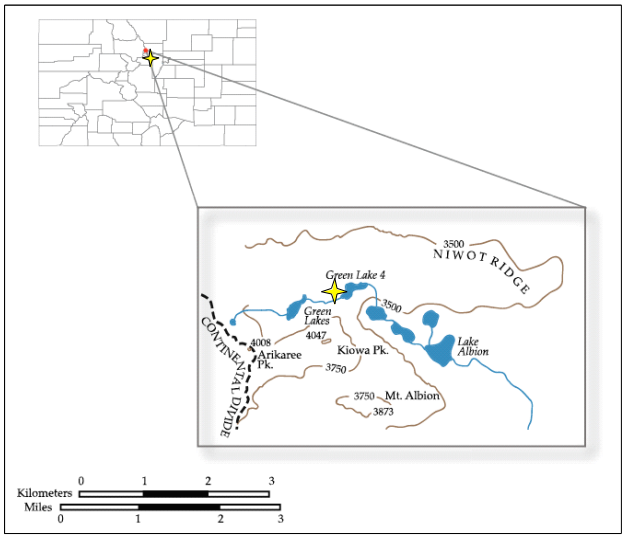
Niwot Ridge Long-Term Ecological Research (NWTLTER)



– GREEN LAKES VALLEY –

Limnological Protocol Monitoring Methods

STANDARD OPERATING PROCEDURES (SOPs)

Green Lake 4: 2000 to Present

- **SAMPLING**:
    - 5 site locations (see colored stars on bathymetric map at end of section):
        - UTM coordinates with topo map at end of document
        - Inlet (western green star)
        - Outlet (eastern green star)
        - In-Lake (black star)
            - Surface
            - 3 meters
            - 9 meters
    - Inlet and outlet can be sampled when ice free- usually around mid to late June. In-lake sampling (surface, 3 meters, and 9 meters) begins as soon as ice is clear enough for safe access in the raft, usually around the first week in July. This is often a week or so before the complete ice out date. Samples taken from the approximate deepest point in the lake (black star). Probes and sampler may get stuck in sediments if not at the deepest point.
    - Field gear:
        - Equipment stored in Action-Packer at the site (yellow star):

Communicate with the previous year's GL4 field tech as some equipment may over-winter at GL4 or at Albion, while some equipment may be located at MRS or INSTAAR

- Action Packer storage tub
- Raft
- Raft Pump
- Paddles (2)
- Life Jackets (2)
- Neoprene Paddling Gloves (2 pair)
- (In the past a rock was used as anchor)
- Van Dorn Sampler
- DO/Temperature meter
- pH/Conductivity/Temp meter
- Light meter (Photometer)
- Secchi disk
- Ropes
- Extra bottles
- AA batteries for meter replacements
- pH buffers (7.00 and 4.00)
- Conductivity buffer (usually 84 μS)

- Equipment to bring up every day:
    - Five 60 mL amber glass bottles (Williams DOM lab)
    - Ten 500 mL brown Nalgenes (Fisher Scientific)
    - Five 250 mL clear plastic bottles (Kiowa Lab)
    - Tape and marker; Field book and pen

Considerations

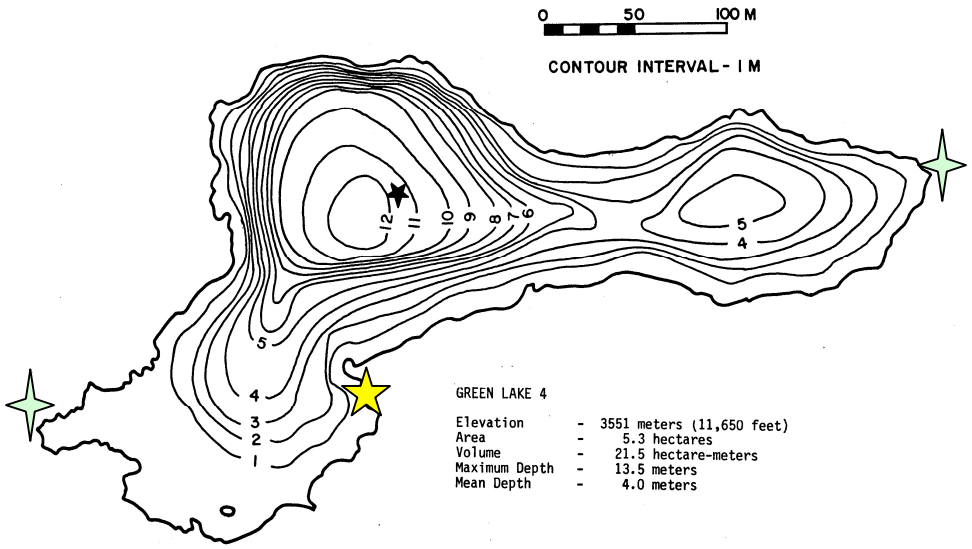
- WEATHER: Safety first. Be aware of developing storms as the high altitude offers little protection for afternoon lightning. Strong winds and subsequent white caps will also contribute to difficulty rowing the raft.
- PERSONAL GEAR: sunscreen, rainjacket, water-resistant pants, hat, sunscreen, sunglasses, extra layers, lunch/snacks, water, hiking boots
- HIKING: two potential routes to Green Lake 4 exist at the waterfall between GL4 and GL5 – to the east (right) is steep but usually without snow while to the west (left) is less steep although across snowfields
- WILDLIFE: Marmots can wreak havoc on equipment and your backpack, especially if there's food in it. 1) Between sampling dates, cover Action-Packer with large, heavy rocks (within reason) to protect equipment inside, and 2) While out on raft sampling, place backpacks and belongings in Action-Packer to keep marmots away.
- RAFT: test for leaks before the field season starts! Fully inflate and while applying pressure, listen for leaks or wipe with soapy water to see where the water is bubbling. Minor tears can be patched, new rafts purchased at McGuckin's Hardware. During the season be cognizant of pulling raft over rocks so to reduce chances of puncture.
- RESEARCH PROPOSAL: The City of Boulder Watershed requests research proposals, due the Spring of each sampling year, access at http://www.colorado.edu/mrs/rform.html

- Collection at each site:
  - pH, DO, conductivity, temperature: pH and conductivity are measured with a YSI 63 hand held meter. DO is also measured with a YSI meter (mg/L). Temperature readings should be the average between YSI 63 and YSI DO. Conductivity (μS/cm) is the reading when the temperature is not blinking. Take a complete temperature profile (measuring at each meter) *every* sampling trip with the YSI 63. Measured increments on cord at 1 m each.
  - Photometer: light readings are taken at deck and every meter. Lower with rope attached to frame, not with cord to sensor, otherwise sensor will not face upward toward the surface.
  - Water Samples – Rinse all bottles 3x with sample water before filling. Take grab samples at surface, take subsamples from Van Dorn sampler at 3 and 9 m depth
    - Phytoplankton and chlorophyll *a*: use 500 mL brown Nalgene bottle
    - Nutrients: use 250 mL clear plastic bottles; <u>labeled green</u>
    - DOC: use 60 mL *burned* amber glass (8 hours at 500 degrees) glass bottles.
  - Secchi depth: Measure weekly *only in-lake samples*. Drop the disc until the black and white sections are indistinguishable, record the depth, then pull it up until they are again distinguishable. The rope measured in ½ meter increments. Secchi depth (m) is the average of those two depths. Try to do this at approximately the same time of day because diurnal cycles effect light attenuation.

- Meter Calibration: Meter guides are located in Diane's lab at INSTAAR.
  - pH: Turn on, press MODE until pH is displayed, rinse probe with DI, place in pH 7 buffer, press UP and DOWN arrows at the same time, if "7.00" does not show up press UP and DOWN again, when the decimal point stops flashing press and hold ENTER, place in pH 4 buffer, press ENTER, when decimal point stops flashing press and hold ENTER, press mode to return to measuring.
  - Conductivity: Turn on, press MODE until conductivity is displayed, place in buffer up to the very top of the probe, make sure to get rid of air bubbles and allow temperature to stabilize, press UP and DOWN arrows at the same time, adjust display to buffer value, press and hold ENTER, press MODE to return to measuring.
  - DO meter should be calibrated to the highest altitude possible, 10,000 ft. The membrane occasionally needs replacement.

- Tape (Kiowa Lab) 1 ¼ around bottle (overlap so tape doesn't fall off when wet) and label with the following:
  - Location: GL4in, GL4out, GL4surf, GL43m, GL49m
  - Date: yymmdd
  - Diane's initials (DM) followed by researcher's initials
  - Mountain Standard Time (MST): subtract 1 hour from daylight savings time
  - Nutrient samples have special instructions according to protocol posted at MRS for Kiowa Lab. Be sure to read that, or ask Kurt Chowanski or Chris Seibold on the whereabouts. Label the front with location and date, right side must be labeled with "U" (unfiltered) or "F" (filtered) and yy, "DMGL4"and have sample number according to binder at Kiowa. For example:

    F05
    DMGL4
    5



FILTERING:

- Chlorophyll *a*: Filter all 500 mL through 47-mm Whatman glass fiber filters (pore size 1.2 μm, Fisher Scientific), rough side down, using a DI washed filtering setup and a hand pump. Discard filtrate, wrap the filter in tin foil, and freeze until analysis.

- Phytoplankton: Preserve sample with 1% Lugol's solution (5 mL in 500 mL). Invert three times before setting aside. Store preserved, ready to analyze samples in the microscope lab at INSTAAR.
  - Lugol's recipe: mix 30 g of KI, 15 g Iodine (crystalline) dissolved in 300 mL of distilled water, and 300 mL glacial acetic acid.
  - Look for chemicals at McKnight's INSTAAR lab, or purchase them at Chem Stores

- Nutrients: From the Kiowa lab, one 125 mL bottle and two 60 mL bottles for each 250 mL sample bottle. Also grab the red binder. All unfiltered samples have green tape and all filtered samples have pink tape. Put pink tape on the five 125 mL bottles and five 60 mL bottles. Use the same labeling procedure outlined above (F goes on pink tape, U on green). Put green tape on five 60 mL bottles. Gelman filters, filtering protocol and filtering equipment are above and around the sink in the MRS lab. Store the two 60 mL samples in the red freezer in the LTER lab. Put the 250 mL and 125 mL bottles in the walk in fridge in the Kiowa lab. They are stored in boxes along the right side shelves. Label the GL4 box DM<your initials>. Again, be sure to record the Kiowa lab number and other sample info in the red binder in the Kiowa lab (ask someone who works there where it is). Access to Kiowa's walk-in at the end of your field day should be arranged ahead of time (doors lock ~4:00pm).

- DOC: Filter through *burned* 47-mm Whatman glass-fiber filters (pore size 1.2 μm, same filters used for chl *a* except burned in tin foil at 500 C for 8 hours). Pour filtrate into <u>new</u>, burned amber DOC bottles (lids are only DI rinsed) and refrigerate until analysis. Discard the filter after each sample.

- **ANALYSIS**:
  - Chlorophyll *a* analysis: Remove chlorophyll from the filters with hot ethanol extraction, and quantify by the spectrophotometric method, including a phaeopigment correction, detailed in Gardner (2003) and attached as supplemental information. In 14 mL vials (obtain from Chem Stores on campus), extract chlorophyll *a* using hot, 100 proof ethanol and then set aside and filter. Take absorbance measurements at 665 and 750 nm both before and after acidification with 40 µL 2N hydrochloric acid. Use the following equation to quantify chlorophyll *a* concentration: chlorophyll *a* (µg/L) = $29.6((665_b - 750_b) - (665_a - 750_a))*(v/Vz)$ where 665_b and 750_b are the absorbencies of the sample at 665 nm and 750 nm before acidification, 665_a and 750_a are the absorbencies at 665 nm and 750 nm after acidification, v is the volume of ethanol solvent in mL, V is the volume of sample originally filtered in L (0.5), and z is the cuvette length in cm (5). 90% ethanol is used as a blank. [Chemicals at Chem Stores on campus if not at INSTAAR lab.]

  - DOC: Shimadzu Total Organic Carbon Analyzer

  - Kiowa Chemistry Lab will analyze nutrient samples for GL4 and return data including nutrients, ions, acid neutralizing capacity, pH, and conductivity by or around March of the following year. Information will be e-mailed via Diane. Details of their protocol can be found at: http://snobear.colorado.edu/Kiowa/Kiowaref/procedure.htmL.

  - Phytoplankton Identification: Volumetric settling of between 10-50 mL of sample, depending on cell concentration, using Hydro-Bios or Utermöhl plate chambers and settling cylinders (see http://www.hydrobios-international.com or Aquatic Research Instruments, hydrobio@aol.com for supply information).

    - Directions for algae settling:
      1) Rinse slides with water, clean with 90% alcohol and kimwipes. Be careful not to break the thin glass bottom plate of the well.
      2) Put adequate "vacuum grease" (i.e., Vaseline) around the edge of the well and place a settling chamber/cylinder on the slide.
      3) Invert the sample 20x (not vigorously) as cells will settle in sample bottle overtime. Pour into column until there is extra sample forming a "bubble" on top and use the Vaseline on a thick glass cover plate to seal the chamber, preventing unwanted evaporation or contamination.

4)
5) Wait 30 hours for 50 mL; 15 hours for 25 mL
6) To remove cylinder, place a thin glass cover plate on the slide, immediately next to the cylinder but not over the small evacuation hole on one end. Push down on the settling cylinder and slide over the hole while doing the same with the thin cover plate, attempting to avoid air seepage. Be quick but careful.
7) Align the cylinder above the evacuation hole, place unit over sink or other receptacle, and remove think glass plate as the sample then drains.
8) Wipe away smudges and excess Vaseline from slide.
9) Identify on an inverted light microscope using a 100x (1000x total) oil immersion lens. Count to 400 cells or 20 fields, whatever comes first.

- Refer to http://culter.colorado.edu/lake-algae/ for photomicrographic records and taxa information.

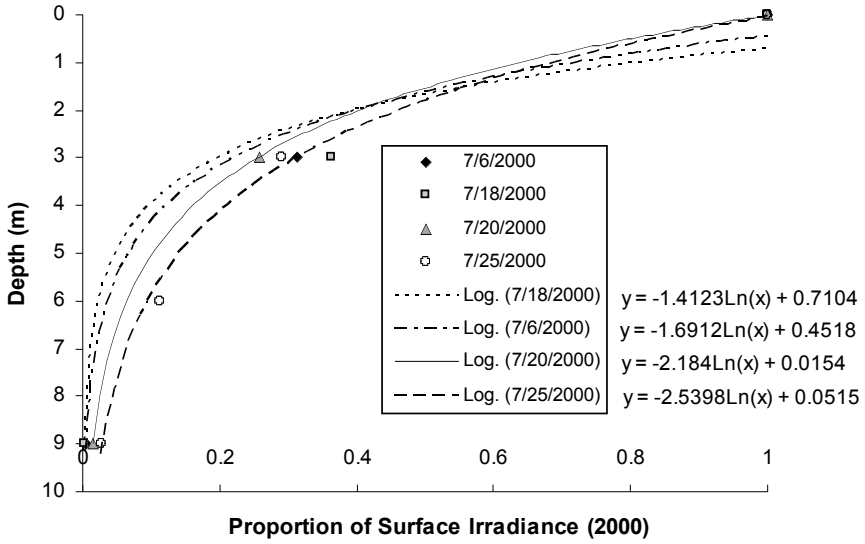
- Also see bibliographic resources such as:

Lee, R. E., 1999: *Phycology*, 3rd Edition. Cambridge: University Press.
Prescott, G. W., 1979: *How to know the freshwater algae*, 3rd Edition. Dubuque: The Pictured Key Nature Series.
Smith, G. M., 1950: *The Fresh-Water Algae of the United States*, 2nd Edition. New York: McGraw-Hill Book Company, Inc.
Wehr, J. D. and Sheath, R. G., (eds.), 2003: *Freshwater Algae of North America*. Boston: Elsevier Science.

- Supplemental data includes hard photographic copies

- **CALCULATIONS**:
  - Residency time (days) = Volume (m^3) / Discharge (m^3/day). Discharge is recorded by stream gauge GL4 outlet and available at http://culter.colorado.edu/NWT/data/datmansearch.htmL. Total volume of the lake is approximately 215,000 m^3 and upon stratification, the volume of the epilimnion (the mixed layer, where flushing is concentrated) is approximately 175,000 m^3.

  - Depth of 1% irradiance is where 99% of the light has been attenuated. This indicates the bottom of the photosynthetic zone. To find this, calculate the percentage of surface light at each depth (light at depth / light at deck; x-axis). Then graph a scatter plot of depth vs. proportion of surface irradiance, and add a logarithmic trend line to each sampling date. Use the equation of the line to solve for 1% attenuation. For example:



Calculate depth of 1% attenuation with substitution of 1% (1/100) for x in the equation of the line. Thus, on 7/6/2000, 1% attenuation yielded 8.24 m; 7/18/2000, 7.21 m.

  - Phytoplankton concentration: cells/mL = (cells/field of view) / [(# fields looked at) * (density number)]. The 'density number' relates the area of the field of view to how much sample was settled. For 10 mL (identifying at 1000x), it is given by:

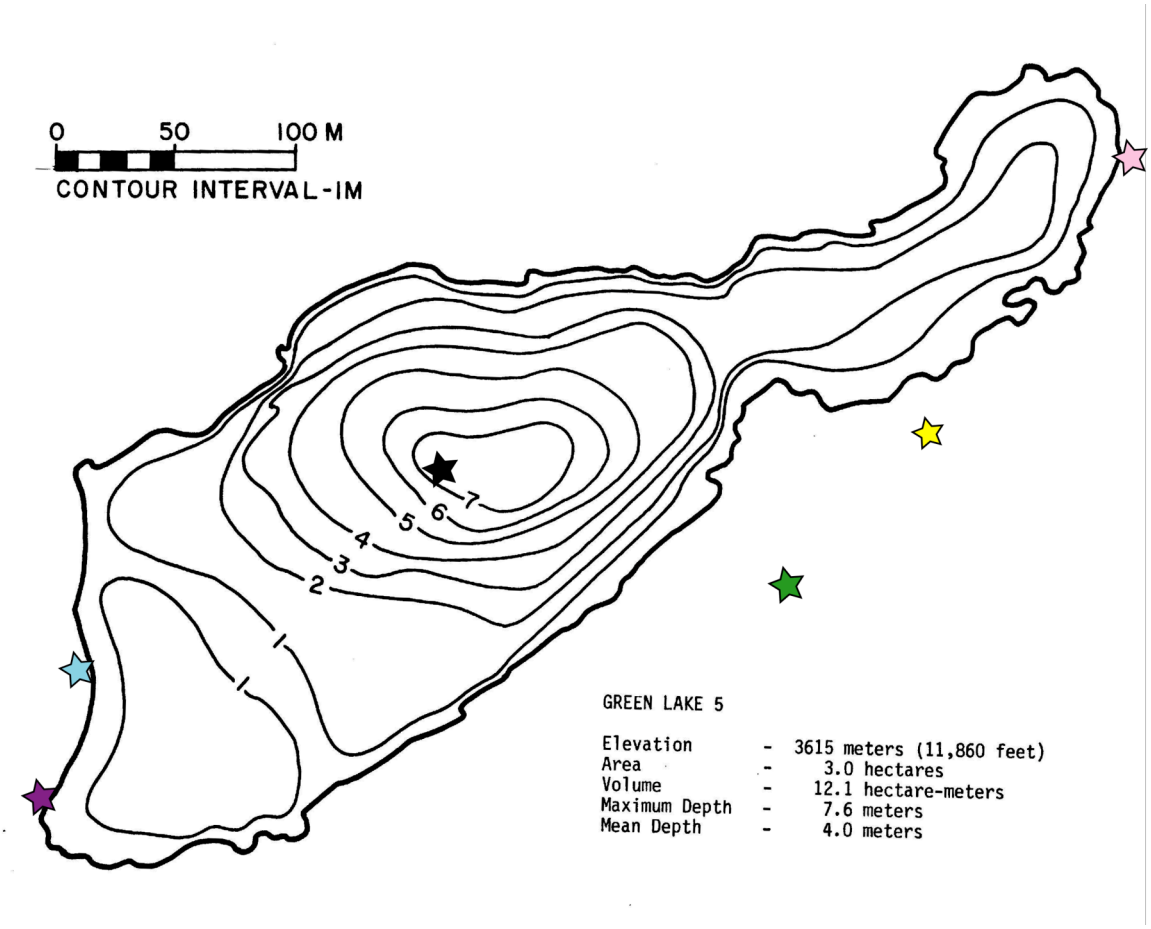
Settling 10 mL:	Nikon Diaphot (bigger scope, A)	Nikon Eclipse (smaller, B)
Hydrobios Slide	0.000875	0.000615
Utermohl Slide	0.0062	0.004375

Therefore to calculate cell density after settling 40 mL, multiply the correct number from above by four.

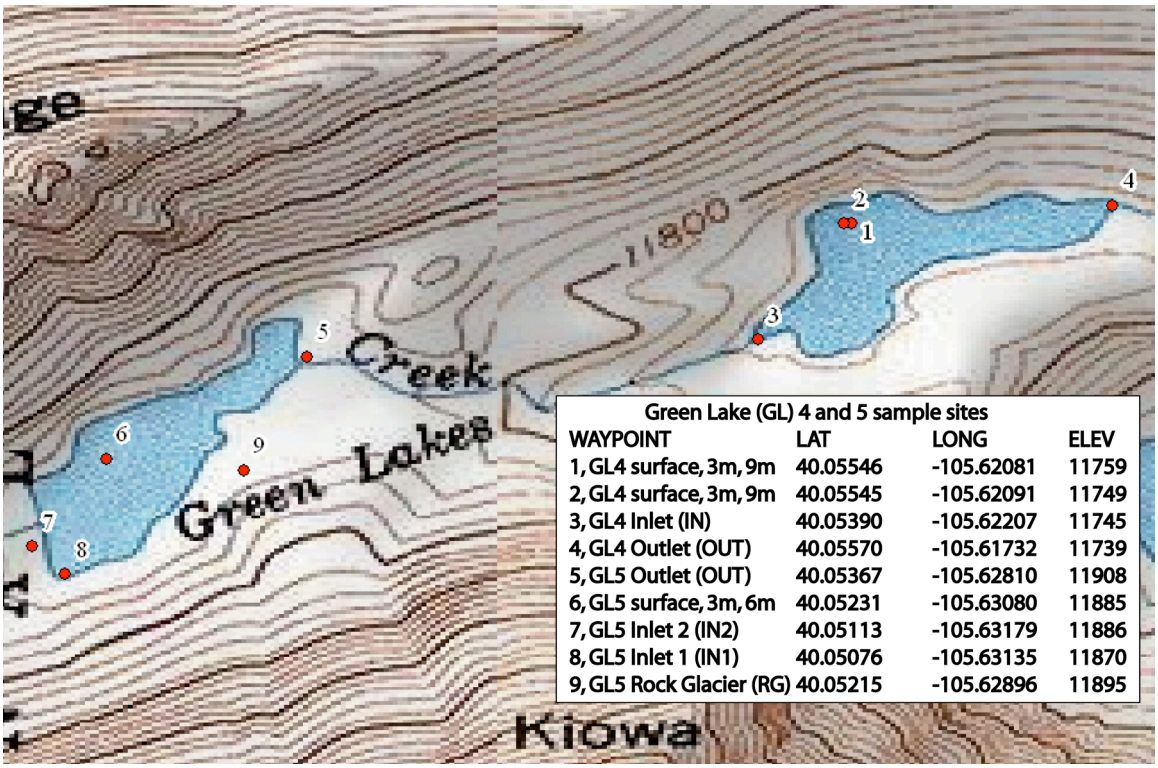
Phytoplankton enumerations recorded as cells/mL in file <GL4algae_2000-present.xls>

Green Lake 5: 2005

- **SAMPLING**:
  - 7 site locations (see colored stars on bathymetric map at end of section):
    - UTM coordinates with topo map at end of document
    - Inlet 1 (purple)
    - Inlet 2 (blue)
    - Rock Glacier (green)
    - Outlet (pink)
    - In-Lake (black)
      - Surface
      - 3 m
      - 6 m
  - Inlet and outlet can be sampled as soon as they are ice free- usually early July. In-lake sampling begins as soon as ice is clear enough to do so, usually mid to late July. This is often a week or so before the complete ice out date recorded by Nel. Samples taken from the approximate deepest point in the lake; raft anchored to rock protruding from west side of lake.
  - Field gear:
    - Equipment stored at the GL5 site (yellow star on map):
      - Action Packer storage tub
      - Raft
      - Raft Pump
      - Life Jackets (2)
      - Van Dorn Sampler
      - Secchi disk
      - Ropes
      - Extra bottles
    - Equipment to bring up from GL4:
      - Paddles (2)
      - Neoprene Paddling Gloves (2 pair)
      - DO/Temperature meter
      - pH/Conductivity/Temp meter
      - Light meter (Photometer)
      - AA batteries for meter replacements
      - pH buffers (7.00 and 4.00)
      - Conductivity buffer (usually 84 μS)
    - Equipment to bring up from MRS:
      - Seven 60 mL amber glass bottles
      - Fourteen 500 mL brown Nalgenes
      - Seven 250 mL clear plastic bottles
      - Tape, marker, field book and pen
  - Label GL5 – site, all else similar
- **FILTERING:** Tape nutrient samples yellow if filtered (F), red if unfiltered (U)
- The remainder (**FILTERING**, **ANALYSIS**, **CALCULATIONS**) similar to GL4


0 50 100 M
CONTOUR INTERVAL-IM
1
2
3
4
5
6
7
GREEN LAKE 5
Elevation - 3615 meters (11,860 feet)
Area - 3.0 hectares
Volume - 12.1 hectare-meters
Maximum Depth - 7.6 meters
Mean Depth - 4.0 meters

UTM Coordinates



Green Lake (GL) 4 and 5 sample sites

WAYPOINT	LAT	LONG	ELEV
1, GL4 surface, 3m, 9m	40.05546	-105.62081	11759
2, GL4 surface, 3m, 9m	40.05545	-105.62091	11749
3, GL4 Inlet (IN)	40.05390	-105.62207	11745
4, GL4 Outlet (OUT)	40.05570	-105.61732	11739
5, GL5 Outlet (OUT)	40.05367	-105.62810	11908
6, GL5 surface, 3m, 6m	40.05231	-105.63080	11885
7, GL5 Inlet 2 (IN2)	40.05113	-105.63179	11886
8, GL5 Inlet 1 (IN1)	40.05076	-105.63135	11870
9, GL5 Rock Glacier (RG)	40.05215	-105.62896	11895

18 October 2007
Flanagan, C. M., Roche, A. E., Miller, M., McKnight, D. M.