BIOLOGICAL INTEGRITY OF THE UPPER STILLWATER RIVER BASED ON THE COMPOSITION AND STRUCTURE OF THE BENTHIC ALGAE COMMUNITY

Prepared for:

State of Montana
Department of Environmental Quality
P.O. Box 200901
Helena, Montana 59620-0901

Project Officer: Patrick Newby
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Prepared by:

Loren L. Bahls, Ph.D.
Hannaea
1032 Twelfth Avenue
Helena, Montana 59601

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SUMMARY

On August 25, 2001, periphyton samples were collected at two stations on the upper Stillwater River near Cooke City for the purpose of assessing whether the river is water-quality limited and in need of TMDLs. The samples were collected following DEQ standard operating procedures, processed and analyzed using standard methods for periphyton, and evaluated following modified USEPA rapid bioassessment protocols for wadeable streams.

Low diatom species diversity and a large percentage of the tolerant diatom *Encyonema minutum* indicate moderate impairment and partial support of aquatic life uses below Daisy Creek. Above the Wilderness boundary, impairment was minor and aquatic life uses were fully supported.

A relatively large number of teratological diatom cells also indicates moderate impairment below Daisy Creek. The cause of these deformities is probably heavy metals. The number of teratological cells was much smaller above the Wilderness boundary, indicating some recovery between the two sites.

All of the major diatom species in the upper Stillwater River are either tolerant of a wide range of trophic conditions or exhibit their most vigorous growth in stronger mesotrophic to eutrophic waters. Among the tolerant species is *Encyonema minutum*, which dominated the diatom assemblage below Daisy Creek.

Periphyton samples were collected from these same two sites in August 1994. A bioassessment based on these samples, using the same methods used here, resulted in the same ratings of biological integrity: "Fair" (moderate impairment) below Daisy Creek and "Good" (minor impairment) above the Wilderness boundary. As in 2001, low diatom diversity was responsible for the less than excellent ratings in 1994.

INTRODUCTION

This report evaluates the biological integrity, support of aquatic life uses, and probable causes of impairment to those uses, in the upper Stillwater River near Cooke City, Montana. The purpose of this report is to provide information that will help the State of Montana determine whether this segment of the Stillwater River is water-quality limited and in need of TMDLs.

The federal Clean Water Act directs states to develop water pollution control plans (Total Maximum Daily Loads or TMDLs) that set limits on pollution loading to water-quality limited waters. Water-quality limited waters are lakes and stream segments that do not meet water-quality standards, that is, that do not fully support their beneficial uses. The Clean Water Act and USEPA regulations require each state to (1) identify waters that are water-quality limited, (2) prioritize and target waters for TMDLs, and (3) develop TMDL plans to attain and maintain water-quality standards for all water-quality limited waters.

Evaluation of use support in this report is based on the species composition and structure of the periphyton (benthic algae, phytobenthos) community at two river sites that were sampled on August 25, 2001. The periphyton community is a basic biological component of all aquatic ecosystems. Periphyton accounts for much of the primary production and biological diversity in Montana streams (Bahls et al. 1992).

Plafkin et al. (1989) and Stevenson and Bahls (1999) list several advantages of using periphyton in biological assessments:

- Algae are universally present in large numbers in all streams and unimpaired periphyton assemblages typically support a large number (>30) of species;
- Algae have rapid reproduction rates and short life cycles, making them useful indicators of short-term impacts;

- As primary producers, algae are most directly affected by physical and chemical factors, such as temperature, nutrients, dissolved salts, and toxins;

- Sampling is quick, easy and inexpensive, and causes minimal damage to resident biota and their habitat;

- Standard methods and criteria exist for evaluating the composition, structure, and biomass of algal associations;

- Identification to species is straightforward for the diatoms, for which there is a large body of taxonomic and ecological literature;

- Excessive algae growth in streams is often correctly perceived as a problem by the public.

- Periphyton and other biological communities reflect the *biological integrity*[1] of waterbodies; restoring and maintaining the biological integrity of waterbodies is a goal of the federal Clean Water Act;

- Periphyton and other biological communities integrate the effects of different stressors and provide a measure of their aggregate impact; and

- Periphyton and other biological communities may be the only practical means of evaluating impacts from non-point sources of pollution where specific ambient criteria do not exist (e.g., impacts that degrade habitat or increase nutrients).

Periphyton is a diverse assortment of simple photosynthetic organisms, called algae, and other microorganisms that live attached to or in close proximity of the stream bottom. Most algae, such as the diatoms, are microscopic. Diatoms are distinguished by having a cell wall composed of opaline glass--hydrated amorphous silica. Diatoms often carpet a stream bottom with a slippery brown film.

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[1] *Biological integrity* is defined as "the ability of an aquatic ecosystem to support and maintain a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitats within a region" (Karr and Dudley 1981).

Some algae, such as the filamentous greens, are conspicuous and their excessive growth may be aesthetically displeasing, deplete dissolved oxygen, interfere with fishing and fish spawning, clog water filters and irrigation intakes, create tastes and odors in drinking water, and cause other problems.

PROJECT AREA AND SAMPLING SITES

The project area is located in Park County near Cooke City, Montana. The Stillwater River heads on the Beartooth Plateau (maximum elevation 12,799 feet) and flows northeasterly to the Yellowstone River. The sampling sites, which lie at about 8,500 feet elevation, are within the Middle Rockies Ecoregion (Woods et al. 1999).

The upper Stillwater River basin lies in a deep glaciated canyon. The study area is upstream of the Beartooth-Absaroka Wilderness. Vegetation is alpine tundra and spruce-fir forest (USDA 1976). The study area lies within the Beartooth Uplift, which is composed of Precambrian granitic gneiss, amphibolite, and subordinate metasedimentary rocks that are resistant to erosion and dissolution and consequently generate groundwater and runoff low in dissolved solids (Gurrieri 1998).

The New World Mining District at the headwaters of the basin is dominated by Tertiary intrusive rocks that cross-cut Cambrian-age sediments with localized Cu, Au, and Ag mineralization along the contacts (Gurrieri 1998). Oxidation of sulfide minerals by groundwater migrating through the McLaren deposit and from surface drainage from historic waste materials, produces water with low pH, high acidity and sulfate, and high concentrations of Al, Cu, Fe, Mn, and Zn that contaminate Daisy Creek (USEPA 1977).

Periphyton samples were collected at two sites on August 25,

2001. The upper site (Y04USR03) is located just below Daisy Creek (Map 1). The lower site (Y04USR04) is located about a mile and a half downstream just above the Wilderness boundary (Map 2). Coordinates of the sampling sites are as follows:

Y04USR03 45 04 05 N/109 59 39 W
Y04USR04 45 05 32 N/109 59 34 W

Land uses in the Stillwater River basin include wilderness, hardrock mining, recreation, and wildlife. The Stillwater River is classified B-1 in the Montana Surface Water Quality Standards.

METHODS

Periphyton samples were collected by Patrick Newby (Water Monitoring Section, MDEQ Monitoring and Data Management Bureau) following standard operating procedures of the MDEQ Planning, Prevention, and Assistance Division.

Using appropriate tools, microalgae were scraped, brushed, or sucked from natural substrates in proportion to the rank of those substrates at the study site. Macroalgae were picked by hand in proportion to their abundance at the site. All collections of microalgae and macroalgae were pooled into a common container and preserved with Lugol's solution.

The samples were examined to estimate the relative abundance and rank by biovolume of diatoms and genera of soft (non-diatom) algae according to the method described by Bahls (1993). Soft algae were identified using Dillard (1999), Prescott (1978), Smith (1950), and Whitford and Schumacher (1984). These books also served as references on the ecology of the soft algae, along with Palmer (1977).

After the identification of soft algae, the raw periphyton samples were cleaned of organic matter using sulfuric acid and postassium dichromate, and permanent diatom slides were prepared using Naphrax, a high refractive index mounting medium, following *Standard Methods for the Examination of Water and Wastewater* (APHA 1998).

Between 405 and 415 diatom cells (810 to 830 valves) were counted at random and identified to species. The four volume series by Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b) was used as the main taxonomic and autecological reference for the diatoms. Lowe (1974), Bahls et al. (1984), van Dam et al. (1994), and Lange-Bertalot (1996) were also used as ecological references for the diatoms.

The diatom proportional counts were used to generate an array of diatom association metrics (Table 1). A metric is a characteristic of the biota that changes in some predictable way with increased human influence (Barbour et al. 1999).

Metric values from the Stillwater River were compared to numeric biocriteria or threshold values developed for streams in the Rocky Mountain Ecoregions of Montana (Table 2). These criteria are based on metric values measured in least-impaired reference streams (Bahls et al. 1992) and on metric values measured in streams that are known to be impaired by various sources and causes of pollution (Bahls 1993).

The criteria in Tables 2 and 3 distinguish among four levels of impairment and three levels of aquatic life use support: no impairment or only minor impairment (full support); moderate impairment (partial support); and severe impairment (nonsupport). These impairment levels correspond to excellent, good, fair, and poor *biological integrity*, respectively.

Quality Assurance. Several steps were taken to assure that the study results are accurate and reproducible.

Upon receipt of the samples, station and sample information were recorded in a laboratory notebook and the samples were assigned a unique number compatible with the Montana Diatom Database, e.g., 1486-02. The first part of this number (1486) designates the sampling site (Stillwater River below Daisy Creek); the second part of this number (02) designates the number of periphyton samples that have been collected at this site to date for which data have been entered into the Montana Diatom Database.

Sample observations and analyses of soft (non-diatom) algae were recorded in a lab notebook along with station and sample information provided by MDEQ. A portion of the raw sample was used to make duplicate diatom slides. After completing the diatom proportional count, the slide used for the count will be deposited in the University of Montana Herbarium in Missoula. The other slide will be retained by *Hannaea* in Helena.

Station information, sample information, and diatom proportional count data have been entered into the Montana Diatom Database, maintained by *Hannaea* in Microsoft Access.

RESULTS AND DISCUSSION

Results are presented in Tables 3 and 4, which are located near the end of this report following the References section. Spreadsheets containing completed diatom proportional counts, with species' pollution tolerance classes (PTC) and percent abundances, are attached as Appendix A.

SAMPLE NOTES

Below Daisy Creek (Y04USR03). Most of the diatoms in this sample were living (contained protoplasm) but a good many are deformed. The bulk of the sample consisted of an orange floc in which fine particles of inorganic sediment were trapped. The matrix of the floc appears to consist of bacterial and algal (including diatom) mucilage. Also present were several short bacterial filaments lying loose in the sample and millions of very tiny (<1 micron diameter) particles (coccoid bacteria?) moving about in Brownian motion.

Above Wilderness Boundary (Y04USR04). Most diatoms appeared to be alive and diatom diversity was good. The same species of *Oscillatoria* present upstream was also found here. The species of *Phormidium* here had very narrow filaments.

NON-DIATOM ALGAE

Besides diatoms, the only genus of algae present below Daisy Creek was a cyanobacterium--*Oscillatoria* (Table 3). *Oscillatoria* includes many species that tolerate a wide range of environmental conditions. The very low genus richness below Daisy Creek suggests impairment. Living diatoms were abundant at this site, suggesting that the impairment was not severe.

Five genera of non-diatom algae were recorded at the site above the Wilderness boundary, including green algae and cyanobacteria (Table 3). This increase in genetic richness indicates a degree of recovery from the site upstream. The filamentous green alga *Mougeotia* ranked second in biomass above the Wilderness boundary. Some species of *Mougeotia* prefer waters with low pH and low conductivity. Diatoms were also abundant at this site and most of the diatoms were living.

DIATOMS

All of the major diatom species in the upper Stillwater River are either tolerant of a wide range of trophic conditions from oligotrophic to eutrophic or exhibit their most vigorous growth in stronger mesotrophic to eutrophic waters (Table 4). Among the tolerant species is *Encyonema minutum*, which dominated the diatom assemblage below Daisy Creek.

Diatom species richness and diversity were depressed at both sites (Table 4). Very low diversity and a large percentage of *Encyonema minutum* indicated moderate impairment and partial support of aquatic life uses below Daisy Creek. Above the Wilderness boundary, impairment was minor and aquatic life uses were fully supported.

A relatively large number of teratological diatom cells also indicates moderate impairment below Daisy Creek (Table 4). Abnormal valves were observed in *Achnanthidium minutissimum* and *Cocconeis placentula*. The cause of these deformities is probably heavy metals. The number of teratological cells was much smaller above the Wilderness boundary, indicating only minor impairment.

The disturbance index (% *Achnanthidium minutissimum*) indicated minor impairment at both sites. *Achnanthidium minutissimum* is tolerant of heavy metals and typically colonizes recently scoured sites. Large numbers of this species indicate a combination of chemical and physical disturbance at both sites.

The pollution index indicated minor impairment from organic loading below Daisy Creek and no impairment above the Wilderness boundary (Table 4). The source of the organic loading below Daisy Creek is unknown, but it may be internal and result from the *in situ* production of algal slimes.

The two Stillwater River sites shared 58.27% of their diatom floras (Table 4). This indicates that the two communities are somewhat similar and that a small amount of environmental change (recovery) occurred between the two sites.

Periphyton samples were collected from these same two sites in August 1994 (Bahls 1995). A bioassessment based on these samples, using the same methods used here, resulted in the same ratings of biological integrity: "Fair" (moderate impairment) below Daisy Creek and "Good" (minor impairment) above the Wilderness boundary. In both cases, low diatom diversity was the reason for the less than excellent ratings.

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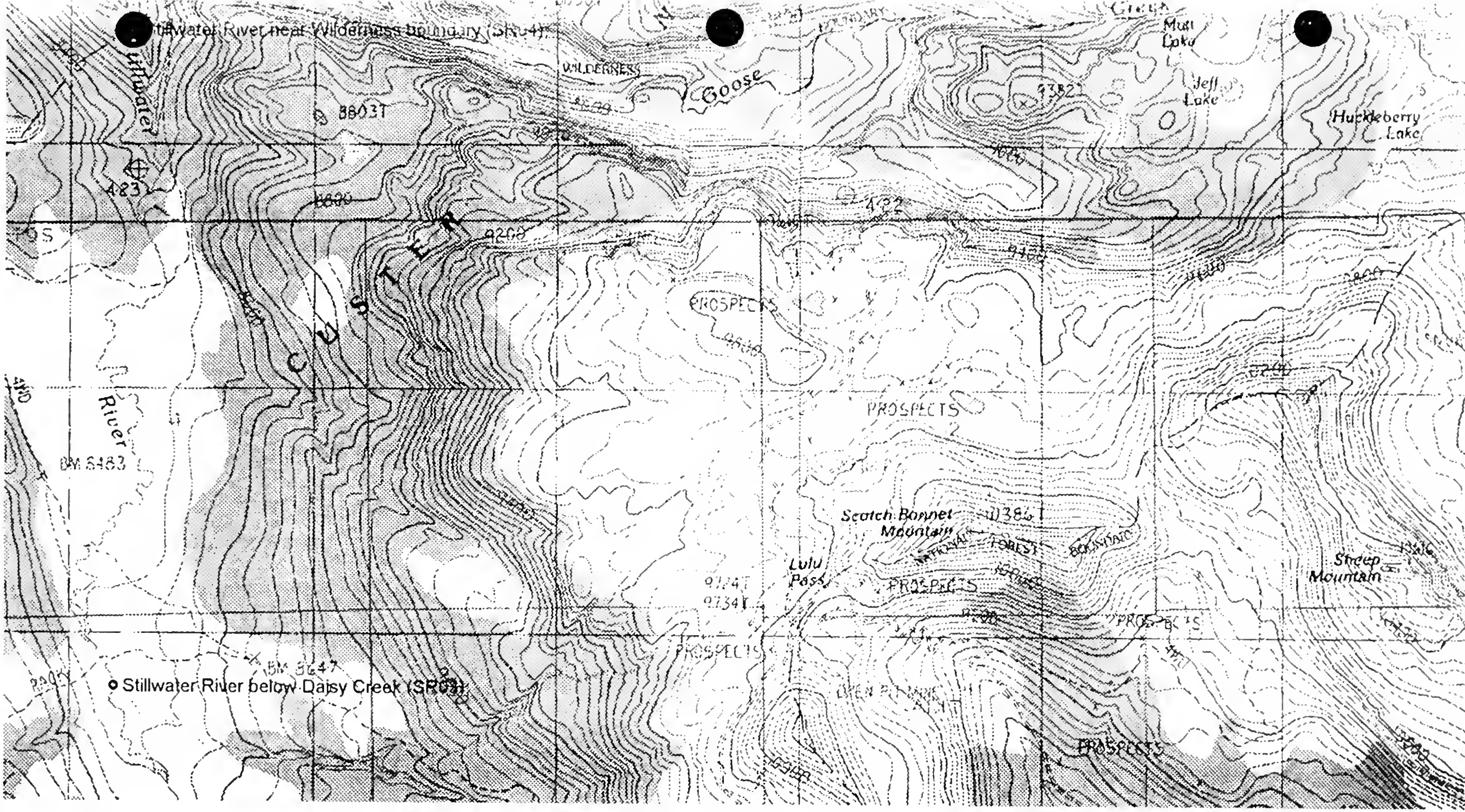

Map 1. Periphyton sampling stations on the upper Stillwater River.

'Cooke City; MT,WY'; Scale: 1" = 0.357Mi 575Mt 1,887Ft, 1 Mi = 2.798" , 1 cm = 226Mt

Table 1. Diatom association metrics used to evaluate biological integrity in Montana streams: reference, range of values in Montana streams, and expected direction of metric response to increasing anthropogenic perturbation or natural stress.

Metric	Reference	Range of Values	Expected Response
Shannon Species Diversity	Bahls 1979	0.00-5.00+	Decrease[1]
Pollution Index[2]	Bahls 1993	1.00-3.00	Decrease
Siltation Index[3]	Bahls 1993	0.00-90.0+	Increase
Disturbance Index[4]	Barbour *et al.* 1999	0.00-100.0	Increase
No. Species Counted	Bahls 1979, 1993	0-100+	Decrease[1]
Percent Dominant Species	Barbour *et al.* 1999	5.0-100.0	Increase
Percent Abnormal Cells	McFarland *et al.* 1997	0.0-20.0+	Increase
Similarity Index	Whittaker 1952	0.0-80.0+	Decrease
Percent Epithemiaceae	Stevenson & Pan 1999	0.0-80.0+	Decrease
Percent Aerophiles	Johansen 1999	0.0-100	Increase

[1] Shannon diversity and species richness may increase somewhat in naturally nutrient-poor mountain streams in response to slight to moderate increases in nutrients or sediment.

[2] Composite numeric expression of the pollution tolerances assigned by Lange-Bertalot (1979) to the common diatom species.

[3] Sum of the percent abundances of all species in the genera *Navicula*, *Nitzschia*, and *Surirella*.

[4] Percent abundance of *Achnanthes minutissima*.

Table 2. Criteria for rating levels of biological integrity, environmental impairment or natural stress, and aquatic life use support in wadeable **mountain** streams of Montana using selected metrics for benthic diatom associations. The lowest rating for any one metric is the overall rating for the study site.

Biological Integrity/ Impairment or Natural Stress/Use Support	Diversity Index (Shannon)	Pollution Index	Siltation Index	Disturbance Index	Number of Species Counted	Percent Dominant Species	Percent Abnormal Cells	Similarity Index[1]
Excellent None/Full Support	>2.99	>2.50	<20.0	<25.0	>29	<25.0	0.0	>59.9
Good/Minor Full Support	2.00-2.99	2.01-2.50	20.0-39.9	25.0-49.9	20-29	25.0-49.9	>0.0-<1.0	40.0-59.9
Fair/Moderate Partial Support	1.00-1.99	1.50-2.00	40.0-59.9	50.0-74.9	10-19	50.0-74.9	1.0-9.9	20.0-39.9
Poor/Severe Nonsupport	<1.00	<1.50	>59.9	>74.9	<10	>74.9	>9.9	<20.0

[1] The Similarity Index or Percent Community Similarity (Whittaker 1952) may be used to compare a study site to an unimpaired upstream control site on the same stream. This metric measures the degree of floristic similarity between diatom associations at the two sites and is the sum of the smaller of the two percent abundance values for each species that is common to both sites. Adjacent riffles on the same stream, without intervening tributaries or environmental perturbations, will generally have at least 60% of their diatom floras in common (Bahls 1993). PCS may also be used to guage the relative amount of impairment or recovery that occurs between adjacent study sites: >59.9% = very similar floras, no change; 40.0-59.9% = somewhat similar floras, minor change; 20.0-39.9% = somewhat dissimilar floras, moderate change; <20.0% = very dissimilar floras, major change.

Table 3. Relative abundance of cells and rank by biovolume of diatoms and genera of non-diatom algae in periphyton samples collected from the Stillwater River on 8/25/01.

Taxa	Relative Abundance and Rank	
	Below Daisy Creek	Wilderness Boundary
Chlorophyta (green algae)		
Cosmarium		rare/6
Mougeotia		frequent/2
Chrysophyta (golden algae)		
Diatoms	abundant/1	abundant/1
Cyanophyta (cyanobacteria)[1]		
Oscillatoria	common/2	common/4
Phormidium		abundant/3
Schizothrix		occasional/5

[1] Formerly known as blue-green algae

Table 4. Percent abundance of major diatom species[1] and values of selected diatom association metrics for periphyton samples collected from the Stillwater River on 8/25/01.

Species/Metric (Autecology)[3]	Percent Abundance/Metric Values[2] Below Daisy Creek	Wilderness Boundary
Achnanthidium minutissimum (tol)	27.04	33.49
Cymbella cistula (eu)		14.70
Encyonema minutum (tol)	61.48	27.45
Encyonema silesiacum (tol)	0.62	8.43
Meridion circulare (eu)	0.62	5.66
Staurosira construens (tol)		3.98
Cells Counted	405	415
Total Species	32	27
Species Counted	26	22
Species Diversity	**1.79**	2.64
Percent Dominant Species	**61.48**	27.45
Disturbance Index	27.04	33.49
Pollution Index	2.28	2.58
Siltation Index	4.07	1.08
Percent Abnormal Cells	**3.46**	0.48
Percent Epithemiaceae	0.00	0.00
Similarity Index	58.27	

[1] A major diatom species is one that accounts for 3% or more of the cells in one or more samples of a sample set

[2] Underlined values indicate good biological integrity, minor impairment, and full support of aquatic life uses; **bold values** indicate fair biological integrity, moderate impairment, and partial support of aquatic life uses; all other values indicate excellent biological integrity, no impairment, and full support of aquatic life uses when compared to criteria for mountain streams in Table 2.

[3] Autecology

eu: best growth in stronger mesotrophic to eutrophic waters

tol: tolerant of a wide range of trophic status from oligo- to eutrophic waters without discernible preference

Sources: Lange-Bertalot, H. 1979. Pollution Tolerance of Diatoms as a Criterion for Water Quality Estimation. Nova Hedwigia, Beiheft 64, pp. 285-304.

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APPENDIX A: DIATOM PROPORTIONAL COUNTS

Sample	Genus/Species/Variety	Pollution Tolerance Class	Count	Percent
148602	Achnanthes lanceolata	2	6	0.74
148602	Achnanthidium affine	3	2	0.25
148602	Achnanthidium minutissimum	3	219	27.04
148602	Cocconeis placentula	3	2	0.25
148602	Diatoma mesodon	3	8	0.99
148602	Encyonema minutum	2	498	61.48
148602	Encyonema silesiacum	2	5	0.62
148602	Eunotia exigua .	3	2	0.25
148602	Eunotia minor	2	2	0.25
148602	Fragilaria vaucheriae	2	14	1.73
148602	Gomphonema angustatum	2	3	0.37
148602	Gomphonema angustum	3	2	0.25
148602	Gomphonema bohemicum	3	4	0.49
148602	Gomphonema minutiforme	3	0	0.00
148602	Hannaea arcus	3	3	0.37
148602	Hantzschia amphioxys	2	0	0.00
148602	Meridion circulare	3	5	0.62
148602	Navicula acceptata	2	0	0.00
148602	Navicula atomus	1	4	0.49
148602	Navicula cincta	1	4	0.49
148602	Navicula cryptocephala	3	0	0.00
148602	Navicula lundii	2	2	0.25
148602	Navicula minima	1	8	0.99
148602	Navicula minuscula	1	0	0.00
148602	Navicula pelliculosa	1	4	0.49
148602	Navicula sp.	2	2	0.25
148602	Nitzschia fonticola	3	2	0.25
148602	Nitzschia frustulum	2	2	0.25
148602	Nitzschia palea	1	4	0.49
148602	Nitzschia paleacea	2	1	0.12
148602	Pinnularia borealis	2	0	0.00
148602	Rhoicosphenia curvata	3	2	0.25

Sample	Genus/Species/Variety	Pollution Tolerance Class	Count	Percent
148702	Achnanthidium minutissimum	3	278	33.49
148702	Caloneis bacillum	2	0	0.00
148702	Caloneis silicula	2	2	0.24
148702	Cymbella affinis	3	0	0.00
148702	Cymbella cistula	3	122	14.70
148702	Diatoma mesodon	3	8	0.96
148702	Encyonema minutum	2	227	27.35
148702	Encyonema reichardtii	3	2	0.24
148702	Encyonema silesiacum	2	70	8.43
148702	Epithemia adnata	2	0	0.00
148702	Eunotia exigua	3	2	0.24
148702	Fragilaria vaucheriae	2	6	0.72
148702	Gomphonema angustum	3	1	0.12
148702	Gomphonema parvulum	1	15	1.81
148702	Hannaea arcus	3	1	0.12
148702	Meridion circulare	3	47	5.66
148702	Navicula cincta	1	0	0.00
148702	Navicula exilis	2	2	0.24
148702	Navicula seminulum	1	2	0.24
148702	Nitzschia fonticola	3	2	0.24
148702	Nitzschia palea	1	2	0.24
148702	Pinnularia borealis	2	1	0.12
148702	Pinnularia subcapitata	3	2	0.24
148702	Staurosira construens	3	33	3.98
148702	Staurosirella pinnata	3	4	0.48
148702	Surirella gracilis	2	1	0.12
148702	Surirella minuta	2	0	0.00