Holding Time for Samples from a Few Selected Lakes and Streams: Effects on Twenty-Five (25) Analytes

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HOLDING TIME FOR SAMPLES FROM A FEW SELECTED LAKES AND STREAMS: EFFECTS ON TWENTY-FIVE (25) ANALYTES

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Water samples collected from three lakes and seven streams in the eastern United States were held in cubitainers at a field laboratory for 12, 24, 48, and 84 hours before aliquots were prepared, preserved and sent to an analytical laboratory. One-factor repeated measures analyses of variance (RMANOVA) were used to test the effects of holding time on 25 chemical analytes, including 3 measures of pH. Regardless of the outcome of the RMANOVA, holding time was not of practical significance if the precision between holding times was less than the precision between standardized samples with no holding time. Holding time had a statisically significant effect on 17 analytes. However, the practical significance of holding time was negligible for all analytes with the exception of Fe, Mn, total Al, and pH. For Fe, Mn, and total Al, the practical significance of the effects of holding time was questionable. Based on these results and results from similar studies, syringes stored at 4 °C are used as the reliable pH measurements for the National Surface Water Survey (NSWS). Furthermore, the costs of water surveys that encompass large geographical areas can be reduced by using less expensive means of sample transport and shipment.

KEY WORDS: Holding time, acid deposition, National Surface Water Survey.

INTRODUCTION

The US Environmental Protection Agency undertook a survey of approximately 2000 lakes and 600 streams in the United States as part of Phase I of the National Surface Water Survey (NSWS). A primary objective of the NSWS is to determine the degree to which surface waters in the United States are at risk as a result of acid deposition.[1] A survey of this magnitude has many associated quality assurance and economic concerns. One concern is that the concentration of certain analytes in samples may change from the time of collection to the time they are prepared for analysis (holding time).

During Phase I of the NSWS, samples were filtered and preserved at a field laboratory, located near the center of a circle encompassing the lakes and streams

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to be sampled, then transported by truck or air charter to an analytical laboratory for chemical analysis. Standard methods of filtration and preservation assured that minimal change would occur from the time samples were preserved to the time they were analyzed at the analytical laboratory. For some analytes (temperature, dissolved oxygen, and pH), there are no techniques for preserving samples longer than 48 hours. Most analytes must be fixed as soon as possible by preservation.[2]

In general, it is possible to prevent microbial-mediated changes in analytes. It is impossible to inhibit physical changes in state. However, chemical changes can usually be inhibited by means of specific measures aimed at stabilization of various analytes. Given the wide array of chemical analytes measured in the present study, no universally applicable method of preservation is available, other than storage at 4°C until the sample can be aliquoted and appropriate preservatives added. Changes in sample concentration as a function of time cannot be ascertained with the necessary precision and accuracy to allow extrapolation of trends beyond the stipulated holding time.[3]

The holding times of samples may vary depending on the distance from the sampling site to the field laboratory, the method of transporting samples to the field or analytical laboratory, weather conditions and terrain. If holding time affects the concentrations of analytes in a sample, then interpretation of data from the NSWS, particularly from samples with different holding times, becomes difficult. If holding time has no effect on the concentration of analytes in a sample, then the costs of water surveys that encompass large geographical areas can be reduced.

This study investigated changes related to 25 chemical analytes in samples that were held 12, 24, 48, and 84 hours prior to being aliquoted and preserved. The results may be useful in future sampling strategies for water surveys covering large and/or remote geographical areas, for which the time between collection and preparation of samples is variable.

MATERIALS AND METHODS

Sample Collection and Chemical Analysis

Water samples were collected on 24 June 1985 from 3 lakes in New York, 3 streams in Pennsylvania, 2 streams in Maryland and 1 stream each in South Carolina and Tennessee. These water bodies represent a wide range of water chemistry types[4] and are representative of lakes and streams in 6 subregions of the eastern United States. This study was part of the National Stream Pilot Survey (NSS), which was a subset of the NSWS.[4,5] The sampling locations are listed in Table 1

At each site, two 19-L (5 US gallons) linear polyethylene cubitainers were filled with water using a hydrostatic pump. The contents were then mixed by rocking the cubitainers for 5 minutes to insure an homogeneous sample. Tygon™ tubing was attached to transfer water from each 19-L cubitainer into eight 3.7-L (1 U.S. gallon) cubitainers. Each 3.7-L cubitainer was rinsed three times, then filled.

Table 1 Locations of lakes and streams

Lake or stream name	County	State	Latitude	Longitude
Big Moose Lake	Herkimer	New York	43° 49′ 45″ N	74° 51′ 00″ W
Fly Pond	Herkimer	New York	43° 44′ 52″ N	74° 54′ 05″ W
West Lake	Herkimer	New York	43° 45′ 20″ N	74° 55′ 15″ W
Bear Creek	Lycoming	Pennsylvania	41° 21′ 31″ N	76° 50′ 50″ W
Cherry Run	Clinton	Pennsylvania	39° 59′ 26″ N	77° 29′ 34″ W
Lick Run	Clinton	Pennsylvania	41° 19′ 02″ N	77° 30′ 52″ W
Lyon Creek	Anne Arundel	Maryland	38° 11′ N	76° 34′ W
Morgan Creek	Kent	Maryland	39° 15′ 20″ N	76° 02′ 30″ W
Six Mile Creek	Pickens	South Carolina	34° 46′ 54″ N	82° 50′ 57″ W
Clear Creek	Anderson	Tennessee	36° 10′ N	84° 00′ W

Approximately 250 mL of sample were bled from each cubitainer to eliminate headspace and facilitate packing. Holding times of 12, 24, 48, and 84 hours were randomly assigned to each cubitainer. Each lake or stream, therefore, was represented by a pair of 3.7-L cubitainers for each holding time. Samples were shipped via charter aircraft from remote sites to the field laboratory and arrived there so that samples could be prepared and preserved within 12 hours of their collection. The cubitainers were kept at aproximately 4 °C until their respective holding time, then aliquoted and preserved in the field laboratory.[5] Preserved sample aliquots were shipped the following day to an analytical laboratory by overnight courier service. Samples were stored at approximately 4 °C during shipment.

At the analytical laboratory, samples were analyzed for 25 analytes including three measures of pH and two measures of dissolved inorganic carbon. Two of the three pH measurements (pH-alkalinity and pH-acidity) were determined from the Gran titration method for acid neutralizing capacity and base neutralizing capacity. The remaining pH (pH-equilibrated) was determined after equilibrating the sample with 300 ppm CO_2. Dissolved inorganic carbon was determined both before and after the sample was equilibrated with 300 ppm CO_2.

A summary of the analytical methods can be found in Table 2. The raw data for the NSS, a summary of the conclusions, and a detailed description of the methods used can be found elsewhere.[4-6] All samples were analyzed within accepted time limits for preserved samples.[2]

Statistical Analysis

Standard single-factor repeated measures analyses of variance[7] were performed (RMANOVA) for each analyte. Specifically, the null hypothesis of equal mean concentrations of a particular analyte of 12, 24, 48, and 84 hours was tested.

One assumption of the RMANOVA model is that the measurement error does

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Table 2 Summary of methodology and detectability[8]

Analyte[a]	Units	Method	Detection
Al, organic monomeric	$mg\,L^{-1}$	Filtration, ion exchange, extraction with 8-hydroxquinoline into MIBK[b], followed by AAS[c] (furnace)	0.005
Al, total extractable	$mg\,L^{-1}$	Exraction with 8-hydroxquinoline into MIBK[b], followed by AAS[c] (furnace)	0.005
Al, total	$mg\,L^{-1}$	AAS[c] (furnace)	
ANC (Acid neutralizing capacity)	$\mu eq\,L^{-1}$	Titration and Gran analysis	$\geqq 10$
BNC (Base neutralizing capacity)	$\mu eq\,L^{-1}$	Titration and Gran analysis	$\geqq 10$
Ca	$mg\,L^{-1}$	AAS[c] (flame)	0.01
Cl^-	$mg\,L^{-1}$	Ion chromatography	0.01
DIC (Dissolved inorganic carbon)	$mg\,L^{-1}$	Instrumental (acidification, CO_2 generation, infrared detection)	0.05
DOC (Dissolved organic carbon)	$mg\,L^{-1}$	Instrumental (ultraviolet-promoted oxidation, CO_2 generation, infrared detection)	0.01
Total fluoride	$mg\,L^{-1}$	Ion-selective electrode and meter	0.005
Fe	$mg\,L^{-1}$	AAS[c] (flame)	0.01
K	$mg\,L^{-1}$	AAS[c] (flame)	0.01
Mg	$mg\,L^{-1}$	AAS[c] (flame)	0.01
Mn	$mg\,L^{-1}$	AAS[c] (flame)	0.01
Na	$mg\,L^{-1}$	AAS[c] (flame)	0.01
NH_4^+	$mg\,L^{-1}$	Automated colorimetry (phenate)	0.005
NO_3^-	$mg\,L^{-1}$	Ion chromatography	0.005
pH, analytical	pH units	pH electrode and meter	—
P, total	$mg\,L^{-1}$	Automated colorimetry (phosphomolybdate)	0.002
SiO_2	$mg\,L^{-1}$	Automated colorimetry (molybdate blue)	0.05
SO_4^-	$mg\,L^{-1}$	Ion chromatography	0.05
Conductance	$\mu S\,cm^{-1}$	Conductivity cell and meter	$\leqq 9$

[a]Dissolved ions and metals are being determined, except where noted.
[b]Methyl isobutyl ketone.
[c]Atomic absorption spectroscopy.

not vary as a function of concentration. To satisfy this assumption, log transformations were required for Ca, Mg, total phosphorus, extractable aluminum, Na, Cl⁻, conductance, air equilibrated dissolved inorganic carbon and acid neutralizing capacity. One stream, Lyon Creek, had extremely unusual data for total aluminum. The RMANOVA for total aluminum was performed both with and without data from this stream.

Of considerable interest to the chemist in the NSWS is whether (1) the precision between measurements from samples held in the field at different times is less than the precision between measurements on stable, standard samples with no holding time; (2) changes in concentration of a particular analyte occur at all lakes and streams in withheld samples; and (3) these changes in concentration are the same (e.g., decreasing) for all lakes and streams. If these three conditions are satisfied, the effects of holding time are of concern ("practical significance") to the chemist.

Forty-one samples obtained from Big Moose Lake, New York were used to estimate analytical precision and evaluate laboratory bias and precision during the NSS. These "natural audit" samples were shown to be chemically stable during NSS, and therefore provided an estimate of the amount of relative variance due to the collection and analysis of samples during NSS.[8] The relative standard deviation (standard deviation/mean) was used to estimate the precision of the NSS natural audit data for all analytes, except pH, for which the absolute standard deviations were used.[8,9] The greater the relative standard deviation, the less the precision and the greater the variance associated with measuring an analyte.

Similarly, precision between two holding times in the present study was estimated by the relative standard deviation (RSD) between two holding times. The absolute standard deviation between holding times was used for pH measurements. If the RSD between two holding times is less than the RSD of the natural audit sample, then the variance due to holding time is less than the variance due to the overall collection and analysis of samples in the NSWS. Therefore, statistically significant effects of holding time become negligible, in the practical sense, when the RSD values between holding times are less than the RSD values of the NSS natural audit data. Evaluating practical significance becomes difficult when concentrations of an analyte are below a given limit of quantitation, or a level below which high precision is not expected.[10] Further, when concentrations of an analyte are low, a small difference between the means at two holding times results in large relative differences.

When determining the practical significance of holding time, one should also consider whether the effects are limited to a few of the lakes or streams or the majority, and whether the effect is the same for all lakes and streams. Suppose that the concentration of a particular analyte changes at a certain holding time for some lakes and streams, while the concentration does not change at the same holding time for the majority of the lakes and streams. Further, suppose that for those lakes and streams that do change, both increases and decreases in concentration of the analyte are observed. In such a case the effects of holding time may be due to site-specific phenomena. The practical significance of holding time in such cases would be questionable.

RESULTS AND DISCUSSION

Plots of the data (Figures 1A–Y) suggest that for most analytes holding time did not have a strong effect over all lakes and streams. Holding time had a statistically significant effect ($p < 0.05$) for 17 analytes (Table 3). The results of groups of analytes are discussed below.

Effect on Total Ca, Mg, K, NH_4^+, Na *(Figures 1A–E)*

Holding time had a statistically significant effect on the concentrations of Ca and Na ($p < 0.05$, Table 3). No significant effect of holding time was observed for Mg, K, and NH_4^+. The average concentration of Ca increased up to 48 hours, then sharply decreased (Table 3, Figure 1A). However, 53.6% of this change for Ca was due to data from Clear Creek alone. For all of these analytes, the RSD between holding times was always less than the RSD of the NSS natural audit data (Table 4). Therefore, the practical significance of holding time for these analytes is negligible.

   The precipitation of $CaCO_3(s)$ has been documented in samples close to the saturation point of Ca in solution.[2] Samples in the present study were well below this point, thus loss of Ca due to precipitation was neither expected nor observed.

Effect on Fe and Mn *(Figures 1F, G)*

Iron and manganese have a tendency to form hydrous oxides upon aging.[11-13] A discussion of these two elements, therefore, is appropriate. The mean concentration of Fe decreased in all lakes and streams between 12 and 84 hours. The concentration of Mn decreased in seven lakes and streams, and did not change for the remaining three lakes and streams between 12 and 84 hours. The effects of holding time appear to be site-specific in nature. Both increases and decreases in concentration of Fe occurred in those lakes and streams exhibiting change. Poor precision between samples having different holding times was expected due to the low concentrations of Mn. As discussed below, the practical significance of the effects of holding time for these two analytes is questionable.

   For Fe, the RSD values for all holding times were greater than the RSD values of the NSS natural audit samples (Table 4). For Mn, the RSD values between samples held between 24 and 48 hours and between 12 and 84 hours were greater than the RSD values of the NSS natural audit samples. However, the RSD values between samples held 12 and 24 hours and between 48 and 84 hours were less than the RSD of the NSS natural audit sample.

   Changes in the mean Fe concentration between holding times was due mainly to changes in Morgan, Lyon and Six Mile Creeks. In all lakes and streams exhibiting changes in concentrations of Fe, both increases and decreases were observed between 12 and 84 hours. It appears, therefore, that the effects of holding time for Fe, particularly between 48 and 84 hours, may have been site-specific in nature and restricted to a minority of lakes and streams. Further, the mean

Table 3 Means and S.E. (as detrmined by RMANOVA) of 25 chemical analytes at four holding times for three lakes and seven streams. Unless stated otherwise, units are $mg L^{-1}$. Probabilities (p) <0.05 for the repeated measured analysis of variance indicate a statistically significant effect of holding time.

	Holding time (h)					
	12	*24*	*48*	*84*	*S.E.*	*p*
Ca	7.012	7.185	7.230	7.019	0.035	<0.0001[a]
Mg	2.571	2.566	2.592	2.596	0.013	0.0042[a]
Na	1.874	1.866	1.864	1.850	0.004	<0.001[a]
K	1.238	1.274	1.254	1.243	0.009	0.0643
Mn	0.054	0.052	0.040	0.039	0.003	0.0096
Fe	0.057	0.050	0.084	0.035	0.010	0.0420
NH_4^+	0.039	0.036	0.035	0.031	0.002	0.1871
$SO_4^=$	5.845	5.743	5.735	5.704	0.022	0.0002
NO_3^-	2.130	2.023	2.021	2.024	0.021	0.0092
Cl^-	3.276	3.269	3.134	3.071	0.50	0.0483[a]
DOC	2.569	2.575	2.457	2.758	0.049	0.0002
DIC	4.842	4.734	5.137	5.017	0.094	0.0505
DICE	4.493	4.476	4.817	4.858	0.073	0.0005[a]
COND[c]	71.42	71.48	71.80	71.61	0.125	0.0465[a]
SiO_2	7.754	7.876	7.798	7.814	0.032	0.797
FTL	0.070	0.071	0.070	0.071	0.000	0.377
PTL	0.074	0.078	0.066	0.073	0.003	0.137
ALTL	0.509	0.736	0.736	0.346	0.126	0.0875
ALTL[b]	0.113	0.145	0.109	0.132	0.009	0.0592
ALEX	0.027	0.030	0.033	0.030	0.001	<0.0001[a]
ALOR	0.012	0.015	0.017	0.015	0.001	0.0342
ANC[d]	438.00	436.75	436.50	434.38	2.075	0.0456[a]
BNC[d]	52.103	59.303	63.053	65.653	2.518	0.0195
pHAC[e]	6.816	6.738	6.663	6.644	0.023	<0.0001
pHAL[e]	6.742	6.703	6.652	6.635	0.020	0.0015
pHEQ[e]	7.326	7.356	7.299	7.308	0.015	0.0514

[a]Repeated measures analysis of variance performed on logarithms of values.
[b]Data from Lyon Creek removed.
[c]$\mu S cm^{-1}$.
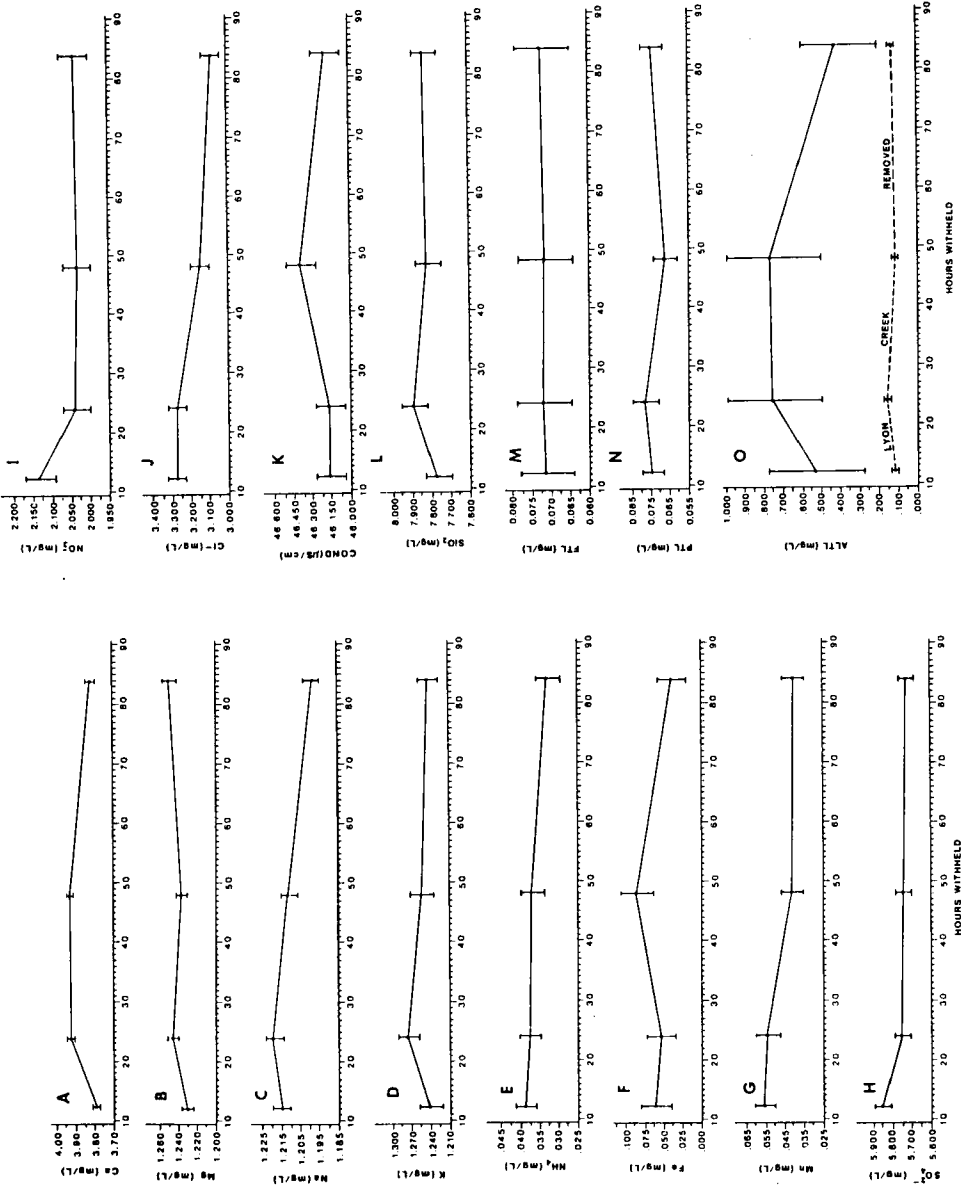[d]$\mu eq L^{-1}$.
[e]pH units.
Abbreviations: FTL = total fluoride, PTL = total phosphorus, ALEX = total extractable aluminum, ALOR = organic aluminum, ALTL = total aluminum, DOC = dissolved organic carbon, DICE = CO_2-equilibrated dissolved inorganic carbon, DIC = dissolved inorganic carbon.

concentration of Fe in NSS audits was $0.134 mg L^{-1}$, which is more than twice the mean concentrations of the withheld samples. The low concentration of Fe in this study may have further contributed to the imprecisions between holding times. The practical significance of holding time for Fe is questionable.

Increasing Fe concentrations in samples with increasing holding time are seldom reported. As mentioned, this observed phenomenon appears to be site-specific. Samples containing large amounts of suspended solids were observed to increase in Fe concentration over time.[14] The phenomenon was restricted to streams in the
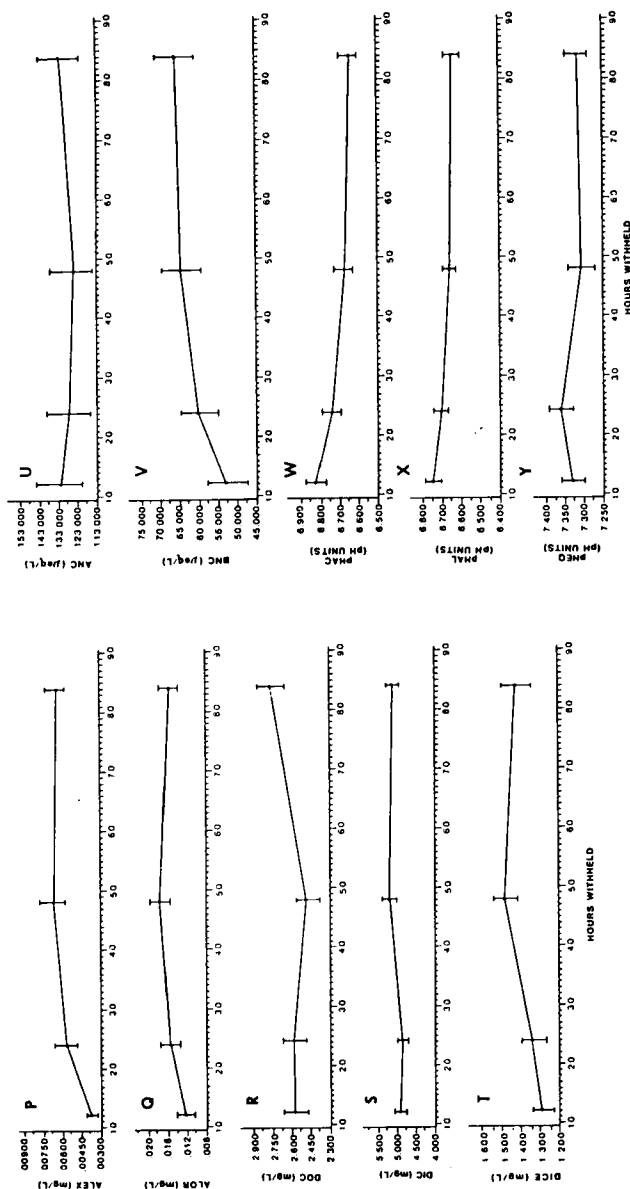
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Figure 1 Plots of mean concentration ±2S.E. of repeated-measures analysis of variance (RMANOVA) versus holding time. Because the RMANOVA was performed on the logarithms of the values, the geometric means and their standard errors are plotted for Ca, Mg, Na, Cl⁻, air-equilibrated dissolved inorganic carbon (DICE), conductance (COND) and total extractable aluminum (ALEX). Abbreviations: DOC = dissolved organic carbon, DIC = dissolved inorganic carbon, FTL = total fluoride, PTL = total phosphorus, ALTL = total aluminium, ALOR = total organic aluminum, ANC = acid neutralizing capacity, BNC = base neutralizing capacity, pHAC = pH-acidity, pHAL = pH-alkalinity, pHEQ = pH-equilibrated.

Table 4 Relative standard deviation (RSD) between the means for each constituent for holding times of 12 to 24, 24 to 48, 48 to 84, and 12 to 84 hours. For pH analytes, absolute standard deviation was used. The RSD of natural audit data from the National Streams Survey (NSS) was one criterion used to determine the practical significance of holding time. For pH analytes, absolute standard deviation of the audits was used.

	12–24 h	24–48 h	48–84 h	12–84 h	NSS
Ca	−0.016	−0.004	0.021	0.001	0.030
Mg	0.001	0.004	−0.001	0.001	0.015
Na	0.003	0.001	0.005	0.009	0.015
K	−0.021	0.011	0.006	0.003	0.021
Mn	0.027	0.185	0.018	0.228	0.054
Fe	0.093	0.359	0.582	0.338	0.010
NH_4^+	0.057	0.020	0.086	0.162	0.440
$SO_4^=$	0.012	0.001	0.004	0.017	0.028
NO_3^-	0.037	0.001	−0.001	0.035	0.070
Cl^-	0.001	0.030	0.014	0.045	0.066
DOC	−0.001	0.053	−0.032	−0.052	0.066
DIC	0.016	−0.057	0.017	−0.025	0.31
DICE	0.003	−0.052	−0.006	−0.055	0.790
COND	−0.001	−0.003	0.002	−0.002	0.010
SiO_2	−0.011	0.007	−0.001	−0.006	0.029
FTL	−0.010	0.010	−0.010	−0.010	0.027
PTL	−0.037	0.118	−0.069	0.010	0.88
ALTL	−0.258	0	0.635	0.269	0.110
ALTL[a]	−0.177	0.198	−0.134	−0.106	0.110
ALEX	−0.074	−0.067	0.067	−0.074	0.230
ALOR	0.157	−0.088	0.088	0.157	0.320
ANC	0.002	0.001	0.004	0.006	−0.270
BNC	0.091	−0.043	−0.029	0.159	0.140
pHAC	0.055	0.053	0.013	0.122	0.05
pHAL	0.028	0.036	0.012	0.076	0.05
pHEQ	−0.021	−0.040	−0.006	−0.013	0.03

[a]Data from Lyon Creek removed.

Abbreviations: DOC = dissolved organic carbon, DIC = dissolved inorganic carbon, DICE = CO_2-equilibrated dissolved inorganic carbon, COND = conductance, FTL = total fluoride, PTL = total phosphorus, ALEX = extractable aluminum, ALOR = organic aluminum, ALTL = total aluminum, ANC = acid-neutralizing capacity, BNC = base neutralizing capacity, pHAC = pH-acidity, pHAL = pH-alkalinity, pHEQ = pH-equilibrated.

present study. In general, streams are characterized by higher total suspended solids than are lakes. Small changes in pH (<0.05 units), possibly caused by CO_2 degassing or diffusion into the sample, may cause changes in the partitioning between the "dissolved" and "particulate" phases of Fe and other metals.[15] The release rates of metals from particulates vary with pH and are directly related to their respective hydrolysis constants.[13]

Similarly, samples from Morgan, Lyon, and Six Mile Creeks had unexpected results for Mn. When data from Morgan, Lyon, and Six Mile Creeks are eliminated, the RSD values of Mn between 24 and 48 hours and between 12 and

84 hours were 0.032 and 0.105, respectively. These values are less than the RSD of the NSS natural audit data for Mn (Table 4).

When the samples from Clear Creek at 12 hours, and all data from Morgan, Lyon and Six Mile Creeks were removed, the maximum change between any two holding times was $0.001 \, \text{mg L}^{-1}$. This low amount of change in concentration between holding times for the majority of lakes and streams suggests that the effect of holding time on the concentration of Mn is site-specific. The mean concentration of Mn in NSS natural audit samples was $0.092 \, \text{mg L}^{-1}$. The mean concentrations of Mn in this study were less than this level (Table 3). Therefore, some of the observed differences in mean concentrations of Mn could be due to the relatively poor precision expected at low concentrations. The practical significance of holding time on the concentration of Mn appears to be questionable.

Michnowsky et al.[16] observed a decrease in acid extractable Fe and Mn in samples with increased holding time. The decrease was attributed to a settling of particulate-bound Fe and Mn which, consequently, was not included in the extraction procedure. Cubitainers were thoroughly mixed in the present study before the aliquot was removed. Subramanian et al.[17] followed the concentrations of Fe and Mn in samples held in linear polyethylene containers over time. Significant losses of both elements were observed in 5 days or less, after which time samples were stable up to 30 days. The cubitainers in the present study were made of similar material. Thus, some adsorptive losses would be expected for Fe and Mn. Benes and Smetana[18] observed a significant loss of Fe above pH 3.0. After 5 days, 98% of the total Fe was lost at pH 6.6. The authors attributed the loss to chemisorption of $Fe(OH)^{2+}$ and $Fe(OH)_2^+$ in the pH range 2–5, and a similar loss of colloidal hydrous ferric oxide above pH 5.0. Loss of Mn due to the formation of hydrous oxide adsorbed onto linear polyethylene was also postulated.

Additionally, organic acids (e.g., humic and fulvic acids) are known to increase the apparent solubility of Fe and Mn in solution as peptized sols above pH 5.0. Adsorption onto the container surface of these organic complexes may be greater than inorganic sols.[17]

Effect on the Anions $SO_4^=$, NO_3^-, and Cl^- (Figures 1H–J)

Holding time had a statistically significant effect on $SO_4^=$, NO_3^-, and Cl^- ($p < 0.05$, Table 3). The mean concentration of all three variables decreased over time (Tables 3, 4). However, the practical significance of these decreases was negligible. In all cases, the RSD values between holding times were less than the RSD of the NSS natural audit samples (Table 4). Between 12 and 84 hours, the mean concentration of $SO_4^=$, NO_3^-, and Cl^- decreased by 2.4%, 5%, and 6.4%, respectively (Table 3). Figures 1H–J suggest no strong trend for any of these analytes with respect to holding time.

The stability of Cl^- in samples is well documented. As a result of its stability, APHA[2] states that no special preservation is needed. Similarly, the Hydrochemistry Team of the German Chemists Association[19] found that preservation

was not required for chloride. The stability of Cl^- is further exemplified in the present study.

Sulfate has also been observed to be stable up to 84 hours. Chan et al.[20] found that the precipitation samples held in polyethylene bags exhibited no significant decrease after 30 days. Conversely, Topol et al.[21] observed significantly lower sulfate concentrations in precipitation samples that were measured weekly as opposed to those derived from daily composite samples.

The stability of nitrate in samples has been reported by several investigators in recent years.[22-24] Within the 84-hour holding time nitrate was found to be quite stable relative to the natural audit sample. Delfino[22] found nitrate to be stable up to 20 days with the only preservation measures being storage at $4\,°C$. Williams[24] and Bowditch et al.[25] also noted the stability of NO_3^- in samples over time. Most of the variability was attributed to daily measurement variation and not due to changes in NO_3^- concentration. The stability of nitrate in parts per billion levels in precipitation samples has been documented.[20,26] In contrast, Topol et al.[21] reported a loss of nitrate in rain and snow samples held for one week. Suarez et al.[27] found nitrate stability to be highly site-specific and dependent upon the matrix of surface water samples.

Effect on Conductance (Figure 1K)

Holding time had a statistically significant effect on conductance ($p < 0.05$, Table 3). The practical significance of the effect of holding time, however, was negligible. The RSD values betwen holding times were always less than the RSD of NSS natural audit data (Table 4). Conductance exhibited no definite trend over time and was stable for all streams (Figure 1N). Samples exhibited a mean increase in only $0.19\,\mu S\,cm^{-1}$ (0.3 %) from 12 to 84 hours (Table 3).

In precipitation samples held for up to 29 days conductance was highly stable.[20] The largest change in conductance reported was an increase of $1.3\,\mu S\,cm^{-1}$ as compared to a mean increase of $0.19\,\mu S\,cm^{-1}$ in the present study. From a statistical standpoint the change was significant, but from a chemical standpoint the change was insignificant, especially if one considers that conductance is the sum of all the major cations and anions in the sample. With all ions free to vary in the sample, the small increase in conductance observed is remarkable.

Effect on SiO_2, Total Flouride, Total Phosphorus (Figures 1L–N)

Holding time did not have a significant effect for these analytes ($p > 0.05$, Table 3). The RSD value between holding times were always less than the RSD of the NSS natural audit data (Table 4). The stability of both SiO_2 and fluoride is expected given the non-reactivity of these two analytes. They are fairly inert species in terms of adsorption onto container surfaces, particularly polyethylene. The APHA[2] does not stipulate any special precautions in measuring these species after extended holding times.

The stability of total phosphorus in the present study corroborates the findings of the German Chemists Association,[19] indicating that no unique preservation is required for this constituent. In contrast, APHA[2] recommends that HCl be added to samples which are to be analyzed for total phosphorus because of the great tendency for adsorptive losses. However, stability in polyethylene containers can be expected up to 84 hours with storage at 4 °C.

Effect on Aluminium (Figures 1O–Q)

Holding time had a statistically significant effect on the concentrations of total extractable (8-oxine) Al (ALEX) and total organic monomeric Al (ALOR), but not for total Al (ALTL) (Table 3). Lyon Creek had unusually high values for ALTL (Figure 1R). When the data for Lyon Creek were removed, holding time had a statistically significant effect on ALTL.

When the data for ALTL from Lyon Creek were removed, the RSD values for all holding times exceeded the RSD of NSS natural audit data (Table 4). Figure 1O suggests stable concentration for ALTL between 12 and 84 hours. The mean concentration of ALTL in NSS natural audit samples was $0.418 \, mg \, L^{-1}$. The quantitation limit was $0.28 \, mg \, L^{-1}$ ALTL for the NSS.[8] Poor precision was expected, therefore, at the low concentrations observed ($<0.15 \, mg \, L^{-1}$) when the data from Lyon Creek were removed. The mean concentration exhibited both slight increases and decreases (Table 3), further suggesting that no overall trend is present. The practical significance of the effects of holding time for ALTL is questionable.

The presence of suspended solids in samples has been noted to cause gradual increases in various trace metals, as discussed earlier for Fe and Mn. A similar phenomenon for total aluminum may have occurred in the Lyon Creek samples. Although no suspended solids or turbidity data were available for this study, stream samples generally have higher levels of these two physical constituents than do lake samples. Chan et al.[20] observed a 30% decrease in total Al after 29 days of storage in polyethylene bags. This decrease represents long-term storage losses. The authors reported a 20% loss of Al from a solution containing $25 \, \mu g \, L^{-1}$ stored in polyethylene containers for 24 hours. At low levels of Al, the relative standard deviation of the analytical method increases dramatically. Thus, losses in Al in some studies may be confounded with poorer precision at the lower range of concentrations. Subramanian et al.[17] reported significant losses of Al from natural and synthetic samples held in Nalgene containers. Most of the adsorption onto the container walls was complete within 5 days.

For ALEX, the RSD between holding times was always less than the RSD of the NSS natural audit data (Table 4). Figure 1P suggests no overall trend in the effect of holding time. With the exception of Big Moose Lake, the maximum change between any two holding times was $0.004 \, mg \, L^{-1}$. These levels are below the detection limit[8] and poor precision is anticipated. For five lakes and streams, the maximum change was $0.002 \, mg \, L^{-1}$. For these reasons, the practical significance of holding time for ALEX is negligible.

Extractable aluminum is intimately related to ALTL, pH, dissolved inorganic

carbon, dissolved organic carbon, and ALOR. Changes in one or more of these will have an effect on ALEX. Changes in ALEX were all less than $0.003 \, mg \, L^{-1}$ between any two holding times for all lakes and streams except Big Moose Lake. Schafran and Driscoll (personal communication) noted an increase in extractable Al from 2.0 to $3.2 \, \mu g \, L^{-1}$ ($\sim 35 \, \mu g \, L^{-1}$) within 4 days for Darts Lake (Adirondack Mountains, NY) samples. There was also a concomitant decrease in pH from 5.13 to 5.04. Although such a decrease in pH was not apparent in the Big Moose Lake sample, such an occurrence would be expected to increase the dissolution of gibbsite, kaolinite, and other aluminum-containing minerals. As a result, Al^{3+} would be released, causing an increase in ALEX. Solubility of clay minerals is also temperature dependent. Solubility decreases with increasing temperature, causing oversaturation with respect to Al^{3+} at higher temperature, provided that pH does not decrease.[28] Thus, some aluminum may be lost[28] because gibbsite precipitates out of solution when the sample temperature increases. The temperature at the time of extraction was not noted in the present study, however.

For ALOR, the RSD between holding times was always less than the RSD of NSS natural audit data (Table 4). The concentration of ALOR for Clear Creek changed more than any other lake or stream during this experiment. Both increases and decreases were noted in concentrations of ALOR in Clear Creek samples, suggesting no overall trend. The changes in concentration exhibited by Clear Creek are probably due to site-specific phenomena. The practical significance of the effects of holding time on the concentration of ALOR is negligible.

The stability of ALOR is highly dependent on pH. The conditional stability constants for many organic complexes involving Al decrease with decreasing pH.[29] The extraction ability of the 8-hydroxyquinoline molecule must be greater than the stability of the inorganic or organic Al complex. Decreases in pH, possibly brought about by diffusion of CO_2 into a sample, could conceivably decrease the conditional stability of organo-Al complexes, resulting in greater extraction by 8-oxine. As shown below, significant decreases for pH occurred.

A gradual increase of $0.011 \, mg \, L^{-1}$ ALOR was observed in Big Moose Lake samples held up to 84 hours. The observed increase in ALEX in Big Moose Lake, possibly attributable to increased dissolution of clay minerals, would alter the equilibrium conditions. The additional free Al would not remain as such, but would distribute itself between free and bound forms. Free binding sites on various organic ligands would partition the free Al between free and bound forms to maintain equilibrium in the system. Humic and fulvic acid contain several types of binding sites with a range of binding intensities.[30] Some Al may bind to sites which are stronger than the extraction ability of the 8-oxine. However, a larger fraction may bind to sites weaker with respect to 8-oxine. This scenario would account for the decrease in ALEX after 48 hours and the gradual increase in ALOR up to 84 hours in samples from Big Moose Lake. Changes in ALOR, ALTL, and ALEX appear to be the result of site-specific phenomena.

Effect on Dissolved Carbon (Figures 1R–T)

Holding time had a statistically significant effect on the concentration of dissolved

organic carbon (DOC) and CO_2-equilibrated dissolved inorganic carbon (DICE), but not on the concentration of dissolved inorganic carbon (DIC) (Table 3). Between 12 and 84 hours, the mean concentrations of DOC and DICE increased by 11.5% and 7.8%, respectively (Table 3). The increase in DICE after 24 hours may have been due to the lack of attaining true equilibrium with the 300 ppm carbon dioxide purge gas in some samples. For these three analytes, the RSD values between holding times were always less than the RSD values of NSS natural audit data (Table 4). Therefore, the practical significance of holding time for these analytes is negligible. Figures 1R, 1S, and 1T suggest no strong trend for these analytes from 12 to 84 hours.

Dissolved inorganic carbon (DIC) levels are highly dependent upon the equilibrium concentration of CO_2 in the sample.[13] Any factors which affect the partial pressure of carbon dioxide will affect the dissolved CO_2 and hence the concentration of DIC in a sample. Between 12 and 84 hours, the mean concentration of DIC increased. Schock and Schock[31] reported the permeability of cubitainers to CO_2. An increase in CO_2, in effect, would cause a decrease in sample pH. Schock and Schock stated that a precipitation of calcium carbonate, which would be measured as a decrease in DIC, would cause a decrease in pH. Cessation of photosynthetic activity in samples could conceivably cause precipitation of $CaCO_3$, due to the lack of CO_2-fixation.[13] However, given the slight increase in the concentration of DIC, the precipitation of calcium carbonate can be discounted. This strongly suggests that the decrease in pH, shown below, is due to the permeation of CO_2 into the cubitainer, and not due to a loss of Ca as $CaCO_3(s)$. An increase in pH and a decrease in DIC would be anticipated if a sample were supersaturated with CO_2 in relation to the equilibrium atmospheric CO_2 partial pressure at the site of analysis. The pH of precipitation samples in storage has been reported to increase with time.[32] This phenomenon was possibly related to gas exchange, since the most dramatic changes occurred after the sample was opened to the atmosphere. The authors could not discount biological activity or dissolution of mineral material as causes for the observed pH changes. Biological activity would be manifested by increased respiration and increased utilization of organic carbon. Rather, a slight increase in dissolved organic carbon was observed in this study. Within the time constraints of the present study, biological activity seems to be of minor importance.

The observed increase in DOC is peculiar. DOC increased by 11.5% between 12 and 84 hours. Subramanian et al.[17] attributed decreases in various trace metal levels in stored samples to adsorption of the organically-bound elemental forms onto container surfaces. The authors did not report a concomitant decrease in DOC. Whitfield and McKinely[33] observed an increase in particulate carbon by approximately 87-131% in samples stored at 5 °C. Dissolved organic carbon is determined on a filtered aliquot. Whether an increase in particulate carbon will result in an increase in DOC is not clear. APHA[2] states that filtration is necessary when only the dissolved TOC (DOC) is of interest, as in the present study. However, filtration can result in a loss or gain of DOC, depending on the physical properties of carbon-containing material in each individual sample.[34] Release of

plasticizers from the container walls may also contribute to the observed increase in DOC.

Effect on Acid Neutralizing Capacity (Figure 1U) and Base Neutralizing Capacity (Figure 1V)

Holding time did not have a significant effect on acid neutralizing capacity (ANC) (Table 3). The RSD values between holding times were always less than the RSD values of the NSS natural audit samples (Table 4). In a similar study, Schock and Schock[30] observed very little change in alkalinity in samples held up to four weeks in cubitainers. Chemical equilibrium calculations[13,35] demonstrate that total alkalinity (ANC) should remain stable as long as hydrogen ion concentration is free to vary, even if samples come in contact with different equilibrium concentrations of atmospheric CO_2. The US EPA[35] recommends that ANC determinations be performed within 24 hours of sample collection. The results of the present study indicate ANC is stable up to 84 hours.

Holding time had a statistically significant effect on base neutralizing capacity (BNC). The RSD between 12 and 84 hours exceeded the RSD for NSS natural audit data (Table 4). However, the RSD for BNC between the remaining holding times were less than the RSD values of the NSS natural audit samples (Table 4). Data from Morgan Creek and Lyon Creek accounted for 60% of the difference in BNC between 12 and 84 hours. When the data from Morgan and Lyon Creeks were removed, the RSD for holding times between 12 and 84 hours was 0.081, which is less than the RSD values of the NSS natural audit samples. The small mean increase in BNC, although not of practical significance, may have been due to an introduction of CO_2 into some samples from Morgan Creek and Lyon Creek.

Effect on pH (Figures 1W–Y)

Holding time had a statistically significant effect on pH-alkalinity and pH-acidity, but not on pH-equilibrated (Table 3). Between 12 and 84 hours, pH-alkalinity and pH-acidity decreased by an average of 0.107 and 0.152 pH units (2.3% and 1.6%), respectively. The introduction of carbon dioxide into some samples, particularly those from Morgan and Lyon Creeks, may have caused this decrease. The standard deviations between holding times of 12 and 84 hours for pH-acidity and pH-alkalinity were greater than the standard deviations for NSS natural audit data (Table 4). Further, the standard deviations between holding times of 12 and 24, and 24 and 48 hours for pH-acidity were greater than the standard deviations for NSS natural audit data. Figures 1W and 1X indicate steadily decreasing levels of pH-acidity and pH-alkalinity from 12 to 84 hours. The standard deviation between holding times of 24 and 48 hours for pH-equilibrated data was greater

than the standard deviation of the NSS natural audit data (Table 4). For the remaining holding times, however, the standard deviation for pH-equilibrated was less than the standard deviation of the NSS natural audit data. Figure 1Y indicates stable levels of pH-equilibrated data from 12 to 84 hours.

The decrease in sample pH with with respect to the audit sample pH is expected because the audit sample has achieved equilibrium with atmospheric CO_2, while actual samples may be either undersaturated or oversaturated with respect to atmospheric CO_2. The direction of pH change depends upon the proximity of the sample's CO_2 concentration to the atmospheric CO_2 partial pressure where the sample container was opened for analysis. Samples undersaturated with respect to the atmospheric partial pressure of CO_2 will decrease in pH, as this gas diffuses into solution. Samples oversaturated will increase in pH as CO_2 degasses out of the sample. Thus, the observed trend in pH reflects an overall response of samples decreasing, due to their initial CO_2 concentrations.

Cubitainers appear to be unsuitable for maintaining stability of pH.[31] Burke et al.[36] successfully maintained stable pH readings for up to 7 days in samples held in syringes stored at 4 °C.

CONCLUSIONS

Although holding time had a statistically significant effect on 17 of 25 analytes, the practical significance of holding time was negligible for all analytes except Fe, Mn, total Al, and pH. Based on the present study and other results, pH measurements made on samples held in syringes stored at 4 °C are used as the reliable pH values in the NSWS. For Fe, Mn, and total Al, the practical significance was questionable because at least two of the following were true: (1) changes in concentration with holding time were restricted to samples from a minority of lakes and streams, and site-specific in nature, (2) the mean concentration exhibited both increases and decreases over time, suggesting no overall trend, or (3) low concentrations may have accounted for relatively poor precision between samples at different holding times. The practical significance must inevitably be determined by the data user. For chemists using the Fe, Mn, total Al, or organic Al data in chemical equilibrium models (e.g., MINEQL or CEOCHEM), the observed effects of holding time on the values of these analytes may not drastically alter the interpretation of the model predictions. Alternatively, the effects of holding time on analyte values may be interpreted as having practical significance with respect to site-specific biological response.

A major uncertainty in this study is the effect of holding samples up to 12 hours, or more than 84 hours, after collection. If samples in polyethylene cubitainers and polypropylene syringes can be kept at 4 °C, transported to an analytical laboratory, filtered, and preserved within 84 hours after collection, the cost of a survey can be decreased by eliminating field laboratories. Furthermore, less expensive modes of sample collection (e.g., ground crews instead of helicopter crews), or sample shipment (e.g., standard air instead of overnight courier) can be employed.

Acknowledgments

Notice

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