BIOLOGICAL INTEGRITY OF EAST ROSEBUD CREEK BASED ON THE COMPOSITION AND STRUCTURE OF THE BENTHIC ALGAE COMMUNITY

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

Project Officer: Patrick Newby
Monitoring and Data Management Bureau

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Prepared by:

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

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SUMMARY

In August 2000, composite periphyton samples were collected from East Rosebud Creek near Roscoe, Montana for the purpose of assessing whether this stream 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.

The very small biovolume of algae at Station 1 indicates very cold and nutrient-poor water entering the study reach. An increase in biovolume and an occasional pollution tolerant alga at Station 2 indicate that a slight increase in nutrients and water temperature occurred between Station 1 and Station 2.

Dominance of the diatom assemblage by *Achnanthes minutissima* indicates moderate stress at Station 1 and minor stress at Station 2. This stress is likely natural in origin and caused by the swift, cold, and nutrient-poor waters entering the study reach from the mountains upstream.

The decline in abundance of *Achnanthes minutissima* from Station 1 to Station 2 was accompanied by an increase in diatom diversity and diatom species richness, and by a small decrease in the pollution index. Such changes also reflect a slight increase in nutrients and water temperature between the two sites.

The siltation index also increased between the two sites, but values at both stations were within acceptable limits for a mountain stream. A few abnormal diatom valves were observed at Station 1, but numbers of deformed valves were within acceptable limits. The two sites had almost two-thirds of their diatom associations in common, indicating that only a small change in water quality occurred between Station 1 and Station 2.

INTRODUCTION

This report evaluates the biological integrity, support of aquatic life uses, and probable causes of impairment to those uses in East Rosebud Creek, a tributary of the Stillwater River in the Yellowstone River Basin of southcentral Montana. The purpose of this report is to provide information that will help the State of Montana determine whether East Rosebud Creek 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 sites that were sampled on August 18, 2000. 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 in Carbon County in southcentral Montana. East Rosebud Creek heads on the Beartooth Plateau in the Absaroka-Beartooth Wilderness and its watershed includes Granite Peak (el. 12,799 feet), Montana's highest peak. East Rosebud Creek is a third-order tributary of Rosebud Creek, which is a tributary of the Stillwater River.

East Rosebud Creek begins in the Middle Rockies Ecoregion and enters the Montana Valley and Foothill Prairies Ecoregion at the upper end of the study reach (Woods et al. 1999). The surface geology of the upper watershed consists of Precambrian metamorphic rocks (Renfro and Feray 1972). Vegetation is alpine tundra at the highest elevations, conifer forest at middle elevations, and grassland along the study reach (USDA 1976).

Periphyton samples were collected at two sites on East Rosebud Creek on August 18, 2000 (Map 1, Table 1). The upper site at the National Forest boundary is located at an elevation of about 5,400 feet. Elevation of the lower site above Roscoe is about 5,200 feet. East Rosebud Creek is classified B-1 in the Montana Surface Water Quality Standards.

METHODS

Periphyton samples were collected by Patrick Newby of the

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 in 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 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 436 and 440 diatom cells (872 to 880 valves) were counted at random and identified to species. The following were used as the main taxonomic and autecological references for the diatoms: Krammer and Lange-Bertalot 1986, 1988, 1991a, 1991b; Patrick and Reimer 1966, 1975. Lowe (1974) was also used as an ecological reference for the diatoms.

The diatom proportional counts were used to generate an array of diatom association metrics (Table 2). 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 East Rosebud Creek were compared to numeric biocriteria or threshold values developed for streams in the Rocky Mountain Ecoregions of Montana (Table 3). 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 Table 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., 2000-01. The first part of this number (2000) designates the sampling site (East Rosebud Creek Station 1); the second part of this number (01) designates the number of periphyton samples that have been collected at this site to date for which data have been entered into the 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.

On completion of the project, station information, sample information, and diatom proportional count data will be entered into the Montana Diatom Database.

RESULTS AND DISCUSSION

Results are presented in Tables 4 and 5, which are located near the end of this report following the Literature Cited section. Spreadsheets containing completed diatom proportional counts, with species' pollution tolerance classes (PTC) and percent abundances, are attached as Appendix A.

SAMPLE NOTES

East Rosebud Creek Station 1. This sample consisted of two small rocks, which I brushed with a toothbrush into the sample container. Algae in this sample were very sparse.

East Rosebud Creek Station 2. This sample contained macrophytes. The diatom assemblage at this site included *Didymosphenia geminata*, a large, stalked, gomphonemoid diatom with striking ornamentation.

NON-DIATOM ALGAE

Both sites on East Rosebud Creek supported green algae, diatoms, and cyanobacteria (Table 4). Green algae accounted for most of the biomass at both sites, while diatoms ranked second and cyanobacteria ranked third. *Euglena*, an indicator of organic pollution, was rare at Station 2.

Algae, including diatoms, were very sparse at Station 1, probably reflecting the cold, nutrient-poor water at this site.

Nevertheless, 8 genera of non-diatom algae were found in the sample collected at Station 1 (Table 4). The number of non-diatom genera increased to 10 at Station 2, perhaps in response to a small increase in nutrients and water temperature at this site. Unimpaired mountain streams in Montana typically support 1 to 10 genera of common non-diatom algae (Bahls 1993).

DIATOMS

All but 2 of the 7 major diatom species in East Rosebud Creek--including the dominant species--were sensitive to pollution (Table 5). The dominant species at both sites was *Achnanthes minutissima*. This opportunistic pioneer species is a good indicator of disturbance or stress, which may be caused by physical factors (e.g., channel scour), biological factors (e.g., invertebrate grazing), or chemical factors (e.g., heavy metal toxicity).

Such stress may be natural or cultural (anthropogenic) in origin. In the case of East Rosebud Creek, this stress is likely derived from the naturally swift, cold, and nutrient-poor waters entering the study reach. Hence, the moderate impairment recorded at Station 1 and the minor impairment recorded at Station 2 can most likely be attributed to natural causes.

The number of diatom species observed and counted, as well as diatom species diversity, increased significantly from Station 1 to Station 2 (Table 5). This was probably in response to small increases in nutrients and water temperature as the stream left the mountains and entered the foothills. Warmer, lower gradient foothill and prairie streams in Montana typically support a more diverse diatom flora than their colder and steeper counterparts in the mountains (Bahls 1993; Bahls et al. 1992).

As expected, the pollution index fell slightly from Station 1 to Station 2, indicating a small increase in nutrients between the two sites (Table 5). The siltation index also increased, but both values were within acceptable limits for mountain streams. Station 1 supported a few deformed valves of *Achnanthes minutissima* and *Synedra rumpens*, but the number of abnormal valves was within acceptable limits. The two sites had almost two-thirds of their diatom floras in common, indicating only a minor change in water quality between the sites.

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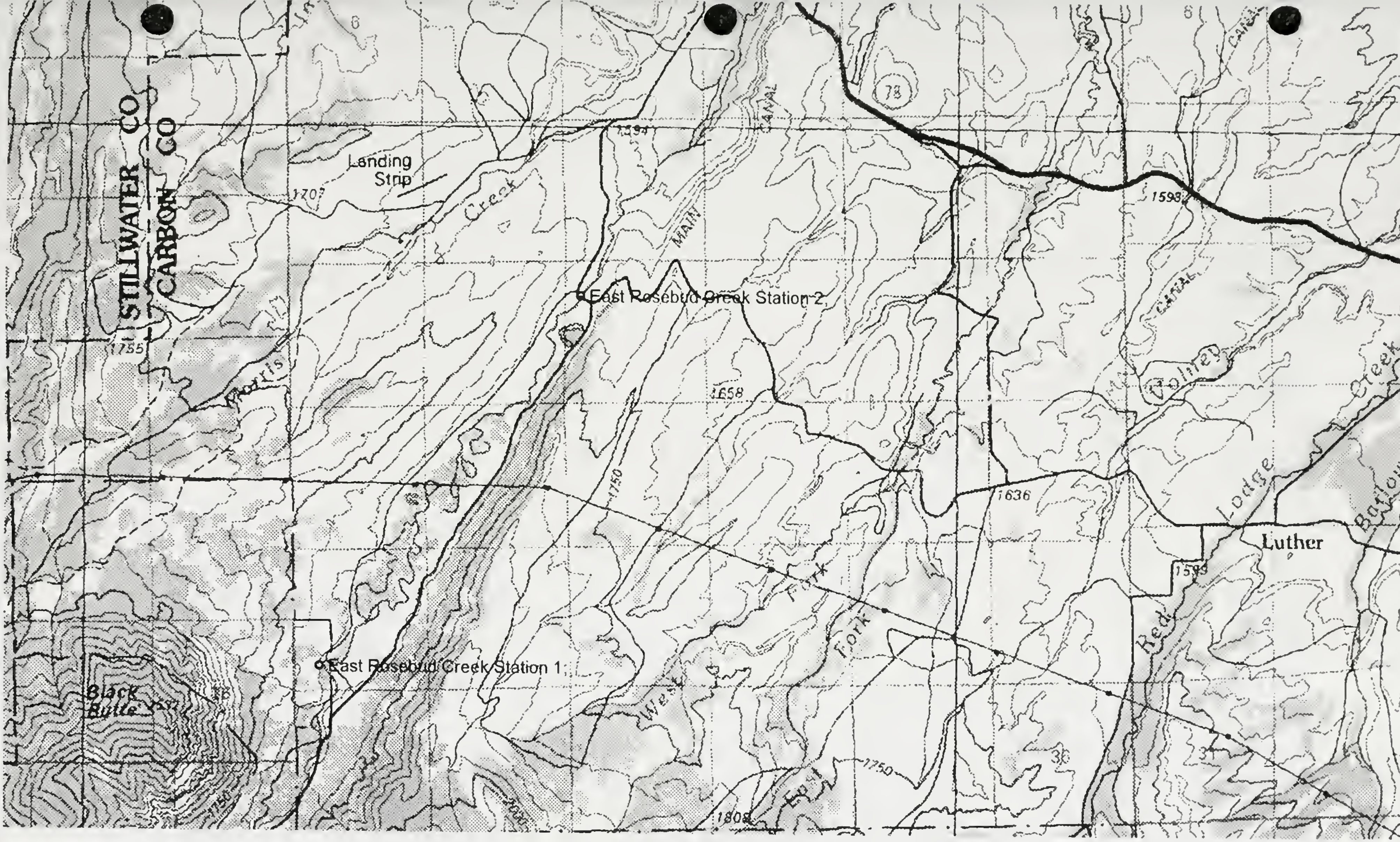

MAP 1.

'Red Lodge 100K; MT,WY'; Scale: 1" = 0.953Mi 1,534Mt 5,031Ft, 1 Mi = 1.049" , 1 cm = 604Mt

Table 1. Location of periphyton stations on East Rosebud Creek: Station codes, sample numbers in the Montana Diatom Database, latitudes and longitudes, and sample dates. Stations are listed in order from upstream to downstream.

Location	Station Code	Sample Number	Latitude/ Longitude	Sample Date
East Rosebud Creek at forest boundary near canyon mouth	Station 1	2000-01	45 16 16/ 109 33 49	08/18/00
East Rosebud Creek above Roscoe	Station 2	2001-01	45 18 31/ 109 31 29	08/18/00

Table 2. 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*, plus the species *Cymbella sinuata*.

[4] Percent abundance of *Achnanthes minutissima*.

Table 3. 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 4. Relative abundance of cells and rank by biovolume of diatoms and genera of non-diatom algae in periphyton samples collected from E. Rosebud Cr. in August 2000.

Taxa	Relative Abundance[1] and (Rank)	
	Station 1	Station 2
Chlorophyta (green algae)		
Ankistrodesmus	rare (9)	occasional (11)
Closterium	rare (6)	occasional (7)
Cosmarium		occasional (8)
Mougeotia	occasional (2)	abundant (3)
Oedogonium		dominant (1)
Spirogyra	occasional (1)	frequent (4)
Staurastrum		occasional (9)
Ulothrix	occasional (5)	
Zygnema	occasional (4)	
Euglenophyta		
Euglena		rare (10)
Chrysophyta (golden algae)		
Diatoms	occasional (3)	abundant (2)
Cyanophyta (cyanobacteria)[2]		
Amphithrix	occasional (8)	
Merismopedia		common (6)
Oscillatoria	occasional (7)	frequent (5)

[1] d = dominant; a = abundant; f = frequent; c = common; o = occasional; r = rare

[2] Formerly known as blue-green algae.

Table 5. Percent abundance of major diatom species[1] and values of selected diatom association metrics for periphyton samples collected from East Rosebud Creek on August 18, 2000.

Species/Metric (Pollution Tolerance Class)[3]	Percent Abundance/Metric Values[2] Station 1	Station 2
Achnanthes minutissima (3)	55.28	43.64
Cymbella silesiaca (2)	3.21	2.50
Fragilaria capucina (2)	0.57	6.93
Fragilaria construens (3)	4.36	11.48
Gomphonema subtile (3)	4.70	0.34
Nitzschia perminuta (3)	1.83	3.41
Reimeria sinuata (3)	5.85	0.34
Cells Counted	436	440
Total Species	43	63
Species Counted	40	51
Species Diversity	3.00	3.55
Percent Dominant Species	**55.28**	<u>43.64</u>
Disturbance Index	**55.28**	<u>43.64</u>
Pollution Index	2.85	2.73
Siltation Index	2.98	14.32
Percent Abnormal Cells	<u>0.57</u>	0.00
Percent Epithemiaceae	0.00	0.00
Similarity Index	62.72	

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

[2] <u>Underlined values</u> 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 3.

[3] 3 = sensitive to pollution; 2 = tolerant of pollution; 1 = most tolerant of pollution.

APPENDIX A: DIATOM PROPORTIONAL COUNTS

Sample	Genus/Species/Variety	Pollution Tolerance Class	Count	Percent
200001	Achnanthes biasolettiana	3	2	0.23
200001	Achnanthes bioretii	3	12	1.38
200001	Achnanthes flexella	3	2	0.23
200001	Achnanthes laevis	3	8	0.92
200001	Achnanthes lanceolata	2	1	0.11
200001	Achnanthes minutissima	3	482	55.28
200001	Amphora inariensis	3	2	0.23
200001	Asterionella formosa	3	1	0.11
200001	Cocconeis placentula	3	8	0.92
200001	Cyclotella stelligera	3	2	0.23
200001	Cymbella hustedtii	3	4	0.46
200001	Cymbella minuta	2	4	0.46
200001	Cymbella naviculiformis	3	2	0.23
200001	Cymbella reichardtii	3	2	0.23
200001	Cymbella silesiaca	2	28	3.21
200001	Diatoma mesodon	3	0	0.00
200001	Eunotia minor	2	2	0.23
200001	Fragilaria brevistriata	3	9	1.03
200001	Fragilaria capucina	2	5	0.57
200001	Fragilaria construens	3	38	4.36
200001	Fragilaria crotonensis	3	7	0.80
200001	Fragilaria leptostauron	3	10	1.15
200001	Fragilaria vaucheriae	2	8	0.92
200001	Gomphonema dichotomum	3	17	1.95
200001	Gomphonema minutiforme	3	2	0.23
200001	Gomphonema minutum	3	14	1.61
200001	Gomphonema olivaceoides	3	4	0.46
200001	Gomphonema parvulum	1	21	2.41
200001	Gomphonema pumilum	3	21	2.41
200001	Gomphonema subtile	3	41	4.70
200001	Hannaea arcus	3	5	0.57
200001	Navicula cari	2	1	0.11
200001	Navicula novaesiberica	2	1	0.11
200001	Nitzschia gracilis	2	2	0.23
200001	Nitzschia linearis	2	4	0.46
200001	Nitzschia palea	3	2	0.23
200001	Nitzschia perminuta	3	16	1.83
200001	Pinnularia microstauron	2	3	0.34
200001	Reimeria sinuata	3	51	5.85
200001	Stauroneis anceps	3	0	0.00
200001	Synedra rumpens	2	23	2.64
200001	Synedra ulna	2	5	0.57
200001	Tabellaria flocculosa	3	0	0.00

Sample	Genus/Species/Variety	Pollution Tolerance Class	Count	Percent
200101	Achnanthes biasolettiana	3	2	0.23
200101	Achnanthes bioretii	3	9	1.02
200101	Achnanthes calcar	3	0	0.00
200101	Achnanthes flexella	3	2	0.23
200101	Achnanthes laevis	3	3	0.34
200101	Achnanthes lanceolata	2	3	0.34
200101	Achnanthes minutissima	3	384	43.64
200101	Achnanthes peragalli	3	0	0.00
200101	Amphora libyca	3	0	0.00
200101	Amphora pediculus	3	2	0.23
200101	Anomoeoneis vitrea	3	5	0.57
200101	Asterionella formosa	3	9	1.02
200101	Cocconeis placentula	3	0	0.00
200101	Craticula cuspidata	2	0	0.00
200101	Cyclotella ocellata	3	1	0.11
200101	Cyclotella stelligera	3	3	0.34
200101	Cymbella cesatii	3	7	0.80
200101	Cymbella cymbiformis	3	2	0.23
200101	Cymbella minuta	2	16	1.82
200101	Cymbella silesiaca	2	22	2.50
200101	Diatoma mesodon	3	3	0.34
200101	Didymosphenia geminata	3	2	0.23
200101	Fragilaria brevistriata	3	25	2.84
200101	Fragilaria capucina	2	61	6.93
200101	Fragilaria construens	3	101	11.48
200101	Fragilaria lapponica	3	4	0.45
200101	Fragilaria leptostauron	3	25	2.84
200101	Fragilaria pinnata	3	21	2.39
200101	Fragilaria vaucheriae	2	8	0.91
200101	Gomphonema minutum	3	0	0.00
200101	Gomphonema olivaceoides	3	9	1.02
200101	Gomphonema parvulum	1	2	0.23
200101	Gomphonema subtile	3	3	0.34
200101	Hannaea arcus	3	7	0.80
200101	Navicula arvensis	1	4	0.45
200101	Navicula capitata	2	5	0.57
200101	Navicula cryptocephala	3	8	0.91
200101	Navicula cryptotenella	2	2	0.23
200101	Navicula declivis	3	0	0.00
200101	Navicula libonensis	2	0	0.00
200101	Navicula menisculus	2	4	0.45
200101	Navicula meniscus	2	1	0.11
200101	Navicula minima	1	4	0.45
200101	Navicula minuscula	1	6	0.68
200101	Navicula perminuta	3	30	3.41
200101	Navicula pseudoscutiformis	3	2	0.23
200101	Navicula pupula	2	0	0.00

Sample	Genus/Species/Variety	Pollution Tolerance Class	Count	Percent
200101	Navicula rhynchocephala	3	2	0.23
200101	Navicula viridula v. linearis	2	15	1.70
200101	Neidium ampliatum	3	0	0.00
200101	Nitzschia dissipata	3	0	0.00
200101	Nitzschia gracilis	2	4	0.45
200101	Nitzschia linearis	2	8	0.91
200101	Nitzschia palea	1	22	2.50
200101	Nitzschia paleacea	2	2	0.23
200101	Nitzschia pura	2	5	0.57
200101	Nitzschia sigmoidea	3	1	0.11
200101	Reimeria sinuata	3	3	0.34
200101	Stauroneis smithii	2	0	0.00
200101	Surirella tenera	3	1	0.11
200101	Synedra rumpens	2	8	0.91
200101	Synedra ulna	2	1	0.11
200101	Tabellaria flocculosa	3	1	0.11