Alternative Site-Specific and Limited-Use Methods Approval for Florida LAKEWATCH

FDEP Aquatic Ecology and Quality Assurance Section

July 2013

The Florida Department of Environmental Protection (DEP) Aquatic Ecology and Quality Assurance Section (AEQAS), at the request of the DEP Division of Environmental Assessment and Restoration, has reviewed method validation information and approved site-specific methods for alternative sample preservation and maximum holding time for total nitrogen (TN), total phosphorus (TP) and chlorophyll *a* (CHLA) samples, and a limited-use alternative method for the laboratory preparation of chlorophyll samples, collected for Florida LAKEWATCH projects, as further explained below. Florida LAKEWATCH (LW) is a surface water monitoring program coordinated by the Department of Fisheries and Aquatic Sciences at the University of Florida, and is located at 7922 NW 71st Street, Gainesville, FL, 32653. This document describes the criteria and references used to evaluate the methods, in support of an approval order for alternative methods as required in Rules 62-160.220(7)(a) and 62-160.330(6)(a), F.A.C. (DEP. 12/3/08). The bases for the approvals are described below, and meet the requirements for alternative method approvals in DEP SOP FA 1000, subparts FA 2210 – FA 2230 (DEP. 3/31/08) and New and Alternative Laboratory Methods, DEP-QA-001/01 (DEP. 2004).

Sample Preservation by Freezing

1. **Alternative Sample Preservation Methods Including Freezing** - The LW method of freezing samples for Total Nitrogen (TN) and Total Phosphorus (TP) is found in "Florida LAKEWATCH Water Chemistry Field Sampling and Laboratory Protocols" (LW. 2013), hereinafter called LWSOP. The chlorophyll preservation and filtering procedure is also described in the LWSOP. All samples are collected and processed according to the LWSOP by LW resident volunteers and stored in home freezers until delivery to a centralized collection point, from which they are collected by LW coordinating staff and brought to the laboratory. Occasionally, samples may be collected by LW coordinating staff, and are delivered directly to the LW lab. Preservation and storage procedures utilized by the LW laboratory are also described in the LWSOP. The maximum holding time for all frozen samples is 5 months from the date of collection to analysis.
    a. Samples for TN and TP are held at ambient temperature until immediately frozen when volunteers return home. If the samples are held for more than one hour while sampling, samples are placed in ice while on board the sampling boat.

b. Chlorophyll *a* (CHLA) samples are collected in bulk sampling containers, which are covered with a dark towel and filtered as soon as possible, but less than 48 hours from time of collection. After filtering the water samples, the filters are packaged according to the LWSOP and frozen.

2. **Basis for Approval of Alternative Preservation Methods Including Freezing** –
   a. LW project coordinators conducted a comparison study of co-collected lake samples using the LW freezing method and APHA preservation procedures (see Canfield *et al*. 2002 and APHA. 1992). Samples were tested for a variety of analytes including TN, TP and CHLA (uncorrected). The study consisted of two approaches to the evaluation of the effect of sample preservation:
      i. Samples were collected from 125 lakes by LW volunteers and preserved using the LWSOP freezing method, and co-collected at the same location and time by LW staff using APHA preservation procedures. In this study, the frozen samples were held for as long as three months at volunteer homes. Samples collected by LW staff were placed in ice and transported to the LW lab for preservation and analysis within recommended holding times. Regressions comparing the LW volunteer vs. LW staff samples indicated the data were highly correlated for TN, TP and CHLA ($r \geq 0.99$). Although some differences were significant for the compared data sets for individual lakes (paired t-test, $p < 0.05$), no bias was observed for either preservation method across all lakes studied.
      ii. Samples were collected from 12 lakes in sufficient volume to immediately analyze five samples without freezing and store the remaining samples frozen until analysis at specified holding times up to 150 days from collection. Analysis of Variance (ANOVA) conducted between samples analyzed immediately and those analyzed after 15, 30, 60, 90, 120, and 150 days in frozen storage showed that results were not statistically different for TN, TP, or chlorophyll *a* ($p < 0.05$). Evaluation of the data using Dunnett's and Wilcoxon/Kruskal-Wallis Rank Sums tests also indicated no significant differences for holding times. Results for unfrozen vs. frozen samples were also highly correlated ($r \geq .94$).
   b. DEP and LW conducted a comparison study of co-collected lake samples using the LW freezing method and the DEP SOP preservation procedures (Hoyer *et al*. 2012). The LW samples were collected by volunteers per the LWSOP and frozen until analysis by the LW lab. The DEP co-collected samples were preserved according to requirements in DEP SOP FS 1000, Part 1006 (DEP. 3/31/08), and analyzed for Total Kjeldahl Nitrogen, Nitrate-Nitrite, TP and CHLA by the DEP Bureau of Laboratories. The LW lab analyzed the frozen samples for TN, TP, and CHLA (uncorrected). LW and DEP results were very highly correlated for TN ($r = 0.95$), TP ($r = 0.98$), and CHLA ($r = 0.98$). A pair-wise comparison showed that the LW and DEP results were not statistically different for TN or CHLA ($p < 0.05$), but were statistically different for TP ($p = 0.0001$). Although the difference in TP

results was statistically significant, the mean difference was 1 µg/L, which will not likely affect the data uses approved below.

c. DEP and Project COAST conducted a comparison study of co-collected estuarine samples. Evaluation of sample analysis results were summarized in an internal DEP report (DEP. 6/7/13). The COAST samples were analyzed for TN and TP by the LW lab. The DEP co-collected samples were preserved according to DEP SOP requirements and analyzed for Total Kjeldahl Nitrogen, Nitrate-Nitrite, TP (and CHLA) by the DEP Bureau of Laboratories. The COAST samples were delivered to the LW lab after collection and freezing, and the LW lab analyzed the frozen samples. While the sample collection and handling procedure used by COAST varies slightly from that used by LW volunteers, both organizations use sample freezing for preservation and storage. COAST and DEP results were very highly correlated for TN ($r = 0.79$) and TP ($r = 0.92$). A pair-wise comparison showed that the COAST and DEP results were not statistically different for TN or TP ($p < 0.05$).

d. The LW alternative preservation methods were evaluated against the approval criteria described in DEP SOP FA 1000, subpart FA 2220 (DEP. 3/31/08). DEP finds the alternative methods of sample preservation to be appropriate for the data quality objectives and usability requirements associated with the data applications listed below. The procedures produce sample analysis results that are equivalent to those generated from samples preserved using DEP SOP procedures, as demonstrated by the studies described above, and are comparable to other data used by DEP. The alternative methods also meet the data validation requirement for adherence to proper preservation procedures as required at Rule 62-160.670(1)(b), F.A.C.

<u>Alternative (Modified) Sample Extraction Method for Chlorophyll</u>

1. **Extraction Method for Chlorophyll Filters** – Frozen filters (CHLA samples) delivered by LW volunteers are extracted in the LW lab using a hot ethanol extraction procedure. This extraction method, which is described in the LWSOP, is intended to replace the approved acetone extraction accepted by DEP, as found in the approved analytical methods cited in Applicability of Chlorophyll *a* Methods, DEP-SAS-002/10 (DEP. 2011).

2. **Basis for Approval of Chlorophyll Extraction Method** –
   a. The LWSOP procedure was compared to the DEP-approved acetone extraction procedure by LW investigators using samples collected from several lakes, and was found to produce sample results that were not statistically different than the DEP-approved technique for the lake samples studied (Canfield, et al. 2002). A pair-wise comparison of lake samples analyzed with both prep methods showed that the results were not statistically different (p

< 0.05), and the acetone and ethanol extraction results were very highly correlated (r = 0.99).

b. DEP and LW conducted a comparison study of co-collected lake samples using the LW CHLA extraction method and the DEP-approved acetone extraction method (Hoyer *et al*. 2012). A pair-wise comparison of these 27 lakes showed that the LW and DEP results were not statistically different ($p < 0.05$), and LW and DEP results were very highly correlated ($r = 0.98$).

c. An initial demonstration of capability (IDOC) to perform the LWSOP CHLA extraction and analytical methods and a method detection limit (MDL) study were provided to DEP by the LW lab. The IDOC and MDL data demonstrated accuracy, precision, and detection capability suitable for DEP data usability purposes.

d. The LWSOP chlorophyll extraction procedure modification to approved DEP methods was evaluated against the approval criteria in DEP-QA-001/01, sections 1.0 - 1.3 and 2.2. as follows:

   i. <u>Sections 1.0 – 1.3</u>

      a) The laboratory certification requirement for this method has been waived by DEP due to the budget constraints on funding available for Florida LW activities, which make participation in the Florida Department of Health environmental laboratory certification program prohibitive for this volunteer program coordinated by a state university research project.
      b) DEP has determined that a method equivalency study is not needed, and the comparability studies summarized above provide acceptable demonstration of the efficacy of the alternative chlorophyll extraction method.
      c) The sample results produced by this extraction modification are found to be appropriate for the data uses described below, based on the information received from LW, as summarized in this review, and meet applicable DEP project and program Data Quality Objectives (DQOs) for these uses. Additionally, DEP agrees with LW that this alternative extraction procedure provides important cost and safety advantages for the LW lab (Canfield *et al*. 2002), which is why DEP reviewed the proposed alternative procedure.
      d) The alternative extraction method is determined to be applicable to the analysis of fresh and marine surface water samples.

   ii. <u>Section 2.2</u>

a) The LW lab has conducted an acceptable MDL study with modifications for the practical adaptation of the MDL protocol to the chlorophyll procedure, demonstrating appropriate detection capability.
b) Acceptable precision and accuracy has been demonstrated by the IDOC validation, using the above adaptation to evaluate percent recovery and percent relative standard deviation (% RSD).
c) The MDL, precision and accuracy determinations, along with the comparability studies summarized in this review, provide sufficient justification for waiving the need for any additional equivalency study of the LW extraction method in comparison to the DEP-approved acetone extraction method, and demonstrate that the ethanol extraction method used by the LW lab is a suitable replacement for the acetone extraction method.
d) All relevant validation information concerning the extraction procedure is included in the LWSOP, MDL study and IDOC.

Scope of Approval

1. **Alternative Sample Preservation Methods Including Freezing** – The alternative sample preservation methods for TN and TP samples, and the preservation procedure for CHLA samples described in the Florida LAKEWATCH Water Chemistry Field Sampling and Laboratory Protocols (LWSOP), are approved as site-specific sampling methods, defined in Rule 62-160.220(4)(a), F.A.C. (DEP. 12/3/08), and as project-specific alternative field procedures, described in DEP SOP FA 1000, subpart FA 2230, section 1 (DEP. 3/31/08). The methods are approved for use for the LW project for the collection, including preservation and storage, of TN, TP and CHLA samples from fresh and marine surface water matrices on all sites or locations sampled by LW project volunteers and coordinating staff. The methods may also be used by other persons collecting samples for the LW project, if applicable. This includes approval of a maximum holding time of 5 months from the date of sample collection for TN, TP and CHLA samples, as stipulated in the LWSOP.

2. **Chlorophyll Extraction Method** – The extraction method for CHLA sample filters described in the LWSOP is approved as a limited-use method, defined in Rule 62-160.330(4)(a), F.A.C. (DEP. 12/3/08) and in DEP-QA-001/01, section 1.2 (DEP. 2004). The method is exclusively approved for use by the LW laboratory for the analysis of CHLA samples collected from fresh and marine surface water matrices, and may not be used by another laboratory without separate review and approval by DEP.

Data Usability

Approval of the above Florida LAKEWATCH alternative methods includes the determination by DEP that sample analytical data for TN, TP and CHLA using the LW sample preservation methods, sample

maximum holding time and the chlorophyll filter extraction method meet all applicable data quality objectives for the use of approved sampling and analysis procedures for the following:

1. Development and implementation of numeric nutrient criteria for proposed water quality standards in Chapter 62-302, F.A.C.;
2. Impairment assessment of surface waterbodies according to requirements in Chapter 62-303, F.A.C.;
3. Development of Total Maximum Daily Loads; and,
4. Development of Basin Management Action Plans.

References

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Hoyer, M. V., N. Wellendorf, R. Frydenborg, D. Bartlett, and D. E. Canfield, Jr. 2012. A comparison between professionally (Florida Department of Environmental Protection) and volunteer (Florida LAKEWATCH) collected trophic state chemistry data in Florida. Lake Reservoir Management. 28: 277-281.

Florida LAKEWATCH Water Chemistry Field Sampling and Laboratory Protocols

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Introduction

The Florida LAKEWATCH program is a successful citizen-monitoring program for Florida waters. LAKEWATCH has provided information about long-term trends in water quality to the citizens and leaders of Florida for over 25 years. LAKEWATCH has also served as a platform for research, education and extension/outreach activities. In response to citizens' requests for lake management assistance, Fisheries and Aquatic Sciences of the University of Florida/Institute of Food and Agricultural Sciences (UF/IFAS) provided faculty, staff, and laboratory support to begin LAKEWATCH in 1986. Public interest and involvement in the program grew to such a degree that the Florida Legislature officially established Florida LAKEWATCH in 1991 (Chapter 1004.49 F.S.). The Legislature also provides an annual appropriation to operate the program.

Florida LAKEWATCH has now expanded to almost 2000 volunteers and currently over 500 lakes, 130 near shore coastal sites, 125 river sites and 5 springs are active in the program making LAKEWATCH Florida's largest and one of the nation's premier citizen volunteer monitoring programs. Since its inception, reliable water chemistry data have been collected on over 1000 aquatic systems located in or offshore of 55 Florida counties.

Since the beginning of the Florida LAKEWATCH program, many agency professionals voiced concerns/criticisms as to whether volunteer monitoring groups were capable of gathering quality data. There were also concerns that LAKEWATCH's method of freezing samples to preserve them was not appropriate for proper analyses. To address both of these concerns, LAKEWATCH personnel conducted a study sampling 125 lakes with professionals and volunteer collecting water at the same time and in all cases the data collected by volunteers were comparable to those collected by professionals (Appendix IV, Canfield et al. 2002). To determine if freezing was a valid means of preserving water samples prior to analysis, estimates of chlorophyll, total phosphorus, total nitrogen, pH total alkalinity, and specific conductance measured on fresh waters were compared to estimates made from water frozen for up to 150 days. All statistical tests showed that freezing was a valid means of preserving samples. A copy of this study is attached to the end of this document.

An additional concern about the study conducted by Canfield et al (2002) was that the chemistry comparison was not conducted with an independent laboratory. Thus, another study was conducted where Florida Department of Environmental Protection (FDEP) professional biologists and Florida LAKEWATCH volunteers sampled 27 Florida lakes simultaneously to measure the concentrations of total phosphorus, total nitrogen, and chlorophyll (Appendix V, Hoyer et al. 2012). Each program used their Standard Operating Procedures for both field and laboratory activities to determine data comparability. Results showed that LAKEWATCH data were nearly equivalent to FDEP's, which were collected using stringent quality assurance (QA) protocols and analyzed in a NELAC (National Environmental Laboratory Accreditation Conference) certified laboratory in compliance with the state's QA rule. The R^2 values for paired comparisons of total phosphorus, total nitrogen and chlorophyll were 0.97, 0.90 and 0.97,

respectively and the slopes were not significantly different from one. Properly trained volunteers should be viewed as partners, and volunteer monitoring can be embraced as a robust tool for obtaining credible, cost-effective data. Studies like this demonstrate that data collected by volunteer monitoring programs are suitable for regulatory decisions, if such programs and agencies work together to ensure necessary data quality and documentation.

The remainder of this manuscript describes other Standard Operating Procedures (SOP) for the Florida LAKEWATCH Program.

Field Sampling Protocols

Training Volunteers

LAKEWATCH has two Regional Coordinators that train volunteers face to face on their lake they how to properly collect water samples. The Regional Coordinator goes out on the lake with the volunteer where they are trained to sample from one to six pre-determined stations (primarily three stations, but occasionally one station on small lakes and up to six stations on large lakes). The stations are generally in open-water areas of each water body and each location (Latitude and Longitude) is recorded with Global Positioning equipment (GPS) so that the stations remain constant over time. At each station, the volunteer is taught and certified to perform the following tasks:

- Fill a sample bottle with surface water (upper 40 cm) that will later be analyzed in the Florida LAKEWATCH laboratory for nutrients (total phosphorus and total nitrogen, monthly or every other month) and quarterly for color and specific conductance.
- Collect a one-gallon jug of water that is used to filter for later chlorophyll analyses.
- Use a Secchi disc provided by LAKEWATCH to measure water clarity.
- Measure the depth of the water at each station.
- Fill out a data sheet with sampling information and unique observations pertinent to their water body on that day.
- Deliver their frozen samples to a LAKEWATCH collection center.

Techniques that volunteers will use to sample the water at each station are described in this document and are taught during a LAKEWATCH training session at a time and place convenient to the volunteer. Volunteers are given a training manual, which contains both a detailed description and a summary of each sampling procedure, for review before future sampling events. Volunteers also receive a laminated sampling reference card (Appendix I) to carry with them when they sample.

Filling Water Bottles for Nutrients

Florida LAKEWATCH supplies water sampling bottles after they are properly washed with acid to insure no outside contamination (See washing methods below). They are available at all collection centers (Appendix II) so the volunteers can pick them up after they deliver their samples from previous sampling activities. For inland fresh waters volunteers use 250 mL plastic Nalgene bottles packaged with white field data sheets and for saline stations they use larger 500 mL bottles packaged with blue field data sheets. Actual water collecting procedures described below are the same for both fresh waters and saline stations. Volunteers use a waterproof marker for labeling all sample bottles.

At each station, a surface water sample is taken for analysis of total phosphorus and total nitrogen is taken as soon as the boat arrives at the sampling location.

Step 1: Each bottle has a pre-printed adhesive label on it for identification. Volunteers use a permanent waterproof marker to label the sample bottle with the following information:

- **Water Body Name:_____**
- **County Name:_____**
- **Month-Day-Year:_____**
- **Station 1, 2, or 3, etc.:_____**

Volunteers are asked to always double check their labels so when the sample reaches the LAKEWATCH laboratory, technicians can properly enter the data for that sampling event.

Step 2: At each sampling location, volunteers uncap the appropriately labeled bottle, being careful not to touch the inside of either the bottle or the lid.

Step 3: Volunteers rinse the lid in the water body's water and set it aside, then give the bottle a thorough rinse by gripping it securely, partially filling it with the water body's water and shaking the water out vigorously. This procedure is then repeated.

Step 4: After conditioning the bottle twice, volunteers grasp the bottom rim of the upright sample bottle with the tips of their fingers. The purpose of this grip is to keep hands as far away from the mouth of the bottle as possible to help prevent possible contamination.

Step 5: Volunteers then **t**urn the bottle upside down and lowered it into the water with its mouth pointing downward as if it were being emptied. This prevents possible inflow of debris from the water's surface.

Step 6: Volunteers then push the bottle down into the water until elbow deep (20 cm to 40 cm deep).

Step 7: Volunteers then turn the bottle to a horizontal position so that it points in the direction in which the boat is drifting as it fills. This lets the bottle fill with water that has not been in contact with the volunteer's hand or bottom material resuspended by the boat in shallow waters, thereby minimizing the chance of contamination.

Step 8: When the bottle is full, the bottle is turned right side up underwater and brought out of the water.

Step 9: Because the sample will be frozen, some water is poured out of the bottle in order to allow some space for the water to expand as it freezes without breaking the plastic bottle.

Step 10: Volunteers then **c**ap the bottle and secures the lid tightly.

Step 11: If volunteers will be spending more than an hour on the water body, they put the bottles on ice in a cooler.

Step 12: When volunteers return home, sample bottles are dried placed in a plastic zip lock bag with the field data sheet and put immediately in their home freezer.

Sampling Water for Chlorophyll

Volunteers prepare one-gallon jugs by rinsing them in tap water. These one-gallon jugs are used for each sampling event as long as no fungus or mold is growing in them. Jugs are stored with the caps off so they can dry out thoroughly between uses. Using a permanent waterproof marker, volunteers write a station number on each of the jugs so they can be easily identified later. At each station, Volunteers then use the following procedure:

Step 1: Remove the jug lid and rinse it in lake water.

Step 2: Rinse the jug by filling it with a couple of inches of water and vigorously shaking the water out and repeat at least once.

Step 3: Turn the jug upside down and push it underwater to elbow depth.

Step 4: Once at the proper depth, volunteers fill the jug by turning its mouth in the direction the boat is drifting. If clumps of vegetation flow into the jug, volunteers empty it and start over. If this is too difficult to force the underwater jug into an upright position to fill, smaller bottles may be substituted.

Step 5: Bring the jug out of the water and cap it.

Step 6: Cover the jug with a dark towel in order to block out light.

Step 7: The volunteer filters the water as soon as possible (less than 48 hours from collection) after all samples are collected (see Filtering Water below).

Measuring Water Clarity

A Secchi disc is used to measure water clarity. Florida LAKEWATCH uses a white eight-inch disc with a cord marked at one-foot intervals attached to its center. A weight on the bottom of the disc helps it sink quickly. Volunteers lower the Secchi disc over the shaded side of the boat watching the disc until it just vanishes from sight. They then measure and record how many feet underwater the disc was when it just vanished.

The volunteers are asked to use the following protocols to get the most reliable Secchi depth measurement:

Step 1: To obtain the most rigorous Secchi reading, volunteers may choose a day when there is full sun and minimal wind.

Step 2: When possible volunteers sample between 9 a.m. and 3 p.m.

Step 3: Volunteers **do not** wear sunglasses while taking the Secchi reading.

Step 4: If the wind is blowing the boat around, volunteers anchor so that the Secchi rope will be vertical when the reading is taken. Volunteers anchor carefully to avoid stirring up bottom sediments that may interfere with water samples and water clarity measurements.

Step 5: Volunteers put the sun at their back and take the reading from the side of the boat that casts a shadow on the water lowering the Secchi disc in the shadow to minimize interference from surface glare.

Step 6: Taking the Secchi reading from the side of the boat that is downwind may also minimize interference from waves.

Step 7: After the Secchi disc is lowered into the water to the vanishing point (where it absolutely cannot be seen at all), volunteers raise and lower it a few times to determine the exact vanishing point of the disc.

Step 9: Volunteers then clip a clothespin onto the Secchi disc rope at the waters surface and check to estimate cloud cover. Cloud cover is recorded on the data sheet.

Step 10: Volunteers then pull the disc into the boat and count the rope markings to read how many feet below the surface of the water the disc was when it vanished from sight (the length of the line from the disc to the clothespin). Volunteers estimate the Secchi reading to the **nearest quarter of a foot.**

Step 11: Volunteers then record the Secchi reading on their data sheet by writing the whole number in the space provided and circling the appropriate quarter foot increment (if needed).

Step 12: The very last measurement taken at the station is the depth of the water. Volunteers lower the Secchi disc until it rests on the bottom, clip a clothespin onto the Secchi disc rope at the waters surface, and pull up the disc and count the feet between the clothespin and the disc. Volunteers estimate the water depth reading to the nearest quarter of a foot and record the depth on the data sheet by writing the whole number in the space provided and circling the appropriate quarter foot increment (if needed).

Volunteers measure the depth of the water last, after taking water samples and measuring water clarity to prevent interference from resuspended bottom sediments.

Filling in the Field Data Sheet (Appendix III)

Step 1: At times the following special notations are needed to record Secchi depth measurements:

- Volunteers write "B" on the data sheet to indicate that the disk went to the bottom and could still be seen.
- Volunteers write "W" on the data sheet to indicate that the Secchi disc disappeared into the weeds growing in the water body. They also record the depth that the disc disappeared into the weeds.

Step 2: Volunteers record the cloud coverage when the Secchi reading was taken. Volunteers do not indicate how much cloud cover there was in the sky at the time the Secchi reading was taken; but record the cloud cover directly over the sun only. For example, if the sky was very cloudy, but the sun popped out from behind the clouds during the time the Secchi disc was being read, a #1 would be entered for the Sun Code #.

Step 3: Volunteers fill out the remainder of the data sheet. They include anything that might possibly influence analytical results. For example, "lime rock washed into the water body from torrential rainfall on roads," "forest fire adjacent to water body" or " sand added to beach area." Comments are written on the back of the data sheet and may include anything unusual or out of the ordinary.

Filtering Water for Chlorophyll Analyses

Step 1: Volunteers learn the names of all the parts of the filtering equipment.

Step 2: They attach one end of the transparent tubing to the hand pump and the other end to the filtering flask.

Step 3: They assemble the filter funnel. There are two types of filter funnels. The first one uses a magnet between the cup and the base. The second type of filter funnel screws together at the base and cup. The base for both types has a stopper on the end. Volunteers grasp the cup base by the rubber stopper and gently twist it downward into the mouth of the filtering flask.

Step 4: Using forceps (tweezers), volunteers pick up one of the 47-mm glass-fiber filters provided by LAKEWATCH. Because it is sometimes difficult to grasp just one filter, as they tend to stick together, volunteers are encouraged to blow gently along the forceps toward the filter papers causing them to flutter apart. If two filters are used inadvertently, they are processed together as if they were one.

Step 5: Holding the filter with the forceps, the volunteer places it in the center of the cup base with the p just one filter, as they tend to stick together, volunteers are encouraged to blow gently along the forceps toward the filter

Step 6: Volunteers center the filter inside the rim of the cup base, by adjusting it from the side with the flat edge of the forceps.

Step 7: Using tap water, volunteers rinse the filter cup every time water from a different jug is filtered.

Step 8: After rinsing the cup in tap water, volunteers place it on top of the cup base. The cup and base are either held together magnetically or screwed down onto the base to form a watertight seal.

Step 9: Volunteers shake the gallon jug that is about to be filtered to make sure the sample is well mixed.

Step 10: Volunteers rinse the graduated cylinder provided by LAKEWATCH with the sample about to be measured.

Step 11: Volunteers measure the sample to be filtered in the rinsed graduated cylinder. Volunteers use a plastic pipette to adjust the water level in the graduated cylinder. Volunteers measure the volume accurately, by adjusting the water level so that the bottom of the meniscus rests on top of the target line on the cylinder. Volunteers filter as much water as necessary to turn the filter paper a green color (up to 3000 mL). If the filter clogs and the water remaining in the filter cup cannot be pumped through, volunteers will pour out all the water, reassemble the filter apparatus with a clean filter paper and start over using less water.

In general, the approach is for volunteers to measure out the amount of water thought necessary, pour it into the filter cup and pump it through. If no color is noticed, they measure out more water and pump that through too. Volunteers pay attention to whether the water is hard to pump and whether the stream of water is diminishing. Either of these

observations is a signal that the filter is getting close to the clogging point and no more water is added.

Step 12: To retrieve the filter, volunteers either gently tip the empty magnetic cup to one side or unscrew the cup so it comes up off the filter and base. The filter paper should remain on the cup base. Sometimes the filter will stick inside the bottom of the cup. If this happens volunteers put a hand under the bottom of the cup to propel the filter out. Alternatively, the edge of the filter can be carefully loosened and peeled off gently with the forceps. However, if the forceps accidentally touch the algae that have accumulated on the filter, any filter on which the algae has been touched by fingers, forceps or any other object the filter is discarded and the filtering process for that station repeated.

Step 13: Assuming the cup comes off the base properly and leaves the filter behind on the base, volunteers use forceps to remove the filter paper from the base by grasping the filter paper only by the white outer rim and do not touch the algae with the forceps.

Step 14: To enclose and protect the algae, volunteers fold the filter exactly in half to protect the algae.

Step 15: Volunteers then put the folded sample on a paper towel and fold the paper towel over the sample and blot it as dry as possible.

Step 16: Volunteers then prepare an envelope by folding a larger paper filter. Using pencil only, volunteers fill all the following information on the pre-attached label:

- Water Body name_____
- County name_____
- Month-day-year_____
- Station number_____
- Volume filtered_____

Step 17: Volunteers then tuck the folded sample filter inside its envelope and fasten the circular edge of the envelope with a plastic coated paperclip, provided in their kit.

Step 18: Volunteers then put the filter sample ant its envelope in the jar of desiccant and gently roll the jar to distribute the crystals around the filter papers.

Step 19: Volunteers store the desiccant jar in their home freezer.

Step 20: Volunteers repeat all steps for the remaining jugs of water and put all the filter papers into the bottle of desiccant and store in the freezer.

Step 21: After all samples are filtered, volunteers rinse the equipment with tap water and let it air dry.

Step 22: Volunteers then check the number of bottles, filters and paper clips to assure there is an adequate supply for next month and store

Step 23: Volunteers deliver their water bottles, data sheet, and desiccant bottle to a collection center that is convenient to them.

Step 24: Volunteers pick up new supplies at the nearest collection center (Appendix II).

Laboratory Protocols

Acid Washing Nalgene Bottles

Prior to any field sampling or laboratory analyses all Nalgene plastic bottles are acid washed

Technicians are required to wear protective gear before starting to acid wash bottles including: a respirator, rubber gloves, protective goggles, and a rubberized apron. Working over a double sink, the technician then carefully pours concentrated sulfuric acid into a bottle until one-half to two-thirds full. After placing a cap on the bottle the technician shakes it vigorously. Following this the cap and bottle are rinsed with tap water twice followed by a double rinse with deionized water, they are allowed to dry, capped and placed in plastic bags for deployment.

After bottles are dried several are filled with deionized water to serve as blanks for both total phosphorus and total nitrogen analyses.

Detergent Washing Glassware

Prior to any laboratory analyses, all glassware is washed with special detergent (Liqui-Nox) designed for laboratory work.

Technicians are required to wear protective gear before starting to detergent wash glassware including: rubber gloves, protective goggles, and a rubberized apron. All glassware is soaked in properly sized buckets with the factory recommended concentration of Liqui-Nox for approximately one day. After this period, the glassware is rinsed multiple times with tap water followed by a double rinse with deionized water. The glassware is allowed to dry and then covered with aluminum foil and stored in cabinets ready for use.

Water Samples Preparation and Pour Out Procedures

Florida LAKEWATCH sends staff on a regular basis to all collection centers (Appendix II) to drop off new sampling supplies and to collect frozen samples to bring back to the laboratory for analyses. The frozen samples are kept in coolers for transport, logged to facilitate processing in chronological order and then placed in large walk-in freezers at the LAKEWATCH laboratory. Samples are removed from freezer in order of the date sampled to decrease holding times. Samples are thawed on trays in the laboratory over night bringing them to room temperature (approximately 25 °C). Frozen samples are thawed and poured out within 24 hours of thawing. These aliquots of samples are stored in the walk-in for 1-10 days before autoclaving and analysis along with the corresponding sample bottles. Technicians also examine the condition of the sample bottles recording any abnormalities like broken bottles or missing data (e.g., date, county) from labels. These notes are turned into lab manager to be entered into individual lake file folders for

later reference. LAKEWATCH maintains individual files folders for each lake, river, spring, and/or coastal area. These folders are kept indefinitely in filing cabinets on site for future reference

Prior to pouring out any samples, working spreadsheets for data entry are built for total phosphorus and total nitrogen analyses. The following information is put on the working spreadsheets, based on the information written on the sample bottles: county, lake, date, and station. Information on the functioning of equipment used to analyze nutrients and/or chlorophyll are also recorded on the spread sheets after analyses are completed. All LAKEWATCH data identification of individual system are based on county name and lake name so spelling have to be accurate for later merging of spreadsheets. Thus, technicians use pull down menus to enter County and Lake names to the working spreadsheets.

Using graduated cylinders technician pours out a 25 mL aliquot of sample into plastic capped glass tubes for TP analysis. A mechanical pipette is used to collect a 10 mL aliquot of sample to put into a plastic capped glass tube for TN analysis. Each glass tube has a sequential number used to follow the sample through analyses and they are placed in racks holding 40 tubes. Technicians incorporate a method blank, laboratory control spike, a matrix spike and sample duplicate within each run (40 samples) for in-laboratory quality assurance/quality control (QA/QC). Principal Investigators periodically spike raw lake or coastal water samples and submit them to the laboratory for analysis without laboratory knowledge to run an additional quality check. Prepared racks with the sample aliquot tubes are stored in a walk-in refrigerator until analyzed, which is between one to 10 days.

Once samples have been poured out, the plastic sample bottles are sealed, placed in labeled pans and also stored in a walk-in refrigerator until chemical analyses have been completed and results have been determined and confirmed. If results are outside of the range of historical data then the samples are analyzed again.

All TP and TN pour out working spreadsheets are archived on laboratory computers in chronological order. The saved files are also backed up and stored on an external hard drive kept by laboratory chemist/manager.

Measuring Total Phosphorus in Both Fresh and Saline Waters

Samples are digested using an autoclave and a persulfate digestion following the methods of Menzel and Corwin (1965).

Sample oxidation: 25 ml of sample are placed in a labeled, screw-capped test tube. Next 4 ml of oxidizing reagent (5.2 g potassium persulfate dissolved per 100 ml DI water) is added. Tubes are capped, inverted, and autoclaved at 15 psi at 121 C for 30 minutes. Pressure is brought down slowly and samples are allowed to cool to room temperature before measurement of TP is made. All blanks, standards, and samples are treated in the same way.

The following standards are made according to current standard methods (APHA 2005) to develop a absorbance versus TP concentration curve used in calculating concentrations of samples based on absorbance: blanks, known concentrations of phosphorus at 0.005, 0.01, 0.05, 0.1, 0.2, 0.4, and 0.6 mg/L. TP stock solution is made from Hach phosphorus standard solution Certified ACS (KH_2PO_4). TP standards are generated from 10mL of Hach standard dissolved in 1000ml DI water giving a concentration of 10mg/L TP stock, which are used to make the various known concentrations.

Sample determination:
TP concentrations are measured with the colormetric procedures of Murphy and Riley (1962). Sample absorbance is determined at a wavelength of 882 nm after being inoculated with 4 ml of mixed coloring reagent. Mixed coloring reagent is composed of 5N sulfuric acid, ammonium molybdate, ascorbic acid, antimony potassium tartrate.

Sample absorbance is determined by LAKEWATCH using a Perkin-Elmer Lambda 2 dual beam spectrophotometer with 50 mm Hellman cylindrical (16 mL) reference and sample cells. The detection program from Perkin-Elmer scans a single wavelength set at 882 nm. LAKEWATCH records data to 1µg/L and the natural waters LAKEWATCH analyzes has generally ranged from 1µg/L to 600 µg/L. LAKEWATCH does not extrapolate calculations outside of our set range of standards and high samples outside the range are brought back into range by repouring original samples and diluting with deionized water for reanalysis.

Technicians enter results into working spreadsheets and verify entry before each subsequent sample. The technician also notes on the spreadsheet whether 1) the standard LAKEWATCH SOP was followed, 2) samples were frozen when the process started, 3) the analysis instrument was functioning properly and 4) if the autoclave functioned properly during digestion. The final TP working spreadsheet is archived on laboratory technician's computer hard drive. A duplicate TP working spreadsheet of the completed run is sent as an attachment to laboratory chemist/manager to calculate concentrations, determine accuracy, request reanalysis, and/or enter results into final spreadsheet for entry into the main LAKEWATCH data set.

First, all concentrations are evaluated to make sure they are in the range of standards. Next, spiked sample recovery, standard recovery, and blanks in the run are evaluated to determine if and how much machine drift occurs. If machine drift is detected greater than 5%, between known samples then all water samples in this run are flagged for reanalysis. The re-analysis allows us to determine if there was contamination on the lab receiving-end. If the re-analyzed result is significantly different then the original value, but within expected range and error of the other samples the second value is assumed to be more reliable and it would be preferentially reported. If the re-analyzed sample is similar or within 10% of the first result then there is essentially no difference between results and the first result is taken as representative of the sample, time, date, and location. It should be noted no data are discarded. Reanalyzed values determined to be more reliable are

recorded and an asterisk indicating they are do-overs is noted in the hard copy in the water body's permanent folder, but no flag is put in the main computer file database.

The Chemist/Manager compares data sheets and lab notes in hard copy files related to status of samples received. This check is to ascertain if sample bottles were damaged in transit or if there would be any extenuating reason to flag results. Results are still recorded and samples that are out of range are diluted and any questionable flagged samples are reanalyzed in a do-over set (analyzed twice).

After these checks, if everything is in order following a regular run or after reanalysis, recorded results are then cross compared by station and date with other parameters to evaluate possible any trends by station and date from month to month for each individual lake and for lakes in a given region. Volunteer data sheets assist in this step by providing relative changes in depth and visibility as well as wet/dry periods.

The final archived data sheet examines the holding time from collection to analyses and if it exceeds five months then a qualifier is added. The final archived data sheet also compares the results with the calculated MDL (minimum detection limit) and PQL (Practical quantitation limit) and qualifiers are added as required. Additionally, blanks, spikes and duplicates are examined for proficiency and qualifiers are added as required.

Measuring Total Nitrogen in Fresh Water

Samples are prepared using an autoclave with a persulfate digestion and then nitrate-nitrogen is determined with second derivative spectroscopy (D'Elia et al. 1977; Simal et al. 1985; Wollin 1987; Crumpton et al. 1992; Bachmann and Canfield 1996).

Sample oxidation: Ten ml of sample are placed in a labeled, screw-capped test tube. Next 1.5 ml of oxidizing reagent (6.0 g low-nitrogen potassium persulfate dissolved per 100 ml 1.5 N sodium hydroxide) is added to each tube. Tubes are capped, inverted, and autoclaved at 15 psi at 121 C for 30 minutes. Pressure is brought down slowly and samples are allowed to cool to room temperature before measurement of nitrate nitrogen is made. All blanks, standards, and samples are treated in the same way.

Measurement of nitrate nitrogen: All blanks, standards, and samples are acidified to pH < 2.0 with 0.2 ml of concentrated sulfuric acid per 10 ml sample prior to measurement. LAKEWATCH uses a Perkin-Elmer Lambda 25 dual beam spectrophotometer with 10mm, 3.0 mL, Suprasil quartz windows spectrophotometer reference and sample cells. LAKEWATCH also uses a UV Windlab software program that scans from 190 nm to 290 nm looking for a peak absorbance around 220 nm. The following standards are made according to current standard methods (APHA 2005) to develop a absorbance versus TN concentration curve to use in calculating concentrations of samples based on absorbance: 3 blanks, 3 sets of known concentrations of N at 0.1, 0.25, and 0.5 mg/L, along with 2 sets of known concentrations 0.75, 1.0, 1.5, and 2.0 mg/L. TN stock solution is made

from urea standard stock Certified ACS (NH2CONH2). TN standards are generated from .1072g urea dissolved in 1000ml DI water giving a concentration of 50 mg/L urea stock used to make the various known concentrations. Averaged values are determined from the multiple sets of known concentrations to generate the linear regression. LAKEWATCH records data to 10µg/L and the natural waters that LAKEWATCH analyzes has generally ranged from 10 µg/L to 2000 µg/L. LAKEWATCH does not extrapolate calculations outside of our set range of standards, high samples outside the range are brought back into range by repouring original samples and diluting with deionized water for reanalysis.

Technicians enter results into working spreadsheets and verify entry before each subsequent sample. The technician also notes on the spreadsheet whether 1) the standard LAKEWATCH SOP was followed, 2) samples were frozen when the process started, 3) the analysis instrument was functioning properly and 4) if the autoclave functioned properly during digestion. The final TN working spreadsheet is archived on laboratory technician's computer hard drive. A duplicate TN working spreadsheet of the completed run is sent as an attachment to laboratory chemist/manager to calculate concentrations, determine accuracy, request reanalysis, and/or enter results into final spreadsheet for entry into the main LAKEWATCH data set.

First, all concentrations are evaluated to make sure they are in the range of standards. Next, spiked sample recovery, standard recovery, and blanks in the run are evaluated to determine if and how much machine drift occurs. If machine drift is detected greater than 5%, between known samples then all water samples in this run are flagged for reanalysis. The re-analysis allows us to determine if there was contamination on the lab receiving-end. If the re-analyzed result is significantly different then the original value, but within expected range and error of the other samples the second value is assumed to be more reliable and it would be preferentially reported. If the re-analyzed sample is similar or within 10% of the first result then there is essentially no difference between results and the first result is taken as representative of the sample, time, date, and location. It should be noted no data are discarded. Reanalyzed values determined to be more reliable are recorded and an asterisk indicating they are do-overs is noted in the hard copy in the water body's permanent folder, but no flag is put in the main computer file database.

The Chemist/Manager compares data sheets and lab notes in hard copy files related to status of samples received. This check is to ascertain if sample bottles were damaged in transit or if there would be any extenuating reason to flag results. Results are still recorded and samples that are out of range are diluted and any questionable flagged samples are reanalyzed in a do-over set (analyzed twice).

After these checks, if everything is in order following a regular run or after reanalysis, recorded results are then cross compared by station and date with other parameters to evaluate possible any trends by station and date from month to month for each individual lake and for lakes in a given region. Volunteer data sheets assist in this step by providing relative changes in depth and visibility as well as wet/dry periods.

The final archived data sheet examines the holding time from collection to analyses and if it exceeds five months then a qualifier is added. The final archived data sheet also compares the results with the calculated MDL (minimum detection limit) and PQL (Practical quantitation limit) and qualifiers are added as required. Additionally, blanks, spikes and duplicates are examined for proficiency and qualifiers are added as required.

Measuring Total Nitrogen in Saline Water

Analyzing for total nitrogen in saline water begins the same as for freshwater incorporating a persulfate digestion using an autoclave. LAKEWATCH then uses a Bran + Luebbe Autoanalyzer 3 continuous flow-analysis system with Automated Analyzer Control and Evaluation software (AACE 6.03) to analyze saline samples. Nitrate (NO^3-N) is reduced to nitrite (NO^2-) by a copperized cadmium redactor. The addition of sulfanilamide under acidic conditions forms a diazo compound with nitrate, which is subsequently coupled with N-1-naphthylene diamine dihydrochloride to form a purple azo dye. Colorimetric quantification is completed using the Bran + Luebbe Autoanalyzer 3 system. The following standards are made according to current standard methods (APHA 2005) to develop a absorbance versus TN concentration curve to use in calculating concentrations of samples based on absorbance: blank and known concentrations of N at 0.1, 0.25, 0.5, 1.0, 1.5, and 2.0 mg/L. LAKEWATCH records saline total nitrogen to 1µg/L and the natural saline waters measured by LAKEWATCH has generally ranged from 1.0 µg/L to 2000 µg/L.

The same in-laboratory QA/QC and data handling procedures used for freshwater nitrogen are used for saline nitrogen.

Chlorophyll Determinations

Chlorophyll filters prepared and frozen by volunteers are removed from desiccant storage bottles and sorted by county, lake, date, and station. Laboratory Technicians examine the condition of sample filters, desiccant, and look for any missing label information or discrepancies. Notes are taken and forwarded to the laboratory Chemist/Manager to be stored in individual lake file folders, for later reference. Similar to TP and TN pour out procedures a chlorophyll working spreadsheet is created which includes the county, lake, date, and station along with the recorded volume of water the volunteer used for each filter. The technician also notes on the spreadsheet whether 1) the standard LAKEWATCH SOP was followed, 2) samples were frozen when the process started, and 3) the analysis instrument was functioning properly. This work spreadsheet is used to track individual filter results through the laboratory.

After spreadsheets are prepared, individual filters, including one blank filter per run, are placed in sequentially numbered screw cap centrifuge tubes used for chlorophyll extraction. Eight mL of 90% ethanol is pipetted into each tube and then capped. Each

tube is inspected to make sure the entire filter is covered with ethanol. One rack of tubes (72 tubes) is placed in hot water bath at 78 C and timed for 5 minutes after reaching temperature. The rack of samples is removed and wrapped in a dark bag and allowed to stand for 24 hours before reading absorbance on a spectrophotometer (Sartory and Grobbelarr 1984). Before centrifuging samples, remove filters with tweezers twisting the excess ethanol off. Once the filters are removed six samples can be centrifuged at a time to separate the filtrate from any precipitate. Method blanks are new filters from stock that are treated in the same manner as sample filters.

After centrifuged samples are done spinning, an aliquot of 3.2 ml of sample is transferred to a glass screw capped test tube with the corresponding number label. This sample is used to determine the absorbance readings at 750nm, 664nm, and 630nm. The sample is then poured back into the glass tube and recapped. After an entire rack of 72 samples has been read, 100 µL of 0.1 N HCL is pipetted into the first test tube, a 90 second timer is started and the contents of the acidified sample are poured into the cuvette and this is placed into the holding cell within the spectrophotometer. After timer goes off the acidified sample is used to determine absorbance readings at 750nm and 665nm. All samples will be treated in the same manner. These additional readings will be used to determine chlorophyll a in the presence of pheophytin.

LAKEWATCH uses a Perkin-Elmer Lambda 20 dual beam spectrophotometer with disposable polystyrene 1cm reference and sample cells. Scanned absorbance values of the blank (750 nm) and three wavelengths (664 nm, 647 nm, and 630 nm) are entered directly into the original chlorophyll spreadsheet by Windlab UV software using the method specifications set up by technician for chlorophyll analysis. After acidification, scanned absorbance values of the blank (750nm) and one wavelength (665nm) are entered into a second spreadsheet corresponding to the sample number tubes. The original chlorophyll spreadsheet and acidified chlorophyll spreadsheet of each completed run is archived in a chronological filing system on lab technician's computer.

Duplicate chlorophyll and acidified chlorophyll working spreadsheets are sent as an attachment to Chemist/Manager to calculate chlorophyll and pheophytin corrected chlorophyll concentrations using standard methods (Method 10200 H; A.P.H.A. 2005). Calculated concentrations are evaluated based on data sheets and lab notes in our hard copy files related to status of samples received. This check is to ascertain if sample filters were not in approved desiccant, or if there is any extenuating reason to flag results. Concentrations are entered into bimonthly data file. LAKEWATCH records chlorophyll concentrations to 1µg/L and the natural waters analyzed by LAKEWATCH have generally ranged between 1.0 µg/L to 350 µg/L. It should be noted no data are discarded, and any suspect results are noted, but no flag is put in the main computer file.

The final archived data sheet examines the holding time from collection to analyses and if it exceeds five months then a qualifier is added. The final archived data sheet also compares the results with the calculated MDL (minimum detection limit) and PQL (Practical quantitation limit) and qualifiers are added as required. Additionally, blanks, spikes and duplicates are examined for proficiency and qualifiers are added as required.

Secchi Transparencies

When water samples are picked up from collection centers, data field sheets containing Secchi depth records are removed from Ziploc bags containing associated water samples. The information on the data sheet is compared with the bottles in the Ziploc bag to verify the county name, lake name and date are all in agreement. Next field datasheet dates are recorded on a master pickup list to facilitate tracking of all samples and aid laboratory technicians in minimizing the holding times for samples dropped off in the walk-in freezer.

The datasheets are then sorted alphabetically and the following information: county, lake, date, depth, and volunteer contact is entered by staff into an active volunteer data file for regional coordinator's reference and communication with volunteers. The original field datasheets are then turned over to laboratory Chemist/Manager who places them in their respective individual folders for record keeping. When chlorophyll concentrations have been determined as described above, the Chemist/Manager pulls corresponding folders and datasheets for associated samples, to verify congruence. Next, manager hand enters Secchi information from data sheet into lab master Chlorophyll-Secchi database and files are returned to storage in filing cabinet. Florida LAKEWATCH maintains individual files for the history of sampling for each lake, river, spring, or coastal area.

Periodic Color and Specific Conductance on Volunteer Collected Samples

After many years of research Florida LAKEWATCH staff realized that color and specific conductance were indicator variables for many limnological changes occurring in Florida lakes. Thus is 2005 LAKEWATCH started analyzing color (PCU) and specific conductance (µS/cm @25°C) on thawed, refrigerated samples collected by volunteers, after TP and TN results have been determined. Quarterly, 100 mL of water used to analyze TP and TN from each station are combined for the determination of specific conductance and color (see methods below). Specific conductance and color are also determined for each individual station located in creeks, rivers, springs and coastal sites.

At the time the samples are poured out for periodic color and specific conductance measurements, a working spreadsheet is created with the same header information as TP and TN working spreadsheets (county name, lake name, date and station) the station column for composite water from all stations for a lake sampled on a given date is designated as station 2 and individual stations for non-composited samples are listed individually.

Supplemental Water Chemistry

To help increase LAKEWATCH's knowledge of Florida Limnology, Regional Coordinators collect extra water from any new lake entering the program for analysis of supplemental water chemistry (pH, total alkalinity, specific conductance, color and chloride: methods listed below). During training sessions, regional coordinators collect

the additional water in two one-liter brown bottles that are placed on ice and then refrigerated for Florida LAKEWATCH to analyze for supplemental chemistry within 48 hours of collection. These samples are collected from each station set up on the lake. This additional testing provides insight into basic biological and chemical processes in our monitored lake systems. These supplemental samples are also collected to help establish a baseline of chemical parameters for an individual lake if it is new to the program. Occasionally Regional Coordinators will collect additional supplemental samples from lakes that have been in the program for a long time or lakes that are being brought back on line after dropping out of the program for a while. These samples provide a series of values for comparison and temporal analyses.

At the time the samples are poured out for measurements of supplemental water chemistry, a working spreadsheet is created with the same header information as TP and TN working spreadsheets (county name, lake name, date and station). Laboratory Technicians type in results as each parameter is analyzed. The working spreadsheet is archived and a duplicate copy sent to the laboratory Chemist/Manager for examination and approval.

pH Analysis

A Fisher Scientific, bench-top Accumet Research model-10 pH meter with AccuTuph + Variable Temperature probe is used to measure pH. The pH meter is calibrated with buffers of pH 4.0, 7.0, and 10.0, before each daily use. The pH detection limits (MDL) based on instrumentation documentation ranges from 0 to 14.0 with a relative accuracy of +/- 0.002 pH.

Total Alkalinity Analysis

Total alkalinity (mg/L as $CaCO_3$) is determined by titration with 0.02 N sulfuric acid (2030 Alkalinity, A.P.H.A. 2005). All unknown samples are titrated to a pH of 4.7 to differentiate if samples are low or high alkalinity. If less than 1mL of 0.02 N sulfuric acid is used to reach this endpoint then the sample is consider low alkalinity and the appropriate method applied (Method 2320B: A.P.H.A. 2005). If greater than 1mL of titrant is used to reach pH of 4.7, the sample is considered high alkalinity and titration continues to pH 4.5 to standardize titrations and avoid interference from silicates, phosphates, and other materials. Our detection limits (MDL) based on method employed ranges from 0.0 to 500.0 with a relative accuracy of +/- 1.0 mg/L.

Specific Conductance Analysis

Specific conductance (µS/cm or mS/cm at 25°C) is measured using standard methods (Conductivity 2510: A.P.H.A. 2005) and a Yellow Springs Instrument Model 3100 bench-top conductance meter with temperature compensation. Our detection limits (MDL) based on instrument documentation ranges from 0 to 499,999 µS/cm (in 5 ranges) with a relative accuracy of +/- 1-5% (depending on the range).

Color Analysis

True Color values (PCU) are determined spectrophotometrically after filtration through a Gelman class A-E filter (Platinum-Cobolt Modified 2120C Spectrophotometric Method; A.P.H.A. 2005). We employ a Spectronic 401 single beam spectrophotometer set at 465 nm to determine absorbance values. The following set range of standards is made from a certified standard Platinum-Cobolt Solution to generate a linear regression from which sample values are derived: blank, known concentrations of Platinum-Cobolt at 5, 10, 15, 20, 25, 30, 40, 50, 75, 100, 125, and 150 PCUs. LAKEWATCH does not extrapolate outside of the range of standards; samples are brought back into range by repouring diluted aliquots for reanalysis.

Chloride Analysis

Using specific conductance measurements to determine the volume of sample to be used for titration, 100 mLs or a diluted volume of supplemental water is used to determine chloride concentrations (mg/L Cl-) (EPA approved modified Mercuric Nitrate Burette Titration Standard Method 4500-Cl- C). Mercuric nitrate is used as the titrant and diphenylcarbazone as the indicator. The mercuric nitrate reacts selectively with all chloride. When all chloride has been complexed, excess mercuric ions combine with indicator to form pink-purple complex indicating endpoint. Concentrations are calculated by a factor related to sample dilution and volume of titrant used to reach endpoint.

Additional Internal Laboratory QA/QC

All glassware and pipettes used to pour out samples are checked for accuracy by weighing deionized water volumes on a Mettler balance. LAKEWATCH Mettler balances are serviced and certified annually.

Spectrophotometers are serviced and calibrated annually by qualified technical personnel. Basic maintenance and cleaning of equipment, is performed daily/weekly by staff in-house.

A Waters Company named ERA prepared ampoules of CRM (certified reference material of simple and complex nutrients) that is used to evaluate repeatability or accuracy of machines. Diluted concentrations of these certified samples are incorporated within our spiking matrix.

Final Data QA/QC

The core LAKEWATCH data including TP, TN, Chlorophyll and Secchi depth reside on three different working data spreadsheets. After the Chemists/Manager finishes all internal laboratory QA/QC procedures a data manager merges all three working spreadsheet by the header information (county name, lake name, date, station) using JMP a statistical software program (SAS 2000). Data that does not merge correctly indicates errors in coding header information (county name, lake name, date and/or station). These

errors are corrected based on the original field sheets. Finally the data are checked line by line with the original worksheets before the data, if verified, are added to the historical database.

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