



Florida LAKEWATCH Water Chemistry Field Sampling and Laboratory Protocols

Florida LAKEWATCH

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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 30 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 1500 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 57 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 scientific 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 (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 (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

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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 multiple Regional Coordinators that train volunteers face to face on their lake 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 later for chlorophyll analyses (primarily monthly).
- Use a Secchi disc provided by LAKEWATCH to measure water clarity (primarily monthly).
- 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. By December 2019, there will also be training videos on the LAKEWATCH web site for volunteers who want a little refresher course on how to sample.

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Filling Water Bottles for Nutrients

Florida LAKEWATCH supplies water sampling bottles after they are properly washed 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 for analysis of total phosphorus and total nitrogen is taken as soon as the boat arrives at the sampling location. Water from these samples is also used to measure color and conductivity quarterly from one center station of the system.

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. This is also important to match bottles up with the data field sheets, which also have the time samples were taken recorded.

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.

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Step 5: Volunteers then turn 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 cap the bottle and secure the lid tightly.

Step 11: After collection volunteers put bottles on ice in a cooler until freezing.

Step 12: When volunteers return home, sample bottles are dried, placed in a plastic zip lock bag with the field data sheet and put in their home freezer as soon as possible (within 4 hours).

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, mold, or algae 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

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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 (water remains at ambient temperature while in transport) 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.

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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

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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 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.

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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 _____

On the Field sheet the volunteer then writes the date and time that each station's water was 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 in its envelope into 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.

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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).

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Laboratory Protocols

Detergent Washing Nalgene Bottles and Laboratory Glassware

Prior to any field sampling or laboratory analyses all Nalgene plastic bottles and laboratory glassware are 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 bottles and glassware are soaked in properly sized buckets with the factory recommended concentration of Liqui-Nox for approximately one day. After this period, the bottles and glassware are 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. On a regular basis, multiple bottles will be filled with deionized water, frozen and used as blanks to monitor the effectiveness of bottle washing techniques.

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. Samples remain frozen in freezers at each collection center and during transport in coolers to Gainesville where they are placed in a walk-in freezer (still frozen) until analyses. LAKEWATCH freezers and refrigerators have temperature data loggers that will be monitored every time samples are added and removed from the freezer. Temperature logs will be kept electronically downloaded monthly. Collections centers are open to the public so there is no strict chain of command for the samples brought to these centers. If temperature sensors indicate freezers/refrigerators are not keep proper temperature samples are moved to additional freezers run by departmental generators. When picked up at a collection center all samples and data sheets present for pickup are recorded in a collection notebook to facilitate sorting on arrival to Gainesville and analyses in chronological order. These collection notebook data are archived in 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 overnight bringing them to room temperature (approximately 25 °C) and aliquots are poured out for analyses of total phosphorus, total nitrogen, color, and conductivity within 24 hours after being taken from the freezer. Then aliquots are stored in the walk-in refrigerator for 1-10 days before autoclaving and analysis. For documentation, date and time will be recorded for pour out, preparation (autoclaving) and analyses on laboratory data sheet and archived on the LAKEWATCH laboratory. Samples are not locked up while in the walk-in refrigerator. The original sample bottle is also stored in the walk-in refrigerator until samples are analyzed. 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,

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and/or coastal area. These folders are kept in filing cabinets and electronically on site for a minimum of five years.

Prior to pouring out any samples, working spreadsheets for data entry are built for total phosphorus, total nitrogen analyses. On a quarterly basis working spreadsheets are also built for color and conductivity analyses. The following information is put on the working spreadsheets, based on the information written on the sample bottles: county, lake, date, and station (time of field sampling for each station is logged on field data sheets and is merged during final data checks). 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 drop 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 and 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 (50 µg/L for total phosphorus and 500 µg/L for total nitrogen), a matrix spike (currently derived from ERA certified concentration of .0363 mg/L with a range of 0.032 to 0.041 mg/L) and sample duplicate within each run (40 samples) for in-laboratory quality assurance/quality control (QA/QC). The amount and concentration of spikes are recorded on the working laboratory data sheets along with calculated recovery percentages. Prepared racks with the sample aliquot tubes are stored in a walk-in refrigerator before digestion until analyzed, which is between one to 10 days (time is documented by comparing pour out dates with analyzed dates).

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.

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 Fresh and Saline Waters

Spectrophotometer:

Samples are digested using an autoclave and a persulfate digestion following the methods of Menzel and Corwin (1965). Method Detection Limits for phosphorus are calculated using EPA method: EPA 821-R-16-006. Sample oxidation: 25 ml of samples and standards 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

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gradually and samples are allowed to cool to room temperature before measurement of TP is made. Heat tape is used to verify autoclave temperature. 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 an absorbance versus TP concentration curve used in calculating concentrations of samples based on absorbance: blanks, known concentrations of phosphorus at 0.01, 0.05, 0.1, 0.2, 0.3, and 0.4 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. New standards are made quarterly or as needed and dates are recorded on a laboratory notebook and working laboratory spreadsheets along with being archived for five years.

TP concentrations are measured with the colorimetric 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 a precision of $1\mu\text{g/L}$ and the natural waters LAKEWATCH analyzes has generally ranged from $1\mu\text{g/L}$ to $400\mu\text{g/L}$. LAKEWATCH does not extrapolate calculations outside of our set range of standards and high samples outside the range are brought back into range by re-pouring original samples and diluting with deionized water for reanalysis.

Technicians enter results into working spreadsheets with date of analysis 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 instrumentation 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.

AutoAnalyzer:

Analyzing for total phosphorus in fresh and saline waters with the autoanalyzer begins the same as for total nitrogen in fresh and saline water incorporating a heated alkaline persulfate digestion using an autoclave (Grasshoff et al. 1983). Sample oxidation: 25 ml of sample are placed in a labeled, screw-capped test tube. Next 4 ml of oxidizing reagent (5 g potassium persulfate, 3 g Boric Acid and 3.8 mL of 10N NaOH dissolved per 100 ml DI water) is added. Tubes are capped, inverted, and autoclaved at 15 psi at 121 C for 30

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minutes. Pressure is brought down gradually, and samples are allowed to cool to room temperature before measurement of TP is made. Heat tape is used to verify autoclave temperature. All blanks, standards, and samples are treated in the same way.

LAKEWATCH then uses a Bran + Luebbe Autoanalyzer 3 continuous flow-analysis system with Automated Analyzer Control and Evaluation software (AAE 7.09) to analyze fresh and saline samples using a dual-channel autoanalyzer that performs nitrate-nitrite by the cadmium reduction method and orthophosphate by the ascorbic acid reduction method. Total nitrogen and total phosphorus can be measured simultaneously. Following the method of Murphy and Riley (1962), this automated procedure for the determination of orthophosphate is based on the colorimetric method in which a blue color is formed by the reaction of orthophosphate, molybdate ion and antimony ion followed by reduction with ascorbic acid at a pH of <1. Colorimetric quantification is completed using the Bran + Luebbe Autoanalyzer 3 system. Sample absorbance is determined at a wavelength of 880 nm (SEAL Analytical Inc. Method No. G-297-03 (MT19) Rev. 7). The following standards are made according to current standard methods (APHA 2005) to develop a absorbance versus TP concentration curve to use in calculating concentrations of samples based on absorbance: blank and known concentrations of P at 0.01, 0.05, 0.075, 0.1, 0.2, 0.3, and 0.4 mg/L. TP stock solution is made from Hach phosphorus standard solution Certified ACS (KH₂PO₄). 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. New standards are made quarterly or as needed and dates are recorded on a laboratory notebook and working laboratory spreadsheets along with being archived for five years.

LAKEWATCH records fresh and saline total phosphorus to a precision of 1 µg/L and the natural fresh and saline waters measured by LAKEWATCH has generally ranged from 1 µg/L to 400 µg/L.

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

All concentrations are evaluated by the Laboratory Manager 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 spike and standard recovery is with 90 to 110 % and if blanks exceed calculated MDL. Recovery is detected less than 90% or greater than 110% for the verification standards or blanks exceed MDL, then all water samples in this run are flagged for reanalysis. The reanalysis allows us to determine if there was contamination on the lab receiving-end. If the reanalyzed samples meet above mention QA/QC evaluations they are approved and added to the main data base.

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

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samples are reanalyzed in a do-over set (analyzed twice). This allows us to determine if there was contamination on the lab receiving-end. If the original samples are not within 90 to 110 % of the reanalyzed samples then the re-analyzed samples are recorded to the main data base. If the original samples are within the 90 to 110% of the reanalyzed samples then the original data entered into the main data base.

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 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 “Q” qualifier is added. The final archived data sheet also compares the results with the calculated MDL (method detection limit) and PQL (practical quantitation limit) and qualifiers are added as required. Currently for total phosphorus the MDL is 5 µg/L and the PQL is 12 µg/L. Additionally, blanks, spikes and duplicates are examined for proficiency and qualifiers are added as required. Samples in a run are qualified with a “V” if the blank exceeds the MDL and the blank result exceeds 10% of the associated sample result. All samples in the run are qualified with a “J” if the Laboratory Control Spikes are not within 90-110% of the expected value. The parent sample of a matrix spike is qualified with a “J” if the spike recovery is not within 90-110% of the expected value. Duplicate samples are qualified with “J” if relative percent difference exceeds plus or minus 20%. Any sample reported with a “J” must include a comment with the reason for the qualifier. Duplicates are poured at intervals of every 20 samples and are offset by ten samples from matrix spikes and LCS that averages 2-3 per run. Thus, ten samples are followed with a MS, followed by 6 samples and an LCS and at the end of a run are placed an LCS, and method blank.

Measuring Total Nitrogen in Fresh and Saline Waters

Analyzing for total nitrogen in fresh and saline waters with the autoanalyzer begins incorporating a heated alkaline persulfate digestion using an autoclave (Grasshoff et al. 1983). Sample oxidation: 25 ml of sample are placed in a labeled, screw-capped test tube. Next 4 ml of oxidizing reagent (5 g potassium persulfate, 3 g Boric Acid and 3.8 mL of 10N NaOH 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 gradually, and samples are allowed to cool to room temperature before measurement of TN is made. Heat tape is used to verify autoclave temperature. All blanks, standards, and samples are treated in the same way.). Method Detection Limits for nitrogen are calculated using EPA method: EPA 821-R-16-006.

LAKEWATCH then uses a Bran + Luebbe Autoanalyzer 3 continuous flow-analysis system with Automated Analyzer Control and Evaluation software (AACE 7.09) to analyze fresh and saline samples. Nitrate ($\text{NO}_3\text{-N}$) is reduced to nitrite ($\text{NO}_2\text{-}$) by a

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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 pink azo dye. Colorimetric quantification is completed using the Bran + Luebbe Autoanalyzer 3 system. Sample absorbance is determined at a wavelength of 520nm (SEAL Analytical Inc. Method No. G-172-96 (MT19) Rev. 18). 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, 0.10, 0.25, 0.5, 0.75, 1.0, 1.5, and 2.0 mg/L. New standards are made quarterly or as needed and dates are recorded on a laboratory notebook and working laboratory spreadsheets along with being archived for five years.

LAKEWATCH records fresh and saline total nitrogen to 1µg/L and the natural fresh and saline waters measured by LAKEWATCH 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 re-pouring 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.

All concentrations are evaluated by the Laboratory Manager 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 spike and standard recovery is with 90 to 110 % and if blanks exceed calculated MDL. Recovery is detected less than 90% or greater than 110% for the verification standards or blanks exceed MDL, then all water samples in this run are flagged for reanalysis. The reanalysis allows us to determine if there was contamination on the lab receiving-end. If the reanalyzed samples meet above mention QA/QC evaluation they are approved and added to the main data base.

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

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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 “Q” 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. Currently for total nitrogen the MDL is 20 µg/L and the PQL is 60 µg/L. Additionally, blanks, spikes and duplicates are examined for proficiency and qualifiers are added as required. Samples in a run are qualified with a “V” if the blank exceeds the MDL and the blank result exceeds 10% of the associated sample result. All samples in the run are qualified with a “J” if the Laboratory Control Spikes are not within 90-110% of the expected value. The parent sample of a matrix spike is qualified with a “J” if the spike recovery is not within 90-110% of the expected value. Duplicate samples are qualified with a “J” if relative percent difference exceeds plus or minus 20%. Any sample reported with a “J” must include a comment with the reason for the qualifier. Duplicates are poured at intervals of every 20 samples and are offset by ten samples from matrix spikes and LCS that averages 2-3 per run. Thus, ten samples are followed with a MS, followed by 6 samples and an LCS. At the end of a run are placed an LCS, and method blanks.

Chlorophyll Determinations

Chlorophyll filters prepared and frozen by volunteers are removed from desiccant storage bottles and sorted by county, lake, date, and station. Filters are kept in dark freezer before analysis and covered with black plastic during preparation. 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, sampling date, and station along with the recorded volume of water the volunteer used for each filter. The time of sampling for each station is recorded on the field sheet. The technician also notes on the spreadsheet whether 1) the standard LAKEWATCH SOP was followed, 2) samples were frozen when the process started, 3) time of analysis and 4) 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 dry blank filter per run, are placed in sequentially numbered screw cap centrifuge tubes used for chlorophyll extraction. A stock solution of 90% ethanol is made in-house from 190 proof (95%) ethanol obtained from Fisher Scientific by measuring 947 mL of 95% ethanol in a volumetric flask and adding 53 mL DI for a total 1000 mL volume. Eight mL of 90% ethanol is added using a Jencons Zippette 10 mL adjustable repipetter set to 8mL 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

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spectrophotometer (Sartory and Grobbelarr 1984). Before centrifuging samples, filters are removed with tweezers while twisting to remove the excess ethanol. 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, used to determine uncorrected chlorophyll and subsequently acidified to read for pheophytin, is transferred to a glass screw capped test tube with the corresponding number label. This sample is poured into the cuvette and placed into the holding cell within the spectrophotometer, to determine the absorbance readings at 750nm, 664nm, 647nm, and 630nm. The sample is then poured back into the glass tube and recapped. A stock solution of 0.1N HCL is made in-house from a standard 1N HCL solution obtained from Fisher Scientific by measuring 10 mL in a volumetric flask and adding 90 mL DI for a total 100 mL volume. After an entire rack of 72 samples has been read, 100 μ L 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 and the cuvettes are rinsed with ethanol between each sample. 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 spreadsheet is sent as an attachment to Chemist/Manager to calculate chlorophyll and pheophytin corrected chlorophyll concentrations using equations report in 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. LAKEWATCH records chlorophyll concentrations to a precision of 1 μ g/L and the natural waters analyzed by LAKEWATCH have generally ranged between 1.0 μ g/L to 350 μ g/L.

The final archived data sheet examines the holding time from collection to analyses and if it exceeds five months then a "Q" qualifier is added. The final archived data sheet also compares the results with the calculated MDL (method detection limit) and PQL (practical quantitation limit) and qualifiers are added to the Chemist work sheets as

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required. Currently for chlorophyll the MDL is 1 µg/L and the PQL is 1 µg/L. Additionally, method and filter blanks are used to determine proficiency. Method blanks are 95% ethanol only in tubes processed as a sample and filter blanks are filters processed in 95% ethanol like a standard sample filter. Samples in the run are qualified on the Chemist work sheet if method blanks are greater than the MDL and the blank sample exceeds 10% of the associated sample. If method blanks do exceed the MDL then additional blanks are run after cleaning all equipment to determine if the spectrophotometer needs to be serviced.

Once a year or after any major instrument maintenance chlorophyll standards are purchased and used to calculate MDL and PQL values. The initial Instrument Detection Limit (IDL) is determined by zeroing the spectrophotometer with a solution of 90% ethanol. Then pure pigment is diluted in 90% ethanol until it yields a response at the selected wavelength between .005 and .008 absorbance units (AU). To determine the Estimated Detection Limit (EDL), at least seven replicate standard pigment diluted samples known to contain the pigments of interest between 2 and 5 times the IDL are analyzed. The EDL is calculated as 3 times the standard deviation of replicate analyses. The chlorophyll standard of 25 µg is taken through a series of 50% dilutions down to 1.5 µg (five concentrations) and seven replicates at each concentration are used to determine standard deviations for calculations of MDL and PQL.

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. These sample pick up logs are archived for five years at the LAKEWATCH laboratory.

The datasheets are then sorted alphabetically and the following information: county, lake, date, depth, time of sampling 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 depth information along with time each sample was taken 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

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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. In 2005 LAKEWATCH started analyzing color (PCU) and specific conductance ($\mu\text{S}/\text{cm}$ @25°C) on thawed, refrigerated samples collected by volunteers, after TP and TN results have been determined (10-14 days from thawing and pouring out). These analyses were conducted quarterly, and 100 mL of water from station 1 for each lake is used to determine the 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 prepared for periodic color and specific conductance measurements, working spreadsheet for each are created with the same header information as TP and TN working spreadsheets (county name, lake name, date and station).

Supplemental Water Chemistry

To help increase LAKEWATCH's knowledge of Florida Limnology, Regional Coordinators collect extra water following standard sampling procedures listed above, 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 and after each daily use. When running more than

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20 samples buffers of pH 4.0 and 10.0 are read as samples. The pH detection limits (MDL) based on instrumentation documentation ranges from 0 to 14.0 with a relative accuracy of ± 0.002 pH. The minimum reportable value is 0.1 pH standard units.

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. The minimum reportable value is 1.0 mg/L as CaCO_3 .

Specific Conductance Analysis

Specific conductance ($\mu\text{S}/\text{cm}$ 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 based on instrument documentation ranges from 0 to 499,999 $\mu\text{S}/\text{cm}$ (in 5 ranges) with a relative accuracy of $\pm 1\text{-}5\%$ (depending on the range). The current MDL and PQL are 2 and 6 $\mu\text{S}/\text{cm}$, respectively. The minimum reportable value is 1 $\mu\text{S}/\text{cm}$ at 25°C .

Instrument is calibrated using conductivity storage solution of a known conductance. Method Blank samples and the storage solution are used within the run to verify readings reliability. Within each run, blanks, standard and duplicates are examined for proficiency and qualifiers are added as required.

Conductivity meter in the laboratory will be calibrated with standards bracketing the normal LAKEWATCH range of conductivities. Calibrations will be documented and kept in LAKEWATCH files.

Color Analysis

True Color values (PCU) are determined spectrophotometrically after filtration through a Gelman class A-E filter (Platinum-Cobalt Modified 2120C Spectrophotometric Method; A.P.H.A. 2005). We employ a Genesys 30 single beam spectrophotometer set at 465 nm to determine absorbance values. The following set range of standards is made from a certified standard Platinum-Cobalt Solution to generate a linear regression from which sample values are derived: blank, known concentrations of Platinum-Cobalt at 5, 10, 15, 20, 30, 40, 50, 100, and 150 PCUs. New standards are made quarterly or as needed and dates are recorded on a laboratory notebook and working laboratory spreadsheets. and archived for five years. Samples outside of the standard limits are diluted and reanalyzed until they are within the standards range.

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Seven blanks are used to establish the calculated MDL (method detection limit) and PQL (practical quantitation limit) for Color analyses. The current MDL and PQL are 1 and 1, respectively. Within each run, blanks, standards and duplicates are examined for proficiency and qualifiers are added as required. Samples in a run are qualified if the blank exceeds the MDL and the blank result exceeds 10% of the associated sample result. Additionally, samples in the run are qualified if the Matrix Spikes are not within 90-110% of the expected value. Duplicate samples are qualified if relative percent difference exceeds plus or minus 20%. Duplicates are poured at intervals of every 20 samples and are offset by ten samples from matrix spikes averaging 2-3 per run. At the end of a run are a duplicate, and two method blanks.

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. The minimum reported value is 1.0 mg/L as Cl.

Additional Internal Laboratory QA/QC

Florida LAKEWATCH is a unique volunteer monitoring program where sample storage after collection protocols is not able to follow NELAC requirements. The majority of LAKEWATCH samples spend time in three different freezers; 1) the volunteer's private freezer, 2) public location freezer for sample drop offs, and 3) University of Florida walk-in freezer. There is no way to certify chain of command for these samples or have complete certainty that temperatures remained constant throughout the samples journey. Once on University property both walk-in freezers and walk-in refrigerators (used after samples are poured out) have alarms systems that are used to maintain temperatures. Temperature data loggers are placed in each freezer and refrigerator and monitored every time samples are added or removed. Data is downloaded monthly and archived electronically for five years.

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 and records are archived for five years.

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

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house. If a spectrophotometer repeatedly shows drift or blank values exceeding MDL's then they are serviced.

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. If CRM material is not accurately analyzed within 95% or greater than 105% for the verification standards, then analyses are conducted again with new standards and/or equipment is recalibrated until CRM samples are accurately analyzed. Diluted concentrations of these certified samples are incorporated within our spiking matrix.

Final Data QA/QC

All data records are archived for a minimum of five years so all LAKEWATCH data values can be reconstructed. These archives contain at least the minimum documentation requirements in the NELAC standards and for Florida Administrative Code Rule Chapter: 62-160. In addition, records of equipment checks and maintenance conducted by service vendors are also archived.

The core LAKEWATCH data including TP, TN, and Chlorophyll reside on three different working data spreadsheets. TP and TN working data sheets are similar with columns for county, lake, date, station analysis tube number, dilution if required, absorbance from the spectrophotometer. These sheets also have columns for the standard concentrations and absorbances for each standard and an excel macro is run to calculate actual concentrations. In each WIN Excel data sheet, there are also formulas to calculate the precision of each set of duplicates. The Scientific Laboratory Manager enters these data into the formulas and qualifies data if precision exceeds acceptance. In addition, the following analysis information is recorded on the sheet for QA/QC purposes:

- Pour out Date, Technician and Time
- Digestion (autoclave) Date, Technician and Time (within 10 days)
- Analysis Date, Technician and Time
- Confirmation of following LAKEWATCH SOP
- Confirmation samples were frozen when processing started
- If used confirmation with heat tape that autoclave functioned properly

Chlorophyll working data sheets (where Secchi data are added) have columns for county, lake, date, station analysis tube number, ethanol volume, volume of water filtered, and absorbance values for each wave length. When completely filled an excel macro is run to calculate actual concentrations. In addition, the following analysis information is recorded on the sheet for QA/QC purposes:

- Extraction (Add Filter to Ethanol for Chlorophyll Extraction) Date, Technician and Time
- Analysis Date, Technician and Time (Within 24 Hours of Extraction)
- Confirmation of following LAKEWATCH SOP
- Confirmation samples were frozen when processing started

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After the macros that are run on each working data sheet, the Chemist makes sure data are qualified if needed with the following qualifiers:

- Q = Holding time not met from time of sample to analysis (5 months)
- U = Final results are below the MDL
- I = Final results are greater than or equal to the MDL and less than the PQL
- J = associated LCS outside acceptable limits of 90% - 110%, or other quality control failure (duplicate RPD > 20%, matrix spike recovery outside acceptable limits of 90% - 110%, result beyond calibration curve)
- V = Analyte detected in method blank at a level greater than 1/10 of sample concentration

After the Chemists/Manager finishes all internal laboratory QA/QC procedures a data manager adds Secchi depth data and time of field sampling recorded on the field data sheets (Appendix III) to the chlorophyll worksheet and merges all working spreadsheets 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, are verified and a final check is included for appropriate qualifiers before adding to the historical database.

MDLs and PQLs for freshwater and saline TP, TN, and Chl are conducted annually or immediately after any equipment for analysis are serviced. The methods used to determine MDLs and PQLs are described in above sections.

Annual Laboratory Control spikes (LCS) are determined for TP and TN analyses by incorporating seven replicates of low and midlevel known concentrations for both freshwater and saline analyses. Currently for TP we are using 10 µg/L and 50 µg/L concentrations, and for TN we are using 100 µg/L and 500 µg/L concentrations. Replicates are analyzed as regular samples on at least two separate analytical dates. The mean, standard deviation, and percent-expected recovery of original concentrations are determined.

A dilution series based on a known concentration of a stock chlorophyll solution are used to determine mean, standard deviation, and percent-expected recovery of calculated concentrations for uncorrected chlorophyll. Currently for chlorophyll we are using a stock solution of 26 µg/L and dilutions calculated as: 13, 6.5, 3.25, 1.6 µg/L. Only mean and standard deviations are determined for corrected chlorophyll and phaeophytin.

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Appendix I: Laminate field sampling methods card used by LAKEWATCH volunteers.



SAMPLING THE WATER



At each sampling station:

A. FILLING THE NUTRIENT BOTTLE

1. Before beginning, use a waterproof marker to label each sample bottle with
 - water body name
 - county
 - month-day-year
 - station number.
2. Without touching inside the bottle or lid, rinse them in the water twice.
3. Hold the bottom of bottle, turn bottle upside down and lower the open end into the water to elbow depth.
4. Fill the bottle by turning it to a horizontal position and pointing its mouth in the direction the boat is traveling.
5. Bring bottle up and pour water out to leave water level 1-inch from top.
6. Cap bottle tightly and put in a shady place or ice chest.

B. FILLING THE ALGAE SAMPLE CONTAINER

1. Rinse the container and lid in the water twice.
2. Invert the container and lower it into the water to elbow depth.
3. Fill the container by turning it to a horizontal position until full.
4. Bring the container up, cap it, and keep it in the dark (cover with towel).

C. MEASURING WATER CLARITY

1. Without wearing sunglasses, lower the Secchi disc into the water on the shady side of the boat (prescription glasses are okay).
2. When the disc vanishes from sight, put the clothespin on the rope at the water line (rope should be vertical).
3. Glance to check the sun cover, haul in the disc, and count rope markings to read how many feet under water the disc was when it vanished from view. Round to the nearest 1/4 foot.
4. Record this number and the sun code on the LAKEWATCH data sheet.

* Note: If your disc is visible on the bottom, write "B" in the "vanishing point" column below. If your disc disappears in the weeds, write "W" in the "vanishing point" column along with the depth at which it disappeared in the weeds.

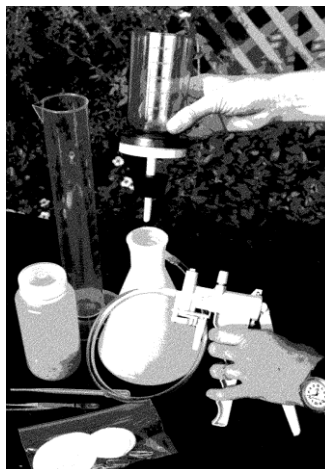
D. FILLING IN THE DATA SHEET

1. Lower the Secchi disc to the bottom and record this depth in the "water depth" column (do this step last so stirred-up bottom sediment does not contaminate the water samples).
2. Fill in the rest of the LAKEWATCH data sheet.
3. Copy data sheet for your records.

Put all nutrient bottles and data sheet in a ziplock bag and freeze them immediately.

FILTERING PROCEDURE

1. Assemble the filtering apparatus by pushing the rubber stopper of the cup base firmly into the flask.
2. Using tweezers, center a small filter (rough side up) on the cup base.
3. Using tap water, rinse the filter cup, and set it on the base.
4. Shake one of the containers and use some of the water to rinse the measuring cylinder. Repeat this rinse for each of the containers as you prepare to filter from it.
5. Measure water from the shaken container into the measuring cylinder.
6. Pour water from the measuring cylinder into the filter cup and pump it through the filter paper. Use all of the measured water. If the filter clogs up before all the measured water can be filtered, throw away the clogged filter and repeat steps 1 through 6—just measure less water this time. If filter paper appears colored after all the measured water is filtered go to step 7. Otherwise, repeat steps 5 and 6 until the filter appears colored then go to step 7.
7. After pumping the cup dry, tip the cup off the base.
8. Loosen the rubber stopper to break vacuum seal (pulling the relief trigger releases the vacuum seal also).
9. Using the tweezers to grip the white part of the filter paper, lift it off the cup base.
10. Being careful not to touch the algae, fold the filter in half with algae on inside. Blot it dry thoroughly on a paper towel.
11. Using a pencil, label a large filter paper with
 - water body name
 - county
 - month-day-year
 - station number
 - amount of water filtered (#mL).
12. Fold the large filter paper in half, tuck the small filter inside, and fasten the open side with a plastic-coated, metal paper clip.
13. Repeat all steps for the remaining containers of water and put all the filter papers into the same bottle of desiccant. Store it in freezer.
14. Rinse equipment with tap water, let it air dry, and check to see there are enough supplies for next month — GOOD WORK!



Remember if you have any questions or need more detail, refer to the blue LAKEWATCH manual or call 1-800-525-3928 (LAKEWAT).

Florida LAKEWATCH SOP (Draft January 2020)



Collection centers as of 1/4/2011

7922 NW 71st Street, Gainesville, FL 32653 Phone: 352-392-4817

Fax: 352-392-4902

Message Line: 1-800-525-3928

E-mail: fl-lakewatch@ufl.edu Web Sit Address: <http://lakewatch.ifas.ufl.edu>



Alachua (also used for Putnam)

Melrose Fire Dept Station 24

301 Cypress St. Melrose, FL 32666

Contact: Michael Heeder (352) 475-2005

Location: on Hwy 26 before traffic light

Bay

St. Andrew Bay Watch/LAKEWATCH

Next to Panama City Marine Institute @ RMA Lab

222 East Beach Dr Panama City, FL 32408

Contact: Patrice Couch Cell# (850) 252-2368

Brevard

Brevard County Extension IFAS

Contact: Jim Fletcher (321) 633-1702

3695 Lake Dr Cocoa, FL 32926

Brevard County Extension IFAS

1455 Treeland Blvd SE Palm Bay, FL 32909

Contact: Karin Strunk (321) 952-4536

Broward

Broward County IGFA Building

300 Gulf Stream Way Dania Beach, FL 33004

Contact: Chris Hess or Veronica Woods (954) 924-4360

Charlotte

Rotonda Collection Center

3754 Cape Haze Dr Rotonda West, FL 33947

Contact: Norene Kugler (941) 697-6788

Open: M-F 8-4

Citrus

Crystal River Preserve State Park

3266 N Sailboat Ave

Crystal River, FL 34428 Contact: Keith (352) 563-0450

Open: 9-5 M-F

East Citrus Community Center

44 going East out of Inverness

9907 E. Gulf to Lake Hwy East Inverness, FL 34452

Contact: Mary or Sal (352) 344-9666

Hours: 9-4 M-F

Clay

Clay County Extension IFAS

2463 SR 16 W Green Cove Springs, FL 32043

Contact: Ray Zerba (904) 284-6355

Keystone Heights City Hall

555 S. Lawrence Blvd Keystone, FL 32656

Contact: front desk (352) 473-4807

Collier

The Conservancy of SW Florida (239) 262-0304

1450 Merrihue Dr Naples, FL 34102

Contact: Kathy Worley (239) 403-4223

Rookery Bay Lab

300 Tower Rd Naples, FL 34113

Contact: Sue Leitholf or Jeff Carter (239) 417-6310

Florida LAKEWATCH SOP (Draft January 2020)

Escambia

Escambia County Extension IFAS
3740 Stefani Rd Cantonment, FL 32533
Contact: Andrew Diller (850) 475-5230

Flagler

Palm Coast Development (City Hall Build)
160 Cypress Point Pkway Palm Coast, FL 32164
Contact: John Moden (sect- Judi) (386) 986-4768
(Freezer out back)

Flagler County Extension IFAS
150 Sawgrass Rd Bunnell, FL 32110
Contact: Desk (386) 437-7464

Franklin

DEP Apalachicola East Lab
350 East Carroll St Eastpoint, FL 32328
Contact: Jessica ext. 121 (850) 653-8063 or (850) 670-4783

Hamilton

Hamilton County Extension IFAS
1143 US Hwy 41 NW Jasper, FL 32052
Contact: Tracy (386)-792-1276

Highlands

Highlands County Extension IFAS
4509 George Blvd Sebring, FL 33875
Contact: Ginger/ Gerry (863) 402-6540

Lake Placid Marine

310 US 27 S Lake Placid, FL 33852
Contact: Alan Underwood (863) 465-2335

Walker Memorial Junior Academy
1525 Avon Blvd Avon Park, FL 33825
Contact: Gordon Davis (863) 453-3131xt 204
Summer: Mon-Fri 8-12

Hillsborough

Hillsborough County Extension IFAS
5339 CR 579 S Seffner FL 33584-3334
Hours: M-F 8-5 Saturday & Sunday closed (813) 744-5519

Austin-Davis Library
17808 Wayne Rd. Odessa, FL 33556-4720
Contact: Librarian (813) 273-3652

Hours: M-12-8, T-10-8, W-10-6, TH-10-6, F-10-6, Sat-10-6

Lutz Library
101 Lutz-Lake Fern Rd. West Lutz FL 33548-7220
Contact: Librarian (813) 273-3652
Hours: M-12-8, T-10-8, W-10-6, TH-10-6, F-10-6, Sat-10-6

Lithia Springs County Park
3932 Lithia Springs Rd Lithia, FL 33547
Contact: Ron Swain (813) 744-5572
Hours: 7 days a week 8-6

Jackson

Beverly Schollian
255 Joshua Rd.
Marianna, FL 32448
(850) 579-4361

Lake

Lake County Water Authority
107 N Lake Ave Tavares, FL 32778
Contact: Gail Goldberg (352) 343-3777 x21

Florida LAKEWATCH SOP (Draft January 2020)

Lake County Dept. of Public Works
609 Disston Ave. Minneola, FL 34755
Contact: Denise (352) 394-2559

Ocala National Forest Fire Dispatch Center
Ranger Office – Clay Coates Ranger (352) 669-3153
18634 538 Rd Altoona, FL 32702
(next to 4-H Camp off Hwy 19)
Open 24 Hrs.

Leon

Alford B. Maclay State Park
3540 Thomasville Rd Tallahassee, FL 32309
Contact: Park Ranger (850) 487-4556

Leon County Extension IFAS
615 Paul Russell Rd Tallahassee, FL 32301
Contact: Jenease Roberts (850) 606-5200

Marion

Silver River State Park (Combination #0812)
1425 NE 58th Ave Ocala, FL 34470
Contact Ranger: (352) 236-7148

Miami-Dade

Tropical Park
7900 SW 40th St. Miami, FL 33135
Contact: Mercedes Suarez (305) 226-8315

Monroe

Sugarloaf Group (Monroe County)
Contact: Bill Bradbury (305) 745-9419

Cudjoe Group
Contact: Randal McDonald (305) 744-0622

Ramrod Group (Monroe County)
Contact: Ron Poller (305) 872-3616

Okaloosa (also used for Walton)

Okaloosa-Walton College/CBA
100 College Blvd Niceville, FL 32578
Contact Onsite: Deanne Simpson (850) 729-5376 x6376
Security Guard: (850) 729-5335 (after hours)
Lauren Nowling (850) 729-6426 Bldg. S

Okeechobee

Okeechobee County Extension IFAS
458 Hwy 98 N Okeechobee, FL 34972-2303
Contact: Pat Miller Ext. Director (863) 763-6469

Orange

Public Works Dept.
301 Maguire Road Ocoee, FL 34761
Contact: Marsha Vandergrift (407) 656-6090 x6000

City of Orlando Stormwater Utility Bureau
Lake Enhancement Building (407) 246-2125
1010 S Woods Ave Orlando, FL 32805
Contact: Katie Kulbaba (407) 246-2241

Orange County Extension IFAS
6021 S Conway Rd Orlando, FL 32812
Contact: Darla Wilkes (407) 254-9200

Florida LAKEWATCH SOP (Draft January 2020)

Osceola

Alligator Lake Inn (Owned by Harmony)
6264 E. Irlo Bronson Memorial Hwy.
St. Cloud, FL 34771
Contact: George Hill (407) 892-3195

Osceola County Extension IFAS
Osceola Heritage Park (321) 697-3000 **M-F 8-5**
1921 Kissimmee Valley Lane Kissimmee, FL 34744
Contact: Eleanor Foerste (407) 846-4181

Palm Beach

SFWMD Laboratory
1480 Skees Rd Palm Beach, FL 33411
Contact: Theresa East (561) 681-2500 xt.4565

FWC Office
8535 N Lake Blvd. West Palm Beach, FL 33412
Contact: Jon Furry or Melvin Burnside (561) 625-5122

Loxahatchee River District
Wild Pine Laboratory (Call First)
2500 Jupiter Park Dr Jupiter, FL 33458-2500
Contact Lorene Bachman (561) 747-5709 or Susan

Pasco

East Pasco Adventist Academy
38434 Centennial Rd Dade City, FL 33525
(352) 567-3646 xt 13
Hours: M-Thr 10-3 pm Wed: 10-1 pm

Land-O-Lakes Community Center
5401 Land-O-Lakes Blvd
Land-O-Lakes, FL 34639
Contact: Cindy or Mandy (813) 929-1229
Hours: M-F 8-5

Pinellas

Mocassin Lake Nature Park
2750 Park Trail Lane Clearwater, FL 33759-2602
Contact: Any staff (727) 462-6024
Hours: Closed Sun & Mon, Sat 9-4:30

Boyd Hill Nature Park
1101 Country Club Way S. St. Petersburg, FL 33705
Contact: Any staff (727) 893-7326
Hours: 7 days 9-5

Polk

Lake Region Lakes Management District (Canal Commission)
804 Ave X SW Winter Haven, FL 33898
Contact: Roger Griffiths/ Carolyn Whitehead (863) 293-1441

Lake Wales Veterinary Hospital
520 East Mountain Lake Cutoff Rd. Lake Wales, FL 33859
Contact: Tom Schotman (863) 676-1451
Open Saturday 8-12

City of Lakeland
407 Fairway Ave
Lakeland, FL 33801
(863) 834-8429

Polk County Extension IFAS
1702 Hwy 17/98
Bartow, FL 33830
Contact: (863) 519-8677
Hours: M-F 8-5

Florida LAKEWATCH SOP (Draft January 2020)

Putnam

St Johns River Water Management District
4049 Reid St Palatka, FL 32177
Contact: Carl Wince (386) 329-4500
(Freezer is outside-Walk in)

Santa Rosa

Santa Rosa Extension IFAS
Located Santa Rosa County Service Center
6263 Dogwood Dr. Milton, FL 32570
Contact: Chris Verlinde (850) 623-3868
Hours: M-F 8-4:30 (office manned Tues)

Sarasota

SWFWMD Regulation Office
6750 Fruitville Rd Sarasota, FL 34240
Contact: Kevin Coughlin x6553 (941)377-3722

Seminole

West Altamonte Facility Public Works Dept.
225 Newberryport Ave Altamonte Springs, FL 32701
Contact: Kathy Parkin (407) 571-8528
Hours: M-F 6-4

Casselberry Stormwater Utilities
95 Triplet Lake Dr Casselberry, FL 32718
Contact: Steve Spencer Crew Chief Leader (407) 262-7725 x 1732

Seminole County Extension IFAS
250 W. County Home Rd Sanford, FL 32773
Contact: Barbara Hughes/Ginny Gregory (407) 665-5551

St. Lucie

St. Lucie County Extension (IFAS)
8400 Picos Rd Ste 101 Ft. Pierce, FL 34945-3045
Contact: James Cummings (561) 462-1660

Volusia

Volusia County Extension IFAS
3100 E SR 44 Deland, FL 32724
Contact: David Griffis (386) 822-5778

Wakulla

Wakulla County Extension IFAS
84 Cedar Ave Crawfordville, FL 32327
Contact: Kathy Frank (850) 926-3931

Walton

Sheriff Sub Station
133 S Sheriff's Dr Santa Rosa Beach, FL 32459
Contact: (On site) Lt. Mitchem (850) 267-2000
(Just north of Tourist development council)
Contact: Joni Barreda or Sarah Kalinoski (850) 729-6456
Open 24 hours.

OWC/CBA (is listed in Okaloosa County)

Walton County Extension IFAS
732 N. 9th St DeFuniak Springs, FL 32433
Contact: Tanessa (850) 892-8172

Washington

Washington County Extension IFAS
1424 Jackson Ave Suite A Chipley, FL 32428-1615
Contact: Melissa DiBattiste or MaryJane Silcox (850) 638-6180

Florida LAKEWATCH SOP (Draft January 2020)

Appendix III: Freshwater field sheet used by LAKEWATCH volunteers

Florida LAKEWATCH Freshwater Data Sheet

Waterbody Name: _____ County: _____

Sampler: _____

Phone: () _____ Sampling Date: _____

Yes___ No___: Surface Water Collected for Total Phosphorus and Total Nitrogen.

Yes___ No___: Surface Water Collected for Chlorophyll and Filtered Within 48 Hours.

Yes___ No___: Secchi Depth Reading Taken

Secchi Disc Measurements:

- For **Secchi depth** and **water depth** measurements, please indicate the number of feet and then estimate and circle the appropriate fraction, if needed.
- If your **disc is visible on the bottom** write **B**, if your **disc disappears in the weeds** write **W**, in the **vanishing point** column and the **depth** at which your disc disappears.

Vanishing Point	Sun Code Number	Sun Code Key <small>Use the codes from below to fill in the Sun Code Number column.</small>	Water Depth	Sampling Time
Sta 1 ft. 1/4 1/2 3/4		1 = full sun	ft. 1/4 1/2 3/4	
Sta 2 ft. 1/4 1/2 3/4		2 = haze over sun	ft. 1/4 1/2 3/4	
Sta 3 ft. 1/4 1/2 3/4		3 = thin cloud	ft. 1/4 1/2 3/4	
Sta 4 ft. 1/4 1/2 3/4		4 = medium cloud cover	ft. 1/4 1/2 3/4	
Sta 5 ft. 1/4 1/2 3/4		5 = heavy cloud cover	ft. 1/4 1/2 3/4	

Date and Time of Chlorophyll Filtration:

Station	Filtering Date	Filtering Time
Sta 1		
Sta 2		
Sta 3		
Sta 4		
Sta 5		

DESCRIBE any unique occurrences since your last sampling date, either in the lake or on the local watershed:

If you wish to record lake levels of your lake, please fill in this last section.

Lake Level Measurements:

Please circle or describe the type of gauge located in the lake and then record the lake level.

Type of Staff Gauge: WMD / City / LCWA / USGS / Other (Please describe): _____

Lake level: _____ **Rain (in.) since last report:** _____

Call LAKEWATCH (1-800-LAKEWAT) if you have any questions on how to get started.

Florida LAKEWATCH SOP (Draft January 2020)

Appendix III: Saline field sheet used by LAKEWATCH volunteers.

Florida LAKEWATCH Saline Data Sheet

Waterbody Name: _____ County: _____

Sampler: _____ Phone: () _____ Sampling Date: _____

Yes___ No___: Surface Water Collected for Total Phosphorus and Total Nitrogen.

Yes___ No___: Surface Water Collected for Chlorophyll and Filtered Within 48 Hours.

Yes___ No___: Secchi Depth Reading Taken.

Secchi Disc Measurements:

- For **Secchi depth** and **water depth** measurements, please indicate the number of feet and then estimate and circle the appropriate fraction, if needed.
- If your **disc is visible on the bottom** write **B**, if your **disc disappears in the weeds** write **W**, in the **vanishing point** column and the **depth** at which your disc disappears.

Vanishing Point	Sun Code Number	Sun Code Key <i>Use the codes from below to fill in the Sun Code Number column.</i>	Water Depth	Sampling Time
Sta 1 ft. 1/4 1/2 3/4		1 = full sun	ft. 1/4 1/2 3/4	
Sta 2 ft. 1/4 1/2 3/4		2 = haze over sun	ft. 1/4 1/2 3/4	
Sta 3 ft. 1/4 1/2 3/4		3 = thin cloud cover	ft. 1/4 1/2 3/4	
Sta 4 ft. 1/4 1/2 3/4		4 = medium cloud cover	ft. 1/4 1/2 3/4	
Sta 5 ft. 1/4 1/2 3/4		5 = heavy cloud cover	ft. 1/4 1/2 3/4	
Sta 6 ft. 1/4 1/2 3/4			ft. 1/4 1/2 3/4	
Sta 7 ft. 1/4 1/2 3/4			ft. 1/4 1/2 3/4	
Sta 8 ft. 1/4 1/2 3/4			ft. 1/4 1/2 3/4	
Sta 9 ft. 1/4 1/2 3/4			ft. 1/4 1/2 3/4	
Sta 10 ft. 1/4 1/2 3/4			ft. 1/4 1/2 3/4	
Sta 11 ft. 1/4 1/2 3/4			ft. 1/4 1/2 3/4	
Sta 12 ft. 1/4 1/2 3/4			ft. 1/4 1/2 3/4	
Sta 13 ft. 1/4 1/2 3/4			ft. 1/4 1/2 3/4	

Date and Time of Chlorophyll Filtration:

Station	Filtering Date	Filtering Time
Sta 1		
Sta 2		
Sta 3		
Sta 4		
Sta 5		
Sta 6		
Sta 7		
Sta 8		
Sta 9		
Sta 10		
Sta 11		
Sta 12		
Sta 13		

DESCRIBE any unique occurrences since your last your sampling date, either in the coastal waters or on the local watershed, on the back of this data sheet.

Call LAKEWATCH (1-800-LAKEWAT) if you have any questions on how to get started.