ's use of periphyton sample data in conducting water quality investigations. One alternative uses Predominant Level III Ecoregion to discriminate among cold-water sites (**Figure 5**). The other uses River Basin, Predominant Level III Ecoregion, and Percent Stream Gradient to discriminate among cold-water sites (**Figure 6**). Both treat warm-water sites similarly; i.e., they use no further discrimination among warm water sites. Overall classification accuracy of the Ecoregion-based construct is about 73%. Overall classification accuracy of the refined classification scheme is 79%. Both are carried forward in the next step in analysis (distribution of metric values within each stream group) and both are presented for MDEQ consideration in water quality investigations.

Figure 4. Dendrogram from hierarchical cluster analysis of cold-water sites in the Columbia River Basin,

Yellowstone River Basin, and Canadian Rockies Ecoregion.

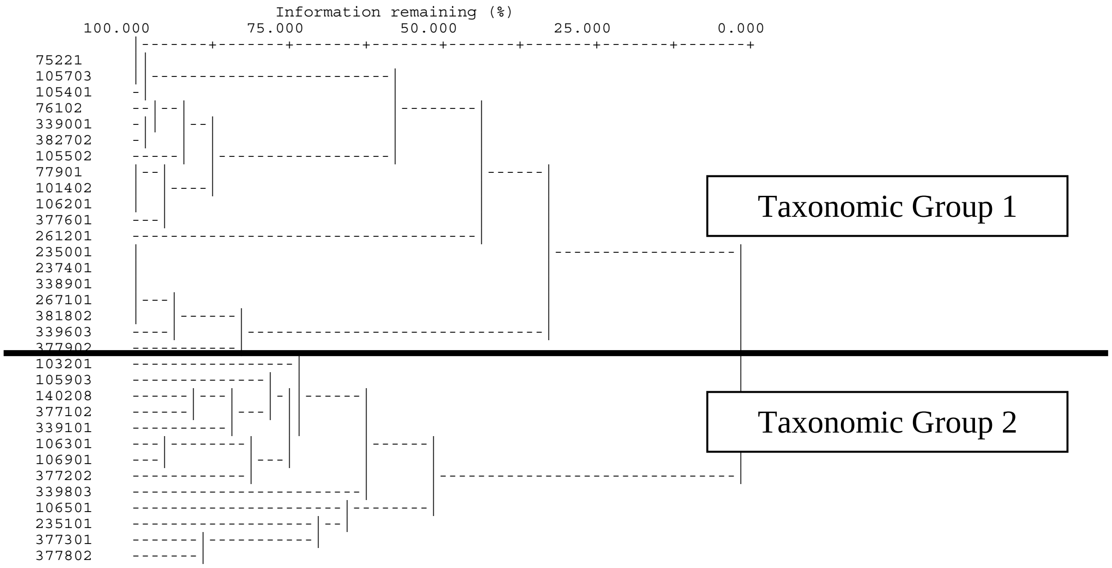


Figure 5. Dichotomous key to stream groupings using MDEQ Fisheries Classification and Predominant Level III Ecoregion (Alternative I).

1. Site in a warm water fishery?
   - a. Yes..Warm-Water Stream Group (I.1a)
   - b. No.. Go To 2
2. Site within the Middle Rockies Ecoregion?
   - a. Yes.. Middle Rockies Stream Group (I.2a)
   - b. No... Northern/Canadian Rockies Stream Group (I.2b)

Figure 6. Dichotomous key to stream groupings using MDEQ Fisheries Classification, River Basin, Predominant Level III Ecoregion, and Stream Gradient (Alternative II).

1. Site in a warm water fishery?
   - a. Yes... Warm-Water Stream Group (II.1a)
   - b. No..Go to 2
2. Site within the Middle Rockies Ecoregion within the Missouri River Basin?
   - a. Yes..Missouri River Basin Middle Rockies Stream Group (II.2a)

b. No.. Go To 3

3. Stream Gradient greater than 5.5%?

a. Yes.....................................High Gradient Columbia/Yellowstone River Basin Group (II.3a)

b. No...................................... Low Gradient Columbia/Yellowstone River Basin Group (II.3b)

<u>Distribution of Metric Values</u>

Figures 7 through 12 present histograms of selected metric values for each of the stream groups identified in **Figures 5 and 6**. Stream group I.1a and II.2a are equivalent in each alternative shown above; otherwise they directly relate to a group shown above. The metrics shown are from Bahls (1993) for determination of biological integrity, impairment, and use support. Bins used in the histogram incorporate breaks used in criteria for rating levels of biological integrity. **Table 1** presents descriptive statistics for each of the metrics by stream group. These summaries provide a "benchmark" supporting use of periphyton sample data in conducting water quality investigations.

<u>Validation of Teply and Bahls (2005)</u>

Teply and Bahls (2005) offered metrics and associated biocriteria as alternatives to the Periphyton Bioassessment Methods for Montana Streams (Bahls 1993). These metrics are based on empirical analysis of periphyton sample data collected since 1995 that indicate taxa observed to increase or decrease in response to environmental stress. Using increaser and decreaser taxa reported by Teply and Bahls (2005), we calculated associated metric scores and determined impairment status based on thresholds

Figure 7. Histograms of values of number of species counted by stream group.

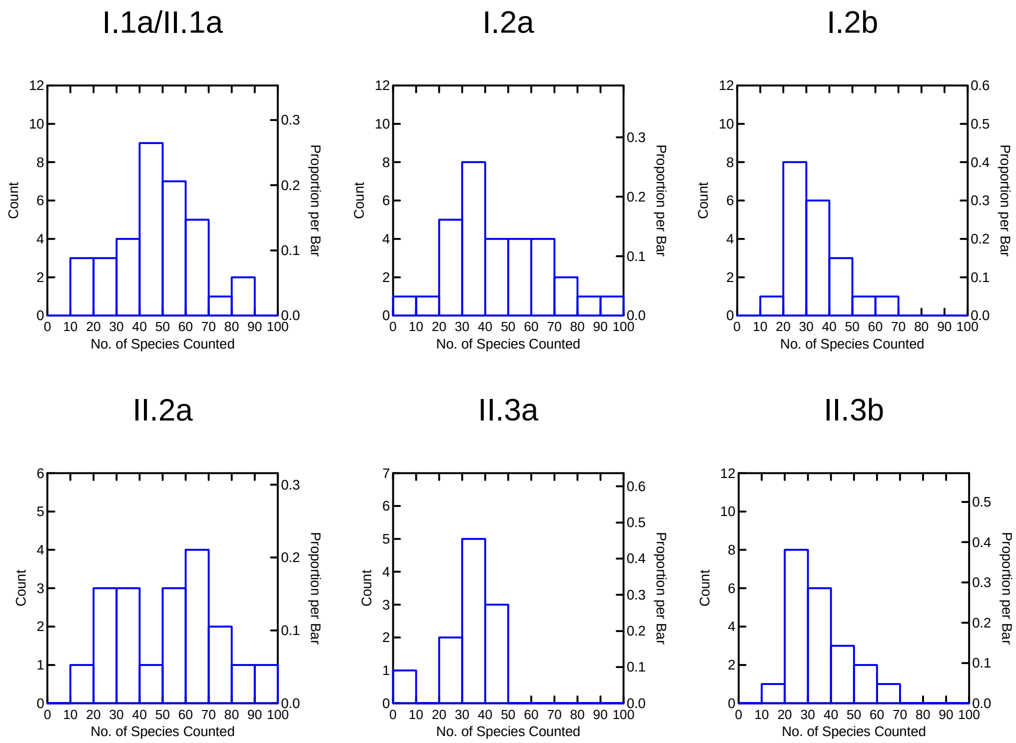


Figure 8. Histograms of values of Shannon's diversity index by stream group.

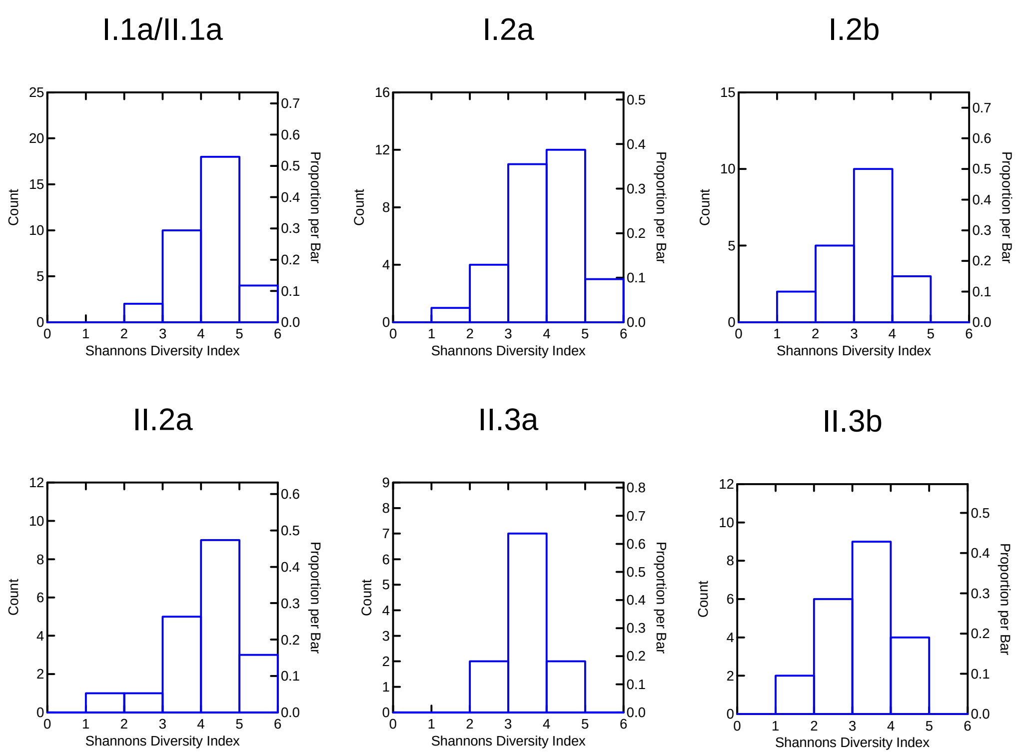


Figure 9. Histograms of values of percent dominant species by stream group.

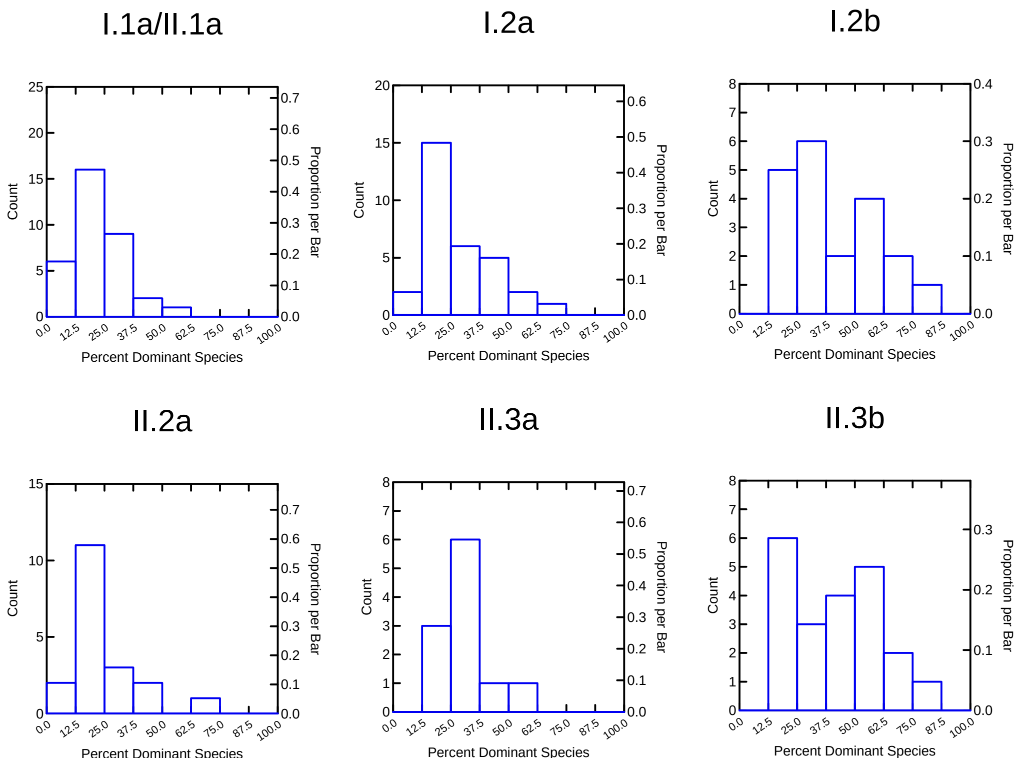


Figure 10. Histograms of values of pollution index by stream group.

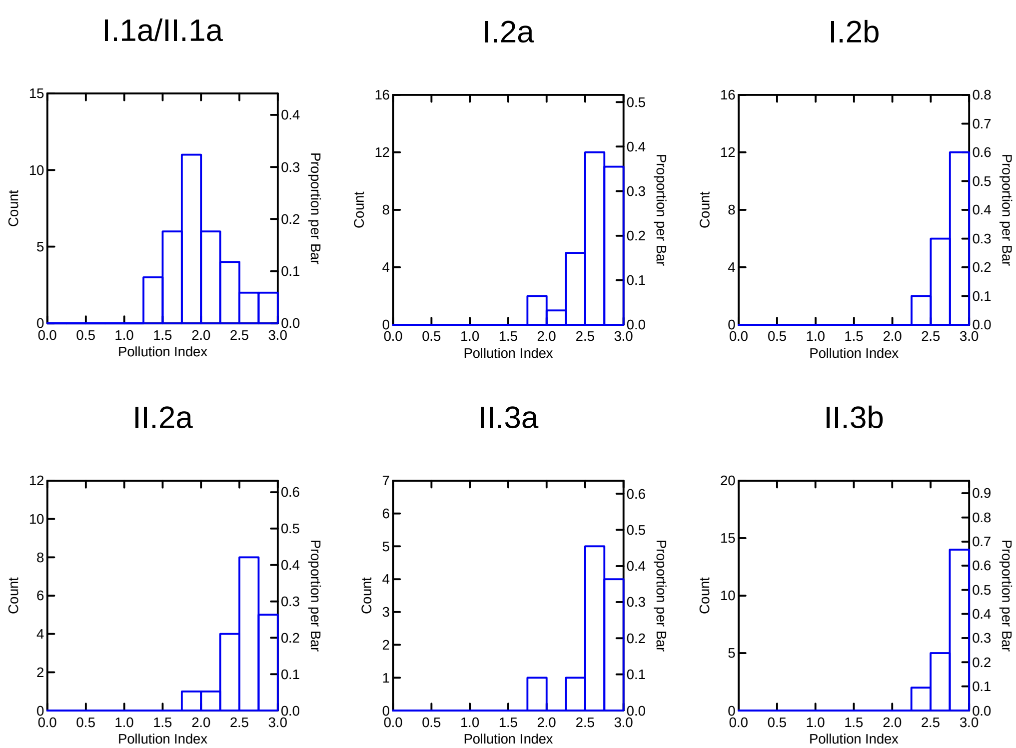


Figure 11. Histograms of values of siltation index by stream group.

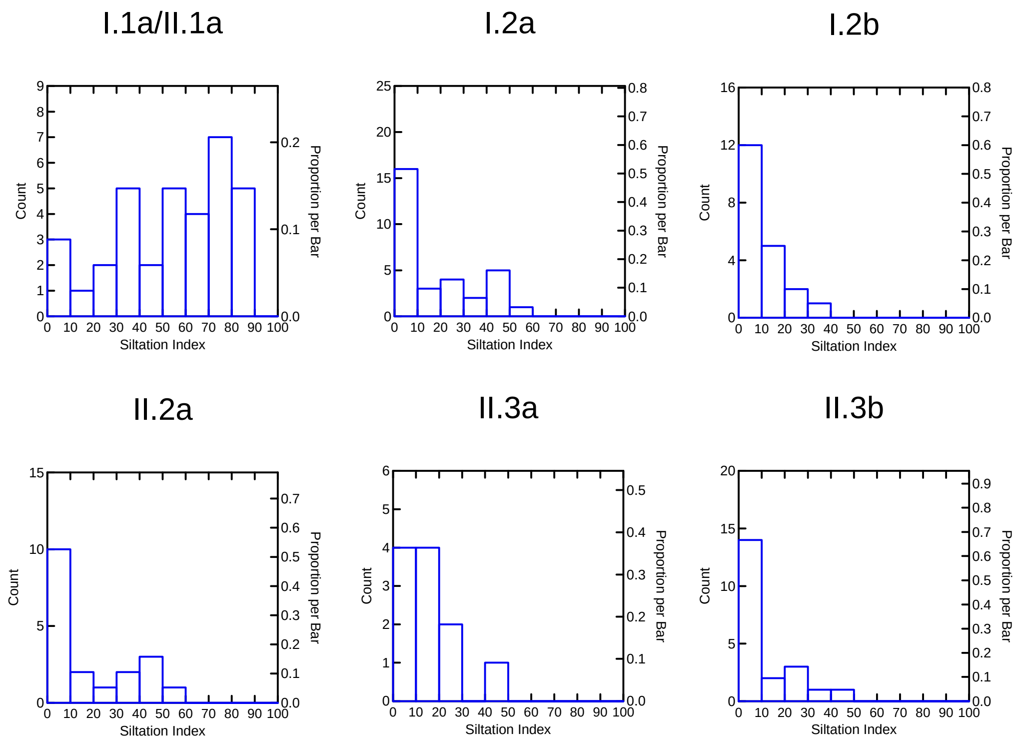


Figure 12. Histograms of values of disturbance index by stream group.

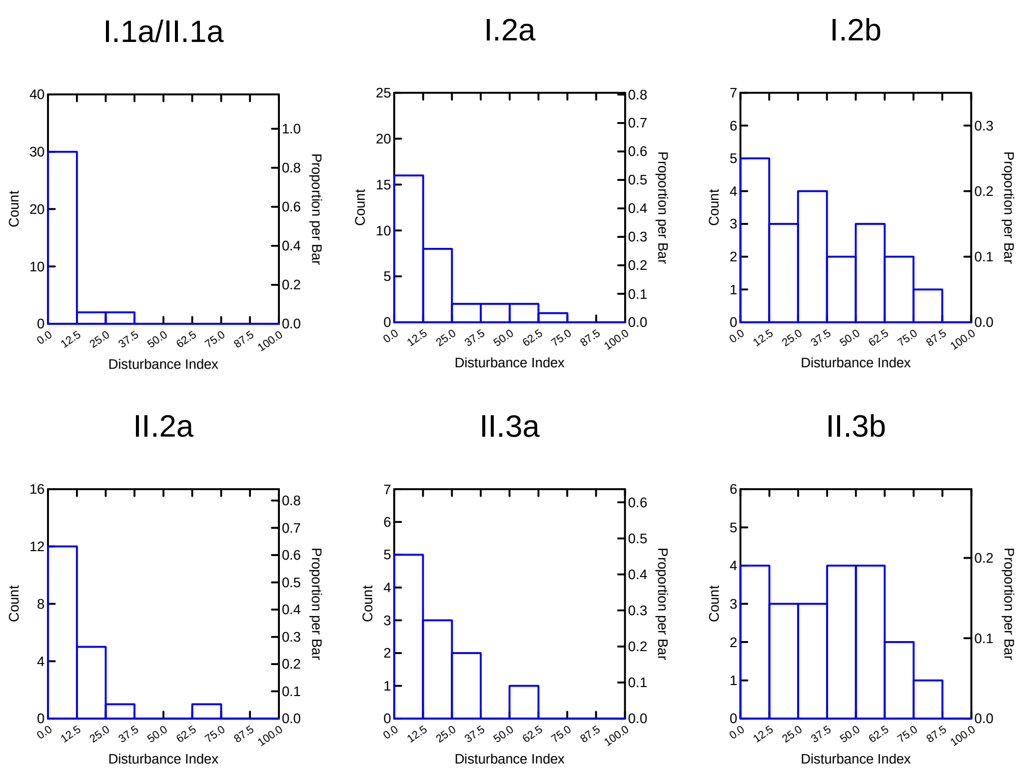


Table 1. Descriptive statistics of selected metrics by stream group.

Statistic	Stream Group					
	I.1a/II.1a	I.2a	I.2b	II.2a	II.3a	II.3b
Total Number of Species Counted						
N	34	31	20	19	11	21
Minimum	13	8	19	18	8	19
Maximum	86	94	60	94	49	60
Mean	47.4	45.0	34.3	51.4	33.7	34.9
Std. Dev.	18.4	19.3	11.1	20.7	11.3	11.3
Shannon's Diversity Index						
N	34	31	20	19	11	21
Minimum	2.61	1.67	1.29	1.67	2.35	1.29
Maximum	5.60	5.65	4.46	5.65	4.19	4.46
Mean	4.194	3.858	3.192	4.086	3.478	3.215
Std. Dev.	0.735	0.882	0.850	0.953	0.597	0.860
Percent Dominant Species						
N	34	31	20	19	11	21
Minimum	9.32	8.83	16.03	8.83	19.02	16.03
Maximum	53.69	74.69	82.43	74.69	61.25	82.43
Mean	22.638	28.588	39.977	25.098	32.068	40.769
Std. Dev.	10.226	15.128	18.216	14.900	11.729	18.590
Pollution Index						
N	34	31	20	19	11	21
Minimum	1.27	1.79	2.41	1.86	1.79	2.42
Maximum	2.94	2.96	2.97	2.96	2.92	2.97
Mean	1.998	2.611	2.743	2.584	2.605	2.763
Std. Dev.	0.389	0.281	0.162	0.266	0.316	0.149
Siltation Index						
N	34	31	20	19	11	21
Minimum	0	0.64	0	1.02	0.64	0
Maximum	89.18	50.36	31.87	50.36	48.55	40.34
Mean	53.173	18.495	8.564	19.552	15.124	9.847
Std. Dev.	25.782	16.403	9.381	16.994	13.772	12.050
Disturbance Index						
N	34	31	20	19	11	21
Minimum	0	0	0.24	0	0	0.24
Maximum	27.51	74.69	82.43	74.69	61.25	82.43
Mean	3.493	18.427	33.074	13.666	19.995	35.864
Std. Dev.	7.957	19.123	23.412	17.200	17.197	22.968

presented by the authors. Ratings were determined using equations relevant to warm-water and cold-water fisheries per the criteria above.

Those taxa lists based on cause-specific relationships (i.e., sediment, nutrient, or metals impairment) perform best; cold-water sites were classified correctly about 85 to 95% of the time and warm-water sites had a 75% classification accuracy. Taxa lists based on general impairment (i.e., not specific to a cause) classified correctly only about 55% of the time. The better performance of cause-specific taxa lists reflects the specificity of a handful of commonly occurring taxa. The better performance of cold-water sites reflects generally lower overall diversity encountered in the mountains – this makes it easier to identify a handful of taxa indicating stress. Overall, these results suggest reliability of taxa lists in Teply and Bahls (2005); however, only among reference sites.

References

Bahls, L.L. 1993. Periphyton Bioassessment Protocols for Montana Streams. Montana Department of Health and Environmental Sciences; Water Quality Bureau; Helena, Montana. Revised January 1993.

Daly, C., Gibson, W.P., M. Doggett, J. Smith, and G. Taylor. 2004. Up-to-date monthly climate maps for the conterminous United States. Proc., 14th AMS Conf. on Applied Climatology, 84th AMS Annual Meeting Combined Preprints, Amer. Meteorological Soc., Seattle, WA, January 13-16, 2004, Paper P5.1, CD-ROM.

Dorn, R.D. 1984. Vascular plants of Montana. Mountain West Publishing. Cheyenne, WY. 276p.

McCune, B. And D. Grace. 2002. Analysis of Ecological Communities. MjM Software. Glenden Beach, Oregon. 300p.

Montana Natural Resources Conservation Service State Office. 2006. Montana Subwatershed

(6th-code) Hydrologic Units. Bozeman, Montana.

Nesser, John A, Ford, Gary L, Maynard, C Lee, Page-Dumroese, Deborah S. 1997. Ecological Unit Subsections, USFS Northern Region. United States Forest Service Rocky Mountain Research Station. Ogden, Utah.

Omernik, J. and G. Griffith. 1986. Total Alkalinity of Surface Waters: A Map or the Western Region. U.S. Environmental Protection Agency, Washington, D.C., EPA/600/J-86/410 (NTIS PB88104302).

Sheskin, D. 1997. Handbook of Parametric and Nonparametric Statistical Procedures. CRC Press. Boca Raton, Florida.

Suplee, M., R.S. de Suplee, D. Feldman, and T. Laidlaw. 2005. Identification and Assessment of Montana Reference Streams: A Follow-up and Expansion of the 1992 Benchmark Biology Study. Montana Dept. of Environmental Quality, Helena, MT. 23p.

Teply, M. and L. Bahls. 2005. Diatom Biocriteria for Montana Streams. Prepared for the Montana Department of Environmental Quality by Larix Systems, Inc. of Helena, Montana. September 2005.

Teply, M. and L. Bahls. 2006. Diatom Biocriteria for Montana Streams – Middle Rockies Ecoregion. Prepared for the Montana Department of Environmental Quality by Larix Systems, Inc. of Helena, Montana. February 2006.

Van Sickle, J., D. D. Huff, and C. P. Hawkins. 2006. Selecting discriminant function models for predicting the expected richness of aquatic macroinvertebrates. Freshwater Biology 61:359-372.

Woods, Alan J., Omernik, James, M., Nesser, John A., Shelden, J., and Azevedo, Sandra H. 1999. Ecoregions of Montana (color poster with map, descriptive text, summary tables, and photographs): Reston, Virginia, U.S. Geological Survey (map scale 1:1,500,000).

Sample No.	Waterbody	Latitude	Longitude	Sample Date
48601	South Fork Spanish Creek, Spanish Peaks Wilderness	45.41	-111.39	7/14/1978
75221	Blackfoot River near mouth	46.9	-113.75	8/15/2000
76102	Belly River at 3-mile campsite	48.96	-113.68	7/25/1996
77901	Gardner River at mouth, Yellowstone National Park	45.02	-110.69	2/11/1981
101402	West Fork Rock Creek above Silver Run	45.15	-109.33	8/5/1992
103201	Crazy Creek (upper) below Mount Patrick Gass, Bob Marshall Wilderness	48.04	-112.9	7/16/1988
105107	Seymour Creek above Lower Seymour Lake Campground	45.99	-113.18	7/8/2001
105401	South Fork Flathead River above Hungry Horse Reservoir	47.8	-113.41	8/31/1990
105502	Waldron Creek near Choteau	47.91	-112.81	6/17/2001
105703	South Fork Sun River below Benchmark and Straight Creek	47.5	-112.89	8/6/1993
105903	Armstrong Spring Creek	45.63	-110.75	7/22/1993
106001	Odell Spring Creek	45.34	-111.71	7/31/1990
106201	Lake Fork Rock Creek	45.07	-109.41	8/22/1990
106301	Fourmile Creek south of Big Timber	45.34	-110.24	9/13/1990
106501	Pine Creek near Livingston	45.5	-110.78	7/26/1990
106602	Calf Creek near White Sulphur Springs	46.84	-110.96	7/29/2001
106901	Big Creek above Gallatin N. F. boundary	45.3	-110.94	7/26/1990
107107	Rock Creek northeast of Hinsdale	48.87	-106.89	9/1/2000
107201	Tule Creek at Highway 13 near Wolf Point	48.18	-105.49	8/23/1990
113604	Woody Island Coulee above Highway 241 bridge north of Turner	48.92	-108.37	9/14/1995
140208	Rock Creek near Clinton	46.69	-113.66	8/16/2000
149701	Madison River near West Yellowstone in Yellowstone National Park	44.65	-111.06	9/13/1994
187001	Milk Creek near mouth	46.16	-104.71	9/28/1999
201501	Gallatin River just below YNP boundary	45.05	-111.15	8/21/2000
206801	Little Lake Creek above USFS road	45.35	-113.6	7/26/2000
213102	O'Fallon Creek	46.73	-105.05	8/8/2000
213302	Little Beaver Creek	46.06	-104.33	8/10/2000
214001	Whitewater Creek	48.6	-107.52	8/25/2000
219003	Willow Creek near mouth north of Hinsdale	48.57	-106.97	8/25/2001
224902	South Cottonwood Creek at trail bridge	45.53	-111.08	9/12/2002
225502	Blackleaf Creek below USFS boundary	48.01	-112.67	6/21/2001
227201	Spring Creek above mouth	46.13	-104.67	8/1/2001

Sample No.	Waterbody	Latitude	Longitude	Sample Date
235001	Silver Creek near mouth	47.4	-115.51	7/23/2001
235101	Deer Creek headwaters	47.31	-115.4	7/24/2001
237001	Cow Creek near Cow Island	47.86	-108.96	6/24/2001
237401	Wyoming Creek	45.05	-109.4	8/15/2001
237701	Little Powder River near Broadus	45.33	-105.31	10/11/2001
237901	Fish Creek	46.25	-109.76	10/15/2001
238001	O'Fallon Creek above Sandstone Creek	46.47	-104.76	10/7/2001
251901	Blackleaf Creek below Blackleaf	48.01	-112.56	7/25/2002
258901	Eagle Creek upper site on IX Ranch	48.1	-109.76	8/22/2002
259101	Eagle Creek near mouth	47.91	-110.05	8/22/2002
261201	Goat Creek Reach 9	47.75	-113.78	9/5/2002
263301	Mill Gulch	45.41	-111.95	9/24/2002
267101	South Fork Flathead River at Spotted Bear	47.98	-113.56	8/13/2002
297901	LaMarche Creek above USFS boundary	45.91	-113.21	7/16/2003
300501	Little Dry Creek near Van Norman School	47.34	-106.36	7/29/2003
302301	East Redwater River on state land	47.75	-104.92	6/19/2003
302501	Pasture Creek below Highway 200	47.7	-105.24	6/20/2003
304501	Beaver Creek near mouth	47.07	-109.59	7/24/2003
338803	Clear Creek (close to station 2186)	48.3	-109.49	9/7/2003
338901	East Fork Bull River	48.12	-115.72	8/19/2003
339001	East Rosebud Creek	45.22	-109.6	8/28/2003
339101	North Fork Teton River (close to station 1056)	47.96	-112.8	8/23/2003
339306	O'Fallon Creek 2 (close to station 2380)	46.47	-104.76	8/27/2006
339403	Rock Creek 1	48.87	-106.89	9/6/2003
339511	Rock Creek 2 on state land near Rock Creek Lodge	48.59	-107	9/6/2004
339603	Roaring Lion Creek	46.19	-114.24	9/1/2003
339803	Seeley Creek	45.09	-109.29	9/28/2003
340003	Wolf Creek at Wolf Point	48.08	-105.67	9/5/2003
340103	West Fork Poplar River	48.69	-105.83	9/6/2003
360602	Cottonwood Creek on Matador Ranch	44.94	-112.42	8/14/2004
360907	Willow Creek at base of Thunderhead Mountain	45.44	-112.82	8/21/2005
361004	Willow Creek on BLM land	45.43	-112.74	9/11/2004

Sample No.	Waterbody	Latitude	Longitude	Sample Date
361101	North Fork Greenhorn Creek at end of Trail 9680	45.12	-112.03	9/2/2004
361405	Bitter Creek just below Chisholm Creek	48.64	-106.9	9/3/2004
361503	Rock Creek on BLM land	48.65	-107.03	9/7/2004
361706	Willow Creek South	48.14	-106.62	9/5/2004
362002	Elk Springs Creek 0.25 miles west of Elk Lake Road	44.64	-111.66	8/20/2004
372101	East Fork Blacktail Deer Creek in Wildlife Management Area	44.86	-112.21	8/20/2005
372601	Cedar Creek on state land	46.79	-104.55	8/30/2005
372701	Custer Creek on state land	46.7	-105.56	9/3/2005
373001	Boxelder Creek on state land	45.84	-104.14	9/2/2005
377102	Cache Creek	46.79	-114.65	9/9/2006
377202	Canyon Creek North Fork	48.42	-115.19	9/4/2006
377301	Chicken Creek above upper Whitefish road	48.62	-114.52	9/9/2006
377601	Deerhorn Creek 1/4 mile above mouth	47.7	-115.09	9/5/2006
377802	Fire Creek	46.88	-114.8	9/8/2006
377902	Fish Creek North Fork	46.91	-114.81	9/7/2006
378302	Fourchette Creek	47.7	-107.77	8/23/2006
378502	Hart Creek	47.56	-106.96	8/25/2006
378602	Hell Creek	47.57	-106.96	8/25/2006
381302	Snap Creek	47.55	-106.29	8/24/2006
381802	Straight Creek	46.91	-114.81	9/7/2006
382702	White Creek	46.79	-114.66	9/9/2006