# Alternative Site-Specific and Limited-Use Method Approvals for the Edward J. Phlips Laboratory and Project COAST

*FDEP Aquatic Ecology and Quality Assurance Section*

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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 holdings 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, as further explained below. Project COAST (COAST) 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. The Edward J. Phlips Laboratory (Phlips Lab) is located at the same address, and is certified by the Florida Department of Health Environmental Laboratory Certification Program (DOH ELCP) for the analysis of TN, TP and CHLA. Project COAST staff share facilities and instrumentation for chlorophyll analyses at the Phlips Lab. 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 Methods

1. **Alternative Sample Preservation Methods Including Freezing** -
    a. The COAST method of freezing samples for Total Nitrogen (TN) and Total Phosphorus (TP) is found in "Standard Operating Procedures for Project Coast Surface Water Sampling" (COAST. June 2013), hereinafter called the COAST Sampling SOP. The chlorophyll filtering and preservation procedure is also described in the COAST Sampling SOP. Chilled (iced) samples are hand-delivered on the same day of collection to the laboratory, or as soon as possible thereafter for samples collected at geographically remote sites. The samples are frozen at the laboratory. The maximum holding time from the date of collection to analysis for all samples is 28 days.

i. Samples for TN and TP analyses are collected unfiltered and immediately placed in ice after collection.
ii. Samples collected for CHLA are filtered immediately upon collection. After filtration, processed filters are packaged according to the COAST Sampling SOP and placed in ice.

2. **Basis for Approval of Alternative Sample Preservation Methods Including Freezing** –
   a. 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 Florida LAKEWATCH Laboratory (LW lab). DEP conducted a separate review and approval of LW lab procedures for TN and TP sample preservation, which includes frozen sample storage (DEP. 7/1/13). The DEP co-collected samples were preserved according to DEP SOP requirements (DEP. 3/31/08) and analyzed for Total Kjeldahl Nitrogen, Nitrate-Nitrite, TP and CHLA by the DEP Bureau of Laboratories. 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 ($p < 0.05$). For corrected CHLA, COAST and DEP results were very highly correlated ($r = 0.90$). However, the slope of the regression line was significantly greater than one, indicating that the COAST CHLA results were likely to be higher than the FDEP result. Paired t-tests indicated no significant difference between DEP and COAST datasets for corrected chlorophyll *a* ($p < 0.05$).

   b. The COAST 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 study and separate LW lab approval described above, and are comparable to other data used by DEP. The alternative methods also meet the data validation requirement for adherence to proper preservation procedures as required at Rule 62-160.670(1)(b), F.A.C.

<u>Alternative (Modified) Sample Extraction Method for Chlorophyll Used by COAST and Phlips Lab Analysts</u>

1. **Extraction Method for Chlorophyll Filters** – Frozen filters (CHLA samples) are extracted in the Phlips Lab using a hot ethanol extraction procedure. This extraction method for chlorophyll , which is described in the Project COAST Laboratory Chlorophyll SOP (COAST. July 2013), and the Phlips Lab Chlorophyll SOP #1 (Phlips. 2011), and is intended to replace the approved acetone extraction accepted by DEP, as found in the approved analytical methods cited in Applicability of Chorophyll *a* Methods, DEP-SAS-002/10 (DEP.2011).

2. **Basis for Approval of Chlorophyll Extraction Method** –

a. DEP and COAST conducted a comparison study of co-collected estuarine samples using the COAST and Phlips Lab CHLA extraction method and the DEP-approved acetone extraction method (DEP.2010). A pair-wise comparison of these 30 estuary sites showed that the COAST and DEP phaeophytin-corrected chlorophyll *a* results were not statistically different ($p < 0.05$), and COAST and DEP results were very highly correlated ($r = 0.90$).

b. Initial demonstration of capability (IDOC) to perform the CHLA extraction and analytical methods, as well as evaluation of the method detection limit were documented by the Phlips lab to meet DOH ELCP certification requirements.

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

i. <u>Sections 1.0 – 1.3</u>

1. The laboratory certification requirement for this method has been waived for Project COAST by DEP due to the budget constraints on funding available for COAST activities, which make accreditation by the DOH ELCP prohibitive for this state university project. The Phlips lab is certified for its CHLA method as detailed in the Phlips Lab CHLA SOP (Phlips. 2011).
2. DEP has determined that a method equivalency study is not needed, and the comparability study summarized above provides acceptable demonstration of the efficacy of the alternative chlorophyll extraction method.
3. 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 COAST and the Phlips Lab, as summarized in this review, and meet applicable DEP project and program Data Quality Objectives (DQOs) for these uses.
4. The alternative extraction method is determined to be applicable to the analysis of fresh and marine surface water samples.

ii. <u>Section 2.2</u>

1. The Phlips Lab has conducted an acceptable method detection limit (MDL) study using the Phlips Lab CHLA SOP .
2. Acceptable precision and accuracy has been demonstrated by the Phlips Lab IDOC study to evaluate percent recovery and percent relative standard deviation (% RSD).

3. The MDL study, IDOC precision and accuracy determinations, and the comparability study summarized in this review, provide sufficient justification for waiving the need for any additional equivalency study of the CHLA extraction method in comparison to the DEP-approved acetone extraction method, and demonstrate that the ethanol extraction method used by COAST and the Phlips Lab is a suitable replacement for the acetone extraction method.
4. All relevant validation information concerning the extraction procedure is included in the COAST CHLA SOP and in the Phlips Lab CHLA SOP and IDOC.

Scope of Approval

1. **Alternative Sample Preservation Methods Including Freezing** – The alternative sample preservation methods for TN, TP and CHLA used by COAST, including freezing of TN and TP samples, as described in the COAST Sampling SOP, are approved as site-specific sampling methods, defined in Rule 62-160.220(4)(a), F.A.C., and as project-specific alternative field procedures, described in DEP SOP FA 1000, subpart FA 2230, section 1. The methods are approved for use for the COAST project for the collection, including preservation and storage, of TN, TP and CHLA samples from fresh and marine surface water matrices collected at all sites or locations sampled by COAST staff. The methods may also be used by other persons collecting samples for the COAST project, if applicable. This includes approval of a maximum holding time of 28 days from the date of sample collection for TN, TP and CHLA samples, as stipulated in the COAST Sampling SOP.

2. **Chlorophyll Extraction Method** – The extraction method for chlorophyll sample filters described in the COAST and Phlips Lab SOPs for CHLA analysis is approved as a limited-use method, as defined in Rule 62-160.330(4)(a), F.A.C. and in DEP-QA-001/01, section 1.2. The method is exclusively approved for use by COAST and the Phlips Lab for the analysis of 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 COAST sample preservation methods and COAST and Phlips Lab CHLA extraction method includes the determination by DEP that analytical data for TN, TP and CHLA from samples collected using the COAST alternative sample preservation procedures and CHLA data from samples analyzed using the COAST and Phlips Lab chlorophyll 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

Project COAST, Department of Fisheries and Aquatic Sciences, University of Florida. June 1, 2013. Standard Operating procedures for Project Coast Surface Water Sampling.

Project COAST, Department of Fisheries and Aquatic Sciences, University of Florida. July 1, 2013. Project COAST Laboratory Chlorophyll SOP.

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.

Department of Environmental Protection. 7/1/13. Alternative Method Approvals Florida LAKEWATCH (Internal Memorandum).

Edward J. Phlips Laboratory, Department of Fisheries and Aquatic Sciences, University of Florida. 1/3/11. Chlorophyll SOP #1.