An evaluation of freezing as a preservation technique for analyzing dissolved organic C, N and P in surface water samples

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ABSTRACT

Techniques for preserving surface water samples are recently in demand because of the increased interest in quantifying dissolved organic matter (DOM) in surface waters and the frequent collection of samples in remote locations. Freezing is a common technique employed by many researchers for preserving surface water samples; however, there has been little evaluation of the effects of freezing on DOM concentrations. Ten streams were sampled in southeast Alaska with a range of dissolved organic carbon (DOC) concentrations (1.5 to 39 mg C L⁻¹) to evaluate the influence of freezing (flash and standard freeze) and filter pore size (0.2 and 0.7 μm nominal pore size) on dissolved organic C, N and P concentrations. We report a significant decrease in DOC (p<0.005) and total dissolved P (p<0.005) concentrations when streamwater samples were frozen, whereas concentrations of dissolved organic N did not significantly decrease after freezing (p=0.06). We further show that when surface water samples were frozen, there was a decrease in the specific ultraviolet absorbance (SUVA) of DOC that is particularly evident with high concentrations of DOC. This finding suggests that spectroscopic properties of DOC have the potential to be used as indicators of whether surface water samples can be frozen. Our results lead us to recommend that surface water samples with high DOC concentrations (>5 mg C L⁻¹) and/or samples with high SUVA values (>3.5–4 L mg-C⁻¹ m⁻¹) should be analyzed immediately and not frozen.

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1. Introduction

Dissolved organic matter (DOM) influences a wide array of physical (Morris et al., 1995), biological (Wetzel, 1992) and chemical (Breault et al., 1996) processes in aquatic ecosystems. As a result, watershed yields of DOM have been examined across a diverse range of environments including tropical (McDowell and Asbury, 1994), desert (Jones et al., 1996), temperate forest (Campbell et al., 2000) and arctic (Petrone et al., 2006). Ideally, water samples are analyzed for dissolved constituents immediately after sample collection and thus, closely reflect actual streamwater solute concentrations. However, it is often desirable or necessary to store water samples before processing because of logistical constraints associated with analyzing samples collected in remote locations or at fine temporal scales during storm sampling. It is therefore important to establish protocols for preserving water samples that will ensure sample integrity and will not alter the accuracy of subsequent dissolved nutrient analyses.

Freezing is a common preservation technique for streamwater samples that cannot be analyzed quickly (Triska et al.,
More recently, Spencer et al. (2007) found that after freezing, DOC precipitation that cannot be re-solubilized with 0.5 N NaOH. Briese (1978) presented evidence from a single location suggesting that freezing water samples with high dissolved organic carbon (DOC) concentrations can result in a loss of DOC through precipitation that cannot be re-solubilized with 0.5 N NaOH. More recently, Spencer et al. (2007) found that after freezing, DOC concentrations in surface water samples decreased by as much as 10%. These findings suggest that more research is necessary to evaluate whether freezing is an appropriate preservation technique when analyzing dissolved organic nutrients. Aquatic humic substances, which form the bulk of DOM in most surface waters, contain organic forms of N (DON) and P (DOP) (Perakis and Chessman, 1998). Thus, it is likely that any loss of dissolved humic substances due to freezing will result in a decrease in concentrations of DON and DOP in addition to a decrease in DOC. Because the streamwater DOM pool is a heterogeneous mixture of organic compounds ranging from simple amino acids to complex high-molecular weight compounds, the loss of DOM through freezing also has the potential to preferentially remove certain DOM fractions, such as aquatic humic substances, from the bulk DOM pool. The specific ultraviolet absorbance (SUVA) of DOC has been shown to be a reliable indicator of the aromaticity of streamwater DOC (Weishaar et al., 2003) and a useful tool for tracking changes in DOM quality at the watershed scale (Hood et al., 2006). Consequently, SUVA has the potential to provide information about whether the freezing-mediated precipitation of particular fractions of DOM has an effect on the chemical character of the streamwater DOM pool.

The purpose of this study was to evaluate freezing as a preservation technique for aqueous samples prior to the analysis of dissolved organic C, N and P in streams. In addition, we evaluated whether filter pore size had any effect on dissolved nutrient concentrations. Our hypothesis was that freezing stream samples would result in lower concentrations of DOC, DON, and TDP and that the effect would be magnified in streams with higher concentrations of aquatic humic substances.

2. Materials and methods

2.1. Sampling and experimental design

Stream water samples were collected from ten streams near Juneau, AK (58.5° N, 134.5° W) in August 2005. Juneau has a hypermaritime climate with mean monthly temperatures ranging from –2 °C to 14 °C and a mean annual precipitation of 1370 mm. Water samples from the ten streams were handled and analyzed in a 2 × 3 × 3 factorial design which included two filter pore sizes (nominal pore size 0.2 and 0.7 μm), three preservation techniques (immediate analysis, flash freezing, and standard freezing) and three replicates for each treatment. Stream DOC concentrations in southeast Alaska have a large range (0.5–40 mg C L⁻¹) and are generally highest in watersheds with a high percentage of wetlands (Edwards et al., 2006). The ten streams sampled in this study encompassed a variety of watershed types including: lowland coniferous forest, wetland-dominated and alpine basins and thus, represented the wide range of streamwater nutrient concentrations found in southeastern Alaska.

2.2. Field and laboratory methods

A one liter grab sample of streamwater was collected from each of the ten streams and was field-filtered through pre-combusted (4 h at 400 °C), Whatman glass fiber filters (nominal pore size 0.7 μm). The glass fiber filters were initially primed with approximately 10 mL of streamwater before the one liter grab sample was collected. Streamwater samples were transported from the field to the laboratory in a cooler packed with ice. Upon return to the laboratory, approximately 300 mL of each one liter grab sample was immediately filtered through Acrodisc syringe filters (0.2 μm nylon membrane) and transferred to three replicate 60 mL high density poly-ethylene (HDPE) bottles. The remaining water from each one liter bottle was divided into three replicate 60 mL HDPE bottles for both freeze treatments and immediate analysis. The HDPE bottles were initially acid-washed for 30 min using 10% hydrochloric acid and were rinsed three times with deionized water. The three replicates for each stream that received the 0.2 and 0.7 μm filtration treatments were refrigerated at 8 °C and analyzed for dissolved nutrients within 48 h.

There were two freezing treatments: storage in a standard freezer (−7 °C) and storage in a flash freezer (−50 °C). Water samples were flash frozen in a VWR cold storage freezer (Model 5461), removed from the freezer after 24 h and stored in the standard freezer at −7 °C until analysis with the standard freeze treatment. Water samples were frozen within 30 min in the flash freezer and within 3 h in the standard freeze. All frozen samples were removed from the freezer after one week and allowed to thaw overnight in a refrigerator before analysis.

All water samples were analyzed for DOC, total dissolved N (TDN), ammonium (NH₄–N), nitrate (NO₃–N), total dissolved phosphorus (TDP) and specific ultraviolet absorbance (SUVA) of DOC. DOC (lower detection limit 0.3–0.5 mg C L⁻¹) and TDN (lower detection limit 0.05–0.10 mg N L⁻¹) were analyzed via high temperature combustion using a Shimadzu TOC/TN-V analyzer. Analytic precision for DOC during the experiment was 0.037 mg C L⁻¹ (mean standard deviation for identical samples re-analyzed) for DOC concentrations less than 5 mg C L⁻¹ and 0.33 mg C L⁻¹ for samples greater than 5 mg C L⁻¹. Ion chromatography ( Dionex ICS — 1500 and 2500) was used to measure NH₄–N (lower detection limit 5 μg NH₄–N) and NO₃–N (lower detection limit 2 μg NO₃–N). DON was calculated as the difference between TDN and dissolved inorganic N (DIN = NH₄–N and NO₃–N) and the calculated error for DON values during analytical runs was 0.18 mg N L⁻¹ (square root of the sum of the squared analytical
errors of TDN and DIN. DOC (10 mg C L\(^{-1}\) of potassium hydrogen phthalate) and TDN (1 mg N L\(^{-1}\) of potassium nitrate) unknowns compared from the different analytical runs were not significantly different as indicated by a t test (p > 0.05). SUVA was measured at 254 nm as described by Weishaar et al. (2003) using a 1 cm, quartz cuvette. Water samples were allowed to warm to room temperature, analyzed on a Genesys 5 spectrophotometer and SUVA was calculated as the UV absorbance at 254 nm and is reported in units of mg-C L\(^{-1}\) m\(^{-1}\).

Persulfate digestion in conjunction with the ascorbic acid method were used to determine TDP concentrations (Valderama, 1981). A 10 cm, quartz flow through cell was used to enable the detection of low TDP concentrations (lower detection limit 0.5–1.0 μg P L\(^{-1}\)) and analytic precision was 0.20 μg P L\(^{-1}\). Previous dissolved P analyses from the streams sampled in this study revealed that >85% of the total dissolved P pool exists in the organic form (unpublished data). Thus, we used TDP as a surrogate for DOP in this study and assume that losses from the TDP pool are indicative of losses of DOP.

2.3. Statistics

The study design was an orthogonal matrix with two vertical levels of filter treatment (0.2 and 0.7 μm) and three levels of preservation treatment (immediate analysis, flash freeze and standard freeze). A series of orthogonal contrasts were performed on concentration values for each treatment from each filtered water fraction using proc mixed (SAS Institute, version 9.1, 2005). The contrasts were completed between the freezing and filter treatments to determine if there were differences between the flash freeze and standard freeze and between the 0.2 and 0.7 μm filter pore sizes. Contrasts between the immediate analysis and freeze treatment were also completed to determine the effect of freezing on dissolved nutrient concentrations. Due to the multiplicity in this design, there was a greater likelihood of type I error. Therefore, a multiplicity adjustment (Holm’s procedure) was applied to p-values generated by the contrasts. The adjusted p-values provide a more conservative estimate of significance and are used as the basis for the interpretation of the results.

3. Results

3.1. Streamwater C, N, and P concentrations

Initial streamwater concentrations of DOC, DON and TDP that were analyzed immediately varied by more than 400% across the ten streams sampled (Table 1). Two of the streams had extremely high DOC and DON concentrations (>20 mg C L\(^{-1}\); >0.4 mg N L\(^{-1}\)) indicative of wetland-dominated watersheds, while three of the streams had low DOC and DON (<3 mg C L\(^{-1}\); <0.1 mg N L\(^{-1}\)) concentrations indicative of recently deglaciated landscapes with poorly developed soils and a large percentage of alpine tundra. The nutrient concentrations reported in this study fall within the range reported in other studies of forested and wetland-dominated watersheds (Mullholland, 2003; Smith et al., 2003; Binkley et al., 2004), with the exception of elevated NO\(_3\)-N concentrations found in regions experiencing high N atmospheric deposition rates (Perakis and Hedin, 2002).

Contrasts revealed no significant difference in concentration between 0.2 and 0.7 μm filter pore size for all nutrients (p > 0.05 for all comparisons; Table 2), thus our hypothesis regarding the influence of filter pore size on organic nutrient analyses was unsupported. This finding suggests that the bulk of DOC in our streams existed in size fractions <2 μm, which is consistent with other riverine studies in Alaska that show the largest concentrations of organic matter lie in the colloidal and dissolved fractions (Guo et al., 2003; Guo and Macdonald, 2006). Additionally, contrasts revealed there was no significant difference between flash freeze and standard freeze treatments (p > 0.05 for all comparisons; Table 2). Therefore, the effects of freezing on dissolved nutrient concentrations were similar regardless of the temperature or rate at which water samples were frozen. Since no significant difference was reported between both freeze treatments and both filter pore sizes, we focus our assessment on comparing the immediate analysis and standard (–7 °C) freezer treatments for samples filtered with the commonly used 0.7 μm filter.

### Table 1 – Mean and range of nutrient concentrations for each of the four preservation treatments

<table>
<thead>
<tr>
<th>Storage technique</th>
<th>Filter pore size (μm)</th>
<th>DOC (mg C L(^{-1}))</th>
<th>DON (mg N L(^{-1}))</th>
<th>TDP (μg P L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Range</td>
<td>Mean Range</td>
<td>Mean Range</td>
<td>Mean Range</td>
</tr>
<tr>
<td>Immediate analysis</td>
<td>0.2 10.6 1.5–39.5</td>
<td>0.18 0.05–0.58</td>
<td>20.5 7.4–42.5</td>
<td></td>
</tr>
<tr>
<td>Immediate analysis</td>
<td>0.7 10.9 1.6–39.4</td>
<td>0.19 0.05–0.59</td>
<td>21.4 7.4–42.4</td>
<td></td>
</tr>
<tr>
<td>Standard freezer (–7 °C)</td>
<td>0.7 8.1 1.5–26.6</td>
<td>0.17 0.05–0.51</td>
<td>17.0 7.3–30.2</td>
<td></td>
</tr>
<tr>
<td>Flash freezer (–50 °C)</td>
<td>0.7 8.2 1.5–27.4</td>
<td>0.17 0.05–0.52</td>
<td>17.1 7.3–31.8</td>
<td></td>
</tr>
</tbody>
</table>

Streamwater samples were collected from ten streams in southeast Alaska.

### Table 2 – Orthogonal contrasts for the four preservation techniques included in this study (df computed from 10 study streams and 3 nutrient analysis) and between the immediate analysis and freeze treatments

<table>
<thead>
<tr>
<th>Contrast</th>
<th>df</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate vs. standard freeze</td>
<td>27</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Flash vs. standard freeze</td>
<td>27</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Filter pore size (0.2 vs. 0.7 μm)</td>
<td>27</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Immediate vs. freeze for DOC</td>
<td>9</td>
<td>0.002*</td>
</tr>
<tr>
<td>Immediate vs. freeze for DON</td>
<td>9</td>
<td>0.06</td>
</tr>
<tr>
<td>Immediate vs. freeze for TDP</td>
<td>9</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

Asterisk (*) indicates significant differences from immediate analyses with 0.7 μm filter at the 95% confidence level.
For the ten streams taken together, contrasts revealed there was a significant loss of DOC ($p=0.002$; Table 2) and TDP ($p=0.002$; Table 2) when samples were frozen. The mean concentration of DOC decreased 2.7 mg C L$^{-1}$ (14%) while the mean concentration of TDP decreased 4.4 μg P L$^{-1}$ (16%). Mean DON concentrations lie within the range of calculated error on DON determinations and as a result, freezing had no significant effect on concentrations of DON ($p=0.06$; Table 2).

The absolute decrease in DOC concentrations associated with freezing ranged from 0.03 to 11.7 mg C L$^{-1}$ (1.5–30%) and was largest in the streams with higher concentrations of DOC (Fig. 1a). The percentage of DOC lost through freezing was significantly correlated with the initial DOC concentrations of the streamwater samples ($p<0.001$; Fig. 1b). When initial streamwater DOC concentrations were greater than 5 mg C L$^{-1}$ ($n=5$), the average loss of DOC after freezing was 20%. However, when initial streamwater DOC concentrations were below 5 mg C L$^{-1}$ ($n=5$), the average DOC loss from freezing was only 7%. The absolute decrease in DON concentrations ranged from below detection to 0.08 mg N L$^{-1}$ (0.1–15.1%) and was largest in the streams with high DON concentrations (Fig. 2).

The absolute decrease in TDP concentrations associated with freezing ranged from below detection to 12.5 μg P L$^{-1}$ (0–27%; Fig. 3) and the percentage of TDP lost from streamwater samples during freezing was significantly correlated with the percentage of DOC lost during freezing ($p=0.02$; Fig. 4).

### 3.2. Chemical character of DOC

The loss of DOC associated with freezing also affected the chemical character of streamwater DOC as evaluated by SUVA. Because SUVA is strongly correlated with aromaticity (Weishaar et al., 2003), a decrease in SUVA is indicative of a decrease in the average aromaticity of the streamwater DOC pool. The absolute decrease in SUVA values associated with freezing ranged from below detection to 0.7 L mg-C$^{-1}$ m$^{-1}$ (0–14%) and was largest in the streams with high SUVA values for DOC (Fig. 5a). The percentage decrease in SUVA after freezing was significantly correlated with the initial concentrations of DOC in the streamwater samples.
(p=0.02; Fig. 5b) and initial SUVA values were significantly correlated with the percentage of DOC lost during freezing (p<0.001; Fig. 5c). The single sample that showed no decrease in SUVA associated with freezing was from an upland watershed dominated by alpine tundra and showed the lowest initial SUVA and concentration of DOC out of the ten streams sampled. The sample with the largest decrease in SUVA drains a carbon rich, forested peatland with elevated DOC concentrations.

4. Discussion

The results of this experiment demonstrate that using freezing as a preservation technique has the potential to substantially decrease surface water concentrations of organic nutrients as measured by standard techniques (high temperature combustion and ion chromatography). Samples in this study were only frozen for one week; however, previous studies have shown that inorganic N and P concentrations are stable in frozen samples for time periods on the order of months to years (e.g. Avanzino and Kennedy, 1993; Bachmann and Canfield, 1996). In particular, our results demonstrated that concentrations of DOC decreased significantly with freezing and that both the absolute magnitude and the proportional loss of DOC increased with increasing DOC concentrations. For all samples above 5 mg C L\(^{-1}\), the loss of DOC during freezing was over 10 times greater than the standard deviation of the analytical precision and for samples below 5 mg C L\(^{-1}\), the loss of DOC during freezing was 6 times greater. The difference between analytic precision and the loss of DOC associated with freezing was significant at the 95% confidence interval as tested by a \(t\) test (p<0.001). Any error contributed by analytic precision would be insignificant compared with the effects of freezing on DOC concentrations.

These results suggest that in streams with low DOC concentrations (<5 mg C L\(^{-1}\)), freezing is potentially a viable option for sample preservation of bulk DOC concentrations. However, as DOC concentration increases, freezing is not recommended for sample preservation of bulk DOC. Additional techniques such as immediate filtration and storage in a cold environment (Sugimura and Suzuki, 1988) or stabilization with inorganic acid (Kaplan, 1994) may be the preferred methods for bulk DOC preservation with high DOC concentrations (>5 mg C L\(^{-1}\)). However, both of these techniques have also been shown to be ineffective as a DOC preservation technique (Sugimura and Suzuki, 1988, Kaplan, 1994).

Fig. 4 - Regression model describing the relationship between percent loss of DOC and percent loss of TDP for samples from ten streams of southeast Alaska that were frozen before analysis. Frozen samples were from the standard freezer, 0.7 \(\mu\)m filter treatment.

Fig. 5 - a) Comparison of SUVA of DOC for samples from ten streams in southeast Alaska that were analyzed immediately and frozen before analysis. b) Regression model describing the relationship between DOC concentrations and the percent loss of SUVA after freezing. c) Regression model describing the relationship between initial SUVA of DOC and percent loss of DOC after freezing. Frozen samples were from the standard freezer, 0.7 \(\mu\)m filter treatment.
In our study, both freeze treatments caused the precipitation of brown particles, which is consistent with the observations by Giesy and Briese (1978). Giesy and Briese (1978) reported that greater than 90% of the total organic carbon lost from solution after freezing was retained by a XM-300 ultrafilter (0.0183 μm pore size) and suggested the primary mechanism for DOC removal is abiotic particle formation during freezing. Moreover, Giesy and Briese (1978) attempted to re-dissolve the brown particles and found less than 50% of the particulate DOC could not be returned to solution with the addition of 0.5 N NaOH and sonification resulted in some particle dispersion, although the size distribution of organic fractions was permanently altered by freezing. The formation of brown particles in our study confirms the idea that regardless of the rate of freezing, the changes in particle density and diameter that occur during freezing (Giesy and Briese, 1978) result in abiotic particle formation in samples with high concentrations of dissolved humic substances.

The observed correlation between the proportional loss of DOC and TDP after freezing suggests that losses of dissolved organic C and P are linked. The co-transport of P by dissolved humic substances has been observed in other studies (Francko, 1986; Jones et al., 1988; Dillon and Molot, 1997), especially in the presence of wetlands where high concentrations of DOM and Fe facilitate the complexation of DOC and P (Dillon and Molot, 1997). Eight of our study streams receive DOC inputs from wetlands, indicating the majority of our study streams have the potential for this DOC–Fe transport relationship.

Unlike TDP, the loss of DON through freezing was not significant. This may be the result of low DON recovery because TDP is measured directly through a persulfate digestion while DON is calculated by difference (TDN – DIN). Several studies have reported negative DON concentrations (e.g. Solinger et al., 2001) and as a result of the need for improved DON recovery, a recent laboratory study was conducted specifically evaluating DON recovery using several different analytical methods (Vandenbruwane et al., 2007). Another potential reason for this finding may be the high DIN concentrations in three of our study streams. In three of the streams where DOC and DON concentrations were low (<3 mg C L⁻¹; <0.1 mg N L⁻¹) and DIN contributed over half of the TDN pool, there was only a small change in DON concentrations with freezing (1%; df=3). For the other seven streams where DIN contributed less than half of the TDN pool and DON was the dominant chemical form of N, the loss of DON with freezing was substantial (>10%; df=7). This finding is consistent with previous studies that have shown a tight link between instream transport of DOC and DON (Qualls et al., 1991; Aitkenhead et al., 1999).

Our results showing a significant decrease in SUVA indicate that freezing alters the chemical quality of DOC and the aromatic fraction of DOC is preferentially removed from solution during freezing. Our findings corroborate the recent research by Spencer et al. (2007) who found that freezing water samples can affect the spectrophotometric properties of DOC through variations in fluorescence intensity and shifts in wavelength fluorescence. Spencer et al. (2007) further demonstrate that changes in pH can affect the spectrophotometric properties of the original DOC. We therefore suggest that for studies specifically evaluating the chemical properties of DOC, water samples with high SUVA values (>3.5–4 L m g⁻¹ m⁻¹) should be analyzed immediately and not frozen or stabilized with inorganic acid.

Due to its analytical simplicity, SUVA is a common tool for evaluating the composition of DOC in watershed scale studies (Striegl et al., 2005; Hood et al., 2006); however, the importance of our findings extends beyond SUVA alone. Our findings suggest that the spectroscopic properties of DOC can potentially be used as an indicator of whether surface water samples can be frozen. In streams draining wetlands that contain substantial concentrations of aromatic DOC, water samples that are frozen are more likely to show a decrease in SUVA. This change in the character of DOC with freezing is particularly important in watershed studies evaluating the ecological role of DOC (i.e. the importance of DOC as an energy source for heterotrophs) within an ecosystem. The findings presented here suggest that employing other techniques for the characterization of streamwater DOM, such as fluorescence spectroscopy and ¹³C NMR, should be done with caution on samples that have been frozen.

On a larger scale, our findings have implications for understanding the riverine transport of DOC with respect to watershed C budgets. For example, in wetland-dominated watersheds where DOC fluxes can be considerable (Dillon and Molot, 1997; Aitkenhead et al., 1999), error associated with DOC flux estimates could be sufficiently large to influence whether a site is a net C source or sink. Worrall et al. (2006) recently underestimated DOC flux from a peatland catchment in the UK because respiratory loss of stream DOC was found to be larger than predicted and concluded that the peatland is a much smaller sink than previously estimated. Consequently, sample preservation and potential error introduced from freezing could have large implications for the measurement and interpretation of watershed C budgets, particularly in watersheds with a high percentage of wetlands. The attempt to predict riverine DOC fluxes begins in the laboratory and the importance of analytic procedures cannot be underestimated given the potential for propagation of error associated with using time-series analyses to estimate DOC fluxes and the importance of estimating watershed C budgets with a changing climate.

5. Conclusions

The streams included in this study are representative of the aquatic ecosystems present near Juneau, AK, although it is important to consider that the chemical changes observed in this study may be somewhat unique to this region. With that in mind, several recommendations for the preservation of water samples for nutrient analysis emerge from this study.

For water samples with low DOC concentrations (<5 mg C L⁻¹) and/or low SUVA values (<3.5–4 L m g⁻¹ m⁻¹), freezing is potentially a viable option for sample preservation of DOC. However, as DOC concentrations and SUVA values increase, water samples should be analyzed immediately. If immediate analysis is not possible, the effects of freezing on organic nutrients should be evaluated on a site-specific basis before freezing is employed as a preservation technique.

Researchers that include water samples using different preservation techniques (frozen vs. refrigeration) in comparative
analysis must recognize the potential for additional variability in organic nutrient concentrations created from sample preservation.

Freezing has the potential to change not only the concentration, but the chemical character of the DOM pool as the most aromatic fractions of DOM are likely to precipitate out of solution during freezing. Since water samples taken from streams that are influenced by wetlands are likely to be dominated by humic DOM, these samples should not be frozen.

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