New Hampshire Center for Freshwater Biology and Lakes Lay Monitoring Program Programmatic Quality Assurance Project Plan (QAPP) Appendices

Appendix A
Center for Freshwater Biology Laboratory Standard Operating Procedures (SOPs)

Lab Location: Room G18 Spaulding Life Sciences
Director: Professor Jeff Schloss
Lab Manager: Robert Craycraft
Additional staff includes undergraduate and graduate students that serve as lab and field technicians and data entry technicians.

A.1. General Laboratory Protocols

A.1.1. Washing Bottles

Color Bottles (60 ml opaque HDPE bottles) – Rinse three times with de-ionized (DI) water and let dry completely before capping them. Make sure the bottles and caps are face down when drying to assure dust particles do not settle inside and contaminate them. If contamination is suspected, rewash the bottles as above.

pH/Alk/Carbon Dioxide/Turbidity/Specific Conductivity bottles (1 liter and 500 ml amber HDPE bottles) – Rinse three times with de-ionized water (DI H_2O). Place both the caps and bottles face down to dry completely before capping them.

Chloride bottles (250 ml rectangular amber HDPE bottles) - Rinse three times with de-ionized water (DI H_2O). Place both the caps and bottles face down to dry completely before capping them.

Chlorophyll bottles (2 liter Amber bottles) – Rinse three times with DI H_2O. Place both the caps and bottles face down to dry completely before capping them.

Total Suspended Solids bottles (2 liter Amber bottles) - Rinse three times with DI H_2O. Place both the caps and bottles face down to dry completely before capping them.

Total Nutrient Bottles (250 ml, 500 ml translucent HDPE Acid Washed Bottles) – These bottles should be rinsed once with DI H_2O and then placed in the acid bath (10% HCl) for ten minutes (make sure the bottles are completely submersed). After 10 minutes, drain the acid from the bottles and rinse three times with DI H_2O and place in the chicken wire drying rack. Always have both the bottles and caps face down to dry. Under no circumstances should these bottles be dried on the peg drying racks (this will contaminate the bottles and they will have to be rewashed). Once dry, cap the bottles and be careful not to touch inside the caps or inside the bottles. If there is any suspicion of contamination, rewash the bottles in the acid bath.

Dissolved Nutrient Bottles (60 ml and 250 ml Amber HDPE) – These bottles should be rinsed once with DI H_2O and then placed in the acid bath (10% HCl) for ten minutes (make sure the bottles are completely submersed). After 10 minutes, drain the acid from the bottles and rinse three times with DI H_2O and place in the chicken wire drying rack. Always have both the bottles and caps face down to dry. Under no circumstances should these bottles be dried on the peg drying racks (this will contaminate the

bottles and they will have to be rewashed). Once dry, cap the bottles and be careful not to touch inside the caps or inside the bottles. If there is any suspicion of contamination, rewash the bottles in the acid bath.

Zooplankton Bottles (500 ml translucent HDPE wide mouth bottles) – Rinse three times with tap distilled water and give a final rinse with DI H_2O. Make sure no particulates are attached inside the bottle or cap. Place both the caps and bottles face down to dry completely before capping them.

Microbiological Bottles: (250 ml translucent wide mouth bottles) – Rinse with tap distilled water. Loosely cap and cover cap with the sterilization paper and rubber band the paper over the cap so that the cap and shoulder of the bottle are protected. Autoclave, carefully tighten cap without disturbing protective paper cover and store upright in the marked cabinet. Alternatively, pre-sterilized and disposable Whirl-Pack bags can be used for microbiological sampling.

Dissolved "true" Color Bottles - Rinse three times with DI H_2O. Place both the caps and bottles face down to dry completely before capping them.

Dissolved Oxygen Bottles - Rinse three times with DI H_2O. Place the bottles on the drying rack pins to dry. Once dry, loosely cap the bottles with the glass stoppers that are tethered to the Biochemical Oxygen Demand (BOD) bottles with nylon line.

A.1.2. Mixing Commonly Used Reagents

5N Sulfuric Acid - In a 500 ml volumetric flask, add 350 ml DI H_2O and place in an ice bath. Once cool, add 70 ml concentrated H_2SO_4 and swirl the flask to mix the reagent and let cool. **(Note: be sure to add the acid slowly as the heat generated can cause the flask to break).** Once cool, slowly add DI H_2O until the solution reaches the neck of the volumetric flask and remove the flask from the ice bath. When the volumetric flask reaches room temperature, add DI H_2O until a volume of 500 ml is reached and cap the flask with Parafilm.

11N Sulfuric Acid - In a 500 ml volumetric flask, add 300 ml DI H_2O and place in an ice bath. Once cool slowly add 155 ml concentrated H_2SO_4 and swirl the flask to mix the reagent. **(Note: be sure to add the acid slowly as the heat generated can cause the flask to break).** Slowly bring the solution to 500 ml and once cool remove from the ice bath. Once cool, slowly add DI H_2O until the solution reaches the neck of the volumetric flask and remove the flask from the ice bath. When the volumetric flask reaches room temperature, add DI H_2O until a volume of 500 ml is reached, then cap the flask with Parafilm and mix gently.

10N Sodium Hydroxide - In a 500 ml volumetric flask, add 250 ml DI H_2O and place in an ice bath on the magnetic stirrer (a white stirrer should be place into the flask at this time). Once cool slowly add 200 grams of concentrated NaOH while the stirrer is spinning slowly. **(Note: be sure to add the base slowly as the heat generated can cause the flask to break).** Once cool, slowly add DI H_2O until the solution reaches the neck of the volumetric flask and remove the flask from the ice bath. When the volumetric flask reaches room temperature, add DI H_2O until a volume of 500 ml is reached and cap the flask with Parafilm.

Phenolphthalein indicator - In a 100 ml volumetric flask, dissolve 1 gram of phenolphthalein crystals in 100 ml 95% ethanol and store the solution in two 60 ml plastic drop

dispensing bottles.

Sources of commonly purchased chemicals and laboratory supplies:

- University of New Hampshire Chemistry Stockroom
- Fisher Scientific
- VWR Scientific

A.1.3. Analyzing Samples on the Cary 50 Spectrophotometer

Turn the Cary 50 spectrophotometer immediately prior to analyzing the samples; the spectrophotometer will run through a set of diagnostic steps that takes approximately 1 minute and then will display the main screen from which various analytical options will be displayed. The Cary 50 uses a xenon source lamp that does not require advanced warm up. Make sure you use the proper cuvette and proper sample blank when analyzing the respective analyte. The Cary 50 is connected to personal computer where Cary software and associated files have been created that facilitate the collection of the absorption data required to measure the parameter of interest (i.e. chlorohyll, dissolved color and phosphorus). Setup the spectrophotometer as follows:

1. Select the appropriate saved file, located on the desktop, to analyze the parameter of interest:
    - Chlorophyll analysis (chlorophyll a)
    - Color Analysis (dissolved "true" color)
    - Phosphorus Analysis (total and soluble reactive phosphorus)
    - Nitrogen Analysis (total nitrogen analysis)
2. Rinse the appropriate cuvette three times with the desired blank solution (Table 1) to ensure the cuvette is clean.
3. Fill the cuvette with the desired blank solution (Table 1). View the interior of the cuvette through the two windows to make sure there are no visible air bubbles in the cuvette that could impede the light path. If necessary, gently tap the cuvette to remove air bubbles.
4. Wipe the cuvette windows with a Kimwipe using a circular motion to ensure all debris (e.g. dust) and smears have been removed.
5. Open the spectrophotometer lid and place the cuvette into the cell holder by pressing down on the cuvette to ensure it is well seated in the cell holder.
6. Close the spectrophotometer lid.
7. Press the "measure zero" key to set the absorption values to zero.
8. Press the "Read" button to measure the absorption values of the blank. All absorption values should read between -0.0010 and 0.0010. If any absorption values deviate from the desired absorption range repeat steps 4 through 8.

Table 1

Analyte	**Cuvette Type**	**Path Length**	**Blank**
Chlorophyll *a*	Near UV Glass	5 cm	90% Acetone w/o $MgCO_3$
Dissolved Color	Quartz Glass	5 cm	DI H_2O
Total Nitrogen *	Quartz Glass	1 cm	DI H_2O
Total Phosphorus	Near UV Glass	10 cm	DI H_2O
Soluble Reactive Phosphorus	Near UV Glass	10 cm	DI H_2O

* Total Nitrogen samples are processed on the Cary 60 using the scanning application. The Total Nitrogen procedure, and the use of the Cary 60, is detailed in section A.4

9. Lift the spectrophotometer lid, remove the cuvette and dispose of waste into the appropriate waste container. *Note Hazardous waste containers are marked with labels provided by the University of New Hampshire Environmental Health and Safety Department.*
10. Follow the protocols for processing the respective analyte: Chlorophyll *a*, Total Phosphorus, Soluble Reactive Phosphorus and Dissolved Color.

A.1.3.a. Chlorophyll a – Blank the Cary 50 spectrophotometer with 90% acetone without $MgCO_3$ (Section A.1.3) and run and record the blank absorption values. All acetone waste will be disposed of in designated acetone waste containers. Chlorophyll *a* samples are analyzed using the following procedure:

a. Rinse the cuvette three times with 90% acetone without $MgCO_3$.
b. Pour the chlorophyll sample into the cuvette using a continuous pouring motion until all solution is transferred into the cuvette to avoid disturbing the pellet at the bottom of the centrifuge tube.
c. View the interior of the cuvette through the two windows to make sure there are no visible air bubbles in the cuvette that could impede the light path. If necessary, gently tap the cuvette to remove air bubbles.
d. Wipe the cuvette windows with a Kimwipe using a circular motion to ensure all debris (e.g. dust) and smears have been removed.
e. Place the cuvette into the cell holder. Press down on the the cuvette to make sure it is well seated in the cell holder.
f. Close the spectrophotometer lid.
g. Press the "measure sample" key and record the pre-acidification absorption values at 663 nm, 664 nm, 665 nm and 750 nm.
h. Lift the spectrophotometer lid, remove the cuvette.
i. Draw up 0.5 ml of 0.1N HCl using a 0.5 ml volumetric pipette.
j. Add the 0.5 ml HCl to the cuvette that contains the chlorophyll *a* sample (note: make sure the pre-acidification absorption values have been recorded onto the datasheet before adding the acid).
k. Cap the cuvette with the white caps and invert the cuvette 10 times; the sample will initially be cloudy when the acid is added and will become clear as the cuvette is inverted).
l. View the interior of the cuvette through the two windows to make sure there are no visible air bubbles in the cuvette that could impede the light path. If necessary, gently tap the cuvette to remove air bubbles.
m. Wipe the cuvette windows with a Kimwipe using a circular motion to ensure all debris (e.g. dust) and smears have been removed.
n. Place the cuvette into the cell holder by gently "rocking" the cuvette to make sure it is well seated. Make sure the cuvette stopper holes are positioned perpendicularly to the base.
o. Close the spectrophotometer lid.
p. Press the "measure sample" key and record the post-acidification absorption values at 663 nm, 664 nm, 665 nm and 750 nm.
q. Dispose of the acetone waste in the designated acetone waste container (note: make sure the absorption values have been recorded before disposing of the waste).
r. Repeat steps a through q until all samples have been processed.
s. Select "save data as" from the program menu.
t. Select the folder "Chl, color, TP and SRP results".
u. Select the sub-folder "Chl".
v. Type in the file name, (e.g. Chl_030121_5cm) to indicate the parameter (chlorophyll), the date of analysis (March 1, 2021) and to indicate the cell path length (5 centimeters).
w. Close out of the program.
x. Turn off the PC.

Chlorophyll *a* standards –Prepackaged chlorophyll standards in 90% acetone can be purchased directly from the Turner Designs Company, catalog number 10-850 for approximately $175. Dilute the stock standard (approximately 155 µg/L) by transferring 15-17 milliliter liquid chlorophyll *a* standard from the turner designs chlorophyll *a* ampoule to a 500 milliliter amber flask and dilute to the 500 ml mark with 90% acetone solution using a chilled pipette tip. Calculate the working chlorophyll *a* concentration using the original concentration of the stock standard contained in the ampoule:

$$\frac{\text{Stock Chlorophyll } a \text{ Concentration} * \text{volume stock standard into 500 ml volumetric flask}}{\text{ml working standard}} \quad 500$$

The working standard should be stored @ -20°C in the dark to avoid chlorophyll degradation and should be used until the expiration date on the ampoule. When analyzing standard reference materials (SRMs) you should assure the spectrophotometer has warmed up for 15 minutes followed by the series of steps detailed below.

0.1N HCl Working Solution -*Note: the preparation of the HCl working solution should be undertaken under a fume hood designated for acid preparation.* Fill an acid washed 1000 ml volumetric flask with approximately 500 ml DI H_2O and place the flask into an ice bath in the fume hood for 15 minutes. Pour approximately 25 ml of 12.1N HCl (FS Cat # A144c-212) into a 50 ml acid washed beaker under the fume hood. Draw up 8.3 ml of the Stock HCl using a class-A glass pipette and dispense the acid into the 1000 ml volumetric flask. Swirl the flask to obtain a homogeneous mixture. Place the 1000 ml volumetric flask into a PP tray without ice and let the diluted acid solution come to room temperature. Once the diluted acid solution reaches room temperature bring the volumetric flask to volume by slowly adding DI H_2O from a Nalgene squirt bottle. Parafilm the 1000 ml volumetric flask and invert the flask 20 times to assure the 0.1N HCl solution is a homogeneous mixture. Store the 0.1N HCl solution in a PP tray on the lab bench.

A.1.3.b. Total Phosphorus & Soluble Reactive Phosphorus - Blank the Cary 50 spectrophotometer with DI H_2O (Section A.1.3) and run and record the blank absorption values. All phosphorus waste will be disposed of in designated acidic waste containers. Total phosphorus and soluble reactive phosphorus samples are analyzed using the following procedure:

a. Rinse the cuvette three times with DI H_2O.
b. Pour the total phosphorus sample into the cuvette using a continuous pouring motion until the solution extends to the top of the cuvette windows.
c. View the interior of the cuvette through the two windows to make sure there are no visible air bubbles in the cuvette that could impede the light path. If necessary, gently tap the cuvette to remove air bubbles.
d. Wipe the cuvette windows with a Kimwipe using a circular motion to ensure all debris (e.g. dust) and smears have been removed.
e. Place the cuvette into the cell holder. Press down on the the cuvette to make sure it is well seated in the cell holder.
f. Close the spectrophotometer lid.
g. Press the “measure sample” key and record the absorption values at 660 nm and 880 nm.
h. Lift the spectrophotometer lid, remove the cuvette.
i. Dispose of the phosphorus waste in the designated acidic waste container.

j. Repeat steps a through i until all samples have been processed. *Note: a DI H_2O spec-check blank should be analyzed after every ten samples and the results should be recorded in the "spec check" column of the datasheet. A DI H_2O "spec-check" blank should always be the last sample analyzed and the results should be recorded on the datasheet.*
k. Select "save data as" from the program menu.
l. Select the folder "Chl, color, TP and SRP results".
m. Select the sub-folder "Total Phoshorus and SRP".
n. Type in the file name, (e.g. SRP_030121_10cm) to indicate the parameter (soluble reactive phosphorus), date of analysis (March 1, 2021) and to indicate the cell path length (10 centimeters).
o. Close out of the program.
p. Turn off the PC.

A.1.3.c. Dissolved "true" Color - Blank the Cary 50 spectrophotometer with DI H_2O (Section A.1.3) and run and record the blank absorption values. Dissolved color samples are analyzed using the following procedure:

a. Rinse the cuvette three times with DI H_2O.
b. Pour the dissolved color sample into the cuvette using a continuous pouring motion until the solution extends to the top of the cuvette windows.
c. View the interior of the cuvette through the two windows to make sure there are no visible air bubbles in the cuvette that could impede the light path. If necessary, gently tap the cuvette to remove air bubbles.
d. Wipe the cuvette windows with a Kimwipe using a circular motion to ensure all debris (e.g. dust) and smears have been removed.
e. Place the cuvette into the cell holder. Press down on the the cuvette to make sure it is well seated in the cell holder.
f. Close the spectrophotometer lid.
g. Press the "measure sample" key and record the absorption values at 440 nm, 456 nm, 493 nm, 750 nm and 880 nm.
h. Lift the spectrophotometer lid, remove the cuvette.
i. Dispose of the dissolved color sample into the waste bucket.
j. Repeat steps a through i until all samples have been processed.
k) Select "save data as" from the program menu.
l) Select the folder "Chl, color, TP and SRP results".
m) Select the sub-folder "color".
n) Type in the file name, (e.g. color_030121_5cm) to indicate the parameter (dissolved color), date of analysis (March 1, 2021) and to indicate the cell path length (10 centimeters).
o) Close out of the program.
p) Turn off the PC.

Notes:

- *Analyze a DI H_2O blank and a 20 CPU dissolved color standard after every ten samples.*

The first four samples analyzed for each day's analytical run should be the 200 CPU, 100 CPU, 20 CPU and 5 CPU dissolved color standards.

Written by Bob Craycraft and Jeff Schloss
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A.2. Soluble Reactive Phosphorus SOP

Reference Source: Standard Methods 20th Edition - 4500 P.E.

Sample Requirements: Non-acidified, filtered (0.45micron) sample

Note: use extreme caution when handling the phosphorus analytical glassware. If there is any doubt of the cleanliness of the glassware, acid wash all applicable materials as described in step three before proceeding any further.

1) Remove a maximum of 29 soluble reactive phosphorus samples from the refridgerator and let the samples warm to room temperature prior to proceeding to step five.

2) In the morning, mix 5N Sulfuric Acid (H_2SO_4) **-** In a 500 ml volumetric flask, add 350 ml DI H_2O and place in an ice bath. Once cool add 70 ml concentrated H_2SO_4 and swirl the flask to mix the reagent. **(Note: be sure to add the acid slowly as the heat generated can cause the flask to break).** Place the heated flask into the ice bath and let the solution cool to room temperature. Once cool, slowly add DI H_2O until the solution reaches the neck of the volumetric flask and place the flask into the ice bath for approximately 15 minutes. After the 15 minute cooing period, Parafilm the flask, invert the flask 10 times, and place the flask in a PP tray without ice until the solution reaches room temperature. Once the flask warms to room temperature, add DI H_2O until a volume of 500 ml is reached and cap the flask with Parafilm. Invert the flask 10 times to obtain a homogeneous mixture, place the flask in a PP tray and store the flask on the laboratory bench until needed.

3) Acid wash (Place in the 10% Hydrochloric Acid (HCl) acid bath for 10 minutes):

84	125 ml Erlenmeyer flasks
1	50 ml graduated cylinder TD
1	100 ml graduated cylinder TD
1	250 ml graduated cylinder TD
6	500 ml volumetric flasks
1	1000 ml volumetric flask
1	100 ml beaker

4) Transfer all pertinent information from the total phosphorus sample bottle to the soluble reactive phosphorus data sheet at this time (i.e. lake, site, date, depth, etc.).

5) Prepare sample flasks as follows: Arrange the 125 ml Erlenmeyer flasks (Corning # 5100-125) alpha-numerically, 1A through 42B, and place the flasks sequentially into the polypropylene sterilizing trays before filling the flasks with samples.

<u>Prepare SRP standards and blank as follows (calibration curve):</u>

Dilute pre-purchased Ricca (cat #5830-16) 50 mg/l stock standard, as 50 mg/l P, to a working standard concentration of 10 mg/l:

1) Set out an acid washed 100 milliliter beaker and a 100 milliliter acid washed volumetric flask.
2) Add approximately 50 milliliters of DD H_2O to the 100 milliliter volumetric flask.
3) Pour approximately 40 milliliters of 50 mg/l stock standard into the 100 milliliter beaker.
4) Draw up five milliliters of 50 mg/l stock standard using the Finnpipetter set to dispense a 5 milliliter volume.
5) Dispense the 50 mg/l standard into a waste bucket.
6) Draw up five milliliter of 50 mg/l stock standard using the Finnpipetter set to dispense a 5 milliliter volume.

7) Dispense the solution into the 100 milliliter volumetric flask.
8) Repeat steps 5 and 6 three additional times.
9) Add DD H_2O until the 100 milliliter volumetric fill line is reached.
10) Cap the 100 milliliter volumetric flask with parafilm.
11) Invert the 100 milliliter volumetric flask ten times.

Dilute the working, 10 mg/l stock solution to the appropriate standard concentrations described in Table 2. *Note: orthophosphate standards are not preserved and should be prepared daily no more than two hours prior to the addition of the mixed reagent.* Dilutions will be performed by pipetting a fixed volume of stock standard, using a combination of the Finnpipetter and MLA fixed volume micro-pipetters, into 500 ml volumetric flasks that are labeled with the respective standard concentrations: 50 μg/l, 20 μg/l, 10 μg/l, 5 μg/l and 1 μg/l. All volumetric flasks are brought to volume with DI H_2O water, covered with Parafilm and inverted ten times to assure the standards are well mixed and homogeneous solutions are achieved. *Note: If higher ranges are expected include a 100* µg/L *standard by diluting five milliliters of 10.0 ml stock standard into a 500 ml volumetric flask and bringing the flask to 500 ml with DI H_2O.*

Table 2. Orthophosphate working standards.

Standard Solution (*u*g P/l) [1]	10 mg/l Working Solution (ml) [2]	Erlenmeyer Flask (ID) [3]
50	2.50	1
20	1.00	2
10	0.50	3,13,23,33 and 41
5	0.25	4
1	0.05	5
(blank) 0	0.0	6 & 42

[1] Standards used to derive calibration curves (prepared within two hours of analysis).
[2] volume of 10 mg/l standard added to a 500 ml volumetric flask and brought to volume with DI H_2O to derive the working standards presented in the leftmost column above.
[3] Erlenmeyer Flasks into which the respective standards and blanks are placed.

Pour out SRP standards as follows:

Invert the 500 ml volumetric flask containing the DI H_2O blank and SRP standards three times immediately prior to pouring the reagents out. Rinse the 50 ml graduated cylinder three times with DI H_2O. Measure 50 ml of each standard and pour the standards into the replicate Erlenmeyer flasks (50 ml into each flask) as denoted in Table 2. Between replicate samples (e.g. 1B and 2A) rinse out the graduated cylinder with 10% HCl and then rinse three times with DI H_2O.

Pour out samples as follows:

Invert each sample twice and rinse the 50 ml graduated cylinder with the sample. Measure out 50 ml of water and pour into the replicate Erlenmeyer Flasks (A & B). Between replicate samples (i.e. 1B and 2A) rinse out the graduated cylinder with 10% HCl and then rinse three times with DI H_2O. *Note: Erlenmeyer flasks 12A and 12B will subsequently be spiked with phosphate standard (as described below) and should contain the same lakewater as was poured into flasks 11A and 11B. Likewise, flasks 32A and 32B will subsequently be spiked with phosphate standard and should contain the same lakewater as was poured into flasks 31A and 31B.*

Spike flasks 12A, 12B, 32A and 32B as follows:
Pour out pre-purchased (cat #5830-16) 50 mg/l stock Ricca standard, as 50 mg/l P, into the cap. Using a .025 ml fixed volume MLA pipetter, draw up a sample of the standard into the pipette and dispense the standard into a waste bucket. Subsequently, draw up .025 ml of phosphate standard from the beaker and successively dispense .025 ml of standard into Erlenmeyer flasks 12A, 12B, 32A and 32B. This will constitute a phosphorus spike of 25.0 µg/L. Swirl each Erlenmeyer flask to obtain a homogeneous mixture.

6) Mix the following reagents:

Ammonium Molybdate	8.00 grams per 200 milliliters DI H_2O
Ascorbic Acid	8.80 grams per 500 milliliters DI H_2O
Potassium Antimonyl Tartrate	0.28 grams per 100 milliliters DI H_2O

7) Mix the combined reagent:
The combined reagent should be mixed in a 1000 ml (acid washed) volumetric flask by measuring the volume of reagents in a graduated cylinder and adding the reagents in the following order (note: the reagents must be added in this order for the proper molecule to form and the graduated cylinder should be rinsed with DI H_2O between the addition of each reagent); mixing the volumetric flask as each reagent is added:
1) 500 ml 5N H_2SO_4
2) 50 ml Antimony Potassium Tartrate
3) 150 ml Ammonium Molybdate
4) 300 ml Ascorbic Acid

Note: the mixed reagent is very unstable and should be made immediately prior to adding the reagent to the sample flasks.

8) Add the mixed reagent using the Eppendorf Repeater M4 pipetter. Rinse the pipette by pipetting a sample of mixed reagent from the beaker and discarding it. After rinsing the pipette tip, add 8 ml of mixed reagent to each successive flask. As the mixed reagent is added, a molecular complex (molybdenum blue) will form in the sample. The concentration of the molybdenum blue complex is proportional to the phosphorus concentration in the sample. While differences in color (low phosphorus concentrations) are not visible to the unaided eye, high phosphorus concentrations become various shades of blue; the bluer the sample the greater the phosphorus concentration.

9) Turn on the Cary 50 spectrophometer and follow the procedure outlined in section A.1.3

10) Begin sample analysis 30 minutes after adding the mixed reagent to the first sample. See Procedures in Section A1.3 and A1.3.b for proper use of the spectrophotometer. Record the absorbencies at 660 nm and 880 nm on the soluble reactive phosphorus data sheet. The spectrophotometer should be blanked with DI H_2O and DI H_2O blanks should also be run after every 10 flasks and should always be run after the final phosphorus sample has been analyzed. Record the blank absorbencies (660 nm and 880 nm) on the SRP datasheet in the "spec chk" column.

11) Phosphorus analysis waste should be rinsed into a yellow 2.5 gallon bucket positioned next to the Cary 50 spectrophotometer. The collected waste should periodically be consolidated into a labeled 20 liter hazardouse waste carboy that will be retrieved by Environmental Health and Safety staff for proper disposal.

12) Glassware cleanup:
Immediately rinse out the flasks and other glassware three times with DI H_2O after the run and place the glassware in the drying rack on the gray Rubbermaid cart. If there is room, place the rinsed glassware into the acid bath and let it sit for one hour, otherwise, fill the flasks with DI H_2O and place out of the way until

room becomes available in the acid bath. When pulling glassware out of the acid bath rinse three times with DI H_2O and place the glassware upside-down in the drying rack.

13) Calculating the SRP Coefficient from the calibration curve data:

After each analytical run enter the five initial SRP standards (samples 1-5) and the corresponding 880 nm absorption values into an Excel spreadsheet as depicted in the first two columns of table 3. Subtract the DI H_2O blank 880 nm absorption value (sample 6) from each of the 880 nm standard absorption values as depicted below. Regress the standards (dependent variable; Y) against the corrected 880nm absorption values (independent variable; X) to generate a statistical output as displayed below. You will now multiply your corrected 880 nm absorption values by the X variable (SRP coefficient) to calculate your SRP concentrations (Table 3). As you can see, the prepared SRP standards compare well with the calculated SRP values. You will now use your SRP coefficient and the corrected 880 nm absorption values of your samples (unknowns) to determine the remaining SRP concentrations. You should record your SRP coefficient, your DI H_2O blank (corrective factor) and your r^2 value on the back of your Orthophosphate (SRP) datasheet. Save the Excel spreadsheet as {date as YYYY/MM/DD}SRP.xls

Table 3: SRP Calibration Curve*

SRP Standard (ppb)	880 nm abs	880 abs (corrected for blanks)	* Calculated SRP (ppb)
100	0.505	0.501	99.2
50	0.262	0.258	51.2
25	0.136	0.132	26.2
10	0.071	0.067	10.6
5	0.034	0.030	4.8
2.5	0.019	0.015	2.4
1	0.013	0.009	1.1
DI H_2O Blank	0.004	0.000	0.0

* based on the SRP coefficient derived from the SRP calibration curve.

Figure 1 – SRP Calibration Curve Regression Output

SUMMARY OUTPUT

Regression Statistics

Multiple R	0.999725572
R Square	0.999451218
Adjusted R Square	0.999341462
Standard Error	0.766073921
Observations	7

ANOVA

	df	SS	MS	F	Significance F
Regression	1	5344.085654	5344.086	9106.092	2.40E-09
Residual	5	2.93434626	0.586869		
Total	6	5347.02			

	Coefficients	Standard Error	t Stat	P-value	Lower 95	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-0.252354078	0.382513977	-0.65973	0.538602	-1.235635952	0.730927796	-1.235635952	0.730927796
X Variable 1	**165.4771839**	1.734091839	95.42585	2.40E-09	161.0195662	169.9348016	161.0195662	169.9348016

Written by Bob Craycraft and Jeff Schloss
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A.3. Total Phosphorus SOP

Reference Source: Standard Methods 20th Edition- 4500 P.E.
Sample Requirements: Acid preserved, whole water sample

Note: use extreme caution when handling the phosphorus analytical glassware. If there is any doubt of the cleanliness of the glassware, acid wash all applicable materials as described in step three before proceeding any further.

1) Remove a maximum of 43 total phosphorus samples from the freezer and let them thaw. In the morning, make up the following reagents as described in the following section A.1.2-"Mixing other reagents" and in Steps 2 of the A.2.-"Soluble Reactive Phosphorus SOP":

5 N H_2SO_4
11N H_2SO_4
10 N NaOH

2) If the stock TP standard working solutions have expired (see section 6k) then acid wash:

7 1 L Volumetric Flasks

3) Acid wash: (Place in the 10% HCl acid bath for 10 minutes):

120 125 ml Erlenmeyer flasks
1 ea. 50 ml, 100 ml and 250 ml graduated cylinder TD
1 plastic 0.5 g measuring scoop (for ammonium peroxydisulfate)
1 1 l volumetric flask
1 ea. 100 ml, 200 ml and 400 ml beakers

4) Transfer all pertinent information from the total phosphorus sample bottle to the total phosphorus data sheet at this time (i.e. lake, site, date, depth, etc.).

5) Preparation of Total Phosphorus stock standard (this step is not required on a daily basis):

A stock phosphorus solution should be prepared monthly using the following procedure:

a) In an acid washed petri-dish pour out approximately 5 grams of anhydrous KH_2PO_4 and place in the oven (Fisher Scientific Model 825F), set at 104˚ C for one hour.
b) After one hour place the petri dish into a desiccator and let the dish/reagent cool to room temperature. Discard any remaining dehydrated KH_2PO_4 after 5 days.
c) Fill a pre-designated (labeled 50 mg/l TP Standard) 1000 ml volumetric flask halfway with DI H_2O.
d) Add four milliliters of concentrated (36N) sulfuric acid to the solution using a 4 ml volumetric pipette and swirl the flask.
e) Carefully weigh out 219.5 mg KH_2PO_4 on the analytical balance and add the reagent to the pre-labeled volumetric flask. Make sure all reagent is dispensed into the flask. Should some reagent spill onto the counter or onto the outside of the volumetric flask, discard the reagent, rinse the flask and repeat steps c through f. *Note: the anhydrous KH_2PO_4 should be placed in the desiccator between weighings to avoid hydration.*
f) Swirl the 1000 ml flask for about 20 seconds to dissolve the KH_2PO_4.
g) Bring the 1000 ml volumetric flask with DI H_2O dispensed from a Nalgene squirt bottle.
h) Add an acid washed magnetic stirring bar and place the 1000 ml volumetric flask onto a magnetic stirrer set at setting #3. Allow the sample to stir for 30 minutes to assure the reagent reaches a homogeneous state.
i) Label the flask with the current date and place the stock solution into the refrigerator.

Any unused portion of this stock reagent should be discarded after 90 days and a new stock phosphorus solution should be mixed at that time.

6) Preparation of working total phosphorus standards and total phosphorus blank.
Note: This step is also not required on a daily basis. However, all total phosphorus working standards and the DI H_2O blank should be prepared on the same day. Dilute the 50 mg/l stock phosphorus standard, from step 5 above (alternatively use purchased Ricca standard), to a 10 mg/l stock phosphorus solution that will be used to obtain the working standard concentrations described in Table 4.

a) Set out an acid washed 100 milliliter beaker and a 100 milliliter acid washed volumetric flak.
b) Add approximately 50 milliliters of DD H_2O to the 100 milliliter volumetric flask.
c) Pour approximately 40 milliliters of 50 mg/l stock standard into the 100 milliliter beaker.
d) Draw up five milliliters of 50 mg/l stock standard using the Finnpipetter set to dispense a 5 milliliter volume.
e) Dispense the 50 mg/l standard into a waste bucket.
f) Draw up five milliliter of 50 mg/l stock standard using the Finnpipetter set to dispense a 5 milliliter volume.
g) Dispense the solution into the 100 milliliter volumetric flask.
h) Repeat steps 5 and 6 three additional times.
i) Add DD H_2O until the 100 milliliter volumetric flask fill line is reached.
j) Cap the 100 milliliter volumetric flask with parafilm.
k) Invert the 100 milliliter volumetric flask ten times.
l) Set out six 1000 ml volumetric flasks, labeled with the respective standard concentrations: 100 µg/L, 40 µg/L, 20 µg/l, 10 µg/l, 2 µg/l and 0 µg/l (DI H_2O blank).
m) Add approximately 500 milliliters of DI H_2O to each of the 1000 ml volumetric flasks.
n) Pipette four milliliters of concentrated H_2SO_4 (36N), using a Finnpipetter set to dispense four milliliters of solution, into each of the six volumetric flasks.
o) Each sample should be swirled to mix the solutions in the respective volumetric flasks.
p) The working standards are then prepared by adding a fixed volume of 10 mg/l working standard, using a combination of the Finnpipetter and fixed volume MLA micro-pipetters, into the respective volumetric flasks (Table 4). *Note: If higher ranges are expected include a 200 µg/L standard by diluting 20.0 ml of working, 10 mg/l, standard into a 1000 ml volumetric flask and bring the flask to volume with acidified DI H_2O.*
q) All volumetric flasks are brought to volume with DI H_2O, covered with Parafilm and inverted

Table 4. Total Phosphorus working standards.

Standard Solution (*u*g P/l) [1]	10 mg/l Working Solution (ml) [2]	Erlenmeyer Flask (ID) [3]
100	10.0	1, 13
40	4.0	2, 23
20	2.0	3, 33
10	1.0	4, 41
2	0.2	5, 53 and 59
(blank) 0	0.0	6, 42 and 60

[1] Standards used to derive calibration curves (prepared within 28 days of use and stored @ 4˚C between uses).

[2] Volume of secondary (10 mg/l) phosphorus stock solution added to the respective 1000 ml volumetric flasks.

[3] Erlenmeyer Flasks into which the respective standards and blanks are placed.

ten times to assure the samples are well mixed and a homogeneous solutions is achieved.

r) The working standards (0 – 200 µg/l) should be refrigerated between uses and discarded after 28 days. The 50 mg/l stock solution should be refrigerated between uses and discarded after 90 days. The 1 mg/l stock standard should be prepared no more than 2 hours prior to the preparation of the working standards. After the working standards are poured, the remaining 1 mg/l stock standard should be discarded into a waste bucket, the flask should be acid washed and subsequently rinsed three times with DI H_2O.

7) Prepare sample flasks as follows:

a) Arrange the 125 ml Erlenmeyer flasks (Corning #5100-125) alpha-numerically, 1A through 60B, and place the flasks sequentially into the polypropylene sterilizing trays before filling the flasks with samples. Invert each sample ten times and rinse the 50 ml graduated cylinder with the sample. Measure 50 ml of sample and pour into the replicated Erlenmeyer Flasks: A & B (50 ml into each flask). Make sure you mix the water sample ten times before pouring out each replicate stream sample (you often have inorganic particulate debris that rapidly settle out). Between replicate samples (i.e. 1B and 2A) rinse out the graduated cylinder with 10% HCl and then with DI H_2O. *Note: Erlenmeyer flasks 12A and 12B will subsequently be spiked with phosphate standard (as described below) and should contain the same lakewater as was poured into sample 11. Likewise, flasks 32 will subsequently be spiked with phosphate standard and should contain the same lakewater sample as was poured into flasks 31.*

b) Pour out the blank as follows: Rinse out the graduated cylinder with 10% HCl and then with DI H_2O three times. Invert the 1000 ml volumetric flask containing the acidified DI H_2O blank ten times. Rinse the 50 ml graduated cylinder with approximately 10 ml of your (acidified) DI H_2O blank. Measure 50 ml of your acidified DI H_2O blank and pour the blank into the replicate Erlenmeyer flasks (50 ml into each flask).

c) Pour out the Standards as follows: Rinse out the graduated cylinder with 10% HCl and then with DI H_2O three times. Invert the 1000 ml volumetric flask containing the first phosphorus standard (200 µg/L) ten times. Rinse the 50 ml graduated cylinder with approximately 10 ml phosphorus standard and then measure 50 ml of sample and place into the replicate Erlenmeyer flasks (50 ml into each flask). Repeat step c for each of the remaining TP standards (40.0 µg/l). *Note: Your standard curve will be generated daily and will consist of five standards that range from 2.0 – 200.0 ppb.*

8) Spike flasks 12, 32 and 52 as follows:

a) Pour out the 50 mg/l stock Phosphorus standard into the stock solution cap.
b) Draw up 50 *u*l of stock standard using a fixed volume micropipetter (50 *u*l MLA Systems D-Tipper, MLA cat # 1054C) and dispense the solution into a waste bucket.
c) Draw up 50 *u*L of stock standard using the fixed volume mircopipetter and dispense the solution into flask 12A. This phosphorus addition constitutes a 50.0 µg/l phosphorus spike.
d) Repeat step c for flasks 12B, 32A, 32B, 52A and 52B.

9) Add 1 ml of 11N H_2SO_4 to each Erlenmeyer flask with the Eppendorf repeater M4 pipetter*(*note: make sure the pipette is set to dispense 1 milliliter of acid) and then add 1 level scoop (*0.5 g* plastic scoop HACH cat # 492-00) of ammonium peroxydisulfate into each flask. Cap each flask with a #6 glass stopper (FS Cat #10-042A) and swirl each flask to assure the reagents are well mixed.

10) Place flasks into the Gettinge Novus I autoclave, adjust the setting to liquids, make sure the temperature is set to 123˚C and set the timer to 45 minutes as indicated below.

a) Select "Sterilize Temperature" under the Cycle Values setting and set it to 123.0˚C by pressing either the "up arrow" or the "down arrow".
b) Select "Sterilize Time" under the Cycle Values setting and set it to 30 minutes by pressing either the "up arrow" or the "down arrow".

c) Select "Liquids" under the Cycle Select setting. **(It is imperative the autoclave switch is set to liquids, otherwise your samples will vaporize as the autoclave heats up and the samples will be lost).**

d) Once you are sure the settings have been properly adjusted press the "start" button. Stay in the room until the autoclave heats up to 123.0˚C to assure the samples are digesting.

11) After approximately 1.5 hours, remove the TP samples from the autoclave using the red thermally insulated gloves.

12) As the samples near room temperature mix the following reagents:

Ammonium Molybdate	8.0 grams per 200 milliliters DI H_2O
Ascorbic Acid	8.8 grams per 500 milliliters DI H_2O
Potassium Antimonyl tartrate	0.28 grams per 100 milliliters DI H_2O

13) Once the phosphorus samples cool to room temperature (25˚C) remove the caps and add one drop phenolphthalein indicator to each flask. Neutralize each sample to a faint pink color by dispensing 1.4 ml 10N NaOH from the Eppendorf repeater M4 pipetter into each flask. *Note: make sure the volume is set to 1.4 ml before beginning.* Following the addition of NaOH to all samples, swirl each flask individually; the pink color should disappear at this point. If the pink color persists, however, consult the laboratory manager.

14) Mix the combined reagent: The combined reagent should be mixed in a 1000 ml (acid washed) volumetric flask by measuring the volume of reagents in a graduated cylinder and adding the reagents in the following order **(Note: the reagents must be added in this order for the proper molecule to form and the graduated cylinder should be rinsed with DI H_2O between the addition of each reagent)**; mixing the volumetric flask as each reagent is added:

500 ml 5N H_2SO_4
50 ml Antimony Potassium Tartrate
150 ml Ammonium Molybdate
300 ml Ascorbic Acid

Note: the mixed reagent is very unstable and should be made immediately prior to adding to the sample flasks.

15) Add the mixed reagent using the Eppendorf repeater M4 pipetter, rinsing the pipette by pipetting a sample of mixed reagent from the beaker and discarding it. After rinsing the pipette tip, add 8 ml of mixed reagent to each successive flask. As the mixed reagent is added a molecular complex (molybdenum blue) will form in the sample. The concentration of the molybdenum blue complex is proportional to the phosphorus concentration in the sample. While differences in color (low phosphorus concentrations) are not visible to the unaided eye, high phosphorus concentrations become various shades of blue; the bluer the sample the greater the phosphorus concentration. See Procedures in Section A.1.5 and A1.3.b. for proper use of the spectrophotometer.

16) Turn on the Cary 50 spectrophometer and follow the procedure outlined in section A.1.3

17) Begin sample analysis 30 minutes after adding the mixed reagent to the first sample. See Procedures in Section A1.3 and A1.3.b for proper use of the Cary 50 spectrophotometer. Record the absorbencies at 660 nm and 880 nm on the total phosphorus data sheet. The spectrophotometer should be blanked with DI H_2O and blanks should also be run after every 10 flasks and should always be run after the final phosphorus sample has been run. Record the blank absorbencies (660 nm and 880 nm) and the DI H_2O blank results on the datasheets.

18) All phosphorus samples should be poured into *a* 2.5 gallon yellow bucket and emptied into the designated 20 liter hazardous acid waste container.

19) Glassware cleanup - Immediately rinse out the flasks and other glassware three times with DI H_2O after the run and place the glassware in the drying rack on the Rubbermaid cart. If there is room, place the rinsed glassware into the acid bath and let sit for one hour, otherwise, fill the flasks with DI H_2O and place out of the way until room becomes available in the acid bath. When pulling glassware out of the acid bath rinse three times with DI H_2O and place the glassware upside-down in the drying rack.

20) Calculating the Total Phosphorus (TP) Coefficient from the calibration curve data:
After each analytical run enter the five initial TP standards (samples 1-5) and the corresponding 880 nm absorption into an Excel spreadsheet as depicted in the first two columns of Table 6. Subtract the DI H_2O blank 880nm absorption value (sample 6) from each of the 880nm standard absorption values as displayed in column 3 of Table 6. Regress the standards (dependent variable) against the corrected 880nm absorption values (independent variable) to generate a statistical output as displayed below. You will now multiply your corrected 880nm absorption values by the X variable (TP coefficient) to calculate your TP concentrations (Table 5). As you can see, the prepared TP standards compare well with the calculated TP values. You will now use your TP coefficient and the corrected 880nm absorption values of your samples (unknowns) to determine the remaining TP concentrations. You should record your TP coefficient, your DI H_2O blank (corrective factor) and your r^2 value on the back of your Total Phosphorus datasheet.

Table 5: Total Phosphorus Calibration Curve

TP Standard (ppb)	880 nm abs	880 abs (corrected for blanks)	* Calculated TP (ppb)
200	1.008	1.005	198.5
40	0.213	0.210	40.9
20	0.110	0.107	20.6
10	0.059	0.056	10.4
2	0.016	0.013	1.9
DI H_2O Blank	0.003	0.000	0.0

* based on the TP coefficient derived from the TP calibration curve below

Figure 2. Total Phosphorus Calibration Curve Regression Output

SUMMARY OUTPUT

Regression Statistics

Multiple R	0.999725572
R Square	0.999451218
Adjusted R Square	0.999341462
Standard Error	0.766073921
Observations	7

ANOVA

	df	SS	MS	F	Significance F
Regression	1	5344.085654	5344.086	9106.092	2.40E-09
Residual	5	2.93434626	0.586869		
Tota	6	5347.02			

	Coefficients	Standard Error	t Stat	P-value	Lower 95	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-0.252354078	0.382513977	-0.65973	0.538602	-1.235635952	0.730927796	-1.235635952	0.730927796
X Variable 1	**198.4771839**	1.734091839	95.42585	2.40E-09	161.0195662	169.9348016	161.0195662	169.9348016

A.4. Total Nitrogen SOP

(via Second Derivative Spectroscopy for Nitrate N)

Reference: Standard Methods 4500NO_3.C

Additional References:

Bachmann, R. and D.E. Canfield. 1996. Use of an alternative method for monitoring total nitrogen concentrations in Florida lakes. Hydrobiologia. 323:1-8.

Crumpton, W.G. 1992. Nitrate and organic N analyses with second-derivative spectroscopy. Limnol. Oceanogr. 37(4): 907-913.

1) Remove up to 22 samples from the freezer and allow to thaw. Acid Wash (10% HCl): 54-Culture Tubes and screw caps, 1-500 ml beaker, 1- 50 ml beaker.

Note: See also sections for nitrogen stock solutions (A through C) if they have expired (shelf-life listed in Section C).

A-Preparation of Nitrogen Stock (1000 ppm N stock solution can also be ordered, Ricca catalog # 5459-16):

A stock potassium nitrate (KNO_3) solution should be prepared every three months and will be used to derive our working nitrogen standards as described below:

1) Place approximately 5 g KNO_3 in the oven at 105˚C for 24 hours.
2) After 24 hours place the dehydrated KNO_3 in a desiccator and allow the reagent to cool to room temperature.
3) Fill a pre-labeled (1000 mg/l Nitrogen Stock), acid washed, 100ml volumetric flask with approximately 50 ml DI H_2O.
4) Carefully measure out .7218g KNO_3 and pour all the crystals into the 100 ml volumetric flask. *Note: should any reagent spill onto the benchtop or onto the outside of the flask, you should rinse the volumetric flask five times with DI H_2O and repeat the steps 3 and 4. Always return the dehydrated KNO_3 to the desiccator after pouring out the reagent.*
5) Add 0.4 ml 36N H_2SO_4, dispensed via an acid washed volumetric pipette, to the 100 ml volumetric flask.
6) Swirl the flask to dissolve the KNO_3 crystals.
7) Bring the flask to volume by slowly adding DI H_2O dispensed via a Nalgene squirt bottle.
8) Parafilm the 100 ml volumetric flask and invert twenty times or until all the KNO_3 has dissolved.
9) Store the stock 1000 mg/l nitrogen standard in the refrigerator until needed. This solution should be discarded after 90 days.

B-Intermediate Nitrogen Stock:

An intermediate Nitrogen stock solution (10 mg/l Nitrogen Stock) should be prepared immediately prior to preparing the working nitrogen standards as described below:

1) Fill a pre-labeled (10 mg/l Nitrogen Stock), acid washed, 1L volumetric flask with approximately 500 ml DI H_2O
2) Draw up 10 ml of 1000 mg/l Nitrogen Stock in an acid washed 10 ml volumetric pipette and discard the solution in a waste bucket.
3) Draw up 10 ml of 1000 mg/l Nitrogen Stock in the 10 ml volumetric pipette and dispense the solution into the 1l volumetric flask (10 mg/l Intermediate Nitrogen Standard).
4) Swirl the volumetric flask to obtain a homogeneous mixture.
5) Bring the 1l flask to volume by slowly dispensing DI H_2O from a Nalgene Squirt bottle.
6) Parafilm the 1l flask and invert 20 times to obtain a homogeneous mixture.

C-Preparation of Working Standards and DI H_2O blank:

The Intermediate Nitrogen Stock Solution should be diluted as outlined below and as summarized in Table 6 to obtain your working standards:

1) Six 500 ml volumetric flasks, labeled with the respective standard concentrations: 2000 µg/l, 1000 µg/l, 500 µg/l, 250 µg/l, 100 µg/l and 0.0 µg/l (DI H_2O blank), should be half filled with DI H_2O.
2) Two milliliters of concentrated H_2SO_4 (36N) should be pipetted, with a Finnpipetter set to dispense two

milliliters of solution, into each of the six volumetric flasks.

3) Each sample should be swirled to mix the solutions in the respective volumetric flasks.
4) The working standards are then prepared by measuring a fixed volume of Intermediate Nitrogen Standard (10 mg/l) into the respective volumetric flasks, as documented in Table 6, using either a 100 milliliter or 25 milliliter graduated cylinder.
5) All volumetric flasks are brought to volume with DI H_2O, covered with Parafilm and inverted ten times to assure the samples are well mixed and a homogeneous solutions are achieved.

The working standards (0 – 2000 µg/l) should be refrigerated between uses and discarded after 28 days. The 1000 mg/l stock solution should be refrigerated between uses and discarded after 90 days. The 10 mg/l intermediate nitrogen solution should be prepared no more than 2 hours prior to the preparation of the working standards. After the working standards are poured, the remaining 10 mg/l intermediate nitrogen standard should be discarded into a waste bucket, the volumetric flask should be acid washed and subsequently rinsed three times with DI H_2O.

Table 6. Nitrogen working standards.

Working Standard Solution (µg/l) [1]	Intermediate Nitrogen Stock Solution (ml) [2]	Culture Tube (ID) [3]
2000	100.0	2
1000	50.0	3, 53
500	25.0	4
250	12.5	5
100	5.0	6
(blank) 0	0.0	1 & 54

[1] Standards used to derive calibration curves (prepared within 28 days of use).
[2] Volume of intermediate nitrogen stock added to a 500ml volumetric flask and brought to volume with DI H_2O to derive the working standards presented in the leftmost column above. The 1000-2000 µg/L standards are prepared by measuring the Intermediate Nitrogen Stock in a 100 milliliter graduated cylinder while the 100, 250 and 500 µg/L standards are prepared by measuring the Intermediate Nitrogen Stock using a 25 milliliter graduated cylinder.
[3] Culture tube into which the respective standards and blanks are placed.

D-Preparing samples, standards and blanks for analysis:

1) Record the appropriate information (i.e. lake, site, date, depth) on the Total Nitrogen datasheet (Appendix C) and proceed as follows:
    a) Invert the first sample (DI H_2O blank) 10 times.
    b) Measure out 30 milliliters of sample using an acid washed 50 ml class-A graduated cylinder.
    c) Pour the sample into the properly labeled test tube (Pyrex 25x150 mm culture tube w/screw cap (cat #9826-25) and place the cap loosely on the sample.
    d) Wash the graduated cylinder with 10% HCl between samples and rinse the graduated cylinder three times with DI H_2O.
    e) Repeat steps a through d with successive samples. *Note: samples 17 through 20 and samples 25 through 28 are laboratory replicates and spikes and should contain water poured from the same samples.*

2) Samples 19, 20, 27 and 28 should be spiked with 1000 mg/l Nitrogen stock standard using the following procedure:
    a) Pour approximately 20 ml of 1000 mg/l nitrogen stock standard into an acid washed 50 ml beaker.

b) Draw up 50 *u*L of stock standard using a fixed volume micropipetter (50 *u*l MLA Systems D-Tipper, MLA cat # 1054C) and dispense the solution into a waste bucket.
c) Draw up 50 *u*L of stock standard using the fixed volume micropipetter and dispense the solution into sample 14. Loosely cap the test tube after adding the spike and gently swirl the sample. This nitrogen addition constitutes a 1664 µg/L nitrogen spike.
d) Repeat step c for samples 15, 40 and 41.

3) Add 4.5 milliliters of potassium persulfate (oxidizing agent preparation described below) to each test tube using the following procedure:
   a) Pour the Alkaline Potassium Persulfate oxidizing agent into an acid washed 500 ml beaker.
   b) Draw up 4.5 ml Alkaline Potassium Persulfate oxidizing agent using a 5.0 ml adjustable Thermo Labsystems Finpipette and discard the solution into a waste bucket.
   c) Draw up 4.5 ml Alkaline Potassium Persulfate oxidizing agent and dispense the solution into test tube #1 (DI H_2O blank). Loosely cap the test tube.
   d) Dispense 4.5 ml Alkaline Potassium Persulfate into each of your remaining test tubes (#2 through #57) as described in step c above.
   e) Tighten each test tube cap after all samples have received an aliquot of oxidizing agent. Gently swirl each sample.

4) Place the samples and the test tube rack (the rack is autoclavable) into a Nalgene Polypropylene autoclavable bin and autoclave the samples for 45 minutes at 123˚C. *Note: be sure the autoclave is set to <u>liquids</u> before initiating the digestion as described in the autoclave use directions.*

<u>E-Oxidizing Agent Preparation:</u>

Dissolve 6.0g Potassium Persulfate in 100 ml 2.0N NaOH = Alkaline Potassium Persulfate solution. (2.0N NaOH solution will neutralize the acid preservative added and maintain a basic environment for the digestion) – All lakewater samples, standards and blanks were preserved with 1 milliliter H_2SO_4/250 milliliters of lakewater.

<u>F-Post-Autoclave treatment:</u>

1. Place test tube-rack into an ice bath and cool the samples to room temperature before proceeding to step 2. *Note: it is imperative that the samples are cooled before the addition of acid – otherwise a volatile reaction will ensue.*
2. Turn on the Cary 60 spectrophotometer if you have not already done so.
3. Add 0.6 milliliters concentrated H_2SO_4 to each test tube using a Wheaton Model 851350 (0.5 – 5 milliliter) adjustable macropipetter.
4. Cap each test tube and invert each test tube three times.
5. Analyze the samples on the Cary 60 scanning spectrophotometer using the procedure outlined in "Spectrophotometric Analysis on the Cary 60 spectrophotometer".

<u>G-Spectrophotometric Analysis on the Cary 60 spectrophotometer</u>

1) Select the icon "Shortcut to Nitrogen.MSW" from the desktop to initiate the Total Nitrogen analysis program. If the icon "Shortcut to Nitrogen.MSW" does not appear on the desktop consult the laboratory manager before proceeding to step two (the total nitrogen analytical settings are included below in the event the "Nitrogen.MSW" file has been lost or modified).
2) Select the Total Nitrogen Cuvette (1 cm path length, rectangular quartz cuvette, (FS cat #14-385-902C) and rinse with DI H_2O three times.
3) Fill the Total Nitrogen cuvette with DI H_2O, gently tap the cuvette to dislodge any air bubbles, wipe the cuvette faces with a Kimwipe and inspect the cuvette to assure no smears or particles will impede the light path. Insert the sample into the spectrophotometer cell holder and close the lid. *Note: the cuvette should be centered in the cell holder and care should be taken to place the cuvette in the same position with each subsequent water sample.*
4) Select "baseline" from the list of options indicated on the computer monitor (this will set all absorbency values between 250nm and 200nm to zero).
5) Empty the cuvette into a waste bucket and fill the cuvette with the contents of test tube #1. Repeat step #3 to assure the sample is ready for analysis (tap the cuvette to dislodge any air bubbles, wipe the cuvette faces with a

Kimwipe and inspect the cuvette to assure no smears or particles will impede the light path and result in errors. Insert the sample into the spectrophotometer cell holder and close the lid).

6) Press "Start" You will then be prompted for a file name – proceed as follows:
    a) Create a new folder in the CaryWinUV sub-directory by clicking on the create folder icon. Name the folder TN "today's date" (e.g. TN021402 which stands for Total Nitrogen analysis that occurred on February 14, 2002).
    b) Double click the folder you just created (e.g. TN021402) to set this folder as the location where your files will be stored.
    c) You are now prompted to enter a file name. Use the format: Site ID, replicate # (e.g. for the first sample on the datasheet, DI H_2O blank, you would enter "DI H_2O blank replicate 1". For a sample collected from Swains Lake, Site A on June 2, 2002, collected at a depth of 5.0 meter you would enter "Swains Lake Site A depth 5 060202". Use the format of month, day, year as indicated in the example. This program will not accept non-alphanumeric characters such as "/", "-" or ".".). If you have a depth that includes a decimal place (e.g. 5.5 meters) enter the depth as 55.
    d) Once you have entered the file name press the "return" key.
    e) You are now prompted for a sample name. Use the same format as used for the file name (e.g. Swains Lake Site A depth 5 060202).
    f) Once you have entered the sample name press the "return" key.
    g) You can now prompted to select "OK" or "cancel". Press "OK" to begin the analysis. The Cary 60 spectrophotometer will scan and record the absorption data between 200 and 250 nm and will cycle through three times – this takes approximately three minutes. At the end of the third cycle all data will automatically be saved to a computer file in the folder (e.g. TN021402) that you created previously.
7) Remove the cuvette from the spectrophotometer chamber, pour the cuvette contents into the waste bucket and rinse three times with DI H_2O. Fill the cuvette with the next sample and repeat step 3 to assure the cuvette has been properly treated/cleaned.
8) Repeat Step 6 typing in the appropriate information for your current sample.
9) Repeat Steps 6 and 7 until all samples have been analyzed.
10) Once all samples have been analyzed we can proceed to the total nitrogen second derivative calculations step described on the next page.

<u>H-Total Nitrogen analytical Settings</u>

Select "setup"
X mode: the scan range should be set from "200" nm to "250" nm.
Cycle count: the cycle count should be set to "3"
Scan Controls - the scan controls should be set to:
1) Average time(s) = "0.25" seconds
2) Data Interval (nm) = "0.5" nm
3) Scan Rate (nm/min) = "120.00" nm/minute

<u>I-Total Nitrogen second derivative calculations</u>

1)
    a) Select the calculator icon from the menu bar (right hand side)
    b) Select "selected graph" from the display options
    c) Select "focused graph"
    d) Set the operation to "Deriv2"
    e) Set the filter size to "17"
    f) Set the interval to "0.5"
    g) Press the "Apply" button

2)
    a) Select trace preferences icon from the menu bar (left hand side)
    b) Select the appropriate file by selecting the "color cell" – object in the cell will become red and indicates it is selected

c) Press "OK"

3)

a) Select the calculator icon again (notice the equation that you generated in step one is displayed).
b) Select the "=" sign
c) At the Y label prompt select "2nd derivative" from the drop down menu.
d) Press "OK" (you have now generated a new "Deriv 2 file" that can be viewed by selecting the traced preferences icon.

4) Repeat steps 2 and 3 until you have generated second derivative files for all your data.

5) Once you have calculated second derivatives for all of your data select "save data as" from the file menu. Name the file "second derivatives" and press the "save" button. All of your second derivative data are now saved and can be archived for further reference.

6) Record the 225.5nm second derivative peaks on the total nitrogen datasheet for each sample analyzed. *Note: 225.5nm represents the wavelength where the maximum second derivative peak typically occurs. However, should the peak be located at another wavelength consult the laboratory manager before recording any measurements on the datasheet.*

J-Total Nitrogen Daily Calibration Curve

After each analytical run the six initial TN standards (samples 2-6) and the 2nd derivative values should be entered into an Excel spreadsheet as depicted in the first two columns of Table 7. Subtract the DI H_2O blank 2nd derivative value (sample 1) from each of the TN Standard 2nd derivative values displayed in the "Corrected 2nd der" column. Regress the standards (dependent variable) against the corrected 2nd derivative values (independent variable) to generate a statistical output as displayed in Figure 3. You will now multiply your corrected 2nd derivative values by the X variable (TN coefficient) to calculate your TN concentrations (Table 7). As you can see, the prepared TN standards compare well with the calculated TN values. You will now use your TN coefficient and the corrected 2nd derivative values of your samples (unknowns) to determine the remaining TN concentrations. You should record your TN coefficient, your 2nd derivative DI H_2O blank (corrective factor) and your r^2 value on the back of your Total Nitrogen datasheet.

Table 7: Total Nitrogen Calibration Curve

Standard	2nd der average	Corrected 2nd der	Calculated TN (*u*g/l)*
DI H_2O Blank	0.000084	0.000000	0
2000	0.004481	0.004397	1995
1000	0.002285	0.002201	999
500	0.001224	0.001140	517
250	0.000650	0.000566	257
125	0.000360	0.000276	125

* based on the Total Nitrogen coefficient derived from the Total Nitrogen calibration curve below.

Figure 3. Regression Output

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.999921079
R Square	0.999842164
Adjusted R Square	0.749842164
Standard Error	9.578222215
Observations	5

ANOVA

	df	SS	MS	F	Significance F
Regression	1	2324633.031	2324633.031	25338.71504	5.46679E-07
Residual	4	366.9693632	91.7423408		
Total	6	2325000			

	Coefficients	Standard Error	t Stat	P-Value
Intercept	0	#N/A	#N/A	#N/A
X Variable 1	453819.5733	1883.197571	240.9835167	1.7789E-09

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A.5. Solids/Percent Organic Matter SOP (Sediment or sludge sample)

Reference: Standard Methods 20th Edition Method 2540.G.

Additional Reference:
USGS 1975 Methods for the Determination of Inorganic Substances in Water and Fluvial Sediments, Techniques of Water-Resources Investigations of the United States Geological Survey, Book 5, Chapter A1 Edited by Marvin J. Fishman and Linda C. Friedman I-5753- Solids, Volatile-on-ignition, Total-in-bottom-material, Gravimetric

A portion of well-mixed sediment sample is dried at up to 105°C. A portion of that dry sample is carefully weighed and then ignited at 550°C. The loss of weight on ignition represents the amount of volatile solids in the sample. The volatile solids of a sample roughly approximate the organic matter content.

Analyze at least 10% of all samples in duplicate.

1-Work-up (sediment drying)

a) Preheat the oven (Fisher Scientific (FS) Model 825F) to 104°C +/-1°C.
b) Transfer the pertinent information (i.e. lake, site, collection date, sample depth, etc.) from the sampling bottle to the Percent Organic Matter Datasheet.
c) Place the evaporating dishes (Pyrex no. 3180) into an acid bath (10% HCl) for 10 minutes and then rinse three times with DI H_2O.
d) Place evaporating dishes into the oven for one hour. Heat dish at 103°C to 105°C for 1 hr in an oven.
e) Cool in the desiccator, weigh, record weights on data sheet, and store in the desiccator until ready for use.
f) Repeat drying (1 d), cooling, weighing, and desiccating steps until weight change is less than 4% or 50 mg, whichever is less. Record the final weight on the datasheet.
g) Arrange the numbered evaporating dishes in sequential order.
h) Pour 25-50 grams of the appropriate benthic sample into the corresponding evaporating dish (as indicated on the datasheet).
i) Place the evaporating dishes into the oven and let the samples dry for 24 hours.
j) Remove the evaporating dishes, place in a large glass dessicator and allow the samples to cool to room temperature. *Note: if the dessicant is not blue but pink consult the laboratory manager to proceed with dessicant replacement and to assure the dessicators have an airtight seal.*
k) Repeat drying (1 hr), cooling, weighing, and desiccating steps until weight change is less than 4% or 50 mg, whichever is less. Record the final weight on the datasheet.

2-Pre-Ash Weight

a) Preheat the muffle furnace.
b) Place up to 9 quartz ashing dishes (FS Cat # 08-072c) into an acid bath (10% HCl) for 10 minutes and then rinse three times with DI H_2O.
c) **Put on the orange thermally insulated gloves labeled "Furnace". These are the only gloves that are resistant to the high furnace temperature and it is imperative that you put them on before proceeding.**
d) Place the quartz ashing dishes into the Thermolyne model 48000 muffle furnace using the crucible tongs (FS Cat # 15-210) and orange protective gloves.
e) Ignite the acid washed ashing dishes at 550°C for 1 hr.
f) Place the quartz ashing dishes into an empty small glass dessicator using the crucible tongs and orange protective gloves. Make sure the desiccant is blue, not pink- otherwise have the lab manager replace the desiccant.

g) Tare the Denver Instruments A-250 analytical balance.

h) Place the first quartz ashing dish on the balance, close the door and let the weight stabilize. Record the weight on the Percent Organic Matter datasheet. Repeat for all other ashing dishes.

i) Remove your first benthic sample from the large glass dessicator and pulverize it (in the evaporation dish) with a pestle until you have obtained a "homogeneous mixture". Make sure you immediately replace the dessicator lid between samples.

j) Carefully pour between 3 and 5 grams of benthic matrix into the Quartz ashing dish. *Note: pour this out carefully to minimize the amount of airborne particles (fine organic and inorganic debris). You may want to save the remainder for Sediment Total Phosphorous or Total Nitrogen digestion and analysis. If so, cover the evaporation dish with a square of aluminum foil shiny side up and replace in the glass dessicator. Start digestion as soon as possible, optimally within 24 hours.*

k) Measure the weight of the ashing dish + the benthic matrix you just added on the analytical balance. Record the total weight of onto the benthic datasheet.

l) Place the filled Quartz ashing dish into an empty dessicator. Make sure you place the ashing dish in the appropriately numbered slot (i.e. sample one should be placed into slot one). This placement is critical since the ashing dishes are not numbered.

m) Carefully wipe off the pestle with a kimwipe. Note: the pestle should not have any moisture on it. If there is any doubt place the pestle into the FS Model 825F oven for 10 minutes and subsequently remove the pestle while wearing the orange, thermally insulated, laboratory gloves.

n) Repeat the Pre-Ash Steps i) through m) until you have poured out up to 9 samples to be ashed.

3-Ashing Procedure

a) **Put on the orange thermally insulated gloves labeled "Furnace".** ***These are the only gloves that are resistant to the high furnace temperature and it is imperative that you put them on before proceeding.***

b) Place the Quartz ashing dishes into the Thermolyne model 48000 muffle furnace using the crucible tongs. Arrange the first three samples on the top shelf from left to right, samples four through six on the bottom shelf in back from left to right, and samples:

Table 8 Position of ashing trays in muffle furnace

	Left	Center	Right
Top Shelf	Sample #1	Sample #2	Sample #3
Bottom Shelf Back	Sample #4	Sample #5	Sample #6
Bottom Shelf Front	Sample #7	Sample #8	Sample #9

c) If the furnace has reached temperature (550°C) skip to e.

d) Turn on the Muffle Furnace and let it warm up to the pre-set temperature of 550°C.

e) Leave the samples in the oven for one hour from the time the temperature reaches 550°C. *Note: you are incinerating the organic matter in the samples and it will smell like something is burning for a short period – longer if there is appreciable organic matter in the sample. Put a note on both laboratory doors (in big bold letters) that indicates we are ashing samples and there is no fire.*

f) You should turn the muffle furnace off after one hour at 550°C.

g) Place a glass dessicator on one of the Rubbermaid carts and wheel it over to the Muffle Furnace.

h) Remove the lid to the dessicator and place it to the side.

i) Using the crucible tongs, carefully pick the ash dishes up one by one and place them in the appropriately numbered slits in the dessicator.

j) Once all samples have been place in the dessicator put on the lid. Note*: you should slide the lid to the side every two minutes (for the first 10 minutes) to release the pressure. If you do not release the*

pressure the samples will cool and will create a vacuum seal that is extremely difficult to "break".

k) After a one-half hour cool down period the ashed samples are ready to be weighed (the change in weight is proportional to the amount of organic matter in the samples).

4-Post-Ashing Weighing Procedure

a) Tare the Denver Instruments A-250 analytical balance.
b) Place the first Quartz ashing dish on the analytical balance, close the door and let the weight stabilize. Record the post-ash weight on the datasheet.
c) Repeat igniting (30 min), cooling, desiccating and weighing steps until the weight change is less than 4% or 50 mg, whichever is less.
d) Remove the Quartz ashing dish from the analytical balance and place it on the laboratory bench for cleaning (all data have been recorded so there is no need to return the sample to the dessicator).
e) Repeat steps "a" through "c" with subsequent samples until all samples have been analyzed and the pertinent data have been recorded.
f) Analyze at least 10 percent of all samples as duplicates.

5-Post Analysis Clean-Up

a) Take both the desiccation dishes and the ashing dishes to the sink and rinse them out with DI H_2O. If necessary, use a laboratory cleaning brush and Alconox to remove any stubborn sediment from the glass and then rinse liberally with DI H_2O to assure all particulate debris had been washed away.
b) Wipe down the analytical balance with a laboratory sponge to remove any dust that had settled out.
c) Turn off the Denver Instruments A-250 analytical balance.

6-Calculations

a) Percent Total Solids:

% Total Solids = 100 x (A - B) / (C - B)

where:
A = weight of dried residue and evaporating dish, in mg,
B = evaporating dish weight dish in mg,
C = weight of wet sample and evaporating dish, in mg.

b) Percent Volatile and Fixed Solids:

% Volatile Solids = 100 x (D - F) / (D - E)

% Fixed Solids = 100 x (F - E) / (D - E)

where:
D = weight of dried residue and ashing dish, in mg,
E = ashing dish weight, in mg,
F = weight of residue and ashing dish after ignition, in mg.

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A.6. Digestion of Samples for Total Phosphorus and/or Total Nitrogen (Sediment samples) SOP

A.6.1-Microwave Digestion-

Due to the caustic nature of the $HCl/HNO_3/HF$ digestion media and specialized equipment (Microwave Digester, Teflon Pressurized Digestion Vessels etc.) we utilize the UNH Instrumentation Center (Department of Chemistry) facilities, equipment (CEM MDS 2000 System) and staff. Make arrangements in advance to bring samples to the lab. Digestions can be run according to any of the appropriate methods such as EPA 3051, 3051A, 3050B, 3015, 3015A, etc.

1- Weigh out proper sample size for technique on tared plastic acid washed disposable weighting dish if not already in evaporation dish or ashing dish. Use dried (105°C) ashed (see SOP A.5. for either), or freeze dried sample depending on request. Carefully transport samples to instrumentation center using a dessicator. Make sure you also bring the proper labeled containers for them to add the digest into when the procedure is complete.

2- When sample are digested run the appropriate Total P or Total N test or both according to the CFB lab SOPs. Generally you will dilute 1 ml of the sample into 49 ml of water to bring the samples down to the proper range and negate any turbidity interference. Do all dilutions under the laboratory hood as the digests used are extremely reactive. Dispose of the samples properly as directed by the lab manager.

A.6.2-Persulfate Method for Simultaneous Determination of Total Nitrogen and Total Phosphorus- (Modified for sediment digestion)

References: Standard Method ("new" online and upcoming 21st edition) 4500 P J. and Simultaneous Determination of Total Nitrogen and Total Phosphorus in Freshwater Samples Using Persulfate Digestion. Hosomi, M; Sudo, R International Journal of Environmental Studies IJEVAW Vol. 27, No. 3/4, p 267-275, 1986.

Sample requirements: Non-acidified fresh or frozen original sample dried, freeze dried or dried then ashed. Through comparison tests between this method, hot acid and microwave digestions we have found that this method is preferable for the determination of both N and P for lake sediments as it does not require HNO_3 or other extremely volatile acids that require great care and special air exhaust areas.

This simultaneous digestion method gives not only a high recovery and reproducibility over a wide range of various nitrogen and/or phosphorus compounds of known nitrogen and phosphorus compositions, but also high recoveries of nitrogen and phosphorus from standard reference materials, stream leaf matter, lake sediments, and algal cultures. No significant difference was found between the determinations of T-N and T-P in freshwater sediment samples by this method and those determined by the standard methods of T-N and T-P, respectively. This method should be useful for the routine analysis of T-N and T-P in freshwater samples containing particulate material and sediment samples.

1- Reagents and Standards:

a. **Sodium hydroxide, 3N**: Slowly dissolve 120 g low-nitrogen NaOH in 800 ml DI water in a 1000-ml volumetric flask placed in ice. Cool and dilute to volume.

b. **Oxidizing reagent**: Dissolve 64 g low-nitrogen (<0.001%N) potassium persulfate, $K_2S_2O_8$, in 500 ml deionized water in a 1 l volumetric flask. Use low heat if necessary. Add 80 ml 3N NaOH, that was just prepared from the low-nitrogen sodium hydroxide, and dilute to 1000 ml. Store in a brown

glass bottle at room temperature.

c. **Urea Standard Stock Solution and Standard:** (Organic N control standard) Dissolved 2.1437 g of urea in 1 l of DI water. 1.0 ml of this solution contains 1 mg of N. Working Calibration Standard Solution: Dilute 2.0 ml of urea stock solution to 100 ml in a volumetric flask with DI water to create a 20 mg/l organic nitrogen control standard. Cover flask with parafilm and invert 10 times to mix. (20 mg/l standard).

d. **Adenosine Triphosphate Stock Solution and Standard**: (Organic P control standard) Dissolve 0.6514 g adenosine triphosphate in deionized water and dilute to 1000 ml; 1 ml = 0.1 mg P. To prepare a calibration standard, dilute 10.0 ml stock solution to 100 ml in a volumetric flask and bring to volume with DI water. Cover flask with parafilm and invert 10 times to mix (10 mg/l standard).

e. **Orthophosphate Control Standard:** Use previously prepared 10 mg/l P stock standard (ie: the 30 ppm phosphate stock standard).

f. **Nitrate Control Standard:** Prepare a 50 mg/l Nitrate Standard by diluting 5 ml of stock potassium nitrate (KNO_3) solution (1000 mg/l N) to 100 ml in a volumetric flask (final concentration 50 mg /l). Cover flask with parafilm and invert 10 times to mix.

g. In addition to the above reagents mix any required reagents and standards for the total nitrogen and total phosphorus determinations.

2- Use the "Sediment Digestion" lab data sheets, as well as the specific Sediment TP or TN datasheets

3- Set up the 250 ml flasks in the autoclave bin so that you have one for each sample as well as 6 extra for controls and blanks. Also have a 500 ml flask ready.

4- Add 48 ml of DI water to all 250 ml flasks except 1A through 3A and 4B. Add 240 ml DI water to the 500 ml flask.

5- Leave flask 1A as the blank.

6- For flask 1B, 2A, 2B, and 3A add 48 ml of the nutrient control standard solutions: Urea, ATP, phosphate and potassium nitrate to each flask respectively. Use a 50 ml graduated cylinder that is acid rinsed and then rinsed with DI water three times between each standard.

7- Weigh out proper sample size for technique chosen on tared disposable weighting paper. Use dried (105°C) and or ashed (see SOP A.5. for either), or freeze dried sample depending on request. Before and after weighting keep samples in the dessicator.

   a. One sample will be weighed out six for a duplicate sample and a matrix spike. For dried samples or freeze dried samples: Weigh 5.0 +/-0.2 grams of the dry sample #1 into the flasks 3B, 4A and 4B. Write the flask number and weight of sample onto the datasheet.
      i. Into flask 4B also add 10 ml of 30 mg/l Stock Phosphate Standard and 10 ml of 50 mg/l Nitrogen Working Standard. Add 28 ml of DI water.
   b. For the remaining dried samples or freeze dried samples: Weigh 5.0 +/-0.2 grams of the dry sample into the flask, write flask number and weight of sample onto the datasheet. Start at flask 5A.

8- Add 10 ml of oxidizing reagent to each flask using the 10.0 ml repeater pipette. Cap each flask with a #6 glass stopper (FS Cat # 10-042A) and swirl each flask to assure the samples are well mixed.

9- Add 50 ml of oxidizing reagent to the 500 ml flask using a graduated cylinder. Cap the flask with a #8 glass stopper and swirl to mix.

10- Place flasks into the Gettinge 533LS autoclave, adjust the setting to liquids, make sure the temperature is set to 123° C and set the timer to 90 minutes as indicated below.

a. Select "Sterilize Temperature" under the Cycle Values setting and set it to 123.0°C by pressing either the "up arrow" or the "down arrow".
b. Select "Sterilize Time" under the Cycle Values setting and set it to 60 minutes by pressing either the "up arrow" or the "down arrow".
c. Select "Liquids" under the Cycle Select setting. (It is imperative the autoclave switch is set to liquids, otherwise your samples will vaporize as the autoclave heats up and the samples will be lost).
d. Once you are sure the settings have been properly adjusted press the "start" button. Stay in the room until the autoclave heats up to 123.0°C to assure the samples are digesting.

11- Turn on the appropriate spectrophotometer: Cary 50 for TP, Cary 60 for TN.

12- Retrieve samples after 2.0 – 2.5 hours. In the meantime set up the appropriate glassware as indicated in sections 13 I and 13 II below.

13- When samples are sufficiently cooled, add 0.2 ml of 3N NaOH using the fixed volume pipettor to each 250 ml flask. Gently swirl the flasks after addition.

14- Add 2.0 ml of 3N NaOH using a fixed volume Class A 2.0 ml volumetric pipette to the 500 ml flask and swirl to mix.

I-For TP analysis prepare sample and standard flasks as follows:

a. Prepare enough 125 ml TP Erlenmeyer flasks (Corning # 5100-125) to account for twice the number of flasks you used in the digestion plus 6 more. Arrange the flasks alpha-numerically, 1A through #B (where # = one half of the total number of flasks used), and place sequentially into the polypropylene sterilizing trays before filling the flasks with samples or standards.
b. Prepare TP standards and blank as follows (for calibration curve):
    i. Label 7 clean 500 ml, volumetric flasks with the respective standard concentrations: 2000 µg/l, 1000 µg/l, 500 µg/l, 100 µg/l, 50 µg/l, and 10 µg/l.
    ii. Using a 25 ml graduated cylinder add 20 ml of digestion blank solution from the 500 ml flask into each volumetric flask.

Prepare Standard Flasks as Follows: Using Class A fixed volumetric pipettes dilute pre-purchased HACH (cat #1436716) 30.0 mg/l stock standard, as 30.0 mg/l PO_4^{-3}, into the appropriate standard concentrations described in Table 9. For the last two standards you will utilize the 1000 *u*g/l solution you will have just mixed.

Note: orthophosphate standards are not preserved and should be prepared daily no more than two hours prior to the addition of the mixed reagent.

c. All volumetric flasks are then brought to volume with DI H_2O, covered with Parafilm and inverted ten times to assure the samples are well mixed and a homogenous solution is achieved. Pour out 50 ml of each of the standards into the appropriate flasks as shown in Table 9.
d. To all TP flasks starting with 5A add 48 ml of DI H_2O.
e. To minimize the impact of turbidity interference from the digested solution, the sediment digestions are diluted 1:25. Draw up 2.0 ml of the blank in digested flask 1A using an adjustable Thermo Labsystems Finipipette (cat # 40270-280) set to 2.0 ml and discard the

solution into a waste bucket. Draw up another 2.0 ml and dispense into flask SRP 5B. Swirl flask to mix.

f. Using a new clean pipette tip repeat the process described above for each of the sediment samples, matrix and standards so that all TP flasks are complete. Between each sample pair use a new clean pipette tip. Swirl flasks to mix well.

g. Run the samples in the flasks according to the TP SOPs listed in section A.3 starting at step 13.

Table 9. TP working standards for Sediment TP.

Standard Solution (µg/l) [1]	HACH Stock Solution (ml) [2]	1000 µg/l Solution (ml)[3]	Erlenmeyer Flask (ID) [4]
(blank) 0	0.0	0.0	1A
2000	20.0	0.0	1B
1000	10.0	0.0	2A
500	5.0	0.0	2B
100	1.0	0.0	3A
50	0.5	0.0	3B
10	0.0	5.0	4A
5	0.0	2.5	4B

[1] Standards used to derive calibration curves (prepared within two hours of analysis).
[2] Volume of stock HACH standard (HACH cat # 1436716) added to a 500 ml volumetric flask and brought to volume with DI water to derive the working standards presented in the leftmost column above
[3] Volume of 1000 µg/l calibration working standard added to a 500 ml volumetric flask and brought to volume with DI water to derive the working standards presented in the leftmost column above. Used for lower standard concentrations.
[4] Erlenmeyer Flasks into which the respective standards and blanks are placed.

II-**For TN analysis:**

a. Prepare sample and standard tubes as described in section A.5 Total Nitrogen. The same calibration standards are used for sediments as are used with water. However, be sure to add 17.0 ml of the digestion blank water to each of the 1 l volumetric flasks before bringing each working standard up to volume.

b. To minimize the impact of turbidity interference from the digested solution, the sediment digestions are diluted 1:30. For all digested sample culture tubes fill with 29.0 ml of DI H_2O. Draw up 1.00 ml of the contents in each digestion flask using an adjustable Thermo Labsystems Finipipette (cat # 40270-280) set to 1.0 ml and discard. Draw up another 1.0 ml and dispense into the appropriate culture tube. Repeat this procedure with all of the samples digested.

c. Use the vortex mixer to blend the sample. Proceed with analysis as describe in section A.5.

15-Calculations: Calculate TP and TN as described in the appropriate SOP sections but remember to substitute the proper working standard solutions for the respective calculation curves.

a. Sediment Total Phosphorus:

$$\text{Sediment TP (mg/kg dry weight)} = \frac{Ctp \times DF \times Vls}{W}$$

Where:

CTP = Concentration of TP in mg/l
DF = the dilution factor (25)
Vtp = volume of extract in liters (0.029 l)
W = weight in kg of dried sample

b. Sediment Total Nitrogen:

$$\text{Sediment TN (mg/kg dry weight)} = \frac{Ctn \times DF \times Vtn}{W}$$

Where:

Ctn = Concentration of TN in mg/l
DF = the dilution factor (30)
V = volume of extract in liters (0.029 l)
W = weight in kg of dried sample

c. Matrix Spikes:

The P matrix spike adds 10 mg/kg {The 10 mg/l Phosphorus Standard Solution / 1000 ml/l = 0.01 mg/ml [(10 ml) (0.01 mg)] /.010 Weight (kg) = Approximately 10.0 mg/kg phosphorous}.

The N matrix spike adds 50 mg/kg {The 50 mg/Nitrogen Standard Solution / 1000 ml/l = 0.05 mg/ml [(10 ml) (0.05 mg)] /.010 Weight (kg) = Approximately 50.0 mg/kg nitrogen}.

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A.7. Phosphorus Fractionation of Sediments / Loosely-Sorbed and Iron Bound Phosphorus SOP (Sediment or sludge sample)

Reference: NELAC SOP by Spectrum Analytical Inc. Provided by ENSR; modified and expanded by CFB

Note: use extreme caution when handling the phosphorus analytical glassware. All glassware is to be acid washed (10 minutes in 10% HCl), rinsed with DI H_2O 3 times and dried with openings facing down on flat drying rack. If there is any doubt of the cleanliness of the glassware, acid wash all applicable materials as described before proceeding any further.

A) **Sample Prepreparation:**

1) Samples to be analyzed should have been collected, iced in cooler, thoroughly mixed and air dried for at least 24 hours. These samples are considered semi-solid and are used in the extraction unless alternative requests have been made (freeze dried, dried at a specific temp and /or milled).
2) If a subsample of the sediment samples have already been dried for 24 hours at 104˚C +/-1˚C go to step B1.
3) The day of the sample analysis (or the day before you want to run this procedure) remove a maximum of 7 sediment samples from the refrigerator. Follow the procedures in SOP A.5.1 for Fixed Solids to determine dry weight basis (percent solids) of each sample.

B) **Glassware and Reagents:**

1) In the morning of analysis make up the following reagents as described in the following section A.3.1-"Mixing other reagents" of the section A.3.-"Total Phosphorus SOP (2005)":
    a. 5 N H_2SO_4
    b. 11N H_2SO_4
    c. 10N NaOH

2) In addition prepare the following solutions:
    a. Ammonium Chloride Solution (1.0 molar): Partially fill a liter volumetric flask with DI H_2O. Add 53.49 grams ammonium chloride. Adjust the pH to 7.0 with concentrate ammonium hydroxide. Perform this step dropwise. Do not keep the ammonium hydroxide bottle open during this procedure. Dissolve and bring up to volume to 1L with DI H_2O. Check the pH of the ammonium chloride before each use. (Used for Loosely-sorbed P).

    b. Buffered Dithionite Solution: Add 9.24 grams $NaHCO_3$ and 19.15 grams $Na_2S_2O_4$ to approximately 500 ml DI H_2O in a 1 liter volumetric flask. Dissolve and dilute to one liter with DI H_2O. Make fresh daily. (Used for Fe-P)

3) Place the following glassware in the acid bath (10% HCl) for 10 minutes:
    a. 12 — 40 ml glass centrifuge tubes (Kimble #45200-40) and screw-caps
    b. 24 — 25mm x 150mm (55 ml) test tubes (Corning #9826-25) and screw caps.
    c. 1 — Stainless spatula
    d. 1 — Ceramic spatula with pestle
    e. 1 — 125 ml Erlenmeyer flasks
    f. 1 ea — 50 ml,100 ml and 250 ml graduated cylinder TD
    g. 1 — 1000 ml volumetric flask
    h. 1 ea — 100 ml, 200 ml and 400 ml beakers
    i. 1 — 10 ml graduated glass pipette

C) <u>Loosely-Sorbed P Extraction Procedure:</u>

1) Tubes and rack positions are numbered to keep track of samples. Set up the sample batch in the orange centrifuge tube rack in the following manner:

Tube 1:	Blank – leave empty.
Tube 2:	2 mg/l Std. – leave empty.
Tube 3:	Sample 1-Weigh 5.0+/-0.2 grams of the semisolid Sample 1 into centrifuge tube, write weight of sample on the datasheet.
Tube 4:	Duplicates of Sample 1, weigh sample one as in Tube 3.
Tube 5:	Duplicate of Sample 1 for matrix spike, weigh sample one as in Tubes 3 and 4.
Tube 6-11:	Samples 2 through 7, weigh 5.0+/-0.2 grams of each of the semisolid samples into each of these centrifuge tubes.
Tube 12:	Blank, leave empty.

2) Make sure you have transferred all pertinent information from the sediment sample label and weight results to the LSP/FEB Phosphorus data sheet at this time (i.e. lake, site, date, depth, etc.).
3) Pour about 20 ml of 30 mg/l NIST phosphorus standard solution (Hach #1436716) that has come to room temperature into an acid washed and triple DI rinsed beaker 50 ml glass beaker. Using a clean 5.0 ml Class A, glass volumetric pipette draw up 5.0 ml of stock standard and dispense the standard into a waste bucket. Subsequently, draw up 5.0 ml of phosphate standard from the beaker and dispense it into Tube 2 (2 mg/l P standard).
4) Using a clean 10.0 ml Class A volumetric pipette draw up 10.0 ml of 30 mg/l stock standard from the beaker and dispense the standard into a waste bucket. Subsequently, draw up 10.0 ml of phosphate standard from the beaker and dispense it into Tube 5 that already contains a sediment portion of sample 1 (4 mg/l spike).
5) Pour about 400 ml of buffered Ammonium Chloride solution into a clean 500 ml beaker. Using a clean 50.0 ml graduated pipette draw up about 50 ml of sample and dispense into the waste bucket. Refill the pipette and carefully bring each graduated centrifuge tube up the 25 ml mark. *Note: If you accidentally slightly overfill the tube do not pour any liquid out. Record the actual volume on the data sheet.*
6) Cap all tubes tight and clip the tubes onto the Barnstead Thermolyne Labquake® Rotating Shaker (Model 415110) and set the lab timer for 2 hours.
7) While waiting for shaking to complete during the last half an hour set up the sample flasks and standards for the phosphorus (SRP) analysis:
8) <u>Prepare Sample and Standard Flasks as Follows:</u>
    a. Arrange thirty-two 125 ml Erlenmeyer flasks (Corning # 5100-125) alpha-numerically, 1A through 16B, and place the flasks sequentially into the polypropylene sterilizing trays before filling the flasks with samples or standards.
    b. Prepare SRP standards and blank as follows (for calibration curve):
        i. Label 7 clean 500 ml volumetric flasks with the respective standard concentrations: 500 µg/l, 100 µg/l, 50 µg/l, 10µg/l, 5 µg/l, 2 µg/l and 0 µg/l. Label 1 clean 250 ml volumetric flask as 1000 µg/l.
        ii. Using a 50 ml graduated cylinder, add 50 ml of buffered Ammonium Chloride solution into each 500 ml flask and 25 ml of the solution into the 250 ml flask.
        iii. Using Class A fixed volumetric pipettes dilute pre-purchased HACH (cat #1436716) 30.0 mg/l stock standard, as 30.0 mg/l PO_4^{-3}, into the appropriate standard concentrations described in Table 8. *Note: For the last four standards you will utilize the 1000 ug/l solution you will have just mixed.*

Note: orthophosphate standards are not preserved and should be prepared daily no more than two hours prior to the addition of the mixed reagent.

iv. All volumetric flasks are then brought to volume with DI H_2O water, covered with Parafilm and inverted ten times to assure the samples are well mixed and a homogeneous solutions is achieved.

c. Set up the yellow tube rack with 12 cleaned culture tubes with caps in row 1.
d. Turn on the Cary 50 Spectrophotometer and let it warm up.

9) After shaking is complete, place tubes into the IEC HN SII (Thermo Electron) centrifuge and spin at 5000 rpm for 15 minutes. Be careful to balance all the samples and start up the centrifuge bringing the rpms up slowly. Do not use the brake—allow the rotor to spin down on its own.

10) To minimize the impact of turbidity interference the sediment elutants are diluted 1:10. Draw up 5.0 ml of the blank in centrifuge tube 1 using an adjustable Thermo Labsystems Finipipette (cat # 40270-280) set to 5.0 ml and discard the solution into a waste bucket. Draw up another 5.0 ml and add to flask 5A. Draw up another 5.0 ml and dispense into flask 5B.

11) Using a new clean pipette tip and without disturbing the soil pellet, repeat the process described above for each of the sediment samples, matrix and standards so that all flasks 5A through 16B are complete. Between each sample pair use a new clean pipette tip.

12) Run the samples in the flasks according to the SRP SOPs listed in section A.2 starting at step 7.

13) While waiting for the mixed reagent to react:
    a. Carefully pipette out the remaining sample in each of the centrifuge tubes and transfer the contents of each tube into the respectively marked culture tube in the yellow rack and cap. These samples are held in the refrigerator until you are sure you do not need to rerun this batch on the basis of QC results.
    b. Using the 10% HCl squirt bottle add about 30 ml of acid to the centrifuge tube #1 and fill the cap with acid, let stand for 10 minutes and then rinse 3 times with DI H_2O and dry inverted.

D) <u>Iron Bound P Extraction Procedure</u>

1) If you have not already done so (step 11 above), without disturbing the soil pellet, carefully discard the supernatant ammonium chloride solution from the previous extraction. Remove as much liquid as possible without discarding any soil sample.

2) Bring up to 25 ml final volume with buffered dithionite solution to each centrifuge tube with sample. This solution must be freshly made. *Note: If you accidentally slightly overfill the tube do not pour any liquid out. Record the actual volume on the data sheet.*

3) Prepare a sample blank by adding the dithionite solution to centrifuge tube #1 bringing it up to 25 ml.

4) Prepare a 10-mgL standard from the 50 ppm phosphate stock standard by adding 5 ml stock standard using a Class A fixed volume 5ml volumetric pipette that has already been filled once with the 5.0 ml of standard that was discarded into the waste bucket. Bring to 25.0 ml with dithionite solution. See *Note* in step 2) above.

5) Cap all tubes tight and clip the tubes onto the Barnstead Thermolyne Labquake® Rotating Shaker (Model 415110) and set the lab timer for one hour.

6) While waiting for shaking to complete during the last half an hour set up the sample flasks and standards for the phosphorus (SRP) analysis: Prepare Sample and Standard Flasks as Follows:

   a. Arrange thirty-two 125 ml Erlenmeyer flasks (Corning # 5100-125) alpha-numerically, 1A through 16B, and place the flasks sequentially into the polypropylene sterilizing trays before filling the flasks with samples or standards.

   b. Prepare SRP standards and blank as follows (for calibration curve):

      i. Label 6 clean 500 ml, volumetric flasks with the respective standard concentrations: 500 µg/l, 100 µg/l, 50 µg/l, 10 µg/l, 5 µg/l, and 0 µg/l. Label 2 clean 250 ml volumetric flasks 2000 µg/l and 1000 µg/l.

      ii. Using a 25 ml graduated cylinder, add 10 ml of buffered dithionite solution into each 500 ml flask and 5 ml into each of the 250 ml flasks.

      iii. Using Class A fixed volumetric pipettes dilute pre-purchased HACH (cat #1436716) 30.0 mg/l stock standard, as 30.0 mg/l PO_4^{-3}, into the appropriate standard concentrations described in Table 11. For the last three standards you will utilize the 1000 *u*g/l solution you will have just mixed.

Note: orthophosphate standards are not preserved and should be prepared daily no more than two hours prior to the addition of the mixed reagent.

      iv. All volumetric flasks are then brought to volume with DI H_2O water, covered with Parafilm and inverted ten times to assure the samples are well mixed and a homogeneous solutions is achieved.

   c. Set up the yellow tube rack with 12 cleaned culture tubes with caps in the third row.
   d. Turn on the GENESYS 6 Spectrophotometer and let it warm up if it has not been on.

7) After shaking is complete, place tubes into the IEC HN SII (Thermo Electron) centrifuge and spin at 5000 rpm for 15 minutes. Be careful to balance all the samples and start up the centrifuge bringing the rpms up slowly. So as not to disturb the sediment, do not use the brake—allow the rotor to spin down on its own.

8) To minimize the impact of turbidity and matrix interference from the dithionite solution, the sediment elutants are diluted 1:50. Draw up 1.0 ml of the blank in centrifuge tube 1 using an adjustable Thermo Labsystems Finipipette (cat # 40270-280) set to 1.0 ml and discard the solution into a waste bucket. Draw up another 1.0 ml and add to the culture tube in flask 1A. Draw up another 1.0 ml and dispense into flask 1B.

9) Using a new clean pipette tip and without disturbing the soil pellet, repeat the process described above for each of the sediment samples, matrix and standards so that all flasks 5A through 16B are complete. Between each sample pair use a new clean pipette tip.

10) Run the samples in the flasks according to the SRP SOPs listed in section A.2 starting at step 7.

11) While waiting for the mixed reagent to react:
   a. Carefully pipette out the remaining sample in each of the centrifuge tubes and transfer the contents of each tube into the respectively marked culture tube in the yellow rack and cap. These samples are held in the refrigerator until you are sure you do not need to rerun this batch on the basis of QC results.

12) Glassware cleanup - Immediately rinse out the flasks and other glassware three times with DI H_2O after

the run and place the glassware in the drying rack on the Rubbermaid cart. If there is room, place the rinsed glassware into the acid bath and let sit for one hour, otherwise, fill the flasks with DI H_2O and place out of the way until room becomes available in the acid bath. When pulling glassware out of the acid bath rinse three times with DI H_2O and place the glassware upside-down in the drying rack.

E) Calculations:

1) Calculate the measured concentration in mg/l phosphate as described in the calibration curve section of the Soluble Reactive Phosphorus SOP (A.2.13). Be sure to use the specific calibration values appropriate for each SRP analysis from Tables 10 and 11 for Loosely-sorbed and Iron bound P respectively.

2) For Loosely-sorbed P: LS-P (mg/kg dry weight) = $\frac{Cls \times DF \times Vls \times S}{W}$

Where:

Cls = Concentration of Loosely-sorbed P in mg/l
DF = the dilution factor (5; if steps C9-C10 are followed)
Vls = volume of extract in liters (0.25l or check datasheet)
S = percent solids of sample
W = weight in kg of semisolid sample

3) For Iron Bound P: Fe-Bound-P (mg/kg dry weight) = $\frac{Cib \times DF \times Vib \times S}{W}$

Where:

Cib = Concentration of Fe-bound P in mg/l
DF = the dilution factor (50; if steps D8-D9 are followed)
Vib = volume of extract in liters (0.025 l or check datasheet)
S = percent solids of sample
W = weight in kg of semisolid sample

4) Matrix Spike Calculation: The spike recovery for the loosely sorbed phosphorus extraction is typically low. During the first extraction procedure the phosphorus is taken up by the iron components found in the sample. The final spike recovery is calculated from the combined recovery of the loosely sorbed and the iron bound constituents. The matrix spike adds 10 mg/kg. The 10 mg/l Phosphorus Standard Solution / 1000 ml/l = 0.01 mg/ml [(10 ml) (0.01 mg)] /.010 Weight (kg) = Approximately 10.0 mg/kg phosphorous}.

Table 10. Orthophosphate working standards for LS-P.

Standard Solution (*u* g/L) [1]	HACH Stock Solution (ml) [2]	1000 *u*g/l Solution (ml)[3]	Volumetric Flask Used	Erlenmeyer Flask (ID) [4]
1000	25.0	0.0	250 ml	1B
500	25.0	0.0	500 ml	2A
100	5.0	0.0	500 ml	2B
50	0.0	25.0	500 ml	3A
10	0.0	5.0	500 ml	3B
5	0.0	2.5	500 ml	4A
2	0.0	1.0	500 ml	4B
Blank (0)	0.0	0.0	500 ml	1A

[1] Standards used to derive calibration curves (prepared within two hours of analysis).
[2] Volume of stock HACH standard (HACH cat # 1436716) added to the designated volumetric flask and brought to volume with DI to derive the working standards presented in the leftmost column above.
[3] Volume of 1000 µg/l calibration working standard added to a 0.5 l volumetric flask and brought to volume with DI H_2O to derive the working standards presented in the leftmost column above. Used for lower standard concentrations.
[4] Erlenmeyer Flasks into which the respective standards and blanks are placed.

Table 11. Orthophosphate working standards for Iron Bound P.

Standard Solution (*u* g/L) [1]	HACH Stock Solution (ml) [2]	1000 u/L Solution (ml) [3]	Volumetric Flask Used	Erlenmeyer Flask (ID) [4]
2000	50.0	0.0	250 ml	1B
1000	25.0	0.0	250 ml	2A
500	25.0	0.0	500 ml	2B
100	5.0	0.0	500 ml	3A
50	0.0	25.0	500 ml	3B
10	0.0	5.0	500 ml	4A
5	0.0	2.5	500 ml	4B
(Blank) 0	0.0	0.0	500 ml	1A

[1] Standards used to derive calibration curves (prepared within two hours of analysis).
[2] volume of stock HACH standard (HACH cat # 1436716) added to a 500 ml volumetric flask and brought to volume with DI H2O to derive the working standards presented in the leftmost column above.
[3] volume of 1000 u/L calibration working standard added to a 500 ml volumetric flask and brought to volume with DI H2O to derive the working standards presented in the leftmost column above. Used for lower standard concentrations.
[4] Erlenmeyer flasks into which the respective standards and blanks are placed.

A.8. Chlorophyll *a* SOP

Chlorophyll *a* Extraction: Go to 2A for periphyton; 2B for filtered lake water

1) Frozen samples will be analyzed within 28 days of collection.

2A) Frozen periphyton filters will be placed in a numerically labeled 15 ml glass centrifuge tubes (Kimax model 45166) with approximately 10 mL of cold 90% acetone with $MgCO_3$ buffer.

3A) Periphyton algal cells will be disrupted using an ultrasonic probe (Kimatica Model CH 6010, Kreins, Luzurne Switzerland) set to 30% power level (200 watts) for 15 seconds while held above an ice-bath. Immediately following the cell disruption the tube is placed into the ice bath up to its solution level to ensure cooling down of the sample.

NOTE: For the types of algae found on the periphyton plates (as well as samples from sediments) we have found this method to deliver the best yields. A 15 second low power disruption time is sufficient for disruption without causing significant heating of the sample.

2B) Frozen Lake sample filters are placed in a Kimax brand glass mortar with approximately 10 ml of cold 90% acetone with $MgCO_3$ buffer. Carefully use the glass mortar to grind and dissolve the filter do not let high levels of frictional heat to occur.

3B) Pour into a numerically labeled 15 ml glass centrifuge tubes (Kimax model 45166). Rinse the mortar with 90% acetone to transfer all of the residue to the centrifuge tube.

4) The algal samples will be brought to 15 ml volume with 90% acetone with $MgCO_3$, capped and allowed to "steep" refrigerated for 4 hours in the dark.

5) After the 4 hour extraction period the first 8 samples will be placed into the Clay Adams Dynac II centrifuge (model 420103).

6) Turn the centrifuge power button to the "on" position.

7) Make sure the "speed" dial is set to zero before proceeding. The centrifuge speed should be raised slowly, as below in step 8, to prolong the life of the centrifuge motor.

8) Set the timer to 20 minutes.

9) Slowly raise the centrifuge speed to 1600-rpm (equivalent to 500 g).

10) After the 20 minute centrifugation period, place the eight centrifuge tubes in a centrifuge tube rack and immediately place the centrifuge rack in the chlorophyll *a* fume hood under subdued lighting.

11) Turn the centrifuge "speed" dial to zero.

12) Proceed with the chlorophyll *a* analytical procedure as outlined in "Analyzing Samples on the Cary 50 Spectrophotometer" until you have analyzed the first 8 chlorophyll *a* samples and recorded the data on the chlorophyll *a* "spec notebook" datasheet.

13) Repeat steps 6 through 11 until all samples have been analyzed. Note: when you centrifuge less than eight samples balance the centrifuge with centrifuge tubes filled with "90% acetone without $MgCO_3$" in the remaining slots.

14) The chlorophyll *a* results for CFB samples will be generated using the monochromatic spectrophotometric equation with a pheophytin correction:

$$\text{Chlorophyll } a = 26.7 * (664_b - 665_a) * \frac{\text{extract volume (ml)}}{\text{Sample volume (L) * path length (cm)}}$$

Or

$$\text{Chlorophyll } a = 26.7 * (664_b - 665_a) * \frac{\text{extract volume (ml)}}{\text{Sample Area (m2, periphyton)}}$$

where b = before acidification
a = after acidification

Chlorophyll samples collected by the volunteer monitors will be analyzed and reported as total chlorophyll without the phaeophytin correction using the mondified monochromatic equation:

$$\text{Total chlorophyll } (mg/m^3) = \frac{\text{absorption coefficient (11.0) * 664b * extract volume (l)}}{\text{Sample volume } (m^3) * \text{path length (cm)}}$$

Preparation:

100% Acetone- Purchase Certified ACS Acetone (Fisher Scientific Cat # A18-4) from our scientific supply vendor.

90% Acetone without $MgCO_3$ – Dilute the stock 100% Acetone (above) to 90% Acetone using the following procedure:

1) Wash the 1 l volumetric flask and funnel, designated for mixing the chlorophyll *a* solvents and stored under the chlorophyll *a* extraction fume hood, three times with DI H_2O.
2) Rinse the 100 ml class-A graduated cylinder, designated for mixing the chlorophyll *a* solvents and stored under the chlorophyll *a* extraction fume hood, three times with DI H_2O.
3) Fill the class-A graduated cylinder to 100 ml with DI H_2O and pour the contents into the 1L volumetric flask.
4) Bring the 1L volumetric flask to volume by slowly adding 100% stock Acetone solution. *Note: use the 100% acetone Nalgene squirt bottle to add the final 20-50 ml of acetone solution to assure accuracy.*
5) Transfer the contents of the 1 l flask into the 2.5 l amber reagent bottle that is labeled "90% Acetone without $MgCO_3$". Invert the 2.5 l reagent bottle 10 times to ensure you have achieved a homogeneous mixture.
6) Rinse the 1 l volumetric flask, the 100 ml graduated cylinder and the funnel with DI H_2O (three times each) before returning the glassware to the chlorophyll *a* fumehood.

90% Acetone with $MgCO_3$ –

1) Wash the 1 l volumetric flask and funnel, designated for mixing the chlorophyll *a* solvents and stored under the chlorophyll *a* extraction fume hood, three times with DI H_2O.
2) Rinse the 100 ml class-A graduated cylinder, designated for mixing the chlorophyll *a* and stored under the chlorophyll *a* extraction fume hood, three times with DI H_2O.
3) Fill the class-A graduated cylinder to 99 ml with DI H_2O and pour the contents into the 1L volumetric flask.
4) Invert the $MgCO_3$ stock solution 20 times and then add 1 ml of $MgCO_3$ stock solution, dispensed via a 1 ml volumetric pipette, to the 1 l volumetric flask.
5) Bring the 1 l volumetric flask to volume by slowly adding 100% stock Acetone solution. Note: use the 100% acetone Nalgene squirt bottle to add the final 20-50 ml of acetone solution to assure accuracy.
6) Transfer the contents of the 1 l flask into the 2.5 l amber reagent bottle that is labeled "90% Acetone with $MgCO_3$". Invert the 2.5 l reagent bottle 10 times to assure you have achieved a homogeneous mixture.
7) Rinse the 1 l volumetric flask, the 100 ml graduated cylinder and the funnel with DI H_2O (three times each) before returning the glassware to the chlorophyll *a* fumehood.

0.1N HCl Solution – *Note: the preparation of the HCl working solution should be undertaken under a fume hood designated for acid preparation.*

1) Fill an acid washed 1000 ml volumetric flask with approximately 500 ml DI H_2O and place the flask into an ice bath in the fume hood for 15 minutes.
2) Pour approximately 25 ml of 12.1N HCl (FS Cat # A144c-212) into a 50 ml acid washed beaker under the fume hood.
3) Draw up 8.3 ml of the Stock HCl using a class-A glass pipette and dispense the acid into the 1000 ml volumetric flask.
4) Swirl the flask to obtain a homogeneous mixture.
5) Place the 1000 ml volumetric flask into a PP tray without ice and let the diluted acid solution come to room temperature.
6) Once the diluted acid solution reaches room temperature bring the volumetric flask to volume by slowly adding DI H_2O from a Nalgene squirt bottle.
7) Parafilm the 1000 ml volumetric flask and invert the flask 20 times to assure the 0.1N HCl solution is a homogeneous mixture.
8) Store the 0.1N HCl solution in a PP tray on the lab bench.

$MgCO_3$ Stock Solution – Prepare the $MgCO_3$ stock solution in the designated 250 ml amber PP bottle. Discard this solution after 6 months.

1) Rinse the 100 ml class-A graduated cylinder, designated for mixing the chlorophyll *a* solvents and stored under the chlorophyll *a* extraction fume hood, three times with DI H_2O.
2) Fill the class-A graduated cylinder to 100 ml with DI H_2O and pour the contents into the designated 250 ml amber PP bottle.
3) Measure out 1gram finely powdered $MgCO_3$ (FS Cat # M27-500)
4) Add the 1 gram $MgCO_3$ to the 250 ml amber PP bottle.
5) Invert the 250 ml PP bottle 20 times to mix the reagent and subsequently store the reagent under the chlorophyll *a* fume hood until needed.

Written by Jeff Schloss and Bob Craycraft
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A.9. Free Carbon Dioxide SOP

Reference Source: Standard Methods 20th Edition- 4500-CO2 C.

Free Carbon Dioxide Titration:
Free Carbon Dioxide samples should only be taken from the 250 ml amber bottles that have been completely filled and sealed or from sealed sample syringes. Use care when pouring the samples since the CO_2 concentration can easily be altered by atmospheric contamination. The procedure for measuring CO_2 is as follows:

1) With a 100 milliliter graduated cylinder, carefully pour out a 100 ml sample by letting the water flow down the side of the cylinder without introducing bubbles into the solution.
2) Pour the sample into a beaker and add six drops of phenolphthalein indicator solution.
3) Carefully fill the burette with 0.00227N NaOH titrant from the 250 ml amber bottle with the flip top. Flick the burette to dislodge trapped air bubbles. Gently squeeze above the clear glass bead in the tubing to allow the titrant to flow out. Bring the fluid level in the burette to the 0.0 milliliter mark while holding the burette vertically making sure no air bubbles are left in the tip.
4) Titrate until the solution appears a faint pink (pH 8.3) and record the number of milliliters titrant added (1 milliliter NaOH titrant = 1 ppm Free CO_2).

Reagent Preparation:
Carbon dioxide-free water: Prepare all stock and standard solutions and dilution water for the standardization procedure with distilled, deionized water that has been freshly boiled for 15 min and cooled to room temperature. The final pH of the water should be ≥ 6.0 and its conductivity should be < 2 µmhos/cm.

0.00227N NaOH: (mix fresh each run) using a volumetric pipette add 22.7 of the 0.1N stock solution of NaOH into a 1000 ml volumetric flask. Bring volume up to 1000 ml.

Phenolphthalein indicator -In a 100 ml volumetric flask, dissolve 1 gram phenolphthalein crystals in 100 ml 95% ethanol and store the solution in two 60 ml plastic drop dispensing bottles.

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A.10. Dissolved Oxygen (Titration; Azide Modification) SOP

Reference Source: Standard Methods 20th Edition- 4500-O2 B.C.

Winkler Dissolved Oxygen Titration:

Dissolved Oxygen samples should be measured as follows *(Note: The first three steps should be undertaken in the field using 300 ml BOD bottles with tapered glass caps):*

Fixing the sample:

1) Add 1 milliliter of reagent #1 ($MnSO_4$) by releasing the liquid onto the neck of the bottle (to minimize air bubbles getting into the sample).

2) Add 1 milliliter of reagent #2 (alkali-iodide-azide) by releasing the liquid onto the neck of the bottle (to minimize air bubbles getting into the sample).

3) Stopper the sampling bottle and mix by inverting the bottle ten times. Allow the resulting material to settle until at least one third of the bottle is clear and mix again as described above. Allow the solid material to settle a second time.

4) When 1/3 of the bottle is clear, carefully add 1 milliliter of concentrated sulfuric acid by allowing the stream of drops to flow down the neck of the bottle. *Note: Reagent #3 is concentrated sulfuric acid and should be handled with care!* Restopper the bottle and invert gently until the solid material has dissolved.

Titration Procedure:

Titrations should always be performed against a white background.

Carefully measure 100 milliliters of your water sample in a graduated cylinder and empty the contents into the beaker. Remember, your water sample contains strong acid and should be handled with care.

5) Carefully fill the burette with Sodium Thiosulfate or PAO from the 250 ml amber bottle with the flip top. Flick the burette to dislodge trapped air bubbles. Gently squeeze above the clear glass bead in the tubing to allow the titrant to flow out. Bring the fluid level in the burette to the 0.0 milliliter mark while holding the burette vertically making sure no air bubbles are left in the tip.

6) Slowly add the titrant, drop by drop, to the water sample while stirring with the glass rod. Titrate until the sample reaches a faint yellow color.

7) When the faint yellow color is reached, 6 drops of starch indicator following which the sample will become a shade of blue and you should proceed to Step 8. If the starch indicator is added too soon the sample will turn black and if it is added too late, the sample will remain clear. In either of the latter cases, another water sample should be measured out and step 6 repeated.

8) Continue titrating until solution becomes clear. Record the milliliters of titrant added; calculate your dissolved oxygen concentration in milligrams per liter by multiplying by 2.0.

9) Rinse the beaker, stirring rod and graduated cylinder well between successive samples.

Reagent Preparation:

Oxygen-free water: Prepare all stock and standard solutions and dilution water for the standardization procedure with distilled, deionized water that has been freshly boiled for 15 min and cooled to room temperature. The final pH of the water should be ≥ 6.0 and its conductivity should be < 2 µmhos/cm.

Manganous sulfate solution: Dissolve 480 g $MnSO_4$ $4H_2O$, 400 g $MnSO_4$ $2H_2O$, or 364 g $MnSO_4$ _ H_2O in

distilled water, filter, and dilute to 1 l. The $MnSO_4$ solution should not give a color with starch when added to an acidified potassium iodide (KI) solution.

Alkali-iodide-azide reagent: Dissolve 500 g NaOH (or 700 g KOH) and 135 g NaI (or 150 g KI) in distilled water and dilute to 1 l. Add 10 g NaN_3 dissolved in 40 ml distilled water. Potassium and sodium salts may be used interchangeably. This reagent should not give a color with starch solution when diluted and acidified.

Sulfuric acid, H_2SO_4, conc: One milliliter is equivalent to about 3 ml alkali-iodide-azide reagent.

Starch: Use either an aqueous solution or soluble starch powder mixtures. To prepare an aqueous solution, dissolve 2 g laboratory-grade soluble starch and 0.2 g salicylic acid, as a preservative, in 100 ml hot distilled water.

Sodium Thiosulfate or PAO solutions (0.025M): are obtained premixed as standard solutions where

1 ml = 2 mg/l DO when 100 ml is titrated.

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A.11. *E. coli* Sampling and Membrane Filter Analytical SOP

FIELD SAMPLE COLLECTION AND HANDLING

1. Collect *E. coli* samples from the bow of the boat to minimize the chances of contamination from debris.
2. Access one 500 milliliter, sterilized Whirl-pack bag and one Whirl-pack bag holder (Figure 1)
3. Place the appropriate site identification information on the 500 milliliter sterilized Whirl-pack bag using a permanent marker: lake, site, date, depth, time and/or other pertinent information as required in a project specific Quality Assurance Project Plan.

Figure 1.

Figure 3.

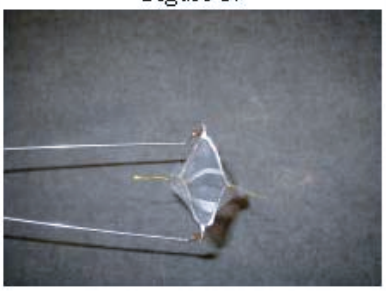

Figure 2.

4. Clamp the two white Whirl-pack tabs to the clips that are affixed to the Whirl-pack holder. The Whirl-pack holder should be facing away from the field technician (the clips will be positioned away from the field technician) and the Whirl-pack holder will be compressed such that the clips are near one another. The 500 milliliter sterilized Whirl-pack bag opening will be facing forward (the bag opening will be facing away from the field technician). See Figure 2.
5. Carefully remove the perforated end of the sterilized Whirl-pack bag being careful not to touch the inside of the bag.
6. Submerse the 500 milliliter sterilized Whirl-pack bag approximately six inches into the water while the bag is closed (the clips will still be near one another)
7. Slowly allow the clips to spread apart and the bag will open (Figure 3).
8. Slowly move the 500 milliliter Whirl-pack bag forward to fill the bag with a water sample.
9. Compress the Whirl-pack bag holder and then raise the 500 milliliter Whirl-pack bag out of the water.
10. Remove the bag from the Whirl-pack holder by holding one of the yellow metal encapsulated wire ends.

11. Grab hold of the second yellow end, being careful not to touch inside the bag, whirl (flip) the bag several times until the bag is sufficiently sealed and intertwine the two yellow tags to ensure the bag remains sealed.
12. Place the *E. coli* bacteria sample in the ice filled cooler for short term storage prior to returning to the laboratory.

LABORATORY APPARATUS, MATERIALS AND MEDIA:

- Pre-sterilized 50 x 9 mm petri dishes with absorbent pads (Pall Corporation Cat # PN 7245 or equivalent)
- Nalgene Pre-sterilized analytical test filter funnels with filters (Nalgene Cat # 145 0045)
- Vacuum Pump
- Burner and Forceps
- 91% isopropyl alcohol
- Fisher Scientific Isotemp waterbath model 5L
- Zip-loc bags
- Coliscan MF (250 MF) *E. coli*/coliform membrane filter medium
- Gettinge Novus I Autoclave
- Sterilized 20 milliliter glass volumetric pipettes (2)
- 1000 ml Erlenmeyer Flasks (3)
- Sterilized distilled water
- Aluminum Foil
- Autoclave (polypropylene) Tray
- Finnpipette repeating pipetter
- Thermo electron labsystems pre-sterilized pipette tips (Thermo electron Cat #: 9402073)
- Dissecting microscope with illuminator
- Autoclavable bags
- White autoclavable steam sterilization tape (Fisher Scientific Cat # 15 -903)
- Positive Control Culture – *E. coli* (Hardy Diagnostic Cat # 5134P). Positive control culture kit manufactured by MicroBioLogics.
- Negative Control Culture – Enterobacter *aerogenes* (Hardy Diagnostic Cat # 5134P). Negative Control Culture kit manufactured by MicroBioLogics.

AUTOCLAVE STERILIZATION OF GLASSWARE AND DISTILLED WATER

Note: glassware shall be sterilized immediately prior to use.

1. Rinse three 1000 milliliter Erlenmeyer Flasks three times each with distilled water.
2. Fill the three 1000 milliliter Erlenmeyer Flasks to the 800 milliliter mark with distilled water.
3. Place Aluminum Foil over each Erlenmeyer Flask.
4. Rinse two 20 milliliter volumetric pipettes three times each with distilled water.
5. Wrap the two 20 milliliter volumetric pipettes in aluminum foil.
6. Place all Erlenmeyer Flasks and volumetric pipetts into an autoclavable Polypropylene tray.
7. Place a strip of autoclavable stream sterilization tape on the polypropylene tray.
8. Place the autoclavable tray into the Gettinge 533LS autoclave.
9. Select “Sterilize Temperature” under the Cycle Values setting and set the temperature to 123.0˚C by pressing the “up arrow” or the “down arrow”.
10. Select “Sterilize Time” under the Cycle Values setting and set the time to 20 minutes by pressing the “up arrow” or the “down arrow”.
11. Select “Liquids” under the Cycle Select setting by pressing the “up arrow” or the “down arrow”.
12. Press the “Start” button and stay in the room until the autoclave heats up to 123.0˚C to ensure that the glassware

is sterilizing.

13. Remove the glassware from the autoclave. *Note: do not remove the aluminum foil until the sterilized water and the sterilized pipettes are needed.*
14. Check the autoclave tape for black horizontal lines to verify that the liquids and the glassware have been sterilized. *If the tape does not contain black horizontal lines, repeat steps seven through fourteen.*

LABORATORY FILTRATION AND ANALYTICAL PROCEDURE

Note: immediately prior to analysis, remove a sufficient volume of Coliscan MF media from the freezer and let the liquid warm up to room temperature.

1. Fill out an *E. coli* laboratory data sheet to account for all samples, positive and negative control cultures and replicates that will be analyzed.
2. Remove a sufficient number of pre-sterilized 50 x 9 millimeter Petri dishes to analyze the day's analytical run as outlined on the *E. coli* laboratory data sheet.
3. Record the pertinent site identification information on each pre-sterilized Petri dish using a permanent marker. Make sure the Petri dishes are labeled to account for laboratory replicates and control cultures as specified on the laboratory data sheet.
4. Loosen the cap of each sterile Petri dish.
5. Draw up two milliliters of Coliscan MF media using the Finnpipette repeating pipetter fitted with a pre-sterilized Thermo electron labsystems pipette tip, lift the lid, dispense the growth medium in the sterile petri dish and replace the lid.
6. Repeat step five until two milliliters of Coliscan MF media has been added to each Petri dish.
7. Remove a sterile Nalgene disposable filter funnel from the bag.
8. Attach the filter funnel to the funnel adapter that is seated in a number 8 rubber stopper by pressing down on the filter funnel until the funnel "snaps" in to place.
9. Remove the cover of the filter funnel.
10. Pour 100 milliliters of sterile distilled water into the filter funnel using gradations on the filter funnel to measure the water volume.
11. Replace the cover and apply vacuum pressure until all water has filtered through.
12. Remove the cover and rinse with 20 milliliters of sterilized distilled water using a sterile 20 milliliter volumetric pipette.
13. Repeat step 12 two additional times.
14. Turn off the pump once all water has passed through the filter.
15. Remove the upper chamber of the filter funnel.
16. Dip the forceps into the 91% isopropyl alcohol and flame the forceps.
17. Carefully remove the filter using the sterilized forceps and place the filter onto the corresponding Petri dish that contains the pertinent sample identification information.
18. Place the used Nalgene disposable filter funnel into an autoclavable bag for sterilization.
19. Remove a sterile Nalgene disposable filter funnel (cat # 09 -740-30D) from the bag.
20. Attach the filter funnel to the funnel adapter that is seated in a number 8 rubber stopper by pressing down on the filter funnel until the funnel "snaps" in place.
21. Invert one of your samples (Whirl-pack bags) five times.
22. Remove the cover of the filter funnel.
23. Pour 100 milliliters of lake water sample into the filter funnel using gradations on the filter funnel to measure the water volume.
24. Replace the cover and apply vacuum pressure until all water has filtered through.

25. Remove the cover and rinse with 20 milliliters of sterilized distilled water using the sterile 20 milliliter volumetric pipette.
26. Repeat step 25 two additional times.
27. Turn off the pump once all water has passed through the filter.
28. Remove the upper chamber of the filter funnel.
29. Dip the forceps into the 91% isopropyl alcohol and flame the forceps.
30. Carefully remove the filter using the sterilized forceps and place the filter onto the corresponding Petri dish that contains the pertinent sample identification information.
31. Place the used Nalgene disposable filter funnel into an autoclavable bag for sterilization.
32. Repeat steps 19 through 31 until eight samples, inclusive of the negative control culture and the positive control culture, have been filtered.
33. Place the eight samples, filter facing down (i.e. inverted Petri dish), into a Zip-loc bag and place the bag in the Fisher Scientific Isotemp water bath.
34. Repeat steps 19 through 33 (steps eight through 18 when filtering a distilled water blank) until you have filtered all samples and blanks listed on your *E. coli* laboratory datasheet that was filled out for the day's analytical run.
35. Incubate the samples for 24 hours at 35 +/-0.2˚C.
36. Remove the samples from the water bath incubator.
37. Place each Petri dish, sequentially, onto the stage of a dissecting microscope at 20 x magnification and count the colony forming units (CFUs); *E.coli* CFUs appear as blue/purple colonies and other coliform CFUs appear pink/magenta.
38. Record the number of *E. coli* CFUs and "other coliform" CFUs on the datasheet.
39. Make sure all Petri dish waste (dishes and covers) and all disposable filter funnels are placed into an autoclavable bag and place the autoclavable bag into a Polypropylene autoclavable tray.
40. Place the autoclavable tray that contains the used filter funnels and used Petri dishes into the Gettinge Novus I autoclave.
41. Select "Sterilize Temperature" under the Cycle Values setting and set the temperature to 123.0˚C by pressing the "up arrow" or the "down arrow".
42. Select "Sterilize Time" under the Cycle Values setting and set the time to 20 minutes by pressing the "up arrow" or the "down arrow".
43. Select "Solids" under the Cycle Select setting by pressing the "up arrow" or the "down arrow".
44. Press the "Start" button and stay in the room until the autoclave heats up to 123.0˚C to ensure that the waste is sterilizing.
45. Remove the autoclavable tray from the autoclave and dispose of the sterilized autoclavable "trash" bag in the dumpster.

EVALUATION CRITERIA

The analytical run will be deemed acceptable when the positive control culture tests positive for *E. coli* bacteria colonies and the negative control tests positive for only non *E. coli* colony forming units following incubation in the growth medium. The laboratory blanks should also be free of any *E. coli* or non *E. coli* colony forming units. A batch run that does not meet these performance standards shall be discarded and another set of samples shall be obtained and shall be cultured for *E.coli* analysis.

Written by Bob Craycraft and Jeff Schloss
Last Updated on May 11, 2007

A.12. Dissolved "true" Color SOP

Color References:

Iain D. Cuthbert and P. Giorgio. 1992. Toward a standard measure of measuring color in freshwater. Limno. Oceanogr. 37(6): 1319-1326.

APHA. 1998. Standard Methods for the Examination of Water and Wastewater: 20th Edition. American Public Health Association. Washington DC.

Color Standard (per Std Methods 2120B.3):

- potassium chloroplatinate K_2PtCl_6
- cobaltous chloride $CoCl_2$ $6H_2O$
- hydrochloric acid HCl

Stock standard solution (500 chloroplatinate units, CPU)

1) Obtain a 500 milliliter acid washed volumetric flask.
2) Fill the volumetric flash with approximately 250 milliliters of de-ionized water directly from the deionization system.
3) Pour out 50 milliliters of concentrated hydrochloric acid, measured to the nearest milliliter, into an acid washed 50 milliliter graduated cylinder (TD) under the fume hood.
4) Slowly empty the contents of the graduated cylinder into the volumetric flask.
5) Swirl the flask gently to obtain a homogeneous solution.
6) Using the Denver Instruments A-250 analytical balance, measure out 0.623 grams of potassium chloroplatinate and carefully transfer the entire 0.623 grams of reagent into the 500 milliliter acid washed volumetric flask.
7) Using the Denver Instruments A-250 analytical balance, measure out 0.50 grams of cobaltous chloride and carefully transfer the entire 0.50 grams of reagent into the 500 milliliter acid washed volumetric flask used in step six above.
8) Carefully rise the inside neck of the 500 milliliter volumetric flask with de-ionized water contained within a Nalgene squirt bottle.
9) Gently swirl the volumetric flask so all of the crystals are either suspended in, or dissolved into solution. Place the flask under the fume hood to cool to room temperature.
10) After one-half hour, fill the flask just below the neck, with de-ionized water, and gently swirl the flask to obtain a homogeneous mixture.
11) Place the flask under the fume hood and allow the flask to cool to room temperature.
12) Bring the flask up to the 500 milliliter mark with de-ionized water from the Nalgene squirt bottle.
13) Place an acid washed stirring bar into the volumetric flask.
14) Place the flask on a Cimeric magnetic stirrer and "stir" the solution until all crystals have dissolved.
15) Seal the volumetric flask with parafilm and place the 500 CPU stock color standard into the refrigerator until use. Discard the stock standard after three months.

Working Standards:

Dilute the stock standard into working standards of 200, 100, 20 and 5 CPU as outlined in Table 1:

Dissolved Color Samples:

Remove dissolved color samples from the freezer and let samples warm to room temperature. True color samples have been filtered through 0.45 *u*m HAWP 047 00 Millipore Membrane filters and, once thawed, are ready for analysis using the following procedure:

Refer to the Cary 50 dissolved color analytical proceedures referenced in sections A.1.3. and A.1.3.c.

Table 1. Working Dissolved "true" Color Standards

Desired Concentration (CPU)	Volumetric Flask Capacity (ml)	Volume of 500 CPU Stock Solution (ml)	De-ionized water
200 CPU	**500 milliliters**	**200 milliliters**	**Bring the volumetric flask to volume, to the "fill" line, with DD H2O.**
100 CPU	**500 milliliters**	**100 milliliters**	
20 CPU	**1000 milliliters**	**40 milliliters**	
5 CPU	**500 milliliters**	**5 milliliters**	

Note: working standards shall be capped with Parafilm and inverted ten times before each use. Working standards shall be stored in the refrigerator, at 4°C, between analytical runs.

Notes:

- *Analyze a de-ionized water blank and a 20 CPU dissolved color standard after every ten samples.*
- *The first four samples analyzed for each day's analytical run should be the 200 CPU, 100 CPU, 20 CPU and 5 CPU dissolved color standards.*

Dissolved "true" color calculations:

The dissolved color concentrations are derived from a color coefficient that is obtained through regression analysis of the day's standards using the equations:

Calculated color = 440 nm absorbance * Color Coefficient (derived through regression analysis)

Written by Bob Craycraft and Jeff Schloss
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A.13. Laboratory Analysis of Zooplankton SOP

1. Purpose and Summary of Method

1.1. The method is used to identify and enumerate species of lake zooplankton collected with plankton net tows. Macrozooplankton are counted from net samples using tows with 253 µm mesh openings, whereas microzooplankton, especially rotifers and nauplii, will be quantitatively defined from samples collected with 80µm mesh nets.

2. Essential Equipment and Supplies

Dissection microscope (magnifications: 10x-80x)
Compound microscope (magnifications: 40x-400x)
Hensen-Stempel pipets (1, 2, and 5 ml)
Folsom Plankton Splitter Circular counting tray (40 ml)
Sedgwich-Rafter counting cell (1 ml vol) with cover slips
Ring nets with 50, 500 and 1000 µm Nitex mesh
Wide-mouth pipet with 50-µm mesh (for condensing samples)
Jeweler's forceps
Microprobe (minutennadeln micro-insect needles with handles)
Microscope slides (standard and depression slides) for high magnification ID
Mounting medium (CMC-9 or CMC-10)

3. Reagents

3.1 Reagents used include:

- Formalin (37% formaldehyde)
- Non-denatured ethanol (95%)
- Chlorox bleach
- Lab detergent (dilute)

4. Procedure

4.1 Microcrustacea (excluding nauplii)

4.1.1 Samples received are condensed using 50 µm mesh, wide mouth pipette, and split into equal halves using the Folsom splitter. One half-split of the original is stored for later use. The other half-split to be used for immediate processing is poured through a 50 µm mesh ring net under a fume hood to remove the ethanol preservative (final conc. 95% non-denatured). The organisms are backwashed from the ring net into glass bottle with distilled water into a glass or HDP plastic container. A drop of very dilute detergent is added to the sample to prevent animals from getting caught in the surface tension and on the sides of the container. All containers used (splitter and sample bottles) must be thoroughly rinsed with distilled water into the container to remove animals that have adhered to the sides of the containers.

4.1.2 Sample volume is determined by weight using a top balance with 0.1 g sensitivity. The volume by weight method (tare for container weight) has accuracy equal or better than the measurement with the volumetric cylinder and avoids the loss of organisms during transfers of the sample.

4.1.3 Sub-samples are taken with a Hensen-Stempel pipet (1, 2 or 5 ml, depending on the original sample size), released, and rinsed into a circular counting tray.

4.1.4 The volumes of the original sample and the sub-sample are recorded. Care must be taken to mix the sample in a random fashion (not swirling) with the Hensen-Stempel pipet, capturing the sub-sample during the mixing process to avoid bias due to sinking of heavier plankton organisms. All Microcrustacea are identified at an appropriate magnification, depending on the morphological features

required for identification. Once identified, organisms are enumerated in a counting tray with a dissecting scope at 10x to 80x.

4.1.5 Repeat steps 4.1.33 and 4 as many times as necessary until 200-400 organisms are counted (excluding rotifers and nauplii).

4.1.6 Rare taxa (< 40 individuals) that are of special interest (e.g. Leptodora kindti, Chaoborus spp) can be counted in additional sub-samples until a minimum of 40 individuals are recorded.

4.1.7 Organisms that cannot be identified in the counting tray will be removed and mounted on microscope slides (standard or depression slides, depending on the size of the organism) and identified under higher magnification with a compound microscope. Slides should be prepared using permanent or semi-permanent slide-mounting techniques (glass cover slip, CMC9 or 10), and properly labeled.

4.1.8 After counting each sub-sample will be rinsed into a sample jar labeled "counted" with additional sample identification and preserved with an appropriate preservative (70% alcohol or 4% formalin/sucrose according to Haney & Hall, 1971).

4.1.9 Jars, vials, and slides from the same sample should be clearly labeled to allow re-association of the sample segments.

4.2 Rotifers

4.2.1 Rotifers and nauplii are counted from samples collected with the 80 µm mesh plankton net.

4.2.2 Subsampling follows the procedure described under Section 4.1.1, except a 1 ml Hensen-Stempel pipet is used, as smaller sub-sample volume is generally required to obtain the necessary 200-400 total count as rotifers tend to occur at higher densities in lakes.

4.2.3 The 1-ml sub-sample for rotifers or nauplii is placed in a Sedgwich-Rafter counting cell and covered with a glass cover slip to maximize the optical resolution. The organisms are then enumerated using 100x magnification. If many more than 400 rotifers are present in the sub-sample, another smaller sub-sample should be taken or, if necessary, the original sample diluted before sub-sampling is done.

4.2.4 Volume of lake water sampled is estimated from the length of the plankton tow and the diameter of the plankton net using the formula for the volume of a cylinder: $V\ (liters)=(\pi*r^2*h)/1000$, where r is the net radius (cm) and h is the tow length (cm).

4.2.5 Samples not finished counting by the end of the day are stored in refrigeration overnight or preserved again if held for more than 24 hours.

4.2.6 Following completion of identifications and enumeration, organisms from the S-R counting cell should be transferred to a labeled vial with appropriate preservative.

4.2.6 Where rotifer identifications require inspection of internal hard parts, such as trophi, specimens mounted on a slide may require clearing of tissue and debris with a drop of hypochlorite solution (commercial Chlorox). All slide mounts should be completed using permanent or semi-permanent techniques and media (CMC9 or 10).

5 Taxonomic References used

Zooplankton are identified to species where possible using Edmondson (1959), Pennak (1978), Smith and Fernando (1978), Stemberger (1979), the on-line Free-living and Parasitic Copepods (Including Branchiurans) of the Laurentian Great Lakes: Keys and Details on Individual Species and the on-line Image-Based Key to the Zooplankton of the Northeast, USA, produced by the University of New Hampshire Center for Freshwater Biology (cfb.unh.edu).

6 Measurement of Length and Calculation of Biomass

Samples used for body length analysis will be sub-sampled with a Hensen-Stempel pipet and transferred to a Sedgwick-Rafter counting cell with a glass cover slip. Digital images are taken with a digital camera attached to either a dissecting or compound microscope. Images are calibrated and body lengths measured using Image-J (NIH Software). Biomass dry weights of crustaceans and rotifers are calculated from body lengths using the regression equations in Dumont et al. (1975).

7 Results of Laboratory Processing; Sample Archiving

All samples should result in completed data sheets with list of taxa and number of individuals of each; the full complement of specimens, in containers of preservative and/or on permanent slide mounts. The identified/preserved sample in jars, vials, or slide mounts; and the unused sample split/fraction. All sample components should be clearly-labelled to associate multiple vials and slides as a single sample. Labels should be as Sample ID "A", jar/vial 1 of x, and Sample ID "A", slide 1 of x; and Sample ID "A", unused sample fraction (1/2 orig-vol).

8 Quality Control

8.1 Subsampling/sample splits

For each laboratory, approximately 10% of the samples will be randomly-selected for evaluation of subsampling precision (consistency of duplicate processing). For these samples, the unused fractions will be treated in an identical manner as the primary fractions (taxonomic identification and enumeration). There will be two precision calculations, one for tracking error for individual samples, and the other for estimating error for the overall dataset. Differences between the two sample fractions are an indication of subsampling consistency, quantified by relative percent difference (RPD) as follows:

$$\text{RPD} = \frac{|n_1 - n_2|}{(n_1 + n_2)/2} \text{ x100}$$

where n_1 is the metric or index value from the first subsample, and n2 is the metric or index value from the second. The magnitude of error expected to be associated with splitting of zooplankton samples is unknown, thus a specific measurement quality objective is not proposed here. For estimating subsampling error for the overall dataset, root mean square error (RMSE) will be calculated. Also called standard error of estimate, this statistic is an estimate of the standard deviation of a population of observations and is calculated by:

$$\text{RMSE} = \sqrt{\frac{\sum_{j=1}^{k}\sum_{i=1}^{n_j}(y_{ij} - \bar{y}_j)^2}{\sum df_{1\ldots k}}}$$

where y_{ij} is the i^{th} individual observation in group j, j = 1…k (Zar 1999). More simply put, the equation can be described as the root of the sums of squared residuals across all subsample pairs, divided by the number of sample pairs. For computational convenience, RMSE is often calculated by taking the root of the mean square error (MSE), which can be output from an analysis of variance (ANOVA).

8.2 Taxonomy

Approximately 10% of the samples will be randomly-selected (for each lab) evaluation of taxonomic precision. Following primary identification and enumeration, the jars, vials, and slides for each of these samples will be sent to the QC taxonomist for complete re-identification and re-enumeration. Differences between the two samples are an indication of taxonomic precision.

Precision of sample counts is determined by calculating percent disagreement in enumeration (PDE) by comparing results from two independent laboratories or taxonomists using the formula:

$$PDE = \frac{|n_1 - n_2|}{(n_1 + n_2)/2} x100$$

where n_1 is the number of organisms in a sample counted by the first laboratory, and n_2, the second. This formula is slightly modified from Stribling et al. (2003) in that the denominator is the average of the sum.

Precision of taxonomic identifications is determined by calculating percent taxonomic disagreement (PTD) by comparing genus-level taxonomic results from two independent taxonomists, using the formula:

$$PTD = \left[1 - \left(\frac{comp_{pos}}{N}\right)\right] \times 100$$

Where $comp_{pos}$ is the number of agreements, and N is the total number of organisms in the larger of the two counts (Stribling et al. 2003).

The measurement quality objectives (MQO) of these performance characteristics are 10% for PDE, and 10% for PTD.

8.3 Corrective Actions

Determination of necessary and appropriate corrective actions (CA) will be made through evaluation of subsampling/splitting and taxonomic QC results. Specifications will be made whether the CA apply to QC samples only, all samples processed by the primary laboratory, selected individual taxa, or all taxa.

9. **References**

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Edmondson, W.T (ed). 1959. Fresh-water Biology. 2nd Ed., Wiley, New York, pp 1248.

Haney, J.F. and D.J. Hall. 1971. A preservation technique for Cladocera. Limnol. Oceanogr. 18(2): 331-333.

Pennak, R.W. 1978. Fresh-water Invertebrates of the United States, . 2nd Ed. Wiley Intersci. Pub. 803 pp.

Smith, K. E., and C. H. Fernando. 1978. A guide to the freshwater calanoid and cyclopoid copepod Crustacea of Ontario. University of Waterloo Biological Series No. 18. 74pp.

Stribling, J. B., S. R. Moulton II, and G.T. Lester. 2003. Determining the quality of taxonomic data. Journal of the North American Benthological Society 22(4):621–631.

Stemberger, R.S. 1979. A guide to rotifers of the Laurentian Great Lakes. U.S. Environmental Protection Agency, Prt. No. EPA 600/4-79-021, 185 pp.

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Written by Jeff Schloss and Bob Craycraft
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A.14. Phytoplankton Collection, Preservation and Analysis SOP

Phytoplankton samples are collected during Center for Freshwater Biology field team visits or as specified in a project specific Quality Assurance Project Plan. Since phytoplankton are responsive to the physical and chemical conditions in the aquatic environment, the composition and abundance of the algal assemblage provides insight into a lake's trophic status and can also be used to screen for potentially toxic cyanobacteria populations. Additionally, phytoplankton populations provide insight into trophic relationships among fish, zooplankton, phytoplankton. These interrelationships can shed light on larger scale ecosystem interrelationships that better our understanding of our aquatic resources. Phytoplankton are thus collected as a component of the standard Center for Freshwater Biology field sampling protocols which, in addition to chemical and physical data collected during field team visits, provide data that held interpret the conditions of New Hampshire's lakes and ponds.

Field Sampling Protocols and Laboratory Preservation

1. Samples are collected in opaque two-liter bottles using either the point or an integrated sampler (composite tube sampler) method outlined in the Center for Freshwater Biology field sampling procedures.
2. The two-liter opaque bottles, upon return to the laboratory and within eight hours of sample collection, are inverted three times and a "whole water sample" is poured into a 40 milliliter Histoplex container (Starplex scientific cat # H402FL) and filled just below the rim.
3. A quarter of a milliliter (0.25 ml) of Lugols solution is added to the sample.
4. The sample is capped and inverted five times.
5. The sample is placed in the refrigerator for storage prior to analysis.

Analytical Methods and Sub-Sample Preparation

Phytoplankton samples are concentrated using Utermöhl chambers that are used in conjunction with a Wild Model M40-81312 inverted microscope. The Utermöhl chambers are particularly useful for concentrating algal cells in New Hampshire's oligotrophic lakes where the phytoplankton densities are generally low. The Utermöhl chambers and an accompanying inverted microscope phytoplankton enumeration method is used for qualitative phytoplankton analyses performed as part of the Center for Freshwater Biology standard operating procedures.

<u>Sub-sample preparation using an Utermöhl chamber:</u>

1. Remove samples from the refrigerator and let the samples reach room temperature.
2. Check the bottom coverslip on the Utermöhl chamber for any particulates, oils or films that would obscure the algae or affect an even distribution during settling. If necessary, rewash the chamber prior to use.
3. Resuspend the algae in the 40 milliliter Histoplex container by "gently" inverting the container ten times. DO NOT vigorously shake the sample container as care must be taken to reduce aeration which can cause air bubble formation in the chambers.
4. Slowly pour a sub-sample into the chamber until it is completely filled and a convex meniscus forms at the top of the chamber.
5. Slide the circular glass plate onto the top of the chamber to remove the convex meniscus and to seal the chamber.
6. Place the Utermöhl chamber in a humidity chamber (dessicator chamber using moist towletts rather than desiccant) and allow the sub-samples to settle for a minimum of eight hours. The humid environment reduces the chances of evaporation and the formation of interfering air bubbles in the Utermöhl chamber. *Note: the chamber will be set on a piece of paper that contains the pertinent site identification information as outlined in a project specific Quality Assurance Project Plan (QAPP).*
7. The preparation may last up to a week without desiccation. Distilled water may be added into settled samples to remove bubbles, however care is required not to disturb the settled material.

Qualitative analysis

Two types of qualitative analyses are performed in the Center for Freshwater Biology lab:

- Identification only (what is it?).
- Identification and enumeration for taxa dominance (what is/are the most common taxa?).

Identification only

Place the Utermöhl chamber on the microscope stage.

1. Record the sample data on a bench sheet (i.e. tracking code, date of analysis).
2. Identify the algae in the sample by slowly panning across horizontal transects at 100x magnification and increase the magnification to 400x when required for accurate identifications.
3. Record the taxa observed on a bench sheet.
4. Scan across at least 50% of the 5 milliliter Utermöhl chamber and record all taxa that were identified.

Dominant taxa analysis

Place prepared slide or chamber on the stage of microscope.

1. Record all sample data on a bench sheet (i.e. tracking code, date of analysis).
2. Identify the algae and count the number of cells and units in a field. A field is the complete field of view of the objective.
3. Continue analyzing fields across a horizontal plane until at least 10 units of each of 10 taxa have been recorded. *Note: Should you view the entire width of a horizontal plane adjust the stage vertically and scan another horizontal transect(s) until you record sufficient algal units.*
4. Tabulate the total number of cells and units for each taxon.
5. Record all data on the phytoplankton datasheet.
6. The bench sheet data are now ready to be entered into the database.

Taxonomic References

- Prescott, G.W. 1973. Algae of the Western Great Lakes Area. Wm. C. Brown Co. Pub., Dubuque, Iowa. 977 pp.
- Smith, G.M. 1950. The Freshwater Algae of the United States. McGraw-Hill Pub., New York, New York. 719 pp.
- Whitford, L.A. and G.J. Schumacher. 1984. A Manual of Freshwater Algae. Sparks Press, Raleigh, NC. 337 pp.

Microscope maintenance

A proper maintenance schedule is required to maintain optimum visual clarity. Microscopes must be covered by a lint free cloth or plastic when not in use. The area around the microscopes should be free of dust and dirt. Objectives and oculars are cleaned using an approved lens cleaner, non abrasive cotton swabs and Kimwipes. Microscopes are serviced by a certified technician as needed.

Utermöhl chamber maintenance

Utermöhl chambers have four parts:

1. A threaded metal base.
2. A threaded Plexiglas chamber that comes in 5 cubic centimeters (ccm) and 10ccm sizes.
3. A replaceable bottom coverslip.

4. A circular glass top plate.

   *note: 1ccm = 1cm^3 = 1 ml.

Utermöhl chambers must be thoroughly cleaned before each use. Cracked or broken bottom coverslips must be replaced.

To clean an Utermöhl chamber:

1. Rinse out the chamber with de-ionized water.
2. Wash the inside of the chamber and both sides of the glass coverslip with a nonabrasive, non-residue soap and a cotton swab.
3. Rinse out the chamber at least three times with de-ionized water.
4. Dry with a nonabrasive, lint free, Kimwipe.
5. Visually inspect the cleaned chamber to ensure that no oils or residues remain.
6. Store clean chambers upside down on a clean surface.

To replace an Utermöhl chamber bottom cover slip:

1. Unscrew the chamber cylinder from the metal base.
2. Carefully remove the damaged cover slip.
3. Wash the chamber with a non-abrasive, non-residue soap and de-ionized.
4. Dry the chamber with a non-abrasive, lint free, Kimwipe.
5. Apply a light coat of petroleum jelly on the threads of the cylinder.
6. Place a new cover slip into the metal base.
7. Screw the cylinder into the ring.
8. Remove any excess petroleum jelly from chamber and coverslip with a nonabrasive, lint free, Kimwipe.
9. Wash the chamber as previously discussed under “To clean an Utermöhl chamber”.

Lugol’s Solution Preparation

A modified Lugol's solution (Vollenweider 1974) is used to preserve phytoplankton and periphyton samples. The modification is glycerin which is added to the traditional Lugol’s solution to help prevent loss of flagella.

The solulution is prepared in a fume hood. The preparer should wear gloves, goggles and a lab coat.

The following amounts of chemicals are used to make about 1 L of the solution:
__40 g Iodine
__80 g Potassium Iodide (KI)
__80 ml Glacial Acetic Acid
__800 ml Distilled Water
__50 ml Glycerin
__50 ml 95% Ethyl Alcohol (ETOH)

To prepare the solution:
__Pour 80 ml of Glacial Acetic Acid into 800 ml of Distilled Water.
__Add 80 g of KI.
__Add 40g Iodine.
__Add 50 ml Glycerin
__Add 50 ml of 95% ETOH.
__Mix thoroughly.

Caution: Iodine will seep through plastic containers and leave stains on shelves and floors.

Lugol’s solution is photosensitive. It must be stored in an opaque container in a cool, dark place.

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A.15. Total Suspended Solids SOP

AWWA. 1998. Standard Methods for the Examination of Water and Wastewater; 20th Edition. American Public Health Association, 1015 Fifteenth Street, NW. Washington, DC. 2540 D.
Total Suspended Solids Dried at 103 – 105°C

1) Numerically label the outer lip of disposable aluminum dishes (FS Cat # 08-732-101) with permanent marker. Make sure you label enough trays to account for the day's lakewater/streamwater samples, blanks, lab duplicates as well as 5 extra trays to account for unexpected circumstances. *Note: laboratory duplicates will be analyzed at a frequency of 5% or one per analytical batch, whichever is more frequent.*

2) Place the trays in the oven (Fisher Scientific Model 825F), set at 104°C, for one hour.

3) Remove the trays from the oven, using forceps (tweezers), and place the trays into a desiccator where the samples will cool to room temperature. Let the samples cool for 10 to 15 minutes. *Note: you should make sure the desiccant is blue before placing the samples into the desiccators. If the desiccator is not blue consult the lab manager who will change the desiccant and assure the desiccators have an "air-tight" seal.*

4) Record the pertinent information on the Suspended Sediment Datasheet (i.e. lake, site, date, depth, etc.).

5) Weigh each tray on the Denver Instruments A-250 analytical balance (to the nearest .00001 gram), record the weight on the total suspended solid (TSS) datasheet and place the trays back into the desiccator as they are weighed.

6) Repeat the cycle of drying, cooling and weighing (steps 2, 3 and 5) until the weight change is less than 4% of the previous weighing or 0.5 mg, whichever is less.

7) Filter Preparation (Whatman 934-AH Filters):
Rinse the Nalgene Filter Holders (Cat No 300-4000) with DI H_2O to assure they are clean before proceeding. Once the filter holders have been properly rinsed, place one 47mm Whatman 934-HA filter onto each white support plate and replace the upper filtration chamber. Tighten the locking ring to assure the filter is securely locked into place. Add 50 milliliters of DI H_2O to each flask and turn on the vacuum pump to pull the water through. Subsequently, filter 50 milliliters of DI H_2O through each filter two additional times.

8) Place one 47mm Whatman 934-HA filter onto each aluminum foil tray using forceps. Bake each tray (each containing a filter) for one hour to dehydrate (dry) the sample.

9) Remove the trays (with filters) from the oven and place each tray into a dessicator and allow the samples to cool for 10 to 15 minutes.

10) Weigh each tray on the Denver Instruments A-250 analytical balance and record the values on the TSS datasheet. Place the trays back into the desiccator as they are weighed.

11) Place each of the aluminum foil tray (with filters) into the oven and re-bake each sample for one hour.

12) Remove the trays (with filters) from the oven and place each tray into a dessicator and allow the samples to cool for 10 to 15 minutes.

13) Re-weigh each tray on the Denver Instruments A-250 analytical balance and record the values on the TSS datasheet. Place the trays back into the desiccator as they are weighed.

14) Repeat the cycle of drying, cooling and weighing (steps 11 through 13) until the weight change is less than 4% of the previous weighing or 0.5 mg, whichever is less.

15) Filtration of Water Samples:

a) Rinse the Nalgene Filter Holders (Cat No 300-4000) with DI H_2O to assure they are clean before proceeding.

b) Once the filter holders have been properly rinsed, place one 47mm Whatman 934-HA filter onto each white support plate and replace the upper filtration chamber.

c) Tighten the locking ring to assure the filter is securely locked into place.

d) Invert your sample bottle to assure the sample contents are homogeneous immediately prior to pouring out the sample.

e) Pour out 250 milliliters of water sample into a 250 ml graduated cylinder, dispense the entire sample volume into the Nalgene Filter holder and turn on the vacuum pump to filter the sample through.

f) Invert your sample bottle again (to assure the sample is homogeneous), pour out another 250 milliliters of lakewater and turn on the vacuum pump to filter the sample through.

g) Repeat step "f" until a total of 1 Liter of sample water has been filtered. *Note: the entire aliquot of sample which is poured into the graduated cylinder must be filtered. If the filter becomes plugged before all the aliquot is filtered, the analyst must discard the filter and begin with a new tarred filter.*

h) Rinse the filter with three successive 10 ml volumes of DI H_2O while allowing complete drainage between each washing. Let the pump run an extra three minutes after the final 10 ml DI H_2O has filtered through to remove as much water as possible before proceeding to the next step.

i) Using forceps, remove the filter and place it into the appropriate sample tray.

j) Place the sample tray into the oven (set at 104°C) for one hour.

k) Repeat steps "a" through "i" until all samples have been filtered and placed in the oven.

l) At least one blank should be prepared by filtering 1 liter of DI H_2O water through the filter. The blank(s) should be analyzed as part of each sample "run" and is/are prepared in accordance with steps "a" through "i above.

16) Preparation of laboratory replicate(s) Laboratory replicates should constitute 5% of the samples or one sample per analytical batch, whichever is greater. The laboratory replicate should represent a second sample prepared from one of the streamwater sampling bottles that were prepared in step 15. The preparation of the laboratory replicate should follow steps 15(a through i) above and the sample should subsequently be placed into the oven (set at 104°C) for one hour.

17) Post Oven Treatment:

a) Remove the TSS samples from the oven, one-by-one while using forceps, and immediately place each sample in a glass desiccator.

b) Allow samples to cool for 10 to 15 minutes.

c) Weigh each sample on the Denver Instruments A-250 Analytical Balance and record the weights on the TSS datasheet. Place each sample into the desiccator as it is analyzed.

d) Place each sample into the oven and re-bake each sample for one hour.

e) Remove each sample from the oven and place the samples into a desiccator and allow the samples to cool for 10 to 15 minutes.

f) Re-weigh each sample on the Denver Instruments A-250 analytical balance and record the values on the TSS datasheet. Place the trays back into the desiccator as they are weighed.

g) Repeat the cycle of drying, cooling and weighing (steps 17d through 17f) until the weight change is less than 4% of the previous weighing or 0.5 mg, whichever is less.

18) Total Suspended Solids Calculation:

$$\text{Milligrams total suspended solids / Liter} = \frac{(A - B) \times 1000}{\text{Sample volume (ml)}}$$

Where:

A = weight of filter + dried residue (mg)
B = weight of filter (mg)

Written by Bob Craycraft and Jeff Schloss
Last Updated on July 23, 2010

A.16. Oakton PC 2700 Benchtop Meter and pH, Specific Conductivity and Chloride SOP

References: Standard Methods 20th Edition Method 4500H+, 2510B, Oakton 2700 Series Benchtop Meters instruction manual (May 2010 REV 0) and Cole-Parmer Laboratory Chloride Ion Electrode Instruction Manual.

Sample requirement: Collected in airtight syringe and sealed or in capped PE bottles, Stored on ice in the dark. Allowed to come to ambient or room temperature before analysis.

The Oakton PC 2700 is fitted with a Beckman Coulter low ionic strength pH electrode (Beckman Model # A57195), Oakton Ion Selective Chloride Electrode (Oakton Model # WD-35802-13) and an Oakton 4 cell conductivity probe (Oakton Model # WD-35608-92). Each probe is calibrated before the initiation of an analytical run using the following calibration procedure:

Specific Conductivity Calibration

1. Turn the meter on.
2. Make sure the conductivity and the temperature probes are secured in the electrode holder.
3. Press the "MODE" button until the "measure conductivity" mode is displayed.
4. Rinse a 100 milliliter beaker, a magnetic stirring bar, the conductivity probe and the temperature probe three times with DI H_2O.
5. Insert the stirring bar into the 100 milliliter beaker.
6. Rinse the 100 milliliter beaker and stirring bar with approximately 25 milliliters of 84 *u*S/cm (Ricca Cat # 5886.84-32) conductivity standard.
7. Fill the 100 milliliter beaker with with 84 *u*S/cm conductivity standard and pour off approximately 20 milliliters over the conductivity probe and temperature probe to remove any residual DI H_2O. If necessary, add 84*u*S/cm conductivity standard until the solution is at/above the 80 milliliter mark.
8. Place the beaker onto the magnetic stirrer.
9. Position the magnetic stirrer dial to a setting between 2 and 3 (sufficient to continuously rotate the stirring bar).
10. Raise and lower the conductivity probe and temperature probe into the conductivity standard three times.
11. Position the conductivity probe in the center of the beaker and one-half inch above the bottom of the beaker. The temperature probe should also be submersed in the beaker.
12. Press the "CAL" button.
13. Wait for the meter to display "Stable" and then press the "ENTER" button. *Note: the conductivity value will also be displayed in bold when the reading is stable*.
14. Remove the temperature and conductivity probes from the standard solution.
15. Rinse the beaker, temperature and conductivity probes three times with DI H_2O.

pH Calibration (*note: the pH and Chloride electrodes cannot be used at the same time since there is only one BNC connector on the PC 2700*)

1. Turn the meter on.
2. Make sure the pH BNC is plugged into the PC 2700.
3. Make sure the pH and temperature probes are secured in the electrode holder.
4. Slide the rubber sleeve to expose the pH electrode solution filling hole.
5. Add 1 Molar saturated AgCl pH electrode filling solution when the level drops one centimeter below the fill hole.
6. Press the "MODE" button until the "measure pH" mode is displayed.
7. Remove the pH probe cap and the rubber plug from the electrolyte solution fill hole. If necessary, add electrolyte solution until the height of the solution in the electrode is within three millimeters of the fill hole.
8. Rinse a 100 milliliter beaker, a magnetic stirring bar, the pH probe and the temperature probe three times with DI H_2O.

9. Insert the stirring bar into the 100 milliliter beaker.
10. Rinse the 100 milliliter beaker and the stirring bar with approximately 25 milliliters pH 7.00 buffer.
11. Fill the 100 milliliter beaker with with pH 7.00 buffer and pour off approximately 20 milliliters over the pH probe and temperature probe to remove any residual DI H_2O. If necessary, add pH 7.00 buffer until the solution is at/above the 80 milliliter mark.
12. Place the beaker onto the magnetic stirrer.
13. Positon the magnetic stirrer dial to a setting between 2 and 3 (sufficient to continuously rotate the stirring bar).
14. Raise and lower the pH probe and temperature probe into the pH 7.00 buffer three times.
15. Position the pH probe in the center of the beaker and one inche above the bottom of the beaker. The temperature probe should also be submersed in the beaker.
16. Press the "CAL" button.
17. Wait for the meter to display "Stable" and then press the "ENTER" button. *Note: the pH value will also be displayed in bold when the reading is stable*.
18. Remove the temperature and pH probes from the buffer solution.
19. Rinse a 100 milliliter beaker, a magnetic stirring bar, the pH probe and the temperature probe three times with DI H_2O.
20. Insert the stirring bar into the 100 milliliter beaker.
21. Rinse the 100 milliliter beaker and the stirring bar with approximately 25 milliliters pH 10.00 buffer.
22. Fill the 100 milliliter beaker with with pH 10.00 buffer and pour off approximately 20 milliliters over the pH probe and temperature probe to remove any residual DI H_2O. If necessary, add pH 10.00 buffer until the solution is at/above the 80 milliliter mark.
23. Place the beaker onto the magnetic stirrer.
24. Position the magnetic stirrer dial to a setting between 2 and 3 (sufficient to continuously rotate the stirring bar).
25. Raise and lower the pH probe and temperature probe into the pH 10.00 buffer three times.
26. Position the pH probe in the center of the beaker and one inch above the bottom of the beaker. The temperature probe should also be submersed in the beaker.
27. Press the "CAL" button.
28. Wait for the meter to display "Stable" and then press the "ENTER" button. *Note: the pH value will also be displayed in bold when the reading is stable*.
29. Remove the temperature and pH probes from the buffer solution.
30. Rinse a 100 milliliter beaker, a magnetic stirring bar, the pH probe and the temperature probe three times with DI H_2O.
31. Insert a stirring bar into the 100 milliliter beaker.
32. Rinse the 100 milliliter beaker and the stirring bar with approximately 25 milliliters pH 4.00 buffer.
33. Fill the 100 milliliter beaker with with pH 4.00 buffer and pour off approximately 20 milliliters over the pH probe and temperature probe to remove any residual DI H_2O. If necessary, add pH 4.00 buffer until the solution is at/above the 80 milliliter mark.
34. Place the beaker onto the magnetic stirrer.
35. Position the magnetic stirrer dial to a setting between 2 and 3 (sufficient to continuously rotate the stirring bar).
36. Raise and lower the pH probe and temperature probe into the pH 4.00 buffer three times.
37. Position the pH probe in the center of the beaker and one inch above the bottom of the beaker. The temperature probe should also be submersed in the beaker.
38. Press the "CAL" button.
39. Wait for the meter to display "Stable" and then press the "ENTER" button. *Note: the pH value will also be displayed in bold when the reading is stable*.
40. Remove the temperature and conductivity probes from the standard solution.
41. Rinse the beaker, temperature and conductivity probes three times with DI H_2O.

Chloride Calibration (*note: the pH and Chloride electrodes cannot be used at the same time since there is only one BNC connector on the PC 2700*)

A. Needed Stock Solutions (the three stock solutions can be purchased as a kit: Oakton SKU # 35802-63):

- Ionic Strength Adjuster (ISA): 5M $NaNO_3$ (Oakton SKU # 35803-51). Alternatively, 5M $NaNO_3$ can be prepared by adding 425 grams reagent grade sodium nitrate to a one-liter volumetric flask and bringing the flask to volume with DI H_2O.
- 1000 ppm chloride standard (Oakton SKU 35803-09). Alternatively, chloride standard can be prepared by adding 1.65 grams reagent grade sodium chloride to a one-liter volumetric flask and bringing the flask to volume with DI H_2O.
- Replacement 1M KNO_3 electrolyte filling solution.

B. High chloride calibration (typically specific conductivity above 150 *u*S/cm)

1. Turn the meter on.
2. Make sure the chloride BNC is plugged into the PC 2700.
3. Make sure the chloride and temperature probes are secured in the electrode holder.
4. Press the "MODE" button until the "measure Ion" mode is displayed.
5. Remove the chloride probe cap and the rubber plug from the electrolyte solution fill hole. If necessary, add electrolyte solution until the height of the solution in the electrode is within three millimeters of the fill hole.
6. Rinse a 150 milliliter beaker, a magnetic stirring bar, the chloride probe and the temperature probe three times with DI H_2O.
7. Insert a stirring bar into the 150 milliliter beaker.
8. Fill the 150 milliliter beaker, that contains a stirring bar, with approximately 25 milliliters 10 ppm chloride standard.
9. Pour out the 10 ppm chloride standard so it flows over the chloride probe and temperature probe to remove any redidual DI H_2O.
10. Measure out 100 milliliters of 10 ppm chloride standard using a 100 milliliter graduated cylinder
11. Dispense the 100 milliliter of 10 ppm chloride standard into the 150 milliliter beaker that contains a magnetic stirring bar.
12. Add 2 milliliters of 5M NaNO3 ionic stength adjuster using a Finnpipetter with a 5 millilter tip.
13. Place the beaker onto the magnetic stirrer.
14. Positon the magnetic stirrer dial to a setting between 2 and 3 (sufficient to continuously rotate the stirring bar).
15. Raise and lower the chloride probe and temperature probe into the 10 ppm chloride standard three times.
16. Position the chloride probe in the center of the beaker and one inch above the bottom of the beaker. The temperature probe should also be submersed in the beaker.
17. Press the "CAL" button.
18. Wait for the meter to display "Stable" and then press the "ENTER" button.
19. Remove the temperature and chloride probes from the 10 ppm standard.
20. Rinse a 150 milliliter beaker, a magnetic stirring bar, the chloride probe and the temperature probe three times with DI H_2O.
21. Insert a stirring bar into the 150 milliliter beaker.
22. Fill the 150 milliliter beaker, containing a stirring bar, with approximately 25 milliliters 100 ppm chloride standard.
23. Pour out the 100 ppm chloride standard so it flows over the chloride probe and temperature probe to remove any redidual DI H_2O.
24. Measure out 100 milliliters of 100 ppm chloride standard using a 100 milliliter graduated cylinder.
25. Dispense the 100 milliliters of 100 ppm chloride standard into the 150 milliliter beaker that contains a magnetic stirring bar.
26. Add 2 milliliters of 5M $NaNO_3$ ionic stength adjuster using a Finnpipetter with a 5 millilter tip.
27. Place the beaker onto the magnetic stirrer.

28. Positon the magnetic stirrer dial to a setting between 2 and 3 (sufficient to continuously rotate the stirring bar).
29. Raise and lower the chloride probe and temperature probe into the 100 ppm chloride standard three times.
30. Position the chloride probe in the center of the beaker and one inch above the bottom of the beaker. The temperature probe should also be submersed in the beaker.
31. Press the "CAL" button.
32. Wait for the meter to display "Stable" and then press the "ENTER" button.
33. Remove the temperature and chloride probes from the 100 ppm standard.
34. Rinse a 150 milliliter beaker, a magnetic stirring bar, the chloride probe and the temperature probe three times with DI H_2O.
35. Insert a stirring bar into the 150 milliliter beaker.
36. Fill the 150 milliliter beaker, containing a stirring bar, with approximately 25 milliliters 1000 ppm chloride standard.
37. Pour out the 1000 ppm chloride standard so it flows over the chloride probe and temperature probe to remove any redidual DI H_2O.
38. Measure out 100 milliliters of 1000 ppm chloride standard using a 100 milliliter graduated cylinder.
39. Dispense the 100 milliliters of 1000 ppm chloride standard into the 150 milliliter beaker that contains a magnetic stirring bar.
40. Add 2 milliliters of 5M $NaNO_3$ ionic stength adjuster using a Finnpipetter with a 5 millilter tip.
41. Place the beaker onto the magnetic stirrer.
42. Positon the magnetic stirrer dial to a setting between 2 and 3 (sufficient to continuously rotate the stirring bar).
43. Raise and lower the chloride probe and temperature probe into the 1000 ppm chloride standard three times.
44. Position the chloride probe in the center of the beaker and one inch above the bottom of the beaker. The temperature probe should also be submersed in the beaker.
45. Press the "CAL" button.
46. Wait for the meter to display "Stable" and then press the "ENTER" button.
47. Remove the temperature and chloride probes from the 1000 ppm standard.
48. Rinse the beaker, temperature and conductivity probes three times with DI H_2O.

C. Low chloride calibration (typically specific conductivity below 150 *u*S/cm):

Prepare low ionic strength, 1M $NaNO_3$, ISA immediately before proceeding: Pipette 40 milliliters 5M $NaNO_3$ ionic strength adjustor into a 200 milliliter flask. Add DI H_2O until the volumetric flask water level reaches the fill mark.

Standard Curve:

1. Turn the meter on.
2. Make sure the chloride BNC is plugged into the PC 2700.
3. Make sure the chloride and temperature probes are secured in the electrode holder.
4. Press the "MODE" button until the "measure Ion" mode is displayed.
5. Remove the chloride probe cap and the rubber plug from the electrolyte solution fill hole. If necessary, add electrolyte solution until the height of the solution in the electrode is within three millimeters of the fill hole.
6. Rinse a 150 milliliter beaker, a magnetic stirring bar, the chloride probe and the temperature probe three times with DI H_2O.
7. Insert a stirring bar into the 150 milliliter beaker.
8. Measure out 100 milliliters of DI H_2O using a 100 milliliter graduated cylinder.
9. Dispense the 100 milliliters of DI H_2O into the 150 milliliter beaker that contains a magnetic stirring bar.
10. Add 1 milliliter of low ionic strength ISA to the beaker.
11. Place the beaker onto the magnetic stirrer.
12. Positon the magnetic stirrer dial to a setting between 2 and 3 (sufficient to continuously rotate the stirring bar).
13. Raise and lower the chloride probe and temperature probe into the DI H_2O three times.
14. Wait for the meter to display "Stable" and then record the millivolt measurement.

15. Sequentially add increments of 1000 ppm chloride standard as indicated in the table below (steps 15A through 15G). After each sequential addition of chloride standard, wait for the reading to stabilize and record the millivolt measurement on the chloride datasheet.

Step	[Chloride] (ppm)	Volume of 1000 ppm chloride standard added (ml)	Result (mV)
15A	1.0	0.1	Record the corresponding mV measurement for each chloride concentration.
15B	2.0	0.1	
15C	4.0	0.2	
15D	6.0	0.2	
15E	9.9	0.4	
15F	29.0	2.0	
15G	48.0	2.0	

16. Rinse the chloride probe, temperature probe, 150 milliliter beaker and stirring bar three times with DI H_2O.
17. Plot a semi-logarithmic calibration curve, log (chloride) vs millivolt using either Microsoft Excel or JMP. The resulting curve and equation will be used to determine the concentration of the unknown lake and stream water samples.

Running Samples (pH and specific conductivity):

1. Rinse a 100 milliliter beaker, a magnetic stirring bar, the pH probe, the conductivity probe and the temperature probe three times with DI H_2O.
2. Insert the stirring bar into the 100 milliliter beaker.
3. Rinse the 100 milliliter beaker and the stirring bar with approximately 25 milliliters of the water sample.
4. Fill the 100 milliliter beaker with the water sample and pour off approximately 20 milliliters over the pH, specific conductivity and temperature probes to remove any residual DI H_2O. If necessary, add additonal water sample until the solution is at/above the 80 milliliter mark.
5. Place the beaker onto the magnetic stirrer.
6. Positon the magnetic stirrer dial to a setting between 2 and 3 (sufficient to continuously rotate the stirring bar).
7. Raise and lower the pH, conductivity and temperature probes into the water sample three times.
8. Position the pH probe in the center of the beaker and one inche above the bottom of the beaker. The temperature probe should also be submersed in the beaker and the conductivity probe should be positioned one-half inch from the bottom of the beaker.
9. Press the “Mode” button to display “measure conductivity”.
10. Wait for the meter to display “Stable” and then record the specific conductivity value on the PC 2700 datasheet.
11. Press the “Mode” button to display “measure pH”.
12. Wait for the meter to display “Stable” and then record the pH value on the PC 2700 datasheet.
13. Repeat steps 1 through 12 until all samples have been analyzed.
14. Rinse a 100 milliliter beaker, a magnetic stirring bar, the pH probe, the conductivity probe and the temperature probe three times with DI H_2O.
15. Slide the sleve over the pH fill hole and place the probe tip into the vial of pH storage solution.

Running Samples (chloride: high and low level):

1. Rinse a 150 milliliter beaker, a magnetic stirring bar, the temperature probe and the chloride probe three times with DI H_2O.
2. Insert the stirring bar into the 150 milliliter beaker.
3. Rinse the 150 milliliter beaker and the stirring bar with approximately 25 milliliters of the water sample.
4. Pour out the water sample so it flows over the chloride probe and temperature probe to remove any residual DI H_2O.
5. Measure out 100 milliliters of water sample using a 100 milliliter graduated cylinder.
6. Dispense the 100 milliliter water sample into the 150 milliliter beaker that contains magnetic stirring bar.
7. Add 2 milliliters of 5M $NaNO_3$ ionic stength adjuster (high chloride) using a Finnpipetter with a 5 millilter tip. *Note: 1 milliliter of 1M $NaNO_3$ ionic strength adjuster is used when the meter is calibrated for low level choride analysis*.
8. Place the beaker onto the magnetic stirrer.
9. Positon the magnetic stirrer dial to a setting between 2 and 3 (sufficient to continuously rotate the stirring bar).
10. Raise and lower the chloride probe and temperature probe into the water sample three times.
11. Position the chloride probe in the center of the beaker and one inch above the bottom of the beaker. The temperature probe should also be submersed in the beaker.
12. Wait for the meter to display "Stable" and then record the chloride value on the PC 2700 datasheet when running high chloride samples. *Note: the millivolt (mV) value is recorded when analyzing low level chloride.*
13. Repeat steps 1 through 12 until all samples have been analyzed.
14. Rinse a 150 milliliter beaker, a magnetic stirring bar, chloride probe and the temperature probe three times with DI H_2O.
15. Slide the sleve over the chloride fill hole and place the cap on the end of the chloride probe.

Calibration Check:

1. **pH -** A calibration check should be analyzed after every 20 samples and generally consists of a pH 7.00 buffer that is within the typical pH range for low elevation New Hampshire lakes and ponds. However, a pH 4.00 or pH 10.00 buffer solution may be substituted when more acidic or more basic samples are analyzed. A calibration check will also be analyzed at the end of each analytical run.
2. **Conductivity -** A calibration check should be analyzed after every 20 samples and generally consists of an 84 uS/cm standard. A calibration check will also be analyzed at the end of each analytical run.
3. **Chloride -** A calibration check should be analyzed after every 20 samples and generally consists of either a 100 ppm chloride standard (high conductivity systems) or a 20 ppm chloride standard (low conductivity systems). A calibration check will also be analyzed at the end of each analytical run.

Written by Bob Craycraft and Jeff Schloss
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Appendix B
Center for Freshwater Biology Standard Operating Procedures (SOPs) for Field Protocols

B.1. Field Measured pH

Reference: Standard Methods 20th Edition Method 4500H+ and Hanna pH meter manual

Sample requirement: Collected in airtight syringe and sealed or in capped PE bottles, Stored on ice in the dark. Allowed to come to ambient or room temperature before analysis.

The pH meter (Hanna Model HI-9025) is fitted with a Beckman Coulter low ionic strength pH electrode (Model A57195).

Notes: Calibration procedure should be undertaken before any samples are processed. Always insert the pH and ATC probes 1.5" into the solutions and leave 0.25" clearance from the bottom). The carry case of the meter includes a built in probe holder to facilitate keeping both probes in proper position while in the sample.

A-Calibration:

1) Make sure both the pH probe and the temperature probe are plugged into the pH meter. Remove the cap and the orange side plug from the pH probe before proceeding any further. Add 1N KCl saturated with AgCl electrode filling solution if necessary (the filling solution should be a few mm below the side plug opening). Place the pH electrode and temperature probe in a lakewater sample and let sit for 10 minutes.
2) After the 10 minute "warm-up" period, pour a pH 7.01 buffer solution into a 100 ml plastic beaker. Lift the pH probes out of the water sample, rinse with DI H_2O and place the probes into the pH 7.01 buffer solution. *Note: a pH 10.01 buffer solution is substituted for the pH 7.01 buffer above when the lake/streamwater pH is expected to exceed 7.01.*
3) Press the CAL button (the CAL and BUF indicators will be displayed on the LCD screen and the BUF should read 4.01). You want to select a different pH buffer (pH 7.01 or pH 10.01) which is done by pressing the up arrow or down arrow buttons on the meter until the BUF reads 7.01 (10.01 when using the more alkaline buffer).
4) Wait 30 seconds or until the LCD display reads READY, and then press the CFM button to confirm the first buffer solution (when the electrode is submerged into the buffer solution, the meter will attempt to stabilize. If the readings fluctuate for more than 10 seconds, the LCD will blink NOT READY. If the reading is stable, READY and CON will blink).
5) Rinse the probes with DI H_2O, pour out a pH buffer 4.01 solution in a clean beaker and submerse the probes into the buffer solution. You should adjust the BUF display to read 4.01 at this point by pressing the down arrow if necessary.
6) Proceed as in step 4. If the calibration symbol (beaker) on the LCD screen blinks then your buffer is contaminated or you used the wrong buffer solution. Repeat step 5 again making sure you are using the correct buffer (4.01).
7) If both the calibration symbol and the probe symbol blink alternately than the slope of the calibration is unacceptable (not within 85%-105%). Try to recalibrate starting from step 2) above. If the slope is still unacceptable you will need to clean or replace the pH probe. See the section below for the probe cleaning instructions (Section D).
8) After successful reading of the second buffer press the CAL buffer to accept the calibration.
9) Once the calibration process is successful, you should rinse the probes with DI H_2O and

submerse the probes in a lake-water sample. Let the pH reading equilibrate for approximately 10 minutes and then begin analyzing your samples.

B-Running Samples:

1) Dispense approximately 50 ml of sample into a clean beaker being careful to fill the beaker slowly allowing the sample to run down the side of the beaker with little agitation. Insert the probes and let the pH reading stabilize for two minutes (or until the readings are stable to the nearest 0.01 unit for 10 seconds). Record your reading on the field data sheet and dispense the remaining sample for the replicate reading. Proceed to the next sample, rinsing with DI H_2O between samples. Repeat the process until all samples are read.

C-Calibration Check:

1) A pH calibration check should be analyzed after every 10 samples and generally consists of a pH 7.01 buffer that is within the typical pH range for low elevation New Hampshire lakes and ponds. However, a pH 4.01 or pH 10.01 buffer solution may be substituted when more acidic or more basic samples are analyzed. The pH buffer is poured into a cleaned beaker and is analyzed after every 10 samples. After running all pH samples fill cleaned beakers with fresh pH 7.01 buffer (pH 10.01 for more alkaline systems) and pH 4.01 buffers. Measure the pH of the buffered solutions and record the readings on the field datasheet. A pH 6.00 QC sample will be analyzed at the beginning and end of the pH analyses by pouring fresh buffer solution into a cleaned beaker and recording the pH measurements on the field sheet. The QC samples shall be collected at a minimum frequency of 5% of the samples and may be measured more frequently (i.e. after every 15-20 samples) when processing large batches of pH samples.

D-Probe (Bulb) Rejuvenation:

NOTE: If step one rejuvenates probe, do not proceed to step two. Likewise, if step 2 works, don't proceed to step 3

1) Soak bulb in 1M HCl for one hour. Rinse thoroughly with deionized water.
2) Soak alternatively in 1M HCl and 1M NaOH for one m inute in each solution, cycling three times. Then soak in pH 4 buffer for 1 hour
3) Clean bulb with 50/50 mixture of acetone and isopropyl alcohol

Note any instrument or probe discrepancies in the field log book and transfer the information logged into the field equipment log book upon return to the lab

Written by Bob Craycraft and Jeff Schloss
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B.2. Field Measured Total Alkalinity

Reference: Standard Methods 20th Edition: 2320 B. (low alkalinity) -modified

This method has been modified in two respects:

1- The ecoregional character of NH lakes makes using a more dilute acid result in the higher sensitivity required to obtain adequate results. Thus, a titrant of .002N H_2SO_4 is used instead of the .02N acid of standard method.

2- While lab analysis typically uses a pH meter, for the field we use a pH indicator solution for efficiency unless the water has high organic color. A mixed bromocresol green-methyl red indicator allows for a sharper equivalence point at the lower pH that t he test requires. It is greenish-blue at pH 5.2, light blue at pH 5.0, light gray at pH 4.8, and light pink at pH 4.5.

Upon special request the protocol can be followed using the Hanna Model HI-9025 pH meter instead of indicator solution. Follow the protocols outlined below but skip step C.2.and substitute pH 4.8 for gray endpoint and pH 4.5 for pink endpoint.

NOTE: This method assumes the water pH is below 8.3 units as occurs in most New Hampshire waters. Phenolphthalein indicator can be used to determine this (no pink when added) or a pH reading by meter can be performed.

A- In-lab Stock and Working Solution Preparation:

1) Stock Titrant (0.1N) – Pipette 2.8 ml concentrated H_2SO_4 (app. 36N) into a 1 Liter flask and bring to volume with DI H_2O.
2) Dilute Titrant (.002N) – Pipette 20 ml (+or- depending on the Stock titrant normality as determined below) into a 1 Liter flask and bring to volume with DI H_2O.
3) Calibration Solution: Dry 3 to 5 grams Na_2CO_3 at 250°C for 4 hours and cool in a dessicator.
    a. Weigh 2.5 +or- 0.2 grams and transfer into a 1 liter volumetric flask. Record the weight.
    b. Fill the flask with CO_2 free (boiled for double distilled water and mix (do not keep this solution longer than one week). This solution is approximately 0.05N.
    c. From the 0.05N Stock solution take 40.00 ml and place in a beaker.
    d. Add 60 ml CO_2 free DI H_2O and place on a magnetic stirrer (make sure the stirring bar has been washed thoroughly prior to placing it into the beaker to avoid contamination).
    e. Fill a 25.0 ml burette with stock alkalinity titrant (0.1N) making sure no air pockets or bubbles remain in the burette.
    f. Slowly titrate to a pH of about 5. Lift out the p H and temperature electrodes, rinse the electrodes into the beaker and boil gently for 3-5 minutes under a watch glass. Cool to room temperature, rinse the cove glass into the beaker and finish titrating to the pH inflection point. Calculate Normality (all data should be recorded on the corresponding data sheet):

$$\text{Normality} = \frac{\text{grams } Na_2CO_3 \text{ in 1L flask x ml } Na_2CO_3 \text{ solution taken for titration}}{53 \text{ x ml titrant (0.1N } H_2SO_4\text{) used}}$$

4) Working Solution: Once the Normality of the stock H_2SO_4 solution is determined 4 Liters of .002N alkalinity titrant should be produced by pipetting 20 ml (+or-) into a 1 Liter flask and bringing to volume with DI H_2O. Repeat this step until 4 Liters of alkalinity titrant are produced and stored in a 4 liter carboy. Make sure the date and lab technician name are included on the carboy label.
5) Indicator Solution: Rinse a 1000 ml volumetric flask three times with DI H_2O and prepare one liter of alkalinity indicator as follows:

a. Fill the 1000 ml volumetric flask with approximately 500 milliliters of 95% Ethyl Alcohol.
b. Weigh out 0.2 grams of methyl red (FS cat # M-296) and transfer the crystals into the 1000 ml volumetric flask.
c. Weigh out 1.0 g of bromocresol green sodium salt (FS cat # B90-10) and transfer the crystals into the 1000 ml volumetric flask.
d. Add 95% Ethyl Alcohol until the volume reaches the 1000 ml volumetric flask fill line.
e. Rinse a magnetic stirring bar three times with DI H_2O and add the stirring bar to the 1000 ml volumetric flask.
f. Place the 1000 ml flask on a magnetic stirrer for one hour or until all crystals have dissolved. The stirrer speed should be set so the bar rotates continuously for the one hour duration.
g. Transfer the alkalinity titrant into a four liter light sensitive (amber) jar.

B- Alkalinity Field Kit

1) Check Contents of Field Kit for all items included and their condition before departing:
    a. 100 ml plastic graduated cylinder
    b. 150 ml plastic beaker or cup
    c. Field burettes (plastic volumetric pipette fitted with ball valve and tip)
    d. Glass stirrers
    e. Indicator solution drop bottle- (Brom Cresol Green Methyl Red)
    f. Titrant solution dispensing bottle (.002N H_2SO_4)
2) Check expiration dates of all chemistry and refresh with new solutions if required.

C-Total Alkalinity Field Measurements:

NOTE: Titrations should always be done against a white background and away from any colored reflections. The graduated cylinder, beaker and stirring rods should be rinsed well between samples. Allow samples to come to room temperature or ambient temperature within their sampling container before processing.

1) Carefully transfer (without agitation) 50 ml or 100 ml of the water sample (from sample bottles or syringes) into the graduated cylinder. Use 50 ml when you expect Alkalinity to be higher than 15 mg/l or when you have a limited amount of sample. Use 100 ml when low alkalinity is expected. Then transfer sample carefully into plastic titrating cup/beaker.

2) Add 8 (for 50 ml sample) or 12 (100 ml) drops of indicator (bromocresol green/methyl red) from the drop dispensing bottle. Stir gently with glass rod.

3) Insert tip of plastic titrant bottle into top of plastic field burette. Squeeze bottle carefully and fill burette with 10-12 ml of titrant (0.002 N H_2SO_4).

4) Hold burette with one hand so that thumb and forefinger can squeeze above the glass bead in the rubber tubing. Be sure to squeeze gently just behind the bead so you release titrant but do not move the bead down the tube.

5) Clear all air bubbles from the burette by gently tapping the pipette while you hold the burette up and down. Clear any trapped air in the tip area by bending the tube so the tip is pointing up and allowing some titrant to flow out of the tip to release the air. Refill the burette if necessary to bring the titrant level to the zero mark and allowing the released titrant to collect into your waste field container.

    DO NOT PUT ANY TITRANT BACK INTO THE TITRANT BOTTLE AT ANY TIME.

6) Add titrant slowly, drop by drop, while stirring with other hand. Titrate until water loses blue coloring and becomes a dull gray color. Record number of milliliters used to reach this first endpoint. Record to the nearest tenth (eg. 5.4 ml); each small line on the burette is 0.1 ml, each half line is 0.5 ml, and each full line is marked with the whole ml number of solution dispensed.

7) Continue titrating until solution becomes a very faint pink. At this point in the titration, the water will become "pinker" as more titrant is added, so it is very important to titrate only until the first signs of loss of

grey and appearance of light pink! Again, record the total number of ml used to reach this second endpoint (in other words, since you began titrating, not since the gray endpoint). For example, if it took 5.4 ml to reach the gray endpoint, plus, 0.4 ml to reach the pink endpoint, the total ml used for the second endpoint would be 5.8 ml. Make sure both endpoints are included on the data sheet.

8) Calculations: We have found that Total Alkalinity results vary slightly due to the analytical method used. For comparison to alkalinities derived from Acid Neutralizing Capacity Gran Titrations (pH inflection point determinations) the grey endpoint (pH 5.1) compares the best. For comparisons to historic measurements made with Methyl-Orange indicator (since shown to influence sample pH and no longer used) the pink endpoint (pH 4.8) is best. To compare to the Standard Method low alkalinity method or the EPA dual endpoint method calculate each (grey and pink) of the alkalinities using equation A and use the second formula (Equation B) below.

$$\textbf{A-Total Alkalinity (mg } CaCO_3\textbf{/L)} = \frac{\textbf{T x N x 50,000}}{\textbf{ml sample titrated}}$$

where:
T is the ml of titrant for the endpoint used
N is the normality of the titrant

Calculate both the grey endpoint and pink endpoint alkalinity and add to the field datasheet. If .002N acid is titrated into 100 ml of sample then each 1.0 ml of titrant used would be equivalent to 1.0 mg $CaCO_3$/l. Thus, the burette reading of titrant used is the Total Alkalinity. If 50 ml of sample is titrated with the same titrant, multiply the burette volume titrated by 2.0.

$$\textbf{B-Total Alkalinity (mg } CaCO_3\textbf{/l)} = \textbf{(2 x G) – P}$$

where:
G is the grey endpoint alkalinity
P is the pink endpoint alkalinity

Written by Bob Craycraft and Jeff Schloss
Last Updated on July 7, 2005

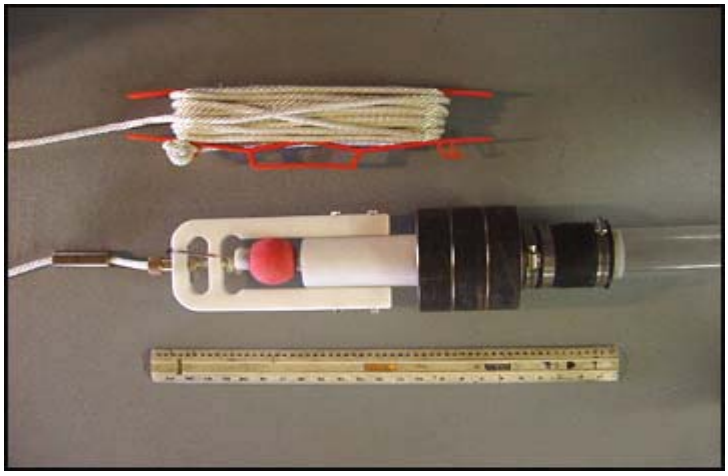
B.3. Use of KB style Core Sampler

1) Ensure that core tube is attached securely to the core head by pushing it fully into the flange.

2) With the unit upright push down on the brass fitting so that the tripping pins open.

3) While still applying pressure to the fitting pull up on the nylon filaments that are attached to the ball seal and place the loops so that they are secured around the tripping pins and release the brass fitting so that the loops are held.

4) Carefully place the unit into the water holding the line reel in one hand, just above the corer, and messenger



in the other. Unreel enough line for the sampling depth being careful to allow the line to run out freely.

5) If over deep water allow the unit to free fall and penetrate the sediments being careful control the line loosely so that after penetration of the sediments the corer will remain upright.

6) Send the messenger down the taunt line and wait for feeling the vibration of the tripping mechanism to occur.

7) Carefully pull the sampler out of the sediments, bring the sampler up to the surface in a smooth manner and just before surfacing the instruments cap the bottom end of the sampler while still under water using the # 11 rubber stopper.

8) Bring the sampler into the boat maintaining it upright, position it over the collection tray and carefully remove the core tube from the core head by smoothly turning the core tube back and forth while maintaining a study down pressure and while someone else is holding the core head stationary. Make measurements of the core depth, notate any observations in the field log books and on the field data sheets and take any photographs of the sediment layering before proceeding.

9) Carefully siphon out the water above the sediment water interface using a siphon tube consisting of a plastic 10 ml volumetric pipette attached to a length of tubing. Keep the pipette tip below the water level but away from the sediment. Depending on the project discard or save the siphoned water sample as directed.

10) If the core is not to be transported intact, carefully remove the bottom stopper and collect the full core or a series of sediment layers as directed by the project specifications. Continue to collect the remaining sample as directed by the project specifications.

11) Before any additional sampling is to continue wash out the core head and core tube with lake water and then rinse with distilled water.

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B.4. Use of the Interstitial Pore Water Sampler

1) Ensure that the delrin (white) probe point moves freely up and down to cover and uncover the small sampling holes. If not remove the two side screws, clean the contact areas and reassemble.
2) Assemble the probe with enough sections to accommodate the depth of the water and the freeboard of the boat you are sampling from. You should allow for the sample penetration depth and have the upper end of the sampler a minimum of 0.5 meters above the water's surface. During assembly make sure you also inset a clean sampling tubing into the sampler so it is even with the sampler bottom and passes through the center opening of each section without twists or pinching.
3) While over the sampling site carefully insert the probe into the water and hold when you reach the bottom sediment.
4) Taking note of the water level against the sampling tube markings insert the sample into the sediments to the target penetration depth and an additional 5cms.
5) To facilitate penetration in harder sediments you can twist the sample clockwise as you apply penetration pressure. If necessary you can attach the sliding hammer assembly to the top of the tube and hammer in the probe.
6) Once the penetration is complete carefully raise the outer tube of the sampler about 5 cm (or until you feel the first sign of resistance. A slight counterclockwise twist will lock the sampler open.
7) Press down on the sampling tubing to ensure it is located at the bottom of the sampler. Secure the tubing to the top of the sampler with vinyl tape so it will remain in place as you sample.
8) Wait about 5-10 minutes for the water to equilibrate in the sampler.
9) Using the portable peristaltic pump attach the sampling tubing to the connector marked "in" and begin pumping out the water in the sampler at the lowest speed noting the start time of the pumping. Collect the sample in the graduated collection bottle.
10) If the sampler is purged of water note the volume and time of collection. If the sampling flow remains consistent then allow for two complete exchanges of the sampler.
11) Allow the required time for the sample to come to equilibrium again using the volume and time measurements made previously. Empty the sample container, turn on the pump and then collect sample into the sample container.
12) Continue these steps until you have collected the required amount of sample.
13) If you are using a hand vacuum pump or syringe in lieu of the peristaltic pump be sure to break the vacuum between sampler purging and collection.
14) After sampling is complete be sure to clean the sampler, check the probe point interface and replace the sampling tubing.

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B.5. Yellow Spring Instruments (YSI) 85 Meter SOPs

Calibrating the YSI 85 Portable Dissolved Oxygen and Conductivity Meter

Dissolved Oxygen Calibration

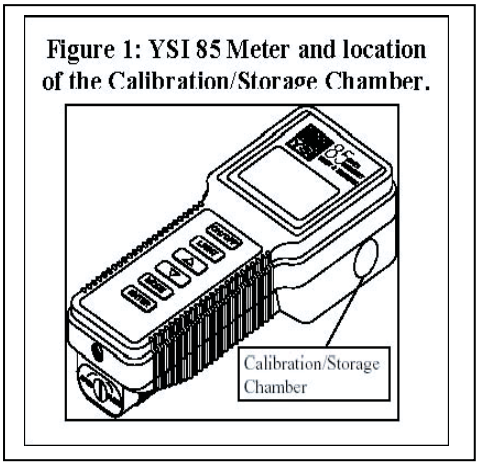


Figure 1: YSI 85 Meter and location of the Calibration/Storage Chamber.

Remember to turn the YSI 85 meter on at least a half an hour before calibration to warm up. The calibration/storage chamber should always be checked to make sure the small yellow sponge is moist prior to the 15 minute warm-up period (see Figure 1). If necessary, carefully add 3 to 6 drops of tap water onto the sponge in the calibration/storage chamber, turn the instrument over to allow any excess water to drain out, and replace the probe into the calibration/storage chamber prior to the 15 minute warm-up period. The wet sponge creates a 100% water saturated air environment for the probe which is essential for an accurate dissolved oxygen calibration. You should also inspect the membrane cap prior to proceeding to ensure that there are no air bubbles in the cap. Membranes should be replaced every three months, or whenever an air bubble forms in the cap. To install a new membrane on your YSI Model 85 dissolved oxygen probe (see Figure 2):

1. Unscrew and remove the probe sensor guard.
2. Unscrew and remove the old membrane cap.
3. Thoroughly rinse the sensor tip with distilled water.
4. Prepare the electrolyte according to the directions on the KCl solution bottle (this will be done in the NLRA office by trained staff/volunteers)
5. Hold the membrane cap and fill it at least ½ full with the electrolyte solution.
6. Screw the membrane cap onto the probe moderately tight. A small amount of electrolyte should overflow.
7. Screw the probe sensor guard on moderately tight.

The YSI 85 meters **must** be calibrated before making dissolved oxygen measurements as described below:

1. Make sure the oxygen cap is free of air bubbles and, if necessary, remove and refill the cap before proceeding using the procedure above.
2. Assure the sponge is wet and the probe is inserted into the storage chamber.
3. Turn the meter on and allow the meter to warm up for 15 minutes.
4. Press the **MODE** button to until dissolved oxygen is displayed in %.
5. Press the **UP ARROW** and the **DOWN ARROW** simultaneously to enter the calibration mode.
6. Use the **UP ARROW** and the **DOWN ARROW** keys to select an altitude of 600 feet (6 should display on the LCD screen) that is approximately the elevation of Newfound Lake.
7. Press the **ENTER** key button once.
8. The letters **CAL** should now be visible in the lower left of the LCD display.
9. Make sure the large display in the LCD display is stable and that the dissolved oxygen is still displayed in % before proceeding.
10. Press **ENTER**.
11. The display should read **SAVE** and will then return to the

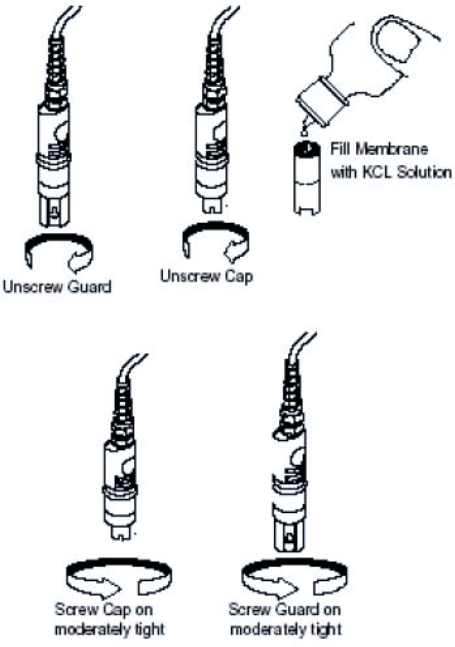


Figure 2: Installation a new Dissolved Oxygen Membrane on the probe.

Normal Operation Mode.
12. You are ready to collect water quality data.

Specific Conductivity Calibration

The specific conductivity is factory calibrated and is highly stable. However, the specific conductivity should be checked against factory calibration standards and re-calibrated at the frequency specified in a project specific Quality Assurance Project Plan (QAPP) to assure the collection of the most accurate data possible. Specific Conductivity shall be calibrated using the following protocol:

1. Remove the conductivity probe from the storage chamber and rinse the probe three times with de-ionized water.
2. Pour approximately eight inches of 1 mS/cm calibration solution into a 100 milliliter graduated cylinder.
3. Rinse the conductivity probe with 1 mS/cm calibration solution directly from the calibration solution bottle.
4. Use the **MODE** button to advance the YSI 85 meter to display specific conductivity (Figure 3). *Note: the small ˚C display in the lower right hand side of the display will flash when you are in the Specific Conductivity mode.* ***Do not*** *record Conductivity readings when the ˚C display is not flashing.*
5. Insert the probe into the graduated cylinder and raise and lower the probe through the solution three times and then lower the probe to a depth of three inches off the bottom of the cylinder and hold the probe at that depth.
6. Allow 60 seconds for the temperature reading to become stable.
7. Press and release the **UP ARROW** and **DOWN ARROW** buttons at the same time. *Note: the CAL symbol will appear at the bottom left of the display to indicate that the instrument is now in calibration mode.*
8. Use the **UP ARROW** or **DOWN ARROW** button to adjust the reading on the display until it matches the value of the calibration solution you are using (i.e. 1 mS/cm).
9. Once the display reads the exact value of the calibration solution being used press the **ENTER** button once. The word **SAVE** will flash across the display for a second indicating that the calibration has been accepted.
10. Rinse the conductivity probe with de-ionized water and return the probe to the storage chamber.

Measuring Temperature, Dissolved Oxygen and Specific Conductivity

STEP 1

Turn the instrument on by pressing the **ON/OFF** key if it wasn't already on and allow the meter to warm up for at least 30 minutes. *Note:* ***make sure*** *the sponge is wet and proceed through the dissolved oxygen calibration procedure as described above*.

STEP 2

Remove the probe from its storage chamber and submerse the probe to a depth of four inches into the stream water. Raise and lower the probe into the water two more times to assure the probe is well rinsed of any residual debris. Hold the probe at a depth of 4 inches below the water surface.

STEP 3

Choose the Dissolved Oxygen mode (See Figure 3) by pressing and releasing the MODE button until the large legend reads mg/l at the far right side of the LCD display.

STEP 4

Gently jiggle the probe in the water until the dissolved oxygen measurement stabilizes to +/- 0.1 mg/l and record the measurement on your datasheet.

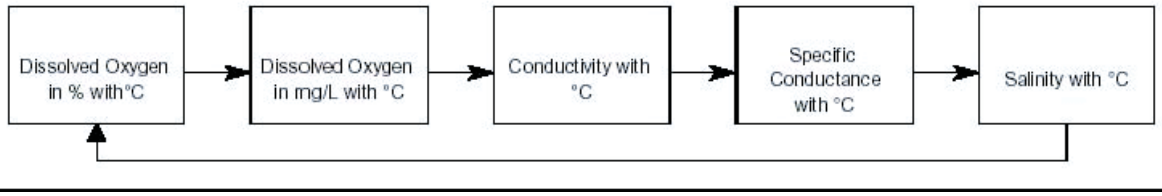
STEP 5

Stop jiggling the probe in the water in preparation of collecting a Specific Conductivity measurement.

STEP 6

Choose the Specific Conductivity mode (See Figure 3) by pressing and releasing the MODE button until the large legend reads υS at the far right side of the LCD display. *Note: the small ˚C display in the lower right hand side of the display will flash when you are in the Specific Conductivity mode. Do not record Conductivity readings when the˚ C display is not flashing.*

Figure 3: Flowchart of YSI Model 85 Parameters.



STEP 7
Once the Specific Conductivity has stabilized to +/- 0.5 υS record the Specific Conductivity measurement and the Temperature measurement on your datasheet.

STEP 8
Repeat steps two through seven and record a second set of measurements on your datasheet.

STEP 9
Remove the probe from the water, place the probe in the storage chamber, turn the meter off and return the meter to the protective storage case.

Written by Bob Craycraft and Jeff Schloss
Last Updated on February 19, 2007

B.6. Specific Conductivity

Reference Source: Standard Methods 20th Edition 2510.

Instructions for YSI Model 30 Specific Conductance Meter:
Measurements may be made by submerging probe into the lake or stream or by placing probe into a sample previously collected.

Initial Meter Check and Calibration (before leaving for field sampling):

1. Turn the meter on by pressing the ON/OFF key. The meter will display all segments of the display screen for a few seconds, followed by a self-test. If the meter is not functioning properly, an error message (LoBat, #Err, Ovr or Undr) will be displayed.
2. If the "°C" is not flashing on and off, press the MODE key until it does. This puts the meter into the temperature compensated mode.
3. Rinse the probe with DI water and blot dry with a Kimwipe.
4. Submerge the probe in the 100 (for lake sampling) or 200 (for stream sampling) µS conductivity standard solution and allow to stabilize for two minutes. Ensure there is enough solution to cover the top opening of the probe.
5. Record the Initial Specific Conductance Calibration Value on the Field Data Sheet. Ensure there are no air bubbles on or inside the probe. A 10% error (a reading of 90-100 µS for the 100 µS standard or a reading of 180-220 µS) is acceptable. If the readings are outside of this rang e then have the laboratory manager recalibrate the unit.
6. Rinse the probe with DI water and return it to the storage chamber.

Field Sampling Procedure:

1. Press the ON/OFF key to turn the meter on. If the "°C" is not flashing on and off, press the MODE key until it does (this puts the meter into the temperature compensated mode). If using a different meter, ensure that it is in specific conductivity mode.
2. Rinse the probe with DI water and blot dry with a Kimwipe.
3. Immerse the probe in the lake, stream or sample container and make sure it is deep enough to cover the entire probe. Do not allow the probe to touch any solid object or the bottom of the container while you are taking readings. It is also important that there are no air bubbles on/in the electrode. To dislodge any bubbles, gently move the elect rode through the water before recording the measurement.
4. Agitate by slowly moving the probe back and forth in the sample for a minimum of two minutes for the temperature and specific conductance readings to stabilize. Record the conductivity reading on the Field Data Sheet.
5. Rinse the probe and return it to the storage chamber between measurements. Please turn the meter off when not in use to conserve battery power.
6. At the end of the day, recheck the meter with the standard and record the value in the "Final Calibration Check" section on the Field Data Sheet.

Written by Jeff Schloss and Bob Craycraft
Last Updated on May 21, 2006

B.7. Stream Flow Measurements

Reference: YSI Son-Tek Users Manual (July 2007).

As the use of this instrument is technical in nature all field crew will need to be trained and certified to use this instrument for any project. An abridged copy of the technical manual will be use for training purposes. The information below summarizes the major steps and references the manual sections used.

The basic operation of the "Flow Tracker ADV" system includes (and manual section reference):

Preparing the system for data collection in the field:

1. Run pre-deployment diagnostic before leaving for the field (sections 3.2 and 6.5.4).
2. Vent the display controller (8.7.5).
3. Mount the probe on the self setting wading rod (8.1).
4. Perform Field diagnostics (3.3.2).
5. Set system parameters (2.4).
6. Start data collection in "Discharge" mode (5).
7. Set your station areas (stream transect points) along a tag line consisting of a fiberglass measuring tape. marked in fractional meters that has been anchored to run perpendicular to the water flow (5.2.1).
8. For shallow station readings use the one point method (0.6 * depth, velocity reading) and for deeper stations use the two point methods (0.2 and 0.8* depth, velocity readings).
9. Set your system parameters for sampling along the transect.
10. Start your data collection by pressing 3 from the Main Menu START DATA RUN (5.3.2).
11. Place the probe at the required depth using the wading rod vernier. Keep the probe X direction perpendicular to water flow. Press MEASURE to start. Enter station depth and distance along the tag line and watch for velocity reading. Press 1 to accept that measurement or 2 to repeat the measurement.
12. If there is more than one measurement (two-point method) adjust the probe to the second required depth as indicated on the readout and press MEASURE.
13. When the station is complete the unit will display the next station number and predict the location, depth and method. If a multiple measurement was used the unit will start at the last reading fractional depth. You can use the SET LOCATION or SET DEPTH buttons to edit or correct these data.
14. Continue pressing the MEASURE key until all readings are taken.
15. At the final reading (stream edge) press the END SECTION key.
16. You can review and edit the collected data by pressing the NEXT STATION and PREVIOUS STATION keys.
17. When all values are checked and edited press the CALC. DISCHARGE key to complete the calculations and close the data file.
18. Press 9 to return to the MAIN MENU. **Always return to the main menu before turning the system off to ensure all data collected has been saved.**

19. The Flowtracker (section 6.0) software is used to download the collected data to our lab computers. Be sure to download all data from the unit before returning to the field.

Written by Jeff Schloss and Bob Craycraft
Last Updated on April 22, 2016

B.8. YSI 6600 Multiparameter Profiling

Reference YSI 6600 Users Manual and YSI 650 MDS Users Manual (March 2009, REV E).

As the use of this instrument is technical in nature all field crew will need to be trained and certified to use this instrument for any project. An abridged copy of the technical manual will be use for training purposes. The information below summarizes the major steps for measurements.

1. Before leaving for the field the instrument and the probes are fully inspected for fouling and damage. All probes are calibrated in the laboratory before field deployment. Battery levels for both the model 6600 sonde unit and the YSI 650 MDS data display/logger are checked to ensure full charges.
2. Before deployment the units are kept out of direct sunlight and the power is turned on for at least 15 minutes. At each site the unit is calibrated for dissolved oxygen, depth and zero fluorescence as directed in the manual.
3. The calibration cup is replaced with the sensor guard cover before deployment and all connections are checked.
4. The datalogger is set to record at measurement intervals between 3 and 5 seconds. After allowing the unit to equilibrate at the surface for 2-5 minutes the 650 MDS is set to start logging and the unit is slowly lowered into the water at a slow rate so readings are logged at approximately every 0.1 meters. The descent rate can be slowed when the 650 MDS display unit indicates the sonde is traveling through the thermocline to compensate for a slower response by the DO and pH sensors.
5. As the bottom is approaching care should be taken to limit movement on the boat so as not to allow the unit to disturb the bottom until all readings are taken.
6. If the bottom is disturbed raise the sonde slightly and allow for the water to settle, watch the display for indication that this has happened.
7. Raise the sonde at the same rate as it was lowered to collect a second set of profile readings from the sonde.
8. When the sonde is raised to the surface replace the sensor guard cover with the calibration cup and store the unit and datalogger out of direct sunlight.
9. Upon returning to the lab take measurements of pH, redox and conductivity standards and note any discrepancies in the equipment logbooks. Wash the units and cables thoroughly before storage.
10. Data can be downloaded from the data logger using the Ecowatch Software.

Written by Jeff Schloss and Bob Craycraft
Last Updated on December 15, 2015

B.9. Lamottee Model 2020 and 2020e Portable Turbidimeter SOPs

LaMotte 2020 Turbidimeter SOP

Calibrating the LaMotte 2020 Turbidimeter

The turbidity instrument's design provides for long-term stability and minimizes the need for frequent calibration. However, the turbidimeter should be calibrated using AMCO™ primary standards at the frequency specified in a project specific Quality Assurance Project Plan. The LaMotte
2020 Turbidimeter will be calibrated as follows:

1. Select a LaMotte AMCO™ 2020 Standard in the range of the samples
   to be tested. *Note: Only use LaMotte* AMCO™ *Standards specific to*
   the 2020 Turbidimeter. Contact LaMotte for replacement standards.
2. Fill a turbidity tube with the standard, cap, wrap a lint-free cloth (Kimwipe) around the tube and wipe clean with circular motions.
3. Open the lid of the Turbidimeter. Align the indexing arrow mark on the tube with the indexing arrow mark on the Turbidimeter and insert the tube into the chamber.
4. Close the lid. Push the **READ** button. If the displayed value is not the same as the value of the reacted standard (within the specification limits), continue with calibration procedure.
5. Push the **CAL** button for 5 seconds until the **CAL** is displayed. Release the button and the display will flash. Adjust the display with the "up" and "down" arrow buttons until the value of the standard is displayed.
6. Push the **CAL** button again to save the calibration. The 2020 display will stop flashing. Calibration is now complete.
7. Turn the unit off by holding the **READ** button down for at least 1 second, or proceed to measure the test samples using the following procedure. *Note: The calibration procedure should be followed once a week, or more often as required by regulations and laws for compliance monitoring. The calibration of the Turbidimeter is independent of the operating mode.*

Measuring Turbidity using the LaMotte 2020 Portable Turbidimeter

Important: The LaMotte 2020 Portable Turbidimeter must be on a flat, stable surface to accurately take a reading. It might be easiest to leave the meter in its case for these testing procedures. Do not hold the meter while taking a reading! Prior to analyzing the water samples, the sampling bottle should be allowed to warm up to room temperature to avoid condensation on the turbidity tubes that will bias the results.

STEP 1
Collect a water sample in clean, 250 milliliter amber sampling bottle by pointing the bottle upstream, submersing the bottle to a depth of four inches and allowing the bottle to fill to the neck. Remove the bottle from the stream, cap the bottle immediately, place the sample bottle in a warm location and let the water temperature increase to room temperature.

STEP 2
Invert the 250 milliliter amber bottle "gently" five times to ensure the sample is well mixed. Fill a turbidity tube to the neck by carefully pouring the sample down the side of the tube to avoid the creation of bubbles. Air bubbles

and/or condensation will result in inaccurate readings and thus you should repeat Step 2 should you encounter either air bubbles or condensation.

STEP 3
While holding the turbidity tube by the black cap, dry the glass cell with a lint free Kimwipe towel. The drying procedure is best accomplished by placing the Kimwipe around the glass turbidity tube and rotating the tube clockwise while holding onto the black cap. This method will both dry the glass turbidity tube and will remove any smears that might otherwise result in inaccurate readings.

STEP 4
Turn the meter on by pressing the "READ" button located at the bottom center of the key pad.

STEP 5
Insert the turbidity tube and make sure that the bottom point of the triangle on the tube lines up with the arrow in front of the turbidity tube holder. Close the lid.

STEP 6
Press the "READ" button and the turbidity will display on the LCD display. Record the displayed value.

STEP 7
Remove the turbidity tube from the meter. Dump the water from the tube.

STEP 8
Repeat steps 2 through 6 and record the second value on your datasheet.

STEP 9
Remove the turbidity tube and rinse the tube with distilled water.

STEP 10
Place the turbidity tube and the turbidity meter in the carrying case. The turbidity meter will automatically turn off following two minutes of non-use.

LaMotte 2020e Turbidimeter SOP

Calibrating the LaMotte 2020e Turbidimeter
The turbidity instrument's design provides for long-term stability and minimizes the need for frequent calibration. However, the turbidimeter should be calibrated using AMCO^{TM} primary standards at the frequency specified in a project specific Quality Assurance Project Plan. The LaMotte 2020 Turbidimeter will be calibrated as follows:

1. Press ***** | **ON** to turn the Turbidimeter on.
2. Press ***** | **OK** to select **Measure**.
3. Rinse a clean turbidity tube three times with the blank.
   Below 1 NTU – The Turbidimeter should be blanked with a 0 NTU Primary Standard or prepared turbidity-free (< 0.1 NTU) water. For the most accurate results, use the same tube for the blank and the sample.
4. Fill the tube to the fill line with the blank. Pour the blank down the inside of the tube to avoid creating bubbles. Cap the tube.
5. Wrap a lint-free cloth (Kimwipe) around the tube, wipe clean with circular motions and put on a positioning ring with the indexing notch tube.
6. Open the Turbidimeter lid. Insert the tube into the chamber. Align the index notch on the positioning ring with the index arrow on the meter.

Close the lid.

7. Press *** | OK** to select **Scan Blank**. Remove the tube.
8. Rinse a clean turbidity tube, or the blanking tube, three times with the standard. Avoid spilling standard on the outside of the tube. **Important:** While the tube is inverted, wipe the lip of the tube to remove droplets of liquid that may be present. This will prevent liquid from being trapped under the ring when the tube is returned to an upright position.
9. Fill the tube to the fill line with the standard. Pour the standard down the inside of the tube to avoid creating bubbles. Cap the tube.
10. Wrap a lint-free cloth (Kimwipe) around the tube and wipe clean with circular motions.
11. Open the turbidimeter lid. Insert the tube into the chamber. Align the index notch on the positioning ring with the index arrow on the meter. Close the lid.
12. Press *** | OK** to select **Scan Sample**.
13. When scan completes, press the "down" arrow and then press *** | OK** to select **Calibrate**.
14. Use the "up" and "down" arrows to change the highlighted digits on the display to match the concentration of the turbidity standard. Press *** | OK** to accept a digit and move to the next digit.
15. When the value on the display matches the concentration of the turbidity standard, press *** | OK** to select **Set**. Or press the "down" arrow and then *** | OK** to return the Turbidimeter to the default setting.
16. Press *** | OK** to proceed to Turbidity analysis. Press **OFF** to turn the meter off or press the "left" arrow to exit to a previous menu or make another menu selection.
17. Proceed to measure the test samples using the following procedure. *Note: The calibration procedure should be followed once a week, or more often as required by regulations and laws for compliance monitoring. The calibration of the Turbidimeter is independent of the operating mode.*

Measuring Turbidity using the LaMotte 2020e Portable Turbidimeter

Important: The turbidimeter must be on a flat, stable surface to accurately take a reading. It might be easiest to leave the meter in its case for these testing procedures. Do not hold the meter while taking a reading! Prior to analyzing the water samples, the sampling bottle should be allowed to warm up to room temperature to avoid condensation on the turbidity tubes that will skew the results.

STEP 1
Collect a water sample in clean, 250 milliliter amber sampling bottle by pointing the bottle upstream, submersing the bottle to a depth of four inches and allowing the bottle to fill to the neck. Remove the bottle from the stream, cap the bottle immediately, place the sample bottle in a warm location and let the water temperature increase to room temperature.

STEP 2
Turn on meter by pressing the "on" button located at the bottom right of the key pad.

STEP 3
Select "measure" on the display screen by pressing the */OK button in the center.

STEP 4
Prompted to "scan blank", fill glass turbidity tube with distilled water. Fill the turbidity tube to the neck by carefully pouring the water down the side of the tube to avoid the introduction of bubbles. Air bubbles and/or condensation will result in inaccurate readings. Repeat Step 4 should you encounter either air bubbles or condensation.

STEP 5
While holding the turbidity tube by the black cap, dry the glass tube with a lint free Kimwipe towel. The drying procedure is best accomplished by placing the Kimwipe around the glass tube and rotating the tube clockwise while holding onto the black cap. This method will both dry the glass turbidity tube and will remove any smears that might otherwise result in inaccurate readings.

STEP 6
Insert the tube into the cell holder and make sure that the vertical white line on the glass turbidity tube lines up with the arrow in front of the turbidity tube holder. Close the lid and press */OK button. Wait for the meter to calibrate.

STEP 7
When prompted to "insert sample", invert the 250 milliliter amber bottle "gently" five times to ensure the sample is well mixed. Fill the turbidity tube to the neck by carefully pouring the sample down the side of the tube to avoid the creation of bubbles. Air bubbles and/or condensation will result in inaccurate readings and thus you should repeat Step 4 should you encounter either air bubbles or condensation.

STEP 8
Insert the turbidity tube into the turbidity tube holder and make sure that the vertical white line on the glass tube lines up with the arrow in front of the tube holder. Close the lid.

STEP 9
Press the */OK button and turbidity will display on the LCD screen. Record the displayed value. Repeat steps 7 and 8 and record the second value on your datasheet.

STEP 10
Remove the turbidity tube and rinse the tube with distilled water.

STEP 11
Place the turbidity tube and the turbidity meter in the carrying case. Turn turbidity meter off by pressing "off" button. *Note: the Lamotte 2020e does not automatically turn off when not in use.*

Written by Bob Craycraft and Jeff Schloss
Last Updated on February 19, 2007

B.10. Yellow Spring Instruments (YSI) Professional Plus Meter SOPs

Calibrating the YSI Professional Plus Multi-parameter Meter

Dissolved Oxygen Calibration

Remember to turn the YSI Professional Plus meter on at least fifteen minutes before calibration to warm up. The calibration/storage chamber should always be checked to make sure the yellow sponge is moist prior to the 15 minute warm-up period. If necessary, carefully add approximately 20 milliliters of tap water onto the sponge in the calibration/storage chamber, turn the calibration chamber over to allow any excess water to drain out, and replace the calibration/storage chamber prior to the 15 minute warm-up period. The wet sponge creates a 100% water saturated air environment for the probe which is essential for an accurate dissolved oxygen calibration. You should also inspect the dissolved oxygen membrane cap prior to proceeding to ensure that there are no air bubbles in the cap. Membranes should be replaced every three months, or whenever an air bubble forms in the cap. To install a new membrane on the polarographic dissolved oxygen probe:

1. Remove the probe from the calibration/storage chamber.
2. Unscrew and remove the old membrane cap.
3. Thoroughly rinse the sensor tip with distilled water.
4. Prepare the electrolyte according to the directions on the KCl solution bottle.
5. Hold the membrane cap and fill it at least ½ full with the electrolyte solution.
6. Screw the membrane cap onto the probe moderately tight. A small amount of electrolyte should overflow.
7. Screw on the probe calibration/storage chamber.

The YSI Professional Plus meters **must** be calibrated before making dissolved oxygen measurements as described below:

1. Make sure the membrane cap is free of air bubbles and, if necessary, remove and refill the cap before proceeding using the procedure above.
2. Assure the sponge is wet, the membrane cap is free of water droplets and insert the probe into the calibration/storage chamber.
3. Loosen the calibration/storage chamber two threads (two 360^{o} rotations) to allow venting.
4. Turn the meter on and allow the meter to warm up for 15 minutes.
5. Press the “Cal” button.
6. Use the up and down arrow buttons to scroll through the calibration options, highlight “DO” and press the “Enter” button.
7. Use the up and down arrow buttons to scroll through the Oxygen Calibration options, select “DO %” and press the “Enter” button.
8. Allow the dissolved oxygen percent saturation reading to stabilize and press the enter key.
9. If your name appears after “User Field 1:” press the “Cal button to complete the calibration procedure. Otherwise, press the “ENTER” button and proceed to the next step.
10. Use the up and down arrow buttons to scroll through the list of laboratory/field technicians, select your name, and press the “Cal” button. *Note: this step will document the technician who calibrated the instrument.*
11. You are ready to collect dissolved oxygen data.

Specific Conductivity Calibration

The specific conductivity is factory calibrated and is highly stable. However, the specific conductivity should be checked against factory calibration standards and re-calibrated at the frequency specified in a project specific Quality Assurance Project Plan (QAPP) to assure the collection of the most accurate data possible. Specific Conductivity shall be calibrated using the following protocol:

1. Remove the calibration/storage chamber and rinse the probe three times with de-ionized water.
2. Screw the protective field sensor guard onto the probe assembly.

3. Rinse the sensor guard three times with de-ionized water.
4. Fill two clean 250 milliliter polypropylene wide mouth sampling bottles, labeled number one and number two, with 100 *u*S/cm calibration standard.
5. Submerse the conductivity probe into the conductivity calibration standard bottle labeled number one.
6. Raise and lower the conductivity probe into the conductivity calibration standard bottle, labeled number one, five additional times.
7. Remove the conductivity probe from the calibration solution.
8. Submerse the conductivity probe into the conductivity calibration standard bottled, labeled number two.
9. Raise and lower the conductivity probe into the conductivity calibration standard bottle, labeled number two, five additional times.
10. Press the "Cal" button.
11. Use the up and down arrow buttons to scroll through the calibration options, highlight "Conductivity" and press the "Enter" button.
12. Use the up and down arrow buttons to scroll through the conductivity calibration options, select "Sp. Conductance" and press the "Enter" button.
13. Use the up and down arrows to scroll through the Sp. Conductivity calibration options and press the "Enter" button when "SPC-*u*Skm" is selected.
14. When the reading stabilizes, press the "Enter" button.
15. If your name appears after "User Field 1:" press the "Cal button to complete the calibration procedure. Otherwise, press the "ENTER" button and proceed to the next step.
16. Use the up and down arrow buttons to scroll through the list of laboratory/field technicians, select your name, and press the "Cal" button. *Note: this step will document the technician who calibrated the instrument and will document the calibration information.*
17. You are ready to collect specific conductivity data.

pH Calibration

The pH calibration should be performed daily to assure the collection of the most accurate data possible. The pH shall be calibrated using the following protocol:

1. Remove the calibration/storage chamber and rinse the probe three times with de-ionized water.
2. Screw the protective field sensor guard onto the probe assembly.
3. Rinse the sensor guard three times with de-ionized water.
4. Fill two clean 250 milliliter polypropylene wide mouth sampling bottles, labeled number one and number two, with pH 7.01 calibration standard.
5. Submerse the pH probe into the pH calibration standard bottle labeled number one.
6. Raise and lower the pH probe into the pH calibration standard bottle, labeled number one, five additional times.
7. Remove the pH probe from the calibration solution.
8. Submerse the pH probe into the conductivity calibration standard bottled, labeled number two.
9. Raise and lower the pH probe into the pH calibration standard bottle, labeled number two, five additional times.
10. Press the "Cal" button.
11. Use the up and down arrow buttons to scroll through the calibration options, highlight "ISE1 (pH)" and press the "Enter" button.
12. When the pH reading stabilizes, press the "Enter" button.
13. Raise the pH probe out of the bottle and rinse the sensor guard and probe three times with de-ionized water.
14. Fill two clean 250 milliliter polypropylene wide mouth sampling bottles, labeled number one and number two, with pH 4.01 calibration standard.
15. Submerse the pH probe into the pH calibration standard bottle labeled number one.
16. Raise and lower the pH probe into the pH calibration standard bottle, labeled number one, five additional times.
17. Remove the pH probe from the calibration solution.
18. Submerse the pH probe into the conductivity calibration standard bottled, labeled number two.

19. Raise and lower the pH probe into the pH calibration standard bottle, labeled number two, five additional times.
20. Leave the probe submersed and when the pH measurement stabilizes press the "Enter" button.
21. Press the "Cal" button to indicate you have completed the pH calibration process.

Oxidation Reduction Potential (ORP) Calibration

The ORP calibration should be performed daily to assure the collection of the most accurate data possible. The ORP probe shall be calibrated using the following protocol:

1. Remove the calibration/storage chamber and rinse the ORP probe three times with deionized water.
2. Screw the protective field sensor guard onto the probe assembly.
3. Rinse the sensor guard three times with de-ionized water.
4. Fill two clean 250 milliliter polypropylene wide mouth sampling bottles, labeled number one and number two, with Zobell Solution.
5. Submerse the ORP probe into the Zobell Solution bottle labeled number one.
6. Raise and lower the ORP probe into the Zobell solution bottle, labeled number one, five additional times.
7. Remove the ORP probe from the Zobell solution.
8. Submerse the ORP probe into the Zobell solution bottle, labeled number two.
9. Raise and lower the ORP probe into the Zobell solution bottle, labeled number two, five additional times.
10. Press the "Cal" button.
11. Use the up and down arrow buttons to scroll through the calibration options, highlight "ISE2 (ORP)" and press the "Enter" button.
12. Use the up and down arrow buttons to select "Accept Calibration".
13. When the ORP reading stabilizes, press the "Enter" button.
14. Press "ESC" when the display reads "Calibration Successful".
15. The ORP has been calibrated and is ready for use.

Chloride Calibration

The chloride calibration should be performed daily to assure the collection of the most accurate data possible. Note: recent submersion in a pH buffer may slow the chloride probe response. Thus, the chloride probe will soak in chloride buffer for 30 minutes before proceeding with the chloride calibration. The chloride probe shall be calibrated using the following protocol:

1. Remove the calibration/storage chamber and rinse the probe three times with de-ionized water.
2. Screw the protective field sensor guard onto the probe assembly.
3. Rinse the sensor guard three times with de-ionized water.
4. Fill two clean 250 milliliter polypropylene wide mouth sampling bottles, labeled number one and number two, with 10.0 milligram per liter chloride calibration standard.
5. Submerse the chloride probe into the chloride calibration standard bottle labeled number one.
6. Raise and lower the chloride probe into the chloride calibration standard bottle, labeled number one, five additional times.
7. Remove the chloride probe from the calibration solution.
8. Submerse the chloride probe into the chloride calibration standard bottled, labeled number two.
9. Raise and lower the chloride probe into the chloride calibration standard bottle, labeled number two, five additional times.
10. Press the "Cal" button.
11. Use the up and down arrow buttons to scroll through the calibration options, highlight "ISE2 (Cl)" and press the "Enter" button.
12. When the chloride reading stabilizes after a minimum of five minutes submersion in the standard, press the "Enter" button.
13. Raise the probe out of the bottle and rinse the sensor guard and probe three times with deionized water.
14. Fill two clean 250 milliliter polypropylene wide mouth sampling bottles, labeled number one and number two, with 1000 milligram per liter calibration standard.
15. Submerse the chloride probe into the chloride calibration standard bottle labeled number one.

16. Raise and lower the chloride probe into the chloride calibration standard bottle, labeled number one, five additional times.
17. Remove the chloride probe from the calibration solution.
18. Submerse the chloride probe into the chloride calibration standard bottled, labeled number two.
19. Raise and lower the chloride probe into the chloride calibration standard bottle, labeled number two, five additional times.
20. Leave the probe submersed a minimum of five minutes and when the chloride value stabilizes press the "Enter" button.
21. The chloride probe has been calibrated and is ready for use.

Measuring Temperature, Dissolved Oxygen, Specific Conductivity, Oxidation Reduction Potential, pH and Chloride

STEP 1
Turn the instrument on by pressing the green ON/OFF button if it wasn't already on and allow the meter to warm up for at least 15 minutes. *Note: make sure the sponge is wet and proceed through the dissolved oxygen calibration procedure (previously described) immediately prior to use.*

STEP 2
Press the "Enter Key" while the "Log One Sample" text is highlighted.

STEP 3
Use the up and down keys to select "Folders". Press the "Enter" key.

STEP 4
Use the up and down arrow keys to select "Add New". Press the "Enter" key.

STEP 5
Use the up and down arrow keys to select letters/numbers to create a new project folder that includes watershed and stream identification information. Press the "Enter" key to accept the folder name.

STEP 6
Use the up and down arrow keys to select "Sites". Press the "Enter" key.

STEP 7
Use the up and down arrow keys to select "Add New". Press the "Enter" key.

STEP 8
Use the up and down arrow keys to select letters/numbers to create a new site name. Press the "Enter" key to accept the site name.

STEP 9
Repeat steps 2 through 8 until ID information exists for all sampling locations to be sampled during this sampling trip.

STEP 10
Remove the probe from its plastic storage chamber and screw on the protective field sensor guard.

STEP 11
Submerse the probe to a depth of four inches into the stream water. Raise and lower the probe into the water five more times to assure the probe is well rinsed of any residual debris. Hold the probe at a depth of 4 inches below the water surface.

STEP 12
Gently jiggle the probe in the water for a minimum of two minutes. After the two minute equilibration period and the stabilization of water quality measurements (i.e. temperature, dissolved oxygen, SPCD, pH, oxidation reduction potential and chloride) proceed to STEP 13.

STEP 13
When you have achieved stable readings, while jiggling the probe, press the "Enter" button while "Log Now" is highlighted.

STEP 14
Use the up and down arrow buttons to select the proper Folder and Site information. *Note: you should continue to jiggle the probe until the data have been logged.*

STEP 15
Press the "Enter" button while "Log Now" is selected. A "sample logged" message will appear at the bottom of the screen to indicate that data have been recorded.

STEP 16
Remain at the sampling site, raise the probe out of the water and submerse it to a depth of 4 inches. Raise and lower the probe to a depth of 4 inches five additional times.

STEP 17
Gently jiggle the probe in the water for a minimum of two minutes. After the two minute equilibration period and the stabilization of water quality measurements (i.e. temperature, dissolved oxygen, SPCD, pH, oxidation reduction potential and chloride) proceed to STEP 18.

STEP 18
When you have achieved stable readings, while jiggling the probe, press the "Enter" button while "Log Now" is highlighted.

STEP 19
Use the up and down arrow buttons to select the proper Folder and Site information. *Note: you should continue to jiggle the probe until the data have been logged.*

STEP 20
Press the "Enter" button while "Log Now" is selected. A "sample logged" message will appear at the bottom of the screen to indicate that data have been recorded.

STEP 21
Place the gray plastic sleeve over the probe housing and proceed to the next sampling station.

STEP 22
Repeat steps 11 through 21 at each subsequent sampling location.

STEP 23
Remove the protective field sensor guard and screw the plastic calibration/storage chamber over the probe assembly. Note: failure to place the plastic calibration/storage chamber over the probe assembly may result in the premature failure of the probes.

B.11. Yellow Spring Instruments (YSI) EXO2 Meter SOPs

Reference YSI EXO Users Manual (August 2014 REV E).

As the use of this instrument is technical in nature all field crew will need to be trained and certified to use this instrument for any project. An abridged copy of the technical manual will be use for training purposes. The information below summarizes the major steps for measurements.

1. Before leaving for the field the instrument and the probes are fully inspected for fouling and damage. All probes are calibrated in the laboratory before field deployment. Battery levels for both the EXO2 Sonde and the EXO Handheld data display/logger are checked to ensure full charges.
2. Before deployment the units are kept out of direct sunlight and the power is turned on for at least 15 minutes. At each site the unit is calibrated for dissolved oxygen, depth and zero fluorescence as directed in the manual.
3. The calibration cup is replaced with the sensor guard cover before deployment and all connections are checked.
4. The datalogger is set to record at measurement intervals between 3 and 5 seconds. After allowing the unit to equilibrate at the surface for 2-5 minutes the data display/logger is set to start logging and the unit is slowly lowered into the water at a slow rate so readings are logged at approximately every 0.1 meters. Lowering can be slowed when the display unit indicates the sonde is traveling through the thermocline to allow for a slower response by the DO and pH sensors.
5. Select the “capture data” key and then press the #6 (change active site) key on the keypad. Scroll through the list of preloaded options until you reach the proper sampling location and select “done”. Press the #4 key to begin logging onto the selected file. Slowly lower the Sonde to collect measurements as indicated above.
6. As the bottom is approaching care should be taken to limit movement on the boat so as not to allow the unit to disturb the bottom until all readings are taken.
7. If the bottom is disturbed raise the sonde slightly and allow for the water to settle, watch the display for indication that this has happened.
8. Raise the sonde at the same rate as it was lowered to collect a second set of profile readings from the sonde.
9. When the sonde is raised to the surface replace the sensor guard cover with the calibration cup and store the unit and datalogger out of direct sunlight.
10. Upon returning to the lab take measurements of pH, redox and conductivity standards and note any discrepancies in the equipment logbooks. Wash the units and cables thoroughly before storage.
11. Data can be downloaded from the data logger using the KOR Software.

Written by Jeff Schloss and Bob Craycraft
Last Updated on December 15, 2015

B.12. Yellow Spring Instruments (YSI) ProODO and ProSOLO Meter SOPs

Make sure your handheld instrument, ProODO or ProSOLO, has a sufficient charge to conduct the water quality sampling; a battery charge in excess of 60% will be deemed sufficient to conduct the water quality sampling. Both handheld instruments are powered by a rechargeable lithium-ion battery pack. When the battery requires charging, plug the micro USB connector into the handheld and charge the instrument using an AC power adaptor, computer USB connector or external USB battery pack.

Calibrating the YSI ProODO and ProSOLO Meters

Dissolved Oxygen Calibration

The calibration/storage chamber should always be checked to make sure the yellow sponge is moist prior to calibrating the optical dissolved oxygen sensor. If necessary, carefully add approximately 20 milliliters of tap water onto the sponge in the calibration/storage chamber, turn the calibration chamber over to allow any excess water to drain out, and replace the calibration/storage chamber. The wet sponge creates a 100% water saturated air environment for the probe which is essential for an accurate dissolved oxygen calibration. You should also inspect the dissolved oxygen cap prior to proceeding to ensure that there are no cap scratches or abrasions that may interfere with accurate oxygen measurements. Membranes should be replaced every two years on the ProSOLO cable (every year on the ProODO cable), or whenever notable scratches or abrasions are evident. To install a new optical dissolved oxygen cap:

1. Remove the old sensor cap assembly from the probe by grasping the probe body with one hand and rotating the sensor cap counterclockwise until it is completely free. *Note: do not use any tools for this procedure*.
2. Carefully remove the o-ring and replace it with the new o-ring included with the replacement sensor cap. *Note: do not use any tools to remove the o-ring*.
3. Ensure the newly installed o-ring is clean. If necessary, wipe the o-ring with a Kimwipe to remove impurities.
4. Apply a thin coat of o-ring lubricant to the newly installed o-ring. Remove any excess lubricant from the o-ring with a Kimwipe.
5. Make sure the end bare end of oxygen probe is completely dry. Gently wipe down the clear surface, where the cap will be positioned, with a Kimwipe to remove any dust and debris.
6. Remove the new oxygen sensor cap from the hydrated container and thread the new sensor cap onto the probe assembly until it is finger-tight. The o-rings should be compressed between the sensor cap and probe. *Do not use any tools for the installation process as it is imperative that the cap is not over-tightened.*
7. place the probe into the calibration sleeve that contains a moist yellow sponge.

Once a new oxygen cap has been installed new calibration coefficients have to be entered into your handheld instrument using the following procedure:

1. Connect the cable assembly to the handheld instrument.
2. Press the “probe” key and use the arrow keys to select the “setup” option.
3. Press the “enter” key to enter the setup mode.
4. Use the arrow keys to select the “ODO” option.
5. Press the “enter” key to enter ODO setup.
6. Scroll down to “Sensor Cap Coefficients” using the arrow keys.
7. Press the “enter” key to display the eight coefficient values that have to be entered into the handheld instrument.
8. Scroll to K1 (the first coefficient) using the arrow keys.
9. Press the “enter” key to allow you to enter the new coefficient.
10. Enter the first (K1) coefficient displayed on the YSI ODO Sensor Cap Instruction Sheet, that came with your newly installed oxygen cap.

11. Use the arrow keys to select the letters/numbers that coincide with the values printed on your instruction sheet.
12. Once you have updated the values, press the “enter” button.
13. Repeat Seps 8 through 11 for the seven additional coefficient values (K2 through K7 and KC).
14. Once you have updated the eight coefficient values, use the arrow keys to highlight “update coefficients”.
15. Press the “enter” button and a message will appear that warns you that you will be overwriting the current sensor cap coefficients.
16. Scroll to “yes”, to acknowledge that you want to overwrite the coefficients.
17. Press the "enter” key to accept the new sensor cap coefficients. *Note: if an error appears, you likely entered one or more of the coefficients incorrectly. Review your entries and overwrite the coefficients (steps 8 through 16).*

The YSI Professional Plus meters **must** be calibrated before making dissolved oxygen measurements as described below:

1. Make sure the membrane cap is free of water droplets.
2. Place the probe in the moist calibration sleve and let the chamber and probe equilibrate for 5 minutes.
3. Press the “Cal” button.
4. Scroll to “ODO” using the arrow keys.
5. Press the "enter” key.
6. Scroll to “DO %” using the arrow keys.
7. Press the "enter” button. The screen should be in calibration mode and “accept calibration” should be highlighted.
8. View the calibration graph and make sure the readings are stable.
9. Press the “enter” key to accept the calibration. The LCD should display “calibration successful” at the bottom of the screen.
10. You are ready to collect dissolved oxygen data.

Specific Conductivity Calibration (ProSOLO only)

The specific conductivity is factory calibrated and is highly stable. However, the specific conductivity should be checked against factory calibration standards and re-calibrated at the frequency specified in a project specific Quality Assurance Project Plan (QAPP) to assure the collection of the most accurate data possible. Specific Conductivity shall be calibrated using the following protocol:

1. Press the “Cal” button.
2. Scroll to “Conductivity” using the arrow keys.
3. Press the “enter” button.
4. Scroll to “Sp. Conductance” using the arrow keys.
5. Press the “enter” botton.
6. Scroll to “calibration value” using the arrow keys.
7. Press the “enter” botton.
8. Use the arrow keys to type in the specific conductivity calibration value of 100.0 uS/cm.
9. Press the “enter” botton.
10. Remove the calibration/storage chamber and rinse the probe three times with de-ionized water.
11. Fill two clean 250 milliliter polypropylene graduated cylinders, labeled number one and number two, with approximately 200 milliliters of 100 *u*S/cm calibration standard.
12. Submerse the conductivity probe into the graduaded cylinder labeled number one.
13. Raise and lower the conductivity probe into the conductivity calibration standard bottle, labeled number one, five additional times.
14. Remove the conductivity probe from the calibration solution.
15. Submerse the conductivity probe into the graduated cylinder labeled number two.
16. Raise and lower the conductivity probe into the graduated cylinder, labeled number two, five additional times.
17. Scroll to “accept calibration” using the arrow keys.

18. View the calibration graph and make sure the readings are stable.
19. Press the "enter" button.
20. Press the "Esc" button to return to the main menu. You are now ready to collect specific conductivity measurements.

Measuring Temperature, Dissolved Oxygen and Specific Conductivity

STEP 1
Turn the instrument on by pressing the green power button if the instrument was not already on. Allow the meter to warm up for at least 5 minutes. *Note: make sure the sponge is wet and proceed through the dissolved oxygen calibration procedure (previously described) immediately prior to use.*

STEP 2
Press the "File" button.

STEP 3
Use the up and down arrow keys to scroll to "Site".

STEP 4
Press the "Enter" button.

STEP 5
Use the up and down arrow keys to select "Add New".

STEP 6
Press the "Enter" button.

STEP 7
Use the up and down arrow keys to select letters/numbers to create a new project folder that includes watershed and stream identification information.

STEP 8
Press the "Enter" button to accept the folder name.

STEP 9
Press the "enter" button to return to the main menu.

STEP 10
Remove the probe from the plastic storage sleve.

STEP 11
Submerse the probe to a depth of 12 inches into the lake/stream water. Raise and lower the probe into the water five more times to assure the probe is well rinsed of any residual debris. Hold the probe at a depth of 12 inches below the water surface and view the temperature, oxygen and (if using the ProSOLO) the specific conductivity values.

STEP 12
After a minimum of 30 seconds, once you have achieved stable readings, press the "Enter" button. *Note: "loading" will display on the bottom of the LCD display, followed by "sample logged", to verify the measurements have been recorded.*

STEP 13

Lower the probe to a depth of 0.5 meters, hold the probe at that depth, and view the temperature, oxygen and (if using the ProSOLO) the specific conductivity values.

STEP 14
After a minimum of 30 seconds, once you have achieved stable readings, press the “Enter” button.

STEP 15
Repeat steps 11 through 21 at each subsequent 0.5 meter increment until you have reached the lake bottom.

STEP 16
Place the protective sleve, containing the moist yellow sponge, over the probe. Note: failure to place protective sleve over the probe assembly may result in the premature failure of the oxygen sensor and oxygen calibration delays in the future.

Written by Jeff Schloss and Bob Craycraft
Last Updated on March 9, 2021

Appendix C

Standard Operating Procedures for PAR Field Measurements
UNH Center for Freshwater Biology

Updated 5/21/06 by Jeff Schloss and Bob Craycraft

LI-1400 DataLogger PAR Measurements

Standard Operating Procedures

Overview

The LI-1400 DataLogger and calibrated quantum sensors provide the capability to quantify Photosynthetically Active Radiation (PAR) both above and below the water surface. The 'air' sensor remains above the water and quantifies downwelling radiance from the sun each time a discrete measurement is taken; this is most often used to normalize readings taken over several minutes to a constant downwelling value. The 'underwater' sensor is deployed on a frame that is lowered into the water. This sensor is generally used to measure a profile of in-water irradiance versus depth so as to estimate the diffuse attenuation coefficient (K_d), a measure of the rate at which photosynthetically active radiation is attenuated as it passes down through the water-column.

In general, the attenuation of light is exponential versus depth. To obtain K_d from a series of light readings with depth, a series of measurements at at least 8 depths is desired (in shallow waters this may not be possible). One obtains K_d by making a linear regression of sample depth versus ln (PAR) and calculating 1/(slope of the regression). Additional information of interest includes the percent of surface radiation reaching the bottom.

Estimates of K_d are considered robust if the r^2 of the regression is >0.95 (generally >0.98). The precision of the method is estimated by taking 3 complete profiles sequentially and calculating the standard error (SE) of the measurement. The SE should be less than 10%.

Before First Sampling of the Day

1. Ensure that the sensors are securely attached to their frames and confirm that the calibrations factors stored in the DataLogger are correct for the sensors in use.
2. Hook up the Underwater BNC connector to Channel I1 labeled "underwater".
3. Hook up the Air BNC connector to Channel I2 labeled "air".
4. Turn the DataLogger 'ON'.
5. Under View, press 'ENTER' to view new data.
6. The first view should say "I1I" which corresponds to the underwater connector. "I2I" corresponds to the air connector.
7. Switch to view I2I, take the cap off of the air sensor, check the reading then cover the sensor with your hand to confirm the reading changes (the reading should decrease with a decrease in light).
8. Switch to view I1I, take the protective covering off of the underwater sensor, check the reading and then cover the sensor with your hand to confirm the reading changes (again the reading should decrease).

At Each Station

1. Turn on the DataLogger.
2. Take out the respective data sheet for the site. Record the time when the underwater sensor is put in the water.
3. Lower the sensor to 10cm. Allow the reading to stabilize (1-2 seconds) and then press 'ENTER'. This logs the data into the DataLogger. Cross off 10cm (and each subsequent depth for which you log data into the DataLogger) on the data sheet.
4. Lower the sensor to the next depth. In shallow areas, record measurements every 25cm as marked on the cable. In deep and/or clearer water areas the sensor can be lowered every 50cm. At least 6-8 depths should be recorded in the DataLogger for each station.

5. When (If) the sensor reaches bottom, write the bottom depth (approximate using the depth markers) on the datasheet and press 'ENTER' to log data into the DataLogger.
6. Raise the underwater sensor out of the water and put the protective cover on. Put the cap on the air sensor also. Turn the DataLogger 'OFF' until reaching the next station.

At End of Sampling

1. Unplug the BNC connectors from the LI-1400.
2. Rinse underwater sensor, frame and cable with freshwater and let dry before storage.

Download Data to Excel in the Laboratory

1. After returning to the lab the data should be retrieved from the DataLogger.
2. Attach the DataLogger to the computer using the serial cable.
3. Open the LI-1400 program and then turn the DataLogger on.
4. Under the remote menu click on 'CONNECT'. Under the connect window, type '2' next to com port number and click 'CONNECT'.
5. Under the remote menu click 'RECEIVE DATA'. Save the data on the computer.
6. Open Microsoft Excel and then open the file you just saved. The file is a delimited file and click 'FINISH'.
7. Download LiCor Data into a new Excel file and **Save As** MPWAP Raw Light Profile (MMDDYY) where the MMDDYY represents the sampling date.
8. Once you are certain that you have successfully downloaded and saved the data the data in the DataLogger should be cleared from memory. This can be done 2 ways:
    a. On the DataLogger, press the 'FCT' key. Arrow to the right twice till clear memory is in the window. Arrow down to clear all, down to date, down to time, and down to clear memory yes/no. Confirm that "clear memory yes" is in the window and then press 'ENTER'. (This may not clear the memory).
    b. In the LI-1400 program, under the remote menu click 'CLEAR DATABASE'. In the clear database window confirm that all is chosen then click 'OK'.
9. Under the remote menu click 'DISCONNECT'. Unplug the DataLogger from the computer, turn it off, make sure that there is no dirt or salt on it and put it away.

Data Processing

1. Open *Light Profile Master* Excel File and save the file as MPWAPAttenuation (MMDDYY)

2. Cut and paste the raw light data into the appropriate rows/columns in the MPWAP *Light Attenuation (MMDDYY)* file making sure to separate each station as indicated by the station labels in column A.

3. Edit the depths Column E so that they reflect the correct depths at which each of the readings at a particular station was taken.

4. Run individual regression analyses for each of the light profiles as follows (using APL as an example):

    a. Click on **Tools**, **Data Analysis**, **Regression**
    b. Select 'Input Y Range' as the range of measured Depth [m] in Column E
    c. Select 'Input X Range' as the range of calculated Quantum [LN] in Column H
    d. Select 'Output Range' as the Yellow Shaded Block in Column J
    e. Select 'OK'; this should insert the regression statistics to the right of the data [Note: Do not include data for which the Quantum [Raw Water] data is < 0.1]
    f. Save File (intermediate save so as to not lose data)

5. At this point, the Diffuse Attenuation Coefficients ($\mathbf{K_d}$) should have been calculated for each station at which you did the regression. $\mathbf{K_d}$ = 1/x-coefficient from the regression.

6. QA/QC: Examine the regression output data:
    a. Acceptable regressions must have an $R^2 > 0.95$ and, for stations with an optimal number of sample depths (>8) should have an $R^2 > 0.98$.
    b. Examine the Quantum [Raw Water] data. These data should show a continuous decrease with depth except for the odd cases where the Quantum [Air] data increased significantly from the preceding reading (e.g. the passing of a cloud). Highlight any questionable reading by applying an 'Orange' fill to the cells in question.

7. In the event that the $R^2 < 0.95$ and there is a data point at the top or bottom of the profile that is clearly bad (these are the most likely places for this to occur because of surface reflection or sediment resuspension) you may choose to run the regression again, omitting the suspect data. In such cases it is imperative that you make a notation in Column I, just below the $\mathbf{K_d}$ calculation block.

Written by Jeff Schloss and Bob Craycraft
Last Updated on May 21, 2006

Appendix D-1

Quality Assurance Plan
UNH Water Quality Analysis Laboratory
(Natural Resources)

QAPP for the Water Quality Analysis Lab at the University of New Hampshire, Department of Natural Resources, Durham, NH

Prepared by: Jeff Merriam
Date of Last Revision: 1/11/2021
Revised by: Jody Potter

I. Laboratory Organization and Responsibility

Dr. William H. McDowell - Director

Jody Potter – Lab Manager/QA manager. Mr. Potter supervises all activities in the lab. His responsibilities include data processing and review (QA review), database management, protocol development and upkeep, training of new users, instrument maintenance and repair, and sample analysis.

Lisle Snyder & Aneliya Cox – Lab Technicians. Ms Cox and Mr Snyder's responsibilities, with the help of undergraduate employees, include sample analysis, logging of incoming samples, sample preparation (filtering when appropriate), daily instrument inspection and minor maintenance.

All analyses are completed by Aneliya Cox, Lisle Snyder, or Jody Potter, and all data from each sample analysis batch (generally 40-55 samples) is reviewed by Jody Potter for QC compliance. All users are trained by the lab manager and must demonstrate (through close supervision and inspection) proficiency with the analytical instrumentation used and required laboratory procedures.

II. Standard Operating Procedures

Standard Operating Procedures for all instruments and methods are kept in a 3-ring binder in the laboratory, and are stored electronically on the Lab manager's computer. The electronic versions are password protected. SOPs are reviewed annually, or as changes are required due to new instrumentation or method development.

III. Field Sampling Protocols

Sample collection procedures are generally left up to the sample originators, however we recommend the guidelines described below, and provide our field filtering protocol on request.

All samples are filtered in the field through 0.7 um precombusted (5+ hours at 450 C) glass fiber filters (e.g. Whatman GF/F). Samples are collected in acid-washed 60-mL HDPE bottles. We prefer plastic to glass as our preservative technique is to freeze. Sample containers are rinsed 3 times with filtered sample, and the bottle is filled with filtered sample. Samples are stored in the dark and as cool as possible until they can be frozen. Samples must be frozen or refrigerated (SiO2) within 8 hours of sample collection. Once frozen, samples can be stored indefinitely (Avanzino and Kennedy, 1993), although they are typically analyzed within a few months.

After collection and freezing, samples are either hand delivered to the lab, or are shipped via an over-night carrier. Samples arriving in the lab are inspected for frozen contents, broken caps, cracked bottles, illegible labels, etc. Any pertinent information is entered into a password protected database (MS Access).

We provide an electronic sample submission form that also serves as a chain of custody form. Submitters should indicate all analyses required for the samples,

preservation (if any), and sample information (name, date, etc …). They should also indicate project name and a description of the project.

IV. Laboratory Sample Handling Procedures

Samples are given a unique 5-digit code. This code and sample information including name, collection date, time (if applicable), project name, collector, logger, the date received at the WQAL, sample type (e.g. groundwater, surface water, soil solution) and any other miscellaneous information, are entered into a password protected database. From this point through the completion of all analyses, we use the log number to track samples. Log numbers are used on sample run queues, spreadsheets, and when importing concentrations and run information into the database

After samples are logged into the WQAL, they are stored frozen in dedicated sample walk-in freezer or refrigerator located next to the lab. These units log temperature and alarms indicate when they are out of range. The paper print-outs are replaced quarterly and kept on file. Samples from different projects are kept separated in cardboard box-tops, or in plastic bags. Samples that may pose a contamination threat (based on the source or presumed concentration range) are further isolated by multiple plastic bags, or isolation in separate freezer space. This is typically not an issue as we primarily deal with uncontaminated samples.

We do not pay special attention to holding time of samples, as frozen samples are stable indefinitely (Avanzino and Kennedy, 1993). However, we do keep track of the date samples arrive at the WQAL, and can report holding times if necessary. After samples are analyzed they are returned to the project's manager for safe keeping or they

are held for a period of time at the WQAL to allow necessary review and analysis of the data by the interested parties (not from a laboratory QC sense, but from a project specific viewpoint). Once the data is analyzed by the project's manager(s), the samples are returned or disposed of, based on the preference of the project's manager.

Samples that arrive unfrozen, with cracked bottles/caps, or with loose caps, are noted in the database and are not analyzed. These samples are disposed of to prevent accidental analysis. The sample originator is notified (generally via e-mail) of which samples were removed from the sample analysis stream. Similarly, if while in the possession of the WQAL, a sample bottle is broken or improperly stored (e.g. not frozen), the sample is removed and the sample originator is notified.

V. Calibration procedures for chemistry

Calibration curves are generally linear, and are made up of 4-7 points. A full calibration is performed at the beginning of each run (a run is generally 40-60 samples) with a reduced calibration (3-5 points) performed at the end of the run. Occasionally calibration data is best fit with a quadratic equation, and this is used if it best describes the data within a specific run.

Standards are made from reagent grade chemicals (typically Fisher Scientific or ACROS) that have been dried and are stored in a dessicator when required. Working stock solutions are labeled with the content description, concentration, initials of the maker, and the date the stock solution was made. Generally stock solutions are kept less than one week; however some stocks (Br, Na, Cl, C for DOC) can be stored for several

months. Standard solutions are kept for less than one week from the date they were made. Stocks and standards are stored tightly covered, in a dark refrigerator in the lab.

Control charts are prepared and evaluated by the lab manager frequently. However data from each run are looked at within days of analyses. Calibration curves, Laboratory Duplicates, Lab Fortified Blanks (LFB), Lab Fortified Sample Matrices (LFM) and Lab Reagent Blanks (LRB) are reviewed and are checked against known concentrations (where applicable) to ensure QC criteria are met for each run of samples.

VI. Data Reduction, validation, reporting and verification

Data reduction and validation are performed in a spreadsheet (MS Excel). The Raw data page of the spreadsheet lists the date of analysis, user, analysis performed, project, any issues or problems noted with the instrument on that date, and the sample queue and the raw data exported from the instruments. Most raw data is exported as an area or an absorbance value. This data is entered into an Excel QC template to guide the user on how to calculate data and QC summary. A second page (typically named "Calculations") is added to the spreadsheet where known concentrations of standards, check standards and reference solutions are added. The calibration curve(s) is calculated and the concentrations are calculated on this page. Calculated concentrations for all standards, LFB, LFM and IPC are compared to the "known" or prepared values. If these are acceptably close (+/- 10% of the "known") no further changes to the calculated concentrations are made. If there is evidence of drift in the response of the instrument during a run, we try to correct for the drift using the responses from the front end calibration curve and the set of standards analyzed at the end of the run. All reference

solutions and replicates must meet certain QC criteria (described below) for a run to be accepted.

Data are then exported to the WQAL database. Exported information includes the unique 5-digit code, calculated concentration, the analysis date, the user, the filename the raw data and calculations are saved in, and any notes from the run regarding the specific sample. Data are sent to sample originators upon completion of all requested sample analyses and following review by the WQAL lab manager. Generally the data include the 5-digit code, the sample name, collection date, and concentrations, in row-column format. Any information entered into the database can be included upon request. Data transfer is typically via e-mail or electronic medium (CD or floppy disk).

All data corrections are handled by the lab manager. Corrections to data already entered into the database are very infrequent. Typically they involve reanalysis of a sample. In this case, the old data is deleted from the database, and the new value is imported, along with a note indicating that it was re-analyzed, the dates of initial and secondary analysis and the reason for the correction.

Hand written or computer printed run sheets are saved for each run and filed, based on the project and the analysis. Spreadsheet files with raw data and calculations are stored electronically by analysis and date. Information in the database allows easy cross-reference and access from individual samples to the raw data and the runsheets. This provides a complete data trail from sample log-in to completion of analysis.

VII. Quality Control

All analyses conducted at the WQAL follow approved or widely accepted methods (Table 1).

Quality Control Samples (QCS) (from Ultra Scientific or SPEC Certiprep) are analyzed periodically (approximately every 10-15 samples) in each sample analysis batch to assure accuracy. The response/unit concentration is also used to monitor day-to-day variation in instrument performance. A difference from the certified concentration of more than 10% requires further investigation of that run. A difference greater than 15% is failure (unless the average of the two samples is less than 10X the MDL), and results in re-analysis of the entire sample queue, unless there is a very reasonable and supported explanation for the inconsistency. Table 2 lists historical average % recoveries. At least 2 QCS are analyzed on each run.

Standards and reagents are prepared from reagent grade chemicals (typically JT Baker) or from pre-made stock solutions. All glassware is acid washed (10% HCl) and rinsed 6 times with ultra pure-low DOC water (18.2 mega-ohm). All analyses (except CHN) use multi-point calibration curves (4-7) points, which are analyzed at the beginning and the end of each run. A Laboratory Reagent Blank (LRB), Laboratory Fortified Blank (LFB) (a standard run as a sample) and Laboratory Duplicate are analyzed every 10 to 15 samples during each run. At least one Laboratory Fortified Sample Matrix (LFM) is analyzed during each run to insure that sample matrices do not affect method analysis efficiency. Field Duplicates are not required by our lab, and are the responsibility of the specific project's manager.

Laboratory Duplicates must fall within 10% relative percent difference (RPD = abs(dup1-dup2)/average of dup1 and dup 2). A difference greater than 5% requires

further investigation of the sample run. A difference greater than 10% is failure (unless the average of the two samples is less than 10X the MDL), and results in re-analysis of the entire sample queue, unless there is a very reasonable and supported explanation for the inconsistency. Long-term averages for relative % difference are included in Table 2.

LFM must show 85% to 115% recovery. A recovery <90% or > 110% requires further investigation of the sample run. A recovery <85% or >115% is failure (unless the sample is less than 10X the MDL), and results in re-analysis of the entire sample queue, unless there is a very reasonable and supported explanation for the inconsistency. Long-term averages for % recovery are included in Table 2.

All QC information from each run is stored in a separate Access database. This includes calibration r^2, error, slope and intercept. The prepared concentration and measured concentration of LFM and calibration standards analyzed throughout the run are also entered. Finally, the lab duplicate measured concentrations are included. All this information can be queried for the project manager. Control charts (PDF) are generated from this database in R and reviewed weekly by the lab manager.

Method Detection Limits are calculated regularly, and whenever major changes to instrumentation or methods occur. Table 2 lists most recently measured MDL values.

VIII. Schedule of Internal/External Audits

Internal audits are not routinely performed, however, QC for each run is thoroughly reviewed by the lab manager before entering data into the database and a review of QC charts, and tables is done at least annually by the lab manager.

External audit samples are analyzed routinely throughout the year. The WQAL takes part in the USGS Round Robin inter-laboratory comparison study twice per year and the Environment Canada Proficiency Testing Program three times per year. The USGS and Environment Canada provide Standard Reference Samples and provide compliance results after analytical testing at the WQAL. Environment Canada is accredited by the American Association for Laboratory Accreditation. These audits are designed to quantify and improve the lab's performance. Poor results are identified and backtracked through the lab to the sources of the issue.

IX. Preventive maintenance procedures and schedules

The laboratory manager, Jody Potter, has 15 years of experience and is highly experienced with all laboratory equipment used within the WQAL. The laboratory manager conducts all maintenance and inspection of equipment based on manufacturer requirements and specifications.

Each day an instrument is used, it receives a general inspection for obvious problems (e.g. worn tubing, syringe plunger tips, leaks). The instruments are used frequently and data is inspected within a few days of sample analysis. This allows instrument (or user) malfunctions to be caught quickly, and corrected as needed.

Each day's run is recorded in the instrument's run log, with the date, the user, the number of injections (standards, samples, and QC samples), the project, and other notes of interests. Maintenance, routine or otherwise, is recorded in the instrument run log, and includes the date, the person doing the maintenance, what was fixed, and any other notes of interest.

X. Corrective Action Contingencies

Jody Potter is responsible for all QC checks and performs or supervises all maintenance and troubleshooting. When unacceptable results are obtained (based on within sample analysis batch QC checks) the data from the run are NOT imported into the database. The cause of the problem is determined and corrected, and the samples are re-analyzed. Problems are recorded in the sample queue's data spreadsheet, or on the handwritten runsheet associated with the run. Corrective actions (instrument maintenance and troubleshooting) are documented in each instrument's run log.

XI. Record Keeping Procedures

Protocols, Instrument Logs, QC charts, databases and all raw data files are kept on the lab manager's computer. These are backed up continuously, with the back up stored off site. The computer is password protected, and is only used by the lab manager. Protocols and the sample database are also password protected. Handwritten run sheets are stored in a filing cabinet in the lab. Instrument run and maintenance logs are combined with the QC data in an access database where instrument performance can easily be compared to instrument repair and the number of analyses, etc. This file is also stored on the lab manager's computer and is password protected.

All information pertinent to a sample is stored in the sample database. From this database we can easily determine the date of analysis and the location of the raw data file if further review is necessary. The amount of information provided to sample originators is dependent on what is required by the project or funding agencies.

Table 1. List of standard operating procedures and description of analyses done at the Water Quality Analysis Laboratory.

Standard Operating Procedure	Analysis	Instrument Used	Description	Protocol Latest Revision	EPA method or other reference
Ion Chromatography Protocol for Anions and Cations Protocol	Anions and Cations	Dionex ICS-1000; IonPac AS22 column Dionex ICS-1000 and ICS 1100; IonPac CS12 column	Anions via ion chromatography w/ suppressed conductivity. Cations via ion chromatography w/ suppressed conductivity	February 7, 2012	Anions EPA #300.0 Cations ASTN D6919-09
Dissolved Organic Carbon Protocol	DOC	Shimadzu TOC-L	High Temperature Catalytic Oxidation (HTCO)	April 4, 2016	EPA 415.3
Total Dissolved Nitrogen Protocol	TDN	Shimadzu TOC-L with TN module	HTCO with chemiluminescent N detection	April 4, 2016	Merriam et al, 1996; ASTM D5176
DOC and TDN combined Protocol	DOC and TDN	Shimadzu TOC-L with TN module	HTCO with chemiluminescent N detection	April 4, 2016	EPA 415.3 and Merriam et al, 1996
Seal AQ2 discrete colorimetric analysis Protocol	Nitrate/Nitrite colorimetric (NO_3/NO_2)	Seal Analytical AQ2 discrete analyzer	Automated Cd-Cu reduction	April 25, 2016	EPA 353.2
SmartChem discrete colorimetric analysis Protocol	Ammonium colorimetric (NH_4)	SmartChem discrete analyzer	Automated Phenate	August 27, 2010	EPA 350.1
Seal AQ2 discrete colorimetric analysis Protocol	Soluble reactive Phosphorous colorimetric (SRP or PO_4)	Seal Analytical AQ2 discrete analyzer	Automated Ascorbic acid	April 20, 2017	EPA 365.3

SmartChem discrete colorimetric analysis Protocol	Silica (SiO_2)	SmartChem discrete analyzer		November, 10, 2005	EPA 370.1
Seal AQ2 discrete colorimetric analysis Protocol	Total Dissolved Phosphorus (TDP) (Filtered sample)	Seal Analytical AQ2 discrete analyzer	Persulfate Oxidation of filtered sample, followed by colorimetric SRP analysis.	April 25, 2016	USGS Test Method 1-4560-03
Seal AQ2 discrete colorimetric analysis Protocol	Total Phosphorus (TP) and Total Nitrogen (TN) (Unfiltered sample)	Seal Analytical AQ2 discrete analyzer	Persulfate Oxidation of unfiltered sample, followed by colorimetric SRP analysis.	April 25, 2016	Resources Investigations Report 03-4174
CHN Protocol	Particulate Carbon (PC) and Nitrogen (PN)	Perkin Elmer 2400 Series II CHN	Filtration of sample followed by Elemental Analysis of the filter and particulates	February 14, 2013	EPA 440.0
Particulate Carbon and Nitrogen filtration	Laboratory Sample Filtration		Filtration of samples for water chemical analysis and particulate analysis	February 14, 2013	EPA 440.0
Acid Washing Protocol	Glass and plastic-ware cleaning		10% HCl rinse and 6 rinses with DDW	July 19, 2012	
Field Filtering Protocol	Sample prep		3-times rinse with filtered sample	July 13, 2015	
Fluorescence	EEMs	Horiba Jobin Yvon Fluoromax 3	Scanning Fluorescence Excitation & Emission on whole water	June 26, 2013	
Absorbance	Abs 254 & SUVA	Shimadzu TOC-L & Shimadzu PDA SPD-M20A	Scanning absorbance spectra on whole water	June 26, 2013	EPA 415.3

pH, Closed cell	pH, Closed cell	Electrode & Thermo Orion 525A	pH in a closed environment under atmospheric CO2 conditions	August 27, 2015	EPA 150.1
pH, aerated	pH, aerated	Electrode and Radiometer ION450	pH equilibrated with atmosphere	January 4, 2013	EPA 150.1
Specific conductance	Specific conductance	Electrode	Specific conductance	May 15, 2017	EPA 120.1
ANC protocol	ANC	Electrode & Radiometer ION450	Gran titration	May 15, 2017	EPA 310.1
Greenhouse Gases	Greenhouse Gases extracted from water	Shimadzu GC-2014	CH4, N2O, & CO2 on GC with FID, ECD, & TCD	December 6, 2012	
Alkalinity protocol	Alkalinity	Electrode & Radiometer ION450	Inflection Point		EPA 310.1

Table 2. Detection limits, acceptable ranges, and recent historical averages for QC samples at the Water Quality Analysis Lab.
[1] Detection limit based on user experience and previous analysis (not statistically calculated). [2] Method Detection Limit (MDL) is the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero.

Analyte	Units	Typical Range	Regression Type	# of Cal. Points	MDL [2]	Lab Duplicate% Relative Difference	Limit	IPC % recovery	Limit
							+/-		+/-
SiO_2	mg SiO2/L	0.05 – 35	Linear	4-7	0.02	0.7	15	97.6	15
PO_4	ug P/L	5 – 200	Linear	4-7	2.00	2.4	15	100.4	15
NH_4	ug N/L	5 – 200	Linear	4-7	5.00	4.8	15	100.6	15
NO_3 Colorimetric	mg N/L	0.025 – 10	Linear	4-7	0.005	3.2	15	99.6	15
Na^+	mg Na/L	0.1 – 15	Quadratic	4-7	0.02	1.2	15	95.5	15
K^+	mg K/L	0.05 – 7	Quadratic	4-7	0.01	1.7	15	101.5	15
Mg^{2+}	mg Mg/L	0.02 – 7	Quadratic	4-7	0.02	1.9	15	95.2	15
Ca^{2+}	mg Ca/L	0.1 – 10	Quadratic	4-7	0.1	2.7	15	98.4	15
Cl^-	mg Cl/L	0.12 – 15	Quadratic	4-7	0.025	1.4	15	94.6	15
NO_3^- by IC	mg N/L	0.02 – 3	Quadratic	4-7	0.004	3.0	15	98.8	15
SO_4^{2-}	mg S/L	0.04 – 8	Quadratic	4-7	0.02	2.3	15	98.4	15
TDN	mg N/L	0.1 – 10	Linear	4-7	0.05	4.1	15	94.7	15
DOC	mg C/L	0.1 – 20	Linear	4-7	0.10	1.8	15	96.9	15
TN	mg N/L	0.05 – 10	Linear	4-7	0.02	3.2	15	97.4	15
TP	ug P/L	20 – 600	Linear	4-7	10.0	2.1	15	100.1	15
TSS	mg/L	0-200	n/a	n/a	1.00		15		15
PC	ug/L	0 – 5	Linear	2	4.0		15		15
PN	ug/L	0 – 2	Linear	2	2.4		15		15

References

Avanzino R.J. and V.C. Kennedy, 1993. Long-term frozen storage of stream water samples for dissolved orthophosphate, nitrate plus nitrite, and ammonia analysis. *Water Resources Research*, 29(10) 3357-3362.

Merriam, J.L, W.H. McDowell, W.S. Currie, 1996. A high-temperature catalytic oxidation technique for determining total dissolved nitrogen. *Soil Science Society of America Journal*, 60(4) 1050-1055.

APPENDIX E
Center for Freshwater Biology Field Team Sampling SOPs

Deep Lake Sites - Sampling Procedure

Field procedures to be used are designed to be consistent with the methods of past studies performed by the University of New Hampshire Center for Freshwater Biology and/or the New Hampshire Department of Environmental Services Limnology Center. Many of these procedures are also referenced in Monitoring Lake and Reservoir Restoration (US EPA 1990). For each lake sampling trip conducted, the historical site (usually the deepest site) as well as a second deep site (if present) will be sampled. Additional sites (deep or shallow) that are historically important or that reflect current concerns may also be sampled if there is sufficient time or need, or if called for in the Memorandum of Understanding.

Sampling of the deep sites will start no earlier than 9:00 AM EST and will continue no later than 3:00 PM EST so the sun angle will not impact the Secchi disk and underwater irradiance measurements.

- The boat will be positioned according to three historical landmark reference sites on shore and an electronic fathometer will be used to find the maximum depth. Alternatively, global positioning system (GPS) coordinates, when available, shall be used to locate the designated sampling location. The boat will be anchored and the depth measured with a calibrated chain to confirm the correct location. The maximum depth will be recorded along with the lake name, site name, sampling date, start time, weather conditions, air temperature and the field technicians present.

- Secchi Disk depth measurements will be collected from both the sunny and the shady side of the boat with and without a viewscope (PVC tube with Plexiglas plate on the end) as indicated in Table 1. The observer shall follow each of the Secchi Disk transparency procedures outlined in Table 1 using the following sampling technique that will involve the collection of triplicate measurements for each treatment. The observer shall lower the Secchi Disk until it disappears from view and note the depth of disappearance. The observer shall then raise the disk until it reappears and note the reappearance depth. The Secchi Disk transparency shall be recorded as the average of the disappearance and the reappearance point.

Table 1. Secchi Disk measurements by treatment.

Secchi Disk *	Side of Boat	Scope	Procedure
Black and White *	Shady Side	Yes	Place the view scope into the water and look through the scope for 30 seconds to allow your eyes to acclimate to the darker conditions before collecting Secchi Disk transparency measurements.
Black and White *	Sunny Side	Yes	
Black #	Sunny Side	Yes	
Black and White *	Shady Side	No	Look over the side of the boat and record Secchi Disk transparency measurements.
Black and White *	Sunny Side	No	

***** Standard 20 cm diameter black and white alternating quadrant Secchi disk (Wildco or equivalent).
# 20 cm diameter all (flat) black Secchi Disk.

- Thermal profiles, and accessory profiling data, will be collected at each sampling station with one of the following:

- The particular instrument used and the parameters measured will be outlined in a site specific project plan (SSPP). Refer to the accompanying standard operating procedures that outline the use of the YSI 6600 Multiparameter instrument, The YSI EXO2 Multiparameter instrument, the YSI Professional Plus instrument, the YSI ProSOLO instrument and the YSI 85 handheld instrument (Table 2). When using the YSI 6600, YSI EXO2 and YSI Professional Plus Multiparameter instruments data will be logged digitally and downloaded to a PC upon return from the field. When using the YSI 85 handheld instrument, the YSI Professional Plus or the YSI ProSOLO, measurements will be collected at 0.1 meters, 0.5 meters and at one half-meter increments to the lakebottom. All data obtained with the YSI 85 handheld instrument will be recorded on the standard Center for Freshwater Biology field data sheet. Data collected using the YSI Professional Plus and the YSI ProSOLO will logged digitally and downloaded to a PC upon return from the field.

Table 2. Thermal Profiling Instruments

Meter	SOP Reference
YSI 85	Appendix B.5
YSI ProSOLO	Appendix B.12
YSI Professional Plus	Appendix B.10
YSI 6600	Appendix B.8
YSI EXO2	Appendix B.11

- Underwater light profiles will be collected from the sunny side of the boat using the SOPs outlined for the LI-1400 Data Logger PAR Measurements. Light measurements shall be collected at 0.5 meter increments.

- Point water samples will be collected using a non-metal (lexan polycarbonate) 3 liter "Van-Dorn" type vertical sampler at 0.5 meters, mid-epilimnion, midmetalimnion, and one meter above the lake bottom. Depths will be determined from the temperature and oxygen profiles that were collected for the respective sampling site. When stratification is not apparent, samples will be collected at 0.5 meters, 1/3 and 2/3 of the water depth and 1.0 meters above the lake bottom. Water will be analyzed for Total Alkalinity, pH, Turbidity, Specific Conductivity, Chloride and Free CO_2 for all depths sampled while supplemental dissolved oxygen (Winkler method) samples will be collected at 0.5 meters and one meter above the lake bottom to QC the digital meter. The 0.5 meter and mid-metalimnetic samples will also be analyzed for chlorophyll *a* and true color, while a mid-metalimnetic whole water phytoplankton sub-sample will be collected for microscopic identification. Additional mid-metalimnetic and bottom water (1 meter above the lake bottom) total phosphorus samples will be collected to determine the extent of internal nutrient loading. On occasional sampling dates, supplemental surface, mid-metalimnion and bottom water grabs for the analysis of selected specific ions including sodium, chloride, nitrate, sulfate, magnesium, calcium, orthophosphate and potassium may be collected. All samples will immediately be placed into the ice filled cooler for transport to the laboratory.

- An integrated (composite) water sample will be taken from the surface to the bottom of the epilimnion to the nearest 0.5 meters) or to a depth of 8.0 meters (whichever is less) by using a weighted clear Tygon tube sampler. This water will be collected into a large opaque 2-liter polyethylene bottle and the sampling shall be repeated until at least one and one-half liters of water has been collected. All of the tube's contents will be transferred into the bottle after each collection to maintain the proper composite matrix. Samples will be collected from the integrated sample for the analysis of chlorophyll *a*, true color, total alkalinity and total phosphorus and all samples will immediately be placed into the ice filled cooler for transport to the laboratory.

- A vertical zooplankton haul (64 micron mesh net) will be made from one meter above the lake bottom when the water at that depth contains at least one part per million (ppm) dissolved oxygen, or from the deepest depth where the dissolved oxygen concentrations is greater than 1 ppm, to the surface. The net will be placed in the water and allowed to sink to the desired sampling depth to the nearest one-half meter. The line shall be extended to its full extent and be allowed to position itself vertically in the water column; there will be tension once the net has fully extended. Once vertical, the net will be raised slowly to the surface at a constant rate until the mouth (ring) rises out of the water. The net shall be raised up and the water shall be allowed to drain out; the contents of the net become concentrated in the plankton bucket at the end. The net shall be rinsed down using a Nalgene squirt bottle filled with distilled water that will wash the organisms into the collection bucket. The sample will be concentrated using the screened net bucket at the end and then transferred to a 500 milliliter wide-mouth sampling bottle. The screened net bucket should then be rinsed twice with distilled water, the contents added to the 500 milliliter wide-mouth sampling bottle. The sample shall then be preserved with formalin sucrose and immediately placed in an ice filled cooler.

- On occasional sampling trips, a water sample will be collected for microbiological analysis in a sterilized Whirl-pac bag just under the surface. Care will be taken to avoid collecting any surface layers or surface scums. The water will be collected in such a way as to prevent any contamination of the sample from the field technician or the watercraft by slowly sweeping the whirl pack bag forward (away from the field technician) and allowing the bag to fill. The whirl pack bag will be sealed and placed in the ice filled cooler for transport to the laboratory.

Shallow Lake Sites - Sampling Procedure

1. Suspect and historical shallow water sites may be sampled depending upon the specific project objectives. If contracted, a shoreline conductivity survey may be used to locate shallow sampling sites; a low draft boat with an electric motor will slowly cruise along the entire shoreline of the lake and as far up any inlet streams as possible without disturbing the bottom. A conductivity probe will be towed off the shoreward side of the boat below the surface of the water and above the lake bottom. Readings will be recorded on a map of the lake and corresponding global positioning system (GPS) coordinates will be recorded. Any significant change in the readings will be noted and samples will be collected just below the surface into the proper sample bottle for pH, total phosphorus and/or specific ions (Na^+ Cl^-, NO_3^-) and into a sterilized whirl-pak bag for bacteria (*E. coli*). Samples will immediately be placed on crushed ice in a field cooler. Samples will be collected as described under the deep lake site sampling procedures to prevent disturbance and contamination. If no areas of suspected pollution are evident

from the shoreline conductivity survey then samples may be taken at each end of the lake, embayed areas, at inlet locations, road runoff entry sites or any visually suspect areas.

2. While cruising the shoreline, major growths of aquatic vascular plants, filamentous algal growth, or surface algal scums may be noted on the map and samples will be taken of any unrecognizable growths for laboratory identification. When major growths are identified, a subjective estimate of their abundance will also be noted on the map.

3. In addition, various potential non-point source pollution threats noticeable from the shore will be documented on the map.

Written by Bob Craycraft and Jeff Schloss
Last Updated on March 9, 2021

APPENDIX F

Volunteer Monitor Field Sampling SOPs and Corresponding Field Sampling Datasheets

Revised March 15, 2021
Bob Craycraft

LAKES LAY MONITORING PROGRAM VOLUNTEER MONITOR LAKE SAMPLING INSTRUCTIONS (2021)

INTRODUCTION

Contact Information

The following instructions and sampling procedures are for use by Lakes Lay Monitoring Program (LLMP) participants. **If any questions arise concerning these sampling procedures, or the program in general, please contact Bob Craycraft (862-3696 or bob.craycraft@unh.edu).**

Any suggestions concerning these directions or the volunteer monitoring activities are encouraged and will help us greatly in our efforts to keep the program current.

Make sure to check for lake specific information (e.g. variations in sampling frequency and lenght of the sampling season) that may be applicable to your lake.

General Information and Things to Remember

Your boat must be securely anchored at the designated sampling station in order to take representative readings. It is important to come as close as possible to the same location from one week to the next. Locate your site with respect to three landmarks (triangulation) or two imaginary lines for consistency. Alternatively, use the pre-designated coordinates to return to the sampling site using global positioning system navigation.

Sampling should be taken at predetermined intervals to provide the data necessary to observe trends which develop over the summer sampling season and which are necessary to detect seasonal water quality fluctuations. A difference of several days is not critical if the weather or circumstances do not permit sampling, but every effort should be made to sample consistently at weekly intervals. Please make plans in advance with your team partner to cover your sampling station if you are unable to sample in a given time period.

All data sheets and zip-lock inserts should be filled out completely. All data on these sheets are important, and without them important information could be lost.

All data sheets must be completed in pencil (pen and felt-tipped markers run when wet, and valuable samples or data can be lost).

All depth measurements should be made from the point where the line touches the water surface, not from the gunwale. This includes measurements collected with the Clinefinder, the Secchi Disk and the composite tube sampler.

Clinefinder digital thermometers remain "on" at all times and have a battery life of approximately two to three years. *Note: battery power should be assessed at the beginning of the sampling season and the batteries replaced if:*

1) *The batteries were not replaced within the last two years.*
2) *The LCD temperature readout appears faint.*

We can supply the three alkaline AA batteries and zip ties that hold the batteries in place or, should you ship the Clinefinder to us, we can replace the batteries for you.

The newer Clinefinders are assembled with T-15 head screws, and we can provide a T-15 screwdriver if needed.

Should a Clinefinder completely lose power, it will display "-Cal" and will have to be returned for recalibration.

When replacing the Clinefinder batteries follow these steps:

- Remove the four screws from the back of the Clinefinder and carefully separate the two clamshell halves to expose the battery compartment. *Note: the internal compartment can pressurize so work the two clamshell halves apart gently to avoid damaging the internal wiring that connects to the two halves.*
- Once open, cut the zip tie, that holds the batteries in place, to facilitate battery removal.
- Replace the three batteries <u>one by one:</u> remove one old battery and replace it with a new battery. Repeat the process two more times. *Note: if you remove all three batteries at once the meter will lose power and will require recalibration*.
- Take a new zip tie and thread it through the rear of the battery compartment and "zip" it into place to secure the batteries.
- Ensure the gasket between the two clam shell haves is in place and reassemble the two clamshell halves.
- Screw in the four screws, being careful not to over-tighten and strip the plastic into which the screws lock.
- The meter should be ready for use with a two-to-three-year battery life.

You may find it convenient to separate your equipment into two crates, one for on-lake procedures and one for on-shore.

On-lake equipment:

- Anchor with enough rope for your sampling location – two anchors is better, to maintain position
- Cooler with ice
- Blank data sheet (optional – a clip board)
- Two pencils
- Secchi disk with line marked at half-meter increments or using a metric fiberglass measuring tape.
- View scope
- Clinefinder digital thermometer
- Composite tube (integrated) sampler
- A two-liter amber/brown bottle to collect the primary water sample

On-shore equipment:

- Filtration unit consisting of receiving funnel, pump, and collection container
- Filter paper
- Tweezers
- Either a 60- or 125-milliliter amber/brown bottle to hold a cyanobacteria sample
- A 60-milliliter white/translucent bottle to hold a dissolved color sample
- A 250-milliliter white/translucent bottle, prewashed with acid and labeled “Corrosive”, to collect a total phosphorus sample
- Graduated cylinder
- Plastic titrating cup (aka beaker)
- Burette – thin graduated plastic tube with dispensing nozzle
- Indicator solution in drip dispensing bottle
- Titrant solution in plastic squeeze bottle

Figure 1. Sampling equipment

Note: items in your kit may look slightly different (data sheets not shown).

PROCEDURES – On the Lake

A. Water Transparency (Secchi Disk Depth)

Function:

To measure the transparency of the lake water at the sampling station.

Rationale:

Water clarity fluctuations correlate to differences in algal populations, silt, and water color.

Important Details:

The view scope (PVC pipe with Plexiglas plate) should always be used while viewing the Secchi Disk. *Never take a Secchi Disk reading while wearing sunglasses or tinted glasses.*

All equipment must be in good working order. The paint on the Secchi Disk should not be chipped or discolored. If the Secchi Disk is chipped or discolored, it can be repainted with flat black and flat white Rustoleum paint (never use gloss finishes when repainting the Secchi Disk). Also, the inside of the view scope should be painted black to reduce glare.

The view scope should be examined prior to each sampling trip. If the Plexiglas plate is coated with dust, clean the plate off with tap water. Dust on the Plexiglas plate will yield artificially low water transparency readings.

The Secchi Disk measurement should be taken as close to noon as possible. This ensures that the maximum amount of sunlight enters the lake. However, any time between 9 AM and 3 PM is acceptable.

The Secchi Disk should always be lowered on the shaded side of the boat.

Procedures:

1. Once anchored at your sampling station, place the view scope into the water so the Plexiglas plate is flush with the water. There should be no air bubbles between the Plexiglas plate and the water; if necessary, tilt the view scope to allow air bubbles to escape.

2. Look through the view scope and wait 30 seconds while your eyes to adjust to the darker lighting. Slowly lower the Secchi Disk until it disappears from view, immediately stop lowering the line and mark the point of disappearance (where the line just touches the water). Lower the disk a few more inches below the point of disappearance. Raise the disk until light can just be seen reflecting upward from the white surface of the disk and mark the point of reappearance.

3. The average between these two points (the point of disappearance and the point of reappearance) is taken as the Secchi Disk transparency.

4. Record the Secchi Disk transparency on the data sheet. Repeat the procedure in order to collect a second Secchi Disk measurement to confirm the first result.

B. Digital Thermometer

Function:

To measure the thermal profile of the water column at the sampling location.

Temperature profiles should at least pinpoint the *thermocline,* the mid-lake layer, that is sandwiched between an upper warm water layer (epilimnion) and a bottom cold water layer (hypolimnion).

More complete temperature profiles are encouraged to better assess the degree of temperature stratification throughout the water column.

Rationale:

The development of thermal layers in lakes is of great importance to the lake's biology and productivity. Therefore, it is important to know if the lake is stratified.

When the lake is stratified, it is important to document the location of the major layers. The boundaries are determined by measuring the temperature at one-half meter intervals and looking for large temperature changes **(Table 1)**.

As a general rule of thumb, the thermocline is identified by recording a continuous decrease in water temperature readings between successive one-half meter increments. A temperature decrease of one degree Celsius per meter depth (or one-half degree Celsius per half meter) over a span of several meters is used to locate the upper and lower limits of the thermocline. Refer to the examples of summer temperature profiles in Table 1.

Important Details:

Clinefinders (Figure 2) remain "on" at all times and have a battery life of approximately two to three years. *Note: battery power should be assessed at the beginning of the sampling season*. See discussion above.

Procedures:

1. Place the Clinefinder in a shady spot in the boat, allow the temperature reading to stabilize, and record the air temperature on the datasheet.

2. Submerse the tip of the Clinefinder cable into the water, wait 15 seconds or until the temperature readings stabilize, and record the temperature in degrees Celsius on your datasheet (the first reading should be recorded at 0.1 meters).

Figure 2. Clinefinder

3. Continue lowering the temperature cable down in ½ meter increments. Let the temperature reading stabilize for 15 seconds, and record the temperature data at the corresponding depth on the datasheet.

Note: it is important to make sure the temperature cable is vertical when recording the temperature readings, otherwise inaccurate data may be recorded.

Table 1: Temperature Profile

Depth (meters)	Temperature (Celsius) May 19	Temperature (Celsius) June 27	Temperature (Celsius) August 5	Temperature (Celsius) Sept 8
0.1 (surface)	13.9	23.4	27.1	21.0
0.5	14.0	23.4	26.9	21.0
1.0	14.0	23.2	26.6	21.0
1.5	14.0	23.2	26.3	21.0
2.0	14.0	22.4	26.1	21.0
2.5	14.0	22.4	26.1	20.7
3.0	14.0	22.3	25.8	20.7
3.5	14.0	22.2	25.1	20.7
4.0	13.0	22.1	24.3	20.7
4.5	11.7	21.1	23.5	20.5
5.0	10.6	19.9	20.6	20.5
5.5	9.6	17.7	17.4	20.5
6.0	9.0	15.3	15.5	20.1
6.5	8.7	12.9	13.1	17.1
7.0	8.5	11.0	11.5	14.5
7.5	8.1	10.7	10.9	13.8
8.0	7.7	10.1	10.2	11.7
8.5	7.7	9.5	9.6	10.0
9.0	7.6	8.9	9.0	9.4
9.5	7.6	8.4	8.5	8.8
10.0	7.5	8.1	8.3	8.4
10.5	7.5	7.8	8.3	8.2
11.0	7.5	7.8	8.3	8.2
11.5	7.4	7.7	8.1	8.1
12.0	7.3	7.6	8.0	8.0

The Gray shaded area indicates the extent of the epilimnion on the respective sampling dates while the thermocline is un-colored and the hypolimnion is shaded light blue.

C. Composite Tube (Integrated) Sampler and the Collection of a Water Sample

Function:

To collect water from the surface down to the maximum extent of the upper warm water layer (epilimnion), with all depths represented equally.

Rationale:

The temperature and light concentrations in the upper warm water layer are conducive to algal growth. Consistent collection of a representative surface water sample, among sampling dates, facilitates the detection of both short-term water quality fluctuations and long-term trends.

Important Details

The depth to which the sample is taken is determined by the temperature profile. The weighted end of the tube should reach down to, but not include, the layer of rapid temperature change (the thermocline).

The upper, relatively constant temperature layer should be measured to the nearest half-meter and should vary by no more than one-half degree Celsius between successive one-half meter increments (see exception below).

Viewing the data from Table 1, we see that the temperature is stable from the surface (0.1 meter) down to 3.0 meters on August 5, changing by less than 0.5 degrees Celsius per half-meter depth. Between 3.0 meters and 9.5 meters, the temperature decreases rapidly (greater than 0.5 degrees Celsius per one-half meter). The zone of rapid temperature decrease between 3.0 meters and 9.5 meters defines the thermocline. So, using this example, the August 5 water sample should be collected down to 3.0 meters, which includes the upper warm water layer but excludes the zone of rapid temperature decrease.

In some instances, particularly during hot periods and calm periods, one can experience false temperature stratification. For example, the June 27 temperature data displayed in Table 1 do not change significantly between successive depths from the surface down to a depth of 4.0 meters, with the exception of a 0.8 degree Celsius difference between 1.5 and 2.0 meters. The true thermocline is actually located at the point where the temperature continues to drop by 0.5 or more degrees Celsius per half meter depth. In the June 27 example, the thermocline is located between 4.0 and 9.5 meters.

Procedures:

1. Rinse the tube sampler by lowering the hose into the lake, to the maximum extent possible, and slowly raise the un-kinked hose to fully flush the sampler. Always uncoil the line that is attached to the weight to allow the hose to descend vertically into the water column.

    a. Place the weighted end of the integrated sampler in the water and slowly lower the hose to the appropriate depth (in the case of our example from August 5 in Table 1, the tube is lowered to 3.0 meters). The rope which is attached to the weighted end should be slack throughout this procedure. *Note: the tube should not be kinked when it is lowered into the water column.*

    b. Once the appropriate depth is reached, kink the tube (fold the tube tightly) about one-half meter above the point where the hose comes in contact with the lake water. Hold the kinked end of the tube at a constant depth and raise the tube up by the attached line which is connected near the weighted end of the tube. This will ensure

that as the bottom of the tube is raised, water will not leak out. *Note: make sure you continue to kink the hose while raising the opposite end of the tube with the attached line.*

c. Place the weighted end of the tube into the mouth of the two liter amber bottle, and un-kink the hose. Slowly raise the hose from the opposite end and let the entire contents of the hose drain into the amber bottle. Be careful not to let water backflow into the unused portion of the tube.

d. Cap your sampling bottle, invert the bottle five times, remove the cap and dump the lake water into the lake. *This ensures the sampling bottle has been rinsed with your water sample*.

2. Place the weighted end of the integrated sampler in the water and slowly lower the hose to the appropriate depth (in the case of our example from Table 1, the tube is lowered to 3.0 meters). The rope, which is attached to the weighted end, should be slack throughout this procedure. *Note: the tube should not be kinked when it is lowered into the water column.*

3. Once the appropriate depth is reached, kink the tube (fold the tube tightly) about one-half meter above the point where the hose comes in contact with the lake water. Hold the kinked end of the tube at a constant depth and raise the tube up by the attached line which is connected near the weighted end of the tube. This will ensure that as the bottom of the tube is raised, water will not leak out. *Note: make sure you continue to kink the hose while raising the opposite end of the tube with the attached line.*

4. Place the weighted end of the tube into the mouth of the two liter amber bottle, and un-kink the hose. Slowly raise the hose from the opposite end and let the entire contents of the hose drain into the amber bottle. Be careful not to let water backflow into the unused portion of the tube. *Note: never touch the end of the tube near the weight; always grab the weight to guide the tube into the sampling bottle.*

5. If the two liter amber bottle is not one-half full at this point, repeat steps 2 through 4 above until the bottle is at least one-half full.

6. Once you have collected an adequate volume of sample water, place the amber bottle in an ice filled cooler.

PROCEDURES – On Shore

D. Filtration of Chlorophyll Sample:

Function:

To filter out all algae from a given quantity of lake water, and provide the sample to UNH for determination of the chlorophyll *a* pigment content.

Rationale:

The filters are of such a fine pore size (0.45 microns) that essentially no algae or bacteria can pass through. Once the sample has been filtered and the filter has been preserved (dried and frozen), the sample provides us with a convenient means of estimating the algal abundance through the analysis of the chlorophyll pigment content.

Important Details:

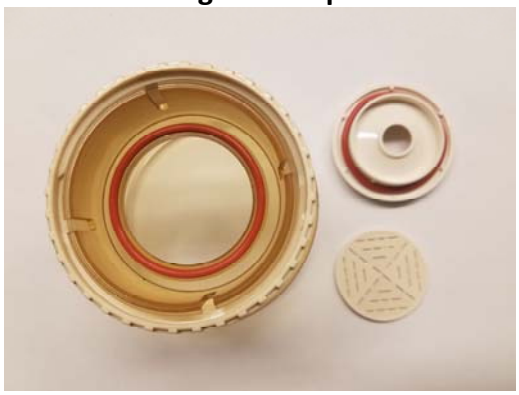
The filtration is best done indoors in an area that is not exposed to direct sunlight. Exposure to direct sunlight can degrade the chlorophyll pigments and must be avoided.

Be sure to fill out all pertinent information on both the large field data sheet and the small data sheet. Information should be recorded in pencil.

The filtration unit contains two gaskets that are critical to the functioning of the system. Should these gaskets become dislodged, they should be re-installed to create a good seal. Otherwise, the vacuum pressure will not be sufficient to pull the lake water through the filter.

There is a plate, upon which the filter sits, that can be dislodged. If the plate falls out, make sure you replace it with the grided side up. If the plate is installed upside-down, water will filter much more slowly through a few narrow slots.

Figure 3. Proper gasket placement and dislodged filter plate.



Procedures:

1. Unscrew the receiving funnel of the filtration unit and place a white filter on the filter holder, using a pair of tweezers or by lifting the filter out of the box with a blue piece of wax paper that separates the white filters from one another. Under no circumstances should the white filters be touched.

2. Replace the receiving funnel. *Note: when screwing the receiving funnel on the filtration setup, be careful not to cross-thread the receiving funnel.*

3. Invert the two liter sampling bottle five times immediately prior to pouring out a water sample.

4. Fill the receiving funnel to the 250 milliliter mark with unfiltered lake water contained in the two-liter amber bottle. If using a manually operated hand pump, squeeze the handle until the vacuum pressure builds and water passes through the white filter. Periodically squeeze the handle, as needed, until all water passes through the white filter. Alternatively, if using an electric pump, plug in/turn on the pump and allow the entire volume of lake water to pass through the filter and collect in the lower chamber.

5. After 250 milliliters of lake water is filtered, empty the bottom chamber. *Caution; do not disturb the filter; additional water will be filtered through the upper chamber in the next step.* If using an electric pump, the filtration units are equipped with an overflow bottle, but this should not be allowed to fill with water. These safety bottles are designed to avoid the water from entering the electric pump. To fully protect the electric pumps, the safety bottle should be kept upright during the filtration process.

6. Repeat steps 3 and 4.

7. Unscrew the collar and take the filter from the filter holder. **Do not discard the filtered water remaining in the lower chamber.** The filter should be carefully handled by the edges (using tweezers or the blue wax paper) and placed upright on the back of a fully labeled (in pencil) paper insert. The filter should be dried in a dark location, such as a cabinet drawer or a shoe box, for a minimum of eight hours while drying the filter overnight is preferable.

8. When the filter is absolutely dry, place it in the Ziplock bag along with the small label sheet and placed in a freezer. The label should contain the following information: Lake, Site, Date, Depth, Monitor, Volume Filtered (total volume = 500 milliliters), Drying Time.

E. Dissolved Water Color

Function:

To measure the color of the water due to dissolved humic substances and other dark-colored organic material.

Rationale:

Water color is a factor that influences the Secchi disk transparency. We are interested in filtered water which doesn't contain any particulate debris.

Procedures:

1. After performing a chlorophyll filtration in Section D, fill a 60 ml translucent plastic bottle approximately half-way with **filtered lake water** (the lake water remaining in the bottom chamber of the filtration apparatus).

2. Dump the lake water out of the 60 milliliter bottle. *You have rinsed the sampling bottle with filtered lake water.*

3. Now fill the 60 milliliter sampling bottle to the neck with filtered lake water and place the labeled bottle in the freezer. The label should contain the following information: Lake, Site, Date, Depth, Monitor.

F. Phosphorus

Function:

To measure the concentration of total phosphorus in the lake water.

Rationale:

Phosphorus is generally the limiting nutrient to growth in New Hampshire lakes. Excess phosphorus leads to increased algal productivity and growth in New Hampshire lakes, and high phosphorus levels can also be an indication of problems around the lake, such as excessive fertilizer runoff and sediment erosion that can be documented as short-term phosphorus "spikes."

Procedures:

1. Invert the two liter amber bottle five times.

2. Fill a pre-labeled 250 ml translucent acid-washed bottle (labeled corrosive) with lake water from the two liter amber bottle. The label should contain the following information: Lake, Site, Date, Depth, Monitor. *Warning, the sampling bottles contain one milliliter of concentrated sulfuric acid so use extreme caution when collecting these samples.* Fill the phosphorus bottle to the neck of the container, cap the bottle and freeze the sample. *Note: be careful not to touch the inside of the cap or bottle as our bodies contain high concentrations of phosphorus that can contaminate the sample.*

G. Cyanobacteria

Function:

To measure the concentration of phycocyanin (a photosynthetic pigment associated with cyanobacteria) in the water sample.

Rationale:

Cyanobacteria can be associated with green water events oftentimes referred to as "blooms," and some species are known to produce liver and nervous system toxins.

Procedures:

1. Invert the two liter amber bottle five times.

2. Fill a pre-labeled amber bottle (either a 60 milliliter or 125 milliliter bottle depending

upon what you were provided) to the neck with lake water that was collected in the 2 liter amber bottle. The label should contain the following information: Lake, Site, Date, Depth, Monitor.

3. Cap the bottle and freeze the sample. *Note: always leave room for expansion since this bottle is frozen and could crack if overfilled.*

H. Alkalinity

Function:

To measure the bicarbonate buffering capacity (resistance to acidification) of lake water.

Rationale:

Alkalinity, or buffering capacity, is an important parameter to assess in New Hampshire where the lakes are often acidic and poorly buffered against atmospheric fall-out. Alkalinity can also serve as an early indicator of lake acidification.

Important Details:

Titrations should always be done against a white background.

Alkalinity samples will be analyzed from water contained in the two liter amber (integrated) sample bottle.

Two endpoints are critical. If more than one monitor in your group is performing the alkalinity titrations, make sure all analysts come to agree on the correct endpoint colors.

Procedures:

1. Invert the two liter amber bottle five times and pour out approximately 50 ml of the lake water sample into a graduated cylinder. Transfer the lake water into plastic titrating cup (aka beaker).

2. Swirl around the sample, in the plastic cup, and pour out the sample. *You have effectively rinsed both the graduated cylinder and beaker.*

3. Invert the two liter amber bottle five time and pour out 100 ml of the lake water sample into a graduated cylinder. Transfer the lake water into plastic titrating cup (aka beaker).

4. Add 12 drops of indicator solution, labeled Alkalinity Indicator, from the dispensing bottle.

5. Insert the tip of plastic titrant bottle, labeled Alkalinity Titrant, into the top of the plastic burette. Squeeze the titrant bottle carefully and fill burette with 10 ml of titrant. Gently tap the tip of the burette to remove any air bubble before proceeding. Refill the burette with alkalinity titrant as needed to replenish any solution that was lost when you dislodged the air bubble.

6. Hold the burette with one hand so that thumb and forefinger can squeeze the glass bead located in the rubber tubing. Add the titrant slowly, drop by drop, while stirring the solution with a stirring rod held in the other hand. Titrate until the water loses the blue coloring and becomes a lavender gray color.

7. Record the number of milliliters (mls) used to reach this first endpoint. Record to the nearest tenth (e.g. 5.4 ml); each line on the burette is 0.1 ml.

8. Continue titrating until the solution becomes a very faint pink. At this point in the titration, the water will become "pinker" as more titrant is added, so it is very important to titrate only until the first signs of pink!

9. Record the total volume of titrant to reach this second endpoint (in other words, the volume of titrant that you used since you began titrating, not since the gray endpoint). For example, if it took 5.4 ml to reach the gray endpoint, plus, 0.6 ml to reach the pink endpoint, the total ml used for the second endpoint would be 6.0 ml. Make sure both endpoints are included on the data sheet.

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LAKES LAY MONITORING PROGRAM

LAY MONITOR DATA SHEET (2021)

SID:_____
ID:_____

MONITOR #1 NAME:_____
MONITOR #2 NAME:_____
MONITOR #3 NAME:_____
LAKE NAME:_____
SITE NAME:_____

AIR TEMP:_____ºC
DATE:_____
SAMPLING TIME: Start_____ **Finish**_____
SITE DEPTH:_____
SECCHI DISK TRANSPARENCY_____**meters**

Weather (circle the best descriptor)

Sky	Clear	Hazy	Cloudy	Overcast
Lake	Calm	Ripples	Waves	White Caps
Wind	Calm	Breezy	Gusty	Windy

Precipitation (Circle the best descriptor below)

None	Past 12 hrs	Past 24 hrs	Past 48 hrs	Past 72 hrs

Temperature Profile

Depth (m)	Temp (°C)	Depth (m)	Temp (°C)
0.1 (Surface)		9.5	
0.5		10.0	
1.0		10.5	
1.5		11.0	
2.0		11.5	
2.5		12.0	
3.0		12.5	
3.5		13.0	
4.0		13.5	
4.5		14.0	
5.0		14.5	
5.5		15.0	
6.0		15.5	
6.5		16.0	
7.0		17.0	
7.5		18.0	
8.0		19.0	
8.5		20.0	
9.0		21.0	

Note: Check surface and bottom temperatures first. Check intermediate depths only if temperature change is found. Indicate the bottom depth.

Comments/Observations (see reverse):

LAKE EUTROPHICATION PUBLIC PERCEPTION SURVEY

IF YOU CIRCLE MORE THAN ONE CHOICE FOR A AND/OR B, WE CANNOT USE THE SURVEY DATA THAT WEEK.

A. Please circle the one number that best describes the physical condition of the lake water today.

1. Crystal clear water.
2. Not quite crystal clear, a little algae visible.
3. Definite algae greenness, yellowness or brownest apparent.
4. High algae levels with limited clarity and/or mild odor apparent.
5. Severely high algae levels with one or more of the following:
   _massive floating scums on lake washed up on shore
   _strong foul odor
   _fish kill.

B. Please circle the one number that best describes your opinion on how suitable the lake water is for recreation and aesthetic enjoyment today.

1. Beautiful, could not be any nicer.
2. Very minor aesthetic problems; excellent for swimming, boating, enjoyment.
3. Swimming & aesthetic enjoyment slightly impaired because of algae levels.
4. Desire to swim & level of enjoyment of the lake substantially reduced because of algae levels.
5. Swimming and aesthetic enjoyment of the lake nearly impossible because of algae levels.

View Scope Comparison Study

We invite interested persons to take part in a study assessing the usefulness of the View Scope when collecting water clarity (Secchi Disk) data. In addition to the weekly water clarity readings that you collect (using the view scope), we are interested in obtaining monthly water clarity data using the four methods described below. Make sure you report weather or lake conditions on front page. **Please take 2 readings under each condition (Sunny/Shady Side, With/Without View Scope) and record each value.**

Take Secchi Disk reading from the shady side of the boat **without** the view scope	Secchi Disk Depth 1)_____meters 2)_____meters
Take the Secchi Disk reading from the sunny side of the boat **without** the view scope	Secchi Disk Depth 1)_____meters 2)_____meters
Take the Secchi Disk reading from the shady side of the boat **with** the view scope	Secchi Disk Depth 1)_____meters 2)_____meters
Take the Secchi Disk reading from the sunny side of the boat **with** the view scope	Secchi Disk Depth 1)_____meters 2)_____meters
Take the Black Disk reading from the sunny side of the boat **with** the view scope	Secchi Disk Depth 1)_____meters 2)_____meters

Comments/Observations
(Continued From Front of Page):

Laboratory Processing

Time: Start_____ Finish_____
Date:_____

[1]**Integrated chlorophyll samples**: Chlorophyll Filter
Lakewater volume filtered: _____ml
Filter drying time: _____hrs
Depth sampled: Surface - _____meters

[2]**Point chlorophyll samples**: Chlorophyll Filter (if taken)
Lakewater volume filtered: _____ml
Filter drying time: _____hrs
Depth sampled: Surface - _____meters

[1]**Integrated color sample**: 60 ml bottle of filtered water
_____ YES _____ NO

[2]**Point color sample**: 60 ml bottle of filtered water (if taken)
_____ YES _____ NO

Specific Conductivity sample: 60 ml bottle
(if taken) Depth of sample:_____meters

Alkalinity Sample Results:
Depth sampled: 0-_____meters
Gray endpoint: _____ml
Pink endpoint: _____ml
(estimate to the nearest 0.1ml)

pH Sample (if taken) Depth sampled:_____
pH Value_____

*** Denotes For FBG Use**

*_____
*_____
*_____
*_____
*_____

Optional Samples

[1]**Integrated Total Phosphorus**: 250ml acid washed bottle
_____YES _____NO *_____

[2]**Point Total Phosphorus**: 250 ml Acid Washed bottle
_____YES _____NO _____Depth *_____

[1]Sample collected in the surface waters using the integrated sampler (weighted garden hose).
[2]Sample collected using the point sampling bottle.. Samples are collected at the request of the CFB.

Turner Trilogy Results (if measured):
Sampling Depth: _____ (meters)
Phycocyanin: _____ (RFU)
Phycocyanin: _____ (*u*g/l)
Chlorophyll *a*: _____ (RFU)
Chlorophyll *a*: _____ (*u*g/l)

Turner Aquafluor Results (if measured):
Sampling Depth: _____ (meters)
Phycocyanin: _____ (*u*g/l)
Phycoerythrin: _____ (*u*g/l)
Chlorophyll *a*: _____ (*u*g/l)

FluoroQuik Fluorometer Results (if measured):
Sampling Depth: _____ (meters)
Phycocyanin: _____ (*u*g/l)
Phycoerythrin: _____ (*u*g/l)
Chlorophyll *a*: _____ (*u*g/l)

Return to: Attn: Bob Craycraft
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Durham NH 03824-3544
Voice: (603) 862-3696
Email: bob.craycraft@unh.edu

Revised 03/03/2021

Integrated Sample		Point Sample	
Chl *a* 663	_____/_____	Chl *a* 663	_____/_____
Chl *a* 664	_____/_____	Chl *a* 664	_____/_____
Chl *a* 665	_____/_____	Chl *a* 665	_____/_____
Chl *a* 750	_____/_____	Chl *a* 750	_____/_____
Diss. Color 440	_____	Diss. Color 440	_____
Diss. Color 460	_____	Diss. Color 460	_____
Diss. Color 493	_____	Diss. Color 493	_____
Diss. Color 750	_____	Diss. Color 750	_____
Diss. Color 880	_____	Diss. Color 880	_____

Volunteer Monitor Signature:_____

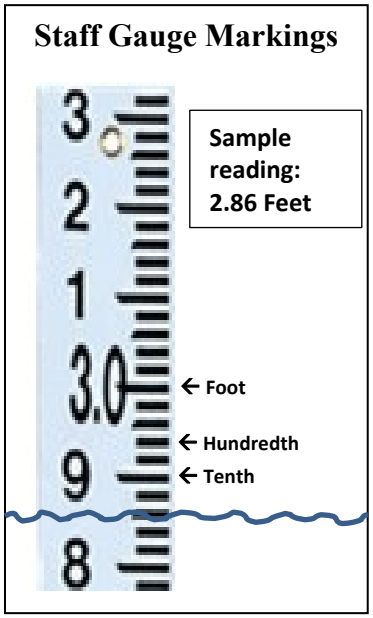
NH LLMP STREAM MONITORING INSTRUCTIONS (2021)

Data Sheet

1- Fill out a data sheet as completely and legibly as possible.
2- Return to the pre-designated sampling locations for consistency. If you are sampling a new location, use a GPS to obtain the coordinates, latitude and longitude, and record any noteworthy site features on the datasheet. Include a sketch of the new sampling location whenever possible.
3- Use a pencil or waterproof ink pen to record your observations.
4- Make sure all volunteers/observers are referenced.
5- Mark an "X" to indicate the collection of Total Phosphorus, Turbidity/Conductivity/pH and Chloride samples.
6- Record comments that may help interpret the results (e.g. sample collected during a heavy rainfall event or collected during an atypically dry month, high or low streamflow, water appears clearer/less clear than normal, etc.). If comments are extensive, they can be added to the back of the datasheet.

Collecting Staff Gauge Readings (when applicable)

1- Clean any attached material at or just below the waterline of the gauge.
2- Read gauge where the water level falls. *Note: binoculars may be needed to read the gauge, from a distance, at some sites.* If the water level fluctuates, select the average height as best you can and record the water level to the nearest hundredth as follows:
   a. Whenever the water line is located directly on the marker (e.g. the foot marker, tenth marker or hundredth marker), that/those value(s) should be recorded. Alternatively, record the water height using the following protocol.
   b. Note the first fully visible foot marker (the large number with the .0 after it) above the waterline. Record one less than that reading since the actual foot marker is submerged.
   c. Note the first visible tenth foot number above the waterline and record one less than that reading, since the actual tenth foot number is submerged.
   d. The hundredth foot digit marks are smaller and unnumbered. Each hundredth mark is 0.02 feet higher than the hundredth mark immediately below. Record the hundredth mark closest to the waterline.
   e. Collect and record a second stream gauge reading by repeating steps 2a through 2d. Whenever possible, have your sampling partner verify the stream gauge readings.



Collecting the Total Phosphorus Samples (250 milliliter translucent bottle)

1- Mark the bottle with site name, date, depth, time and volunteer name before taking the sample.
2- **Handle the bottle with care; it contains acid preservative**.
3- Carefully remove the cap. Be careful and avoid contact with the inside of the cap and the bottle opening.
4- Position yourself at a stable area, where the water is flowing, and face upstream.
5- Carefully place the bottle approximately four inches under the water, while pointing the bottle upstream and without disturbing the stream bottom. *Note: Alternatively, a large plastic scoop can be used to collect the water sample (see below)*
6- Only fill the bottle to the "shoulder". *Note: do not rinse this bottle with steam water. These bottles have been thoroughly cleaned in the laboratory and contain acid preservative.*
7- Carefully cap the bottle without touching inside the cap or the bottle opening.
8- Slowly and gently invert the bottle a few times to ensure the sample is preserved.

9- Place the sample in an ice filled cooler.

Collecting the Turbidity/Conductivity/pH samples (500 milliliter brown bottle)

1. Mark the bottle with site name, date, depth, time and volunteer name before taking the sample.
2. Carefully remove the cap. Be careful and avoid contact with the inside of the cap and the bottle opening.
3. Position yourself at a stable area, where the water is flowing, and face upstream
4. Carefully place the bottle approximately four inches under the water, while pointing the bottle upstream and without disturbing the stream bottom. *Note: Alternatively, a large plastic scoop can be used to collect the water sample (see below).*
5. Cap the bottle, invert serval times, and dump the water downstream of your sampling location.
6. Carefully place the bottle approximately four inches under the water, while pointing the bottle upstream and without disturbing the stream bottom. *Note: Alternatively, a large plastic scoop can be used to collect the water sample (see below).*
7. Fill the bottle to the top and leave no air space.
8. Carefully cap the bottle, without touching inside the cap or the bottle opening.
9. Place the sample in an ice filled cooler.

Collecting the Chloride samples (250 milliliter rectangular brown bottle)

1. Mark the bottle with site name, date, depth, time and volunteer name before taking sample.
2. Carefully remove the cap. Be careful and avoid contact with the inside of the cap and the bottle opening.
3. Position yourself at a stable area, where the water is flowing, and face upstream
4. Carefully place the bottle approximately four inches under the water, while pointing the bottle upstream and without disturbing the stream bottom. *Note: Alternatively, a large plastic scoop can be used to collect the water sample (see below).*
5. Cap the bottle, invert serval times, and dump the water downstream of your sampling location.
6. Carefully place the bottle approximately four inches under the water, while pointing the bottle upstream and without disturbing the stream bottom. *Note: Alternatively, a large plastic scoop can be used to collect the water sample (see below).*
7. Fill the bottle to the top and leave no air space.
8. Carefully cap the bottle, without touching inside the cap or the bottle opening.
9. Place the sample in an ice filled cooler.

Use of the Large Plastic Scoop (when applicable):

1. Rinse the scoop five times with stream water by submersing the scoop and discarding the water downstream of your sampling location.
2. Use the scoop to collect a water sample and pour the sample into the 250 milliliter total phosphorus sample bottle, the 500 milliliter brown sampling bottle and the 250 milliliter rectangular brown sampling bottle.

Project:_____ Tributary Datasheet (Date:_____20__)

Location	Depth (meters)	Time (24:00)	Latitude (dd.ddddd)	Longitude (dd.ddddd)	Total Phosphorus	Turbidity pH, Alkalinity Conductivity	Chloride	Sampler(s)/Notes/Comments	YSI Field Meter Temperature (°C)	YSI Field Meter Dissolved Oxygen (mg/l)	YSI Field Meter Specific Conductivity (uS/cm)

Note: Total phosphorus samples are collected in 250 milliliter translucent bottles, preserved with sulfuric acid. 500 milliliter brown sampling bottles collect stream water that is analyzed for specific conductivity, pH and turbidity. 250 milliliter brown rectangular sampling bottles collect stream water that is analyzed for chloride. All filled sampling bottles are placed in an ice filled cooler.

Relinquished By:	Date	Time	Received By:
Relinquished By:	Date	Time	Received By:

Appendix G
Center for Freshwater Biology Standard Operating Procedures
Field and Lab Sheets

CFB DATA SHEET (20__)

DLS #:_____

LAKE:_____ SITE:_____

WHO:_____ Time: Start_____ Finish_____

DATE:_____ Zmax:_____meters Zsd:_____meters AirTemp_____°C

Depth (meters)	pH	CO_2 (mg/L)	SPCD (υS/cm)	Alk (mg/L)	TP (ppb)	Chl *a* (ppb)	Diss. O_2 (mg/L)	Color (ptu)	Turb #1 (NTU)	Turb #2 (NTU)	Chloride (mg/l)
Surf. 0.5				/							/
1/2 epi_____m				/							/
1/2 meta____m				/							/
Bot-1m_____m				/							/
Depth_____m				/							/
Cyano: 0-3.0m				/							/
Int. 0-_____m				/							/

Depth (meters)	Temp (°C)	Diss O_2 (mg/L)	Diss O_2 (% sat)	SPCD (*u* S/cm)	Light (rel %)
0.1					
0.5					
1.0					
1.5					
2.0					
2.5					
3.0					
3.5					
4.0					
4.5					
5.0					
5.5					
6.0					
6.5					
7.0					
7.5					
8.0					
8.5					
9.0					
9.5					
10.0					
10.5					
11.0					
11.5					
12.0					
12.5					
13.0					
13.5					
14.0					
14.5					
15.0					
15.5					
16.0					

Depth (meters)	Temp (°C)	Diss O_2 (mg/L)	Diss O_2 (% sat)	SPCD (*u* S/cm)	Light (rel %)
16.5					
17.0					
17.5					
18.0					
18.5					
19.0					
19.5					
20.0					
20.5					
21.0					
21.5					
22.0					
22.5					
23.0					
23.5					
24.0					
24.5					
25.0					
25.5					
26.0					
26.5					
27.0					
27.5					
28.0					
28.5					
29.0					
29.5					
30.0					
30.5					
31.0					
31.5					
32.0					
32.5					

	Depth (meters)	pH	CO_2 (mg/L)	SPCD (υS/cm)	TP (ppb)	Alk (mg/L)	Chl *a* (ppb)	Diss. O_2 (mg/L)	Color (ptu)
Duplicate Sample	Depth_____m					/			
Replicate Sample	Depth_____m					/			

Depth Int: 0-_____meters

chl a663:_____/_____
chl a664:_____/_____
chl a665:_____/_____
chl a750:_____/_____
chl volume:_____ml
color a440:_____
color a460:_____
color a493:_____
color a750:_____
color a880:_____

Depth: 0.5 meters
chl a663:_____/_____
chl a664:_____/_____
chl a665:_____/_____
chl a750:_____/_____
chl volume:_____ml
color a440:_____
color a460:_____
color a493:_____
color a750:_____
color a880:_____

Depth: mid-meta_____m
chl a663:_____/_____
chl a664:_____/_____
chl a665:_____/_____
chl a750:_____/_____
chl volume:_____ml
color a440:_____
color a460:_____
color a493:_____
color a750:_____
color a880:_____

Depth:_____meters
chl a663:_____/_____
chl a664:_____/_____
chl a665:_____/_____
chl a750:_____/_____
chl volume:_____ml
color a440:_____
color a460:_____
color a493:_____
color a750:_____
color a880:_____

View Scope Comparison Study

WEATHER (circle best descriptor)

Sky: **Clear** **Hazy** **Cloudy** **Ovecast**

Lake: **Calm** **Ripples** **Waves** **Choppy**

Wind: **Calm** **Breezy** **Gusty** **Windy**

Take Secchi Disk reading from the **shady** side of the boat **without** the view scope Secchi Disk Depth (meters)	Take Secchi Disk reading from the **shady** side of the boat **with** the view scope Secchi Disk Depth (meters)	Take Secchi Disk reading from the **sunny** side of the boat **without** the view scope Secchi Disk Depth (meters)	Take Secchi Disk reading from the **sunny** side of the boat **with** the view scope Secchi Disk Depth (meters)	Take Black Secchi Disk reading from the **sunny** side of the boat **with** the view scope Secchi Disk Depth (meters)
Viewer #1:_____				
1)_____meters 2)_____meters 3)_____meters	1)_____meters 2)_____meters 3)_____meters	1)_____meters 2)_____meters 3)_____meters	1)_____meters 2)_____meters 3)_____meters	1)_____meters 2)_____meters 3)_____meters
Viewer #2:_____				
1)_____meters 2)_____meters 3)_____meters	1)_____meters 2)_____meters 3)_____meters	1)_____meters 2)_____meters 3)_____meters	1)_____meters 2)_____meters 3)_____meters	1)_____meters 2)_____meters 3)_____meters
Viewer #3:_____				
1)_____meters 2)_____meters 3)_____meters	1)_____meters 2)_____meters 3)_____meters	1)_____meters 2)_____meters 3)_____meters	1)_____meters 2)_____meters 3)_____meters	1)_____meters 2)_____meters 3)_____meters

Comments (continued from front of sheet):

SPCD Readings (post processing)
_____*u*S Standard = _____*u*S

dd H_2O TP blank _____ ppb

pH Meter Readings (post processing)
pH buffer 7.01 = _____
pH buffer 6.86 = _____
pH buffer 4.01 = _____

Zooplankon Tow: 0 - _____ meters
Mesh size:_____*u*m Net Diameter:_____cm

Turner Trilogy			
	Phycocyanin (RFU)	Chlorophyll a (RFU)	Chlorophyll a (ug/l)
Depth:_____	_____	_____	_____
Depth:_____	_____	_____	_____
Depth:_____	_____	_____	_____
Depth:_____	_____	_____	_____

Duplicate	**chl a663**	**chl a664**	**chl a665**	**chl a750**	**volume (ml)**
Depth: 0 - _____meters	/	/	/	/	
Depth:_____meters	/	/	/	/	
Duplicate	**color a440**	**color a493**	**color a750**	**color a880**	**color (CPU)**
Depth: 0 - _____meters					
Depth:_____meters					

Circle the Fluorometer that was used to generate the data (below). Note: the Cyanofluor records data as RFU

Fluor*Quik/Aquafluor
Depth:_____(meters)
Phycocyanin:_____ (*u*g/l)
Chlorophyll *a*:_____(*u*g/l)

Fluor*Quik/Aquafluor
Depth:_____(meters)
Phycocyanin:_____ (*u*g/l)
Chlorophyll *a*:_____(*u*g/l)

Fluor*Quik/Aquafluor
Depth:_____(meters)
Phycocyanin:_____ (*u*g/l)
Chlorophyll *a*:_____(*u*g/l)

Fluor*Quik/Aquafluor
Depth:_____(meters)
Phycocyanin:_____ (*u*g/l)
Chlorophyll *a*:_____(*u*g/l)

Instrument: Lamotte 2020e turbidometer

Date	Calibration	Inspection (Specify)	Corrective Action	Acceptance Criteria	Person Responsible (Signature)
	1 and 10 NTU standards	Check batteries, visual inspection of meter and cell holders		No visible damage to the meter or cell holders	Field Coordinator
	1 and 10 NTU standards	Check batteries, visual inspection of meter and cell holders		No visible damage to the meter or cell holders	Field Coordinator
	1 and 10 NTU standards	Check batteries, visual inspection of meter and cell holders		No visible damage to the meter or cell holders	Field Coordinator
	1 and 10 NTU standards	Check batteries, visual inspection of meter and cell holders		No visible damage to the meter or cell holders	Field Coordinator
	1 and 10 NTU standards	Check batteries, visual inspection of meter and cell holders		No visible damage to the meter or cell holders	Field Coordinator
	1 and 10 NTU standards	Check batteries, visual inspection of meter and cell holders		No visible damage to the meter or cell holders	Field Coordinator
	1 and 10 NTU standards	Check batteries, visual inspection of meter and cell holders		No visible damage to the meter or cell holders	Field Coordinator
	1 and 10 NTU standards	Check batteries, visual inspection of meter and cell holders		No visible damage to the meter or cell holders	Field Coordinator
	1 and 10 NTU standards	Check batteries, visual inspection of meter and cell holders		No visible damage to the meter or cell holders	Field Coordinator
	1 and 10 NTU standards	Check batteries, visual inspection of meter and cell holders		No visible damage to the meter or cell holders	Field Coordinator

Turbidity Analysis Sheet (20__)

Transferred	Site ID	Analyst	Turbidity Rep 1 (NTU)	Turbidity Rep 2 (NTU)	Date of Analysis

Page Control #:_____

Instrument: Oakton PC 2700

Date	Calibration	Inspection (Specify)	Corrective Action	Acceptance Criteria	Person Responsible (Signature)
	pH: 4.01, 7.00, 10.01 SPCD: 84 *u* S Chloride: standard curve; 1, 2, 4, 6, 9.9, 29 and 48 mg/l	Inspect probes, cable and meter for damage and check the battery power. Make sure the ISE (pH or Chloride) electrode filling solution is at the proper level.		No visible damage to the meter, probes or cables; pH fill hole plug removed and electrode filling solution level is near the fill hole.	Field Coordinator
	pH: 4.01, 7.00, 10.01 SPCD: 84 *u* S Chloride: standard curve; 1, 2, 4, 6, 9.9, 29 and 48 mg/l	Inspect probes, cable and meter for damage and check the battery power. Make sure the ISE (pH or Chloride) electrode filling solution is at the proper level.		No visible damage to the meter, probes or cables; pH fill hole plug removed and electrode filling solution level is near the fill hole.	Field Coordinator
	pH: 4.01, 7.00, 10.01 SPCD: 84 *u* S Chloride: standard curve; 1, 2, 4, 6, 9.9, 29 and 48 mg/l	Inspect probes, cable and meter for damage and check the battery power. Make sure the ISE (pH or Chloride) electrode filling solution is at the proper level.		No visible damage to the meter, probes or cables; pH fill hole plug removed and electrode filling solution level is near the fill hole.	Field Coordinator
	pH: 4.01, 7.00, 10.01 SPCD: 84 *u* S Chloride: standard curve; 1, 2, 4, 6, 9.9, 29 and 48 mg/l	Inspect probes, cable and meter for damage and check the battery power. Make sure the ISE (pH or Chloride) electrode filling solution is at the proper level.		No visible damage to the meter, probes or cables; pH fill hole plug removed and electrode filling solution level is near the fill hole.	Field Coordinator
	pH: 4.01, 7.00, 10.01 SPCD: 84 *u* S Chloride: standard curve; 1, 2, 4, 6, 9.9, 29 and 48 mg/l	Inspect probes, cable and meter for damage and check the battery power. Make sure the ISE (pH or Chloride) electrode filling solution is at the proper level.		No visible damage to the meter, probes or cables; pH fill hole plug removed and electrode filling solution level is near the fill hole.	Field Coordinator
	pH: 4.01, 7.00, 10.01 SPCD: 84 *u* S Chloride: standard curve; 1, 2, 4, 6, 9.9, 29 and 48 mg/l	Inspect probes, cable and meter for damage and check the battery power. Make sure the ISE (pH or Chloride) electrode filling solution is at the proper level.		No visible damage to the meter, probes or cables; pH fill hole plug removed and electrode filling solution level is near the fill hole.	Field Coordinator

Analyist:_____

Oakton PC 2700 pH and SPCD Analysis Sheet (20__)

Transferred	Site ID	pH Rep 1 (std units)	pH Rep 2 (std units)	SPCD Rep 1 (uS/cm)	SPCD Rep 2 (uS/cm)	Date of Analysis

Page Control #_____

Oakton PC 2700: Chloride Analysis Sheet (20__)

Transferred	Site ID	Chloride Rep 1 mV	Chloride Rep 2 mV	Chloride Rep 1 mg/L	Chloride Rep 2 mg/L	Date of Analysis
	Chloride Standard: 1.0 ppm					
	Chloride Standard: 2.0 ppm					
	Chloride Standard: 4.0 ppm					
	Chloride Standrd: 6.0 ppm					
	Chloride Standard: 9.9 ppm					
	Chloride Standard: 29.0 ppm					
	Chloride Standard: 48.0 ppm					
	Chloride Standard: 20 ppm					

Page Control #:_____

Analyst:_____

Oakton PC 2700: Chloride Analysis Sheet (20__)

Transferred	Site ID	Chloride Rep 1 mV	Chloride Rep 2 mV	Chloride Rep 1 mg/L	Chloride Rep 2 mg/L	Date of Analysis
	Chloride Standard: 20 ppm					
	Chloride Standard: 20 ppm					
	Chloride Standard: 20 ppm					

Page Control #:_____

Alkalinity Analysis Sheet (20__)

Transferred	Site ID	Analyst	Alkalinity gray end pt. @ pH 5.1 Rep 1 (mg/l)	Alkalinity gray end pt. @ pH 5.1 Rep 2 (mg/l)	Alkalinity pink end pt. @ pH 4.6 Rep 1 (mg/l)	Alkalinity pink end pt. @ pH 4.6 Rep 2 (mg/l)	Date of Analysis

Page Control #:_____

Carbon Dioxide Sheet (20__)

Transferred	Site ID	Analyst	Carbon Dioxide Rep 1 (mg/L)	Carbon Dioxide Rep 2 (mg/L)	Date of Analysis

Page Control #:_____

Winkler Dissolved Oxygen Sheet (20__)

Transferred	Site ID	Analyst	Dissolved Oxygen Rep 1 (mg/L)	Dissolved Oxygen Rep 2 (mg/L)	Date of Analysis

Page Control #:_____

Total Suspended Solids (20__)

Date Processed: _____ **Data Analyst: _____**

	Sample ID	Collection Date	Dish ID	Weight Dish (grams)	Weight Dish + Filter Without Filtrate weight 1 / weight 2 (grams)	Volume Filtered (liters)	Weight Dish + Filter With Filtrate weight 1 / weight 2 (grams)	TSS (mg/liter)
1	Blank				/		/	
2					/		/	
3	(laboratory replicate)				/		/	
4					/		/	
5					/		/	
6					/		/	
7					/		/	
8					/		/	
9					/		/	
10					/		/	
11					/		/	
12					/		/	
13					/		/	
14					/		/	
15					/		/	
16					/		/	
17					/		/	
18					/		/	
19					/		/	
20					/		/	
21					/		/	
22					/		/	
23					/		/	
24					/		/	
25					/		/	
26					/		/	
27					/		/	
28					/		/	

E. coli Lab Analysis Datasheet

Date Processed: _____ **Data Analyst: _____**

Sample ID	Collection Date	Collection Time	Time Filtered	Volume Filtered (ml)	Time Counted	*E.coli* (CFU/100 ml)	Other Coliforms (CFU/100 ml)	Comments
Blank								

Page Control 20__:_____

Spectrophotometer:_________________

Chlorophyll *a* "spec" Datasheet (20__)

ID #	Data Trans	Lake	Site	Date	Depth (meters)	Volume Filtered	a663	a664	a665	a750	[chl *a*] (ppb)	Date Processed
							/	/	/	/		
							/	/	/	/		
							/	/	/	/		
							/	/	/	/		
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							/	/	/	/		

Page Control (20__) ____ of _____

Dissolved Color "spec" Datasheet (20__)

Spectrophotometer:

Trans-ferred	Lake	Site	Collection Date	Depth (meters)	a440	a460	a493	a750	a880	Color (CPU)	Comments	date processed
	dd H_2O Blank											
	200 cpu standard											
	100 cpu standard											
	20 cpu standard											
	5 cpu standard											
	20 cpu standard											
	dd H_2O Blank											
	20 cpu standard											
	dd H_2O Blank											

Current Color Coefficients based on _____standard curve: 5.0 - 200 cpu

a440nm Color Coefficient: _____
a493nm Color Coefficient: _____

a440nm Calibration Curve r^2:_____
a493nm Calibration Curve r^2:_____

Page Control 20___: _____

Dissolved Color "spec" Datasheet (20__)

Data Trans	Lake	Site	Collection Date	Depth (meters)	a440	a460	a493	a750	a880	Color (CPU)	Comments	date processed

Page Control 20__:______ **Color Coefficient based on ____________________ standard curve.**

Total Nitrogen Datasheet (20__)

Data Analyst:_____

Date Processed:_____

Cycle: cycle count = 3, cycle time = 0.5 minutes Scan Controls: average time = 0.25 seconds, data internavl = 0.5 nm, scan rate = 120 nm/min

Sample	Lake ID	Date	Wavelength	2nd Der. #1	2nd Der. #2	2nd Der. #3	Average 2nd Deriv	[TN] (ug/L)
1	dd H2O blank							
2	2000 ug/L Standard							
3	1000 ug/L Standard							
4	500 ug/L Standard							
5	250 ug/L Standard							
6	100 ug/L Standard							
7	(replicate)							
8								
9	(replicate)							
10								
11	(replicate)							
12								
13	(replicate)							
14								
15	(replicate)							
16								
17	(replicate)							
18								
19	(replicate spike _____)							
20								
21	(replicate)							
22								
23	(replicate)							
24								
25	(replicate)							
26								

Nitrogen Coefficient:_____ **Calibration Curve r^2:**_____

Page Control 20__: _____

Total Nitrogen (20__) Date:_____

Cycle: cycle count = 3, cycle time = 0.5 minutes Scan Controls: average time = 0.25 seconds, data internavl = 0.5 nm, scan rate = 120 nm/min

Sample	Lake ID	Date	Wavelength	2nd Der. #1	2nd Der. #2	2nd Der. #3	Average 2nd Deriv	[TN] (ug/L)
27								
28	(replicate spike ____ ppb)							
29								
30	(replicate)							
31								
32	(replicate)							
33								
34	(replicate)							
35								
36	(replicate)							
37								
38	(replicate)							
39								
40	(replicate)							
41								
42	(replicate)							
43								
44	(replicate)							
45								
46	(replicate)							
47								
48	(replicate)							
49								
50	(replicate)							
51								
52	(replicate)							
53	1000 ug/l standard							
54	dd H_2O Blank							

Page Control (20__): _____

CFB ORTHOPHOSPHATE ANALYSIS DATA SHEET (20__)

CURRENT DATE:_____ ANALYZED BY:_____

Sample ID# / Site Description	a660	a880	SRP (ppb)	Spec chk	Transfered
1A 50.0 ppb SRP Standard				/	
1B 50.0 ppb SRP Standard				/	
2A 20.0 ppb SRP Standard				/	
2B 20.0 ppb SRP Standard				/	
3A 10.0 ppb SRP Standard				/	
3B 10.0 ppb SRP Standard				/	
4A 5.0 ppb SRP Standard				/	
4B 5.0 ppb SRP Standard				/	
5A 1.0 ppb SRP Standard				/	
5B 1.0 ppb SRP Standard				/	
6A dd H_2O blank				/	
6B dd H_2O blank				/	
7A				/	
7B				/	
8A				/	
8B				/	
9A				/	
9B				/	
10A				/	
10B				/	
11A				/	
11B				/	
12A Lab Spike 25.0 ppb				/	
12B Lab Spike 25.0 ppb				/	
13A SRP Standard: 10.0 ppb				/	
13B SRP Standard: 10.0 ppb				/	
14A				/	
14B				/	
15A				/	
15B				/	
16A				/	
16B				/	
17A				/	
17B				/	
18A				/	
18B				/	
19A				/	
19B				/	
20A				/	
20B				/	
21A				/	
21B				/	
22A				/	
22B				/	

Sample ID# / Site Description	a660	a880	SRP (ppb)	Spec chk	Transfered
23A SRP Standard: 5.0 ppb				/	
23B SRP Standard: 5.0 ppb				/	
24A				/	
24B				/	
25A				/	
25B				/	
26A				/	
26B				/	
27A				/	
27B				/	
28A				/	
28B				/	
29A				/	
29B				/	
30A				/	
30B				/	
31A				/	
31B				/	
32A Lab Spike 25.0 ppb				/	
32B Lab Spike 25.0 ppb				/	
33A SRP Standard: 5.0 ppb				/	
33B SRP Standard: 5.0 ppb				/	
34A				/	
34B				/	
35A				/	
35B				/	
36A				/	
36B				/	
37A				/	
37B				/	
38A				/	
38B				/	
39A				/	
39B				/	
40A				/	
40B				/	
41A SRP Standard: 1.0 ppb				/	
41B SRP Standard 1.0 ppb				/	
42A dd H_2O blank				/	
42B dd H_2O blank				/	

Date Chemicals were made:

5N H_2SO_4_____

Potassium antimonyl tartrate_____

Ammonium Molybdate_____

Ascorbic Acid_____

SRP Coefficient (Based on 1 - 50 ppb standards)

a880 SRP Coefficient = _____ Calibration Curve r^2 = _____

CFB TOTAL PHOSPHORUS ANALYSIS DATA SHEET (20__)

CURRENT DATE:_____ **ANALYZED BY:_____**

Sample ID# / Site Description	a660	a880	Mean a660	TP (ppb)	Spec chk	Transfered
1A 100 ppb TP Standard					/	
1B 100 ppb TP Standard					/	
2A 40 ppb TP Standard					/	
2B 40 ppb TP Standard					/	
3A 20 ppb TP Standard					/	
3B 20 ppb TP Standard					/	
4A 10 ppb TP Standard					/	
4B 10 ppb TP Standard					/	
5A 2 ppb TP Standard					/	
5B 2 ppb TP Standard					/	
6A dd H_2O blank					/	
6B dd H_2O blank					/	
7A					/	
7B					/	
8A					/	
8B					/	
9A					/	
9B					/	
10A					/	
10B					/	
11A					/	
11B					/	
12A Lab Spike: 50 ppb					/	
12B Lab Spike: 50 ppb					/	
13A TP Standard: _____ ppb					/	
13B TP Standard: _____ ppb					/	
14A					/	
14B					/	
15A					/	
15B					/	
16A					/	
16B					/	
17A					/	
17B					/	
18A					/	
18B					/	
19A					/	
19B					/	
20A					/	
20B					/	
21A					/	
21B					/	
22A					/	
22B					/	
23A TP Standard: _____ ppb					/	
23B TP Standard: _____ ppb					/	

Sample ID# / Site Description	a660	a880	Mean a660	TP (ppb)	Spec chk	Transfered
24A					/	
24B					/	
25A					/	
25B					/	
26A					/	
26B					/	
27A					/	
27B					/	
28A					/	
28B					/	
29A					/	
29B					/	
30A					/	
30B					/	
31A					/	
31B					/	
32A Lab Spike: 50 ppb					/	
32B Lab Spike: 50 ppb					/	
33A TP Standard: _____ ppb					/	
33B TP Standard: _____ ppb					/	
34A					/	
34B					/	
35A					/	
35B					/	
36A					/	
36B					/	
37A					/	
37B					/	
38A					/	
38B					/	
39A					/	
39B					/	
40A					/	
40B					/	
41A TP Standard: _____ ppb					/	
41B TP Standard _____ ppb					/	
42A dd H_2O blank					/	
42B dd H_2O blank					/	

Date Chemicals/Standards Made:

5N H_2SO_4_____

11N H_2SO_4_____

10N NaOH_____

Potassium antimonyl tartrate_____

Ammonium Molybdate_____

Ascorbic Acid_____

Current TP Coefficients (Based on 2 - 100 ppb standards)

a880 TP Coefficient = _____ Calibration Curve r^2 = _____

CFB TOTAL PHOSPHORUS ANALYSIS DATA SHEET (20__)

CURRENT DATE:_____ ANALYZED BY:_____

Sample ID# / Site Description	a660	a880	Mean a660	TP (ppb)	Spec chk	Transfered
43A					/	
43B					/	
44A					/	
44B					/	
45A					/	
45B					/	
46A					/	
46B					/	
47A					/	
47B					/	
48A					/	
48B					/	
49A					/	
49B					/	
50A					/	
50B					/	
51A					/	
51B					/	
52A Lab Spike: 50 ppb					/	
52B Lab Spike: 50 ppb					/	
53A TP Standard: _____ ppb					/	
53B TP Standard: _____ ppb					/	
54A					/	
54B					/	
55A					/	
55B					/	
56A					/	
56B					/	
57A					/	
57B					/	
58A					/	
58B					/	
59A TP Standard: _____ ppb					/	
59B TP Standard: _____ ppb					/	
60A dd H_2O Blank					/	
60B dd H_2O Blank					/	

Instrument: Denver Instruments A-250 Analytical Balance

Date	Calibration	Inspection (Specify)	Corrective Action	Acceptance Criteria	Person Responsible (Signature)
	200 g standard	Make sure weighing plate is seated correctly and is clear of debris		Weighting plate is correctly seated and is clear of debris	Lab Coordinator
	200 g standard	Make sure weighing plate is seated correctly and is clear of debris		Weighting plate is correctly seated and is clear of debris	Lab Coordinator
	200 g standard	Make sure weighing plate is seated correctly and is clear of debris		Weighting plate is correctly seated and is clear of debris	Lab Coordinator
	200 g standard	Make sure weighing plate is seated correctly and is clear of debris		Weighting plate is correctly seated and is clear of debris	Lab Coordinator
	200 g standard	Make sure weighing plate is seated correctly and is clear of debris		Weighting plate is correctly seated and is clear of debris	Lab Coordinator
	200 g standard	Make sure weighing plate is seated correctly and is clear of debris		Weighting plate is correctly seated and is clear of debris	Lab Coordinator
	200 g standard	Make sure weighing plate is seated correctly and is clear of debris		Weighting plate is correctly seated and is clear of debris	Lab Coordinator
	200 g standard	Make sure weighing plate is seated correctly and is clear of debris		Weighting plate is correctly seated and is clear of debris	Lab Coordinator
	200 g standard	Make sure weighing plate is seated correctly and is clear of debris		Weighting plate is correctly seated and is clear of debris	Lab Coordinator
	200 g standard	Make sure weighing plate is seated correctly and is clear of debris		Weighting plate is correctly seated and is clear of debris	Lab Coordinator

Instrument: Denver Instruments XE-510 Scale

Date	Calibration	Inspection (Specify)	Corrective Action	Acceptance Criteria	Person Responsible (Signature)
	200 g standard	Make sure weighing plate is seated correctly and is clear of debris		Weighting plate is correctly seated and is clear of debris	Lab Coordinator
	200 g standard	Make sure weighing plate is seated correctly and is clear of debris		Weighting plate is correctly seated and is clear of debris	Lab Coordinator
	200 g standard	Make sure weighing plate is seated correctly and is clear of debris		Weighting plate is correctly seated and is clear of debris	Lab Coordinator
	200 g standard	Make sure weighing plate is seated correctly and is clear of debris		Weighting plate is correctly seated and is clear of debris	Lab Coordinator
	200 g standard	Make sure weighing plate is seated correctly and is clear of debris		Weighting plate is correctly seated and is clear of debris	Lab Coordinator
	200 g standard	Make sure weighing plate is seated correctly and is clear of debris		Weighting plate is correctly seated and is clear of debris	Lab Coordinator
	200 g standard	Make sure weighing plate is seated correctly and is clear of debris		Weighting plate is correctly seated and is clear of debris	Lab Coordinator
	200 g standard	Make sure weighing plate is seated correctly and is clear of debris		Weighting plate is correctly seated and is clear of debris	Lab Coordinator
	200 g standard	Make sure weighing plate is seated correctly and is clear of debris		Weighting plate is correctly seated and is clear of debris	Lab Coordinator
	200 g standard	Make sure weighing plate is seated correctly and is clear of debris		Weighting plate is correctly seated and is clear of debris	Lab Coordinator

Denver Instruments Scale/Balance Standard Weight Measurements

Denver Instruments XE-510 Scale

Date/Time	200g Standard (grams)	1.00 g Standard (grams)

Denver Instruments A-250 Analytical Balance

Date/Time	200g Standard (grams)	1.00 g Standard (grams)

Instrument: Yellow Spring Instruments EXO2

Date	Calibration	Inspection (Specify)	Corrective Action	Acceptance Criteria	Person Responsible (Signature)
	pH, ORP, DO, SPCD Turbidity, Temp, chl phycocyanin, fDOM	inspect probes, ODO cap cable and meter for damage, check batteries		No visible damage to the meter, probes or cable, ODO cap not scratched or chipped.	Field Coordinator
	pH, ORP, DO, SPCD Turbidity, Temp, chl phycocyanin, fDOM	inspect probes, ODO cap cable and meter for damage, check batteries		No visible damage to the meter, probes or cable, ODO cap not scratched or chipped.	Field Coordinator
	pH, ORP, DO, SPCD Turbidity, Temp, chl phycocyanin, fDOM	inspect probes, ODO cap cable and meter for damage, check batteries		No visible damage to the meter, probes or cable, ODO cap not scratched or chipped.	Field Coordinator
	pH, ORP, DO, SPCD Turbidity, Temp, chl phycocyanin, fDOM	inspect probes, ODO cap cable and meter for damage, check batteries		No visible damage to the meter, probes or cable, ODO cap not scratched or chipped.	Field Coordinator
	pH, ORP, DO, SPCD Turbidity, Temp, chl phycocyanin, fDOM	inspect probes, ODO cap cable and meter for damage, check batteries		No visible damage to the meter, probes or cable, ODO cap not scratched or chipped.	Field Coordinator
	pH, ORP, DO, SPCD Turbidity, Temp, chl phycocyanin, fDOM	inspect probes, ODO cap cable and meter for damage, check batteries		No visible damage to the meter, probes or cable, ODO cap not scratched or chipped.	Field Coordinator
	pH, ORP, DO, SPCD Turbidity, Temp, chl phycocyanin, fDOM	inspect probes, ODO cap cable and meter for damage, check batteries		No visible damage to the meter, probes or cable, ODO cap not scratched or chipped.	Field Coordinator
	pH, ORP, DO, SPCD Turbidity, Temp, chl phycocyanin, fDOM	inspect probes, ODO cap cable and meter for damage, check batteries		No visible damage to the meter, probes or cable, ODO cap not scratched or chipped.	Field Coordinator
	pH, ORP, DO, SPCD Turbidity, Temp, chl phycocyanin, fDOM	inspect probes, ODO cap cable and meter for damage, check batteries		No visible damage to the meter, probes or cable, ODO cap not scratched or chipped.	Field Coordinator
	pH, ORP, DO, SPCD Turbidity, Temp, chl phycocyanin, fDOM	inspect probes, ODO cap cable and meter for damage, check batteries		No visible damage to the meter, probes or cable, ODO cap not scratched or chipped.	Field Coordinator

YSI EXO2 Post-Deployment Log (20__)

Date____________________
pH 10.00 buffer =
pH 7.00 buffer =
DO % Saturation =
Rhodamine Std PC RFU =
Rhodamine Std CHL RFU =
ORP 228mV =
SPCD 100 uS/cm standard =

Date____________________
pH 10.00 buffer =
pH 7.00 buffer =
DO % Saturation =
Rhodamine Std PC RFU =
Rhodamine Std CHL RFU =
ORP 228mV =
SPCD 100 uS/cm standard =

Date____________________
pH 10.00 buffer =
pH 7.00 buffer =
DO % Saturation =
Rhodamine Std PC RFU =
Rhodamine Std CHL RFU =
ORP 228mV =
SPCD 100 uS/cm standard =

Date____________________
pH 10.00 buffer =
pH 7.00 buffer =
DO % Saturation =
Rhodamine Std PC RFU =
Rhodamine Std CHL RFU =
ORP 228mV =
SPCD 100 uS/cm standard =

Date____________________
pH 10.00 buffer =
pH 7.00 buffer =
DO % Saturation =
Rhodamine Std PC RFU =
Rhodamine Std CHL RFU =
ORP 228mV =
SPCD 100 uS/cm standard =

Date____________________
pH 10.00 buffer =
pH 7.00 buffer =
DO % Saturation =
Rhodamine Std PC RFU =
Rhodamine Std CHL RFU =
ORP 228mV =
SPCD 100 uS/cm standard =

Date____________________
pH 10.00 buffer =
pH 7.00 buffer =
DO % Saturation =
Rhodamine Std PC RFU =
Rhodamine Std CHL RFU =
ORP 228mV =
SPCD 100 uS/cm standard =

Date____________________
pH 10.00 buffer =
pH 7.00 buffer =
DO % Saturation =
Rhodamine Std PC RFU =
Rhodamine Std CHL RFU =
ORP 228mV =
SPCD 100 uS/cm standard =

Date____________________
pH 10.00 buffer =
pH 7.00 buffer =
DO % Saturation =
Rhodamine Std PC RFU =
Rhodamine Std CHL RFU =
ORP 228mV =
SPCD 100 uS/cm standard =

Date____________________
pH 10.00 buffer =
pH 7.00 buffer =
DO % Saturation =
Rhodamine Std PC RFU =
Rhodamine Std CHL RFU =
ORP 228mV =
SPCD 100 uS/cm standard =

Date____________________
pH 10.00 buffer =
pH 7.00 buffer =
DO % Saturation =
Rhodamine Std PC RFU =
Rhodamine Std CHL RFU =
ORP 228mV =
SPCD 100 uS/cm standard =

Date____________________
pH 10.00 buffer =
pH 7.00 buffer =
DO % Saturation =
Rhodamine Std PC RFU =
Rhodamine Std CHL RFU =
ORP 228mV =
SPCD 100 uS/cm standard =

Instrument: Yellow Spring Instruments 6600

Date	Calibration	Inspection (Specify)	Corrective Action	Acceptance Criteria	Person Responsible (Signature)
	pH, ORP, DO, SPCD Turbidity, Temp, chl phycocyanin	inspect probes, cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator
	pH, ORP, DO, SPCD Turbidity, Temp, chl phycocyanin	inspect probes, cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator
	pH, ORP, DO, SPCD Turbidity, Temp, chl phycocyanin	inspect probes, cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator
	pH, ORP, DO, SPCD Turbidity, Temp, chl phycocyanin	inspect probes, cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator
	pH, ORP, DO, SPCD Turbidity, Temp, chl phycocyanin	inspect probes, cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator
	pH, ORP, DO, SPCD Turbidity, Temp, chl phycocyanin	inspect probes, cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator
	pH, ORP, DO, SPCD Turbidity, Temp, chl phycocyanin	inspect probes, cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator
	pH, ORP, DO, SPCD Turbidity, Temp, chl phycocyanin	inspect probes, cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator
	pH, ORP, DO, SPCD Turbidity, Temp, chl phycocyanin	inspect probes, cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator
	pH, ORP, DO, SPCD Turbidity, Temp, chl phycocyanin	inspect probes, cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator

YSI 6600 Post-Deployment Log (20__)

Date____________________
pH 10.00 buffer =
pH 7.00 buffer =
pH 6.86 buffer =
pH 5.00 buffer =
pH 4.00 buffer =
ORP 228mV =
SPCD 100 uS/cm standard =

Date____________________
pH 10.00 buffer =
pH 7.00 buffer =
pH 6.86 buffer =
pH 5.00 buffer =
pH 4.00 buffer =
ORP 228mV =
SPCD 100 uS/cm standard =

Date____________________
pH 10.00 buffer =
pH 7.00 buffer =
pH 6.86 buffer =
pH 5.00 buffer =
pH 4.00 buffer =
ORP 228mV =
SPCD 100 uS/cm standard =

Date____________________
pH 10.00 buffer =
pH 7.00 buffer =
pH 6.86 buffer =
pH 5.00 buffer =
pH 4.00 buffer =
ORP 228mV =
SPCD 100 uS/cm standard =

Date____________________
pH 10.00 buffer =
pH 7.00 buffer =
pH 6.86 buffer =
pH 5.00 buffer =
pH 4.00 buffer =
ORP 228mV =
SPCD 100 uS/cm standard =

Date____________________
pH 10.00 buffer =
pH 7.00 buffer =
pH 6.86 buffer =
pH 5.00 buffer =
pH 4.00 buffer =
ORP 228mV =
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Instrument: Yellow Spring Instruments 85

Date	Calibration	Inspection (Specify)	Corrective Action	Acceptance Criteria	Person Responsible (Signature)
	DO and SPCD	inspect probes, O_2 cap cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator
	DO and SPCD	inspect probes, O_2 cap cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator
	DO and SPCD	inspect probes, O_2 cap cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator
	DO and SPCD	inspect probes, O_2 cap cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator
	DO and SPCD	inspect probes, O_2 cap cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator
	DO and SPCD	inspect probes, O_2 cap cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator
	DO and SPCD	inspect probes, O_2 cap cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator
	DO and SPCD	inspect probes, O_2 cap cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator
	DO and SPCD	inspect probes, O_2 cap cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator

Instrument: Yellow Spring Instruments Professional Plus

Date	Calibration	Inspection (Specify)	Corrective Action	Acceptance Criteria	Person Responsible (Signature)
	pH, ORP, DO, SPCD Chloride, Temp	inspect probes, cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator
	pH, ORP, DO, SPCD Chloride, Temp	inspect probes, cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator
	pH, ORP, DO, SPCD Chloride, Temp	inspect probes, cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator
	pH, ORP, DO, SPCD Chloride, Temp	inspect probes, cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator
	pH, ORP, DO, SPCD Chloride, Temp	inspect probes, cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator
	pH, ORP, DO, SPCD Chloride, Temp	inspect probes, cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator
	pH, ORP, DO, SPCD Chloride, Temp	inspect probes, cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator
	pH, ORP, DO, SPCD Chloride, Temp	inspect probes, cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator
	pH, ORP, DO, SPCD Chloride, Temp	inspect probes, cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator
	pH, ORP, DO, SPCD Chloride, Temp	inspect probes, cable and meter for damage, check batteries		No visible damage to the meter, probes or cable; O_2 cap filled with solution	Field Coordinator

YSI Professional Plus Post-Deployment Log (20__)

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Instrument: Yellow Spring Instruments ProSolo

Date	Calibration	Inspection (Specify)	Corrective Action	Acceptance Criteria	Person Responsible (Signature)
	SPCD, DO, Temp	inspect probes, ODO cap, cable and meter for damage, check power		No visible damage to the meter, probes or cable; battery at >= 60% charge	Field Coordinator
	SPCD, DO, Temp	inspect probes, ODO cap, cable and meter for damage, check power		No visible damage to the meter, probes or cable; battery at >= 60% charge	Field Coordinator
	SPCD, DO, Temp	inspect probes, ODO cap, cable and meter for damage, check power		No visible damage to the meter, probes or cable; battery at >= 60% charge	Field Coordinator
	SPCD, DO, Temp	inspect probes, ODO cap, cable and meter for damage, check power		No visible damage to the meter, probes or cable; battery at >= 60% charge	Field Coordinator
	SPCD, DO, Temp	inspect probes, ODO cap, cable and meter for damage, check power		No visible damage to the meter, probes or cable; battery at >= 60% charge	Field Coordinator
	SPCD, DO, Temp	inspect probes, ODO cap, cable and meter for damage, check power		No visible damage to the meter, probes or cable; battery at >= 60% charge	Field Coordinator
	SPCD, DO, Temp	inspect probes, ODO cap, cable and meter for damage, check power		No visible damage to the meter, probes or cable; battery at >= 60% charge	Field Coordinator
	SPCD, DO, Temp	inspect probes, ODO cap, cable and meter for damage, check power		No visible damage to the meter, probes or cable; battery at >= 60% charge	Field Coordinator
	SPCD, DO, Temp	inspect probes, ODO cap, cable and meter for damage, check power		No visible damage to the meter, probes or cable; battery at >= 60% charge	Field Coordinator
	SPCD, DO, Temp	inspect probes, ODO cap, cable and meter for damage, check power		No visible damage to the meter, probes or cable; battery at >= 60% charge	Field Coordinator

Appendix H
Site Specific Project Plan (SSPP) Sample

SITE SPECIFIC PROJECT PLAN FOR:

Mirror Lake Community Watershed Plan-Phase I

Operated Under:
New Hampshire Center for Freshwater Biology and Lakes Lay Monitoring Program Programmatic QAPP

RFA# XXXXX
(Approval Date)

Final Draft
(Insert Date)

Prepared by:
Robert Craycraft and Jeffrey Schloss
UNH Center for Freshwater Biology and UNH Cooperative Extension
38 College Road, 133 Spaulding Hall
Durham NH 03824

Reviewed by:

CFB Project Manager: _____
Signature/Date
Jeffrey Schloss

CFB QA Officer: _____
Signature/Date
Robert Craycraft

WQ Analysis Lab QA Officer: _____
Signature/Date
Jody Potter

Program Quality Assurance Coordinator: _____
Signature/Date
Jillian McCarthy

NHDES Quality Assurance Manager: _____
Signature/Date
Vincent Perelli

Received by:
US EPA Project Manager: _____
Signature/Date
Leah O'Neill, US EPA Region I

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Distribution List

Table 1 lists people who will receive copies of the approved Site Specific Project Plan (SSPP) under the **New Hampshire Center for Freshwater Biology and Lakes Lay Monitoring Program Programmatic QAPP RFA # XXX**.

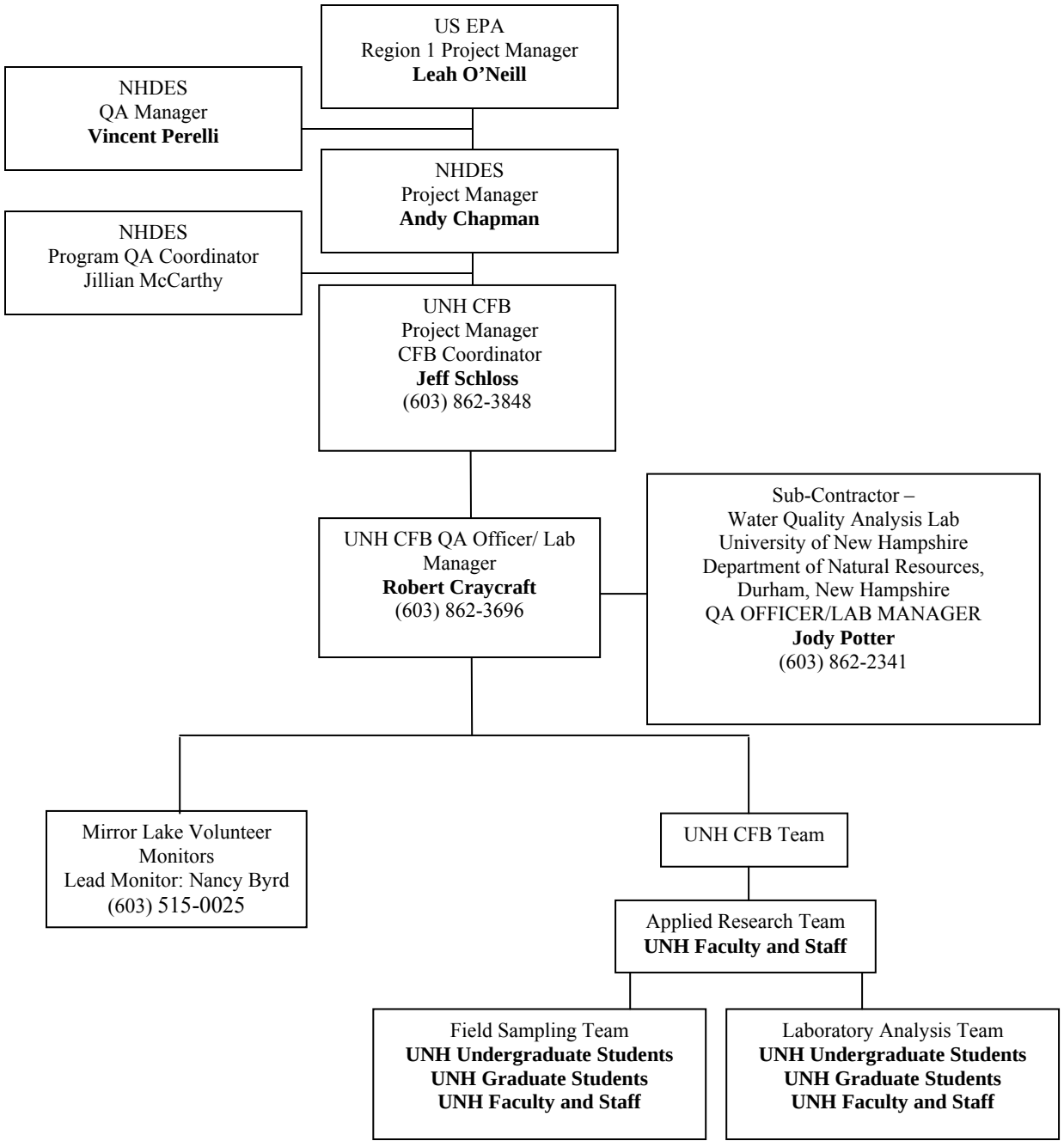
Table 1. QAPP Distribution List

QAPP Recipient Name	Project Role	Organization	Contact Information: Telephone Numbers and email Addresses
Jeffrey Schloss	Project Co-Manager/ Field Team Manager	UNH Center for Freshwater Biology/	(603) 862-3848 jeff.schloss@unh.edu
Robert Craycraft	CFB Laboratory Quality Assurance Officer/ Laboratory Manager	UNH Cooperative Extension	(603) 862-3696 bob.craycraft@unh.edu
Jody Potter	UNH WQ Lab Quality Assurance Officer/ Laboratory Manager	UNH Dept. Natural Resources, WRRC	(603) 862-2341 Jody.Potter@unh.edu
Nancy Byrd	Lead Mirror Lake Volunteer Monitor	Mirror Lake Protective Association (Tuftonboro New Hampshire)	(603) 515-0025 nancy@pangean.com An electronic copy of the QAPP will be posted on the CFB website. Paper copies will be provided to volunteer monitors upon request.
Mirror Lake Volunteers	Mirror Lake Volunteer Lake and Stream Monitors	Mirror Lake Protective Association (Tuftonboro New Hampshire)	An electronic copy of the QAPP will be posted on the CFB website. Paper copies will be provided to volunteer monitors upon request.
Jillian McCarthy	Program QA Coordinator	NHDES, Watershed Management Bureau	603-271-8475 jmccarthy@des.state.nh.us
Vince Perelli	NHDES QA Manager	NHDES, Planning, Prevention, & Assistance Unit	603-271-8989 vperelli@des.state.nh.us

Project Organization

Figure 1 is an organizational chart outlining the parties involved in this investigation and the communication pathways.

Figure 1: Organizational Chart.



Personnel Qualifications and Experience

Table 2 displays the personnel credentials of the University of New Hampshire Center for Freshwater Biology supervisory staff and affiliated UNH Water Resource Research Center Water Quality Analysis Laboratory staff.

Table 2. Personnel Qualifications and Experience

Name and Affiliation	Responsibilities	Education and Qualifications
Jeffrey Schloss UNH Center for Freshwater Biology, Cooperative Extension Department of Zoology	Project Co-Manager / UNH CFB Project Coordinator	BS Marine Zoology; BA Economics MS Marine and Aquatic Biology PhD candidate; Extension Professor; Water Resources Specialist
Robert Craycraft UNH Center for Freshwater Biology Cooperative Extension	CFB Laboratory Manager/QA Officer	BS Biology; Educational Program Coordinator NH Lakes Lay Monitoring Program
Jody Potter UNH Water Quality Analysis Laboratory Water Resources Research Center	Laboratory Manager/ QA Officer	BS Water Resource Management; MS Water Resource Management; UNH Water Quality Analysis Laboratory Manager
CFB Student Technicians	Lab and Field Support	Trained by Project Manager and Laboratory Manager
Volunteer Mirror Lake Monitors	Collect field samples	Trained by Project Manager and QA Officer

Site Information

Mirror Lake is a 378 acre lake located within the towns of Wolfeboro and Tuftonboro, Carroll County, New Hampshire. The Mirror Lake Watershed is approximately 2,160 acres (Appendix A) with approximately 250 residences of which 105 are lakefront and 71 of these are "seasonal" (vacation and/or weekend or summer only). Proposed sampling, discussed under project rationale, focuses on the collection of water quality data at one centrally located, deep, sampling site, six near-shore sampling locations and four stream sampling sites (Appendix A and Appendix B).

Project Rationale

Problem Definition

Mirror Lake and the Mirror Lake beach are listed in the final 2008 303(d) list as being impaired by hepatatoxic cyanobacteria blooms. Historical phosphorus data, collected by several programs (Table 4) and at varying times throughout the years, have included elevated total phosphorus concentrations in both Mirror Lake and the tributary inlets. However, the sources of the phosphorus are listed as unknown. The Mirror Lake Protection Association (MLPA) received a 319 Watershed Assistance grant to facilitate the development of a watershed based plan that will identify non-point source phosphorus sources (and estimated loadings) to the Mirror Lake surface waters and sediments. Direct and indirect watershed phosphorus sources, identified in the 319 Watershed Assistance grant application, are likely to include (bold bullet points highlight water quality elements directly targeted in this SSPP):

- **The tributary entering Mirror Lake at Lang Pond Road.**

- Runoff associated with land uses bordering Abenaki Pond, the tributary, and Mirror Lake watershed and lakefront residential properties.
- Mirror Lake residential septic system leachate.
- **Runoff from Lang Pond Road and State Route 109.**
- **Internal phosphorus loading from sediments in deeper areas of Mirror Lake that have acquired elevated concentrations of phosphorus over time and are subject to low dissolved oxygen during periods of stratification.**

An important goal of the Mirror Lake watershed based plan is to quantify the phosphorus sources and to establish a plan for source control (management measures), monitoring recovery, and remediation (if needed) within an adaptive management framework. The work proposed in this SSPP focuses on the in-lake phosphorus concentrations, including internal nutrient loading (deep sampling site) as well as runoff from Lang Pond Road and State Route 109 (near-shore sampling sites). Supplemental tributary sampling of the primary tributary inlets located along Lang Pond Road (tributary sampling), a secondary tributary inlet (Bowles Inlet) and at the tributary outlet will also be undertaken concurrently (on the same day) with the near-shore sampling to document the difference between in-lake and tributary total phosphorus concentrations in accordance with the **New Hampshire Center for Freshwater Biology and Lakes Lay Monitoring Program Programmatic QAPP RFA # XXX**. Additional stream monitoring data will also be collected by the New Hampshire Department of Environmental Services (NH DES) Volunteer Lake Assessment Program, in accordance with the NHDES Volunteer Lake Assessment Program Generic QAPP, on a monthly basis; the NH DES will collect data in select stream reaches to characterize the tributary phosphorus load using methods directly comparable to the UNH CFB. Modeling by a contracted natural resource scientist will help quantify relative phosphorus contributions from watershed sources (i.e. septic leachate, tributary loading, internal nutrient loading). The modeling methodology will be included in a separate QAPP or SSPP (as appropriate).

Data collected by the CFB will be shared with both the NH DES and the consulting natural resource professional to assist in the water quality modeling effort. Table 3 provides a description of sample parameters collected in conformance with this SSPP and the corresponding sampling rationale.

Table 3. Parameters of Concern

Sampling Parameters
Total Phosphorus
Soluble Reactive Phosphorus
Secchi Disk Transparency
Turbidity
Temperature
Specific Conductivity
Total Alkalinity
pH
Dissolved Oxygen
Carbon Dioxide
Oxidation Reduction Potential (ORP)
Chlorophyll *a*
Phycocyanin
True Color

Sampling Parameters
Zooplankton
Phytoplankton

Historical Data

Historical Data pertinent to this study, their intended use and their limitations are summarized in Table 4.

Table 4. Non-Direct (Historical) Measurement Criteria and Limitations Table

Non-direct Measurement (Secondary Data)	Data Source	How Data will be Used	Limitations on Data Use
Water Quality Data: Total Phosphorus, Secchi Transparency, pH, Specific Conductivity. Alkalinity, Dissolved Oxygen, selected ions	NH DES, UNH CFB , UNH Lakes Lay Monitoring (historical and recently collected), DES Volunteer Lake Assessment Program	Comparison purposes	Comparable DQO requirements and validated performances to this study; otherwise will notate differences and qualify the data
Bathymetric maps, topographic maps orthophotos, GIS coverages	NH DES, NH Fish and Game and UNH GRANIT GIS data coverages	Sub-watershed delineations: land cover assessments: Sample location mapping	Date of data collection and map creation. GIS metadata standards

Project Description and Schedule

Study Design

Water quality sampling will be undertaken and will emphasize the collection of in-lake water quality parameters from both a deep centrally located sampling site and from supplemental near-shore sampling sites that characterize the in-lake water quality. Vertical profiling will be undertaken at the deep, centrally located, sampling station to characterize the water quality variations among thermal strata and to identify the degree of oxygen depletion and internal phosphorus loading when Mirror Lake is thermally stratified. Data will also be collected at target near-shore sampling points around the periphery of Mirror Lake, to help characterize the phosphorus load and spatial phosphorus variability during the study period. Emphasis will be placed on the collection of total phosphorus, Secchi Disk Transparency, dissolved oxygen, and chlorophyll *a* data (eutrophication parameters) while accessory data will include temperature, phycocyanin concentrations, pH and oxidation reduction potential measurements, turbidity, specific conductivity, dissolved "true" color, carbon dioxide and soluble reactive phosphorus concentrations (refer to table 3 for a description of target parameters). Zooplankton and Phytoplankton samples will also be collected.

Data Collection Protocols / Procedures and Requirements

Sampling outlined in Table 6 (A&B) will be performed in conformance with the SOPs outlined in the New Hampshire Center for Freshwater Biology and Lakes Lay Monitoring Program Programmatic QAPP. Tributary and near-shore sampling will consist of point (grab) samples collected at a standardized depth of 0.1 meters and 0.5 meters, respectively. In-lake samples collected at Site 3 Deep Point will consist of an epilimnetic composite sample while a point sample will be collected one-meter off the lake-bottom on all sampling dates. Supplemental metalimnetic samples will be collected by the CFB during the monthly site visits to better characterize the physical, chemical and biological variability among the thermal strata. CFB sampling may include supplemental point water samples, using the standardized sampling procedure and at the discretion of the project manager, at intermittent depths as conditions warrant. Instances during which supplemental sampling depths may be added include the summer stratification period when highly variable total phosphorus concentrations, associated with internal nutrient loading, may be documented among depths or mid-lake cyanobacteria populations that can stratify at discrete locations within the water column. Tables 5 and 6 (A&B) identify the minimum number of sampling dates and the minimum number of samples to be collected during the study period.

Data Analysis

Data will be graphed to provide a visual data interpretation and to facilitate statistical analysis. Monthly physical/chemical profiling data, collected by the CFB, will be graphed by depth to exhibit seasonal changes in the physical and chemical characteristics among the thermal strata. Total phosphorus and chlorophyll *a* data will be compared among sampling sites using box-and-whisker plots that provide a statistical assessment of the data (Reckhow et. al., 1993). A trend analysis will be performed on annual Secchi Disk and chlorophyll *a* data, collected since 1991, using box-and-whisker plots. Box-and-whisker plots are appropriate when a minimum of 10 water quality measurements are collected and thus all box-and-whisker plots will be performed on data-sets that contain a minimum of 10 measurements.

Table 5. Sampling Location, Sampler and Sampling Duration [1]

Location	Sampler	Sampling Duration	Minimum # Sampling Dates
3 Deep Point (deep in-lake site)	Volunteers & CFB	Bi-weekly (June – October)	14
4 Hersey Cove (near-shore site)	Volunteers & CFB	Bi-weekly (June – October)	14
5 Bowles Inlet (near-shore site)	Volunteers & CFB	Bi-weekly (June – October)	14
7 Beach Inlet near Lang Pond Road (near-shore site)	Volunteers & CFB	Bi-weekly (June – October)	14
8 Libby Cove (near-shore site)	Volunteers & CFB	Bi-weekly (June – October)	14
9 Mirror Lake Drive (near-shore site)	Volunteers & CFB	Bi-weekly (June – October)	14
10 109 Launch	Volunteers	(June – October)	14

(near-shore site)	& CFB		
Lang Pond North (tributary site)	CFB	(June – October)	4
Lang Pond South (tributary site)	CFB	(June – October)	4
Bowles Inlet (tributary site)	CFB	(June – October)	4
Mirror Lake Outlet @ Route 109 (tributary site)	CFB	(June – October)	4

[1] Refer to Table 6 (A&B) for more detailed information.

Reference: Reckhow, K.H., K. Kepford, and W. Warren Hicks. 1993. Methods for the Analysis of Lake Water Quality Trends. EPA 841-R-93-003

Table 6(A). CFB Sample Load Breakdown (presented as minimum requirements)

Analysis	Minimum # of Sampling Dates	Minimum # of Samples per Site	Total # of Locations Sampled (minimum)	Field Duplicates or Replicates (% of Samples QC'd)	Field and Bottle Blanks	Minimum # of Samples to Lab (or Total Readings Taken)
Field Measurements (Monthly Deep, In-Lake, Sampling by the CFB Field Team)						
Depth Temperature Dissolved Oxy. Conductivity pH ORP Turbidity Chlorophyll	4	2	1	2 profiles / site (100%)	NA	Measured in field (profiles[1])
Secchi Disk Transparency	4	3	1	3 replicates / site (200%)	NA	18
Field Samples (Monthly Deep, In-Lake, Sampling by the CFB Field Team)						
Total Phosphorus	4	3	1	1 duplicate / trip (* 10%)	1 / trip (10% [2])	20
Chlorophyll *a*	4	3	1	1 duplicate / trip (* 10%)	1 / trip (10% [2])	20
Dissolved "true" Color	4	3	1	1 duplicate / trip (* 10%)	1 / trip (10% [2])	20
Dissolved Oxy. (Winkler titration)	4	2	1	1 duplicate / trip (* 10%)	NA	12
Carbon Dioxide and Alkalinity	4	4	1	1 duplicate / trip (* 10%)	1/ trip (10% [2])	24
Zooplankton	4	1	1	1 duplicate / trip (* 10%)	1/ trip (10% [2])	12
Whole water Phytoplankton	4	2	1	1 duplicate / trip (* 10 %)	1/trip (10% [2])	16
Field Samples (Monthly near-shore sampling by the CFB Field Team)						
Total Phosphorus	4	1	6	1 duplicate / trip (10% [2])	1 / trip (10% [2])	32
Chlorophyll a	4	1	6	1 duplicate / trip (10% [2])	1 / trip (10% [2])	32
Dissolved "true" Color	4	1	6	1 duplicate / trip (10% [2])	1 / trip (10% [2])	32

Field Samples (Monthly tributary sampling by the CFB Field Team)						
Total Phosphorus	4	1	4	1 duplicate / trip (10% [2])	1 / season (10% [2])	24

(based on EPA NE Worksheet 22a and 22b)

[1]- Profiles at deep sites are measured starting at 0.1 meters depth and the multi-parameter sonde is set to record approximately every 0.2 meters.

[2] - Indicates the minimum collection frequency for field duplicates, replicates and field bottle blanks. Under most circumstances, the collection frequency of QC data will exceed 10%.

Table 6(B). Volunteer Monitor Sample Load Breakdown (presented as minimum requirements)

Analysis	# of Sampling Dates (minimum)	# of Samples per Site	Total # of Locations Sampled (minimum)	Field Duplicates or Replicates (Minimum % of Samples QC'd)	Field and Bottle Blanks	Minimum # of Samples to Lab (or Total Readings Taken)
Field Measurements (Bi-weekly In-lake Sampling by the Volunteer Monitors)						
Temperature	10	1	1	1 profile / season (10% [1])	NA	11
Secchi Disk Transparency	10	2	1	1 replicate / site (100 %)	NA	20
Field Samples (Bi-weekly In-lake Sampling by the Volunteer Monitors)						
Total Phosphorus	10	1	1	1 duplicate / trip (10% [1])	1 / season (10% [1])	31
Chlorophyll *a*	10	1	1	1 duplicate / trip (10% [1])	1 / season (10% [1])	21
Dissolved "true" Color	10	1	1	1 duplicate / trip (10% [1])	1 / season (10% [1])	21
Alkalinity	10	1	1	1 duplicate / trip (10% [1])	NA	20
Field Samples (Bi-weekly near-shore sampling by the volunteer monitors)						
Total Phosphorus	10	1	6	1 duplicate / trip (10% [1])	1 / season (10% [1])	71
Chlorophyll a	10	1	6	1 duplicate / trip (10% [1])	1 / season (10% [1])	71
Dissolved "true" Color	10	1	6	1 duplicate / trip (10% [1])	1 / season (10% [1])	71

(based on EPA NE Worksheet 22a and 22b)

[1] - Indicates the minimum collection frequency for field duplicates, replicates and field bottle blanks. Under most circumstances, the collection frequency of QC data will exceed 10%.

Final Products and Reporting

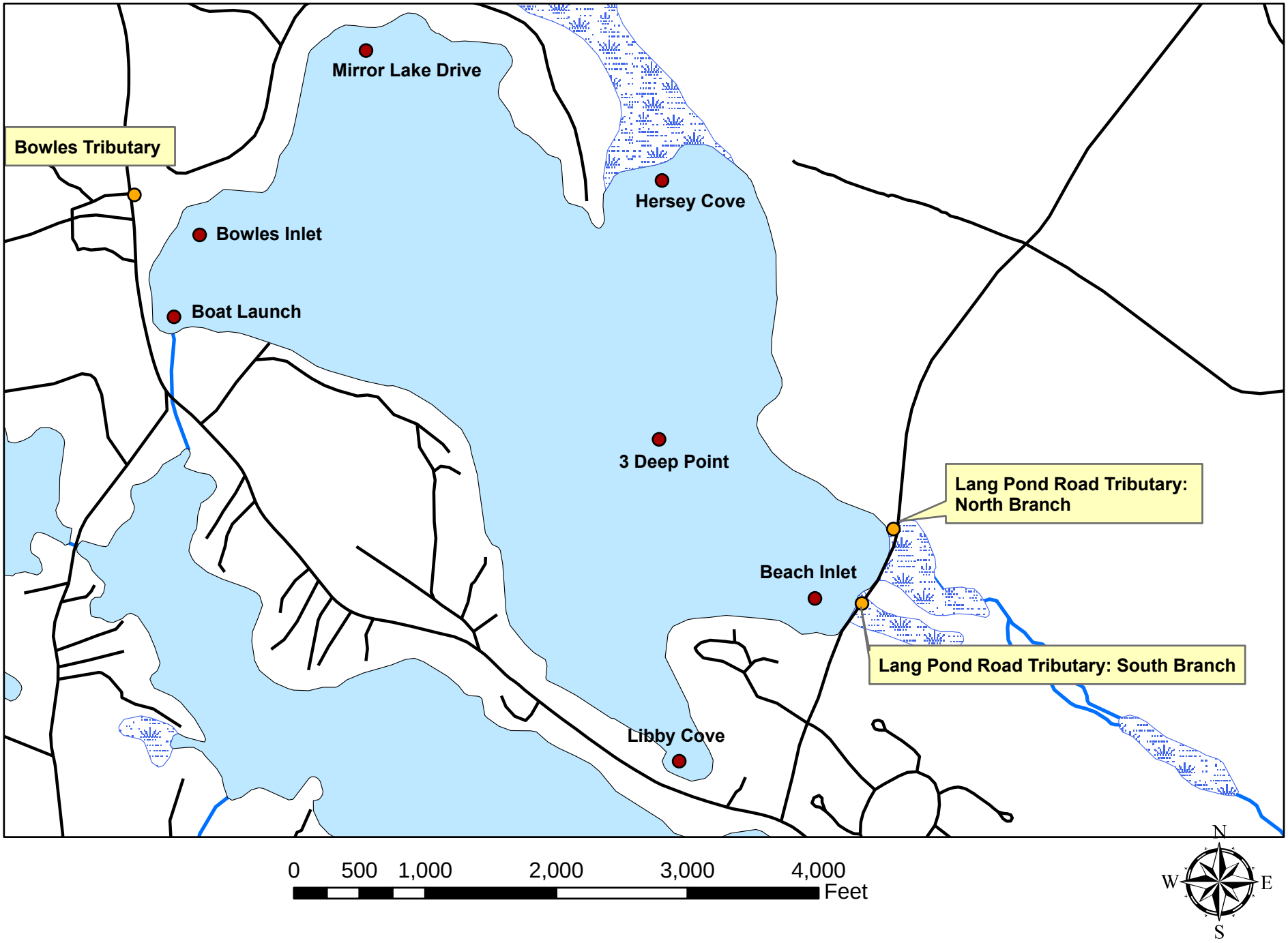
The final products for this project include the following:

- Final interpretive report including a data listing and interpretive graphics.

- Final data summary, including QC data, entered into Microsoft Excel.

All products will be submitted by the Center for Freshwater Biology in both electronic and paper copies, to the NHDES Watershed Assistance Section, the Mirror Lake Protective Association, the Town of Tuftonboro NH and the Town of Wolfeboro NH.

Mirror Lake Sampling Sites



APPENDIX B: GPS COORDINATES & SITE DESCRIPTION

Site	Latitude mm dd ss	Longitude mm dd ss	Site Description
3 Deep Point (deep sampling site)	71°15'40.74"	43°37'19.26"	Deepest Point in the lake that provides insight into the overall condition of Mirror Lake.
Mirror Lake Drive (near-shore site)	71°16'10.92"	43°37'49.38"	Near-shore sampling location near Mirror Lake Drive and a small intermittent stream. Provides insight into Mirror Lake's reaction to local NPS pollution loading.
Bowles Inlet (near-shore site)	71°16'28.26"	43°37'34.86"	Near-shore sampling location north of the Boat Launch (outlet) and near and intermittent stream inlet. Provides insight into Mirror Lake's reaction to local NPS pollution loading.
Boat Launch (near-shore site)	71°16'30.96"	43°37'28.68"	Near-shore sampling location adjacent to Route 109, a public boat launch site, and near the Mirror Lake Dam (outlet). Provides insight into Mirror Lake's reaction to local NPS pollution loading.
Libby Cove (near-shore site)	71°15'38.82"	43°36'55.02"	Near-shore, cove, sampling location in the southernmost section of Mirror Lake adjacent to Route 109. Provides insight into Mirror Lake's reaction to local NPS pollution loading.
Beach Inlet (near-shore site)	71°15'20.82"	43°37'09.60"	Near-shore sampling location that receives outwash of the Lang Pond Road tributary (south). Provides insight into Mirror Lake's reaction to local NPS pollution loading.
Hersey Cove (near-shore site)	71°15'41.82"	43°37'39.24"	Near-shore sampling location that is influenced by a large connected wetland complex north of the site. The wetland is interconnected with 19 Mile Brook which receives treated effluent from a Rapid Wastewater Infiltration Disposal System.
Bowles Tributary Inlet	71°16'35.0"	43°37'37.9"	Small (approximately 3 foot channel) tributary inlet that drains a sub-watershed north-west of Mirror Lake.
Lang Pond Road Tributary (north)	71°15'16.56"	43°37'12.4"	North branch of the largest tributary inlet that enters Mirror Lake from the East. Will provide insight into nutrient loading at the sub-watershed scale.
Lang Pond Road Tributary (south)	71°15'19.8"	43°37'06.8"	North branch of the largest tributary inlet that enters Mirror Lake from the East. Will provide insight into nutrient loading at the sub-watershed scale.
Mirror Lake Outlet	71°16'33.2"	43°37'24.2"	Tributary outlet that drains into Lake Winnipesaukee. Provides insight into phosphorus export out of Mirror Lake

Appendix I

Standard Operating Procedures for Onset data logger Light and Temperature Measurements

Updated 1/25/08 by Bob Craycraft and Jeff Schloss

HOBO® Pendant® Temperature/Light Data Logger (UA-002-xx) Manual

HOBO Pendant Temperature/Light Data Logger

Models: UA-002-08
UA-002-64

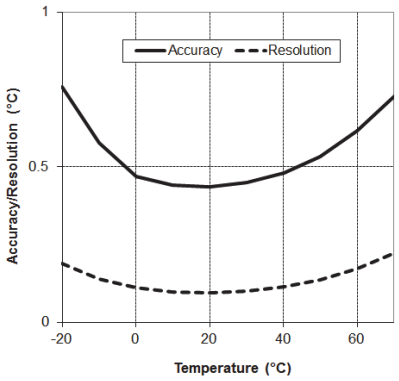
Required Items:

- HOBOware 2.x or later
- USB cable (included with software)
- Pendant Optic USB Base Station & Coupler (BASE-U-1)
- Optic USB Base Station (BASE-U-4) or HOBO Waterproof Shuttle (U-DTW-1) & Coupler (COUPLER2-A)

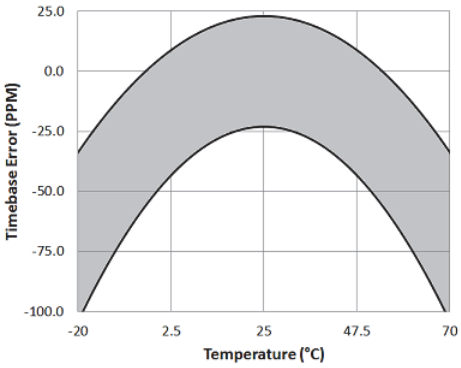
The HOBO Pendant Temperature/Light Data Logger is a waterproof, two-channel logger with 10-bit resolution and can record up to approximately 3,500 (8K model) or 28,000 (64K model) combined temperature and light readings or internal logger events. The logger uses a coupler and optical base station with USB interface for launching and data readout by a computer.

Specifications

Measurement Range	Temperature: -20° to 70°C (-4° to 158°F) Light: 0 to 320,000 lux (0 to 30,000 lumens/ft^2)
Accuracy	Temperature: ± 0.53°C from 0° to 50°C (± 0.95°F from 32° to 122°F), see Plot A Light intensity: Designed for measurement of relative light levels, see Plot D for light wavelength response
Resolution	Temperature: 0.14°C at 25°C (0.25°F at 77°F), see Plot A
Drift	Less than 0.1°C/year (0.2°F/year)
Response Time	Airflow of 2 m/s (4.4 mph): 10 minutes, typical to 90% Water: 5 minutes, typical to 90%
Time Accuracy	±1 minute per month at 25°C (77°F), see Plot B
Operating Range	In water/ice: -20° to 50°C (-4° to 122°F) In air: -20° to 70°C (-4° to 158°F)
Water Depth Rating	30 m from -20° to 20°C (100 ft from -4° to 68°F), see Plot C
NIST Traceable Certification	Available for temperature only at additional charge; temperature range -20° to 70°C (-4° to 158°F)
Battery Life	1 year typical use
Memory	UA-002-08: 8K bytes (approximately 3.5K combined temperature and light readings or events) UA-002-64: 64K bytes (approximately 28K combined temperature and light readings or events)
Materials	Polypropylene case; stainless steel screws; Buna-N o-ring
Weight	18 g (0.6 oz)
Dimensions	58 x 33 x 23 mm (2.3 x 1.3 x 0.9 inches)
CE	The CE Marking identifies this product as complying with all relevant directives in the European Union (EU).

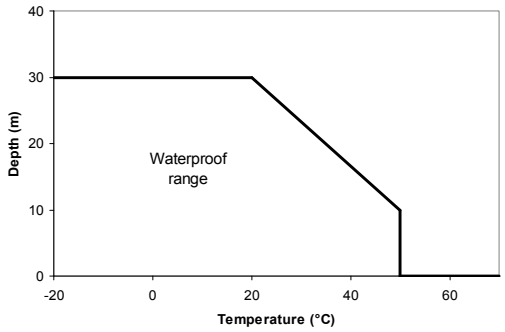


Plot A

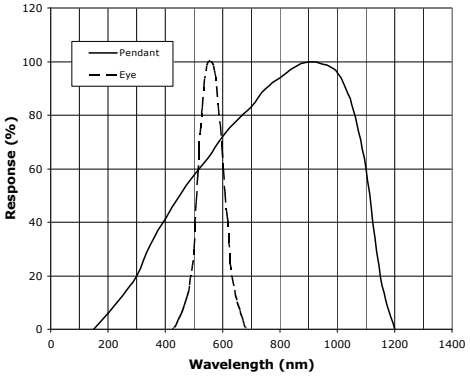


Plot B

Specifications (continued)



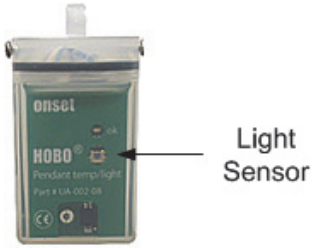
Plot C



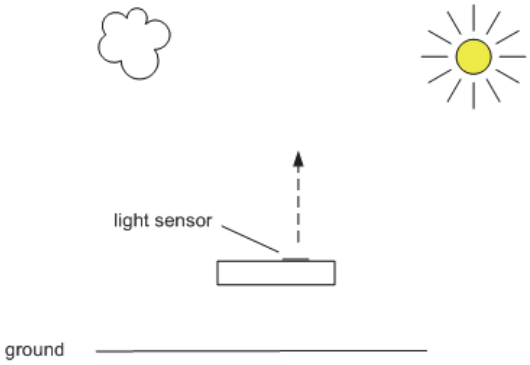
Plot D

Deployment/Mounting

The Light Sensor response is roughly cosine dependent with the angle from vertical. Therefore, whether outdoors or underwater, the logger must be mounted horizontally so that the sensor is pointing straight up towards the sky.



Attach the logger to a flat surface using glue, a tie, or a rubber band, making sure that the sensor is pointing up.



Connecting the Logger to a Computer

The HOBO Pendant logger requires either of the following to connect to the computer:

- Pendant Optic USB Base Station & Coupler (BASE-U-1); HOBOware 2.1 or later

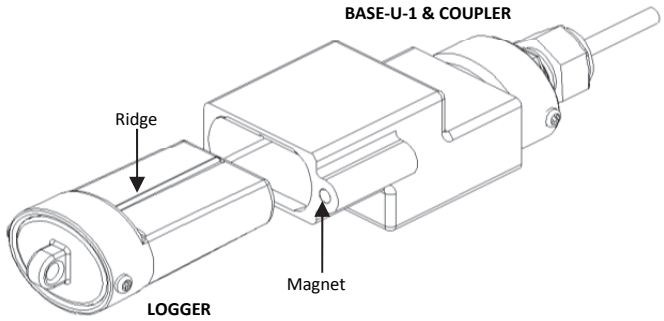
  OR

- Optic USB Base Station (BASE-U-4) or HOBO Waterproof Shuttle (U-DTW-1); coupler (COUPLER2A); HOBOware 2.2 or later

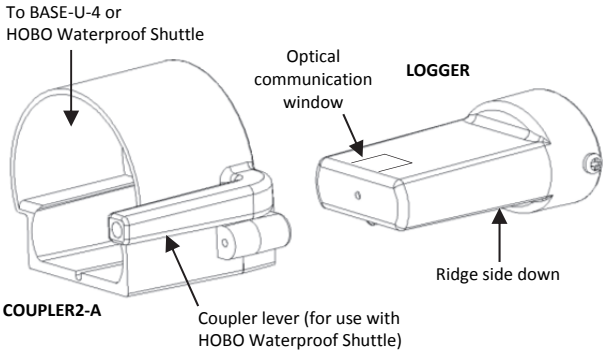
If possible, avoid connecting at temperatures below 0°C (32°F) or above 50°C (122°F).

1. Plug the USB connector on the base station into an available USB port on your computer.
2. Insert the logger and the base station into the coupler, as shown in the following diagrams.

   For BASE-U-1, make sure that the logger is inserted in the end of the coupler that has the magnet, and that the ridges on the base station and logger are aligned with the grooves in the coupler.



   For BASE-U-4 or the HOBO Waterproof Shuttle, firmly insert the optical end of the base station into the D-shaped end of the coupler, and make sure that the ridge on the logger is aligned with the groove in the coupler.



3. If you are using the HOBO Waterproof Shuttle, briefly press the coupler lever to put the shuttle into base station mode.
4. If the logger has never been connected to the computer before, it may take a few seconds for the new hardware to be detected.

5. Use the logger software to set up the alarms, launch, and read out the logger.

You can read out the logger or check its status while it continues to log, stop it manually with the software, or let it record data until the memory is full. Refer to the software user's guide for complete details on launching, reading out, and viewing data from the logger.

Important: Do not cover the optical communication window in the logger (shown in diagram above) with a label or sticker as that may interfere with the communications with the base station or shuttle.

Triggered Start

This logger can be configured to start at your command, using the magnet in the coupler to trigger a start.

1. Use HOBOware to launch the logger with Using Coupler selected. Remove the logger from the coupler.
2. Bring the logger and an empty coupler or strong magnet to the deployment location.

Important: Any magnet can trigger a start. This can be helpful, but it can also cause a premature start. Keep the logger away from strong magnetic fields until you are ready to begin logging.

3. When you are ready for the logger to start logging, insert the logger into the empty coupler (or place it next to a strong magnet) and remove it after three seconds.

Important: The logger will not launch if the base station is in the coupler.

4. Verify that the logger's light is blinking at least every four seconds.

Sample and Event Logging

The logger can record two types of data: samples and internal logger events. Samples are the measurements recorded at each logging interval (for example, temperature every minute). Events are independent occurrences triggered by a logger activity, such as Bad Battery or Host Connected. Events help you determine what was happening while the logger was logging.

Operation

A light (LED) on the front of the logger confirms logger operation. The following table explains when the light blinks during logger operation.

When:	The light:
The logger is logging	Blinks once every one to four seconds (the shorter the logging interval, the faster the light blinks); blinks when logging a sample
The logger is awaiting a start because it was configured to start logging At Interval, On Date/Time, or Using Coupler	Blinks once every eight seconds until logging begins

Light Measurement

The logger measures light intensity in units of lumens per square foot (US) or lux (SI). The light sensor in the Pendant logger measures a much broader spectrum of light wavelengths than are visible to the human eye. Plot D shows that the logger's response extends farther into ultraviolet and infrared wavelengths than the eye's response. This means the logger is useful for sensing wavelengths not visible to the eye, but it also may mean that the logger's readings will not correspond exactly to measurements made with a device having different spectral sensitivity. The light sensor is most useful for determining relative changes, rather than absolute values of intensity.

The logger has a very wide dynamic range of light sensitivity extending from complete darkness to somewhat beyond full daylight. The resolution steps are smaller at low light levels than at high light levels to allow useful measurements across this broad range of intensities. Intensity readings are maximum for light hitting the sensor directly on-axis and are reduced for light coming in at an angle. Readings can also be reduced by abrasions or dirt on the case above the light sensor.

Protecting the Logger

The logger can be damaged if the water depth rating is exceeded. The depth rating is approximately 30 m (100 ft) at temperatures below 20°C (68°F), but is less in warmer water. Refer to Plot C for details.

Do not store the logger in the coupler. Remove the logger from the coupler when you are not using it. When the logger is in the coupler or near a magnet, it consumes more power and will drain the battery prematurely.

Keep the logger away from magnets. Being near a magnet can cause false coupler events to be logged. It can also launch the logger prematurely if it was waiting for a trigger start.

Note! Static electricity may cause the logger to stop logging. To avoid electrostatic discharge, transport the logger in an anti-static bag, and ground yourself by touching an unpainted metal surface before handling the logger. For more information, search for "static discharge" in the FAQ section on onsetcomp.com.

Periodically inspect the desiccant and dry it if it is not bright blue. The desiccant pack is located in the cap of the logger. To dry the desiccant, remove the desiccant pack from the cap and leave the pack in a warm, dry location until the bright blue color is restored. (Refer to the *Battery* section for instructions on removing and replacing the logger cap.)

Temperature Range	Desiccant Maintenance Schedule
Less than 30°C (86°F)	Approximately once per year
30° to 40°C (86° to 104°F)	Approximately every six months
Over 40°C (104°F)	Approximately every three months

Battery

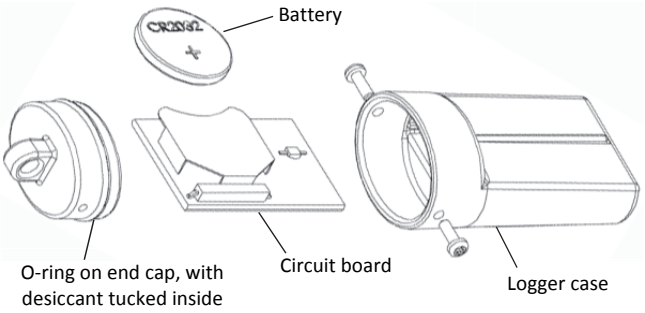
The logger requires one 3-Volt CR-2032 lithium battery. Battery life varies based on the temperature and the frequency at which the logger is recording data (the logging interval). A new battery typically lasts one year with logging intervals greater than one minute. Deployments in extremely cold or hot temperatures, or logging intervals faster than one minute, may significantly reduce battery life. Continuous logging at the fastest logging rate of one second will deplete the battery in as little as two weeks.

Replacing the Battery

Before you begin: You will need a small Philips head screwdriver and silicone-based O-ring grease, such as Parker Super-O-Lube, to complete these steps (no petroleum-based lubricants). The logger should be wiped clean and dried completely before opening it.

To replace the battery:

1. Avoid electrostatic discharge while handling the logger and internal circuit board; ground yourself by touching an unpainted metal surface. Hold the circuit board by its edges and avoid touching electronics.
2. Working on a clean, dry surface, remove the two screws that secure the end cap to the case and remove the cap.
3. Examine the desiccant pack that is tucked into the cap. If the desiccant is not bright blue, put the desiccant pack in a warm, dry place until the blue color is restored. Or, for faster drying, the desiccant can be dried for two hours in a 70°C (160°F) oven.
4. Gently tap the case to loosen the circuit board and remove it from the case.



5. Carefully push the battery out of the holder with a small, nonmetallic blunt instrument.
6. Insert a new battery, positive side facing up.
7. Return the circuit board and label to the case, carefully aligning the circuit board with the grooves in the case so that the battery faces the ridged side of the case.
8. Remove the O-ring from the end cap. Use the thumb and finger of one hand to hold the cap from the top and bottom, and use the thumb and fingers on your other hand to slide the O-ring to form a loop as shown. Use this loop to roll the O-ring off the cap.

9. Inspect the O-ring for cracks or cuts and replace it if any are detected (the O-ring is included in the Pendant replacement parts kit, UA-PARTSKIT).
10. Using your fingers (not cloth or paper), spread a small dot of silicone-based grease on the O-ring, just enough to moisten it all the way around and making sure that the entire O-ring surface is completely coated with grease. As you work the grease into the O-ring, make sure there is no grit or debris on the O-ring.
11. Place the O-ring back on the end cap, making sure it is fully seated and level in the groove. Make sure the O-ring is not pinched or twisted and that no dirt, lint, hair, or any debris is trapped on the O-ring. This is necessary to maintain a waterproof seal.
12. Very slightly grease the inside rim of the case, especially around the screw holes with the silicone grease, just enough to moisten the inside edges without touching any circuitry. Make sure that there is no excess lubricant that could get onto the logger electronics or label. Make sure there is no debris on this surface.
13. Check that the desiccant pack is tucked into the cap.
14. Carefully push the end cap into the lubricated case until the screw holes are aligned. Visually check that the O-ring forms a uniform seal all around.
15. Re-fasten the screws. Tighten the screws until you feel them hit the bottom of the screw holes, but not so tight that they distort the clear housing.

WARNING: Do not cut open, incinerate, heat above 85°C (185°F), or recharge the lithium battery. The battery may explode if the logger is exposed to extreme heat or conditions that could damage or destroy the battery case. Do not dispose of the logger or battery in fire. Do not expose the contents of the battery to water. Dispose of the battery according to local regulations for lithium batteries.

Patent # 6,826,664
9556-K MAN-UA-002

HOBO® Waterproof Shuttle (Part # U-DTW-1)

Inside this package:

- HOBO Waterproof Shuttle
- USB host cable
- Set of couplers:
  - For UA Pendant (Part # COUPLER2-A)
  - For U20 Water Level (Part # COUPLER2-B)
  - For U22 Water Temp Pro v2 and U24 Conductivity (Part # COUPLER2-C)
  - For UTBI TidbiT v2 (Part # COUPLER2-D)
  - For U23 HOBO Pro v2 (Part # COUPLER2-E)

Doc # 10264-G
MAN-U-DTW-1
Onset Computer Corporation

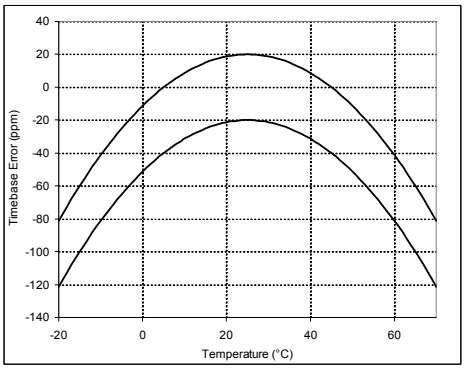
Thank you for purchasing a HOBO Waterproof Shuttle. The HOBO Waterproof Shuttle performs several major functions:

- Reads out all logger information (serial number, deployment number, data, etc.) from loggers in the field for transfer to host computer, and stores each logger's data in a "bank"
- Nonvolatile memory preserves data, even if batteries are depleted
- Relaunches the logger, resetting the logger's time to the shuttle's time and synchronizing the logging interval on relaunch
- Can be used as an optic-to-USB base station

Although the HOBO Waterproof Shuttle is easy to use, Onset strongly recommends that you spend a few minutes reading this manual and trying out the procedures described here before taking the shuttle into the field.

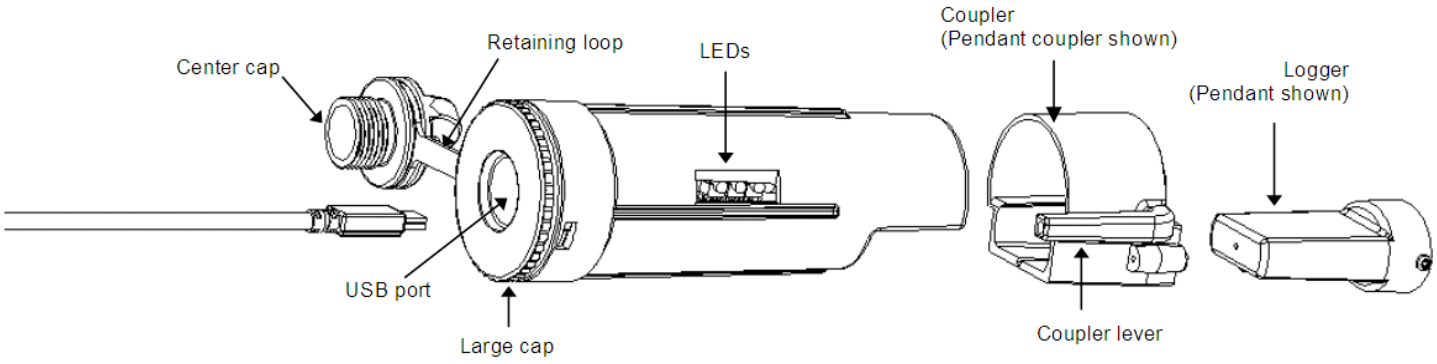
Specifications

Compatibility	All HOBO U-Series loggers with optic USB. Not compatible with the HOBO U-Shuttle (U-DT-1).
Requirements	HOBOware 2.2+; compatible logger and matching coupler
Data capacity	63 logger readouts of up to 64K each
Operating temperature	0° to 50°C (32° to 122°F)
Storage temperature	-20° to 50°C (-4° to 122°F)
Wetted materials	Polycarbonate case, EPDM o-rings and retaining loop
Waterproof	To 20 m (66 feet)
Time accuracy	± 1 minute per month at 25°C (77°F); see Plot A
Logger-to-shuttle transfer speed	Reads out one full 64K logger in about 30 seconds
Shuttle-to-host transfer speed	Full shuttle offload (4 MB) to host computer in 10 to 20 minutes, depending on computer
Batteries	2 AA alkaline batteries required for remote operation
Battery life	One year or at least 50 complete memory fills, typical use
Weight	150 g (4 oz)
Dimensions	15.2 x 4.8 cm (6.0 x 1.9 inches)
CE	The CE Marking identifies this product as complying with the relevant directives in the European Union (EU). To maintain CE compliance, this product must be used with the supplied USB cable or equivalent (less than 3 m long).



Plot A

HOBO Waterproof Shuttle features



Preparing to go on location

Before using the shuttle for the first time, you must launch it with HOBOware 2.2 or greater. You must also launch any compatible loggers that were last launched with an earlier version of HOBOware, or have never been launched at all.

1. Use HOBOware 2.2 or greater to launch each logger you wish to read out and relaunch with the shuttle later. (Read "Using the shuttle as a base station" for instructions if you do not have another base station for the loggers.) The shuttle cannot relaunch loggers that were last launched with an earlier version of HOBOware. (You only have to do this once for each logger.)
2. Plug the large end of a USB interface cable into a USB port on the computer. (Avoid using a USB hub, if possible.)
3. Unscrew the center cap on the shuttle. If the cap is too tight to loosen by hand, insert a screwdriver through the lanyard hole and rotate counterclockwise until the cap is loosened.
4. Plug the small end of the USB interface cable into the USB port in the shuttle. (If the shuttle has never been connected to the computer before, it may take a few seconds for the new hardware to be detected.)
5. Follow the instructions in the *HOBOware User's Guide* to access the **Manage Shuttle** dialog. Make sure the battery level is good, and change the batteries now if they are weak.

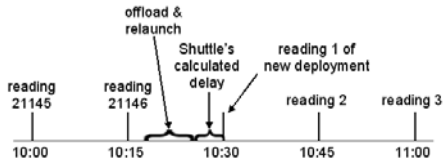
   Important: *If you change the batteries in the field, the shuttle's clock will stop, and the shuttle will not read out loggers again until you relaunch it in HOBOware.*
6. If you are using the shuttle for the first time, launch the shuttle as described in the *HOBOware User's Guide*. Launching synchronizes the shuttle's clock to the host computer and initializes the shuttle's header.

   Important: *The shuttle's clock is used to set the logger's clock at relaunch. For most accurate results, make sure the host computer's clock is correct before launching the shuttle. If you need to adjust the computer's clock, quit HOBOware, set the computer's clock, then reopen HOBOware and launch the shuttle.*
7. If you have used the shuttle before, make sure there are enough banks available to accommodate the loggers you plan to read out.
8. Disconnect the USB cable from the shuttle and replace the center cap securely.

Reading out and relaunching loggers in the field

After you have ensured that the shuttle's batteries are good, there is sufficient memory available, and the shuttle's clock is synchronized, follow these steps to read out and relaunch a logger in the field:

1. Make sure the shuttle's large cap and center cap are closed securely. Tighten the center cap until it is just flush with the large cap, or until the O-ring is no longer visible.
2. Make sure the communication end of the shuttle is clean. Attach the correct coupler for the logger, and ensure that it is seated properly.
3. Insert the logger into the coupler, following the instructions that came with the coupler.
4. Momentarily press the coupler lever (pressing hard enough so the lever bends). Readout should begin immediately. The amber LED blinks continuously while readout and relaunch are in progress. Do not remove the logger when the amber LED is blinking.
5. After reading out the logger, the shuttle synchronizes the logger's clock to the shuttle's internal clock and relaunches the logger, using the description, channels to log, logging interval, and other settings that are already in the logger. (If the logger was launched with multiple logging intervals, the final defined logging interval will be used.) The logger is launched with a slight delay that causes its readings to be synchronized with those of the previous deployment, as shown in the following diagram.



Important: *If the logger was launched with multiple logging intervals, there will be no synchronizing delay. The logger will start immediately with the last defined logging interval.*

6. When the relaunch has completed, the green LED blinks for 15 minutes, or until you momentarily press the coupler lever to stop it (press hard enough so the lever bends). If the red LED blinks instead, there was an error, and the logger may have stopped. Refer to "Troubleshooting" in this manual for details.
7. Remove the logger from the coupler.

Checking shuttle status in the field

The shuttle's memory has 63 "banks." One logger readout can be stored in each bank. To check the shuttle's memory and batteries in the field, remove the logger and press the coupler's lever for at least three seconds (pressing hard enough so the lever bends). When you release the lever, the green LED blinks once for each unoccupied bank in the shuttle's memory. (Press the lever momentarily to stop the blinking, pressing hard enough so the lever bends.)

If the shuttle's batteries are running low, all of the shuttle banks are full, or the clock has not been set, the red LED blinks. (Press the lever momentarily to stop the blinking, pressing hard enough so the lever bends) Use HOBOware to check the shuttle's battery level, available memory, and clock. You may need to change the batteries, or offload the datafiles to the host computer and delete them from the shuttle to free up memory before you can continue reading out loggers.

Offloading data to the host computer

You can offload the data stored in the shuttle even when the batteries are depleted. Take the following steps:

1. Connect the shuttle to a host computer running HOBOware.
2. Follow the instructions in the *HOBOware User's Guide* to offload the new datafiles or access the **Manage Shuttle** dialog. The **Manage Shuttle** dialog shows you how many banks are occupied, and whether they have already been offloaded and saved to the host computer.
3. Offload and save data from the banks of your choice. Refer to the *HOBOware User's Guide* for details on saving datafiles offloaded from the shuttle.
4. Review the list of banks and delete any that are no longer needed. Make sure the battery level is good, and change the batteries now if they are weak. (If you change the batteries in the field, the shuttle's clock will stop, and the shuttle will not read out loggers.) Update the shuttle's clock, if necessary.
5. When finished, disconnect the shuttle from the computer and close the center cap securely.

Using the shuttle as a base station

You can use the shuttle as a base station for any U-Series logger with an optic USB interface. (This function is available even when the batteries are depleted.) To use the shuttle as a base station:

1. Connect the shuttle to the host computer running HOBOware.
2. Attach a compatible logger and coupler.
3. Momentarily press the coupler's lever (pressing hard enough so the lever bends).
4. The amber LED blinks momentarily, then the green LED should glow steadily to indicate that the logger is ready to communicate with HOBOware. (If the red LED blinks instead, the logger was not found. Make sure the logger and coupler are aligned and seated properly, and that there is no dirt or strong sunlight interfering with communications.)
5. When finished, remove the logger from the coupler. The green LED stops glowing when you disconnect the logger or the USB cable.

Important: *The Waterproof Shuttle cannot be used as a base station with Pendant logger models UA-001 and UA-003 (including rain gauges RG3 and RG3-M) with serial numbers less than 988278. These loggers require a BASE-U-1 for communication with the host computer.*

Indicator lights

Green "OK" LED

The green "OK" LED blinks when HOBOware recognizes it as a base station; when it finishes reading out and relaunching a logger; and when you press the coupler lever to check the shuttle's status (see "Checking shuttle status in the field" for details). Momentarily press the coupler lever to stop the blinking (pressing hard enough so the lever bends).

The green LED glows steadily when the shuttle is being used as a base station.

Amber "Transfer" LED

The amber "Transfer" LED blinks when the shuttle is reading out a logger and relaunching it. Do not remove the logger when the Transfer light is lit.

Red "Fail" LED

The red "Fail" LED blinks whenever the shuttle encounters an error condition. Refer to "Troubleshooting" for details.

All LEDs

All LEDs blink in unison when the shuttle has just been powered up, either by installing fresh batteries or (if batteries are not installed) by connecting to the computer's USB port.

Troubleshooting

This section describes problems you may encounter while using the shuttle.

Shuttle is not recognized by host computer

If HOBOware does not recognize the shuttle when you connect it to the computer, simply disconnect and reconnect the shuttle.

Red "Fail" LED blinks

The red "Fail" LED blinks (for 15 minutes, or until you press the coupler lever, pressing hard enough so the lever bends) whenever the shuttle encounters an error. There are several conditions that might cause an error:

- **Shuttle is full:** If the red LED blinks when you try to read out a logger, check whether all of the banks are full, as described in "Checking shuttle status in the field." Or, use HOBOware to check the shuttle's memory.
- **Shuttle batteries are low:** If you cannot read out any loggers at all, check the logger's status, as described in "Checking shuttle status in the field," or use HOBOware to check the shuttle's batteries. The batteries may simply need to be replaced.
- **Compatibility:** The shuttle cannot read out or relaunch loggers that were last launched from HOBOware prior to version 2.2. You will need to read out these loggers on the host computer and relaunch them in HOBOware 2.2 or greater before you can use them with the shuttle.
- **Shuttle clock is not set:** The shuttle has experienced a power failure that caused the clock to reset. You must use HOBOware to offload the files that are already on the shuttle, then relaunch the shuttle before you can read out another logger.
- **Can't communicate with logger:** Remove the logger and coupler. Inspect them and the shuttle to ensure that all are free of dirt that could block the optic communication sensor. Carefully reassemble the shuttle, coupler, and logger, and make sure they are all seated properly. Shield the shuttle from strong sunlight, if applicable, which can interfere with optic communications.
- **Other logger problems:** If you can read out some loggers but not others, or if you cannot read out any loggers even with fresh batteries in the shuttle, check the loggers in HOBOware. Make sure their batteries are at acceptable levels and that there is no "corrupted header" message.

Amber "Transfer" LED stays on without blinking

The amber light is magnetically activated when you press the coupler lever. If it glows steadily at any other time, the magnet in the lever may be too close to the magnetic switch in the shuttle, or another strong magnet may be present. Try bending the lever away from the coupler to reduce the magnet's effect.

LEDs do not function

If the LEDs are not functioning at all, the batteries may be completely exhausted. To test this, attach the shuttle to the host computer and check the battery level. The shuttle should be able to communicate with the host computer, blink its LEDs normally, and perform as a base station even when the batteries are missing or depleted.

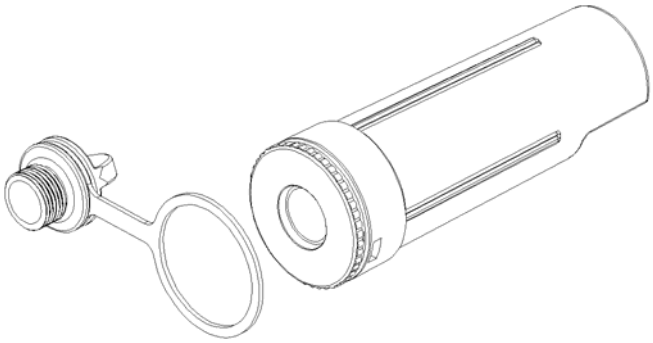
Replacing the shuttle's batteries

The shuttle's batteries should last about one year or at least 50 complete memory fills in typical conditions. When the shuttle's batteries run low (2.2 V or less), any logger data that is already in the shuttle will remain safe, but the shuttle will not read out another logger until its batteries are replaced.

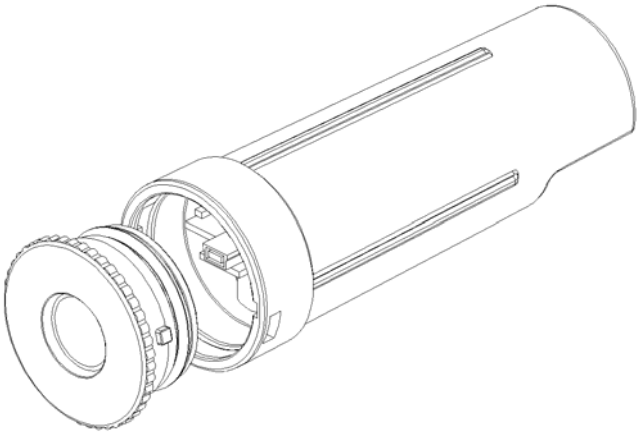
To avoid battery problems, always check the shuttle's batteries in HOBOware before going into the field, and replace them if needed. If you cannot replace the bad batteries right away, you should remove them as soon as possible to ensure that they do not leak and damage the shuttle.

To change the shuttle's batteries:

1. Work over a clean surface to provide a safe platform for the disassembly.
2. Unscrew the center cap on the shuttle. If the cap is too tight to loosen by hand, insert a screwdriver through the lanyard hole and rotate counterclockwise until the cap is loosened.
3. Use the center cap to help you carefully pull the rubber loop free of the large cap. The large cap cannot be removed while the rubber loop is in place.

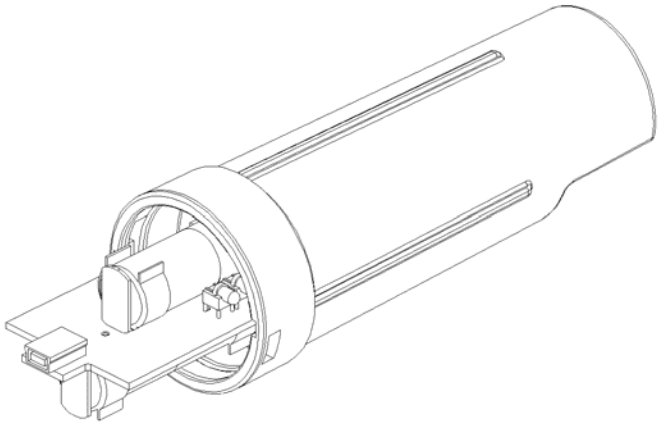


4. Turn the large cap counter-clockwise slightly, then pull it off.



(continued)

5. Turn the shuttle over and tap it gently. The circuit board should slide into your hand.



6. Remove the old batteries and install two new ones in the correct orientation. Both batteries should be turned the same way, with their positive ends facing the USB port on the board. (When the second battery makes contact, all of the shuttle's LEDs will blink in unison.)
7. Put the board back into the case, taking care not to bend the communication LEDs. Align the circuit board with the runners in the case. The USB port should face the open end of the shuttle, and the LEDs should show through the window on the label.
8. Close the shuttle's case. Line up the tabs on the large cap with the slots on the case, press gently, and turn slightly clockwise until the large cap is closed securely.
9. Replace the rubber loop and center cap. Tighten the center cap until it is just flush with the large cap, or until the O-ring is no longer visible.
10. Using HOBOware, offload any datafiles that are on the shuttle and launch the shuttle before going into the field again. The shuttle will not read out and relaunch loggers until the clock has been synchronized.

WARNING: Do not install batteries backwards, recharge, put in fire, expose to extreme heat, or mix with other battery types, as the batteries may explode or leak. Contents of an open or leaking battery can cause chemical burn injuries. **Replace all used batteries at the same time.** Recycle or dispose of batteries according to applicable federal, state, and local regulations.

Appendix I

CFB Lab QA Plan

Updated 3/11/2021 by Bob Craycraft and Jeff Schloss

QAPP for the Center for Freshwater Biology at the University of New Hampshire, Durham, NH.

I. Laboratory Organization and Responsibility

Jeffrey Schloss - Director

Robert Craycraft – Lab Manager/QA manager. Mr. Craycraft supervises all activities in the lab. His responsibilities include data processing and review (QA review), database management, protocol development and upkeep, training of new laboratory technicians, instrument maintenance and repair, and sample analysis.

All analyses are completed by Robert Craycraft and laboratory technicians, and all data from each sample analysis is reviewed by Robert Craycraft for QC compliance. All laboratory technicians are trained by the lab manager and must demonstrate (through close supervision and inspection) proficiency with the analytical instrumentation used and required laboratory procedures.

II. Standard Operating Procedures

Standard Operating Procedures for all instruments and methods are kept in a 3-ring binder in the laboratory, and are stored electronically on the Lab manager's computer. The electronic versions are password protected. SOPs are reviewed annually, or as changes are required due to new instrumentation or method development.

III. Field Sampling Protocols

All data collected by the University of New Hampshire Center for Freshwater Biology and Lakes Lay Monitoring Program will adhere to field sampling protocols outlined in the New Hampshire Center for Freshwater Biology and Lakes Lay Monitoring Program Programmatic QAPP. When samples are processed for other clients (e.g. University of New Hampshire Faculty) the field sampling protocols will be left up to the sample originators.

IV. Laboratory Sample Handling Procedures

Samples are tracked by sample ID information: lake, site, date, and time. The project name, sampler's name and date received at the CFB lab, sample type (e.g. surface water, benthic sediment, etc) and any other miscellaneous information, are also entered

into a password protected database. From this point through the completion of all analyses, we use the log number to track samples. Lake ID information is used on sample run queues and spreadsheets.

After samples are analyzed and the results have been reviewed by the laboratory manager and have meet the acceptance criteria for the analyte of interest, the samples are disposed of.

Chain of custody forms are used when samples are collected by the Center for Freshwater Biology and the Lakes Lay Monitoring Program (see page J-13). Chain of custody forms are not required for samples collected by external clients although chain of custody forms may be submitted dependent upon the client's protocol.

The Center for Freshwater Biology uses pre-sterilized whirl-pack bags that are ready for use while nutrient bottles used for Total Phosphorus, Total Nitrogen and Orthophosphate are washed with Double Distilled Water, placed in a 10% hydrochloric acid bath for ten minutes. Total Phosphorus and Total Nitrogen bottles are subsequently rinsed three times with double distilled water while Orthophosphate bottles are rinsed six times. Sample bottles used for Alkalinity, pH, carbon dioxide, turbidity, true color, specific conductivity, chloride and total suspended solids are rinsed three times with deionized water. Sample containers, preservative and holding times are outlined in Table 1 (*Note: Anion and Cation data are analyzed through the WRRC laboratory using their analytical instrumentation and laboratory SOPs*).

Table 1. Sampling Method Requirements for Water Samples

Parameter	Sample Holding Container[1]	Preservative	Maximum Holding Time
Alkalinity	1 l Opaque HDPE[1] plastic	On ice	<24 hours
pH[1]			
Carbon Dioxide			
Turbidity			
Specific Conductivity			
Dissolved Oxygen	300 ml Wheaton glass BOD bottle	On ice w/ manganous sulfate, alkali-iodide-azide and H_2SO_4	<24 hours
Chlorophyll *a*	2 l Opaque HDPE plastic	On ice	< 24 hours[2]
True color			
Chloride	250 ml Opaque HDPE	On ice	< 28 days

Parameter	Sample Holding Container[1]	Preservative	Maximum Holding Time
Total Phosphorus	Individual 250 ml Plastic HDPE for each	Acidified w/ H_2SO_4 to pH <2 / iced in field / frozen within 8 hours of sample collection	<90 days[3]
Total Nitrogen	Individual 250 ml Plastic HDPE for each	Acidified w/ H_2SO_4 to pH <2 / iced in field / frozen within 8 hours of sample collection	<90 days[3]
Total Suspended Solids	Individual 2 l Opaque HDPE for each	On ice	< 7 days[7]
Ortho-Phosphate [6]	Individual 250 ml Plastic HDPE for each	Filtered [4] / iced / and refrigerated at 4ºC	< 7 days
Cations/Anions [6]: Nitrate, Sodium, Potassium, Sulfate Magnesium, Calcium, Chloride	60 ml Plastic HDPE	Filtered / iced / frozen within 8 hours of sample collection	3 months[5]
E. *coli* [8]	Sterilized 500 ml Whirl-Pack bag	On ice	< 24 hours

Notes for Table 1:

[1] HDPE-High Density Polyethylene. pH samples that are not analyzed in the laboratory within eight hours of sample collection will be qualified as being analyzed within 24 hours of sample collection.

[2] Chlorophyll and color samples are filtered within 24 hours of sample collection and the color samples are then refrigerated with a 90 day holding time and the chlorophyll a samples are frozen with a 90 day holding time. An assessment of chlorophyll holding time conducted by Axler, R.P and C.J. Owen (1994) documented no significant loss of pigments up to four months. An internal UNH CFB assessment of dissolved color samples, refrigerated over a 90 day span, documented "true" color stability relative to samples analyzed within 24 hours of collection. The phytoplankton samples are collected in 40 ml Histoplex containers within 8 hours of collection, preserved with Lugol's solution, refrigerated and quantified microscopically within six months of preservation.

[3] Expected target holding times are indicated for nutrient analysis; however documentation in the literature on unacidified samples (Canfield et al 2002) and UNH CFB analyses on acid preserved samples have shown samples remain stable for over 150 days.

[4] Ortho-phosphate samples will be field filtered using a Nalgene filterholder receiver and a Nalgene hand pump. 250 milliliters of streamwater will be filtered through a 0.45 micron HAWP 04700 Millipore membrane filter and the filtrate will be transferred to a 250 milliliter acid washed sampling bottle and immediately placed on ice. Orthophosphate samples will be held for a maximum of seven days at 4ºC and orthophosphate samples that are not analyzed within 48 hours of data collection shall be qualified as exceeding a two day holding period.

[5] The UNH WQA lab will usually process these samples within 3 months of receipt. Holding times for nitrate-plus-nitrite has been show to be at least eight years if samples are immediately kept cold (4ºC) and frozen within a couple of hours (Avanzino, R. J., and V. C. Kennedy. 1993). The UNH WRRC has generated similar data that indicate the rapid refrigeration and subsequent freezing of chloride and sulfate, as well as most cations, produce a holding time of at least one year. The ion chromatography procedure does not facilitate acidification of cation samples and the cation samples will not be acidified prior to freezing.

[6] Samples that are frozen prior to analysis shall be flagged and a qualifier shall be included in the final data summary that identifies the samples that were frozen.

[7] Total suspended solid samples that are not analyzed within 24 hours of sample collection shall be qualified as exceeding a one day holding time.

[8] *E. coli* samples that are not analyzed in the laboratory within eight hours of sample collection will be qualified as bein analyzied within 24 hours of sample collection.

Samples that arrive unfrozen/not refrigerated, with cracked bottles/caps, or with loose caps, are noted in the database and are flagged with a qualifier that indicates the deficiency. The sample originator is notified (via e-mail or telephone) of which samples deviated from sample transport SOPs to determine whether or not the samples should be

analyzed. Similarly, if a sample in the possession of the CFB laboratory is broken or improperly stored (e.g. not frozen or refrigerated) the sample originator is notified.

V. Calibration procedures for chemistry

Calibration curves are generated for Total Phosphorus, Total Nitrogen, Orthophosphate and Dissolved Color analyses and are made up of 4-5 points. A full calibration is performed at the beginning of each run (a run is generally 20-50 samples) while additional standards are run at a frequency of approximately 10% throughout the analytical run. Calibration data are fit with a linear equation.

Standards are made from reagent grade chemicals (typically purchased through Fisher Scientific). Working stock solutions are labeled with the content description, concentration, initials of the preparer, and the date the stock solution was made. With the exception of Orthophosphate stock that is made up no more than two hours prior to analysis, the remaining stock solutions (Total Nitrogen and Total Phosphorus) can be stored under refrigeration for 90 days. Standard solutions are kept for no more than 28 days from the date they were made. Stock and working standards are stored tightly covered, in a dark refrigerator.

Control charts are prepared and printed every few months. However, data from each run are looked at within days (generally the next day but within a week) of analyses. Calibration curves, Laboratory Duplicates, Lab Fortified Blanks (LFB), Lab Fortified Sample Matrices (LFM) and Lab Reagent Blanks (LRB) are reviewed and are checked against known concentrations (where applicable) to ensure QC criteria are met for each sample run.

VI. Data Reduction, validation, reporting and verification

Data reduction and validation are performed in a spreadsheet (MS Excel). Each analytical run is entered onto an Excel file that is specific to the analyte of interest (e.g. Total Phosphorus, Orthophosphate, etc) and each file contains multiple worksheets that contain data from individual analytical runs. The worksheets list the date of analysis, Sample ID (i.e. lake, site, date, etc), raw absorption values, and any other issues or problems noted with the instrument on that date. All Laboratory Duplicates, Lab Fortified Blanks (LFB), Lab Fortified Sample Matrices (LFM) and Lab Reagent Blanks (LRB) are entered on the respective worksheet. Each worksheet contains calculations that includes

the day's calibration curve, laboratory precision (as relative percent difference), percent recovery for LFM and calculations for unknown samples. If these are acceptably close (+/- 15% of the "known") no further changes to the calculated concentrations are made. If there is evidence of drift in the response of the instrument during a run (i.e. LFB < 85% or >115%) the samples are deemed unacceptable and are re-run with the next set of samples. All reference solutions and replicates must meet certain QC criteria (described below) for a run to be accepted.

Summary results are then compiled onto accompanying datasheets and entered onto CFB database files that contain the accepted data. Data are sent to sample originators upon completion of all requested sample analyses and following review by the CFB lab manager. Generally, the data include, the sample name, collection date, and concentrations/results, in row-column format. Any information entered into the database can be included upon request. Data transfer is typically in digital format (i.e. e-mail correspondence) while printed summary output is provided upon request.

All data corrections are handled by the lab manager. Corrections to data already entered into the database are very infrequent. Typically they involve reanalysis of a sample. In this case, the old datum is deleted from the database, and the new value is imported, along with a note indicating that it was re-analyzed, the dates of initial and secondary analysis and the reason for the correction.

Hand written or computer printed run sheets are saved for each run and filed, based on the analysis while spreadsheet files with raw data and calculations are stored electronically by analysis and date.

VII. Quality Control

All analyses conducted at the CFB laboratory follow approved or widely accepted methods (Table 2).

Quality Control Samples (QCS) (i.e. Fisher Scientific or Ricca Chemicals) are analyzed periodically (approximately every 10 samples) in each sample analysis batch to assure accuracy. The response/unit concentration is also used to monitor day-to-day variation in instrument performance. A difference from the certified concentration of more than 10% requires further investigation of that run. A difference greater than 15%

is failure (unless the average of the two samples is less than 10X the MDL), and results in re-analysis of the entire sample queue, unless there is a very reasonable and supported explanation for the inconsistency. Table 2 lists historical average % recoveries. At least 4 QCS are analyzed during each run.

Standards and reagents are prepared from reagent grade chemicals (Fisher Scientific) or from pre-made stock solutions (Ricca). All glassware is acid washed (10% HCl) and rinsed 3 times with deionized (> 10 mega-ohm) water. All analyses use multi-point calibration curves (4-5) points, which are analyzed at the beginning of each run. A Laboratory Reagent Blank (LRB), Laboratory Fortified Blank (LFB) (a standard run as a sample) and Laboratory Duplicate are analyzed approximately every 10 samples during each run. At least one Laboratory Fortified Sample Matrix (LFM) is analyzed during each run to insure that sample matrices do not affect method analysis efficiency. Field Duplicates are not required by our lab, and are the responsibility of the specific project's manager.

Laboratory Duplicates must fall within 10% relative percent difference (RPD = abs (dup1-dup2)/average of dup1 and dup2). A difference greater than 10% requires further investigation of the sample run. A difference greater than 15% is failure (unless the average of the two samples is less than 10X the MDL), and results in re-analysis of the entire sample queue, unless there is a very reasonable and supported explanation for the inconsistency.

LFM must show 85% to 115% recovery. A recovery <85% or > 115% requires further investigation of the sample run. A recovery <85% or >115% is failure (unless the sample is less than 10X the MDL), and results in re-analysis of the entire sample queue, unless there is a very reasonable and supported explanation for the inconsistency.

Method Detection Limits are calculated periodically, and whenever major changes to instrumentation or methods occur. Table 3 lists most recently measured MDL values.

VIII. Schedule of Internal Audits

Internal audits are not routinely performed, however, QC for each run is thoroughly reviewed by the lab manager before entering data into the database and a review of QC charts, and tables is done at least annually by the lab manager.

IX. Preventative maintenance procedures and schedules

The laboratory manager, Robert Craycraft, has 30 years of experience and is experienced with the equipment used within the CFB lab. The laboratory manager conducts all inspections of equipment based on manufacturer requirements and specifications. Maintenance is performed by the laboratory manager and when necessary by the University of New Hampshire Instrumentation Center staff (housed in the Chemistry Department) who regularly service analytical instrumentation for the University of New Hampshire campus community.

Each day an instrument is used, it receives a general inspection for obvious problems (e.g. blown bulbs, properly seated cell holders). The instruments are used frequently and data are inspected within a few days of sample analysis. This allows instrument (or user) malfunctions to be caught quickly, and corrected as needed.

Each day's run is recorded in the instrument's run log, with the date, the user, the number of standards, samples, and QC samples), the project, and other notes of interests. Maintenance, routine or otherwise, is recorded in the instrument run log, and includes the date, the person doing the maintenance, what was fixed, and any other notes of interest.

X. Corrective Action Contingencies

Robert Craycraft is responsible for all QC checks and performs or supervises all maintenance and troubleshooting. When unacceptable results are obtained (based on within sample analysis batch QC checks) the data from the run are NOT compiled into the database. The cause of the problem is determined and corrected, and the samples are re-analyzed. Problems are recorded in the sample queue's data spreadsheet, or on the handwritten runsheet associated with the run. Corrective actions (instrument maintenance and troubleshooting) are documented in each instrument's run log.

XI. Record Keeping Procedures

Protocols, Instrument Logs, QC charts, databases and all raw data files are kept on the lab manager's computer. These are backed up daily, with the back up stored off site. The computer is password protected, and is only used by the lab manager and trained laboratory technicians. Protocols and the sample database are also password protected. Handwritten run sheets are stored in three ring notebooks in the lab. Instrument run and maintenance logs are combined with the QC data to form one large Excel file where

instrument performance can easily be compared to instrument repair and the number of analyses, etc. This file is also stored on the lab manager's computer and is password protected.

All information pertinent to a sample is stored in the sample database. From this database we can easily determine the date of analysis and the location of the raw data file if further review is necessary. The amount of information provided to sample originators is dependent on what is required by the project or funding agencies.

Table 2. List of standard operating procedures and description of analyses performed at the Center for Freshwater Biology Laboratory.

Standard Operating Procedure	Analysis	Instrument Used	Analytical Method Description and Method Citation	Protocol Latest Revision
Soluble Reactive Phosphorus	PO_4^{-3}	Cary 50 UV/Vis scanning spectrophotometer	Standard Methods 4500-P.E.	March 11, 2021
Total Phosphorus	TP	Cary 50 UV/Vis scanning spectrophotometer	Acid Digestion; Standard Methods 4500-P.E.	March 11, 2021
Total Nitrogen	TN	Cary 60 UV/Vis scanning spectrophotometer	Second Derivative Spectroscopy; Standard Methods 4500-$NO3^-$C (21^{st} edition proposed method) and Crumpton (1992)	March 11, 2021
Chlorophyll *a*	Chl a	Cary 50 UV/Vis scanning spectrophotometer	Standard Methods 10200 H.2	March 11, 2021
Free Carbon Dioxide	CO_2	NA	Titration via Standard Methods 4500 CO2-C	July 7, 2005
Dissolved Oxygen (Titration; Azide Modification)	O_2	NA	Winkler Titration via Standard Methods 4500-02 B.C.	July 7, 2005
E. Coli	E. *coli*	Fisher Scientific Isotemp waterbath model 5L	Membrane filtration, Standard Methods 9213 D.2,3	May 11, 2007
Dissolved "true" Color	Dissolved Color	Cary 50 UV/Vis scanning spectrophotometer	Standard Methods 2120-B	March 11, 2021
Total Suspended Solids	TSS	Fisher Scientific Model 825F oven and Denver Instruments A-250 analytical balance	Filtered and oven dried at 103-105^oC; Standard Methods 2540 D.	July 23, 2010

Standard Operating Procedure	Analysis	Instrument Used	Analytical Method Description and Method Citation	Protocol Latest Revision
Field Measured pH	pH	Hanna Model HI 9025 Meter / Beckman Coulter Low Ionic Strength pH Probe.	Field Measured pH	July 23, 2010
Field Measured Total Alkalinity	Total Alkalinity	NA	Low Alkalinity Titration to pH 4.5; Standard Methods 2320B	July 7, 2005
Lamottee Model 2020 and 2020e Portable Turbidimeter	Turbidity	Lamottee 2020e	Lamotte Model 2020e Turbidimeter. Instrument Manual; USEPA 180.1	February 19, 2007
Oakton PC 2700 Benchtop Meter	pH	Oakton PC 2700 Benchtop Meter	Oakton PC 2700 Meter / Low Ionic Strength Probe. Instrument Manual; Standard Methods 4500H^+	March 8, 2021
Oakton PC 2700 Benchtop Meter	Specific Conductivity	Oakton PC 2700 Benchtop Meter	Oakton PC 2700 meter /Oakton Model # WD-35608-92 4 cell conductivity probe, Standard Methods 2510 B.	March 8, 2021
Oakton PC 2700 Benchtop Meter	Chloride	Oakton PC 2700 Benchtop Meter	Oakton PC 2700 Meter / Oakton Model # WD-35802-13 Chloride Electrode; Instrument manuals.	March 8, 2021
Analyzing Samples on the Cary 50 UV/Vis scanning spectrophotometer	NA	Cary 50 UV/Vis scanning spectrophotometer	NA	March 11, 2021

Table 3. Detection limits and acceptable ranges for QC samples at the CFB Lab.

[2] Method Detection Limit (MDL) is the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. The Quantitation limit is calculated as >/=2.5 times the MDL.

Analyte (Sample Source)	SOP Method	Desired Precision	Desired Accuracy	Analytical / Achievable Method Detection Limit[1]	Analytical / Achievable Laboratory Quantitation Limit[2]	Typical Measurement Range
Total Phosphorus (Stream and Lake Water)	Appendix A.3	RPD ≤ 20% (Field) RPD ≤ 15% (Lab)	+/- 15% RPD ≤ 15% $r^2 \geq 0.995$	0.8 μg/L	2.0 μg/L	2 – 500 μg/L P
Soluble Reactive Phosphorus (Stream and Lake Water)	Appendix A.2	RPD ≤ 20% (Field) RPD ≤ 10% (Lab)	+/- 10% RPD ≤ 10% $r^2 \geq 0.995$	0.3 ug/L	1.0 ug/L	1 – 500 ug/l P
Total Nitrogen (Stream and Lake Water)	Appendix A.4.	RPD ≤ 20% (Field) RPD ≤ 15% (Lab)	+/- 15% RPD ≤ 15% $r^2 \geq 0.995$	30 ug/L	100 ug/L	100 – 3000 ug/l N
Turbidity (Stream and Lake Water)	Appendix B.9	RPD ≤ 5% (Field) RPD ≤ 5% (Lab)	+/- 1.0 NTU	0.01 NTU	NA	0 – 50 NTU
pH (Stream Water)	Appendix B.1	RPD ≤ 0.2 std units (Field)	+/- 0.2 pH units	NA	NA	2 –12 pH Units
Dissolved Oxygen (Lake Water)	Appendix A.10 (titration)	RPD ≤ 10% (Field) RPD ≤ 5% (Lab)	+/- 15%	0.2 mg/L	0.5 mg/L	0.5 – 15 mg/L
Chlorophyll (Lake Water)	Appendix A.8	RPD ≤ 20% (Field) RPD ≤ 10% (Lab)	+/- 15% of Turner Standard	NA	NA	0 – 50 μg/L
Total Alkalinity (Lake Water)	Appendix B.2	RPD ≤ 15% (Field)	+/- 15%	0.2 mg/L	0.5 mg/L	0.5 – 20 mg/L $CaCO_3$
Carbon Dioxide (Lake Water)	Appendix A.9 (titration)	RPD ≤ 15% (Field) RPD ≤ 10% (Lab)	+/- 15%	0.2 mg/L	0.5 mg/L	0.5 – 30 mg/L
Dissolved "true" Color (Lake Water)	Appendix A.12	RPD ≤ 20% (Field) RPD ≤ 10% (Lab)	+/- 10% of 20 CPU Standard	2.0 CPU	5.0 CPU	5 – 500 CPU
Total Suspended Sediment (Stream and Lake Water)	Appendix A.15	RPD ≤ 20% (Field) RPD ≤ 10% (Lab)	DD H_2O Filter Blank < 0.3 mg/L	0.3 mg/L	2.0 mg/L	2 – 100 mg/L
E. coli (Stream and Lake Water)	Appendix A.11	RPD ≤ 20% (Field) RPD ≤ 10% (Lab)	0 counts/100 ml (sterilized blank)	NA	NA	0 – 500 CFU/100ml

Analyte (Sample Source)	SOP Method	Desired Precision	Desired Accuracy	Analytical / Achievable Method Detection Limit[1]	Analytical / Achievable Laboratory Quantitation Limit[2]	Typical Measurement Range
Chloride (Lake and Stream Water)	Appendix A.16	RPD ≤ 20% (Field) RPD ≤ 10% (Lab)	10 % of 20 ppm standard	NA	NA	0 – 100 mg/L
Specific Conductivity (Lake and Stream Water)	Appendix A.16	RPD ≤ 10% (Field) RPD ≤ 5% (Lab)	+/- 5%	NA	NA	0 – 1000 *u*S/cm

UNH Center for Freshwater Biology

Chain of Custody

Project:_____

Phone #: (603) 862-3696

SAMPLING		SAMPLE IDENTIFICATION	MATRIX		Composite (C)/Grab (G)	Total # Containers	ANALYSES REQUESTED									SAMPLER'S NAME	COMMENTS
Date	Time		Lake/stream water	Benthos/sediment			Total Phosphorus	Soluble Reactive Phosphorus	Chl, color	CO_2, Alk, Turbidity	Cond, pH	Chloride	Dissolved Oxygen (Winkler)				

Relinquished By:	Date	Time	Received By:
Relinquished By:	Date	Time	Received By:

COMMENTS:

Analyses/Sample containers and preservative (samples delivered to lab on ice):

Total Phosphorus - 250ml translucent HDPE bottle with acid preservative

Soluble Reactive Phosphorus - 250ml translucent HDPE bottle with filtered water

Chlorophyll & Color - 2L amber HDPE bottle

CO_2, Alkalinity, turbidity - 500ml amber HDPE bottle

Conductivity, pH - 500ml amber HDPE bottle

Chloride - 250ml amber HDPE bottle

Dissolved Oxygen - 300ml Wheaton BOD bottle with Maganous Sulfate, Alkali-iodide-azide and sulfuric acid

ThermoScientific Custody Seals, Fisher Scientific Catalog Number 05-719-337