

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 (CACH-E-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 °C, and analyze within 28 days.
 - d. Total organic carbon (TOC): Ice in field, store at 2-6 °C, and analyze within 28 days.
 - e. Total Nitrogen (TN): Ice in field, add HCl acid within 48 hours, store at 2-6 °C, 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 ₂ SO 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 ₂ SO 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 °C, analyze within 28 days	Yes***	Yes	Yes
Total phosphorus (TP)			Yes	Yes	No
Total organic carbon (TOC)	Acid-preserve with HCl, H ₂ SO ₄ , or H ₃ PO ₄ , and ice within 15 minutes, analyze within 28 days	Iced in field, stored in refrigerators at 2-6 °C, 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 NO₂+NO₃ and TKN, which are the components of TN.

*** HCl was used instead of H₂SO₄ 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 (NO₂) 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. CACHÉ-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 R² 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 ($\alpha < 0.05$)	Paired Wilcoxon Result ($\alpha < 0.05$)	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 .

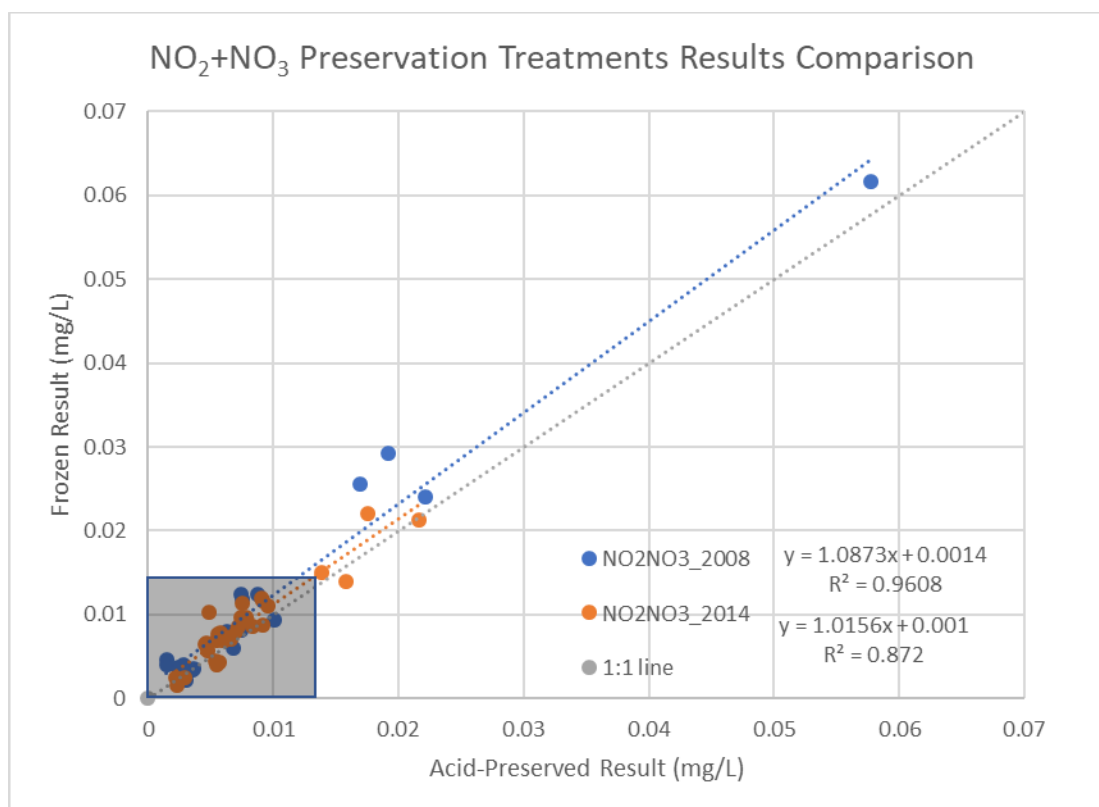


Figure 1. 2008 and 2014 study comparison study results for NO_2+NO_3 . The gray box is the area below the 2014 PQL. None of the 2014 results were below the applicable MDL. Linear regression lines, equations, and R^2 are displayed for each dataset. The line of equality is included for reference.

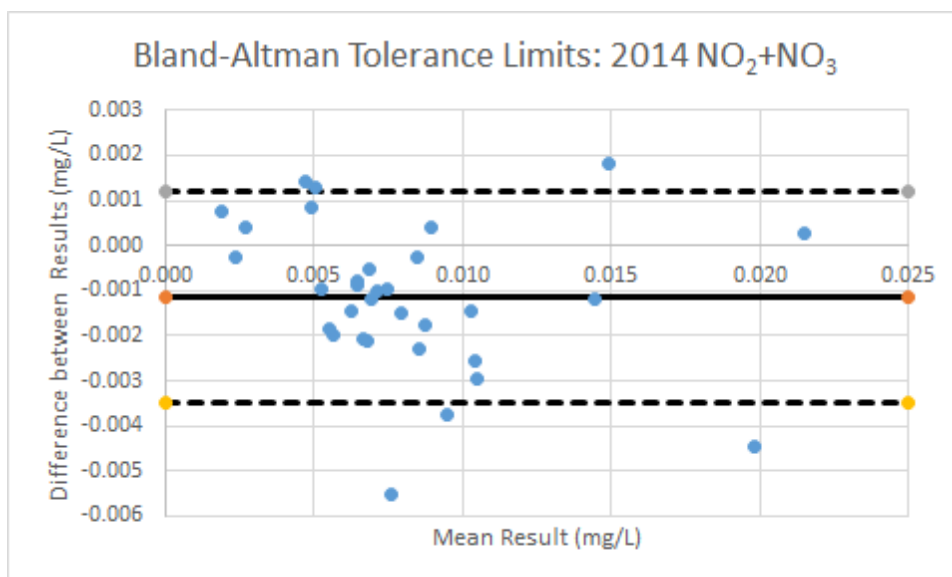


Figure 2. Bland & Altman tolerance limits for NO_2+NO_3 results from the 2014 comparison study. The plot represents the mean result on the x-axis and the difference between the results on the y-axis for each sample pair. The difference was calculated as the result from the DEP SOP method minus the result 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 .

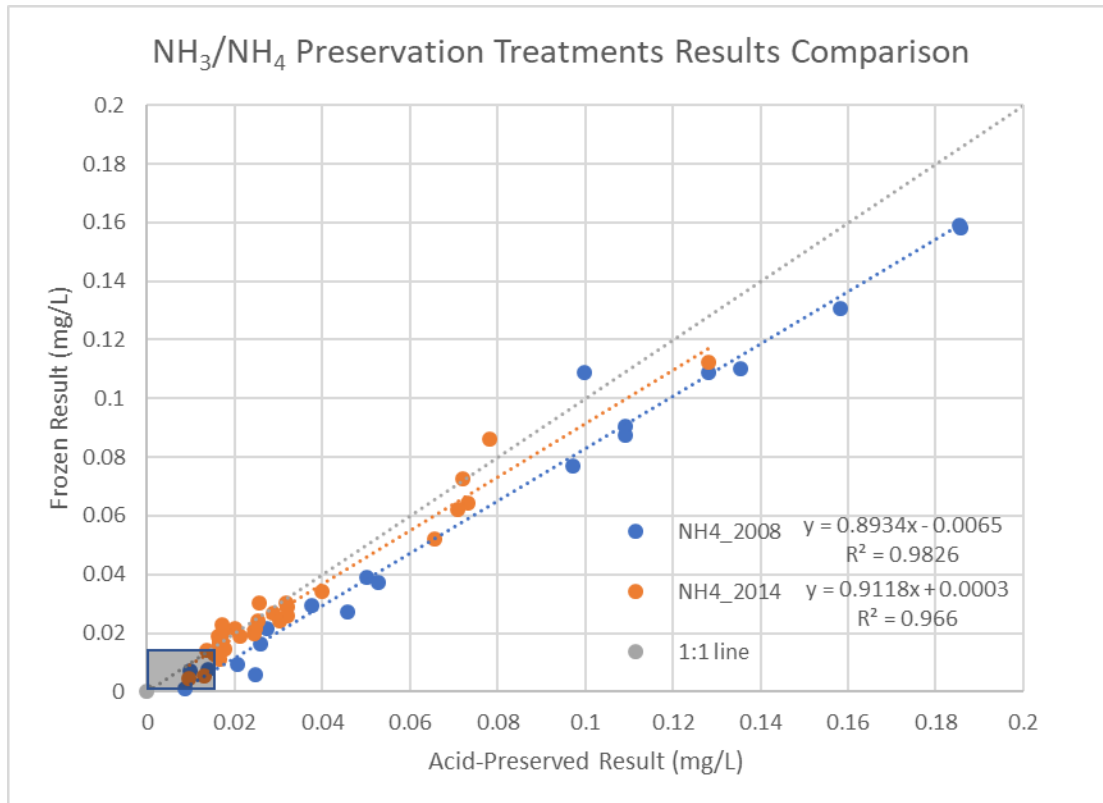


Figure 3. 2008 and 2014 study comparison study results for NH₄. The gray box is the area below the 2014 PQL. None of the 2014 results were below the applicable MDL. Linear regression lines, equations, and R² are displayed for each dataset. The line of equality is included for reference.

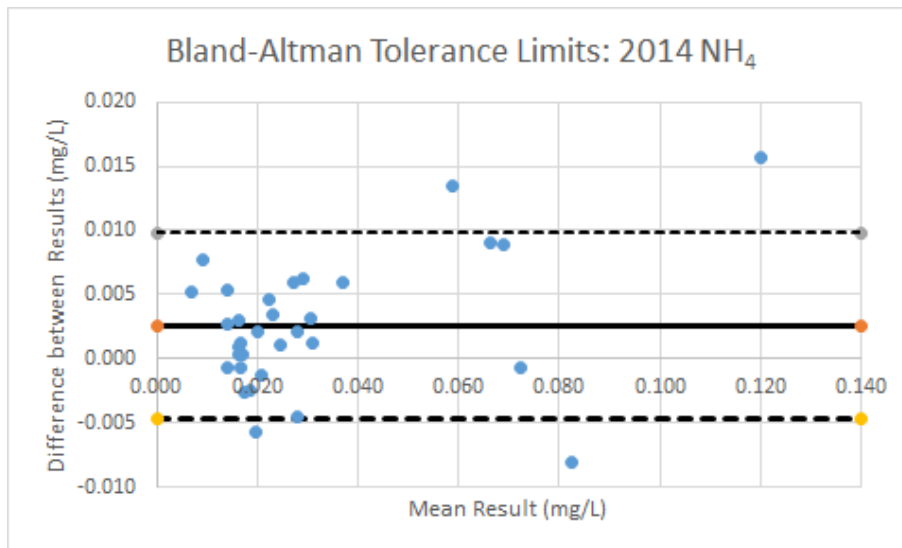


Figure 4. Bland & Altman tolerance limits for NH₄ results from the 2014 comparison study. The plot represents the mean result on the x-axis and the difference between the results on the y-axis for each sample pair. The difference was calculated as the result from the DEP SOP method minus the result from 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 NO₂ 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 NO₂.

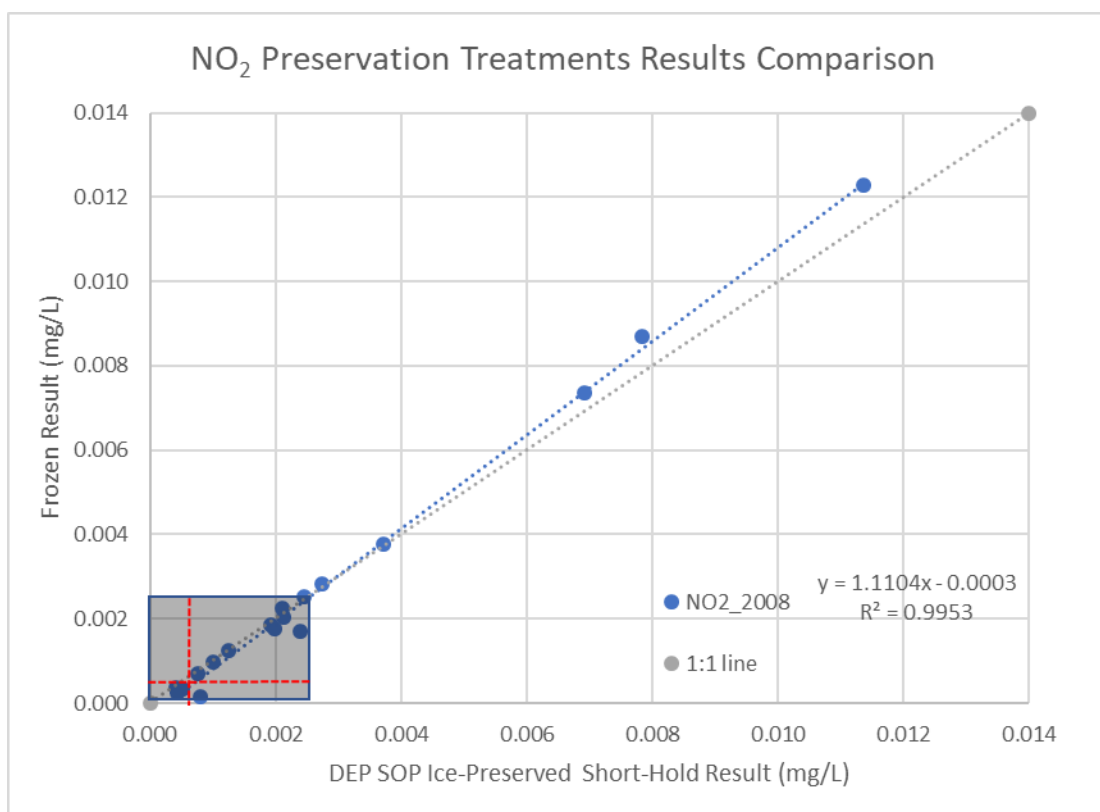


Figure 5. 2008 study comparison study results for NO₂. The gray box is the area below the 2008 PQL, and the red lines show the 2008 MDL. The linear regression line, equation, and R² are displayed. The line of equality is included for reference.

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.

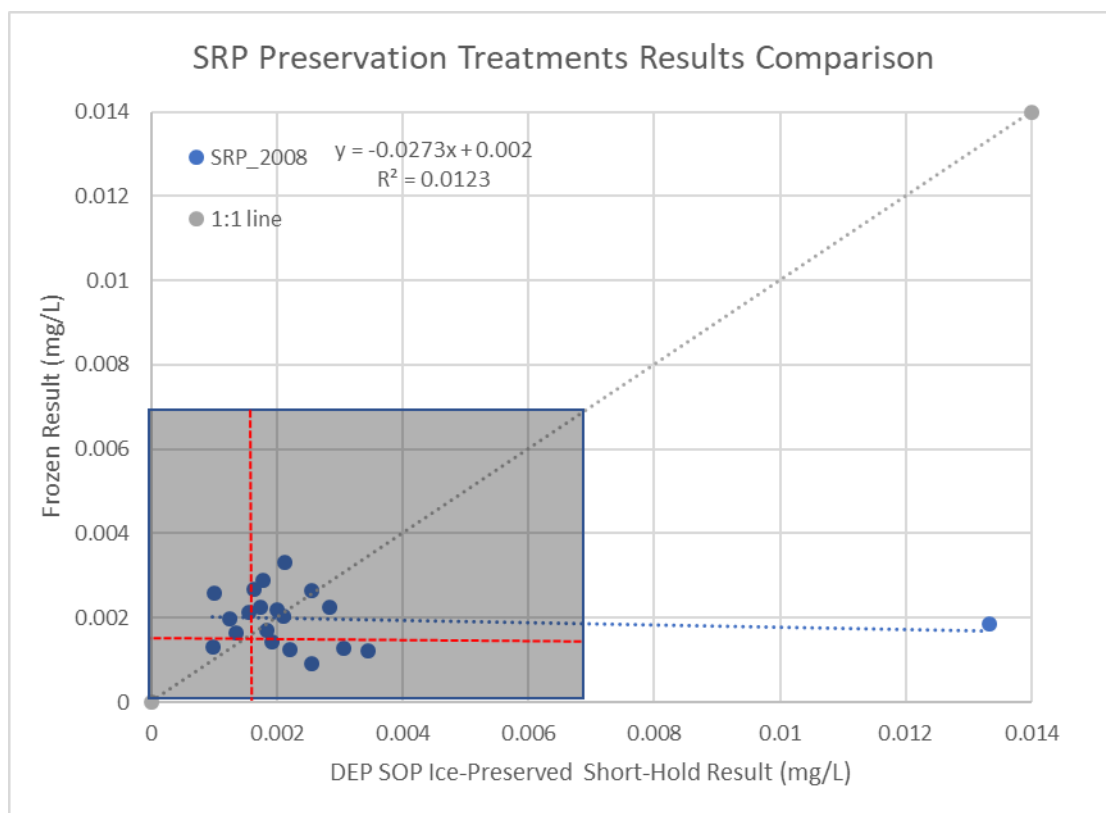


Figure 6. 2008 study comparison study results for SRP. The gray box is the area below the 2008 PQL, and the red line show the 2008 MDL. The linear regression line, equation, and R^2 are displayed. The line of equality is included for reference.

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.

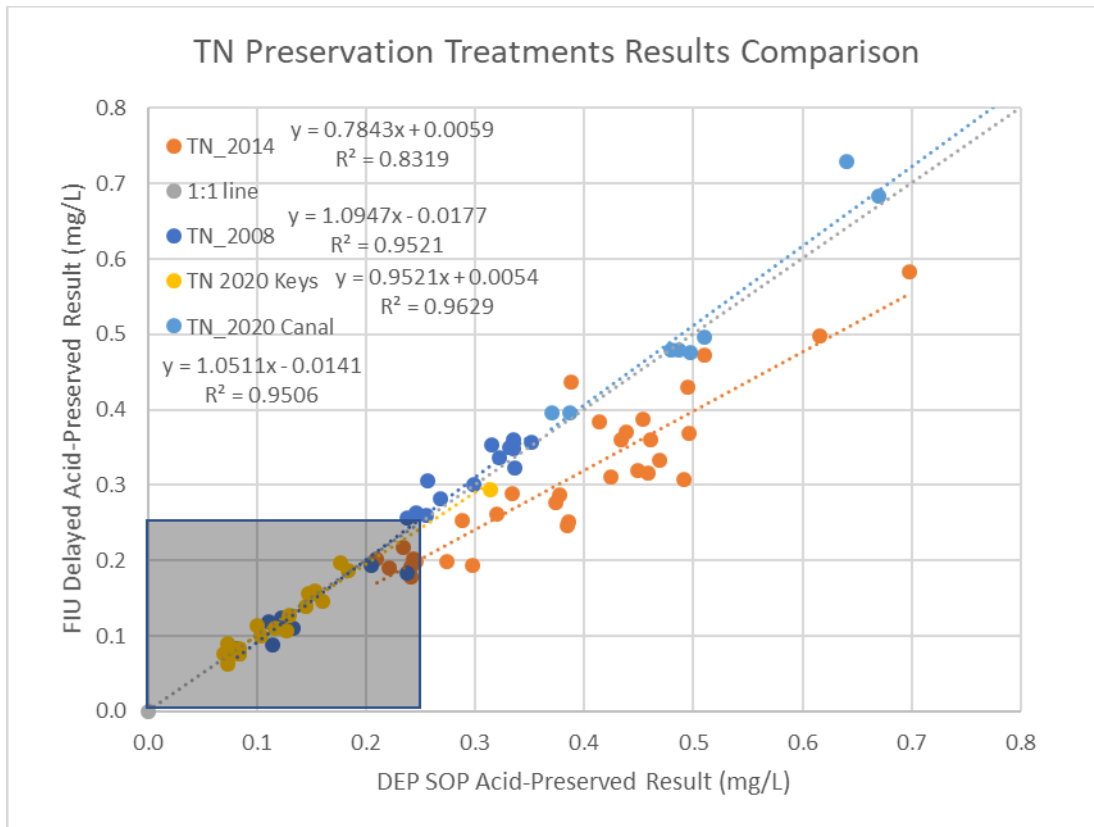


Figure 7. 2008, 2014, and 2020 study comparison study results for TN. The gray box is the area below the 2014 PQL. No results were below the applicable MDL. The linear regression lines, equations, and R² are displayed. The line of equality is included for reference. Note that in 2008, HCl was used instead of H₂SO₄ for the DEP SOP acid-preservation treatment.

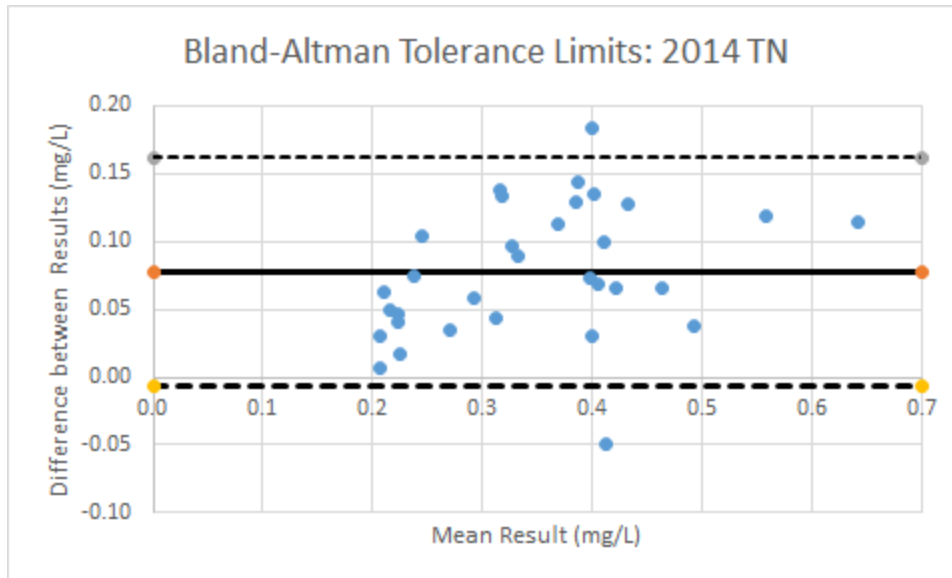


Figure 8. Bland & Altman tolerance limits for TN results from the 2014 comparison study. The plot represents the mean result on the x-axis and the difference between the results on the y-axis for each sample pair. The difference was calculated as the result from the DEP-SOP method minus the result from 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.

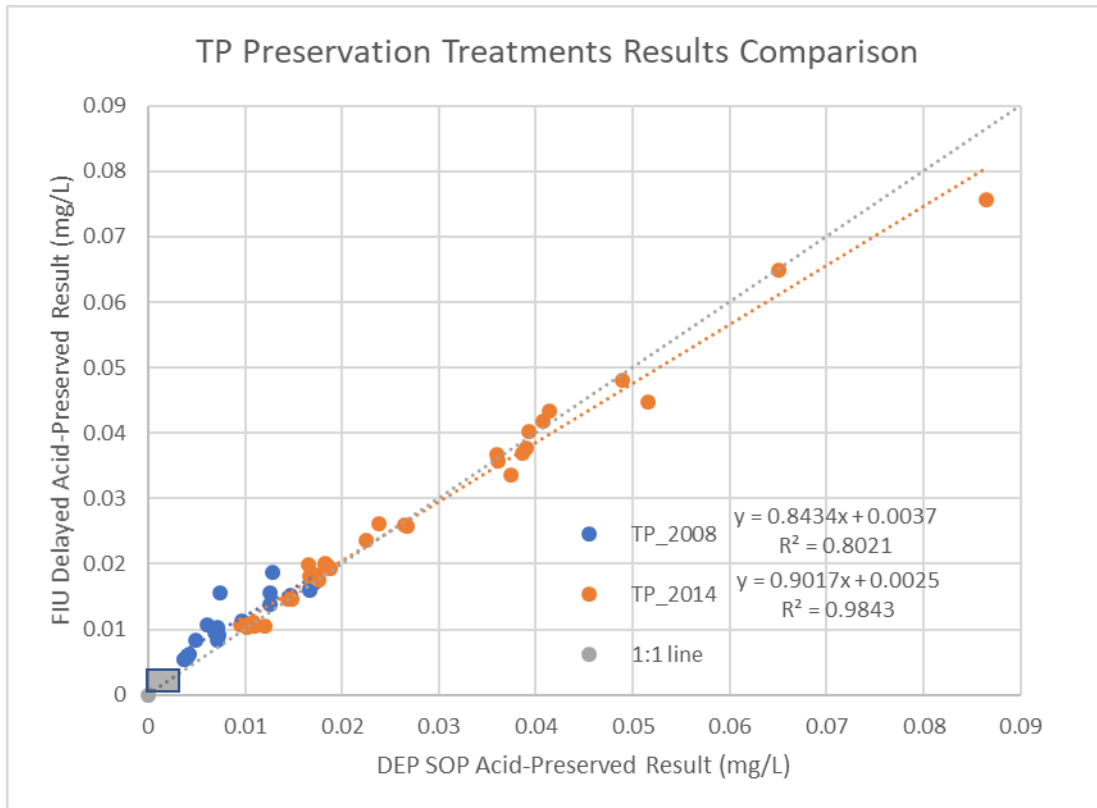


Figure 9. 2008 and 2014 study comparison study results for TP. The gray box is the area below the 2014 PQL. None of the 2014 results were below the applicable MDL. Linear regression lines, equations, and R^2 are displayed for each dataset. The line of equality is included for reference.

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.

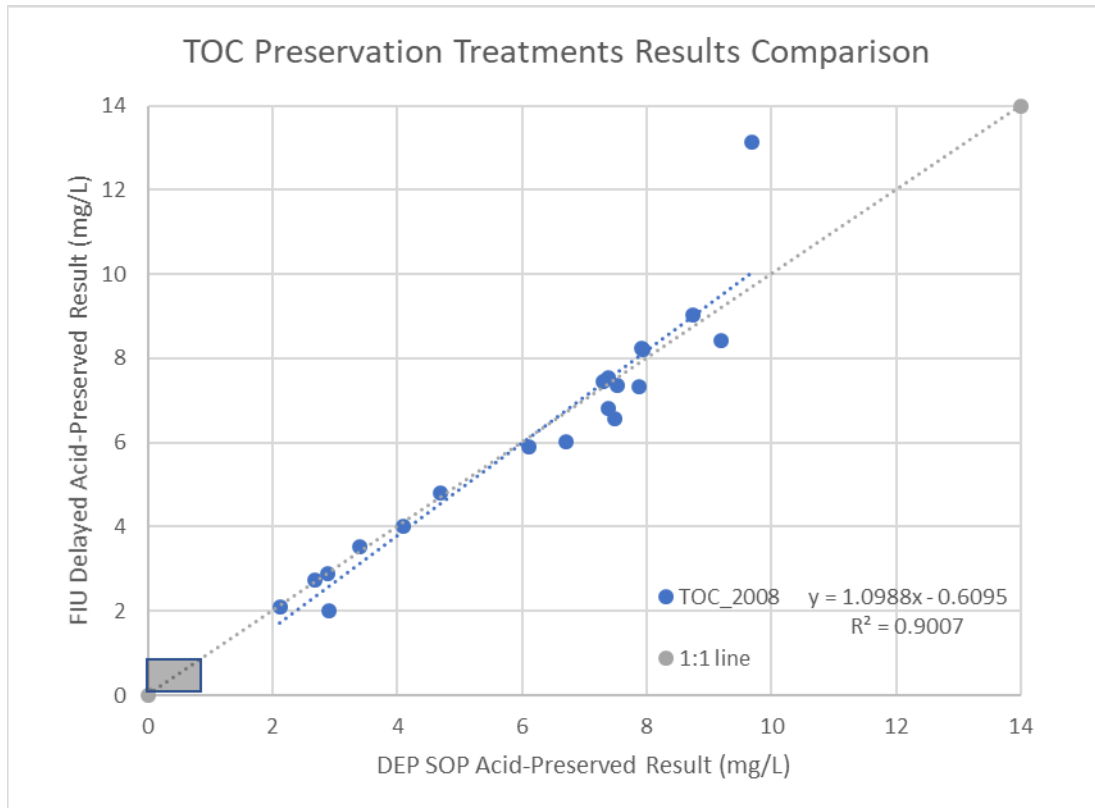


Figure 10. 2008 study comparison study results for TOC. The gray box is the area below the 2008 PQL. None of the 2014 results were below the applicable MDL. The linear regression line, equation, and R^2 are displayed. The line of equality is included for reference.

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 $\text{NO}_2 + \text{NO}_3$ 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 $\text{NO}_2 + \text{NO}_3$, NH_3/NH_4 , TN, TP, and TOC, but are not approved for SRP.

References

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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 NO₂+NO₃ 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 NH₄ 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
15	0.194	*	0.204
16	0.111	*	0.133
17	0.124	*	0.122
18	0.083	*	0.082
19	0.119	*	0.111
20	0.088	*	0.114
	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