FLORIDA DEPARTMENT OF
Environmental Protection

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2600 Blair Stone Road
Tallahassee, FL 32399-2400

Ron DeSantis
Governor

Jeanette Nuñez
Lt. Governor

Noah Valenstein
Secretary

VIA EMAIL
Read Receipt Requested

January 4, 2021

Henry O. Briceño
Florida International University
Institute of Environment
Southeast Environmental Research Center
6 SE 2nd Avenue
Miami, FL 33131
E-mail: bricenoh@fiu.edu

Re: Alternative Limited-Use Methods Approval for the Florida Keys National Marine Sanctuary Water Quality Monitoring Project, Conducted by Florida International University Southeast Environmental Research Center; Center for Aquatic Chemistry and Environment - Nutrient Analysis Core Facility, DOH ID E76930

Dear Dr. Briceño,

The Aquatic Ecology and Quality Assurance Section (AEQAS) of the Department of Environmental Protection (department) has reviewed the method validation information submitted by Florida International University Southeast Environmental Research Center (SERC) and has approved methods for limited-use alternative maximum holding time and preservation for nitrite (NO_2) and limited-use alternative preservation for nitrate-nitrite (NO_2+NO_3), ammonia/ammonium (NH_3/NH_4), total nitrogen (TN), total phosphorus (TP), and total organic carbon (TOC) samples. The alternative method submittal for alternative preservation methods for soluble reactive phosphorus (SRP) samples was not approved. This letter provides an approval order for alternative methods as required in Rule 62-160.220(7)(a), Florida Administrative Code (F.A.C.).

Scope of Approval

The alternative methods for maximum holding time and preservation of NO2 and alternative preservation methods for NO2+NO3, NH3/NH4, TN, TP, and TOC, as described in the field protocol titled, "Florida National Marine Sanctuary Survey Field Sampling Protocol" (Revision 7, 12/02/2020), are approved as limited-use field methods, as defined in Rule 62-160.120(13), F.A.C., and as designated in paragraph 62-160.220(6)(a), F.A.C., and DEP SOP FA 1000, subpart FA 2230, section 1.

A copy of this approval letter and the following supporting documents, located on the AEQAS Alternative Method Approvals webpage, detail the specific field procedures, evaluation of comparison studies, and data usability recommendations for this approval.

- The technical document titled, "Alternative Limited-Use Methods Approval for the Florida Keys National Marine Sanctuary Water Quality Monitoring Protection Project, Conducted by Florida International University Southeast Environmental Research Center" (December 2020).
- The field protocol titled, "Florida National Marine Sanctuary Survey Field Sampling Protocol" (Revision 7, 12/02/2020).

The department's evaluation of these alternative methods was based on data collected for the Florida Keys National Marine Sanctuary Water Quality Protection Program's Water Quality Monitoring Project (WQMP) and the "Water Quality Monitoring Project for Demonstration of Canal Remediation Methods: Florida Keys." The use of these alternative methods is limited to samples analyzed by the SERC's Water Quality Monitoring Laboratory (known as the Center for Aquatic Chemistry and Environment - Nutrient Analysis Core Facility, DOH ID E76930) for samples of non-potable marine waters in the Florida Keys region for the specific matrices, analytes, methods and sample concentration ranges evaluated for this approval, per subsection 62-160.220(6a), F.A.C. These alternative methods may not be used for other matrices, analytes, methods or sample concentration ranges or by another laboratory without separate review and approval by the department.

Data Usability

Approval of the above alternative methods includes the determination that sample analytical data for NO_2 using the WQMP sample maximum holding times and sample analytical data for NO_2+NO_3, NH_3/NH_4, TN, TP, and TOC using WQMP sample preservation methods described above meet all applicable data quality objectives for the use of approved sampling 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 waters 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.

For additional information or questions about these approvals, please contact Jessica Patronis (AEQAS) at (850) 245-8980 or Sarah Noble (AEQAS) at (850) 245-8533.

Effective Date of Approval and Notice of Rights

Pursuant to requirements in paragraph 62-160.220(7)(a), F.A.C., the department will send a notice of our approval of your alternative method to subscribers enrolled to receive our Quality of Science e-Newsletter. We will also post our approval letter and your alternative method SOP on our AEQAS webpage designated for alternative method approvals.

This action is final and effective on the date mailed (via e-mail) unless a sufficient petition for an administrative hearing is timely filed under sections 120.569 and 120.57 of the Florida Statutes as provided below. If a sufficient petition for an administrative hearing is timely filed, this agency action becomes only proposed agency action, subject to the result of the administrative review process. Therefore, on the filing of a timely and sufficient petition, this action will not be final and effective until further order of the department. Because an administrative hearing may result in the reversal or substantial modification of this action, the petitioner is advised not to proceed until the deadlines noted below for filing a petition for an administrative hearing or request for an extension of time have expired. Mediation is not available.

A person whose substantial interests are affected by the department's action may petition for an administrative proceeding (hearing) under sections 120.569 and 120.57 of the Florida Statutes. The petition must contain the information set forth below and must be filed (received by the clerk) in the Office of General Counsel of the Department at 3900 Commonwealth Boulevard, Mail Station 35, Tallahassee, Florida 32399-3000. The petitioner shall also mail a copy of the petition to the Florida International University Institute of Environment, Southeast Environmental Research Center, at the address indicated above at the time of filing.

Under rule 62-110.106(4), F.A.C., a person whose substantial interests are affected by the department's action may also request an extension of time to file a petition for an administrative hearing. The department may, for good cause shown, grant the request for an extension of time. Requests for extension of time must be filed with the Office of General Counsel of the Department at 3900 Commonwealth Boulevard, Mail Station 35, Tallahassee, Florida 32399-3000, or via electronic correspondence at Agency_Clerk@dep.state.fl.us, before the applicable deadline. A timely request for extension of time shall toll the running of the time period for filing a petition until the request is acted upon. If a request is filed late, the department may still

grant it upon a motion by the requesting party showing that the failure to file a request for an extension of time before the deadline was the result of excusable neglect.

If a timely and sufficient petition for an administrative hearing is filed, other persons whose substantial interests will be affected by the outcome of the administrative process have the right to petition to intervene in the proceeding. Intervention will be permitted only at the discretion of the presiding officer upon the filing of a motion in compliance with rule 28-106.205, F.A.C.

In accordance with rules 28-106.111(2) and 62-110.106(3)(a), F.A.C., petitions for an administrative hearing by the petitioner must be filed within 21 days of receipt of this written notice. Petitions filed by any persons who were not entitled to written notice under section 120.60(3) of the Florida Statutes must be filed within 21 days of publication of the notice, if published, or within 21 days of receipt of the written notice, whichever occurs first. Under section 120.60(3) of the Florida Statutes, however, any person who has asked the department for notice of agency action may file a petition within 21 days of receipt of such notice, regardless of the date of publication.

The failure of any person to file a petition for an administrative hearing within the appropriate time period shall constitute a waiver of that person's right to request an administrative determination (hearing) under sections 120.569 and 120.57 of the Florida Statutes.

A petition that disputes the material facts on which the department's action is based must contain the following information: (a) The name and address of each agency affected and each agency's file or identification number, if known; (b) The name, address, any e-mail address, any facsimile number, and telephone number of the petitioner, if the petitioner is not represented by an attorney or a qualified representative; the name, address, and telephone number of the petitioner's representative, if any, which shall be the address for service purposes during the course of the proceeding; and an explanation of how the petitioner's substantial interests will be affected by the agency determination; (c) A statement of when and how the petitioner received notice of the agency decision; (d) A statement of all disputed issues of material fact. If there are none, the petition must so indicate; (e) A concise statement of the ultimate facts alleged, including the specific facts that the petitioner contends warrant reversal or modification of the agency's proposed action; (f) A statement of the specific rules or statutes that the petitioner contends require reversal or modification of the agency's proposed action, including an explanation of how the alleged facts relate to the specific rules or statutes; and (g) A statement of the relief sought by the petitioner, stating precisely the action that the petitioner wishes the agency to take with respect to the agency's proposed action.

A petition that does not dispute the material facts on which the department's action is based shall state that no such facts are in dispute and otherwise shall contain the same information as set forth above, as required by rule 28-106.301, F.A.C. Under paragraphs 120.569(2)(c) and (d) of the Florida Statutes, a petition for administrative hearing must be dismissed by the agency if the petition does not substantially comply with the above requirements or is untimely filed.

This determination constitutes an order of the department. Subject to the provisions of paragraph 120.68(7)(a) of the Florida Statutes, which may require a remand for an administrative hearing, the applicant has the right to seek judicial review of the order under section 120.68 of the Florida Statutes, by the filing of a notice of appeal under rule 9.110 of the Florida Rules of Appellate Procedure with the Clerk of the Department in the Office of General Counsel, 3900 Commonwealth Boulevard, Mail Station 35, Tallahassee, Florida, 32399-3000, or via electronic correspondence at Agency_Clerk@dep.state.fl.us; and by filing a copy of the notice of appeal accompanied by the applicable filing fees with the appropriate district court of appeal. The notice of appeal must be filed within 30 days from the date when the final order is filed with the Clerk of the department.

Executed in Tallahassee, Florida.

STATE OF FLORIDA DEPARTMENT OF
ENVIRONMENTAL PROTECTION

David Whiting, Deputy Director
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Copies furnished to:

Kenneth Hayman, DEP, Office of General Counsel
Julie Zimmerman, DEP, Watershed Information Network Section
Gus Rios, DEP, South District Marathon Office
Steven Blackburn, EPA Region 4, Oceans, Wetlands and Streams Protection Branch
Chris Anderson, FWC
File

CERTIFICATE OF SERVICE

The undersigned duly designated clerk hereby certifies that this determination, including all copies, was e-mailed before the close of business on, January 5, 2021 , to the above listed

persons.

FILING AND ACKNOWLEDGMENT

FILED, on this date, pursuant to 120.52(7)
Florida Statutes, with the designated department Clerk,
receipt of which is hereby acknowledged.

Clerk Date

Alternative Limited-Use Methods Approval for the Florida Keys National Marine Sanctuary Water Quality Monitoring Project, Conducted by Florida International University Southeast Environmental Research Center

FDEP Aquatic Ecology and Quality Assurance Section
December 2020

Summary of Approval

The Florida Department of Environmental Protection (DEP) Aquatic Ecology and Quality Assurance Section (AEQAS) was asked to review method validation information submitted in support of program-specific field sample preservation methods used by the Southeast Environmental Research Center (SERC) of Florida International University (FIU) for samples collected and analyzed for the Florida Keys National Marine Sanctuary Water Quality Protection Program's (FKNMS-WQPP) Water Quality Monitoring Project (WQMP). Table 1 lists the analytes included in the review and a summary of required and alternative preservation methods.

The WQMP is a long-term water quality monitoring project executed by SERC's Water Quality Monitoring Laboratory. The laboratory, Center for Aquatic Chemistry and Environment - Nutrient Analysis Core Facility (CAChE-NACF), which is within SERC and is located at 11200 SW 8th Street, OE 148 University Park, Miami, Florida 33199, analyzes the samples and is certified by the Florida Department of Health Environmental Laboratory Certification Program (DOH ELCP), DOH ID E76930, for the analytes collected for the WQMP. Details about the sampling procedures for the WQMP can be found in "*Florida National Marine Sanctuary Survey Field Sampling Protocol"* (revision date, December 2020).

This document describes the criteria and references used to evaluate the alternative methods used by SERC and determine if approval is warranted, as required in Rule 62-160.220(4), Florida Administrative Code (F.A.C.). The basis for the approvals or disapprovals is described in DEP SOP FA 1000, subparts FA 2210 – FA 2230 (revision date, January 2017).

Scope of Approval and Disapproval

The approval determination for the alternative preservation and holding time methods used for the SERC WQMP and the Water Quality Monitoring Project for Demonstration of Canal Remediation Methods: Florida Keys (Canal Project), which was conducted using the same field preservation methods as the WQMP and from which one of the method comparison studies (2014) was conducted, are described below. The use of this alternative method approval is limited to the WQMP, the Canal

Project, and other projects using the CAChE-NACF laboratory for samples of non-potable marine waters in the Florida Keys region for the specific matrices, analytes, methods and sample concentration ranges evaluated for this approval, per subsection 62-160.220(6a), F.A.C.

1. Alternative Preservation Methods are approved for the following parameters:
    a. Nitrate-Nitrite (NO_2+NO_3): Filter and ice in field, freeze if not analyzed within 48 hours, and analyze within 28 days.
    b. Ammonia/Ammonium (NH_3/NH_4): Filter and ice in field, freeze if not analyzed within 48 hours, and analyze within 28 days.
    c. Total phosphorus (TP): Ice in field, add HCl acid within 48 hours, store at 2-6 ℃, and analyze within 28 days.
    d. Total organic carbon (TOC): Ice in field, store at 2-6 ℃, and analyze within 28 days.
    e. Total Nitrogen (TN): Ice in field, add HCl acid within 48 hours, store at 2-6 ℃, and analyze within 28 days.
2. Alternative Preservation and Holding Times are approved for the following parameters:
    a. Nitrite (NO_2): Filter and ice in field, freeze if not analyzed within 48 hours, and analyze within 28 days
3. Alternative Preservation Method is not approved for the following parameter:
    a. Soluble reactive phosphorus (SRP): Filter and ice in field, freeze if not analyzed within 48 hours, and analyze within 28 days.

Even though the CAChE-NACF employs a minor modification in its chlorophyll *a* method for the WQMP and the Canal Project (the lab follows method EPA 445.0, but begins extraction in acetone before freezing the filters), chlorophyll *a* data were not included in the scope of this alternative method review because this modification is considered minor and because FIU successfully showed that this modification produced comparable results to the unmodified method in a 1997 study.

Basis for Approval and Disapproval

AEQAS reviewed data from three separate studies conducted by the CAChE-NACF (in 2008, 2014, and 2020) to compare the DEP SOP-required preservation methods and the WQMP methods to determine if results were comparable between the two methods.

The most recent study with data relevant to a given analyte was evaluated for statistical analyses, but all applicable studies were considered as supporting information per criteria in subsection 62-160.220(4), F.A.C., and DEP SOP FA 2220. The alternative method was considered comparable to the required method if comparison datasets were not statistically significantly different according to paired tests, and the predominance of relative percent difference (RPD) results for sample pairs met acceptance criteria for lab duplicates (RPD < 20%). However, if the datasets were significantly different, the datasets are considered comparable if the differences between methods were too small to impact DEP's use of the data and the majority of RPD results for sample pairs met acceptance criteria for lab duplicates. Evaluation methods and comparison results are provided in detail below.

Data Usability

The DEP Division of Environmental Assessment and Restoration (DEAR) requested this review of SERC's alternative preservation and holding time procedures because DEAR would like to use data from the WQMP in its Clean Water Act 303(d) assessments through the DEP Impaired Waters Rule (IWR), Chapter 62-303, F.A.C. The IWR requires that data used for the assessments adhere to requirements in the QA

Rules, Chapter 62-160, F.A.C. Data produced for the WQMP or the Canal Project using the approved alternative methods, for the duration of these studies for which the methods were used, may be used by DEAR for 303(d) assessments and other assessment uses, including TMDL development, BMAP evaluation, and standards derivation. Alternative methods are not approved for use by DEP Drinking Water, Wastewater, or Waste regulatory programs.

Table 1. Summary of alternative preservation methods requested and availability of study data. DEP SOP requirements are from DEP SOP Table FS 1000-4, and the alternative preservations are from the Florida National Marine Sanctuary Survey Field Sampling Protocol.

Analyte	DEP SOP-required Preservation	Alternative Preservation for FIU Florida Keys Monitoring	Comparison Included in 2008 Study	Comparison Included in 2014 Study	Comparison Included in 2020 Study
Nitrate-Nitrite (NO_2+NO_3)	Acid-preserve with H_2SO_4 and ice within 15 minutes, analyze within 28 days	Filtered and iced in field, frozen if not analyzed within 48 hours (always the case for these projects), analyzed within 28 days	Yes	Yes	No
Ammonia / Ammonium (NH_3/NH_4)			Yes	Yes	No
Nitrite (NO_2)	Ice within 15 minutes, analyze within 48 hours		Yes	No*	No
Soluble reactive phosphorus (SRP)	Filter and ice within 15 minutes, analyze within 48 hours		Yes	No*	No
Total nitrogen (TN)	Acid-preserve with H_2SO_4 and ice within 15 minutes, analyze within 28 days**	Iced in field, HCl acid added within 48 hours of sample submission, stored in refrigerators at 2-6 ℃, analyze within 28 days	Yes***	Yes	Yes
Total phosphorus (TP)			Yes	Yes	No
Total organic carbon (TOC)	Acid-preserve with HCl, H_2SO_4, or H_3PO_4, and ice within 15 minutes, analyze within 28 days	Iced in field, stored in refrigerators at 2-6 ℃, analyze within 28 days	Yes	No	No

* This analyte was included in the study, but preservation was not conducted correctly for the DEP SOP-required preservation treatment.

** DEP SOP Table FS 1000-4 does not contain TN as a test. The required preservation provided in the table is for NO_2+NO_3 and TKN, which are the components of TN.

*** HCl was used instead of H_2SO_4 for the DEP SOP acid-preservation treatment for TN in 2008.

Evaluation of Preservation Method Comparison Studies

Three separate studies were conducted, in 2008, 2014, and 2020, to determine if sample data were comparable between the DEP SOP preservation requirements in DEP SOP FS 1000 and the alternative preservation and holding times for the WQMP. These studies were conducted at the CAChE-NACF, which meant all samples were analyzed under comparable laboratory conditions. Table 2 contains laboratory methods and detection limits for each study. The 2008 study included 20 samples from the WQMP sites, the 2014 study included 32 samples from the Canal Project, and the 2020 study included 20 samples from the WQMP sites and 10 samples from a different Florida Keys canal study titled, "WATER QUALITY MONITORING PROGRAM FOR ISLAMORADA, VILLAGE OF ISLANDS: Tracking Canal Water Changes after Connection to Wastewater Collection System." The preservation methods are described in Table 1. Results from all three comparison studies were considered for evaluation to determine comparability, but greater emphasis was placed on the 2014 study for most analytes because it includes more samples, spans a wider range of concentrations, and more likely reflects analysis conditions for samples analyzed in recent years. While more recent, the 2020 study was conducted as a supplemental study due to unfavorable results for TN in the 2014 study and only included comparison of preservation methods for analysis of TN.

More information about the 2008 study can be found in 2009 and 2012 memos to DEP titled, "Alternative Method for SERC Use Only," which are available upon request. The 2014 comparison study included comparison between the DEP and CAChE-NACF laboratories as well as between different preservation methods within the CAChE-NACF laboratory. For the purposes of this alternative method approval, AEQAS focused on the comparison of sample treatments within the CAChE-NACF laboratory in order to rule out variability between labs. Due to error in the 2014 study, the nitrite (NO_2) and soluble reactive phosphorus (SRP) samples were acid-preserved for the DEP SOP preservation treatment rather than just being preserved on ice and analyzed within 48 hours, so those results could not be used for this alternative method approval evaluation. TOC was not collected during the 2014 study. More details about the 2014 comparison study can be found in, "Plan of Study: 2014 Lab and Method Comparison Study between FDEP and SERC for Florida Keys Canal Restoration Project," which is available upon request. More details about the 2020 study can be found in the June 2020 document titled, "Procedures for TN water sample collection, FIU TN alternative method study – preservation method and matrix effect."

Table 2. CAChE-NACF lab methods and method detection limit (MDL) and practical quantitation limit (PQL) levels for 2008, 2014, and 2020. Analytes that do not have an MDL or PQL listed in the table were not included in that year's comparison study.

Analyte	Lab SOP Name	Reference Method	2008 MDL (mg/L)	2008 PQL (mg/L)	2014 MDL (mg/L)	2014 PQL (mg/L)	2020 MDL (mg/L)	2020 PQL (mg/L)
NO_2+NO_3	CACHE-NACF SOP-004	EPA 353.4	0.0039	0.0172	0.0008	0.0134	-	-
NH_3/NH_4	CACHE-NACF SOP-004	EPA 349.0	0.0049	0.0217	0.0015	0.014	-	-
NO_2	CACHE-NACF SOP-004	EPA 353.4	0.0006	0.0024	-	-	-	-
SRP	CACHE-NACF SOP-004	EPA 365.1	0.0016	0.0068	-	-	-	-
TN	CACHE-NACF SOP-006	ASTM D5176	0.05	0.22	0.04	0.25	0.04	0.25
TP	CACHE-NACF SOP-008	EPA 365.1	0.0019	0.0078	0.0003	0.0031	-	-
TOC	CACHE-NACF SOP-007	SM #5310B & FDEP SOP NU-062-1.8	0.16	0.75	-	-	-	-

Data Review Methods

The department used various techniques to compare the sample results between the two sets of preservation and holding time procedures, per specifications in DEP SOP FA 2240, section 4. Sample results for each analyte were first visualized using scatterplots to compare results from required (x axis) and alternative preservation methods (y axis). If the two methods produced equal results, the points would be close to the line of equality that was included in the graph. If most or all points lie on one side of the line of equality, then there is a bias in the method, with one preservation method consistently producing higher results than the other. Linear regression equations and R^2 values for the graphs are presented for informational purposes but were not themselves criteria for acceptance of the alternative method.

Following recommendations from Niu (2014), the department then conducted pairwise comparisons to determine if the paired results generated by the required preservation and alternative preservation were statistically significantly different (the department used the paired t-test if the results were normally distributed, and the Wilcoxon signed rank test if non-normal). If the pairs were not statistically significantly different, the alternative method would be considered comparable to the required method.

Because statistical significance does not necessarily equate to ecological differences or differences great enough to impact resource management decisions, Bland and Altman limits of agreement were calculated to show the 95% confidence range of differences if the paired results were significantly different. The differences were calculated by subtracting the alternative preservation result from the required preservation result. A negative result means the alternative method is higher than the required preservation and a positive result means the alternative method is lower than the required preservation. To determine if the differences are acceptable for the intended use of the data (per DEP

SOP FA 2240, section 4.4), these ranges were evaluated in the context of the method's sensitivity and how the data may be used.

The department also calculated the relative percent difference (RPD) for each sample pair. The RPD is a measure of precision for 2 data points and is calculated as the difference between the results in the sample pair divided by their mean. The RPD is typically used in labs to evaluate laboratory duplicates, and < 20% is a common acceptance criterion for nutrient duplicates. SERC's acceptance criteria for paired sample results in the 2008 comparison study was < 20% RPD. RPD values may be greater for results below the practical quantitation limit (PQL). The department considered RPDs < 20%, < 30% and < 40% for precision evaluations for each analyte.

All lines of evidence are considered in the determination of whether the alternative method produces results comparable to the required method, with consideration of the following criteria for approval of alternative procedures from DEP SOP FA 2220 and subsection 62-160.220(4), F.A.C.:

- Alternative and modified procedures must be appropriate for the Data Quality Objectives (DQOs) established by the department for the data uses for which the alternative or modified procedure is proposed.
- Where applicable, the alternative or modified procedure must be demonstrated to be equivalent to or exceed the performance of the DEP SOP that the alternative or modified procedure is proposed to replace.
- Approval will not be granted if the procedure produces data unusable by DEP for the fulfillment of DQOs, or if the procedure produces data that are not comparable to or are otherwise incompatible for use with existing DEP data generated by other approved procedures.
- Approval will not be granted if the alternative or modified procedure is shown to produce data at obvious risk of being invalidated according to Rule 62-160.670, F.A.C., or according to data validation criteria established by DEP as specific DQOs for the affected project(s), DEP program activities, or data uses.

Comparison Results

The sample concentrations for the three studies were within the range expected for marine waters surrounding the Florida Keys, and therefore are representative of the typical concentrations measured for the WQMP and the Canal Project. Results of statistical tests are provided in Table 3, and results for each analyte are described in the sections below. All data and RPD results are in Appendix A.

Table 3. Results of statistical comparisons between DEP SOP-required preservation and alternative preservation for WQMP. These analyses do not include data pairs for which either result was lower than the applicable MDL. Bland-Altman tolerance limits were only calculated if the paired results were statistically significant.

Analyte	Dataset Analyzed	Normally Distributed	Paired T-test Result (α<0.05)	Paired Wilcoxon Result (α<0.05)	Bland & Altman's Limits of Agreement (mg/L)
NO_2+NO_3	2014	Yes	Significantly different (p=0.000547)	N/A	-0.0011 +/- 0.0023
NH_4	2014	Yes	Significantly different (p=0.002915)	N/A	0.0026 +/- 0.0073
NO_2	2008	No	N/A	Not significantly different (p=0.5416)	Not conducted
SRP	2008	Yes	Not significantly different (p= 0.5166 [0.1122 if outlier excluded])	N/A	Not conducted
TN	2008	No	N/A	Not significantly different (p=0.1796)	Not conducted
TN	2014	Yes	Significantly different (p=0.000000001)	N/A	0.0774 +/- 0.0841
TN	2020	No	N/A	Not significantly different (p=0.77)	Not conducted
TP	2014	No	N/A	Not significantly different (p=0.4105)	Not conducted
TOC	2008	No	N/A	Not significantly different (p=0.5459)	Not conducted

Nitrate + Nitrite

Results for NO_2+NO_3 are shown in Figure 1. There is generally good agreement between sample pairs, but the paired results for the 2014 study were statistically significantly different. The Bland & Altman limits of agreement show that the SERC preservation method will typically produce results that range from 0.001 mg/L lower to 0.003 mg/L higher than the DEP SOP-required preservation method (Figure 2). However, on average, the alternative preservation method yielded results that were 0.001 mg/L higher NO_2+NO_3 than the DEP SOP-required preservation method.

For the 2014 study, RPDs were < 20% for 18 of 32 sample pairs, < 30% for 25 of 32 sample pairs, and <40% for 31 of 32 sample pairs. Most of the results (28 of 32 sample pairs in 2014 and 16 of 20 sample pairs in 2008) were below the lab PQL, which is the range where somewhat lower precision is expected.

Although the 2014 paired results were statistically significantly different, there is very low risk that the magnitude of the difference will impact DEAR's use of the data. There is currently not a specific water quality criterion for NO_2+NO_3 in marine waters, and NO_2+NO_3 comprises a small portion of the TN in marine waters surrounding the Florida Keys. Greater variability is expected in the range of low-level detections, and these are the typical concentrations encountered in the Florida Keys. Therefore, the datasets are sufficiently comparable to support limited-use approval of the SERC alternative preservation method for NO_2+NO_3.

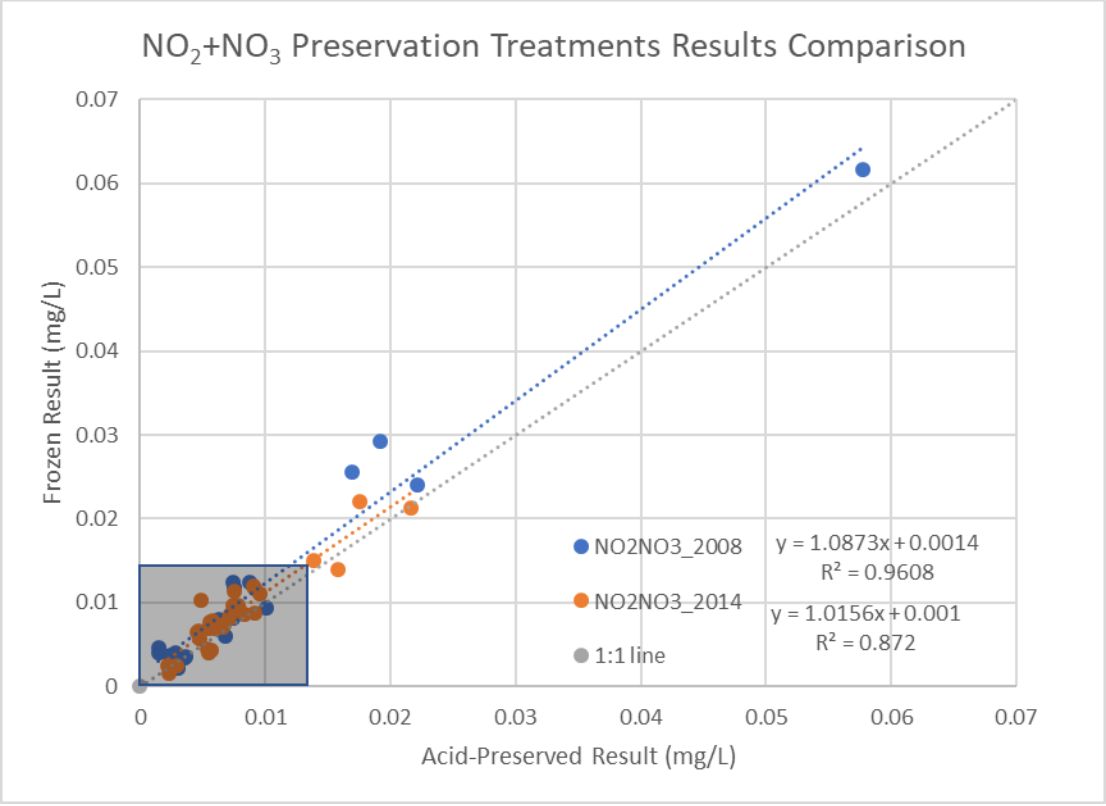

Figure 1. 2008 and 2014 study comparison study results for NO_2+NO_3. The gray box is the area below the 2014 PQL. None of the 2014 results were below the applicable MDL. Linear regression lines, equations, and R^2 are displayed for each dataset. The line of equality is included for reference.

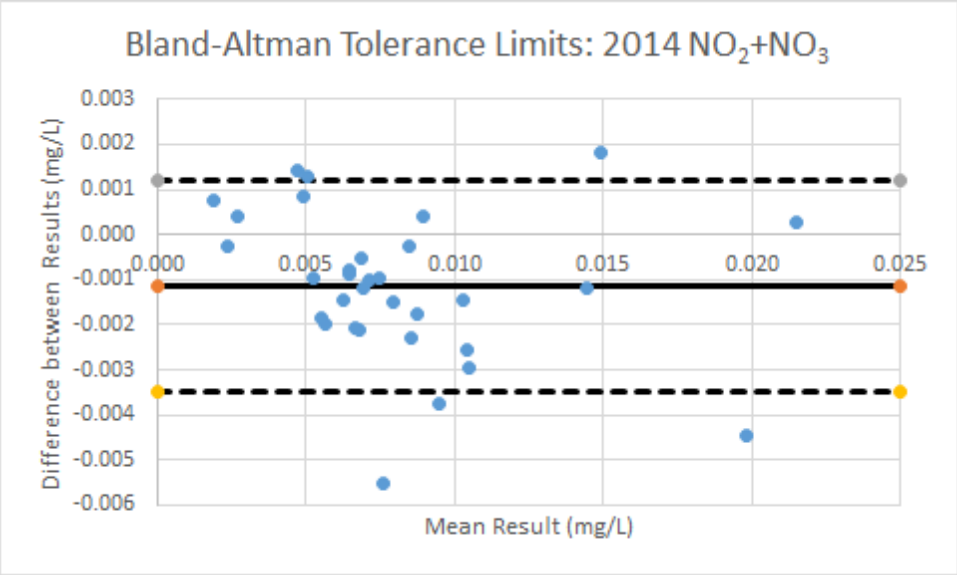

Figure 2. Bland & Altman tolerance limits for NO_2+NO_3 results from the 2014 comparison study. The plot represents the mean result on the x-axis and the difference between the results on the y-axis for each sample pair. The difference was calculated as the result from the DEP SOP method minus the result from the alternative method. The solid line is the mean difference, and the dashed lines represent the upper and lower tolerance limits, or the 95% confidence interval of the differences.

Ammonia/Ammonium

Results for NH_3/NH_4 are shown in Figure 3. There is generally good agreement between sample pairs, but the paired results were statistically significantly different. The Bland & Altman limits of agreement show that the SERC preservation method will typically produce results that range from 0.010 mg/L lower to 0.005 mg/L higher than the DEP SOP-required preservation method (Figure 4). However, on average, the alternative preservation method yielded results that were 0.0025 mg/L lower NH_3/NH_4 than the DEP SOP-required preservation method.

For the 2014 study, RPDs were < 20% for 24 of 32 sample pairs, < 30% for 29 of 32 sample pairs, and <40% for 30 of 32 sample pairs. Most of the results were greater than the lab PQL, so good precision is expected. The 2008 study results showed more bias, with the alternative method preservation method yielding consistently lower results.

Although the 2014 paired results were statistically significantly different, there is low risk that the magnitude of the difference will impact DEAR's use of the data. There is currently not a specific water quality criterion for NH_3/NH_4 in marine waters, and the range of differences observed would comprise a small portion of the TN in marine waters surrounding the Florida Keys. Therefore, the datasets are sufficiently comparable to support limited-use approval of the SERC alternative preservation method for NH_3/NH_4.

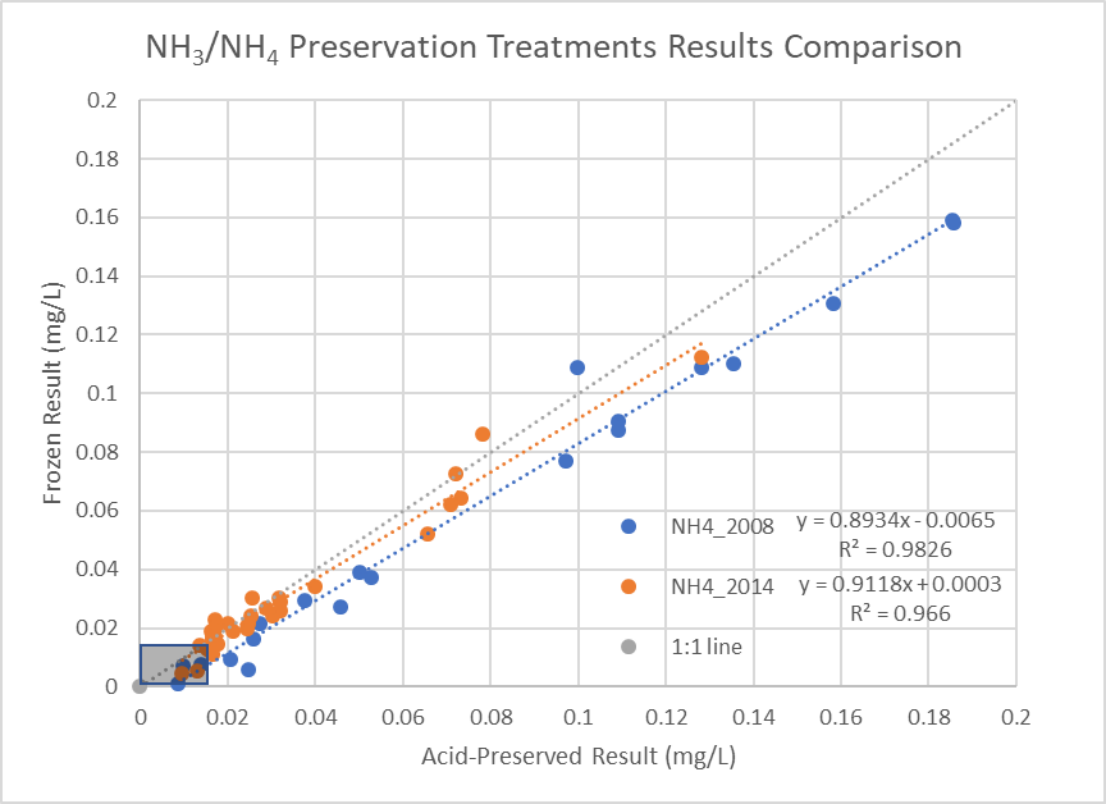

Figure 3. 2008 and 2014 study comparison study results for NH_4. The gray box is the area below the 2014 PQL. None of the 2014 results were below the applicable MDL. Linear regression lines, equations, and R^2 are displayed for each dataset. The line of equality is included for reference.

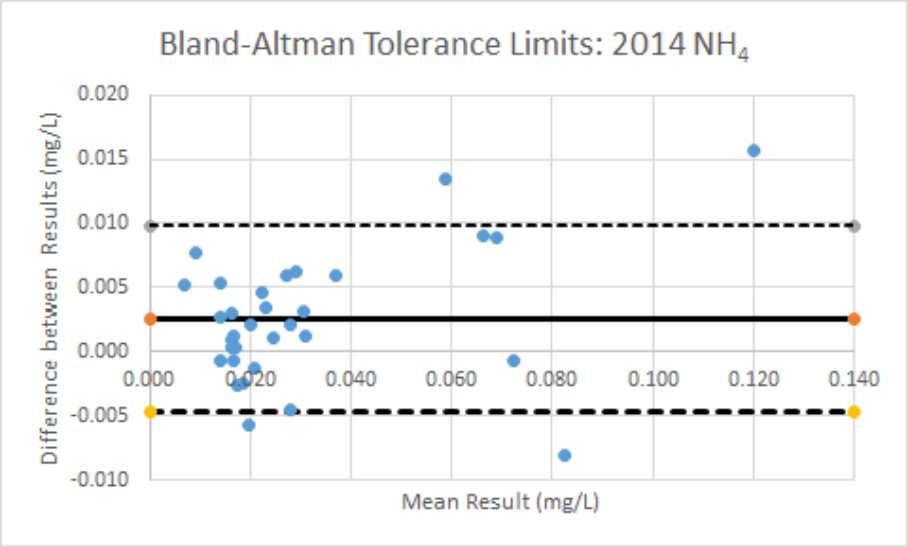

Figure 4. Bland & Altman tolerance limits for NH_4 results from the 2014 comparison study. The plot represents the mean result on the x-axis and the difference between the results on the y-axis for each sample pair. The difference was calculated as the result from the DEP SOP method minus the result from the alternative method. The solid line is the mean difference, and the dashed lines represent the upper and lower tolerance limits, or the 95% confidence interval of the differences.

Nitrite

Results for NO_2 are shown in Figure 5. There is good agreement between sample pairs, and the paired results were not statistically significantly different. Seventy-five percent (15 of 20) of the results were less than the lab PQL, and six of those were less than the lab MDL. For the 2008 study, RPDs for sample pairs greater than the MDL were < 20% for 13 of 14 sample pairs and <40% for the remaining sample pair.

This dataset is smaller than is generally required for alternative field method comparisons (DEP SOP FA 2240, section 4). However, the sample pairs were not statistically significantly different, and the concentrations in the study reflect the range expected in the Florida Keys; therefore, the datasets are sufficiently comparable to support limited-use approval of the SERC alternative preservation method for NO_2.

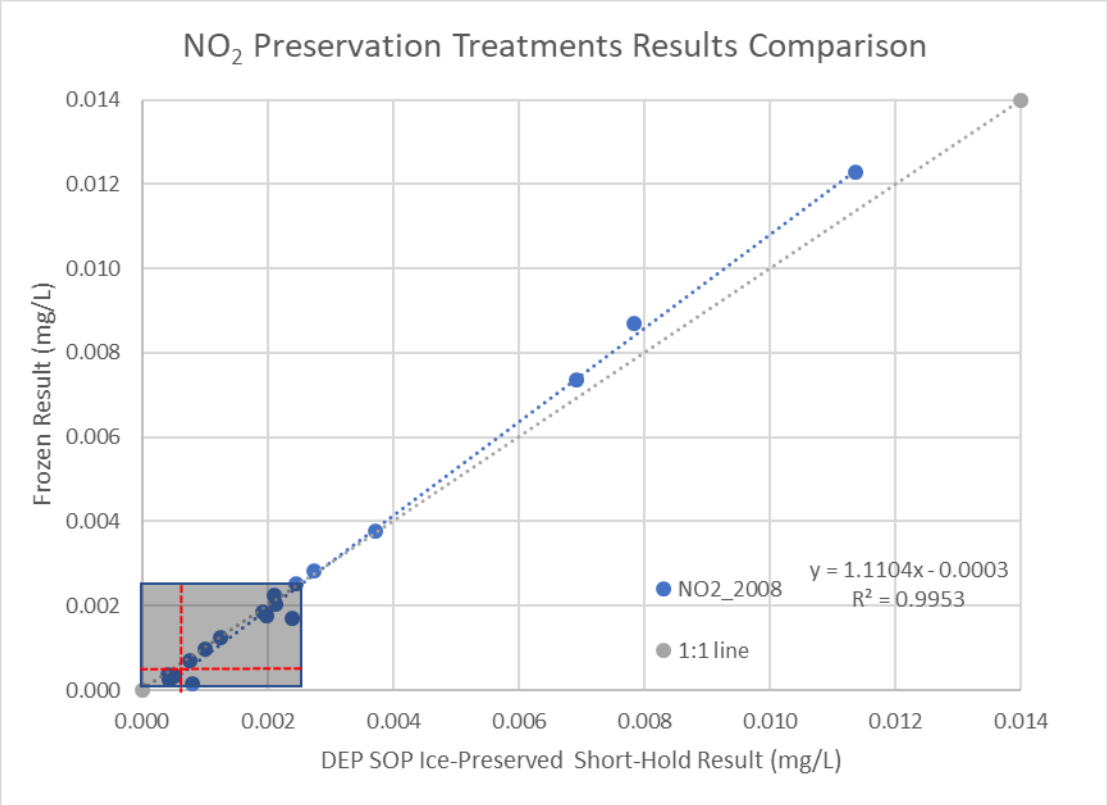

Figure 5. 2008 study comparison study results for NO_2. The gray box is the area below the 2008 PQL, and the red lines show the 2008 MDL. The linear regression line, equation, and R^2 are displayed. The line of equality is included for reference.

Soluble Reactive Phosphorus

Results for SRP are shown in Figure 6. All of the results except for one outlier were less than the lab PQL, so this comparison dataset lies completely in a zone of low precision. The sample pairs greater than the MDL were not statistically significantly different with or without the outlier pair. For the 10 sample pairs greater than the MDL, RPDs were < 20% for 4, between 20% and 30% for 2, and > 40% for 4 sample pairs. These results reflect low precision, which can be expected for these low-level detections. Both methods yielded similarly low levels of detection; however, the dataset does not contain sufficient range of values for alternative method approval.

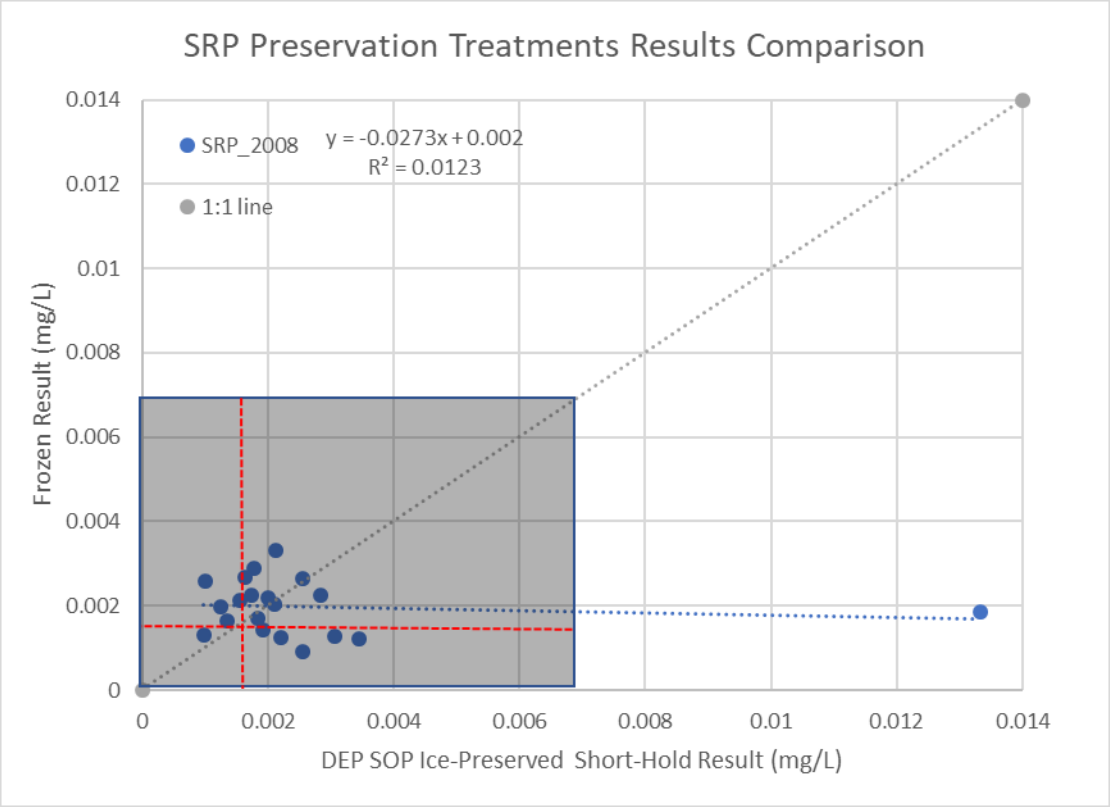

Figure 6. 2008 study comparison study results for SRP. The gray box is the area below the 2008 PQL, and the red line show the 2008 MDL. The linear regression line, equation, and R^2 are displayed. The line of equality is included for reference.

Total Nitrogen

Results for TN are shown in Figure 7. Paired results from the 2008 and 2020 studies were not statistically significantly different and plotted consistently around the line of equality (Figure 7). For the 2008 study, RPDs were < 20% for 18 of 20 sample pairs and <30% for the remaining 2 sample pairs. For the 2020 study, RPDs were ≤20% for all 30 sample pairs. The 2008 and 2020 datasets are sufficiently comparable to support limited-use approval of the SERC alternative preservation method for TN.

The paired results from the 2014 study were statistically significantly different, and there is a clear bias, with the SERC alternative preservation method yielding consistently lower results. The Bland & Altman

limits of agreement show that the SERC preservation method produced results that were up to 0.16 mg/L lower than the DEP SOP-required preservation method (Figure 8), which would be an unacceptable difference if data were used by DEP for waterbody assessment. Most of the 2014 results were greater than the lab PQL, so the results are in a zone of expected good precision. RPDs were < 20% for 15 of 32 sample pairs, < 30% for 23 of 32 sample pairs, and < 40% for 28 of 32 sample pairs.

To investigate whether the low biased results of the 2014 comparison study were isolated to that study or an indication of a systemic error in the lab, FIU analyzed their long-term data set (2008 to 2019) using a one-way ANOVA analysis. Results indicated statistically significant lower results for TN over the years spanning 2013 to 2015, but the standard deviations for that period did not differ from the overall dataset. Further, other analytes exhibited a similar pattern, suggesting the low values in 2014 were caused by environmental variation, rather than laboratory bias. Reasons for the differences between methods for the 2014 study are unknown, but the 2014 results do not appear to be representative of the preservation method effects based on the acceptable comparability of the 2008 and 2020 studies.

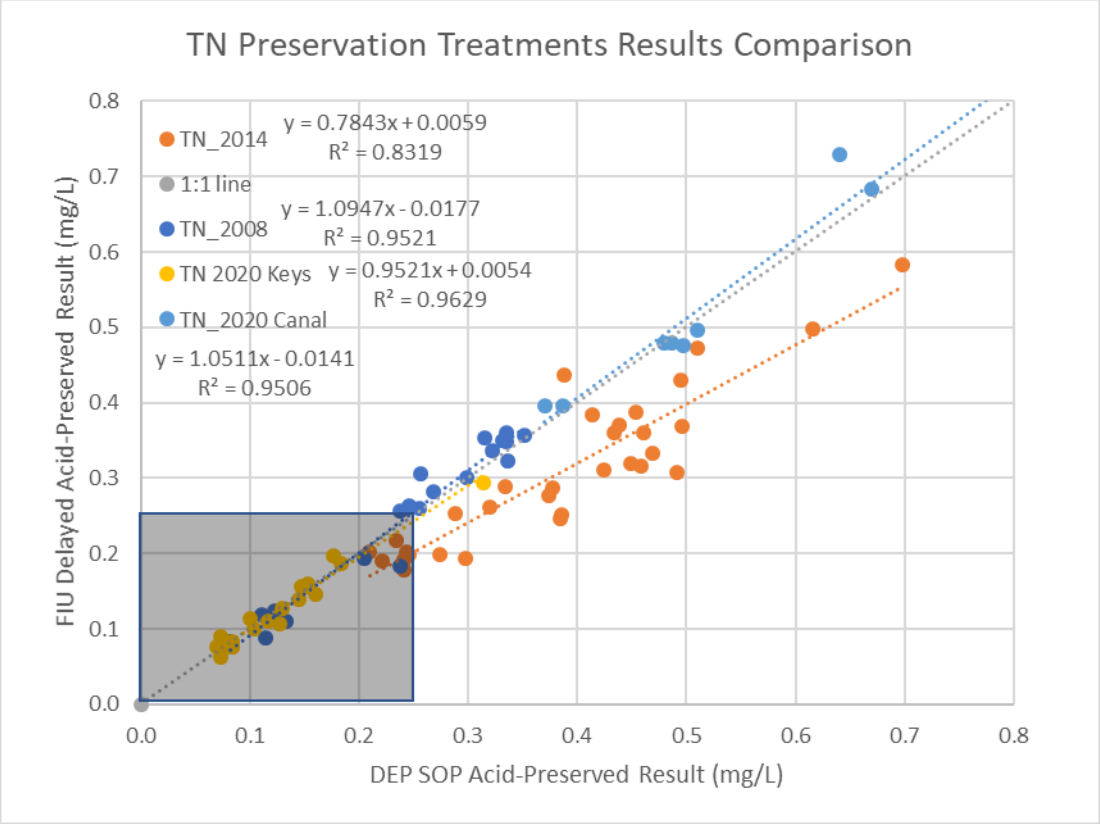

Figure 7. 2008, 2014, and 2020 study comparison study results for TN. The gray box is the area below the 2014 PQL. No results were below the applicable MDL. The linear regression lines, equations, and R^2 are displayed. The line of equality is included for reference. Note that in 2008, HCl was used instead of H_2SO_4 for the DEP SOP acid-preservation treatment.

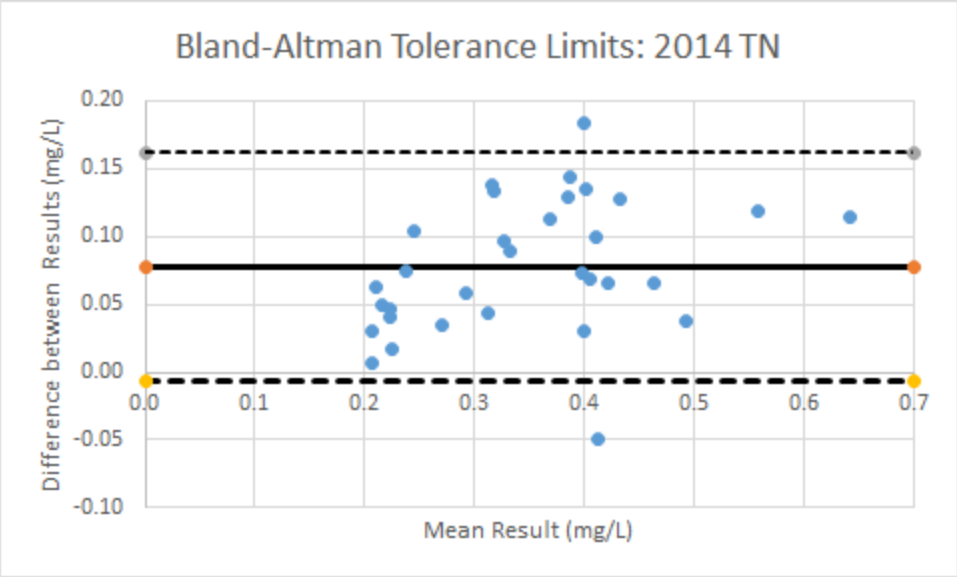

Figure 8. Bland & Altman tolerance limits for TN results from the 2014 comparison study. The plot represents the mean result on the x-axis and the difference between the results on the y-axis for each sample pair. The difference was calculated as the result from the DEP-SOP method minus the result from the alternative method. The solid line is the mean difference, and the dashed lines represent the upper and lower tolerance limits, or the 95% confidence interval of the differences.

Total Phosphorus

Results for TP are shown in Figure 9. There is good agreement between sample pairs, and the paired results were not statistically significantly different. All of the results were greater than the lab PQL, so the results are in a zone of expected good precision. For this 2014 study, all RPDs were < 20%. The 2008 results also show good agreement between sample pairs.

The 2014 paired results were not statistically significantly different, and the precision within sample pairs met expectations for lab duplicate results for all pairs. Therefore, the datasets are sufficiently comparable to support limited-use approval of the SERC alternative preservation method for TP.

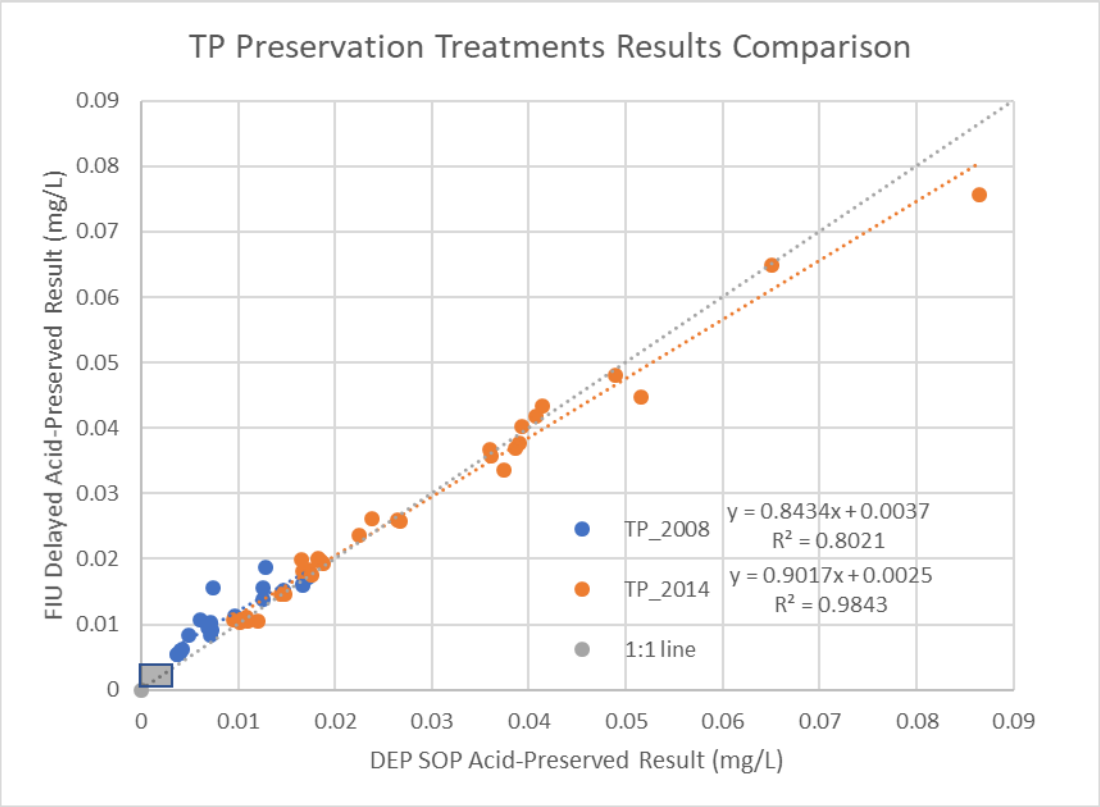

Figure 9. 2008 and 2014 study comparison study results for TP. The gray box is the area below the 2014 PQL. None of the 2014 results were below the applicable MDL. Linear regression lines, equations, and R^2 are displayed for each dataset. The line of equality is included for reference.

Total Organic Carbon

Results for TOC are shown in Figure 10. There is good agreement between sample pairs, and the paired results were not statistically significantly different. All of the results were greater than the lab PQL, so the results are in a zone of expected good precision. For the 2008 study, RPDs for samples pairs were < 20% for 18 of 20 sample pairs, and < 40% for the remaining two sample pairs.

The paired results were not statistically significantly different, and the precision within sample pairs met expectations for lab duplicate results for nearly all pairs. Therefore, the datasets are sufficiently comparable to support limited-use approval of the SERC alternative preservation method for TOC.

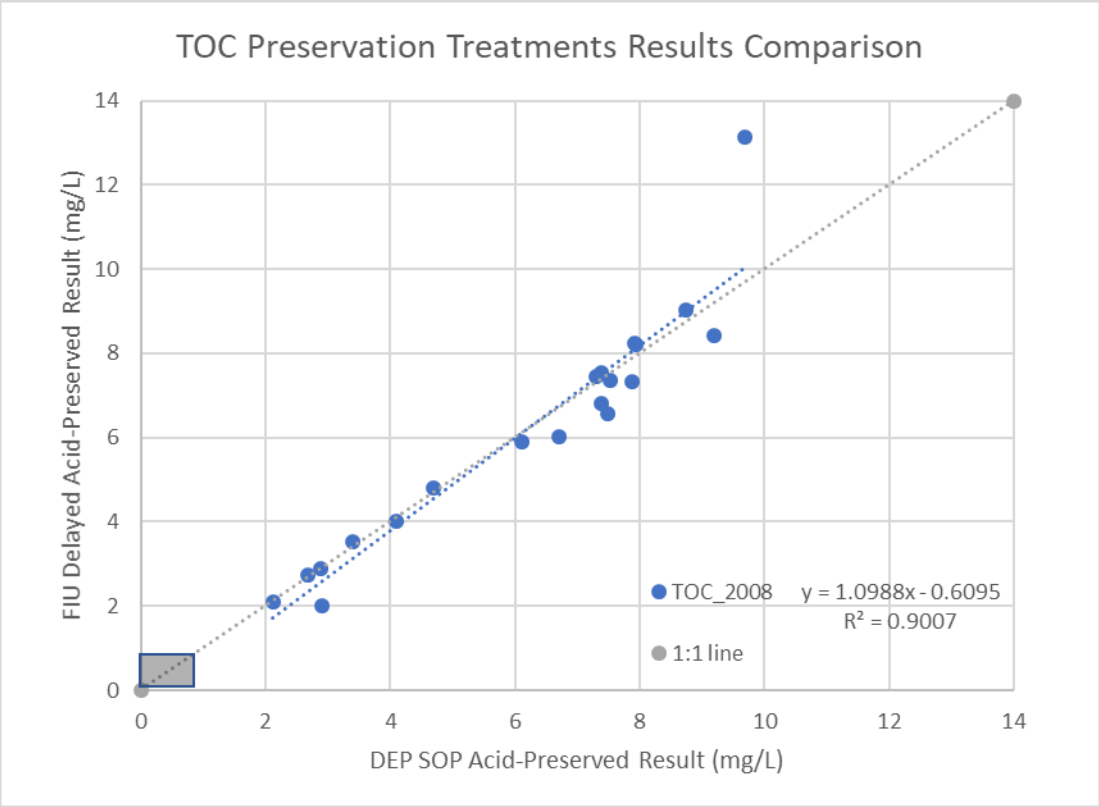

Figure 10. 2008 study comparison study results for TOC. The gray box is the area below the 2008 PQL. None of the 2014 results were below the applicable MDL. The linear regression line, equation, and R^2 are displayed. The line of equality is included for reference.

Conclusions

The nutrient concentrations found in the study were typical for these sampling programs, so the department was able to make a determination regarding approval or disapproval of alternative methods. Sample pairs were not statistically significantly different for NO_2, TP, and TOC, and plots of those datasets showed that paired results were close to the line of equality. For SRP, nearly all data points were either below the MDL or were very low detections; therefore, the dataset was not sufficient to support alternative method approval. Sample pairs were statistically significantly different for NO_2+NO_3 and NH_3/NH_4, but the differences were deemed acceptable for the intended uses of the data. In the 2014 study, sample pairs were statistically significantly different for TN, and the differences were deemed unacceptable for the intended uses of the data. However, sample pairs were not statistically significantly different for TN in the 2008 or 2020 studies and a low bias was not indicated in the environmental data; therefore, DEP concluded that the 2014 study was not representative of the differences between preservation methods. Therefore, alternative holding time and preservation methods are approved for NO_2 and alternative preservation methods are approved for NO_2+NO_3, NH_3/NH_4, TN, TP, and TOC, but are not approved for SRP.

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Appendix A

Dataset from 2020 comparison study, FIU TN FDEP Method vs. FIU Method

Site	Sample ID TN FDEP Method	TN FDEP Method	Sample ID TN FIU Method	TN FIU Method	Difference TN FDEP - TN FIU	RPD
Keys	AC84926	0.313	AC84378	0.293	0.020	6.6
Keys	AC84927	0.147 *	AC84379	0.157 *	-0.010	6.6
Keys	AC84928	0.117 *	AC84380	0.110 *	0.007	5.9
Keys	AC84929	0.083 *	AC84381	0.077 *	0.007	8.3
Keys	AC84914	0.127 *	AC84386	0.107 *	0.020	17.1
Keys	AC84915	0.130 *	AC84387	0.127 *	0.003	2.6
Keys	AC84916	0.103 *	AC84388	0.100 *	0.003	3.3
Keys	AC84917	0.070 *	AC84389	0.077 *	-0.007	9.1
Keys	AC84918	0.145 *	AC84390	0.140 *	0.005	3.5
Keys	AC84919	0.153 *	AC84391	0.160 *	-0.007	4.3
Keys	AC84920	0.153 *	AC84392	0.157 *	-0.003	2.2
Keys	AC84921	0.160 *	AC84393	0.147 *	0.013	8.7
Keys	AC84922	0.073 *	AC84394	0.063 *	0.010	14.6
Keys	AC84923	0.077 *	AC84395	0.077 *	0.000	0.0
Keys	AC84930	0.083 *	AC84402	0.083 *	0.000	0.0
Keys	AC84931	0.083 *	AC84403	0.077 *	0.007	8.3
Keys	AC84924	0.183 *	AC84406	0.187 *	-0.003	1.8
Keys	AC84925	0.177 *	AC84407	0.197 *	-0.020	10.7
Keys	AC84932	0.100 *	AC84532	0.113 *	-0.013	12.5
Keys	AC84933	0.073 *	AC84533	0.090 *	-0.017	20.4
Canal	AC84852	0.387	AC85988	0.397	-0.010	2.6
Canal	AC84853	0.370	AC85989	0.397	-0.027	7.0
Canal	AC84856	0.510	AC85990	0.497	0.013	2.6
Canal	AC84857	0.487	AC85991	0.480	0.007	1.4
Canal	AC84860	0.480	AC85992	0.480	0.000	0.0
Canal	AC84861	0.497	AC85993	0.477	0.020	4.1
Canal	AC84864	0.900	AC85994	0.860	0.040	4.5
Canal	AC84865	0.850	AC85995	0.945	-0.095	10.6
Canal	AC84868	0.670	AC85996	0.683	-0.013	2.0
Canal	AC84869	0.640	AC85997	0.730	-0.090	13.1

* Value is below the PQL; PQL: 0.25 mg/L

** Value is below the MDL; MDL: 0.04 mg/L

Dataset from 2014 comparison study, FIU NO2NO3 acidified vs. not acidified

Row Labels	N+NA	N+N	Difference FIU_N+NA-FIU_N+N	RPD
266 A-B	0.0029 *	0.0025 *	0.0004	14.22
266 A-S	0.0022 *	0.0025 *	-0.0002	10.45
266 B-B	0.0023 *	0.0015 *	0.0008	39.31
266 B-S	0.0047 *	0.0057 *	-0.0010	18.73
277 A-B	0.0048 *	0.0104 *	-0.0055	72.52
277 A-S	0.0079 *	0.0096 *	-0.0017	19.97
277 B-B	0.0072 *	0.0087 *	-0.0015	18.80
277 B-S	0.0074 *	0.0097 *	-0.0023	26.92
278 A-B	0.0176	0.0220	-0.0044	22.42
278 A-S	0.0216	0.0213	0.0003	1.35
278 B-B	0.0091 *	0.0117 *	-0.0026	24.52
278 B-S	0.0076 *	0.0113 *	-0.0038	39.83
282 A-B	0.0158	0.0140	0.0018	12.19
282A-S	0.0090 *	0.0120 *	-0.0029	28.08
287 A-B	0.0055 *	0.0040 *	0.0014	29.95
287 A-S	0.0056 *	0.0077 *	-0.0021	31.09
290 A-B	0.0055 *	0.0070 *	-0.0014	23.16
290 A-S	0.0070 *	0.0080 *	-0.0009	12.62
290 B-B	0.0096 *	0.0110 *	-0.0014	14.07
290 B-S	0.0092 *	0.0088 *	0.0004	4.60
293 A-B	0.0053 *	0.0045 *	0.0009	17.31
293 A-S	0.0057 *	0.0044 *	0.0013	26.01
458 A-B	0.0060 *	0.0069 *	-0.0009	13.55
458 A-S	0.0066 *	0.0076 *	-0.0010	13.93
459 A-B	0.0064 *	0.0076 *	-0.0012	17.34
459 A-S	0.0061 *	0.0069 *	-0.0008	12.34
459 B-B	0.0066 *	0.0071 *	-0.0005	7.43
459 B-S	0.0058 *	0.0079 *	-0.0021	30.78
472 A-B	0.0084 *	0.0086 *	-0.0003	3.00
472 A-S	0.0139	0.0151	-0.0012	8.25
472 B-B	0.0047 *	0.0066 *	-0.0020	34.97
472 B-S	0.0046 *	0.0065 *	-0.0019	33.82

* Value is below the PQL; PQL: 0.0134 mg/L

** Value is below the MDL; MDL: 0.0008 mg/L

Dataset from 2014 comparison study, FIU NH4 acidified vs. not acidified

Row Labels	NH4-A	NH4-N	Difference NH4-A - NH4-N	RPD
266 A-B	0.0211	0.0189	0.0021	10.73
266 A-S	0.0096 *	0.0044 *	0.0052	74.71
266 B-B	0.1280	0.1124	0.0156	12.99
266 B-S	0.0783	0.0863	-0.0080	9.75
277 A-B	0.0302	0.0243	0.0059	21.63
277 A-S	0.0317	0.0305	0.0013	4.05
277 B-B	0.0289	0.0268	0.0021	7.49
277 B-S	0.0320	0.0289	0.0031	10.16
278 A-B	0.0166	0.0112 *	0.0054	38.48
278 A-S	0.0166	0.0157	0.0010	5.89
278 B-B	0.0253	0.0242	0.0011	4.39
278 B-S	0.0256	0.0301	-0.0045	16.12
282 A-B	0.0734	0.0645	0.0089	12.88
282A-S	0.0720	0.0728	-0.0007	1.04
287 A-B	0.0131 *	0.0054 *	0.0077	82.79
287 A-S	0.0245	0.0199	0.0046	20.71
290 A-B	0.0322	0.0259	0.0062	21.46
290 A-S	0.0399	0.0340	0.0059	15.91
290 B-B	0.0709	0.0620	0.0090	13.53
290 B-S	0.0656	0.0521	0.0135	22.90
293 A-B	0.0171	0.0227	-0.0057	28.43
293 A-S	0.0248	0.0213	0.0035	15.17
458 A-B	0.0178	0.0147	0.0030	18.69
458 A-S	0.0172	0.0160	0.0013	7.61
459 A-B	0.0161	0.0188	-0.0026	15.07
459 A-S	0.0165	0.0171	-0.0006	3.85
459 B-B	0.0201	0.0213	-0.0012	5.89
459 B-S	0.0166	0.0162	0.0004	2.18
472 A-B	0.0171	0.0168	0.0003	1.65
472 A-S	0.0172	0.0197	-0.0025	13.72
472 B-B	0.0136 *	0.0142	-0.0006	4.47
472 B-S	0.0155	0.0127 *	0.0027	19.24

* Value is below the PQL; PQL: 0.014 mg/L

** Value is below the MDL; MDL: 0.0015 mg/L

Dataset from 2014 comparison study, FIU TN acidified vs. not acidified

Row Labels	FIU_TNA	FIU_TN	Difference FIU_TNA-FIU_TN	RPD
266 A-B	0.4963	0.3689	0.1274	29.45
266 A-S	0.4685	0.3339	0.1346	33.56
266 B-B	0.6978	0.5837	0.1141	17.81
266 B-S	0.6160	0.4977	0.1183	21.25
277 A-B	0.4242	0.3114	0.1128	30.66
277 A-S	0.4910	0.3083	0.1828	45.74
277 B-B	0.4484	0.3201	0.1284	33.40
277 B-S	0.3742	0.2778	0.0965	29.59
278 A-B	0.3839	0.2461 *	0.1378	43.74
278 A-S	0.3852	0.2515	0.1337	42.01
278 B-B	0.3199	0.2623	0.0576	19.78
278 B-S	0.3335	0.2895	0.0440	14.11
282 A-B	0.3884	0.4372	-0.0488	11.83
282A-S	0.4141	0.3843	0.0299	7.48
287 A-B	0.4334	0.3601	0.0733	18.48
287 A-S	0.3771	0.2878	0.0893	26.87
290 A-B	0.4602	0.3602	0.1000	24.37
290 A-S	0.4532	0.3874	0.0658	15.64
290 B-B	0.4951	0.4298	0.0653	14.12
290 B-S	0.5104	0.4726	0.0378	7.69
293 A-B	0.4585	0.3156	0.1430	36.93
293 A-S	0.4388	0.3709	0.0678	16.76
458 A-B	0.2406 *	0.1915 *	0.0492	22.76
458 A-S	0.2096 *	0.2030 *	0.0066	3.19
459 A-B	0.2459 *	0.1991 *	0.0469	21.07
459 A-S	0.2339 *	0.2167 *	0.0171	7.61
459 B-B	0.2405 *	0.1785 *	0.0620	29.58
459 B-S	0.2209 *	0.1910 *	0.0299	14.51
472 A-B	0.2975	0.1932 *	0.1042	42.49
472 A-S	0.2737	0.1996 *	0.0741	31.30
472 B-B	0.2436 *	0.2029 *	0.0408	18.25
472 B-S	0.2882	0.2534	0.0348	12.85

* Value is below the PQL; PQL: 0.25 mg/L

** Value is below the MDL; MDL: 0.04 mg/L

Dataset from 2014 comparison study, FIU TP acidified vs. not acidified

Row Labels	FIU_TPA	FIU_TP	Difference FIU_TPA-FIU_TP	RPD
266 A-B	0.0490	0.0480	0.0009	1.87
266 A-S	0.0407	0.0418	-0.0011	2.61
266 B-B	0.0864	0.0757	0.0108	13.28
266 B-S	0.0650	0.0648	0.0001	0.23
277 A-B	0.0173	0.0183	-0.0010	5.44
277 A-S	0.0176	0.0176	0.0000	0.18
277 B-B	0.0183	0.0201	-0.0017	9.10
277 B-S	0.0182	0.0198	-0.0016	8.64
278 A-B	0.0167	0.0181	-0.0013	7.65
278 A-S	0.0165	0.0200	-0.0035	19.22
278 B-B	0.0239	0.0262	-0.0023	9.17
278 B-S	0.0225	0.0236	-0.0011	4.74
282 A-B	0.0267	0.0257	0.0010	3.67
282A-S	0.0265	0.0259	0.0005	2.10
287 A-B	0.0362	0.0358	0.0004	0.98
287 A-S	0.0386	0.0370	0.0016	4.26
290 A-B	0.0390	0.0377	0.0013	3.32
290 A-S	0.0393	0.0402	-0.0010	2.45
290 B-B	0.0414	0.0434	-0.0020	4.75
290 B-S	0.0516	0.0447	0.0069	14.34
293 A-B	0.0374	0.0337	0.0037	10.51
293 A-S	0.0360	0.0366	-0.0006	1.72
458 A-B	0.0110	0.0105	0.0005	4.63
458 A-S	0.0108	0.0111	-0.0003	2.94
459 A-B	0.0121	0.0106	0.0015	13.03
459 A-S	0.0102	0.0103	0.0000	0.27
459 B-B	0.0101	0.0107	-0.0006	5.56
459 B-S	0.0095	0.0106	-0.0011	11.09
472 A-B	0.0186	0.0197	-0.0011	5.98
472 A-S	0.0188	0.0193	-0.0005	2.67
472 B-B	0.0145	0.0145	0.0000	0.30
472 B-S	0.0149	0.0147	0.0002	1.09

* Value is below the PQL; PQL: 0.0031 mg/L

** Value is below the MDL; MDL: 0.0003 mg/L

Dataset from 2008 comparison study, FIU NO_2+NO_3 acidified vs. non-acidified

Sample ID	NN_1		NN_3		RPD
1	0.0065	*	0.0068	*	4.6
2	0.0074	*	0.0081	*	8.3
3	0.0169	*	0.0255		40.6
4	0.0192		0.0293		41.5
5	0.0087	*	0.0124	*	35.3
6	0.0063	*	0.0080	*	23.5
7	0.0076	*	0.0118	*	44.1
8	0.0074	*	0.0125	*	50.8
9	0.0101	*	0.0094	*	6.8
10	0.0068	*	0.0059	*	13.9
11	0.0024	**	0.0037	**	41.0
12	0.0015	**	0.0047	*	103.5
13	0.0015	**	0.0040	*	88.5
14	0.0222		0.0240		8.1
15	0.0578		0.0616		6.4
16	0.0037	**	0.0036	**	2.5
17	0.0030	**	0.0033	**	10.2
18	0.0030	**	0.0022	**	32.4
19	0.0035	**	0.0034	**	3.2
20	0.0028	**	0.0041	*	36.5
	Acid/ice within 28 days		Frozen within 48 hours from freezer removal		

* Value is below the PQL; PQL: 0.0172 mg/L

** Value is below the MDL; MDL: 0.0039 mg/L

Dataset from 2008 comparison study, FIU NH_4 acidified vs. non-acidified

Sample ID	NH4_1	NH4_3	RPD
1	0.0527	0.0374	34
2	0.0998	0.1088	9
3	0.1855	0.1589	15
4	0.1856	0.1580	16
5	0.1353	0.1103	20
6	0.1582	0.1308	19
7	0.1282	0.1089	16
8	0.1093	0.0907	19
9	0.1090	0.0875	22
10	0.0972	0.0770	23
11	0.0274	0.0215 *	24
12	0.0260	0.0163 *	46
13	0.0375	0.0294	24
14	0.0501	0.0392	25
15	0.0459	0.0272	51
16	0.0206 *	0.0093 *	75
17	0.0140 *	0.0075 *	60
18	0.0086 *	0.0009 **	163
19	0.0248	0.0058 *	124
20	0.0098 *	0.0070 *	33
	Acid/ice within 28 days	Frozen within 48 hours from freezer removal	

* Value is below the PQL; PQL: 0.0217 mg/L

** Value is below the MDL; MDL: 0.0049 mg/L

Dataset from 2008 comparison study, FIU NO2 analyzed w/in 48 hours vs. Frozen

Sample ID	NO2_2		NO2_3		RPD
1	0.0013	*	0.0012	*	0.6
2	0.0021	*	0.0022	*	5.8
3	0.0069		0.0074		6.3
4	0.0078		0.0087		10.6
5	0.0027		0.0028		4.0
6	0.0020	*	0.0018	*	12.0
7	0.0019	*	0.0018	*	4.1
8	0.0024		0.0017	*	32.8
9	0.0010	*	0.0010	*	1.4
10	0.0008	*	0.0007	*	8.6
11	0.0005	**	0.0003	**	39.4
12	0.0004	**	0.0003	**	38.1
13	0.0004	**	0.0004	**	12.4
14	0.0037		0.0038		1.3
15	0.0114		0.0123		7.7
16	0.0021	*	0.0020	*	5.1
17	0.0008	*	0.0002	**	136.2
18	0.0004	**	0.0003	**	32.9
19	0.0004	**	0.0002	**	57.0
20	0.0024		0.0025		2.8
	Ice within 48 hours		Frozen within 48 hours from freezer removal		

* Value is below the PQL; PQL: 0.0024 mg/L

** Value is below the MDL; MDL: 0.0006 mg/L

Dataset from 2008 comparison study, FIU SRP analyzed within 48 hours vs. frozen

Sample ID	SRP_2		SRP_3		RPD
1	0.001829	*	0.001705	*	7.02
2	0.003069	*	0.001271	**	82.86
3	0.002558	*	0.000899	**	95.96
4	0.002	*	0.002186	*	8.89
5	0.002201	*	0.001256	**	54.71
6	0.002093	*	0.002031	*	3.01
7	0.003457	*	0.001225	**	95.36
8	0.013328		0.001845	*	151.37
9	0.00155	**	0.002139	*	31.93
10	0.001922	*	0.001426	**	29.63
11	0.001349	**	0.001643	*	19.69
12	0.000977	**	0.001302	**	28.57
13	0.001008	**	0.002589	*	87.93
14	0.001256	**	0.001984	*	44.98
15	0.001628	*	0.002682	*	48.92
16	0.002124	*	0.003317	*	43.87
17	0.002837	*	0.002248	*	23.17
18	0.001736	*	0.002248	*	25.68
19	0.002544	*	0.002635	*	3.53
20	0.001783	*	0.002899	*	47.68
	Ice within 48 hours		Frozen within 48 hours from freezer removal		

* Value is below the PQL; PQL: 0.0068 mg/L

** Value is below the MDL; MDL: 0.0016 mg/L

Dataset from 2008 comparison study, FIU TN acidified vs. non-acidified

Sample ID	TN_2ppm		TN_3ppm		RPD
1	0.307		0.256		18.1
2	0.261		0.255		2.1
3	0.300		0.299		0.4
4	0.348		0.335		3.6
5	0.357		0.351		1.6
6	0.336		0.322		4.2
7	0.351		0.332		5.5
8	0.361		0.335		7.5
9	0.353		0.315		11.5
10	0.323		0.337		4.2
11	0.282		0.268		5.0
12	0.263		0.246		6.8
13	0.257		0.238		7.8
14	0.183	*	0.238		26.1
15	0.194	*	0.204	*	5.2
16	0.111	*	0.133	*	18.2
17	0.124	*	0.122	*	1.8
18	0.083	*	0.082	*	1.5
19	0.119	*	0.111	*	7.4
20	0.088	*	0.114	*	25.3
	Ice, within 28 days		Ice, prep (HCl) within 48 hours		

* Value is below the PQL; PQL: 0.22 mg/L

** Value is below the MDL; MDL: 0.05 mg/L

Dataset from 2008 comparison study, FIU TP acidified vs. non-acidified

Sample ID	TP_1ppm		TP_2ppm		RPD
1	0.0049	*	0.0084		51.5
2	0.0040	*	0.0057	*	33.6
3	0.0037	*	0.0054	*	38.1
4	0.0037	*	0.0054	*	37.7
5	0.0040	*	0.0057	*	36.0
6	0.0042	*	0.0062	*	37.7
7	0.0062	*	0.0108		54.8
8	0.0069	*	0.0096		32.9
9	0.0072	*	0.0091		22.3
10	0.0074	*	0.0155		70.9
11	0.0072	*	0.0103		35.0
12	0.0072	*	0.0083		14.6
13	0.0096		0.0113		15.5
14	0.0126		0.0157		22.0
15	0.0172		0.0172		0.1
16	0.0167		0.0160		4.6
17	0.0167		0.0162		3.0
18	0.0125		0.0138		9.4
19	0.0147		0.0153		3.6
20	0.0128		0.0188		38.0
	Acid/ice within 28 days		Ice prep within 48 hours		

* Value is below the PQL; PQL: 0.0078 mg/L

** Value is below the MDL; MDL: 0.0019 mg/L

Dataset from 2008 comparison study, FIU TOC acidified vs. non-acidified

Sample ID	TOC_2ppm	TOC_3ppm	RPD
1	6.558	7.494	13.32
2	6.03	6.7125	10.71
3	5.907	6.1135	3.44
4	6.8145	7.387	8.06
5	8.421	9.1955	8.79
6	7.3605	7.5215	2.16
7	13.145	9.696	30.20
8	9.0215	8.7375	3.20
9	7.333	7.8815	7.21
10	7.556	7.382	2.33
11	8.2305	7.926	3.77
12	7.4395	7.296	1.95
13	8.1995	7.9325	3.31
14	4.817	4.6975	2.51
15	4.023	4.103	1.97
16	3.5145	3.397	3.40
17	2.02	2.907	36.01
18	2.8995	2.874	0.88
19	2.7345	2.6835	1.88
20	2.0995	2.1155	0.76
	Ice prep within 48 hours	Acid within 28 days	

* Value is below the PQL; PQL: 0.75 mg/L

** Value is below the MDL; MDL: 0.16 mg/L

Florida National Marine Sanctuary Survey Field Sampling Protocol (last modified 12/2/2020)

A. Field Sampling Preparation and Checklist

SERC performs water-column sampling and measurement of the physical parameters listed in Table 1.0. Preceding a sampling event, the field personnel are required to ensure that all supplies and equipment are prepared for the sampling event. Table 2.0 lists the field sampling equipment used for sampling each matrix, while the following list is an equipment checklist prepared for the sampling team.

Water sampling equipment may include plastic sample containers, syringes, filter holders, Niskin Bottle samplers, buckets, YSI multi-parameter data loggers, Seabird© Conductivity, Temperature and Depth (CTD) profilers, and LaMotte turbidity meters.

TABLE 1.0

SERC Sampling Capabilities

Parameter Group	Sample Source
Field Parameters Temperature Salinity/Conductivity Dissolved Oxygen	Surface Water/Water Column (Seabird SBE-19 or SBE-19plus seacat profiler)
PAR	Surface/Water Column (LiCor PAR irradiance Spherical Quantum Sensor) model# 193SA
CDOM	Surface/Water Column (WETLabs ECO-CDOM Sensor)
Turbidity	Surface Water/Bottom Water (LaMotte 2020 WE Turbidity meter)
Field Parameters Salinity/Conductivity	Surface Water (YSI EXO2 multi-parameter logger)

Table. 2.0. Field Sampling Equipment

Field Sampling Equipment	Construction	Use	Parameter Groups	Notes
Syringes	140 ml plastic (Latex-free)	Collection	Soluble nutrients, Suspended matter, Chlorophyll	None
Bottles	60 ml plastic (HDPE)	Collection	Soluble nutrients	Filtered samples only
Bottles	125 ml plastic (HDPE)	Collection	Total nutrients	Non-filtered
Micro-centrifuge Tubes	2.0 ml plastic (HDPE)	Filter storage	Suspended matter, Chlorophyll-a	Chlorophyll-a preserve with acetone
Wide-mouth Amber Bottle	250 ml plastic (HDPE)	Storage	Suspended matter, Chlorophyll-a	Dark storage for Chlorophyll-a containers
Bucket	2-5 gallon plastic (LDPE)	Collection	All surface parameters	None
Niskin Sampler (model 1010)	2.5 L Teflon coated PVC	Collection	All surface/bottom parameters	None
In-line Filter Holders	2.5 cm plastic (Gellman)	Filtration	Soluble nutrients, Suspended matter, Chlorophyll-a	Attach to end of syringe
Filters	2.5 cm Whitman GF/F glass fiber	Filtration	Soluble nutrients, Suspended matter, Chlorophyll-a	Use filter forceps to place into and sample tube
Acetone	90%, ACS grade	Preservation	Chlorophyll-a	Store in a 60 ml HDPE bottle in a Ziploc bag
Disposable pipettes	Plastic (polyethylene)	Dispensing	Chlorophyll-a	Used to dispense 90% acetone
Forceps	Metal	Handling	Chlorophyll-a	None
DI water	1 L plastic (HDPE) 250 ml plastic (HDPE) squeeze bottle	Equipment blanks Rinsing	All parameters	None

KimTech Kimwipes®	Cellulose	Cuvette wipes	Turbidity	Used to clean Turbidity cuvettes
Field Sampling Equipment	**Construction**	**Use**	**Parameter Groups**	**Notes**
Coolers	Plastic	Transportation	All parameters	None
Field datasheets	Field data sheets Instrument calibration sheets Field equipment checklist	Documentation	All parameters	None
Pens	Waterproof	Documentation	All parameters	None
Labeling tape	Waterproof	Documentation	All parameters	None
Ice		Preservation	Total and Soluable nutrients, Chlorophyll-a	Need sufficient quantity to ensure even temperature distribution
DI water	1 L plastic (HDPE) 250 ml plastic (HDPE) squeeze bottle	Equipment blanks Rinsing	All parameters	None
Site charts	Waterproof	Reference	All parameters	None
Field instruments	Seabird SBE-19 or SBE19plus CTD YSI EXO2 Mult-parameter Data Logger LaMotte 2020 WE Turbidity Meter	Field measurements	All parameters Salinity Only Turbidity Only	Data Collection Salinity check for laboratory analysis Data Collection

Field Equipment Checklist:

Water Sampling Equipment

- LIMS Labeled sample bottles (narrow-mouth plastic)
    - 60 ml plastic (HDPE)
    - 125 ml plastic (HDPE)
- 140 ml clean plastic syringes (extra for backup)
- 2.0 ml Micro-centrifuge tubes pre-labeled
- 250 ml Wide-mouth amber bottle for dark storage
- 2.5 cm Gellman in-line filter holders pre-loaded with 2.5 cm Whatman GF/F glass fiber filters
- 1 box 2.5 cm Whitman GF/F glass fiber filters
- 2 boxes of Kimwipes® for cleaning turbidity cuvettes
- 2 Filter forceps
- 2-5 gallon plastic bucket with rope
- 3 Niskin Bottle water samplers (surface, bottom, and a backup)
- DI water (4) 1L for equipment blanks and rinsing

Field Measurement Equipment

- Seabird SBE19 CTD multi-parameter data logger\profiler
- Seabird SBE19*plus* CTD multi-parameter data logger\profiler
- YSI EXO2 multi-parameter data logger\profiler with handheld display
- LaMotte 2020 WE Turbidity meter

Sample Preservation

- 90% Acetone
- Disposable polyethylene pipettes
- Ice
- Coolers
- NIST – calibrated thermometer

Boat Supplies

- Depth finder
- GPS Chartplotter (and 1-2 additional GPS handheld units)
- VHF Radio (and additional handheld VHF Radio)
- Cell phone
- Satellite phone (if traveling outside cell phone coverage area)
- Hard copy with site list and waypoints of sampling locations
- Coast Guard required equipment and safety gear
- Charts
- Tool box

Field Equipment Checklist (continued)

Miscellaneous Equipment

- Clipboard with waterproof field data and calibration sheets
- Waterproof Pens
- Waterproof label tape
- Deionized water squeeze bottle (filled)
- Watch
- QAP plan (available in the field for reference)
- Blank Water\Control Water

1. Field Sampling Equipment Calibration

a. Seabird SBE19 CTD multi-parameter data logger\profiler Seabird SBE19plus CTD multi-parameter data logger\profiler

The SEB 19 and SBE19*plus* are designed to measure conductivity, temperature, and pressure in both marine and freshwater environments at depths up to 7000 meters. These instruments operate in a profile mode for acquiring vertical profiles. The SBE 19 and 19*plus* run continuously, sampling at 4 scans per second (4 Hz) and averaging the scan to log a reading every 0.5 second. These instruments are self-powered and self-contained and includes conductivity and temperature sensors and a precision, semiconductor, strain gauge pressure sensor. Nine D-sized alkaline batteries provide 60 hours of operation in profile mode and these are replaced prior to each sample survey. Setup, diagnostics, and data extraction are performed without opening the housing through a data cable. Data output is in raw data form or engineering units (.hex or XML).

Specifications: (based on factory specifications sheet)

Conductivity (S/m)
- Measurement Range: 0 to 9
- Initial Accuracy: 0.0005
- Typical Stability: 0.0003/month
- Resolution: 0.00005

Temperature (◦C)
- Measurement Range: -5 to +35
- Initial Accuracy: 0.005
- Typical Stability: 0.0002/month
- Resolution: 0.0001

Pressure Strain Gauge (m)
- Measurement Range: 0 to 20/100/35/600/1000/2000/3500/7000 meters.
- Initial Accuracy: 0.1% of full-scale range
- Typical Stability: 0.1% of full-scale range/year
- Resolution: 0.002% of full-scale range

The SBE 19 and 19*plus* are returned to the factory every 1000 hours of use or less to perform a calibration check. The criteria for calibration are based on the amount of sensor drift between diagnostic checks. If the sensor has drifted outside the range of "Typical Stability", the sensor is then calibrated. If the sensor fails calibration it is replaced, and new calibration coefficients are generated*. Sea-Bird sensors are calibrated by subjecting them to known physical conditions and measuring the sensor responses.* Coefficients are then computed, which may be used with appropriate algorithms to obtain engineering units. The conductivity, temperature, and pressure sensors on the SBE 19 and SBE 19*plus* are supplied fully calibrated, with coefficients printed on their respective Calibration Certificates and stored in the instrument configuration (.xmlcon or .con) file. Calibration reports and associated .con files are generated by the factory and saved electronically for reference. Factory calibration reports of sensor drift are evaluated to determine if the drift between calibration events is within FDEP SOP verification acceptance criteria in (Table FT 1000-1). If any of the sensors have drifted outside the criteria, the data collected with that instrument will be evaluated to determine if a qualifier is needed. A "J" qualifier and comment would then be attached to each data point for which the instrument was not within the accepted criteria.

Auxiliary Sensors:

SBE 43 Oxygen Sensor (mg/L)

Measurement Range: 0.00 – 20.00 mg/L
Initial Accuracy: 2% of saturation
Typical Stability: 0.5% per 1000 hours (with clean membrane)

The SBE 43 is calibrated at six different temperature points ranging from 2 – 30 °C. At each temperature point oxygen/nitrogen is diffused into the bath and three different oxygen points are taken (7, 4, and 1 ml/L) and the results are displayed in the calibration report.

ECO-CDOM sensor (mg/mˆ3) WET labs sensor

Measurement Range: 0.00 – 50.00 mg/mˆ3 or 0 – 500 ppb
Excitation: 370 nm
Emission: 460 nm
Sensitivity: 0.09 ppb QS

Note: This sensor is delivered from WET Labs calibrated and must returned to WET Labs for calibration. After calibration Seabird adds the calibration coefficients to the CTD software.

LiCor (LI-193) Spherical Quantum Sensor – The LI-193 measures PAR underwater from all directions at depths up to 350 meters. It uses a diffusive sphere to direct light through glass optical filters to the silicon photodiode. The filters create nearly uniform sensitivity to

light between 400 and 700 nm, which closely corresponds to light used by most terrestrial and aquatic plants and algae.

Specifications:

Absolute Calibration: ± 5% in air traceable to NIST
Sensitivity: Typically, 7 µA per 1,000 µmol s^{-1} m^{-2} in water
Linearity: Maximum deviation of 1% i[tp 10,000 µmol s^{-1} m^{-2}
Stability: < ± 2% change over a 1-year period
Response Time: 10 µS
Angular Response: < ± 4% error up to ± 90˚ from normal axis
Operating Temperature Range: - 40 ˚C to 65 ˚C
Detector: High stability silicon photovoltaic detector (blue enhanced)

Note: This sensor is delivered from LiCor calibrated and must be returned to LiCor for calibration. After calibration Seabird adds the calibration coefficients to the CTD software.

b. YSI EXO2 multi-parameter data sonde –

A Multiparameter Water Quality Sonde with 7 sensor ports (including a central wiper port) and optional depth sensor, is used to take surface water quality readings. Salinity readings are calculated from conductivity and temperature sensors.

Sensor Specifications

Conductivity (mS/cm)
- Range: 0 to 200 mS/cm
- Accuracy: (0 to 100) ± 0.5% of reading or 0.001 mS/cm
  (100 to 200) ± 1% of reading
- Response: T63<2 sec
- Resolution: 0.0001 to 0.01 mS/cm (range dependant)

Temperature (˚C)
- Range: -5 to 35 ˚C
  35 to 50 ˚C
- Accuracy: ± 0.01 ˚C2
  ± 0.05 ˚C2
- Response: T63<1 sec
- Resolution: 0.001 ˚C

Salinity (ppt)
- Range: 0 to 70 ppt
- Accuracy: ± 1.0% of reading or 0.1 ppt
- Response: T63 < 2 sec
- Resolution: 0.01 ppt

The sonde is calibrated in the laboratory following FDEP FT-series SOP criteria.

Note: Sonde and standards are equilibrated to room temperature prior to calibration.

- The sonde can be connected to the PC via the “wet-mate” connector cable or Bluetooth connection. To “awaken” sonde via Bluetooth, use the sonde magnet.
- Once connected to the PC, open the KOR EXO software and select the *Calibration Menu*, a list of the installed sensors will appear, select the sensor you are calibrating.
- Rinse the probe with analyte-free water and then with the appropriate standard solution.
  Note: When selecting the appropriate standard solution select a standard that best represents the conditions in the field.
- Place the sonde probes inside calibration cup with the appropriate standard solution *Note: some sensors require a two-point calibration.*
- Selecting the parameter will initiate the probe’s calibration in the standard. Allow the sensor readings to stabilize.
- Enter the standard value, the reading should match the standard value when stabilized.
  Note: EXO sensors have SmartQC© technology, the sensor will read Green, Yellow or Red after stabilizing.
  A Green score means the sensor is calibrated properly and all parameters used to assess its performance state are within factory-defined limits. A Yellow score means that the sensor will still perform within factory-defined limits, but that during calibration enough of an adjustment was required to suggest that the sensor is drifting from those limits or may soon require some adjustments. A Red score means that the sensor is not performing within factory-specified limits or a component of the sensor is due to be replaced.
- Once stable, confirm probe is reading within the acceptable margin of error and Select **Apply** to accept the calibration point. Repeat for each calibration point and select **Complete** when all points have been calibrated.
- A calibration summary report is generated with QC score, and the report is saved for records. If a calibration error occurs, repeat the calibration, or preform a factory reset (see manual for details).

- After the Initial Calibration (IC) a Continuing calibration verification (CCV) is preformed, and the sonde is placed in two different standards that bracket the expected range of sample measurements and the values are recorded. These values must meet the calibration acceptance criteria listed in FDEP SOP table FT-1000-1).

Tank Test – A Continuing Calibration Verification (CCV) is performed with each sample survey. Prior to each sample survey all three calibrated instruments (Seabird SBE19, *SBE19plus,* and YSI EXO2) are placed in a large, clean 55-gallon calibration chamber with known physical conditions. The salinity of the tank is adjusted using Instant Ocean and the salinity is verified using the calibrated EXO2 sonde. An aerator is used to fully saturate the water and a certified NIST thermometer is used to determine accurate temperature. The internal clocks of all three instruments are synchronized.
Note: Instrument clocks are synced using the United States Naval Observatory Master Clock (USNO).
The instruments are programmed to log data every second and allowed to run for approximately 30 minutes. The resulting data is then analyzed for consistency between instruments as well as to verify they are reading within FDEP SOP acceptance criteria (see Table FT1000-1). If any of the instruments do not meet this criterion, they are not used in the sampling survey. A post survey verification is preformed after the sample survey to ensure instruments were working properly during the survey. If the instrument fails post verification, a "J" qualifier and comment would then be attached to each data point for which the instrument was not within the accepted criteria. The tank test results, both pre and post, are saved as an excel file and stored for reference.

c. **LaMotte Turbidity 2020 WE turbidity meter –** is a portable, microprocessor controlled, direct reading nephelometer. Turbidity is measured directly by either EPA Method 180.1 or ISO Method 7027. The 2020 uses a multi-detector micro optical configuration that assures long term stability of calibrations, high precision and accuracy and low detections limits. [from manual] EPA turbidity uses a tungsten filament light source that meets or exceeds EPA specifications.

Specifications:

Range: 0 – 4000 NTU/FNU, 1-10, 500 ASBC, 0-150 EBC
Resolution: 0.01 NTU/FNU 10.00-10.99
0.1 NTU/FNU 11.00-109.9
1.0 NTU/FNU 110-4000
Accuracy: From 0-2.5 NTU (accuracy is ±0.05 NTU
From 2.0-100 NTU/FNU (accuracy is ±2%)
From 100 NTU (accuracy is ±3%)
Detection limit: 0.05 NTU/FNU
Reproducibility: 0.02 NTU/FNU or 1%
Light Source: Tungsten (EPA) complies with EPA 180.1. Standard 860 LED (ISO) complies with ISO 7027

The meter is calibrated prior to each sampling survey using factory standards: 0.0, 1.0, and 10.0 NTU and the results are recorded on the calibration sheet along with the following information: date, initials of technician, and lot# of standards used. Calibration checks using (0.0, 1.0, 10.0 NTU) in "run" mode, are performed at the beginning and end of each sample day and the results recorded on the field sheets. Batteries are re-charged after each sampling event and sample cuvettes are rinsed with DI water and air dried.

2. Sampling Equipment and Cleaning Procedures

Water sampling equipment such as Niskin samplers will be used to collect water samples from the appropriate depths. Additional water sampling equipment include 140 ml plastic piston syringes, 2.5 cm plastic (Gellman) in-line filter holders with 2.5 cm Whatman GF/F glass fiber filters. A Seabird SBE19 and SBE19*Plus* CTD data logger/profiler and YSI EXO2 multi-parameter sonde will be used to collect physio-chemical parameters in the field. Immediately following a sampling trip, all reusable field sampling and measurement equipment is cleaned in the laboratory according to the following conditions:

1. Wash all surfaces thoroughly with fresh water.
2. Rinse thoroughly with distilled water.
3. Let air dry completely, or dry with Kimwipes®.
4. Place equipment in field storage boxes or in plastic bags for storage and transport to the field.

a. **Filter holders** used for the collection of suspended matter and chlorophyll-a samples are rinsed three times with distilled water, then allowed to air dry. Clean dry filter holders are kept in a clean plastic bag until next sampling event.

b. **Data Loggers:**

i. **Seabird SBE 19 and SBE *19plus CTD* loggers/profilers –** the pumping mechanisms is flushed with tap water and then DI water after use. Battery volts and memory are checked prior to each sample day. Batteries are replaced when the voltage is below 11 volts (full is 13.1 volts). The memory is cleared at the end of each survey.

ii. **EXO2 logger –**The sonde and probes are rinsed with tap water and then DI water and stored back in the calibration cup with a small amount of water.

B. Field Measurements

The Field Manager or Crew Chief is responsible for all corrective actions in the field during sampling campaigns. To ensure collection of undisturbed samples, the boat is advanced toward a sampling station from the downstream direction whenever possible.

Field measurements at each station include temperature, conductivity/salinity, dissolved oxygen, PAR, turbidity, and depth. At each site a cast of all these parameters will be logged in a surface to bottom profile from the boat. Water column profiles collecting the above parameters every 0.5 second are preformed using Sea-Bird model SBE-19 or SEB 19*plus* CTD data loggers. All users must be properly trained in the use of this instrument, and a copy of the instrument manual taken out into the field during each sampling trip for reference.

1. <u>CTD Cast:</u>
   The magnetic switch is turned to ON position. At this point, the instrumentation will start collecting data. The time and station number are recorded on the field data sheet. The CTD is placed overboard on the sun-lighted side of the boat, at approximately 1-2 meters depth. Once the pump turns on the davit operator will allow the CTD to “soak” 3 minutes to allow the instrument to completely flush with ambient water. The CTD is raised to verify the pump is activated and then submerged again, making sure the PAR sensor is just below the surface; then will begin the slow steady decent to the seafloor. When the CTD reaches the bottom, the davit operator starts the ascent back to the surface. The CTD is recovered, the pumping mechanism is verified and the CTD is turned to the OFF position and secured to the deck

2. <u>EXO2:</u>
   The EXO2 is lowered over the side of the boat at the level of sample collection (within 0.5 m of the surface), and the readings are allowed to stabilize. The salinity reading is recorded on the field sheet and the sonde is removed from the

water and rinsed with analyte-free water and returned to storage cup. *Note: A single salinity reading is taken to note salinity ranges for laboratory analysis, these readings are NOT used in the final report.*

3. LaMotte Turbidity meter:
   A small portion of the unfiltered sample water (125 ml bottle), is used to rinse a clean sample cuvette three times. The cuvette is then filled to the "fill line" and wiped clean with a lint-free Kimwipe®. The sample cuvette is then placed in the Turbidity chamber aligning the index notch with the index arrow, and the reading is recorded on the field sheet. *Note: Both surface and bottom samples are read. (See below in Field Sampling techniques)*

C. Field Sampling Techniques

Water samples are collected using either the actual sample container or Niskin sampler from either side of the boat, as in the case for a small boat, or from a sampling platform, as in the case of the large vessel. It is important that samples are collected away from the boat's engine. Only those persons trained in the proper method of sample collection from a boat or vessel will conduct the sampling.

Three types of water samples are collected: suspended matter samples for chlorophyll-*a* determination, filtered water samples for determination of dissolved nutrients, and unfiltered samples for determination of turbidity, total phosphorus, total nitrogen, total silicate, and total organic carbon. Water samples are collected according to the following procedures:

1. Unfiltered Samples

a. Surface samples:

- Samples are collected by removing the container cap of a125 ml plastic HDPE bottle and slowly submerge the container, opening first, into the water.
- The sample bottle is inverted so the opening is upright and pointing upstream into the oncoming direction of water flow. The sample container is then rinsed three times with sample water prior to filling, allowing water to run slowly into the container until filled. *Note: Rinsate is collected in a small bucket and then discarded after sampling is completed.*
- The sample container is then returned quickly to the surface.
- A small portion of this sample is used to measure turbidity using a LaMotte 2020 WE turbidity meter (see below procedure). The remainder of the sample is stored in a cooler on ice.

b. *Bottom samples: (Niskin Bottle)*

- The site water depth is noted and the Niskin bottle is cocked and lowered to appropriate depth taking care not to disturb the sediments.
- The sample device is rinsed with ample amounts of site water prior to collecting the first sample.
- At the desired depth, the messenger weight is sent down to trip the closure mechanism, and the sample is slowly retrieved to the boat.
- Labeled sample bottles are rinsed three times with site water prior to filling via the discharge tube. *Note: Rinsate is collected in a small bucket and discarded after sample collection.*
- A small portion of the sample water is used to measure turbidity and the labeled sample bottles are then stored on ice.

2. Filtered Samples

a. Surface samples

- Samples are collected using a clean 140 ml syringe. The syringe is rinsed three times with ambient sample water and then filled beyond 140 ml.
- Excess water is discharged to ensure full 140 ml in syringe.
- A Gillman in-line filter holder containing a clean 2.5 cm Whitman GF/F glass fiber filter is placed on the end of the syringe.
- 10 ml of sample water is forced through the filter prior to rinsing sample bottle (60ml).
- The sample bottle is rinsed three times with a small amount of filtered water from the syringe.
- The sample bottle is then filled to the shoulder to allow for expansion during freezing.
- The sample is then placed on ice until return to shore, where it is placed upright in the freezer and freezing time is noted on field sheets.
- The remainder of the water in the syringe is forced through the filter along with a small amount of air to dry the filter. The filter is then collected for Chlorophyll-a analysis and placed in a pre-labeled micro-centrifuge tube with 1.5 ml of 90% acetone and placed in the dark on ice. The amount of water filtered is noted on field sheets.

b. Bottom samples (Niskin Bottle)

- Samples are collected using a clean 140 ml syringe via the discharge tube of the Niskin bottle. The syringe is rinsed three times with ambient sample water and then filled beyond 140 ml.
- Excess water is discharged to ensure full 140 ml in syringe.
- A Gillman in-line filter holder containing a clean 2.5 cm Whitman GF/F glass fiber filter is placed on the end of the syringe.
- 10 ml of sample water is forced through the filter prior to rinsing sample bottle (60ml).
- The sample bottle is rinsed three times with a small amount of filtered water from the syringe.
- The sample bottle is then filled to the shoulder to allow for expansion during freezing.
- The sample is then placed on ice until return to shore, where it is placed upright in the freezer and freezing time is noted on field sheets.

3. **Chlorophyll a sample:**
   Suspended matter samples collected for chlorophyll-a determination are preserved in the field by adding 1.5 ml of acetone to the HDPE micro-centrifuge tube with a disposable pipet. The chlorophyll-a samples are then stored in a cooler in the dark on ice. Equipment blanks for chlorophyll-a determination are preserved with the same amount of acetone and stored in the dark on ice. The acetone preservative used is of ACS reagent-grade or better; obtained fresh from the laboratory stocks on a daily basis and transported to the field in an HDPE bottle.

4. **Field Equipment Blank Checks and QC**
   SERC's field quality control includes the collection of duplicate samples at every 10^{th} sample site (10% of total samples collected). In addition, according to FDEP-FQ1000, both field blanks and equipment blanks are collected during each sample event. *Note: There are four "Surveys" per year (ie: K100) and there are approximately 10 "Sampling Events" or Sample days, within a Survey.*
   Field blanks and Equipment blanks are collected at the beginning and the end of the sampling event/day (C1-1 and C1-2). For Field blanks, analyte-free water is poured directly into the control sample container (unfiltered surface samples). For the equipment blank, analyte-free water is run through the equipment used for sampling such as the Niskin bottle (bottom samples) and the sample syringe (filtered and chlorophyll-a samples) and placed in appropriate bottles and preserved according to each analysis. The filter is saved and placed in a micro-centrifuge tube along with 1.5 mls of 90% acetone and serves as the chlorophyll-a blank. The collections of blank samples are recorded on the field data sheet along with the process times.

Field instrument checks are completed at the beginning of each sampling event, and at the end of the field-sampling event. Field equipment not functioning properly are not used to collect data until they are brought back to the laboratory for repair. Duplicate field equipment and probes are kept on hand during field sampling trips.

Note: Two types of analyte-free water are produced in the laboratory: 1) deionized-distilled water and 2) double-deionized water. The deionized-distilled water is used for washing equipment and glassware, while the double-deionized water is used as reagent water.

Method: Tap water is first deionized using a Culligan system containing activated carbon and 2-mixed bed ion exchange beds followed by filtering through a 0.45 um polypropylene filter cartridge. The water is then either distilled through a Corning Mega-Pure 11 Liter Automatic Water Still to produce the deionized-distilled water or further deionized with a Barnstead model D8911 HN Ultrapure mixed bed deionization cartridge to produce the double-deionized water. Both types of water have proven to be analyte-free for the nutrients analyzed. The quality of this water is frequently checked with laboratory method and field equipment blanks. Containers used to store analyte-free water are kept dedicated to this use.

D. Sample Handling and Custody Requirements

1. Sample Preservation, Holding Times, and Sample Volume

Sample containers, sizes, preservatives, and maximum holding times, by parameter are included in Table 3. Note, there are two columns listing sample holding times prior to analysis. SERC-CAChE/NAL lab recognizes that samples should be analyzed as soon as possible after sample collection. Therefore, SERC has instituted its own maximum holding times as goals. The SERC holding times are almost always met; however, due to the intense sampling schedule of the Florida Keys National Marine Sanctuary Survey (FKNMS), the filtered samples are frozen and analyzed within the maximum holding time. If for any reason the samples are held beyond their maximum holding times, those samples are identified, and the data are flagged with a “Q” qualifier.

Table 3: Sample Preservation and Holding Times

Sample Type/Parameter	Container/Size	Preservation Method	FIU Holding Time (based on CAChE-NACF SOPs)	EPA Maximum Allowable Holding Times
Ammonia	Plastic, 60 ml	Filtered and iced in the field, Frozen upon return from field	48 hours after thawing, 28 days after collection SOP-004	28 days EPA350.1
Nitrite	Plastic, 60 ml	Filtered and iced in the field, Frozen upon return from field	48 hours after thawing, 28 days after collection SOP-004	28 days EPA353.4
Nitrate + Nitrite	Plastic, 60 ml	Filtered and iced in the field, Frozen upon return from field	48 hours after thawing, 28 days after collection SOP-004	28 days EPA353.4
Soluble Reactive Phosphate	Plastic, 60 ml	Filtered and iced in the field, Frozen upon return from field	48 hours after thawing, 28 days after collection SOP-004	28 days EPA365.1
Total Nitrogen	Plastic, 120 ml	Iced in field, refrigerated	Add HCl as preservative within 48 hours of sample submission, 28 days SOP-006	28 days ASTMD5176
Total Phosphorus	Plastic, 120 ml	Iced in field, refrigerated	Acid digest the samples within 48 hours of sample submission, 28 days SOP-008	28 days EPA365.1
Total Organic Carbon,	Plastic, 120 ml	Iced in field, refrigerated	28 days SOP-007	28 days NU-062-1.8
Turbidity	Plastic, 120 ml	Read in the Field within 15 min of collection	Within 15 minutes of collection SOP-FT1600	48 hours EPA 180.1
Chlorophyll-a	GF/F Glass Fiber Filter, 1.8 HDPE Micro-centrifuge tube	10% Acetone, Frozen at -15˚C, Stored in the dark	28 days* SOP-003 * The holding time in SOP-003 is 21 days. Data will not be flagged if analyzed between 21-28 days.	28 days EPA 445.0

2. Sample Custody

Sample custody is the responsibility of the sampling team and of the Chief Chemist. The sampling team is responsible for labelling, collecting, documenting in the field notebook, and transporting samples to the laboratory. The Chief Chemist is responsible for proper log-in and storage of the samples within the laboratory, and that the samples are analyzed within their appropriate holding times. Samples are not discarded until the analytical results are checked and approved by the Quality Assurance Officer. All documentation/logs are signed/initialed by appropriate personnel.

The SERC CAChE-NACF Laboratory is controlled by a Laboratory Information Management System (LIMS). Prior to leaving for the field, each sample container is labelled with a unique sample identification LIMS bar coded number, and colored tapes to differentiate surface and bottom samples.

3. Field Custody

Field Data Sheets are used to record field information during sampling (Figure 1). Once QA checked, the sheets are kept in project-specific files. Entries into the field notebook include: name and number of sampling trip; date of sampling trip; general weather and water conditions (waves and tides); name of individuals in sampling team; location and number of sample; and sample collection start time and end times. Additional recorded information includes, turbidity readings (surface and bottom), surface salinity and the volume of water filtered. Each sheet is signed by the sampling team. If an error is made in the field notebook, corrections are made by drawing a line through the error and entering the correct information next to the error.

Fig. 1. Field Data Sheet

FIELD DATA SHEET (V.5) 15-row (revised 2020-07-01)

Weather Time: ____:____ Temp. ____° C / ____° F, humidity _____ %, cloud cover _____ %, wind spd _____ units _____ & direction _____ , sea height _____ & direction _____							Vessel used: _____ Depth calibrated at sea level (daily) _____	
Sampling Event		**Date of Collection**	**Names**				**Equipment Used/ Serial#**	**Equipment Calibration**
			sampled by:				CTD or YSI Serial #	Date:
			read by:				CTD or YSI Sonde #	hour: attach equipment calibration sheet
Station Number	**Equip. Rinsed**	**Start Time**	**Salinity (ppt)**	**Turbidity Surface (NTU)**	**Turbidity bottom (NTU)**	**Vol. filtered (ml)**	**End Time**	COMMENTS
		: :					: :	
		: :					: :	
		: :					: :	
		: :					: :	
		: :					: :	
		: :					: :	
		: :					: :	
		: :					: :	

4. Laboratory Custody

Legal chain-of-custody (COC) procedures are followed for samples collected as part of this project. Upon transport of the samples to the CAChE-NACF laboratory at FIU, the field sampling personnel log-in the samples on the COC form (Figure 2). All the sample bottles are inspected for integrity, proper documentation (labelling), and preservation (bagged in cooler with wet ice). Any samples bottles found broken, leaking, not properly marked, or not properly preserved are rejected from analysis, and noted on the bottom of the Sample Checklist.

Fig. 2. COC sheet

CAChE Nutrient Analysis Laboratory Core
OE 148 (Office)/VH-303 (Lab), University Park, Miami, FL 33199
305-348-3095 or 305-348-2836

MATRIX			
FW	Freshwater	S	Soil
SW	Saltwater	O	Other

SDG# **200722**

PROJECT NAME:	K100	**LIMS RECORD:**	AC84360-AC84565	**ACCOUNT NO.:**	800007547
DELIVERED BY:	Sandro Stumpf	**RECEIVED BY:**		**DATE AND TIME:**	

ITEM #	BOTTLE ID	SAMPLE UNIQUE ID	MATRIX	COLLECTION DATE and Time		# OF REPLIC.	ANALYSES REQUIRED* FILT. NUTR.	TP: (W)ater (S)oil	SI	TN	(T)OC (D)OC	CHL-a	TN/TC
1	AC84360	K216BIF	SW	7/7/2020			X	W	X	X	T		
2	AC84361	K216SIF	SW	7/7/2020	10:41		X	W	X	X	T	X	
3	AC84364	K218BIF	SW	7/7/2020			X	W	X	X	T		
4	AC84365	K218SIF	SW	7/7/2020	11:23		X	W	X	X	T	X	
5	AC84366	K219BIF	SW	7/7/2020			X	W	X	X	T		
6	AC84367	K219SIF	SW	7/7/2020	11:46		X	W	X	X	T	X	
7	AC84370	K222BIF	SW	7/7/2020	:		X	W	X	X	T		
8	AC84371	K222SIF	SW	7/7/2020	12:24		X	W	X	X	T	X	
9	AC84374	K224BIF	SW	7/7/2020	:	1	X	W	X	X	T		
10	AC84375	K224SIF	SW	7/7/2020	13:01	1	X	W	X	X	T	X	
11	AC84376	K225BIF	SW	7/7/2020	:		X	W	X	X	T		
12	AC84377	K225SIF	SW	7/7/2020	12:47		X	W	X	X	T	X	
13	AC84382	K228BIF	SW	7/7/2020	:		X	W	X	X	T		
14	AC84383	K228SIF	SW	7/7/2020	13:33		X	W	X	X	T	X	
15	AC84434	K264BIF	SW	7/7/2020	:		X	W	X	X	T		