

Grand County Water Information Network
Algae Monitoring Project
FINAL REPORT

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For the
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Project Grant Program

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Granby Sanitation District (GSD)
Three Lakes Water and Sanitation District (TLWSD)
Colorado Department of Public Health and Environment (CDPHE),

Other entities participating in the project and/ or benefiting from the results are:

The Towns of Fraser, Granby, Grand Lake, Hot Sulphur Springs, and Kremmling,
Colorado Division of Wildlife (CDOW)
Three Lakes Watershed Association (TLWA)
Greater Grand Lake Shoreline Association (GGLSA)
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Grand County Water Information Network Algae Monitoring Project Draft Report

Executive Summary

The Grand County Water Information Network (GCWIN), located in Grand Lake, Colorado, obtained funding through the Colorado Watershed Protection Fund (CWPF) Project Grant for the GCWIN Algae Monitoring Project (Project). The purpose of the Project was to quantify the extent of the existing algae problem and potential health risks in Grand County through a watershed-specific approach. The Project goals were to:

- 1) quantify algal productivity (cell count by genre)
- 2) find the most rapid analytical method available to determine toxin levels in drinking water resources, and
- 3) map the locations and concentrations of algae in Grand County

The Project met the first and third goals. No rapid and quantifiable analytical method was found to determine toxin levels in drinking water. However, a method was found that can help determine the presence of toxin-releasing Cyanobacteria which will help optimize future algal toxin testing. The Project also resulted in an increased awareness and level of education about algal toxins which has helped create funding for additional testing.

The primary algae and algal toxin concerns in the Three Lakes area were:

- 1) drinking water concerns due to the algal toxins;
- 2) recreational concerns due to the extensive use of the lakes for fishing, swimming, kayaking, water skiing, jet skiing, and sailing; and
- 3) aquatic habitat and wildlife concerns.

The primary algae and algal toxin concerns in the Fraser River and Colorado River Basins were:

- 1) drinking water concerns due to taste and odor problems from the algae and potential algal toxins;
- 2) water quality concerns regarding water used for livestock; and
- 3) aquatic habitat and wildlife concerns.

Empirical data were greatly needed to quantify the extent of algae colonies. Tracking and quantifying the increased algal growth in Grand County's lakes and rivers can be used to help decision-makers implement watershed improvement goals and objectives and improve in-situ water quality. The Project Sampling Design was based on the estimated worst case scenario for algal growth facing Grand County during the Project. The worst case scenario would be one in which algae continued to rapidly grow and spread exponentially each year throughout Grand County waterways. If the worst case scenario was true there would be increased cell counts and toxin levels every year posing a greater health risk to the public and those using water from Grand County as their source water. This was not found to be true for the years monitored.

Initially, 15 monitoring sites were selected throughout Grand County for 2005 and 2006. Limited results are included for 2007. Algal productivity was quantified by cell count and monitored temporally and spatially. Phytoplankton (algae) data collected for 2004, 2005, 2006, and 2007 alone indicated a large variation in the cell counts between years and monitoring locations. This temporal and spatial variation was noted in the Phycocyanin values measured at the Grand Lake Adams Tunnel West Portal Picnic site during August 12, 2007. Water samples analyzed with an Aquafluor and calibrated with a solid standard were compared with

the phytoplankton samples to determine whether Cyanobacteria were present in water samples. Use of the Aquafluor during routine water quality monitoring was found to be a successful tool in determining whether Cyanobacteria populations were present. This method can be used to measure for the presence of Cyanobacteria in the Fraser and Colorado Rivers as well as any of the lakes or reservoirs in Grand County. Monitoring costs for algal toxin sampling may be reduced by using the Aquafluor in conjunction with phytoplankton sample prior to an algae bloom and during a bloom to help determine whether toxin producing Cyanobacteria are present.

Additionally, GCWIN helped test the *Immunostrip*, a new rapid analytical lab product designed to determine toxin concentrations within 20 minutes. However, the product was determined not to be useful for our application because lake toxin concentrations are below the 10 ug/L threshold of determination by the method.

Ongoing collection of empirical data is greatly needed to continue to quantify the extent of the algae colonies and concentrations of toxin when present and determine how best to reduce algal growth in Grand County.

Introduction and Background

During the spring, summer, and fall of 2004, Grand County experienced excessive algae growth in the Fraser and Colorado Rivers, their tributaries, and the Three Lakes (Grand Lake, Shadow Mountain and Granby Reservoirs). Complaints from ranchers, fishermen, boaters, homeowners, and concerned citizens were directed to GCWIN and Grand County. Nutrients, warm temperatures in May – June, and low stream flows without spring flushing flows were noted by the Colorado Department of Public Health and Environment (CDPHE) as contributing to the algae blooms on the Fraser River.

Algae are small naturally occurring single-cell colonies or filamentous plants containing chlorophyll. Needed for a healthy aquatic habitat, they are found in all waterways including rivers and lakes (Holdren, et. al., 2001). Algae thrive in waters that are rich in nutrients, especially phosphorous and phosphates. Warmer water temperatures and sunlight also increase algae growth. In rivers, algae need high flows during spring runoff to flush the decaying algae (periplankton) downstream and decrease areas of decaying cells that can become anoxic (Nuttle, 2004). Free floating algae in rivers and lakes are called phytoplankton.

Cyanobacteria, or blue green algae, are found world-wide and have been historically noted during research on Grand Lake, Colorado in 1953 (Pennak, 1955). Cyanobacteria are bacteria that act like algae because they contain chlorophyll and can produce oxygen through photosynthesis. In Grand County, the majority of Cyanobacteria genera present in the lakes, rivers, and reservoirs do not produce algae toxins or pose a threat to humans and wildlife. The main potential health concern determined was that the predominant Cyanobacteria *Anabaena* found in Grand County is capable of producing toxins that pose risks to humans and animals from drinking water and recreation in contaminated waters. *Aphanizomenon* and *Microcystis*, also know toxin-producing Cyanobacteria, have been found periodically in the watershed every year. Low levels of Microcystin toxin were found in the Shadow Mountain Reservoir and Grand Lake samples throughout September and October 2004, but fortunately, were below the World Health Organization's drinking water guideline of 1 ug/L. Health effects, such as allergies to algae and the toxins are fairly common and can cause skin reactions such as itching, rashes, swelling, nausea, and headaches. The most common toxin can accumulate over a long period, or develop rapidly, depending on dose. Other health risks range

from liver damage and tumor formation to paralysis, asphyxiation, and death for high concentrations (Carmichael, 2001).

In response to the excessive algae growth in 2004, the Grand County Water Information Network (GCWIN) coordinated the Algae Monitoring Project, a locally based, countywide collaborative effort designed to identify and track Cyanobacteria growth and algal toxin production in Grand County. Monitoring sites were primarily in the Three Lakes area, with selected sites on the Colorado River, the Fraser River and chosen tributaries. Cyanobacteria capable of producing toxins were found in the Fraser River and the Colorado River. The GCWIN Technical Committee reviewed the 2004-phytoplankton and toxin results in January 2005, and an algal toxin-monitoring program was determined essential to identify any potential public and safety health risks.

Purpose and Need

The purpose of the Project as determined by the GCWIN Technical Committee was to quantify the existing algae problem and potential health risks through a watershed-specific approach. The primary concern in both the Three Lakes area and the Fraser and Colorado River basins was drinking water safety. Many houses on the shores of Grand Lake and Shadow Mountain Reservoir draw their water directly from those water bodies. The Town of Granby draws their water directly from the lower Fraser River, and the Town of Hot Sulphur Springs draws its water from the Colorado River downstream of the Fraser River and Windy Gap Reservoir. Further downstream on the west end of the county, the Town of Kremmling draws a portion of their water directly from the Colorado River after the confluence of Muddy Creek and the Blue River and just before the mouth of Gore Canyon. Obtaining a rapid means to identify the presence of Cyanobacteria and any associated algal toxin would benefit municipal water treatment facilities, and anyone using the Fraser River and Colorado Rivers for their drinking water supply. Wellhead protection for the Town of Fraser due to the proximity to the Fraser Lion's Ponds and the surrounding area was another concern due to the invasive nature of Cyanobacteria.

Information Needs: The GCWIN Technical Committee determined that more information was needed. These needs include: 1) quantifying algal productivity (cell count by genera); 2) finding the most rapid analytical method available to determine if toxin was present in drinking water resources; and 3) mapping the locations and concentrations of algae in Grand County. Empirical data was greatly needed to quantify the extent of the alga colonies and concentrations of toxin, when present. Empirical data was needed to better understand and determine best management practices (BMPs) for control of the excessive algal growth. The Algae Monitoring Project was sponsored by GCWIN, Grand County, NCWCD, USFS, US Bureau of Reclamation (USBR), Granby Sanitation District (GSD), and the Three Lakes Water and Sanitation District (TLWSD). Funding provided by the CWPF was used to help met the goals of the Project.

Monitoring Plan

The Algae Monitoring Project Sampling Design – Appendix A

This was based on results from 2004 with input from in-state, national algae experts, and the GCWIN Technical Committee which includes Members from the USBR, USGS, USFS, BLM, CDPHE, CDOW, and CDOT. The sampling design was based on the worst case scenario, which would be one where the algae continued to rapidly grow and spread exponentially throughout the rivers and lakes in Grand County. The

results would be increased cell counts and toxin levels posing a greater health risk to the public. The same basic sampling design was used for 2005, 2006 and 2007. However, more lake and reservoir sites were added to the design to better understand spatial variability prior to the 2006 Shadow Mountain Reservoir Drawdown and the possible long term and short term effects on water quality, algae productivity, and algal toxins after removing the aquatic weeds.

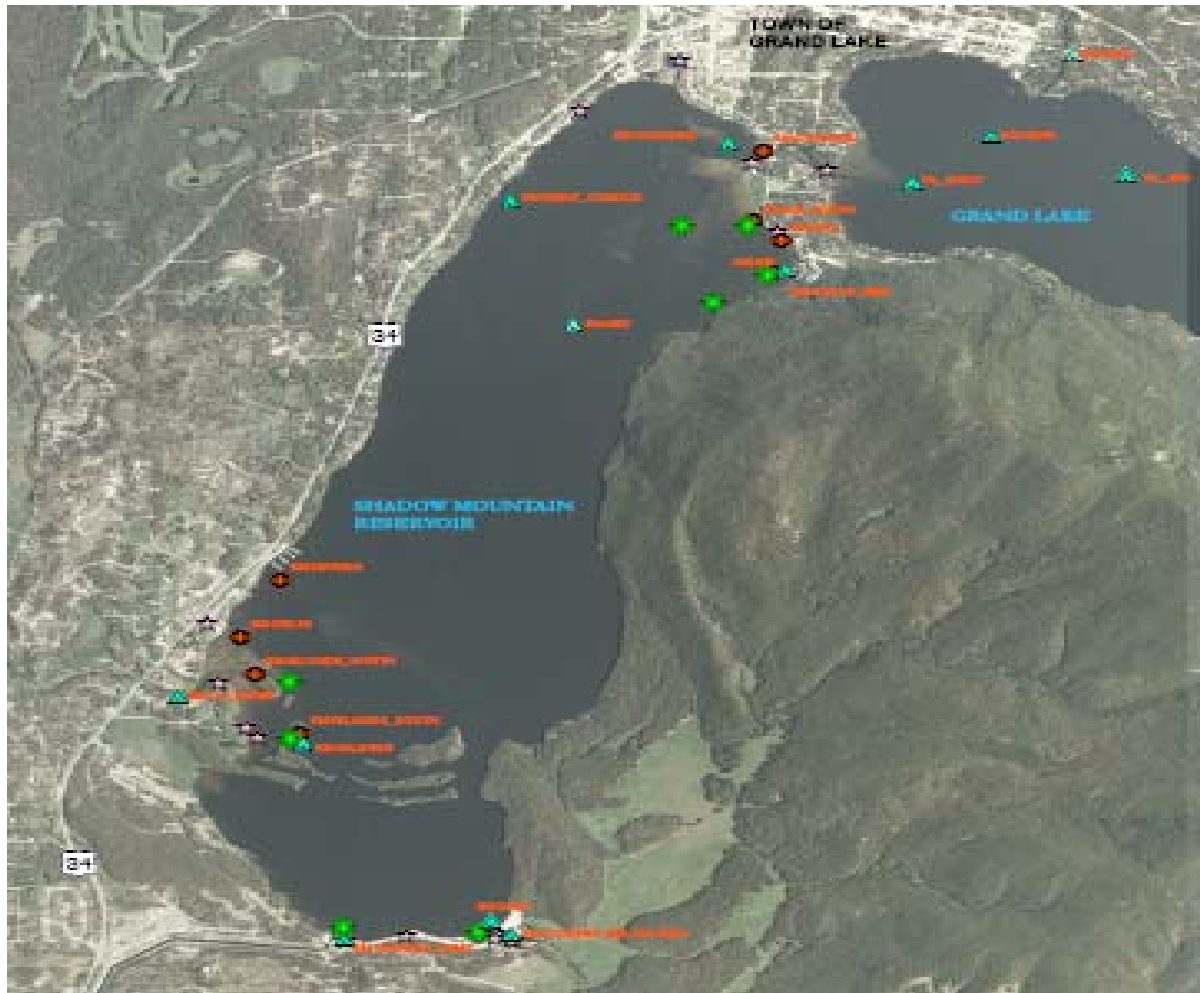


Figure 1: Monitoring sites Shadow Mountain Reservoir and Grand Lake.

Methods and Sampling Procedures - Appendix B

The number of sampling parameters and constituents evolved over the course of the study as analytical methods improved and more sampling and analysis equipment became available for the project. Phytoplankton samples and algal toxin samples were collected at most sites whether river or lake/reservoir. YSI sondes data was taken at most of the lake and reservoir sites during 2005 – 2006 as well as secchi disk measurements and phycocyanin data for 2006 and 2007. Due to initial calibration problems, there is limited phycocyanin data available for 2006. Written sampling methodology and procedures for operating the YSI sondes and collecting phytoplankton and algal toxin samples are presented in this appendix.

Algal Toxin Sampling Procedures

Monitoring protocols for collecting algal toxin samples were written up by Clements in 2005, and revised in 2006, after being researched and discussed with experts across the nation (Westrick, 2003 and Boyer, 2004 and 2005). All written monitoring protocols are presented in Appendix B. All personnel read and followed the protocols when sampling. Discrete water samples were collected using a Van Doren sampler that was lowered by a rope to the desired depth. When duplicate samples were needed for either Quality Control (QC) or for multiple laboratories to analyze, discrete samples were collected at the desired depth and then poured into a clean bucket to create a composite sample. The bucket, sample bottles, and Van Doren sampler were rinsed with the sample water three times before collecting the actual samples.

Algal Toxins Analysis

Algal toxins were analyzed using four methods. The ELISA(Enzyme-Linked ImmunoSorbent Assay) and PPIA(Protein Phosphatase Inhibition Assay) are the most sensitive analytical methods while the HPLC(High Pressure Liquid Chromatography) and LCMS(Liquid Crystal Mass Spectrometry)are more selective to the Microcystin-LR toxin.

All toxin samples were tested either at the Syracuse University of New York or at the Lake Superior State University. Cost for analyses is expensive and took at least 48 hours to obtain any results. A more rapid test was needed to be beneficial and obtain results sooner.

GCWIN helped test an ImmunoStrip, a new rapid analytical method and lab product by Agdia, which was to help determine toxin concentrations within 20 minutes. However, after a year of testing, the lab product was determined not to be beneficial to the Three Lakes area. Originally, test strips with a base sensitivity of 1.0 ug/L were used by the project but it was found that they reacted slower at altitude. The final product produced tested for toxin concentrations at a base sensitivity of 10 ug/L, which is greater than any known toxin levels recently found in the Three Lakes.

Results and Discussion

Communication

Algal toxin results were compared to the World Health Organization and the limited results from the 2004 samples. Brief updates when new data was available were emailed to all interested parties, and the results are being added into the GCWIN web on-line database in 2008. In accordance with this evaluation plan, during the 2007 event of increased potential health risk, the results were distributed immediately.

In 2004 and 2007, results from the algae taxonomy and toxin analyses were used to notify homeowners on Grand Lake who use Grand Lake for drinking water of the potential health risks. GCWIN staff used the information to notify the Grand County Board of Commissioners, the USFS, the Grand Lake and Granby Board of Trustees, and all GCWIN Members. Additionally in 2004, a special meeting was held for the general public in the Town of Grand Lake. GCWIN used their Three Lakes Technical Committee Meetings in 2004, 2005, 2006, and their Annual Summer Meeting in 2007 to update Members and the general public about the algae blooms and water quality in the Three Lakes watershed. The 2004, 2005, 2006, and 2007 Algae Monitoring Project's results were emailed periodically to GCWIN Members and interested parties throughout the program.

The increased amount of algae throughout Grand County may result from cumulative factors such as weather, drought, available nutrients, and hydrologic conditions. Determining which factors can be managed is critical in the overall control and spread of the algae. The physical factors contributing to the alga growth, such as possible nutrient sources; past and present water quality; and hydrologic conditions still need to be determined. Tracking and quantifying the increased alga growth in Grand County's lakes and rivers helps decision-makers such as the US Forest Service and Grand County implement watershed improvement goals and objectives and improve in stream water quality.

Quantifying Algal Productivity – Appendix C

Samples were analyzed for over 80 species of algae and bacteria. Those of most interest were *Anabaena*, *Aphanizomenon*, and *Microcystis*. Those results are presented in Appendix C along with correlating analyses for toxins. As seen from the scatter plot below, there is no real correlation between amount of bacteria, as measured in cell counts per milliliter, and amount of toxin. Although a regression analysis determined a slope of 1 and a y-intercept of virtually zero (2.8×10^{-17}), the correlation is poor at best. Additionally, only nine samples were analyzed both for cell counts and toxins, thus a small data set adds to the inaccuracy.

Water flows from Lake Granby through Shadow Mountain Reservoir and into Lake Granby where it exits through the Alva Adams tunnel to the East Slope. There was little to no consistency between high cell counts seen from one lake to the next. An explanation for this is that many of the sample sites were at beaches on the water's edge, which may create areas of low turnover. Those are, however, important areas for testing as those are where many people will interact with the lake waters.

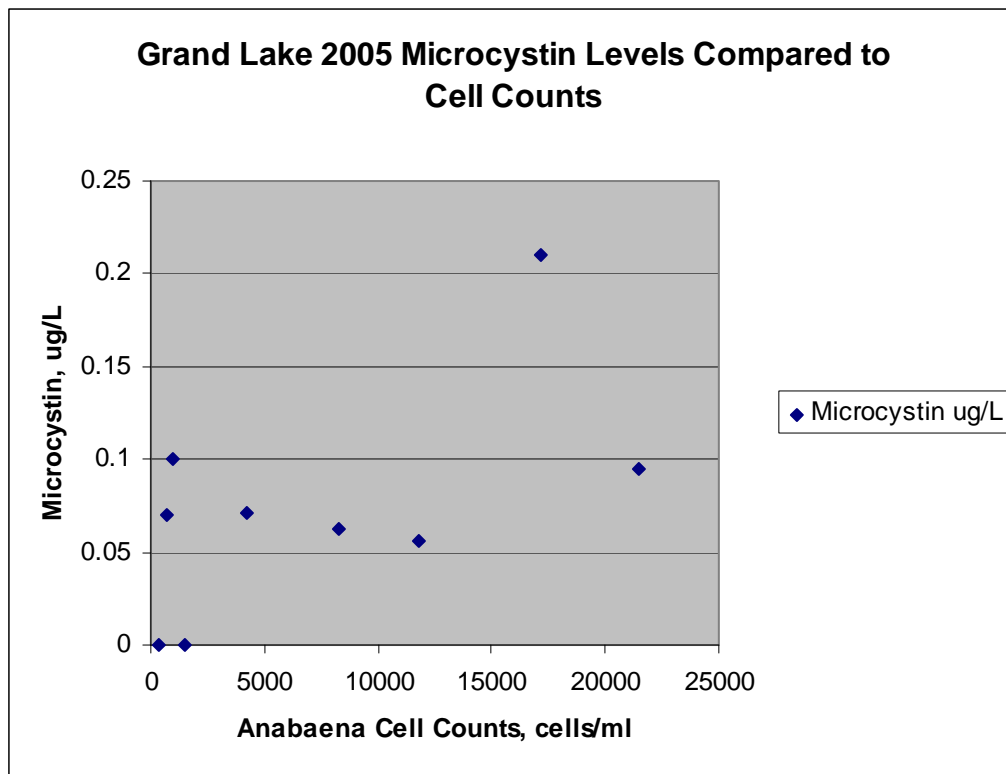


Chart 1: Correlation of Microcystin levels to *Anabaena* cell counts

Phycocyanin Data for 2006 and 2007

An Aquafluor, a portable spectrophotometer set for Chlorophyll and Phycocyanin was purchased for the Project. While the pigment chlorophyll is found in all algae, only the Phycocyanin is found in Cyanobacteria. Using the Aquafluor and a solid standard for calibration can help determine whether Cyanobacteria are present in water samples. This method can be used to measure for the presence of Cyanobacteria in the Fraser and Colorado Rivers as well as any of the lakes or reservoirs in Grand County. Monitoring costs for algal toxin sampling may be reduced by using the Aquafluor in conjunction with phytoplankton sample prior to an algae bloom and during a bloom to help determine whether toxin producing Cyanobacteria are present. Table 1 shows the Phycocyanin values for Grand Lake Mid-Lake site during 2007.

Table 1: Phycocyanin values for Grand Lake Mid-Lake site during 2007.

<u>Date</u>	<u>Time</u>	<u>Site</u>	<u>Site Name</u>	<u>Phycocyanin ug/L</u>	<u>Absorption nm</u>
3-Jul	13:40	GL-Mid	Grand Lake - Mid	0.005	11.445
16-Jul	11:45	GL-Mid	Grand Lake - Mid	-1.176	2.904
24-Jul	13:00	GL-Mid	Grand Lake - Mid	2.689	30.85
31-Jul	11:08	GL-Mid	Grand Lake - Mid	3.137	34.09
6-Aug	13:00	GL-Mid	Grand Lake - Mid	6.006	54.83
14-Aug	13:26	GL-Mid	Grand Lake - Mid	6.231	56.458
20-Aug	15:50	GL-Mid	Grand Lake - Mid	3.538	36.986
28-Aug	10:52	GL-Mid	Grand Lake - Mid	2.381	28.618
5-Sep	12:05	GL-Mid	Grand Lake - Mid	0.848	17.535
11-Sep	10:20	GL-Mid	Grand Lake - Mid	0.193	12.805
25-Sep	11:00	GL-Mid	Grand Lake - Mid	0.651	16.116
5-Oct	10:00	GL-Mid	Grand Lake - Mid	-1.199	2.738
9-Oct	13:00	GL-Mid	Grand Lake - Mid	0.432	14.53
16-Oct	10:30	GL-Mid	Grand Lake - Mid	-1.088	3.543

Due to the fact that there are no correlations between Phycocyanin concentrations and the various algal toxins, an increase in Phycocyanin or Cyanobacteria populations do not indicate that there is an increased health risk. However, the increased Cyanobacteria populations may contribute to the overall carbon loading to the C-BT system. A study done by the Big Thompson Watershed Forum in 2007 determined that the largest contribution of organic carbon in the C-BT system was being transported through the Adams Tunnel from Grand Lake (Loftis, 2007). Though carbon was not monitored for by the GCWIN CWPf study, Phycocyanin values taken from the Grand Lake Adams Tunnel West Portal Picnic Area during the 2007 August and September algae bloom can be compared to the Grand Lake Mid-Lake values. Table 2 shows that the populations of Cyanobacteria were larger at the Grand Lake Adams Tunnel West Portal Picnic Area than at the Grand Lake Mid Lake site during sampling.

Table 2: Phycocyanin values for Grand Lake Adams Tunnel West Portal Picnic Area during the 2007 August and September algae bloom. The first two samples were taken one minute apart.

Date	Time	Site	Site Location	Phycocyanin ug/L	Absorption nm
8/12/2007	7:58	GL-AT-PIC	Adams Tunnel Picnic Area	11.97	97.47
8/12/2007	7:59	GL-AT-PIC	Adams Tunnel Picnic Area	6.97	61.79
8/14/2007	15:39	GL-AT-PIC	Adams Tunnel Picnic Area	6.563	58.855
8/20/2007	18:41	GL-AT-PIC	Adams Tunnel Picnic Area	2.49	29.39
9/2/2007	7:05	GL-AT-PIC	Adams Tunnel Picnic Area	2.78	31.508

The greatest Phycocyanin concentration of 11.97 ug/L was a grab sample collected from an early morning scum present prior to the first motor boat wave mixing the surface water on August 12, 2007. The concentration of the next sample taken one minute later after the motorboat wave had mixed the water is not quite half the value of the sample taken prior to the wave. Observations in Grand Lake noted in the phytoplankton data indicated that the greatest numbers of phytoplankton and Cyanobacteria are usually found below the water's surface between 1-2 meters and are rarely at the surface. However, samples were generally not taken prior to 8:00 AM or 9:00 AM in 2004-2007.

When working out in the field, it is easy to forget that Cyanobacteria are just organisms controlled by several limiting factors; most of which we were not able to control during the project. Field parameters such as water temperature, pH, flowing or stagnant water, available nutrients, intensity and duration of UV light (sun versus no sun), and even elevation were all factors that are known to contribute to algae growth, and in particular Cyanobacteria growth (Westrick, 2003).

Chart 2, below, shows a slight correlation between Phycocyanin levels as measured by the Fluoroscope, and Cyanophyta cell concentrations. The cell concentrations were measured by the cell count analysis. With a slope of 43.5 and y-intercept of 180, the correlation is poor, especially when looking at the range of the scatter plot. However, the fluoroscope appears to be very good at indicating the presence of Phycocyanin, which may help optimize expensive toxin analyses in the future.

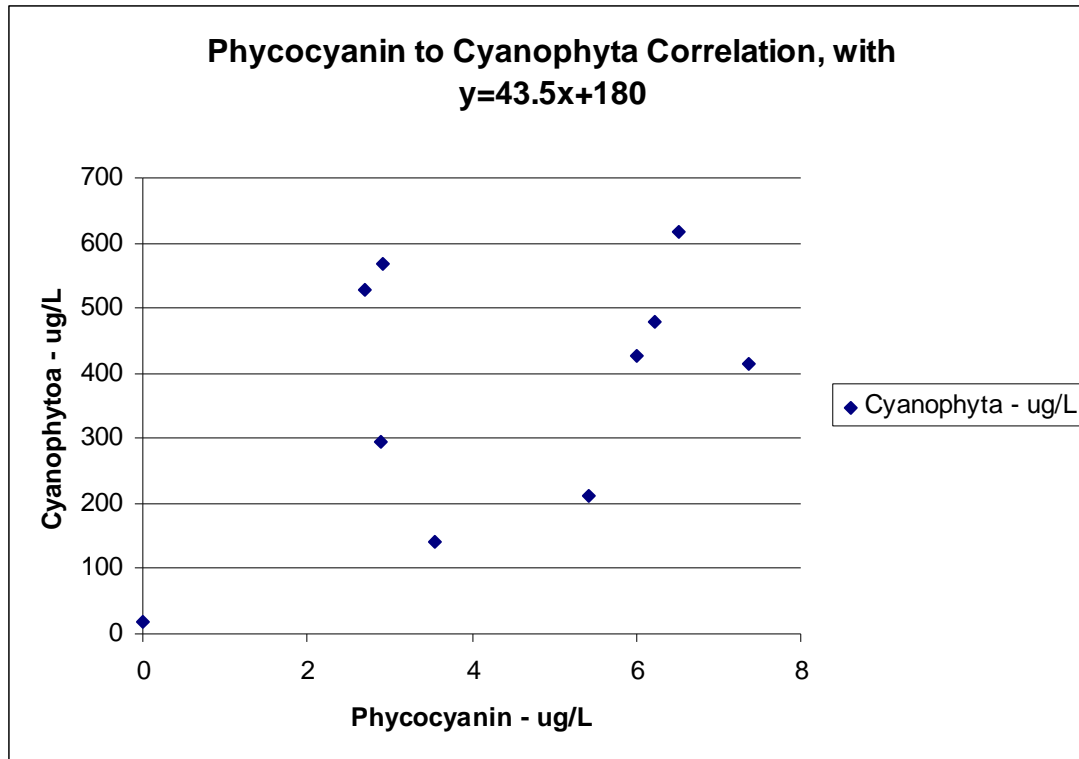


Chart 2: Phycocyanin and Cyanophyto correlation

Aquafluor and Secchi Disc Sampling

Use of the Aquafluor during routine water quality monitoring was found to be a successful tool in determining whether Cyanobacteria populations were present and could be used to help determine when algal toxin samples need to be taken early in the summer. At this time it can only be recommended as a tool to indicated the possible presence and not amounts of Cyanobacteria.

Looking at some of the Secchi disc analyses however, it is possible there are other correlations with Phycocyanin data. Algae populations are one of the components of water turbidity, which has a negative influence on water clarity.

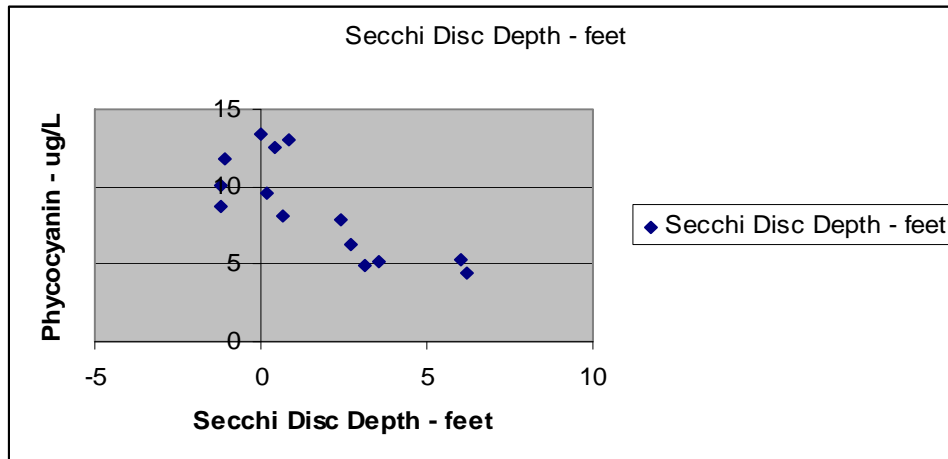
Secchi Disk Data for 2007

Water transparency was measured using an Aqua Scope II and a tape measure. Each measurement took into account one lowering value and one raising value. Those two numbers were then averaged to get the Secchi depth reading. When possible, two people each took measurements and the values were averaged together. Table 3 shows Secchi disk measurements in Grand Lake for 2007.

Table 3: 2007 Secchi disk measurements for Shadow Mountain Reservoir and Grand Lake

Date	Time	Site ID	Site Name	Type	<u>Depth Feet</u>	Weather
3-Jul	13:40	GL-Mid	Grand Lake - Mid	Average	13.35	Sunny
16-Jul	11:45	GL-Mid	Grand Lake - Mid	Average	10.12	Cloudy
24-Jul	13:00	GL-Mid	Grand Lake - Mid	Average	6.33	Sunny/Prtly Cloudy
31-Jul	11:08	GL-Mid	Grand Lake - Mid	Average	4.96	Sunny w/ high clouds
6-Aug	13:00	GL-Mid	Grand Lake - Mid	Average	5.25	Cloudy, rain off and on
14-Aug	13:26	GL-Mid	Grand Lake - Mid	Average	4.48	Cloudy and windy
20-Aug	15:50	GL-Mid	Grand Lake - Mid	Average	5.15	Sunny
28-Aug	10:52	GL-Mid	Grand Lake - Mid	Average	7.9	Sunny
5-Sep	12:05	GL-Mid	Grand Lake - Mid	Average	13.09	Prtly cloudy/sunny
11-Sep	10:20	GL-Mid	Grand Lake - Mid	Average	9.63	Sunny
25-Sep	11:00	GL-Mid	Grand Lake - Mid	Average	8.12	Sunny
5-Oct	10:00	GL-Mid	Grand Lake - Mid	Average	8.7	Sunny
9-Oct	13:00	GL-Mid	Grand Lake - Mid	Average	12.6	Sunny/Prtly Cloudy
16-Oct	10:30	GL-Mid	Grand Lake - Mid	Average	11.75	Sunny

The chart below shows a possible correlation between Phycocyanin and Secchi disk measurements. Although there are many factors contributing to lake clarity, algae formation is definitely one of the key contributors.



Sediment Core Data for 2006

Microcystin toxin was analyzed for and found in 6-8 inch composite sediment samples taken from sites on the Shadow Mountain Reservoir lake-bed during the 2006 Drawdown. All of the Shadow Mountain Reservoir sediments had detectable Microcystin toxin except for the Solar Bee North control site which was covered with small cobbles, had a very low levels of carbon and nitrogen, and little if any vegetation or aquatic weeds present. Microcystin toxin concentrations were greater in sediment samples containing higher levels of organic material and carbon present, such as the Island South and Island North sites which contained 6.0 ng/g and 1.5 ng/g of toxin, respectively.



Figure 2: Sediment flowing into Shadow Mountain Reservoir after the Grand Ditch breach, June 2003. The islands are visible in the distance.



Figure 3: Looking east toward the first island in Shadow Mountain Reservoir. The Island North Site is located on the opposite side of the island near the river channel. The Island South Site is located to the right just out of the photo.

Conclusion

Algal toxin analysis is very expensive. Without additional funding from the CWPf, adequate monitoring of the Fraser and Colorado Rivers would not have occurred. Long-term strategies for funding future algae monitoring need to be developed and re-evaluated once the full extent of the problem is determined as needs of the program change each year. In the long-term, the bulk of the funding will most likely fall to the water providers who deal directly with the taste, odor, and toxin removal during water treatment. Short-term funding will include some federal, state, and private grants. Long-term funding options include federal hazardous algae programs and Environmental Protection Agency (EPA) grants. Other options include the water providers who deal directly with the taste, odor, and toxin removal during water treatment. Implementing a water quality fee from the water providers is another possibility as well as private foundation grants.

The Grand County Algae Monitoring Project funded in part by the CWPf grant directly benefited anyone who drinks or uses water in Grand County. The recently developed Grand County Algae Toxin Plan addresses rapid notification to the public of any increased health risk. The Algae Monitoring Project directly benefited the Towns, County, State, and Federal decision-makers who deal directly with land management issues by producing water quality data including phytoplankton and toxin data used to help track the year to year variations in algal blooms in Granby Reservoir, Grand Lake, Shadow Mountain Reservoir and the Fraser and Colorado Rivers.

Next Steps

- 1) Continue monitoring for Algal toxins in 2009.
- 2) Continue the Secchi disc monitoring program in 2009. For 2008 over 600 measurements were taken at 14 sites, a considerable increase over previous years.
- 3) Look for additional data that can be taken with the fluoroscope. There is work to be done on the Phycocyanin/toxin correlation and this can be used to optimize algal toxin monitoring in 2009.

GCWIN Goals for 2009

1) Complete the redesign and loading of the database.

The current database design was not completed nor was all the data loaded into the database. Searches were limited to site by site only basis. NCWCD is working with GCWIN to redesign the database onto a platform that will allow for increased future growth. Watershed data is valuable only if it can be communicated to those who need it.

2) Work with the county to continue Secchi Disc, Temperature and Algal Toxin Monitoring

All three of these programs benefit water users in Grand County and the East Slope. As a neutral party, funded both by East and West slope entities, GCWIN needs to focus on getting good science integrated into the water monitoring programs.

3) Work for grants to make GCWIN more sustainable.

Areas we are interested in include: education, pollution prevention, and West Grand issues such as water table drawdown.

Current Monitoring and the Algae Task Force

Grand County's Alga Toxin Monitoring Program

NCWCD Nutrient Study and Routine Monitoring by the USBR

Monitoring for the WQCD Water Clarity Standard for Grand Lake

Grand County's Volunteer Secchi Disk Program

Monitoring for Quagga and Zebra Mussels in the Three Lakes and C-BT system by the CDOW and USBR

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Appendix A

Algae Monitoring Plan & Costs – FY 2005

Grand County Water Information Network Algae Monitoring Plan for FY2005

Background

The Grand County Water Information Network Algae Monitoring Project (Algae Monitoring Project) is a collaborative effort to help define the current water quality status throughout the Three Lakes area, the Colorado and Fraser Rivers, and their tributaries for FY2005. The increased appearance of algae, especially blue-green algae, is a fairly recent phenomenon in Grand County. During the spring, summer, and fall of 2004, Grand County experienced excessive algae growth in the Fraser River, the Colorado River, their tributaries, and the Three Lakes. Complaints from ranchers, fishermen, boaters, homeowners, and concerned citizens were directed to the Grand County Water Information Network (GCWIN). Nutrients, warm temperatures in May –June, and low stream flows without spring flushing flows were noted by the Colorado Department of Public Health and Environment (CDPHE) as contributing to the algae blooms on the Fraser River.

The main potential health concern is that the predominant Cyanobacteria, or blue-green algae, found in Grand County could produce toxins that pose human and animal health risks to drinking water and recreation in contaminated waters. Low-levels of Microcystin toxin were found in the Shadow Mountain Reservoir and Grand Lake samples throughout September and October 2004, but fortunately, were below the World Health Organization's guideline of 1 ug/L. Monitoring for toxins to identify any potential health risks for public health and safety is essential in 2005.

Purpose and Need

The purpose of the algae monitoring project is to quantify the existing algae problem and potential health risks through a watershed-specific approach. The existence of excessive algae and low-level toxins indicate that excessive nutrient loading is presently occurring to many waterways throughout Grand County. The primary concerns and information needs are as follows:

The primary algae and algae toxin concerns in the Three Lakes area are: 1) *drinking water concerns* due to the algal toxins; 2) *recreational concerns* due to the extensive use of the lakes for fishing, kayaking, water skiing, jet skiing, sailing, and limited swimming; and 3) *aquatic habitat and wildlife concerns*. The primary algae and algal toxin concerns in the Fraser River and Colorado River Basins are: 1) *drinking water concerns* due to taste and odor problems from the algae and potential algal toxins; 2) *water quality concerns regarding water used for agriculture, irrigation, and livestock purposes*; and 3) *aquatic habitat and wildlife concerns*.

The Town of Granby draws their water directly from the Fraser River, and the Town of Hot Sulphur Springs draws its water from the Colorado River. The Town of Kremmling will be drawing their water from the Colorado River in the near future. Wellhead protection for the Town of Fraser and the surrounding area is another concern due to the invasive nature of Cyanobacteria.

Information Needs: The GCWIN Technical Committee determined that more information regarding the algae was needed. These needs include: 1) quantifying algal productivity (cell count by species); 2) a rapid analytical method to determine if toxin is present in drinking water resources; and 3) mapping the locations and concentrations of algae in Grand County. Empirical data are greatly needed to quantify the extent of the alga colonies and concentrations of toxin, when present. Empirical data are needed to understand and best mitigate the causes of the excessive alga growth.

The Algae Monitoring Project Sampling Design is based on results from 2004 and input from in-state and national algae experts. The sampling design is based on the worst case scenario, which would be one where the algae continued to rapidly grow and spread throughout the rivers and lakes in Grand County. The results would be increased cell counts and toxin levels posing a greater health risk to the public.

Project Schedule:

Secure funding for project:	January - May 2005
Monitor and analyze for algae/toxins:	05/15/05 through 10/30/05
Weekly emailed reports:	05/15/05 through 10/30/05
Written report:	12/31/05

Evaluation

Rapid notification is needed for users of affected water supplies in the event that the toxin concentrations increase. Evaluation of the project is on-going, and the results will be distributed throughout the sampling period to all interested parties (e.g. USFS, Grand County, CDPHE, Towns, NCWCD, CRWCD, DW, etc). Brief weekly updates will be emailed to all interested parties, and the results will be added into the GCWIN web accessible database. In the event of an increased potential health risk, results will be distributed immediately.

In 2004, results from the alga taxonomy and toxin analyses were used to notify homeowners on Grand Lake who use Grand Lake for drinking water, of the potential health risks. Also, the CDPHE took the information and informed the East Slope drinking water suppliers of the situation and potential health risks. GCWIN staff used the information to notify the Grand County Board of Commissioners, the USFS, the Grand Lake and Granby Board of Trustees, and all GCWIN Members. In addition, a special meeting was held for the general public in the Town of Grand Lake. A follow-up general public meeting is scheduled at Grand Lake in June 2005. The 2005 Algae Monitoring Project's results will be used in a similar manner of informing interested parties of potential health risks.

Collecting and analyzing algal samples in the period of May – October 2005, fulfills the first and second information needs outlined by the GCWIN Technical Committee. Algal toxin concentrations will be compared to the World Health Organization and EPA recommended guidelines and the limited results from the 2004 samples. Algae cell counts, chlorophyll, temperature, D.O., and conductivity will be compared to the 2004 results when available.

The results and weekly reports will be analyzed into a final report, fulfilling the third information need of tracking the algae in Grand County. The final report, expected by 12/31/05, will be used to evaluate the extent of this rapidly growing problem in Grand County. The final report will combine the empirical data with the physical factors contributing to the alga growth, such as nutrient sources; past and present water quality; and hydrologic conditions. Tracking and quantifying the increased alga growth in Grand County's lakes and rivers will help decision-makers implement watershed improvement goals and objectives and improve in stream water quality.

Project Budget - Breakdown of Costs

Total Budget

Price of phytoplankton biovolume analysis		
	\$110/sample x 114 =	\$12,540
Mileage to Berthoud		
212 miles x 21 trips = 4,452 miles		
	4,452 miles x \$0.375/mile =	\$1,670
Price using the Elisa Method		
	\$20/sample x 103 samples =	\$2,060
Price using PPIA and HPLC Methods		
	\$150/sample x 63 samples =	\$9,450
Equipment Costs:		
(coolers, ice packs, nalgene bottles, filters)		\$1,040
FedEx shipping of filters for algal toxins		
	Average of \$58/sample x 20 samples =	\$1,120
Elisa Microplate Reader		\$4,000
<u>Contingency</u>		<u>\$3,000</u>
TOTAL		\$34,880

<u>Three Lakes</u>		<u>Costs</u>
1) Number of phytoplankton analyses	52	\$6,500
2) Samples analyzed y ELISA	41	\$820
3) Samples analyzed by PPIA & HPLC	33	<u>\$4,950</u>
<i>Three Lakes Analytical Costs Portion</i>		<i>\$12,270</i>

<u>Fraser & Colorado River</u>		
1) Number of phytoplankton analyses	62	\$6,820
2) Samples analyzed y ELISA	62	\$1,240
3) Samples analyzed by PPIA & HPLC	30	<u>\$4,500</u>
<i>Fraser & Colorado River Analytical Costs Portion</i>		<i>\$12,560</i>

<u>Shared Project Costs</u>		
1) Mileage to Berthoud		\$1,670
2) Equipment Costs		\$1,040
3) ELISA Microplate Reader		\$4,000
4) FedEx shipping		<u>\$1,120</u>
<i>Shared Project Costs Portion</i>		<i>\$7,830</i>

Grand County Algae Monitoring Project – Monitoring Sites

	<u>Site ID</u>	<u>Site Name</u>
1	Shad-USGS <i>SM-DAM-USGS USBR</i>	Shadow Mountain Lake – USGS site by south end of reservoir near dam <i>Shadow Mountain Reservoir by Dam</i>
2	Shad-mid <i>SM-MID</i>	Shadow Mountain Lake – mid-lake site over old river bed <i>Shadow Mtn Reservoir at Mid-Reservoir</i>
3	Grand L <i>9013900?</i>	Grand Lake <i>Grand Lake at Grand Lake/USGS</i>
4	L Granby <i>400806105474700</i>	Lake Granby – USGS site north of dam <i>Lake Granby (east) near Granby, CO/USGS</i>
5	Fras.R-1 <i>FR-1</i> <i>FR1</i>	Fraser River above Windy Gap at FR-1 <i>FRASER RIVER</i> <i>Fraser River upstream of WP and downstream of turnoff to Mary Jane</i>
6	Fras. R-2 <i>FR-abvGSD</i>	Fraser River above Granby Sanitation Plant at River Watch Site 54 <i>Fraser River above Granby Sanitation District</i>
7	Fras. R-3 <i>FR-Hwy40GR</i>	Fraser River @Granby (Hwy 40) <i>Fraser River at Hwy 40 at Granby</i>
8	Fras R-4 <i>FR-blwCrk</i>	Fraser River below Crooked Creek @ the mouth of the Fraser Canyon <i>Fraser River below Crooked Creek</i>
9	Fras. R-5 <i>FR-10</i>	Fraser River @ Tabernash <i>FRASER NEAR TABERNASH</i>
10	Fras. Lions <i>Fras. Lions-AP</i> <i>Fras. Lions-KP</i>	Fraser Lion's Ponds in Fraser <i>Fraser Lions Club Adult Pond</i> <i>Fraser Lions Club Kids Pond</i>
11	Ranch <i>R1</i>	Ranch Creek below Cabin Creek near Devil's Thumb <i>Ranch Creek NR Tabernash</i>
12	Tenmile <i>12167</i>	Tenmile Creek above the mouth <i>TENMILE CK. NEAR GRANBY</i>
13	CO R-1 <i>COR-blwWG</i>	Colorado River below Windy Gap @ DOW <i>Colorado River below Windy Gap @ Hitching Post</i>
14	CO R-2 <i>COR-abvHSS</i> <i>COR-abvHSR</i>	Colorado River above Hot Sulphur Springs <i>Colorado River above Hot Sulphur Springs Water Treatment Plant</i> <i>Colorado River above Hot Sulphur Springs Resort</i>
15	CO R-3 <i>WS-CO-004</i>	Colorado River above Kremmling <i>Colorado River at Kremmling (at Bridge on Colo 9)</i>

Note: Alternate Site IDs and Site Names are in italics and are the estimated site location that corresponds to the current GCWIN monitoring site list located at co.grand.co.us/waterquality/waterdata.

Project Sampling Schedule:

Phytoplankton Sampling Schedule FY 2005

<u>Date</u>	<u>Shad- USGS</u>	<u>Shad- mid</u>	<u>Grand L</u>	<u>L Granby</u>	<u>Fras R- 1</u>	<u>Fras R- 2</u>	<u>Fras R- 3</u>	<u>Fras R- 4</u>	<u>Fras R- 5</u>	<u>Fr Lions</u>	<u>Tenmile</u>	<u>CO R-1</u>	<u>CO R-2</u>	<u>CO R-3</u>
5/23/2005		1	1	1		1					1			
6/6/2005		1												
6/20/2005	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7/5/2008		1	1											
7/11/2005		1	1											
7/18/2005	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7/25/2005		1	1											
8/1/2005		1	1											
8/8/2005		1	1											
8/15/2005	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8/22/2005		1	1											
8/29/2005		1	1											
9/6/2005	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9/12/2005		1	1											
9/19/2005		1	1		1	1	1	1	1	1	1	1	1	1
9/26/2005		1	1											
10/3/2005		1	1											
10/10/2005		1	1		1	1	1	1	1	1	1	1	1	1
10/17/2005	1	1	1	1										
10/21/2005		1	1											
10/28/2005		1	1											

Total Samples	5	21	20	6	6	7	6	6	6	6	7	6	6	6
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Total Samples to be Analyzed = 114

Algal Toxin Analysis Schedule

FY 2005

	ELISA Method
	PPIA & HPLC Methods

Date	L Granby	Shad-mid	Grand L	Ranch	Fras R-1	Fras R-2	Fras R-3	Fras R-4	Fras R-5	Fr Lions	Tenmile	CO R-1	CO R-2	CO R-3
5/23/2005		1		1		1					1			
6/6/2005		1												
6/20/2005		1		1	1	1	1	1	1	1	1	1	1	1
7/5/2008		1	1											
7/11/2005		1	1											
7/18/2005		1	1	1	1	1	1	1	1	1	1	1	1	1
7/25/2005		1	1											
8/1/2005		1	1											
8/8/2005		1	1	1	1	1	1	1	1	1	1	1	1	1
8/15/2005	1	1	1											
8/22/2005		1	1											
8/29/2005		1	1	1	1	1	1	1	1	1	1	1	1	1
9/6/2005	1	1	1											
9/12/2005		1	1											
9/19/2005		1	1	1	1	1	1	1	1	1	1	1	1	1
9/26/2005		1	1											
10/3/2005		1	1											
10/10/2005		1	1	1	1	1	1	1	1	1	1	1	1	1
10/17/2005		1	1											
10/21/2005		1	1											
10/28/2005		1	1											

Total Samples	2	21	18	7	6	7	6	6	6	6	7	6	6	6
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Appendix B

Sampling & Analytical Methods

Algal Toxin Collections

Send all samples to:

Bureau of Reclamation

Mike Simonavice

6th ave. and Kipling St.

Denver Federal Center, Bldg. 67, 86-68220

Denver, CO 80225

303 445-2196

1. Collect 1 L of water at 0.5m or right under the surface in scummy areas.
2. Filter on Whatman 934 AH Glass Fiber, 47mm, filters
3. Fold filter in half and place in aluminum foil and then in coin envelope
4. Send on dry ice overnight to above address. If collecting on multiple days freeze filters and send on dry ice ASAP.
5. Water sample can be sent but we prefer filters. Collect 1L of sample, freeze on dry ice and send immediately.
6. Extract of algal toxin can be analyzed on LCMS. We will send extract to Dr. Greg Boyers Lab for an additional fee.

Extract in screw top tube (wet ice) will be sent to Boyer's Lab for LCMS analysis by overnight express per customer request. These samples will be sent on wet ice if sample is sent immediately in overnight express mail or extract will be sent on dry ice if sample is kept in our freezer (-20°C) for a few days. Extract for reruns will also be kept in -20°C freezer for a few days since it is very stable.

Customer can also collect an additional filter and store in -20°C freezer and send to any lab for LCMS analysis, if they so choose. Or customer can collect a duplicate 1L sample and keep it frozen for LCMS analysis. These samples will be sent by customer if further analysis is warranted.

Additional fees will be charged to customer for dry ice and shipment to Dr. Greg Boyer's lab in Syracuse, New York if LCMS analysis is requested.

All data will be confidential. Data will be sent in excel spreadsheets via e-mail. Phone calls to customer can also be made if algal toxin levels are high. These "high" levels should be specified by customer.

2006 GCWIN Algae Monitoring Procedures

YSI Sondes:

Equipment needed:

YSI Sondes

Hand held data logger

Cable

Unscrew small metal cap on end of sondes and place in secure place. Attach sondes to the cable and the data logger. Unscrew protective plastic cover on the bottom of the probe. Take out the damp sponge and screw plastic cover back on. Now loosen the top black bottom of the plastic cover and replace with just one or two threads screwed down. You are now ready to calibrate the sondes for DO.

To **Calibrate for DO** (must be done prior to monitoring each time): Turn on data logger. The first screen that comes up is the **Main Menu**. Check to see that correct date and time are showing. Move the cursor down with the down arrow to **Sonde menu** and press the enter arrow key. Chose Calibrate from the options. Chose Dissolved Oxygen, and then DO %. The cursor will flash on the barometric pressure. If barometric pressure is different than what is shown on the bottom right corner, then enter numbers in manually (pressing enter using the enter arrow key). Hold the sondes out of direct sunlight as much as possible with your body while the sondes calibrates automatically for 60 seconds. After calibrating the sondes will go to the field screen. Hit the enter arrow to accept the calibration. Now hit the Esc button until you are back at the main menu (the probe will connect and disconnect its self automatically in order to go back to the main menu).

Now you are ready to screw on the open-cut plastic cover and begin sampling. Screw on the open-cut plastic cover to protect probes. Place the sondes in water.

On the data logger, go to the **Main Menu** and select Run. The data logger will connect. Select run and press enter. The probes will go into a cleaning cycle, indicated at the top of the screen.

After the cleaning cycle, the screen will read run at the top. Lower the probe to the desired depth starting at 0.5 m. Once the pH, temp, and DO are stable, press enter. The site selection will come up on the screen. I believe Shadow Mountain is SM06NEW, Grand Lake is GL06NEW, and Lake Granby is GR06NEW. Use the up and down arrows to select the right site and press enter. After the data point has been recorded, the data logger will return to the previous screen. In **Grand Lake**, take measurements in 1-meter increments starting at 1 m to 25 m. After 25 m, take measurements in 5-meter increments. We're been going down to 50 meters depending on the depth meter. In **Shadow Mountain**, take measurements in 1-meter increments starting at 1 m to 5 m.

0-5 Meter Phytoplankton Samples:

Equipment needed:

Swimming pool hose

Bucket

Sample bottle – 250 mL or 500 mL

Rinse the bucket out with lake water two or three times to remove any dirt or debris.

Rinse the swimming pool hose by first removing the plug or cork at the one end of hose. Holding onto the rope, lower the end of the hose connected to the rope into the water. Let the hose extend the full-length (6 meters). Place plug or cork into the end of the hose.

Holding onto the corked end of hose, pull on the rope to bring the hose up into the boat. Place the end of the hose into the bucket and remove the cork. The water should rush out of the hose into the bucket. Repeat the rinse step at least three times before collecting the sample.

Collect the sample at the 5 meter depth if possible (each duct tape marking is 1 meter). If the sampling location is not 5 meter deep, take the hose to the nearest meter and record depth on sample bottle. After placing the sample from the hose into the bucket, swirl the bucket to mix the sample. Rinse the sample bottle with the sample water by filling the bottle approximately half full. Cap the bottle, and then shake the bottle vigorously before emptying the contents. Rinse three times. Swirl the bucket once again before filling the bottle with the sample water just to the bottom of the neck. Add 18 drops of Lygols (acidified iodine) to the 500 mL bottles. 250 mL bottles only need 9 drops of Lygols. Record the time on the sample bottle.

1 Meter Phytoplankton Samples:

Equipment needed:

Van Doran Sampler

Sample Bottle

Take the Van Doran Sampler and open the blue spigots on the top and bottom of the sampler. The spigots are open when the blue levers are in line with the spigots.

Next, with your left hand, open the top lid by pulling the top and metal loop out and down. With your right hand, depress the metal lever on top of the handle down. Slide the metal loop attached to the top lid into the slot on the left side of the handle. Release the metal lever on top to secure the metal loop from the top of the lid.

Next, with your right hand, pull out and up on the metal loop attached to the bottom lid. Slide the metal loop over the metal stud on the top right side of the handle. Both ends of the sampler should be open now.

Gently, lower the sampler into the water and move up and down a few times to flush the water column through the sampler. Attach the metal messenger (weight) to the rope (if it isn't already attached) by rotating the bottom of the weight to the open position and slide the rope inside. Be careful since the weight is spring-loaded and will snap shut. After the messenger is on the rope, slide the messenger down the rope into the water. The weight will trigger the handle to release the metal loops and close the lids.

Once the lids are closed on the sampler, pull the sampler up and let the water flush through the attached tubing rinsing the sampler. Repeat the rinse process three times.

To take a sample, first, close the bottom spigot lever before opening up the lids. Lower the sampler down to the 1-meter mark on the attached rope. Once the sampler is vertical in the water column, release the messenger to trigger the lids. Haul the sampler up and out of the water. Rest the sampler on the edge of the boat to keep pressure on the bottom of the sampler if needed. Open and close the bottom spigot while rinsing the sample bottle with sample water three times. Fill the bottle to the bottom of the neck and add 18 drops of Lygol's per 500 mL of sample water. Add 9 drops of Lygol's per 250 mL. Record the time on the sample bottle.

Note: The same sample water used to fill the 1 meter bottles can be used to fill the toxin bottles. Rinse the toxin bottles three times with the sample water to be used. Fill the toxin sample bottoms to below the neck of the bottle to allow for the water to expand when frozen.

When filling the small plastic toxin tubes with the blue lids, *do not* rinse the tubes. Instead, fill toxin tubes only to the 12 mL level (marked with a black line).

Algal Toxin Samples:

Equipment needed:

Van Doran Sampler

Sample bottles

Sample tubes

*** Please note that algal toxin samples are collected in three different sizes:

1 liter bottle, small amber bottles, and plastic tubes with blue caps.

Do not rinse the plastic tubes as they have a preservative (ascorbic acid) in them.

All other bottles are rinsed prior to collecting the sample.

If the sampler hasn't been rinsed in the lake to be sampled, use the procedures above for opening and rinsing the Van Doran Sampler. If the sampler has already been rinsed in the lake being sampled, lower the sampler to the desired depth (usually 1-meter) and collect the sample. Rinse the sample bottles three times. Fill the toxin sample bottles to below the neck of the bottle to allow for the water to expand when frozen. Record the sample time on the bottle.

When filling the small plastic toxin tubes with the blue lids, *do not* rinse the tubes. Instead, fill toxin tubes only to the 12 mL level (marked with a black line) to allow for the water to expand when frozen. Record the sample time on the tube. Keep the samples as cold as possible while in the field before freezing.

Microcystin ImmunoStrip® Prototype

Prototype tests for the detection of Microcystins

CONTENTS

Size 00200	Item	Quantity
	ImmunoStrip™	200 strips
	Sample Tubes	200 tubes
	Instructions	1
	100ul pipette	2
	Pipette tips	250

STORAGE

Keep the strips tightly sealed in the container with the desiccant at all times. Keep the sample tube lids tightly sealed until use. Store the ImmunoStrip® and sample tube containers in the refrigerator (4°C) between uses.

YOU WILL NEED

- Well water
- Micro tube rack

SAFETY

Microcystins are considered hazardous and classified as hepatotoxins. Always wear gloves and protective garments when handling samples.

This kit is a lateral flow prototype intended for the detection of microcystin toxin, an algal toxin in fresh water. Microcystins have become a growing concern for the public and private sectors due to the known harmful impacts on the physiological state of aquatic and mammalian species.

The prototype is formatted to operate as a rapid competitive immunoassay. Unlike direct sandwich assay lateral flow devices, the Microcystin ImmunoStrip® will not produce a line if the sample is positive. A test line will only appear with negative samples.

TEST PROCEDURE

1. To extract the toxin from the cyanobacteria, aliquot the desired volume into the extraction vials (At least 300ul). Snap the cover on the vials and place the vials in the extraction floatation device.



Microcystin ImmunoStrip® Prototype

Prototype tests for the detection of Microcystins

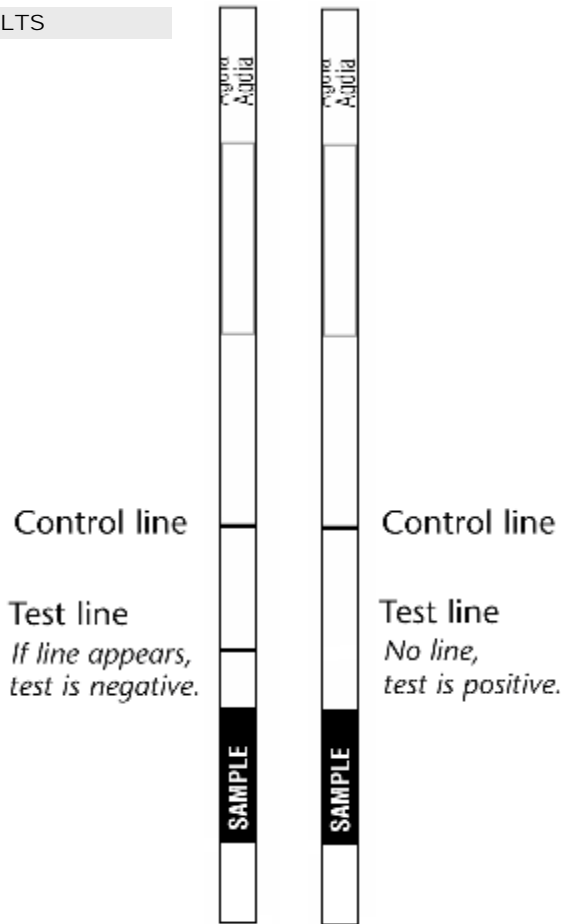
INTENDED USE

2. Using a 'hot pot' or container of water (a pan or beaker will also work), place the floatation device with the extraction vials into the container of water. Turn the heat source to high and watch the container until the water boils. Depending on the heat source, it should take about 10 minutes.
3. Once the water starts to boil, turn off the heat. Using water tight and heat protective gloves or forceps, remove the floatation device with the vials. Make sure that water has not leaked into the vials.
4. Let the vials cool so that they can be handled.
5. Carefully remove the snap lid on the extraction vials.
6. Firmly push one of the yellow pipette tips included in the kit onto the 100ul pipette also included in the kit. The tip should not fall off if it is on correctly.
7. Carefully remove the snap lid on the sample vial. Pipette 200ul (Two aliquots using the kit pipette) of extracted sample into the sample vials and gently swirl the vial.
8. Carefully place the Microcystin ImmunoStrip® into the vial and incubate until all of the liquid has been drawn into the ImmuoStrip®. Placing the sample vial in a microtube rack will insure that the vial does not tip over during incubation.
9. Once the sample liquid has been absorbed by the ImmunoStrip®, refer to the results section below for interpretation of the ImmunoStrip®.

Microcystin ImmunoStrip® Prototype

Prototype tests for the detection of Microcystins

RESULTS



Maximum reaction occurs in 30 minutes or until all of the liquid has been absorbed by the stick at which time the ImmunoStrip should be removed from the sample tube. The control line assures that the test is working properly. If the control line does not appear, the test is invalid.

The illustration below shows results expected for negative and positive samples. If the sample is positive, the test line will NOT appear. If the sample is negative, the test line will appear.

If you wish to keep the strips as permanent records, be aware of the possibility that the sample and absorbent pads may contain microcystins and should be handled with gloves. Be sure to store the strips so that the absorbed liquid is contained and potential microcystin exposure from the ImmunoStrip® is eliminated.

Microcystin ImmunoStrip® Prototype

Prototype tests for the detection of Microcystins

LIMITATIONS

The following is a description of factors that could limit test performance or interfere with proper test results.

- Expiration: Extended stability tests have not been performed on the prototype, therefore a shelf life can not be guaranteed for this product.
- Samples: Laboratory algal cultures were used to develop this product. It is unknown how the prototype will work environmental algal matrix.
- Temperature: Optimal test results will occur when the test is run in an environment where the temperature is between 60° and 95° F (15° and 35° C).
- Storage: Test results may be weak or the test may fail if the storage instructions are not followed properly. If the ImmunoStrip package is left open too long, the strips may absorb moisture. This may affect test results.
- Sample volume: Strip performance is very dependent on the proper sample volume. The strip will not perform according to specifications with a volume other than 200ul.

Appendix C

Data Summaries and Analyses

2005 Toxin Analyses										
				Microcystin by PPIA				Anatoxin-a by HPLC		
<u>Sample #</u>	<u>Lake Name</u>	<u>Date Received</u>	<u>Collection Date</u>	<u>n</u>	<u>Mean (ug/L)</u>	<u>SD</u>	<u>CV</u>	<u>n</u>	<u>ug/L</u>	<u>Detection limit (ug/L)</u>
05-984	Grand Lake	9/15/2005	9/8/2005	4	0.210	0.109	52	2	nd	0.008
05-1201	Grand Lake	9/21/2005	9/16/2005	2	0.095	0.019	20	2	nd	0.007
05-1202	Shadow Mountain	9/21/2005	9/16/2005	2	0.084	0.017	20	2	nd	0.007
05-1237	Grand Lake	10/19/2005	10/16/2005	2	0.100	0.000	0	2	nd	0.007
05-1238	Shadow Mountain	10/19/2005	10/16/2005	1	<0.061			2	nd	0.007
05-1239	Shadow Mountain	10/19/2005	9/22/2005	2	0.105	0.022	21	2	nd	0.008
05-1240	Shadow Mountain	10/19/2005	9/27/2005	3	0.081	0.018	23	2	nd	0.007
05-1241	Grand Lake	10/19/2005	9/27/2005	2	0.063	0.007	10	2	nd	0.007
05-1242	Grand Lake	10/19/2005	10/6/2005	2	0.071	0.023	32	2	nd	0.007
05-1243	Grand Lake	10/19/2005	9/22/2005	2	0.056	0.016	28	2	nd	0.007
05-1264	Grand Lake	10/27/2005	10/20/2005	2	0.070	0.002	2	2	nd	0.007
05-1265	Shadow Mountain	10/27/2005	10/20/2005	2	<0.060			2	nd	0.007
05-1298	Shadow Mountain	12/8/2005	10/30/2005	2	<0.060			2	nd	0.007
05-1299	Grand Lake	12/8/2005	10/30/2005	2	<0.060			2	nd	0.007
05-1300	Grand Lake	12/8/2005	11/6/2005	2	<0.060			2	nd	0.007

2004/2005:

Anabaena Cell Counts & Microcystin Toxin Levels for Shadow Mtn.& Grand Lake.

Anabaena, Microcystis, and Aphanizomenon cell counts are in cells/mL.

All samples were collected at 1 meter unless otherwise noted.

Microcystin toxin levels are in ug/L. All Anatoxin-a toxin levels were below detection limits of either 0.007 or 0.008 ug/L as run by HPLC.

The yellow cells - toxins analyses run by PPIA. The orange cells - toxin analyses run by ELISA.

Date	Type	Shadow Mountain				Grand Lake				Lake Granby			
		2004	ug/L	2005	ug/L	2004	ug/L	2005	ug/L	2004	ug/L	2005	ug/L
June 6, 8	Anabaena			172 (0-5 m)				0 (0-5 m)				22 (0-5 m)	
	Aphaniz.			153 (0-5 m)				0 (0-5 m)				13 (0-5 m)	
July 1	Anabaena			0				0					
	Aphaniz.			68				0					
	Microcystis			348				0					
July 7, 8	Anabaena			12 (0-5 m)				4 (0-5 m)				2,210 (0-5 m)	
	Aphaniz.			42 (0-5 m)				0 (0-5 m)				170 (0-5 m)	
	Microcystis			0 (0-5 m)				0 (0-5 m)				0 (0-5 m)	
July 20	Anabaena			93				2					
July 22	Anabaena	43,350				199				232			
July 26	Anabaena			93				0					
Aug. 4, 5	Anabaena			1,785	< 0.16			255				0	< 0.16
	Aphaniz.			0				0				0	
	Microcystis			0				0				0	
Aug. 10	Anabaena	42,457 avg.				538				229			
	Aphaniz.									150			
Aug. 15	Anabaena			21,080				612				0	
Aug. 23	Anabaena	57,000	0.05										

Date	Type	Shadow Mountain				Grand Lake				Lake Granby			
		2004	ug/L	2005	ug/L	2004	ug/L	2005	ug/L	2004	ug/L	2005	ug/L
Aug. 27	Anabaena					5,661							
Aug. 28	Anabaena			29,376	< 0.16			3,536	< 0.16				
Aug. 31	Aphaniz. Microcystis			0									
	Anabaena	15,928	0.02	0		5,763							
Sept. 1	Anabaena			9,027	< 0.16			13,770				18	
	Aphaniz. Microcystis			0				2				15	
Sept. 8	Anabaena	8,325	0.05	32,436	< 0.16	4,921	< 0.06	17,136	< 0.16	246			
	Aphaniz.												
Sept. 14	Anabaena	994	0.38			1,339	0.29						
Sept. 16	Aphaniz.	8,000			< 0.16	0			< 0.16				
	Anabaena			18,870 (0-5m)	0.08			21,471	0.1				
Sept. 19	Aphaniz. Microcystis							382					
	Anabaena	555 avg.	0.08			2,856	0.08						
Sept. 22	Aphaniz.	26			< 0.16	0			< 0.16			0 (0-5 m)	
	Anabaena			13,464	0.11			11,781	0.06				
Sept. 27	Aphaniz. Microcystis			0	< 0.16			0	< 0.16				
	Anabaena			8,468	0.08			8,236	0.06				
	Aphaniz. Microcystis												

		Grand Lake								Lake Granby			
Date	Type	Shadow Mountain				Grand Lake				Lake Granby			
		2004	ug/L	2005	ug/L	2004	ug/L	2005	ug/L	2004	ug/L	2005	ug/L
Oct. 3	Anabaena					493	0.19						
Oct. 6	Anabaena							4,233 6,120 (0-5m)	< 0.16 0.07				
Oct. 16	Aphaniz. Microcystis							0 0					
Oct. 16	Anabaena			22	< 0.16 < 0.06 1			935	< 0.16 0.10 0				
Oct. 20	Aphaniz. Microcystis			0 0	< 0.16			0 0	< 0.16				
Oct. 20	Anabaena	0		11	< 0.06	190		663	0.07 0				
Oct. 30	Aphaniz. Microcystis	35 0		0 0	< 0.16	0 0		0 0	< 0.16				
Oct. 30	Anabaena			37	< 0.06			1,530 (0-5m)	< 0.06				
Nov. 6	Aphaniz. Microcystis			0 0				0 0	< 0.16				
Nov. 6	Anabaena							340	< 0.06				
Nov. 18	Aphaniz. Microcystis							68 (0-5m)	< 0.16				
	Aphaniz. Microcystis							0 0					

Other Samples

Willow Creek Reservoir
Surface grab samples

Oct. 15
Oct. 29
Nov. 6

Aphanizomenon too numerous to count
Aphaniz. cell ct. 338,334 cells/ml, <0.16 ug/L Microcystin
Aphanizomenon cell count 258,450 cells/ml

CO River below
WG

Oct. 27
Nov. 6
Nov. 6
Nov. 6

Anabaena cell count 510 cells/ml, <0.16 ug/L Microcystin
Anabaena cell count 10 cells/ml, <0.16 ug/L Microcystin
Anabaena cell count 3 cells/ml
Anabaena not detected, < 0.16 ug/L Microcystin

2007 Grand County Phycocyanin Values

All values produced using a Turner Designs Aquafluor.
Instrument calibrated, and standard set to 525.5 ug/L by College of
Environmental Science, Syracuse, NY.

Date	Time	Station ID	Station Name	Phycocyanin Ave. (ug/L)	Phycocyanin Average Absorption Reading
7/3/2007	16:10	GR-Dam	Granby Reservoir Dam	0.134	12.376
7/16/2007	9:15	GR-Dam	Granby Reservoir Dam	0 (-0.449)	8.163
7/24/2007	10:45	GR-Dam	Granby Reservoir Dam	0 (-0.659)	6.642
7/31/2007	boat failure	GR-Dam	Granby Reservoir Dam	no sample	
8/6/2007	USBR	GR-Dam	Granby Reservoir Dam	no sample	
8/14/2007	10:27	GR-Dam	Granby Reservoir Dam	1.547	22.595
8/20/2007	USBR	GR-Dam	Granby Reservoir Dam	no sample	
8/28/2007	9:34	GR-Dam	Granby Reservoir Dam		
9/5/2007	USBR	GR-Dam	Granby Reservoir Dam	no sample	
9/11/2007	16:04	GR-Dam	Granby Reservoir Dam	0 (-1.344)	1.689
9/17/2007	USBR	GR-Dam	Granby Reservoir Dam	no sample	
9/25/2007	13:35	GR-Dam	Granby Reservoir Dam	0.198	12.84
10/5/2007	12:37	GR-Dam	Granby Reservoir Dam	1.279	20.655
10/9/2007	10:17	GR-Dam	Granby Reservoir Dam	0.121	12.279

7/3/2007	11:45	SM-Mid	Shadow Mountain Mid-Reservoir	0 (-0.350)	8.878
7/19/2007	10:03	SM-Mid	Shadow Mountain Mid-Reservoir	2.205	27.351
7/24/2007	15:31	SM-Mid	Shadow Mountain Mid-Reservoir	6.52	58.57
7/31/2007	9:50	SM-Mid	Shadow Mountain Mid-Reservoir	2.911	32.455
8/6/2007	16:18	SM-Mid	Shadow Mountain Mid-Reservoir	7.36	64.64
8/14/2007	14:25	SM-Mid	Shadow Mountain Mid-Reservoir	5.418	50.575
8/20/2007	13:03	SM-Mid	Shadow Mountain Mid-Reservoir	2.90	32.36
8/28/2007	12:50	SM-Mid	Shadow Mountain Mid-Reservoir	1.702	23.715
9/5/2007	13:04	SM-Mid	Shadow Mountain Mid-Reservoir	0.375	14.118
9/11/2007	12:30	SM-Mid	Shadow Mountain Mid-Reservoir	0 (-1.004)	4.149
9/25/2007	10:37	SM-Mid	Shadow Mountain Mid-Reservoir	0.827	17.383
10/5/2007	11:04	SM-Mid	Shadow Mountain Mid-Reservoir	0 (-0.99)	4.23
10/9/2007	15:03	SM-Mid	Shadow Mountain Mid-Reservoir	0.157	12.545
10/16/2007	13:20	SM-Mid	Shadow Mountain Mid-Reservoir	0 (-1.197)	2.754

Date	Time	Station ID	Station Name	Phycocyanin Ave. (ug/L)	Phycocyanin Average Absorption Reading
7/3/2007	13:40	GL-Mid	Grand Lake Mid-Lake	0.005	11.445
7/16/2007	11:45	GL-Mid	Grand Lake Mid-Lake	0 (-1.176)	2.904
7/24/2007	13:00	GL-Mid	Grand Lake Mid-Lake	2.689	30.85
7/31/2007	11:30	GL-Mid	Grand Lake Mid-Lake	3.137	34.09
8/6/2007	13:05	GL-Mid	Grand Lake Mid-Lake	6.006	54.83
8/14/2007	13:32	GL-Mid	Grand Lake Mid-Lake	6.231	56.458
8/20/2007	15:51	GL-Mid	Grand Lake Mid-Lake	3.538	36.986
8/28/2007	10:32	GL-Mid	Grand Lake Mid-Lake	2.381	28.618
9/5/2007	12:08	GL-Mid	Grand Lake Mid-Lake	0.848	17.535
9/11/2007	11:16	GL-Mid	Grand Lake Mid-Lake	0.193	12.805
9/25/2007	11:10	GL-Mid	Grand Lake Mid-Lake	0.651	16.116
10/5/2007	10:18	GL-Mid	Grand Lake Mid-Lake	0 (-1.199)	2.738
10/9/2007	12:26	GL-Mid	Grand Lake Mid-Lake	0.432	14.53
10/16/2007	11:17	GL-Mid	Grand Lake Mid-Lake	0 (-1.088)	3.543

8/12/2007	7:58	GL-AT-PIC	Grand Lake Adams Tunnel West Portal Picnic Area	11.90	97.47
8/12/2007	7:59	GL-AT-PIC	Grand Lake ATWP-PIC	6.97	61.79
8/14/2007	15:39	GL-AT-PIC	Grand Lake ATWP-PIC	6.564	58.855
8/20/2007	18:41	GL-AT-PIC	Grand Lake ATWP-PIC	2.49	29.39
9/2/2007	7:05	GL-AT-PIC	Grand Lake ATWP-PIC	2.781	31.508

Absorp x	Conc y
33.1	3
83.7	10
228.3	30