
BLACKFOOT WATERSHED WATER QUALITY STATUS AND TRENDS MONITORING PROJECT

2004 CHLOROPHYLL DATA EVALUATION

Prepared for:

BLACKFOOT CHALLENGE
P.O. Box 103
Ovando, MT 59854

Prepared by:

PBS&J
801 N. Last Chance Gulch
P.O. Box 239
Helena, MT 59624

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1.0 Background and Introduction

In 2004, the Monitoring Work Group of the Blackfoot Challenge's Habitat and Water Quality Restoration Committee implemented the *Blackfoot Watershed Water Quality Status and Trends Monitoring Program* to provide information for tracking the health of the Blackfoot watershed (Land & Water Consulting 2003). Informational needs that will be addressed by this program include: 1) evaluating the cumulative benefits of restoration projects and TMDLs, 2) detecting the cumulative impacts of land development activities, 3) identifying priority tributaries and mainstem river segments for restoration, and 4) providing a basis for reporting to the public and restoration sponsors on the condition of the Blackfoot watershed.

The *Blackfoot Watershed Water Quality Status and Trends Monitoring Program* includes the annual collection of periphyton (attached algae), macroinvertebrates (aquatic insects), and chlorophyll *a* samples in mid- to late-August at each of 12 fixed monitoring stations on the mainstem Blackfoot River and selected tributaries (**Table 1-1**). Data from the program are evaluated on a periodic basis and will be used to describe the status, spatial patterns, and time trends in water quality in the Blackfoot watershed.

This report evaluates data for chlorophyll *a* samples collected during August 2004. Chlorophyll *a* concentration data provide a convenient surrogate measure of algal densities, or standing crops, in the Blackfoot watershed. The amount of algae growing in a stream may provide an indication of nutrient (nitrogen and phosphorus) enrichment from natural or man-caused sources. Excessive levels of algae can interfere with the beneficial uses of a stream, including fisheries and associated aquatic life, recreation and aesthetics, and others. Evaluating trends in chlorophyll concentrations over time may allow the early detection of nutrient enrichment problems.

Table 1-1. 2004 Blackfoot watershed chlorophyll *a* sampling locations and dates.

SITE NUMBER	STREAM NAME / SITE LOCATION	DATE SAMPLED
B-1	Blackfoot River below Alice Creek	8/24/2004
B-2	Landers Fork near Lincoln	8/24/2004
B-3	Blackfoot River at Dalton Mountain Rd. near Lincoln	8/24/2004
B-4	Blackfoot River above Nevada Creek near Helmville	8/24/2004
B-5	Nevada Creek below Nevada Creek Reservoir	8/25/2004
B-6	Nevada Creek near mouth	8/25/2004
B-7	Blackfoot River at Raymond Bridge	8/25/2004
B-8	North Fork Blackfoot River above Dry Gulch	8/26/2004
B-9	Monture Creek near Ovando	8/29/2004
B-10	Blackfoot River at Scotty Brown Bridge	8/29/2004
B-11	Clearwater River at Clearwater	8/26/2004
B-12	Blackfoot River near Bonner	8/26/2004

2.0 Methods

Field Sampling

In late-August 2004, 10 replicate 28.6 cm² samples of periphyton were collected for chlorophyll *a* analysis at each of 12 fixed monitoring stations in the Blackfoot watershed, including six mainstem river locations plus the Landers Fork, Nevada Creek (2 stations), North Fork, Monture Creek, and the Clearwater River. Chlorophyll *a* concentrations are expressed as milligrams per square meter (mg/M²) of stream bottom surface area.

Previous visual observations of periphyton growth in the Blackfoot River watershed indicated highly variable growth patterns within and between the various sample sites. The decision to collect 10 replicate samples for chlorophyll *a* analysis at each monitoring location reflected this expected variability and was based on statistical analysis of data sets from other rivers in Montana (Land & Water 2004), recommendations from Dr. Vicki Watson based on long-time experience in the greater Clark Fork watershed, and cost considerations.

Samples were collected using a 28.6 cm² cylindrical PVC plastic sampling frame attached to a harness assembly composed of a 4-inch disc of PVC fabric and three 1-inch wide buckle straps (**Figure 2-1**). The PVC frame was equipped with a ring of closed cell foam that allowed the frame to be secured to a stream bottom cobble with a water tight seal. Ten randomly selected 4 to 8-inch diameter stream bottom cobbles were individually collected from riffle habitats at each sampling site and placed on the streambank. The sampling frame was carefully attached to the surface of each rock and a small volume (about 5 ml) of methanol was initially decanted into the frame. A cordless rotary tool equipped with a round steel brush and a small stiff-bristled artist's brush was used to remove the attached algae from the rock. The alcohol/algae slurry was then carefully transferred to a screw capped 50 ml disposable plastic centrifuge tube with the use of a disposable plastic pipette. More methanol was applied to the sampling frame and the brushing process was repeated until all visible algae were removed from the surface of the rock within the confines of the sampling frame. When the methanol no longer developed a visible greenish or yellowish color and no more visible organic material remained on the surface of the stone, the extraction process was deemed to be complete. Immediately upon collection, each replicate chlorophyll sample container was wrapped in aluminum foil to exclude light and was placed in a cooler containing dry ice. Samples were delivered to the laboratory within 24 hours of collection for analysis.

Figure 2-1. Sampling frame used to collect 2004 Blackfoot watershed chlorophyll *a* samples.



Laboratory Analysis

The methanol used in the field to remove the algal material from the natural substrates is highly efficient at extracting photosynthetic pigments from the algal biomass without the need for tissue grinding in the laboratory (Lee Harbour, MT DEQ, pers. comm.). The methanol is capable of extracting chlorophyll across the intact plant cell membranes. However, one drawback to this method is that the samples need to be analyzed quickly following collection (i.e. within 48 hours) because the chlorophyll begins to degrade under longer holding times.

Laboratory sample treatment following the initial field-extraction of pigments in methanol included centrifugation to remove particulate material. The samples were then made up to a standard 30 milliliter volume using more methanol. The clarified extract was analyzed with a narrow-band spectrophotometer to measure absorption prior to and following acidification. Pre-acidification optical density (OD) absorption values were read at 664 nanometers (nm) for chlorophyll *a* and 750 nm to correct for turbidity. Absorption values were again recorded 90 seconds after acidification with 0.1 mL of hydrochloric acid (0.1 N) using 665 nm for total pheophytin and 750 nm to correct for turbidity. Calculations to determine chlorophyll *a* and pheophytin *a* concentrations followed Standard Methods 10200H (APHA 1998). The analytical method used the monochromatic calculations required by Montana DEQ for pheophytin *a* (ARM 17.30.602 Definitions (5)). All laboratory analyses were completed within 48 hours of sample collection. Raw laboratory data were entered into a series of spreadsheets which calculated and summarized the sample results on a per unit-area basis and also presented laboratory quality assurance results.

3.0 Results

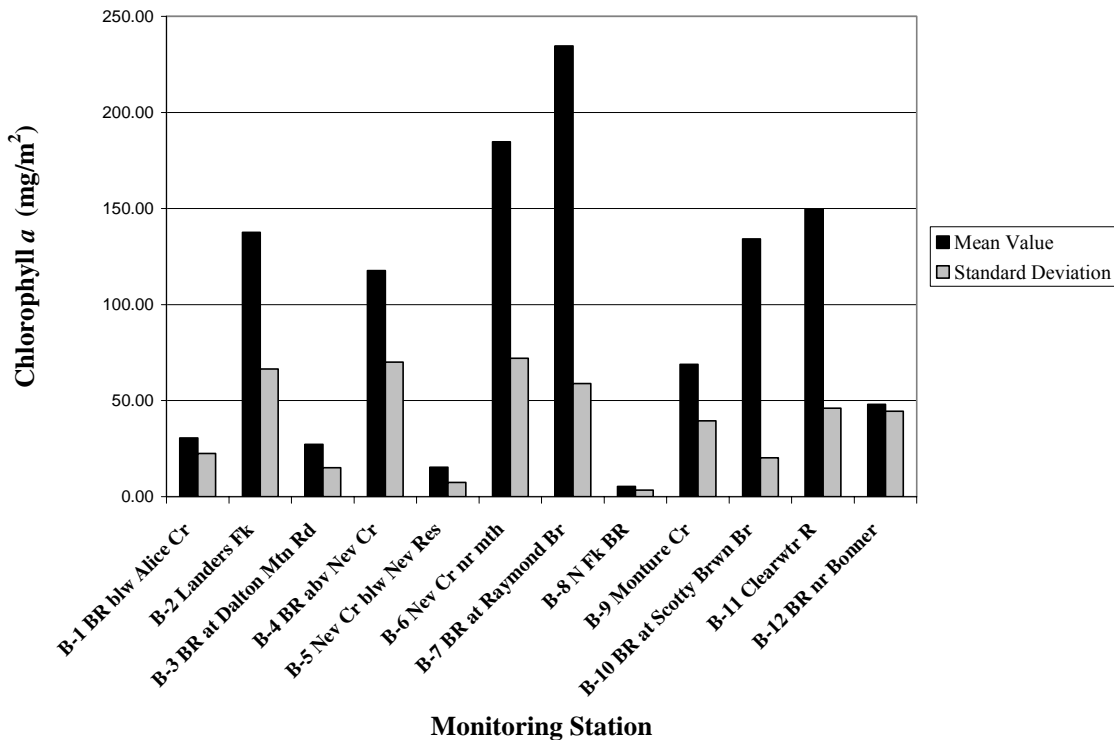
Chlorophyll *a* samples were collected during August 23-29, 2004. Chlorophyll *a* concentration ranges and mean, median, and standard deviation values for each of the 12 Blackfoot watershed monitoring stations are shown in **Tables 3-1**. Concentration means and standard deviation values are shown in the bar graph in **Figure 3-1**. Boxplots showing the median chlorophyll *a* concentration and percentile distribution of the individual replicate data are shown in **Figure 3-2**. Individual chlorophyll data for each monitoring station are included in **Appendix A** to this report.

Table 3-1. 2004 Blackfoot watershed chlorophyll *a* data summary.

Monitoring Station	Range of Chlorophyll <i>a</i> Concentrations (mg/M ²) (N=10)	Mean Chlorophyll <i>a</i> Concentration (mg/M ²)	Median Chlorophyll <i>a</i> Concentration (mg/M ²)	Std. Dev. of Chlorophyll <i>a</i> Concentrations (mg/M ²)
B-1 Blackfoot River below Alice Creek	11.1 – 75.0	30.6	22.3	22.6
B-2 Landers Fork near Lincoln	72.9 – 253.9	137.6	116.8	66.5
B-3 Blackfoot River at Dalton Mountain Road near Lincoln	7.4 – 51.2	27.2	21.9	15.0
B-4 Blackfoot River above Nevada Creek near Helmville	52.0 – 269.2	117.7	95.1	70.1
B-5 Nevada Creek below Nevada Creek Reservoir	5.7 – 25.8	15.3	16.0	7.4
B-6 Nevada Creek near mouth	95.7 – 327.3	184.7	174.0	72.0
B-7 Blackfoot River at Raymond Bridge	174.0 – 329.1	234.6	213.4	58.9
B-8 North Fork Blackfoot River above Dry Gulch	1.4 – 12.1	5.4	4.1	3.4
B-9 Monture Creek near Ovando	12.6 – 149.2	68.9	59.2	39.5
B-10 Blackfoot River at Scotty Brown Bridge	107.0 – 159.7	134.2	135.7	20.2
B-11 Clearwater River at Clearwater	52.6 – 219.5	149.6	150.9	46.1
B-12 Blackfoot River near Bonner	8.7 – 136.8	48.0	30.2	44.4

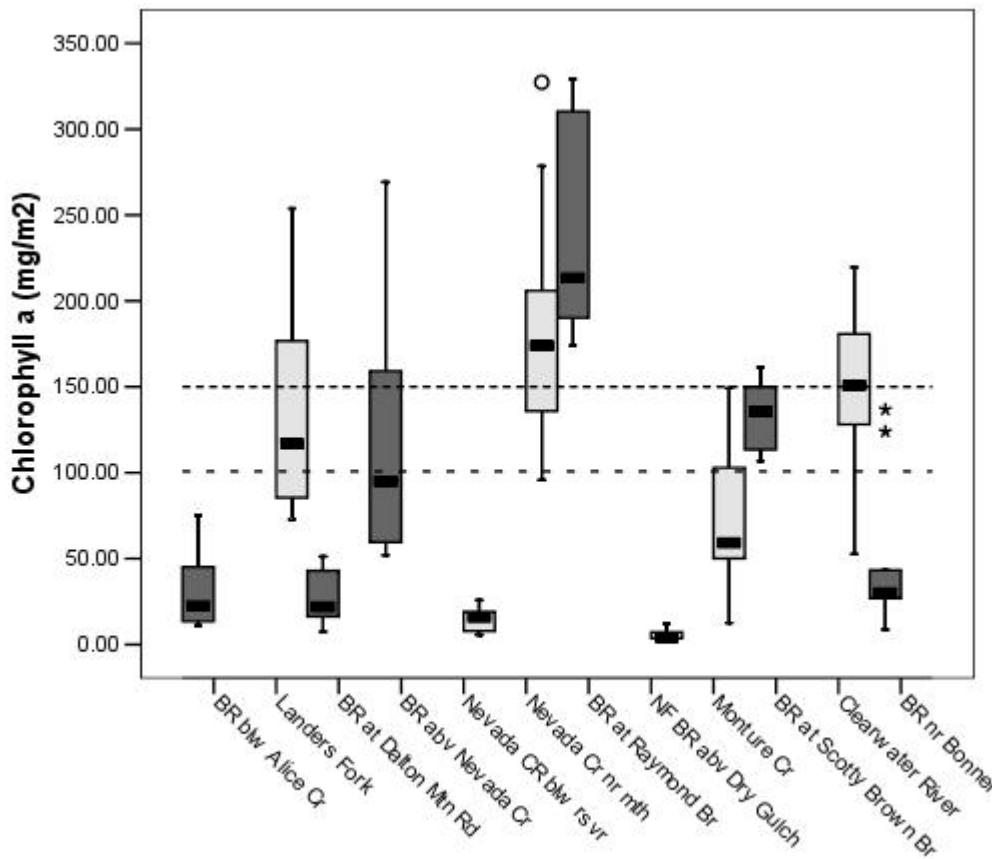
Mean chlorophyll *a* concentrations in the mainstem Blackfoot River ranged from a low of 27.2 mg/M² at the Dalton Mountain Road to a high of 234.6 mg/ M² at the Raymond Bridge below Nevada Creek. Blackfoot River tributaries had mean chlorophyll *a* concentrations ranging from a low of 5.4 mg/M² in the North Fork to a high of 184.7 mg/M² in Nevada Creek near its mouth. Fairly large standard deviations in chlorophyll *a* concentrations at many of the stations confirmed that benthic algae were naturally patchy in their distribution. Extremes in variation between sample replicates were noted at sites B-1 and B-12 where the standard deviation values approached or exceeded the mean and median concentration values (**Table 3-1**). The highest individual chlorophyll *a* concentration was measured at station B-7, the Blackfoot River at Raymond Bridge (329.1 mg/M²), while the single lowest value was documented at station B-8, the North Fork Blackfoot River above Dry Gulch (1.4 mg/M²).

Figure 3-1. Algal standing crops in the Blackfoot watershed (measured as chlorophyll *a*) during 2004.



The spatial (upstream to downstream) distribution of chlorophyll *a* data for the Blackfoot River and the selected tributaries are illustrated by the box plots in **Figure 3-1**. The top and bottom margins of the “box” represent the 75th and 25th percentile values of the data (10 replicates at each site), the median value is shown as the heavy cross line within the box, and the error bars depict the 90th (upper) and 10th (lower) percentile values of the data distribution.

Figure 3-2. Boxplots showing the percentile distribution of individual 2004 chlorophyll *a* data (N=10) for Blackfoot watershed water quality status and trends monitoring stations. (Dotted lines represent threshold values for the Clark Fork River¹)



¹ – 100 mg/M² is the maximum allowable summer mean chlorophyll *a* concentration for the Clark Fork River above the Flathead River. 150 mg/M² is the maximum allowable summer peak concentration (ARM 17.30.631).

It is useful to review the relevant water quality criteria for chlorophyll *a* concentrations in streams as a basis for interpreting the Blackfoot watershed data. The State of Montana has not adopted numeric criteria for algae or chlorophyll *a* in the Blackfoot River. However, standards have been adopted for the Clark Fork River from its headwaters to the Flathead River confluence. These standards are 100 mg/M² chlorophyll *a* as a summer mean concentration, and 150 mg/M² as a summer peak concentration (ARM 17.30.631). These values have been included for reference purposes on the chlorophyll data box plots in **Figure 3-2. Table 3-2** (modified from EPA 2000) summarizes other reference values that have been recommended to prevent “nuisance” densities of algae and to avoid nutrient-related water quality degradation in streams. These threshold values do not necessarily reflect upper limits for protection of all aspects of aquatic life-related water uses and biological integrity. Other literature suggests that 100 mg/M² for chlorophyll *a* would be indicative of moderately enriched conditions. According to EPA,

“unenriched, light-limited, or scour-dominated stream systems typically have benthic chlorophyll *a* values much less than 50 mg/M² (EPA 2000).

Table 3-2. Algal biomass criteria limits¹ recommended to prevent nuisance conditions and water quality degradation in streams (excerpted from U.S. EPA 2000).

Chlorophyll <i>a</i> conc. (mg/M ²)	Impairment Risk	Source
100-200	Nuisance growth	Welch et al. 1988, 1989
100-200	Nuisance growth	Dodds et al. 1997
200	Eutrophy	Dodds et al. 1997
100, 150 ²	Nuisance growth	Clark Fork River, Tri-State Council, MT
200	Nuisance growth	Biggs 2000
100	Reduced invertebrate diversity	Nordin 1985
100	Nuisance growth	Quinn 1991
≈100	Nuisance growth	Sosiak

¹Expressed as chlorophyll *a* concentrations.

²100 mg/M² as a summer average concentration and 150 mg/M² as a summer maximum concentration.

From these various reference values, it can be assumed that benthic algal standing crops containing chlorophyll *a* concentrations in excess of about 100 mg/M² are reflective of nuisance growth. Mean chlorophyll *a* concentrations at six of 12 Blackfoot watershed monitoring sites, including B-2 (Landers Fork), B-4 (Blackfoot River above Nevada Creek), B-6 (Nevada Creek near mouth), B-7 (Blackfoot River at Raymond Bridge), B-10 (Blackfoot River at Scotty Brown Bridge), and B-11 (Clearwater River), all exceeded this threshold value. In addition, maximum recorded chlorophyll *a* concentrations at eight of the 12 stations (B-2, B-4, B-6, B-7, B-9, B-10, B-11, and B-12) exceeded the recommended maximum level. Only the Blackfoot River below Alice Creek (B-1), the Blackfoot River at Dalton Mountain Road (B-3), Nevada Creek below Nevada Creek Reservoir (B-5), and the North Fork Blackfoot River (B-8) were consistently below the threshold limit in all 10 replicate samples. Stream bottom composition at the Blackfoot River at Dalton Mountain Road site, and in Nevada Creek below Nevada Creek Reservoir (small cobbles, gravel or silt), may have somewhat limited algal growth potential in those stream segments. The North Fork showed the lowest levels of chlorophyll *a*, while the Blackfoot River below Nevada Creek at the Raymond Bridge showed the highest average and maximum chlorophyll *a* concentrations.

Of the tributaries monitored, lower Nevada Creek sustained the highest chlorophyll levels, followed in descending order by the Clearwater River, Landers Fork, Monture Creek, upper Nevada Creek, and the North Fork. Nevada Creek is known to be impacted by agricultural practices, hydromodification, and historical mining, all of which may contribute to nutrient enrichment and elevated water temperatures thereby promoting the growth of benthic algae. The Clearwater River drains a series of warm lakes and has a significant amount of residential development in its watershed that have the potential to impact water quality. The Landers Fork drainage sustained a large wildfire in 2003, which may have contributed to a release of nutrients capable of stimulating algae growth. Monture Creek drains national forest lands in its upper reaches but flows through

livestock pastureland in its lower section. The upper Nevada Creek site is immediately downstream of a large irrigation reservoir which may attenuate nutrients to some degree. The streambed was also not particularly conducive to supporting benthic algae colonies due to a lack of firm attachment sites (silty bottom lacking rocks or cobbles).

The spatial pattern for chlorophyll in the mainstem Blackfoot River was one of gradually increasing concentrations from the headwaters downstream to the Raymond Bridge, followed by a declining trend downstream to the lower river stations. Largest increases in the Blackfoot River were noted from the Dalton Mountain Road station to the station located above Nevada Creek (increase in mean concentrations of 90.5 mg/M²), and from above to below Nevada Creek (mean increase of 116.9 mg/M²). Largest mean decreases were noted from above to below the North Fork and Monture Creek (100.4 mg/M²). The North Fork and Monture Creek inflows are likely to dilute in-stream nutrient concentrations in the Blackfoot River thereby reducing algal growth potential.

Streamflow conditions, which can influence water temperatures and algal growth, were somewhat below normal in the Blackfoot watershed in 2004. Subsequent years' monitoring will help to put the 2004 chlorophyll data in perspective.

4.0 Discussion

The 2004 Blackfoot watershed chlorophyll *a* monitoring program provided insight into basin-wide conditions, spatial patterns, impairment problems, and pollution sources. Key findings are discussed in the following paragraphs.

In-stream Status and Spatial Trends

Chlorophyll *a* concentrations measured in the Blackfoot watershed during 2004 showed considerable variability between stations, and between replicate samples collected at the same station. This was due to the naturally patchy distribution of algae across the available substrate. However, the sample size of ten replicates per site provided a sound basis for evaluating central tendencies as well as differences between sites.

Within the 12 monitoring stations that were sampled, chlorophyll *a* concentrations ranged from very low in the North Fork Blackfoot River, to very high in lower Nevada Creek and in the Blackfoot River below Nevada Creek, relative to the potential for negative impacts on beneficial water uses. The Landers Fork, the Blackfoot River above Nevada Creek and at the Scotty Brown Bridge, and the Clearwater River, also contained elevated levels of chlorophyll *a*. The Blackfoot River below Alice Creek, at the Dalton Mountain Road and near Bonner, Nevada Creek below Nevada Creek Reservoir, the North Fork, and Monture Creek had very low to relatively low levels of chlorophyll.

Mean chlorophyll *a* concentrations measured in the Landers Fork, lower Nevada Creek, Clearwater River, and in the Blackfoot River above Nevada Creek, at Raymond Bridge, and at Scotty Brown Bridge exceeded the state water quality standard set for the Clark

Fork River. The Clark Fork standard is 100 mg/M² for summer mean chlorophyll *a* concentrations. Mean chlorophyll *a* concentrations at two Blackfoot basin stations – Nevada Creek near mouth and the Blackfoot River at Raymond Bridge -- surpassed the state water quality standard of 150 mg/M² for summer peak chlorophyll *a* concentrations in the Clark Fork River. Only the Blackfoot River below Alice Creek (B-1), the Blackfoot River at Dalton Mtn Road Bridge (B-3), Nevada Creek below Nevada Creek Reservoir (B-5), and the North Fork Blackfoot River (B-8) were consistently below the Clark Fork threshold limits in all 10 replicate samples.

Field observations suggested that chlorophyll levels in the Blackfoot River at the Dalton Mountain Road and in Nevada Creek below Nevada Creek Reservoir may have been artificially limited by an absence of suitable (stable) substrate for algal colonization. In other words, the measured chlorophyll levels at those sites may not have been representative of the actual level of nutrient enrichment. A review of the available water chemistry data for these sites may help address this question.

The spatial pattern for chlorophyll concentrations in the mainstem Blackfoot River showed a gradually increasing trend downstream to the Raymond Bridge, concurrent with suspected sources of nutrient inputs (see discussion in next section). This increasing trend was followed by declining concentrations downstream to the lower-most monitoring station near Bonner. Declining chlorophyll *a* concentrations appeared to be associated with inflows from cleaner tributaries, notably the North Fork and Monture Creek.

Sources of Elevated In-Stream Chlorophyll Concentrations

Apparent enrichment in the Landers Fork in summer 2004 may have been a result of nutrient releases in the watershed associated with the 2003 wildfires. It will be interesting to monitor time trends at this station as the watershed recovers.

High levels of algal growth in Nevada Creek and in the Blackfoot downstream of Nevada Creek in 2004 are likely to be influenced by agricultural practices in the Nevada Creek sub-basin. Chlorophyll *a* concentrations in lower Nevada Creek were an order of magnitude greater than levels seen just below the Nevada Creek Reservoir.

Chlorophyll levels in the Clearwater River may have been stimulated by warm water releases from the Clearwater chain of lakes, as well as residential development and heavy recreational use in the lakes.

Apparent sources of enrichment in the Blackfoot River between the Dalton Mountain Road Bridge and the Nevada Creek confluence, indicated by a large in-stream increase in chlorophyll *a* concentrations, are unknown. One factor may have been that upstream concentrations were under-represented due to the possible substrate limitations discussed earlier. Agricultural land use activities, including intensive irrigation, may have been additional contributing factors to the observed spatial trends in this reach of the river.

Recommendations for Future Monitoring

Chlorophyll *a* monitoring should be continued on an annual basis in the Blackfoot watershed, contingent on available funding. The approach used in 2004 appeared to be well suited to the conditions encountered. Monitoring should occur in mid- to late-August and ten replicate samples should be collected, as was done in 2004. The issue of possible substrate limitations should be examined for sites B-3 and B-5 and consideration given to relocating the sampling sites a short distance upstream (B-3) or downstream (B-5).

5.0 Literature Cited

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6.0 Appendices

APPENDIX A –

2004 BLACKFOOT WATERSHED CHLOROPHYLL SAMPLING RESULTS