

Draft: Nutrient Holding Time Study

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EXECUTIVE SUMMARY:

The California Statewide Surface Water Ambient Monitoring Program (SWAMP) collects water samples for nutrient analysis to help assess the overall health of a water body. Transferring samples from remote water bodies to the laboratory for analysis within standard analytical method required holding times is not always feasible because of logistical and budget constraints. Acid preservation is useful for extending the holding times of samples with high concentrations of nutrients, but acid preservation may compromise the integrity of samples containing low levels of nutrients. The purpose of this study is to review extending the SWAMP required holding times for water samples analyzed for nutrients using two preservation techniques: refrigeration and freezing. The nutrients analyzed included soluble reactive phosphorus (SRP), nitrite (NO_2^-), nitrate + nitrite ($\text{NO}_3^- + \text{NO}_2^-$), ammonia (NH_4^+), nitrate (NO_3^-), total nitrogen (TN), and total phosphorus (TP). Sampling was conducted at six different sites to ensure a variety of sample matrices (EC's ranged from 30 $\mu\text{S}/\text{cm}$ to 2714 $\mu\text{S}/\text{cm}$) and nutrient concentrations (e.g. TN ranged from 0.151 mg/L to 34.843 mg/L). All collection was completed between 23 June 2008 and 24 July 2008 and included field measurements for temperature, pH, dissolved oxygen, specific conductivity, and turbidity.

Upon collection, samples being analyzed for SRP, NO_2^- , NH_4^+ , and $\text{NO}_3^- + \text{NO}_2^-$ were filtered with a 0.45 μm Millipore nitrocellulose membrane filter, TN and TP samples were not filtered. Samples were split into two sets of four replicates that were designated to be analyzed at 48 hour, 4 day, 7 day, and 28 day holding times; one set was immediately placed on dry ice and remained frozen until analysis while the other set was immediately placed on wet ice and refrigerated until analysis. The 48 hour holding time was used as the reference point for comparison. Samples were analyzed at the Land, Air, and Water Resources Department at UC Davis. Methods were used with differing reporting limits so some samples were spiked to ensure the change could be measured in various sample matrices.

Seven replicates of each sample were analyzed at 48-hour, 4-day, 7-day, and 28-day holding times with the mean value of the seven replicates measured at the 48 hour holding time utilized as the reference point for the extended holding time comparison. An analyte was considered stable if the percent difference between the mean value analyzed at a given time (either 4 days, 7 days, or 28 days in this study) and the mean reference value, taken at less than 48 hours, was less than 10% as outlined in the peer reviewed Study Design.

Three concentration ranges for multiple nutrients in both frozen and refrigerated ambient water samples of differing matrices were statistically evaluated at confidence levels of 95% for each holding time. For Soluble Reactive Phosphorous (SRP) the results show that the holding time for frozen samples may be extended up to four days at all concentration ranges. Results for Ammonia, Total Nitrogen, and Total Phosphorous show that samples for these constituents should be analyzed within 48 hours. Results for remaining constituents measured (Nitrite, Nitrate + Nitrite, and Nitrate) for both frozen and refrigerated samples were statistically inconclusive due to a combination of failed lab and/or field Quality Assurance; although frozen samples with mid to high concentrations ranges did appear more stable. Table ES-1 shows the concentration ranges for each nutrient and their stability at each holding time. Table ES-2 shows the specific conductivities of the sampling sites.

Table ES-1. Summary of Nutrient Holding Time Results

	Range of Constituent Concentration	Site Number#	Current Hold Time	Comparable Concentrations					
				Frozen Preservation			Refrigerated Preservation		
				4 Days	7 Days	28 Days	4 Days	7 Days	28 Days
SRP	Below Reporting Limit (0.050mg/L)	1, 2, 4, 5	48 hours	Yes	No	No	Yes	No	No
	0.050mg/L - 0.060mg/L	3		Yes	Yes	Yes	Yes	Yes	No
	0.400mg/L - 0.500mg/L	6		Yes	Yes	Yes [^]	Yes [^]	Yes [^]	Yes [^]
Nitrite	0.019mg/L - 0.029mg/L	2	48 hours	Yes	No	No	No	No	No
	0.028mg/L - 0.036mg/L	1, 3		No	No	No	No	No	No
	0.138mg/L - 0.144mg/L	5		Yes	Yes	Yes	NA [^]	NA [^]	NA [^]
	0.239mg/L - 0.248mg/L	6		NA [^]	NA [^]	NA [^]	NA [^]	NA [^]	NA [^]
Nitrate + Nitrite	0.017mg/L - 0.024mg/L	4	48 hours	Yes	No	No	NA [^]	NA [^]	NA [^]
	0.041mg/L - 0.049mg/L	1, 2		Yes	Yes	Yes	Yes	Yes	No
	1.474mg/L - 1.506mg/L	3		NA [^]	NA [^]	NA [^]	NA [^]	NA [^]	NA [^]
	24mg/L - 26mg/L	5		Yes	Yes	Yes	Yes	Yes	Yes
	30mg/L - 33mg/L	6		Yes [°]	Yes [°]	Yes [°]	Yes [°]	Yes [°]	Yes [°]
Ammonia	0.014mg/L - 0.021mg/L	3, 4	48 hours	No	No	No	Yes	No	No
	0.021mg/L - 0.032mg/L	1, 2, 5		No	No	No	No	No	No
	0.099mg/L - 0.119mg/L	6		Yes	Yes	Yes	Yes	Yes	No
Nitrate	0.010mg/L - 0.024mg/L	1, 2, 4	48 hours	No	No	No	No	No	No
	1.438mg/L - 1.473mg/L	3		Yes	Yes	Yes	Yes	Yes	Yes
	23mg/L - 26mg/L	5		Yes	Yes	Yes	NA [^]	NA [^]	NA [^]
	30mg/L - 33mg/L	6		Yes ^{^°}	NA ^{*°}	NA ^{*°}	NA ^{*°}	NA ^{*°}	NA ^{*°}
Total N	0.146mg/L - 0.204mg/L	1, 2, 4	7 days (US EPA Recommended)	No Frozen Samples Analyzed			No	Yes	No
	2.0mg/L - 2.5mg/L	3					Yes	Yes	Yes
	24mg/L - 27mg/L	5					Yes	Yes	Yes [°]
	32mg/L - 35mg/L	6					Yes [°]	Yes [°]	Yes ^{°^}
Total P	0.030mg/L - 0.053mg/L	1, 2, 4, 5	48 hours	No Frozen Samples Analyzed			No	No	No
	0.156mg/L - 0.182mg/L	3					NA [^]	NA [^]	NA [^]
	0.822mg/L - 2.561mg/L	6					Yes [°]	Yes [°]	Yes [°]
^Spike recovery outside control limits. Spike added less than one half sample concentration. LCS and Method Blank are in control.			°Field Blanks did not meet SWAMP QA/QC however data not considered invalid because the sample concentration was 10X greater than the field blank result						
#See Table 2		*Lab QA Failed	= no recommendation of hold time extension			= failed QA			

Table ES-2. Specific Conductivities of Sampling Sites

Site Number	Site Location	SC (uS/cm)
1	Upper Truckee River	30
2	West Fork Carson River	50
3	San Joquin River at Airport Way	576
4	Sacramento River at Freeport	142
5	Franklin Creek	1749
6	Orcutt Creek	2714

GLOSSARY:

Background Sample	Sample analyzed for constituents not evaluated in the holding time study to help define the matrix of the water bodies used
FB	Field Blank
FC	Franklin Creek
MDL	Minimum Detection Limit
MS	Matrix Spike
NH ₄ ⁺	Ammonia
NO ₂ ⁻	Nitrite
NO ₃ ⁻	Nitrate
NO ₃ ⁻ + NO ₂ ⁻	Nitrate + Nitrite
OC	Orcutt Creek
QA	Quality Assurance
QAPrP	Quality Assurance Program Plan
Reference Sample	Sample collected and analyzed at the 48 hour hold time
RL	Reporting Limit
SJR	San Joaquin River
SRF	Sacramento River at Freeport
SRP	Soluble Reactive Phosphorus
SWAMP	Surface Water Ambient Monitoring Program
TN	Total Nitrogen
TP	Total Phosphorus
UTR	Upper Truckee River
WFCR	West Fork Carson River

INTRODUCTION

The purpose of this study is to evaluate the holding times for water samples currently required by the California Statewide Surface Water Ambient Monitoring Program (SWAMP) for nutrient analysis. While the SWAMP Quality Assurance Program Plan (QAPrP) does not require specific methods be used to analyze samples it does require water samples to be analyzed within analytically specified holding times. Current holding times required by the QAPrP for sample analysis for soluble reactive phosphorus (SRP), nitrite (NO_2^-), nitrate + nitrite ($\text{NO}_3^- + \text{NO}_2^-$), ammonia (NH_4^+), nitrate (NO_3^-), and total phosphorus (TP) are 48 hours. Data from this study was used to evaluate whether the holding times currently required by SWAMP can be extended without compromising the integrity of the samples if the samples are immediately preserved either by refrigeration or freezing.

SWAMP collects samples for nutrient analysis to help assess the overall health of a water body. Phosphorus and nitrogen naturally exist in several forms within aquatic ecosystems and are commonly referred to as nutrients. Nutrients naturally exist in surface waters as a food source necessary for plant and animal growth (Mueller 1996). When nutrients are overabundant in an aquatic ecosystem eutrophication occurs which causes algal blooms that lead to unpleasant odors and tastes, decreased dissolved oxygen levels, and fish kills (Mueller 1996).

Transferring samples from the water body to the laboratory for analysis within the required holding time is not always feasible because of logistical and budget constraints. Some sampling locations are so remote that samplers are required to hike in, take the sample, hike out, and then either take the sample to a nearby laboratory or ship the sample to the laboratory via overnight delivery. Even if the water body is accessible, the samples may still need to be shipped overnight to the laboratory. Acid preservation is useful for extending the holding times of samples with high concentrations of nutrients, but acid preservation may compromise the integrity of samples containing low levels of nutrients.

Previous studies have evaluated the holding times of nutrient samples that were collected using autosamplers. Samples tested for nitrogen or phosphorus more than two days after collection that did not have any form of preservation showed significant changes in analyte concentration (Kotlash 1998). A study containing pre-acidified sample bottles in automated samples compared refrigerated preservation to non-refrigerated preservation and showed that nitrate, nitrite, TKN, and TP concentrations were stable over a seven day period in both refrigerated and non-refrigerated sample sets; ammonia samples, however, did not show stability longer than two days after sampling (Burke 2002). The current study evaluated nutrient concentrations over a 28 day period in samples that were only preserved with refrigeration or freezing.

EXPERIMENTAL DESIGN

Sampling Locations

Two locations in each of three different regions of California were chosen for a total of six sampling sites. The sites were selected to provide a variety of matrices as well as ranges in nutrient concentrations. Samples in the Lahontan Region represented relatively clean water at low nutrient concentrations and were collected from West Fork Carson River below Willow Creek (WFCR) and Upper Truckee River at South Upper Truckee Road (UTR). Samples in the Lahontan Region were spiked with 0.025 mg/L of SRP, NO_2^- , NH_4^+ , and NO_3^- because of low nutrient levels historically exhibited by those water bodies. Samples in the Central Valley Region represented midrange quality and concentration and were collected from the San Joaquin River (SJR) at Vernalis and the Sacramento River (SRF) at Freeport; these water bodies have historically exhibited low- to mid-level nutrient concentrations. Samples from the Central Coast Region were collected from Orcutt Creek (OC) in San Luis Obispo and Franklin Creek (FC) in Carpentry; these water bodies have historically exhibited high-level nutrient concentrations. Appendix A discusses the general water quality and historic ranges of nutrient concentrations for the sampling sites of this study. Tables A1 and A2 summarize the general water quality and the historic ranges of nutrient concentrations, respectively, for the sampling sites of the study. Samples for this study were collected between 23 June 2008 and 24 July 2008.

Sample Collection

In addition to samples collected to evaluate preservation techniques, a “background” sample was collected at each site to provide general water quality information. Background samples were analyzed for: SiO_2 , Na, K, Mg, Ca, Cl, SO_4 , HCO_3 , DOC, turbidity, TSS, VSS, and NVSS. At each collection site field measurements were taken for temperature, pH, DO, SC, and turbidity. Results of the background analyses and field measurements are presented and discussed in Appendix A.

Samples from West Fork Carson River (WFCR) were collected by triple rinsing a 5 gallon plastic sampling bucket with sample water and then filling the bucket with sample water. A 1L sample was taken to establish background information on the water body. Sample water was then transferred from the 5 gallon bucket into a 2.5 gallon carboy where it was spiked with 0.025 mg L^{-1} of NO_3^- -N, NO_2^- -N, NH_4^+ -N, and SRP to assure detectable concentrations. Sample was then collected for total phosphorous and total nitrogen analyses into four 125 ml high-density polyethylene (HDPE, Nalgene[®]) containers and then stored on wet ice to be stored at 4°C. Sample water was then filtered with a 0.45 μm Millipore nitrocellulose membrane filter using a hand held aspirator before being transferred to eight 125 ml high-density polyethylene (HDPE, Nalgene[®]) containers. Four of the containers were stored on wet ice and four were stored on dry ice to be transferred to 4°C and -20°C, respectively.

Samples from Upper Truckee River (UTR) were collected by triple rinsing a sample pump and using it to triple rinse a 5 gallon plastic sampling bucket with sample water. The sampling bucket was then filled with sample water. A 1L sample was taken to establish background information on the water body. Sample water was transferred from the 5 gallon bucket into a 2.5 gallon carboy where it was spiked with 0.025 mg L^{-1} of NO_3^- -N, NO_2^- -N, NH_4^+ -N, and SRP to assure detectable concentrations. Sample was then collected for total phosphorous and total nitrogen analyses into four 125 ml high-density polyethylene (HDPE, Nalgene[®]) containers and then stored on wet ice to be stored at 4°C . Sample water was then filtered with a $0.45 \mu\text{m}$ Millipore nitrocellulose membrane filter with a hand held aspirator before being transferred to eight 125 ml high-density polyethylene (HDPE, Nalgene[®]) containers with four stored on wet ice and four stored on dry ice to be transferred to 4°C and -20°C , respectively.

Samples from San Joaquin River (SJR) and Sacramento River at Freeport (SRF) were collected by triple rinsing a plastic churn splitter with sample water and then filling the churn splitter with sample water. A 1L sample was taken to establish background information on the water body. Sample was then collected for total phosphorous and total nitrogen analyses into four 125 ml high-density polyethylene (HDPE, Nalgene[®]) containers and then stored on wet ice to be stored at 4°C . Sample water was then filtered with a $0.45 \mu\text{m}$ Millipore nitrocellulose membrane filter using a hand held aspirator before being transferred to eight 125 ml high-density polyethylene (HDPE, Nalgene[®]) containers with four stored on wet ice and four stored on dry ice to be transferred to 4°C and -20°C , respectively.

Samples from Orcutt Creek (OC) and Franklin Creek (FC) were collected by triple rinsing a sample pump and using it to triple rinse the plastic churn splitter with sample water. The churn splitter was then filled with sample water. A 1L sample was taken to establish background information on the water body. Sample was then collected for total phosphorous and total nitrogen analyses into four 125 ml high-density polyethylene (HDPE, Nalgene[®]) containers and then stored on wet ice to be stored at 4°C . Sample water was then filtered with a $0.45 \mu\text{m}$ Millipore nitrocellulose membrane filter with a hand held aspirator before being transferred to eight 125 ml high-density polyethylene (HDPE, Nalgene[®]) containers and with four stored on wet ice and four stored on dry ice to be transferred to 4°C and -20°C , respectively.

Analytical Preparation and Methods

All samples were analyzed by the Department of Land, Air, and Water Resources (LAWR) at the University of California, Davis under the supervision of Randy Dahlgren. Two forms of preservation were used for filtered samples: refrigeration (at 4°C) and freezing (at -20°C); samples for refrigeration were placed on wet ice in the field and samples for freezing were placed on dry ice in the field. Seven replicates of each sample were analyzed at 48-hour, 4-day, 7-day, and 28-day holding times with the mean value of

the seven replicates measured at the 48 hour holding time utilized as the reference point for the extended holding time comparison. The stannous chloride method (SM4500-P D) was used to spectroscopically determine SRP. Nitrate + Nitrite ($\text{NO}_3^- + \text{NO}_2^-$) was evaluated using the vanadium chloride method which was originally developed for blood serum analysis (Miranda 2001) and later applied to other sample types. The vanadium chloride method was shown to be comparable to EPA method 353.2 which uses granulated copper-cadmium instead of vanadium chloride for the reduction of nitrate to nitrite in a study done by Timothy Doane and William Horwath (Doane 2003). EPA method 353.2 was used to determine NO_2^- . Ammonia (NH_4^+) was evaluated using the Berthelot reaction using sodium salicylate instead of phenol. A study done by Verdouw shows that using sodium salicylate in place of phenol is comparable to the Nessler method (EPA method 350.2) (Verdouw 1977).

All samples for total nitrogen (TN) and total phosphorus (TP) were refrigerated and seven replicates of each were evaluated at 48-hour, 4-day, 7-day, and 28-day holding times. Samples for TN or TP were not filtered. Oxidation of both TN and TP was done using 1% potassium persulfate solution (SM4500N-C) before determination of analyte amounts. The vanadium chloride (VCl_3) method (Doane 2003) was used to spectroscopically determine TN. The stannous chloride method (SM4500-P D) was used to spectroscopically determine TP. Method detection limit and reporting limit for each constituent analyzed are shown in Table 1.

Data Analysis Method

Seven replicates of each sample were measured at each holding time to evaluate the precision of the results. The average value and standard deviation were calculated and used for statistical analysis of results to determine Confidence Intervals. See Appendix B for a detailed discussion of the statistical analysis of the data.

The mean values of results measured at 4-days, 7-days, and 28-days were compared with the mean value of results measured within 48-hours of sample collection (reference value) to determine stability of each analyte over time. An analyte was considered stable if the percent difference between the mean value analyzed at a given holding time (either 4-days, 7-days, or 28-days in this study) and the mean reference value (taken at less than 48-hours) was less than 10% per the original peer reviewed Nutrient Holding Time Study Design. Results that were reported below the minimum detection limit were not used in drawing conclusions for the study.

Quality Assurance/Quality Control

Laboratory quality assurance/quality control (QA/QC) followed the SWAMP protocols set by the California State Water Resources Control Board. The protocol includes implementation of standard laboratory procedures including replicates, spikes, reference materials, setting of control limits, criteria for rejection, and data validation methods. Laboratory blanks, method blanks, matrix spikes, and laboratory control standards were

used for each sample set to verify accuracy of methods, instruments, and handling of samples by laboratory staff. Field blanks were used to verify sample collection methods. For a complete discussion of QA/QC sample results please refer to Appendix C.

QA/QC concerns in this study included analyte recovery below the minimum detection limit, failed field blanks, and failed matrix spikes.

RESULTS

Soluble Reactive Phosphate

Soluble reactive phosphate (SRP) samples that were preserved with freezing or refrigeration remained stable up to 4 days. Table 2 summarized the stability of SRP at different ranges at the different holding times. Table 3 shows the soluble reactive phosphorus data for both freezing and refrigeration preservations. Specific QA/QC results for SRP are discussed in Appendix C. Samples with an SRP concentration greater than 0.050 mg/L (the reporting limit) showed analyte stability up to seven days after sampling. Samples that contained SRP concentrations greater than ten times the reporting limit showed analyte stability up to 28 days. With the current data there is no information on samples with SRP concentrations between 0.060 mg/L and 0.400 mg/L or samples with more than 0.500 mg/L.

Figure 1 shows the percent difference between the mean reference value (48-hour holding time) and the mean results taken at 4 day, 7 day, and 28 day holding times from samples preserved by freezing. Mean results from all six locations had percent differences of less than 10% for samples analyzed at 4 days. At 7 days percent differences for five locations were less than 10% with Franklin Creek having a percent difference of 11.8%. The mean result for Franklin Creek at seven days was 0.019 mg/L, well below the 0.050 mg/L reporting limit. At 28 days Franklin Creek results had a percent difference of 21.74%.

Figure 2 shows the percent difference between the mean reference value (48 hour holding time) and the mean results taken at 4-day, 7-day, and 28-day holding times from samples preserved by refrigeration. Mean results from all six sampling locations had percent differences that were less than 10% at 4 days. At 7 days only three of the mean results had percent differences that were greater than 10%: Upper Truckee River (17.24%), Sacramento River at Freemont Ford (18.52%), and Franklin Creek (11.11%). At 28 days West Fork Carson River had a percent difference of -12.00% and Franklin Creek had a percent difference of -22.22%. Mean results whose percent differences were greater than 10% at 7 days all had reported concentrations between the method detection limit (0.005 mg/L) and the reporting limit (0.050 mg/L).

Nitrite

Samples taken from the Sacramento River at Freeport exhibited nitrite concentrations below the minimum detection limit (0.010 mg/L) therefore no conclusions could be drawn from that data. Table 4 summarizes the stability of nitrite in samples at different ranges over time and identifies QA/QC issues which are discussed in Appendix C. Table 5 shows the nitrite data for both freezing and refrigeration preservations.

Figure 3 shows the percent difference between the mean reference value (48 hour holding time) and the mean results taken at 4-day, 7-day, and 28-day holding times from samples preserved by freezing. Only the results of data sets that passed field and laboratory QA protocols are shown.

Figure 4 shows the percent difference between the mean reference value (48 hour holding time) and the mean results taken at 4-day, 7-day, and 28-day holding times from samples preserved by refrigeration. Only the results of data sets that passed field and laboratory QA protocols are shown.

Ammonia

Ammonia data did not show consistent analyte stability past 48-hours. Samples with ammonia concentrations near the reporting limit of 0.020 mg/L resulted in percent differences of greater than 10%. Samples from Orcutt Creek had ammonia concentrations around 0.100 mg/L and these samples showed analyte stability up to 28 days when frozen and up to 7 days when refrigerated. Table 6 summarizes the stability of ammonia at different concentrations at each holding time. Table 7 summarizes the ammonia data for both freezing and refrigeration preservations and identifies any QA/QC concerns. All quality assurance samples passed for the ammonia analyses. QA/QC results are discussed in Appendix C. Ammonia concentrations from five of the six sites range from 0.018 mg/L to 0.026 mg/L while ammonia concentrations at the sixth site were greater than 0.100 mg/L. Greater analyte stability was shown in samples that contained concentrations of ammonia greater than five times the reporting limit of 0.02 mg/L.

Figure 5 shows the percent difference between the mean reference value (48 hour holding time) and the mean results taken at 4-day, 7-day, and 28-day holding times from samples preserved by freezing. Only one site had samples that consistently had percent differences that were less than 10%. Ammonia concentrations from five sites ranged from 0.014 mg/L to 0.032 mg/L and none showed analyte stability over the 28-day period. The WFCR and FC sites showed stability up to 4 days. The OC site results ranged from 0.114 mg/L to 0.119 mg/L and showed stability up to 28 days.

Figure 6 shows the percent difference between the mean reference value (48-hour holding time) and the mean results taken at 4-day, 7-day, and 28-day holding times from samples preserved by refrigeration. As can be seen no site had samples that consistently had percent differences that were less than 10%. Ammonia concentrations from five of the sites ranged from 0.006 mg/L to 0.030 mg/L. OC concentrations ranged from 0.099 mg/L to 0.119 mg/L. UTR had a percent difference of 16.7% at 4 days. WFCR, SJR,

and SRF showed stability through 4-days, while FC and OC showed stability through 7-days.

Nitrate + Nitrite

Table 8 summarizes the stability of nitrate + nitrite at different concentrations at each holding time. Table 9 shows the nitrate + nitrite data for both freezing and refrigeration preservations. Due to Lab QA failures the data for Nitrate + Nitrite is considered inconclusive to determine stability at 48 hours. QA/QC results are discussed in Appendix C. Sample concentrations ranged from 0.017 mg/L to 0.046 mg/L, 1.474 mg/L to 1.506 mg/L, 24 mg/L to 26 mg/L, and 30 mg/L to 33 mg/L with no results between 1.5 mg/L and 24 mg/L. Results for the SJR site failed QA requirements at the 48-hour holding time for both frozen and refrigerated samples. Results for the SRF site failed QA requirements at the 48-hour holding time for the refrigerated samples.

Figure 7 shows the percent difference between the mean reference value (48 hour holding time) and the mean results taken at 4 day, 7 day, and 28 holding times from samples preserved by freezing. Only the results of data sets that passed field and laboratory QA protocols are shown.

Figure 8 shows the percent difference between the mean reference value (48 hour holding time) and the mean results taken at 4 day, 7 day, and 28 holding times from samples preserved by refrigeration. Only the results of data sets that passed field and laboratory QA protocols are shown.

Nitrate

Nitrate (NO_3^-) was calculated by subtracting the mean results of the Nitrite analysis from the mean results of Nitrate + Nitrite analysis. Table 10 summarizes calculated nitrate stability at different concentrations at each holding time. Due to laboratory QA failures no conclusions could be drawn.

Figure 9 shows the percent difference between the mean reference value (48 hour holding time) and the mean results taken at 4 day, 7 day, and 28 day holding times from samples preserved by freezing. Only the results of data sets that passed field and laboratory QA protocols are shown.

Figure 10 shows the percent difference between the mean reference value (48 hour holding time) and the mean results taken at 4 day, 7 day, and 28 day holding times from samples preserved by refrigeration. Only the results of data sets that passed field and laboratory QA protocols are shown.

Total Nitrogen

Refrigeration was the only preservation method used for total nitrogen and the samples were not filtered. Table 11 summarizes total nitrogen stability at different concentrations at each holding time. Table 12 shows the data for TN. QA/QC results are discussed in Appendix C. All samples contained TN concentrations greater than the RL (0.05 mg/L) and showed analyte stability over time. TN concentrations ranged from 0.146 mg/L to 35 mg/L.

Figure 11 shows the percent difference between the mean reference value (48 hour holding time) and the mean results taken at 4-day, 7-day, and 28-day holding times. Only two samples have percent differences that were more than 10%: one from the samples analyzed at 4 days from WFCR (10.98%) and one from samples analyzed at 28 days from UTR (25.15%).

Total Phosphorus

Table 13 summarizes total phosphorus stability at different concentrations at each holding time. Table 14 shows the total phosphorus data preserved by refrigerating. Due to lab QA failures data for Total Phosphorous is considered inconclusive. Results for the FC site failed QA requirements at each holding time. QA/QC results are discussed in Appendix C. TP concentrations ranged from 0.030 mg/L to 0.045 mg/L, 0.156 mg/L to 0.182 mg/L, and 0.822 mg/L to 2.561 mg/L with a RL of 0.05 mg/L.

Figure 12 shows the percent difference between the mean reference value (48 hour holding time) and the mean results taken at 4 day, 7 day, and 28 day holding times. Only the results of data sets that passed field and laboratory QA protocols are shown.

CONCLUSIONS

Soluble Reactive Phosphate: This study indicates that the holding time of SRP can be extended from the SWAMP required holding time of 48 hours up to 4 days for samples that are either refrigerated or frozen.

Nitrite: Due to the laboratory QA failures as well as the low analyte concentration in SRF samples the data for nitrite is considered inconclusive and no holding time extension is recommended for nitrite. Results show that samples containing nitrite concentrations greater than five times the RL show the potential for increased analyte stability over time.

Ammonia: The holding time for ammonia cannot be extended from the less than 48 hour holding time that is currently recommended by the US EPA for either preservation method.

Nitrate + Nitrite: Due to Lab QA failures the data for nitrate + nitrite is considered inconclusive and no holding time extension is recommended for this constituent. This study covered a limited range of concentrations and further studies should be done to determine the stability of nitrate + nitrite at concentrations between 0.5 mg/L and 24 mg/L.

Nitrate: Due to QA data failures the results for nitrate are inconclusive. Additional studies should be done to evaluate a broader range of concentrations.

Total Nitrogen: Total Nitrogen samples cannot have their holding time extended due to high percent differences at the 4 day and 28 day holding times.

Total Phosphorous: Due to lab QA failures data for Total Phosphorous is considered inconclusive. Additional studies should be done to evaluate a broader range of Total Phosphorous concentrations in samples as well as additional sample preservation methods, such as freezing the sample upon collection. Table 15 summarizes the findings of the nutrient holding time study.

NEXT STEPS

Although the current study showed some promising results, definitive findings were marred by QA/QC issues with field collection and laboratory analyses as well as limitations on the range of matrices investigated and analytical methodologies utilized. A number of future study design considerations were identified as follows:

- Samples should be taken from more locations to show analyte stability over time in different water matrices and a wider range of nutrient concentrations.
- Samples should also be analyzed at a 10 day holding time as well as one year after sample collection to verify analyte stability.
- Methods that are more commonly used in the analysis of nutrient constituents for SWAMP Projects should also be tested at each holding time.
- Field duplicates should be included to evaluate quality assurance in addition to the QA/QC samples already incorporated.
- More replicates should be included to increase statistical significance.
- Low-level nutrient samples should be spiked at specific levels within the calibration curve at concentrations of expected optimal instrumentation performance.

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