

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.

Alternative (Modified) Sample Extraction Method for Chlorophyll

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. Sections 1.0 – 1.3
 - 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. Section 2.2



- 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

American Public Health Association. 1992. Standard Methods for the Examination of Water and Wastewater. 18th Edition.

Canfield, D. E. Jr., C. D. Brown, R. W. Bachmann, and M. V. Hoyer. 2002. Volunteer lake monitoring: testing the reliability of data collected by the Florida LAKEWATCH program. Lake and Reservoir Management 18: 1-9.

Department of Environmental Protection. 2/1/04. New and Alternative Laboratory Methods, DEP-QA-001/01.

Department of Environmental Protection. 12/3/08. Quality Assurance. Chapter 62-160, Florida Administrative Code.

Department of Environmental Protection. 3/31/08. Standard Operating Procedures for Field Activities, DEP-SOP-001/01.

Department of Environmental Protection. 10/24/11. Applicability of Chlorophyll *a* Methods, DEP-SAS-002/10.

Department of Environmental Protection. 6/7/13. Evaluation of COAST data in comparison with DEP data from duplicate samples. Internal Report.

Florida LAKEWATCH, Department of Fisheries and Aquatic Sciences, School of Forest Resources and Conservation/IFAS, University of Florida. April 2013. Florida Lakewatch Water Chemistry Field Sampling and Laboratory Protocols.



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.

