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Sources of Uncertainty in Stream Nutrient Sampling Below a Point Source

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SOURCES OF UNCERTAINTY IN STREAM NUTRIENT SAMPLING BELOW A POINT SOURCE

by

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Abstract

The goal of this study was to determine what aspects of sampling and sample storage could lead to uncertainty when taking samples in a stream below a point source. Sources of uncertainty studied were the locations where the samples were taken to assess if nutrients were adequately mixed within a cross-section, different filtration techniques, dilution errors, analytical uncertainty, and freezing time. Bootstrapping analyses were used to determine whether mixing and dilution errors led to uncertainty, while one-way ANOVAs were used to evaluate filtration techniques and storage time. Sample spikes to determine percent recovery of nutrients and repeat sample analyses are routinely performed as part of the lab quality assurance/quality control plan (QA/QC), and are used here to evaluate analytical uncertainty. Comparison of coefficients of variation (CV) of samples collected within a cross section at four locations, above, at, and below a point source, revealed that mixing of nutrients within a cross section appeared to be different at the different locations. The filtration devices analyzed were an electric pump and a manual syringe. These two devices gave statistically similar results in ammonium, nitrate, and soluble reactive phosphorus concentrations ($p>0.05$). Dilution error was determined by comparing seven diluted samples with the original sample with which they were made. Dilutions proved to have the highest uncertainty relative to other treatments. The diluted samples were consistently higher than the original sample for all nutrients and were more variable than lab QA/QC duplicates for ammonium and soluble reactive phosphorus. Analytical uncertainty was found to be less than uncertainty associated with sample collection and storage except for unanticipated protocol failure. For this study, QA/QC data beyond 20% were considered fails, and the samples required reanalysis. In most cases the percent recovery of spiked samples was within 20% and coefficients of variation of samples repeatedly analyzed

were much less than 20%. However, ammonium, total nitrogen, and total phosphorus incurred the most failures. Freezing samples appeared to be an adequate storage method. Samples frozen for 12 weeks showed statistically significant declines in total nitrogen (TN) and total phosphorus (TP) concentrations ($p<0.05$), however these declines were less than 9% of the initial values. This is within the range of variation seen for analytical duplicates.

Acknowledgements

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Introduction

Nutrient samples are often collected below point sources, such as wastewater treatment plants, to ascertain if nutrient quantities exceed in-stream water quality standards. When analyzing these samples, it is imperative that the samples at the time of analysis are representative of the samples at the original time of sampling. In order to ensure that nutrient samples are reliable, this study tested five sources of uncertainty that had the potential to cause unreliable nutrient measurements. These sources included the location within a cross-section that samples were taken in order to assess whether inadequate mixing was occurring within the stream, different filtration techniques, dilution errors, analytical uncertainty, and freezing time.

This research emerged due to observed anomalies in previous nutrient sampling completed below a point source, Silver Creek Water Reclamation Facility, in Silver Creek, Utah. When these previous analyses were completed, comparisons were made between total phosphorus and constituent phosphorus concentrations (e.g., soluble reactive phosphorus (SRP)). The amount of SRP measured was greater than the amount of total phosphorus measured. Additionally, similar anomalies were found when comparing constituent dissolved nitrogen concentrations and total nitrogen concentrations. This led to the recognition of sampling and/or analytical errors, but did not reveal the source of the error. This research was conducted in order to determine what potential sources of uncertainty could have led to these anomalies.

Sampling location and incomplete mixing

When taking nutrient samples within a cross section of a stream, choosing the location from which to sample is critical. When using grab sampling as the sampling technique, the sample must be representative of the cross section. Incomplete mixing can occur below point-

source discharges, such as waste water treatment plants, as well as from tributary inputs and groundwater seepage (Martin, Smoot, & White, 1992, p. 866). If a sample is taken from a point where mixing is incomplete, estimation of suspended sediment and associated trace element or nutrient concentrations could be inaccurate (Horowitz et al., 1990). When designing a sampling plan, it is best to determine if there are sources that could lead to incomplete mixing and cross-sectional variability (Martin et al., 1992). Concentrations of particulate nutrients can be highly variable among field replicate samples (Whitfield and McKinley,1981), in part because of unequal distribution of nutrient particles in space. Therefore, this research sought to examine the extent to which incomplete mixing was problematic across sampling locations below a point-source dicharge from a waste water treatment plant. If samples for dissolved nutrients were not consistently collected at the same location as total nutrients, this source of uncertainty may have led to an overestimation of dissolved nutrient concentrations due to mixing variability in a cross-section.

Filtration Techniques

Another possible source of uncertainty in sampling could arise from filtration techniques employed. Filtration of water samples is often necessary in order to obtain certain dissolved nutrient constituent concentrations, such as nitrite, nitrate, ammonium, and soluble reactive phosphorus. Filtration is also beneficial as it removes particles in the water sample that could lead to adsorption onto the container surface (Clementson & Wayte, 1992; Maher & Woo, 1998). It can also remove phytoplankton and other microorganisms that utilize nutrients in the water for biological reasons (Clementson & Wayte, 1992; Degobbis, 1973; Maher & Woo, 1998). However, filtration under too high of a vacuum could cause algal cells to lyse, if algae is present,

which introduces intracellular contents, e.g., phosphorus, into the sample (Lambert, Maher, & Hogg 1992; Worsfold et al., 2005). There is also a possibility that the filter itself can cause sample contamination from filter surfactants leaching into the sample (Clementson & Wayte, 1992; Norrman, 1993). Some studies have reported that incomplete retention of glass-fiber filters can occur leading to the presence of bacteria and various types of plankton in the sample (Norrman, 1993; Stockner, Klut, & Cochlan, 1990; Yen, Lenz, Gassie, & Hartline, 1994). Lambert et al. (1992) also found that the effective pore size changed during filtration as material accumulated on the filter surface. Filtration has proven beneficial, but not without some potential sources that lead to uncertainty in nutrient measurements. Therefore, filtration was also examined to determine the extent to which it led to uncertainty in measurement below a point source.

Dilution Errors/Analytical Uncertainty

Due to the ability of laboratories to detect whether analysis equipment is reliable, based on standards, sample spikes, and duplicate standards, analytical uncertainty is usually assessed as part of the routine laboratory quality control (QC) protocol. Analytical uncertainty is included in this study to determine if there were specific laboratory analyses that had higher analytical uncertainty. Dilution errors were also included in this study to determine the amount of variation incurred under the handling of different lab personnel. According to Harmel et al. (2006), proper methodology and personnel expertise are vital to limit laboratory analysis errors. Meyer (2002) offers tips on minimizing uncertainty in sample preparation, including using adequate working techniques, working with large volumes, minimizing the number of working steps, making sample and reference measurements in close time proximity and using the same instrument, using an internal standard, preparing an artificial matrix, and performing replicated analyses.

Sample analyses are equally as important as sample collection and storage for determination of water quality, therefore analytical uncertainty was also examined in this research.

Sample Preservation

The use of freezing as a preservation technique has been examined in many studies involving various types of water samples, i.e., freshwater, seawater, ocean water, estuarine water, and lake water. For each water type, the effectiveness of freezing varies according to different studies. The type of water sampled, and whether the sample was filtered before storage, are major factors in determining the effectiveness of a storage technique (Maher & Woo, 1998).

For freshwater samples, different studies have reported varying results as to the effectiveness of freezing. Some of these studies reported that freezing was an ideal storage method for total phosphorus analysis and freezing after filtration was a preferred method for dissolved organic phosphorus analysis (Maher & Woo, 1998; Worsfold et al., 2005). Other studies found that freezing was an adequate preservation technique for only some nutrient constituents, for example Fellman, D'Amore, and Hood (2008) found that freezing had no significant effects on concentration of DON [dissolved organic nitrogen] (p. 308). However, some studies have found that freezing can cause changes in particulate nitrogen and carbon concentrations before filtration, and orthophosphate, dissolved organic carbon, and total dissolved phosphorus concentrations (Avanzino & Kennedy, 1993; Fellman et al., 2008; Whitfield & McKinley, 1981). Because of these varying results, Gardolinski et al. (2001) concluded that a standard storage protocol could not be designed for natural water due to differing biological and physico-chemical characteristics of sample matrices (p. 3677).

According to many different studies concerning sampling of ocean and estuarine water, the majority concluded that freezing is an effective storage technique for the preservation of

water nutrients. The nutrients observed include inorganic phosphate, soluble reactive phosphate, particulate phosphorus fractions, ammonia, nitrite, nitrate, and soluble reactive silicate (Clementson & Wayte, 1992; Degobbis, 1973; Dore et al., 1996; Marvin & Proctor, Jr., 1965; Thayer, 1970; Venrick & Hayward, 1985). However, some studies found that freezing is only effective for a certain amount of time, for example four months, and after this period, a slight decrease in phosphate and nitrate is observed (Clementson & Wayte, 1992; Kremling & Wenck, 1986). Studies also found that the variance between samples increased with increasing storage time (Kremling & Wenck, 1986; MacDonald & McLaughlin, 1982). Dore et al. (1996) concluded that the reason studies do not recommend freezing as an effective storage technique is due to "relative large errors at the lowest concentrations tested" (p. 174).

When sampling lake water, studies have found that freezing samples as soon as possible after collection can suppress bacterial activity that could cause changes in nutrient concentrations (Heron, 1962). Freezing was found to be an effective storage technique over a six month period for lake water samples being analyzed for total phosphorus (Lambert et al., 1992).

Based on the literature review completed, here, it can be seen that there are many potential problems that can arise during water sampling. Based on this previous research, it was determined that closer examination of sampling location, filtration techniques, dilution errors/analytical uncertainty, and sample preservation was warranted.

Methods

Sampling Location

Samples were collected in Silver Creek near the Silver Creek Water Reclamation Facility near Park City, Utah (40.7349591°, -11.2809016°) on June 11, 2013. Discharge was 3.5 cubic feet per second (CFS) and water temperature was 16.4°C at the flow gauge operated by the U.S. Geological Survey (Gauge # 10129900). These values were typical of baseflow water conditions during the year, and represent early summer conditions in terms of visible periphyton and filamentous algae observed at the creek.

We chose four locations along Silver Creek where samples were collected to test whether inadequate mixing could cause different nutrient concentrations depending on where in the cross section the samples were collected. These locations included one stream site about 13 meters above the confluence with the wastewater treatment plant effluent (Above WWTP, approximately 13 meters), one site in the wastewater effluent (Point Source), one stream site approximately 103 meters below the confluence (Below (I) WWTP) and another stream site approximately 717 meters below the confluence (Below (II) WWTP) (Figures 1a and 1b). Seven 1,000 mL grab samples were collected at each of the four cross-sections. Each sample was collected at a different location within the cross section, both at varying distances across the cross section and at varying depths (Figure 2). From each 1,000 mL sample, two sub-samples were taken. One sub-sample was 120 mL that was not filtered, and was used to analyze for total nitrogen (TN) and total phosphorus (TP). The second sub-sample was filtered with a syringe and was analyzed for nitrite+nitrate-N (NO_3+NO_2), ammonium-N (NH_4), and soluble reactive phosphorus (SRP). Samples were collected in the following order to prevent contamination: Below (II) WWTP, Below (I) WWTP, Point Source, Above WWTP.

If incomplete mixing was occurring at a given sample location, we predicted that the samples collected at different locations in the cross section would be highly variable, as measured by the coefficient of variation. The coefficients of variation were calculated for each cross section and compared to determine if they were statistically different. Three parametric tests were run to compare coefficients of variation among locations, including the Modified Bennet's test, the Wald Test, and the Modified Miller Test (Jafari & Kazemi, 2013). Since these tests compared all four sites together, another statistical test was required to determine which sites, between the four, actually were statistically different. A nonparametric bootstrap was used to compare the coefficients of variation between two individual sites. Critical significance level was set at 0.05.

From a five-gallon bucket collected Below (II) WWTP (Figure 1a) in the middle of the cross-section, five 120 mL grab samples were taken and five samples were filtered with a syringe. These samples were repeat samples to determine how variable nutrient concentrations were when samples were collected from the same location. The variance of the bucket-collected samples was compared with the variance of the seven samples collected across a cross-section to act as a control. We assumed the bucket-collected samples had less variance than the samples collected across a cross-section. The unfiltered samples were analyzed for TN and TP, and the filtered samples were analyzed for NH_4, NO_3+NO_2, and SRP.

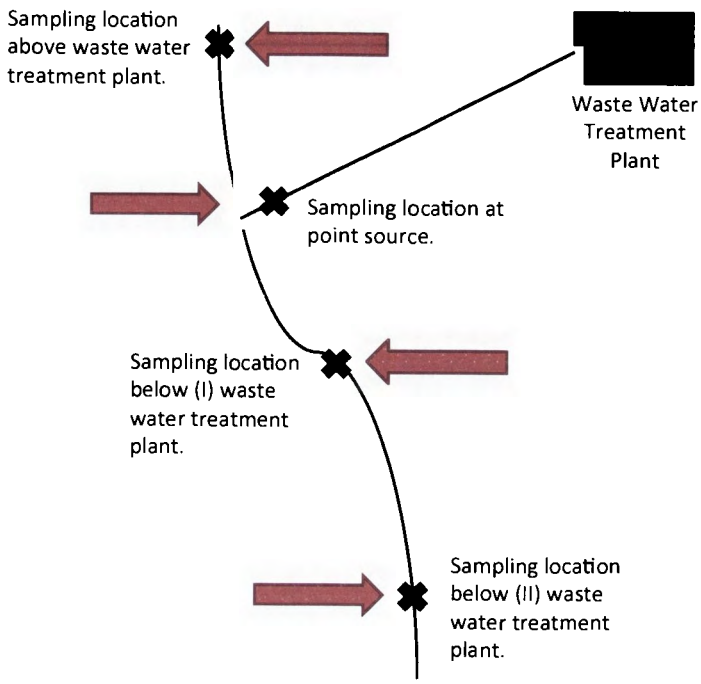


Figure 1a. Schematic (not to scale) showing sample locations for testing inadequate mixing.

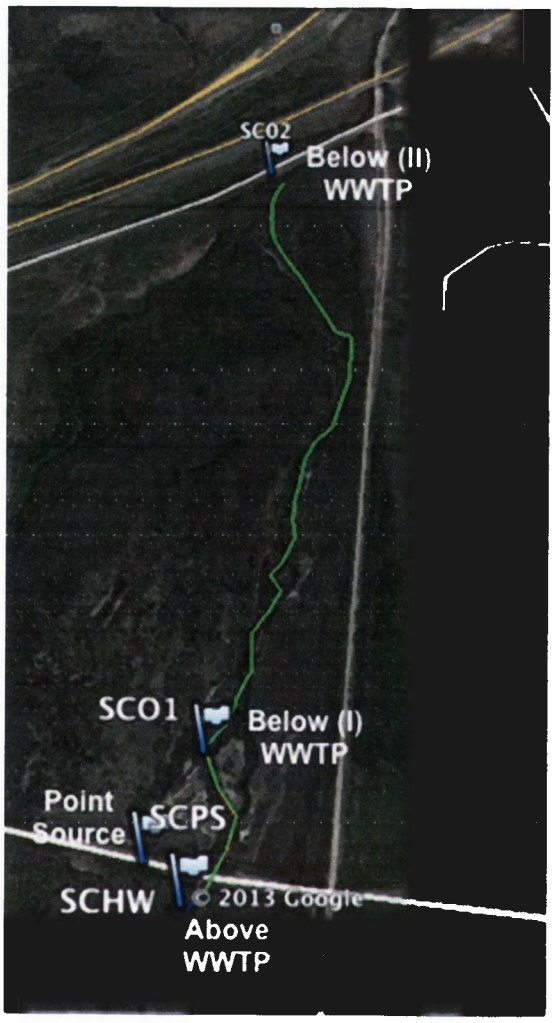


Figure 1b. Google Earth image of sampling locations. Note stream flow (green) is from north (Above WWTP) to south (Below (II) WWTP).

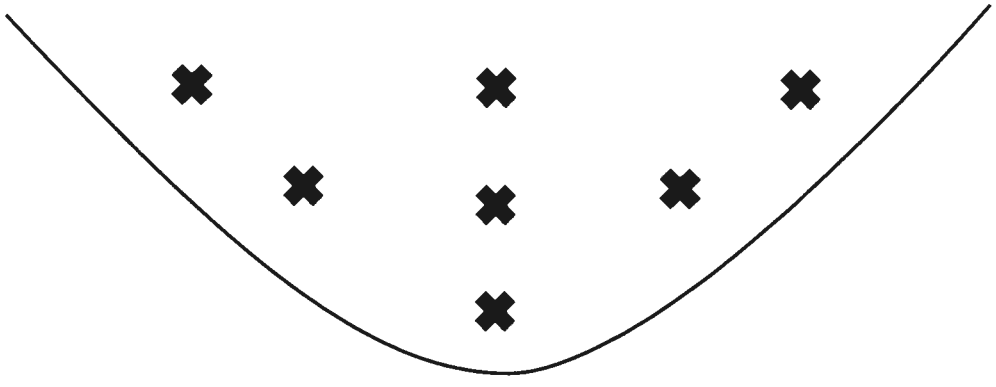


Figure 2. Approximate locations in cross section of stream where grab samples were obtained.

Filtration Techniques

The samples collected for analyzing whether differences in nutrient concentrations exist when filtering with a syringe or an electric pump were collected at the farthest location downstream (Below (II) WWTP) (Figure 1a). A 5-gallon bucket was collected in the middle of the stream at this location, separate from the bucket described under Sampling Location. From this bucket, which remained continuously mixed with a hand mixer, 12 samples were collected using a battery-operated peristaltic pump (Geopump, Golden Colorado, Figure 3), and 12 samples were collecting using a manual syringe. Each of these samples was analyzed for NH_4, NO_3+NO_2, and SRP. One-way ANOVAs were used to compare the average concentrations of each nutrient for both filtering methods.

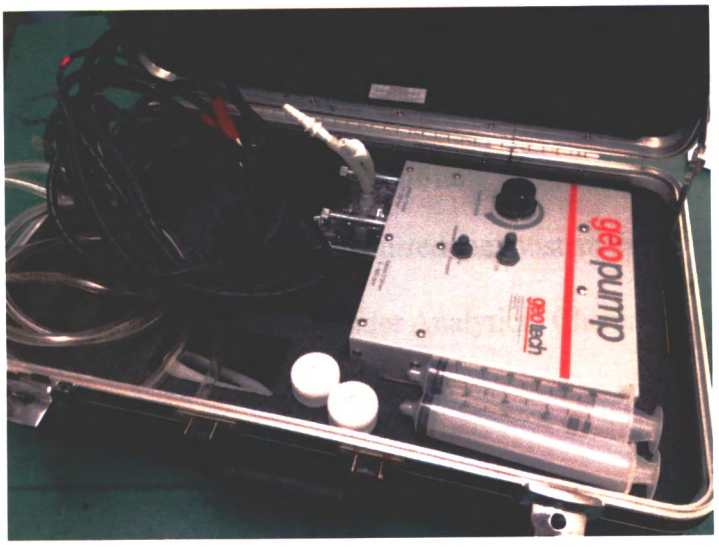


Figure 3. Peristaltic pump and syringes used to contrast filtering methods.

Dilution Error

From the 5-gallon bucket used to collect five grab samples and five filtered samples, as described in the Sampling location section, two unfiltered samples and two filtered samples were

used to make dilutions to determine how variable nutrient concentrations were when making dilutions. Five 1:100 dilutions were made for each of the four samples. This was done by adding 99 ml of water to 1 ml of each sample using volumetric flasks. The dilution protocol used here was the same as the protocol followed before nutrient analysis. The dilutions made on the unfiltered samples were analyzed for TN and TP, and the dilutions made on the filtered samples were analyzed for NH_4, NO_3+NO_2, and SRP. These concentrations were compared to the original samples, which were also diluted before analysis.

Analytical Uncertainty

Lab quality assurance and control (QA/QC) uses results from spiked samples, duplicates, and certified reference materials to assess lab analyses. For this study, spiked sample data not within 20% and duplicate sample data beyond 20% were considered fails, and the samples required reanalysis. For each nutrient, the number of fails was tallied for spiked samples and duplicate samples to determine what nutrients incurred the most analytical failure. The analytical techniques used here are described under Analytical Chemistry below.

Storage Time

Samples to assess the reliability of freezing were collected from Below (II) WWTP. A 4,000 mL grab sample was collected from the middle of the stream and transported back to the lab. Twenty-eight 100 ml samples were collected from this 4,000 mL sample and placed in the freezer. Because these samples were unfiltered, they were analyzed for TN and TP. Seven samples were analyzed after one week of freezing, seven samples were analyzed after three weeks of freezing, seven samples were analyzed after six weeks of freezing, and seven samples

were analyzed after twelve weeks of freezing. The average concentrations were compared using one-way ANOVAs to test for differences that resulted from freezing samples for different amounts of time.

After the seven samples were analyzed after one week, they were placed back in the freezer and analyzed again at three weeks, six weeks, and twelve weeks of freezing. The samples analyzed after three weeks were also placed back in the freezer and analyzed again at six weeks and twelve weeks of freezing. The same was done for the samples analyzed after six weeks (i.e., they were placed back in the freezer after analysis and analyzed again at twelve weeks). This was done to determine whether multiple thawing and freezing events affected nutrient concentrations.

Analytical Chemistry

All samples were analyzed in the Aquatic Biogeochemistry Lab at Utah State University using standard protocols summarized below. All analytical instruments were calibrated using standard reference materials (APHA 1998). Analytical quality control included use of reagent blanks, spikes, check standards, and duplicate samples. Method detection limits were calculated as the product of the standard deviation of a minimum of seven replicates of a mid-range standard and the *t*-value from a one-sided *t* distribution (APHA 1998).

TN was quantified using a potassium persulfate digestion (Nydahl 1978) followed by cadmium reduction for measurement of NO_3+NO_2 (APHA 1998, EPA method 353.2). Measures of TP were made using a potassium persulfate digestion followed by an ascorbic acid molybdenum reaction for soluble reactive phosphorus (SRP, Murphy and Riley 1962, EPA

method 365.1). Both colorimetric analyses were done on automated analytical system with FASPac II data acquisition software (Astoria Pacific International, Portland OR).

NH_4-N concentration in filtered water samples was measured using an automated alkaline phenolhypochlorite reaction followed by spectrophotometric analysis (EPA method 350.1, APHA 1998, Solorzano 1969) on an automated analytical system with FASPac II data acquisition software (Astoria Pacific International, Portland OR). NO_3+NO_2 on filtered samples was measured using cadmium reduction, and SRP on filtered samples was measured using the ascorbic acid molybdenum reaction as for digested TN and TP samples described above.

Results

Sampling Location

The average nutrient concentrations for the seven samples representing the cross-section collected furthest downstream the wastewater treatment plant (Below (II) WWTP) were compared with five replicate samples (Figures 4-8). Because the five samples were collected from a mixed bucket, these samples were expected to have less variance than samples collected in the cross section. This was observed for ammonium (Figure 6) where the CV in the bucket was less than the CV of the seven samples collected across the cross-section (Table 1). This was not observed for TP or SRP where mean values and CV were similar between the cross-section and the bucket (Figures 4 and 8, and Table 1). This was also not observed for TN or NO_3+NO_2 (Figures 5 and 7). For these nutrients, the samples collected across the cross-section appeared to be less variable than the samples taken from the mixed bucket. The mean concentrations were not statistically different between the samples collected across the cross-section and the samples collected from the bucket.

Table 1. Coefficients of variation of samples collected from a bucket and across the cross section at location Below (II) WWTP.

	Cross-Section	Bucket
Total P	10%	8%
Total N	7%	22%
NH_4	62%	30%
NO_3	2%	15%
SRP	3%	6%

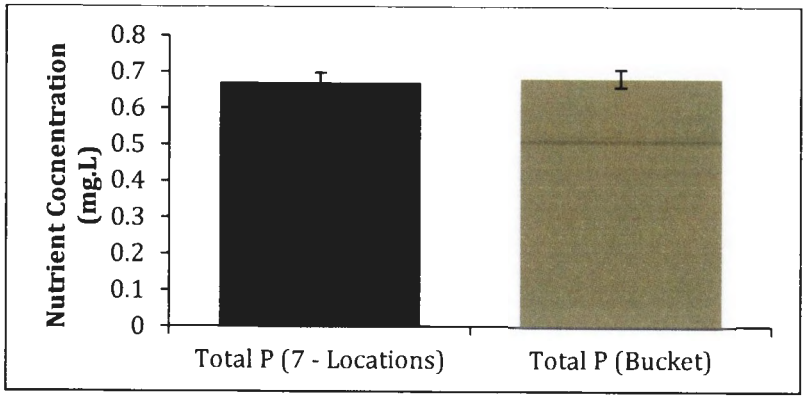


Figure 4. Comparison of mean TP concentrations (±1 standard error) between seven samples collected at various locations within the cross section, and five samples collected from a mixed bucket. Mean concentrations were not significantly different (ANOVA, p=0.66).

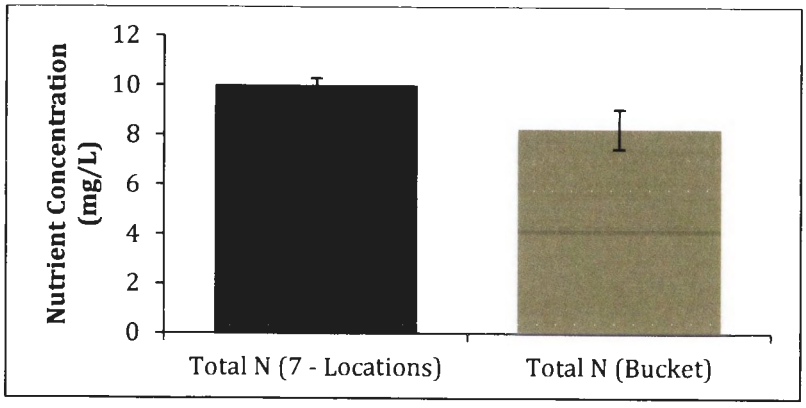


Figure 5. Comparison of mean TN concentrations (±1 standard error) between seven samples collected at various locations within the cross section, and five samples collected from a mixed bucket. Mean concentrations were not significantly different (ANOVA, p=0.08).

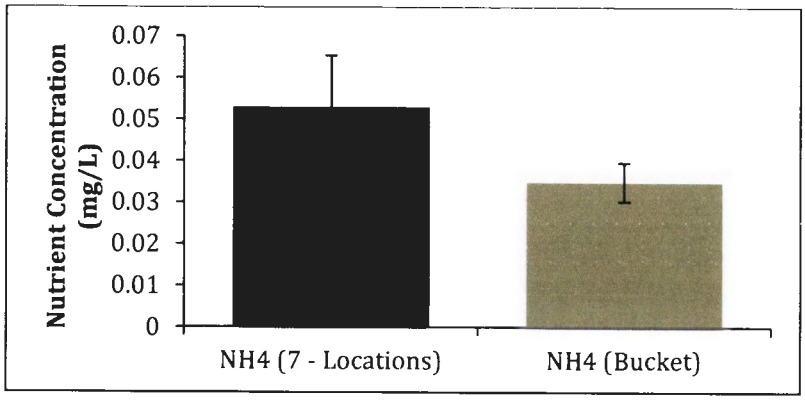


Figure 6. Comparison of mean NH_4 concentrations ($\pm$1 standard error) between seven samples collected at various locations within the cross section, and five samples collected from mixed bucket. Mean concentrations were not significantly different (ANOVA, p=0.27).

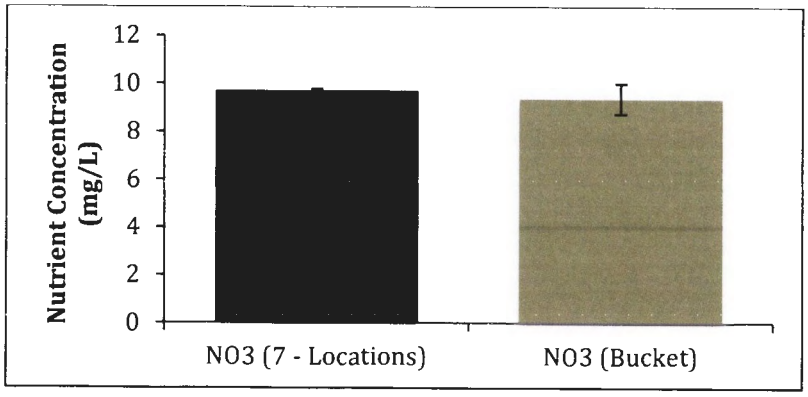


Figure 7. Comparison of mean NO_3 concentrations ($\pm$1 standard error) between seven samples collected at various locations within the cross section, and five samples collected from mixed bucket. Mean concentrations were not significantly different (ANOVA, p=0.53).

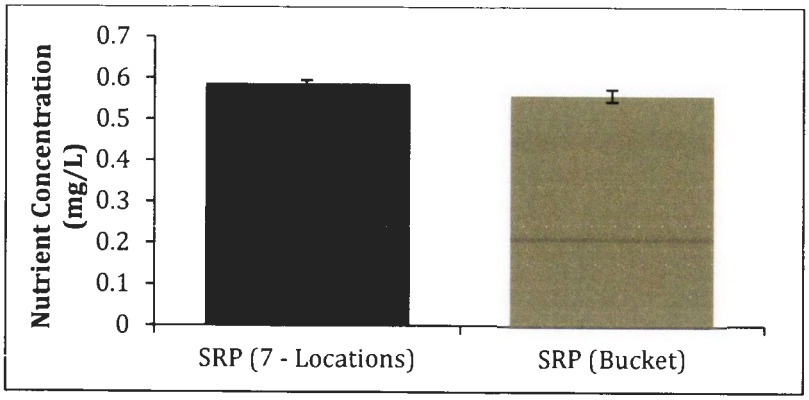


Figure 8. Comparison of mean SRP concentrations (±1 standard error) between seven samples collected at various locations within the cross section, and five samples collected from mixed bucket. Mean concentrations were not significantly different (ANOVA, p=0.08).

If incomplete mixing is an important source of uncertainty, we expected that sites below the point source would have higher variation in nutrient concentrations compared to samples collected above the point source, and the five bucket-collected samples. Surprisingly, NO_3 above the point source was the most variable, with a CV of 69%, which may have been due to high NO_3 groundwater influences above the point source. CV for this nutrient at other sites was much lower (<2.5%). In fact, the CV at the location above the point source was higher than at other locations for nutrients TP, NO_3, and SRP (Table 3 and Appendix A). This could be explained by the channel morphology/geometry above the point source inflow, as the stream flow is slower and could contain stratified pools. As expected, the CV for TP and NH_4 was greater at the two sites below the point source (Table 3 and Appendix A). The CV for TN was greater at the site nearest the point source compared to the site farther downstream. The CV appeared to be lowest for samples collected at the point source in all nutrients compared to the other three locations.

Table 2. Mean nutrient concentrations (± 1 standard error) of samples collected across the cross-section at four locations

	TN (mg/L)	TP (mg/L)	NH4 (mg/L)	NO3 (mg/L)	SRP (mg/L)
Above WWTP	0.38 ± 0.01	0.017 ± 0.001	0.014 ± 0.001	0.004 ± 0.001	0.008 ±0.001
Point Source	14.92 ± 0.83	1.19 ± 0.01	0.038 ± 0.002	16.84 ± 0.11	1.08 ± 0.01
Below I WWTP	12.10 ± 0.83	0.89 ± 0.03	0.037 ± 0.01	12.45 ± 0.12	0.79 ± 0.01
Below II WWTP	10.02 ± 0.25	0.67 ± 0.03	0.053 ± 0.01	9.71 ± 0.06	0.59 ± 0.01

Table 3. Coefficients of variation of samples collected across the cross-section at four locations.

	TN	TP	NH4	NO3	SRP
Above WWTP	6%	22%	25%	69%	31%
Point Source	17%	2%	13%	2%	1%
Below I WWTP	18%	9%	37%	2%	4%
Below II WWTP	7%	10%	62%	2%	3%

Table 4. P-values computed from the Modified Bennet's test, the Wald test, and the Modified Miller test used to compare coefficients of variation for each type of nutrient analysis.

	TN	TP	NH4	NO3	SRP
Bennet's	0.061	<0.001	0.030	<0.001	<0.001
Wald	0.063	<0.001	0.045	<0.001	<0.001
Miller	0.069	<0.001	0.046	<0.001	<0.001

Filtration Techniques

The method of filtering did not appear to influence NH_4, NO_3, or SRP concentrations. The average NH_4 concentration was 1.5 times higher using a manual syringe compared to the peristaltic pump, and the observed analytical variation was also higher (Figure 9). The difference between the average NH_4 concentrations filtered with the syringe and with the pump was also greater than the method detection limit. However, the nutrient concentrations were not significantly different (one-way ANOVA, p=0.098). Filtering also did not appear to impact analyses of NO_3 and SRP. The mean concentrations were 8.45 mg/L for both the pump and the

syringe for NO_3 (one-way ANOVA, p=0.89, Figure 10) and 0.56 mg/L for the pump and 0.57 mg/L for the syringe for SRP (one-way ANOVA, p=0.57, Figures 11). The CV's of nutrient measurements for the peristaltic pump and syringe are summarized in Table 5.

Table 5. Coefficients of variation for samples collected with a pump and a syringe.

	Pump	Syringe
NH4	44%	60%
NO3	2%	3%
SRP	4%	5%

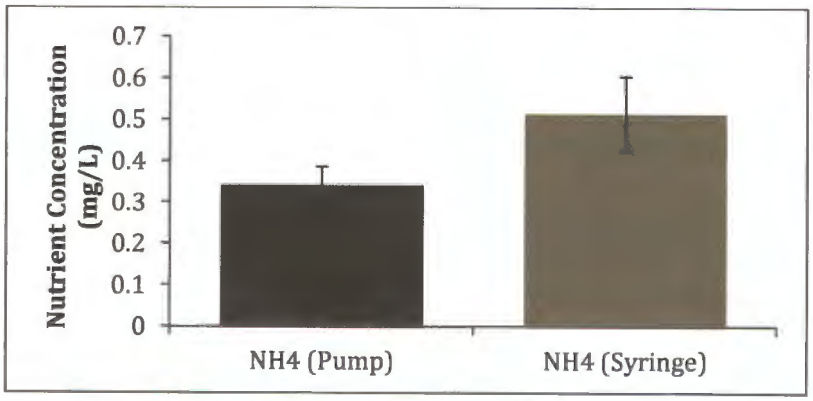


Figure 9. Comparison of mean ammonium concentrations (±1 standard error) between samples filtered with an electric geopump and with a syringe.

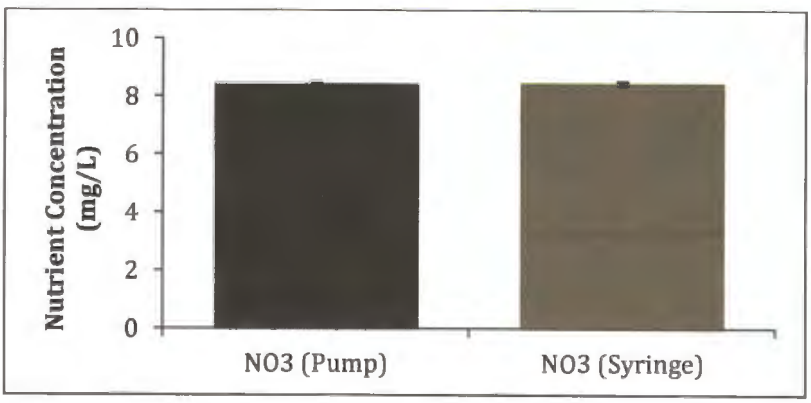


Figure 10. Comparison of mean nitrate concentrations (±1 standard error) between samples filtered with an electric geopump and with a syringe.

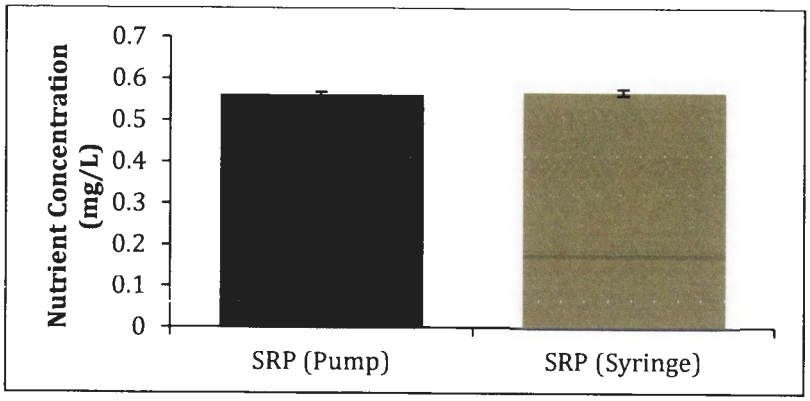


Figure 11. Comparison of mean soluble reactive phosphorus concentrations (±1 standard error) between samples filtered with an electric geopump and with a syringe.

Dilution Error

Comparisons of the dilutions I made and the dilutions made in the lab showed that the lab dilutions resulted in consistently lower nutrient concentrations for every nutrient (Figures 12-16). Standard errors for the dilutions I made were also consistently higher than standard errors for the lab dilutions, except for NO_3. This may be due to less experience with making dilutions.

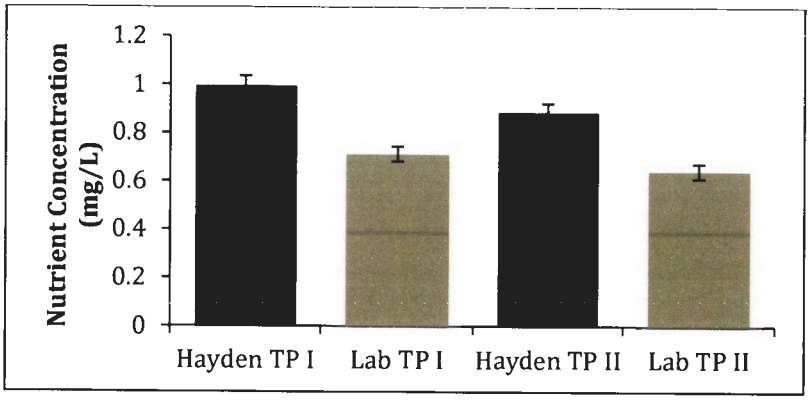


Figure 12. Comparison of mean total phosphorus concentrations (±1 standard error) between samples I diluted and samples diluted in the lab.

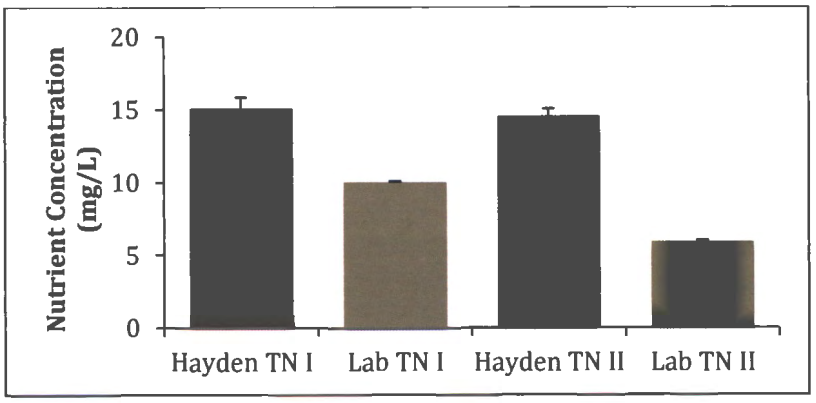


Figure 13. Comparison of mean total nitrogen concentrations (±1 standard error) between samples I diluted and samples diluted in the lab.

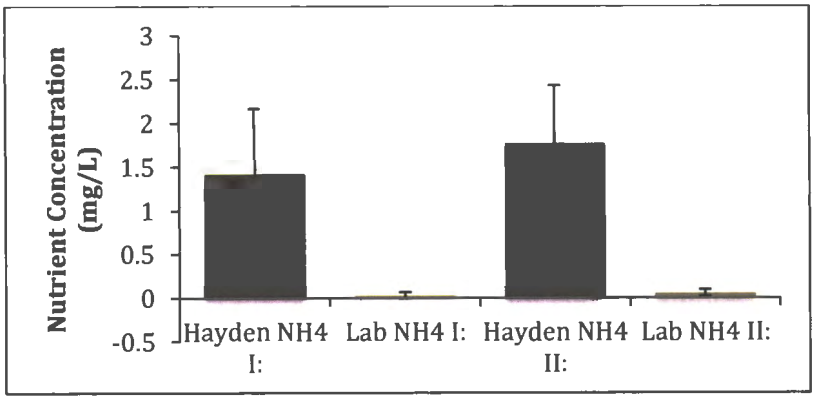


Figure 14. Comparison of mean ammonium concentrations (±1 standard error) between samples I diluted and samples diluted in the lab.

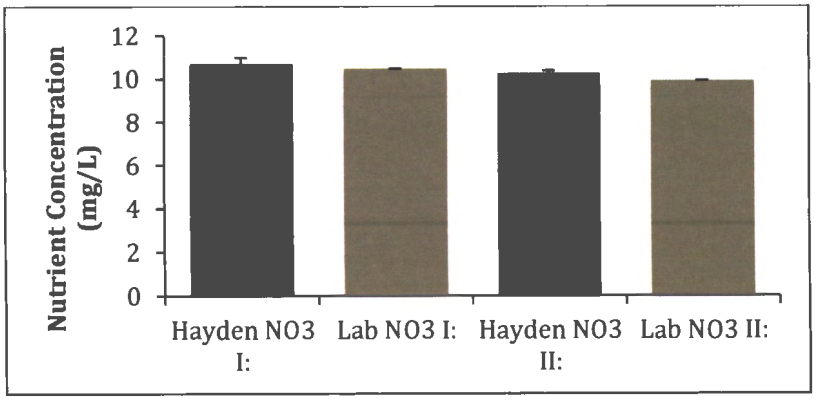


Figure 15. Comparison of mean nitrate concentrations (±1 standard error) between samples I diluted and samples diluted in the lab.

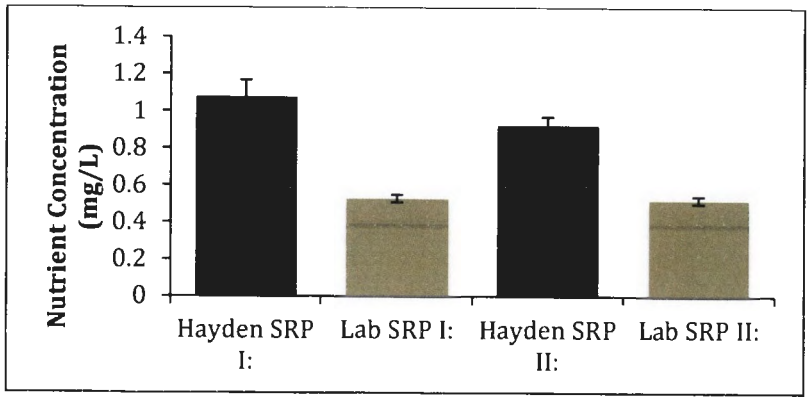


Figure 16. Comparison of mean soluble reactive phosphorus concentrations (±1 standard error) between samples I diluted and samples diluted in the lab.

Analytical Uncertainty

TN, TP, and NH_4 incurred the greatest number of fails, but in most cases, the percent recovery was within 20% and coefficients of variation were much less than 20%. The average CV for duplicate samples and average percent recovery for spiked samples were calculated using only analyses that did not fail.

Table 6. Average coefficients of variation for sample duplicates and number of fails encountered for each nutrient

Note: NH4 failed three of four duplicate sample analyses, and therefore did not have enough values to average.

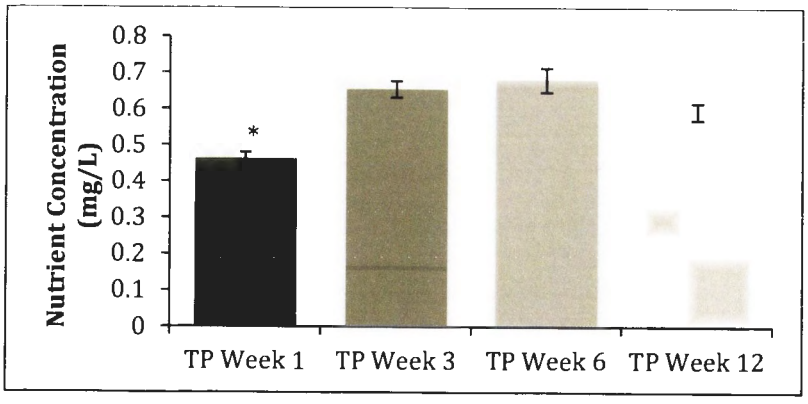
	Average CV	Number of Fails
TN	4.3%	1
TP	5.6%	0
NH_4	-	3
NO_3	1.2%	0
SRP	6.5%	0

Table 7. Average percent recovery for spiked samples and number of fails encountered for each nutrient.

	Average % Recovery:	Number of Fails:
TN	101.5%	2
TP	100.4%	2
NH_4	93.5%	1
NO_3	93.4%	0
SRP	98.8%	0

Frozen Storage Time

Freezing samples appeared to be an adequate storage method. Samples frozen for 12 weeks showed statistically significant declines in TN and TP concentrations ($p<0.05$) (Figures 17 and 18), however these declines were less than 9% of the initial values. This is within the range of variation seen for analytical duplicates. The large concentration differences observed between TP concentrations of week one and the weeks following were, in large part, due to protocol failure (Figure 17). The samples analyzed on week one were locked in an autoclave overnight. The low concentrations on week one are attributed to this.



Note: Star represents statistical significance due to lab protocol failure (i.e. samples being locked in an autoclave for loo long).

Figure 17. Average total phosphorus concentrations (±1 standard error) for samples analyzed at one week, three weeks, six weeks, and twelve weeks of freezing.

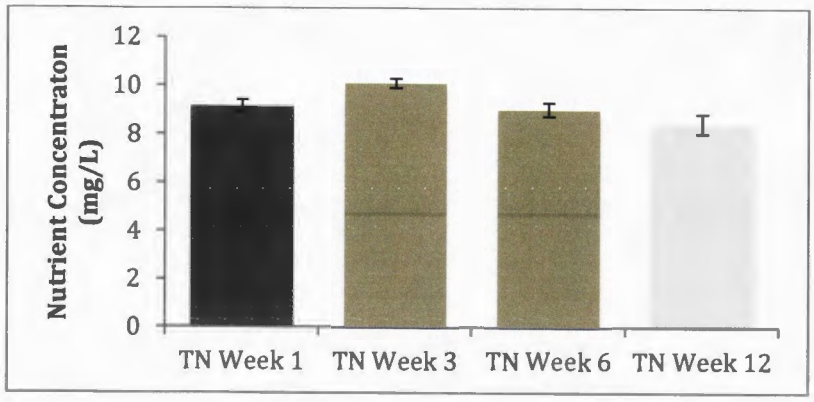


Figure 18. Average total nitrogen concentrations (±1 standard error) for samples analyzed at one week, three weeks, six weeks, and twelve weeks of freezing.

When samples were thawed for initial analysis, put back in the freezer, and then thawed again for reanalysis, TN concentrations appeared to decrease, but TP seemed unaffected (Tables 8 and 9). The values in Tables 8 and 9 were calculated by taking the differences of concentration averages of samples opened once, twice, and three times.

Table 8. Total phosphorus method detection limit and differences between samples thawed multiple times.

Total Phosphorus	mg/L
Method Detection Limit	0.43
Difference between samples opened twice and opened once	0.04
Difference between samples opened three times and opened twice	-0.002

Table 9. Total nitrogen method detection limit and differences between samples thawed multiple times.

Total Nitrogen	mg/L
Method Detection Limit	0.10
Difference between samples opened twice and opened once	-0.55
Difference between samples opened three times and opened twice	-0.71

Discussion

A range of CV's was observed among sampling location samples and nutrient types for each cross-section tested. Mixing patterns appeared to be different for each nutrient between the four locations. Unexpectedly the highest CV's were generally observed above the point source. This may be due to slower stream flow, or stratified pools above the point source. This could also be due to higher errors associated with analyses at lowest concentrations (e.g. Dore et al. 1996). According to Horowitz et al. (1990), poor selection of sampling locations within a cross-section could lead to inaccurate nutrient concentrations, due to over- or underestimation of these concentrations from variable mixing patterns. The coefficients of variation seemed to be lowest at the point source, and highest above the wastewater treatment plant, except for total nitrogen and ammonium concentrations. This could potentially lead to the hypothesis that higher concentrations confer less analytical variation; therefore concentration differences are masked by the overall high concentration.

When deciding whether to use a peristaltic pump or a syringe when taking nutrient samples around a point source, caution should be used when analyzing for NH_4, even though a statistical significance was not observed between nutrient concentrations. From this data, it is not evident which filtering method is more reliable. Filtering with a manual syringe may result in a higher likelihood of contamination due to the small size of the filters and greater likelihood that they could accidently be touched. When filtering with a peristaltic pump, discretion should be used to assure that a tear or larger hole is not created in the filter because of too much pressure (Worsfold et al., 2005). Lambert et al. (1992) also observed the formation of filter cakes during filtration that led to changes in the effective pore size of the filter. Filters should be observed after use to determine if either of these events have occurred. It is important to note that the NH_4

concentrations for both filtering methods were observed to be an order of magnitude greater than NH_4 concentrations at the same distance from the point source as seen in the samples collected for analyzing sampling location, and from the replicate samples collected from a bucket.

Dilutions made by the laboratory chemist before analysis of nutrients were consistently lower than the dilutions I made, except for NO_3+NO_2-N. Variation was also greater in the dilutions I made, which is likely due to less experience. It is possible that the differences observed between the dilutions I made and the dilutions made in the lab could be due to thawing samples multiple times before analysis. When making the dilutions, the samples were taken out of the freezer and thawed, and then placed back in the freezer. For analysis, the samples had to the thawed again. This is consistent with the data obtained from storage analysis, except TP concentrations also decreased, which was not observed in the storage analysis. It is also important to note that the difference between lab-diluted samples and the samples I diluted was sometimes an order of magnitude greater than the differences observed from multiple-freeze-thaw tests.

Analytical uncertainty proved to be less than uncertainty observed during sample collection and storage, except for unanticipated protocol failure. Protocol failure occurred due to samples being locked in an autoclave overnight before analysis, which caused some of the samples to completely evaporate. NH_4 concentrations seemed to be the most variable in the laboratory, as measured by duplicates that exceeded 20% difference in three of four analytical runs.

Freezing samples appeared to be an adequate storage method. Samples frozen for 12 weeks showed statistically significant declines in TN and TP concentrations, however these declines were less than 9% of the initial values. This is within the range of variation seen for

analytical duplicates. These results are consistent with studies done by Avanzino and Kennedy (1993), Dore et al. (1996), and Venrick and Hayward (1985). According to Gordolinski et al. (2001), a standard storage protocol cannot be designed due to different chemical and biological characteristics of different sample matrices. This is one reason why some studies have found freezing to be an inadequate storage technique for some nutrients. The study done by Fellman et al. (2008) is an example of a study that determined that freezing was not an adequate storage technique for total dissolved phosphorus.

According to this study, appropriate sampling methods should be used when collecting samples within a cross-section. One option is a composite sampling technique using an automatic water sampler to get a representative sample for the whole cross section (Facchi et al., 2007; Worsford et al., 2005). Martin et al. (1992) also observed different mixing patterns due to point-source discharges, and recommended collecting grab samples at a "representative point" in a stream, if possible, or employing automatic water samplers. This may only be necessary in locations that have low nutrient concentrations, where the coefficients of variation seemed to be the greatest; indeed our analyses showed highest variation at the location above the point source. Whitfield & McKinley (1981) observed that variability among field replicate samples was a great source of uncertainty in their study. This is consistent with the results of this study. Filtration methods should also be chosen appropriately when collecting samples for NH_4 analysis. Finally, samples should not be thawed and frozen multiple times before analysis as this has been shown to decrease nutrient concentrations.

With these considerations in mind, the anomalies from the previous research were re-observed. The previous research was conducted in late summer 2011, and TP concentrations were more than an order of magnitude greater than when I collected samples in 2013. Stream

flow was also more variable during sampling in 2011, varying from 1.5 CFS to 3.5 CFS within 6 hours (USGS gauge). Comparisons of data between these sample events should consider this context. Thawing samples multiple times could have accounted for a lower total nutrient concentration than constituent concentration if the multiple thawing only occurred in the samples analyzed for total concentrations, but not constituent concentrations. Multiple thawing events could affect total nutrient concentrations differently than constituent concentrations, but this was not considered in this study. These anomalies could have also been due to a labeling error. The filtering technique is not a likely reason for the observed anomalies in the prior study because the anomalies are not consistent in time: samples where constituent nitrogen concentrations were higher than total nitrogen occurred early in the study (3 instances), while constituent phosphorus samples were higher later in the sample collection (7 instances). In addition, NH_4 concentrations were low in comparison to NO_3+NO_2 and TN concentrations and my research found that NH_4 could be influenced by filtration technique, and was generally the most variable constituent.

Conclusion and Future Research

Comparison of coefficients of variation (CV's) of samples collected within a cross section at four locations, above, at, and below a point source, revealed that the mixing patterns of nutrients within a cross section appeared to be different at the different locations. This makes choosing a representative grab sample difficult, as the locations for taking such samples would be different in each cross-section. If determining a location for taking a representative grab sample proves to be too difficult, employing an automatic sampler, which would account for flow rates when taking samples, may be wise. The filtration devices analyzed were an electric pump and a manual syringe. These two devices gave statistically similar results in NH_4, NO_3+NO_2, and SRP concentrations ($p>0.05$). The data collected here do not indicate which filtering device proved to be the most reliable in filtering samples for NH_4 concentrations, but syringe filtered samples were 1.5 times greater than electric-pump filtered samples, even though this was not a statistically significant difference, and could be a source of future research. Dilution error was determined by comparing seven diluted samples with the original sample with which they were made. Dilutions proved to have the highest uncertainty relative to other treatments. The diluted samples were consistently higher than the original sample for all nutrients and were more variable than lab QA/QC duplicates for ammonium and soluble reactive phosphorus. These differences may have been due to multiple thawing and freezing events, and is another area for future research. Analytical uncertainty was found to be less than uncertainty associated with sample collection and storage except for unanticipated protocol failure. For this study, QA/QC data beyond 20% were considered fails, and the samples required reanalysis. In most cases the percent recovery of spiked samples and coefficients of variation of samples repeatedly analyzed were much less than 20%. However, NH_4, TN, and TP incurred the most

failures. Freezing samples appeared to be an adequate storage method. Samples frozen for 12 weeks showed statistically significant declines in TN and TP concentrations ($p<0.05$), however these declines were less than 9% of the initial values. This is within the range of variation seen for analytical duplicates. However, freezing may only be an adequate storage technique for a certain amount of time if the nutrient concentrations continued to decline with time.

While this research was informative, there are additional areas of research that are needed. Specifically, thawing samples multiple times proved to decrease TN concentrations, but not TP concentrations. This research did not test whether NH_4, NO_3+NO_2, or SRP also decreased as a result of multiple thaws. More research in the future is needed to test the effects of multiple thaws on these constituent nutrient concentrations. Additionally, a repeat sampling event could also be performed to determine if higher nutrient concentrations do confer less analytical variation as hypothesized above. This research is believed to add to the literature concerning sources of uncertainty in nutrient sampling, both in the findings revealed and in the areas of future research identified.

Reflective Writing

My experience completing this thesis was an overall positive experience. I would highly recommend that future students planning on completing an Honors thesis in Biology, or any other science, begin the research process early during their undergraduate studies. I began searching for research positions the summer of my freshman year, and took a laboratory technician position at the Utah Water Research Laboratory under Dr. Bethany Neilson the fall semester of my sophomore year. I was only a laboratory technician for one semester, as Dr. Neilson suggested an idea for an undergraduate research project, and recommended that I gain some laboratory experience in Dr. Michelle Baker's Aquatic Biogeochemistry Laboratory. Dr. Neilson helped me craft my thesis topic the spring semester of my sophomore year. During the summer after my sophomore year, I was a participant in the iUTAH (innovative Urban Transitions and Aridregion Hydro-sustainability) undergraduate research fellows (iFellows) program. In this program, I collaborated with iFellows in bi-monthly meetings, where we discussed our individual research projects, and also heard from panels of researchers who discussed their research careers. We also discussed poster and oral presentation techniques, and the research program culminated in a poster session at the annual iUTAH meeting on the University of Utah campus, where I presented preliminary research results obtained that summer. During the process of this research program, I collected sources for writing a literature review, as well as organized a sample collection plan with the advice of Drs. Baker and Neilson as to where the samples would be collected, the techniques that would be used for sample collection, and the number of samples that would be collected for each source of uncertainty tested. Before collecting the samples for my project, I assisted graduate students in Dr. Baker's laboratory with fieldwork, where I learned the techniques that I used in collecting my samples. I was also able to

learn from their organization of supplies needed to conduct sampling, and the value of accurate record keeping.

I collected the samples for this thesis in early June 2013, accompanied by Dr. Baker and graduate students Elizabeth Ogata and Andrew Hobson. The sample collection site was chosen based on the previous research anomalies that led to my thesis. This team was very helpful in every aspect of the sample collection process, as they had a great deal of experience doing this type of research. After the sample collection process, I was shown how to complete analysis forms in order for my samples to be run by the Aquatic Biogeochemistry Laboratory chemist. I also converted all my written sample records to computer records for better archiving.

Once I began receiving the sample analyses towards the end of the summer and into the beginning of fall semester 2013 of my junior year, Dr. Baker introduced me to a statistical program used within Excel that was very helpful in the statistical analysis of my samples. I used this program to conduct the simple statistical analyses, such as one-way ANOVAs. I consulted with Ms. Susan Durham, the ecology center statistician, for the more difficult statistics, such as the nonparametric bootstrapping analyses. Ms. Durham was very helpful in researching statistical techniques that could be used in analyzing the samples collected for the sampling location source of uncertainty. She was also very helpful in explaining the statistical techniques she found. I took the course Statistics for Scientists (STAT 3000) the fall semester of my sophomore year, which I found to be very helpful in understanding the statistics used in this thesis.

I enrolled in the Biology program Undergraduate Research course (BIOL 5800) the fall semester of my junior year, during which time I conducted the statistical analyses discussed

above. At the end of this semester, I participated in a poster presentation alongside others enrolled in BIOL 5800, and gained valuable experience presenting my research results.

From the sample statistical analyses, I wrote a report in January 2014 for Dr. Neilson to submit to the Utah Department of Water Quality, who provided financial support for this project. This report served as the starting point of my written thesis. I was also able to present my results at a poster presentation at the National Conference for Undergraduate Research in Lexington, Kentucky in April of 2014. This conference afforded me additional experience in presenting my research, and discussing my results in a conference setting.

In the fall semester of 2014, my senior year, I enrolled in the Biology thesis course (BIOL 5810), and wrote a thesis proposal to submit to the Honors office. After the proposal was submitted, I began expanding my written report submitted to the Utah Division of Water Quality, to include a literature review with the sources collected during the summer when I initially began my research. At the end of this semester, I submitted an initial draft to Dr. Baker. Dr. Baker and I exchanged a few drafts spring semester of 2015 before sending a draft to Ms. Durham and Dr. Neilson to receive additional comments. At the end of the process of exchanging drafts, my defensible thesis was submitted to the second member of my thesis committee, Nancy Mesner. With comments from both thesis committee members, I finalized my Honors thesis. My thesis defense occurred at the Student Research Symposium on April 9, 2015, where I gave an oral presentation.

Throughout this process, the most difficult aspects included the statistical analyses of my results, and writing these results in an informative, concise manner. My research mentors, Drs. Baker and Neilson, were very helpful in this process in answering the many questions that arose throughout experimentation and analysis. The organization of my sampling plan was an easy

aspect of this process, because I was able to observe graduate students conduct similar fieldwork. I believe my experience writing this thesis was positive due to spreading the process over two years. I was able to complete different portions of my thesis in a timely manner without being overwhelmed by time constraints, and I gained experience presenting my results during multiple poster sessions. I highly recommend that future students planning on writing a scientific thesis begin the process early in their undergraduate career.

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Author Biography

Alexandria 'Hayden' Campbell majored in Biology with a cellular/molecular emphasis and minored in chemistry. During her undergraduate career, she received the Thomas M. Farley Chemistry Award given to top General Chemistry students, as well as seven academic scholarships. She worked as a research assistant in two laboratories at Utah State University, including the Utah Water Research Laboratory under Bethany Neilson and the Aquatic Biogeochemistry Laboratory under Michelle Baker. She also volunteered for Rocky Mountain Home Care & Hospice. Hayden graduated with Honors in Biology in Spring 2015. She is excited about a future career in health care.

Appendix A. Descriptive Statistics

TP:

CV	22.4%	2.3%	8.7%	10.2%
	Above	Point Source	Below I	Below II
Above	X	0.023	0.114	0.179
Point Source	X	X	0.019	0.026
Below I	X	X	X	0.654
Below II	X	X	X	X

TN:

CV	6.5%	16.8%	18.1%	6.6%
	Above	Point Source	Below I	Below II
Above	X	0.086	0.069	0.794
Point Source	X	X	0.841	0.018
Below I	X	X	X	0.027
Below II	X	X	X	X

NH4:

CV	25.2%	13.1%	37.0%	61.8%
	Above	Point Source	Below I	Below II
Above	X	0.068	0.354	0.068
Point Source	X	X	0.093	0.262
Below I	X	X	X	0.403
Below II	X	X	X	X

NO3:

CV	69.0%	1.8%	2.4%	1.6%
	Above	Point Source	Below I	Below II
Above	X	0.012	0.013	0.011
Point Source	X	X	0.127	0.473
Below I	X	X	X	0.072
Below II	X	X	X	X

SRP:

CV	31.3%	1.3%	3.9%	3.2%
	Above	Point Source	Below I	Below II
Above	X	0.015	0.016	0.013
Point Source	X	X	0.029	0.032
Below I	X	X	X	0.273
Below II	X	X	X	X

Note: (a) x is included in the table where no comparison is needed (i.e. the CV for above does not need to be compared to itself) or where value is already provided in the table and (b) red values indicate a statistical significance.

P-values computed from nonparametric bootstrap analyses comparing two locations.